Assembly of a Protein into Nanoscopic Amyloid Pores

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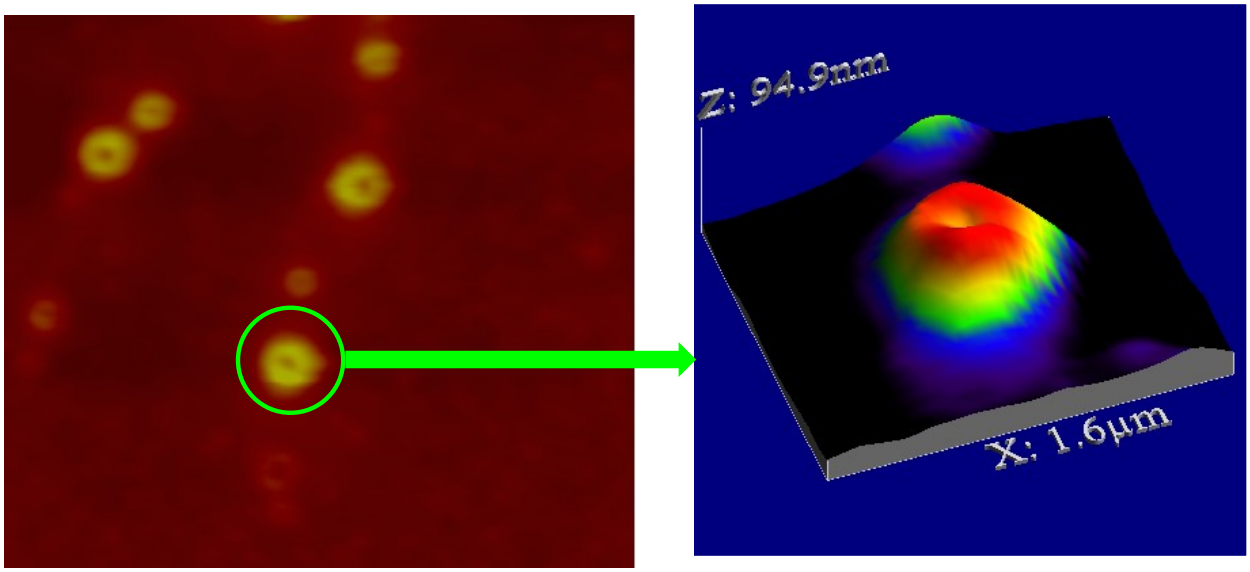
Protein misfolding and aggregation leading amyloid fibril formation have been implicated in a number of human diseases. Amyloid forming protein and peptides have been associated with a number of debilitating human diseases like Alzheimer's disease, Parkinson's disease, type 2 diabetes and transmissible spongiform encephalopathies (prion diseases). A line of studies has revealed that the common amyloid intermediate that is seen in neurodegenerative disorders is a pore-like protofibril. The amyloid pore hypothesis suggests that the pores disrupt the cell membranes by a mechanism, speculated to be similar to that of pore-forming bacterial toxins, although other types of membrane disruption mechanisms have been also proposed. However, the ultra-structural organization of individual protein molecules within the supramolecular amyloid assembly remains elusive. In order to elucidate the molecular events involved in amyloid assembly, we have used a glycoprotein named ovalbumin (present in chicken egg-white), a model noninhibitory member of the serpin (serine protease inhibitors) super-family. Serpinopathies are described as range of physiological diseases that occur due to the misfolding and self-assembly of serpins such as neuroserpin which lead to Alzheimer-like dementia and liver cirrhosis. The structural similarity with serpins as well as the ease of availability made ovalbumin an attractive candidate for investigating the mechanistic aspects of serpinopathies. In our study, we have used a wide array of spectroscopic and imaging techniques such as fluorescence, Raman spectroscopy, circular dichroism spectroscopy (CD), and atomic force microscopy (AFM) for obtaining the in-depth insights into both molecular and nanoscale structural transitions of ovalbumin during morphological transformation of the soluble precursor into amyloid annular pores. A progressive change in the conformation was observed from α-helical molten-globule state to cross β-rich amyloid pores. The AFM images unveiled a stepwise morphological transition from spherical oligomers to nanoscopic annular pores with an average diameter of ~50 nm. In-depth molecular details into the secondary structural changes of the protein during amyloid assembly and pore formation were obtained using Raman spectroscopy that revealed a progressive sequestration of protein molecules into the amyloid pores consisting of antiparallel β-sheets in the core. These results provide important molecular clue to the structural and morphological changes that can result into aberrant disease-associated amyloids. This work has appeared in the *Journal of Physical Chemistry Letters* **[**"Nanoscopic Amyloid Pores Formed via Stepwise Protein Assembly" M. Bhattacharya, N. Jain, P. Dogra, S. Samai & S. Mukhopadhyay. ***J. Phys. Chem. Lett.*** 2013, 4, 480-85. http://dx.doi.org/10.1021/jz3019786**].**



Atomic force microscopy images of amyloid pores recorded at the Mukhopadhyay lab.