Applying cellular crowding models to simulations of virus capsid assembly *in vitro*

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***Short Abstract* — A key challenge in modeling cellular reaction networks is understanding how models from *in vitro* data might mislead us about the system *in vivo*. We examine one aspect of this challenge, characterizing effects of molecular crowding on self-assembly, using virus capsids as a model system. We learn assembly models from *in vitro* data and use simple crowding models to infer changes in intersubunit rate parameters. We then examine how such adjustments affect assembly pathways. Simulations suggest complex effects, including an interplay of crowding with nucleation-limited to enhance assembly to that suggested by uncrowded *in vitro* models.**

***Keywords* — Self-Assembly, Stochastic Simulation, Macromolecular Crowding, Nucleation, Virology.**

I. Motivation

Virus capsids have proven a powerful model system for understanding highly complex macromolecular assembly. Nonetheless, many features of the capsid assembly process, such as detailed binding kinetics and pathways, remain inaccessible to direct experimental observation. Given the limited sources of experimental data, theory and simulation methods have played an essential role in developing models of how capsid assembly functions in detail. A variety of simulation methods have been employed to tackle aspects of capsid assembly [1,2,3]. We recently showed how simulation-based optimization could allow one to fit such models to light scattering data from in vitro capsid assembly systems to provide quantitative models one can use to explore experimentally unobservable details of assembly pathways for specific virus capsids [4,5].

Even a perfectly faithful model of assembly *in vitro* may yield limited insight into the natural assembly of the virus, however, because the *in vitro* assembly environment itself is quite different from the environment of a living cell in which a virus would normally assemble. One significant difference known to affect many assembly processes is macromolecular crowding [6], which is at far greater levels during *in vivo* assembly. It remains unknown, however, if or how changes in crowding might affect the choice among many possible assembly trajectories available to any given virus. Simulation provides an avenue for asking this question.

II. Modeling and Methods

We apply a previously developed system for rule-based stochastic modeling of self-assembly [3] to sample capsid assembly trajectories using three systems for which we previously fit parameters to experimental light scattering data using a simulation-based optimization approach: Cowpea Chlorotic Mottle Virus (CCMV), Human papillomavirus (HPV) and Hepatitis B virus (HBV) [5]. To model the effects of macromolecular crowding levels, we apply an approach combining regression models with Green's Function Reaction Dynamics (GFRD) particle simulations to infer corrections to reaction rates for various crowding levels [7]. We then sample assembly trajectories for both corrected and uncorrected rates, examining changes in predicted assembly dynamics and pathway usage for each system at varying simulated crowding levels.

III. Conclusion

We find that crowding can both enhance and suppress assembly depending on the crowding level and system examined. A striking difference is evident between two models exhibiting nucleation-limited growth and one exhibiting non-nucleation-limited growth. In the non-nucleation limited case, increased crowding promotes off-pathway, kinetically trapped intermediates, slowing the rate and yield of assembly. Nucleation-limited growth, however, can buffer these effects, allowing realistic cellular crowding levels to enhance rather than suppress growth. Excessive crowding will eventually undermine growth by causing the nucleation-limited assembly to break down. The results suggest surprising ways in which viruses may have adapted to function efficiently in crowded environments.

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