Adsorption of α-Synuclein on Lipid Bilayers:
Modulating the Structure and Stability of Protein Assemblies

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Supporting Information

1. Fluidity of the observed lipid phase separation

To determine the mobility of the PC-rich and PA-rich regions, fluorescence recovery after photobleaching (FRAP) experiments were performed. The FRAP measurement system is discussed in detail elsewhere.[1] Briefly, a Nikon TE2000 fluorescence microscope equipped with a 40x oil immersion objective, a NBD filter set (Chroma Technology Corp.) and a silicon avalanche photodiode (APD) Single Photon Counting Module (SPCM-AQR-16-FC, PerkinElmer, Inc., Vaudreuil, Quebec) was used to focus, collect and count the emitted fluorescence respectively. A 15 mW Argon ion laser (488 nm Melles Griot) was used to both bleach and monitor the lipid bilayer. To reduce further photobleaching of the fluorophore during the recovery period the laser intensity was reduced 100,000-fold using a 5X (focal transmission of $1x10^5$) neutral density filter (NE50B, Thorlabs, Inc., Newton, NJ). A LabVIEW program was used to acquire the counts from the APD, control the filter wheel and trigger the shutter (Uniblitz ®, VA, Inc., Rochester, NY).

In Figure 1A-D the fluorescence recovery can be observed. Separation was induced by the addition of 5.4 μM α-synuclein at pH 5.0. An image of the bilayer was acquired before bleaching, as shown in Figure 1B. The bilayer was then bleached to background levels in one second using an Argon ion laser operating at 15 mW. The laser power was attenuated by 100,000-fold to 150 nW, and the recovering fluorescence was monitored using an avalanche photodiode (Figure 1A). At this laser power it is possible to monitor the bilayer indefinitely without further bleaching the sample.[1] The intensity was normalized by acquiring data prior to bleaching. To show the bleached area, the bilayer was bleached again with the laser and an image quickly acquired with a CCD camera (Figure 1C). Another image was acquired ~10 minutes later (Figure 1D). The first thing to note is that the recovery was nearly perfect and rapid, indicating that all of the lipids were fluid. Due to compositional differences, the diffusion of the NBD moiety will be different in the two regions. Fitting the recovery curve precisely is therefore non-trivial. By fitting to an exponential, a rough measurement of the diffusion coefficient can be determined.[2] Using this approach, we obtained a diffusion coefficient of ~3

$\mu m^2/s$, a value typical of fluid lipid bilayers. As detailed elsewhere,[3] if an entire dark PA-rich region (the size of which is similar to the laser bleach spot) is bleached, there is a clear exchange of lipids between the PA-rich and PC-rich regions with an estimated diffusion coefficient of ~1 $\mu m^2/s$. If recovery in the PA-rich regions only occurred because of transport through those regions the diffusion would not be fully two-dimensional, and therefore, it would be at least an order of magnitude slower. Consequently, the results show that there is exchange of lipids between the PA-rich and PC-rich regions. This experiment was repeated at pH 7.4 and similar behavior was observed.

2. Addition of 0.26, 2.6 and 5.4 μM α-synuclein at pH 5.0 and 7.4

In Figures 1 and 2 of the main text we examined the effect of sequential addition of α-synuclein at pH 5.0 and 7.4, respectively. Here, we discuss the effects of exposing protein-free bilayers to different concentrations of α-synuclein. The PC/PA bilayers were prepared as discussed in the Materials and Methods section. Figure 2A shows a PC/PA bilayer at pH 5.0 before the addition of protein. The bright spots in the lipid images are caps (an illustration and a higher magnification image are shown in Figures 2B and 2C). The bilayer bends away from the surface when exposed to asymmetric screening environments [4]. The bilayers are formed in a 250 mM NaCl buffer solution, and before addition of protein the NaCl concentration is reduced to 100 mM. In the images shown in the main text caps are present, but barely visible. They play no role in any of the discussed phenomena. We note however that when higher magnification images are acquired it is clear that α-synuclein binds less avidly to the caps than the surrounding PC-rich regions (caps are not observed within the limit of resolution in the PA-rich regions; they are destroyed with the creation of the PA-rich regions).

