Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supplementary Figure 1

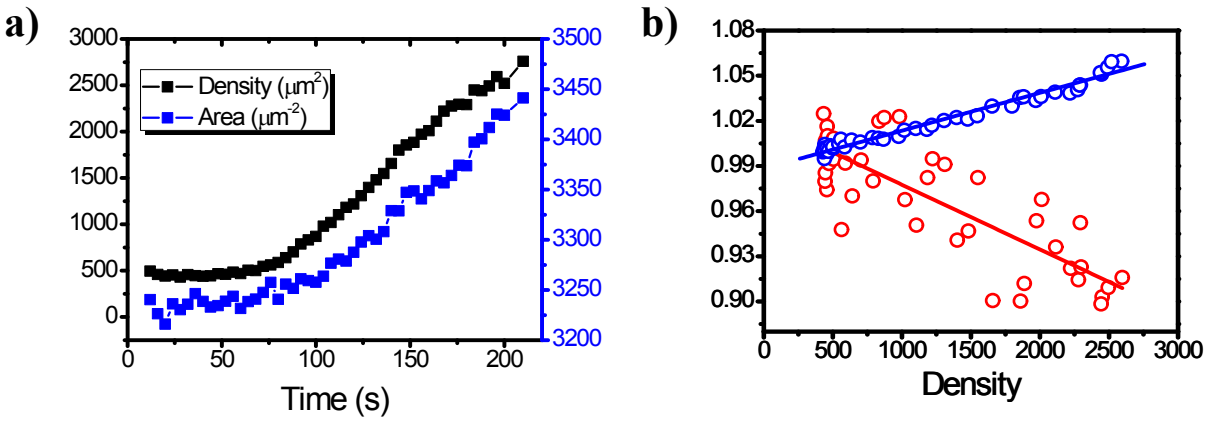


Figure S1. Membrane expansion is accompanied with a dilution of lipid dye

a) The binding of α-synuclein (black) and corresponding GUV area expansion (blue) follow the same trend. b) The expansion in membrane area during protein binding (blue) is accompanied with a decrease in lipid dye intensity (red), consistent with a protein induced lipid thinning effect. The lipid is labeled with TexasRed-DHPE and its fluorescence intensity is corrected for protein channel bleed through.

