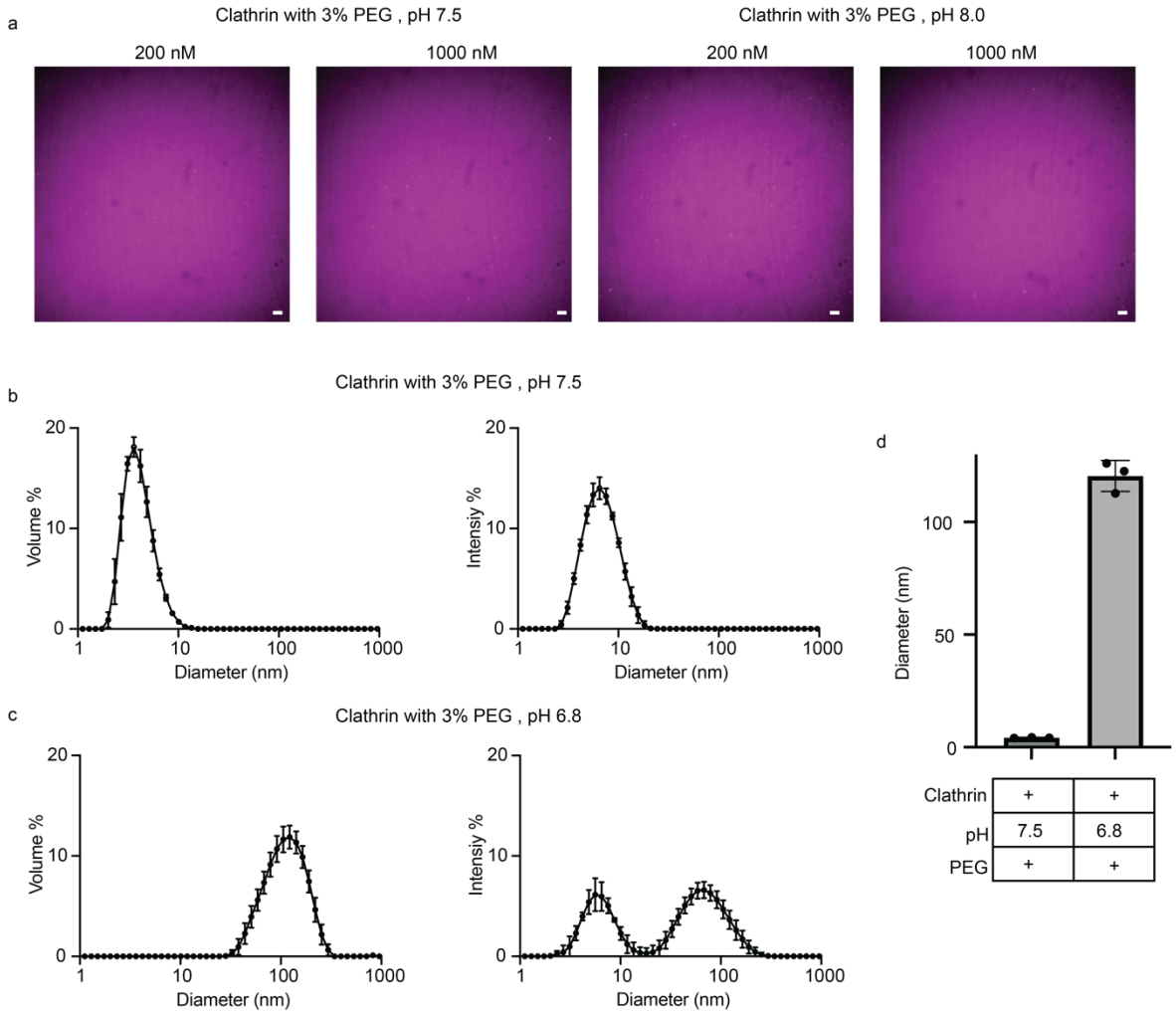
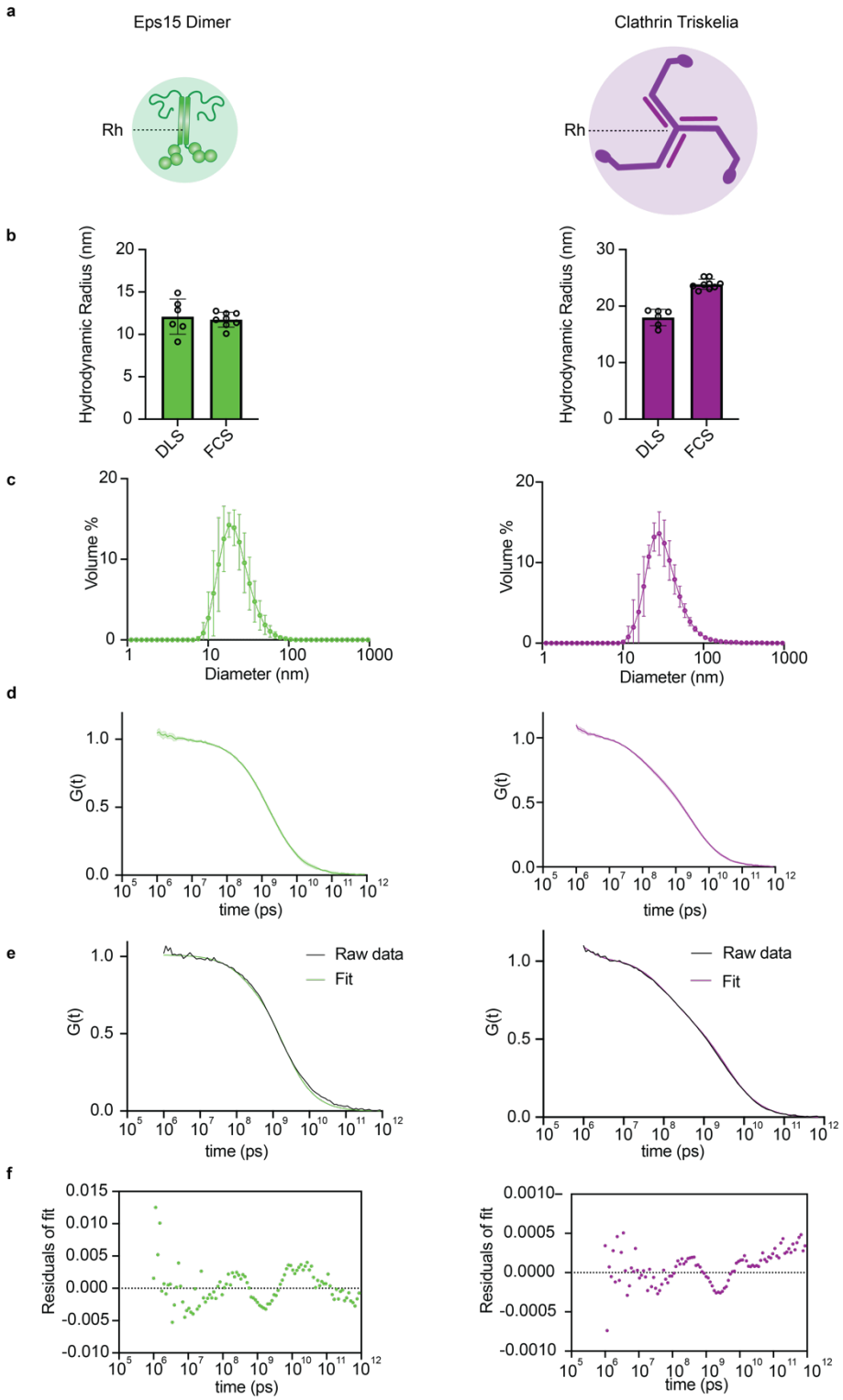
Supplementary Material