When varying concentrations of α-synuclein (in 50 mM MES hydrate, 0.1 mM EDTA, and 100 mM NaCl, pH 5.0) were added to the bulk solution, a similar separation of the lipids was observed in all three cases (Figures 2D, F, H). The extent of depletion of the NBD fluorophore (attached to the tail of a PC lipid) from the dark regions varied with the protein concentration: 18±1% at 0.26 μM, 23±1% at 2.6 μM and 27±4% at 5.4 μM. The addition of 0.26 μM α-synuclein (protein:lipid ratio 1:10) induced clustering of the negatively charged PA (Figure 2D) and resulted in preferential binding of the protein to the PA-rich regions (Figure 2E). The addition of 2.6 μM α-synuclein (protein:lipid ratio 1:1) induced clustering of additional negatively charged PA (Figure 2F). The branches in the PA-rich regions were thicker and again the protein bound at these sites preferentially (Figure 2G). At this point, protein binding to the PC-rich regions was saturated. In the presence of 5.4 μM α-synuclein (protein:lipid ratio 2:1), a greater extent of separation was observed (Figure 2H). Here we observed two distinct morphologies when viewing the protein (Figure 2I). α-Synuclein bound more extensively to some of the PA-rich regions (black arrow) as opposed to others (white arrow). In the regions of enhanced binding, the protein-rich regions did not overlay exactly with the PA-rich regions. Examining multiple samples, the area fraction of the PA-rich region was determined. For 0.26, 2.6 and 5.4 μM these values were: 18±5%, 29±8%, 37±8% respectively.

The experiments shown in Figure 2 were repeated at pH 7.4. Figure 3A shows a PC/PA bilayer before the addition of protein. In this image the caps are barely visible. However, their presence is evident in the protein images (Figure 3C, E, G): because the protein binds less avidly to the

caps, the latter appear as black dots that contrast strikingly with the lighter background. This is confirmed with higher magnification images (not shown). Protein-free PC/PA bilayers were exposed to α-synuclein at 0.26 μM (Figure 3B, C), 2.6 μM (Figure 3D, E) and 5.4 μM (Figure 3F, G). The extent of depletion of the NBD fluorophore (attached to the tail of a PC lipid) from the PA-rich regions was: 23±2% at 0.26 μM, 23±3% at 2.6 μM and 26±3% at 5.4 μM. Calculating the area coverage of the PA-rich regions was more complicated in this case: the separated regions were widely spaced from one another (i.e. the images shown contain regions where reorganization is visible, but it is also possible to acquire images of similar size in which no reorganization is visible). Many spots on many samples were interrogated. For bilayers treated with 0.26 μM α-synuclein we could only estimate an upper bound of 6% for the area coverage. Bilayers treated with 2.6 μM and 5.4 μM α-synuclein had PA-rich regions with area coverages of 16±3% and 19±3%, respectively. Compared to pH 5.0, the PA-rich regions were much more compact. At all concentrations the protein bound more avidly to the PA-rich regions than the PC-rich regions. The more protein added, the more difficult it became to see in the protein images the fine features which are observable in the lipid images. The binding appeared to saturate on the PC-rich regions but continued to occur on the PA-rich regions, as observed at pH 5.0.

3. Protein adsorption and desorption on zwitterionic bilayers

The experiments outlined in Figures 8 and 9 of the main text were repeated on a 99 mol% DOPC/1 mol% NBD-PC bilayer. The bilayer was uniform and fluid at all times. The protein bound uniformly and did not cluster over time, at either pH or at any ionic strength. Compared to PA-rich regions on mixed bilayers there was little protein adsorption on PC bilayers. To improve the accuracy of our measurements an avalanche photodiode (APD) was used instead of a CCD camera. The experimental set-up was as follows: A Nikon TE2000 fluorescence microscope equipped with a 40x oil immersion objective, an Alexa filter set (Chroma Technology Corp.) and a silicon avalanche photodiode (APD) Single Photon Counting Module (SPCM-AQR-16-FC, PerkinElmer, Inc., Vaudreuil, Quebec) was used to focus, collect and count the emitted fluorescence. An arc lamp was used to monitor the protein, and a LabVIEW program was used to acquire the counts from the APD. To minimize photobleaching of the fluorophore during the counting period, the lamp intensity was reduced using a 5X neutral density filter (focal transmission of $1x10^5$) (NE50B, Thorlabs, Inc., Newton, NJ). Results are shown in Figure 4. Each data point represents the average of six individual experiments, and the error bars represent the standard deviation of the mean.

