Large Self-Assembled Clathrin Lattices Spontaneously Disassemble Without Sufficient Adaptor Proteins

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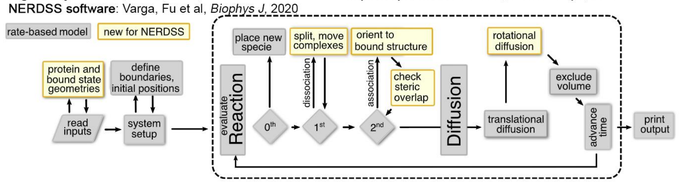
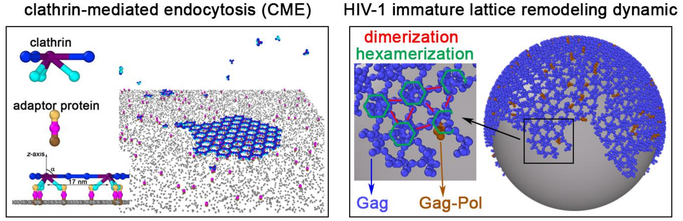
Abstract: Clathrin-coated structures can grow surprisingly large, yet often fail to form productive vesicles. We use reaction-diffusion simulations and theory to differentiate mechanisms of stable vs unstable clathrin assembly on membranes, showing that abortive assembly events happen spontaneously when adaptor availability is low. We find that the critical concentration of adaptors needed to nucleate lattices can be lowered by the forming of droplet-like AP-2 clusters on the membrane. Using a continuum thin-film model, we find that the energy gained from flat to curved structures largely offsets the bending energy. Our model predicts how adaptor density controls stabilization of clathrin-coated structures against spontaneous disassembly, and shows ATPases are not required for lattice remodeling, which is a critical advance towards predicting productive vesicle formation.

Reaction-diffusion (RD) methods development in simulations of cell biology and applications to self-assembly

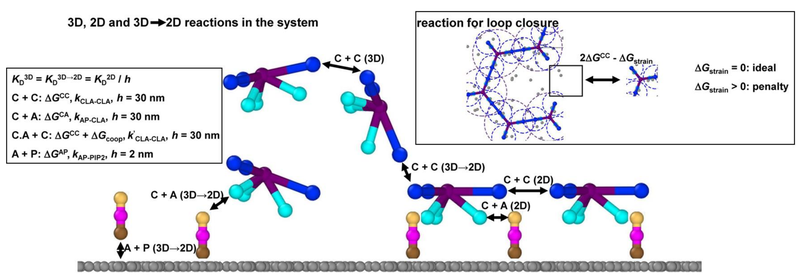
FPR algorithms: Johnson & Hummer, *Phys. Rev. X*, 2014; **2D**: Yogurtcu & Johnson, *J. Chem. Phys*, 2015
Rigid body: Johnson, *JPCB*, 2018; **Implicit lipid model**: Fu et al, *J Chem Phys*, 2019
NERDSS software: Varga, Fu et al, *Biophys J*, 2020



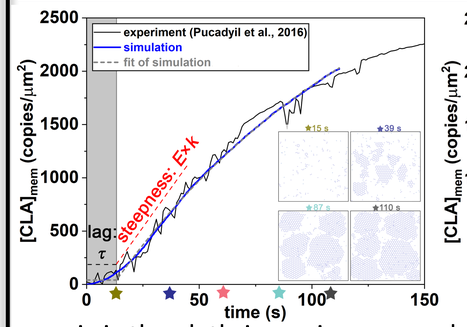
clathrin-mediated endocytosis (CME) HIV-1 immature lattice remodeling dynamic

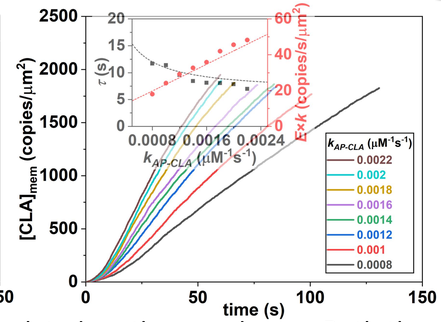


Modeling spatial and temporal dynamics of clathrin assembly and lattice formation on membranes in CME using RD simulations



