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***Design and synthesis
of metal complexes and covalent organic frameworks
for technological, catalytic and biological applications***

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Abstract

This study explores the synthesis, characterization, and applications of metal-containing coordination compounds and covalent-organic frameworks (COFs) in electrochemical sensing, catalysis, and biology. Novel Cu(II), Cu(I), and Ag(I) coordination complexes incorporating phosphane and phenoxy-ketimines ligands were synthesized, and their chemical and antibacterial properties were evaluated. Additionally, CuI/Schiff base complexes were employed as catalysts for the three-component A³-coupling reaction to synthesize propargylamines. Further investigations into pyrazolyl-derived ligands led to the development of novel Cu(I)/Cu(II) complexes with modified acetylacetone ligands, assessed for their chemical structures.

In the field of electrochemical sensing, a “signal-inversion” photoelectrochemical aptasensor was designed based on a semiconductive Cu_{2.46}Co_{0.54}(HITP)₂ MOF and integrated with the manganese ferrite nanozyme-regulation for the selective detection of fumonisin B1. Additionally, a donor-acceptor triazine-containing COF-based electrochemiluminescent immunosensor was developed for trace detection of zearalenone, utilizing a signal “on-off-on” strategy and a CuFe₂O₄@PDA quencher. Finally, a trinuclear copper cluster-based COF with a high content of Cu-N₂ single-atom sites was synthesized and employed in a photoelectrochemical aptasensor for zearalenone detection, incorporating multivariate signal amplification strategies to enhance sensitivity.

These findings contribute to advancing coordination chemistry and electrochemical biosensing, providing innovative materials for catalytic applications and food safety monitoring.

Key words

Copper and silver complexes; coordination chemistry; covalent organic frameworks; antibacterial; A³-coupling reaction; electrochemical sensing.

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Fig. 7.1 (a) Schematic illustration of the synthesis procedure of $\text{Cu}_3\text{L}_3\text{-TAPT-COF}$. (b) TEM, (c) HR-TEM (inset: selected area electron diffraction), (d) HAADF-STEM image, (e) EDS mapping [Cu (yellow), C (red), and N (green)], (f) PXRD pattern, (g) eclipsed stacking, high-resolution XPS spectra of (h) Cu 1s, (i) C 1s, and (j) N 1s of $\text{Cu}_3\text{L}_3\text{-TAPT-COF}$.

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Fig. 7.9 (a) PEC responses of each step for the construction of the Cu_3L_3 -TAPT-COF-based PEC aptasensor and the detection of ZEN (20 ng mL^{-1}), including (i) the bare GCE, (ii) Cu_3L_3 -TAPT-COF/GCE, (iii) ssDNA/ Cu_3L_3 -TAPT-COF/GCE, (iv) MCH/ssDNA/ Cu_3L_3 -TAPT-COF/GCE, and (v) Hs/ ZnLn_2S_4 /MCH/ssDNA/ Cu_3L_3 -TAPT-COF. (b) The quantitative analysis of different concentrations of ZEN (0.1 pg mL^{-1} - 20 ng mL^{-1}).

Fig. 7.10 (a) The selectivity of Cu_3L_3 -TAPT-COF-based PEC aptasensor using FB1, OTA, AFB1, AFM1, Glu, Gly, and as interferents (10 pg mL^{-1}). (b) The reproducibility of the developed PEC aptasensor for the inspection of different concentrations of ZEN (1.0 , 5.0 , and 20 ng mL^{-1}). (c) The stability of the given aptasensor for the detection of ZEN (0.1 ng mL^{-1}) (under 95% confidence band).

List of Schemes

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List of Abbreviation

2D cMOF	two-dimensional conductive MOF
Ab	antibody
acs	aluminum copper sulfide
AFM1	aflatoxin-M1
AFs	aflatoxins
Ag NPs	silver nanoparticles
Apt	aptamer
ATR	attenuated total reflection
bcu	body-centered cubic
BDC	benzene dicarboxylate
BET	Brunauer-Emmett-Teller
bex	branch exchange
bor	boracite topology
BPO	benzoyl peroxide
BSA	bovine serum albumin
C ₂ linkers	two-carbon linkers
CB	conduction band
ceq	cequalite
COF	covalent organic framework
cpi	clock per instruction
ctn	cubic truncated network
Cu ₃ L ₃	trinuclear copper cluster
DAB	3,3-diaminobenzidine
dia	diamond topology
DMA	N,N-dimethylacetamide
DMF	N,N-dimethylformamide
DON	deoxynivalenol
DPPH	1,1-Diphenyl-2-picrylhydrazyl radical
DRS	diffuse reflectance spectrum
E. coli	Escherichia coli
EA	Elemental Analyses
EABPR	enzyme-assisted biocatalytic precipitation reaction
EC	electrochemical
ECL	electrochemiluminescence
EDS	energy dispersive spectrometer
E_g	band-gap energy
ELISA	enzyme-linked immunosorbent assay
EPR	electron paramagnetic resonance

ER	estrogen receptor
ESI-MS	electrospray ionization mass spectra
ESP	electrostatic potential
FB1	fumonisin B1
FB1	aflatoxin B1
fcc	face-centered cubic
fjh	faujasite-type hexagonal
FT-IR	Fourier Transform-Infrared
GCE	glassy carbon electrode
Glu E	glutamic acid
Gly	glycine
H ₂ O ₂	hydrogen peroxide
hcb	hexagonal
HCl	hydrochloric acid
hea	heazlewoodite
HITP	2,3,6,7,10,11-hexaminotriphenylene
HR-TEM	high-resolution TEM
HSV-1	herpes simplex virus type 1
hxl	hexagonal lattice
ICP-MS	inductively coupled plasma-mass spectrometry
IUPAC	International Union of Pure and Applied Chemistry
kgd	rhombille tiling
kgm	kagome
LOD	Limit of detection
LOX	Lipoxygenases
MBC	bactericidal concentration
MBC ₅₀	Half-maximal bactericidal concentration
mbr	medium broad
MCH	6-mercapto-1-hexanol
MCOF	metal-covalent organic frameworks
MIC	minimum inhibitory concentration
MIC ₅₀	Half-maximal inhibitory concentrations
MIL	Materials of Institute Lavoisier
MOF	metal-organic framework
MP	Melting Point
NMR	Nuclear Magnetic Resonance
OD ₆₀₀	optical density 600
OMS	open metal site
OTA	ochratoxin A
OTB	ochratoxin B
PA	propargyl amine

PBS	phosphate-buffered saline
pcb	polycatenated borromean
PCN	porous coordination network
pcu	primitive cubic
PEC	photoelectrochemical
PL	photoluminescence
PPh ₃	triphenylphosphine
PTA	1,3,5-triaza-7-phospaadamantane
pts	platinum sulfide
PXRD	X-ray diffractometry
RET	resonance energy transfer
ROS	reactive oxygen species
S. aureus	Staphylococcus aureus
sbr	strong broad
scMOF	semiconductive MOF
sDNA	single-stranded DNA
soc	square-octahedral
sql	square lattice
stp	stilpnomelane
TAPB	1,3,5-tris (4-aminophenyl) benzene
TAPT	4,4',4''-(1,3,5-triazine-2,4,6-triyl)trianiline
tbo	tetragonal boron
T _d	tetrahedral units
TEM	transmission electron microscope
TFPT	2,4,6-tris (4-formyl-phenyl)-1,3,5-triazine
THF	tetrahydrofuran
TPA	tri-n-propylamine
tth	through-the-hole
UiO	Universitetet i Oslo
VB	valence band
XPS	X-ray photoelectron spectroscopy
ZEN	zearalenone
ZIF	zeolitic imidazolate framework

Chapter I: Introduction

1.1 Metal

1.1.1 Copper

1.1.1.1 Characteristics of Copper

Copper, a chemical element with an atomic number 29 and the symbol Cu, is a metal that has been a part of our civilization (**Fig. 1.1**).

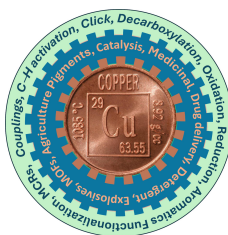


Fig. 1.1 The properties of copper.

As a transition metal, Cu exhibits excellent physical and chemical properties. In terms of physical properties, copper has a red metallic luster, is soft, has good malleability and ductility, and can be easily processed into thin sheets or drawn into fine wires. Its electrical and thermal conductivity is second only to that of silver, making it widely used in the electrical and electronic industries. Additionally, copper has a density of 8.96 g cm^{-3} , a melting point of $1084.62 \text{ }^{\circ}\text{C}$, and a boiling point of $2562 \text{ }^{\circ}\text{C}$, and it exhibits resistance to corrosion by most non-oxidizing acids. Chemically, copper is stable in dry air but readily reacts with oxygen, carbon dioxide, and water in wet environments to form a green patina (basic copper carbonate). Copper does not react with diluting hydrochloric or sulfuric, but dissolves in strong oxidizing acids such as concentrated sulfuric acid and nitric acid, forming the corresponding copper salts and gases. It also readily reacts with halogens and sulfur to form copper halides and sulfides.

Copper is an essential micronutrient for all organisms living in oxygen-rich environments. As a redox-active metal, it readily swings from the reduced Cu(I) to oxidized Cu(II) states in both chemical and physiological processes. Among them, Cu(I) is stable in the solid state or complexes, while Cu(II) is more stable in aqueous solutions. In compounds, Cu(I) ions exhibit a d^{10} electronic configuration, rendering them diamagnetic. The oxidation state of d-block elements significantly influences the stability of their corresponding complexes. Notably, all Cu(II) complexes are inherently paramagnetic due to unpaired electrons. These physical and chemical properties make

copper widely applicable in electrical engineering, materials science, catalysis, and biomedicine ^[1].

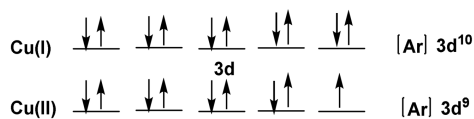


Fig. 1.2 Electronic configurations of Cu(I) and Cu(II) ions.

1.1.1.2 Copper Applications

As an essential nutrient, Cu is directly involved in various biological procedures including cell proliferation, respiration, angiogenesis, neuropeptide processing, and free radical detoxification. The metabolic process of copper in biology is related to the interconversion between Cu(I) and Cu(II) states, which is contributed by donating or accepting single electrons.

Applications of copper in catalysis

Copper can catalyze organic reactions through its ability to undergo redox transformations, participate in electron transfer processes, and form coordination complexes with organic substrates. The key features contributing to copper's catalytic ability are often associated with its variable oxidation states and the availability of different coordination environments. This redox activity allows copper to act as an electron shuttle, facilitating electron transfer reactions in various organic transformations. On the other hand, copper ions can act as Lewis acids, accepting electron pairs from organic substrates, or as Lewis bases, donating electron pairs. This versatility in electron acceptance or donation is crucial in catalyzing reactions that involve the activation of substrates through coordination, especially in carbonyl-containing functional group transformations. Finally, copper can form complexes with organic molecules, creating a conducive environment for reactions. The coordination of substrates to copper centers can stabilize reaction intermediates, lower activation energies, and consequently influence the reaction pathway.

Copper species are highly versatile catalysts for various organic transformations due to their ability to promote sustainable reactions, including coupling reactions, click chemistry, C-H activation, reduction and oxidation reactions, electrochemical reactions, asymmetric reactions and multicomponent reactions. The mechanism of C-H activation follows a systematic progression through four distinct steps in the transformation of a C-H activation substrate with a suitable reaction partner ^[2]. These steps include: (I) the C-H activation itself, (II) functionalization of the resulting organometallic species, (III)

product release, and (IV) regeneration of the active copper catalyst (**Fig. 1.3**). This sequence elucidates the key stages in the complex pathway of C-H activation. Copper catalysts exhibit high regio- and chemo-selectivity and excellent functional group tolerance. Consequently, this methodology has been successfully applied in synthesizing various heterocycles, pharmaceuticals, and natural products.

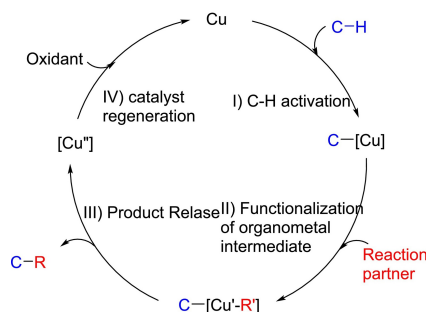


Fig. 1.3 Generalized mechanistic pathway for C-H activation catalysis.

Copper compounds as antibacterial agents

Copper-based compounds have been widely studied for their antibacterial properties, demonstrating effectiveness against both common and drug-resistant bacteria. Their antimicrobial activity primarily arises from the ability of copper ions to disrupt bacterial cell membranes, ultimately leading to cell death. This disruption occurs through multiple mechanisms, including interactions with membrane lipids and proteins, which compromise membrane integrity and destabilize bacterial cells (**Fig. 1.4**).

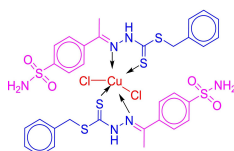


Fig. 1.4 Hydrazone-carbodithioate-Cu(II) complex.

Copper complexes in cancer therapy

The role of Cu in the progression of cancer has long been a topic of study, as Cu ions may be involved in the activation of cell proliferation-related signaling pathways. Evidence has suggested that cancer cells generally have a higher demand for Cu compared to healthy, resting cells. Furthermore, increased Cu concentrations have been reported in tumor tissues and/or serum obtained from patients with various types of cancers, including breast lungs, gastrointestinal, oral thyroids, gall bladder, gynecologic pancreatic, and prostate cancer. In addition, elevated levels of serum Cu have been correlated with both tumor stage and disease progression in patients with colorectal, lung, and breast cancer ^[3] (**Fig. 1.5**).

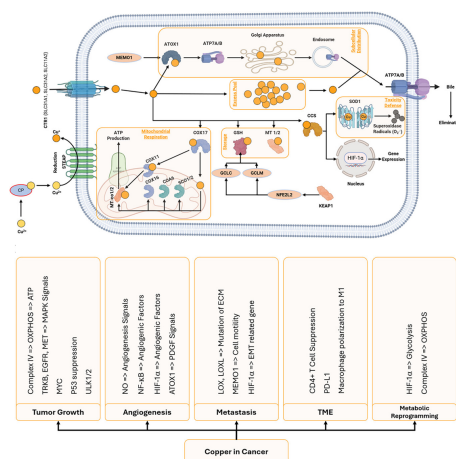


Fig. 1.5 Copper homeostasis and utilization and the key factors of copper involved in tumorigenesis.

A lot of studies investigated the putative effects of Cu on the growth of cancer cells. For instance, daily administration of Cu sulfate has been shown to increase tumor growth in a rat model of chemically induced mammary tumorigenesis [4]. Evidence has shown that Cu could activate metastasis-related enzymes and signal cascades, thus promoting the spread of cancer. For instance, the cuproenzyme Lipoxygenases (LOX) is involved in the invasion and metastasis of tumor cells. In estrogen receptor (ER) negative breast cancer, high expression of LOX is associated with bone metastasis and leads to the formation of tumor-driven osteolytic lesions [3]. The altered regulation of Cu homeostasis in tumors, coupled with the important role that copper may play in promoting the progression of cancer, has led to the development of Cu-coordinating compounds for use in anticancer therapies [5]. Two main strategies for targeting Cu homeostasis have been proposed, including the use of Cu chelators to decrease Cu bioavailability, and the use of Cu ionophores to deliver Cu into cells to increase intracellular Cu levels [4]. In addition, mixtures of copper chelators with copper salts have been found to act as efficient proteasome inhibitors and apoptosis inducers, specifically in cancer cells. Moreover, following the worldwide success of platinum(II) compounds in cancer chemotherapy, several families of individual copper complexes have been studied as potential antitumor agents. These investigations, revealing the occurrence of mechanisms of action quite different from platinum drugs, head toward the development of new anticancer metallodrugs with improved specificity and decreased toxic side effects [6].

In recent years, Cu-based nanomaterials have garnered significant attention in the biomedical field and diverse biological effects and have been successfully utilized in various advanced therapeutic and imaging applications, including chemodynamic

therapy, radiodynamic therapy, immunotherapy, and sonodynamic therapy (**Fig. 1.6**).



Fig. 1.6 The application of Cu and Cu-based nanomaterials in cancer therapy.

1.1.2 Silver

1.1.2.1 Characteristics of silver

Silver (Ag), a transition metal, is renowned for its exceptional physical and chemical properties. Physically, it exhibits a characteristic silvery-white metallic luster, high ductility, and malleability, allowing it to be easily processed into thin sheets or fine wires. Silver possesses the highest electrical and thermal conductivity among all metals, making it indispensable in the electronics industry and precision instrument manufacturing. It has a density of 10.49 g cm^{-3} , a melting point of $961.78 \text{ }^{\circ}\text{C}$, and a boiling point of $2162 \text{ }^{\circ}\text{C}$. Additionally, silver is chemically stable and does not oxidize in dry air.

Chemically, silver is relatively inert, showing no reaction to oxygen or water, which allows it to exist in nature as a pure element. However, it readily reacts with sulfur and sulfides, forming black silver sulfide (Ag_2S) upon prolonged exposure to trace amounts of hydrogen sulfide in the air, leading to surface tarnishing. Silver is insoluble in diluted hydrochloric and sulfuric acids but dissolves in oxidizing acids, such as nitric acid, producing silver nitrate (AgNO_3) and nitrogen dioxide (NO_2) gas. Furthermore, silver reacts with halogens (e.g., chlorine, bromine, iodine) to form insoluble silver halide precipitates, such as silver chloride (AgCl). Silver ions (Ag^+) exhibit strong antibacterial properties, making them valuable in medical applications and water treatment. Owing to its outstanding optical, electrical, and chemical characteristics, silver finds extensive applications in electronic components, mirror coatings, catalysis, precious metal alloys, and photographic materials.

1.1.2.2 Silver applications

Compared to other common transition metal ions, silver exhibits a broader range of coordination numbers (ranging from 2-6 or even higher) and diverse geometries, including linear, triangular planar, and octahedral structures. Additionally, it

demonstrates a significantly stronger affinity for soft-based donor ligands. Based upon the versatile superior properties of Ag-based complexes, their potentiality were further explored in a variety of applications, including gas adsorption and separation, catalysis, sensing and adsorption, energy-related applications, and antimicrobial and anticancer activity [7].

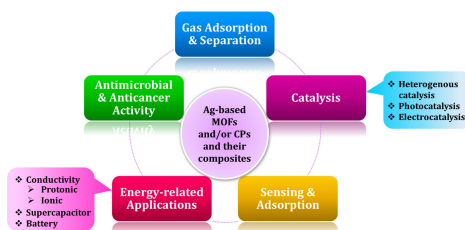


Fig. 1.7 Silver applications.

Applications of silver in catalysis

Ag-based coordination networks featuring closed shell d^{10} Ag-metal ions provide great promise in many Lewis acidic catalytic reactions by their abundant OMSs. Ag(I) ions as soft π -Lewis acids display unique interaction between Ag^+ and $\text{C}\equiv\text{C}$ bonds, which enhances their catalytic activity in the sequential cyclization of propargylamines. The versatile coordination geometries of Ag(I) including linear, seesaw, Y-type, T-type, square pyramidal, and trigonal bipyramidal also provide better compatibility for a wide range of substrates when forming Ag- π intermediates. Besides, the presence of two or more Ag-Ag interactions stimulates substantial interest in developing multinuclear platforms for cooperative catalysis, thus making them a good alternative in many organic catalytic transformations (**Fig. 1.7**).

Furthermore, Ag-based frameworks are also capable of displaying excellent photocatalytic activity and photosensitivity due to their narrow band gap and are often used as efficient photocatalysts for promoting the separation of electron-hole pairs and enhancing the visible light absorption using the surface plasmon resonance effect. In addition, silver possesses relatively higher electrocatalytic activity than platinum group metals. The higher reactivity, cost-effectivity, and greater abundance make it worthy as an alternative catalyst for oxygen reduction reaction in alkaline electrolyte solution.

Silver compounds as antibacterial agents

Silver has a long history in the medicinal field. In 2004, Youngs's group synthesized the first-generation Ag(I)-carbene complexes (**Fig. 1.8**) and found their minimum inhibitory concentration (MIC) against *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), and *Pseudomonas aeruginosa* (*P. aeruginosa*) better

than AgNO₃, even at a much lower concentration. This first success led to a flourishing development in Ag(I) antibacterial agents [8].

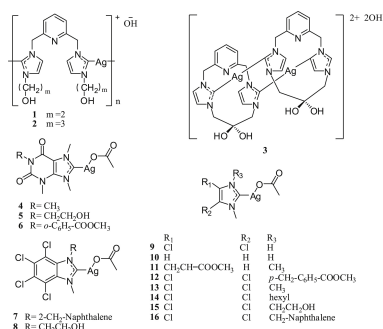


Fig. 1.8 The silver-complexes prepared by Youngs's group were investigated as antibacterial agents.

In addition, silver nanoparticles (Ag NPs) exhibit interactions with various bacterial strains, highlighting their significant potential for antibacterial applications. Researchers have extensively discussed the antibacterial mechanisms of Ag NPs, as shown in (Fig. 1.9). Ag NPs are known to anchor themselves to bacterial cell walls and penetrate them due to their unique physicochemical and biological properties. This penetration can damage the membrane, potentially causing cell content leakage and bacterial death. Variations in cell wall thickness, mainly composed of peptidoglycan, account for the difference in susceptibility between Gram(+/-) bacteria to Ag NPs. The bacterial cell membrane carries a negative charge due to carboxyl, phosphate, and amino acid groups. This feature results in an electrostatic attraction between the positively charged Ag NPs and negatively charged bacterial cell membranes, facilitating the binding and penetration of Ag NPs into the bacteria [9].

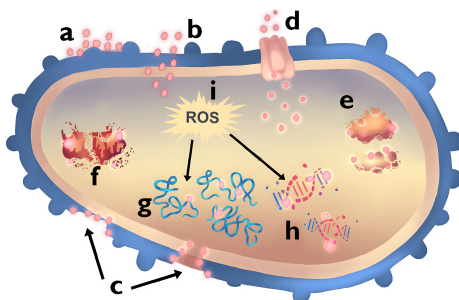


Fig. 1.9 Mechanisms of antibacterial action of Ag NPs: (a) adhesion of Ag NPs to the cell wall, (b) cross the membrane; (c) damage to the cell wall and membrane induced by Ag NPs; (d) entry of Ag NPs into the bacterial cell is facilitated by porin proteins, which serve as conduits for their passage; (e) disassembly of ribosomes triggered by Ag NPs; (f) mitochondrial dysfunction caused by Ag NPs; (g) protein denaturation induced by Ag NPs; (h) DNA damage inflicted by Ag NPs; and (i) reactive oxygen species (ROS) production and oxidative stress induction resulting from Ag NPs interaction..

Silver compounds as virucidal and anticancer agents

In 2021, Kirillov, Smoleński et al. reported a quinolinic acid-based complex, [Ag(Hquin)(μ-PTA)]*n*·H₂O, which exhibited remarkable antibacterial, antiviral, and

antiproliferative activity ^[10]. The framework displayed excellent virucidal efficacy against enveloped herpes simplex virus type 1 (HSV-1) and non-enveloped adenovirus 36 (Ad-36) with $\geq 99.99\%$ killing efficiency, which indicated that $[\text{Ag}(\text{Hquin})(\mu\text{-PTA})]_n \cdot \text{H}_2\text{O}$ can be employed as clinically important drug or disinfectant material. The obtained MIC values of $20 \mu\text{g mL}^{-1}$ for *Escherichia coli* and *Pseudomonas aeruginosa*, and $30 \mu\text{g mL}^{-1}$ for *Staphylococcus aureus* highlighted the significant antibacterial activity of $[\text{Ag}(\text{Hquin})(\mu\text{-PTA})]_n \cdot \text{H}_2\text{O}$. By the combination of biocidal metal nodes and water-soluble aminophosphine linker with quinolinat ligand as an important metabolic intermediate, the present compound extends the family of bioactive silver(I) coordination polymers (**Fig. 1.10**).

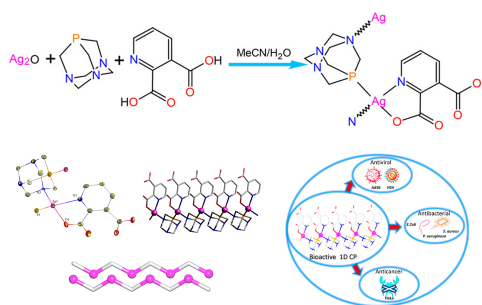


Fig. 1.10 Simplified representation for the synthesis of $[\text{Ag}(\text{Hquin})(\mu\text{-PTA})]_n \cdot \text{H}_2\text{O}$.

Silver(I) coordination compounds are effective against various bacteria, fungi, protozoa, and several cancer cell lines, evidencing in the latter case anticancer mechanisms that share the same biological pathways as the antibacterial ones and for these reasons are completely different from those at the basis of cisplatin cytotoxicity. To date, only a few studies have been reported on the effects of silver(I) complexes on tumor xenograft murine models, resulting in decreased growth of the tumor mass or cell death. The cytotoxic mechanisms of silver ions are based on a series of damages caused by Ag^+ to the bacterial or cancer cells ^[11].

1.2 Ligands

1.2.1 Schiff base ligands

As previously noted, Hugo Schiff reported the first synthesis of imines or azomethine in 1864 ^[12], introducing a new class of organic compounds. In his honor, these groups of compounds, imines, are frequently known as the Schiff bases. The simplicity of synthesis and complexation of Schiff bases has attracted the interest of researchers since of their excellent thermal, mechanical, electrical, optical,

optoelectronic, and fiber-forming capabilities. Conjugated Schiff base polymers and oligomers have been extensively investigated during the last two decades. In 1866, Schiff, employing Fuchsin as a Schiff reagent, analyzed various aldehyde-fuchsin; these compounds had two dye molecules and three aldehyde molecules. According to Schiff's suggestions, similar coloring properties were exhibited by acetaldehyde-fuchsin and Gomori's aldehyde-Fuchsin. In addition, Fuchsin was reportedly widely applied for industrial dyes over the last years of the 19th century. The chemical structure of the Basic Fuchsin Schiff base is presented in **Fig. 1.11** [13, 14].

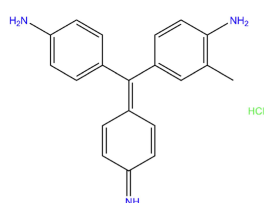


Fig. 1.11 Chemical structure of Fuchsin.

One of the most studied Schiff base ligands is a Salen ligand system, first reported in 1933 by Pfeiffer et al [15]. Condensing salicylaldehyde compounds with diamine derivatives produces 2 nitrogen and hydroxyl group molecules, resulting in Salen Schiff base compounds [16]. Since these compounds exhibit tetradentate coordination modes, they are categorized as Salen ligands, and some of them are shown in **Fig. 1.12**. Furthermore, the Salen Schiff bases may stabilize metallic ions in various oxidation states, providing potentially promising catalyst candidates.

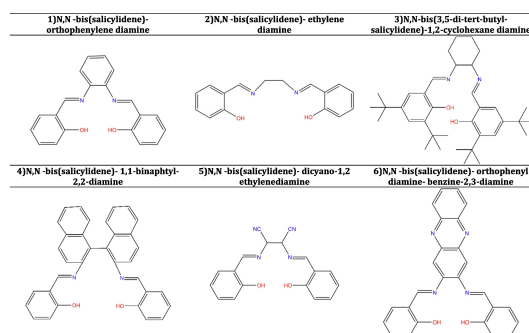


Fig. 1.12 Chemical structures of some Schiff bases.

In the classic Schiff bases synthesis, a carbonyl compound and an amine are condensed at varied processing parameters and solvents. Thus, dehydrating factors speed up the condensation process. Since its discovery, the Schiff base ligands reactions involving acceptable aldehydes and ketones using suitable amines have been frequently employed [17]. As demonstrated in **Fig. 1.13**, Schiff bases synthesis includes water elimination during the aldehyde or ketone condensation process (either aliphatic or aromatic) with a primary amine [18]. Mostly, this process achieves high yields of aryl

ketone and arylamine, but mostly low yields for alkylamine. Many Schiff bases have been synthesized using this process, which has seen significant applications ^[19].

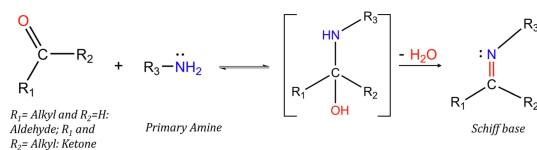


Fig. 1.13 Typical formation mechanism of a Schiff base.

1.2.2 -Substituted Acetylacetone ligands

β -Diketone compounds represent a very important class of reagents in synthetic chemistry ^[20] with a well-established role in the synthesis of heterocyclic compounds. The biological effects of these compounds are of great interest, and they have also been investigated as potential antiviral agents ^[21]. In this regard, it is often noted that even modestly sterically hindered β -diketones offer improvements over the parent acetylacetone ^[22]. The presence of steric bulk on β -diketones is of high interest for their peculiar coordination behavior useful to improve their catalytic activity and selectivity ^[23].

On the other hand, α -monosubstituted β -diketones exhibit a range of reactivities depending on the nature of the substituents attached to the α -carbon atom. The presence of the α -substituent significantly influences the behavior of these compounds, affecting their stability, reactivity, and reaction pathway. There are three possible tautomeric isomers for symmetric and α -monosubstituted β -diketones (**Fig. 1.14**). Many factors influence the keto-enol tautomeric equilibrium, and among them it was found that electronegative β -substituents (R groups in **Fig. 1.14**) sharply increase the degree of enolization, whereas α -substituents (Z group in **Fig. 1.14**) cause a marked decrease in the percentage of enol. However, the cause is mainly steric-repulsion between the α -moiety and the other β -substituents, which makes the chelate ring of the cis-enolic form unstable. On the other hand, for 3-substituted-2,4-pentanediones, the keto form is predominant in the presence of bulky substituents in the 3-position. Ditopic ligands based on 3-monosubstituted acetylacetone with two distinctly different coordination sites allow for assembling cations to coordination polymers, and thus add degree of freedom to these complex solids ^[24]. Substituted acetylacetonates have often been used in this context and 3-ciano ^[25], 3-diazo ^[26], 3-halogen ^[27], 3-caged phosphine ^[28], 3-(4-methylthiophenyl) ^[29] and 3-pyrazolyl ^[30] substituents, capable of supporting secondary interactions through exocyclic atoms with metal cations, have been described, and the

subject has recently been reviewed.

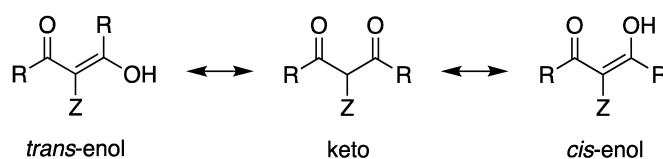


Fig. 1.14 Keto-enol tautomeric equilibria in β -dicarbonyl compounds.

1.2.3 Phosphane ligands

With the synthesis of the first stable organophosphorus species featuring phosphorus-carbon multiple bonds at the beginning of the 1960s, the research field of these so-called “low-coordinate phosphorus compounds” emerged rapidly and even nowadays still attracts a lot of interest ^[31]. The generic structures of 1-phosphinines, 1,4-diphosphinines and 1,3,5-triphosphinines were shown in **Fig. 1.15**.

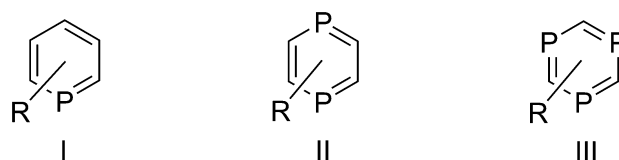


Fig. 1.15 Generic structures of 1-phosphinines, 1,4-diphosphinines and 1,3,5-triphosphinines.

1,3,5-Triaza-7-phosphaadamantane (PTA) is a water-soluble phosphane ligand with a cage structure containing one phosphorus atom and multiple nitrogen heterocycles, endowing it with good hydrophilicity and hydrogen bonding interactions. Its pK_a value is high ($\sim 8\text{--}9$), indicating moderate basicity and facilitating the formation of stable metal complexes ^[32]. Triphenylphosphine (PPh_3) is a lipophilic ligand with a strong π -acceptance ability that stabilizes low-valent metal centers. PTA is frequently used to construct water-soluble metal complexes due to its high-water solubility and moderate basicity, and it has demonstrated significant potential in antitumor, antibacterial, and biocatalytic applications (**Fig. 1.16**) ^[33]. Due to its strong lipophilicity, PPh_3 enhances the cell membrane permeability of metal complexes, making it a key focus in the development of targeted mitochondrial anticancer drugs, free radical scavengers, and biocatalytic systems. As common phosphane ligands, both have demonstrated distinct advantages in various biomedical applications ^[34, 35].

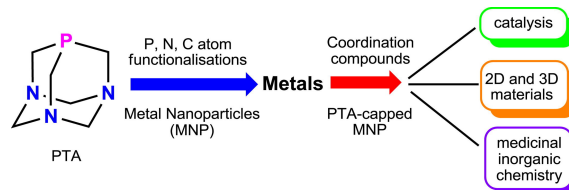


Fig. 1.16 The water-soluble ligand PTA and its application in coordination chemistry.

Studies have shown that compounds conjugated to lipophilic cations, such as

Ph_3P^+ , can accumulate in the mitochondria driven by plasma membrane potential and mitochondrial membrane potential. Thus, Ph_3P^+ has been used as a mitochondrial targeting moiety. Furthermore, the generation of new coordination networks by applying the water-soluble cage-like PTA is a promising research direction. The presence of four potential donor atoms (one P and three N) within the adamantane cage provides a good platform for creating various coordination environments around metal centers. Recent examples of water-soluble silver-PTA derivatives demonstrate that PTA can be applied as a convenient *N,P*-building block in the construction of bioMOFs or CPs (**Fig. 1.17**) [36].

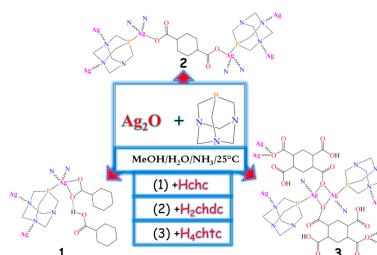


Fig. 1.17 Simplified synthetic procedure and structural formula for compounds.

Recently, Pellei's group investigated the potential of small ester derivatives of bis(pyrazolyl)acetates species to form Cu(I) complexes and their potential applications as anticancer agents (**Fig. 1.18**). Bis(pyrazol-1-yl)acetic acid ($\text{HC}(\text{pz})_2\text{COOH}$) and bis(3,5-dimethyl-pyrazol-1-yl)acetic acid ($\text{HC}(\text{pz}^{\text{Me}_2})_2\text{COOH}$) were converted into the isopropyl ester derivatives L^{OiPr} and $\text{L}^{2\text{OiPr}}$, respectively, and they were used for the preparation of Cu(I) phosphane complexes. In particular, the lipophilic PPh_3 and the hydrophilic PTA, able to stabilize copper in +1 oxidation state, were selected as co-ligands to confer a different hydrophilic-lipophilic balance to the corresponding complexes [37].

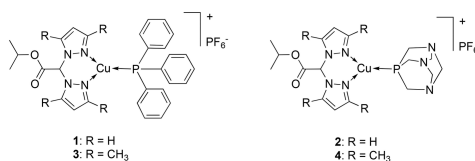


Fig. 1.18 The chemical structures of Cu complexes as potential anticancer agents.

1.3 Metal complexes

Metal complexes represent chemical entities formed through the coordination of metallic elements with ligands via coordinate covalent bonds. Typically, the metallic constituents in such complexes are drawn from the category of transition metals, classified into the d-block and f-block elements within the periodic table. The d-block

metals encompass elements belonging to the 3d, 4d, and 5d series, while the f-block metals comprise the lanthanides and actinides. Ligands, on the other hand, encompass a diverse array of species, including simple ions, molecules, or chelating groups, which establish attachments with central metallic elements, often in the form of metal ions or atoms. These ligands function as Lewis bases, serving as electron-pair donors in the formation of coordinate covalent bonds. In this chemical interaction, the central metal element acts as Lewis acid, accepting electron pairs from the ligands. Ligands can manifest as cations (e.g., NO^+ and N_2H_5^+), anions (e.g., CN^- , Cl^- , and OH^-), or neutral molecules (e.g., H_2O and CO). They may occur as identical ligands within a complex, as exemplified by carbon monoxide (CO) in the iron pentacarbonyl complex $[\text{Fe}(\text{CO})_5]$ or water (H_2O) in the hexa-aqua nickel(II) complex $[\text{Ni}(\text{H}_2\text{O})_6]^{2+}$. Alternatively, complexes can incorporate diverse ligands, such as CO and NO in the cobalt tricarbonyl nitrosyl complex $\text{Co}(\text{CO})_3(\text{NO})$. Depending on the mode of attachment, ligands can be categorized as monodentate (binding via a single atom) or bidentate/polydentate (involving attachment through two or more atoms).

1.3.1 Application of metal complexes in the field of catalysis

The coordination of ligands with different transition metals leads to the formation of stable complexes that can serve as active catalytic species in various organic transformations. Their unique physicochemical properties, electronic configurations, and steric arrangements make them highly attractive for diverse applications. In recent years, metal complexes have garnered significant attention due to their broad applications in catalysis, materials science, and medicinal chemistry [38]. For example, the Schiff-base complexes of V, Mn, Fe, Co, Ni, Cu, and Zn ions were utilized as catalysts for the peroxidation of several alkenes, including limonene, cyclohexene, styrene, trans-stilbene, verbenone, cis-stilbene, linear alkene, cyclooctene, α -methyl styrene, and α -pinene. The different Schiff-bases and various types of oxidants were explored [39]. In addition, because of their distinct structural characteristics and chemical capabilities, Schiff-base complexes function well as catalysts for different organic processes, including asymmetric synthesis, aldol reactions, and Diels-Alder reactions [19]. Jain's group reported a method for one-pot synthesis of propargylamine via a multicomponent coupling reaction. The aminoacidic ionic liquid Schiff base, derived from DL-threonine and salicylaldehyde, was complexed with Cu(II) ions and employed as an efficient, reusable catalyst for the A^3 -coupling reaction of aldehydes, amines, and

alkynes (**Fig. 1.19**). The synthesized Schiff-based catalyst, exhibited stable activity over multiple cycles, and demonstrated excellent catalytic performance ^[40].

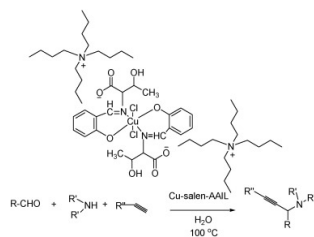


Fig. 1.19 Copper catalyzed A³-coupling reaction.

1.3.2 Application of metal complexes in the field of biology

The application of coordination compounds in the medical field dates to the XVI century with some isolated reports about the therapeutic importance of metals and their compounds in the treatment of various diseases, including cancer. Nowadays, the number of commonly prescribed metal-containing compounds is very broad and includes antimony (antiprotozoal), bismuth (antiulcer), iron (antimalarial), gold (antiarthritic), silver (antimicrobial), platinum (anticancer), and vanadium (antidiabetic), among others. Most biological molecules, such as DNA or proteins, are rich in electron density, while metal ions lack electrons. For that reason, we can observe a marked trend in which metal ions interact with various molecules of biological interest. Furthermore, metal ions also have a great affinity for various tiny molecules, such as molecular oxygen, which are essential to life. During the last sixty years, several classes of new metal derivatives, mainly organometallic and coordination compounds have been intensively investigated as possible anticancer and/or antitumor agents based on a great variety of metals. Among these, transition metals, transition metal compounds can interact with cellular structures, particularly with DNA, thereby inducing damage and cell death. Coordination compounds of transition metals have been designed specifically to inactivate therapeutically important targets through various mechanisms. These mechanisms include the generation of ROS, enzyme inhibition, as well as intercalation of DNA or the formation of DNA adducts. The above interactions lead finally to cell death, depending on the oxidation state of the metal and the steric effect of the ligands.

1.4 Electrochemical (EC) sensors

A sensor is an instrument that detects specific physical or chemical changes,

generating an output typically in the form of electrical or optical signals. Generally, a sensor comprises four main components: the receptor, the transducer, the signal-processing electronics, and the signal display unit ^[41]. The receptor, which is the sensing part of the sensor, is a sampling area where the surface chemistry occurs. It typically includes a chemo-selective coating that induces a change in the property of the receptor, such as redox potential, pH, temperature, light, and so on, upon adsorption of the analyte. This change is then recorded by the transducer, which is the measuring part of the sensor. The transducer converts the energy variation associated with the receptor's property change into a proper analytical signal, that may be electrical, optical, thermal, or chemical. The signals processing electronics, the third sensor component, interprets the signal generated by the transducer and performs the necessary operations to make it worthwhile for display and analysis. Finally, the signal display unit presents the data generated by the sensor in a meaningful and understandable way. Differences in the sensing element give rise to various sensor types, such as fluorescence, luminescence, and electrochemical ^[42].

Electrochemical (EC) sensors employ electrodes as transducers to detect chemical changes. A fundamental design structure of a typical electrochemical sensor is shown in **Fig. 1.20**. EC sensors are grounded in the principles of electrochemistry, and primarily function to detect and quantify specific analytes in a sample through interactions between the analyte and an electrode, resulting in a discernible electrical signal ^[43]. Furthermore, if the receptor is of biological origin (e.g., DNA, antibodies, and enzymes), the device is referred to as a biosensor. EC biosensors can be divided into two main categories based on the nature of the biological recognition process, i. e. biocatalytic sensors and affinity sensors. Biocatalytic sensors utilize catalytic activity to generate the signal, as in the case of enzymatic, DNAzyme, or functionalized surfaces that can perform redox reactions under certain conditions of biological components. Affinity sensors, instead, use the selective and strong binding of biomolecules such as antibodies, membrane receptors, and oligonucleotides, to produce a measurable electrical signal with a target analyte ^[44]. Known for superiorities in high sensitivity, low cost, fast response, simple equipment, and easy miniaturization, EC sensors have been recognized as versatile analytical tools over the past few decades and remain competitive in the face of growing demands in life analysis, environmental monitoring, and food inspection ^[45].

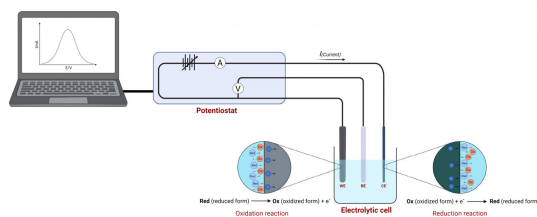


Fig. 1.20 Overview of the fundamental design of an electrochemical sensor.

1.4.1 Photoelectrochemical (PEC) sensor sensing technique

The Photoelectrochemical (PEC) identification strategy is a brand of developing pioneering analysis platforms based on converting photo energy to electrical energy. Compared with traditional EC sensing platforms, the PEC sensor method has combined the electrocatalytic process and photocatalytic reaction, which is regarded as a newly emerged approach for analyzing pollutants [46]. The main components of a typical PEC sensor include an excitation light source, a detection system with electrolytes and electrodes, and a signal acquisition system, as shown in **Fig. 1.21**. Generally, PEC sensors record two types of detection signals: potential and current. Voltage-type PEC sensors have the advantages of easy measurement and detection; however, they can easily react with reducing substances in the solution to produce false-positive or false-negative signals, which reduces their anti-interference ability. Therefore, in recent years, current-based PEC sensors have been widely used for signal generation, which responses rapidly to light and are suitable for fast and dynamic monitoring [47, 48].

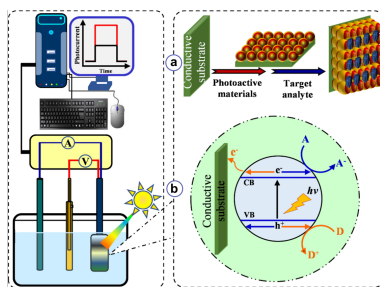


Fig. 1.21 The PEC sensing (a) working principle and the (b) mechanism.

The photoelectrochemical process consists of the absorption of incident photons, electron-hole excitation, and charge separation followed by charge carrier collection and interfacial electron transfer processes. The interactions between the sensing material and light result in photocurrent/photovoltage generation. Photoelectrochemical sensors are designed most often for work in standard three-electrode systems [49]. The photoelectric occurs takes place on the working electrode, which is coupled with the counter and reference electrodes. Charge carriers photogenerated in a semiconductor-based electrode can generate anodic or cathodic photocurrents by moving toward a

conductive substrate or toward the counter electrode, respectively. Charge carriers pass through the electrolyte, where the donor/acceptor species undergo reduction or oxidation reactions. The analytes present in electrolytes can also participate in charge transfer by changing the charge transfer path. Depending on the unique properties of the molecule, the observed current/voltage signal may be disturbed in several ways. The photocurrent/photovoltage signal may be amplified, attenuated, or even polarization direction changes are possible. The overall process consists of partial charge transfers at the analyte/sensing material and then at the sensing material/conducting substrate interfaces. Thus, the only complex characterization of sensing material and analyte properties, knowledge of environment properties, and understanding of all processes occurring at several interfaces allow the design of sensors of proper photoelectrochemical performance. Three main mechanisms can be distinguished as operating bases of photoelectrochemical sensing: charge transfer, energy transfer, and reactant determinant mechanisms, as shown in **Fig. 1.22** [50].

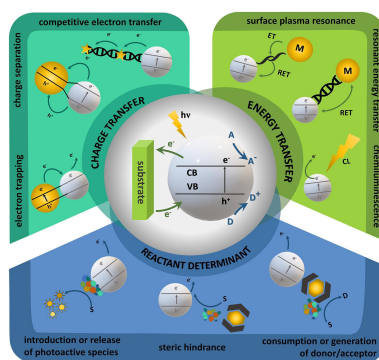


Fig. 1.22 Strategies for creating photoelectrochemical sensors.

1.4.2 Electrochemiluminescence (ECL) sensing technique

Electrochemiluminescence (ECL) is a light emission triggered by electrochemical stimulation. Under voltage, the luminophore near the electrode surface undergoes an electrochemical oxidation reaction and forms the corresponding reaction intermediates. After a subsequent chemical reaction, the intermediates are transformed into the luminescent excited state, which emits luminescence while returning to the ground state [51]. In this process, the electrical signal was converted into a light signal and output as a detection signal. Electrochemiluminescence works on two mechanisms: the annihilation and co-reactant pathways. In the former, a single emitter produces the radical species. On the other hand, the electrochemical reaction between an emitter and a co-reactant generates radical species in the latter pathway. Annihilation ECL was

initially discovered and investigated. The process of this pathway can be shortly described as follows: the emitter (R) can be oxidized to form a radical cation ($R^{\bullet+}$) and reduced to form a radical anion ($R^{\bullet-}$) by applying step potential at a working electrode, and then both of them are annihilated via an exergonic electron transfer reaction to produce the excited state of the emitter (R^*) which will later relax to the ground state by emitting luminescence.

In 1977, Bard found that the simultaneous electrochemical oxidation of oxalate and luminophores could trigger light emission, the first report of co-reactants being involved in ECL generation [52]. The co-reactant pathway can be described as follows. First, the co-reactant is initially electrochemically oxidized or reduced to a radical intermediate. Then it converts to a strong reducing or oxidizing species to react with oxidized or reduced luminophores close to the electrode. Last, the excited states could be generated via an electron transfer process to emit light. Unlike the annihilation pathway, the ECL by the co-reactant path can be produced through only one-directional potential scanning on working electrodes due to co-reactants in solutions.

Based on the different redox abilities of species, it can be divided into two types (Fig. 1.23): the “oxidative-reduction route” and the “reductive-oxidation route.” The former type represents the situation in which the generation of ECL was from a strong reducing species after electrochemical oxidation of co-reactants, where the classic co-reactants include oxalate, cerium(III) sulfate, and tri-n-propylamine (TPA). In contrast, the latter refers to the situation in which the ECL generation was caused by strong oxidizing intermediates from electrochemical reducing products of co-reactants, where the representative co-reactants are benzoyl peroxide (BPO), hydrazine, and potassium persulfate ($K_2S_2O_8$) [53].

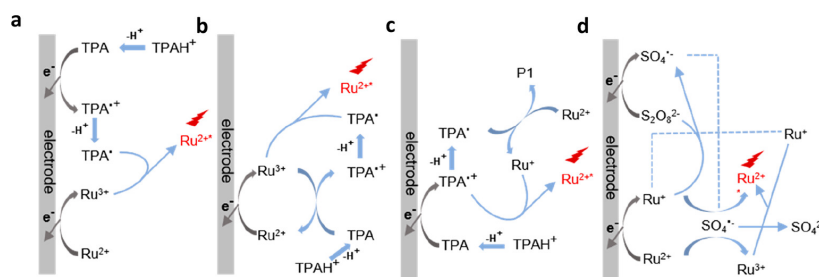


Fig. 1.23 Three different reaction routes in $Ru(bpy)_3^{2+}/TPA$ ECL system.

As a delicate combination of optical and electrochemical methods, ECL inputs the electrical signals into an optical reader, avoiding the interference of charging and discharging currents that occurred in the traditional electrochemical measurement. More importantly, ECL completes the measurement without the need for excitation

light; hence, the background light's influence during the measurement is removed. Therefore, compared with fluorescence and other optical methods, ECL has the advantage of a near-zero background that significantly improves the detection sensitivity. In addition, the intermediates involved in the ECL high-energy electron transfer reaction are controlled by electrical signals and confined near the electrode surface. The surface-confined feature allows for the precise modulation of the ECL duration and location with high spatiotemporal resolution. The integration of high-resolution imaging components enables ECL imaging technology to visualize the spatial distribution and quantitative information of molecules. Thus, ECL-based analysis exhibits significant characteristics, such as high sensitivity, strong versatility, and low detection cost, and has become a dominant method in clinical tests. **Fig. 1.24** provides a summary overview of nanomaterials-driven ECL aptasensors for detecting food pollutants, such as pesticides, heavy metal ions, toxins, antibiotics, and pathogenic bacteria.

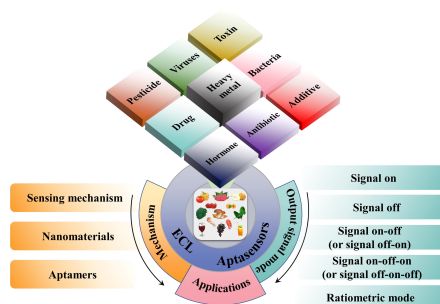


Fig. 1.24 Summary of nanomaterials-driven ECL aptasensors for detecting food pollutants.

1.5 Metal-organic frameworks (MOFs)

1.5.1 Characteristics of MOFs

Metal-organic frameworks (MOFs) are porous materials formed by the coordination of the metal ions or metal-oxo clusters to a molecular scaffold of organic ligands (e.g. imidazolate, bodily, porphyrinic, pyridinic, acidic), known as nodes and linkers in the framework ^[54], respectively (**Fig. 1.25**).

In 1995, Yaghi et al. first proposed the MOF concept and synthesized coordination compounds exhibiting 2D structures from a rigid organic ligand and the transition metal Co ^[55]. Over the past two decades, various MOFs series have been designed and synthesized including Universitet i Oslo (UiO), porous coordination network (PCN), and Materials of Institut Lavoisier (MIL).

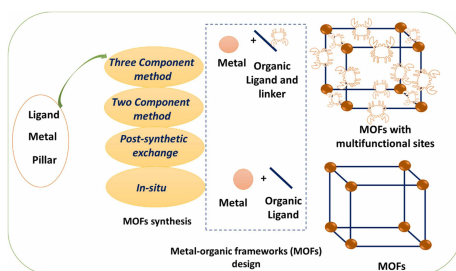


Fig. 1.25 The choice of synthetic methods with metal salts, ligands, and linkers determines the framework's structures.

The primary concept of MOFs synthesis revolves around linking the organic linker molecule with the metal center to develop a porous framework with the objective of not disrupting the integrity of the linker molecule during the synthesis procedure. The surface area and pore size variation of MOFs are highly subjective to the temperature changes and reaction conditions. A general schematic representation of the different synthetic routes for MOFs has been shown in **Fig. 1.26**, including solvothermal/hydrothermal synthesis, electrochemical synthesis, microwave-assisted synthesis, sonochemical synthesis, mechano-chemical synthesis, dry-gel conversion, diffusion synthesis, and ionothermal synthesis [56].

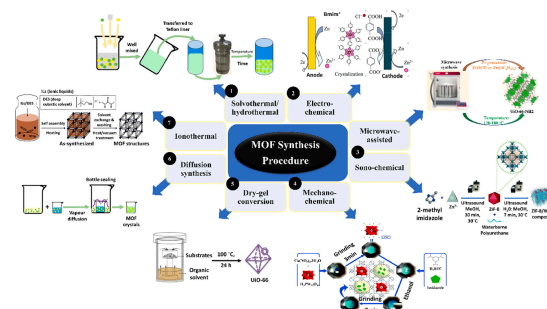


Fig. 1.26 A general schematic representation of the prime MOFs synthesis routes.

Within the last few decades, exponential growth has been witnessed in the syntheses, characterizations, and applications of MOFs. A critical part of successful MOFs construction is ensuring the framework's robustness. Herein, considering MOF-5 (the milestone in the MOF family) as an example, the reasons for the stability of the MOF were shown in **Fig. 1.27**. The fundamental building block of MOF-5, $\text{Zn}_4\text{O}(\text{CO}_2)_6$, consists of a cubic arrangement in which metal cluster nodes and organic linkers are interconnected through benzene dicarboxylate (BDC) units. This structural unit is composed of four ZnO_4 tetrahedra sharing a common oxygen vertex, while six carboxylate carbon atoms serve as secondary building units, forming an octahedral coordination environment around the central Zn_4O cluster. From a topological perspective, a basic network topology can be formed if the metal clusters are considered vertices and the organic linkers are simplified as edges. With the continuous efforts of

researchers, an increasing number of rigid nodes and organic linkers have been developed. Several classical metal cluster nodes and organic ligands are listed in **Fig. 1.27** [57].

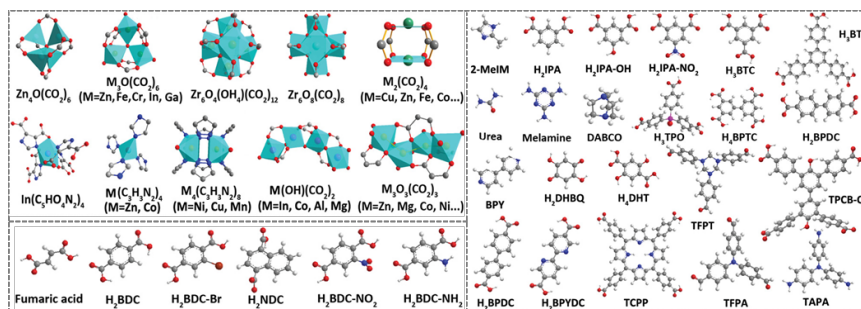


Fig. 1.27 Schematic representation of various MOFs' conceptual design using different metal nodes and organic linkers.

1.5.2 Application of MOFs materials in the field of electrochemical sensors

MOFs have multidimensional coordination with high surface-volume, tunable structures, and interface properties that have been used as promising candidates for environmental, pharmaceutical, food, and industrial applications (**Fig. 1.28**) [58]. The tailorable framework of MOFs enables the design and development of materials suited explicitly for molecule capture and selective recognition. MOFs exhibit key structural advantages, such as their periodic network structures, unsaturated metal coordination sites, high surface areas, and cavity structures. These characteristics make MOFs ideal as electrocatalytic electrode materials, significantly enhancing signal response and detection sensitivity. The porous structure of MOFs allows for high-efficiency mass transfer and concentration of analytes, amplifying their signal response.

