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Physical chemistry in biomedical research: From cuvettes towards cellular insights

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Physical chemistry offers the tools necessary for understanding molecular interactions in the world around us and plays an essential role in biomedical research to help reveal the complex mechanisms of disease. One significant challenge in biomedical research is that the chemistry of human health takes place in a complicated cellular environment full of large molecules, like proteins and lipids, which can all play critical roles in the development of disease. And while the large size of these molecules can make them difficult to study, their complex interactions and roles in human health make them fascinating to investigate using the techniques of physical chemistry. Importantly, physical chemistry offers a collection of sophisticated experimental and theoretical methods that allow rigorous, quantitative analysis of molecular-level details that could potentially influence the development of therapeutic strategies and diagnostic tools for many human diseases. Additionally, by quantifying the relationships observed, the error associated with these measurements can be understood, leading to improved experimental techniques and increased reproducibility of results. Ultimately, a broad range of physical methods can work in concert to gain a unique first-principles perspective of the molecular details that drive mechanisms of disease in cellular environments.

Much of the work done in our own lab is focused on understanding the mechanisms and cellular consequences of amyloid formation, the aberrant aggregation of proteins into β -sheet rich fibrils and a hallmark of many neurodegenerative diseases.¹ Specifically, we study amyloid formation of α -synuclein (α -syn), a small neuronal protein implicated in Parkinson's disease.² The debilitating symptoms of Parkinson's disease result from the loss of cells in the *substantia nigra* region of the brain and the disease is characterized by the formation of Lewy bodies, intracellular inclusions found in the brain with high concentrations of α -syn in amyloid form.² Despite much study, the mechanisms responsible for cell death and the aggregation of α -syn into amyloid are still not well understood, leaving many unanswered questions to explore, particularly with respect to a cellular environment. The native biological function of α -syn is ill-defined, but it is known that α -syn is membrane-associated *in vivo*.^{3,4} Our group and others have shown previously through *in vitro* experiments that α -syn, which is disordered in solution, can adopt an α -helical conformation upon binding to membranes.^{5,6} The presence of membranes or membrane

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mimics, like phospholipid vesicles or sodium dodecyl sulfate micelles, is also known to modulate α -syn amyloid formation *in vitro*.^{7–10} In addition, many studies have shown that α -syn membrane-binding can lead to instability and remodeling of the membrane,^{9,11–13} and disease-related mutations of α -syn have been shown to directly impact membrane-binding capabilities.^{14,15} Biologically, maintaining membrane integrity is important for healthy cellular function, and therefore understanding how α -syn can bind to or disrupt cellular membranes may offer insight into the potential physiological function of α -syn, as well as its pathological relevance in Parkinson's disease.

In our lab, we have used a broad range of biochemical, spectroscopic, and microscopic techniques to gain a better understanding of the conformational dynamics and membrane interactions that dictate the functional and dysfunctional roles of α -syn. Circular dichroism and electron microscopy experiments have shown that α -syn monomers with no detectable helical structure can directly remodel and tubulate membranes composed of zwitterionic phosphatidylcholine (PC), the most abundant phospholipid in all mammalian membranes, and that membrane remodeling inhibits amyloid formation.⁹ These results bolster the biological importance of membrane curvature generation by α -syn, which is thought to play a physiological role in synaptic vesicle trafficking.¹⁶ We have also explored α -syn-membrane interactions with emerging physical methods like neutron reflectometry (NR). Using NR, we have simultaneously measured protein and membrane structure and provided the first quantitative evidence that α -syn induces membrane thinning,¹¹ a phenomenon linked to bilayer disruption and pathogenesis. Taken together, results from NR, fluorescence, and computational experiments show that as few as the first four residues can associate with the bilayer, allowing α -syn to bind to the membrane without forming a full, extended α -helix.¹¹ Biologically, this is important because it suggests that even with limited amounts of free lipid surface, like on natural membranes of synaptic vesicles, the N-terminus of α -syn could serve as a membrane anchor for binding. Coupling NR and native chemical ligation, we have achieved region-specific insight into α -syn-membrane interactions by identifying which specific residues interact with the lipid membrane.¹⁷

