

Supplemental material

Chen et al., <https://doi.org/10.1083/jcb.201804136>

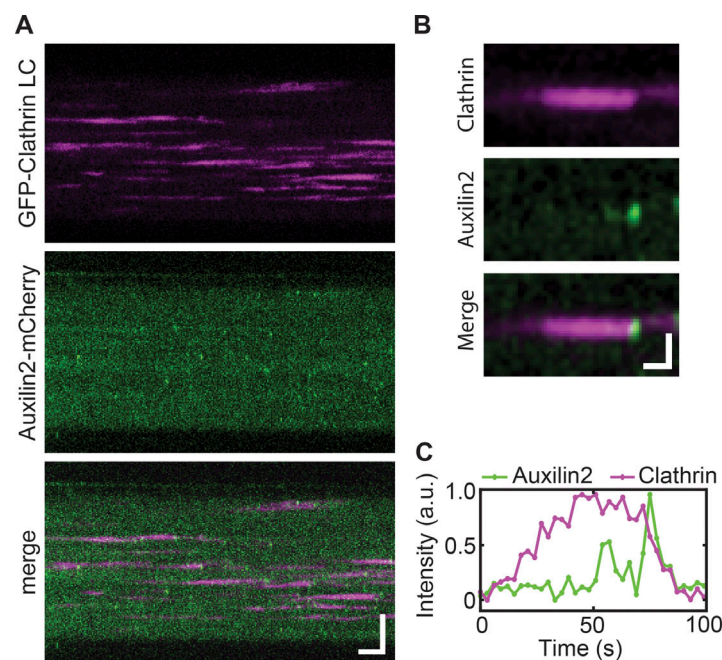


Figure S1. **Auxilin2 recruitment during CCP assembly in HeLa cells.** (A) Kymograph generated from a HeLa cell stably expressing GFP-Clathrin light chain and Auxilin2-mCherry. Images were captured under TIRF illumination for both channels. (B) Kymograph of a single CCP forming and pinching off with the corresponding Auxilin2-mCherry kymograph. (C) Normalized intensity profile of clathrin and Auxilin2 of the single CCP from B. Scale bars, kymograph vertical, (A) 5 μ m, (B) 1 μ m; kymograph horizontal, (A) 60 s, (B) 10 s.

