

a cytoprotective effect. Intermolecular FRET was used to characterize the topology, packing and dimensions of IAPP oligomers. FRET measurements provided experimental constraints for atomistic modeling and MD simulations both of IAPP monomers and oligomers. Simultaneously, we have used fluorescence correlation spectroscopy (FCS) to explore the interactions of IAPP with lipid bilayers, probing the thermodynamic landscape of membrane-catalyzed IAPP oligomer assembly. Single-molecule techniques provide unique experimental insight into the processes governing the membrane binding and aggregation of IAPP and similar amyloidogenic peptides.

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N&B and Cross-N&B Analysis Detect Oligomerization of Huntingtin in Live Cells

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Aggregation of misfolded proteins is a hallmark of several neurodegenerative diseases such as Huntington's disease (HD). HD is caused by a mutation of Huntingtin caused by an elongation of a polyglutamine (polyQ) sequence in the protein. Here we describe the application of the recently developed Number and molecular Brightness method (N&B) to monitor the aggregation process of Huntingtin exon1 (Httex1). N&B measures the molecular brightness of the protein aggregates in the entire cell non-invasively based on the fluctuation dynamics at each pixel of an image. This analysis provides a map of aggregation with pixel resolution.

We observed the behavior of Httex1-97QP-EGFP this is a construct with 97 polyQ repeats corresponding to Juvenile onset of the disease.

We performed experiments in ST14A cells transfected with Httex1-97QP-EGFP. We establish that the process of nucleation leading to inclusion formation has four phases: i) Initially only monomers are present; ii) Following an increase in protein concentration ($\sim 1 \mu\text{M}$), due to protein accumulation, small oligomers (8-15 proteins) form throughout the cell; iii) At higher protein concentrations, an inclusion is formed in the cytoplasm; iv) The inclusion recruits most of the Httex1 protein in the cell, including those in the nucleus, leaving only monomers at very low concentration.

We also performed cross-N&B analysis to measure the size of the oligomeric species. Cross-N&B recovers the stoichiometry of the complexes from the simultaneous fluctuations of the fluorescence intensity in two image channels. The experiments were done on ST14A cells co-transfected with Httex1-97QP-EGFP and Httex1-97QP-mCherry. These experiments confirmed the mechanism of aggregation observed by N&B and the range of size of the oligomers. Work supported by NIH-P41-RRO3155, P50-GM076516, NIH NS045283 (J. L. M.), Optical Biology Shared Resource of the Cancer Center Support Grant CA-62203 at University of California, Irvine.

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Amyloid- β and α -Synuclein Aggregate in Live Cells at Concentrations Far Below their *in vitro* Solubility Limits

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Amyloid protein aggregates are believed to be the key cause of neurodegenerative diseases such as Alzheimer's and Parkinson's, but the mechanism of their biological action is far from understood. Different studies have implicated different aggregated forms of these proteins as the key toxic elements. Significantly, these studies typically use concentrations in the μM range to get the proteins to aggregate, but this is at least an order of magnitude higher than the concentrations observed *in vivo*. Here we examine the aggregation of amyloid beta ($\text{A}\beta$) and α -synuclein on cell membranes and inside live cells at sub- μM concentrations, using coupled fluorescence correlation spectroscopy, confocal microscopy and time correlated single photon counting techniques. For $\text{A}\beta$, we find that only small aggregates ($< 6 \text{ nm}$ hydrodynamic radius) form at 350 nM concentration *in vitro*, but large aggregates of $\text{A}\beta$ are present on the cell membrane, together with much smaller species (monomers or small oligomers). When the *in vitro* concentration is lowered to 150 nM, the solution structures are even smaller ($< 4 \text{ nm}$, but significantly, still at least dimeric) and no large multimers form on the membrane, though the smaller species are still present. We conclude that $\text{A}\beta$ aggregates at much lower concentrations on the membrane, possibly because of the lower free energy of association required for such aggregation in a two dimensional system. For α -synuclein, we find that a 550 nM solution which does not form any aggregates in the extra-cellular medium, develops large aggregates in the cytoplasm. Our results challenge the hypotheses that specific aggregate structures (e.g. donut shaped, spherical or proto-fibrillar aggregates) that form in the solution phase are responsible for the physiological effects of $\text{A}\beta$ or α -synuclein.