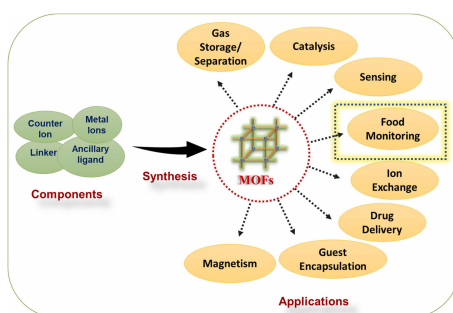


Fig. 1.28 MOFs applications in various scientific fields.

Additionally, the specific size and form of the accessible channels and cavities contribute to their high selectivity for specific analytes, further improving detection accuracy. Metal ions and organic ligands in MOFs are crucial in catalyzing electrochemical processes. The performance of MOF-based materials in electrochemical sensing is often influenced by the type of metal ions incorporated. For

example, Wei et al. designed HNTs/ZIF-67 and HNTs/MIL-88A 1D MOF composites (ZIF: Zeolitic imidazolate framework) with active surfaces for *in situ* growth of halloysite nanotube minerals by electrostatic adsorption (**Fig. 1.29**) [59]. In addition, MOFs' synthetic control provided promising candidates for target applications. It exhibited materials with tunable, well-ordered, active surfaces and high-porosity frameworks [60].

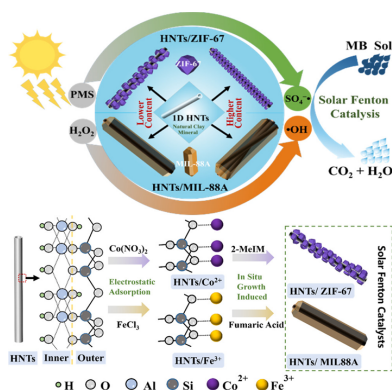


Fig. 1.29 The preparation process for the designed HNTs/ZIF-67 and HNTs/MIL88A catalysts.

1.6 Covalent-organic frameworks (COFs)

1.6.1 Characteristics of COFs

Covalent-organic frameworks (COFs), which are formed by covalent bonds of elements that are predominantly C, N, O, and B in the absence of metal centers, exhibit superior chemical and thermal stability compared with MOFs. In 2005, Yaghi et al. [61] designed and synthesized the first COFs, COF-1 and COF-5, which were prepared by self-condensation of 1,4-phenylenediboric acid and co-condensation of 1,4-phenylenediboric acid with triphenylene-2,3,6,7,10,11-hexaol under solvothermal conditions, respectively. Their crystal structures consist entirely of strong bonds among B, C, and O atoms to form rigid pores ranging from 7 to 27 Å. COF-1 and COF-5 exhibit high thermal stability up to 500-600 °C, permanent porosity, and high specific surface areas (711 and 1590 m² g⁻¹, respectively).

The diverse linkage chemistry of COFs is the basis not only for investigating the structure-property relationship, but also for expanding the applications of COFs (**Fig. 1.30**). Early studies focused on the COFs linked by dynamic covalent bonds. The reversibility of the linkages allows the self-correction of defects formed during the reaction, resulting in COFs with ordered structures and thermodynamic stability. Diverse categories of knots are generated for linking units in COFs, including boroxine,

boronic ester, imine, hydrazone, azine, β -ketoenamine, imide, borazine, 1,4-dioxin, C=C bond, phenazine, triazine, urea, squaraine, and double-linkage. In addition to linkages, the topology of COFs is also an important source of structural diversity of COFs. Generally, COFs are synthesized by the polymerization of monomers with symmetric reactive groups after specific geometries, which are mainly based on dynamic covalent chemistry. Therefore, organic units, denoted as building blocks, are the key components to form COF frameworks with highly ordered open channels.

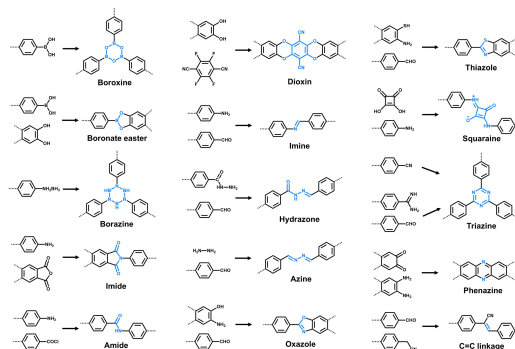


Fig. 1.30 Timeline of various linkages for COF formation.

Organic units can form reversible covalent bonds, promoting the crystallization process of COFs. The bridge manners or geometrical structures of building blocks decide the spatial geometry of COF, which can be divided into 2D or 3D structures [62]. Most COFs consist of several building blocks with specific geometries. According to different symmetric knots and linkers, the topologies of COFs can be divided into at least 9 kinds of topologies of 2D COFs, including hexagonal (**hcb**), hexagonal lattice (**hxl**), kagome (**kgm**), square lattice (**sql**), clock per instruction (**cpi**), branch exchange (**bex**), rhombille tiling (**kgd**), and through-the-hole (**tth**) [63]. Amongst, the linear linker and square knot can yield a 2D sql network through $[C_4+C_2]$ combination. Two C_2 linkers (two-carbon linkers) and knots can create **kgd** or **kgm** topology's structure, which is composed of hexagonal and trigonal pores. Further, merging C_3 symmetric units and C_2 linker molecules can form the **hxl** topology's structure with trigonal pores.

In addition, more complicated backbones and structures can be developed by using multi-component strategies with different knots and linkers. For example, the structure of 2D COFs can possess different stacking modes in space, such as AA, AB, and ABC [64]. Further, 3D COFs can be created by introducing tetrahedral units (T_d) or orthogonally as building blocks [65]. When four connected monomers encounter tetrahedral T_d monomers, the abundant changeable topologies of 3D COFs can be obtained such as cubic truncated network (**ctn**), boracite topology (**bor**), diamond

topology (**dia**), and platinum sulfide (**pts**) topology ^[63]. Six connected monomers with trigonal prism D_{3h} monomers can form the stilpnomelane (**stp**), cequalite (**ceq**), aluminum copper sulfide (**acs**), and heazlewoodite (**hea**) topologies. Similarly, D_{3d} and C_3 symmetric six connected monomers can achieve primitive cubic (**pcu**) and square-octahedral (**soc**) topologies. Eight-connected building blocks can produce body-centered cubic (**bcu**) and polycatenated borromean (**pcb**) topologies. In addition, the topological types of 3D COFs still increase, including face-centered cubic (**ffc**), faujasite-type hexagonal (**fjh**), and tetragonal boron (**tbo**) topologies generated by $[C_4+C_3]$ combination and **srs** created by $[C_3+C_3]$ reactions ^[63] (**Fig. 1.31**).

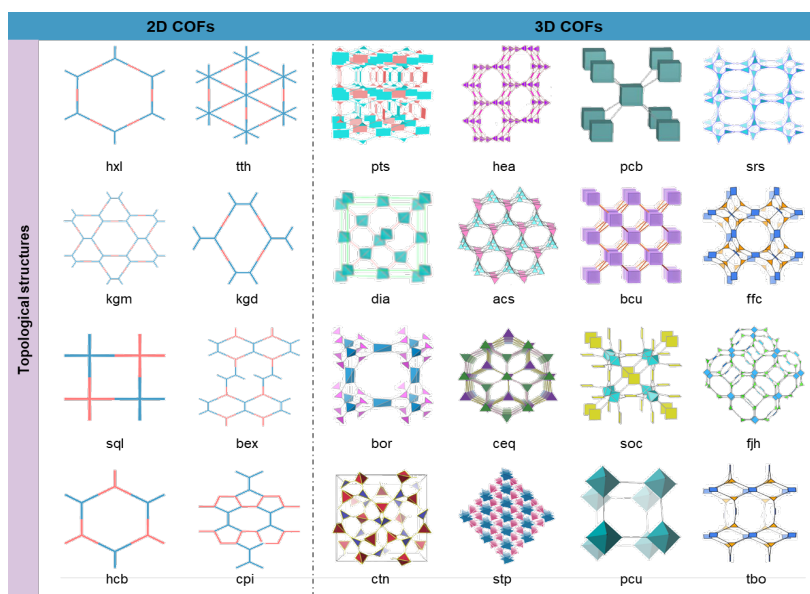


Fig. 1.31 Reported topologies of 2D COFs and 3D COFs.

1.6.2 Application of COF Materials in the field of electrochemical sensors

As novel materials, COFs exhibit diverse characteristics essential for the design of electrochemical sensors. Their robust framework architecture imparts low solubility in most common electrolytes, a critical property for aqueous analysis. By tuning pore size and hydrophobicity, COFs enhance electrolyte mass transport within the structure and regulate electrochemical responses through selective molecular adsorption, thereby minimizing interference in complex aqueous matrices.

Additionally, their stability in both alkaline and acidic media makes them well-suited for analyzing challenging samples or those requiring specific conditions. Furthermore, COFs feature intricate, highly ordered structures with tunable pore sizes and diverse topologies. The self-assembled two-dimensional pores, formed through interlayer interactions, facilitate efficient charge transport. COFs have exhibited great

potential in chemical sensing in detecting various chemicals (**Fig. 1.32a**), including heavy metals, explosives, and hazardous substances. Furthermore, COFs have been employed in biomolecule sensing, enabling the detection of biomarkers and facilitating applications in medical diagnostics and biotechnology. Additionally, COFs have proven valuable in environmental sensing, aiding in the monitoring and detection of pollutants in the air, water, and soil [66].

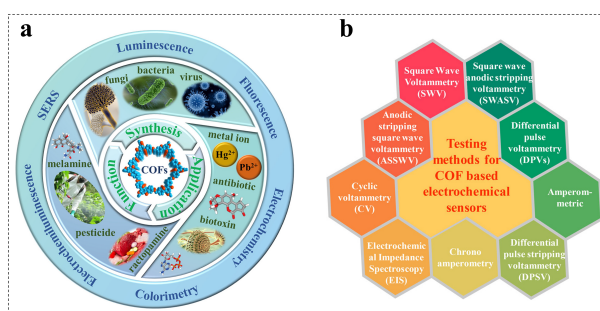


Fig. 1.32 (a) Application of COF-based electrochemical sensors and (b) testing methods for COF-based electrochemical sensors.

COF materials, owing to their high functionality, well-ordered π - π conjugated systems, independent open pores, and large specific surface areas, promote rapid electron transfer and energy storage. Additionally, COFs provide electrodes with high specific surface areas and densely exposed catalytic active sites, while their interconnected pores facilitate analyte diffusion and interaction with active sites. Consequently, electrodes constructed from COFs or incorporating electrochemically active molecules have been recognized as ideal platforms for electrochemical sensing analysis. According to the difference in the output signal, the electrochemical sensor can be divided into conductometry, potentiometry, amperometry, analytical voltammetry, impedance method, and so on; the testing methods for COF-based electrochemical sensors are displayed in **Fig. 1.32b** [67].

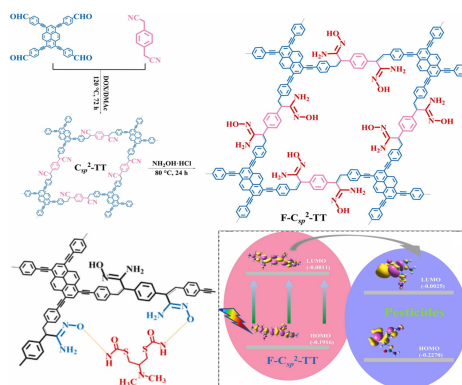


Fig. 1.33 A sp² carbon-connected COF-based sensor for Cartap and water content sensing.

For example, Recently, Zhang et al. synthesized a sp² carbon-connected COF (F-

Csp²-TT) using 1,3,6,8-tetrakis(4-ethynyl benzaldehyde)-pyrene through Knoevenagel condensation reaction followed by the post-modification of amidoxime (**Fig. 1.33**). The C=C bond in F-Csp²-TT reduced steric hindrance and enhanced the luminescent performance of COFs. The hydroxyl and amino groups loaded on F-Csp²-TT interacted with Cartap through hydrogen bonding, resulting in a luminescent response. Cartap could be visually detected using a smartphone-based sensing platform. The fluorescent sensor fabricated with F-Csp²-TT enabled the detection of Cartap in real samples, with a LOD of 0.51 µg L⁻¹ in agricultural and food samples and a recovery rate of 95.90% to 119.3%. Furthermore, this sensor can also be used for sensing water content, but it does not exhibit a significant response to other interferents such as common metal ions and amino acids. Therefore, it demonstrates high selectivity and detection stability for Cartap, showing the unique advantages and potential applications of F-Csp²-TT in pesticide detection [68].

1.7 Metallo-COFs (MCOFs)

1.7.1 Characteristics of MCOFs

MCOFs (metal-covalent organic frameworks) hold high promise in combining the advantages of MOFs and COFs, thus realizing a balanced mix of crystallinity, porosity, stability, tunability, and functionality. The methods for the introduction of metal ions into COFs through coordination, known as metalation, include both pre-synthetic and post-synthetic routes.

In pre-synthetic metalation, the complexes are used directly as the monomers for the construction of COFs, and the amount and position of the metals in the COFs can be precisely controlled. As for the method of post-synthetic metalation, through either direct coordination or ligand exchange, metal ions can be anchored to COF frameworks while maintaining the original crystallinity of COFs. During this process, the regularly arranged non-metal atoms in COFs act as a well-defined net that becomes a reticular chemical ligand for accurate capture of metal ions.

Depending on the position of the metal ions in the metalated COFs, metalated COFs can be divided into three categories (**Fig. 1.34**): (i) metal ions are embedded in the COFs as nanoparticles (COF-366-Co); (ii) metal ions are coordinated to the linkages of the COFs (COF-505); and (iii) metal ions are coordinated to the COF monomeric units (JNM-1).

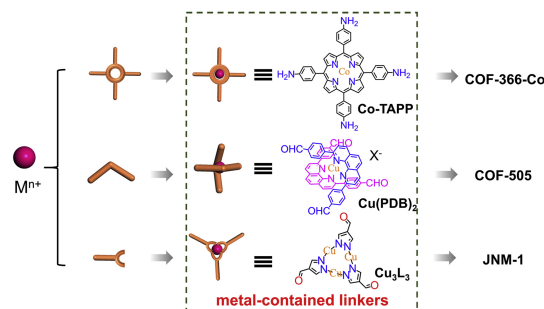


Fig. 1.34 Three reported metal-contained linkers and the related COFs.

The timeline of remarkable progress in the fabrication of MCOFs is shown in Fig. 1.35: the MCOFs display various properties that cover almost all possible application fields, which is being increasingly recognized as a new class of organic-inorganic hybrid materials.

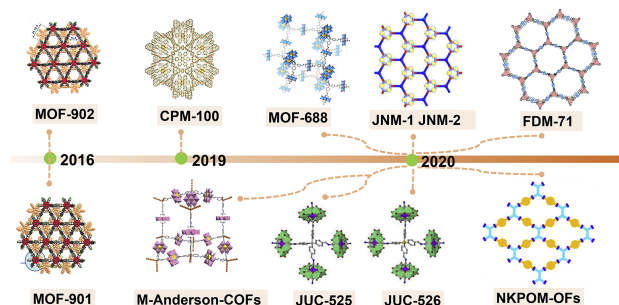


Fig. 1.35 Timeline of the remarkable progress made in the fabrication of MCOFs.

1.7.2 Application of MCOF Materials in the field of electrochemical sensors

The integration of metals and COFs to form MCOF composites not only inherits their unique features (such as periodic network structures, high specific surface area, tunable pore sizes, and excellent chemical and thermal stability), but also creates a synergistic effect between them to afford multifunctional properties for specific applications involving adsorption and separation, heterogeneous catalysis, sensing, energy storage, and photodynamic therapy. As it is well known, electrode modification materials play an important role in improving sensitivity in EC sensors. Wang's group reported a novel porphyrin (Cu)-based covalent organic framework named COF(Cu) by condensing pre-metalated 5,10,15,20-tetrakis (para-aminophenyl) porphyrin copper (II) and 2,3,6,7-tetra (4-formylphenyl) tetrathiafulvalene ligands (Fig. 1.36). By combining the advantages of porphyrin with tetrathiafulvalene, it may be possible to create a COF with an intrinsically effective charge-transfer channel. In addition, the Cu-N₄ type in the COF(Cu) can be regarded as the single-site electrocatalyst. Benefiting from these advantages, the COF(Cu) based electrochemical sensor demonstrated an

outstanding response to gallic acid (GA). Under the optimal conditions by square wave voltammetry technique, the COF(Cu) modified electrode showed a wide linear range (0.01-1000 μM), a low detection limit (2.81 nM), good reproducibility, acceptable selectivity as well as high stability. Moreover, the established approach was adopted to detect GA in real tea samples with good recoveries, indicating that the COF(Cu) based electrochemical sensor may pave the way for the application in food analysis [69].

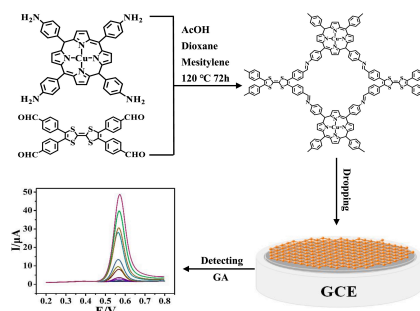


Fig. 1.36 Illustration of the synthetic route of COF(Cu) and the electrochemical detection of GA.

1.8 Research ideas and main content of this chapter

Based on extensive research on the properties and performance of metals, ligands, and metal compounds, coupled with electrochemical sensing strategies and literature on biological reach, my specific research activities can be summarized:

1: As part of the continuous investigation of the chemical and biological properties of copper- and silver-containing coordination compounds, we synthesized and evaluated novel Cu(II), Cu(I), and Ag(I) complexes incorporating phosphane and phenoxy-ketimine ligands. Specifically, we utilized 2-(1-(benzylimino)ethyl)phenol (HL^{BSMe}) (Oletimol) and 2-((benzylimino)(phenyl)methyl)phenol (HL^{BSPh}) as ligands, alongside the lipophilic triphenylphosphine (PPh_3) and the hydrophilic 1,3,5-triaza-7-phosphadiazane (PTA) as co-ligands.

2: We designed and synthesized a series of novel Schiff bases and employed their Cu complexes as catalysts in the synthesis of propargylamine via the three-component A^3 -coupling reaction of aldehydes, amines, and alkynes.

3: As an extension of the studies on pyrazolyl derivatives and as part of our continuous investigation of the chemical and biological properties of copper-containing coordination compounds, we performed a study on the syntheses, characterization, and reactivity of new 3-monosubstituted acetylacetonate ligands, HL^{acPz} and $\text{HL}^{\text{acPzMe}}$, and related Cu(II) and Cu(I) complexes containing phosphane co-ligands, that could be evaluated for their biological effects and their catalytic activity and selectivity.

4: The photoelectrochemical properties of mixed-metal MOF were investigated: we developed a novel “signal-inversion” photoelectrochemical (PEC) aptasensor based on the photoelectric active CuCo-based metal-organic framework with 2,3,6,7,10,11-hexaiminotriphenylene (HITP) as ligand [denoted as $\text{Cu}_{2.46}\text{Co}_{0.54}(\text{HITP})_2$] for sensitively detecting Fumonisin B1 (FB1) using the enzyme-assisted biocatalytic precipitation reaction. $\text{Cu}_{2.46}\text{Co}_{0.54}(\text{HITP})_2$ was a highly efficient PEC signal indicator and a biological platform for immobilizing single-stranded DNA (ssDNA). Integrating the enhanced photoelectric conversion efficiency of $\text{Cu}_{2.46}\text{Co}_{0.54}(\text{HITP})_2$ and the superior electrocatalytic activity of MnFe_2O_4 will achieve the sensitive detection of FB1 in food and complex environments.

5: The ECL mechanism of COF and their application in the construction of ECL biosensors was explored: we utilized a novel signal “on-off-on” ECL immunosensing strategy for the accurate and sensitive detection of trace zearalenone (ZEN) by using a triazine-containing COF as luminophore and a complex of CuFe_2O_4 and polydopamine ($\text{CuFe}_2\text{O}_4@\text{PDA}$) as the quencher. The triazine-containing COF, which was synthesized by reacting 2,4,6-tris (4-formyl-phenyl)-1,3,5-triazine (TFPT) and 1,3,5-tris (4-aminophenyl) benzene (TAPB) (denoted as TFPT-TAPB-COF), exhibited a donor-acceptor (D-A) conjugated structure, and high intramolecular charge transfer, achieving an enhanced ECL emission ability in the presence of $\text{K}_2\text{S}_2\text{O}_8$, and combining the trimodal ECL quenching mechanism and competitive immunosensing strategy to verify the feasibility of TFPT-TAPB-COF-based ECL immunosensor for detecting ZEN in food samples.

6: Based on previous research on MOFs and COFs, the photoelectrochemical properties of MCOFs were studied: a novel amplified PEC aptasensor was developed based on the photoelectronic active and donor-acceptor (D-A) conjugated COF for the efficient detection of ZEN. The D-A-conjugated COF synthesized through the reaction between trinuclear copper cluster (Cu_3L_3) and 4,4',4''-(1,3,5-triazine-2,4,6-triyl)trianiline (TAPT) (denoted as $\text{Cu}_3\text{L}_3\text{-TAPT-COF}$) comprised rich Cu- N_2 single-atom sites. It was simultaneously employed as a PEC electrode and bioplatfor for anchoring single-stranded DNA. Combining the target-modulated competitive binding method and the multivariate signal-amplified strategy significantly enhances the sensor's detection sensitivity, enabling accurate analysis and detection of ZEN in food and complex environmental samples.

Chapter II: New Cu(II), Cu(I) and Ag(I) complexes of phenoxy-ketimine Schiff base ligands: synthesis, structures and antibacterial activity

2.1 Introduction

In recent years, antibiotic resistance in hospitals, communities, and the environment has increasingly grown, becoming a significant global public health challenge. The World Health Organization (WHO) classified antimicrobial-resistant bacterial pathogens as critical in 2017 ^[7]. Despite ongoing efforts, the global antibiotic pipeline remains limited in innovation. The rise in antimicrobial resistance and the misuse of antimicrobials have created a need for new antimicrobial compounds. Medicinal inorganic chemistry offers opportunities for designing therapeutic agents beyond the scope of organic compounds. A successful approach in antimicrobial chemotherapy has been the use of metallo-drugs, which show potential in treating multidrug-resistant infections more efficiently. Schiff bases, known for their versatile metal-chelating properties, have been used to coordinate almost all metal ions and exhibit various biological activities, including anticancer and antimicrobial properties ^[19,39]. In most cases, their metallic derivatives display higher antimicrobial activity than the parent ligands. Among Schiff bases, phenoxy-imines and phenoxy-ketimines are particularly notable. These 2-imidoylphenols exist in tautomeric forms due to bonding the easy proton exchange between the phenoxy oxygen atom and the imine nitrogen atom favored also by the relative position of the two groups. Schiff bases and their copper(II) derivatives have demonstrated promising catalytic, anticancer, and biological properties, serving as useful models in biological systems. Several Cu(II) complexes of N, O-donor Schiff base ligands exhibit favorable antimicrobial activities. Despite extensive research on copper(II) Schiff base complexes ^[35], reports on Cu(I) analogs remain scarce, likely due to their tendency to disproportionate into copper metal and Cu(II) compounds in the absence of stabilizing co-ligands ^[6]. Schiff bases, as chelating N-donors, and phosphines, as P-donors, have recently been employed in synthesizing photoactive neutral and cationic heterodetic Cu(I) complexes. Additionally, silver(I) and related metal complexes exhibit broad-spectrum antimicrobial activity, with numerous studies focusing on silver(I) Schiff base complexes. Recent interest in Cu(I) and Ag(I) Schiff base complexes incorporating phosphines as co-ligands

highlights their biological potential.

Metal complexes of N,O-bidentate phenoxy-ketimine Schiff bases have gained research attention. However, copper(I)- and silver(I)-based complexes supported by these ligands, including phenoxy-imines, remain unexplored.

Therefore, as part of the chemical and biological properties of copper- and silver-containing coordination compounds, we report here the syntheses, characterization and biological evaluation of new Cu(II), Cu(I) and Ag(I) complexes containing phosphanes and the phenoxy-ketimines ligands, 2-(1-(benzylimino)ethyl)phenol (HL^{BSMe}) (Oletimol) and 2-((benzylimino)(phenyl)methyl)phenol (HL^{BSPh}) (**Fig. 2.1**). The ligands were selected to modify the steric and electronic properties of the resulting metal complexes using phenyl and methyl groups on the C-C(R)=N imino moiety, respectively. In designing the novel phenoxy-ketimines metal complexes, the lipophilic triphenylphosphine (PPh_3) and the hydrophilic 1,3,5-triaza-7-phosphaadamantane (PTA) were selected as co-ligands, to stabilize copper and silver in their +1 oxidation state and to confer different solubility properties to the corresponding metal complexes.

The antimicrobial properties of the new copper and silver complexes as well as of the corresponding uncoordinated ligands and co-ligands were evaluated against Gram-negative bacteria (*Escherichia coli*) and Gram-positive (*Staphylococcus aureus*). The antimicrobial data have been compared with the control antibiotic ciprofloxacin to evaluate their relative efficacy.

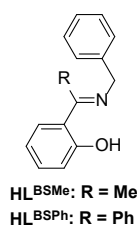


Fig. 2.1 Structure of phenoxy-ketimine ligands used in this study.

2.2. Experimental Section

2.2.1. Materials and Instruments

All reagents were obtained from commercial suppliers and used as received. Melting Points (MP) were performed by an SMP3 Stuart Scientific Instrument (Bibby Sterilin Ltd., London, UK). Elemental Analyses (C, H, N, S) (EA) were performed with a Fisons Instruments EA-1108 CHNS-O Elemental Analyzer (Thermo Fisher Scientific Inc., Waltham, MA, USA). Fourier-Transform Infrared (FT-IR) spectra were recorded

from 4000 to 700 cm^{-1} on a PerkinElmer Frontier Instrument (PerkinElmer Inc., Waltham, MA, USA), equipped with Attenuated Total Reflection (ATR) unit using universal diamond top-plate as sample holder. Abbreviation used in the analyses of the FT-IR spectra: br = broad, m = medium, mbr = medium broad, s = strong, sbr = strong broad, vs = very strong, w = weak, wbr = weak broad. Nuclear Magnetic Resonance (NMR) spectra for the nuclei ^1H , ^{13}C , and ^{31}P were recorded with a Bruker 500 Ascend Spectrometer (Bruker BioSpin Corporation, Billerica, MA, USA; 500.13 MHz for ^1H , 125.78 MHz for ^{13}C , 202.46 MHz for ^{31}P and 470.59 MHz for ^{19}F). Tetramethylsilane (SiMe_4) was used as an external standard for the ^1H - and ^{13}C -NMR spectra, while 85% H_3PO_4 was used for the ^{31}P -NMR spectra. The chemical shifts (δ) are reported in ppm and coupling constants (J) are reported in hertz (Hz). Abbreviation used in the analyses of the NMR spectra: br = broad, d = doublet, dbr = broad doublet, m = multiplet, s = singlet, sbr = broad singlet, t = triplet. ElectroSpray Ionization Mass Spectra (ESI-MS) were recorded in positive- (ESI-MS(+)) or negative-ions (ESI-MS(-)) mode on a Waters Micromass ZQ Spectrometer equipped with a single quadrupole (Waters Corporation, Milford, MA, USA), using methanol or acetonitrile mobile phase. The compounds were added to reagent-grade methanol or acetonitrile to give approximately 0.1 mM solutions. These solutions were injected (1 μL) into the spectrometer fitted with an autosampler. The pump delivered the solutions to the mass spectrometer source at a flow rate of 200 $\mu\text{L min}^{-1}$ and nitrogen was employed both as a drying and nebulizing gas. The capillary voltage was typically 2500 V. The temperature of the source was 100 $^\circ\text{C}$, while the temperature of the desolvation was 400 $^\circ\text{C}$. In the analysis of ESI-MS spectra, the confirmation of major peaks was supported by a comparison of the observed and predicted isotope distribution patterns, the latter calculated using the IsoPro 3.1 computer software (T-Tech Inc., Norcross, GA, USA).

2.2.2 Synthesis

2.2.2.1 Synthesis of the ligand HL^{BSMe} (1)

The ligand (E)-2-(1-(benzylimino)ethyl)phenol, HL^{BSMe} (1) was prepared by the reaction of 1-(2-hydroxyphenyl)ethan-1-one (1.100 mmol, 0.150 g) and benzylamine (1.000 mmol, 0.107 g) in methanol (30 mL). The mixture was stirred at room temperature for 4 h, and monitored via TLC, until the complete consumption of acylphenol. The mixture was evaporated at reduced pressure and dissolved in CH_2Cl_2

(25 mL) and 1M solution of NaOH (4 x 8 mL). The organic phase was then dried with Na₂SO₄, evaporated, and dried at reduced pressure, giving the product HL^{BSMe} in 80% yield. Solubility: CH₃OH, CH₃CH₂OH, CH₃CN, CHCl₃, Acetone, EtOAc, DMSO. M.p.: 118-119°C. FT-IR (cm⁻¹): 3211wbr, 3108vw, 3061w, 3034w, 2918w, 2877w, 2814w (C-H); 1610s, 1572m (C=N); 1492m, 1451s, 1413m, 1376m, 1350m, 1330m, 1303s, 1263m, 1232m, 1181w, 1161m, 1129m, 1074w, 1055m, 1024m, 974m, 950m, 941m, 928m, 900m, 860m, 831m, 743vs, 733vs, 698vs, 639m, 584m, 559m, 525m, 501m. ¹H-NMR (CDCl₃, 293K): δ 2.51 (s, 3H, CH₃), 4.87 (s, 2H, CH₂), 6.83-7.61 (m, 9H, CH_{ar}), 15.78 (sbr, 1H, OH). ¹H-NMR (CD₃CN, 293K): δ 2.48 (s, 3H, CH₃), 4.83 (s, 2H, CH₂), 6.83-7.71 (m, 9H, CH_{ar}), 16.23 (sbr, 1H, OH). ¹H-NMR (Acetone-d₆, 293K): δ 2.52 (s, 3H, CH₃), 4.88 (s, 2H, CH₂), 6.80-7.72 (m, 9H, CH_{ar}), 15.87 (sbr, 1H, OH). ¹H-NMR (DMSO-d₆, 293K): δ 2.51 (s, 3H, CH₃), 4.83 (s, 2H, CH₂), 6.78-7.72 (m, 9H, CH_{ar}), 16.37 (sbr, 1H, OH). ¹³C (100 MHz, CDCl₃) 172.2, 163.8, 138.4, 132.6, 128.7, 128.1, 127.5, 127.2, 119.4, 118.7, 1172, 53.4, 14.9. ESI-MS(+) (major positive ions, CH₃CN), *m/z* (%): 226 (100) [L^{BSMe} + H]⁺. Elemental Analysis (%) calculated for C₁₅H₁₅NO: C, 79.97; H, 6.71; N, 6.22: found: C, 80.25; H, 6.54; N, 6.36.

2.2.2.2 Synthesis of the ligand HL^{BSPH} (2)

The ligand (E)-2-((benzylimino)(phenyl)methyl)phenol, HL^{BSPH} (2) was prepared following the procedure described for ligand 1, using (2-hydroxyphenyl)(phenyl)methanone (1.100 mmol, 0.218 g) and was obtained in 72% yield. Solubility: CH₃OH, CH₃CH₂OH, CH₃CN, CHCl₃, Acetone, EtOAc, DMSO. M.p.: 89-91°C. FT-IR (cm⁻¹): 30653w, 3026w, 2988w, 2966w, 2922w, 2858w (C-H); 1604s, 1563m; 1498m, 1446m (C=C/C-N); 1378m, 1355w, 1350w, 1299m, 1266m, 1227m, 1203m, 1165m, 1153m, 1126m, 1094m, 1075m, 1049m, 1028m, 993m, 948m, 912m, 856w, 829m, 798m, 752vs, 711m, 697s, 676m, 627m, 588m. ¹H-NMR (CDCl₃, 293K): δ 4.59 (s, 2H, CH₂), 6.68-7.56 (m, 9H, CH_{ar}), 15.64 (sbr, 1H, OH). ¹H-NMR (CD₃CN, 293K): δ 4.55 (s, 2H, CH₂), 6.70-7.60 (m, 9H, CH_{ar}), 15.42 (sbr, 1H, OH). ESI-MS(+) (major positive ions, CH₃CN), *m/z* (%): 288 (100) [L^{BSPH} + H]⁺. Elemental Analysis (%) calculated for C₂₀H₁₇NO: C, 83.59; H, 5.96; N, 4.87: found: C, 83.42; H, 5.73; N, 4.92.

2.2.2.3 Synthesis of [Cu(HL^{BSMe})(PTA)₂](PF₆) (3)

Tetrakis(acetonitrile)copper(I)hexafluorophosphate (0.500 mmol, 0.186 g) and 1,3,5-triaza-7-phosphadaamantane (1.000 mmol, 0.157 g) were dissolved in CH₃CN

(30 mL) and the reaction was stirred for 2 h at room temperature. Then, the ligand HL^{BSMe} (0.500 mmol, 0.113 g) was added, and the reaction was stirred for 24 h at room temperature. The precipitate obtained was filtered and dried at reduced pressure giving the complex [Cu(HL^{BSMe})(PTA)₂]PF₆ in 66% yield. M.p.: 191-193 °C Solubility: CH₃CN, DMSO. FT-IR (cm⁻¹): 3200vbr (OH); 3062w, 3034w, 3268wbr, 2919wbr, 2879w (C-H); 1618m, 1574w (C=N); 1496w, 1449m, 1415w, 1376w, 1350w, 1294m, 1239m, 1161w, 1130w, 1107w, 1055w, 1040s, 969s, 949s, 895w; 831vs (PF₆); 742vs, 733s, 698s, 639m, 583s, 556vs, 525m, 501m. ¹H-NMR (CD₃CN, 293K): δ 2.47 (s, 3H, CH₃), 4.09 (s, 12H, NCH₂P), 4.53-4.58 (AB q, 12H, NCH₂N), 4.83 (s, 2H, CH₂), 6.83-7.71 (m, 9H, CH_{ar}), 16.24 (sbr, 1H, OH). ³¹P H v-NMR (CD₃CN, 293K): δ -144.62 (septet, J_{P-F} = 706 Hz), -91.64 (sbr). ESI-MS(+) (major positive ions, CH₃CN), *m/z* (%): 226 (100) [HL^{BSMe} + H]⁺, 287 (20) [Cu(HL^{BSMe})]⁺, 377 (10) [Cu(PTA)₂]⁺, 445 (100) [Cu(HL^{BSMe})(PTA)]⁺. ESI-MS(-) (major negative ions, CH₃CN), *m/z* (%): 145 (100) [PF₆]⁻. Elemental Analysis (%) calculated for C₂₇H₃₉CuF₆N₇OP₃: C 43.35, H 5.25, N 13.11; found: C 44.14, H 5.39, N 13.05

2.2.2.4. Synthesis of [Ag(HL^{BSMe})(PTA)]NO₃ (4)

Silver nitrate (0.500 mmol, 0.085 g) and 1,3,5-triaza-phosphadaamantane (0.500, 0.078 g) were dissolved in CH₃OH (30 mL) and the reaction was stirred for 2 h at room temperature. Afterwards, the ligand HL^{BSMe} (0.500 mmol, 0.113 g) was added, the reaction was stirred at room temperature for 24 h and the solution was evaporated at reduced pressure and dried under reduced pressure. The product [Ag(HL^{BSMe})(PTA)]NO₃ was obtained in 58% yield. M.p.: 206-208 °C. Solubility: CH₃OH, CH₂Cl₂, CHCl₃, CH₃CN, DMSO. FT-IR (cm⁻¹): 3210br (OH); 3060w, 3033w, 2917w, 2878w (C-H); 1616m, 1573w (C=N); 1494w, 1449m, 1425m, 1375m, 1334s; 1292s (NO₃); 1240s, 1161m, 1129w, 1107m, 1074w, 1042w, 1013vs, 970vs, 947vs, 929m, 900m, 861w, 829w, 806s, 790sh, 743vs, 732vs, 698vs, 639m, 602s, 583s, 560s, 525m, 501m. ¹H-NMR (DMSO-d₆, 293K): δ 2.48 (s, 3H, CH₃), 4.15 (s, 6H, NCH₂P), 4.40-4.58 (AB q, 6H, NCH₂N), 4.83 (s, 2H, CH₂), 6.77-7.72 (m, 9H, CH_{ar}), 16.38 (s, 1H, OH). ³¹P ¹H-NMR (DMSO-d₆, 293K): δ -86.03 (s). ³¹P ¹H-NMR (CDCl₃, 293K): δ -93.61 (s). ESI-MS(+) (major positive ions, CH₃OH), *m/z* (%): 226 (100) [HL^{BSMe} + H]⁺, 248 (20) [HL^{BSMe} + Na]⁺, 423 (50) [Ag(PTA)₂]⁺, 491 (40) [Ag(HL^{BSMe})(PTA)]⁺. ESI-MS(-) (major negative ions, CH₃OH), *m/z* (%): 145 (100) [AgNO₃ + NO₃]⁻. Elemental Analysis (%) calculated for C₂₁H₂₇AgN₅O₄P: C 45.67, H 4.93, N 12.68;

found: C 46.15, H 5.12, N 13.31 modification.

2.2.2.5 Synthesis of $[\text{Cu}(\text{L}^{\text{BSMe}})_2]$ (5)

Copper(II) acetate (0.25 mmol, 0.045 g) and the ligand HL^{BSMe} (0.50 mmol, 0.113 g) were solubilized in CH_3OH (20 mL). The reaction was stirred at reflux for 4 h, giving a small dark green precipitate. The mother liquors were dried at reduced pressure obtaining the green-blue solid $[\text{Cu}(\text{L}^{\text{BSMe}})_2]$ in 39% yield. M.p.: 201-203 °C Solubility: CH_3OH , CH_3CN , CH_2Cl_2 , CHCl_3 , DMSO. FT-IR (cm^{-1}): 3268wbr, 3062w, 3034w, 2878w (C-H); 1599vs, 1572sh (C=N); 1496m, 1443vs, 1421vs, 1379m, 1353m, 1329m, 1304m; 1264m, 1232m, 1181w, 1161m, 1130m, 1074w, 1053m, 1025m, 974m, 950m, 941m, 929m, 901w, 861m, 831m, 743vs, 733vs, 697vs, 627s, 584m, 559m, 524s, 501s. ESI-MS(+) (major positive ions, CH_3CN), m/z (%): 226 (100) $[\text{HL}^{\text{BSMe}} + \text{H}]^+$, 287 (40) $[\text{Cu}(\text{L}^{\text{BSMe}})]^+$, 329 (30) $[\text{Cu}(\text{L}^{\text{BSMe}}) + \text{CH}_3\text{CN}]^+$, 511 (90) $[\text{Cu}(\text{L}^{\text{BSMe}})_2 + \text{H}]^+$, 576 (80) $[\text{Cu}(\text{L}^{\text{BSMe}})_2 + \text{Na} + \text{CH}_3\text{CN}]^+$. ESI-MS(-) (major negative ions, CH_3CN), m/z (%): 240 (100) $[\text{L}^{\text{BSMe}}]^-$. Elemental Analysis (%) calculated for $\text{C}_{30}\text{H}_{28}\text{CuN}_2\text{O}_2$: C 70.36, H 5.51, N 5.47; found: C 68.79, H 5.41, N 5.25.

2.2.2.6 Synthesis of $[\text{Cu}(\text{HL}^{\text{BSPH}})(\text{PPh}_3)_2]\text{PF}_6 \cdot 2\text{CH}_3\text{CN}$ (6)

Tetrakis(acetonitrile)copper(I)hexafluorophosphate (0.500 mmol, 0.186 g) and triphenylphosphine (1.000 mmol, 0.262 g) were dissolved in CH_3CN (30 mL) and the reaction was stirred for 3 h at room temperature. The ligand HL^{BSPH} (0.500 mmol, 0.144 g) was added, and the reaction was stirred for 24 h at room temperature. The solvent was removed at reduced pressure and the solid obtained was washed with Et_2O . A precipitate was obtained by filtration giving the complex $[\text{Cu}(\text{HL}^{\text{BSPH}})_2(\text{PPh}_3)]\text{PF}_6 \cdot 2\text{CH}_3\text{CN}$ in 83% yield. M.p.: 161-164 °C Solubility: CH_3OH , CH_3CN , CHCl_3 , CH_2Cl_2 , DMSO. FT-IR (cm^{-1}): 3200vbr (OH); 3070w, 3050w, 2938w, 2916w (C-H); 2301wbr, 2271wbr (CH_3CN); 1605m, 1572w (C=N); 1497w, 1480m, 1450w, 1434s, 1369w, 1346w, 1328w, 1304m, 1258m, 1245w, 1181w, 1150w, 1112w, 1095m, 1072w, 1047w, 1035w, 999w, 972w, 923w, 879w, 859msh; 832vs (PF_6); 775m, 766m, 744vs, 716m, 694vs, 646m, 618w, 557vs, 541m, 527m, 516s, 504vs. ^1H -NMR (CD_3CN 293K): δ 1.99 (s, 6H, CH_3CN), 4.55 (s, 2H, CH_2), 6.71-7.69 (m, 44H, CH_{ar}), 15.41 (s, 1H, OH). ^1H -NMR (CDCl_3 293K): δ 2.08 (s, 6H, CH_3CN), 4.61 (s, 2H, CH_2), 6.70-7.71 (m, 44H, CH_{ar}). $^{31}\text{P}\{^1\text{H}\}$ -NMR (CD_3CN , 293K): δ -144.62 (septet, $J_{\text{P-F}}$ = 706 Hz), -0.30 (sbr). $^{31}\text{P}\{^1\text{H}\}$ -NMR (CDCl_3 , 293K): δ -144.25 (septet, $J_{\text{P-F}}$ = 713 Hz), -0.61

(s). ESI-MS(+) (major positive ions, CH₃CN), *m/z* (%): 366 (100) [Cu(PPh₃) + CH₃CN]⁺, 589 (100) [Cu(PPh₃)₂]⁺. ESI-MS(-) (major negative ions, CH₃CN), *m/z* (%): 145 (100) [PF₆]⁻. Elemental Analysis (%) calculated for C₆₀H₅₃CuF₆N₃OP₃: C 65.36, H 4.85, N 3.81; found: C 65.25, H 4.88, N 3.66.

2.2.2.7 Synthesis of [Cu(HL^{BSPH})(PTA)₂](PF₆)₂·2H₂O (7)

Tetrakis(acetonitrile)copper(I)hexafluorophosphate (0.500 mmol, 0.186 g) and 1,3,5-triaza-7-phosphadaamantane (1.000 mmol, 0.157 g) were dissolved in CH₃CN (30 mL) and the reaction was stirred for 2 h at room temperature. The ligand HL^{BSPH} (0.500 mmol, 0.144 g) was added giving a yellow mixture. After 24 h at room temperature, the solvent was evaporated under reduced pressure obtaining the complex [Cu(HL^{BSPH})(PTA)₂](PF₆)₂·2H₂O in 72% yield. M.p.: 225-228 °C. Solubility: CH₃OH, CH₃CH₂OH, CH₂Cl₂, CHCl₃, CH₃CN, DMSO. FT-IR (cm⁻¹): 3345wbr, 3188wbr (OH); 3063wbr, 3028w, 2916w, 2878w (C-H); 1605s, 1571m (C=N); 1497m, 1441m, 1419m; C=N); 1346w, 1328w, 1303m, 1257m, 1242s, 1171w, 1150m, 1111m, 1081w, 1045w, 1015s, 970s, 949s, 923m, 876w; 832vs (PF₆); 775s, 765s, 751vs, 715vs, 698vs, 646s, 611m, 582s, 555vs, 506s. ¹H-NMR (CD₃CN, 293K): δ 2.24 (s, 4H, H₂O), 4.06 (s, 12H, NCH₂N), 4.52-4.60 (AB q, 14H, NCH₂P and CH₂), 7.31-7.61 (m, 14H, CH_{ar}), 15.44 (s, 1H, OH). ³¹P{¹H}-NMR (CD₃CN, 293K): δ -144.58 (septet, J_{P-F} = 706 Hz), -89.39 (sbr). ESI-MS(+) (major positive ions, CH₃CN), *m/z* (%): 261 (90) [Cu(PTA) + CH₃CN]⁺, 288 (20) [HL^{BSPH} + H]⁺, 377 (10) [Cu(PTA)₂]⁺, 507 (15) [Cu(HL^{BSPH})(PTA)]⁺. ESI-MS(-) (major negative ions, CH₃CN), *m/z* (%): 145 (100) [PF₆]⁻. Elemental Analysis (%) calculated for C₃₂H₄₅CuF₆N₇O₃P₃: C 45.42, H 5.36, N 11.59; found: C 44.69, H 5.03, N 12.24.

2.2.2.8 Synthesis of [Ag(HL^{BSPH})(PPh₃)₂](NO₃) (8)

Silver nitrate (0.500 mmol, 0.085 g) and triphenylphosphine (1.000 mmol, 0.262 g) were dissolved in CH₃CN (30 mL) and the reaction was stirred for 2 h at room temperature. Afterwards, the ligand HL^{BSPH} (0.500 mmol, 0.144 g) was added, and the reaction was stirred at room temperature for 24 h. The precipitate obtained was filtered and dried under reduced pressure giving the product [Ag(HL^{BSPH})(PPh₃)₂](NO₃) in 40% yield. M.p.: 187-189 °C. Solubility: CH₃OH, CH₂Cl₂, CHCl₃, CH₃CN. FT-IR (cm⁻¹): 3059wbr, 3028w, 2918w (C-H); 1605m, 1571w (C=N); 1497w, 1489w, 1479m, 1436m, 1397s, 1346w, 1328w; 1296s (NO₃); 1257m, 1179w, 1150m, 1112w, 1095m, 1072w,

1047m, 1025m, 997m, 971w, 918s, 864w, 849w, 833w, 825w, 818w, 775m, 766m, 751vs, 742vs, 715s, 693vs, 646m, 618w, 567w, 547w, 513ssh, 503vs. $^1\text{H-NMR}$ (CDCl_3 , 293K): δ 4.58 (s, 2H, CH_2), 6.68-7.70 (m, 44H, CH_{ar}), 15.67 (s, 1H, OH). $^1\text{H-NMR}$ (DMSO-d_6 , 293K): δ 4.52 (s, 2H, CH_2), 6.71-7.63 (m, 44H, CH_{ar}), 15.46 (s, 1H, OH). $^{31}\text{P}\{^1\text{H}\}$ -NMR (CDCl_3 , 293K): δ 14.68 (sbr). ^{31}P 1H-NMR (CD_3CN , 233K): δ 8.69 (d, $J(\text{Ag-}^{31}\text{P}) = 478$ Hz). ESI-MS(+) (major positive ions, CH_3CN), m/z (%): 288 (10) $[\text{HL}^{\text{BSPH}} + \text{H}]^+$, 371 (30) $[\text{Ag}(\text{PPh}_3)]^+$, 410 (30) $[\text{Ag}(\text{PPh}_3) + \text{CH}_3\text{CN}]^+$, 630 (100) $[\text{Ag}(\text{PPh}_3)_2]^+$. ESI-MS(-) (major negative ions, CH_3OH), m/z (%): 231 (100) $[\text{AgNO}_3 + \text{NO}_3]^-$. Elemental Analysis (%) calculated for $\text{C}_{56}\text{H}_{47}\text{AgN}_2\text{O}_4\text{P}_2$: C 68.51, H 4.83, N 2.85; found: C 69.43, H 4.87, N 2.90.

2.2.2.9 Synthesis of $[\text{Ag}(\text{HL}^{\text{BSPH}})(\text{PTA})]\text{NO}_3$ (9)

Silver nitrate (0.500 mmol, 0.085 g) and 1,3,5-triaza-7-phosphadaamantane (0.500 mmol, 0.078 g) were dissolved in CH_3CN . After 2 h the ligand HL^{BSPH} (0.500 mmol, 0.145 g) was added and the reaction was stirred at room temperature for 22 h and refluxed for a further 4 h. Successively, the solvent was removed under reduced pressure and the yellow solid product $[\text{Ag}(\text{HL}^{\text{BSPH}})(\text{PTA})]\text{NO}_3$ was obtained in 55% yield. M.p.: 232-234 °C. Solubility: CH_3OH , CH_3CN , CHCl_3 , CH_2Cl_2 , DMSO. FT-IR (cm^{-1}): 3200wbr (OH); 3064wbr, 3029w, 2916w (C-H); 1605s, 1595sh, 1571m (C=N); 1516w; 1497w, 1489w, 1450m, 1441m, 1425m, 1416m, 1374m, 1329s; 1294s (NO_3); 1257s, 1241s, 1170w, 1150m, 1110m, 1081w, 1072w, 1045m, 1013s, 970vs, 947vs, 919s, 865w, 851m, 825m, 775s, 766s, 751vs, 715vs, 698vs, 646s, 605s, 582s, 564s, 547m, 506s. $^1\text{H-NMR}$ (DMSO-d_6 , 293K): 4.16 (s, 6H, NCH_2N), 4.52-4.58 (dd, 6H, NCH_2P and CH_2), 6.71-7.61 (m, 14H, CH_{ar}), 15.45 (sbr, 1H, OH). ^{31}P 1H-NMR (CDCl_3 , 293K): δ -94.43 (s). $^{31}\text{P}\{^1\text{H}\}$ -NMR (CD_3CN , 233K): δ -86.22 (sbr). ESI-MS(+) (major positive ions, CH_3CN), m/z (%): 158 (90) $[\text{PTA} + \text{H}]^+$, 260 (80) $[\text{Ag}(\text{PTA})]^+$, 288 (100) $[\text{HL}^{\text{BSPH}} + \text{H}]^+$, 423 (50) $[\text{Ag}(\text{PTA})_2]^+$, 553 (10) $[\text{Ag}(\text{HL}^{\text{BSPH}})(\text{PTA})]^+$. ESI-MS(-) (major negative ions, CH_3CN), m/z (%): 231 (70) $[\text{AgNO}_3 + \text{NO}_3]^-$. Elemental Analysis (%) calculated for $\text{C}_{26}\text{H}_{27}\text{AgN}_5\text{O}_4\text{P}$: C 50.83, H 4.76, N 11.50; found: C 51.27, H 5.12, N 11.91.

2.2.2.10 Synthesis of $[\text{Cu}(\text{L}^{\text{BSPH}})_2]$ (10)

Copper(II) acetate (1.000 mmol, 0.200 g) was dissolved in CH_3OH obtaining an intense blue solution. At the same time, the ligand HL^{BSPH} (2.000 mmol, 0.115 g) was

solubilized in CH₃CN. The two solutions were mixed, and it was stirred at room temperature for 1 h and then refluxed for 23 h. After 24 h, the dark green precipitate formed was filtered and dried under reduced pressure giving the complex [Cu(L^{BSPH})₂] in 77% yield. M.p.: 228-230 °C. Solubility: CH₃OH, CH₃CH₂OH, CH₂Cl₂, CH₃Cl, EtOAc, CH₃CN, DMSO, Acetone. FT-IR (cm⁻¹): 3081w, 3026w, 2941w (C-H); 1599s, 1569s (C=N); 1530vs; 1491m, 1456m, 1441vs, 1342vs, 1257s, 1242s, 1204w, 1179w, 1143vs, 1120m, 1076m, 1052m, 1040w, 1025m, 998w, 962m, 936s, 915m, 805s, 778m, 765m, 749vs, 720s, 703vs, 694vs, 691vs, 642w, 630m, 618m, 582m, 537m, 518s. ESI-MS(+) (major positive ions, CH₃CN), *m/z* (%): 288 (100) [HL^{BSPH} + H]⁺, 349 (70) [Cu(L^{BSPH})₂]⁺, 390 (100) [Cu(L^{BSPH}) + CH₃CN]⁺, 636 (20) [2HL^{BSPH} + H]⁺, 658 (20) [2HL^{BSPH} + Na]⁺, 741 (25) [2HL^{BSPH} + Na + 2CH₃CN]⁺. Elemental Analysis (%) calculated for C₄₀H₃₂CuN₂O₅: C 75.51, H 5.07, N 4.40; found: C 75.38, H 5.12, N 4.41.

2.2.3 Crystallographic data collection and refinement

A batch of very good crystals were obtained by slow recrystallization of the [Cu(L^{BSPH})₂] complex from a toluene solution. The specimens were of prismatic shape and intensely coloured in green. The selected item was fixed to a glass capillary with Loctite glue and mounted on the head of a four-circle, kappa-geometry single crystal OD Rigaku diffractometer. The data collection was performed under a graphite-monochromated Cu Kα radiation ($\lambda=1.54184$ Å) at room temperature [295.1(3) K], and reflections were collected with the ω -scans technique. The preliminary screening of the sample allowed us to plan the data collection in the 1024×1024 pixel mode and 2×2 pixel binning. Intensity spots were recorded with an Atlas CCD area detector recently obtained by Prof. K Rissanen (University of Jyväskylä, Finland) in replacement of the former EOS camera. The crude data diffraction data were collected working with the *CrysAlisPro* software, Version 1.171.43.141a, and treated for Lorentz and polarization effects. An empirical correction for absorption based on a multi-scan approach using equivalent reflections was applied by means of the *SCALE3 ABSPACK* algorithm, also accessible *via* the *CrysAlisPro* software. Two reference frames were collected every 50 frames to ensure crystal and equipment stability, showing no sign of sample deterioration or motion.

The complex crystallizes in the monoclinic system and the structure was solved in the *I2/a* space group; the unit cell parameters have been refined by working on the least-squares refinement of 18133 strong reflections gathered during the whole experiment.

The structure solution was performed by direct phasing and refined by full-matrix least squares methods based on F_o^2 through the OLEX2 program interface with the SHELXT and SHELXL programs. The asymmetric unit contains half of a molecule, with the copper ion lying on a proper 2-fold axis, and the whole cell hosts four units of the complex, very efficiently packed (no solvent accessible voids). In the final stages of the refinement non-H atoms were allowed to vibrate anisotropically, whereas the H atoms were placed in calculated positions and refined as a riding model, with their displacement parameters calculated as 1.2 times the U_{eq} of the ‘parent’ atom.

Representations of the asymmetric unit of $[\text{Cu}(\text{L}^{\text{BSPH}})_2]$ and of the entire complex with and without the disordered rings, highlighting the selected numbering scheme, have been prepared with the *Mercury* software. Complete listings of atomic coordinates, bond lengths, bond angles and anisotropic thermal parameters are available as supporting Information, in the form of the .cif file, that has been deposited at the Cambridge Crystallographic Data Center (CCDC) with deposition number 2433689. These data can be obtained free of charge via www.ccdc.cam.ac.uk/structures.

2.2.4 Antibacterial screening

The synthesized Schiff bases HL^{BSMe} (**1**) and HL^{BSPH} (**2**), the related metal complexes **3-10**, and the free phosphane co-ligands triphenylphosphine (**11**) and, 3,5-triaza-7-phosphadaamantane (**12**) were evaluated for their *in vitro* antibacterial activity against Gram-negative bacteria (*E. coli* China-bio-00021) and Gram-positive bacteria (*S. aureus* ATCC 25923), with ciprofloxacin serving as standard antibacterial agent.