At pH 5.0 (Figure 4, open squares), 2.6 μM α-synuclein was added to a PC bilayer in 100 mM NaCl. As in Figure 8 of the main text, the bulk solution was altered in three steps: (i) increasing the NaCl concentration to 1000 mM at pH 5.0, (ii) increasing the pH to 7.4 at 1000 mM NaCl, and (iii) decreasing the NaCl concentration to 100 mM at pH 7.4. As discussed we propose that there is ~1 monolayer adsorption on PC bilayers. The protein is less helical than when bound to PA-rich regions on a mixed bilayer, which means that there are fewer solvated hydrophobic residues and thus, less to gain energetically from the formation of protein-protein contacts. It is expected that a variety of conformations are adopted by the membrane-bound protein. With each manipulation of the environment desorption is observed; as a percentage of the total bound protein less desorption is observed in this experiment than in Figure 8 of the main text.

At pH 7.4 (Figure 4, open circles) 2.6 μM α-synuclein was added to a PC bilayer in 100 mM NaCl. As in Figure 9 of the main text the bulk solution was altered in two steps: (i) increasing the NaCl concentration to 1000 mM, and (ii) decreasing the NaCl concentration to 10 mM. As compared with pH 5.0 there was less initial adsorption, an effect attributed to charge-charge repulsion of neighboring C-termini. It is expected that the membrane-bound protein adopts a variety of conformations because the PC bilayer has a much lower propensity to stabilize an α-helical structure than PA-rich regions on a mixed bilayer. Some aSyn conformers are displaced from the PC bilayer when the ionic strength is increased (Figure 4). Similar to the results obtained at pH 5.0, less desorption is observed from a PC bilayer (as a percentage of the total bound protein) than from PC/PA membranes (Figure 9 of the main text). These findings provide further support for the assumption that on PC membranes there is ~1 monolayer coverage, while on PA-rich regions there is multilayer coverage.

References:

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(3)Cambrea, L. R.; Haque, F.; Schieler, J. L.; Rochet, J. C.; Hovis, J. S. *Biophys J* **2007**, *93*, 1630-8.
(4)Cambrea, L. R.; Hovis, J. S. *Biophys J* **2007**, *92*, 3587-94.

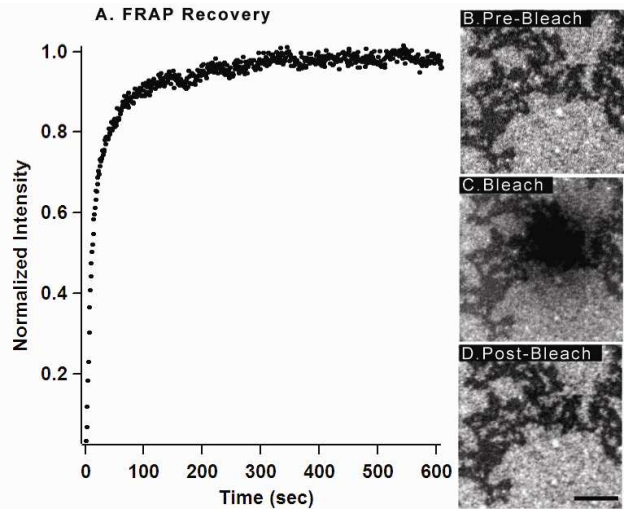

Figure 1: Fluorescence recovery after photobleaching (FRAP): The bilayer was composed of 30 mol% DOPA/69 mol% DOPC/1 mol% NBD-PC, and 5.4 μM α-synuclein was added at pH 5.0. (A) Plot of fluorescence recovery as a function of time; an avalanche photodiode was used to collect the light, and the laser power was 150 nW. Epi-fluorescence images of the bilayer prior to bleaching (B), immediately after bleaching (C), and 20 minutes later (D). A 40X, 1.3 NA objective was used to acquire the images. The scale bar represents 20 μm.

