α-Synuclein-Induced Tubule Formation in Lipid Bilayers

Anjan P. Pandey [†], Farzin Haque[†], Jean-Christophe Rochet[‡] and Jennifer S. Hovis[†]**

[†]Department of Chemistry, [‡] Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, IN 47907.

Supporting Information

Effect of incubation time. To investigate the effect of incubation time on the tubulation process, protein was added to the bulk solution, and images were acquired at the same spot on the bilayer immediately thereafter (~0 min), and after 5, 10, and 15 minutes. The bilayer contained 25 mol% PG. Figure S1 shows that for all four variants, tubules were seen essentially immediately. The largest extent of growth occurred from 0 to 5 minutes, regardless of the identity of the α-synuclein variant.

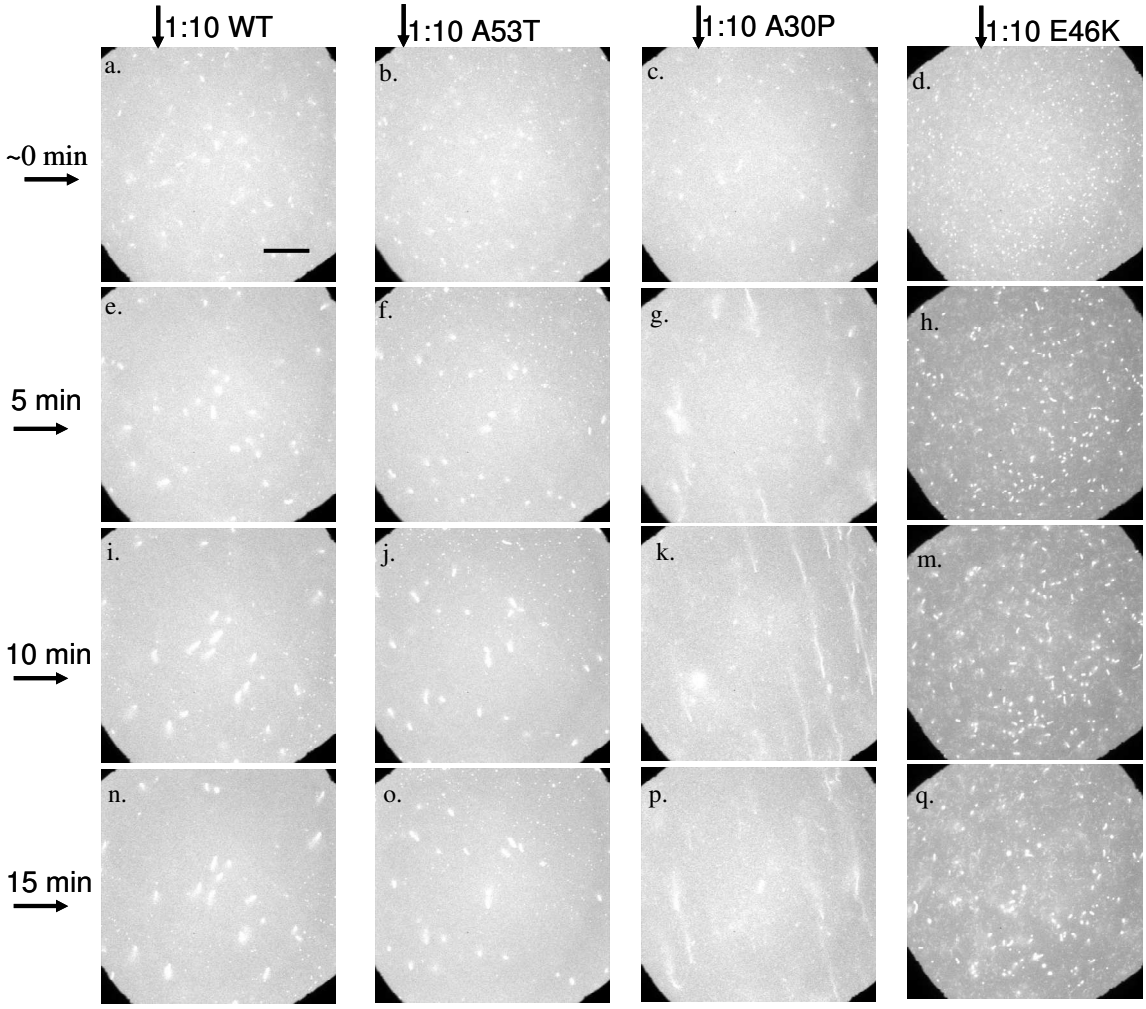

Figure S1: Time evolution of tubule formation: Epi-fluorescence images of bilayers containing 25 mol% PG at different time intervals (indicated by horizontal arrows) after the addition of 2.6 μM WT, A53T, A30P, or E46K (indicated by vertical arrow). All the images in each column were acquired at the same spot. The scale bar represents 40 μm

Effect of addition of unlabeled protein: In previous work, we looked at the adsorption of 2.6 μM WT α-synuclein on bilayers containing 30, 40 and 50 mol% PG.[1] After α-synuclein addition the bilayers remained flat, and no tubules were observed. When 40 or 50 mol% PG was present the bilayers reorganized into PC-rich and PG-rich regions. In the presence of 30 mol% PG no de-mixing was observed in the membrane. This earlier work was done with protein that was labeled on the N-terminus (at ~20 mol%) with Alexa 647. If unlabeled WT α-synuclein was used the features observed at high anionic lipid content were reproduced: uniform, flat bilayer at 35 mol% (Figure 2a); phase-separated, flat bilayer at 50 mol% (Figure 2b).

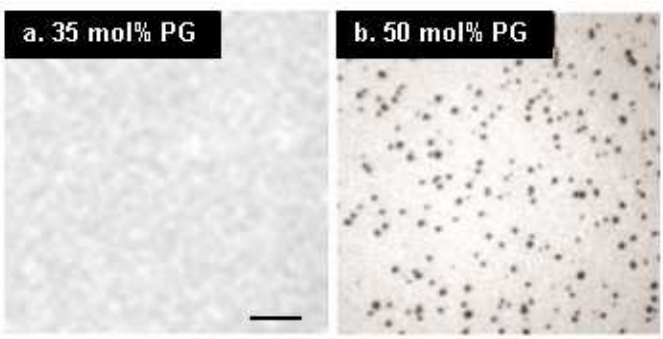

Figure S2: Adsorption of unlabeled protein on bilayers containing different amounts of PG: Epi-fluorescence images of bilayers with (a) 35 and (b) 50 mol% PG after the addition of 2.6 μM WT. Each bilayer contained 0.25 mol% NBD-PC. The scale bar represents 10 μm.

(1) Pandey, A. P., Haque, F., Rochet, J.-C, Hovis, J.S. *Biophys J* **2009**, *96*, 540.