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Visualising 2-simplex formation in metabolic reactions

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ABSTRACT

Understanding in silico the dynamics of metabolic reactions made by a large number of molecules has led to the development of different tools for visualising molecular interactions. However, most of them are mainly focused on quantitative aspects. We investigate the potentiality of the topological interpretation of the interaction-as-perception at the basis of a multiagent system, to tackle the complexity of visualising the emerging behaviour of a complex system. We model and simulate the glycolysis process as a multiagent system, and we perform topological data analysis of the molecular perceptions graphs, gained during the formation of the enzymatic complexes, to visualise the set of emerging patterns. Identifying expected patterns in terms of simplicial structures allows us to characterise metabolic reactions from a qualitative point of view and conceivably reveal the simulation reactivity trend.

1. Introduction

Metabolic reactions have been widely studied with the aid of computational approaches. In recent years modelling and computer simulations played a fundamental role in systems biology [4,5]; in particular, agent-based modelling and simulation methods have been successfully applied to the study of molecular interactions in metabolic pathways [2,8,22].

In multiagent system (MAS) simulations, agents are autonomous and asynchronous; therefore, their interactions can be placed in faithful correspondence with those occurring between the components of a biological system. Moreover, a MAS simulation has a light computational load and can handle compositionality more effectively than other approaches like PDE-based molecular dynamics.

However, understanding and representing as a whole the agent dynamics characterising qualitatively a metabolic reaction made by a large number of molecules constitutes a big issue [29,30].

The core idea of our work is to analyse the space of potential reactions of a simulated metabolic process with the topological data analysis, one of the most effective methods to extract patterns, which are hidden relations approximating a concept whose qualitative information (global properties) can be formally described [13,18,20,21,27]. This technique consists in building simplicial complexes, i.e. finite collections of objects, each of which could be seen as a n-bodies relation, and selecting the most meaningful one.

Weight Rank Clique Filtration and Persistent Homology are the two computational methods used to *map* simulation data into simplicial complexes, and to *visualise* the significant simplicial structures in the specific domain of metabolic reactions [9,11,19,28].

This approach allows us to define a new visualisation paradigm based on the concept of *interaction-as-perception*; whenever a molecule perceives another one to interact with, a potential link between the two is established. In this way we can derive the graph of perceptions at a given step; on those graphs, we apply the topological data analysis to capture the 3-body interactions through the interpretation of 2-simplices as observable structures, which are convex hulls of three points. We use the 2-simplex formation as a valid semantic to represent the global dynamics of the system.

Such a result has been possible thanks to two tools in the past developed at our lab: Hermes, the agent-based coordination middleware [10], and Orion, an agent-based spatial simulator [1,12,16].

2. Materials and methods

2.1. Multiagent modelling and simulation

Our work is based on a spatial simulator (Orion) for metabolic

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pathways built over Hermes (`emanuelamerelli.eu/hermes`), a MAS coordination middleware written in Java.

Each molecule of the modelled system is represented by an agent that runs continuously (i.e. it is persistent), perceiving and reacting to the environment changes.

In the model at the basis of our MAS simulator, each molecule is represented as a sphere, whose radius is calculated through the Richards formula [23], starting from its weight and placed in a three-dimensional space. Molecules are simulated with three types of agents: enzymes, complexes and metabolites. Enzymes and complexes are active entities (or agents), which can move and perceive cognate metabolites so as to interact with them (see Fig. 3 for a 3D representation of an instant of simulation).

The measuring unit of space is the picometre (10^{-12} m) while the time unit is 10^{-4} second, which corresponds to one tick of the simulation clock.

The movement of a molecule, in each step of simulation time, is given by a vector applied to the centre of its sphere; its module is calculated from the ambient diffusion coefficient D via the following Equations (1) and (2), while its direction is calculated randomly basing on polar coordinates.

$$D = \frac{k_B T}{6\pi\eta r} \tag{1}$$

where, k_B is the Boltzmann constant, T is the temperature, η the viscosity of the environment and r is the radius of the molecule.

$$\langle x^2 \rangle = 2Dt \tag{2}$$

where, assuming Brownian motion, $\langle x^2 \rangle$ is the average value of the square of the distance covered in a time t.

Two parameters are necessary to perform the reactions of the metabolic pathway correctly:

- K_m, which measures the affinity of an enzyme for a specific substrate;
- k_{cat}, which represents the number of molecules transformed by an enzyme in 1 s.