Our simulation match the *in vitro* kinetic measurements

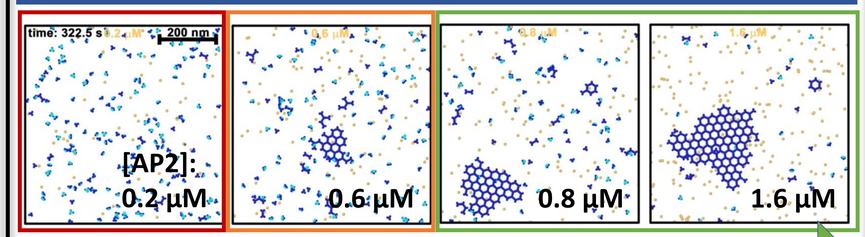




y-axis is the clathrin copies accumulated on the membrane. Both the simulation and experimental data can produce two timescales (growth rate k and lag time τ). The initial growth is approximately linear, with a steepness given by kE, with E the maximal extent of clathrin on the membrane.

	In vitro	"Physiologic-like"
Volume to area ratio:	991 μm	1 μm
AP2 concentration:	9000 μm^{-2}	120 μm^{-3}(0.2μM)

"Physiologic-like" conditions do not support nucleation of lattices on membranes

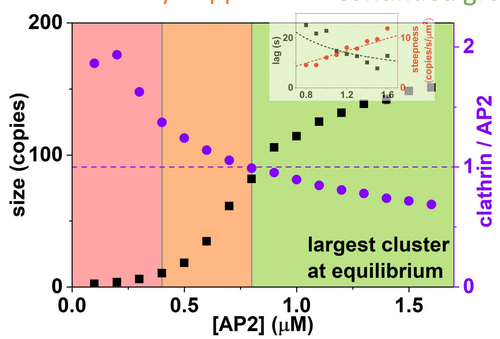


[AP2]: 0.2 μM — fail at cell condition

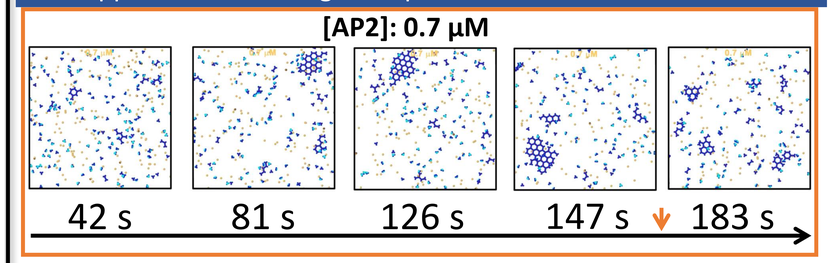
0.6 μM — spontaneous disassembly happen

0.8 μM

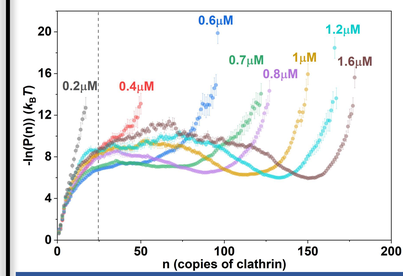
1.6 μM — nucleation and continued growth

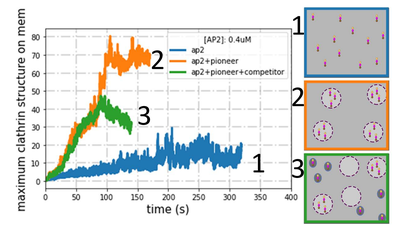


Remodeling and disassembly of stochastically assembled lattices can happen without energetic input from ATPases

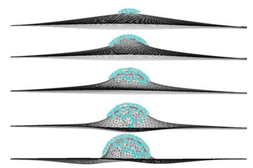


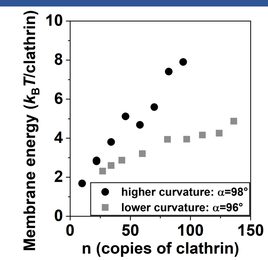
Clathrin lattices start as monomers that face an initial barrier (~25 clathrin) to growth; Droplet-like AP-2 clusters helps to catalyze nucleation





Quantify the cost of bending the membrane under curved clathrin lattices using a continuum thin-film model





Summary:

- Our results quantitatively and visually demonstrate the inherent dynamic remodeling of clathrin lattice, showing that lattice will assemble, but spontaneously disassemble, dependent on the density of adaptor. Concentration, stoichiometry, and dimensional reduction control the stability.
- Our approach provides insight and tools to study the kinetics of self-assembly on surface that is directly transferrable to steps in viral assembly and protein aggregation diseases.

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preprint: https://doi.org/10.1101/2021.04.19.440502 software & model: https://github.com/mjohn218/NERDSS