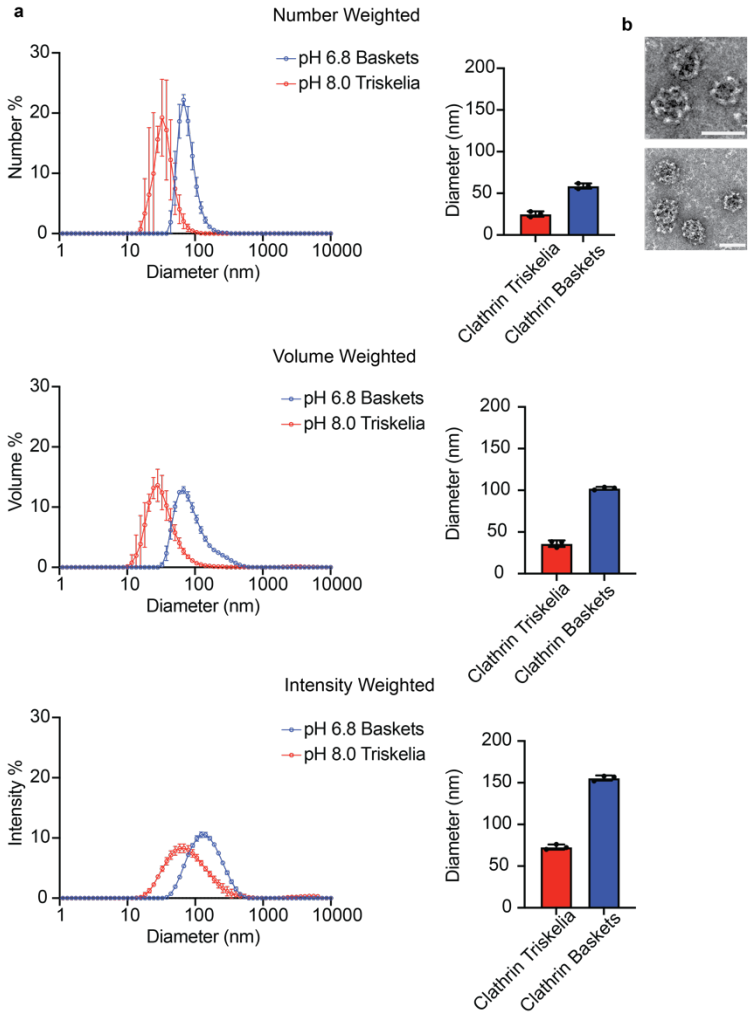


Supplementary Figure 1. 3% w/v PEG does not trigger clathrin assembly and clathrin does not form condensates. **(a)** Confocal microscopy images showing a lack of clathrin assembly or condensate formation, scale bar is 5 μm. Similar micrographs were obtained across n=3 biological replicates. **(b)** DLS data of volume and intensity weighted distributions at pH 7.5 with 3% w/v PEG. (**c)** DLS data of volume and intensity weighted distributions at pH 6.8 with 3% w/v PEG. **(d)** Bar chart showing the average volume-weighted diameter of clathrin at pH 7.5 and pH 6.8 in the presence of 3% w/v PEG. Distributions and the bar chart are the average diameter ± s.d from n=3 biological replicates with 15 DLS measurements per replicate. Source data are provided as a Source Data file.