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Amyloid Beta Oligomer Studied by Newly Developed Single Molecule Analysis Method

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Amyloid beta (Ab) is 4 kDa peptide which forms aggregates such as oligomers and fibrils. They have been considered to cause Alzheimer's diseases (AD). Recent results have suggested that soluble Ab oligomers are the causative agent of AD since such oligomers are more cytotoxic than fibrils. It was also suggested that Ab oligomers affect not only cell death but also early stage of cell dysfunction and cause memory loss.

However, the formation mechanism of these soluble oligomers is still unknown. In this study, we developed new single molecule analysis method that can analyze Ab oligomer distribution. For this purpose, we combined total internal reflection fluorescence microscopy (TIRFM) with photon counting histogram (PCH) (Terada, N. et al. (2007) *Biophys. J.* 92, 2162). Using TIRFM, fluorescent intensity of monomer is obtained from discrete photobleaching. Using PCH method, the number of protomers in oligomers and concentrations are obtained from histograms of photons from fluorescent molecules diffusing through the confocal volume.

A model Ab oligomer was prepared by sonication treatment of Ab fibrils made from FITC labeled Ab monomer. The number of Ab monomer in a single Ab oligomer was estimated by TIRFM, which agrees well with the results of PCH analysis. Thus we concluded that PCH can be applied to analyze Ab oligomer formation. Next, Ab oligomers were formed at physiological Ab concentration (LeVine III, H. (2004) *Anal. Biochem.* 335, 81). Oligomer formation was observed with TIRFM analysis. PCH analysis is now in progress.

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Investigating Amyloid Nucleation and Growth with Fluctuation Microscopy

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The nucleation and growth mechanisms in amyloid forming materials are of high interest due to their importance in human diseases and also due to their potential applications as functional nano-materials. While much is known about the β -sheet secondary structure of amyloids, the nucleation mechanism and self-assembly processes remain poorly understood. Multiple intermediate species have been proposed to play important roles in the self assembly process, yet these states remain poorly defined or have not been unambiguously identified in solution. We have used single molecule fluorescence spectroscopy and two-photon fluorescence imaging to investigate early events in amyloid nucleation and growth. Our observations have demonstrated that unstructured protein aggregates play a key role in the earliest phases of self assembly.

3391-Pos

Detecting and Characterizing Amyloid- β_{1-40} Oligomers using Single Molecule Fluorescence

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The devastating symptoms of Alzheimer's Disease (AD) have been attributed to the behavior of aggregated Amyloid- β ($\text{A}\beta$) peptides. Cleaved from the extra-cellular portion of the transmembrane receptor, amyloid precursor protein (APP), $\text{A}\beta$ has a very high propensity to aggregate into fibrils with a cross- β -sheet structure. Though fibrillar plaques have been seen as a hallmark of AD, there has recently been increasing evidence suggesting that smaller soluble $\text{A}\beta$ oligomers are the agents of neuronal toxicity. Molecular level characterization of these oligomers using conventional biochemical techniques has been very difficult as they are both short-lived and heterogeneous.

In this work we have used a single molecule fluorescence microscopy technique to follow the formation and resolve distributions of these oligomers. Two-color coincidence detection (TCCD) has the ability to detect such transient complexes even when they comprise only 0.1% of the population (1, 2). In this work, we have detected and characterized the species ranging from dimers to 50-mers formed during the oligomerization of monomeric $\text{A}\beta_{1-40}$ (the 40-amino acid portion of $\text{A}\beta$) as well as those resulting from breakage of $\text{A}\beta_{1-40}$ fibrils. Through experiments tracking the formation of oligomers throughout the $\text{A}\beta$ aggregation process in real-time, we have gained novel insights into the mechanisms of oligomer formation and fibril breakage.

1. A. Orte, R. Clarke, S. Balasubramanian, D. Klenerman, *Anal. Chem.* **78**, 7707 (2006).

2. A. Orte et al., *Proc. Natl. Acad. Sci. U. S. A.* **105**, 14424 (2008).