An enzyme can form a complex with an encountered metabolite on the basis of a list constructed over the k_{cat}/K_m ratio (specificity constant). If a complex is final (i.e. it represents the last step of a reaction), the system waits an amount of time obtained from the value of k_{cat} before converting it into the related products.

To find a cognate metabolite, each actor checks its presence inside a small neighbourhood surrounding itself.

The input of the simulator is an SBML (Systems Biology Markup Language) file, retrieved from the literature and filled with experimental data; it contains information about the molecules involved in the metabolic pathway and their initial concentrations. Data related to the reactions carried out (including the values of K_m and k_{cat}) are also taken from this SBML file and stored in an external XML database; such an approach allows us to chose a specific subset of reactions we want to focus on. The XML database also contains the values of weight and radius of interaction of each molecule.

The simulation produces as output an XML file filled, at each instant of the simulation, with the type and number of molecules contained in the simulated environment.

The model chosen to gain the data necessary for the simulation is "Smallbone2013 - Glycolysis in S.cerevisiae - Iteration 18" [24], accessible on the BioModels database at https://www.ebi.ac.uk/biomodels/MODEL1303260018. We opted for this model because it contains a complete set of experimental data (including enzymatic concentrations) about the well-studied organism Saccharomyces cerevisiae.

The reaction simulated for the aims of this study is the phosphorylation of glucose (GLC) catalysed by hexokinase (HXK), which produces glucose 6-phosphate (G6P) and adenosine diphosphate (ADP); the Smallbone2013 model takes into account the contribution of isoenzymes (for the hexokinase, they are HXK1, HXK2 and GLK1); therefore we considered the following three reactions:

$$\mathrm{GLC} + \mathrm{ATP} \xrightarrow{\mathrm{HXK1}} \mathrm{G6P} + \mathrm{ADP}$$

$$\mathrm{GLC} + \mathrm{ATP} \xrightarrow{\mathrm{HXK2}} \mathrm{G6P} + \mathrm{ADP}$$

$$\mathrm{GLC} + \mathrm{ATP} \xrightarrow{\mathrm{GLK1}} \mathrm{G6P} + \mathrm{ADP}$$

For such reactions, the Smallbone2013 model provides the experimental data summarised in Table 1.

2.2. Simplicial data analysis

Topological data analysis is a promising technique for finding hidden patterns in (big) data. It is based on topology, a branch of mathematics that studies the shapes of spaces. According to topology, a space can be characterised by some quantities, called *topological invariants*, that identify the space. In particular, those invariants can be thought as $n-$ dimesional holes. Given a set of points P (our data), a topological space is built over P, whose elements are equipped with a notion of proximity that characterises a coordinate-free metric.

As we work in a discrete domain, the focus is on a topological spaces called simplicial complexes.

Simplicial complexes are made up by building blocks called simplices: as depicted in Fig. 1, points are 0-simplices, line segments are 1-simplices, filled triangles are 2-simplices, filled tetrahedra are 3-simplices and so on.

A filtration is a collection of nested simplicial complexes. Performing a filtration can be seen as wearing lenses for examining the dataset: different lenses consent to extract different kinds of information from the topological space; different filtrations give rise to different conversions of the data points into simplicial complexes. In this paper, we use the Weight Rank Clique Filtration.

Topological invariants are obtained by using Persistent Homology. It is the computational counterpart of homology, an algebraic object that counts the number of n-dimensional holes in a topological space, also called Betti numbers. The set of Betti numbers is composed by β_0, the number of connected components in a generic topological space S; β_1, the number of holes in S; β_2, the number of voids in S and so on; in Fig. 1 it is possible to distinguish β_0 and β_1.

Table 1
Initial concentrations and kinetics parameters from Smallbone2013 model [24].

ID	*Conc.* (mM/l)	k_{cat} (s^{-1})	K_{GLC} (mM)	K_{ATP} (mM)
enzymes				
HXK1	0.017	10.2	0.15	0.293
HXK2	0.061	63.1	0.2	0.195
GLK1	0.045	0.0721	0.0106	0.865
metabolites				
GLC	6.28	/	/	/
ATP	4.29	/	/	/
ADP	1.29	/	/	/
G6P	0.77	/	/	/