The antimicrobial properties of the compounds were evaluated using the standard broth microdilution method in 96-well cell culture plates following the reported method [70]. Firstly, the compounds were dissolved in PBS buffer containing 1% DMSO solution and stirred for 20 min until forming a homogeneous suspension (1.0 mg mL^{-1}). Subsequently, a series of compounds solutions ($50 \text{ }\mu\text{L}$) were prepared in a 96-well plate using the double dilution method, followed by the addition of bacterial suspensions. The final compound concentrations were 3.125, 6.25, 12.5, 25, 50, 100, and $200 \text{ }\mu\text{g mL}^{-1}$, and the final bacterial concentration was $5 \times 10^6 \text{ CFU mL}^{-1}$. The group without an antimicrobial agent was set as the control. Then, the 96-well plate was incubated at $37 \text{ }^\circ\text{C}$ for 24 h and the optical density (OD_{600}) values of each well were recorded at different times (0, 2, 4, 6, 8, 10, 12, 20, and 24 h) using a microplate reader (Thermo Scientific). According to the principle that the turbidity of the culture increases with the

proliferation of bacteria, the growth state of bacteria can be evaluated by detecting the absorbance value of the bacterial suspension at 600 nm.

In addition, the synthesized compounds were evaluated for their *in vitro* antibacterial activity against two bacterial strains using the plate count method. Typically, 100 μL of bacterial suspension (10^6 CFU mL^{-1}) was mixed with 100 μL of compound solution at different concentrations (25, 50, 100, 200, and 400 $\mu\text{g mL}^{-1}$). Subsequently, 100 μL of the mixed solution was spread on LB agar plates. After incubation at 37 $^{\circ}\text{C}$ for another 24 h, the surviving bacteria on each plate were counted. The antibacterial ratio was calculated using the following formula, where C_b and C_e are the numbers of colonies in the blank group and the experimental group, respectively:

$$\text{Antibacterial efficiency} = \frac{C_b - C_e}{C_b} \times 100\%$$

2.2.5 Statistical analysis

The collected data were analyzed using GraphPad Prism 10 or Origin software. Statistical significance was defined as $P < 0.05$, and the results were presented in three decimal places.

2.3 Results and discussion

2.3.1 Synthesis and characterization

The phenoxy-ketimines ligands HL^{BSMe} and HL^{BSPH} were prepared using modified literature methods [71], by the condensation reaction of the 2-acylphenols 2'-1-(2-hydroxyphenyl)ethan-1-one or (2-hydroxyphenyl)(phenyl)methanone, respectively, with stoichiometric amounts of the benzylamine in methanol, and isolated as yellow solids in very good yield and high purity (**Fig. 2.2**).

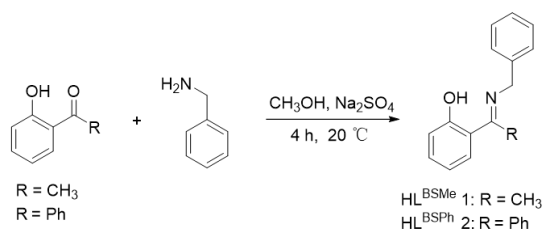


Fig. 2.2 Synthesis of HL^{BSMe} (1) and HL^{BSPH} (2) ligands.

The ligands were fully characterized by ^1H -NMR, ^{13}C -NMR, FT-IR spectroscopy, and ESI-MS spectrometry. Both 1,3,5-triaza-phosphaadamantane copper(I) complexes $[\text{Cu}(\text{HL}^{\text{BSMe}})(\text{PTA})_2]\text{PF}_6$ (3) and $[\text{Cu}(\text{HL}^{\text{BSPH}})(\text{PTA})_2]\text{PF}_6 \cdot 2\text{H}_2\text{O}$ (7) were synthesized in

CH₃CN using, as starting materials, the ligand HL^{BSMe} or HL^{BSPH}, the metal acceptor [Cu(CH₃CN)₄]PF₆ and the PTA co-ligands in the stoichiometric ratio 1:1:2, respectively. Analogously, triphenylphosphine copper(I) complex [Cu(HL^{BSPH})(PPh₃)₂]PF₆·2CH₃CN (**6**) was synthesized in CH₃CN using, as starting material the ligand HL^{BSPH}, the metal acceptor [Cu(CH₃CN)₄]PF₆ and the PPh₃ co-ligands in the stoichiometric ratio 1:1:2 (**Fig. 2.3**).

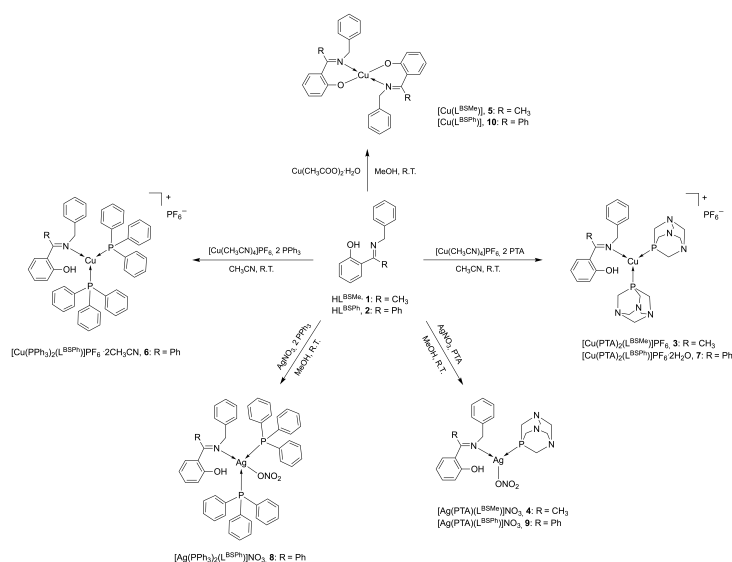


Fig. 2.3 Synthesis of compounds **3-10**.

Elemental analyses and spectroscopic studies such as FT-IR, ¹H-, ³¹P-NMR and ESI-MS confirm the stoichiometry of the synthesized complexes **3**, **6** and **7**. All the expected absorption bands were observed in the FT-IR spectra. The most characteristic feature of the complexation is the disrupt of the O···H···N hydrogen bond and the presence of a very broad ν(OH) absorption band at about 3200 cm⁻¹. The complexes exhibit weak bands in the range 2878-3070 cm⁻¹ typical of C-H stretching. The strong absorptions at 1571-1618 cm⁻¹ are due to the stretching of the aromatic and imine bonds (C=C and C=N), while very intense absorptions in the range 831-832 cm⁻¹ are attributable to the stretching of the PF₆⁻ counterion. The ¹H-NMR spectra, recorded in CD₃CN for complexes **3**, **6** and **7**, confirm the stoichiometric ratio between Schiff-base ligand and phosphane co-ligands. They showed single set of resonances for the skeletal ligands, indicating that the Schiff-ligand protons are equivalent. The signals of phenolic OH protons appeared as broad peaks in the range 15.41-16.24 ppm, the signal of the CH₂Ph protons is visible at δ 4.55-4.83 ppm, while the signal of the ketimine methyl CH₃C=N proton for complex **3** appeared at δ 2.47 ppm, with a slight shift with respect to the signal of the free ligand due to the coordination to the copper acceptor. The

aromatic hydrogens of ligand and triphenylphosphine co-ligands for $[\text{Cu}(\text{HL}^{\text{BSPH}})(\text{PPh}_3)_2]\text{PF}_6 \cdot 2\text{CH}_3\text{CN}$ (**6**) are detectable in the range 6.71-7.69 ppm, while in the spectra of $[\text{Cu}(\text{HL}^{\text{BSMe}})(\text{PTA})_2]\text{PF}_6$ (**3**) and $[\text{Cu}(\text{HL}^{\text{BSPH}})(\text{PTA})_2]\text{PF}_6 \cdot 2\text{H}_2\text{O}$ (**7**) recorded in CD_3CN , the NCH_2P protons of PTA co-ligands are visible as singlets at δ 4.06-4.09 ppm and the related NCH_2N protons show characteristic AB quartets in the range 4.52-4.60 ppm. The ^{31}P -NMR spectrum of $[\text{Cu}(\text{HL}^{\text{BSPH}})(\text{PPh}_3)_2]\text{PF}_6 \cdot 2\text{CH}_3\text{CN}$ (**6**) performed in CD_3CN gives broad singlet peak at -0.30 ppm, downfield shifted with respect to the value of the free triphenylphosphine in the same solvent ($\delta = -4.85$ ppm). Likewise, a broad singlet is visible in the spectra of the PTA complexes **3** and **7**, recorded in CD_3CN , at -91.64 and -89.66 ppm, downfield shifted with respect to the value of the free PTA in CD_3CN (δ -102.07 ppm). Moreover, the spectra of complexes **3**, **6** and **7** show the distinctive septets due to the presence of the PF_6^- counterion at about -144.60 ppm with $J_{\text{F-P}} = 706$ Hz. ^1H -NMR and $^{31}\text{P}\{^1\text{H}\}$ -NMR data of complexes **3**, **6** and **7** are summarized in **Table 2.1**. The ESI-MS study was performed by dissolving complexes **3**, **6** and **7** in acetonitrile and recording the spectra in ion-positive and ion-negative mode. In the ESI-MS(+) spectrum of **3**, the major peak at m/z 445 can be attributed to the species $[\text{Cu}(\text{HL}^{\text{BSMe}})(\text{PTA})]^+$. Similarly, the ESI-MS(+) spectrum of **7** showed at m/z 507 the peak due to the species $[\text{Cu}(\text{HL}^{\text{BSPH}})(\text{PTA})]^+$, confirming the formation and the stability of the complexes.

Table 2.1 Relevant nonbonding interactions for $[\text{Cu}(\text{L}^{\text{BSPH}})_2]$ (**10**).

Compounds	δ (ppm) CH_3	δ (ppm) PTA	δ (ppm) CH_2	δ (ppm) Ar	δ (ppm) OH	δ (ppm) $^{31}\text{P}\{^1\text{H}\}$
HL^{BSMe} (1)	2.48 s ^a	/	4.83 s ^a	6.83-7.71 m ^a	16.23 s ^a	/
HL^{BSPH} (2)	/	/	4.55 s ^a	6.70-7.60 m ^a	15.42 s ^a	/
$[\text{Cu}(\text{HL}^{\text{BSMe}})(\text{PTA})_2]\text{PF}_6$ (3)	2.47 s ^a	4.09 s, 4.50-4.61 AB q ^a	4.83 s ^a	6.82-7.71 m ^a	16.25 s ^a	-91.64 sbr ^a
$[\text{Ag}(\text{HL}^{\text{BSMe}})(\text{PTA})]\text{NO}_3$ (4)	2.48 s ^b	4.15 s, 4.41-4.58 AB q ^b	4.83 s ^b	6.77-7.72 m ^b	16.38 s ^b	-86.03 s ^b -82.00 sbr ^{c*}
$[\text{Cu}(\text{HL}^{\text{BSPH}})(\text{PPh}_3)_2]\text{PF}_6 \cdot 2\text{CH}_3\text{CN}$ (6)	/	/	4.55 s ^a	6.71-7.69 m ^a	15.41 s ^a	-0.30 sbr ^a
$[\text{Cu}(\text{HL}^{\text{BSPH}})(\text{PTA})_2]\text{PF}_6 \cdot 2\text{H}_2\text{O}$ (7)	/	4.08 s, 4.50-4.60 AB q ^a	4.55 s ^a	6.70-7.62 m ^a	15.45 s ^a	-89.66 sbr ^a
$[\text{Ag}(\text{HL}^{\text{BSPH}})(\text{PPh}_3)_2]\text{NO}_3$ (8)	/	/	4.52 s ^b	6.71-7.63 m ^b	15.46 s ^b	8.69 d ($J(\text{Ag}-^{31}\text{P})$ = 478 Hz) ^{**}
$[\text{Ag}(\text{HL}^{\text{BSPH}})(\text{PTA})]\text{NO}_3$ (9)	/	4.16 s, 4.42-4.58 AB q ^b	4.52 s ^b	6.71-7.63 m ^b	15.45 s ^b	-94.43 s ^d -86.09 sbr ^{**}

The copper(II) complexes $[\text{Cu}(\text{L}^{\text{BSMe}})_2]$ (**5**) and $[\text{Cu}(\text{L}^{\text{BSPH}})_2]$ (**10**) were synthesized using the copper(II) acetate salt $\text{Cu}(\text{CH}_3\text{COO})_2$ and the ligands HL^{BSMe} and HL^{BSPH} , respectively, in the stoichiometric ratio 1:2 (**Fig. 2.3**). The reaction was performed in methanol at reflux for the synthesis of complex **5** and in acetonitrile at room temperature

for the synthesis of **10**. Isolated complexes are air-stable, soluble in CH₃OH, CH₃CN, CH₂Cl₂, CHCl₃, DMSO and they have been characterized by means of FT-IR spectroscopy and ESI-MS spectrometry. Successful complexation was indicated by a slight shift of the imine bonds ($\nu(\text{C}=\text{N})$) in the IR spectra. In particular, the imine and aromatic bands in complexes **5** and **10** are observed at 1588-1597 cm⁻¹ and 1569-1599 cm⁻¹ respectively, whereas in the corresponding ligand precursors HL^{BSMe} and HL^{BSPH}, the bands appear at 1572-1610 and 1563-1604 cm⁻¹, respectively. The ESI-MS spectra of complexes **5** and **10** were performed in CH₃CN solution. In the ESI-MS(+) of complex **5**, peaks at m/z 287 and 329 are due to the species [Cu(L^{BSMe})]⁺ and [Cu(L^{BSMe}) + CH₃CN]⁺, respectively; the major peaks at m/z 511 and 576, respectively, are due to the adducts [Cu(L^{BSMe})₂ + H]⁺ and [Cu(L^{BSMe})₂ + Na + CH₃CN]⁺, respectively, confirming the presence of two ligands that coordinate copper. For complex **10** signals at m/z 349 and 390, are attributable to the fragments [Cu(L^{BSPH})]⁺ and [Cu(L^{BSPH}) + CH₃CN]⁺.

The silver complexes [Ag(HL^{BSMe})(PTA)]NO₃ (**4**) and [Ag(HL^{BSPH})(PTA)]NO₃ (**9**) were synthesized by treatment of AgNO₃ with one equivalent of 1,3,5-triaza-phosphadaamantane (PTA) and one equivalent of the corresponding HL^{BSMe} or HL^{BSPH} ligands in methanol and acetonitrile solution, respectively. Analogously, the triphenylphosphine silver(I) complex [Ag(HL^{BSPH})(PPh₃)₂]₂NO₃ (**8**) was synthesized in CH₃CN using, as starting material the ligand HL^{BSPH}, the metal acceptor silver nitrate and the PPh₃ co-ligands in the stoichiometric ratio 1:1:2. Isolated complexes are air-stable and have been characterized using ¹H-, ³¹P-NMR and FT-IR spectroscopy, as well as ESI-MS spectrometry. In IR spectra, a slight shift of the imine bonds ($\nu(\text{C}=\text{N})$) was observed indicating complexation. Complexes **4**, **8**, and **9** exhibit a series of bands in the 1479-1292 cm⁻¹ region, of the IR spectrum that can be attributed to the stretching modes of the NO₃ group. These bands are consistent with those previously reported for analogous silver(I) phosphane complexes. However, the multiplicity of the observed bands prevents an unambiguous assignment of the nitrate group's coordination mode - whether monodentate or bidentate - to the metal center.

The ¹H-NMR spectrum of **4** and **9** in DMSO-d₆ and **8** in CDCl₃ showed a set of resonances in agreement with the proposed structure. The slightly shifted resonance at δ 15.45-16.38 ppm corresponds to the OH phenolic proton, whereas the NCH₂P protons of PTA co-ligands are visible as singlets at δ 4.15-4.16 ppm and the related NCH₂N

protons show characteristic AB quartets in the range 4.40-4.58 ppm. In the spectrum of complex **8** the aromatic hydrogens of the ligand and triphenylphosphine co-ligands are detectable in the range 6.71-7.63 ppm. The ^{31}P -NMR spectra of the PTA complexes **4** and **9** recorded in CDCl_3 , a singlet is visible at -93.61 and -94.43 ppm, downfield shifted concerning the value of the free PTA (δ -102.50 ppm). Broad signals are also in the spectra recorded in CD_3OD and CD_3CN at 233K, indicating the impossibility of stopping or slowing the phosphane exchange process, to show doublets in which the coupling of ^{31}P to the ^{107}Ag and ^{109}Ag are resolved. In the ^{31}P -NMR spectrum of the triphenylphosphine complex **8** recorded in CDCl_3 at room temperature, a singlet is visible at 14.68 ppm, downfield shifted concerning the value of the free PPh_3 (δ -5.34 ppm). In the spectrum of **8** recorded in CD_3CN at 233K, a doublet with $J(\text{Ag}-^{31}\text{P})$ coupling constant of 478 Hz, being of the same order of magnitude as those reported for analogous silver(I) bis(triphenylphosphine) species. The ESI-MS study was performed by dissolving complexes **4**, **8**, and **9** in methanol or acetonitrile and recording the spectra in ion-positive and ion-negative modes. ^1H -NMR and $^{31}\text{P}\{^1\text{H}\}$ -NMR data of complexes **4**, **8**, and **9** are summarized in **Table 2.1**. In the ESI-MS(+) spectrum of **4** and **9** the peaks at m/z 491 and 553 can be attributed to the species $[\text{Ag}(\text{HL}^{\text{BSMe}})(\text{PTA})]^+$ and $[\text{Ag}(\text{HL}^{\text{BSPH}})(\text{PTA})]^+$, confirming the formation and the stability of the complexes, respectively. In all the ESI-MS(+) spectra we detected peaks attributable to the fragments with $[\text{Ag}(\text{PTA})]^+$, $[\text{Ag}(\text{PTA})_2]^+$ and $[\text{Ag}(\text{PPh}_3)_2]^+$.

2.3.2 X-ray Crystallography

Despite several efforts, we were able to crystalline samples of only one of the afore mentioned compounds. In particular, the molecular structure of the $[\text{Cu}(\text{L}^{\text{BSPH}})_2]$ complex (**10**) was determined by single-crystal diffraction analysis on an item extracted from a batch of very good, dark green prismatic specimens, obtained by slow recrystallization of the compound from a toluene solution. The investigation revealed that the neutral complex crystallizes in the monoclinic system; the solid-state structure was solved in the centrosymmetric $I2/a$ space group (No. 15). The asymmetric unit (**Fig. 2.4**) consists of one half of the molecule, that is, the copper ion and a single molecule of the anionic 2-(benzylimino(phenyl)methyl)phenolate (L^{BSPH}) ligand, which chelates the central Cu atom through the O, N atoms. The phenyl ring of the benzylimino residue of the ligand (C15/C20) is disordered over two possible arrangements; the alternate positions of disordered atoms have been named with a final 'A'.

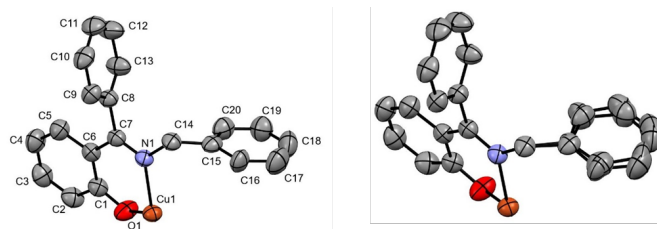


Fig. 2.4. Left: a drawing of the asymmetric unit $[\text{Cu}(\text{L}^{\text{BSP}^{\text{h}}})_2]$, showing the numbering scheme; disorder not shown. Right: the same view highlights the disorder of the phenyl ring C15/C20. Hydrogen atoms omitted; thermal ellipsoids at 50% probability.

Upon chelation, the ligand and the metal form a six-membered metallacycle (Cu1, O1, C1, C6, C7, N1) showing an *envelope* conformation, with the Cu1 atom at the flap' and departing by 0.55 Å from the mean plane (p1) of the other five atoms, that are instead coplanar within 0.05 Å, given the sp^2 nature of the C6-C7-N1 atoms of the ligand. In the chelating $\text{L}^{\text{BSP}^{\text{h}}}$ molecule, the mean plane of the phenol ring (C1/C6) is almost coplanar with p1 (angle of 1.39°); instead, the mean planes passing through the phenyl rings of the benzyl moiety (C15/C20 or C15A/C20A, respectively, p2 and p2') or emerging from the methine bridge (C8/C13, p3) are approximately orthogonal to the p1 plane, making with the latter angles of 79.1° (79.6° for p2'), and 75.2°, respectively. The p3 and p2 planes are also roughly orthogonal concerning each other (angles of 69.9° and 72.2° for p2 and p2', respectively). The copper coordination environment is completed by a second $\text{L}^{\text{BSP}^{\text{h}}}$ molecule (**Fig. 2.5**), in which both the O1 and N1 atoms are *trans*-positioned to their symmetry-generated counterparts. The $[\text{Cu}(\text{L}^{\text{BSP}^{\text{h}}})_2]$ complex (**10**) has a Calix shape when observed through the O_2N_2 coordination plane and the four coordinating atoms around the central copper atom define a moderately distorted square planar environment (τ_4 and τ_4' indexes of 0.21 and 0.18, respectively), with O1-Cu1-O1* and N1-Cu1-N1* (* = $3/2 - x, y, 1 - z$) angles of 160.77(9)° and 170.07(7)°, respectively, the first quite far from the ideal 180° value. The departure is due to the engagement of the O1 atom in an intermolecular non-canonical hydrogen bond with the H18 atom of a nearby phenyl ring (see further below).

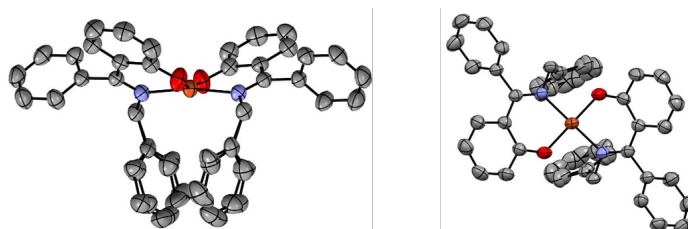


Fig. 2.5. Two orthogonal views of the $[\text{Cu}(\text{L}^{\text{BSP}^{\text{h}}})_2]$ complex. Left: overall view of the complex looking through the coordination plane, highlighting its calix shape; right: view of the Cu(II) coordination plane. Hydrogen atoms omitted; thermal ellipsoids at 50% probability.

Table 2.2 Relevant nonbonding interactions for [Cu(L^{BSPH})₂] (**10**).

A atom	D atom	P atom ^a	A····D (Å)	A····D-P (°)	Symmetry op. ^b
O1	H18	C18	2.81	171.8	$3/2 - x, y, 1 - z$
O1	H18A	C18	2.85	159.9	$3/2 - x, y, 1 - z$
C15/C20	H11	C11 ^c	2.78 ^d	168.8 ^d	$1 - x, -1/2 + y, 1/2 - z$
C15A/C20A	H11	C11 ^c	2.78 ^d	165.0 ^d	$1 - x, -1/2 + y, 1/2 - z$
C1	H12	C12 ^c	2.91	147.7	$1 - x, 1 - y, 1 - z$
C2	H12	C12 ^c	2.91	145.7	$1 - x, 1 - y, 1 - z$
C15/C20	C15/C20 ^e		4.20 ^f	26.6 ^g	$1/2 - x, y, 1 - z$
C15A/C20A	C15A/C20A ^e		4.03 ^f	23.4 ^g	$1/2 - x, y, 1 - z$

^aAtom to which D atom is bound; ^bsymmetry operations related to asymmetric unit = x, y, z ; ^cC-H···· π interactions; ^ddistances and angles referring to selected ring centroids; ^e π ···· π interactions; ^fdistance between ring centroids; ^gangle between mean planes encompassing phenyl rings.

Concerning bond distances and angles, the 2-(benzylimino(phenyl)methyl)-phenolate ligand does not show unusual metric values when compared to existing data. An investigation in the CCDC database for molecules showing the benzyl(diphenylmethylene)amine moiety returned about 50 entries. A comparison of bond distances next to the C=N link with those found in this work showed that the object with the best overall similarity, that is, with the smaller sum of deviations for examined distances, is the (already reported) ligand itself, besides a few other compounds. Interestingly, molecules in which the C=N distance is close to the one reported here (1.299(2) Å) are those in which a phenolic moiety was also present. The C=N bond participates in some degree of conjugation with the nearby phenyl rings, in known compounds as well as in the present complex. The C6-C7 and C7-C8 bonds are a little shorter than the ideal 1.54 Å (1.4654(19) and 1.5009(19) Å, compared with mean values of 1.494 and 1.492 Å for known molecules); the same is true for the N1-C14, C14-C15 distances in the benzyl residue (1.4787(17), 1.483(10) Å against corresponding mean values of 1.450 and 1.517 Å in reported compounds). These data suggest that bond conjugation in the ligand is little affected by coordination. A second search in the CCDC repository was made to seek transition metal complexes showing a ligand similar to L^{BSPH}. The search returned a very limited number of Mn, Co, Ni, and Cu compounds showing a tetradentate dimeric form of the L^{BSPH} ligand, all prepared by the same research group; to the best of our knowledge, **10** is the first Cu(II) complex of the L^{BSPH} ligand reported to date. In known molecules, the O and N donor atoms are

always *trans* to each other, opposite to what happens in the $[\text{Cu}(\text{L}^{\text{BSPH}})_2]$ complex. The Metal-O and Metal-N distances in these molecules fall in the ranges 1.82-1.91 Å, 1.85-2.03 Å, respectively, with differences basically due to the ionic radii of the elements. In fact, the mean values for the Cu-O, Cu-N distances in the Cu(II) known complexes (1.879 and 1.949 Å, respectively) are in very good/good agreement with the 1.8839(11) and 1.9773(12) Å found here.

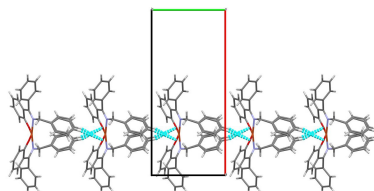


Fig. 2.6 Nonbonding contacts for $[\text{Cu}(\text{L}^{\text{BSPH}})_2]$; projection down the crystallographic c axis, highlighting the one-dimensional motif generated by the $\text{O1}\cdots\text{H18}$, $\text{O1}\cdots\text{H18A}$ hydrogen bonds (in cyan) and propagating along the crystallographic b axis. The $\pi\cdots\pi$ pairing of the C15/C20, C15A/C20A rings with the corresponding symmetry-generated counterparts is also visible. Other contacts were omitted for clarity.

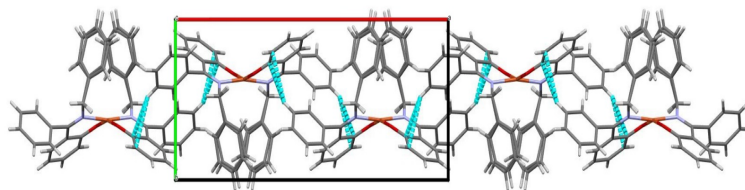


Fig. 2.7 Nonbonding contacts for $[\text{Cu}(\text{L}^{\text{BSPH}})_2]$; projection down the crystallographic c axis, highlighting the head-to-tail one-dimensional chain generated by the $\text{C1}(\text{C2})\cdots\text{H12}$ (in cyan) and propagating along the crystallographic a axis. Other contacts were omitted for clarity.

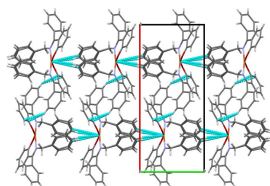


Fig. 2.8 Nonbonding contacts for $[\text{Cu}(\text{L}^{\text{BSPH}})_2]$; projection down the crystallographic c axis, highlighting (in cyan) the 2D nonbonding interaction grid formed into the ab plane due to crosslinking between the one-dimensional motifs described in Figures III and IV. Other contacts were omitted for clarity.

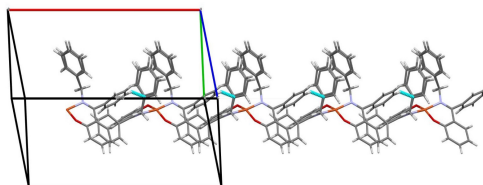


Fig. 2.9 Nonbonding contacts for $[\text{Cu}(\text{L}^{\text{BSPH}})_2]$; projection down the 0,1,1 plane showing the one-dimensional chain generated by the (almost) orthogonal $\text{C-H}\cdots\pi$ interaction between H11 and the C15/C20, C15A/C20A rings (only the $\text{C15}\cdots\text{H11}$ contact highlighted in cyan), propagating along the same plane. Other contacts were omitted for clarity.

The examination of relevant nonbonding interactions (metric details listed in **Table 2.2**), mostly hydrophobic contacts, shows that the complex units are well-packed and that the unit cell does not show solvent-accessible voids. Looking at the asymmetric unit (one half of the complex), just one intermolecular hydrogen bond connects H18 (or

H18A) with the O1 atom of a nearby molecule at $3/2 - x, -1 + y, 1 - z$ (at 2.81 / 2.85 Å, respectively); this interaction also shows in the symmetry-generated half of the molecule at $1/2 - x, y, 1 - z$ (**Fig. 2.6**). Incidentally, the two symmetry-related halves of the complex have their C15/C20 (or C15A/C20A) rings in roughly eclipsed positions, so they are also coupled by somehow weak $\pi \cdots \pi$ interactions, with distances between planes encompassing the alternate arrangements of the rings of 4.20, 4.03 Å, respectively, (compared with 3.35 Å in graphite). The H18 $\cdots$ O1 contact creates a one-dimensional motif that propagates along the crystallographic *b* axis, and it is coupled to a second orthogonal chain due to the C-H $\cdots$ π contacts made by C1 / C2 atoms and the H12 atom of another unit at $1 - x, 1 - y, 1 - z$ (**Fig. 2.7**). The two chains cross-link each other and create a 2D grid in the *ab* plane (**Fig. 2.8**).

Table 2.3 Summary of crystal and data collection parameters for [Cu(L^{BSP_h})₂] (**10**).

Empirical formula	C ₄₀ H ₃₂ CuN ₂ O ₂
Formula weight	636.21
Temperature/K	295.1(3)
Radiation	Cu K α (λ = 1.54184)
Crystal system	monoclinic
Space group	<i>I</i> 2/ <i>a</i>
<i>a</i> /Å	20.4267(4)
<i>b</i> /Å	8.7751(2)
<i>c</i> /Å	18.3240(3)
α /°	90
β /°	101.135(2)
γ /°	90
Volume/Å ³	3222.68(11)
<i>Z</i>	4
ρ_{calc} /cm ³	1.311
μ /mm ⁻¹	1.246
<i>F</i> (000)	1324.0
Crystal size/mm ³	0.42 × 0.4 × 0.2
2 θ range for data collection/°	8.824 to 155.422
Index ranges	-20 ≤ <i>h</i> ≤ 25, -11 ≤ <i>k</i> ≤ 11, -23 ≤ <i>l</i> ≤ 23
Reflections collected	36327
Independent reflections	3416 [<i>R</i> _{int} = 0.0377]
Data/restraints/parameters	3416/0/247
Goodness-of-fit on <i>F</i> ²	1.034
Final <i>R</i> indexes [<i>I</i> ≥ 2 σ (<i>I</i>)]	<i>R</i> ₁ = 0.0331, <i>wR</i> ₂ = 0.0927
Largest diff. peak/hole / e Å ⁻³	0.17/-0.31

Table 2.4 Bond Lengths (Å) for [Cu(L^{BSP_h})₂] (**10**).

Atom	Atom		Atom	Atom	
Cu1	O1	1.8839(11)	C10	C11	1.376(3)
Cu1	O1 ¹	1.8839(11)	C11	C12	1.370(3)
Cu1	N1 ¹	1.9773(12)	C12	C13	1.385(3)
Cu1	N1	1.9773(12)	C14	C15	1.483(10)
O1	C1	1.303(2)	C14	C15A	1.536(11)
N1	C7	1.299(2)	C15	C16	1.360(8)
N1	C14	1.4787(17)	C15	C20	1.369(8)
C1	C2	1.420(2)	C16	C17	1.382(9)
C1	C6	1.417(2)	C17	C18	1.347(12)
C2	C3	1.363(3)	C18	C19	1.322(13)
C3	C4	1.384(3)	C19	C20	1.386(8)
C4	C5	1.373(2)	C20A	C19A	1.3900
C5	C6	1.412(2)	C20A	C15A	1.3900
C6	C7	1.4654(19)	C19A	C18A	1.3900
C7	C8	1.5009(19)	C18A	C17A	1.3900
C8	C9	1.390(2)	C17A	C16A	1.3900
C8	C13	1.390(2)	C16A	C15A	1.3900
C9	C10	1.380(2)			

¹symmetry operation: 3/2 - x, y, 1 - z.**Table 2.5** Bond Angles (°) for [Cu(L^{BSP_h})₂] (**10**).

Atom	Atom	Atom		Atom	Atom	Atom	
O1	Cu1	O1 ¹	160.77(9)	C13	C8	C9	118.83(14)
O1 ¹	Cu1	N1	90.20(5)	C10	C9	C8	120.10(16)
O1	Cu1	N1 ¹	90.20(5)	C11	C10	C9	120.77(16)
O1 ¹	Cu1	N1 ¹	91.46(5)	C12	C11	C10	119.51(17)
O1	Cu1	N1	91.46(5)	C11	C12	C13	120.53(19)
N1 ¹	Cu1	N1	170.07(7)	C12	C13	C8	120.25(17)
C1	O1	Cu1	124.80(10)	N1	C14	C15	113.9(7)
C7	N1	Cu1	125.32(10)	N1	C14	C15A	108.1(7)
C7	N1	C14	120.91(12)	C16	C15	C14	122.7(9)
C14	N1	Cu1	113.63(9)	C16	C15	C20	116.7(7)
O1	C1	C2	117.14(14)	C20	C15	C14	120.6(9)
O1	C1	C6	124.98(13)	C15	C16	C17	121.4(8)
C6	C1	C2	117.88(14)	C18	C17	C16	120.1(9)
C3	C2	C1	121.81(16)	C19	C18	C17	119.9(6)
C2	C3	C4	120.51(15)	C18	C19	C20	120.5(7)
C5	C4	C3	119.43(17)	C15	C20	C19	121.3(7)
C4	C5	C6	122.06(16)	C19A	C20A	C15A	120.0
C1	C6	C7	122.71(13)	C18A	C19A	C20A	120.0
C5	C6	C1	118.30(13)	C19A	C18A	C17A	120.0
C5	C6	C7	118.95(13)	C16A	C17A	C18A	120.0

Atom	Atom	Atom		Atom	Atom	Atom	
N1	C7	C6	122.33(13)	C17A	C16A	C15A	120.0
N1	C7	C8	120.34(12)	C20A	C15A	C14	120.9(8)
C6	C7	C8	117.32(12)	C16A	C15A	C14	118.6(8)
C9	C8	C7	119.69(14)	C16A	C15A	C20A	120.0
C13	C8	C7	121.47(13)				

¹symmetry operation: $3/2 - x, y, 1 - z$.

Finally, a third chain is formed by a second, T-shaped C-H $\cdots\pi$ contact involving the C15/C20 (or C15A/C20A) ring with the H11 atom of a nearby molecule at $1 - x, -1/2 + y, 1/2 - z$ (**Fig. 2.9**; only C15 $\cdots$ H11 approach shown). The H11 atom in fact points almost straight towards the C15/C20 ring centroid, with a contact length of 2.78 Å (also towards the C15A/C20A ring) and C11-H11-ring centroid angle of 168.8° and 165.0° for C15/C20, C15A/C20A rings, respectively. This chain propagates along the [011] plane and diagonally intersects the above described 2D motif, thus sealing a full 3D contact network. Additionally, the crystallographic data collection revealed out that the phenyl ring (C15/C20) belonging to the benzylimino moiety of the ligand can assume two possible arrangements (involved atoms labelled with a final 'A'). The sum of the pertinent occupation factors for each disordered position were constrained to unity, with finally refined sofs of 0.57/0.43 for untagged and tagged atoms, respectively. A regular hexagon geometry with bond distances of 1.3900 Å was imposed on the disordered positions of the phenyl ring *via* the AFIX 6 constraint to ensure refinement convergence and to prevent some atoms from assuming non-positive definite displacement parameters. A summary of crystal and data collection parameters is given below in **Table 2.3**; full listings of bond lengths and angles in **Tables 2.4** and **2.5**.

2.3.3 *In vitro* antibacterial activity

First, the resazurin-based microtiter dilution assay (RMDA) method was used to evaluate the synergic effect of Schiff bases (**1** and **2**), metal-complexes (**3-10**), and phosphane co-ligands (**11** and **12**) against Gram-negative bacteria (*E. coli*) (**Fig. 2.10**) and Gram-positive (*S. aureus*) (**Fig. 2.11**). The minimum inhibitory concentration (MIC) and Half-maximal inhibitory concentrations were reported (MIC₅₀) in **Table 2.6** and higher MIC values indicate lower antibacterial activity of the complex. Generally, the growth of bacteria follows four phases: lag phase, logarithmic phase, stationary phase and decline phase. When bacteria are exposed to external interferences or inhibitory factors, their growth may be significantly delayed or even completely suppressed,

ultimately leading to growth cessation. Based on the bactericidal growth curves, the free Schiff base ligands and phosphane co-ligands exhibited negligible toxicity against both tested bacterial species, while all the metal complexes demonstrated varying degrees of antibacterial activity. Especially, the silver complexes $[\text{Ag}(\text{HL}^{\text{BSMe}})(\text{PTA})]\text{NO}_3$ (**4**) and $[\text{Ag}(\text{HL}^{\text{BSPh}})(\text{PTA})]\text{NO}_3$ (**9**) showed significant bactericidal effect. The MIC values of compound **4** against *E. coli* and *S. aureus* were 0.050 and 0.100 mg mL⁻¹, respectively, while the MIC values of compound **9** were 0.025 and 0.050 mg mL⁻¹, respectively. Interestingly, the MIC values of compound **4** were higher in comparison to the values of **9**; however, the bacterial growth curves indicated that the growth rate of bacteria treated with compound **4** was slower at concentrations below 0.025 mg mL⁻¹. Comparing compounds **4** and **9**, the primary structural difference lies in the structure of the Schiff base ligands with the presence of a methyl or a phenyl substituent, respectively. Previous studies have suggested that the bactericidal activity of silver compounds is primarily due to two distinct mechanisms, including the strong affinity to associate with R-SH groups and their ability to generate reactive oxygen species (ROS). Silver ions can readily bind to SH groups, such as those in cysteine residues, which can disrupt cellular metabolism and physiology by directly interfering with enzymatic functions or by destabilizing disulfide (S-S) bonds critical for maintaining protein structure. However, effective binding to SH groups requires silver ions to be sufficiently accessible and reactive [72].

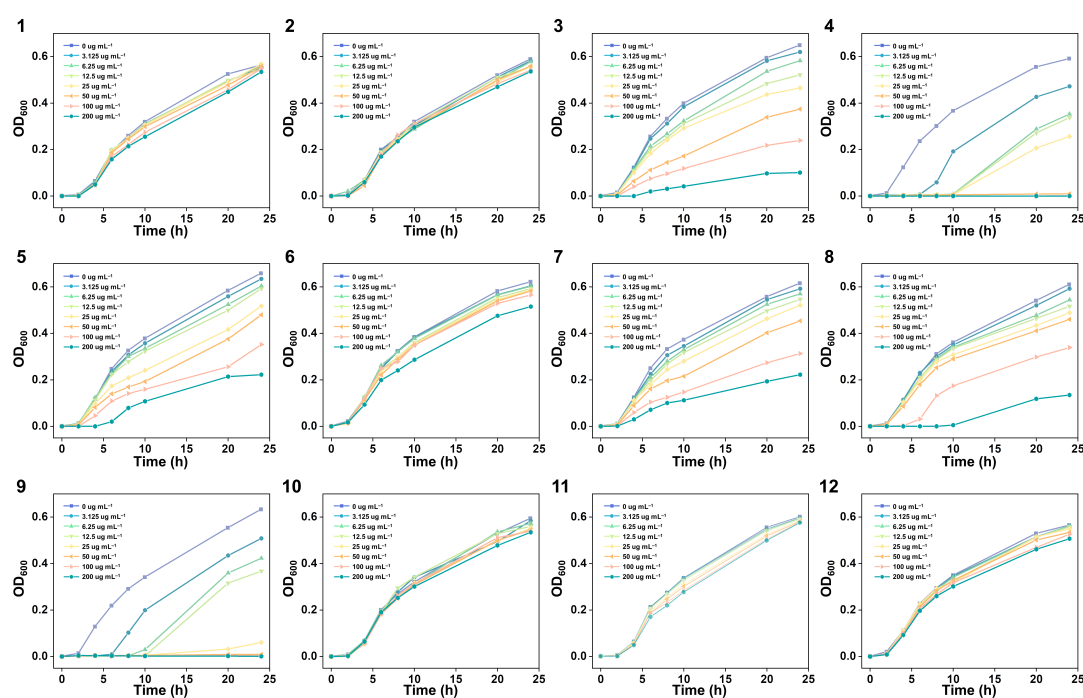


Fig. 2.10 Bacterial growth curves of *E. coli* after treatment with Schiff base ligands (**1** and **2**), metal complexes (**3-10**), and phosphane co-ligands (**11** and **12**). The data represent the means of three independent experiments.

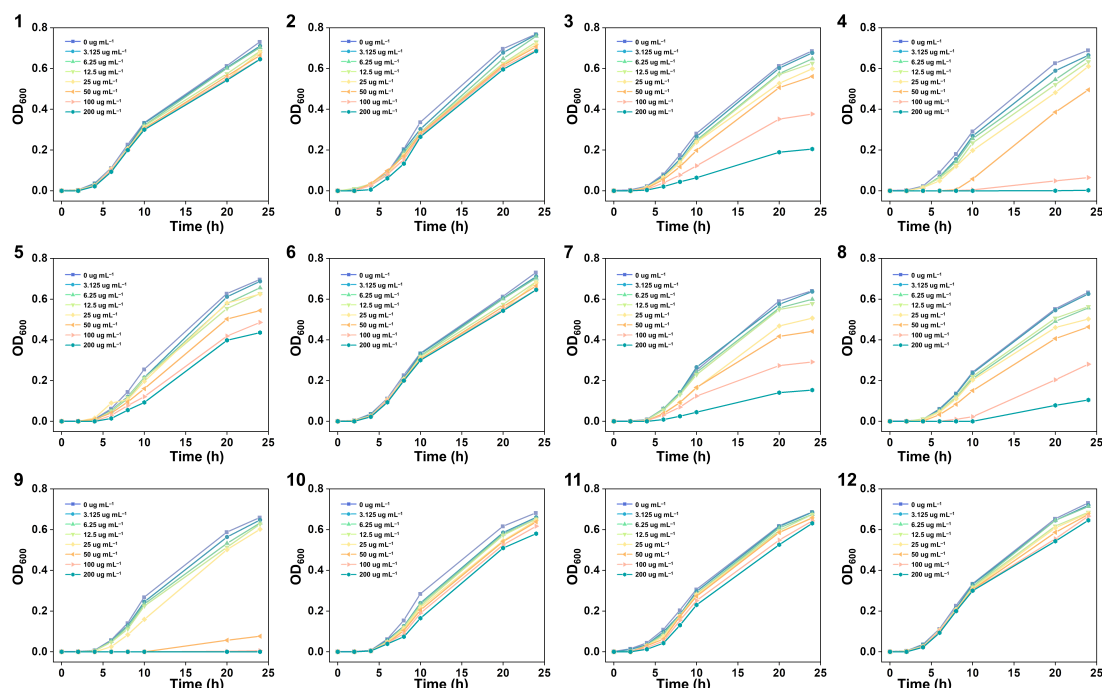


Fig. 2.11 Bacterial growth curves of *S. aureus* after treatment with Schiff base ligands (**1** and **2**), metal complexes (**3-10**), and phosphane co-ligands (**11** and **12**). The data represent the means of three independent experiments.

Table 2.6 MIC and MIC₅₀ values of Schiff base ligands **1** and **2**, metal complexes **3-10**, and co-ligands **11** and **12** *in vitro* antimicrobial screening.

N.	Tested compounds	<i>E. coli</i>		<i>S. aureus</i>	
		MIC	MIC ₅₀	MIC	MIC ₅₀
1	HL ^{BSMe}	/	/	/	/
2	HL ^{BSPH}	/	/	/	/
3	[Cu(HL ^{BSMe})(PTA) ₂] ₂ PF ₆	/	0.057 (0.076)	/	0.116 (0.149)
4	[Ag(HL ^{BSMe})(PTA)]NO ₃	0.050 (0.090)	0.012 (0.021)	0.100 (0.181)	0.061 (0.110)
5	[Cu(L ^{BSMe}) ₂]	/	0.110 (0.210)	/	0.381 (0.738)
6	[Cu(HL ^{BSPH})(PPh ₃) ₂] ₂ PF ₆ ·2CH ₃ CN	/	/	/	/
7	[Cu(HL ^{BSPH})(PTA) ₂] ₂ PF ₆ ·2H ₂ O	/	0.114 (0.130)	/	0.084 (0.100)
8	[Ag(HL ^{BSPH})(PPh ₃) ₂] ₂ NO ₃	/	0.098 (0.100)	/	0.080 (0.079)
9	[Ag(HL ^{BSPH})(PTA)]NO ₃	0.025 (0.040)	0.010 (0.016)	0.050 (0.081)	0.036 (0.058)
10	[Cu(L ^{BSPH}) ₂]	/	/	/	/
11	PPh ₃	/	/	/	/
12	PTA	/	/	/	/

MIC and MIC₅₀ calculated in mg mL⁻¹ (mM in parentheses); “/”: more than 0.381 mg mL⁻¹ and 0.738 mM.

The bactericidal activity of the compounds at different concentrations was further evaluated using the plate colony count method against *E. coli* (**Fig. 2.12**) and *S. aureus* (**Fig. 2.13**). The minimum bactericidal concentration (MBC) and Half-maximal bactericidal concentration (MBC₅₀) were calculated and included in **Table 2.7**. The results demonstrate that the antimicrobial activity of the metal complexes **3-9** is

significantly enhanced compared to the free Schiff base ligands, as explained by Tweedy's chelation theory. Chelation reduces the polarity of the metal ion, primarily due to the electron-donating properties of Schiff base ligands, allowing the metal ion to share part of its charge through orbital overlap. Furthermore, this process increases the lipophilicity of the central metal atom, facilitating its penetration through the lipid layer of the microorganism, and blocking the interaction of enzymes on the cell membrane, thereby inhibiting the growth of the microorganism ^[73]. Subsequently, Cu(I) and Ag(I) coordination compounds were investigated to study the influence of the nature of metal center in bioactivity. A comparison of the antimicrobial properties of compounds **3** and **4**, **6** and **8**, as well as **7** and **9** reveals that silver complexes exhibit superior antimicrobial effects against both *E. coli* and *S. aureus* strains compared to their copper-based counterparts. Among these metal complexes, compound **9** was found to be most active against the bacterial strains with MBC values of 0.100 and 0.200 mg mL⁻¹ for *E. coli* and *S. aureus*, respectively. Also compound **4** was prominently active with MBC values of 0.200 mg mL⁻¹ against both tested bacterial strains. Compound **8** displayed moderate inhibitory effects with MBC₅₀ values of 0.322 and 0.294 mg mL⁻¹, however lower than the MBC₅₀ values of 0.566 and 1.125 mg mL⁻¹ for compound **6**. As previously mentioned, Ag(I) ions can alter the bacteria cell wall structure and cell constituent by irreversibly binding to cysteine residues, thereby disrupting essential enzyme systems and ultimately inhibiting bacterial proliferation ^[72]. Furthermore, a possible correlation between the biological activity of the compounds and their lipophilicity and hydrophilicity was discussed. By comparing compounds **6** and **7**, as well as **8** and **9**, it was observed that the antibacterial activity of the complexes with PTA as a co-ligand was superior to those with PPh₃ as a co-ligand. Notably, the lipophilic triphenylphosphine and the hydrophilic 1,3,5-triaza-7-phosphaadamantane have been chosen as co-ligands because they can stabilize copper and silver in +1 oxidation state and can give also a different hydrophilic-lipophilic balance to the corresponding complexes. Antibacterial data show that the PTA co-ligands confer to the related silver complexes the most appropriate balance between hydrophilicity and lipophilicity, what is in fact a major challenge in the development of antimicrobial agents ^[74]. The presence of lipophilic groups enhances the ability of compounds to penetrate the bacterial lipophilic outer membrane which contains murein, thereby facilitating interactions with the bacterial replicative system. However, several studies have also highlighted the importance of water solubility as a key parameter in antibacterial activity.

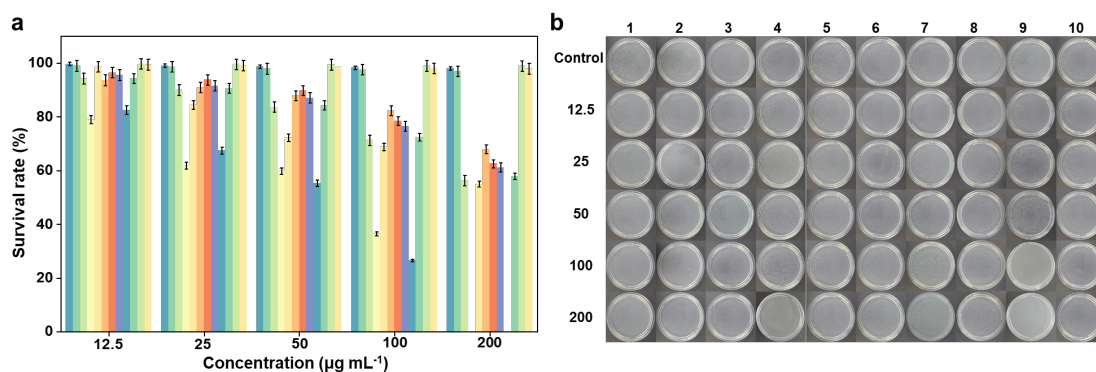


Fig. 2.12 (a) Corresponding bacterial viability of compounds **1-12** with different concentrations and (b) bacterial colony images of *E. coli* after treatment with the compounds. Colonies were quantified with Image J software (1.50d) and presented as mean values from duplicate independent experiments. Error bars denote standard deviation. The significance between the control and treated sample levels was established at $P < 0.05$. Data were presented as the average values from three independent experiments, and error bars represent the standard deviations (SD) of three replicates.

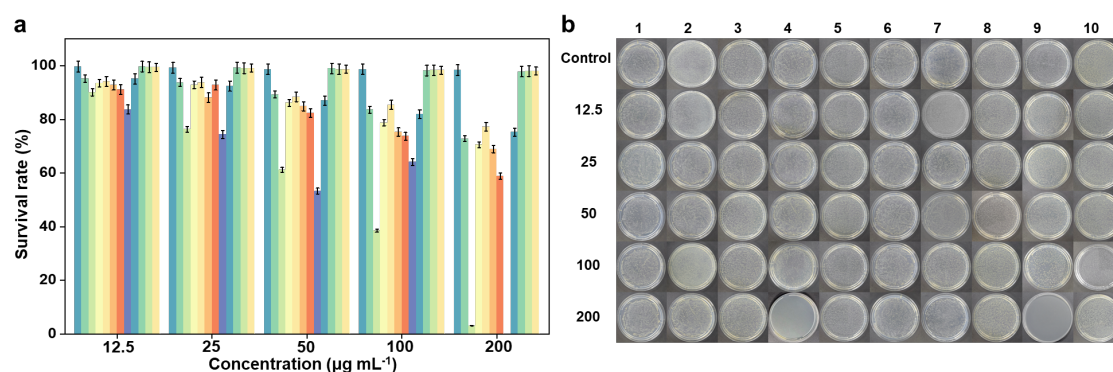


Fig. 2.13 (a) Corresponding bacterial viability of compounds **1-12** with different concentrations and (b) bacterial colony images of *S. aureus* after treatment with the compounds. Colonies were quantified with Image J software (1.50d) and presented as mean values from duplicate independent experiments. Error bars denote standard deviation. The significance between the control and treated sample levels was established at $P < 0.05$. The significance between the control and treated sample levels was established at $P < 0.05$. Data were presented as the average values from three independent experiments, and error bars represent the SD of three replicates.

Table 2.7 MBC and MBC₅₀ values of Schiff base ligands **1** and **2**, metal complexes **3-10**, and co-ligands **11** and **12** *in vitro* antimicrobial screening.

N.	Tested compounds	<i>E. coli</i>		<i>S. aureus</i>	
		MBC	MBC ₅₀	MBC	MBC ₅₀
1	HL ^{BSMe}	/	/	/	/
2	HL ^{BSPH}	/	/	/	/
3	[Cu(HL ^{BSMe})(PTA) ₂] ₂ PF ₆	/	0.262 (0.350)	/	0.642 (0.858)
4	[Ag(HL ^{BSMe})(PTA)]NO ₃	0.200 (0.362)	0.048 (0.086)	0.200 (0.362)	0.062 (0.112)
5	[Cu(L ^{BSMe}) ₂]	/	0.243 (0.474)	/	0.578 (1.128)
6	[Cu(HL ^{BSPH})(PPh ₃) ₂] ₂ PF ₆ ·2CH ₃ CN	/	0.566 (0.513)	/	1.125 (1.020)
7	[Cu(HL ^{BSPH})(PTA) ₂] ₂ PF ₆ ·2H ₂ O	/	0.319 (0.377)	/	0.551 (0.651)
8	[Ag(HL ^{BSPH})(PPh ₃) ₂] ₂ NO ₃	/	0.322 (0.327)	/	0.294 (0.299)
9	[Ag(HL ^{BSPH})(PTA)]NO ₃	0.100 (0.163)	0.046 (0.075)	0.200 (0.326)	0.068 (0.110)
10	[Cu(L ^{BSPH}) ₂]	/	0.282 (0.443)	/	0.875 (1.375)
11	PPh ₃	/	/	/	/
12	PTA	/	/	/	/

MBC and MBC₅₀ calculated in mg mL⁻¹ (mM in parentheses); “/”: more than 1.125 mg mL⁻¹ and 1.375 mM.

The antibacterial performance of the synthesized compounds was systematically evaluated concerning four critical determinants: (i) ligands chelation effects on bioavailability, (ii) properties of metal ions, and (iii) hydrophobic-lipophilic balance. Compared to the broad-spectrum antibiotic ciprofloxacin (Fig. 2.14), although the synthesized metal complexes are less effective than the standard drug, compounds **4** and **9** have demonstrated significant potential as antibacterial agents. In addition, compared with other silver Schiff base metal complexes reported for antibacterial applications, compounds **4** and **9** exhibit similar or lower MIC values [72, 75].

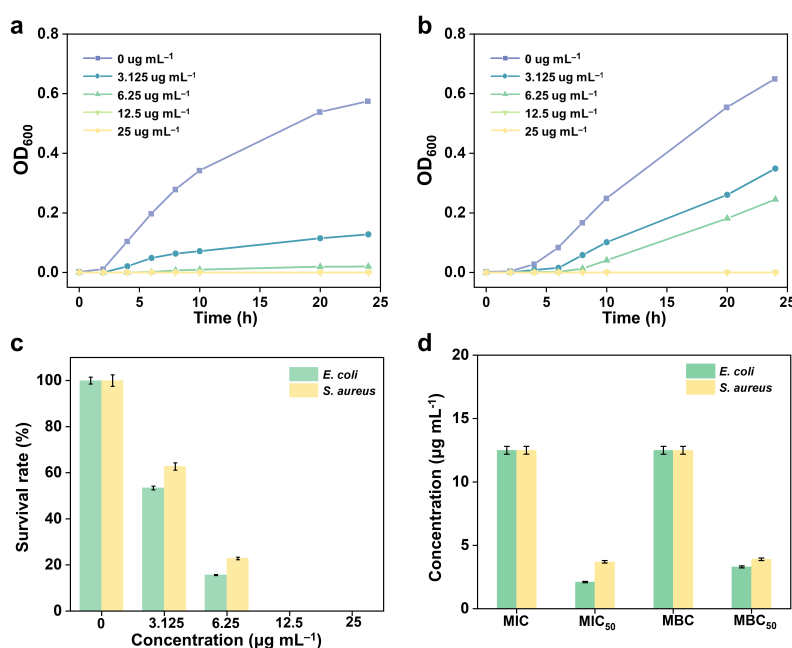


Fig. 2.14 Bacterial growth curves of (a) *E. coli* and (b) *S. aureus* after treatment with ciprofloxacin. (c) Corresponding bacterial viability of *E. coli* (green) and *S. aureus* (yellow) after treatment with ciprofloxacin. (d) The summary of antimicrobial activity of ciprofloxacin. The significance between the control and treated sample levels was established at $P < 0.05$. Data were presented as the average values from three independent experiments, and error bars represent the SD of three replicates.