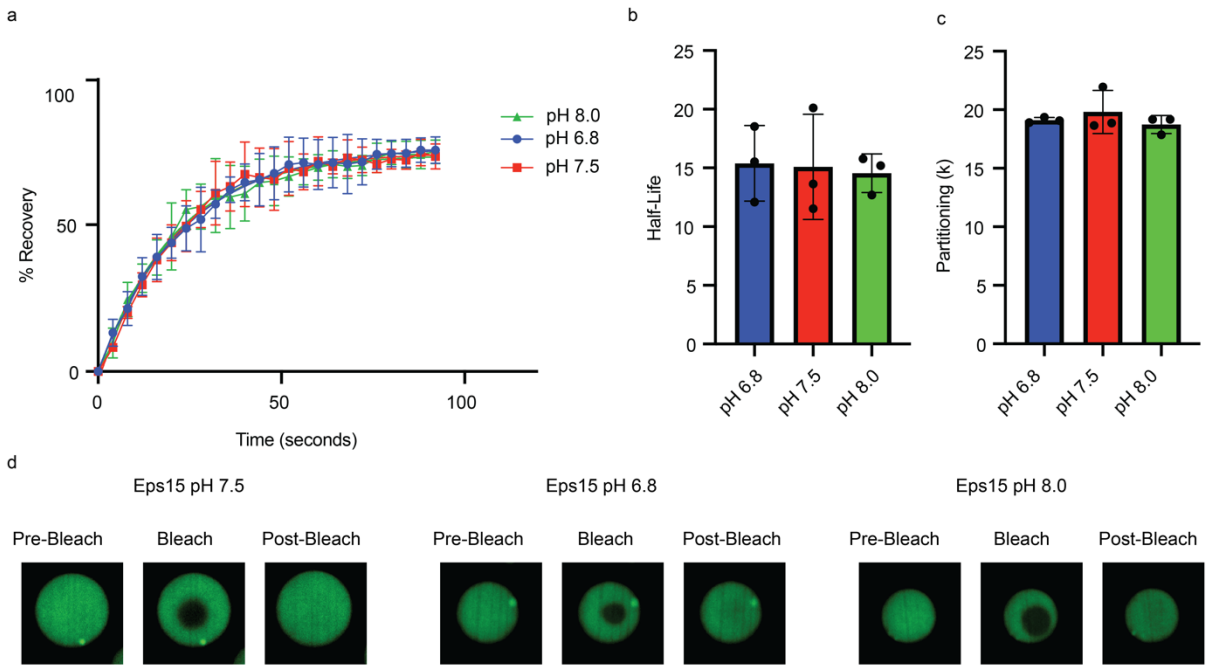


Supplementary Figure 2. Size determination of Eps15 and Clathrin Triskelia. **a)** Cartoon depiction of Eps15 and Clathrin Triskelia showing their radius of hydration. **b)** Bar chart comparing the determined radius of hydration for Eps15 and clathrin using DLS and FCS. Bars represent the mean radius of hydration ± s.d

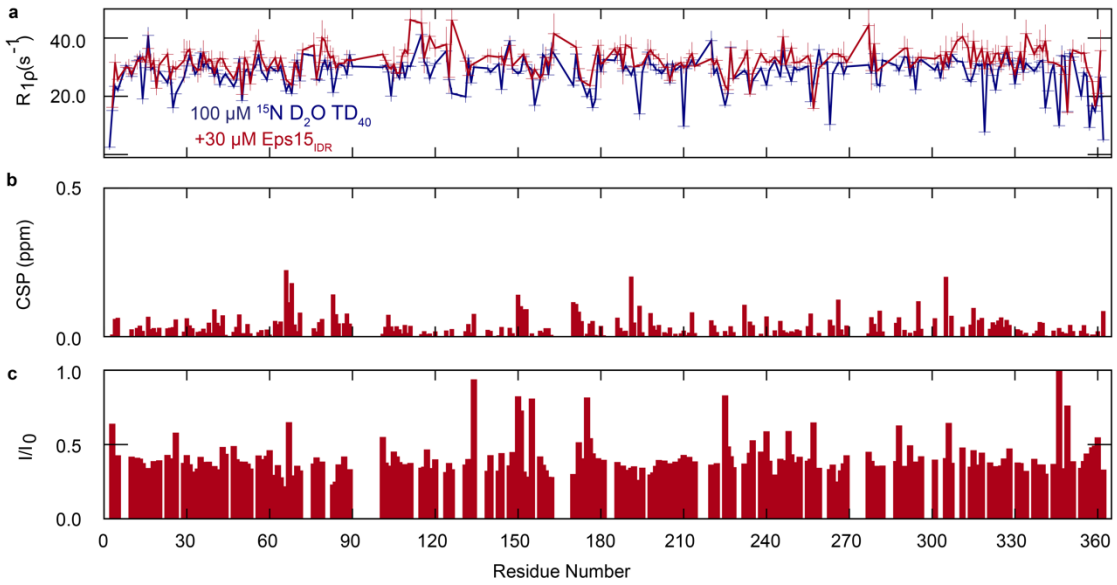
across n=6 biological replicates for DLS with 15 measurements per replicate and n=8 (Eps15) or n=9 (Clathrin) technical replicates for FCS. **c)** Volume weighted diameter-distributions from DLS for Eps15 and clathrin. Here the individual points represent the mean hydrodynamic diameter ± s.d. across n=3 separate biological replicates. **d)** FCS curve showing average measurements and standard deviation across n=8 technical replicates. **e)** Example replicate and fit for FCS measurements. **f)** Residual values for FCS curve fitting. Source data are provided as a Source Data file.



