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Peer-Review Record:

Emergent Chemical Behavior in Variable-Volume Protocells

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Reviewer 1: Anonymous
Reviewer 2: Anonymous
Editor: Fabio Mavelli and Pasquale Stano (Guest Editor of Special Issue "Protocells—Designs for Life")

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First Round of Evaluation

Round 1: Reviewer 1 Report and Author Response

Novel concepts in establishing "minimal" models for chemical oscillators in internal compartments of proto-cellular agents and semi-permeability of membranes. Produces a dynamic model for chemical evolution rather than traditional deterministic, static, systems that requires centralised information to direct evolution, such as a constant gradient.

Very well presented, clear and graphically illustrated experimental findings that make a clear case for the model.

Also a very thorough understanding of the limitations of the system are presented as well as proposals for even more complex instabilties that may underpin lifelike events.

Overall a beautifully written, wonderfully presented, paper with clear concepts, methods and proposals that significantly contribute to original knowledge in the field.

Response: This review does not require any action on our part.

Round 1: Reviewer 2 Report and Author Response

The article "Emergent Chemical Behaviour in Variable-Volume Protocells" focuses on the consequences that a changing volume may have on a set of reactions encapsulated within a semipermeable vesicle; in particular, this kind of compartment can degenerate existing bistable reactions, or promote emergent bistability from very simple reactions, which are not bistable in bulk conditions. Remarkably, even reaction systems that have no chemical species in common could become indirectly coupled to each other through the volume they share, by means of a sort of osmotic coupling. The authors discuss these issues by means of differential equations and simulations.

The article is clear and well-written. The mathematical methods used to deal with the problem are based on approximations and require the support of a huge number of simulations; on the other side, the approximations themselves constitute an interesting feature of the article and allow interesting discussions on the involved physical situations. As the authors acknowledge, the article is the first step toward a more complete understanding of an interesting class of possible chemo-physical phenomena.

I have few question/comments:

- The authors write: "In these initial stages, the emergence of bistability will serve as a proxy for the emergence of other chemical novelties in the vesicle reactor model." The authors probably means that the existence of more than one stable regime could consent regime changes, but this situation do not indicates how these changes appear: in the article, the change is stimulated by means of a deliberated injection of chemical substances from outside. So, I think that the appearance of novelties requires a different order of considerations, whereas the bistability plays possibly the role of novelties amplification and consolidation (in particular conditions). The same observation can be done for the word "innovation", used on the abstract and at Line 45. In order to avoid misunderstandings, I think that the authors should avoid these expressions, ad use the words "regime change", or similar concepts.

Response: Here, the reviewer did not like our use of the particular words "novelty" and "innovation".

- *Use of the word novelty*
  - *Instances of "chemical novelty" in the manuscript replaced by "emergent chemical behaviour".*
  - *On Page 8, after Equation 8, we removed sentence:*
    The emergence of bistability will serve as a proxy for the emergence of other chemical novelties in the vesicle reactor model.

Replaced whole paragraph, to read:

In particular, in these initial stages, we will focus on the emergence of bistability in the vesicle reactor model—a dynamical feature deducible directly from the number and stability of the fixed points present (i.e., two asymptotically stable points separated by an unstable saddle point). We also expect that more complicated dynamical regimes could also be present in the model, like multi-stability or global phase space features such as limit cycles giving rise to sustained oscillations. However, investigation of these regimes will be deferred to later work: for the time being, the "emergent chemical behavior" referred to in the title will be restricted to bistability.

We think this is a clearer explanation, and also uses the word "regime" suggested by the reviewer.

- *Use of the word innovation*

We kept the 2 occurrences of the word "innovation" in the abstract and the introduction. An innovation is defined as a "new method, idea or product" in the dictionary. We use the word to refer to new emergent chemical behaviour that the whole vesicle system exhibits (e.g., expanded steady states), which did not exist before. We think the use of this word is acceptable.

- Note 4 presents a very important issue: why the inner chemical environment is different from the external one, if the running chemical reactions are the same? The presence of particular features of the vesicle interior or of entrapped catalysts seem a too easy and *ad hoc* hypothesis. A useful reference proposing an explanation of this symmetry breaking (without requiring different conditions in internal and external environments) could be "R. Serra, M. Villani *Mechanism for the formation of density gradients through semipermeable membranes* Physical Review E 87, 2013".

Response: we added a new footnote, to explain our standing on this issue:

Reference 36: In this work, concentrations outside the vesicle are set as system parameters. However, we make no commitment to the type of environment the vesicle is embedded in or how these concentrations are maintained. Our purpose is simply to show that bistability can exist in the model for certain sets of outside concentrations. Exploration of the model in explicit environments is deferred to future work.

- How the authors derive the range [0.9, 101/3] for the so called "reduced surface" index? In Figure 1d, Lines 21/3 and 41/3 have some particular meanings, or are plotted mainly to ease the figure comprehension? Could the authors add some indications about the derivation of these upper limits?

Response: Reference 11 has been added after Equation 10, to point the reader to where to find information about phi limits was first discussed. Repeating this information here would complicate the paper. The lines Phi = 2^(1/3) and 4^(1/3) in Figure 1d have now been explained.

- The authors write (case 1): "of the 5000 parameter set tested under constant surface area, 82% (4098) gave a single fixed point, 321 15.7% (785) gave two fixed points, and 2.3% (117) no fixed points." How this statistics compare with a similar one, performed on a similar chemical situation without container? Could the authors add the results of the same within a not confined environment?

Response: It is difficult to make a direct and meaningful comparison like this. Without a container, the reaction system has less parameters, and thus the sampled parameter space is smaller. The aim in the paper, was to simply show that encapsulating a Schlogl model which was bistable in bulk condition, seemed to destroy this bistability.

Other minor issues:

- Line 89: "where the vesicle has again fixed surface area, but now variable volume" should be: "where the vesicle has again fixed surface area, but not variable volume".
- Adamala, K.; Szostak, J. ompetition betweenmodel protocells driven by an encapsulated catalyst. Nat. Chem. 2013, 5, 495–501. → "ompetition" should be: "Competition".

Response: The minor issues were fixed.