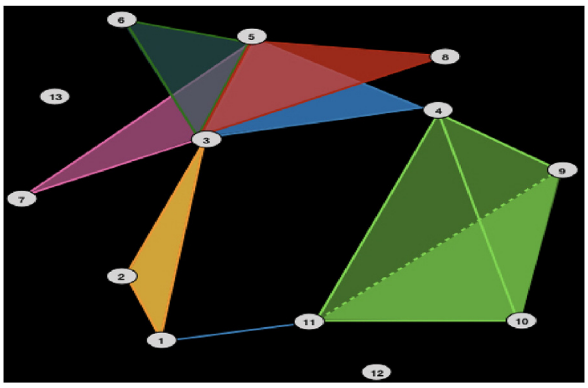


Fig. 1. A graphical example of a simplicial complex and its building blocks, called simplices. Each simplex is represented by a different colour: the light-green tetrahedron is a 3-simplex; the yellow, the red, the blue, the dark-green and the violet triangles are 2-simplices; the dark-blue line is a 1-simplex and the grey points are 0-simplices. In the figure, the two 0-simplices, labelled 12 and 13, and the large structure made by 1-, 2- and 3- simplices, are the three connected components (that implies $\beta_0 = 3$). A 1-dimensional hole (made by the points 1, 3, 4 and 11) is also represented as part of the large structure (implying $\beta_1 = 1$). Moreover, it is possible to notice a "booklet-like" simplicial structure made by the red, the dark-green, the violet and the blue 2-simplices, sharing a common "backbone" edge, which plays a central role in this work, as discussed in Section 3.

Along the filtration, persistent homology calculates k-dimensional Betti intervals: a k-dimensional Betti interval $[t_{start}, t_{end}]$ defines the time at which a k-dimensional hole appears in the simplicial complex t_{start}, while t_{end} is the time at which it disappears. The holes that are still present at $t_{end} = t_{max}$ correspond to persistent topological features.

A graphical representation of those intervals in S is called persistence barcode and it is associated with a filtration. An equivalent representation is given by persistence diagrams.

2.2.1. Weight Rank Clique Filtration

Weight Rank Clique Filtration (WRCF) is a particular filtration that is designed for operating on graphs: it allows us to build a simplicial complex starting from a weighted undirected graph. An example of a simplicial complex obtained from an undirected graph is shown in Fig. 2.

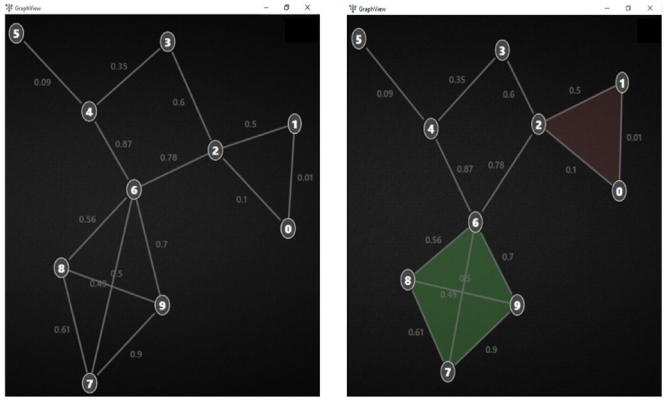


Fig. 2. A comparison between a weighted undirected graph **(a)** and a simplicial complex **(b)**, given by our visualisation tool after the filtration procedure. The two representations are based on the same set of nodes but, in (a), the set of 6, 8, 7 and 9 nodes represents a 4-clique, while in (b) a 3-simplex, the green tetrahedron. WRCF acts on the weights of the graph and builds the higher order structure whose mining is different from the sum of the local interaction simplicial. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