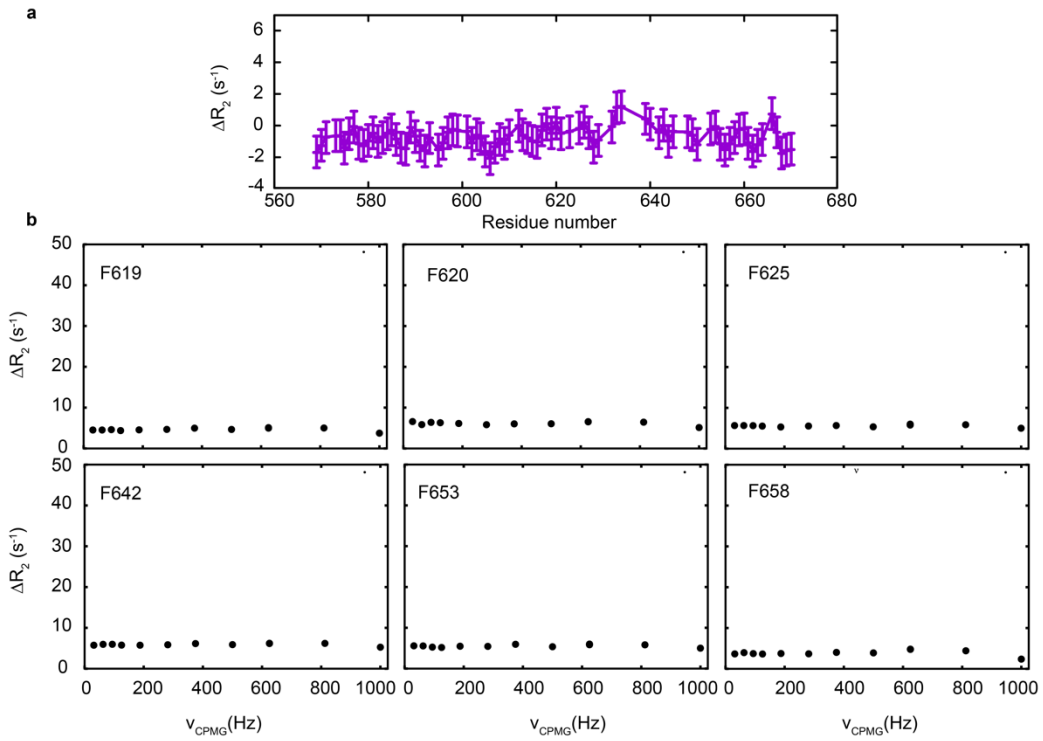
Supplementary Figure 3. DLS size comparison of clathrin baskets and triskelia. **a)** DLS data of volume, intensity, and number weighted distributions for clathrin baskets or triskelia along with weighted averages. All data is the average hydrodynamic diameter from 3 biological replicates with 15 measurements per replicate ± s.d. **b)** TEM images of clathrin baskets. Similar electron micrographs of clathrin baskets were obtained across n=3 biological replicates. Scale bars are 100 nm. Source data are provided as a Source Data file.



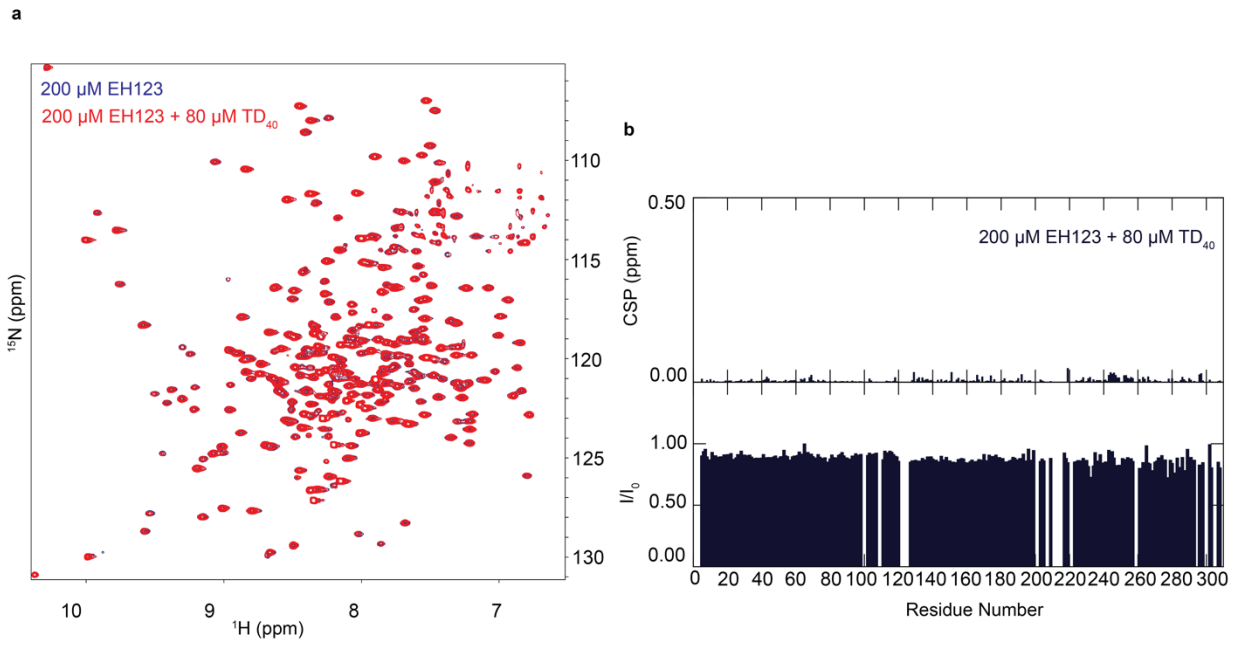
Supplementary Figure 4. Eps15 recovery at various pH. a) FRAP Recovery curves of Eps15 condensates at various experimental pH. Lines are average % fluorescent recovery from n=3 biological replicates, error bars are s.d. b) Bar chart showing the average half-lives ± s.d from corresponding curves in a). Half-lives were extracted by fitting a single-phase association to the curves in a) using GraphPad Prism version 10.3.0, error bars are s.d. c) Bar chart showing the average partitioning ± s.d of Eps15 into condensates at various pH across n=3 biological replicates. d) Confocal microscopy images showing the recovery after photobleaching for Eps15 condensates at the various pH values with similar results obtained across 3 biological replicates for each condition. Source data are provided as a Source Data file.