2.4 Conclusion

In this study, we report the synthesis, characterization and antibacterial evaluation of Cu(I) and Ag(I) complexes supported by two phenoxy-ketimines ligands. The single-crystal XRD allowed to describe the molecular structure of $[\text{Cu}(\text{L}^{\text{BSPH}})_2]$, the first reported complex of Cu(II) with the HL^{BSPH} ligand, where the copper ion has a slightly distorted square planar environment. The antimicrobial properties of the new copper and silver complexes as well as of the corresponding uncoordinated ligands were evaluated against *E. coli* and *S. aureus*. The results revealed that all the complexes exhibited moderate to excellent antibacterial efficacy. Among them, the Ag(I) complexes displayed the highest antibacterial activity. Specifically,

[Ag(HL^{BSP_h})(PTA)]NO₃ demonstrated the most promising antibacterial performance, with MIC values of 0.025 and 0.05 mg mL⁻¹ against *E. coli* and *S. aureus*, respectively, while the corresponding MBC values were 0.100 and 0.200 mg mL⁻¹.

This study provides new insights into the design of antimicrobial agents, highlighting the potential for rationally designing and synthesizing novel, highly efficient antibacterial compounds. To further enhance the antibacterial activity of the silver complexes of phenoxy-ketimine Schiff base ligands, future research should focus on optimizing the synthesis of the most promising candidates, including fine-tuning the electronic and steric effects of both primary and auxiliary ligands, as well as modifying the solubility properties of the related complexes. In addition, detailed mechanistic studies could reveal specific pathways and interactions, facilitating the design of more effective compounds. Future investigations should also prioritize an in-depth exploration of structure-activity relationships to elucidate the role of distinctive phosphanes and of different substituents on the phenoxy-ketimines ligands in biological activity.

Chapter III: CuI/Schiff base catalyzed for One-Pot A³-coupling under solvent-free conditions

3.1 Introduction

A catalyst is a substance that accelerates the reaction without being consumed or permanently altered, which provides an alternative reaction pathway with lower activation energy, making the process more efficient. The catalyst plays a crucial role in many chemical and biological processes. In industry, catalysts are indispensable for producing fuels, pharmaceuticals, and polymers, improving reaction rates and selectivity while reducing energy consumption. In energy systems, energy technology depends on catalysts in the processing of conventional fuels and emerging forms of renewable energy, for instance, where hydrogen is produced via photocatalytic or electrocatalytic routes. In biological systems, enzymes act as natural catalysts, facilitating vital biochemical reactions essential for life [76].

With increasing environmental concerns, green catalysts have gained prominence in catalysis, addressing the conflict between chemical industry expansion and sustainable development. Green catalysts optimize raw material utilization, eliminate toxic reagents and solvents, and minimize waste or harmful by-products. Among catalytic technologies, heterogeneous catalysis has attracted significant attention due to its advantages in catalyst recovery, reusability, and scalability. Unlike homogeneous catalysts, heterogeneous systems facilitate separation and reduce metal contamination, aligning with green chemistry principles. Since heterogeneous catalysis occurs at solid-gas or solid-liquid interfaces, it plays a pivotal role in chemical production, energy conversion, and environmental sustainability. Improvements in heterogeneous catalysis can result in more favorable kinetics and high selectivity, in turn improving energy efficiency.

The multicomponent reaction (MCR) strategy for the construction of important molecular scaffolds is trending nowadays as these reaction strategies are atom economic in nature and very strict in by-product formation that significantly contributes towards “Green Chemistry” [77]. Recently, such strategies have been widely applied in numerous organic transformations such as triazole formation, propargylamine formation, etc. The MCR strategy for propargylamine synthesis consists of coupling of aldehyde, alkyne and amine, and is commonly termed as A³-coupling reaction. Over

the past two decades, A³-coupling reactions have garnered significant attention in organic chemistry due to their ability to generate diverse valuable heterocycles in a single step. For example, the resultant propargylamine derivatives are key intermediates for the formation of varieties of important heterocyclic compounds such as pyrroles, pyridines, oxazoles etc. and numerous biologically active molecules such as β -lactams, conformationally restricted peptides, isosteres, fungicides, herbicides and therapeutic drug molecules pargyline (neurodegenerative disease), rasagyline (Parkinson's disease) and selegiline (Alzheimer's disease) [77]. However, among A³-coupling reactions, the volatility of most alkynes limits the applicability of this method to some extent. Additionally, this method's major drawback was its high moisture sensitivity, necessitating strictly anhydrous reaction conditions. Currently, numerous methods have been developed for A³-coupling reactions using various transition metal catalysts, including Cu-, Au-, Ag-, Ni-, Fe-, Zn-, and bimetallic Cu-/Ru- salts [76].

Schiff bases, as a type of ligand, play an important role in coordination chemistry. An imine-N donor was utilized as an anchoring group in the study of C-Cl, C-F, and C-H bond activation. However, their application in combination with transition metals for A³-coupling reactions remains comparatively underexplored.

Notably, among A³-coupling reactions, the multicomponent reactions with more challenging primary amines as substrates have been more rarely reported. In this context, we successfully developed a novel catalyst-CuI/Schiff base, for directly synthesizing propargylamines in a one-pot multicomponent reaction of amines, aldehydes, and alkynes. The reaction proceeds efficiently under mild, solvent-free conditions, achieving high yields within 4 h. Moreover, this approach requires only a small amount of an inexpensive and readily available catalyst (2.5 to 10 mol% CuI/Schiff base). This method not only streamlines the synthesis of propargylamines but also aligns with the principles of green chemistry.

3.2 Experimental Section

3.2.1 Materials and Instruments

The synthetic method and instruments are referenced in **2.2** experimental section, **Chapter II**.

3.2.2 Synthesis

3.2.2.1 Synthesis of Schiff base

The synthetic method of Schiff base was referenced in **Chapter II**, and this chapter only changes the group on ketones and amines.

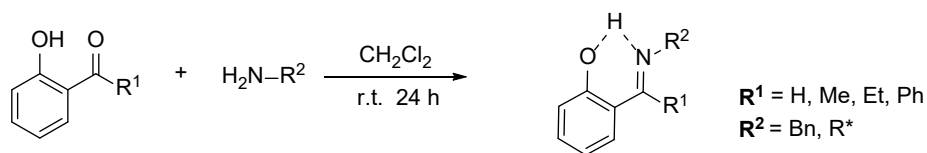
3.2.2.2 General procedure for CuI/Schiff base catalyzed A³-coupling reaction

The A³-coupling reaction was performed in three sequential steps. First, copper iodide and the Schiff base ligand were combined at room temperature and stirred for 12 h under a nitrogen atmosphere, allowing Cu(I) to be coordinated with the Schiff base. Then, the solvent was evaporated under a nitrogen flow, after which benzaldehyde, benzylamine, and magnesium sulfate were added, and the reaction continued for 30 minutes. Next, phenylacetylene and benzoic acid were introduced, and the reaction mixture was heated to 80 °C for 4 h. Finally, copper was removed from the reaction mixture through successive treatments with hydrochloric acid (0.5 M) and an ammonia buffer solution (0.5 M), and the product yield was determined by gas chromatography.

3.3 Results and Discussion

3.3.1 Synthesis and characterization

As previously described in **Chapter II**, the synthetic methodology employed in this chapter remains consistent (**Scheme 3.1**). In brief, Schiff bases are formed through a condensation reaction between an aldehyde or ketone and an amine. Initially, the carbonyl compound undergoes nucleophilic addition by the amine, forming a carbinolamine intermediate. Subsequently, this intermediate undergoes dehydration, eliminating water molecules, to yield the corresponding imine (Schiff base).



Scheme 3.1 Mechanism of Schiff base synthesis.

In addition, different substituent groups on ketones and amines were introduced to synthesize eight distinct Schiff base structures, as illustrated in **Fig. 3.1**. In contrast to **Chapter II**, this chapter investigates explicitly the catalytic performance of these Schiff bases in the A³-coupling reaction of aldehydes and alkynes. Based on our group's previous research on A³-coupling reaction conditions, which explored various

parameters such as catalyst loading, solvent, temperature, and reaction time, we established the optimal conditions as follows: the molar ratios of amines, aldehydes, and alkynes were 1:1:1.1, the reaction temperature was 80 °C, and the reaction time was 4 h under solvent-free conditions. The catalytic performance of the CuI/Schiff base catalyst was subsequently evaluated through the A³-coupling reaction of benzylamine, benzaldehyde, and phenylacetylene under these optimized conditions (**Scheme 3.2**).

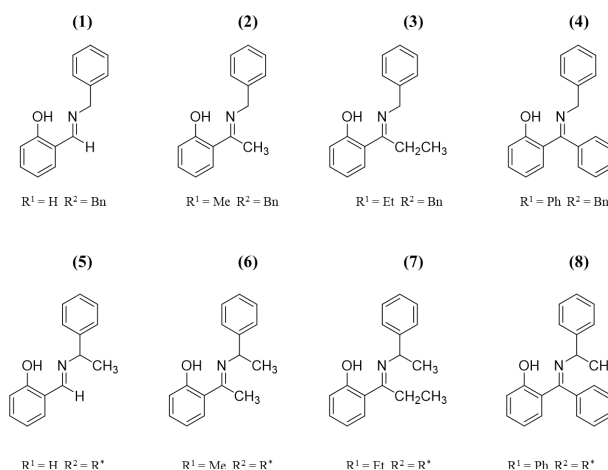
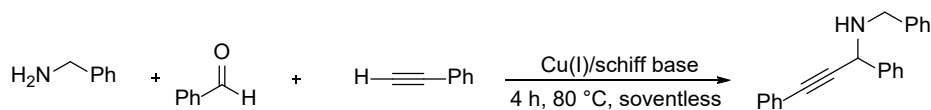


Fig. 3.1 Structure of Schiff base ligands used in this study.



Scheme 3.2 A³-coupling reaction of benzylamine, benzaldehyde, and phenylacetylene with CuI/Schiff base as catalysts.

Table 3.1 Summary of A³-coupling reaction.

Entry	R ¹	R ²	Catalyst (mol %)	Yield ^a (%)	Entry	R ¹	R ^{2b}	Adduct (mol %)	Yield ^a (%)
1	H	Bn	2.5	29	17	H	R [*]	2.5	12
2	H	Bn	5	64	18	H	R [*]	5	36
3	H	Bn	7.5	62	19	H	R [*]	7.5	62
4	H	Bn	10	58	20	H	R [*]	10	65
5	Me	Bn	2.5	65	21	Me	R [*]	2.5	29
6	Me	Bn	5	49	22	Me	R [*]	5	44
7	Me	Bn	7.5	50	23	Me	R [*]	7.5	48
8	Me	Bn	10	75	24	Me	R [*]	10	60
9	Et	Bn	2.5	9	25	Et	R [*]	2.5	38
10	Et	Bn	5	39	26	Et	R [*]	5	53
11	Et	Bn	7.5	40	27	Et	R [*]	7.5	55
12	Et	Bn	10	65	28	Et	R [*]	10	60
13	Ph	Bn	2.5	49	29	Ph	R [*]	2.5	7
14	Ph	Bn	5	47	30	Ph	R [*]	5	24
15	Ph	Bn	7.5	40	31	Ph	R [*]	7.5	53
16	Ph	Bn	10	74	32	Ph	R [*]	10	58

Notes: The pilot reaction was performed with benzaldehyde (1.0 mmol), benzylamine or R^{*}NH₂ (1.0 mmol), phenylacetylene (1.3 mmol). The catalyst is obtained by stirring equal amounts of CuI and Schiff base in acetonitrile under nitrogen atmosphere at room temperature for 4 h. ^a Gas chromatographic yields. ^b R^{*} stands for (R)-1-phenylethyl group; R^{*}-NH₂ stands for (R)-1-phenylethylamine.

For comparative analysis, the results of the A³-coupling reaction were summarized in **Table 3.1**, with yields reported as the percentage of propargylamine produced. A control experiment performed without copper iodide and Schiff base catalyst showed no detectable formation of propargylamine after 4 h of reaction time. This observation aligns with previous findings [78], indicating that copper is essential for the reaction to proceed, and confirms that the A³-coupling likely occurs exclusively through copper-catalyzed activation of the terminal alkyne C-H bond. As illustrated in **Fig. 3.2**, no clear correlation is observed between catalytic activity and catalysts **1-4** content. The highest yields of propargylamine obtained from catalysts **1** to **4** were 64%, 75%, 65%, and 74%, respectively. This variability in catalytic efficiency may be attributed primarily to electronic effects. The electron-donating methyl substituent increases the electron density on the coordinated nitrogen atom, enhancing its coordination ability and stability with copper. Consequently, the activity of the copper catalyst is improved, leading to higher yields. Although an ethyl substituent also possesses electron-donating properties, its more considerable steric hindrance may inhibit catalyst performance, resulting in lower yields than methyl substitution. Furthermore, previous studies have highlighted the significance of weak interactions in catalytic processes. In particular, the enhanced catalytic efficiency of the phenyl substituent may result from the π - π interactions between the phenyl group of the catalyst and the aromatic substrate [79], thereby increasing the catalyst-substrate affinity and improving reaction outcomes.

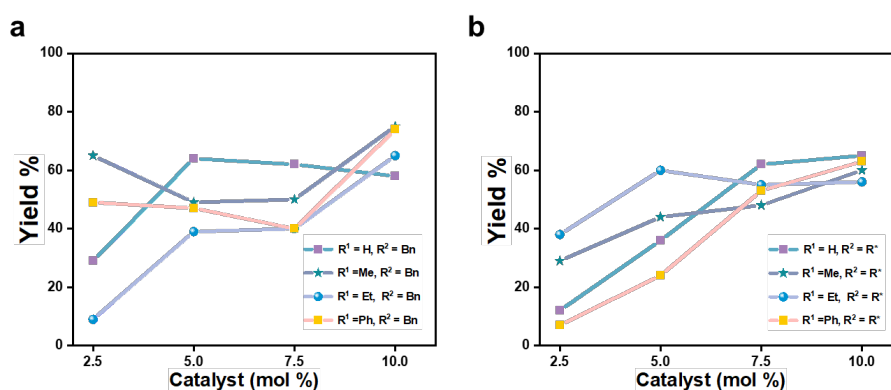


Fig. 3.2 The results of the A³-coupling reaction with CuI/Schiff base as the catalyst.

The catalytic activity of catalysts **5-8** increased with the amount of catalyst added, however, no significant differences were observed in their highest catalytic activities, yielding propargylamine in 65%, 60%, 60%, and 63%, respectively. This can be rationalized by steric hindrance effects. Previous studies have demonstrated that the steric effects of large heterocyclic groups significantly influence catalytic processes by

controlling spatial accessibility around the active metal center. Specifically, when R^* [(R)-1-phenylethyl] as the functional group, increasing the substituent size (R^1) from methyl to ethyl enhances steric hindrance, restricting substrate access to the active site and marginally decreasing reaction efficiency. Conversely, although the aryl group is larger, its planar conjugated structure facilitates strong π - π interactions between the catalyst and substrate, slightly increasing the yield compared to alkyl substitutions. In summary, when the R^1 substituent is identical, Schiff bases containing functional group benzyl exhibit higher catalytic activity than those with functional group (R)-1-phenylethyl group.

Overall, these results highlight that substituent variation significantly influences the electronic and steric properties of Schiff base copper catalysts, directly impacting their catalytic activities. This relationship underscores the critical importance of comprehensively evaluating structural factors when optimizing catalyst design.

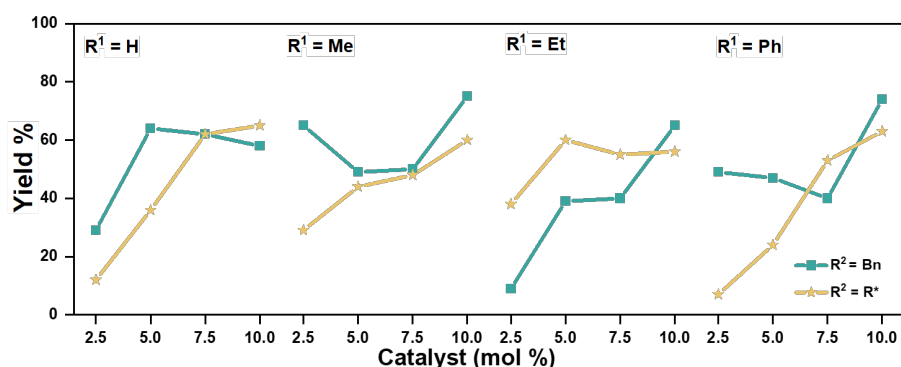
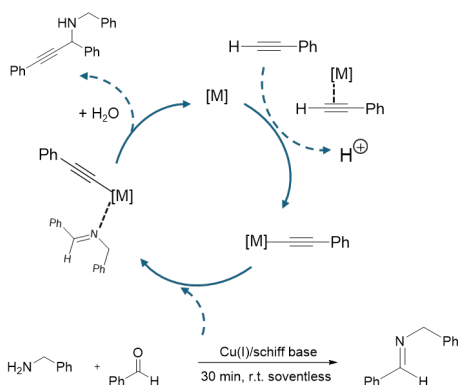


Fig. 3.3 The results of the A^3 -coupling reaction with CuI/Schiff base as the catalyst.

Based on control experiments and literature studies, the possible mechanisms of the A^3 -coupling reaction catalyzed by CuI/Schiff base were depicted in **Scheme 3.3**. This tailored approach significantly expands the scope and applicability of the A^3 -coupling reaction and other metal-catalyzed C-H activation reactions. The proposed catalytic cycle follows a sequence similar to the one outlined in the Scheme, consisting of the following steps:

- 1: A monomeric metal species forms a π -complex with the terminal alkyne.
- 2: Activation of the C(sp)-H bond occurs, whereby the coordinating metal weakens the terminal hydrogen, facilitating its removal by a mild base, such as the amine component or an anion, to generate the active metal acetylide intermediate.
- 3: The acetylide intermediate nucleophile intermediate attacks the imine or iminium intermediate.
- 4: Decomplexation occurs, releasing the propargyl amine (PA) product and

regenerating the metal catalyst, allowing it to re-enter the catalytic cycle.



Scheme 3.3 Proposed reaction mechanism for the A³-coupling reaction.

3.4 Conclusion

We have successfully developed a catalytic application of CuI/Schiff base systems for the synthesis of propargylamines through the three-component coupling of benzylamine, benzaldehyde, and phenylacetylene via C-H activation, achieving moderate to good yields (approximately 60-75%). To further expand the applicability of this catalytic system, ongoing studies are exploring the scope and generality of the CuI/Schiff base-promoted A³-coupling reaction by employing a variety of structurally diverse aldehydes, amines, and alkynes as reaction substrates.

Chapter IV: New copper complexes with N, O-donor ligands based on pyrazole moieties supported by 3-substituted acetylacetone scaffold

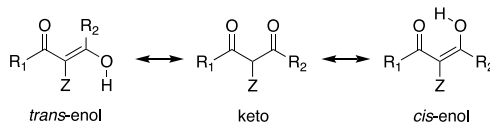
4.1 Introduction

β -Diketone compounds represent an essential class of reagents in synthetic chemistry and have a well-established role in the synthesis of heterocyclic compounds. The biological effects of these compounds are of great interest, and they have also been investigated as potential antiviral agents. Although β -diketones represent one of the oldest classes of chelating ligands, their coordination chemistry continues to attract much interest, due to the ability of related metal complexes to support several unique and important catalytic reactions. In this regard, it is often noted that even modestly sterically hindered β -diketones offer improvements over the parent acetylacetone. The presence of steric bulk on β -diketones is highly interesting for their peculiar coordination behavior useful to improve their catalytic activity and selectivity [79].

β -diketones are known to form complexes with almost every metal and despite the enormous amount of work devoted to the synthesis and characterization of copper(II) β -diketonate complexes [80], there are relatively few reports devoted to the corresponding Cu(I) complexes showing to undergo disproportionation to copper metal and copper(II) compounds in the absence of stabilizing ligands. In particular, very little attention has been paid to the study of triorganophosphane adducts of copper(I) β -diketonates of general formula (β -diketonate)Cu(PR₃)_n, although a rich structural diversity can also be expected there. On the other hand, very few studies on the biological activity of group 11 metal complexes of β -diketones have been reported in the literature to date [81] and copper(I)-based anticancer complexes supported by β -diketonate ligands remain an unexplored research field. Recently, as part of the investigation of the chemical and biological properties of coinage metal complexes [82, 83], we reported the first study on the syntheses, characterization and biological evaluation of new Cu(I) complexes containing triorganophosphanes and the anion of several sterically hindered the β -diketone ligands.

On the other hand, α -monosubstituted β -diketones exhibit a range of reactivities depending on the nature of the substituents attached to the α -carbon atom [84]. The presence of the α -substituent significantly influences the behavior of these compounds,

affecting their stability, reactivity, and reaction pathways ^[85]. Generally, there are three possible tautomeric isomers for α -monosubstituted β -diketones (**Scheme 4.1**).



Scheme 4.1. Keto-enol tautomeric equilibria.

Generally, electronegative β -substituents (R_1 and R_2 groups in **Scheme 4.1**) increase sharply the degree of enolization, whereas α -substituents (Z group in **Scheme 4.1**) cause a marked decrease in the percentage of enol. However, the cause is mainly steric-repulsion between the α -moiety and the other β -substituents, which makes the chelate ring of the cis-enolic form unstable ^[86]. On the other hand, for 3-substituted-2,4-pentanediones the keto form is predominant in the presence of bulky substituents in 3-position. Ditopic ligands based on 3-monosubstituted acetylacetone with two distinctly different coordination sites allow for assembling cations to coordinate polymers and thus add a degree of freedom to these complex solids. Substituted acetylacetonates have often been used in this context and 3-ciano, 3-diaz, 3-pyridyl, 3-halogen, 3-caged phosphine, 3-(4-methylthiophenyl), and 3-pyrazolyl substituents, capable of supporting secondary interactions through exocyclic atoms with metal cations, have been described, and the subject has recently been reviewed.

Therefore, as an extension of the studies on pyrazolyl derivatives and as part of our continuous investigation of the chemical and biological properties of copper-containing coordination compound, we report here for the first time a study on the syntheses, characterization and reactivity of new 3-monosubstituted acetylacetone ligands, HL^{acPz} and HL^{acPzMe} (**Scheme 4.2**), and related Cu(II) and Cu(I) complexes containing phosphane co-ligands.

Since these ligands have two different donor sites of different Pearson hardness (a soft neutral nitrogen atom of the pyrazolyl moiety and a hard oxygen donor atom of the acetylacetone moiety), they may be considered versatile coordinating ligands. However, in contrast to the previously mentioned ditopic 3-monosubstituted acetylacetone ligands, HL^{acPz} and HL^{acPzMe} may also act as bidentate ligands at either the N or the O donor sites. In an endeavor to extend the range of metal chelates in which the β -diketones attain a conformation other than the usual encountered, herein we report the crystal structure of compound $[Cu(HL^{acPz})(PPh_3)_2]PF_6$ that reveals the formation of a chelate complex, wherein the HL^{acPz} ligand coordinates in bidentate κ^2N, O fashion to the

copper(I) ion.

4.2 Experimental Section

4.2.1 Materials and Instruments

All reagents were obtained from commercial suppliers and used as received. Melting Points (MP) were performed by an SMP3 Stuart Scientific Instrument (Bibby Sterilin Ltd., London, UK). Elemental Analyses (C, H, N, S) (EA) were performed with a Fisons Instruments EA-1108 CHNS-O Elemental Analyzer (Thermo Fisher Scientific Inc., Waltham, MA, USA). Fourier-Transform InfraRed (FT-IR) spectra were recorded from 4000 to 700 cm^{-1} on a PerkinElmer Frontier Instrument (PerkinElmer Inc., Waltham, MA, USA), equipped with Attenuated Total Reflection (ATR) unit using universal diamond top-plate as sample holder. Abbreviation used in the analyses of the FT-IR spectra: br = broad, m = medium, mbr = medium broad, s = strong, sbr = strong broad, vs = very strong, w = weak, wbr = weak broad. Nuclear Magnetic Resonance (NMR) spectra for the nuclei ^1H , ^{13}C , and ^{31}P were recorded with a Bruker 500 Ascend Spectrometer (Bruker BioSpin Corporation, Billerica, MA, USA; 500.13 MHz for ^1H , 125.78 MHz for ^{13}C , 202.46 MHz for ^{31}P and 470.59 MHz for ^{19}F). Tetramethylsilane (SiMe_4) was used as an external standard for the ^1H - and ^{13}C -NMR spectra, while 85% H_3PO_4 was used for the ^{31}P -NMR spectra. The chemical shifts (δ) are reported in ppm and coupling constants (J) are reported in hertz (Hz). Abbreviation used in the analyses of the NMR spectra: br = broad, d = doublet, dbr = broad doublet, m = multiplet, s = singlet, sbr = broad singlet, t = triplet. Electrospray Ionization Mass Spectra (ESI-MS) were recorded in positive- (ESI-MS(+)) or negative-ions (ESI-MS(-)) mode on a Waters Micromass ZQ Spectrometer equipped with a single quadrupole (Waters Corporation, Milford, MA, USA), using methanol or acetonitrile mobile phase. The compounds were added to reagent-grade methanol or acetonitrile to give approximately 0.1 mM solutions. These solutions were injected (1 μL) into the spectrometer fitted with an autosampler. The pump delivered the solutions to the mass spectrometer source at a flow rate of 200 $\mu\text{L}/\text{min}$ and nitrogen was employed both as a drying and nebulizing gas. The capillary voltage was typically 2500 V. The temperature of the source was 100°C, while the temperature of the desolvation was 400°C. In the analyses of ESI-MS spectra, the confirmation of major peaks was supported by a comparison of the observed and predicted isotope distribution patterns, the latter calculated using the IsoPro 3.1

computer software (T-Tech Inc., Norcross, GA, USA).

4.2.2 Synthesis

4.2.2.1 Synthesis of HL^{acPz} (**1**)

To an ethanol solution (20 mL) of 3-benzylidene-2,4-pentadienone (5.313 mmol, 1.000 g) with an excess of triethylamine (6.206 mmol, 0.628 g), pyrazole (5.313 mmol, 0.362 g) was added and a yellow clear solution was obtained. The reaction was carried out overnight under magnetic stirring at reflux, and the yellow color was intensified. Then, the solution was left to cool to room temperature, and it was dried at reduced pressure obtaining an orange oil product. The oil was solubilized in diethyl ether and precipitated with *n*-hexane until a white precipitate was formed. The white solid product HL^{acPz} (**1**) was recovered by filtration (70% yield). Crystals of ligand **1**, suitable for Single Crystal X-ray Diffraction, were obtained by slow evaporation of a *n*-hexane solution of **1**. M.P.: 98-100 °C. FT-IR (cm⁻¹): 3108w, 3056w (C-H); 1732s, 1699m (C=O); 1515w, 1505w, 1497w, 1454w, 1418m, 1394m, 1356s, 1323w, 1282sh, 1263s, 1231m, 1198m, 1168m, 1140s, 1096s, 1069w, 1046w, 1037w, 965m, 916w, 893m, 878w, 869w, 757vs, 729vs, 702s. ¹H-NMR (CDCl₃, 293 K): δ 2.03 (s, 3H, CH₃), 2.22 (s, 3H, CH₃), 5.32 (d, 1H, CH, *J* = 11.28 Hz), 6.01 (d, 1H, CH, *J* = 11.28 Hz), 6.22 (t, 1H, 4-CH_{pz}, *J* = 2.06 Hz), 7.32-7.41 (m, 6H, CH_{ar} and 5-CH_{pz}), 7.50 (dbr, 1H, 3-CH_{pz}). ¹H-NMR (DMSO, 293 K): δ 2.07 (s, 3H, CH₃), 5.15 (s, 3H, CH₃), 5.58 (d, 1H, CH, *J* = 11.55 Hz), 6.04 (d, 1H, CH, *J* = 11.28 Hz), 6.18 (t, 1H, 4-CH_{pz}, *J* = 5.12 Hz), 7.26-7.50 (m, 6H, CH_{ar} and 5-CH_{pz}), 7.85 (d, 1H, 3-CH_{pz}, *J* = 5.12 Hz). ¹H-NMR (Acetone-d₆, 293 K): δ 2.07 (s, 3H, CH₃), 5.18 (s, 3H, CH₃), 5.51 (d, 1H, CH, ³*J* = 11.44 Hz), 6.08 (d, 1H, CH, ³*J* = 11.44 Hz), 6.19 (t, 1H, 4-CH_{pz}, *J* = 2.06 Hz), 7.28-7.35 (m, 3H, CH_{ar}), 7.44 (dbr, 1H, 5-CH_{pz}), 7.51-7.53 (m, 2H, CH_{ar}), 7.69 (d, 1H, 3-CH_{pz}, *J* = 2.31 Hz). ¹³C{H}-NMR (CDCl₃, 293 K): δ 29.9, 30.9 (CH₃), 64.2 (CH), 72.6 (CH), 106.1 (4-CH_{pz}), 127.5, 128.7, 128.9, 129.6, 137.7, 139.4 (CH_{ar}, 3- and 5-CH_{pz}), 200.0 (C=O), 200.3 (C=O). ¹³C¹H-NMR (Acetone-d₆, 293 K): δ 29.5, 30.2 (CH₃), 63.8 (CH), 71.4 (CH), 105.5 (4-CH_{pz}), 127.8, 128.2, 128.5, 129.8, 138.7, 138.8 (CH_{ar}, 3- and 5-CH_{pz}), 199.5 (C=O), 199.8 (C=O). ESI-MS(+) (major positive ions, CH₃CN), *m/z* (%): 211 (40) [3-benzylidene-2,4-pentanedione + Na]⁺, 257 (95) [HL^{acPz} + H]⁺, 279 (100) [HL^{acPz} + Na]⁺. ESI-MS(-) (major negative ions, CH₃CN), *m/z* (%): 187 (100) [3-benzylidene-2,4-pentanedione - H]⁻, 213 (25) [HL^{acPz} - CH₃CO]⁻. Elemental Analysis (%) calculated

for C₁₅H₁₆N₂O₂: C 70.29, H 6.29, N 10.93; found C 70.65, H 6.36, N 9.79.

4.2.2.2 Synthesis of HL^{acPzMe} (**2**)

To an ethanol solution (20 mL) of 3-benzylidene-2,4-pentanedione (5.313 mol, 1.000 g) with an excess of triethylamine (6.206 mmol, 0.628 g), 3,5-methylpyrazole (5.313 mmol, 1.000 g) was added obtaining a clear yellow solution. The reaction was carried out overnight under magnetic stirring at room temperature observing no colour variation. Then the solution was dried at reduced pressure obtaining a colorless oil with yellow reflections. The oil was solubilized with diethyl ether and precipitated with *n*-hexane. The white solid was filtered and dried to give HL^{acPzMe} (**2**) in 73% yield. Crystals of ligand **2**, suitable for Single Crystal X-ray Diffraction, were obtained by slow evaporation of a diethyl ether/*n*-hexane solution of **2**. M.P.: 89-92 °C. FT-IR (cm⁻¹): 3034w, 2980w, 2916w (C-H); 1729s, 1699s (C=O); 1601w, 1586w, 1552m, 1496w, 1458m, 1422m, 1381m, 1350s, 1311w, 1294w, 1256sh, 1244s, 1234sh, 1192m, 1174m, 1134m, 1088w, 1071w, 1022m, 1002w, 972w, 951m, 930w, 898m, 864w, 853w, 813w, 776m, 763s, 717sh, 709vs. ¹H-NMR (CDCl₃, 293 K): δ 2.00, 5.18, 2.21, 2.22 (s, 12H, CH₃), 5.41 (d, 1H, CH, *J* = 11.11 Hz), 5.75 (s, 1H, 4-CH_{pz}), 5.82 (d, 1H, CH, *J* = 11.13 Hz), 7.28-7.37 (m, 5H, CH_{ar}). ¹³C{H}-NMR (CDCl₃, 500 MHz): δ 10.9, 13.6, (3- and 5-CH₃), 30.3, 31.6 (CH₃), 60.6 (CH), 72.4 (CH), 105.6 (4-CH_{pz}), 127.5, 128.2, 128.8, 129.0, 129.7, 138.0, 139.3, 147.3 (CH_{ar}, 3- and 5-C_{pz}), 199.9, 200.9 (C=O). ESI-MS(+) (major positive ions, CH₃CN), *m/z* (%): 211 (50) [3-benzylidene-2,4-pentanedione + Na]⁺, 285 (100) [HL^{acPzMe} + H]⁺, 307 (45) [HL^{acPzMe} + Na]⁺. ESI-MS (major negative ions, CH₃CN), *m/z* (%): 187 (45) [3-benzylpentane-2,4-dione - H]⁻. Elemental Analysis (%) calculated for C₁₇H₂₀N₂O₂: C 71.81, H 7.09, N 9.85; found C 73.45, H 7.29, N 9.70.

4.2.2.3 Synthesis of [Cu(HL^{acPz})(PPh₃)₂](PF₆) (**3**)

The ligand HL^{acPz} (1.000 mmol, 0.256 g) was added to an acetonitrile solution (40 mL) of PPh₃ (2.000 mmol, 0.525 g) and Cu(CH₃CN)₄PF₆ (1.000 mmol, 0.372 g) and stirred at room temperature for 12 h. The solvent was removed at reduced pressure, giving a yellow oil that was washed with diethyl ether, giving the white complex [Cu(HL^{acPz})(PPh₃)₂](PF₆) in 89% yield. M.P.: 153-157 °C. Crystals of compound **3**, suitable for Single-Crystal X-ray Diffraction, were obtained by slow evaporation of an acetone/ethyl acetate solution of **3**. FT-IR (cm⁻¹): 3063wbr (C-H); 1727w, 1687m (C=O); 1586w, 1480w, 1454w, 1435m, 1417w, 1363w, 1327w, 1279w, 1229w, 1194w,

1184w, 1172w, 1153m, 1120w, 1097m, 1070w, 1027w, 998w, 982w, 958w, 876w; 831vs (PF₆); 787w, 750s, 729m, 695s. ¹H-NMR (Acetone-d₆, 293 K): δ 2.19 (s, 3H, CH₃), 2.84 (s, 3H, CH₃), 5.53 (d, 1H, CH, *J* = 11.40 Hz), 6.10 (d, 1H, CH, *J* = 11.42 Hz), 6.21 (t, 1H, 4-CH_{pz}, *J* = 2.01 Hz), 7.33-7.57 (m, 36H, CH_{ar} and 5-CH_{pz}), 7.72 (d, 1H, 3-CH_{pz}, *J* = 2.20 Hz). ¹³C{H}-NMR (Acetone-d₆, 293 K): δ 29.7, 30.4 (CH₃), 64.2 (CH), 71.2 (CH), 106.2 (4-CH_{pz}), 127.7, 128.4, 128.7, 128.9, 129.1, 129.7, 130.7, 131.2, 131.4, 133.5, 138.1; 199.6, 199.8 (C=O). ³¹P{H}-NMR (CDCl₃, 293 K): δ -0.86 (s), -144.21 (sept, *J*(¹⁹F-³¹P) = 712 Hz, PF₆). ESI-MS(+) (major positive ions, CH₃CN), *m/z* (%): 581 (30) [Cu(HL^{acPz})(PPh₃)]⁺, 587 (100) [Cu(PPh₃)₂]⁺, 603 (30) [Cu(L^{acPz})(PPh₃) + Na]⁺, 849 (10) [Cu(PPh₃)₃]⁺, 865 (5) [Cu(L^{acPz})(PPh₃)₂ + Na]⁺. ESI-MS(-) (major negative ions, CH₃CN), *m/z* (%): 145 [PF₆]⁻. Elemental analysis (%) calculated for C₅₁H₄₆CuF₆N₂O₂P₃: C 61.91, H 4.69, N 2.83; found: C 60.46, H 4.72, N 2.58.

4.2.2.4 Synthesis of [Cu(HL^{acPz})₂(L^{acPz})₂] (4)

The ligand HL^{acPz} (4.000 mmol, 1.025 g) and copper(II) acetate monohydrate (Cu(CH₃CO₂)₂·H₂O (1.000 mmol, 0.199 g) were dissolved in CH₃OH (40 mL), and the reaction was stirred for 12 h at room temperature. The mixture was filtered, and the solution was dried at reduced pressure obtaining a blue oil. Diethyl ether was added, and the precipitate was filtered off. The solution was dried at reduced pressure to obtain the blue complex [Cu(HL^{acPz})₂(L^{acPz})₂] in 58% yield. M.P.: 87-88 °C. FT-IR (cm⁻¹): 3107w, 3056w, 3031wbr, 3004wbr, 2916wbr (C-H); 1732s, 1700s 1657w (C=O); 1616w, 1517w, 1497w, 1453w, 1418m, 1394m, 1355s, 1322w, 1282sh, 1263s, 1230sh, 1917m, 1168s, 1140s, 1095s, 1069w, 1045m, 1038m, 1030sh, 966m, 916w, 893m, 869w, 879w, 757s, 728s, 701s. ESI-MS(+) (major positive ions, CH₃CN): *m/z* (%): 318 (30) [Cu(L^{acPz})]⁺, 360 (50) [Cu(L^{acPz}) + CH₃CN]⁺, 575 (10) [Cu(L^{acPz})₂ + H]⁺. ESI-MS(-) (major negative ions, CH₃CN): 255 (100) [L^{acPz}]⁻. Elemental analysis (%) calculated for C₆₀H₆₂CuN₈O₈: C 66.31, H 5.75, N 10.31; found: C 67.84, H 6.14, N 9.18.

4.2.2.5 Synthesis of [Cu(HL^{acPzMe})(PPh₃)₂]PF₆·2CH₃CN (5)

The ligand HL^{acPzMe} (0.500 mmol, 0.142 g) was added to an acetonitrile solution (20 mL) of PPh₃ (1.000 mmol, 0.262 g) and Cu(CH₃CN)₄PF₆ (0.500 mmol, 0.186 g) and stirred at room temperature for 24 h. The solvent was removed at reduced pressure to obtain the white complex [Cu(HL^{acPzMe})(PPh₃)₂]PF₆·2CH₃CN in 73% yield. M.P.:

130-133 °C. FT-IR (cm⁻¹): 3049w, 2988w, 2938w, 2902w (C-H); 2301w, 2271w (C≡N); 1730m, 1700m (C=O); 1667w, 1586w, 1554w, 1479m, 1458m, 1434s, 1394m, 1382m, 1331sh, 1311m, 1257m, 1246m, 1236sh, 1174mbr, 1160m, 1135m, 1121m, 1094s, 1067m, 1057m, 1027m, 1000m, 973w, 953w, 922vw, 898w, 879m, 859s; 832vs (PF₆); 791m, 777m, 744vs, 693vs. ¹H-NMR (CDCl₃, 293 K): δ 2.06 (s, 6H, CH₃), 2.21 (s, 12H, CH₃), 5.44 (sbr, 1H, CH), 5.77 (sbr, 1H, CH), 5.86 (s, 1H, 4-CH_{pz}), 7.19-7.41 (m, 35H, CH_{ar}). ¹³C{H}-NMR (CDCl₃, 500 MHz): δ 1.98 (CH₃CN), 11.0, 13.6 (3- and 5-CH₃), 30.4, 31.5 (CH₃); 60.6, 72.0 (CH); 105.8 (4-CH_{pz}); 118.7 (CH₃CN); 127.5, 128.3, 129.0, 129.7, 130.5, 131.1, 131.3, 132.0, 132.1, 133.4, 133.5, 137.8, 139.8, 147.6 (CH_{ar}, 3- and 5-C_{pz}); 200.1, 200.9 (C=O). ³¹P{H}-NMR (CDCl₃, 293 K): δ -0.12 (s), -144.23 (sept, *J*(¹⁹F-³¹P) = 712 Hz, PF₆). ESI-MS(+) (major positive ions, CH₃CN), *m/z* (%): 366 (70) [Cu(PPh₃) + CH₃CN]⁺, 589 (100) [Cu(PPh₃)₂]⁺. ESI-MS(-) (major negative ions, CH₃CN), *m/z* (%): 145 (100) [PF₆]⁻. Elemental analysis (%) calculated for C₅₇H₅₆CuF₆N₄O₂P₃: C 62.26, H 5.13, N 5.10; found: C 61.69, H 5.16, N 4.86.

4.2.2.6 Synthesis of [Cu(HL^{acPzMe})₂(L^{acPzMe})₂] (6)

The ligand HL^{acPzMe} (4.000 mmol, 1.137 g) and (Cu(CH₃CO₂)₂·H₂O (1.000 mmol, 0.199 g) were dissolved in CH₃OH (40 mL), and the reaction was stirred for 24 h at room temperature. The solution was dried at reduced pressure obtaining a blue oil; diethyl ether was added and the precipitate was filtered off. The solution was dried at reduced pressure to obtain the blue complex [Cu(HL^{acPzMe})₂(L^{acPzMe})₂] in 50% yield. M.P.: 89-93 °C. FT-IR (cm⁻¹): 3062w, 3031w, 3002wbr, 2919w (C-H); 1734s, 1703vs, 1659s (C=O); 1615m, 1556s, 1494m, 1455s, 1420s, 1382s, 1355vs, 1311m, 1288sh, 1245vs, 1213s, 1194sh, 1173s, 1086m, 1023m, 1005sh, 973m, 955m, 927m, 900m, 866m, 844w, 787m, 759s, 704vs. Elemental analysis (%) calculated for C₆₈H₇₈CuN₈O₈: C 68.12, H 6.56, N 9.35; found: C 69.89, H 6.80, N 9.16.

4.2.2.7 Synthesis of 4-phenyl-4-(1*H*-pyrazol-1-yl)butan-2-one, ^{PhPz}MEK (7)

An excess of sodium hydroxide (1.200 mmol, 0.048 g) was added to an ethanol solution (20 mL) of HL^{acPz} (1.000 mmol, 0.284 g), giving a pale-yellow solution. The reaction was stirred for 72 h at room temperature and the solution was dried at reduced pressure. The residue was washed with diethyl ether and hexane, the precipitate was filtered off and the solution was dried at reduced pressure, giving a yellow oil of ^{PhPz}MEK. M.P.: oil. FT-IR (cm⁻¹): 3106w, 3086w, 3061w, 3028w, 3003w, 2912w, 2833w

(C-H); 1715s (C=O); 1659m, 1602m, 1510sh, 1494m, 1454m, 1435sh, 1417m, 1396s, 1358s, 1283m, 1255m, 1204m, 1160m, 1090m, 1044m, 1022m, 959m, 917m, 875m, 861m, 845m, 749vs, 697vs, 628sh, 618s. ^1H -NMR (CDCl_3 , 293K): δ 2.19 (s, 3H, CH_3), 3.11 (dd, 1H, CH_2 , $J = 4.96$ Hz, $J = 17.28$ Hz), 3.93 (dd, 1H, CH_2 , $J = 9.05$ Hz, $J = 17.27$ Hz), 5.89 (m, 1H, CH), 6.26 (s, 1H, 4- CH_{pz}), 7.25-7.34 (m, 5H, CH_{ar}), 7.43 (m, 1H, 5- CH_{pz}), 7.56 (dbr, 1H, 3- CH_{pz}). $^{13}\text{C}\{\text{H}\}$ -NMR (CDCl_3 , 500 MHz): δ 30.4 (CH_3), 48.7 (CH_2), 60.8 (CH), 105.7 (4- CH_{pz}), 126.6, 128.0, 128.8, 129.7, 139.1, 140.5 (CH_{ar} , 3- and 5- C_{pz}), 205.2 (C=O). ESI-MS(+) (major positive ions, CH_3CN), m/z (%): 215 (100) [$^{\text{PhPz}}\text{MEK} + \text{H}$] $^+$. Elemental analysis (%) calculated for $\text{C}_{13}\text{H}_{14}\text{N}_2\text{O}$: C 74.35, H 7.49, N 11.56; found C, H, N.

4.2.2.8 Synthesis of 4-(3,5-dimethyl-1H-pyrazol-1-yl)-4-phenylbutan-2-one, $^{\text{PhPzMe}_2}\text{MEK}$ (8)

An excess of sodium hydroxide (1.200 mmol, 0.048 g) was added to an ethanol solution (20 mL) of $\text{HL}^{\text{acPzMe}}$ (1.000 mmol, 0.284 g), giving a pale-yellow solution. The reaction was stirred for 12 h at room temperature and the solution was dried at reduced pressure. The residue was washed with chloroform, the precipitate was filtered off and the solution was dried at reduced pressure, giving an oil. The latter was solubilized in diethyl ether and *n*-hexane was added; white crystals of $^{\text{PhPzMe}_2}\text{MEK}$ suitable for Single-Crystal X-ray Diffraction were obtained from a slow evaporation of the solution at a low temperature. M.P.: 98-100 °C. FT-IR (cm^{-1}): 3123w, 3064w, 3008w, 2987w, 2947w, 2914w, 2898w, 2863w (C-H); 1714vs (C=O); 1601w, 1585w, 1552m, 1498m, 1459s, 1440m, 1425s, 1395s, 1370s, 1354s, 1323m, 1302w, 1274s, 1253m, 1200w, 1182m, 1162s, 1122w, 1060w, 1029s, 1018m, 1000w, 975m, 930w, 873m, 816w, 797vs, 775m, 762s, 710vs. ^1H -NMR (CDCl_3 , 293 K): δ 2.19 (s, 6H, CH_3), 2.26 (s, 3H, CH_3), 3.05 (dd, 1H, CH_2 , $J = 4.76$ Hz, $J = 17.31$ Hz), 3.99 (m br, 1H, CH_2), 5.73 (m, 1H, CH), 5.80 (s, 1H, 4- CH_{pz}), 7.17-7.32 (m, 5H, CH_{ar}). $^{13}\text{C}\{\text{H}\}$ -NMR (CDCl_3 , 500 MHz): δ 11.0, 13.6 (3- and 5- CH_3), 30.6 (CH_3), 49.0 (CH_2), 56.9 (CH), 105.4 (4- CH_{pz}), 126.3, 127.5, 128.7, 139.7, 141.0, 147.1 (CH_{ar} , 3- and 5- C_{pz}), 205.9 (C=O). ESI-MS(+) (major positive ions, CH_3CN), m/z (%): 243 (100) [$^{\text{PhPzMe}_2}\text{MEK} + \text{H}$] $^+$. Elemental analysis (%) calculated for $\text{C}_{15}\text{H}_{18}\text{N}_2\text{O}$: C 74.35, H 7.49, N 11.56; found C 74.82, H 7.73, N 11.83.

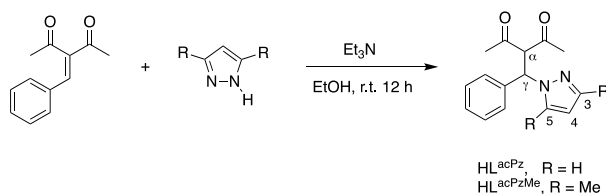
4.2.2.9 Crystallographic data collection and refinement

Single crystals suitable for the X-ray experiment of the compounds **1**, **2**, and **8** were obtained by slow evaporation of *n*-hexane or diethyl ether/*n*-hexane solutions;

likewise, crystals of the complex **3** were obtained by slow evaporation of an acetone/ethyl acetate solution. In all cases, several specimens were screened before selecting the most appropriate items, which were picked up with a nylon loop and mounted on the top of the goniometer head of a Rigaku-OD Gemini E diffractometer, equipped with a 2K×2K EOS CCD area detector and sealed tube Enhance Cu X-ray source. The raw data diffraction for all compounds was collected at room temperature [range: 298(1)-299.4(8) K] using the ω -scans technique, using graphite-monochromated Cu K α radiation ($\lambda=1.54184$ Å) in a 1024×1024-pixel mode and 2×2-pixel binning. Data collection, reduction, and finalization were performed using the *CrysAlisPro* software, Versions 1.171.41.123a (**1**) and 1.171.42.49 (**2**, **3**, **8**). Data collections were usually planned to allow for a certain degree of redundancy, except for compound **8**, where reflection intensities were low and the time for the experiment would have been exceedingly long. The raw data were corrected for Lorentz / polarization effects. An empirical absorption correction was also performed using a multi-scan approach, with the scaling algorithm *SCALE3 ABSPACK*, using equivalent reflections. Accurate unit cell parameters were obtained by the least-squares refinement of 8464 (**1**), 19353 (**2**), 23975 (**3**), and 2282 (**8**) strongest reflections chosen throughout the whole data collection. The crystal and equipment stability was checked by monitoring two reference frames for every 50 frames for all compounds. A manual data reduction was performed at the end of the data collection to appropriately account for limited sample wobbling, but no significant change in peak intensities was observed during all experiments.

The structures were solved by direct phasing and refined by full-matrix least squares based on F_o^2 with the SHELXT and SHELXL programs through the OLEX2 program interface ^[87]. For all compounds, non-H atoms were allowed to vibrate anisotropically in the last cycles of refinement. In compounds **1**, **2**, and **8** the positions of the H atoms were obtained by different Fourier maps; in complex **3**, H atoms were placed in calculated positions and refined as a riding model, with their displacement parameters calculated as 1.2 (or 1.5 for the methyl groups) times the U_{eq} of the pertinent carrier carbon atom. The asymmetric unit of compound **2** contains two independent molecules that define an enantiomeric pair, with C1 and C1A atoms showing, respectively, *S* and *R* configurations. The cationic complex **3** crystallizes as a hexafluorophosphate salt. The PF₆[−] anion is disordered over two positions, whose site

occupation factors were constrained to sum to unity; finally refined sofs were 0.472/0.528 for F1/F6 and F1A/F6A, respectively. The involved atoms have also been modelled introducing RIGU restraints. The analysis of the diffraction data of **8** showed that this compound crystallizes in the form of a non-merohedral two-component twin (second component individuated by a rotation of -179.992° about the $[1.00\ 0.00\ 0.00]$ direction in the reciprocal space). The two components account, respectively, for 77.61% and 22.39% of the diffraction peaks. Twin data finalization of this compound showed that the R_{int} value of the data about the minor component was significantly worse than that of the major component; for this reason, the structure of **8** was solved using only the data of the major component. ORTEP representations of all compounds (**Figs. 4.1-4.4**) have been prepared using the *Mercury* program.



Scheme 4.2 Synthesis of HL^{acPz} (**1**) and $\text{HL}^{\text{acPzMe}}$ (**2**) ligands.

4.3 Results and discussion

4.3.1 Synthesis and characterization

The ligands HL^{acPz} (**1**) and $\text{HL}^{\text{acPzMe}}$ (**2**) were prepared, using a modified literature method ^[88], from the reaction of 3-benzylidenepentane-2,4-dione and pyrazole or 3,5-dimethylpyrazole, respectively, in ethanol solution with triethylamine as a basic catalyst, and isolated as white solids in very good yield and high purity (**Scheme 4.2**). The precursor 3-benzylidenepentane-2,4-dione can be synthesized by Knoevenagel condensation of an equimolar amount of acetylacetone and benzaldehyde in the presence of piperidine as a catalyst.

Ligands **1** and **2** are soluble in methanol, diethyl ether, chloroform, acetonitrile, DMSO, THF, dichloromethane, ethyl acetate, and acetone. They were fully characterized by multinuclear NMR spectroscopy, FT-IR, ESI-MS, and elemental analysis. A batch of good quality crystals of **1** and **2**, suitable for X-ray analysis, was obtained by slow evaporation of a n-hexane and diethyl ether/ n-hexane solution, respectively.

The IR spectra of **1** and **2** have been carried out on solid samples and shows all the

expected absorption bands. Weak bands in region 2916-3108 cm^{-1} are assigned to C-H stretching vibrations. The ligands also show two sharp strong bands in the region 1699-1732 cm^{-1} attributable to the stretching vibrations of the two carbonyl groups. The bands at *ca.* 1500 cm^{-1} correspond to C=C/C=N rings stretching. The ^1H -NMR spectra of HL^{acPz} and $\text{HL}^{\text{acPzMe}}$ in CDCl_3 suggest the presence of the keto form in the solution. In the ^1H -NMR spectrum of **1**, the 3-CH and 4-CH protons of the pyrazole ring are present at 7.50 and 6.22 ppm, respectively. The 5-CH of pyrazole and the CH aromatic protons of the phenyl ring are present in the range of 7.32-7.41 ppm. The α -CH and γ -CH protons are present at 5.32 ($J = 11.28$ Hz) and 6.01 ppm ($J = 11.28$ Hz). The singlets at 2.03 and 2.22 ppm are attributable to the methyl groups bonded to the b-dicarbonyl moieties. In the ^{13}C -NMR spectrum in CDCl_3 the C=O signals, are detectable at about 200.0 ppm, while the α -CH and γ -CH carbons are visible at 72.6 and 64.2 ppm; the 4-CH of the pyrazole ring and the CH_3 carbons are present at 106.1 and 29.9-30.9 ppm, respectively. In the ^1H -NMR spectrum of **2** recorded in CDCl_3 solution, the CH aromatic protons are present in the range 7.28-7.37 ppm and the α -CH and γ -CH proton is present at 5.82 ppm ($J = 11.13$ Hz) and 5.41 ppm ($J = 11.11$ Hz), respectively. The singlets in the range 2.00-2.22 ppm are attributable to the methyl groups bonded to the b-dicarbonyl moieties and the 3- and 5- CH_3 of pyrazole, while the 4-CH proton is present as a singlet at 5.75 ppm. In the ^{13}C -NMR spectrum, the C=O signals are detectable at 199.9 and 200.9 ppm, while the α - and γ -CH are at 72.4, and 60.6 ppm. The 4-CH carbon of the pyrazole ring is detectable at 105.6 ppm, while the 3- and 5- CH_3 of the pyrazole and the CH_3 of the b-dicarbonyl moieties are present at 10.9-13.6 and 30.3-31.6, respectively. The ESI-MS study has been performed by dissolving **1** and **2** in CH_3CN and recording the spectra in positive- and negative-ion modes. The molecular structure of **1** has been confirmed by the presence of the molecular peaks at m/z 257 and 279 respectively, attributable to the $[\text{HL}^{\text{acPz}} + \text{H}]^+$ and $[\text{HL}^{\text{acPz}} + \text{Na}]^+$ species. Analogously, in the positive-ion spectrum of **2** peaks at m/z 285 and 307 respectively are attributable to the $[\text{HL}^{\text{acPzMe}} + \text{H}]^+$ and $[\text{HL}^{\text{acPzMe}} + \text{Na}]^+$ species.