Supplementary Figure 2

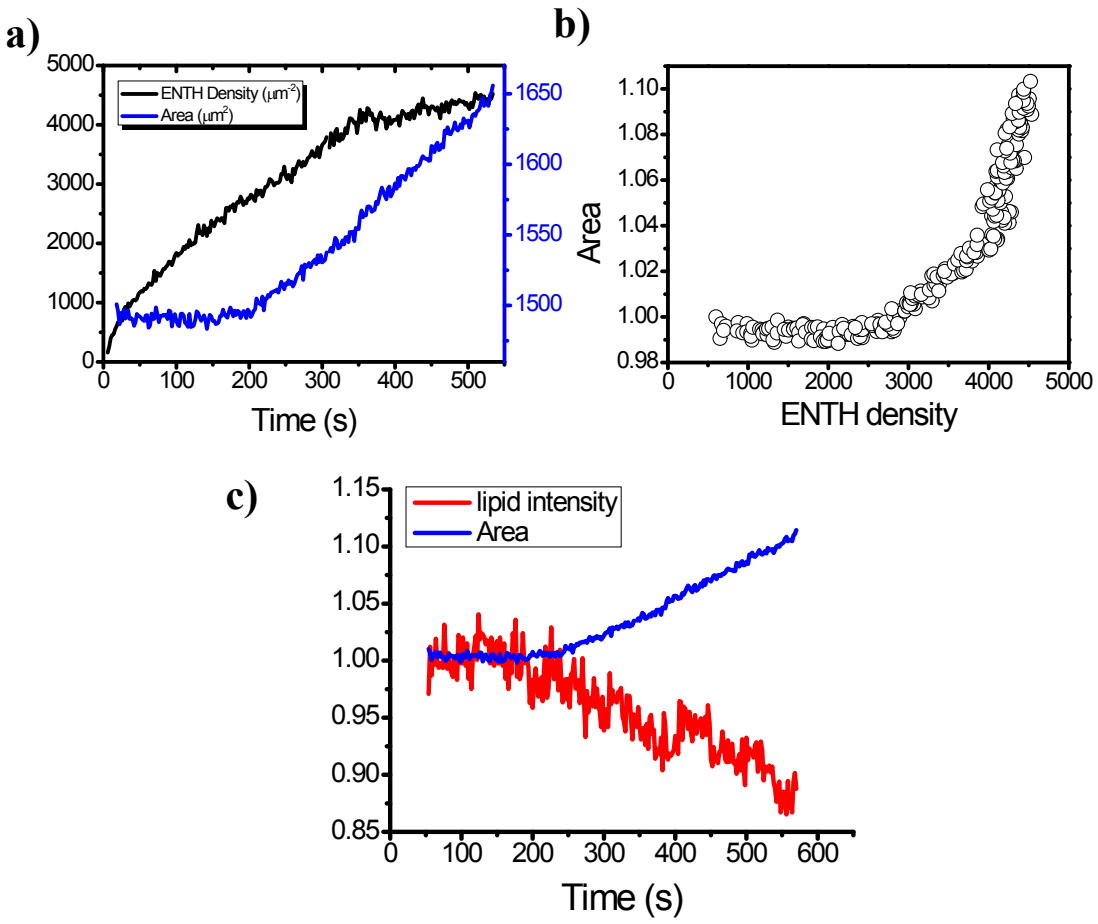


Figure S2. Membrane expansion induced by ENTH_GFP domain

a) Membrane binding of ENTH_GFP (black) and corresponding change in GUV membrane area (blue). b) A nonlinear relation between the ENTH_GFP density on membrane and the amount of area expansion. c) The expansion in membrane area is accompanied with a dilution of lipid dye in the membrane similar as in Figure S1b.

Bulk ENTH_GFP concentration, 200nM. GUV composition: 2% $PI4,5P_2$, 98%POPC.

Supplementary Figure 3

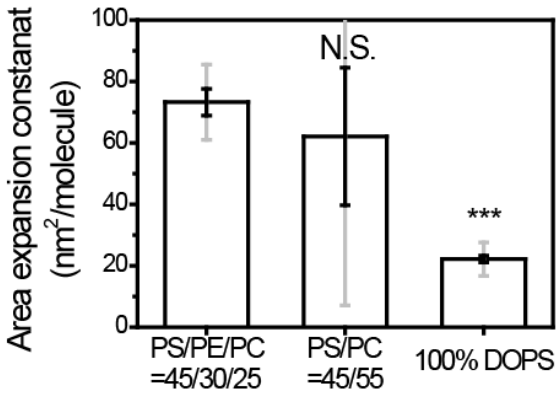
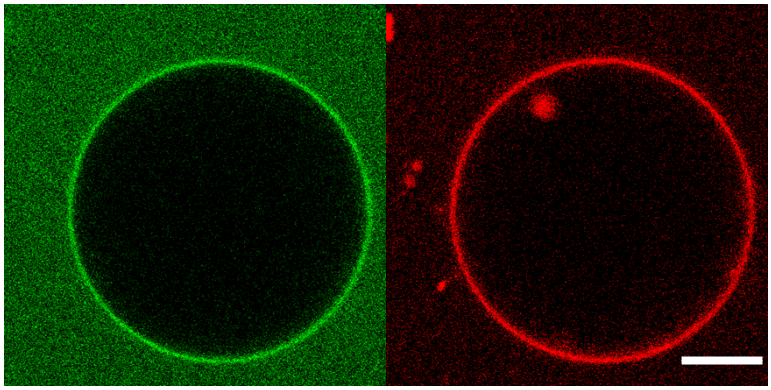


Figure S3. Comparison of area expansion constant on different lipid composition

Data on GUVs of DOPS/DOPE/DOPC=45/30/25 and pure DOPS GUVs are the same as in Fig. 3. In the case with GUVs of DOPS/DOPC=45/55 (average of 6 GUVs), the weak membrane binding (protein density<200μm^{-2}) of α-synuclein leads to a very high uncertainty in determining the area expansion constant on this lipid composition. Student t-test, ***p<0.001, N.S. p>0.1. The comparison is carried out under the same bulk protein concentration (250nM).