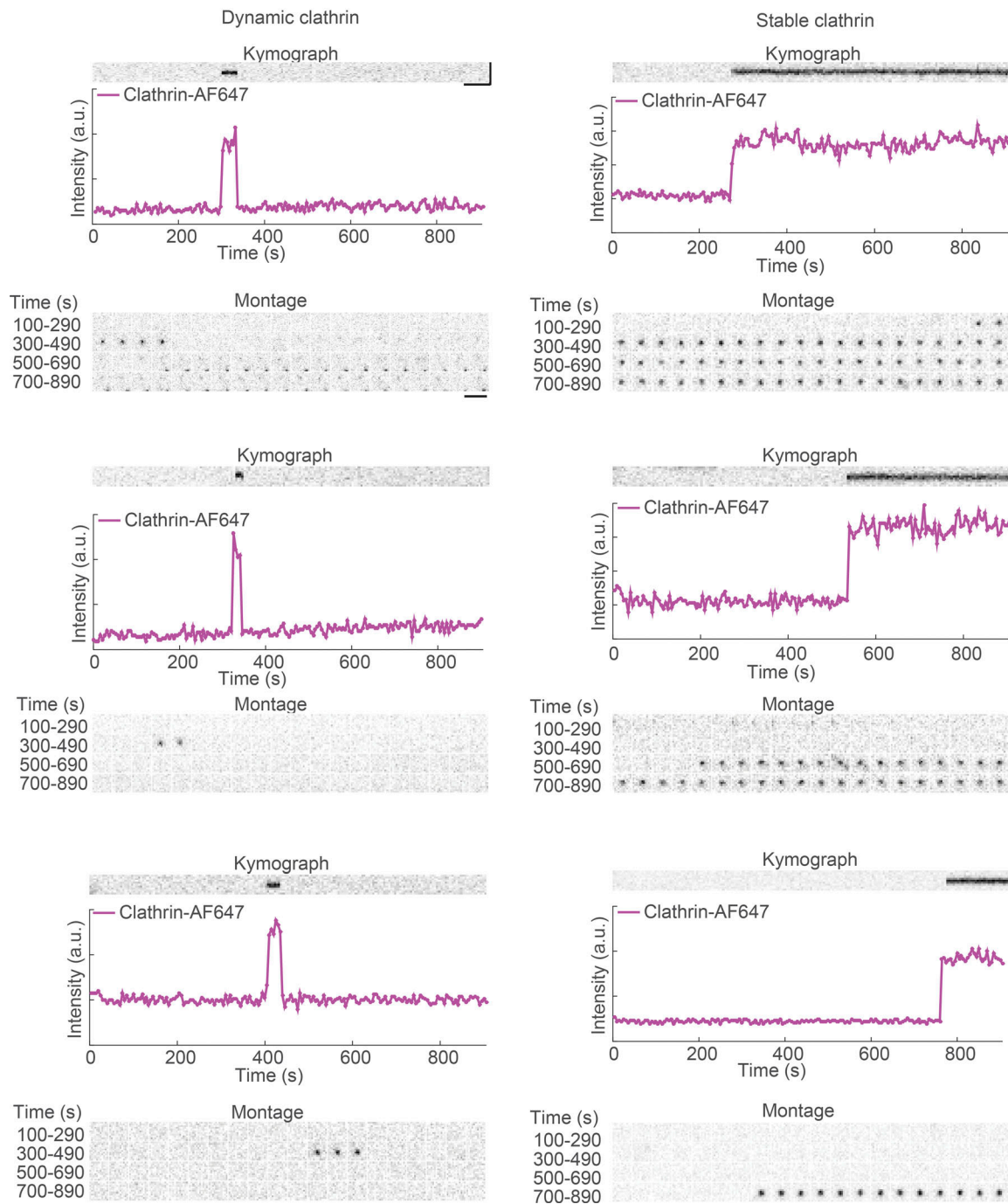


Figure S2. **Representative profiles of dynamic and stable clathrin single molecules.** Profiles for dynamic and stable single-molecule Clathrin-AF647. Kymograph (top), intensity profile (middle), and montage (bottom) of single molecules of Clathrin-AF647. Scale bars, 1 min (kymograph, horizontal); 2 μ m (kymograph, vertical); and 2 μ m (montage).