The Cu(I) complex $[\text{Cu}(\text{HL}^{\text{acPz}})(\text{PPh}_3)_2]\text{PF}_6$ (**3**) was prepared from the reaction of PPh_3 , $\text{Cu}(\text{CH}_3\text{CN})_4\text{PF}_6$ and the ligand **1**, in CH_3CN solution. Complex **3** is soluble in CHCl_3 , CH_3CN , DMSO, and acetone. Compound **3** was fully characterized by multinuclear NMR spectroscopy, FT-IR, ESI-MS, and elemental analysis, and a batch of good quality crystals, suitable for X-ray analysis, was obtained by slow evaporation

of an acetone/ethyl acetate solution. The IR spectrum carried out on a solid sample of **3** shows all the expected bands for the β -diketone ligand and the triphenylphosphine co-ligand. The absorptions due to the C=O stretching are at 1687-1727 cm^{-1} ; they don't significantly vary concerning the same absorptions of the carbonyl group detectable in the spectrum of the free ligand (1699-1732 cm^{-1}). In a lower frequency region, complex **3** shows a broad strong band at 831 cm^{-1} due to the stretching vibrations of the PF_6^- anion. The $\delta(\text{PF}_6)$ bending vibration is observed as a narrow strong band at 560 cm^{-1} . In the ^1H -NMR spectra of **3**, recorded in acetone- d_6 solution at room temperature, the 3-CH and 4-CH protons of the pyrazole ring are present at 7.72 and 6.21 ppm, while the 5-CH proton of the pyrazole ring and the CH aromatic protons of the phenyl rings are present in the range 7.33-7.57 ppm. The α -CH and γ -CH bridging protons are present at 5.53 ppm ($J = 11.40$ Hz) and 6.10 ppm ($J = 11.42$ Hz). The singlets at 5.19 and 2.84 ppm are attributable to the methyl groups of the acetylacetone fragment; the signal at 2.84 is shifted concerning the signal of the free ligand in the same solvent. In the ^{13}C -NMR spectrum in acetone- d_6 the C=O signals, are detectable at about 200 ppm. The ^{31}P H-NMR spectrum of the Cu(I) complex **3**, recorded in CDCl_3 solution at room temperature, gave a broad singlet at -0.86 ppm downfield shifted to the value of the free phosphane ($\delta = -5.36$ ppm). In the spectrum, the characteristic septet centered at -144.21 ppm is due to the PF_6^- counterion.

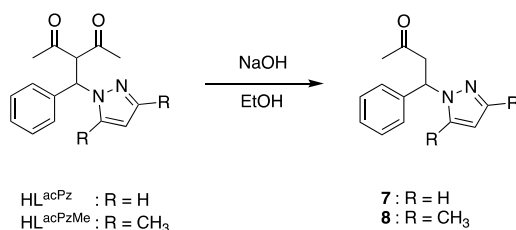
Compound $[\text{Cu}(\text{HL}^{\text{acPzMe}})(\text{PPh}_3)_2]\text{PF}_6 \cdot 2\text{CH}_3\text{CN}$ (**5**) was prepared similarly to compound **4** from the reaction of PPh_3 , $\text{Cu}(\text{CH}_3\text{CN})_4\text{PF}_6$ and the ligand **2**, in CH_3CN solution and it was fully characterized. Complex **5** is soluble in CHCl_3 , CH_2Cl_2 , CH_3CN , ethyl acetate, THF, DMSO and acetone. The IR spectrum carried out on a solid sample of **5** shows all the expected bands for the β -diketone ligand and the triphenylphosphine co-ligand. The absorptions due to the C=O stretching are at 1700-1730 cm^{-1} ; they don't significantly vary to the same absorptions of the carbonyl group detectable in the spectrum of the free ligand (1699-1729 cm^{-1}). Very strong bands at 832 and 557 cm^{-1} are due to the stretching vibrations and the $\delta(\text{PF}_6)$ bending vibrations, respectively, of the PF_6^- anion. In the ^1H -NMR spectra of **5**, recorded in CDCl_3 solution at room temperature, the 3- and 5- CH_3 protons of the pyrazole ring are present at 2.21 ppm, while the 4-CH proton of the pyrazole ring and the CH aromatic protons of the phenyl rings are present at 5.86 and at in the range 7.19-7.41 ppm, respectively. The α -CH and γ -CH bridging protons are present at 5.44 and 5.77 ppm. The singlets at 5.19 and 2.84

ppm are attributable to the methyl groups of the acetylacetone fragment; the signal at 2.84 is shifted to the signal of the free ligand in the same solvent. In the ^{13}C -NMR spectrum in CDCl_3 the $\text{C}=\text{O}$ signals, are detectable at about 200 ppm. The ^{31}P -NMR spectrum of **5**, recorded in CDCl_3 solution at room temperature, gave a singlet at -0.12 ppm downfield shifted to the value of the free phosphane. In the spectrum, the characteristic septet centered at -144.21 ppm is due to the PF_6^- counterion. The ESI-MS study was performed by dissolving **3** and **5** in CH_3CN and recording the spectra in positive- and negative-ion mode. The molecular structure of **3** was confirmed by the presence of the peak at m/z 865, attributable to the molecular peak $[\text{Cu}(\text{L}^{\text{acPz}})(\text{PPh}_3)_2 + \text{Na}]^+$; in addition, peaks at m/z 581 and 603 are attributable to the $[\text{Cu}(\text{HL}^{\text{acPz}})(\text{PPh}_3)]^+$ and $[\text{Cu}(\text{L}^{\text{acPz}})(\text{PPh}_3) + \text{Na}]^+$ species, being positive fragments of the dissociation of the PPh_3 coligand from the complex. In both the compounds the peaks at m/z 587 are due to the fragments of the dissociation of the ligand $[\text{Cu}(\text{PPh}_3)_2]^+$. The ESI-MS spectra of **3** and **5** in CH_3CN recorded in negative-ion mode show peaks at m/z 145 attributable to the PF_6^- aggregate, confirming the presence of the counterion (PF_6^-).

The copper(II) complexes $[\text{Cu}(\text{HL}^{\text{acPz}})_2(\text{L}^{\text{acPz}})_2]$ (**4**) and $[\text{Cu}(\text{HL}^{\text{acPzMe}})_2(\text{L}^{\text{acPzMe}})_2]$ (**6**) were prepared from the reaction of $\text{Cu}(\text{CH}_3\text{CO}_2)_2 \cdot \text{H}_2\text{O}$ with HL^{acPz} (**1**) and $\text{HL}^{\text{acPzMe}}$ (**2**), respectively, in methanol solution at room temperature, starting from a 1:4 stoichiometric ratio between metal and ligands. The syntheses were also performed starting from a 1:2 stoichiometric ratio obtaining the same products. The Cu(II) compounds **4** and **6** are soluble in Et_2O , CH_2Cl_2 , CHCl_3 , EtOAc , MeCN , DMSO , acetone and are air stable even as solutions; compound **6** is soluble in methanol too. The authenticity of compounds **4** and **6** was confirmed by elemental analysis, IR spectroscopy, and Electrospray mass spectra. The infrared spectra showed all the bands required by the presence of the chelating donors. In the spectra of compounds **4** and **6** weak absorptions due to the CH stretching were observed at 2916-3107 cm^{-1} . The carbonylic asymmetric stretching of **4** and **6** is detectable as strong absorptions at 1700-1734 cm^{-1} , together with medium signals at 1667 and 1659 cm^{-1} , respectively, in the typical range of β -diketonate systems ^[89]. The ESI-MS study was conducted by dissolving the Cu(II) complexes compounds in acetonitrile and recording the spectra in positive- and negative-ion modes. In the positive-ion spectrum of **4**, it's possible to detect peaks at m/z 318 and 360 attributable to the $[\text{Cu}(\text{L}^{\text{acPz}})]^+$ and $[\text{Cu}(\text{L}^{\text{acPz}})^+ \text{CH}_3\text{CN}]^+$ species, confirming the complex formation.

Both α -unsubstituted and α -monosubstituted β -diketones are well-studied in retro-Claisen reactions [85]. The retro-Claisen reactions of β -diketones are extremely attractive, not only because these reactions are distributed widely in biological chemistry [90] but also because this C-C bond cleavage protocol provides efficient access to synthetically useful ester and ketone compounds, simultaneously [91].

In ethanol solution, HL^{acPz} and $\text{HL}^{\text{acPzMe}}$ react with strong bases such as NaOH or *t*-BuOK by retro-Claisen C-C bond cleavage reaction giving rise to the formation of the species 4-phenyl-4-(1*H*-pyrazol-1-yl)butan-2-one, PhPz^{MEK} (**7**) and 4-(3,5-dimethyl-1*H*-pyrazol-1-yl)-4-phenylbutan-2-one, $\text{PhPz}^{\text{Me}_2\text{MEK}}$ (**8**) (**Scheme 4.3**). The reactions presumably proceed by the nucleophilic addition of bases to keto carbonyl group furnishing the key tetrahedral intermediate as a hemiketal anion, which might introduce C-C bond cleavage leading to the retro-Claisen reaction, where an enolate anion intermediate is potentially involved in the reaction process [85].



Scheme 4.3 Retro-Claisen reaction.

The reaction was found to be highly dependent on the basicity of the base. Weak bases (such as Et_3N) gave poor yield or even no reaction, while strong bases afforded a complete transformation. It's interesting to note that compound **7** was obtained in ethanol solution by reaction of HL^{acPz} with strong bases only in small amounts, in the presence of unreacted ligand. Compounds **7** and **8** were fully characterized by multinuclear NMR spectroscopy, FT-IR, ESI-MS, and elemental analysis and in addition, a batch of good quality crystals of **8**, suitable for X-ray analysis, was obtained by slow evaporation of a diethyl ether/*n*-hexane solution.

The IR spectra of **7** and **8** have been carried out on solid samples and show all the expected absorption bands. Weak bands in the region $2833\text{--}3106\text{ cm}^{-1}$ are assigned to C-H stretching vibrations, while the presence of only one strong band at about 1715 cm^{-1} , due to the stretching vibration of the carbonyl group, confirms the deacetylation of **1** and **2** by the retro-Claisen reaction. In the ^1H -NMR spectrum of **7** and **8** recorded in CDCl_3 solution, the *CH* aromatic protons are present in the range 7.17–7.34, and the singlets at 5.19 and 2.26 ppm are attributable to the methyl bonded to the carbonyl

group, respectively in **7** and **8**. The *CH* bridging proton is present as a double doublet at 5.73-5.89 ppm. The diastereotopic protons of the methylene are detectable as double doublets at 3.05-3.11 ppm and 3.93-3.99 ppm. In the ^{13}C -NMR spectra of **7** and **8**, recorded in CDCl_3 the diagnostic CH_2 signals, are detectable at 48.7 and 49.0 ppm, respectively. In the ESI-MS spectra of **7** and **8**, recorded in acetonitrile in positive-ion mode, it's possible to detect the molecular peaks at m/z 215 and 243 attributable to the protonated species $[\text{PhPzMEK} + \text{H}]^+$ and $[\text{PhPzMe}_2\text{MEK} + \text{H}]^+$, respectively.

4.3.2 X-ray Crystallography

A summary of the data collection parameters and crystal data for the HL^{acPz} (**1**) and $\text{HL}^{\text{acPzMe}}$ (**2**) ligands, the $\text{PhPzMe}_2\text{MEK}$ molecule (**8**), and the $[\text{Cu}(\text{PPh}_3)_2(\text{HL}^{\text{acPz}})]\text{PF}_6$ complex (**3**) are reported in **Table 4.1**. **Table 4.2** lists a selection of bond lengths and angles of these compounds, while ORTEP representations of the pertinent molecular structures are shown in **Figs. 4.1-4.4**.

The selected specimens of **1**, **2**, and **8** (**Table 4.1**) were all found to belong to the monoclinic crystal system; compound **8**, besides, crystallized as a non-merohedral, two-component twin, with the two components having the same cell parameters. The twin data finalization showed that the whole reflection dataset was unequally divided into a major and a minor component (0.7761/0.2239 ratio). The R_{int} values for the two sets of reflections were significantly different, with the R_{int} value for the data of the minor component significantly worse. Accordingly, the molecular structure of **8** has been solved by analyzing only data about the major component. Compounds **1**, **2**, and **8** share a common 4-phenyl-4-(1H-pyrazol-1-yl)butan-2-one moiety, and their structures can be discussed together. An examination of the CCDC repository shows that the above fragment has not yet been documented, so, to the best of our knowledge, these molecules and complex **3** below are the first examples of structures containing this rather unusual synthon, while only a few compounds show only some degree of similarity with those described here. Among these, ethyl N-(2-acetyl-3-oxo-1-phenylbutyl)carbamate ^[92] incorporates the 3-phenylmethyl-2,4-pentanedione moiety bound to a sp^2 nitrogen that is also present in the **1** and **2** ligands, while few bis-pyrazolyl derivatives show the pyrazole ring bound to a short alkyl-carbonyl chain. **1**, **2**, and **8** are optically active molecules because the C1 atom of all compounds shows four different substituent groups.

Table 4.1 Summary of data collection parameters and crystal data for compounds **1**, **2**, **3** and **8**.

Compound	1	2	3	8
Radiation (all experiments)	Cu K α (λ = 1.54184)			
Empirical formula	C ₁₅ H ₁₆ N ₂ O ₂	C ₁₇ H ₂₀ N ₂ O ₂	C ₅₁ H ₄₆ N ₂ O ₂ F ₆ P ₃ Cu	C ₁₅ H ₁₈ N ₂ O
Formula weight	256.30	284.35	989.35	242.31
Temperature / K	298.1(4)	299.4(8)	298(1)	298.8(8)
Crystal system	monoclinic	monoclinic	orthorhombic	monoclinic
Space group	C2/c	P2 ₁ /c	Pna2 ₁	P2 ₁ /c
<i>a</i> / Å	26.9711(5)	8.3223(2)	20.9587(2)	9.1203(7)
<i>b</i> / Å	9.7500(2)	12.4916(3)	9.96560(10)	12.8652(8)
<i>c</i> / Å	10.5745(2)	30.5285(5)	22.7132(2)	12.5423(9)
α / °	90.00	90.0	90.00	90.0
β / °	95.406(2)	91.296(2)	90.00	108.296(8)
γ / °	90.00	90.0	90.00	90.0
Volume/Å ³	2768.39(9)	3172.90(12)	4744.02(8)	1397.25(18)
<i>Z</i>	8	8	4	4
ρ_{calc} Mg / m ³	1.230	1.191	1.385	1.152
μ / mm ⁻¹	0.668	0.629	2.164	0.575
<i>F</i> (000)	1088	1216	2040	520
Crystal size / mm ³	0.80 × 0.40 × 0.10	0.44 × 0.40 × 0.24	0.40 × 0.30 × 0.22	0.44 × 0.30 × 0.06
Reflections collected	20260	53329	41569	5370
Independent reflections / <i>R</i> _{int}	2658 / 0.0374	6320 / 0.0276	8023 / 0.0393	2484 / 0.0338
Restraints / parameters	0 / 237	0 / 539	1 / 643	0 / 236
Goodness-of-fit on <i>F</i> ²	1.049	1.049	1.038	0.984
Final <i>R</i> (<i>R</i> ₁ ; <i>wR</i> ₂) indexes [<i>I</i> > 2 σ (<i>I</i>)]	0.0473, 0.1113	0.0468, 0.1307	0.0336, 0.0838	0.0446, 0.1136
Largest diff. peak / hole / e Å ⁻³	0.187 / -0.175	0.222 / -0.233	0.317 / -0.182	0.149 / -0.120
Goodness-of-fit = $[\sum (w(F_o^2 - F_c^2)^2) / (N_{\text{obsvns}} - N_{\text{params}})]^{1/2}$, based on all data; $R_1 = \sum F_o - F_c / \sum F_o $; $wR_2 = [\sum w(F_o^2 - F_c^2)^2 / \sum w(F_o^2)]^{1/2}$.				

Table 4.2 Selected bond lengths (angstroms) and angles (°) for molecules **1**, **2**, **8**, (top), and complex **3** (bottom).

Bond	1	2	8	Angle	1	2	8		
N1-N2	1.3412(18)	1.3606(15)	1.3645(15)	1.357(2)	N2-N1-C2	103.65(14)	104.76(12)	104.73(11)	104.30(16)
N1-C2	1.333(2)	1.3293(17)	1.3298(17)	1.334(3)	N1-N2-C4	112.21(14)	112.45(11)	112.25(11)	112.47(15)
N2-C1	1.4694(19)	1.4637(15)	1.4621(15)	1.465(2)	N1-N2-C1	120.53(12)	119.53(10)	119.45(10)	119.84(15)
N2-C4	1.338(2)	1.3513(17)	1.3496(17)	1.352(2)	N2-C1-C5	109.98(12)	109.65(10)	109.59(10)	109.70(15)
C1-C5	1.531(2)	1.5363(18)	1.5362(18)	1.522(3)	N2-C1-C10*	110.68(12)	111.46(10)	112.36(10)	112.90(15)
C1-C10*	1.520(2)	1.5213(18)	1.5211(18)	1.518(2)	C1-C5-C6	110.1(9)	111.23(10)	111.70(10)	113.97(17)
C2-C3	1.371(3)	1.388(2)	1.393(2)	1.384(3)	C1-C5-C8	110.32(13)	110.41(10)	110.27(11)	
C3-C4	1.359(3)	1.373(2)	1.366(2)	1.372(3)	C2-C3-C4	104.79(16)	106.70(13)	106.73(13)	106.17(19)
C5-C6	1.536(2)	1.5411(17)	1.5388(17)	1.504(3)	C5-C6-C7	116.89(15)	118.13(11)	117.63(12)	116.7(2)
C5-C8	1.532(2)	1.5380(17)	1.5342(17)		C6-C5-C8	107.35(12)	107.02(10)	106.88(10)	
C6-O1	1.1953(19)	1.2016(16)	1.2026(17)	1.211(2)	C5-C6-O1	120.18(15)	119.26(12)	119.57(13)	121.29(19)
C8-O2	1.195(2)	1.1964(18)	1.1948(19)		C5-C8-O2	120.39(14)	120.77(13)	120.66(14)	

Bond	3	Angle	3				
Cu1-P1	2.2385(9)	C5-C8	1.551(7)	N1-Cu1-P1	110.40(9)	N2-C1-C5	110.9(3)
Cu1-P2	2.2578(9)	C8-O2	1.195(7)	N1-Cu1-P2	109.52(10)	C1-C5-C6	112.7(4)
Cu1-N1	2.039(3)	C2-C3	1.369(6)	O1-Cu1-P1	119.80(10)	C5-C6-O1	122.1(4)
Cu1-O1	2.307(3)	C3-C4	1.351(7)	O1-Cu1-P2	93.11(9)	N1-N2-C4	109.4(4)
N1-N2	1.347(5)	P1-C16	1.833(4)	P1-Cu1-P2	128.25(4)	N2-N1-C2	105.8(3)
N1-C2	1.323(5)	P1-C22	1.825(4)	N1-Cu1-O1	87.81(13)	C16-P1-C28	106.79(17)
N2-C4	1.348(6)	P1-C28	1.828(4)	Cu1-P1-C16	114.70(12)	C28-P1-C22	102.83(18)
N2-C1	1.470(5)	P2-C34	1.824(3)	Cu1-P2-C34	117.08(12)	C34-P2-C46	103.63(17)
C1-C5	1.545(5)	P2-C40	1.829(4)	Cu1-N1-N2	129.9(3)	C46-P2-C40	106.5(2)
C5-C6	1.525(6)	P2-C46	1.820(4)	Cu1-O1-C6	136.9(3)	F-P3-F (av.)	103.9
C6-O1	1.203(6)	P3-F (av.)	1.540	N1-N2-C1	121.4(3)		

*C12 for compound **2** with two independent molecules in an asymmetric unit.

The X-ray experiments for **1** (Fig. 4.1a) and **8** (Fig. 4.2a) reveal that the asymmetric unit of these compounds is made of a single molecule, in which C1 has an *S* configuration. The asymmetric unit of compound **2** (Fig. 4.1b) shows instead two independent molecules, defining an enantiomeric pair, with C1 and C1A atoms showing, respectively, *S* and *R* configurations. The atoms common to **1**, **2**, and **8** are reasonably superimposable (RMS value within 0.315-0.489 Å, making allowance for the inversion of the *R* enantiomer of **2**) by using the overlay routine in *Mercury* [93]. In **1** and **2**, the two -C(O)-CH₃ groups branching from C5 have a nearly eclipsed reciprocal arrangement. In all compounds, the carbonyl oxygen atoms are always *syn*-placed to the C1 methine hydrogen and the molecular pucker brings the mean planes encompassing the phenyl and the pyrazolyl moieties to form similar angles with each other; 73.8° in **1**, 71.8 and 67.5° in the two enantiomers of **2** and 61.6° in **8**. In the latter, the propan-2-one chain bound to C1 is also planar and makes angles of 87.7 and 54.1°, respectively, with the mean planes of the pyrazolyl and phenyl moieties.

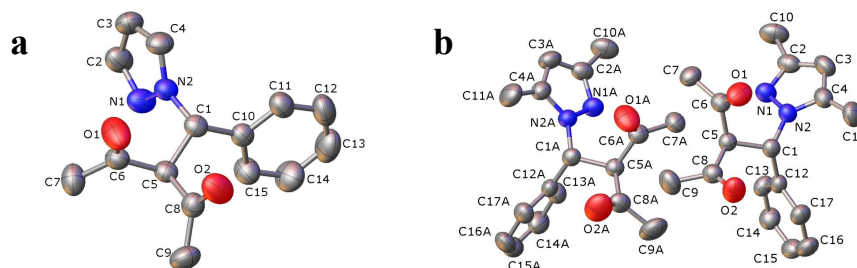


Fig. 4.1 ORTEP representation of (a) the HL^{acPz} ligand (**1**) and (b) the two independent molecules in the asymmetric unit of the HL^{acPzMe} ligand (**2**), with the atomic numbering scheme. Ellipsoids drawn at 50% probability level; H atoms not shown.

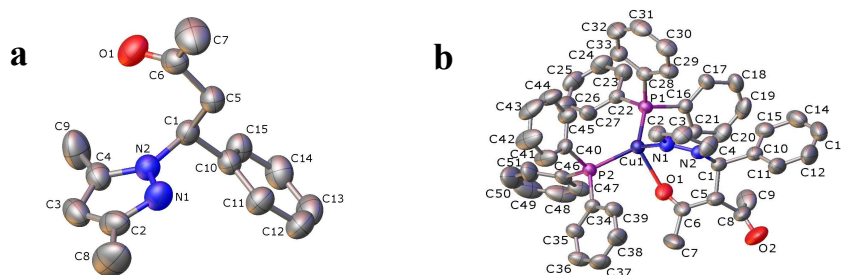


Fig. 4.2 ORTEP representation of (a) the PhPzMe₂MEK molecule (**8**) and (b) the [Cu(PPh₃)₂(HL^{acPz})]⁺ cation of the complex (**3**), with the atomic numbering scheme. Ellipsoids drawn at 50% probability level; H atoms and the disordered PF₆⁻ anion not shown.

A comparison (Table 4.2) of the selected structural parameters in the common fragments of the three molecules indicates that the distribution of the bond lengths in the pyrazolyl rings and the 2,4-pentanedione/propan-2-one chains is comparable, and it is also comparable with those reported for the few molecules sharing some similarities with the compounds described here. In particular, the C=O bonds (mean length of 1.199

Å) have a clear double bond character, and the bonds made by the C1 atom (mean length of 1.526 Å) have a single-bond character. Likewise, the distribution of the average bond lengths about the pyrazolyl rings (1.356, 1.332, 1.384, 1.368, and 1.465 Å for, respectively, N1-N2, N1-C2, C2-C3, C3-C4, N2-C4 distances) matches those found in reported compounds (1.366, 1.334, 1.394, 1.367, 1.357 and 1.462 Å), thus suggesting that there is not conjugation outside the ring and that N1-C2 and C3-C4 distances have the greater double-bond character. As for the above compounds, the exploration of the CCDC database reveals only one bis-triphenylphosphine copper(I) complex having some similarity with the one presented here.

The complex **3** crystallizes in the orthorhombic crystal system; in its structure (**Fig. 4.2b**), the Cu(I) ion is surrounded by two unidentate PPh₃ molecules and one bidentate HL^{acPz} neutral ligand, which attacks the copper ion using its N1, O1 atoms. The resulting complex [Cu(PPh₃)₂(HL^{acPz})]⁺ is cationic and crystallizes with the help of a (disordered) hexafluorophosphate anion. The central metal has a distorted tetrahedral environment (τ_4 and τ_4' indexes values of 0.794 and 0.768, respectively^[94]), dominated at one side by the large P1-Cu1-P2 angle of 128.25(4), to make allowance for the bulky triphenylphosphine ligands and at the other side by the narrow N1-Cu1-O1 chelation angle of 87.81(13). Notably, upon coordination, the HL^{acPz} ligand forms a seven-membered metallacycle, in which, besides C1, also C5 becomes optically active, with both centers having an *R* configuration. In its bound form, only the O6 atoms remain *syn*-oriented to the methine C1 hydrogen, while O5 becomes *anti*-oriented. Accordingly, the two -C(O)-CH₃ groups branching from C5 are also reciprocally *anti*-oriented to the C5 atom. The atoms participating in the seven-membered ring, besides Cu1, O1, and N1, are N2, C1, C5, and C6. The ring has a distorted *boat* (*C_s*) arrangement, due to the unequal Cu1-O1, and Cu1-N1 distances as well as to the constraints imposed by the pyrazolyl ring. The Cu1, N1, O1, and C6 atoms are coplanar within 0.06 Å; another plane encompasses N1, N2, C5, and C6, coplanar within 0.02 Å, with C1 out of this last plane by 0.78 Å. The mean planes passing through the phenyl and the pyrazolyl rings in **3** make with each other a dihedral angle of 70.2° (compared with 73.8° in the free ligand), so the HL^{acPz} ligand satisfies the copper coordination needs by properly adjusting only its 2,4-pentanedionato moiety. The two triphenylphosphine ligands have the expected propeller shape, with the planes of the three phenyl rings making with each other angles of 64.2, 73.4, 88.1° for the rings branching from P1, and 78.1, 82.8, 87.3°

for the rings branching from P2, respectively. Notably, the atoms of the C22/C27 ring, P1, and Cu1 are also coplanar within 0.03 Å. The C6-O1 and N1-N2 bond distances (**Table 4.2**) slightly elongate upon coordination (+0.008 and +0.006 Å, respectively), while the N1-C2 and C3-C4 distances shrink a bit, slightly increasing their double-bond character (-0.010 and -0.008 Å, respectively).

By examining the metrical data of the above noted (1H-pyrazole-3,5-dicarboxylato)-bis(triphenylphosphine)-copper(I), we note that Cu-P distances in **3**, (2.2385(9) / 2.2578(9) Å) compare quite well with those in the reported complex (2.226 / 2.265 Å) and are little shorter than the average of 2.272 Å for 950 bis-triphenylphosphine copper(I) complexes in the CCDC repository. On the other side, Cu-N (2.039(3) Å) and Cu-O (2.307(3) Å) distances are instead shorter and longer (-0.05 and +0.147 Å, respectively) than the corresponding ones and also appreciably longer (+0.104 and +0.127 Å) of the known means for Cu(I) complexes showing Cu-N(pyrazolyl) and Cu-O=C bonds (1.935 Å/290 compounds and 5.180 Å/260 compounds, respectively). The longer Cu-N and Cu-O bonds (compared to averages for known compounds) may hint in part to a lesser donating ability of the N1 and especially O6 atoms, as well as to the steric hindrance brought by the bulky triphenylphosphine ligands. o the nonbonding contacts, a list of the tightest interactions for the compounds described in this work is given in Table 3 (involved distances equal the sum of the pertinent van der Waals radii minus 0.05 Å and almost all of them fall below 2.7 Å). An inspection of the packing diagrams of compounds **1** and **8** shows a similar contact network. In **1**, the two noticeable contacts involve O1 and N1 that bind, respectively the H15 and H11 atoms of two different nearby molecules. (at $x, 1 - y, -1/2 + z$ and $x, 1 - y, 1/2 + z$). The two contacts help to consolidate a one-dimensional chain that propagates along the crystallographic *c*-axis. In **8**, the three shortest contacts (range: 2.55-2.66 Å) all involve the O1 atom which binds, again, the H1 / H15 and H14 of two different proximal molecules. The links with H1 and H15 establish a dimeric couple, the third one (symmetry operation: $1 + x, y, z$) in turn propagates a one-dimensional chain of the dimer along the crystallographic axis. In **2**, the two independent molecules in the asymmetric unit are bound through an O1A····H7B link (2.53 Å); O1A is also involved in contact with the H14A of another molecule (at $1 + x, y, z$). Likewise, O1 establishes two different contacts with the H7AC and H14 atoms 14 (at $1 + x, y, z$ and $-1 + x, y, z$), and all these links create again a one-dimensional chain propagating along the crystallographic axis. This motif is also reinforced by an O2····H16 contact that

joins together two proximal chains. The more extended nonbonding contact network of complex **3** depends on the disordered PF_6^- anion. In the latter, almost all the atoms of the alternate position of the anion establish contacts with several hydrogen atoms sustaining a full 3D network in which the hexafluorophosphate anions and the complex cations pile up in alternate layers along the crystallographic *c*-axis.

Table 4.4 Most efficient nonbonding interactions in molecules **1**, **2**, **8**, (top) and complex **3** (bottom).

Molecule	Acceptor atom (A) atom	Donor (D) atom	Parent (P) atom*	Distance A...D (Å)	Angle A-D-P (°)	Symmetry op.
1	O1	H15	C15	2.51	141.5	$x, 1-y, -1/2+z$
	N1	H11	C11	2.69	171.6	$x, 1-y, 1/2+z$
2	O1A	H7B	C7	2.53	147.1	x, y, z
	O1A	H14A	C14A	2.60	143.7	$1+x, y, z$
	O1	H7AC	C7A	2.52	156.1	$1+x, y, z$
	O1	H14	C14	2.56	152.0	$-1+x, y, z$
	O2	H16	C16	2.62	175.0	$2-x, 2-y, 1-z$
	O1	H15	C15	2.55	153.2	$1-x, 1-y, -z$
8	O1	H1	C1	2.65	157.3	$1-x, 1-y, -z$
	O1	H14	C14	2.66	136.7	$1+x, y, z$
	O1	H14	C14	2.56	135.3	$1/2+x, 3/2-y, z$
3	F1A	H18	C18	2.44	160.7	$1/2+x, 3/2-y, z$
	F2A	H9B	C9	2.51	142.8	x, y, z
	F2	H9B	C9	2.56	130.7	x, y, z
	F2	H42	C42	2.44	145.6	$1-x, 1-y, 1/2+z$
	F3	H11	C11	2.51	158.2	$x, 1+y, z$
	F3	H32	C32	2.63	160.6	$1/2-x, 1/2+y, 1/2+z$
	F4A	H38	C38	2.56	130.0	$x, 1+y, z$
	F4	H5	C5	2.46	154.5	$x, 1+y, z$
	F5A	H21	C21	2.57	148.4	x, y, z
	F6A	H42	C42	2.55	146.6	$1-x, 1-y, 1/2+z$
	C15	H37	C37	2.79	153.4	$1-x, 1/2-y, 1/2+z$

*Atom to which donor atom is bound.

4.4. Conclusions

In this study, we report the synthesis and characterization of two new 3-monosubstituted acetylacetone ligands, 3-(phenyl(1*H*-pyrazol-1-yl)methyl)pentane-2,4-dione (HL^{acPz} , **1**) and 3-((3,5-dimethyl-1*H*-pyrazol-1-yl)(phenyl)methyl)pentane-2,4-dione ($\text{HL}^{\text{acPzMe}}$, **2**). They were employed for the preparation of the copper(II) and copper(I) phosphane complexes of general formula $[\text{Cu}(\text{PPh}_3)_2(\text{HL}^{\text{acX}})]\text{PF}_6$ (**3** and **5**) and $[\text{Cu}(\text{HL}^{\text{acX}})_2(\text{L}^{\text{acX}})_2]$ (**4** and **6**). HL^{acPz} and $\text{HL}^{\text{acPzMe}}$ react with strong bases by retro-Claisen C-C bond cleavage reaction giving rise to the formation of the species 4-phenyl-4-(1*H*-pyrazol-1-yl)butan-2-one (**7**) and 4-(3,5-dimethyl-1*H*-pyrazol-1-yl)-4-phenylbutan-2-one (**8**), providing efficient access to synthetically useful ketone compounds. All species were fully characterized both in the solid state and in solution.

The X-ray experiments for **1** and **8** reveal that the related asymmetric unit is made of a single molecule, in which C1 has an *S* configuration. The asymmetric unit of compound **2** shows instead two independent molecules, defining an enantiomeric pair. The molecular structure reveals that complex **3** crystallizes in the orthorhombic crystal system, where the copper(I) ion is surrounded in a distorted tetrahedral environment by two unidentate PPh₃ molecules and one bidentate HL^{acPz} neutral ligand, which attacks the metal through its N1, O1 atoms.

This study establishes a new and easily accessible class of copper compounds, provides new insights into the chemistry group 11 metal compounds, and opens new opportunities for further research in this field.

Chapter V: Ultrasensitive “signal-inversion” photoelectrochemical aptasensor based on semiconductive MOF integrated with the manganese ferrite nanozyme-regulation for the selective detection of fumonisin B1

5.1 Introduction

During food production, storage, and transportation, foodstuffs can be polluted by pathogenic bacteria, mycotoxins, heavy metal ions, or antibiotics [95]. Mycotoxins, the secondary metabolites produced by fungi species, have been regarded as one of the most threatening food pollutants for humans and livestock. As one of the most dangerous mycotoxins, fumonisin B1 (FB1) is often found in agricultural products or grains and exhibits high neurotoxicity, hepatotoxicity, and nephrotoxicity [96]. The maximum tolerable level of FB1 formulated by the World Health Organization is set as $2.0 \mu\text{g kg}^{-1}$. Thus, the sensitive determination of FB1 is essential for ensuring food safety and protecting the health of human beings.

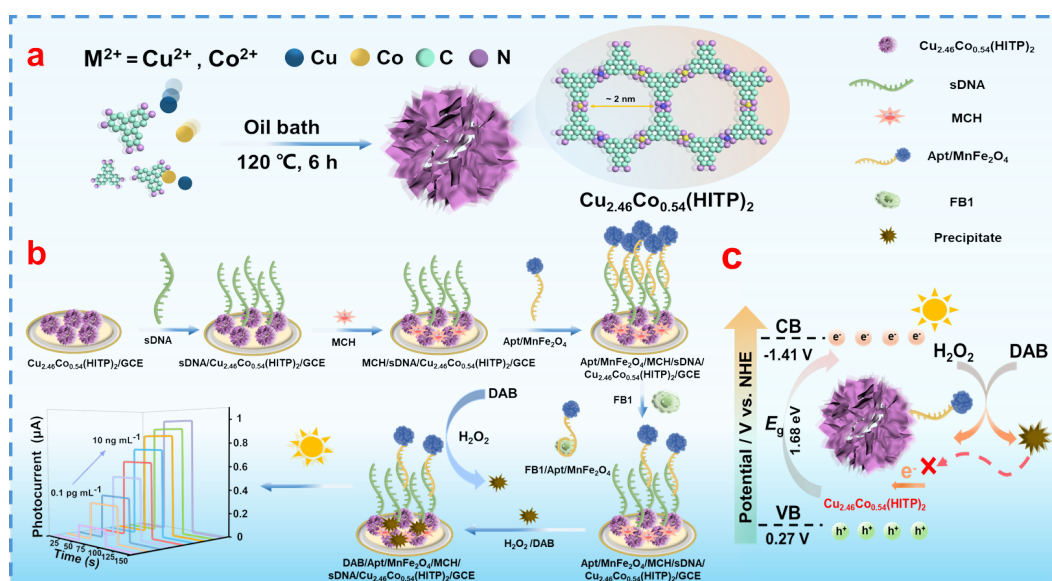
As a kind of important porous organic framework, metal-organic frameworks (MOFs) demonstrate enlarged specific surface areas and pore sizes, tunable nanostructures, and multifunctionality [97], showing widespread applications in biosensing fields. MOFs and MOF-related nanomaterials have been regarded as excellent electrode materials for fabricating PEC biosensors in light of the features of high photoabsorption ability, enhanced separation of photocarriers, and adjustable band gaps [98]. Among many MOFs, two-dimensional conductive (2D cMOFs) or semiconductive MOFs (scMOFs), which are synthesized using linking ligands with highly planar and conjugated structures containing multitrophic *ortho*-substituted $-\text{NH}_2$, $-\text{OH}$, $-\text{SH}$, or $-\text{SeH}$ functional groups, have aroused the increasing attention [99]. Besides the features of most MOFs, semiconductive MOFs (scMOFs) demonstrate unique physicochemical properties and structural characteristics, such as photovoltaic efficiency, tunable bandgap, structural and chemical robustness, and fast electron transfer because of their high conjugated, planar, and graphene-like structure. These characteristics of scMOFs render enhanced photoelectronic properties, tunable structures, and multifunctionality positions for immobilizing probes, thus showing the great potential application in the PEC biosensing field. Consequently, 2D cMOFs or scMOFs show superior biosensing ability because of their high affinity toward probes via π - π stacking, Van der Waal force, and hydrophobic interaction [100] and great

potential for the development of PEC biosensors.

Moreover, diverse biosensing design strategies, such as polymerase chain reaction, rolling circle amplification, DNA walker, catalytic hairpin assembly, hybridization chain reaction, ratiometric strategies, and enzyme-assisted biocatalytic precipitation reaction (EABPR), have been combined with the PEC technique to boost further the sensitivity of biosensors. In the case of the EABPR strategy, noble metal-based nanozymes with excellent catalytic efficiency and specificity integrate EABPR with the PEC technique; thus, they can markedly enhance biosensing efficiency and selectivity for the analysis of AFB1 or FB1, such as Au@Pd@Pt^[101] and PtPd-CoSnO₃^[102]. However, these noble metal-based nanozymes usually suffer from high cost, low earth abundance, or fussy synthesis procedures. These shortcomings can be overcome by employing various enzyme-like nanomaterials, such as metal oxides, carbon-based materials, and single atom-based nanomaterials, for the construction of PEC biosensors. Spinel ferrites can be considered promising enzyme-like nanomaterials with precise and tunable crystal structures, well-defined structures, and strong tolerance to acid/base media by taking advantage of their inherent catalytic ability. Combining 2D scMOFs with spinel ferrite nanozymes is highly promising for manufacturing an advanced PEC aptasensor and improving the analysis efficiency of FB1.

Herein, we established a novel “signal-inversion” PEC aptasensor for the ultrasensitive determination of FB1 using Cu_{2.46}Co_{0.54}(HITP)₂ (HITP: 2,3,6,7,10,11-hexaiminotriphenylene) with enhanced photoelectric activity as an efficient signal indicator with the aid of the EABPR strategy (**Scheme 5.1**). The photoelectric active Cu_{2.46}Co_{0.54}(HITP)₂ synthesized using HITP as ligand and Cu²⁺ and Co²⁺ ions as coordination sites (**Scheme 5.1a**) was simultaneously used to amplify the PEC signal. As depicted in **Scheme 5.1b**, Cu_{2.46}Co_{0.54}(HITP)₂ also served as a bioplatfor for anchoring the single-stranded DNA (ssDNA). Afterward, the MnFe₂O₄-labeled aptamer probe (represented as Apt/MnFe₂O₄), in which the FB1-target aptamer possesses a complementary sequence toward ssDNA, was added into the sensing system and tended to hybridize with ssDNA/Cu_{2.46}Co_{0.54}(HITP)₂. It thus resulted in the deposition of the Apt/MnFe₂O₄ probe onto the modified electrode, further declining the photocurrent. When detecting FB1, the binding interaction of FB1 with aptamer, which formed the G-quadruplex via the specific recognition between them, led to the release of the FB1/Apt/MnFe₂O₄ complex. Further, in the presence of H₂O₂, the colorless 3,3'-diaminobenzidine (DAB) was catalyzed and changed to brown insoluble precipitate

(oxDAB) by the residual Apt/MnFe₂O₄ hybridized with sDNA/Cu_{2.46}Co_{0.54}(HITP)₂. As illustrated in **Scheme 5.1c**, the generated precipitate caused by the oxidation of DAB by MnFe₂O₄ in the presence of H₂O₂ inhibited the electron transferring from the VB to the CB of the Cu_{2.46}Co_{0.54}(HITP)₂-modified electrode, resulting in a sharp decrease in the photocurrent. Notably, the inhibition ability of the electron transfer by the precipitate at the electrode/electrolyte decreased with the increase of the FB1 concentration, causing the photoelectric signal to enlarge inversely. Given that the Cu_{2.46}Co_{0.54}(HITP)₂-based “signal-inversion” PEC aptasensor integrated the structure features of Cu_{2.46}Co_{0.54}(HITP)₂ and the superior catalytic performance of MnFe₂O₄, the enhanced aptasensing performance toward FB1 was achieved with an ultralow detection limitation of 65 fg mL⁻¹ in a wide linear range of 0.1 pg mL⁻¹ to 10 ng mL⁻¹. This work provides a newly designed “signal-inversion” PEC aptasensor by combining the competitive biosensing strategy with the help of the EABPR technique for the sensitive and selective inspection of FB1 in food products and complicated environments.



Scheme 5.1 (a) Synthesis of Cu_{2.46}Co_{0.54}(HITP)₂. (b) The construction procedure of the PEC aptasensor. (c) The mechanism of the electron transfer with the Cu_{2.46}Co_{0.54}(HITP)₂ and the mixture of DAB and H₂O₂.

5.2 Experimental section

5.2.1 Reagents and materials

Copper sulfate pentahydrate (CuSO₄·5H₂O), cobalt sulfate heptahydrate (CoSO₄·7H₂O), sodium chloride (NaCl), potassium chloride (KCl), dibasic sodium phosphate (Na₂HPO₄), potassium dihydrogen phosphate (KH₂PO₄), potassium

ferricyanide ($\text{K}_3\text{Fe}(\text{CN})_6$) and potassium hexacyanoferrate ($\text{K}_4\text{Fe}(\text{CN})_6 \cdot 3\text{H}_2\text{O}$) were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Anhydrous sodium acetate (CH_3COONa), N,N-dimethylformamide (DMF), N,N-dimethylacetamide (DMA) hydrochloric acid (HCl) and hydrogen peroxide (H_2O_2) were obtained from Tianjin Fuyufen Chemical Co. DAB and HITP were from Aladdin Chemical Reagent Co. All other chemicals were analytical grade and used without further purification. 6-mercapto-1-hexanol (MCH, 14.4 M), enzyme-linked immunosorbent assay (ELISA), ochratoxin A (OTA), ochratoxin B (OTB), deoxynivalenol (DON), zearalenone (ZEN), aflatoxin B1 (AFB1), fumonisin B1 (FB1), single strand DNA (ssDNA), and FB1-targeted aptamer (FB1-Apt) were obtained from Shanghai Sangyo Biotechnology Co. The base sequences of ssDNA and aptamer were shown as bellows:

ssDNA: 5'- C6 CTC GTA GCC TGT GGG GGG AGT TAA TCT ATC ATT TCT TAT GGT CGA ATA AGT TAA TTA-3'.

Aptamer: 5'- C6-TCT AAC GTG AAT GAT AGA TGA ACT TAT TCG ACC ATA CAC GTC TGC ATT AAC TTA TTC GAC CAT ATT CCA TTA CGC TAA TTA ACT TAT TCG ACC ATA-3'

5.2.2 Basic characterizations

The crystal structure was measured by powder X-ray diffractometry (PXRD) using a Rigaku D/Max-2500 X-ray diffractometer ($\lambda = 0.15406 \text{ nm}$). The morphologies were characterized using a JSM-6490LV field emission scanning electron microscope (FE-SEM, Japan) and a JEOL JEM-2100 transmission electron microscope (TEM, Japan). X-ray photoelectron spectroscopy (XPS) analyses were performed on a Bruker TENSOR 27 spectrometer using Al $\text{K}\alpha$ X-ray irradiation (1486.6 eV photons) with an excitation voltage of 120 W. Optical measurements were performed using a UV-visible spectrophotometer (Hitachi UV U3900). The Brunauer-Emmett-Teller (BET) surface areas, pore volume, and pore size were obtained by analyzing the N_2 adsorption-desorption isotherms with Micromeritics ASAP 2020 built-in software. Metal content in $\text{Cu}_{2.46}\text{Co}_{0.54}(\text{HITP})_2$ was determined using a Thermo Scientific iCAP 6500 model inductively coupled plasma-mass spectrometry (ICP-MS). Raman spectra were obtained on a Renishaw Via Raman spectrometer with a solid-state laser (excitation at 532 nm) in 50 - 4000 cm^{-1} . The electron paramagnetic resonance (EPR) image was tested on a Bruker E500.

5.2.3 Synthesis of $\text{Cu}_{2.46}\text{Co}_{0.54}(\text{HITP})_2$

$\text{Cu}_{2.46}\text{Co}_{0.54}(\text{HITP})_2$ was synthesized according to our previous work, but with slight modifications ^[103]. Typically, 3.5 mg $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (14 μmol) and 2.4 mg $\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$ (14 μmol) were homogeneously dispersed in the mixed solvent of N, N'-dimethylformamide and N, N'-dimethylacetamide (10 mL, $v : v = 1:1$), followed by adding 4.0 mL of anhydrous sodium acetate (2.0 M) and stirring for 0.5 h. Simultaneously, 5.0 mg HITP (9.3 μmol) was dispersed in 2 mL of Milli-Q water. Afterward, the HITP solution was added to the above mixture and further heated in an oil bath at 75 °C for 5 h. The obtained precipitate was washed with acetone thrice and dried under vacuum at 60 °C for 12 h. The atomic contents of Cu and Co atoms in the synthesized MOF determined by inductively coupled plasma mass spectrometry are 9.86% and 2.03%, respectively. According to the ratio of the atomic percent of Cu and Co atoms in the MOF and the sum coordination number of 3 ^[104], the ratio of the coordination number of Cu to Co was around 2.46:0.54. Therefore, the bimetallic sCMOF was named $\text{Cu}_{2.46}\text{Co}_{0.54}(\text{HITP})_2$. Similarly, the monometallic MOFs, i.e., $\text{Cu}_3(\text{HITP})_2$ and $\text{Co}_3(\text{HITP})_2$ MOFs, were synthesized using a similar method, but without adding $\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$ and $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, separately.

5.2.4 Construction of the $\text{Cu}_{2.46}\text{Co}_{0.54}(\text{HITP})_2$ -based PEC aptasensor

Firstly, 10 mg of $\text{Cu}_{2.46}\text{Co}_{0.54}(\text{HITP})_2$ was added to 5 mL of phosphate-buffered saline (PBS; 0.01 M, pH 7.4) containing 10 μL Nafion and stirred for 30 min until forming the homogeneous suspension (2.0 mg mL^{-1}). Subsequently, 10 μL of the $\text{Cu}_{2.46}\text{Co}_{0.54}(\text{HITP})_2$ dispersion (2.0 mg mL^{-1}) was coated onto the pre-treated glassy carbon electrode (GCE) and completely dried at room temperature (denoted as $\text{Cu}_{2.46}\text{Co}_{0.54}(\text{HITP})_2/\text{GCE}$). Secondly, the $\text{Cu}_{2.46}\text{Co}_{0.54}(\text{HITP})_2/\text{GCE}$ was incubated with sDNA (0.8 μM) for 12 h, followed by washed with PBS thrice to remove the loosely adsorbed sDNA and dried in air (denoted as sDNA/ $\text{Cu}_{2.46}\text{Co}_{0.54}(\text{HITP})_2/\text{GCE}$). Then, the blocking step was performed by immersing the sDNA/ $\text{Cu}_{2.46}\text{Co}_{0.54}(\text{HITP})_2/\text{GCE}$ in 150 μL of the 6-mercapto-1-hexanol solution (MCH; 1.0 mM) for 1 h to eliminate the non-specific adsorption (MCH/sDNA/ $\text{Cu}_{2.46}\text{Co}_{0.54}(\text{HITP})_2/\text{GCE}$). Thirdly, the MCH/sDNA/ $\text{Cu}_{2.46}\text{Co}_{0.54}(\text{HITP})_2/\text{GCE}$ was incubated with the Apt/ MnFe_2O_4 (10 μL , 1.0 μM) suspension for 70 min, leading to the hybridization of sDNA and the aptamer strand and generating the double strand sDNA/Apt complex (denoted as

Apt/MnFe₂O₄/MCH/sDNA/Cu_{2.46}Co_{0.54}(HITP)₂/GCE). Notably, all experiments were performed at 4 °C, while the electrode surface was washed with PBS (0.01 M, pH 7.4) after each step to eliminate unbound material or DNA strands.

5.2.5 Quantitative analysis of FB1

The concentration titration method was utilized for the detection of FB1 using the developed PEC aptasensor. Typically, the constructed aptasensor was separately incubated in the series of FB1 solutions with different concentrations (0.0001, 0.001, 0.01, 0.1, 0.5, 1, 2, 5, 10 ng mL⁻¹) for 70 min at 4 °C. As such, the formed G-quadruplex between FB1 and Apt/MnFe₂O₄ was released from the electrode. After that, the developed aptasensor anchored with residual Apt/MnFe₂O₄ probes was immersed in the mixed solution containing H₂O₂ (0.15 mM) and DAB (1.0 mM) for 40 min. With the help of EABPR, the obtained PEC response was recovered. Distinctly, the recovery intensity of the PEC signal was proportional to the FB1 concentration owing to the gradual decrease of the formed precipitate. According to the recommendation of the International Union of Pure and Applied Chemistry (IUPAC), the LOD of the constructed aptasensor was calculated based on the $3S_B/m$ equation, where S_B and m were the standard deviations of the blank ($n = 11$) and the slope of the linear calibration curve, respectively.

5.3 Results and discussion

5.3.1 Chemical component and surface morphology of Cu_{2.46}Co_{0.54}(HITP)₂

As depicted in **Fig. 5.1a**, the transmission electron microscope (TEM) image of Cu_{2.46}Co_{0.54}(HITP)₂ demonstrates the shape of nanoflakes gathered with nanorods. No obvious lattice can be observed in the high-resolution TEM (HR-TEM) image (**Fig. 5.1b**), hinting the poor crystallinity degree. The energy dispersive spectrometer (EDS) mapping image of Cu_{2.46}Co_{0.54}(HITP)₂ (**Fig. 5.1c**) suggests the homogeneous dispersion of Cu, Co, C, and N elements within the whole region. In the Cu_{2.46}Co_{0.54}(HITP)₂ MOF pattern (**Scheme 5.1a**), Cu nodes are dominated in the MOF network, whereas a fraction of Cu sites is replaced by the Co atom. In particular, the coordination of Co²⁺ ions with HITP can generate the unsaturated coordination of Cu atoms, which can markedly regulate the electron density of Cu atoms. Thus, photoabsorption ability, transfer of photocarriers and electrons, and bioaffinity toward sDNA can be promoted. Moreover,

the N₂ adsorption isotherm of Cu_{2.46}Co_{0.54}(HITP)₂ shows a type-I adsorption curve (**Fig. 5.1d**). The Brunauer-Emmett-Teller surface area of Cu_{2.46}Co_{0.54}(HITP)₂ is approximately 136 m² g⁻¹, along with an average pore size of 0.345 nm.

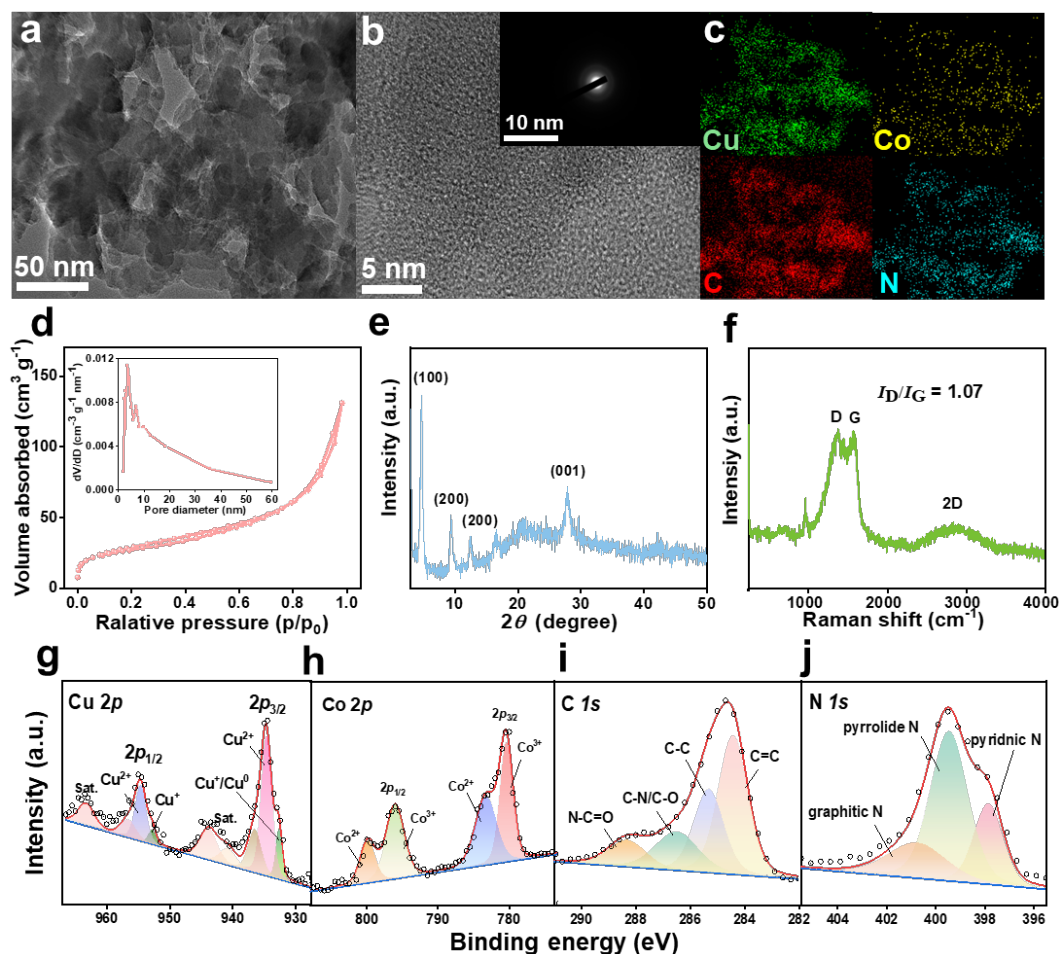


Fig. 5.1 (a) TEM, (b) HR-TEM, (c) EDS mapping images of Cu_{2.46}Co_{0.54}(HITP)₂ [Cu (green), Co (yellow), C (red), and N (cyan)]. (d) The N₂ adsorption-desorption isotherms, (e) PXRD pattern, (f) Raman spectrum, and the high-resolution (g) Cu 2p, (h) Co 2p, (i) C 1s and (j) N 1s XPS spectra of Cu_{2.46}Co_{0.54}(HITP)₂.

The powder X-ray diffractometry (PXRD) pattern of Cu_{2.46}Co_{0.54}(HITP)₂ (**Fig. 5.1e**) shows the diffraction peaks at $2\theta = 4.6^\circ$, 9.7° , and 27.8° , which correspond to the (100), (200) and (001) planes, respectively. The presence of the wide peak at $2\theta = 27.8^\circ$ implies that the orderliness of the crystalline pattern grown along the c-axis direction is lower than the regularity of the AB plane. This characteristic is a typical feature of 2D layered materials. The Raman spectrum of Cu_{2.46}Co_{0.54}(HITP)₂ (**Fig. 5.1f**) shows two apparent peaks at 1368 and 1576 cm⁻¹ because of the D and G bands of graphitic carbon, respectively. In addition, the peak at 2903 cm⁻¹ is assigned to the 2D band. Therefore, the Cu_{2.46}Co_{0.54}(HITP)₂ exhibits a graphene-like structure, thereby benefiting the improvement of photoabsorption and electron transfer. The intensity ratio of the D and G bands (I_D/I_G) value of Cu_{2.46}Co_{0.54}(HITP)₂ is around 1.07, hinting at the disorganized

and hierarchical stack structure with a certain degree of defects in the bimetallic MOF.

