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

Supplementary Information:

Order-disorder skewness in alpha-synuclein: a key mechanism to recognize membrane curvature

Marcelo Caparotta,[†] Diego M. Bustos,[‡,†] and Diego Masone[*,‡,¶]

†*Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Cuyo (UNCuyo), 5500, Mendoza, Argentina*

‡*Instituto de Histología y Embriología de Mendoza (IHEM) - Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Universidad Nacional de Cuyo (UNCuyo), 5500, Mendoza, Argentina*

¶*Facultad de Ingeniería, Universidad Nacional de Cuyo (UNCuyo), 5500, Mendoza, Argentina*

E-mail: diego.masone@ingenieria.uncuyo.edu.ar

Phone: +54 261 449 4117. Fax: +54 261 449 4117

Umbrella Sampling Convergence Analysis

Free energy profiles were recovered using the Weighted Histogram Analysis Method (WHAM) from 51 windows runs (100ns each, with the system in proper equilibrium at all times) which are a set of configurations representative of the complete equilibrium ensemble of interest. Convergence was assessed by applying WHAM on consecutive trajectory blocks of 10 ns. Supporting figure 1 shows the resulting profile at different times for the DPPC:DOPC:CHOL lipid bilayer with full-length α-synuclein adsorbed on its surface. It can be observed that between 80ns and 90ns of simulation time per window, free energy changes between profiles are already sufficiently small ($\sim 2k_BT$).

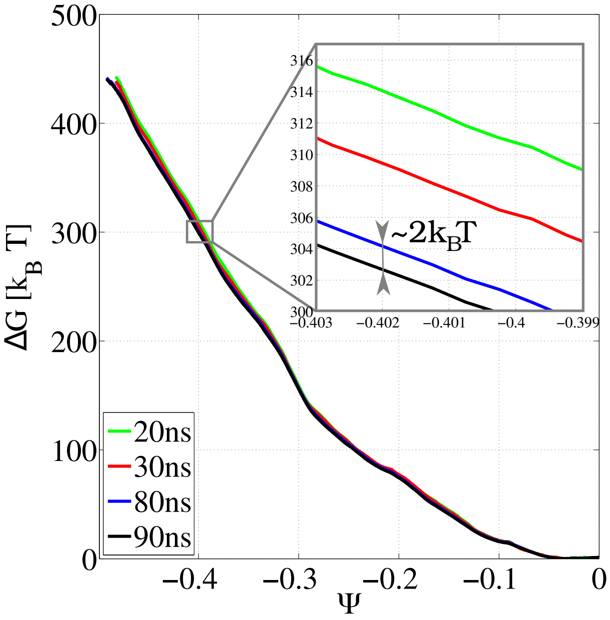

Figure 1: **Umbrella Sampling Convergence 1.** Successive application of the weighted histogram analysis method for recovering free energy profiles and to check for convergence. DPPC:DOPC:CHOL bilayer with full-length α-synuclein.

Supporting figure 2 shows the free energy profile recovered at different times for the DPPC:DOPC:CHOL lipid bilayer with truncated 1-100 α-synuclein adsorbed on its surface. In all cases, uncertainties were estimated using bootstrapping analysis with the *gmx wham* tool available in Gromacs.

In supporting figure 3 it is observed that truncated 1-100 α-synuclein shows the lowest RMSF and Lindemann index for both situations: flat and curved bilayer (see cyan and magenta diamonds). This indicates that truncated α-synuclein is unable to respond to membrane bending as much as full-length α-synuclein does. On the contrary, full-length α-synuclein shows different patterns as the bilayer bends (see red and black circles for the ordered region and green and blue stars for the disordered one).

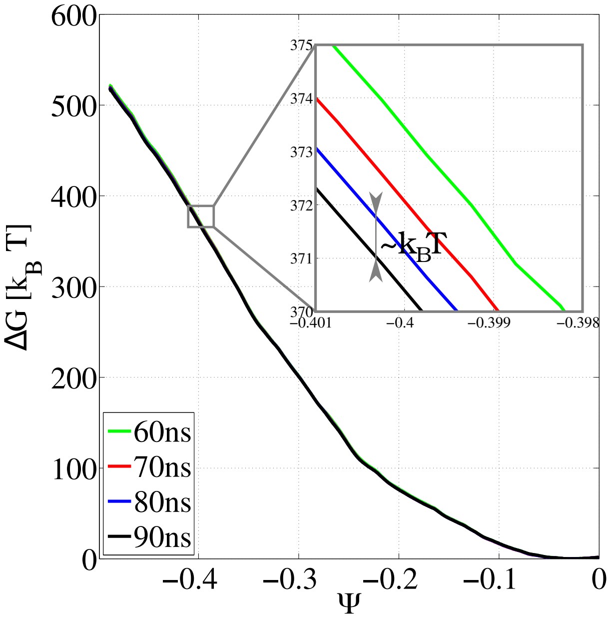

Figure 2: **Umbrella Sampling Convergence 2.** Successive application of the weighted histogram analysis method for recovering free energy profiles and to check for convergence. DPPC:DOPC:CHOL bilayer with truncated 1-100 α-synuclein.

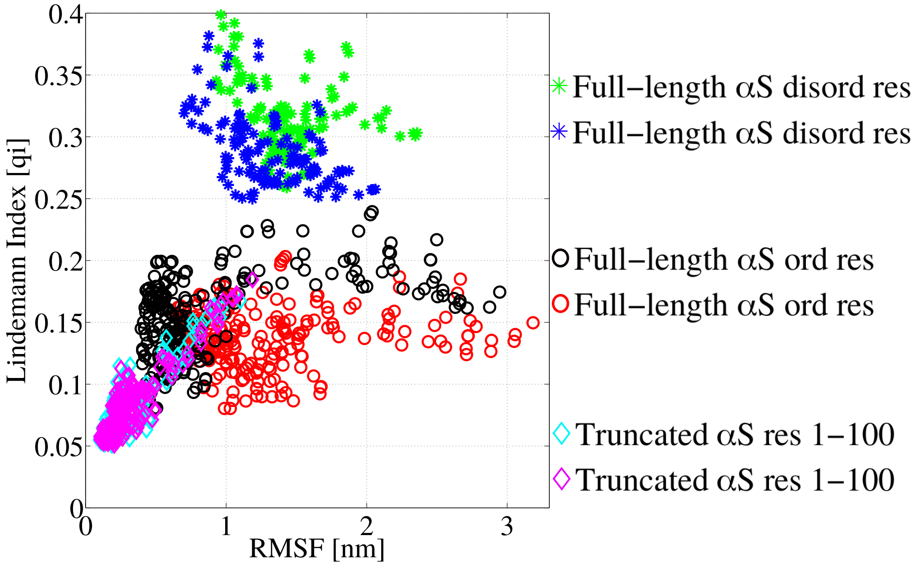

Figure 3: **Scatter plot mapping Lindemann index to RMSF along 5 μs of unbiased simulations for full-length and truncated α-synuclein**. Calculated for α-synuclein adsorbed on a DPPC:DOPC:CHOL lipid bilayer for different amounts of induced curvature (green, red and cyan for the flat bilayer and black, blue and magenta for a highly curved one). Symbols indicate: ordered region (circles), disordered region (stars) and truncated protein (diamonds).