



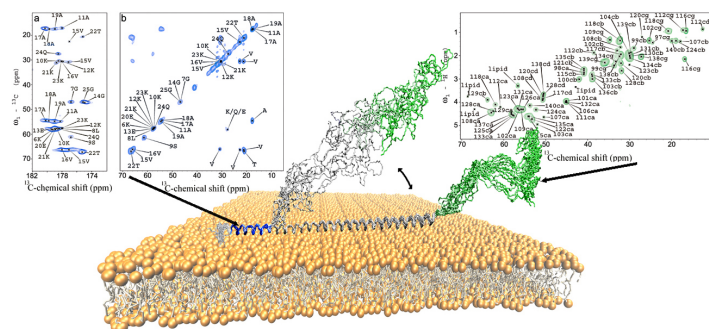
# Probing the interaction between $\alpha$ -synuclein and lipid membranes by NMR Spectroscopy



Giuliana Fusco<sup>||</sup>, Alfonso De Simone<sup>†</sup>, Gopinath Tata<sup>§</sup>, Vitaly Vostricov<sup>§</sup>, Michele Vendruscolo<sup>||</sup>, Christopher M. Dobson<sup>||</sup>, Gianluigi Veglia<sup>§</sup>

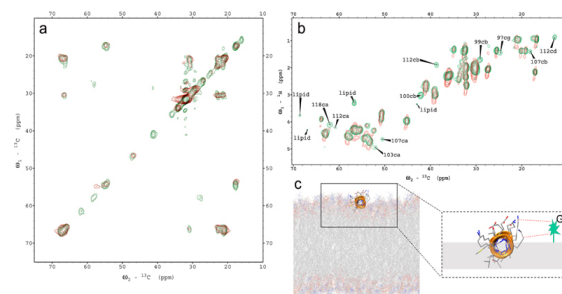
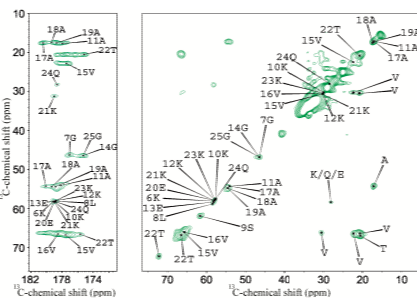
<sup>||</sup>Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge, CB2 1EW, UK <sup>†</sup>Department of Life Sciences, Imperial College London, South Kensington, London, SW7 2AZ, UK <sup>§</sup>Department of Chemistry & Department of Biochemistry, Biophysics & Molecular Biology, University of Minnesota, 6-155 Jackson Hall, USA

$\alpha$ -synuclein ( $\alpha$ S) is a protein involved in neurotransmitter release in presynaptic terminals, and whose aberrant aggregation is associated with Parkinson's disease. In dopaminergic neurons,  $\alpha$ S exists in a tightly regulated equilibrium between water-soluble and membrane-associated forms. Using a combination of solid-state (Figures 1-3) and solution-state NMR spectroscopy (Figure 4), we characterised the conformations of  $\alpha$ S bound to lipid membranes mimicking the composition and physical properties of synaptic vesicles. The study evidences key regions of the protein possessing distinct structural and dynamical properties and having specific roles in determining the way the protein partitions between membrane-bound and unbound states. The N-terminal segment of *ca* 25 residues adopts a well-defined, motionally restricted helical conformation and acts as a membrane-anchor. This region appears conserved using lipid mixtures having significantly different affinity (Figures 1, 3). The C-terminal residues remain in an unstructured state (Figure 1) and exhibits weak and transient interactions with the membrane surface (Figure 2). A key role has, however, been identified for the central segment of the  $\alpha$ S sequence, which behaves as a sensor of the properties of the membrane in such a way that it determines the affinity of membrane binding (Figure 4). Taken together, our data define the nature of the interactions of  $\alpha$ S with biological membranes and provide insights into their roles in the function of this protein as well as in the molecular processes leading to its aggregation.