Steady-state and time-resolved fluorescence measurements have also offered tremendous insight into α -syn-membrane interactions and the mechanisms of aggregation. From excited-state lifetime measurements of site-specific tryptophan probes, we have learned that α -syn can adopt many local conformations when binding to membrane surfaces.^{11,18–20} Anisotropy and fluorescence decay measurements of site-specifically labeled α -syn variants have shown that local structural reorganization of the N- and C-termini is necessary to break certain intra- and intermolecular electrostatic interactions and form the amyloid state.²⁰ More recently, we are biosynthetically incorporating terminal alkynes into the sequence of α -syn at specific residue locations.²¹ These small, nonnative probes provide very strong, environmentally sensitive, Raman peaks in the spectroscopically “quiet” cellular region near 2100 cm⁻¹, and therefore offer a unique spectroscopic signature that can be utilized for studying α -syn aggregation and amyloid formation inside a cell. The conformation of α -syn *in vivo* is generally thought to be disordered,^{22–24} but recent reports suggest it may exist as a multimer in its functional form.^{25–27} Experiments that can characterize the structure of α -syn *in vivo* and study aggregation in real-time are therefore key to understanding the link between amyloid and disease.

Our broad physical approach has taught us a great deal about the conformational dynamics, membrane-binding, and aggregation properties of α -syn. But our work has also led us to consider many new questions about how our *in vitro* data inform on α -syn pathogenesis within a cellular environment. For example, do membrane interactions give rise to specific consequences that result in cellular death? What is happening locally to the membrane structure upon α -syn binding? How does the protein structure change as membrane reorganization occurs? Does water play an important role during reorganization of the membrane? How does solvation of the protein change or fluctuate? What is the time-scale for membrane reorganization within a cellular environment? How do specific properties of the lipid membrane (hydrocarbon chain length, level of saturation, head group charge, etc.) change interactions with α -syn?

A fundamental question is what is the role of amyloid formation in cell death and the development of neurodegenerative disease? Though amyloid fibrils are a hallmark of several neurodegenerative diseases,^{1,2} it is not yet established if fibrils are a toxic agent or a benign end-product in a series of events that lead to cellular death. Evidence points to the potential of fibrils causing cell death, but there is also substantial evidence that oligomers preceding fibril formation are the toxic species.^{28–31} Amyloid fibrils are rich in cross- β -sheet structure,^{32,33} but it is unknown if the β -sheet conformation induces cellular death or is just a stable final structure? If a β -sheet conformation does indeed lead to cell death, where is this conformational change initiated inside the cell? Does this occur at a membrane interface or in the cytosol? Additionally, fibril polymorphs have recently been shown to be important in disease and patient phenotypes in Alzheimer's disease.³⁴ Cellular studies that identify and observe fibril polymorphs *in vivo* could be incredibly valuable toward understanding if amyloid polymorphism also affects the presentation and progression of Parkinson's disease symptoms.^{30,35}

Of course, amyloid formation of α -syn and its role in neurodegeneration is just one example of a complex process that is well-suited for studying from a physical chemistry perspective. Many more are awaiting rigorous study using physical methods. The problems of disease are bigger than any one approach and require a collection of experimental techniques to understand the biology taking place and the molecular mechanisms that degrade human health. We must therefore continue the development of physical chemistry methods for studying biological problems. Further, we must also encourage students who are taught to think deeply about the complexities of quantum mechanics, statistical mechanics, and thermodynamics to turn their well-trained and creative minds toward investigating the complex mechanisms of human disease. Physical chemistry reveals molecular details that are not accessible through other means, and by using what has been learned from cuvette-based studies to design and interpret experiments in a cellular context, we will help in discovering new treatment strategies and eventual cure of many human diseases.

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