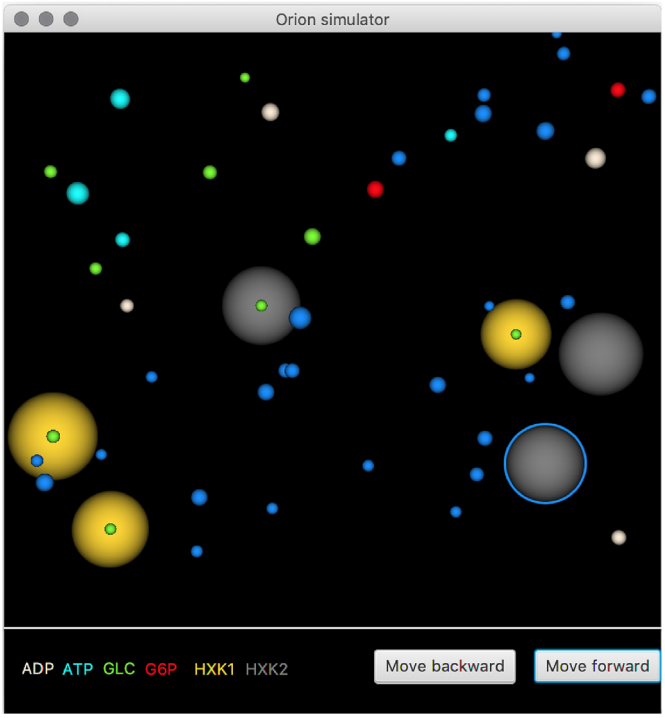


Fig. 3. 3D portion of the MAS simulation environment captured from the Orion simulator prototype interface. Each molecule is represented as a sphere; the HXK2 isoenzyme identified by the blue circle perceives all the metabolites highlighted in blue. The yellow and grey spheres with green centres represent dual-complexes formed by a GLC metabolite docked to an HXK1 and to an HXK2 enzyme respectively. The GLK1 isoenzyme is not visible in the above represented simulation.

Graphs are mathematical objects that lie in two dimensions: using simplicial data analysis we derive from a graph the relative simplicial complex that can be in any dimension. To perform the Weight Rank Clique Filtration and the visualisation, we used a tool that is currently under development at the Bioshape and Data Science Lab of the University of Camerino. This tool exploits the Javaplex library for the computation of homology and the GraphSharp library for visualisation [25].

2.3. Interaction-as-perception paradigm

The output of the simulator has been adapted to carry out a topological interpretation of the modelled molecular interactions. To achieve this result, we defined an *interaction-as-perception* paradigm applied to the agent dynamics of our metabolic simulator. The idea at the basis of this approach is that the perception between cognate partners could be interpreted as an abstraction for a complex formation.

Turning to the details, we generated, along with the standard output of the simulator (as described in Section 2.1), additional information about every interaction performed at each time step. In particular, we gain the identifier of all the molecules involved in such an interaction and the value of the related k_{cat}/K_m ratio. Basing on these data, we can define the following classes of perception:

- **Direct unstable perception**, of an enzyme for one of the possible cognate metabolites identified in its surroundings.

- **Direct fixed perception**, of an enzyme for an already docked metabolite (so as to form a dual-complex).
- **Indirect unstable perception**, of the metabolite forming the dual-complex for an external one perceived by the cognate enzyme; the enzyme mediates this kind of perception which, by convention, has the fixed value of 0.001.
- **Indirect fixed perception**, of a metabolite for another metabolite docked to the same enzyme.

By analysing the dynamics of the MAS simulations from the above-defined perspective, we can observe the following behaviours:

- A *free enzyme* can make no perception (if there is no other compatible molecule in its surroundings) or just direct unstable perceptions.
- A *dual-complex* (formed when an enzyme binds one of the perceived metabolites) always carries out an inner fixed perception - of the enzyme for the docked metabolite. Two additional kinds of perceptions are generated for every external compatible metabolite it identifies, i.e. the direct and the indirect unstable perceptions performed respectively by the enzyme and by the metabolite composing the dual-complex.
- A *saturated enzyme* can show just the direct fixed perceptions of the enzyme for the docked metabolites and an indirect fixed perception between the two metabolites (if more than one is present, as in the case of the reaction we analysed). This condition is maintained for the duration of the delay given by the k_{cat} value of the reaction (when it runs out, the enzyme returns free and two new metabolites are released in the simulation environment).

These three different behaviours identify the states of the automaton highlighting the cycle of an enzymatic reaction. As shown in Section 3, the iteration of this cycle drives the evolution of the reaction through phases of higher/lower stability, a property that we highlight through a quantitative analysis the topological representation (2-simplex) of intermolecular perceptions (see Fig. 5a). The 2-simplex structures provide an higher order global representation of interaction compared to that of a classical MAS model. In the latter, each molecular interaction is 2-body, defined according to the biochemical reactions (like those shown in Section 2.1), and generates a new agent (a new complex or a final product); conversely, in the topological setting, all the potential interactions between molecules are 3-body and represented as a whole on the basis of the interaction-as-perception paradigm.

3. Results

By applying our MAS simulation to study the metabolic reactions catalysed by hexokinase isomers (see Section 2.1 for details), we can observe how the molecules in the simulated environment move and interact at each time instant.

To analyse the dynamic evolution of each reaction from a topological point of view, we need to abstract from the standard spatial simulation output, basing on an interaction-as-perception paradigm. According to such an approach, an enzyme perceives a cognate metabolite whether a metabolite enters its interaction volumes or a docking between the two molecules actually happens.

The network of intermolecular perceptions (modelled on the basis of the above-described approach) can be interpreted in terms of simplicial complexes formation, where, every time an enzyme perceives a cognate metabolite, an edge among the two molecules is defined.