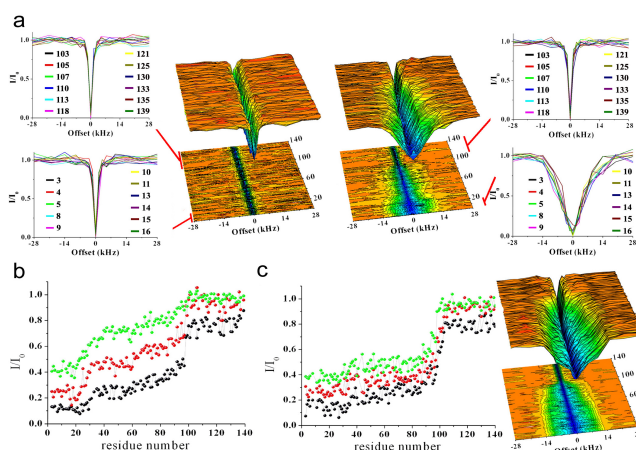


**Figure 1. Magic angle spinning (MAS) measurements evidence three regions in  $\alpha$ S in determining its interaction with lipid bilayers.** The experiments identified three different regimes of structural dynamics in the membrane associated state of  $\alpha$ S, when bound to lipid vesicles that mimic synaptic vesicles for physical properties and composition (DOPE:DOPS:DOPC mixtures). The N-terminal region (blue) is visible in Dipolar Assisted Rotational Resonance (DARR) experiments, indicating that it is rigidly bound and anchored to the membrane. The central region (grey), showing intermediate dynamics and therefore being invisible in both Cross Polarization (CP) and Insensitive Nuclei Enhanced Polarization Transfer (INEPT) experiments, play a key role in modulating the affinity of  $\alpha$ S for membranes. Finally a C-terminal fragment (green) maintains its unstructured nature and remains essentially uncorrelated with the membrane surface.)

**Figure 3. MAS spectra of  $\alpha$ S bound to POPG SUV.**  $^{13}\text{C}$ - $^{13}\text{C}$  DARR correlation spectrum recorded using 100 ms mixing time at a temperature of -19 °C. The left panel shows the carbonyl region and the right panel the aliphatic region.



**Figure 2. Topology of the membrane bound state of  $\alpha$ S.** The DOPE:DOPS:DOPC mixture was doped with 2% of 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine-N-DTPA (gadolinium salt). Comparison between DARR and INEPT spectra with (red) and without (green) the spin label are shown in panels a and b respectively. c) Schematic description of hydrophilic residues populating the water/headgroup interface and the hydrophobic residues pointing toward the inner core of the membrane



**Figure 4. Strength of membrane association by chemical exchange saturation transfer experiments (CEST).** CEST experiments were recorded using a protein concentration of 300 mM and 0.06% (0.6 mg ml<sup>-1</sup>) of DOPE:DOPS:DOPC lipids in a ratio of 5:3:2 and assembled in SUVs. a) CEST surface for unbound (left) and bound (right)  $\alpha$ S; the upper and lower inserts report individual CEST profiles for residues at the N- and C-termini, respectively. b) CEST saturation along the  $\alpha$ S sequence. Black lines refer to the averaged CEST profiles measured using offsets at  $\pm 1.5$  kHz. Similarly, profiles for  $\pm 3$  kHz and  $\pm 5$  kHz are shown in red and green, respectively. c) The interactions between  $\alpha$ S and POPG SUVs probed by CEST. Labels as in panel b. The data reported in this figure were measured using 350 Hz as bandwidth.

## Reference

- 1 Bodner CR et al. *J. Mol. Biol.* **390**, 775-790 (2009).
- 2 Lokappa SB et al. *J. Biol. Chem.* **286**, 21450-21457 (2011).
- 3 Eliezer D et al. *J. Mol. Biol.* **307**, 1061-1073 (2001).
- 4 Breydo L et al. *Biochem. Biophys. Res. Commun.* **372**, 261-285 (2008).
- 5 Ulmer TS et al. *J. Biol. Chem.* **280**, 9595-9603 (2005).