Ries Group

A nanoscopic reconstruction of the mammalian endocytic machinery

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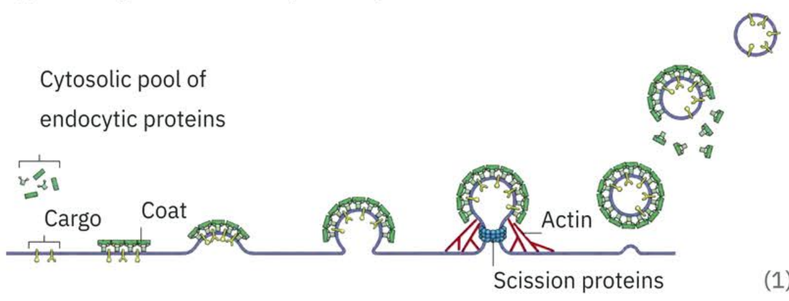
Background

Clathrin-mediated endocytosis

- Uptake of extracellular material by membrane invagination.
- Reshaping of the plasma membrane (PM) at nanoscale.
- Regulated by over 50 endocytic components.



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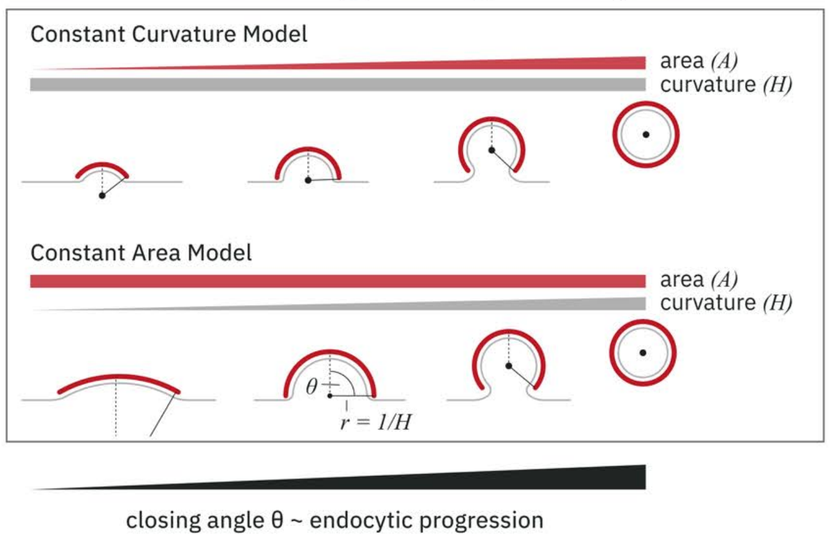
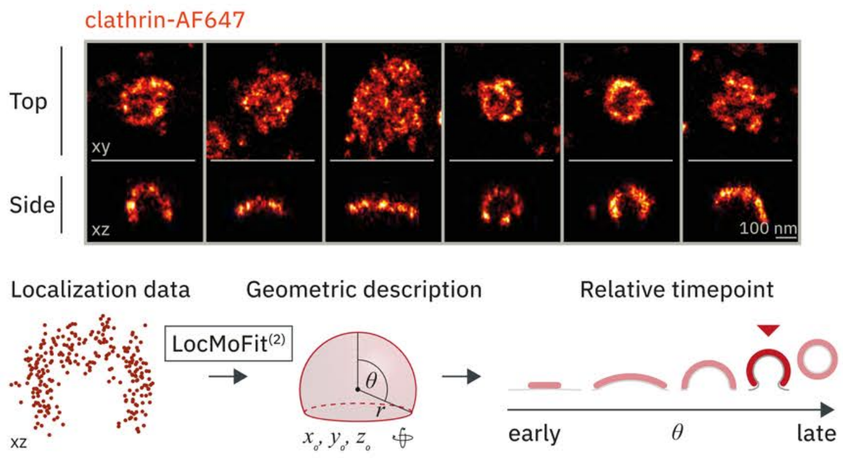
Question: What is the underlying mechanism of membrane bending by clathrin?