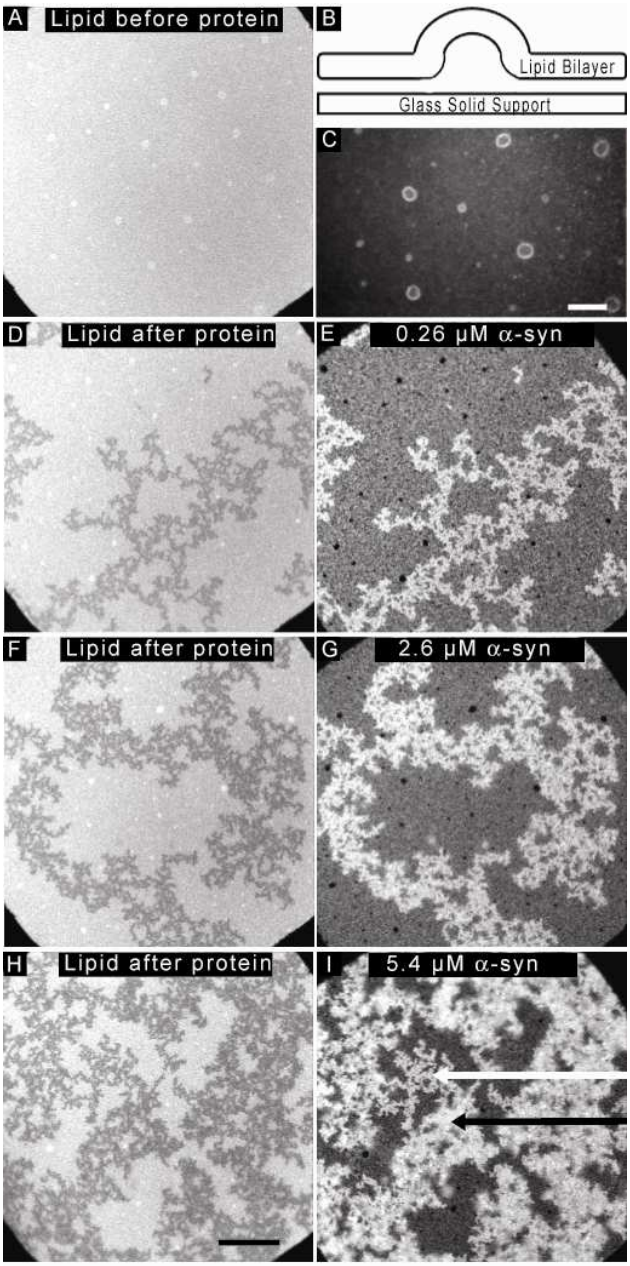

Figure 2: Epi-fluorescence images of 30 mol% DOPA/69 mol% DOPC/1 mol% NBD-PC bilayers at pH 5.0. Bilayer image was taken before the addition of the protein (A). The bright spots are caps, shown schematically in (B) and evident at higher magnification in (C) (scale bar: 10 μm). Lipid bilayers after the addition of 0.26 μM, 2.6 μM and 5.4 μM α-synuclein to protein-free bilayers: (D, F, H) lipid images, (E, G, I) protein images. The white and black arrows in (I) identify regions of greater (black arrow) and lesser (white arrow) adsorption. The images are contrasted to clearly show the light and dark regions compared to the background levels in the corner. A 40X, 1.3 NA objective was used to acquire the images in panels A, C-E. A 100X, 1.3 NA objective was used to acquire the image in panel B. The scale bar in (H) represents 40 μm (applies to panels A and D-H).