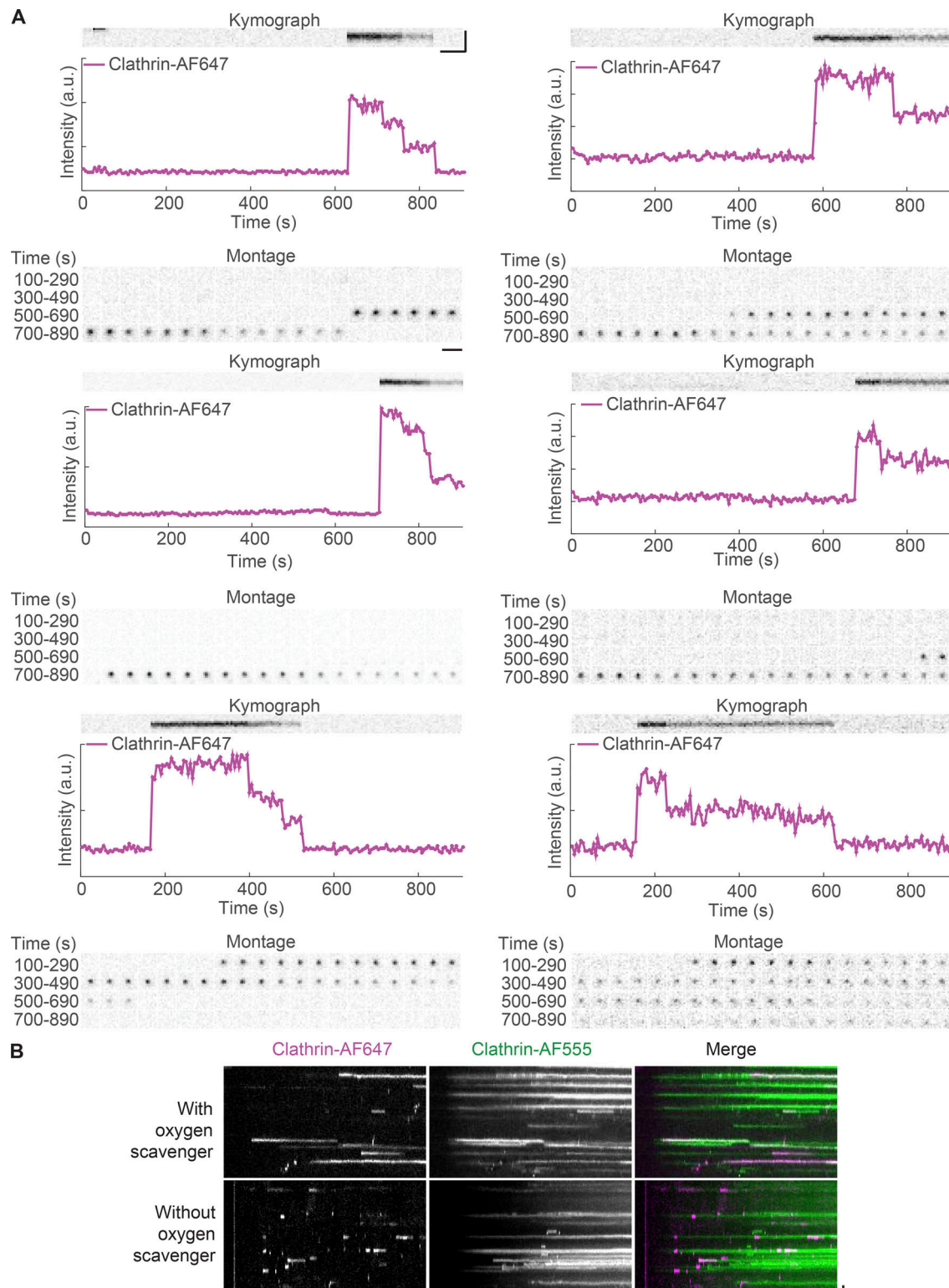


Figure S3. **Representative profiles of photobleached clathrin single molecules.** (A) Profiles of single molecules of Clathrin-AF647 undergoing possible photobleaching. (B) Kymographs of single molecules of Clathrin-AF647 and corresponding CCP (Clathrin-AF555). The oxygen scavenger system was included (top panel, and all other experiments unless otherwise noted) or omitted (bottom panel) in the reaction mix during live imaging. Scale bars, 1 min (kymograph, horizontal); 2 μ m (kymograph, vertical); and 2 μ m (montage).

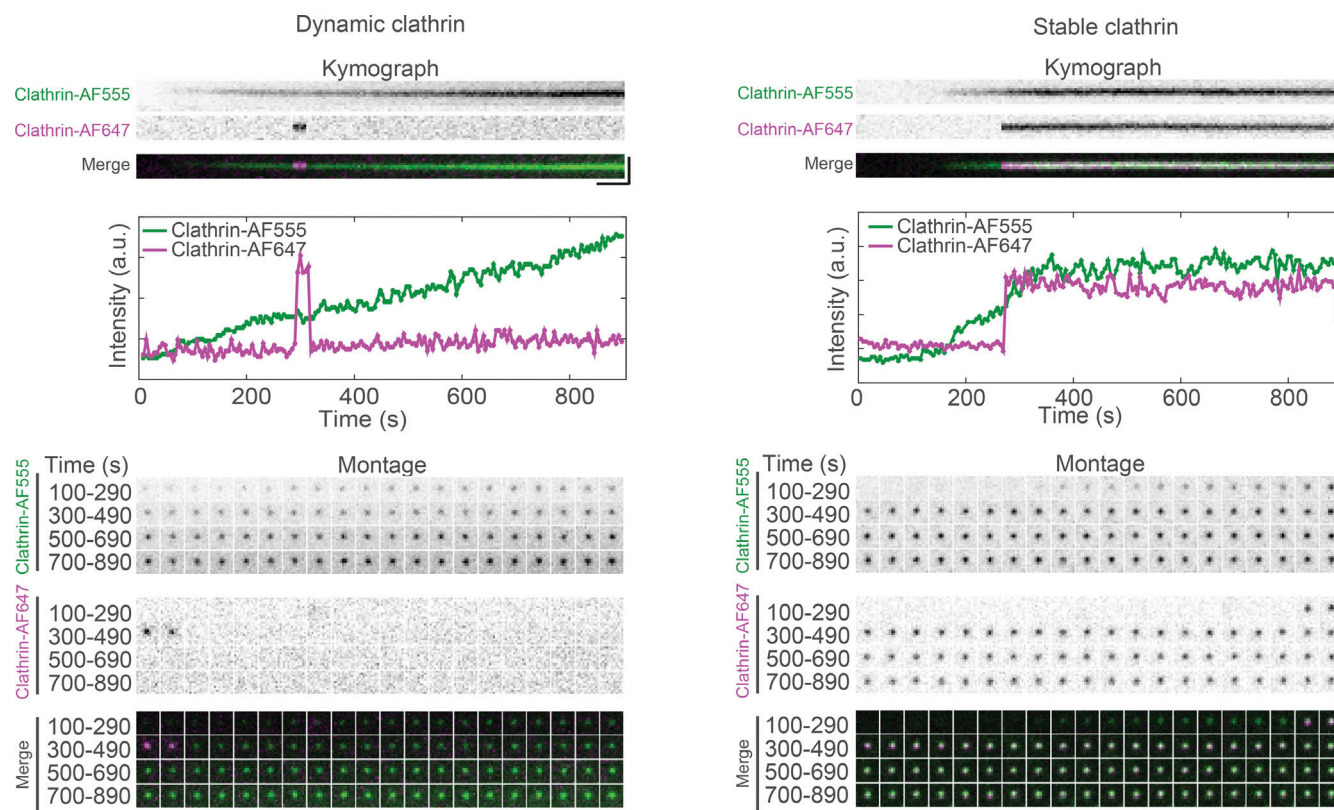


Figure S4. **Representative profiles of clathrin single molecules and bulk clathrin.** Profiles for single molecules of Clathrin-AF647 with its corresponding CCP (Clathrin-AF555). Kymograph (top), intensity profile (middle), and montage (bottom) of Clathrin-AF555 and single molecules of Clathrin-AF647. Scale bars, 1 min (kymograph, horizontal); 2 μ m (kymograph, vertical); and 2 μ m (montage).