Further, the Cu 2*p* of the X-ray photoelectron spectroscopy (XPS) spectrum (**Fig. 5.1g**) can be deconvoluted into two groups of Cu 2*p*_{3/2} (935.35 eV) and Cu 2*p*_{1/2} (952.29 eV), which are composed of Cu⁺ (932.71 and 952.83 eV) and Cu²⁺ (934.67 and 956.78 eV) components. The mixed valence states of Cu⁺/Cu²⁺ facilitate the electron transfer and photoabsorption ability. The Co 2*p* XPS spectrum (**Fig. 5.1h**) can be divided into Co³⁺ (780.36 eV of Co 2*p*_{3/2}; 796.03 eV of Co 2*p*_{1/2}) and Co²⁺ (783.28 eV of Co 2*p*_{3/2}; 799.78 eV of Co 2*p*_{1/2}) species. The C 1*s* XPS spectrum (**Fig. 5.1i**) is composed of C=C (284.44 eV), C-C (285.34 eV), C-N/C-O (286.56 eV), and N-C=O (288.38 eV) groups, in which the high content of C=C reveals the highly conjugated structure of Cu_{2.46}Co_{0.54}(HITP)₂. Moreover, the N 1*s* spectrum includes (**Fig. 5.1j**) pyridinic N, pyrrolic N, and graphitic N components. These results further clarify the successful synthesis of Cu_{2.46}Co_{0.54}(HITP)₂.

5.3.2 Photoelectric performances of Cu_{2.46}Co_{0.54}(HITP)₂

The UV-vis diffuse reflectance spectrum (UV-vis DRS) of Cu_{2.46}Co_{0.54}(HITP)₂ (inset, **Fig. 2.2a**) shows the apparent photoabsorption within the full spectrum range from 200 to 2000 nm. The Tauc plots, conduction band (CB) and valence band (VB) positions of Cu_{2.46}Co_{0.54}(HITP)₂ were deduced from the following equations:

$$(\alpha h\nu)^{1/n} = A (h\nu - E_g)$$

$$E_{CB} = N - 0.5E_g - E_c$$

$$E_g = E_{VB} - E_{CB}$$

where α , h , ν , and A represent the absorption coefficient of the material, Planck's constant, incident light frequency, and a coefficient, respectively, while E_{CB} and E_{VB} are the potentials of the CB bottom and VB top, respectively. N represents the electronegativity of the semiconductor. In **Fig. 5.2a**, the optical band-gap energy (E_g) of Cu_{2.46}Co_{0.54}(HITP)₂ is calculated to be 1.68 eV. Thereby, the narrow band gap of Cu_{2.46}Co_{0.54}(HITP)₂ affords fast electron mobility, further improving the PEC response. The flat band potential of Cu_{2.46}Co_{0.54}(HITP)₂ derived from a Mott-Schottky plot (**Fig. 5.2b**) is approximately -1.41 V relative to that of the Ag/AgCl electrode (vs. Ag/AgCl). Further, due to the positive slope of the curve, Cu_{2.46}Co_{0.54}(HITP)₂ is a n-type semiconductor. The PEC signals of Cu_{2.46}Co_{0.54}(HITP)₂, Cu₃(HITP)₂, and Co₃(HITP)₂ were evaluated to compare their separation efficiencies of the e⁻ - h⁺ couples. As illustrated in **Fig. 5.2c**, it demonstrates that the Cu_{2.46}Co_{0.54}(HITP)₂/GCE (curve *i*) has

a larger photocurrent of 6.87 μA than those of $\text{Cu}_3(\text{HITP})_2/\text{GCE}$ (curve *ii*, 2.61 μA) and $\text{Co}_3(\text{HITP})_2/\text{GCE}$ (curve *iii*, 0.46 μA). Thereby, $\text{Cu}_{2.46}\text{Co}_{0.54}(\text{HITP})_2$ exhibits the enhanced PEC, which could be due to the synergistic effect of different metal nodes. Moreover, $\text{Cu}_{2.46}\text{Co}_{0.54}(\text{HITP})_2/\text{GCE}$ (curve *i*, Fig. 5.2d) illustrates the small diameter of the semicircle, giving the smaller resistance of charge transfer (R_{ct} , 424.6 Ω) than those of $\text{Cu}_3(\text{HITP})_2/\text{GCE}$ (curve *ii*, 561.3 Ω) and $\text{Co}_3(\text{HITP})_2/\text{GCE}$ (curve *iii*, 692.2 Ω). This finding again verifies that the bimetallic MOF displays fast electron transfer, conducive to the improvement of carrier transport.

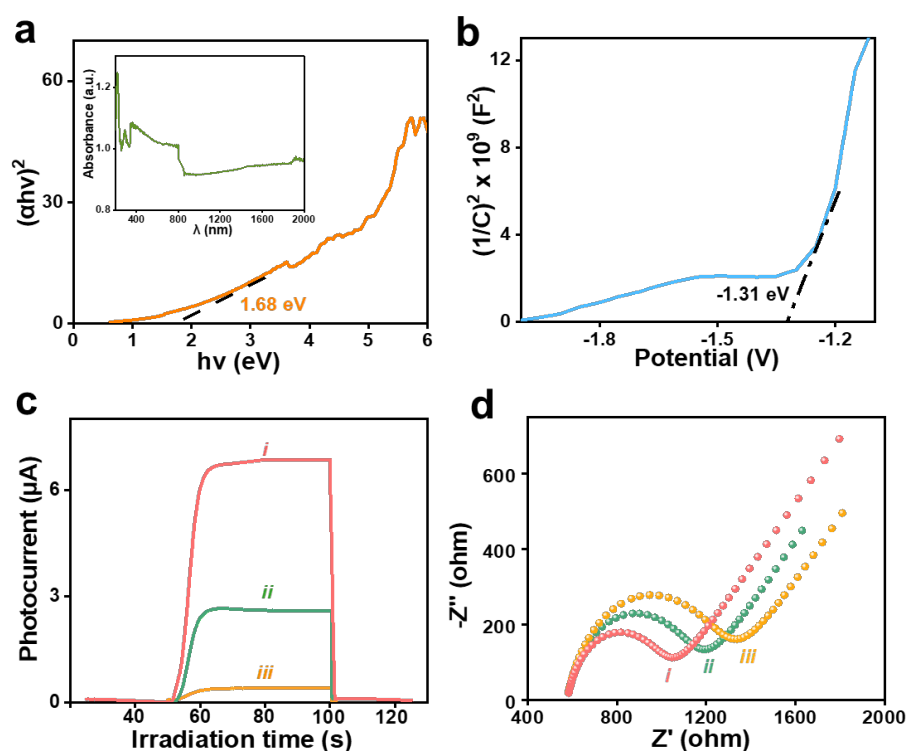


Fig. 5.2 (a) Band gap (Inset: UV-vis diffuse reflectance spectrum), (b) Mott-Schottky plot, (c) Photocurrent responses and (d) EIS Nyquist plots of (i) $\text{Cu}_{2.46}\text{Co}_{0.54}(\text{HITP})_2$, (ii) $\text{Cu}_3(\text{HITP})_2$, and (iii) $\text{Co}_3(\text{HITP})_2$.

5.3.3 Aptasensing mechanism and construction of the $\text{Cu}_{2.46}\text{Co}_{0.54}(\text{HITP})_2$ -based “signal-inversion” PEC aptasensor

The $\text{Cu}_{2.46}\text{Co}_{0.54}(\text{HITP})_2$ -based PEC aptasensing strategy is illustrated in **Scheme 5.1b**. The GCE is modified with $\text{Cu}_{2.46}\text{Co}_{0.54}(\text{HITP})_2$ and employed as the working photoanode, the immobilization of the probe, i.e., single-strand DNA, was performed on the $\text{Cu}_{2.46}\text{Co}_{0.54}(\text{HITP})_2$ -modified electrode. Unlike other kinds of photoactive materials (such as $\text{TiO}_2/\text{BiOI}/\text{BiOBr}$ [105], $\text{CuWO}_4@\text{rGO}$ [106], and $\text{Au NPs}/\text{PTCDA}@C_{60}$ [107], $\text{Cu}_{2.46}\text{Co}_{0.54}(\text{HITP})_2$ exhibits large specific area and pore size, rich functionality, single atomic M-N₄ sites, and high d- π conjugated and graphene-like structure. The ssDNA not only can be anchored on the $\text{Cu}_{2.46}\text{Co}_{0.54}(\text{HITP})_2$ surface but also can be

penetrated the MOF interior through the integrated interactions of π - π stacking, electrostatic interaction caused by the positively charged $\text{Cu}_{2.46}\text{Co}_{0.54}(\text{HITP})_2$ MOF and negatively charged sDNA, Van der Waal force and coordination action between metal ions (Cu or Co) and amino group bearing on sDNA. As a result, the detection sensitivity toward the analyte can be significantly improved. After the blocking of the residual binding sites with MCH, the hybridization between sDNA and the Apt/ MnFe_2O_4 occurs considering their complementary bases. As a result, the highly selective recognition of FB1 can be realized via the competitive complementarity between FB1 and MnFe_2O_4 -labeled aptamer, resulting in the formation of the G-quadruplex. It thus causes the release of the Apt/ MnFe_2O_4 . Afterwards, DAB is then electrocatalytically oxidized to insoluble precipitate by the remnant MnFe_2O_4 on the modified electrode in the presence of H_2O_2 . Thus, the photocurrent response declines, showing a “signal-off” state. The amount of the MnFe_2O_4 precipitate decreases with the increase in the FB1 concentration because of the competitive reaction. This phenomenon leads to the recovery of the PEC signal, which is inversely proportional to the FB1 level in the system. As a result, the conversion of the original “signal-off” mode to the “signal-on” one can be realized. The “signal-inversion” mechanism can be achieved at the present PEC aptasensor for the sensitive analysis of FB1.

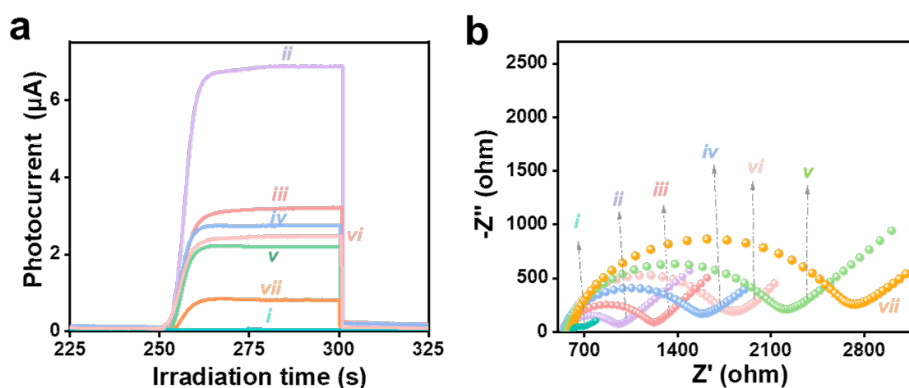


Fig. 5.3 (a) Photocurrent responses and (b) EIS Nyquist plots of each step of the construction procedure of the $\text{Cu}_{2.46}\text{Co}_{0.54}(\text{HITP})_2$ -based PEC aptasensor and the detection of FB1.

The manufacturing procedure of the $\text{Cu}_{2.46}\text{Co}_{0.54}(\text{HITP})_2$ -based aptasensor is determined by the PEC (**Fig. 5.3a**) and electrochemical techniques (**Fig. 5.3b**). $\text{Cu}_{2.46}\text{Co}_{0.54}(\text{HITP})_2/\text{GCE}$ (curve *ii*, Fig. 2a) exhibits the markedly enhanced PEC signal relative to that of the bare GCE (curve *i*) owing to the boosted photoelectric performance, with the photocurrent being 6.94 μA . This value is greatly higher than those of other reported PEC electrode materials, such as Cu-TCPP@MOFs^[108], CdTeQDs/AuNRs^[109], Cu-MOF@CuPc-TA-covalent-organic framework^[110],

CdS@PCN-224^[111], and Pdts/CdS/CS,^[112]. After the sDNA immobilization, the photocurrent response remarkably decreases to 3.21 μA (curve *iii*) due to the inhibition effect on the photoinduced electron transfer by the insulated sDNA. After blocking with MCH (curve *iv*) and connecting with Apt/MnFe₂O₄ (curve *v*), the photocurrent gradually decreases to 2.68 and 2.36 μA , separately, owing to the obstruction of photocarrier transfer. Subsequently, when detecting FB1 (5.0 ng mL⁻¹), the photocurrent of the modified electrode increases to 2.49 μA (curve *vi*) because of the release of the Apt/MnFe₂O₄ probe specifically bound with FB1, resulting in the dissociation of the partial sDNA/Apt double strand. Finally, the apparent decrease in the PEC response to 1.05 μA (curve *vii*) is obtained when concurrently incubating with the mixture of DAB and H₂O₂. This finding can be attributed to the steric hindrance caused by the in situ generated precipitates. Therefore, the whole procedure of the obtained PEC aptasensor can be clearly determined step by step according to the variation of the photocurrent.

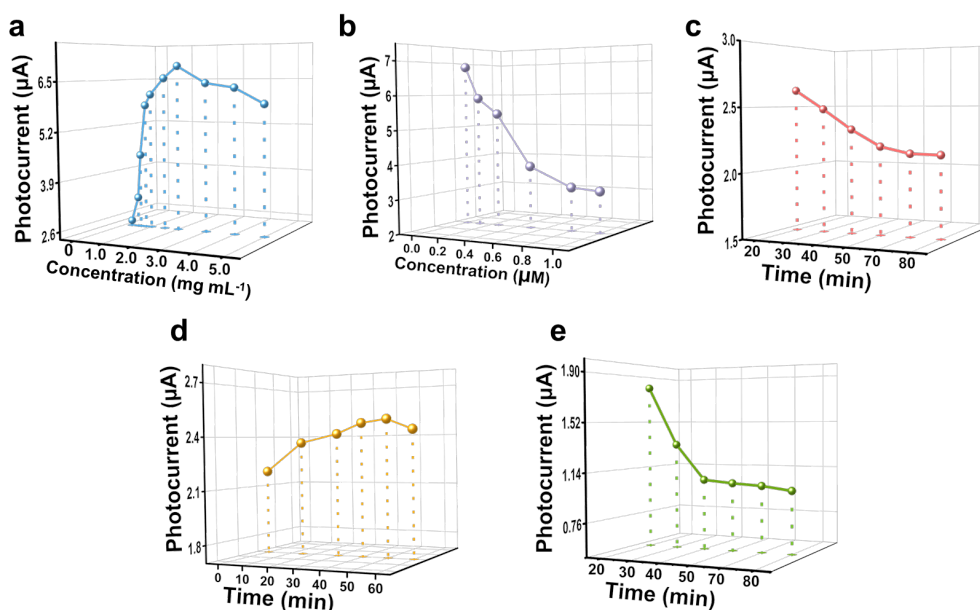


Fig. 5.4 Photocurrents of the optimized experimental conditions.

5.3.4 Quantitative analysis of FB1

To improve the analytical performance of the constructed aptasensor, some experimental conditions were optimized, including the concentration of $\text{Cu}_{2.46}\text{Co}_{0.54}(\text{HITP})_2$ suspension, the concentration of sDNA, the incubation time of Apt/MnFe₂O₄ suspension, the incubation time of FB1, and the catalytic precipitation time of H₂O₂/DAB solution. **Fig. 5.4a** shows the photocurrent of $\text{Cu}_{2.46}\text{Co}_{0.54}(\text{HITP})_2/\text{GCE}$ increases initially with increasing the $\text{Cu}_{2.46}\text{Co}_{0.54}(\text{HITP})_2$ concentration. When the $\text{Cu}_{2.46}\text{Co}_{0.54}(\text{HITP})_2$ suspension dosage exceeds 2.0 mg mL^{-1} ,

the photocurrent signal declines. The too thick MOF layer is apt to be removed from the electrode surface. Consequently, the dosage of 2.0 mg mL^{-1} is the best choice. Further, **Fig. 5.4b** indicates the photocurrent of the sDNA/Cu_{2.46}Co_{0.54}(HITP)₂/GCE decreases with the increase of the sDNA concentration. When the concentration of sDNA is larger than $0.8 \text{ }\mu\text{M}$, the observed photocurrent approaches a platform. As depicted in **Fig. 5.4c**, the photocurrent of the Apt/MnFe₂O₄/MCH/sDNA/Cu_{2.46}Co_{0.54}(HITP)₂/GCE decreases along with the binding time toward the Apt/MnFe₂O₄ probe, approaching to be stable after 70 min. When detecting FB1 (5.0 ng mL^{-1}), **Fig. 5.4d** hints the attained photocurrent of the remained MCH/sDNA/Cu_{2.46}Co_{0.54}(HITP)₂/GCE increases along with the binding time going on. After 40 min, the obtained photocurrent of the electrode tends to be stable. Moreover, **Fig. 5.4e** suggests that the photocurrent of the electrode declines by prolonging the incubation time in the mixed solution of DAB and H₂O₂, reaching equilibrium after 70 min. Based on these discussions, the optimized conditions are shown as follows: the dosage of Cu_{2.46}Co_{0.54}(HITP)₂ of 2.0 mg mL^{-1} , the concentration of sDNA of $0.8 \text{ }\mu\text{M}$, the incubation time of Apt/MnFe₂O₄ of 70 min, the binding time of FB1 of 40 min, and the reaction time of DAB and H₂O₂ of 70 min.

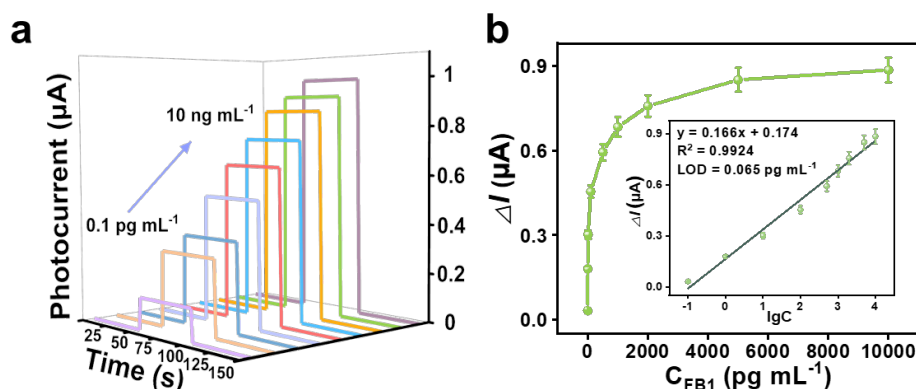


Fig. 5.5 (a) The quantitative analysis of different concentrations of FB1 (0.1 pg mL^{-1} - 10 ng mL^{-1}) by using the Cu_{2.46}Co_{0.54}(HITP)₂-based PEC aptasensor and (b) the variation of the photocurrent responses (ΔI) caused by the detection of different concentrations of FB1 using the developed PEC aptasensor (Inset: the linear fitting of the ΔI versus the $\text{LogCon}_{\text{FB1}}$).

Under these optimal experiment conditions, the quantitative analysis of FB1 using the Cu_{2.46}Co_{0.54}(HITP)₂-based PEC aptasensor was performed according to the concentration titration method. As depicted in **Fig. 5.5a**, the photocurrent increases with the increase in the FB1 concentration from 0.1 pg mL^{-1} to 10 ng mL^{-1} . Large amounts of Apt/MnFe₂O₄ were released from the electrode when adding FB1 at a high concentration, thereby reducing the catalytic reaction amount of DAB in the presence of H₂O₂. The variation in the photocurrent ($\Delta I = I_{\text{FB1}} - I_0$) is linear with the logarithm

of the FB1 concentration ($\text{LogCon}_{\text{FB1}}$), in which I_{FB1} and I_0 represent the presence or absence of FB1 in the analytical system, respectively.

Table 5.1 Comparison of the proposed $\text{Cu}_{2.46}\text{Co}_{0.54}(\text{HITP})_2$ -based PEC aptasensor with other reported biosensors for the detection of FB1.

Material	Detection Method	Linear range (ng mL^{-1})	LOD (pg mL^{-1})	Refs.
$\text{Cu}_2\text{MoS}_4/\text{CdS}/\text{In}_2\text{S}_3$	PEC	0.0001 – 100	0.072	[113]
GO/CdS	PEC	0.01 – 1000	4.7	[114]
$\text{Ru}@\text{SiO}_2$ NPs	ECL	0.001 – 100	0.35	[115]
TDN	Electrochemical	0.001 – 1000	0.087	[116]
AuNPs/GS/TH	Electrochemical	0.001 – 1000	1	[117]
cDNA/Exo I	Electrochemical	0.001 – 1000	0.15	[118]
TDN	Electrochemical	0.0005 – 0.5	0.479	[119]
Ag/ CeO_2	CV	0.01 – 100	1.5	[120]
PANI/MIPs	CV	0.001 – 500	0.32	[121]
$\text{Cu}_{2.46}\text{Co}_{0.54}(\text{HITP})_2$	PEC	0.0001 – 10	0.065	This work

The relationship is illustrated in **Fig. 5.5b**. The found ΔI sharply increases from 0.03 μA to 0.89 μA , thereby approaching an equilibrium when the FB1 concentration exceeds 10 ng mL^{-1} . A well-defined linear relationship can be obtained between the ΔI and the $\text{LogCon}_{\text{FB1}}$ (inset, **Fig. 5.5b**), represented by $\Delta I (\mu\text{A}) = 0.166\text{LogCon}_{\text{FB1}} + 0.174$ with the correlation coefficient of R^2 of 0.9924. Therefore, the LOD of the developed $\text{Cu}_{2.46}\text{Co}_{0.54}(\text{HITP})_2$ -based PEC aptasensor is calculated to be 65 fg mL^{-1} within a range of 0.1 pg mL^{-1} to 10 ng mL^{-1} . Compared with the state-of-the-art aptasensors for analyzing FB1 (**Table 5.1**), the constructed $\text{Cu}_{2.46}\text{Co}_{0.54}(\text{HITP})_2$ -based PEC aptasensor exhibits low LOD and wide detection range. This feature of the attained aptasensor can be attributed to the following factors: (i) the advantages of a highly porous network, large pore size, the residual amino group of HITP ligand, good conductivity, and tunable bandgap of $\text{Cu}_{2.46}\text{Co}_{0.54}(\text{HITP})_2$ facilitate sDNA immobilization and boost the photoelectronic conversion, thereby amplifying the PEC response and declining the background noise; (ii) MnFe_2O_4 derived from MnFe-Prussian blue analogous comprised mixed metal oxide species, rich oxygen vacancies, and lattice defects, thereby providing the outperformed electrocatalytic ability toward DAB with the aid of H_2O_2 ; (iii) integrating the competition sensing principle and the G-quadruplex formation between FB1 and aptamer can efficiently realize the signal conversion, greatly enhancing the selectivity and sensitivity of the developed aptasensor.

The selectivity of the given aptasensor was assessed as it is one of the essential factors, which allows the promising potential in practical applications. As illustrated in **Fig. 5.6a**, the analysis of different interferents even with the 100-fold concentration of FB1 only gives insignificant photocurrents, which is remarkably lower than that of FB1. The photocurrent for the detection of the mixture of interferents and FB1 is only 4.1% higher than that of the sole FB1. Thereby, the proposed aptasensor shows a high selectivity toward the analysis of FB1. Moreover, the reproducibility of the developed aptasensor was appraised by five of the same constructed aptasensors. **Fig. 5.6b** indicates the close photocurrent for the detection of FB1 with the concentrations of 0.01, 0.1, and 0.5 ng mL⁻¹, giving the low relative standard deviations (RSDs) of 3.2%, 3.9%, and 2.8%, respectively. These results hint at the excellent reproducibility of the developed aptasensor. The stability of the PEC biosensor was inspected by exposure to the on-off irradiation in PBS (0.01 M, pH 7.4) containing FB1 (10 ng mL⁻¹) by continuously recording for 11 runs within 1100 s (**Fig. 5.6c**). The obtained photocurrent for the detection of FB1 shows a low RSD of 3.68%, together with a slight decrease in the photocurrent of 9.2%. This finding reveals the good stability of the Cu_{2.46}Co_{0.54}(HITP)₂-based PEC aptasensor.

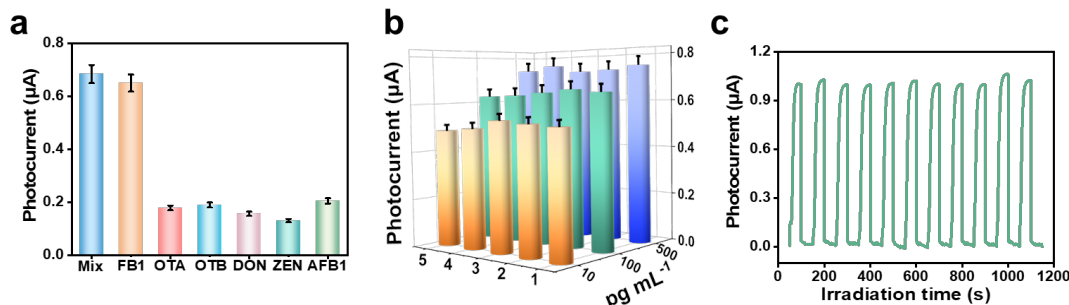


Fig. 5.6 (a) The selectivity of Cu_{2.46}Co_{0.54}(HITP)₂-based PEC aptasensor using OTA, OTB, DON, ZEN, and AFB1 as interferents (0.5 ng mL⁻¹). (b) The reproducibility of the developed PEC aptasensor for the inspection of different concentrations of FB1 (0.01, 0.1, and 0.5 ng mL⁻¹) and (c) the stability of the given aptasensor for the detection of FB1 (10 ng mL⁻¹).

5.3.5 Practicality of the Cu_{2.46}Co_{0.54}(HITP)₂-based PEC aptasensor

The applicability of the constructed PEC aptasensor was investigated using the standard addition method, for which corn and flour were utilized as real samples. As illustrated in **Tables 5.2** and **5.3**, the obtained recoveries range from 96.4 to 103.5%, and 98.7 to 108.0%, along with the low RSD of less than 4.3% and 5.5% for the detection of FB1 in the corn and flour samples, respectively. These results are also comparable with those detected by the ELISA method (**Tables 5.4** and **5.5**). The results of the Student's *t*-test in different concentrations (**Tables 5.2-5.5**) supported that no

significant systematic error existed in our analysis based on a comparison of the obtained $|t|$ value and $|t|_{\text{Critical},3} = 2.21$ in three replicate measurements. All results confirm that the proposed aptasensor displays excellent and acceptable practicability.

Table 5.2 Analytical results of FB1 containing corn using the developed $\text{Cu}_{2.46}\text{Co}_{0.54}(\text{HITP})_2$ -based aptasensor.

Added amounts	Detected amounts	Recovery (%)	RSD (%)	<i>t</i> -test
0.1	0.0987	98.7	4.3	0.53
1	1.03	103.0	3.2	1.57
10	10.1	101.0	4.1	0.42
100	96.4	96.4	2.7	2.31
1000	997	99.7	3.5	0.15
2000	2018	100.9	3.9	0.39
5000	5023	100.5	2.1	0.38
10000	10125	101.3	4.2	0.51

$n=3$ and the units were pg mL^{-1} , unless specified noted.

Table 5.3 Analytical results of FB1 containing flour using the developed $\text{Cu}_{2.46}\text{Co}_{0.54}(\text{HITP})_2$ -based aptasensor.

Added amounts	Detected amounts	Recovery (%)	RSD (%)	<i>t</i> -test
0.1	0.108	108.0	5.5	2.33
1	1.021	102.1	3.2	1.11
10	9.87	98.7	3.9	0.59
100	100.23	100.2	4.2	0.095
1000	986.5	98.7	2.3	1.03
2000	2010	100.5	4.0	0.22
5000	5035	100.7	4.7	0.26
10000	9987	99.9	3.5	0.06

Table 5.4 Comparative results of FB1 containing corn using the developed $\text{Cu}_{2.46}\text{Co}_{0.54}(\text{HITP})_2$ -based aptasensor and ELISA method.

Method	Added amounts	Detected amounts	Recovery (%)	RSD (%)	<i>t</i> -test
PEC	1000	997	99.7	3.5	0.15
	2000	2018	100.9	3.9	0.39
	5000	5023	100.5	2.1	0.38
	10000	10125	101.3	4.2	0.51
ELISA	1000	1022	102.2	3.12	1.20
	2000	1819	90.9	2.68	6.43
	5000	5.246	104.9	3.89	2.09
	10000	10173	101.7	4.07	0.72

Table 5.5 Comparative results of FB1 containing flour using the developed $\text{Cu}_{2.46}\text{Co}_{0.54}(\text{HITP})_2$ -based aptasensor and the ELISA assay.

Method	Added amounts	Detected amounts	Recovery (%)	RSD (%)	<i>t</i> -test
PEC	1000	986.5	98.7	2.3	1.03
	2000	2010	100.5	4.0	0.22
	5000	5035	100.7	4.7	0.26
	10000	9987	99.9	3.5	0.06
ELISA	1000	1161	116.1	4.21	5.70
	2000	1872	93.6	5.03	2.35
	5000	5221	104.4	3.43	2.14
	10000	9782	97.8	4.34	0.89

5.4 Conclusion

In summary, photoactive bimetallic semiconductive $\text{Cu}_{2.46}\text{Co}_{0.54}(\text{HITP})_2$ was employed as the electrode material for constructing a new “signal-inversion” PEC aptasensor to detect FB1 by coupling with MnFe_2O_4 nanoenzyme mediated EABPR. Given that $\text{Cu}_{2.46}\text{Co}_{0.54}(\text{HITP})_2$ exhibited large specific surface and pore size, high functionality, enhanced conductivity, narrowed bandgap, and promoted photoabsorption ability, the significantly strengthened PEC response and biosensing amplified effect were simultaneously achieved. Moreover, MnFe_2O_4 demonstrated superior electrocatalytic performance owing to active bimetal sites, rich oxygen vacancies, and lattice defects, thereby showing the efficient oxidation toward DAB in the presence of H_2O_2 . Compared with most of the reported FB1 biosensors, the developed $\text{Cu}_{2.46}\text{Co}_{0.54}(\text{HITP})_2$ -based PEC aptasensor provided a wide detection range from 0.1 pg mL^{-1} to 10 ng mL^{-1} and an ultralow LOD of 65 fg mL^{-1} . Moreover, the aptasensor displayed high selectivity, good reproducibility and stability, and outstanding practicality for the precise determination of FB1 in diverse real samples. This work can broaden the application of MOFs in the sensitivity analysis of mycotoxin in food products or foodstuffs.

Chapter VI: Donor-acceptor conjugated and triazine-containing covalent organic framework: Construction of a signal “on-off-on” electrochemiluminescence immunosensor for efficiently detecting zearalenone

6.1 Introduction

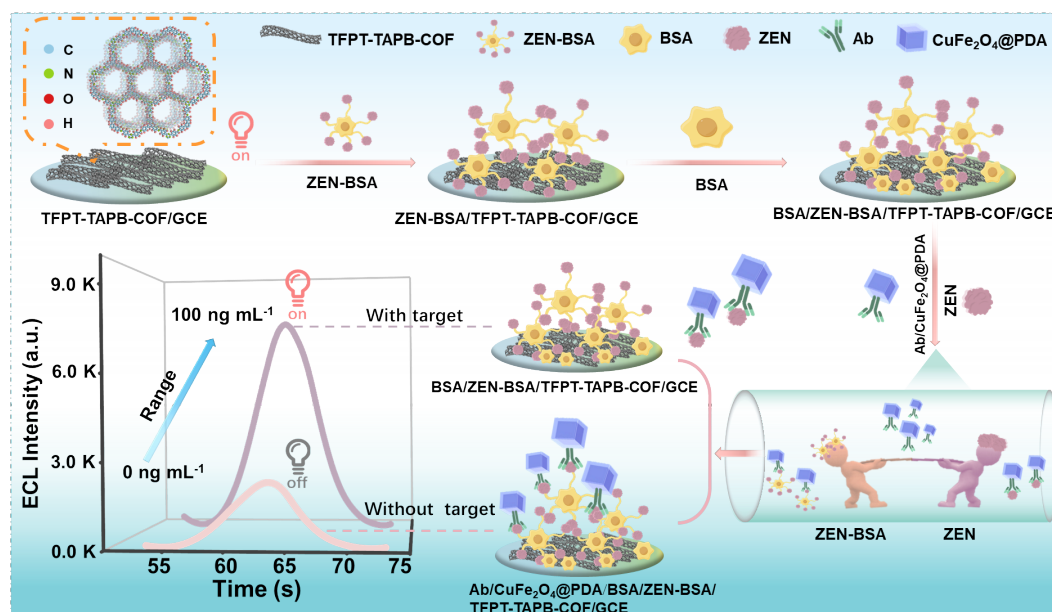
As a T-2 toxin, zearalenone (ZEN), which is metabolized by fungi, is often present in grains or food products. Eating food that contains a high level of ZEN can cause serious health issues. Accordingly, different countries and organizations have imposed strict regulations on the maximum content of ZEN in grains, such as $60\ \mu\text{g kg}^{-1}$ for wheat, corn, and flour set by China and $100\text{-}200\ \mu\text{g kg}^{-1}$ for unprocessed cereals set by the European Union. Therefore, determining ZEN with an ultralow level in food products is essential. In general, various signal modes, namely, “signal-off,” “signal-on,” or “on-off-on,” are utilized in constructing ECL biosensors. Particularly, compared with the two other modes, the signal “on-off-on”-based ECL biosensors can minimize background signal interference and avoid false positive results. About the inspection of small molecule contaminants, such as mycotoxins, the competitive immunoassay format is generally preferred and more reliable because small molecule contaminants typically make the performance of typical sandwich assays difficult. However, quenching efficiency is unsatisfactory given the small size of mycotoxins. Increasing the output signal with an increase in analyte level can enhance detection sensitivity. For the superior signal “on-off-on” model-based ECL biosensor, two requirements, namely, excellent quenchers and emitters, should be met. A signal-on ECL biosensor can be constructed through a highly efficient quencher in a competitive format. Although several quenchers, such as ferrocene and its derivatives, methylene blue, H_2O_2 , and polydopamine (PDA), have been employed in the fabrication of ECL biosensors, the single quenching strategy is not efficient for blocking ECL response, producing an unsatisfactory result. Therefore, the multimodal quenching method combined with different quenchers is regarded as a promising alternative for further amplifying ECL signals. Among the aforementioned quenchers, PDA that contains benzoquinone or catechol units can be used as a free radical scavenger and quencher of ECL signals and an energy transfer acceptor. Moreover, CuFe_2O_4 spinel can generate Cu^{2+} to quench ECL signals via electron transfer as carriers of PDA. In particular, the electronegativity of PDA can be weakened by CuFe_2O_4 , facilitating the capture and scavenging of

electrogenerated radical anions. Accordingly, the trimodal quenching of resonance energy transfer (RET), electron transfer, and radical scavenging can achieve the ultrasensitive and selective detection of ZEN in food products when using the ECL technique with the CuFe₂O₄@PDA hybrid as the quencher. However, this technique has not been reported to date.

The other key for the manufacture of an excellent signal “on-off-on”-based ECL biosensor is searching for an efficient emitter. Given their features of having large specific surface areas, periodic cylindrical π -arrays, and inherent pores, COFs have demonstrated extensive applications in the biosensing field. COF-based bioplatfroms frequently exhibit boosted bioaffinity toward a variety of probes [e.g., aptamers, antibodies (Abs), or DNA] via π - π stacking, van der Waals force, or covalent binding interaction in consideration of their high functionality, regular skeletons, and excellent optical/electronic performance. Therefore, integrating COFs with different analysis methods, such as antigen-Ab immunoassay techniques, can realize the sensitive detection of analytes. COFs have also demonstrated extensive applications in the analysis of mycotoxins, such as aflatoxins (AFs) ^[122], norfloxacin ^[123], cholesterol, and T4 polynucleotide kinase, through chromatography, optical spectrum, fluorescence, or EC due to the strong host-guest interaction. Furthermore, various COFs have been utilized in the construction of ECL biosensors because they can enhance the excitation of internal and external ECL luminophores and exhibit high immobilization ability toward luminophores. However, COFs are difficult to use directly as emitters because most common imine-lined COFs only produce nonradiative transition. The aggregation-caused quenching effect considering π - π stacking between different layers can also result in the decrease/non-emission of COFs. Considerable effort, such as the synthesis of imine-linked COFs, donor and acceptor (D-A) COFs, or sp²-conjugated COFs, has been focused on this issue. In particular, rendering the conjugated joint in COFs with a fully π -conjugated network across a 2D COF lattice can promote photochemical reactions and electron delocalization. Consequently, manufacturing ECL biosensors by using COFs as emitters tends to be a promising alternative for the sensitive and accurate detection of ZEN.

In the current work, a newly designed reversal signal “on-off-on” ECL immunosensor was developed using a triazine-based COF synthesized with 2,4,6-tris(4-formyl-phenyl)-1,3,5-triazine (TFPT) and 1,3,5-tris(4-aminophenyl) benzene

(TAPB) as building units (denoted as TFPT-TAPB-COF) as emitter for the sensitive and selective detection of ZEN based on the trimodal quenching effect and a competitive immunosensing strategy (**Scheme 3.1**). The systematic characterization revealed that TFPT-TAPB-COF exhibited an intramolecular donor-acceptor (D-A) structure, offering a tunable local charge distribution and high charge transfer efficiency, and thus, demonstrating an enhanced ECL emission ability in the presence of $K_2S_2O_8$. By combining the trimodal quenching mechanism caused by the $CuFe_2O_4@PDA$ complex and the competitive immunosensing strategy, output signal reversion with an increase in ZEN level was realized at the constructed TFPT-TAPB-COF-based ECL immunosensor. The presented ECL biosensor exhibited an ultralow detection limit of 7.9 fg mL^{-1} within a wide range of 10 fg mL^{-1} to 100 ng mL^{-1} . This work not only provides deep insight into the construction mechanism of a triazine-containing COF-based ECL biosensor but also proposes a new ECL immunosensing approach for the efficient analysis of mycotoxins.



Scheme 6.1 Schematic illustration of the constructed reversal signal “on-off-on” ECL immunosensor based on TFPT-TAPB-COF with the aid of the trimodal strategy for the ZEN detection.

6.2 Experimental section

6.2.1 Reagents and materials

Ferric chloride hexahydrate, copper sulfide pentahydrate, polyvinylpyrrolidone, and sodium chloride were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). 1,1-Diphenyl-2-picrylhydrazyl radical (DPPH), $K_2S_2O_8$, bovine

serum albumin (BSA, 96-99%), and DA were purchased from Shanghai McLean Biochemical Co. Further, N, N',-dimethylformamide, tetrahydrofuran, dimethyl sulfoxide, N, N'-dimethylacetamide, TFPT, TAPB were purchased from Aladdin Chemical Reagent Co. ZEN, ZEN-bovine serum albumin (ZEN-BSA), Antibody (Ab), fumonisin B1 (FB1), ochratoxin A (OTA) and aflatoxin B1 (AFB1), aflatoxin-M1 (AFM1), glutamic acid (Glu E), glycine (Gly), and enzyme-linked immunosorbent assay (ELISA), were obtained from Sangon Biotechnology Co. All chemicals and reagents used throughout the experiments were of analytical reagent grade. All solutions were prepared with Milli-Q water ($\geq 18.2 \text{ M}\Omega \text{ cm}$).

6.2.2 Synthesis of TFPT-TAPB-COF

TFPT-TAPB-COF was synthesized via the condensation reaction through the hydrothermal method. Typically, 19.70 mg TFPT (0.05 mmol) and 17.60 mg TAPB (0.05 mmol) were dispersed in 2 mL of the mixed solvent of 1,4-dioxane and mesitylene (v:v = 3:2) under continuous stirring for 20 min until forming the homogeneous suspension. The mixed suspension was placed into a 10 mL Pyrex tube, followed by adding 0.2 mL of acetic acid (6.0 M) as the catalyst. After being treated ultrasonically until generating the fluffy solid, the resultant was rapidly frozen at 77 K (liquid N₂ bath) and degassed through three freeze-thaw cycles. Subsequently, the tube was sealed and heated at 120 °C for 3 days. After cooling to room temperature, the mixture was filtered and washed several times with acetone to remove unreacted monomers. Finally, the result was dried in an oven at 80 °C. The collected product was then purified by tetrahydrofuran (THF) Soxhlet extraction for 1 day, obtaining the yellow powder (26.94 mg, 76% yield).

6.2.3 Fabrication of the ECL immunosensor based on TFPT-TAPB-COF

Firstly, 10 mg TFPT-TAPB-COF was dispersed in Milli-Q water (10 mL) under continuous stirring until uniform suspension (1.0 mg mL^{-1}) was obtained. Secondly, 10 μL of the obtained TFPT-TAPB-COF suspension (1.0 mg mL^{-1}) was coated onto the pre-treated glassy carbon electrode (GCE), followed by drying in air at room temperature (represented as TFPT-TAPB-COF/GCE). Then, the TFPT-TAPB-COF/GCE was incubated in the ZEN-BSA solution (100 μL , $7 \text{ }\mu\text{g mL}^{-1}$) overnight at 4 °C, followed by rinsed with phosphate-buffered saline (PBS; 0.01 M, pH 7.4) thrice to remove the unabsorbed ZEN complex and dried again (denoted as ZEN-BSA/TFPT-

TAPB-COF/GCE). Thirdly, the ZEN-BSA/TFPT-TAPB-COF/GCE was immersed in the BSA solution (100 μL , 1.0 wt%) to block the unbound sites for 1 h, followed by washing with PBS (0.01 M, pH 7.4) thrice and dried again. The resulting BSA/ZEN-BSA/TFPT-TAPB-COF/GCE was then stored at 4 $^{\circ}\text{C}$ for further use.

6.2.4 Quantitative detection of ZEN using the TFPT-TAPB-COF-based ECL immunosensor

To attain the optimal immunosensing performance of the TFPT-TAPB-COF-based ECL immunosensor, some critical experimental parameters were optimized, including the dosage of TFPT-TAPB-COF, the concentration of $\text{CuFe}_2\text{O}_4@\text{PDA}$, the binding time of ZEN with Ab/ $\text{CuFe}_2\text{O}_4@\text{PDA}$, and the concentration of ZEN-BSA. Under the optimized conditions, the quantitative determination of ZEN using the constructed ECL immunosensor was performed by separately detecting ZEN with different concentrations (0.01, 0.1, 1.0, 10, 100, 500, 5×10^3 , 5×10^4 , and 1×10^5 pg mL^{-1}), which was mixed with the Ab/ $\text{CuFe}_2\text{O}_4@\text{PDA}$ quencher system. According to the criterion recommended by IUPAC, the limit of detection (LOD) of ZEN was appraised according to the $3S_b/m$ equation, where S_b and m are the standard deviation of the blank ($n = 11$) and the slope of the linear calibration curve, respectively. In addition, the selectivity of the developed ECL immunosensor was analyzed by detecting diverse interferents, including FB1, OTA, AFB1, AFM1, Glu E, and Gly with 100-fold concentration higher than that of ZEN (50 ng mL^{-1}). The reproducibility of the given ECL immunosensor was achieved by detecting ZEN with diverse concentrations (10 pg mL^{-1} , 0.1 ng mL^{-1} , and 0.5 ng mL^{-1}) using five same electrodes. The stability of the obtained ECL immunosensor was probed by performing 15 consecutive scanning cycles and continuously detecting ZEN using the same immunosensor for 15 days and measured once per day.

6.3 Results and discussion

6.3.1 Immunosensing mechanism of the reversal signal “on-off-on” ECL biosensor

As illustrated in **Scheme 6.1**, the synthesized D-A conjugated TFPT-TAPB-COF demonstrates improved ECL emission performance and achieves the initial “on” state due to its structural features (e.g., extended π -conjugated skeletons, D-A conjugated

structure, and high intramolecular charge transfer) in the presence of $K_2S_2O_8$. Meanwhile, TFPT-TAPB-COF can absorb large amounts of ZEN conjugated with BSA via hydrophobic interaction, hydrogen bond, van der Waals force, or electrostatic effect. The ECL emission efficiency of the TFPT-TAPB-COF-modified electrode declines because of the obstruction of electron transfer. Subsequently, when the TFPT-TAPB-COF-based ECL immunosensor is incubated with the mixture solution of ZEN and the Ab/CuFe₂O₄@PDA complex, immunosensing competitiveness occurs between ZEN and ZEN-BSA anchored on TFPT-TAPB-COF. Particularly, the maximum amount of the Ab/CuFe₂O₄@PDA complex binds with the immobilized BSA-ZEN conjugates on the surface of TFPT-TAPB-COF/GCE without target ZEN. Then, the ECL response of the electrode is effectively quenched by the CuFe₂O₄@PDA complex through the triple-quenching mechanism of energy resonance transfer, electron transfer, and electrogenerated radical scavenging, resulting in a minimum ECL signal. Consequently, the signal is in an “off” state. As ZEN concentration increases, competitive immunoreaction reduces the amount of immunoprobes available to quench the luminescent electrode, turning the signal “on”. Finally, the signal “on-off-on”-based ECL immunosensor that uses CuFe₂O₄@PDA as a trimodal quencher and triazine-COF as an emitter can be employed to detect ZEN quantitatively.

6.3.2 Basic characterization of TFPT-TAPB-COF

TFPT-TAPB-COF obtained by reacting TFPT with TAPB through the solvothermal method (**Fig. 6.1a**) is composed of rich triazine moieties and π -electrons, benefiting the improvement of optoelectronic performance. The transmission electron microscopy (TEM) image (**Fig. 6.1b**) confirms this result. However, these nanotubes are aggregated with nanoflakes, indicating partial exfoliation and a layered structure. Such a structure benefits the enhancement of the ECL emission ability of TFPT-TAPB-COF. Two lattice spacings of 1.75 nm and 1.72 nm are observed in the high-resolution TEM (HR-TEM) image of TFPT-TAPB-COF (**Fig. 6.1c**) due to the (100) plane. The powder X-ray diffraction (PXRD) pattern of TFPT-TAPB-COF (**Fig. 6.1d**) indicates diffraction peaks at $2\theta = 4.32^\circ$ and 6.02° , which are attributed to the (100) and (110) crystal planes, respectively. The sharp and intense diffraction peak at $2\theta = 4.32^\circ$ hints at the high crystallinity of the fully conjugated TFPT-TAPB-COF. The PXRD pattern of TFPT-TAPB-COF aligns well with the simulated masked AA superposition model (**Fig. 6.1e**). The TFPT-TAPB-COF structure was analyzed by Pawley refinement,

showing the unit cell parameters of $a = 26.135 \text{ \AA}$, $b = 26.128 \text{ \AA}$, and $c = 3.773 \text{ \AA}$; $\alpha = \beta = 90^\circ$ and $\gamma = 119.961^\circ$, with factors of $R_p = 7.40\%$ and $R_{wp} = 9.39\%$. The C 1s X-ray photoelectron spectroscopy (XPS) spectrum of TFPT-TAPB-COF (**Fig. 6.1f**) incorporates six groups: C=C (284.2 eV), C-C (284.8 eV), C-N (285.5 eV), C-O (286.9 eV), N-C=O (289.3 eV), and $\pi-\pi^*$ (291.7 eV). The coexistence of C=C and $\pi-\pi^*$ clarifies the highly conjugated structure of TFPT-TAPB-COF, boosting electron transfer and delocalization. Pyridinic N (398.9 eV) and pyrrolic N (399.7 eV) are involved in the N 1s XPS spectrum (**Fig. 6.1g**), along with a weak group of NO_x (402.9 eV).

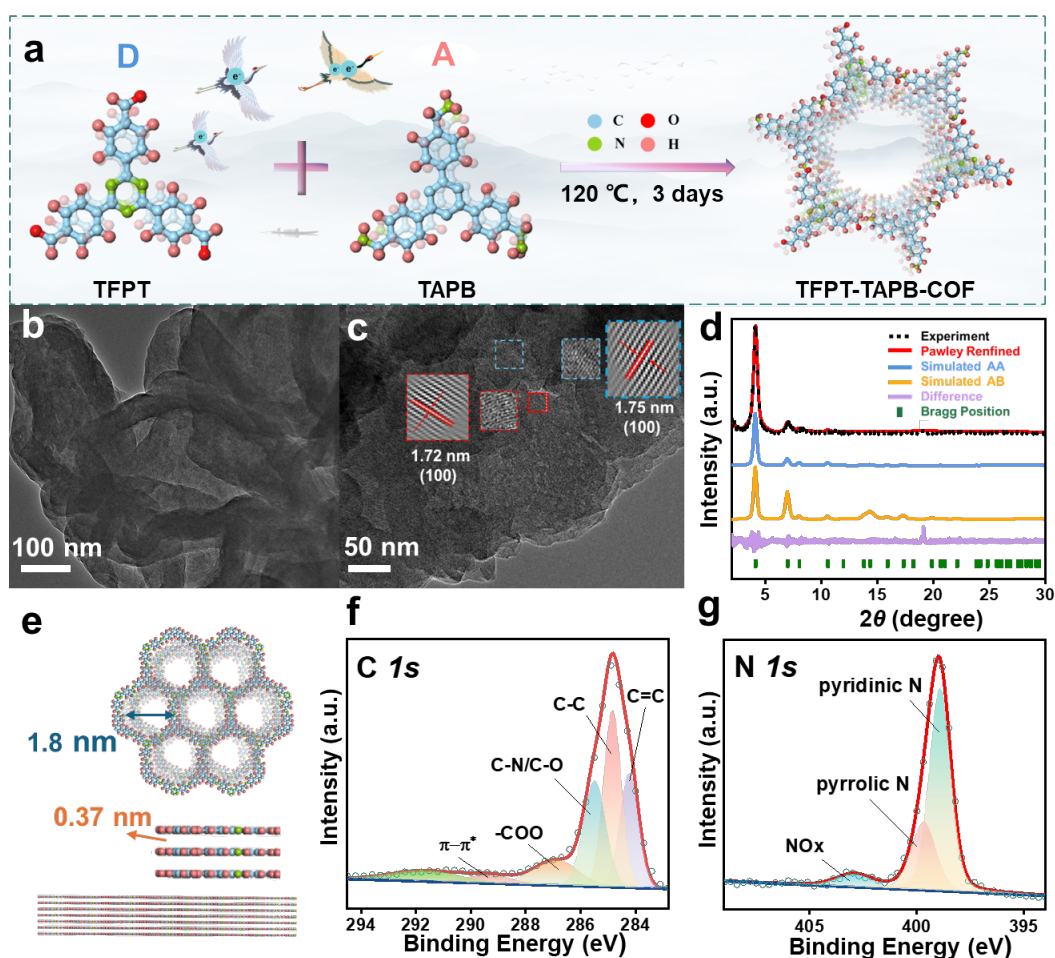


Fig. 6.1 (a) Schematic illustration of the synthesis procedure of TFPT-TAPB-COF. (b) TEM, (c) HR-TEM images, (d) the PXRD pattern and (e) eclipsed stacking of TFPT-TAPB-COF. The high-resolution (f) C 1s and (g) N 1s XPS spectra of TFPT-TAPB-COF.

6.3.3 Electron structure and electrochemical properties of TFPT-TAPB-COF

To verify the construction principle of the D-A system of TFPT-TAPB-COF, the molecular geometries of the ground and excited states were obtained using the density functional theory calculation at the B3LYP/6-31G (d) level. The molecular orbital

model of electrons was analyzed with Multiwfn software [124].

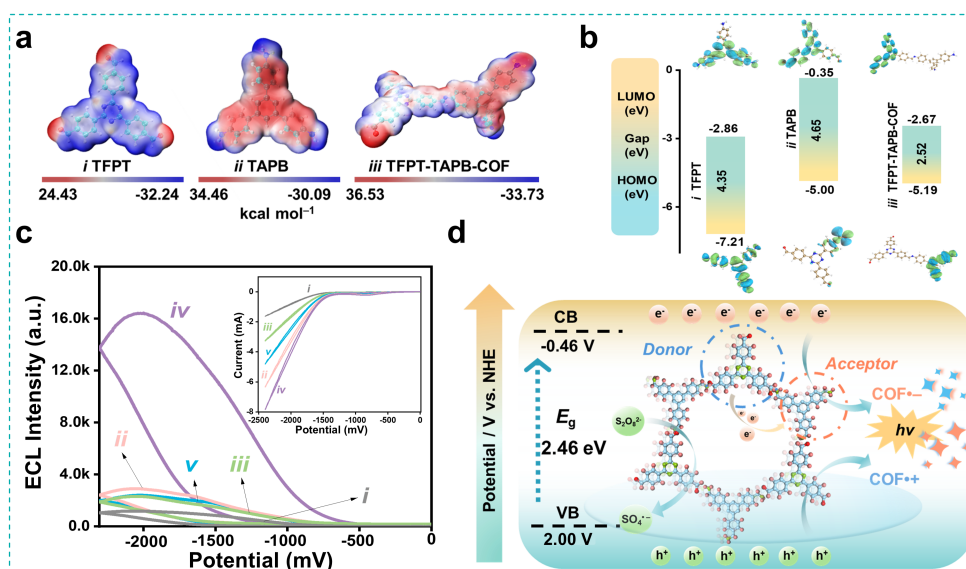


Fig. 6.2 (a) ESP distributions, (b) the values of HOMOs, LUMOs, and calculated energy gaps of (i) TFPT, (ii) TAPB, and (iii) TFPT-TAPB-COF. (c) The ECL potential-intensity curve (inset: corresponding cyclic voltammograms) of (i) the bare GCE, (ii) TFPT/GCE, (iii) TAPB/GCE, (iv) TFPT-TAPB-COF/GCE and (v) TFPT/TAPB/GCE. (d) ECL emission mechanism of TFPT-TAPB-COF micro-reaction chambers. The buffer solution of ECL experiments was PBS (0.1 M, pH 7.4) with 10 mM K₂S₂O₈ between 0 V and -2.4 V; PMT: 700 V, potential scan rate: 100 mV s⁻¹. The procedure was used throughout all ECL detection processes unless otherwise stated.

As shown in **Fig. 6.2a**, intermolecular interaction can be analyzed theoretically through the molecular electrostatic potential (ESP) distribution patterns. ESP distribution reflects the uneven feature of charge density distribution. The blue and red areas show the sites that are most electron-rich and most electron-poor, respectively. For TFPT, more negative charges exist on the molecular surface than positive ones. In the case of TAPB, however, a large region of positive charges is presented on the surface. The positive region of the electrostatic potential on one molecular surface tends to interact with the negative region of another molecule, facilitating electron transfer between TFPT-TAPB-COF molecules.

As depicted in **Fig. 6.2b**, the highest occupied molecular orbital is located on the main chain of the TFPT subunit as an electron-donor unit, while the lowest unoccupied molecular orbital is concentrated on the TAPB subunit as an acceptor (**Fig. 6.2b**). Furthermore, the energy gap of TFPT-TAPB-COF is calculated to be 2.52 eV, which is lower than those of TFPT (4.35 eV) and TAPB (4.65 eV). The finding indicates that the D-A structure of COF narrows the bandgap, leading to more electrons and holes being excited. Charge is believed to transfer from TFPT to TAPB within the D-A structure of TFPT-TAPB-COF.