Changes in simplicial structures go along the evolution of the simulated reaction, according to the following general observations:

- at the beginning of the simulation, every molecule in the simulated volume do not perceive nor interact; therefore the topological environment is filled with sparse nodes (see Fig. 4a);
- in the first simulation instants, since enzymes start to perceive the related substrate, we can observe the formation of isolated enzyme-metabolite edges (1-simplices) as well as of "dandelion-like" structures (Fig. 4b), made by a central hub (the enzyme) connected to multiple nodes (metabolites);
- dockings between an enzyme and a single metabolite are caught in our representation by the formation of stable isolated 1-simplices composed by the two nodes;
- each metabolic complex may perceive the presence of the metabolite needed to saturate the enzyme; in this case, we can both observe the presence in the environment of isolated triangles (2-simplices) and "booklet-like" complexes, each made by an edge placed at the centre of a star of 2-simplices and linking the half-saturated enzyme to its bound metabolite (as shown in Fig. 4c). Every triangle of this type is a potential stable link connecting the central complex and the opposite vertex;
- the potential condition described above is resolved when a fully saturated enzyme forms and can be identified by a stable 2-simplex; each final complex lingers in the simulation volume for a time given by the experimental value of the related k_{cat}, therefore, after such a delay, three new isolated nodes appear in place of a 2-simplex (Fig. 4d), i.e. the ones represented the enzyme and the products of the catalysed reaction.

All the simplicial complexes we can observe during the time evolution of the simulation have a direct correlation with the perception-based structures described in Section 2. In Table 2 we summarise such relations by tracing each simplicial structure, identified in the previous description, back to the interaction-as-perception paradigm.

Representing through the above-described simplicial approach the dynamics of the MAS simulation allows us to highlight some fundamental properties of metabolic reactions progression over time. Specifically, we can observe that changes in system's reactivity are affected by the fluctuation of 2-simplices concentration.

A simulated reaction alternates states of high reactivity and states of semi-stability that can be correlated to the number of 2-simplices identifiable in the environment. Stars of 2-simplices determine the instability of the system; therefore, we observe high concentrations of these structures during the reactive phases. As shown in Fig. 5, considering a long temporal horizon, blocks of reactive phases are clearly distinguishable from the ones almost saturated with stable 2-simplices (representing final molecular complexes).

Inside these higher reactive blocks, the formation of stable 2-simplices causes the transition from a reactivity phase to another, in most cases identifiable by two opposite and overlapped spikes of the graph. Indeed, a new stable 2-simplex forms when a star of 2-simplices resolves its instability (by choosing one of the possible associated peripheral nodes); such an event determines the immediate drop of the system's 2-simplices overall amount correlated to just one unit increase of stable 2-simplices.

As we can observe in Fig. 5, such a behaviour determines a progressive decrease in 2-simplex stars amount, and therefore in block's reactivity, over time.

We also highlight that a transition between a stable phase and a reactive block is related to the k_{cat} value of the reaction, since it determines the time interval through which a stable 2-simplex maintains its conformation.