Supplementary Figure 4

a)



b)

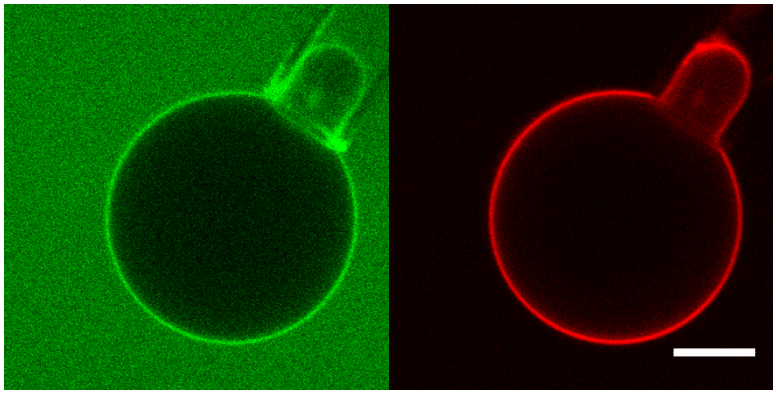


Figure S4. Membrane binding of α-synuclein does not lead to pore formation on the GUV

a) Representative confocal image of pure DOPS GUVs (50μM) co-incubated in 8μM α-synuclein.
b) Representative confocal image of an individual micropipette-aspirated GUV (DOPS/DOPE/DOPC=45/30/25) transferred into 500nM α-synuclein with an applied membrane tension =0.2mN/m.
Green: protein channel. Red: lipid channel. Scale bar: 10μm. In both cases, green fluorophore labeled proteins are not permeable to the inside of the GUVs. Furthermore, the GUV in b) remain intact under a high membrane tension. Both are evidence to support that there are no α-synuclein size pores on the GUV.

Supplementary Figure 5

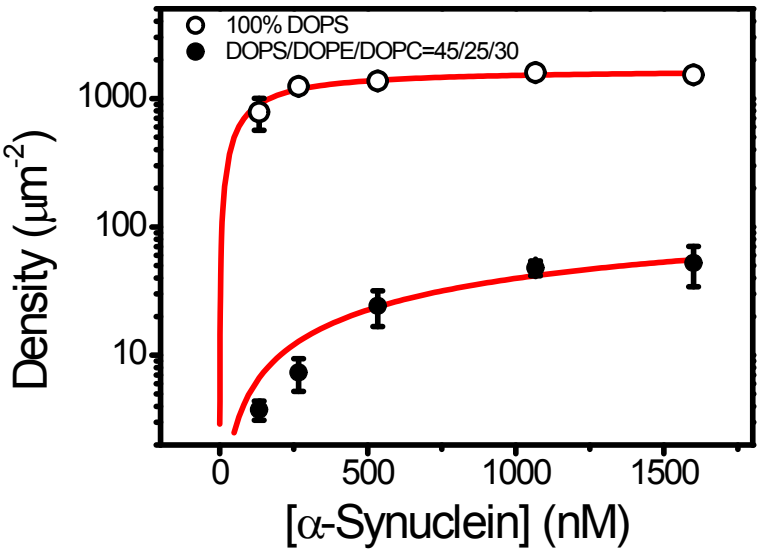


Figure S5. Comparison of GUV binding isotherm

α-Synuclein binding isotherm on GUVs with 100%DOPS (open). Fitting the isotherm to $\rho = \rho_{max}/(1+K_D/[P])$, the resulted binding constant is K_D=120±30nM, maximum protein density on membrane is ρ_{max}=1700±70μm^{-2}. α-Synuclein binding isotherm on GUVs with DOPS/DOPE/DOPC=45/30/25 (closed) has significantly less binding towards α-synuclein, with a dissociation constant K_D=3000±1000nM, maximum protein density on membrane ρ_{max}=200±100μm^{-2}. Lipid concentration: 50μM. Both isotherms are average of two independent trials with error bars representing standard deviation, each trail includes 15~20 GUVs per protein concentration. Buffer: 7 mM Hepes, 50 mM NaCl, pH 7.