Clathrin remodeling during endocytosis



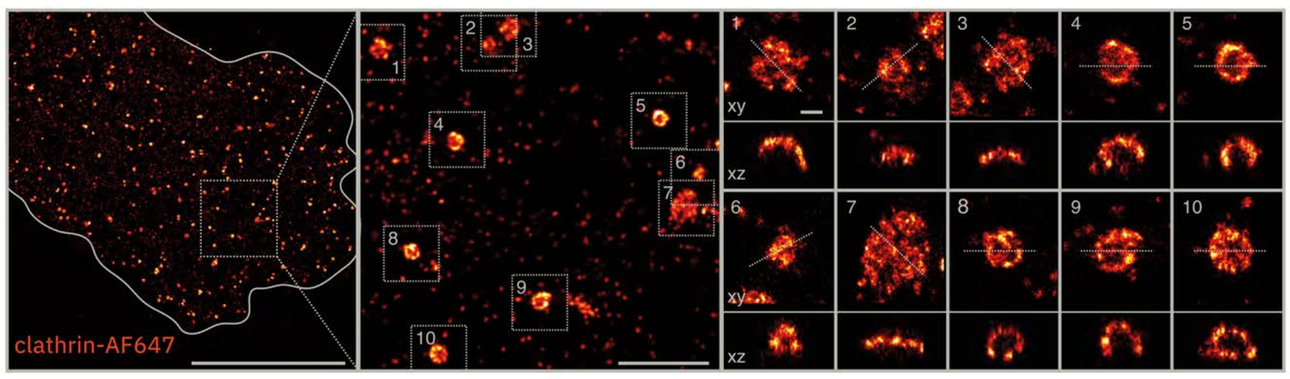
Project overview

3D Single-molecule localization microscopy to study the dynamic remodeling of the clathrin coat based on static high-resolution snapshots



Results

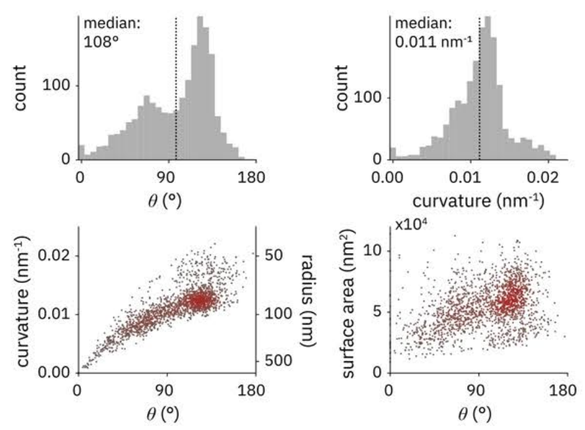
Single-molecule localization microscopy of clathrin



- 3D high-resolution snapshots of 1798 endocytic sites at the basal membrane of SK-MEL-2 cells
- Geometric description by LocMoFit: position (x_o, y_o, z_o), orientation ($\oplus$), endocytic progression (θ), size/radius (r)

Clathrin coat geometry

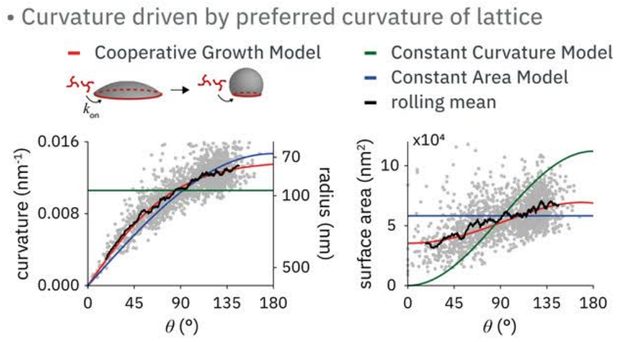
Increase of clathrin coat curvature and surface area



Coat growth and remodeling

Cooperative growth model assumptions:

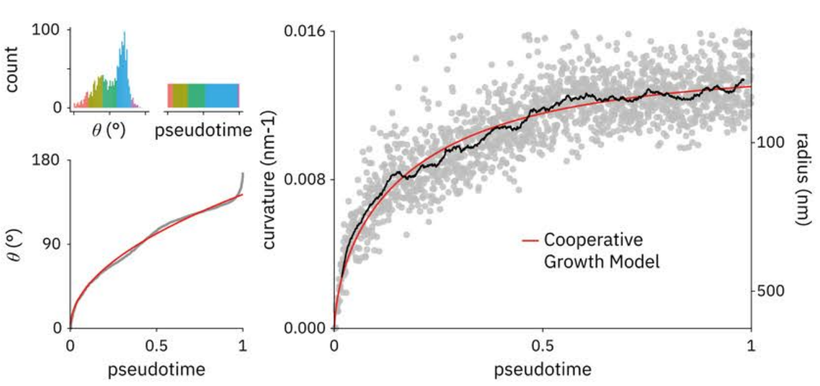
- Clathrin coat growth at lattice edge
- Curvature driven by preferred curvature of lattice