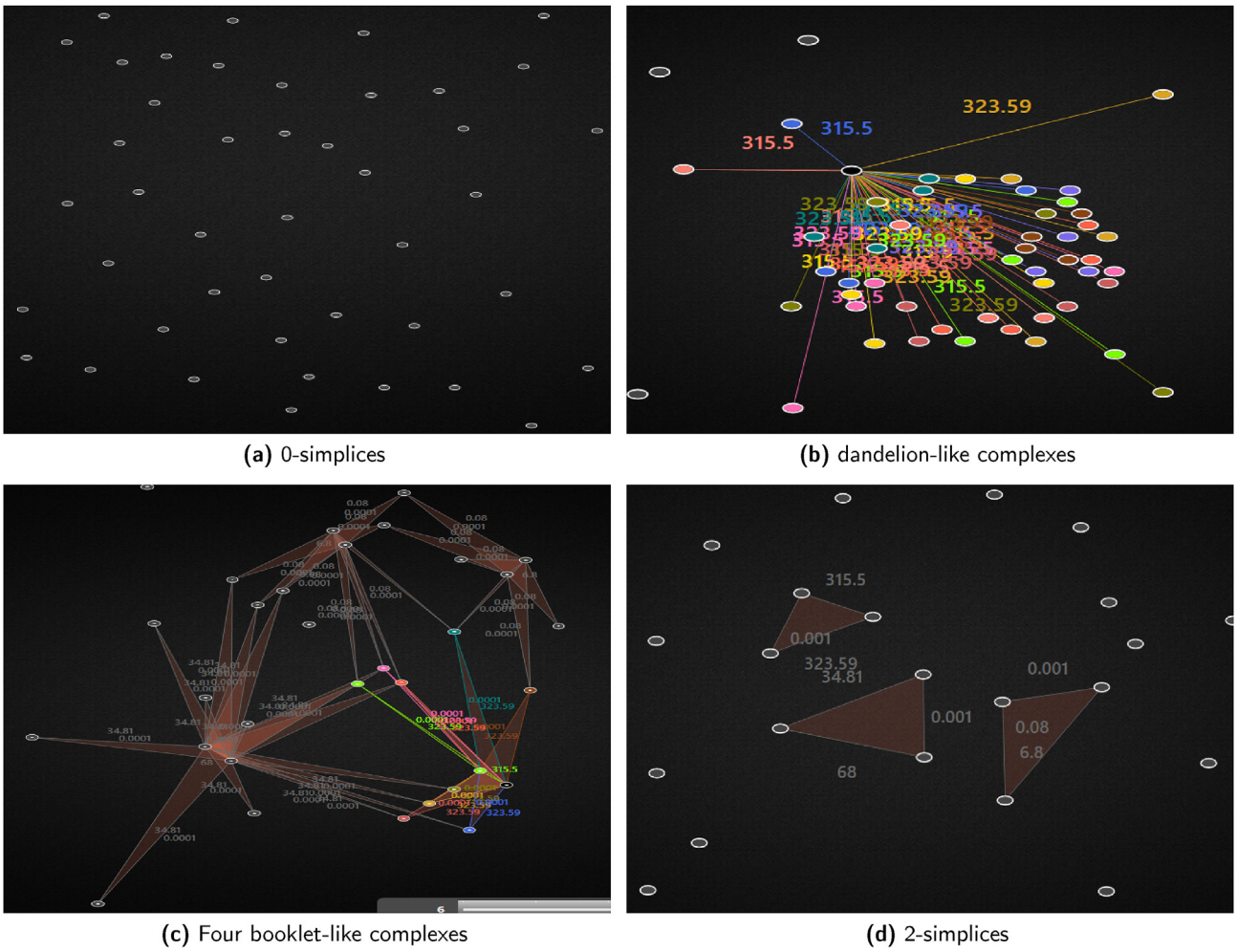


Fig. 4. This figure shows the most significant structures we can identify through our topological analysis of the simulation. **(a)** 0-simplices representing all the molecules at the beginning of the simulation; **(b)** a "dandelion-like" structure made by a central node (enzyme) linked to the nodes representing the compatible substrate in its neighbourhood; **(c)** "booklet-like" structure composed by a central hub made by two linked nodes (corresponding to a "dual-complex" enzyme-metabolite) each forming an edge with an external node, i.e a metabolite that can complete the enzyme saturation; **(d)** isolated 2-simplices correlated to the saturated enzymes identifiable in this portion of the environment. In figures (b), (c) and (d), the value above each edge, i.e. its weight, represents the specificity (k_{cat}/K_m ratio) of the enzymes for the cognate metabolite connected by the arch itself.

After such a delay elapsed, the product is released, and the enzyme starts to look for a new substrate, pushing the system towards a new reactive block.

In Section 2.3, we mentioned a three-state automaton as a formal representation of the studied enzymatic reaction. The progression through phases of the simulation as described above is directly related to the cyclical iteration of the three states of a reaction, identified by the molecular structures that cause them, i.e. free enzymes, dual complexes and saturated enzymes (see Fig. 5a).

4. Discussion

In the present work, we use a MAS simulation to generate the dynamics of a complex system, while the Weight Rank Clique Filtration and Persistent Homology to try to visualise and understand the global properties of that system.