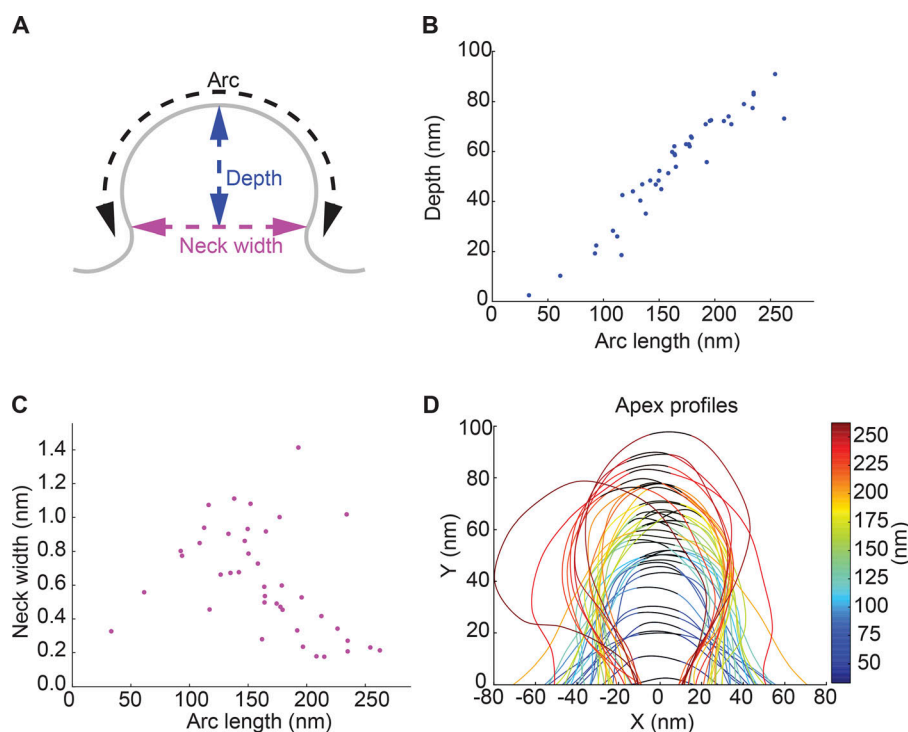
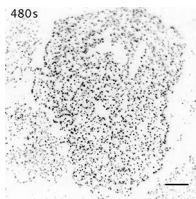
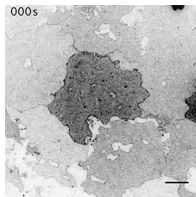


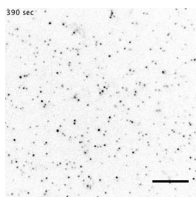
Figure S5. **Changes of CCP depth and neck width during CCP development.** (A) Schematic diagram of the CCP membrane. (B) Increase in CCP depth during CCP development. (C) Changes in neck width during CCP development. (D) CCP apex profiles. The apex is highlighted in black.



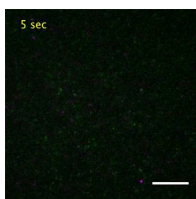
Video 1. **TIRF video of CCP assembly on membrane sheets.** CCP assembly induced by cytosol. Clathrin-AF555 was added to cytosol to visualize CCP. Images were taken at 5 s/frame. Scale bar, 10 μ m.



Video 2. **TIRF video of TfR-pHluorin sequestration.** Membrane sheet expressing TfR-pHluorin was incubated with cytosol. Images were taken at 5 s/frame. Scale bar, 10 μ m.



Video 3. **TIRF video of a single molecule of clathrin during CCP assembly.** Clathrin-AF647 (0.5 nM) was added to cytosol to trace single-molecule dynamics. Images were taken at 5 s/frame and played at 30 frames/s. Scale bar, 10 μ m.



Video 4. **Two-color TIRF video of clathrin turnover during CCP assembly.** Clathrin-AF647 (0.5 nM, magenta) was added to cytosol to trace single-molecule dynamics. Clathrin-AF555 (95 nM, green) was added to cytosol to mark CCP. Images were taken at 5 s/frame and played at 10 frames/s. Scale bar, 10 μ m.