

Membrane elasticity and polymerization energy modulate the shape of clathrin coats

Mohammed Saleem^{1§*}, Sandrine Morlot¹, Annika Hohendahl¹, John Manzi², Martin Lenz³, Aurelien Roux¹

¹Biochemistry Department, University of Geneva, CH-1211 Geneva, Switzerland

²Institute Curie, CNRS, UMR168 - Physico-Chimie Curie, Paris, France

³Univ. Paris-Sud; CNRS ; LPTMS ; UMR 8626, Orsay 91405 France

[§]current address: Department of Life Sciences, National Institute of Technology, Rourkela

*Communicating Author: saleemm@nitrkl.ac.in

Clathrin mediated endocytosis is the most widely used means of vesicular trafficking and membrane bending is the first step involved, believed to be facilitated by clathrin polymerization. The polymerization of clathrin is thought to force the membrane to adopt the shape of the clathrin coat by scaffolding mechanism. However the variety of clathrin lattice shapes found *in vivo* has challenged this model. The mechanism of membrane bending by clathrin is still highly debated¹⁻³. Addressing this question, in this study, we have reconstituted clathrin budding *in vitro* with giant unilamellar vesicles (GUVs), purified adaptors and clathrin. By changing the osmotic conditions, we found that clathrin coats caused extensive budding of GUVs under low membrane tension, while polymerizing as a flat lattice under moderate tension. High tension and bending rigidity of the membrane fully inhibited polymerization. We hypothesize that membrane tension could oppose polymerization energy of clathrin. Using theoretical modeling, we predict that a transition between different shapes of clathrin coats depends on membrane tension and clathrin polymerization energy. We then experimentally validated the theoretical model by estimating the transition values by measuring the polymerization energy of clathrin for the first time. The measured membrane tension and clathrin polymerization energy were found to be of the same order as *in vivo* tension regimes, suggesting a physiologically critical control of the shape of clathrin mediated budding by membrane elasticity.

References

1. Ford, M.G., Barbara M.F. Pearse, Mathew K. Higgins, Yvonne Vallis, David J. Owen, Adele G, Colins R Hopkins, Philip R Evans, Harvey T McMahon. Simultaneous binding of PtdIns(4,5)P2 and clathrin by AP180 in the nucleation of clathrin lattices on membranes. *Science* **2001**, 291:1051-1055.
2. Boulant, S., Kural, C., Zeeh, J.C., Ubelmann, F. & Kirchhausen, T. Actin dynamics counteract membrane tension during clathrin-mediated endocytosis. *Nature Cell Biol* **2011**, **13**:1124-1131.
3. Dannhauser, P.N. & Ungewickell, E.J. Reconstitution of clathrin-coated bud and vesicle formation with minimal components. *Nature Cell Biol* **2012**, **14**:634-639.