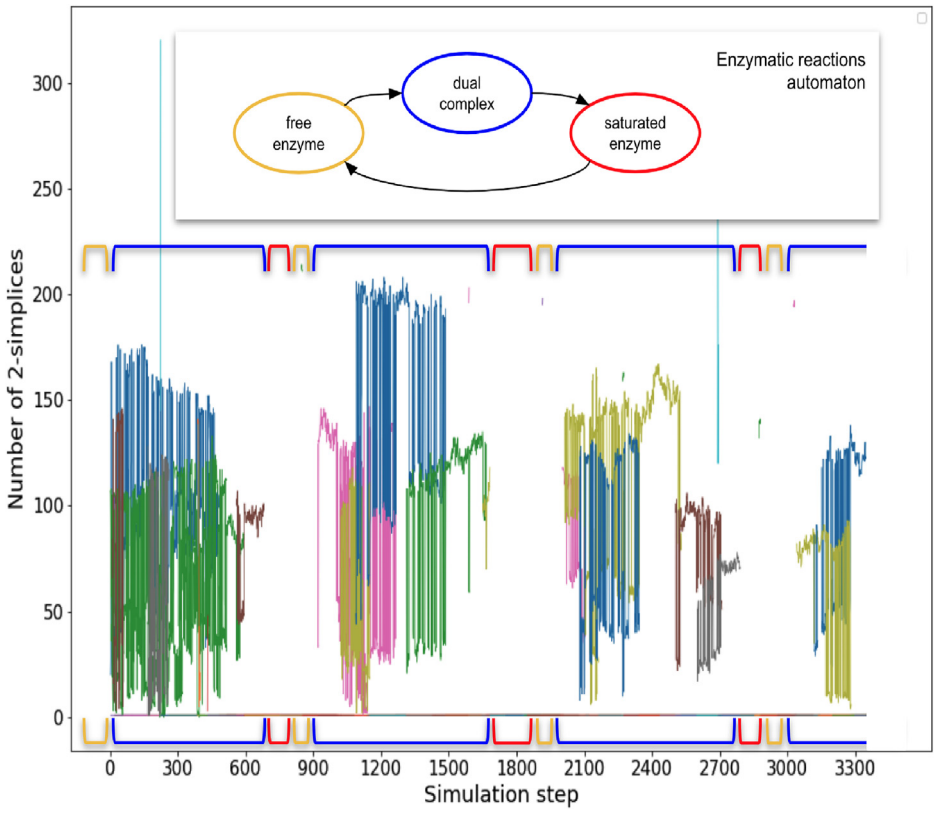
Thanks to the interaction-as-perception paradigm, the visualisation clearly highlights the formation of the simplicial structures characterising the system.

Such structures are directly correlated to the dynamical evolution of molecular complex formation and allow us to identify specific patterns that underline the in silico behaviour of a metabolic reaction.

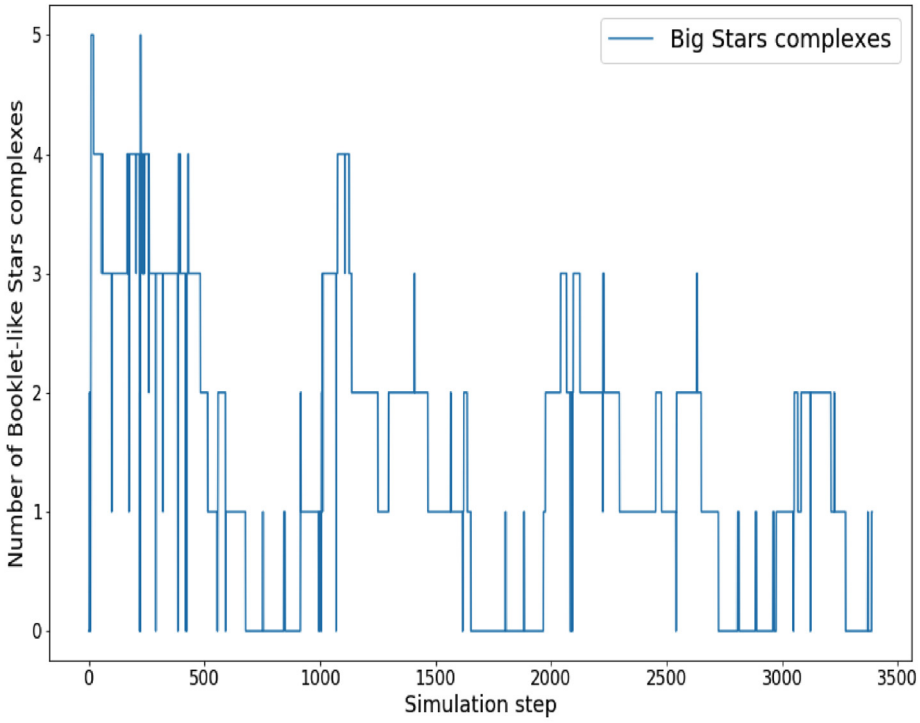
Moreover, those instruments gave us some insights, in terms of topological invariants, of what happens in the simulated systems.

One of the major results is the pattern of the Betti numbers, β_0 and β_1. It indicates that after the formation of a stable 2-simplex, all the molecules represented by the nodes linked in such a structure are no longer involved in any perception: thus β_0, the connected components, has its maximum values while β_1, the planar holes, has its minimum values (See Fig. 6).