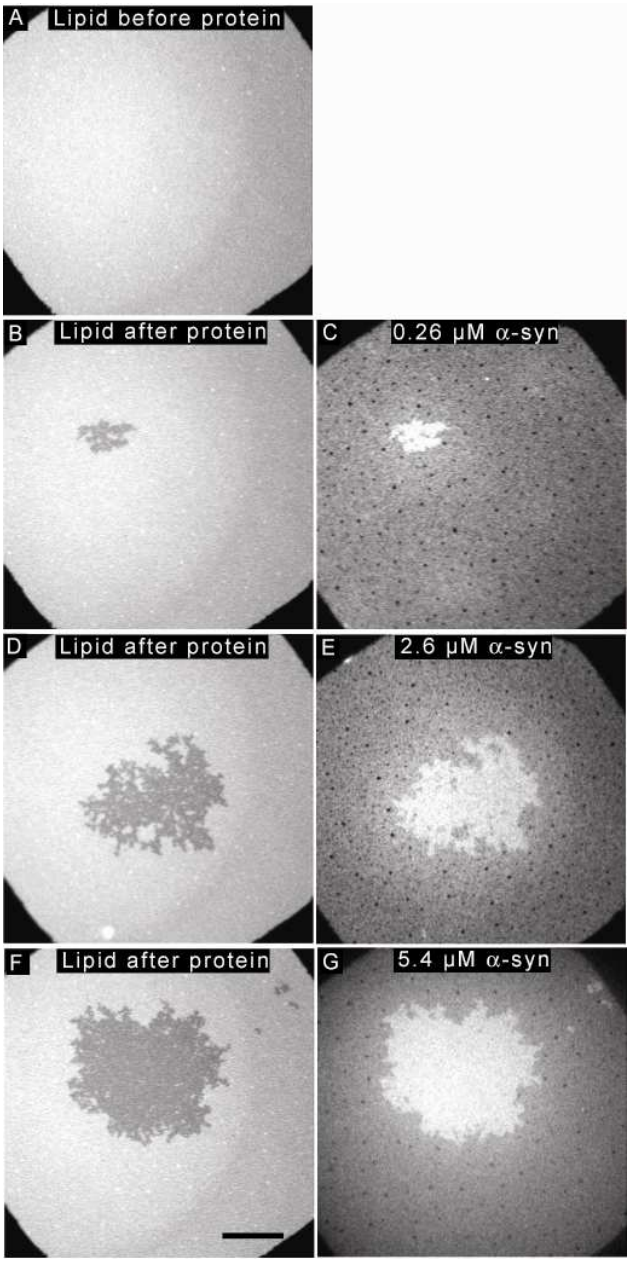

Figure 3: Epi-fluorescence images of 30 mol% DOPA/69 mol% DOPC/1 mol% NBD-PC bilayers at pH 7.4. Bilayer image was taken before the addition of the protein (A). Lipid bilayers after the addition of 0.26 μM, 2.6 μM and 5.4 μM α-synuclein to protein-free bilayers: (B, D, F) lipid images, (C, E, G) protein images. A 40X, 1.3 NA objective was used to acquire the images. The scale bar represents 40 μm.

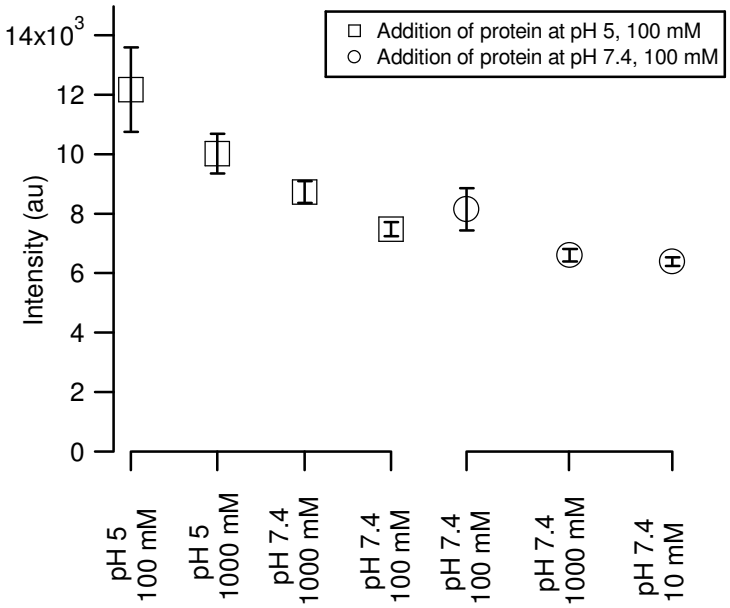

Figure 4: Quantification of the amount of bound protein to zwitterionic surfaces; a 40X, 1.3 NA objective and an avalanche photodiode was used to collect the fluorescence. The protein was added in the presence of 100 mM NaCl at either pH 5.0 (open squares, left) or pH 7.4 (open circles, right), and the bulk solution was sequentially exchanged as indicated on the x-axis for each of the two starting conditions. Each data point represents the average of six samples. The error bars represent the standard deviation of the mean.