Curvature initiation after 51% of surface area is acquired

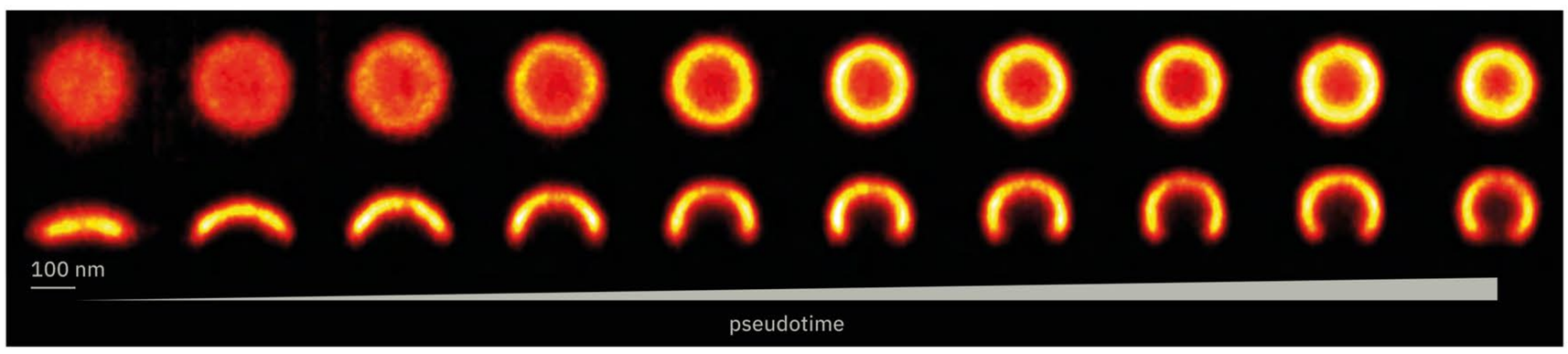
Clathrin coat remodeling over pseudotime

Assumption of equal sampling → assignpseudo time based on θ rank



High-resolution averages of the mammalian clathrin coat

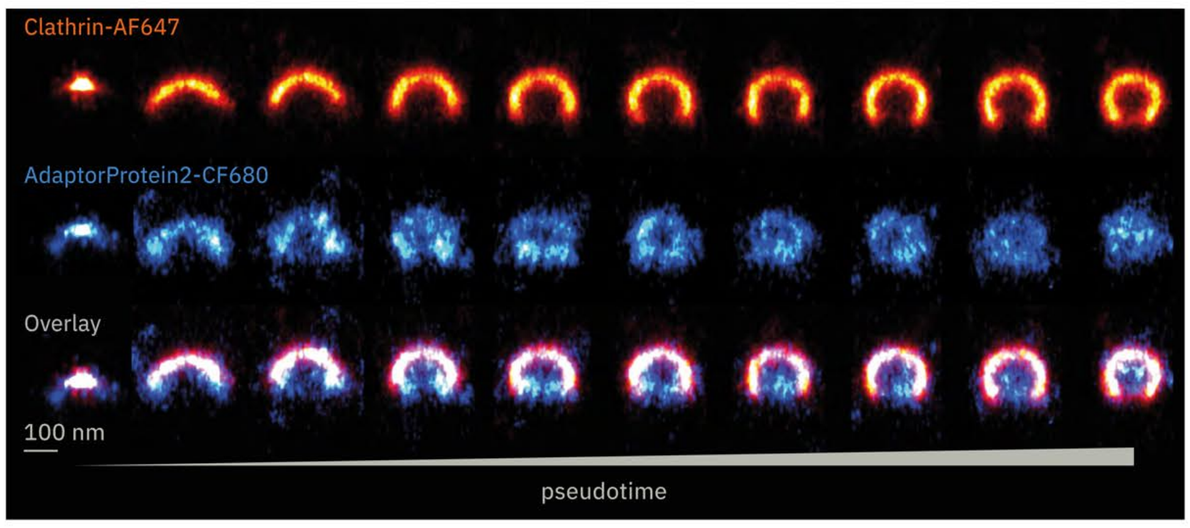
Parameters resulting from LocMoFit are used to bin snapshots (179 sites per bin) to generate high-resolution averages of distinct endocytic stages



Conclusions

- Single-molecule localization microscopy to visualize the nanoscale architecture of the clathrin coat in 3D.
- Geometric characterization of clathrin using a novel maximum-likelihood-based framework (LocMoFit).
- Sorting of static clathrin snapshots along their endocytic progression or θ.
- Continuous increase in curvature and surface area of the coat throughout the endocytic process.
- Formulation of a novel cooperative growth model, based on well-founded assumptions about the process:
  - Growth at the rim of the lattice with a constant on-rate k_{on}.
  - Preferred curvature of the lattice as driving force for curvature generation.
- 51% of clathrin coat growth happens on flat membranes, before curvature is initiated.

Outlook - Extension to multi-color imaging



Investigating the recruitment of endocytic components to the clathrin coat:

- Dual-colour imaging of clathrin together with a target protein.
- Geometric characterization of the clathrin coat in one channel using LocMoFit.
- Mapping of the target protein in the second channel to the developing clathrin coat.
- Reconstruction of the dynamic recruitment to the coat.

References

(1) adapted from Kaksonen, M., Roux, A., Nat Rev Mol Cell Biol (2016)

(2) Wu, Y. et al., Biorxiv (2021)

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