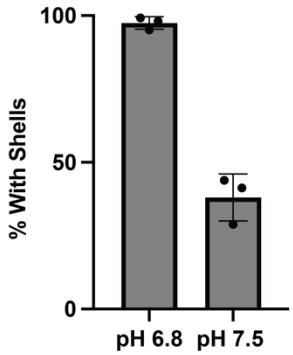
Supplementary Figure 5: Interaction of ^{15}N ^{2}D TD_{40} with $Eps15_{IDR}$. **(a)** ^{15}N $R_{1\rho}$ of 100 µM TD_{40} in the absence (blue) and the presence (red) of 30 µM $Eps15_{IDR}$ recorded at a ^{1}H frequency of 600 MHz and a temperature of 25°C. Relaxation rates were derived from a fit of the peak intensities against the relaxation delay. Errors of the fitted rates are shown and derived from the experimental uncertainty. **(b)** Chemical shift perturbations (CSPs) extracted from 200 µM TD_{40} ^{1}H-^{15}N TROSY spectra in the presence versus the absence of 60 µM $Eps15_{IDR}$ (corresponding spectra are shown in Fig. 2). **(c)** Peak intensity ratios (I/I_0) extracted from 200 µM TD_{40} ^{1}H-^{15}N TROSY spectra in the presence versus the absence of 60 µM $Eps15_{IDR}$ (corresponding spectra are shown in Fig. 2), showing an overall drop in peak intensities to around 45% of the original intensity across the sequence of TD_{40}. Source data are provided as a Source Data file.



Supplementary Figure 6. Relaxation dispersion of 100 µM ^{15}N Eps15$_{569-671}$ with 10 µM TD$_{40}$. **(a)** ΔR_2 calculated from the effective R_2 values at CPMG frequencies (ν_{CPMG}) 31 Hz and 1000 Hz. The error is the sum of the errors from the individual R_2 values, which were derived from the experimental uncertainty. **(b)** Exemplary CPMG curves of the different phenylalanines in the interacting regions showing flat curves and thus the absence of intermediate exchange in the microsecond to millisecond range. Errors were determined based on standard deviations of repeat measurements. The minimum error was set to 0.5. The CPMG relaxation dispersion data were recorded at a ^{1}H frequency of 1200 MHz. Source data are provided as a Source Data file.