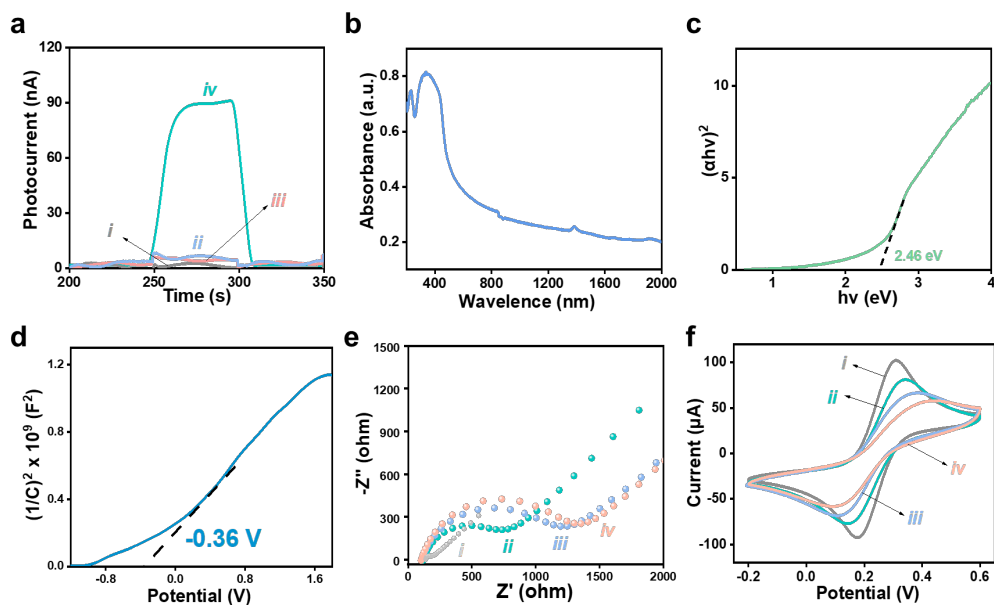


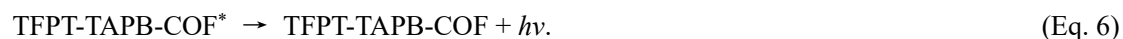
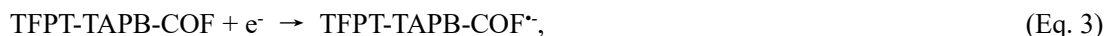
Fig. 6.3 (a) Photocurrent responses of (i) the bare GCE, (ii) TFPT/GCE, (iii) TAPB/GCE, and (iv) TFPT-TAPB-COF/GCE. (b) UV-vis diffuse reflectance spectrum, (c) band gap, and (d) Mott-Schottky plot of TFPT-TAPB-COF. The PEC experiments were carried out in PBS (0.1 M; pH 7.4) buffer solution. (e) EIS Nyquist plots and (f) Cyclic voltammograms of (i) the bare GCE, (ii) TFPT-TAPB-COF/GCE, (iii) TFPT/GCE and (iv) TAPB/GCE at the scan rate of 100 mV s⁻¹ in PBS (0.01 M, pH 7.4) contained 5 mM [Fe(CN)₆]^{3-/4-}.

Electrochemical measurements reveal TFPT-TAPB-COF exhibits fast electron transfer ability compared with building blocks (**Fig. 6.3**). The PEC results (**Fig. 6.3a**) indicate that the bare GCE (curve *i*) does not show any photocurrent, implying the absence of photoelectric conversion performance. By contrast, TFPT-TAPB-COF/GCE exhibits an obvious photocurrent response of 120.00 nA, while the monomer-modified electrodes show an inapparent response. TFPT-TAPB-COF demonstrates an effective separation ability for the photoinduced electron and holes because of its narrow band gap and D-A conjugated structure, which will be discussed next. According to the Mott-Schottky spectrum of TFPT-TAPB-COF (**Fig. 6.3b**), TFPT-TAPB-COF has a narrow band gap of 2.46 eV (**Fig. 6.3c**) and a negative slope (-0.36 eV) can be obtained (**Fig. 6.3d**), suggesting its n-type semiconductive feature. From the Kubelka-Munk transformed reflectance spectrum. Thereto, the D-A conjugated structure generated between TFPT (D) and TAPB (A) can remarkably enhance the light trapping ability, accelerating the separation of holes and photoinduced electrons in the donor and acceptor units, respectively. As depicted in **Fig. 6.3e**, the EIS Nyquist plot of TFPT-TAPB-COF/GCE comprises two parts: a semicircle and a straight line with an oblique angle of 45°. According to the analog circuit, the R_{ct} of TFPT-TAPB-COF/GCE (curve *ii*) is around 0.37 k Ω , larger than that of the bare GCE (curve *i*, 78.50 Ω) but smaller than those of TFPT/GCE (curve *iii*, 0.51 k Ω) and TAPB/GCE (curve *iv*, 0.53 k Ω). These

findings unveil that electron transfer is inhibited by TFPT-TAPB-COF because of its relatively inferior conductivity. Compared with the monomers of TFPT and TAPB, however, electron transfer is accelerated by TFPT-TAPB-COF, which is due to the generation of a D-A conjugated structure. The cyclic voltammograms of all samples (**Fig. 6.3f**) show consistent results, i.e., TFPT-TAPB-COF/GCE exhibits a larger peak current than the monomers.

The ECL response of TFPT-TAPB-COF/GCE (curve *iv*) is markedly high (16544 a.u.), triggered at -0.8 V and peaked at -2.0 V (**Fig. 2c**). However, no distinct ECL signal is found for the bare GCE (curve *i*), the monomer-modified GCE (curves *ii* and *iii*), and the GCE modified with mixed monomers at a mole ratio of 1:1 (curve *v*). This result indicates that the intraarticular charge transfer formed within the monomers can effectively activate the ECL signals of TFPT-TAPB-COF. The optimal conditions for obtaining the maximum ECL response of TFPT-TAPB-COF/GCE are recorded at a K₂S₂O₈ concentration of 10 mM between 0 V and -2.4 V, with PBS (0.1 M, pH 7.4), at a scan rate of 100 mV s⁻¹.

Based on the preceding discussion, the ECL generation mechanism of TFPT-TAPB-COF is proposed as follows:



During cathodic scanning, the apparent reduction peak at a potential of -0.83 V corresponds to the electrolytic reduction of S₂O₈²⁻, producing the strong oxidative intermediate radical SO₄^{·-} (Eq. 1). Subsequently, SO₄^{·-} is injected into the valence band molecular orbital of TFPT-TAPB-COF, yielding the cationic radical COF^{·+} (Eq. 2). Meanwhile, the anion radical TFPT-TAPB-COF^{·-} (Eq. 3) can be formed by injecting electrons into the conduction band of TFPT-TAPB-COF. Thereafter, electron transfer between COF^{·+} and COF^{·-} produces the excited state COF*, while COF^{·-} is electrically reduced to more COF* by the strongly oxidizing intermediate SO₄^{·-}. When the excited state returns to the ground state, a strong ECL signal can be produced at the TFPT-TAPB-COF electrode. For a deep insight into the working mechanism of the D-A system of TFPT-TAPB-COF on ECL emission, a building block contained in TFPT-

TAPB-COF is regarded as the model. After excitation, the holes and electrons concentrate and effectively separate in the donor and acceptor portions, respectively, effectively activating the ECL signal of TFPT-TAPB-COF (**Fig. 2d**).

6.3.4 Construction of the TFPT-TAPB-COF-based ECL immunosensor

UV-vis adsorption spectrum, photoluminescence (PL) emission spectrum, and free radical capture experiments were performed to probe the quenching mechanism of CuFe₂O₄@PDA toward TFPT-TAPB-COF/GCE. **Fig. 6.4a** indicates that the normalized ECL emission spectrum of TFPT-TAPB-COF demonstrates a maximum emission at around 575 nm. The UV-vis absorption spectrum of CuFe₂O₄@PDA illustrates a high photoabsorption within the UV-vis light range, which can be well matched with the ECL emission spectrum of TFPT-TAPB-COF. Distinctly, RET can occur between the energy donor of TFPT-TAPB-COF and the energy acceptor of CuFe₂O₄@PDA.

Generally, Cu²⁺ ions released from CuFe₂O₄ can trap electrons that escape from the excited state of the COF emission via electron transport, showing a quenching ability toward the ECL emitter. **Fig. 6.4b** shows the efficient release of Cu²⁺ ions from CuFe₂O₄@PDA, reaching 5.22% at 48 h. This effect plays a certain role in the quenching of the ECL of TFPT-TAPB-COF. In addition, the scavenging ability of CuFe₂O₄@PDA toward free radicals caused by TFPT-TAPB-COF was probed using a DPPH immunoprobe (**Fig. 6.4c**), CuFe₂O₄@PDA shows a scavenging efficiency of 72.38%, remarkably higher than those of PDA (65.01%) and CuFe₂O₄ (29.85%). This finding verifies the high quenching efficiency of CuFe₂O₄@PDA toward the ECL signal of TFPT-TAPB-COF. As illustrated in the inset of **Fig. 6.4c**, PDA has high electronegativity (-19.79 mV), resulting in strong electrostatic repulsion to SO₄^{•-}. The CuFe₂O₄@PDA complex shows lower electronegativity (-14.60 mV) than CuFe₂O₄. It can provide a strong driving force for adsorbing free radicals, facilitating the trapping and removal of SO₄^{•-}. Thereto, the combination of CuFe₂O₄ and PDA can greatly promote the quenching effect on the emission ability of TFPT-TAPB-COF. As shown in **Fig. 6.4d**, TFPT-TAPB-COF shows an obvious photoluminescence intensity (curve *i*). After the mixture with CuFe₂O₄@PDA, however, the photoluminescence intensity of TFPT-TAPB-COF sharply decreases (curve *ii*). That is, the photoluminescence emission of TFPT-TAPB-COF is markedly hampered by the CuFe₂O₄@PDA complex. To better investigate the influence of CuFe₂O₄@PDA and CuFe₂O₄ as quenching probes,

the ECL spectra were provided for the construction of the TFPT-TAPB-COF-based ECL immunosensor using CuFe_2O_4 and $\text{CuFe}_2\text{O}_4@\text{PDA}$ as quenching probes, respectively. As shown in **Fig. 6.4e**, the initial ECL intensity of the COF/GCE was 16000 a.u. (curve *ii*). Upon the sequential addition of ZEN-BSA (curve *iii*, 12567 a.u.) and BSA (curve *iv*, 10385 a.u.), the ECL signal gradually decreased. When CuFe_2O_4 and $\text{CuFe}_2\text{O}_4@\text{PDA}$ were used as immunoassay probes, the ECL signal further decreased to 7482 a.u. (curve *v*) and 3101 a.u. (curve *vi*), respectively. Notably, at this stage, the ZEN concentration was 0, enabling the probes to completely bind to the BSA/ZEN-BSA/TFPT-TAPB-COF/GCE surface. Therefore, $\text{CuFe}_2\text{O}_4@\text{PDA}$ exhibits a significant ECL quenching effect.

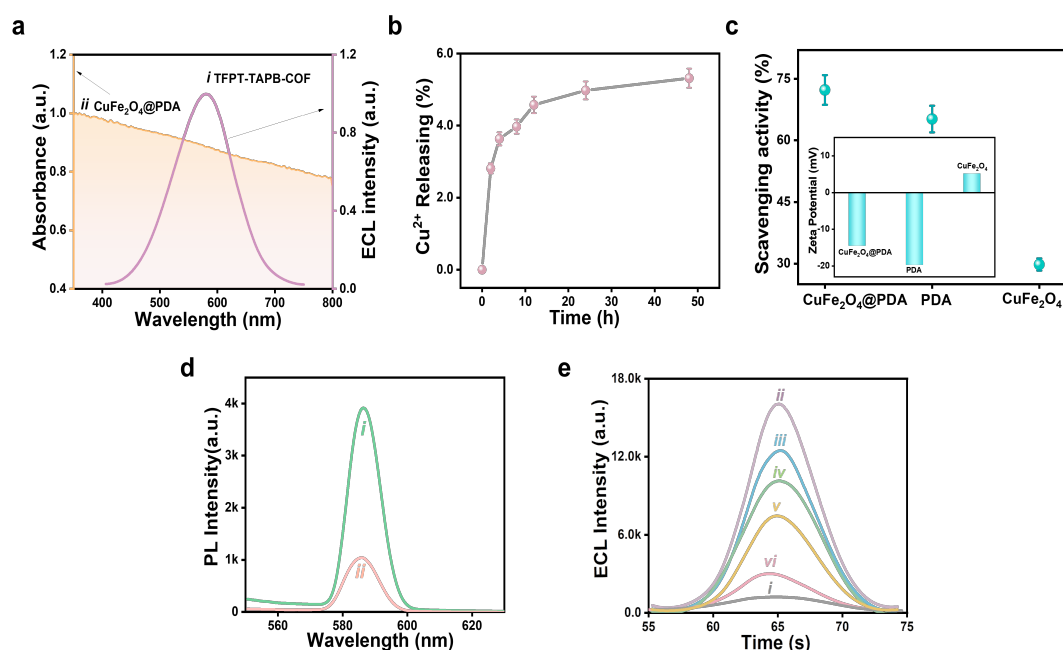
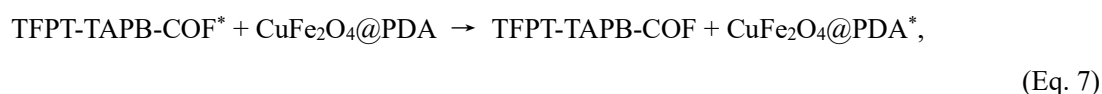
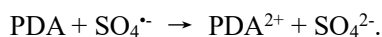


Fig. 6.4 (a) The normalized (*i*) ECL emission spectrum of TFPT-TAPB-COF and (*ii*) the UV-vis spectrum of $\text{CuFe}_2\text{O}_4@\text{PDA}$. (b) The amount of Cu^{2+} released from $\text{CuFe}_2\text{O}_4@\text{PDA}$. (c) 1,1-Diphenyl-2-picrylhydrazyl radical (DPPH) radical scavenging activity of $\text{CuFe}_2\text{O}_4@\text{PDA}$, PDA and CuFe_2O_4 (Inset: zeta potentials of $\text{CuFe}_2\text{O}_4@\text{PDA}$, PDA and CuFe_2O_4). (d) The PL intensity of TFPT-TAPB-COF and $\text{CuFe}_2\text{O}_4@\text{PDA}/\text{TFPT-TAPB-COF}$. (e) ECL responses of the construction of the TFPT-TAPB-COF-based ECL immunosensor, including (*i*) the bare GCE, (*ii*) TFPT-TAPB-COF/GCE, (*iii*) ZEN-BSA/TFPT-TAPB-COF/GCE, (*iv*) BSA/ZEN-BSA/TFPT-TAPB-COF/GCE, (*v*) Ab/ CuFe_2O_4 /BSA/ZEN-BSA/TFPT-TAPB-COF/GCE, and (*vi*) Ab/ $\text{CuFe}_2\text{O}_4@\text{PDA}$ /BSA/ZEN-BSA/TFPT-TAPB-COF/GCE. Error bars represent the standard deviations of three replicates.

Based on the analysis of the influence factors on the ECL response of TFPT-TAPB-COF, the quenching mechanism of $\text{CuFe}_2\text{O}_4@\text{PDA}$ toward the TFPT-TAPB-COF/GCE is provided in **Fig. 6.5a**, which is shown as follows:





(Eq. 9)

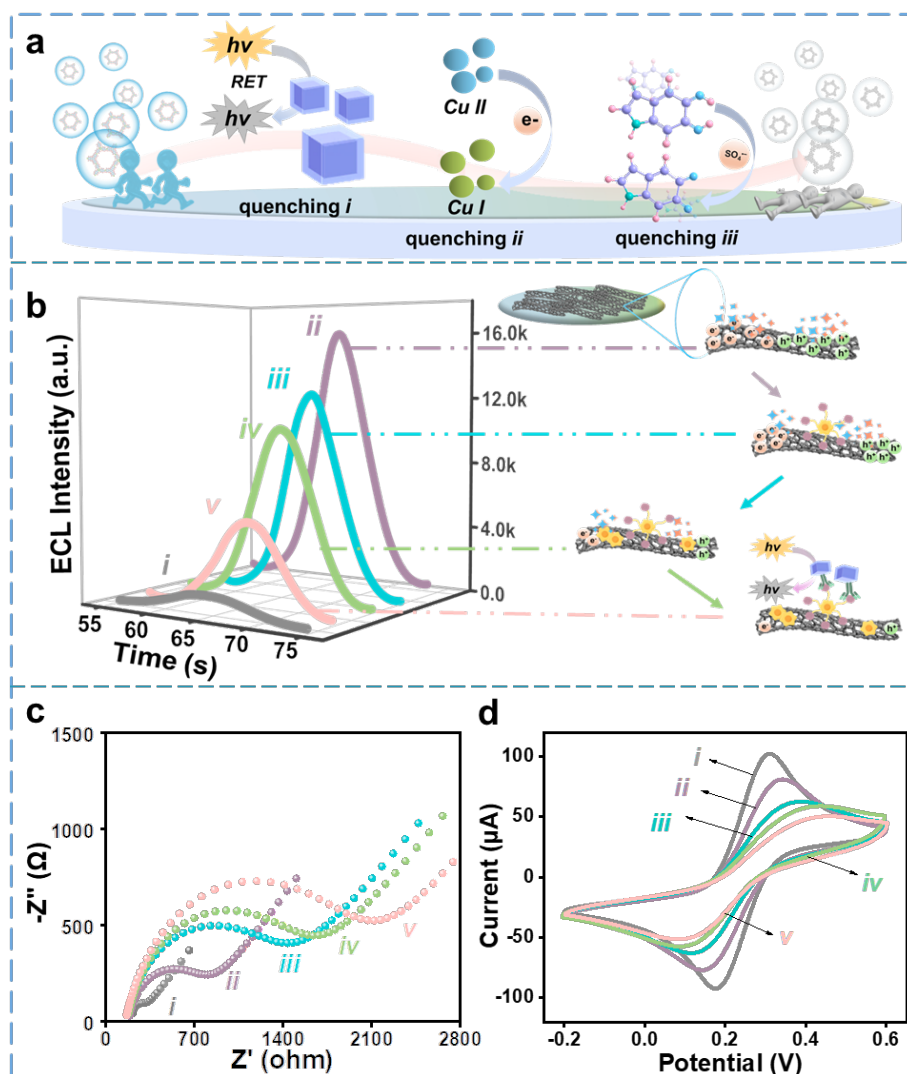


Fig. 6.5 (a) Schematic illustration of the quenching mechanism of CuFe₂O₄@PDA towards TFPT-TAPB-COF. (b) ECL responses, (c) EIS Nyquist plots, and (d) cyclic voltammograms of each step of the construction of the TFPT-TAPB-COF-based ECL immunosensor and the detection of ZEN.

The whole construction procedure of the TFPT-TAPB-COF-based ECL immunosensor for ZEN detection was inspected using ECL (**Fig. 6.5b**) and EC techniques (**Figs. 6.5c** and **3d**). As illustrated in **Fig. 6.5b**, when ZEN-BSA (curve *iii*, 12567 a.u.) and BSA (curve *iv*, 10385 a.u.) were immobilized on TFPT-TAPB-COF/GCE, the ECL signal was gradually weakened due to hindered electron transfer at the electrode/electrolyte interface. The amount of diffusion of the co-reacting reagent to the electrode surface was also considerably reduced. After BSA/ZEN-BSA/TFPT-TAPB-COF/GCE was incubated with the mixture of Ab/CuFe₂O₄@PDA and ZEN (10 pg mL⁻¹), specific identification between the unbound Ab/CuFe₂O₄@PDA complex and ZEN-BSA/TFPT-TAPB-COF/GCE occurred, resulting in a significant decrease in the ECL response of the modified GCE (curve *v*, 5158 a.u.). As mentioned earlier, the triple

quenching effect caused by $\text{CuFe}_2\text{O}_4@\text{PDA}$, i.e., RET, electron transfer, and the scavenging of electrogenerated radicals, toward the modified electrode occurs, resulting in a sharp decrease in ECL response. Therefore, the detection of ZEN can be realized using the manufactured TFPT-TAPB-COF-based ECL immunosensor. The EIS Nyquist plots (Fig. 6.5c) show that ZEN-BSA/TFPT-TAPB-COF/GCE (curve *iii*) has a larger R_{CT} of 1.042 $\text{k}\Omega$ than TFPT-TAPB-COF/GCE (curve *ii*, 372.5 Ω). This result can be ascribed to the further hindrance of electron transfer caused by the steric effect of the adsorbed ZEN over the modified GCE. Blocking the immunosensor with BSA further leads to increased R_{ct} (curve *iv*, 1.399 $\text{k}\Omega$) due to the inhibition of electron transfer at the interface of the electrode/electrolyte. Ab/ $\text{CuFe}_2\text{O}_4@\text{PDA}$ /BSA/ZEN-BSA/TFPT-TAPB-COF/GCE exhibits an even larger R_{ct} (curve *v*, 1.813 $\text{k}\Omega$). This appearance can be explained by the obstruction of charge transfer by Ab/ $\text{CuFe}_2\text{O}_4@\text{PDA}$. The cyclic voltammograms recorded at each step for the fabrication of the TFPT-TAPB-COF-based ECL immunosensor (Fig. 6.5d) present analogous results, indicating the successful assembly of the immunosensor.

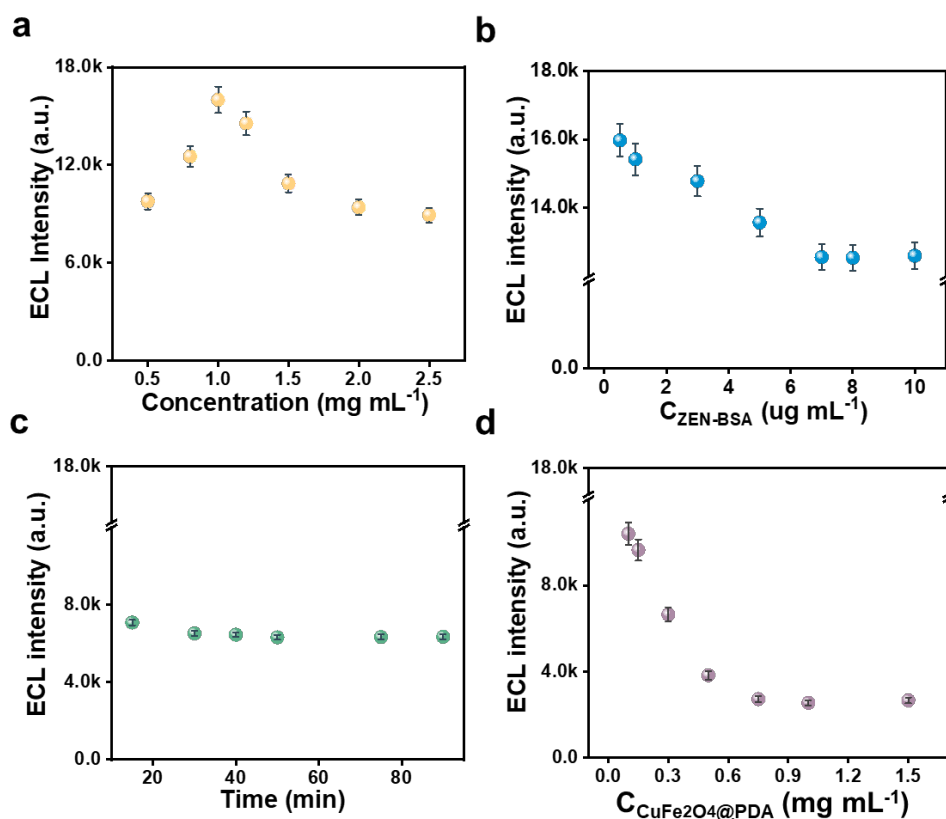


Fig. 6.6 The influence of (a) the concentration of TFPT-TAPB-COF, (b) the concentration of ZEN-BSA, (c) the incubation time of the modified electrode with the mixture of ZEN and Ab/ $\text{CuFe}_2\text{O}_4@\text{PDA}$, and (d) the concentration of Ab/ $\text{CuFe}_2\text{O}_4@\text{PDA}$ on the ECL response of the TFPT-TAPB-COF/GCE in 0.1 M PBS (pH 7.4) with 10 mM $\text{K}_2\text{S}_2\text{O}_8$ within a scan potential of 0 V to -2.4 V at the scan rate of 100 mV s^{-1} . Error bars represent the standard deviations of three replicates, and the results were rounded to two significant digits.

6.3.5 Quantitative analysis of ZEN by using the constructed ECL immunosensor

To achieve the maximum immunosensing performance for ZEN by using the constructed TFPT-TAPB-COF-based ECL immunosensor, some experimental conditions were optimized (**Fig. 6.6**) as follows: COF dosage of 1.0 mg mL^{-1} , ZEN concentration of $7.0 \text{ } \mu\text{g mL}^{-1}$, binding time of ZEN with Ab/CuFe₂O₄@PDA of 50 min, and Ab/CuFe₂O₄@PDA concentration of 0.75 mg mL^{-1} .

Fig. 6.6a depicts that the ECL response of the TFPT-TAPB-COF/GCE increases with the increase of the dosage of TFPT-TAPB-COF from 0.5 to 1.0 mg mL^{-1} , but decreases when further increases the usage from 1.0 to 2.5 mg mL^{-1} . With increasing the COF concentration, nevertheless, the ECL signal tends to decline because of the self-quenching effect easily triggered by π - π stacking interactions in TFPT-TAPB-COF. In addition, the too-thick COF layer coated on the GCE surface is apt to be removed, leading to the instability of the ECL signal. Thereby, TFPT-TAPB-COF with a concentration of 1.0 mg mL^{-1} was chosen for the fabrication of the ECL immunosensor. As illustrated in **Fig. 6.6b**, the influence of the concentration of the ZEN-BSA complex on the ECL response of the constructed ECL biosensor. Apparently, the obtained ECL response of the ZEN-BSA/TFPT-TAPB-COF/GCE decreases with increasing the concentration of ZEN-BSA. It can be due to the enhanced quenching effect of ZEN-BSA on the emission performance of TFPT-TAPB-COF. When the ZEN-BSA concentration exceeds $7.0 \text{ } \mu\text{g mL}^{-1}$, the ECL signal of the ZEN-BSA/TFPT-TAPB-COF/GCE approaches a platform, hinting at the saturated adsorption of ZEN-BSA. Thus, the optimal ZEN-BSA used to develop the ECL immunosensor was set as $7.0 \text{ } \mu\text{g mL}^{-1}$. Moreover, the binding time of ZEN with Ab/CuFe₂O₄@PDA was also investigated to optimize the detection signal toward ZEN using the developed ECL immunosensor. As illustrated in **Fig. 6.6c**, the ECL response caused by the analysis of ZEN using the proposed ECL biosensor increases along with the binding time. With prolonging the binding time, more ZEN molecules specifically bind with Ab/CuFe₂O₄@PDA, resulting in the less unbound Ab/CuFe₂O₄@PDA complex. Thereby, the quenching effect seems to be weak, leading to a large ECL response toward the detection of ZEN. When the binding time was longer than 1 h, the obtained ECL response was stable, which was then set as the optimal binding time for the manufacture of the ECL immunosensor. The effect of different concentrations of Ab/CuFe₂O₄@PDA

on the ZEN immunosensor was also appraised to determine the saturating concentration of the immune response (**Fig. 6.6d**). The ECL response approaches equilibrium for the detection of ZEN when the Ab/CuFe₂O₄@PDA concentration exceeds 0.75 mg mL⁻¹. The Ab/CuFe₂O₄@PDA concentration of 0.75 mg mL⁻¹ was then chosen for the development of the immunosensor for the analysis of ZEN.

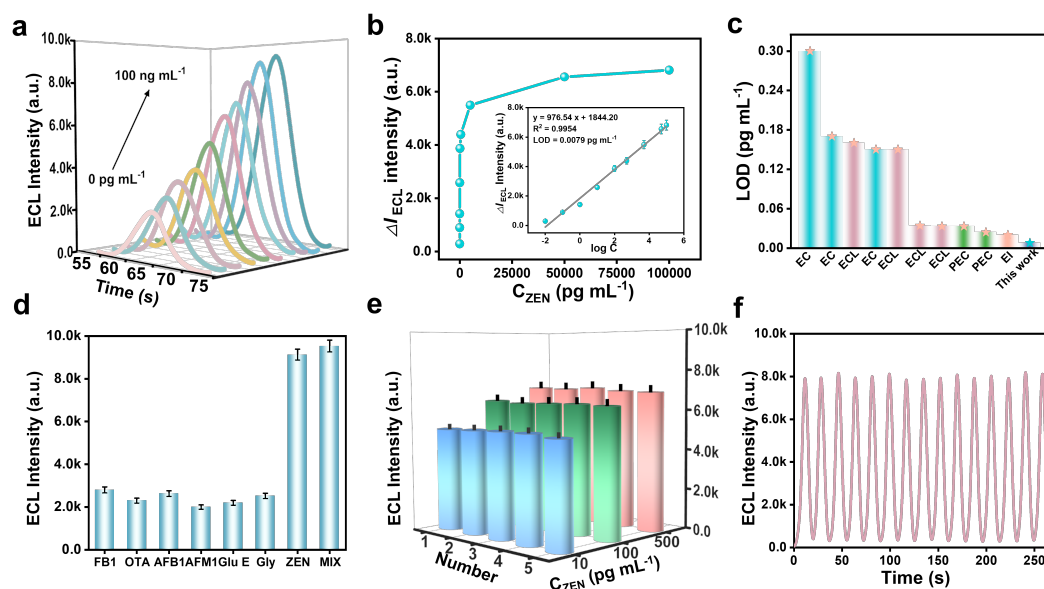


Fig. 6.7 (a) The quantitative analysis of different concentrations of ZEN (10 fg mL⁻¹ - 100 ng mL⁻¹) and (b) the variation of the ECL signals responses ($\Delta I = I - I_0$) caused by the detection of different concentrations of ZEN (0.01, 0.1, 1.0, 10, 100, 500, 5×10^3 , 5×10^4 , and 1×10^5 pg mL⁻¹) by using the developed ECL immunosensor (Inset: the linear fitting of the ΔI versus $\log C_{ZEN}$). (c) Comparison of the proposed ECL immunosensor with other reported biosensors for the detection of ZEN. (d) The selectivity of TFPT-TAPB-COF-based ECL immunosensor using FB1, OTA, AFB1, AFM1, Glu E, Gly, and as interferents (1.0 ng mL⁻¹). (e) The reproducibility of the developed ECL immunosensor for the inspection of different concentrations of ZEN (0.01, 0.1, and 0.5 ng mL⁻¹). (f) The stability of the given immunosensor for the detection of ZEN (5.0 ng mL⁻¹). Data were presented as the average values from three independent experiments, and error bars represent the standard deviations (SD) of three replicates.

The LOD of the developed ECL immunosensor based on TFPT-TAPB-COF toward the detection of ZEN was investigated under optimal conditions through the concentration titration method. As depicted in **Fig. 6.7a**, the ECL response obtained from the analysis of ZEN increases with an increase in ZEN concentration. The recorded ECL response, which is inversely proportional to the quenching efficiency of CuFe₂O₄@PDA, is closely related to ZEN concentration in the detection system. **Fig. 6.7b** shows that the variation in ECL response ($\Delta I = I - I_0$, where I and I_0 represent the ECL response of the presence or absence of ZEN in the analytical system, respectively) dramatically increases within increasing ZEN level. Given ΔI as the function of the logarithm of ZEN concentration ($\log C_{ZEN}$), a well-defined linear relationship is deduced (inset, **Fig. 6.7b**), which can be represented by ΔI (a.u.) = 976.54 $\log C_{ZEN}$ + 1844.20, with a correlation coefficient (R^2) of 0.9954. Accordingly, the LOD of the developed

ECL immunosensor for the detection of ZEN is calculated to be 7.9 fg mL^{-1} ($S/N = 3$) within a wide range of 10 fg mL^{-1} to 100 ng mL^{-1} . The TFPT-TAPB-COF-based ECL immunosensor has a lower LOD and a wider detection range than some reported immunosensors (**Fig. 6.7c** and **Table 6.1**) [64, 125-132]. This finding can be ascribed to the following aspects. (i) TFPT-TAPB-COF with regularly distributed triazine moieties demonstrates an improved emission ability in the presence of SO_4^{2-} . The large specific surface area, high porosity, and maintained amino group of TFPT-TAPB-COF renders a boosted interaction with ZEN-BSA, improving detection sensitivity. (ii) The integration of CuFe_2O_4 and PDA can strengthen the quenching effect toward TFPT-TAPB-COF, increasing detection sensitivity and analysis range. (iii) The possible competitiveness between free ZEN and ZEN-BSA/TFPT-TAPB-COF can promote the analysis accuracy of the proposed ECL immunosensor.

Table 6.1 Comparison of the proposed ECL immunosensor with other reported biosensors for the detection of ZEN.

Method	Material	Linear range (pg mL^{-1})	LOD (pg mL^{-1})	Refs
ECL	Pd-GO	0.5 – 500000	0.16	[133]
ECL	GSH-ABEI@g-C ₃ N ₄ NS	0.1 – 10000	0.033	[125]
ECL	Zr-MOF/Au@MoS ₂	0.1 – 100000	0.034	[126]
ECL	PCN-222@AuNPs/NSG	0.0005 – 1000	0.15	[127]
EC	MoS ₂ -rGO	1.0 – 100000	0.3	[128]
EC	MWCNT/PEI@AuNPs	0.1 – 100	0.15	[129]
EC	PEI-MoS ₂ -MWCNTs	0.5 – 50000	0.17	[130]
PEC	CdS/EDA NWN	0.1 – 100000	0.024	[64]
PEC	ZnO-NGQDs	0.1 – 100000	0.033	[131]
EI	PEI-rGO/Pt@Au	1.0 – 1000000	0.02	[132]
ECL	TFPT-TAPB-COF	0.01 – 100000	0.0079	This work

6.3.6 Selectivity, reproducibility, and stability of the immunosensor

The selectivity of ECL immunosensors is an essential factor for the accurate inspection of ZEN. **Fig. 6.7d** shows that detecting interferents with 100 times the concentration of ZEN (50 ng mL^{-1}) exhibits an insignificant variation in ECL response. By contrast, the detection of ZEN using the developed immunosensor demonstrates an evident change in ECL signal that is comparable with the detection of a mixture of ZEN and interferents. Hence, the proposed ECL immunosensor achieves excellent selectivity for the analysis of ZEN in complicated environments. **Fig. 6.7e** depicts the comparable

ΔI caused by the detection of ZEN with different concentrations of 0.5 pg mL⁻¹, 0.1 ng mL⁻¹, and 0.5 ng mL⁻¹ by using five similar electrodes, illustrating low relative standard deviations (RSDs) of 2.64%, 4.32%, and 2.42%, respectively. An influential factor for obtaining an excellent immunosensor is stability. After the incubation of ZEN (5.0 ng mL⁻¹) and Ab/CuFe₂O₄@PDA (0.75 mg mL⁻¹), the modified electrodes provide a stable ECL signal (**Fig. 6.7f**), with an RSD of 1.31% after 15 consecutive scanning cycles.

6.3.7 Assessment of practicality

The practicality of the developed ECL immunosensor was assessed for the detection of ZEN in diverse real samples (corn, wheat flour, and tea). **Tables 6.2-6.4** indicate that recovery for the analysis of ZEN in corn, wheat flour, and tea by using the constructed immunosensor range from 97.8% to 112.0%, 97.0% to 108.0%, and 97.7% to 108.1%, respectively, accompanied by low RSDs that range from 2.13% to 5.24%, 2.56% to 4.91%, and 2.10% to 4.28%, respectively. To verify the reliability of this method, the samples were detected via enzyme-linked immunosorbent assay (ELISA) for the analysis of ZEN under the same conditions (**Tables 6.5-6.7**). Furthermore, *t*-test and *F*-test were performed to compare the ELISA method with the present method at a confidence level of 95% ($\alpha = 0.05$). As indicated in **Table 6.8**, the calculated *t*-values and *F*-values did not exceed the theoretical values ($|t|_{\text{critical}, 4} = 2.776$; $F_{(2,2)} = 19$). These findings indicate no statistically significant difference between the two approaches, further verifying the accuracy and reliability of the constructed TFPT-TAPB-COF-based ECL immunosensor.

Table 6.2 Detection results of ZEN in corn samples tested with the TFPT-TAPB-COF based immunosensor.

Added	Detected	Recovery (%)	RSD (%)
0.01	0.0107	107.0	4.36
0.1	0.0978	97.8	3.99
1	1.12	112.0	4.45
10	9.89	98.9	4.52
100	108.90	108.9	2.20
500	500.41	100.1	4.17
5000	5140	102.8	5.24
50000	48975	98.0	2.13
100000	99270	99.3	3.55

n = 3 and the units were pg mL⁻¹, unless specified noted.

Table 6.3 Detection results of ZEN in wheat flour samples tested with the ECL immunosensor.

Added	Detected	Recovery (%)	RSD (%)
0.01	0.0097	97.0	4.17
0.1	0.105	105.0	2.56
1	0.99	99.0	4.91
10	10.8	108.0	4.23
100	98.7	98.7	3.39
500	512.3	102.5	3.12
5000	4870	97.4	3.75
50000	50230	100.5	4.85
100000	102000	102.0	4.05

Table 6.4 Detection results of ZEN in tea samples tested using the ECL immunosensor.

Added	Detected	Recovery (%)	RSD (%)
0.01	0.0099	99.0	3.95
0.1	0.0108	108.0	2.15
1	1.03	103.0	4.28
10	10.81	108.1	3.12
100	98.75	98.8	2.68
500	488.63	97.7	3.89
5000	5122	102.4	3.90
50000	51025	102.1	2.10
100000	99885	99.8	4.24

Table 6.5 Comparative results of ZEN containing corn using the developed TFPT-TAPB-COF based immunosensor and the ELISA assay.

Methods	Added amounts	Detected amounts	Recovery (%)	RSD (%)
ECL	5000	5140	102.8	5.24
	50000	48975	98.0	2.13
	100000	99270	99.3	3.55
ELISA	5000	4856	97.1	3.86
	50000	49339	98.7	4.78
	100000	105860	105.9	2.59

Table 6.6 Comparative results of ZEN containing wheat flour using the developed TFPT-TAPB-COF based immunosensor and the ELISA assay.

Methods	Added amounts	Detected amounts	Recovery (%)	RSD (%)
ECL	5000	4870	97.4	3.75
	50000	50230	100.5	4.85
	100000	102000	102.0	4.05
ELISA	5000	5155	103.1	5.16
	50000	48934	97.9	3.34
	100000	109210	109.2	3.92

Table 6.7 Comparative results of ZEN containing tea using the developed TFPT-TAPB-COF based immunosensor and the ELISA assay.

Methods	Added amounts	Detected amounts	Recovery (%)	RSD (%)
ECL	5000	5122	102.4	3.90
	50000	51025	102.1	2.10
	100000	99885	99.8	4.24
ELISA	5000	5284	105.7	4.28
	50000	53600	107.2	4.73
	100000	98563	98.6	2.65

Table 6.8 Statistical comparison of the results obtained by the developed TFPT-TAPB-COF based immunosensor and the ELISA assay.

Sample	Added amounts	<i>t</i> -test	<i>F</i> -test
corn	5000	1.50	4.27
	50000	0.05	0.01
	100000	1.42	7.44
flour	5000	1.44	0.21
	50000	0.77	0.55
	100000	1.93	0.34
tea	5000	0.98	0.61
	50000	1.60	0.03
	100000	0.44	6.88

The values in the table for *t*-test and *F*-test correspond to the calculated values at a significance level of $\alpha = 0.05$ and $n = 3$, and the results were reported to two decimal places.

6.4 Conclusion

A newly designed reversal signal “on-off-on” and triple quenching modal-based ECL immunosensor was constructed using triazine-based COF as luminophore and

CuFe₂O₄@PDA as the quencher for the accurate and sensitive detection of ZEN in food products. By integrating the trimodal quenching strategy and the competitive immunosensing method, the recorded ECL signal was proportional to the level of ZEN in the system, realizing high accuracy for the detection of ZEN. Based on the features of TFPT-TAPB-COF and the trimodal quenching effects of CuFe₂O₄@PDA, the established ECL immunosensor exhibited the following advantages. (i) The D-A conjugated TFPT-TAPB-COF not only demonstrated an enhanced ECL response but also exhibited high immobilization ability toward ZEN-BSA, indicating the high sensitivity and rapid response of the constructed ECL immunosensor. (ii) The trimodal quenching effect caused by CuFe₂O₄@PDA considerably reduced background noise, enhancing the accuracy of the ECL biosensor. (iii) The integration of the competitive immunosensing method and ECL signal quenching enabled the ECL biosensor to exhibit a signal that was positively correlated with the concentration of ZEN, declining the signal interference of other types of analytes. Given its low LOD, high selectivity, good stability, and excellent reproducibility, the developed ECL immunosensor demonstrated a promising application in the analysis of ZEN in different food products. This work provides a new designable ECL biosensing method by integrating different strategies to efficiently detect mycotoxins in food items.

Chapter VII: Trinuclear copper cluster-based COF with a high content of Cu-N₂ single-atom sites: A multivariate signal-amplified photoelectrochemical aptasensor for the sensitive detection of mycotoxins

7.1 Introduction

Diverse mycotoxins, such as aflatoxin, ochratoxin, ergot alkaloid, and zearalenone (ZEN), have been found in food products or grains, causing serious harm to humans, animals, and plants, such as cancerization and nervous system diseases. ZEN, the most widespread pollutant in grains and food stocks, frequently contaminates various crops. Therefore, the sensitive determination of ZEN is essential for guaranteeing food safety. In general, the PCE of COFs may be decreased due to the fast recombination of photoinduced electrons and holes, the low stability of transient species, and energy losses during charge excitation and migration. Utilizing a highly photoelectronic active COF for the construction of a superior biosensor remains a considerable challenge. Various approaches, such as the incorporation of a photosensitizer into COF networks, the regulation of bandgaps of COFs by changing the ligands ^[134], and the introduction of the D-A conjugated structure into COFs, have been used to enhance PCE. Among different COFs, Metallo-COFs (MCOFs), which are synthesized using the functional building blocks that contain cyclic trinuclear copper (CTC) clusters, exhibit excellent photocatalytic and electrocatalytic performance due to their copper single-atom sites, highly conjugated structure, tunable bandgap, and enhanced separation of photoinduced carriers. Based on the above overview, developing a novel PEC biosensor based on CTC-COF with superior electronic properties and high PCE is highly promising for efficiently detecting ZEN. To date, however, such biosensors have not yet been reported.

To considerably improve the detection signal of biosensors, diverse amplification strategies, such as polymerase chain reaction, enzyme-assisted signal amplification, rolling circle amplification, target-modulated competitive binding method, and hybrid chain reaction ^[135], have been used. Furthermore, different signal amplification strategies have been combined to construct PEC biosensors for remarkably boosting detection sensitivity. To realize the ultrasensitive detection of ZEN, we first fabricated a novel multivariate signal-amplified PEC aptamer biosensor (aptasensor) based on the D-A-conjugated copper-cyclic trinuclear unit (CTU)-COF via the exonuclease III (Exo III)-assisted strategy coupled with the p-n heterojunction quenching mode (Scheme 1).

The new n-type D-A-conjugated COF semiconductor synthesized via the reaction of tris(2-4-carboxaldehyde-pyrazolato-N, N')-tricopper (Cu_3L_3) and 4,4',4''-(1,3,5-triazine-2,4,6-triyl)-trianiline (TAPT) (denoted as Cu_3L_3 -TAPT-COF) was composed of a large number of Cu-N₂ single-atom sites, which markedly facilitated fast photocarrier transfer. Thereto, Cu_3L_3 -TAPT-COF demonstrated high bioaffinity toward single-stranded DNA (ssDNA) and enhanced PEC response. With the application of the multivariate signal-amplified strategy, the Cu_3L_3 -TAPT-COF-based PEC aptasensor realized efficient and accurate detection of ZEN from complicated food products, exhibiting an ultralow limit of detection (LOD) of 24 fg mL⁻¹ within a wide range (from 0.1 pg mL⁻¹ to 20 ng mL⁻¹) and other superior sensing performance. This work extends the application of D-A-conjugated COF to the sensitive inspection of mycotoxins and provides deep insight into the construction of an excellent PEC aptasensor compared with the use of multivariate signal-amplified methods.

7.2 Experimental section

7.2.1 Chemicals and reagents

All chemical reagents and raw materials were acquired from commercial sources. Tris(2-4-carboxaldehyde-pyrazolato-N, N')-tricopper (Cu_3L_3), 4,4',4''-(1,3,5-triazine-2,4,6-triyl) trianiline (TAPT), $\text{Zn}(\text{Ac})_2 \cdot 2\text{H}_2\text{O}$, $\text{InCl}_3 \cdot 4\text{H}_2\text{O}$, and ruthenium hexamine trichloride (RuHex) were purchased from Sigma-Aldrich. Mesitylene, dioxane, acetic acid, citric acid monohydrate, thioacetamide, and sodium hydroxide were purchased from Macklin. Ethylene glycol, ethanol absolute, tetrahydrofuran, and dimethylformamide were from Tianjin Damao Chemical Preparations Factory. Exonuclease III (Exo III), 6-mercapto-1-hexanol (MCH, 14.4 M), zearalenone (ZEN), fumonisin B1 (FB1), aflatoxin B1 (AFB1), aflatoxin-M1 (AFM1), glutamic acid (Glu), glycine (Gly), ochratoxin A (OTA), ZEN-aptamer, and enzyme-linked immunosorbent assay (ELISA) were obtained from Sangon Biotechnology Co.

The base sequences of oligonucleotides are shown as bellows (5' - 3'):

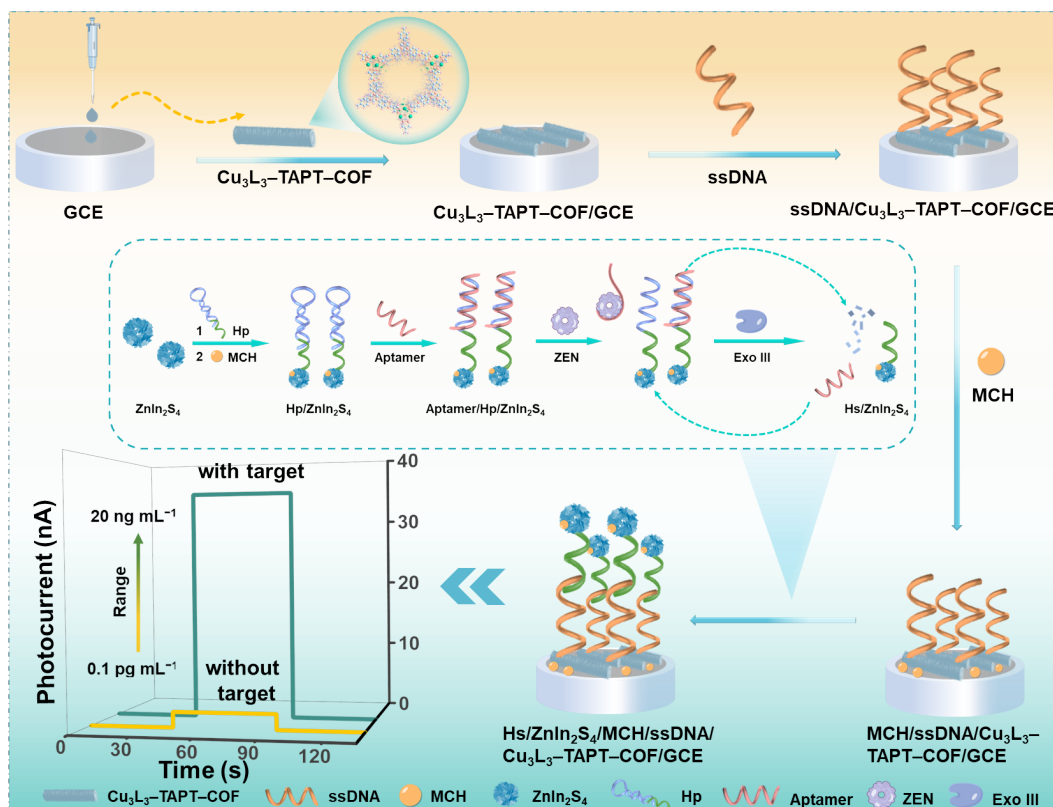
Hairpin DNA (Hp): TTTTTT CGC GCG TGT CAT TCC CTG CAT CGG TTC
ACG GTA GCA TTTTTT

ZEN-aptamer: AGC AGC ACA GAG GTC AGA TGT CAT TCT ATG GTA CAT
TAC TAT CTC TGT AAT GTG TGC TAC CGT GAA

Hairpin probe surpluses strand (Hs): TTTTTT CGC GCG TGT CAT TCC CTG

CAT CGG TTC ACG GTA GCA

ssDNA: TTTTTTTTTT TGC TAC CGT CC



Scheme 7.1 Schematic diagram of the construction of the multivariate signal-amplified PEC aptasensor based on $\text{Cu}_3\text{L}_3\text{-TAPT-COF}$ for detection of ZEN.

7.2.2 Synthesis of $\text{Cu}_3\text{L}_3\text{-TAPT-COF}$

The synthesis of D-A-conjugated $\text{Cu}_3\text{L}_3\text{-TAPT-COF}$ was based on previous work with several modifications (**Fig. 7.1a**). First, 23.7 mg of Cu_3L_3 (0.05 mmol) and 26.5 mg of TAPT (0.075 mmol) were introduced into a 15 mL Schlenk tube, followed by the addition of a mixed solvent composed of mesitylene (0.5 mL), dioxane (0.5 mL), and 0.1 mL of acetic acid (6 M). Then, the tube underwent flash-frozen treatment at -196.15°C in liquid nitrogen, followed by degassing with three freeze-pump-thaw cycles. When the tube was warmed to room temperature, it was heated at 120°C and allowed to react for 3 days. Subsequently, the tube was cooled to room temperature. The resulting product was collected and washed separately with tetrahydrofuran and dimethylformamide via centrifugation. Then, the product was washed with anhydrous acetone thrice. After drying at 100°C under vacuum for 12 h, a pale green powder was obtained and denoted as $\text{Cu}_3\text{L}_3\text{-TAPT-COF}$.

7.2.3 Manufacture of the Cu₃L₃-TAPT-COF-based PEC aptasensor

First, 3 mg of Cu₃L₃-TAPT-COF was dispersed in 2.0 mL of Milli-Q water under continuous stirring until a homogeneous suspension was formed. Thereafter, 10 μ L of the Cu₃L₃-TAPT-COF suspension (1.5 mg mL⁻¹) was coated onto the pretreated glass carbon electrode (GCE), followed by drying in the air (represented by Cu₃L₃-TAPT-COF/GCE). After rinsing three times with phosphate-buffered saline (PBS; 0.01 M, pH 7.4), Cu₃L₃-TAPT-COF/GCE was dried again in the air. Thereafter, Cu₃L₃-TAPT-COF/GCE was incubated in ssDNA solution (100 μ L, 500 nM) for 2 h, followed by washing three times with PBS (0.01 M, pH 7.4) to remove the unbound ssDNA (represented by ssDNA/Cu₃L₃-COF/GCE) and then dried again. Subsequently, the ssDNA/Cu₃L₃-COF/GCE was incubated with 10 μ L of 6-mercapto-1-hexanol solution (MCH; 1.0 mM) for 0.5 h to block nonspecific sites, followed by rinsing three times with PBS (0.01 M, pH 7.4; denoted as MCH/ssDNA/Cu₃L₃-COF/GCE).

7.2.4 Preparation of ZnIn₂S₄, Hp/ZnIn₂S₄, aptamer/Hp/ZnIn₂S₄, and Hs/ZnIn₂S₄ complex

ZnIn₂S₄ was prepared based on previous work. Then, 0.2 mg of ZnIn₂S₄ was thoroughly dispersed in 2.0 mL of Milli-Q water. Thereafter, the ZnIn₂S₄ suspension (2.0 mL, 0.1 mg mL⁻¹) was mixed with the hairpin probe (Hp) strand solution (2.0 mL, 0.5 μ M) for 4 h to form the Hp/ZnIn₂S₄ complex. After centrifuging and washing with PBS (0.01 M, pH 7.4) to remove the loosely bound Hp strands, the obtained precipitates were dispersed again in 2.0 mL of PBS. To block nonspecific binding sites, 0.5 mL of MCH (1.0 mM) was subsequently added to the above suspension and allowed to react for 0.5 h. The cleaning process was repeated. Subsequently, the Hp/ZnIn₂S₄ complex (0.5 μ M) was mixed with the aptamer solution (2.0 mL, 0.5 μ M) and allowed to react for 1 h. Given the complementary sequences of the aptamer and Hp, aptamer/Hp hybridized double strands were formed on the ZnIn₂S₄ surface (denoted as aptamer/Hp/ZnIn₂S₄). In the presence of ZEN, however, the generation of the G-quadruplex between the aptamer and ZEN resulted in the release of a part of the aptamer from aptamer/Hp/ZnIn₂S₄ because the binding interaction between ZEN and the aptamer was higher than the hybridization action between the aptamer and Hp. This extrapolation was also confirmed later. When introducing Exo III, the double-stranded aptamer/Hp on ZnIn₂S₄ was then cleaved, resulting in the formation of the hairpin probe

surplus (Hs)/ZnIn₂S₄ residual (denoted as Hs/ZnIn₂S₄). The resultant Hs/ZnIn₂S₄ then served as the quencher for blocking the PEC signal of the MCH/ssDNA/Cu₃L₃-TAPT-COF/GCE.

7.2.5 Detection of ZEN by using the Cu₃L₃-TAPT-COF-based PEC aptasensor

The quantitative analysis of ZEN by using the Cu₃L₃-TAPT-COF-based PEC aptasensor was performed using the concentration titration method. First, the aptamer/Hp/ZnIn₂S₄ complex was separately mixed with ZEN with different concentrations (0.1, 1.0, 5.0, 10, 100, 1000, 5000, and 20000 pg mL⁻¹), retaining the series of concentrations of the Hs/ZnIn₂S₄ complex with the assistance of Exo III catalysis. Subsequently, the MCH/ssDNA/Cu₃L₃-TAPT-COF/GCE was separately incubated in the obtained Hs/ZnIn₂S₄ suspension with different concentrations. Notably, the concentration of the Hs/ZnIn₂S₄ complex was inversely proportional to the ZEN level. A high concentration of ZEN resulted in a small amount of Hs/ZnIn₂S₄ residual, successively decreasing the quenching efficiency of ZnIn₂S₄ on the PEC signal of the MCH/ssDNA/Cu₃L₃-TAPT-COF/GCE. LOD was attained using the $3S_b/m$ equation, where S_b is the standard deviation of the blank ($n = 11$), and m is the slope of the linear calibration curve, respectively, following the criterion recommended by the International Union of Pure and Applied Chemistry. The selectivity of the PEC aptasensor was investigated for the determination of interferents, including fumonisin B1 (FB1), ochratoxin A (OTA), aflatoxin B1 (AFB1), aflatoxin M1 (AFM1), glutamic acid (Glu), glycine (Gly) (1.0 ng mL⁻¹), and their mixture with ZEN with a 100-fold concentration of ZEN (10 pg mL⁻¹). The reproducibility of the manufactured aptasensor was evaluated by recording the photocurrents for the analysis of ZEN (1.0, 5.0, and 20 ng mL⁻¹) by using six of the same developed aptasensors. Furthermore, long-term stability was probed by recording the photocurrents caused by the detection of ZEN (0.1 ng mL⁻¹) per day and continuously for 16 days.

7.2.6 Real samples

Diverse real samples of corn and flour were used to assess the practicality of the developed PEC aptasensor. Before the use of real samples, pretreatment was performed by previous work [136]. Thereafter, different ZEN concentrations were spiked into real samples, and their levels were determined using the proposed PEC aptasensor. ZEN

levels in the real samples were deduced by the calibration curve and compared with the added ones. Moreover, an ELISA assay was performed to detect the ZEN content of diverse real samples to further determine the accuracy of the developed PEC aptasensor.

