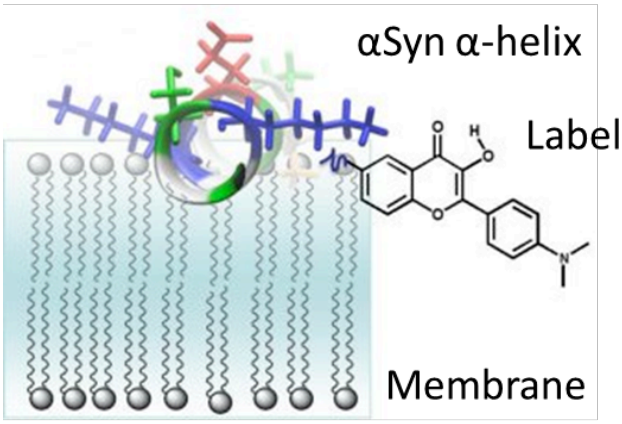
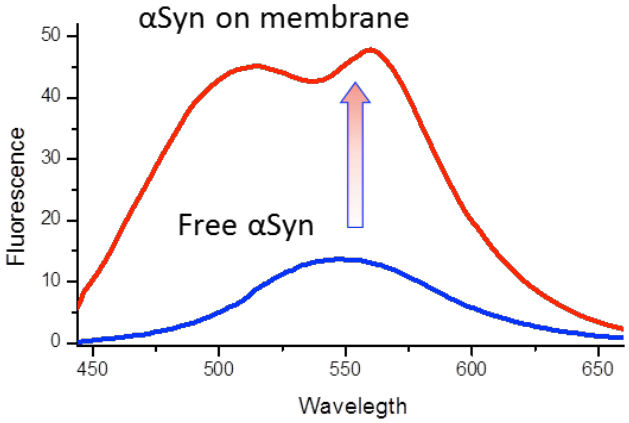
Parkinson´s Disease is the second most common neurodegenerative disease affecting more than 1% of people of over 65 years. It is characterized by the loss of functional dopaminergic neurons and the intracellular accumulation of aggregated, fibrillar amyloid forms of the protein α-synuclein (αSyn). Although the precise causes of cellular death are still unclear, misfolding leading to the formation of toxic, oligomeric forms of αSyn are almost certainly involved. The physiological functions of αSyn are tentatively presumed to be associated with vesicle trafficking and fatty acid metabolism, and the membrane-binding capacity of the protein is thought to modulate both its physiological and pathological roles. αSyn is a small (140 amino acids) protein lacking a distinct secondary structure when free in solution but is able to adopt α-helical conformation upon membrane binding or a β-sheet structure upon amyloid fibrillization.

For studying the αSyn membrane binding, we constructed a new Cys-reactive fluorescent probe (ESIPT: excited state intermolecular proton transfer) with high sensitivity to the polarity and H-bonding capacity of the microenvironment and introduced it into the AS molecule at each of several alternative positions spanning the protein sequence. These constructs were used in a series of equilibrium and kinetic (stopped-flow) studies to establish the key membrane parameters that determine the binding and conformation of AS. We also used it to compare the immersion of different αSyn domains into membrane and correlated results with CD and EPR spectroscopy data in order to estimate the conformation and orientation of αSyn on membranes. To study the dissociation of αSyn from membranes we devised an assay based on forming complexes of αSyn with liposomes of certain affinity for the protein, then challenging with liposomes formed with lipids affording higher affinity. The ESIPT probes monitored the changes in environment polarity upon migration of the protein between the liposomes.

We have determined that αSyn binds either negatively charged membranes or rigid neutral membranes of high curvature. Binding to neutral membranes likely depends on the presence of membrane defects and the maximal protein to lipid ratio is lower in that case. The protein density on membranes also affects the conformation of αSyn. At high protein to lipid ratio it binds mostly through the N-terminus with less involvement of the hydrophobic NAC region. We have also shown that binding of αSyn to membranes is reversible and that the protein can migrate between membranes varying in lipid

composition.





The potential impact of this study is great because it reveals the propensity of αSyn for binding to a number of intracellular compartments and documented this property by quantitative techniques. Thus, one has gained quantitative kinetic data for the binding of αSyn to membranes, information not previously available in the literature. One must assume that the consequences will vary depending upon whether a physiological (e.g. synaptic transmission) or pathological (impairment of protein quality control and of cellular energy metabolism) is involved. For example, exposure of αSyn to an acidic microenvironment (as in liposomes) is likely to induce its amyloid transformation, e.g. to fibrillar forms. By themselves, such structures may not be injurious to the cell yet their physical and secondary interactions may well be We have not investigated the consequences of post-translational modifications of αSyn but these are undoubtedly very important as well. In addition, the interactions of αSyn with other proteins (and membranes) is likely to be just as (or even more) important as with itself. Has one gained new insights into drug development strategies from our study? Not directly although the perception that the intracellular targetting of αSyn, in addition to its level of expression, may be constitute a focus for selection strategies.