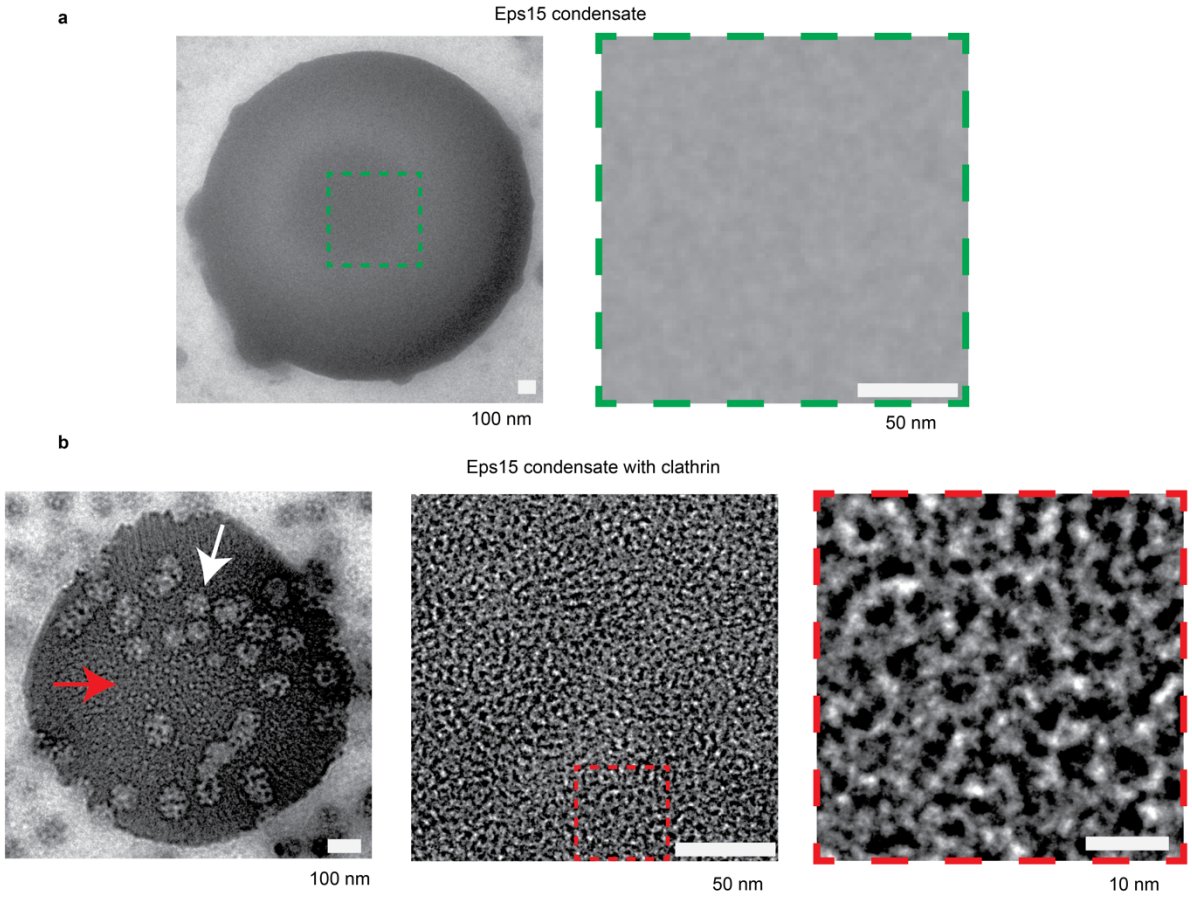


Supplemental Figure 7. Lack of interaction between TD_{40} and EH123. **(a)** ^{1}H-^{15}N TROSY spectrum of 200 μM of the EH123 domains of Eps15 alone (blue) and in the presence of 80 μM TD_{40} (red), showing no signs of interaction. **(b)** Chemical shift perturbations (CSPs) in the presence versus the absence of TD_{40} are zero throughout EH123 and intensity ratios of peaks in the presence versus the absence of TD_{40} are 1, both demonstrating that no interaction takes place between TD_{40} and EH123. Source data are provided as a Source Data file.

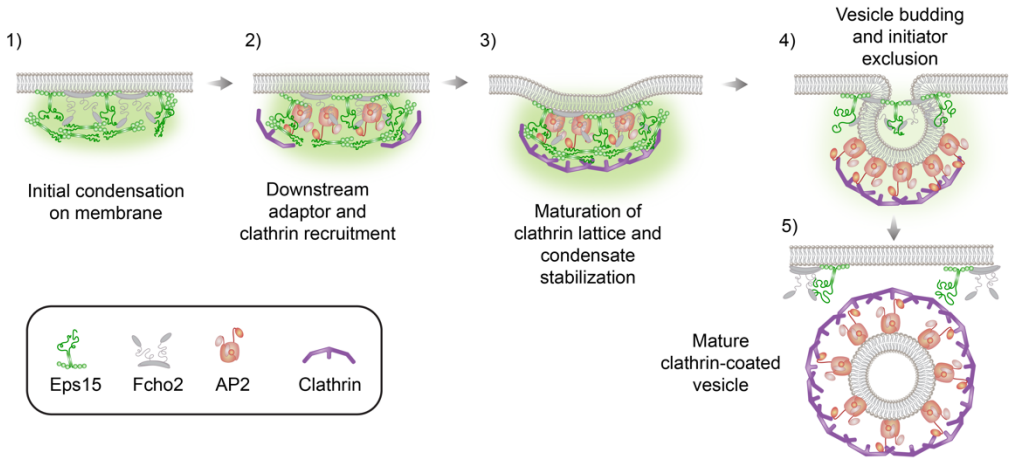


Supplementary Figure 8: Quantification of clathrin shells around Eps15 condensates at pH 6.8 vs pH 7.5