7.3 Results and discussion

7.3.1 Biosensing mechanism of ZEN by using the Cu₃L₃-TAPT-COF-based PEC aptasensor

As illustrated in **Scheme 7.1**, the n-type Cu₃L₃-TAPT-COF with a narrow bandgap, high photoabsorption ability, and enhanced PCE simultaneously acted as PEC electrode and bioplatfrom, which represented the first signal amplification. Meanwhile, large amounts of ssDNA strands were immobilized on the Cu₃L₃-TAPT-COF-modified electrode via the combined effects of π - π stacking, van der Waals force, and electrostatic interaction. Moreover, the p-type ZnIn₂S₄ semiconductor was utilized as the carrier for immobilizing Hp strands, which exhibit complementary sequences of the ZEN-targeted aptamer, creating the Hp/ZnIn₂S₄ complex. When aptamer exists, double-stranded DNA (dsDNA) was formed on ZnIn₂S₄ through the hybridization of Hp (blue region) and aptamer (aptamer/Hp/ZnIn₂S₄), resulting in the change of HP to expose the enzyme cleavage site of HP at the 3' terminus (blue region). Furthermore, the release of the generated ZEN-aptamer G-quadruplex due to specific recognition resulted in the separation of the partial aptamer from the aptamer/Hp/ZnIn₂S₄ complex. When Exo III was added, the mononucleotides from the blunt 3' terminus of the remaining aptamer/Hp double strands that anchored to ZnIn₂S₄ were cleaved, eventually releasing the aptamer and resulting in the generation of the Hs/ZnIn₂S₄ complex. The released aptamer can continue to bind with other Hp/ZnIn₂S₄ complexes to achieve the second signal amplification, while the remained Hs/ZnIn₂S₄ complex hybridizes with ssDNA/Cu₃L₃-TAPT-COF due to the complementary base sequences of Hs and ssDNA. As a result, the p-n heterojunction between Cu₃L₃-TAPT-COF and ZnIn₂S₄ is established. Given that the p-type semiconductor can compete with n-type semiconductors, which consume visible light energy and electron donors, the quenching of the PEC response of the modified GCE electrode occurs. Notably, the amount of the remaining aptamer/HP/ZnIn₂S₄ complex decreases with increasing the ZEN concentration, further declining the Hs/ZnIn₂S₄ level. Accordingly, the quenching efficiency of ZnIn₂S₄ on the Cu₃L₃-TAPT-COF-based PEC signal is remarkably

weakened. Consequently, the attained PEC response can be positively correlated with the ZEN concentration.

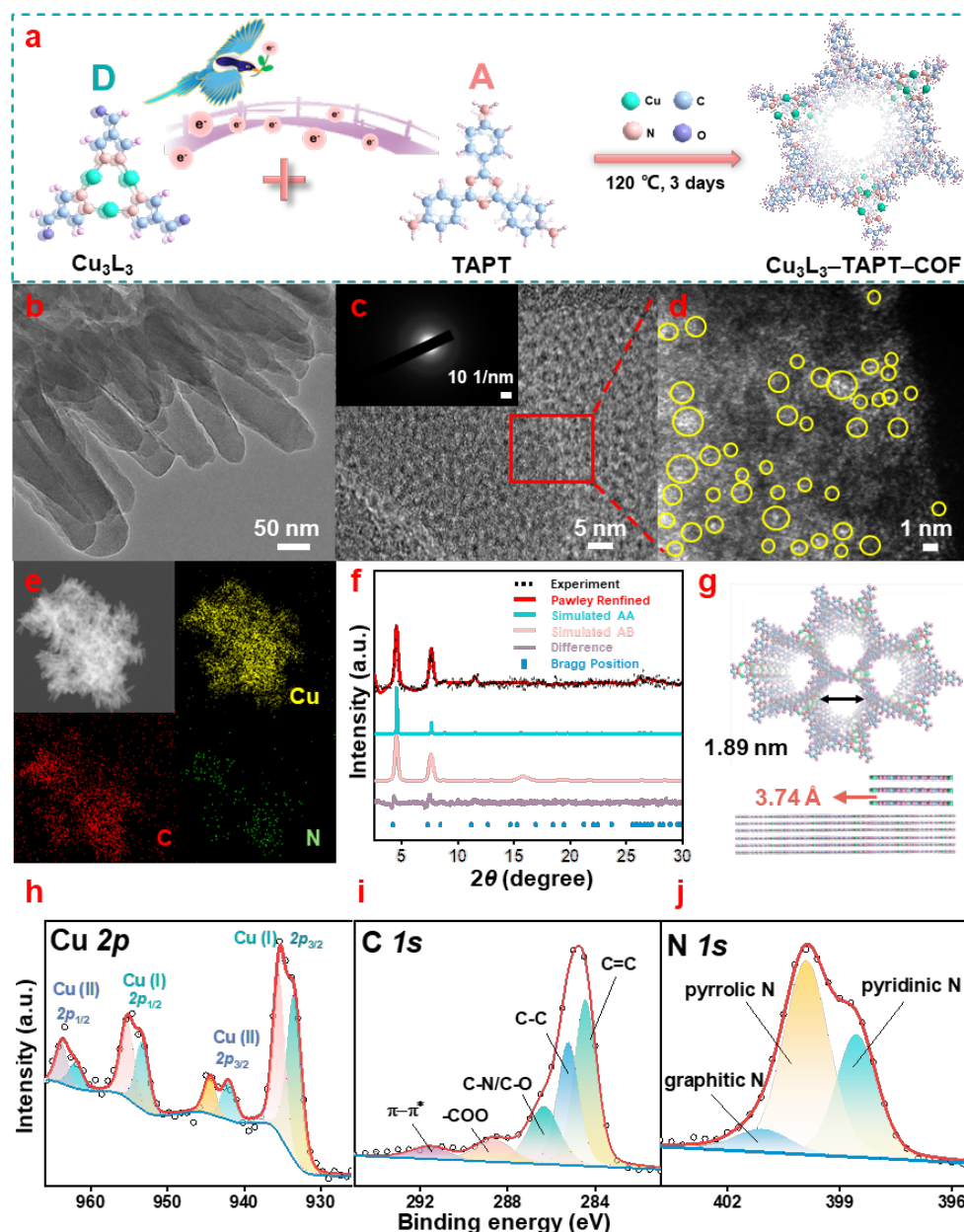


Fig. 7.1 (a) Schematic illustration of the synthesis procedure of $\text{Cu}_3\text{L}_3\text{-TAPT-COF}$. (b) TEM, (c) HR-TEM (inset: selected area electron diffraction), (d) HAADF-STEM image, (e) EDS mapping [Cu (yellow), C (red), and N (green)], (f) PXRD pattern, (g) eclipsed stacking, high-resolution XPS spectra of (h) Cu 1s, (i) C 1s, and (j) N 1s of $\text{Cu}_3\text{L}_3\text{-TAPT-COF}$.

7.3.2 Basic characterizations of $\text{Cu}_3\text{L}_3\text{-TAPT-COF}$

The nanostructure and chemical components of $\text{Cu}_3\text{L}_3\text{-TAPT-COF}$ were characterized using diverse techniques. The transmission electron microscopy (TEM) image of $\text{Cu}_3\text{L}_3\text{-TAPT-COF}$ confirms this finding, i.e., $\text{Cu}_3\text{L}_3\text{-TAPT-COF}$ is aggregated by ultrathin nanosheets (**Fig. 7.1b**). No clear lattice spacing is observed in the high-resolution TEM (HR-TEM) image of $\text{Cu}_3\text{L}_3\text{-TAPT-COF}$ (**Fig. 7.1c**). Furthermore, the

aberration-corrected high-angle annular dark-field scanning TEM (HAADF-STEM) image of Cu₃L₃-TAPT-COF reveals high-density isolated bright dots encircled by yellow color; these dots are attributed to the existence of single-atomic Cu-N₂ sites (**Fig. 7.1d**)^[137]. Distinctly, single-atomic Cu-N₂ sites in Cu₃L₃-TAPT-COF can promote PCE and electron transfer. In addition, the energy-dispersive X-ray spectroscopy (EDS) mapping image of Cu₃L₃-TAPT-COF demonstrates the even distribution of copper, carbon, and nitrogen elements within a selected region (**Fig. 7.1e**). Notably, the atomic content of copper in Cu₃L₃-TAPT-COF, which is determined via inductively coupled plasma mass spectrometry, is around 18.7%. This finding reveals the extremely high content of single-atomic Cu-N₂ sites in the developed D-A-conjugated Cu₃L₃-TAPT-COF. This phenomenon is rarely observed in other MOFs.

Furthermore, the powder X-ray diffraction (PXRD) pattern of Cu₃L₃-TAPT-COF displays an intense diffraction peak at $2\theta = 7.39^\circ$, along with a series of weaker peaks at $2\theta = 7.65^\circ$, 8.42° , 11.49° , and 26.07° , which are attributed to the (100), (110), (200), (120), and (001) diffraction planes, respectively (**Fig. 7.1f**). Theoretical simulation by using Materials Studio software was also performed, in which the eclipsed stacking (AA) and staggered stacking (AB) structures of Cu₃L₃-TAPT-COF were simulated. The experimental PXRD pattern can be matched well with the calculated PXRD profile of the AA stacking mode with acceptable parameters ($R_p = 10.78\%$ and $R_{wp} = 13.41\%$). The space group is P6/M, and the lattice parameters are $a = b = 22.724 \text{ \AA}$, $c = 3.69 \text{ \AA}$, $\alpha = \beta = 90^\circ$, and $\gamma = 120^\circ$, for a unit cell of Cu₃L₃-TAPT-COF (**Fig. 7.1g**). Accordingly, the D-A-conjugated Cu₃L₃-TAPT-COF demonstrates the regular AA stacking mode, which is conducive to the improvement of photoinduced electron and charge transfer.

The Cu 2*p* X-ray photoelectron spectroscopy (XPS) spectrum of Cu₃L₃-TAPT-COF comprises two groups: Cu 2*p*_{3/2} (935.1 eV) and Cu 2*p*_{1/2} (952.3 eV), as shown in **Fig. 7.1h**. Both groups include Cu⁺ (933.6 eV and 953.3 eV) and Cu²⁺ (935.5 eV and 955.3 eV) species, along with their satellite peaks. These findings reveal the mixed valent states of copper in Cu₃L₃-TAPT-COF, which is conducive to the regulation of bandgap and electron transfer. In addition, the C 1*s* XPS spectrum can be divided into C-C (287.4 eV), C-N (285.2 eV), C-O (286.3 eV), N-C=N (288.6 eV), and π - π^* (291.5 eV) (**Fig. 7.1i**). The N 1*s* XPS spectrum involves pyridinic N (398.6 eV), pyrrolic N (399.9 eV), and graphitic N (401.1 eV) (**Fig. 7.1j**). These results indicate the high conjugation degree of Cu₃L₃-TAPT-COF, enhancing photoabsorption ability.

7.3.3 Electron structure of Cu₃L₃-TAPT-COF

To study the D-A-conjugated structure of Cu₃L₃-TAPT-COF, density functional theory (DFT) at the B3LYP/6-31G (d, p) level was adopted to probe the electron structures of Cu₃L₃, TAPT, and Cu₃L₃-TAPT-COF, for which the frontier orbitals of these fragments were analyzed utilizing a multifunctional wavefunction analyzer (Multiwfn).

A substantial electron donation from the π -system of Cu₃L₃ to the delocalized TAPT acceptor zone is found due to the electron-withdrawing effect of the acceptor unit (TAPT) and the electron-donating effect of the donor unit (Cu₃L₃) (**Fig. 7.2a**). As depicted in **Fig. 7.2b**, the energy gap of Cu₃L₃-TAPT-COF, deduced from the electron distribution of the highest occupied molecular orbital and the lowest unoccupied molecular orbital, is calculated to be 2.69 eV, which is significantly narrower than those of Cu₃L₃ (7.56 eV) and TAPT (6.19 eV), this finding is consistent with other molecules with the D-A architecture in the reported results. In addition, the photocurrent of Cu₃L₃-TAPT-COF/GCE (curve *iv*) reaches as high as 650.3 nA (**Fig. 7.2c**), this result can be ascribed to the D-A effect of Cu₃L₃-TAPT-COF. This effect markedly enhances the effective separation and migration of charge carriers, and this condition is conducive to the inhibition of the recombination of photogenerated hole-electron pairs and the facilitation of PCE. Based on the theoretical calculations and experiment results, the establishment of the D-A conjugation structure in Cu₃L₃-TAPT-COF and the transfer direction of intralayer charge from Cu₃L₃ to TAPT is explicitly expected.

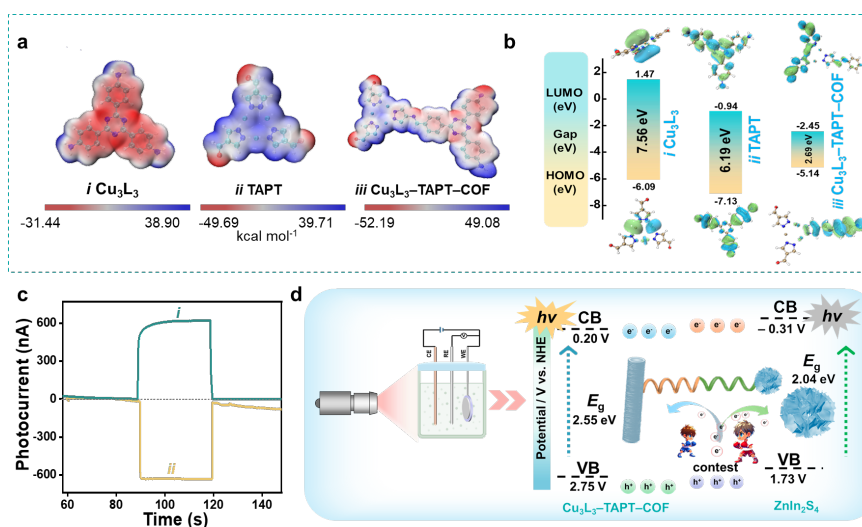


Fig. 7.2 The calculated (a) electrostatic potential (ESP) distributions and (b) the HOMOs and LUMOs of (i) Cu₃L₃, (ii) TAPT, and (iii) Cu₃L₃-TAPT-COF. (c) The PEC performances of (i) Cu₃L₃-TAPT-COF/GCE and (ii) ZnIn₂S₄/GCE. (d) The quenching mechanism of the PEC system based on the p-n heterojunction (The PEC assay was acquired in PBS (0.1 M, pH=7.4) at a bias voltage of 0 V).

7.3.4 Quenching mechanism of the photocurrent toward the p-n heterojunction

To further reveal the quenching effect of ZnIn_2S_4 on the PCE of $\text{Cu}_3\text{L}_3\text{-TAPT-COF}$, PEC measurements were performed. As illustrated in **Fig. 7.2c**, $\text{Cu}_3\text{L}_3\text{-TAPT-COF/GCE}$ (curve *i*) has an anodic photocurrent of 650.3 nA, while $\text{ZnIn}_2\text{S}_4/\text{GCE}$ (curve *ii*) exhibits a cathodic photocurrent of 655.5 nA. The electron transfer of the p-n heterojunction quenching mechanism is presented in **Fig. 7.2d**, specifically, the optical properties of $\text{Cu}_3\text{L}_3\text{-TAPT-COF}$ and ZnIn_2S_4 were investigated (**Fig. 7.3**). UV-vis DRS (**Fig. 7.3a**) was used to measure the absorbance and optical band gap (**Fig. 7.3b**) of $\text{Cu}_3\text{L}_3\text{-TAPT-COF}$ and ZnIn_2S_4 , which were obtained from the Tauc diagram using the formula, where α , h , ν , A , and E_g represent the absorption coefficient, Planck's constant, the light frequency, the proportionality constant, and the energy band gap, respectively. Further, the exponent n depends on the type of the semiconductor, the value of 2 for direct transition semiconductor and 1/2 for indirect transition semiconductor.

$$(\alpha h\nu)^n = A (h\nu - E_g)$$

The Mott-Schottky (M-S) test (**Fig. 7.3c**) was performed to confirm the band structure accurately. The Fermi levels (E_f) values (vs. NHE) of $\text{Cu}_3\text{L}_3\text{-TAPT-COF}$ and ZnIn_2S_4 were assessed based on the following equations. Specifically, E_{CB} and E_{VB} are the potentials of the CB (conduction band) bottom and VB (valence band top) top, respectively.

$$E_{\text{CB}} = E_{\text{VB}} - E_g,$$
$$E_{\text{NHE}} = E_{\text{Ag/AgCl}} + 0.197$$

Based on the above calculations, the optical E_g of $\text{Cu}_3\text{L}_3\text{-TAPT-COF}$ is about 2.55 eV, whereas the flat-band potential E_f value (vs. NHE) is 0.20 eV and the valence band (VB) is 2.75 eV. Moreover, the optical E_g , the flat-band potential E_f (vs. NHE), and the VB of ZnIn_2S_4 are 2.04 eV, - 0.31 eV, and 1.73 eV, respectively. Particularly, the slope of the M-S curve of $\text{Cu}_3\text{L}_3\text{-TAPT-COF}$ is positive, indicating the n-type semiconductor characteristic. However, the slope of ZnIn_2S_4 is negative, hinting at its p-type semiconductor feature. Accordingly, upon excitation by visible light, $\text{Cu}_3\text{L}_3\text{-TAPT-COF}$ can be excited, leading to the generation of the electron-hole (e^-/h^+) pairs. The holes in the valence band (VB) of $\text{Cu}_3\text{L}_3\text{-TAPT-COF}$ are quenched by electrons, which transfer from the electrolyte. Meanwhile, the photogenerated electrons transfer from the VB to the conduction band (CB) of $\text{Cu}_3\text{L}_3\text{-TAPT-COF}$ and reach the GCE surface, where they are efficiently converted into detectable electrical signals. In particular, the introduction

of the D-A structure into Cu₃L₃-TAPT-COF remarkably facilitates photogenerated carrier transport, generating a fast and stable photocurrent. Given that photogenerated holes act as major carriers, the p-type semiconductor tends to interact with electron acceptors rather than electron donors. Consequently, gaining electrons is easier for the p-type semiconductor than the n-type semiconductor. As a p-type semiconductor, ZnIn₂S₄ with a narrow bandgap exhibits the advantages of competing with substrates under visible light irradiation, while electron transfer to the electrolyte is faster than hole transfer. This characteristic decreases the number of electrons obtained by the substrate, causing the low photocurrent response of the modified electrode. In addition, the energy band positions of Cu₃L₃-TAPT-COF and ZnIn₂S₄ were staggered and matched readily (**Fig. 2d**). Consequently, photoinduced electrons in the CB of Cu₃L₃-TAPT-COF can transfer to the VB of ZnIn₂S₇. It can efficiently suppress the recombination of the photoinduced carriers, thus obviously inhibiting the anodic photocurrent.

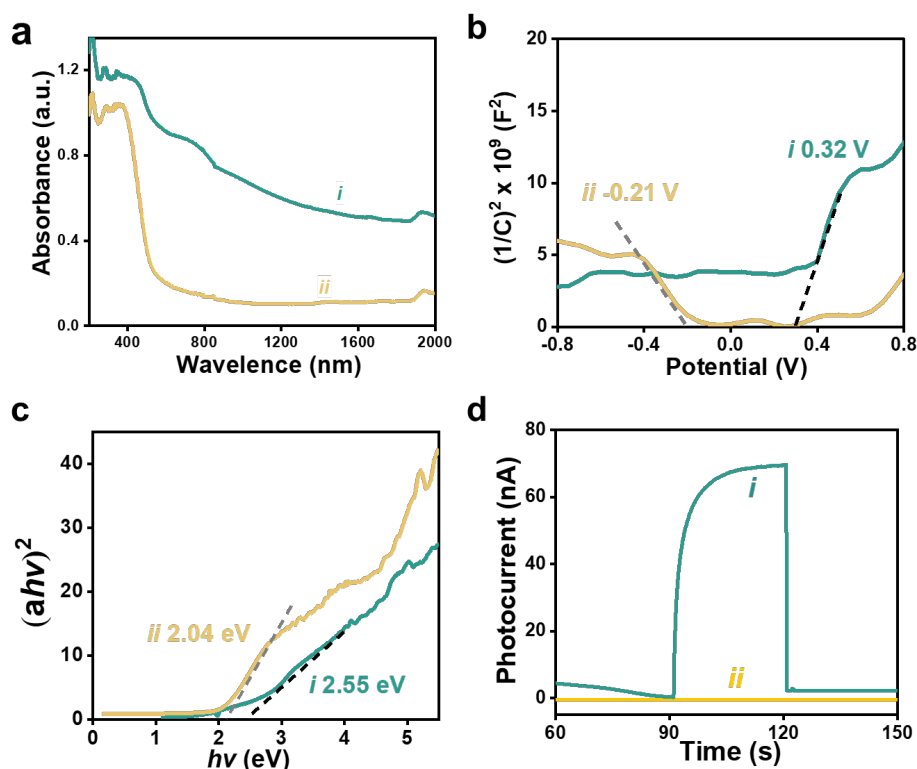


Fig. 7.3 (a) The UV-vis DRS spectra, (b) band gaps, and (c) M-S plots of (i) Cu₃L₃-TAPT-COF and (ii) ZnIn₂S₄. (d) PEC responses of (i) MCH/ssDNA/Cu₃L₃-TAPT-COF/GCE the bare GCE and (ii) Hs/ZnIn₂S₄/MCH/ssDNA/Cu₃L₃-TAPT-COF.

7.3.5 Construction of the Cu₃L₃-TAPT-COF-based PEC aptasensor

To verify the feasibility of the Cu₃L₃-TAPT-COF-based PEC aptasensor, the affinity ability of diverse bioprobes was determined, while the dissociation constant (K_d)

was calculated through the Hill equation. The K_d values between Hp/ZnIn₂S₄ and aptamer (K_{d1}), aptamer and ZEN (K_{d2}), Cu₃L₃-TAPT-COF/GCE and ssDNA (K_{d3}), and ssDNA/Cu₃L₃-TAPT-COF/GCE and Hs/ZnIn₂S₄ (K_{d4}) were deduced. The results reveal that the K_{d2} caused by the specific binding between aptamer and ZEN (0.08 fM) is significantly smaller than K_{d1} (1.61 pM). It hints that the separation of the G-quadruplex formed between aptamer and ZEN from the electrode surface is highly feasible (**Figs. 7.4a and 7.4b**). Further, the K_{d3} caused by the immobilization of ssDNA on Cu₃L₃-TAPT-COF (0.12 pM) is lower than the K_{d4} (0.33 pM). Consequently, the Hs/ZnIn₂S₄ is apt to hybridize with ssDNA anchored on Cu₃L₃-TAPT-COF, leading to the formation of the Hs/ZnIn₂S₄/Cu₃L₃-TAPT-COF/GCE complex (**Figs. 7.4c and 7.4d**). These results demonstrate the rationality of the p-n heterojunction quenching strategy.

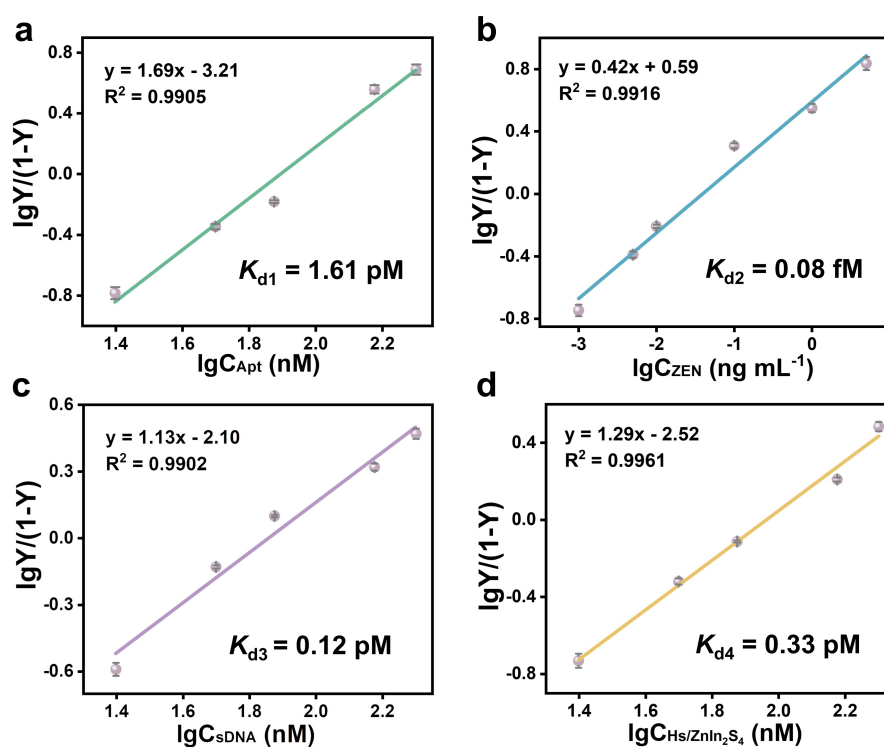


Fig. 7.4 Hill plots of (a) Hp/ZnIn₂S₄ and aptamer, (b) aptamer/Hp/ZnIn₂S₄ and ZEN, (c) Cu₃L₃-TAPT-COF/GCE and ssDNA, and (d) ssDNA/Cu₃L₃-TAPT-COF/GCE and Hs/ZnIn₂S₄.

Moreover, the anchored amount of ssDNA on the Cu₃L₃-TAPT-COF/GCE surface and the amount of Hp loaded on ZnIn₂S₄ were investigated. The results show that the surface density of ssDNA is approximately $5.49 (\pm 0.5) \times 10^{15}$ molecules per cm² (**Fig. 7.5a**), indicating the effective immobilization of ssDNA. Moreover, the amount of Hp loaded on ZnIn₂S₄ is around 1080 nmol mg⁻¹ (**Fig. 7.5b**), also hinting at the strong interaction between Hp and ZnIn₂S₄. These results guarantee the broad detection range of the developed Cu₃L₃-TAPT-COF-based PEC aptasensor toward ZEN.

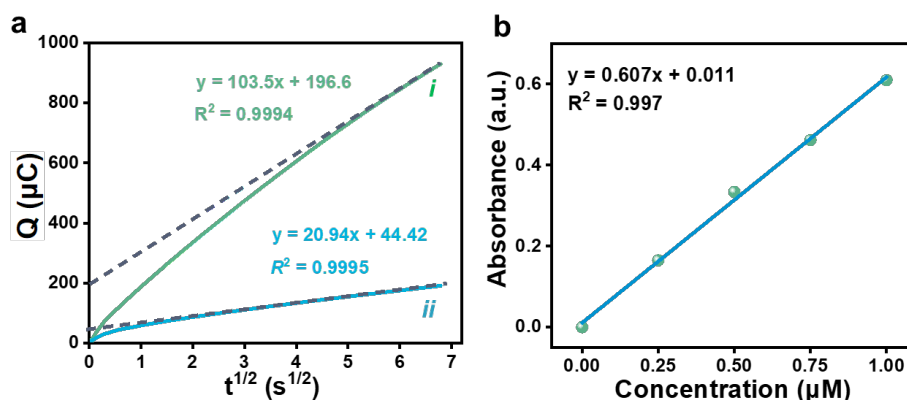


Fig. 7.5 (a) Chronocoulometric curves for the MCH/Cu₃L₃-TAPT-COF/GCE in (i) the presence ($100 \mu\text{M}$) and (ii) the absence of RuHex in 0.1M KCl at an applied potential (-0.3 V) and (b) the calibration curve of the aptamer concentration.

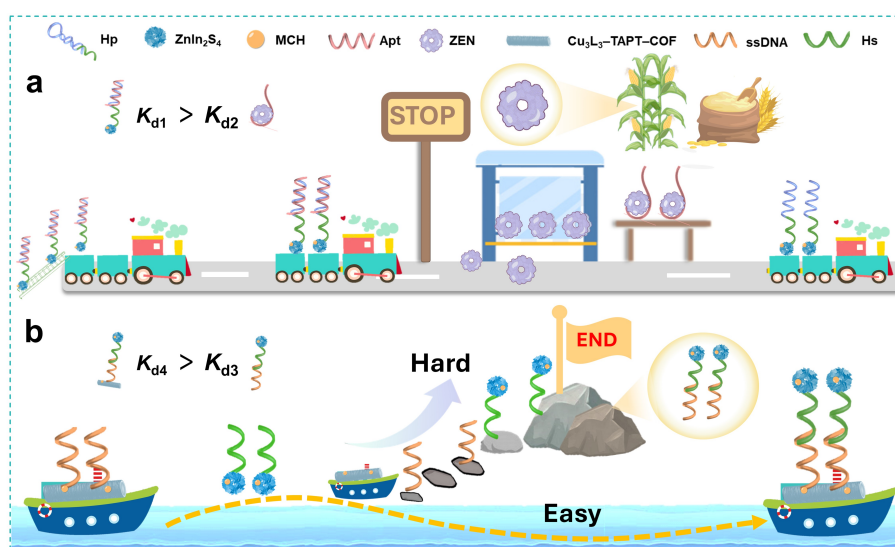


Fig. 7.6 Schematic illustration for the feasibility of the Cu₃L₃-TAPT-COF-based PEC aptasensor for the detection of ZEN.

Based on these discussions, the feasibility of constructing the Cu₃L₃-TAPT-COF-based PEC aptasensor was investigated under the assessment of the affinity abilities of the coupling interaction between different bioprobes and targets. The results reveal the high feasibility of the developed PEC aptasensor (**Fig. 7.6**). Moreover, the stepwise construction of the Cu₃L₃-TAPT-COF-based aptasensor was inspected using the PEC technique because of the superior PCE, enhanced photoabsorption ability, and amplified PEC response. As mentioned earlier, Cu₃L₃-TAPT-COF/GCE provides an enlarged photocurrent of 650.3 nA . Distinctly, the photocurrent of Cu₃L₃-TAPT-COF/GCE is markedly higher than those of some reported COFs, such as PA-T COF, P-bpy-COF, and TAPT-Tp-COP, again verifying the superior photoabsorption ability and high PCE of Cu₃L₃-TAPT-COF/GCE. Nevertheless, ssDNA/Cu₃L₃-TAPT-COF/GCE exhibits a declined photocurrent of 116.6 nA , which is largely attributed to the inhibition of charge transfer and photoabsorption and the steric effect of the anchored ssDNA. Subsequently,

MCH/ssDNA/Cu₃L₃-TAPT-COF/GCE illustrates the evenly reduced PEC response (76.1 nA) because the adsorbed MCH molecules hamper photoabsorption and photocarrier transfer. Moreover, quenching the PEC response of ssDNA/Cu₃L₃-TAPT-COF/GCE by using Hs/ZnIn₂S₄ further causes the decrease of photocurrent when detecting ZEN in the system. Therefore, the whole manufacturing procedure of the Cu₃L₃-TAPT-COF-based PEC aptasensor can be sensitively and quantitatively inspected by the variation of PEC response (**Fig. 7.7a**).

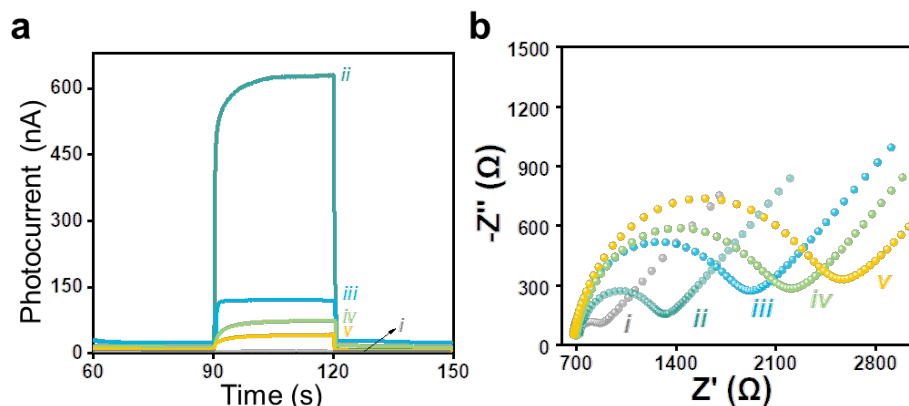


Fig. 7.7 (a) PEC responses and (b) EIS Nyquist plots of each step for the construction of the Cu₃L₃-TAPT-COF-based PEC aptasensor and the detection of ZEN (20 ng mL⁻¹), including (i) the bare GCE, (ii) Cu₃L₃-TAPT-COF/GCE, (iii) ssDNA/Cu₃L₃-TAPT-COF/GCE, (iv) MCH/ssDNA/Cu₃L₃-TAPT-COF/GCE, and (v) Hs/ZnIn₂S₄/MCH/ssDNA/Cu₃L₃-TAPT-COF.

As depicted in **Fig. 7.7b**, electrochemical impedance spectroscopy (EIS) Nyquist plots at each step of the construction of the Cu₃L₃-TAPT-COF-based aptasensor and the detection of ZEN were recorded. The bare GCE exhibits a small resistance of charge transfer (R_{ct}) of 100.5 Ω , suggesting its excellent electrochemical performance. Furthermore, Cu₃L₃-TAPT-COF/GCE presents a larger R_{ct} (476.5 Ω) than that of the bare GCE. This finding reveals that Cu₃L₃-TAPT-COF demonstrates inferior electrochemical activity than GCE due to its organic component. However, the R_{ct} of the Cu₃L₃-TAPT-COF-modified electrode is markedly smaller than those of reported COFs, such as Tp-BD-DBd COF, TAPB-DMTP-COF, and TAPP-COF, verifying its relatively fast electron transfer ability. Moreover, the immobilization of ssDNA and the adsorption of MCH onto the surface of Cu₃L₃-TAPT-COF/GCE continuously lead to an increase in R_{ct} values to 1.17 k Ω and 1.30 k Ω , respectively, due to the poor conductivity of biomolecules. In addition, the formation of the p-n heterojunction results in the further increase of R_{ct} (1.74 k Ω) on account of the steric-hindrance effect.

7.3.6 Quantitative analysis of ZEN

Under controlled variable conditions, the experimental conditions were also

screened to obtain the optimal signal response for ZEN detection, including the dosage of Cu₃L₃-TAPT-COF of 1.5 mg mL⁻¹, the concentration of ssDNA of 500 nM, the binding time for ZEN and aptamer/Hp/ZnLn₂S₄ of 40 min, the concentration of Exo III of 15 U mL⁻¹, the incubation time for Exo III of 1.5 h, and the incubation time for Hs/ZnLn₂S₄ and ssDNA/Cu₃L₃-TAPT-COF/GCE of 1 h (**Fig. 7.8**).

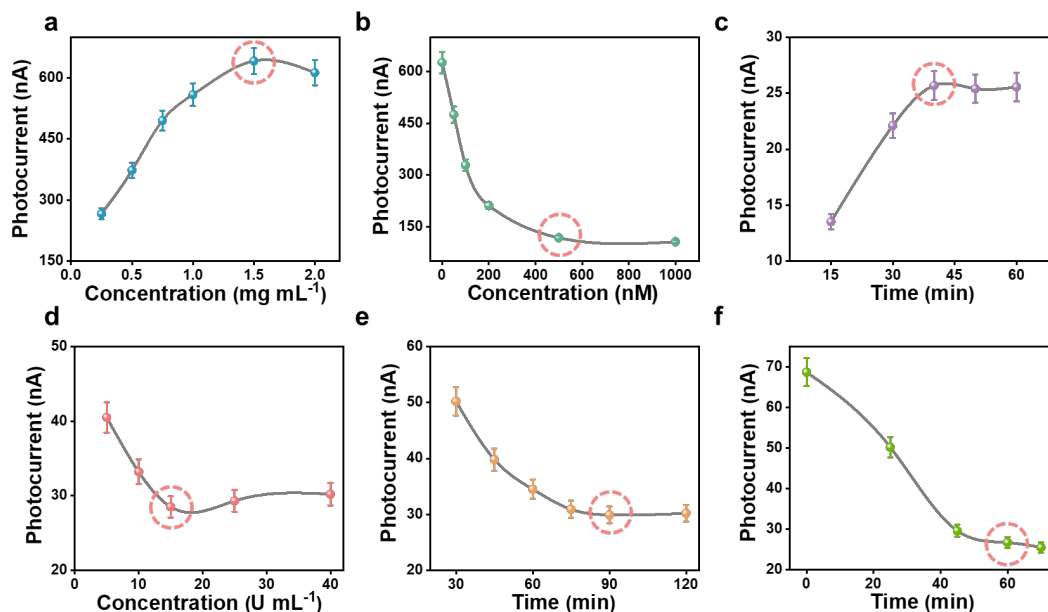


Fig. 7.8 The effects of the concentration of (a) Cu₃L₃-TAPT-COF and (b) ssDNA on the photocurrent of the Cu₃L₃-TAPT-COF/GCE. The effects of (c) the binding time of ZEN and aptamer/Hp/ZnLn₂S₄, (d) the concentration of Exo III, (e) the incubation time of the Exo III-assisted recycling process, and (f) the incubation time for Hs/ZnLn₂S₄ and ssDNA/Cu₃L₃-TAPT-COF/GCE on the photocurrent responses of the PEC aptasensor for the detection of ZEN.

Considering the enhanced analysis ability toward ZEN by using the manufactured PEC aptasensor, the quantitative analysis of ZEN is highly promising and performed by separately detecting ZEN with diverse concentrations (0.1, 1.0, 5.0, 10, 100, 1000, 5000, and 20000 pg mL⁻¹). As depicted in **Fig. 7.9a**, the photocurrent that results from the detection of ZEN increases with increasing ZEN concentration from 0.1 pg mL⁻¹ to 20 ng mL⁻¹. Such an increase can be attributed to the gradual decrease in quenching ability caused by the formation of the p-n ZnLn₂S₄@Cu₃L₃-TAPT-COF heterojunction. It occurs through the generation of dsDNA due to the hybridization of ssDNA immobilized on Cu₃L₃-TAPT-COF and Hs anchored onto ZnLn₂S₇. **Fig. 7.9b** depicts the caused variation of the photocurrent before and after the detection of ZEN ($\Delta I = I - I_0$) by using the Cu₃L₃-TAPT-COF-based PEC aptasensor, in which I and I_0 are the photocurrent of the modified electrode when detecting ZEN with a certain content and ssDNA/Cu₃L₃-TAPT-COF/GCE, respectively. ΔI dramatically decrease with increasing ZEN concentration from 0.1 pg mL⁻¹ to 20 ng mL⁻¹ but becomes stable when exceeding

20 ng mL⁻¹. The calibration curve is defined as highly linear and represented by ΔI (nA) = 7.09 + 10.35LogC_{ZEN} with a correlation coefficient ($R^2 = 0.9915$) (inset, **Fig. 7.9b**). Therefore, LOD is calculated to be 24 fg mL⁻¹ within the range of 0.1 pg mL⁻¹ to 20 ng mL⁻¹ at the signal-to-noise ratio of 3.

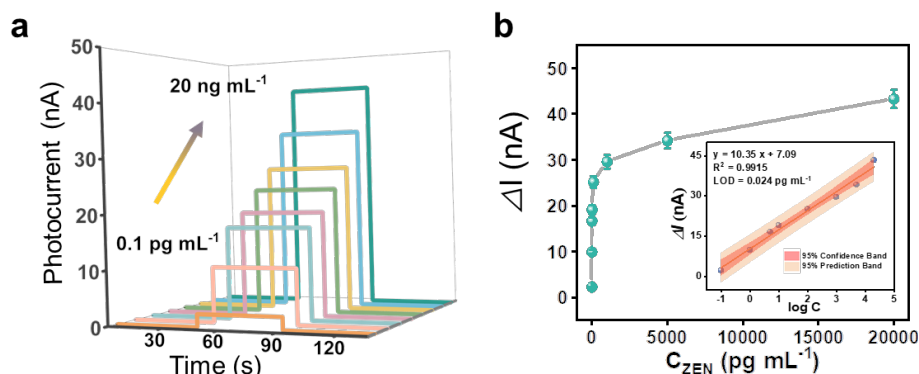


Fig. 7.9 (a) PEC responses of each step for the construction of the Cu₃L₃-TAPT-COF-based PEC aptasensor and the detection of ZEN (20 ng mL⁻¹), including (i) the bare GCE, (ii) Cu₃L₃-TAPT-COF/GCE, (iii) ssDNA/Cu₃L₃-TAPT-COF/GCE, (iv) MCH/ssDNA/Cu₃L₃-TAPT-COF/GCE, and (v) Hs/ZnLn₂S₄/MCH/ssDNA/Cu₃L₃-TAPT-COF. (b) The quantitative analysis of different concentrations of ZEN (0.1 pg mL⁻¹ - 20 ng mL⁻¹).

Table 7.1 Comparison of the proposed PEC aptasensor with other reported biosensors for the detection of ZEN.

Method	Material	Linear range (pg mL ⁻¹)	LOD (pg mL ⁻¹)	Refs.
ECL	PCN-222	5 – 1000000	0.17	[138]
ECL	NP-PtCo	5 – 25000	2.1	[139]
ECL	Au@MoS ₂ /Zr-MOF	0.1 – 100000	0.034	[140]
ECL	Au NPs@PBAs	0.5 – 100000	0.37	[141]
PEC	ZnO-NGQDs	0.1 - 100000	0.03	[131]
PEC	CdS/MoS ₂	1 – 1000	0.6	[142]
PEC	WO ₃ /BiVO ₄ @Ag ⁺	1 – 120000	0.28	[143]
CV	PEI-MoS ₂ -MWCNTs	0.0005 – 5	0.17	[144]
CV	hcPt@AuNFs/PEI-rGO	0.0005 – 50	0.11	[145]
DPV	MoS ₂ -rGO	1 – 100000	0.3	[146]
PEC	Cu₃L₃-TAPT-COF	0.1 – 20000	0.024	This work

As indicated in **Table 7.1**, the sensitivity of the developed Cu₃L₃-TAPT-COF-based PEC aptasensor is higher than those of reported ones for ZEN detection, such as ZnO-NGQDs, CdS/MoS₂, and WO₃/BiVO₄@Ag⁺. This appearance can be ascribed to the following aspects. (i) Cu₃L₃-TAPT-COF with a narrow bandgap, enhanced separation ability of photoinduced carriers, large porosity, specific surface, and single-atom sites exhibit enhanced PCE performance. (ii) Large amounts of ssDNA strands

anchored over the Cu₃L₃-TAPT-COF-modified electrode through the integrated effect of π - π stacking, van der Waals force, and possible Cu-N coordination interaction between ssDNA and Cu₃L₃-TAPT-COF provides high detection sensitivity. (iii) The binding of target ZEN with aptamer in an aqueous solution endows the PEC aptasensing system with high feasibility. (iv) Combining the target-modulated competitive binding method, Exo III-assisted signal amplification strategy, and p-n heterojunction quenching modal can robustly ensure detection accuracy and improve analysis selectivity.

7.3.7 Selectivity, stability, reproducibility, and practicality of the developed PEC aptasensor

The selectivity of the Cu₃L₃-TAPT-COF-based PEC aptasensor is the key for the effective analysis of ZEN in real samples. As indicated in **Fig. 7.10a**, the inspection of interferents (1.0 ng mL⁻¹) only provokes small photocurrents, which is significantly lower than that of ZEN detection (10 pg mL⁻¹). Moreover, the determination of the mixture of all interferents and ZEN exhibits a comparable PEC response to that of ZEN analysis. These results distinctly suggest that the given PEC aptasensor exhibits high selectivity when analyzing ZEN from a complicated environment. Furthermore, **Fig. 7.10b** presents the assessment results of the reproducibility of the proposed aptasensor. The results indicate that low relative standard deviations (RSDs) of 1.55%, 2.86%, and 3.32% are attained for the inspection of ZEN with 1.0, 5.0, and 20 ng mL⁻¹, respectively, suggesting the excellent reproducibility of the provided PEC aptasensor. In addition, the long-term stability of the used PEC aptasensor was also investigated. **Fig. 7.10c** shows the photocurrent generated by the determination of ZEN (0.1 ng mL⁻¹) each day, and it was recorded continuously for 16 days. The Figure demonstrates that the last signal maintains 90.7% of the initial one, with an RSD of 7.3 %. Therefore, the developed PEC aptasensor exhibits high stability for the analysis of ZEN in real samples. Considering the high sensitivity and selectivity, good reproducibility, and excellent stability of the developed PEC aptasensor, it can be extensively applied to the sensitive analysis of ZEN in food products. As indicated in **Tables 7.2** and **7.3**, the detection of ZEN in corn by using the Cu₃L₃-TAPT-COF- based PEC aptasensor achieves recoveries ranging from 96.4% to 109.4%, with low RSDs ranging from 1.7% to 7.3%. For the detection of ZEN in flour, the recoveries range from 97.8% to 110.0%, while RSDs range from 2.9% to 7.9%. In addition, the results of the *t*-test indicate no

significant systematic error based on a comparison of the $|t|$ values obtained with $|t|_{\text{critical}}$, $t_2 = 7.3$ in three replicate measurements. These results indicate that the proposed aptasensing strategy has demonstrated wide applications for the detection of ZEN in different food products. To further verify the accuracy of the PEC aptasensor in practical application, ELISA was also performed to determine ZEN in food products (Tables 7.4 and 7.5), and its result is close to the results obtained in the current work.

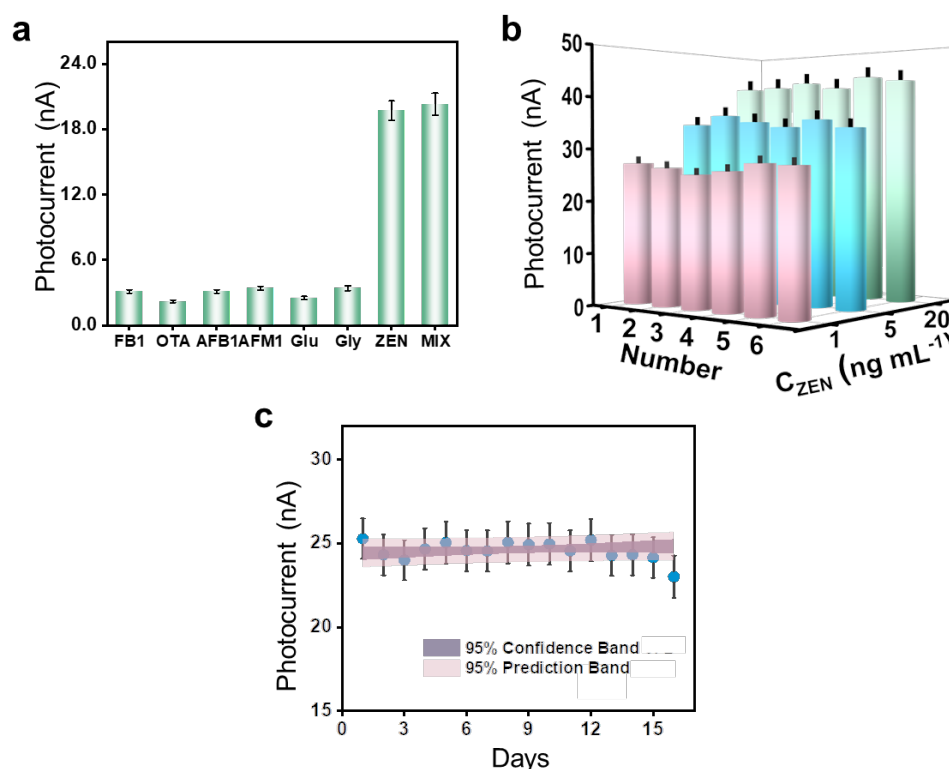


Fig. 7.10 (a) The selectivity of Cu₃L₃-TAPT-COF-based PEC aptasensor using FB1, OTA, AFB1, AFM1, Glu, Gly, and as interferents (10 pg mL⁻¹). (b) The reproducibility of the developed PEC aptasensor for the inspection of different concentrations of ZEN (1.0, 5.0, and 20 ng mL⁻¹). (c) The stability of the given aptasensor for the detection of ZEN (0.1 ng mL⁻¹) (under 95% confidence band).

Table 7.2 Analytical results of ZEN containing corn using the developed Cu₃L₃-TAPT-COF-based aptasensor.

Added amounts	Detected amounts	Recovery (%)	RSD (%)	<i>t</i> -test
0.1	0.099	99.0	4.3	0.41
1	0.973	97.3	1.9	2.69
5	5.2	104.6	2.1	3.17
10	9.6	96.4	2.3	3.14
100	103.6	103.6	1.7	3.54
1000	1054.2	105.4	3.5	2.54
5000	5196.7	103.9	3.8	1.72
20000	20890.5	104.4	2.5	2.95

$n = 3$ and the units were pg mL⁻¹, unless specified noted.

Table 7.3 Analytical results of ZEN containing flour using the developed Cu₃L₃-TAPT-COF-based aptasensor.

Added amounts	Detected amounts	Recovery (%)	RSD (%)	<i>t</i> -test
0.1	0.109	109.0	4.9	2.92
1	1.05	110.0	2.0	4.12
5	4.7	94.8	3.3	3.35
10	9.6	96.5	2.9	2.49
100	97.8	97.8	3.9	0.99
1000	985.6	98.6	4.2	0.60
5000	5143.7	102.8	3.5	1.38
20000	21455.2	107.2	3.2	3.67

Table 7.4 Comparative results of ZEN containing corn using the developed Cu₃L₃-TAPT-COF-based aptasensor and ELISA method.

Methods	Added amounts	Detected amounts	Recovery (%)	RSD (%)	<i>t</i> -test
PEC	1000	1054.2	105.4	3.5	2.54
	5000	5196.7	103.9	3.8	1.72
	20000	20890.5	104.4	2.5	2.95
ELISA	1000	1064.5	106.4	4.6	2.28
	5000	4870.3	97.4	1.7	2.71
	20000	18980.0	94.9	2.4	3.86

Table 7.5 Comparative results of ZEN containing flour using the developed Cu₃L₃-TAPT-COF-based aptasensor and ELISA method.

Method	Added amounts	Detected amounts	Recovery (%)	RSD (%)	<i>t</i> -test
PEC	1000	985.6	98.6	4.2	0.60
	5000	5143.7	102.8	3.5	1.38
	20000	21455.2	107.2	3.2	3.67
ELISA	1000	1063.1	106.3	2.8	3.56
	5000	4935.0	98.7	1.5	1.52
	20000	20658.5	103.3	3.8	1.45

7.4 Conclusion

To summarize, a novel PEC aptasensor was developed by introducing the target-modulated competitive binding method in the design of a multivariate signal-amplified (photoelectronic active COF, Exo III-assisted, p-n heterojunction quenching) strategy for ultrasensitive, high accuracy, and fast response of ZEN detection. DFT and basic characterizations revealed that the n-type Cu₃L₃-TAPT-COF network exhibited D-A conjugation, AA stacking, and ultrathin nanosheet structure, with a narrow bandgap, enhanced separation, and transfer of photoinduced electrons and holes. In particular, a

high content of Cu-N₂ single-atom sites was embedded into porous Cu₃L₃-TAPT-COF, remarkably facilitating electron transfer and binding interaction toward ssDNA. Given the high-affinity ability of ssDNA with Cu₃L₃-TAPT-COF, ZEN with the aptamer, and Hs with ssDNA, and the successful generation of the p-n heterojunction between Cu₃L₃-TAPT-COF and ZnIn₂S₄, the constructed Cu₃L₃-TAPT-COF-based PEC aptasensor exhibited an ultralow detection limit of 24 fg mL⁻¹ within a wide range of 0.1 pg mL⁻¹ to 20 ng mL⁻¹, along with high selectivity, good accuracy, excellent stability, and promising practicability. Therefore, this work provides a new aptasensing strategy for the sensitive and selective inspection of mycotoxins by using photoelectronic active COF as a bioplatform. It offers deep insight into the aptasensing mechanism by integrating diverse signal amplification methods.

Publications

1. Jo' Del Gobbo, Carlo Santini, Alessandro Dolmella, **ZhenZhen Li**, Miriam Caviglia, Maura Pelli, **"New copper complexes with N,O-donor ligands based on pyrazole moieties supported by 3-substituted acetylacetone scaffold"**, *Molecules* 29 (2024) 621. doi.org/10.3390/molecules29030621.
2. **ZhenZhen Li**, Yifei Wang, Xinxin Ji, Chuanpan Guo, Shuai Zhang, Qiaojuan Jia, Minghua Wang, Linghao He, Miao Du, Zhihong Zhang, **"Ultrasensitive "signal-inversion" photoelectrochemical aptasensor based on semiconductive MOF integrated with the manganese ferrite nanzyme-regulation for the selective detection of fumonisin B1"**, *Sensors and Actuators: B. Chemical* 420 (2024) 136473. doi.org/10.1016/j.snb.2024.136473.
3. **ZhenZhen Li**, Yifei Wang, Maura Pelli, Shuai Zhang, Minghua Wang, Serena Gabrielli, Cristina Cimorelli, Chuanpan Guo, Miao Du, Zhihong Zhang, **"Trinuclear copper cluster-based COF with a high content of Cu-N₂ single-atom sites: A multivariate signal-amplified photoelectrochemical aptasensor for the sensitive detection of mycotoxins"**, *Chemical Engineering Journal* 505 (2025) 159603. doi.org/10.1016/j.cej.2025.159603.
4. **ZhenZhen Li**, Yifei Wang, Serena Gabrielli, Cristina Cimorelli, Chuanpan Guo, Miao Du, Maura Pelli, and Zhihong Zhang, **"Donor-acceptor conjugated and triazine-containing covalent-organic framework: Construction of a signal "on-off-on" electrochemiluminescence immunosensor for efficiently detecting zearalenone"**, *Sensors and Actuators: B. Chemical* 433 (2025) 137539. doi.org/10.1016/j.snb.2025.137539.
- 5: Miriam Caviglia, **ZhenZhen Li**, Carlo Santini, Jo' Del Gobbo, Cristina Cimorelli, Miao Du, Alessandro Dolmella, Maura Pelli, **"New Cu(II), Cu(I) and Ag(I) complexes of phenoxy-ketimine Schiff base ligands: synthesis, structures and antibacterial activity"**, *Molecules* 30 (2025) 1893. doi.org/10.3390/molecules30091893.
- 6: Mingyang. Xu¹, **ZhenZhen Li**¹, **"Constructing S-scheme heterojunction between porphyrinyl covalent organic frameworks and Nb₂C MXene for photocatalytic H₂O₂ production"**. *Chinese Journal of Catalysis* 70 (2025) 431. doi.org/10.1016/S1872-2067(24)60247-0.
- 7: Chuanpan Guo, Yifei Wang, **ZhenZhen Li**, **"A Donor-Acceptor Covalent Triazine Framework-based "On-Off-On" Electrochemiluminescence Aptasensor for Detecting Aflatoxin B1 with the Assistance of Nicking Endonuclease-powered DNA Walking Machine"**,

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8. **ZhenZhen Li**, Miriam Caviglia, Cristina Cimorelli, Serena Gabrielli, Maura Pellei, Carlo Santini, Miao Du: “**Synthesis of selected Schiff bases and their copper and silver complexes**”. *TUMA 2023*. Francavilla al Mare (CH), June 22-23, 2023. Proceedings P9. **Best poster award TUMA 2023**.

9. Jo’ Del Gobbo, Miriam Caviglia, **ZhenZhen Li**, Serena Gabrielli, Cristina Cimorelli, Alessandro Dolmella, Carlo Santini, Maura Pellei, “**New and efficient copper(II) complexes as catalysts for allylic oxidation and Ullman-type reactions**”. *XIV International School of Organometallic Chemistry*. Camerino (MC), 7-11 September 2023. Proceedings, p. 58, PISBN 9788867680634.

10. Miriam Caviglia, **ZhenZhen Li**, Carlo Santini, Jo’ Del Gobbo, Luca Barigelli, Cristina Cimorelli, Miao Du, Alessandro Dolmella, Maura Pellei, “**Synthesis, characterization and antibacterial activity of new Cu(II), Cu(I) and Ag(I) metal complexes with phenoxy-ketimine Schiff base ligands**”. *50th Conference of the Divisione di Chimica Inorganica (INORG2025)*. Napoli, September 9-12 2025. Accepted.

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