Even if we do not claim to infer from these results any direct biological meaning, we hypothesise that both the above-mentioned patterns reveal the reactivity trend of the modelled reaction, turning out to be an effective validation tool for a biochemical reaction simulation. Indeed, we can compare the highlighted trends with the ones obtained by applying our visualisation method to other well-proven modelling approaches (e.g. based on PDE or SDE) or even directly to experimental data; it might allow us to identify how the simulated process differs from the one chosen as benchmark, and consequently make the necessary adjustments to make them fit.



(a)



(b)

Fig. 5. **(a)** Changes over time of the number of 2-simplices associated to each edge representing a dual complex; they are plotted along with the number of the stable 2-simplices (correlated to saturated enzymes). The aim of this plot is to provide a global view of how, on a long temporal horizon, highly reactive blocks alternate with time intervals dominated by stable 2-simplices. Each block is correlated to the automaton states representing the three steps of the enzymatic reaction, respectively dominated by high concentrations of free enzymes (yellow state), dual complexes (blue state) and saturated enzymes (red state). Their iteration drives the evolution of each reactivity block shown in the plot, as identified by the square brackets coloured as the related state of the automaton. Due to the large number of complexes represented, a complete legend describing all of them would impact the readability of the figure. **(b)** Changes in the number of booklet-like complexes over time. By comparing this plot to the one shown in (a) it is possible to observe that they describe a similar trend of the reaction. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 2
Correlation between interaction-as-perception paradigm and simplicial structures.

Interaction as perception (MAS Simulation)		Simplicial Data Analysis
Molecule	Perception	Structure
free enzyme	no perception	0-simplex (isolated node)
	direct unstable perception	1-simplex\dandelion-like structure
dual-complex	no perception	1-simplex
	direct unstable perception (external) indirect unstable perception (external) direct fixed perception (internal)	2-simplex booklet-like structure
saturated enzyme	direct fixed perception (internal) indirect fixed perception (internal)	stable 2-simplex

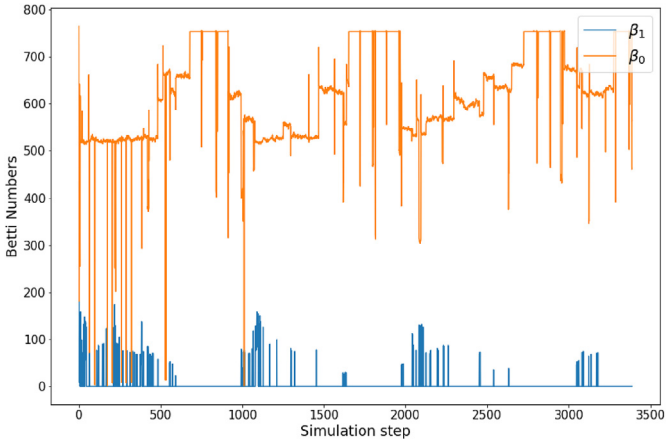


Fig. 6. The evolution of the Betti numbers, β_0 and β_1, during the simulation.

5. Conclusions

Agent-based computational models and simplicial data analysis are well suited methods for simulating and visualising the dynamics of complex systems, which are characterised by high number of entities interacting in a bounded space. Moreover, they allow us to represent some specific features of the system to be compared with empirical observations or experimental data in a future work. By studying the emerging behaviour of a MAS simulation with simplicial data analysis we have advanced the visualisation capabilities of the Orion simulator. The visualisation allowed us to identify the simplicial structures associated with the reaction space over time. This result might reveal to be a useful validation tool for the MAS simulation itself. Indeed, it opens to the possibility of performing the same simplicial data analysis on empirically retrieved data, so as to verify the faithfulness of the simulation to the actual biological process [17].

At the same time, identifying patterns in the reactivity associated with molecular interactions graph might provide computational support for studying therapies based on drug targeting and enzyme inhibition [6,7,14,26]. However, we want to point out that at the current stage of the study we are still validating and testing the proposed simulator.

As further developments, we are working on other validation approaches that could be combined with those mentioned above, and in particular, the ones involving innovative applications of formal methods in the analysis of biological processes [3,15].

Declaration of competing interest

The authors declare no competing interests.

CRediT authorship contribution statement

Marco Piangerelli: Data curation, Writing - original draft, Conceptualization, Methodology, Software. **Stefano Maestri:** Data curation, Writing - original draft, Conceptualization, Methodology, Software. **Emanuela Merelli:** Conceptualization, Methodology, Supervision, Writing - review & editing.

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