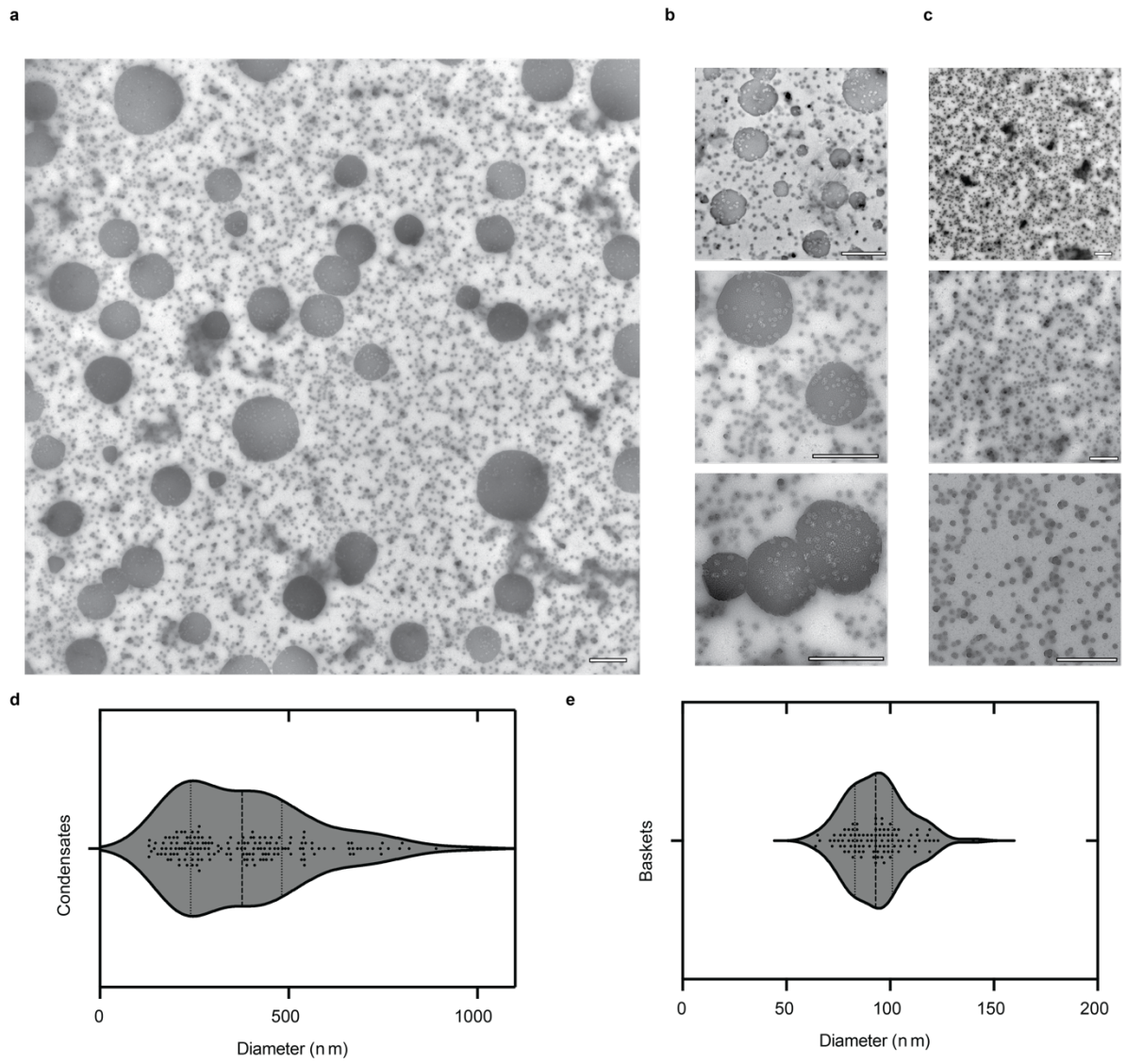
Bar chart comparing the amount of clathrin shells when pre-formed clathrin baskets were added to Eps15 condensates at pH 6.8 vs. the addition of clathrin triskelia to Eps15 condensates at pH 7.5. Eps15 condensates were formed with 7 μM Eps15 to which clathrin was added to achieve a working concentration of 200nM. The experimental buffer is pH 6.8 or pH 7.5 TNEET with 3% w/v PEG for the respective conditions. Bar chart shows mean % of condensates with shells ± s.d from n=3 biological replicates with at least 200 condensates counted per replicate 15 minutes after clathrin addition. For pH 7.5 this data is identical to that shown in Fig.6f. Source data are provided as a Source Data file.



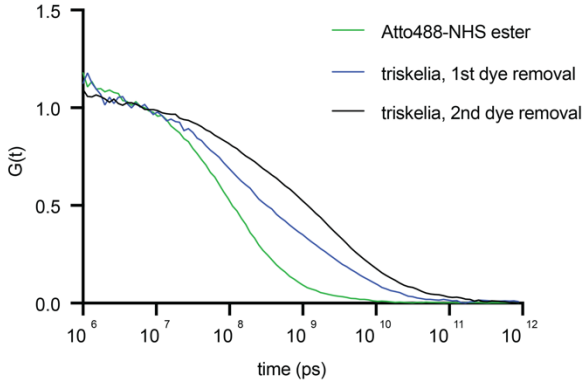
Supplementary Figure 9. TEM comparison of Eps15 condensates and Eps15 + clathrin condensates **a**) TEM images showing condensates formed by 7 μM Eps15 with a zoom in revealing the smooth surface. **b)** Representative view of sub-micron sized condensates of 7 μM Eps15 formed in the presence of 1 μM clathrin showing increasing magnification on the textured clathrin lattice (red arrow) on the surface of Eps15 condensates formed with clathrin. Mature clathrin baskets are indicated by the white arrow. For both conditions the experiment was repeated 3 times with similar results. Source data are provided as a Source Data file.



Supplementary Figure 10. Proposed model for reciprocal assembly of protein condensates and clathrin lattices during endocytosis**.**1) Initial condensation on membrane: Eps15 and Fcho2 undergo phase separation at the plasma membrane, creating a scaffold that recruits adaptor proteins such as AP2 .2) Downstream adaptor and clathrin recruitment: The condensed phase concentrates AP2 which enhances clathrin recruitment. 3) Maturation of clathrin lattice: clathrin begins to polymerize leading to condensate stabilization and limiting uncontrolled growth. 4) Vesicle budding and initiator exclusion: The growing clathrin lattice ultimately excludes remaining initiators Eps15 and Fcho2, producing 5) a mature, clathrin-coated vesicle. Future work will be required to validate, invalidate, and expand upon these proposed steps.



