Optimizing the assembly of stacked rings

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***Short Abstract* — Many macromolecular machines, like GroEL and the proteasome, consist of ring-like structures that stack on top of one another. It has been shown that these structures can suffer from a phenomenon we term "assembly deadlock," which occurs when smaller intermediates are exhausted from the system before all of the full structures have formed. In this work, we used mathematical models to systematically study the impact of deadlock on stacked ring assembly *in vitro* and *in vivo*. Our models predicted that certain patterns of interaction affinities would maximize assembly yield. Analysis of solved structures of stacked rings confirmed these predictions.**

***Keywords* — Self-assembly, Deadlock**

I. Introduction

MANY macromolecular machines inside the cell adopt a "stacked ring" architecture, with multiple rings of protein subunits bound to one another. Examples of such structures include the proteasome, bacterial proteases like ClpXP, and the chaperonin GroEL [1,2]. The majority of these machines must adopt their fully assembled quaternary structure in order to function, making the assembly process vital for cellular function and survival [1].

We recently demonstrated that ring-like structures can suffer from assembly deadlock when small intermediates are exhausted from the system but large, unfinished structures persist. These large intermediates cannot react with one another due to steric clashes, and drive down assembly yields both *in vitro* and *in vivo* [3]. Others have found evidence that stacked rings may suffer from a similar phenomenon [4,5], but it is currently unclear to what extent deadlock has driven the evolution of stacked ring assembly pathways.

In this work, we use a set of efficient simulation methodologies developed in our lab to systematically study the assembly of stacked rings.

II. Results

We have developed a mathematical framework for modeling the assembly of ring-like structures [3]. Our approach is inspired by the binary encoding of Saiz and Vilar [6] as well as rule-based modeling techniques like BNGL [7]. Briefly, we first define a target structure (e.g. a three- or seven-member stacked ring) and then enumerate all possible assembly intermediates that could form. Using a few basic assembly principles, we then enumerate all possible chemical reactions, generating a Chemical Reaction Network (CRN) that we can simulate using Ordinary Differential Equations (ODEs) [3]. This deterministic approach is far more efficient than existing stochastic methods [4,5], allowing us to consider a large number of structures and parameter sets.

Using this model, we found that assembly deadlock is considerably worse for stacked rings compared to simple ring-like structures [1-5]. This behavior arises from the fact that many intermediates in this case are rings themselves, and as such are too stable to dissociate on biologically relevant time scales [3,6]. Our results indicate that deadlock can have a massive impact on assembly yields both for *in vitro* self-assembly experiments and more realistic "*in vivo*" models that include the synthesis and degradation of subunits [3].

Our model predicts that assembly yields will be maximized both *in vitro* and *in vivo* when the interaction affinity *between* the rings is much higher than that *within* the rings. Using buried surface area as a rough proxy for binding affinity [3], we found that the vast majority of stacked ring structures in the PDB adopt this architecture, confirming our prediction.

We also developed a formal definition of an "assembly pathway" based on observed fluxes in the complete CRN. Using this definition, we found that the pathways employed by optimal structures were different in our *in vitro* models compared to our *in vivo* ones. This implies that *in vitro* experiments using purified subunits may provide limited insight into the assembly mechanisms relevant within cells [1].

III. Conclusion

Stacked rings are a very common type of structure observed for molecular machines. While this architecture is very thermodynamically stable, it is also very prone to dynamic assembly deadlock [3-6]. Our work indicates that these structures have evolved specific patterns of interaction affinities in order to minimize the impact of deadlock.

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References

[1] Marques, AJ *et al.* (2009) "Catalytic Mechanism and Assembly of the proteasome," *Chem Rev* **109**, 1509-36.
[2] Braig, K *et al.* (1994) "The crystal-structure of the bacterial chaperonin GroEL at 2.8 Å," *Nature* **371**, 578-86.
[3] Deeds, EJ, Bachman JA and Fontana, W (2012) "Optimizing ring assembly reveals the strength of weak interactions" *PNAS* **109**, 2348-53.
[4] Sweeney, B, Zhang, T and Schwartz, R (2008) "Exploring the Parameter Space of Complex Self-Assembly through Virus Capsid Models," *Biophys J* **94**, 772-83.
[5] Villar, G *et al.* (2009). "Self-Assembly and Evolution of Homomeric Protein Complexes," *Phys Rev Lett* **102**, 118106.
[6] Saiz L and Vilar JMG (2006) "Stochastic dynamics of macromolecular-assembly networks" *Mol Syst Biol* **2**, 2006.0024
[7] Hlavacek, WS *et al.* (2003) "The complexity of complexes in signal transduction," *Biotech Bioeng* **84**, 783-94.