

while curvature sensing is generally conserved throughout the membrane binding region of aSyn, curvature generation requires specific binding domains. These results will help to form a model for the interplay between aSyn membrane binding activity and membrane remodeling and may have implications for understanding both aSyn's native role as well as its contribution to PD.

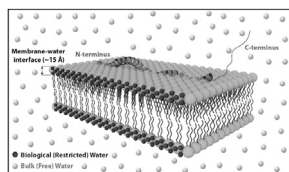
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Site-Specific Hydration Dynamics Illuminates the Key Structural Features of Membrane-Bound α -Synuclein

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α -Synuclein is an intrinsically disordered protein that is preferentially expressed in presynaptic nerve terminals. It undergoes a large-scale conformational rearrangement upon binding to synaptic vesicle membranes. In order to obtain the structural insights into the membrane-bound α -synuclein at the residue-specific resolution, we incorporated single tryptophan at various locations along the sequence. These tryptophans were used as site-specific fluorescence markers to characterize the structural and dynamical aspects of α -synuclein. The spatial localization of various parts of the protein near the membrane surface was elucidated utilizing a unique and sensitive fluorescence readout, namely, red-edge excitation shift (REES), which originates when a fluorophore is located in a highly ordered micro-environment. The extent of REES observed at different locations allowed us to directly identify the residues that are localized at the membrane-water interface comprising a thin (~ 15 Å) layer of motionally-constrained water molecules (Figure 1). Additionally, we have

been able to distinguish subtle but important structural differences of α -synuclein bound to different lipids membranes. We believe that the structural modulations of α -synuclein on the membrane could potentially be related to its physiological functions as well as to the onset of Parkinson's diseases.



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Determination of Primary Nucleation Mechanisms of α -Synuclein Amyloid Aggregation

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Protein conformational diseases represent a class of pathologies in which specific peptides or proteins form aberrant self-assemblies that constitute the hallmark of several neurodegenerative diseases. Specifically, the formation of intra-neuronal inclusions of the protein α -synuclein (α Syn) is associated with the pathogenesis of Parkinson's disease (PD).

A great interest is in the early stages of α Syn aggregation, for which soluble monomeric proteins are converted into fibrillar nanostructures. It has been shown that at these stages many parallel and competing pathways take contemporaneously place and it is currently very difficult to address on these mechanisms by using standard techniques of molecular investigations. In order to overcome the limitations of standard approaches, we employed ensemble-averaged kinetic studies coupled with microdroplet technology in order to characterize the primary nucleation early stages of α Syn amyloid formation and therefore to elucidate the fundamental mechanisms underlying this phenomenon. Testing different aggregation conditions, we have been able to understand that the primary nucleation mechanism underlying α Syn aggregation is not homogeneous, whereas it is catalysed by different factors, including air/water surface interactions.

The full characterization of all the processes involved in the aggregation mechanism of α Syn will be fundamental for devising new and innovative therapeutic strategies against PD. Indeed, based on our analysis, we expect that it will be possible to design and screen pharmacological compounds able to selectively inhibit the nucleation steps that trigger either the overall of the process or specifically the formation of the toxic aggregated species.

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Oligomerisation of Alpha-Synuclein at Physiological Concentrations

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Alpha-synuclein is a small intracellular protein naturally abundant in the brain at low-micromolar concentrations. Its fibrillar aggregates are the major constituents of intracellular inclusions, known as Lewy bodies, which are the pathological hallmarks of Parkinson disease and related neurodegenerative disorders. However, increasing evidence suggest that oligomers, rather than fibrils, are the

most toxic and damaging to brain neurons. Single molecule FRET can be used to detect and characterise the low levels of heterogeneous oligomers formed during protein aggregation. In this presentation, I will discuss the recent results of in-vitro studies of alpha-synuclein oligomer formation at physiologically-relevant concentrations using single-molecule FRET spectroscopy and show how these experiments reveal the key microscopic reactions taking place during the aggregation of alpha-synuclein.

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Single-Molecule Spectroscopy Reveals Polymer Effects of Disordered Proteins in Crowded Environments

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It is currently unclear how the crowded cellular milieu affects the structural distributions of intrinsically disordered proteins (IDPs). Here we employ single-molecule Förster resonance energy transfer (FRET) to quantify the effect of molecular crowding on four disordered proteins with very different degrees of charge-induced expansion. For a variety of crowding agents (PEG, PVP, Dextran, PVA), an increasing collapse of the polypeptide chains is observed with increasing concentrations of crowder, as expected from simple considerations, such as scaled particle theory. However, we also observe an increasing collapse with increasing size of the crowder, the opposite of what scaled particle theory predicts.

Interestingly, the observations can be rationalized quantitatively within the framework of Flory-Huggins theories that take into account the polymeric properties of both the disordered proteins and the crowder. The results provide a step towards understanding the behavior of IDPs and denatured proteins in the presence of polydisperse co-solutes as those characteristic of the cellular environment.

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Single-Molecule Characterisation of Alpha-Synuclein Oligomers

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The pathological hallmark of Parkinson's disease is the presence of insoluble protein deposits in the brain, which are formed when specific protein molecules misfold and aggregate into highly ordered fibrils. In Parkinson's disease, the deposits are primarily made up of alpha-synuclein, a protein whose major function is not fully known. Rather than the fibrils themselves being toxic, evidence now points towards the smaller, soluble oligomers formed in the initial stages of the process as being the culprit. We have developed a novel single-molecule fluorescence technique to detect and characterise the oligomers of alpha-synuclein. Using this methodology, we are able to identify the cytotoxic species, and apply these species to primary neuronal cultures to investigate their damaging effects.

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Repeats in the α -Synuclein Sequence Determine its Conformation on Membranes and Influence Aggregation Properties

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Alpha-Synuclein (aS) is a 140 aa intrinsically disordered and amyloidogenic protein. Its physiological functions are unclear, but are believed to be connected to the interaction with synaptic vesicles or membranes of other organelles.

The sequence of aS shows partial 11 amino acid periodicity that induces atypical 3/11 helix conformation on membranes. Fluorescence studies of tryptophan mutants of aS point to a flexible break at residues 52-54 between two helical domains. Deletion of this flexible 4 aa fragment between two groups of 11 aa repeats does not significantly affect aS membrane binding but strongly decreases the protein aggregation and fibril formation propensity. Moreover, the deletion mutant inhibits aggregation of wildtype aS, likely by hindering the fibril growth, since the mutant does not appear to co-aggregate into fibrils with wt aS.

Introducing additional 11 amino acid repeats into the aS sequence increases affinity of the modified protein to membranes and slows down the protein aggregation. We believe that the 11 amino acid repeats in the aS sequence play a key role in aS's ability to switch between a helical conformation on membranes and β -sheets in fibrils.