Supplementary Figure 11. TEM of Eps15 condensates formed in the presence of clathrin. **a**) Representative view of sub-micron sized condensates of 7 μM Eps15 formed in the presence of 1 μM clathrin. **b**) Images with an emphasis on large condensates. **c**) Images with an emphasis on small basket structures. This experiment was conducted 3 times with similar results. **d**) and **e**) Violin plots representing the size distribution of either clathrin condensates or basket structures viewed in TEM. Plots represent the measurement of the diameter from 100 structures measured for each population across n=3 separate biological replicates. The central dashed line shows the median size value for the distribution and the additional dashed lines represent the upper (75^{th}) and lower (25^{th}) percentiles. Each point indicates the diameter of an individual condensate or basket. Source data are provided as a Source Data file.



Supplementary Figure 12. Expanded data on Clathrin FCS. **(a)** FCS curves showing average measurements and standard deviations for free dye alone (n=3 technological replicates), Clathrin triskelia after dye removal (n=10 technological replicates) and clathrin triskelia after an additional dye round of dye removal (n=8 technological replicates). Source data are provided as a Source Data file.

Molecule	N_p	T	**Measured τ_{D1} (µs)**	**Measured τ_{D2} (µs)**	**τ_{trp} (µs)**	F
Atto488-NHS ester	22.3 ± 0.2	0.24 ± 0.10	95 ± 2	X	1.03 + 0.55	X
GFP	19.1 + 0.5	0.27 + 0.06	348 + 9	X	1.58 + 0.44	X
Eps15	5.6 + 0.2	0.09 + 0.02	1776 + 133	X	84.82 + 51.95	X
Triskelia, 1st dye removal	17.8 + 0.6	0.20 + 0.04	92 + 7	3820 + 441	fixed 1.03	0.40 + 0.02
Triskelia, 2nd dye removal	23.8 + 1.8	0.15 + 0.02	107 + 4	3613 + 134	fixed 1.03	0.63 + 0.02

Supplementary Table 1. FCS regression parameters for Atto488, GFP, Eps15, and clathrin triskelia after 1 or 2 rounds of free dye removal. "X" indicates that the parameter was not included in the regression equation for the respective molecule. Here N_p corresponds to the average number of particles diffusing through the confocal volume, τ_{D1} and τ_{D2} are the particle diffusion time constants, T is the fraction of intersystem crossing, and τ_{trp} is the triplet state time constant, and F is the fraction of component 2.

Fig.	3% (w/v) PEG used	Rationale
Fig.1	Yes, except for panel d)	To form Eps15 condensates PEG is required. For the DLS in panel d) we are trying to show the size of clathrin triskelia vs. baskets which would be clouded by excessive PEG in solution and we also show with Fig.S1 that PEG does not induce clathrin assembly or cause clathrin condensate formation.
Fig.2	Yes except for panel d)	To form Eps15 condensates PEG is required. The NMR experiments in panel d) are looking at fundamental interactions between Eps15 and the terminal domain of clathrin heavy chain.
Fig.3	No	NMR experiments are looking at molecular interactions between Eps15 and the terminal domain of clathrin heavy chain. We are not forming condensates here.
Fig.4	Yes, except part of panel a (as shown) and all of panel b	In panel a-b of this figure we are showing that clathrin does not assemble at physiological pH (7.5) unless in the presence of Eps15 condensates. We show Eps15 alone (in a non-condensed form) does not trigger clathrin assembly. Then in panels c-f we are demonstrating that Eps15 condensates do induce clathrin assembly.
Fig.5	Yes	We are monitoring clathrin interactions with Eps15 condensates. To form Eps15 condensates PEG is required.
Fig.6	Yes	We are monitoring clathrin interactions with Eps15 condensates and additional adaptor proteins. To form Eps15 condensates PEG is required.
Fig.7	Yes	We are monitoring clathrin interactions with Eps15 condensates. To form Eps15 condensates PEG is required.
Fig.S1	Yes	We are demonstrating as a control that clathrin does not polymerize or form condensates in the presence of 3% w/v PEG
Fig.S2	No	We are using FCS to determine the size of individual molecules of Eps15 and clathrin, not condensates.
Fig.S3	No	We are trying to show the size of clathrin triskelia vs. baskets which would be clouded by excessive PEG in solution and we also show with Fig.S1 that PEG does not induce clathrin assembly or cause clathrin condensate formation.
Fig.S4	Yes	To form Eps15 condensates PEG is required. We are checking if the condensate network of Eps15 is sensitive to pH.
Fig.S5	No	NMR study of molecular interactions, no condensates.
Fig.S6	No	NMR study of molecular interactions, no condensates.
Fig.7	No	NMR study of molecular interactions, no condensates.
Fig.S8	Yes	Quantification of clathrin shells around Eps15 condensates. To form Eps15 condensates PEG is required.
Fig.S9		We are monitoring clathrin interactions with Eps15 condensates. To form Eps15 condensates PEG is required.
Fig.S10	No	Theoretical mechanism graphic, not an experiment. In cells the crowded environment of the cytosol mimics our in vitro use of 3% w/v PEG.
Fig.S11	Yes	We are monitoring clathrin interactions with Eps15 condensates. To form Eps15 condensates PEG is required.
Fig.S12	No	We are using FCS to determine the size of individual molecules of Eps15 and clathrin, not condensates.

Supplementary Table 2. Clarification of which experiments use 3% w/v PEG and which do not. We include

this table to clarify which experiments utilize 3% w/v PEG and which do not. In general PEG is used in experiments in which we are investigating interactions with protein condensates as 3% w/v PEG (which mimics the crowded environment of the cytosol) is required for condensate formation.