

HYBRID OPTICAL AND SCANNING PROBE MICROSCOPIES FOR VISUALIZATION OF BIOMOLECULES ON SURFACES

[Vinod Subramaniam](#)

Biophysical Engineering Group, Faculty of Science and Technology, University of Twente, PO Box 217, 7500AE Enschede, The Netherlands

ABSTRACT: Effective visualization and quantification of dynamic biological processes involving (multiple) molecular interactions is a key challenge of molecular and cellular imaging. Successfully addressing this challenge requires a multifaceted approach including probe development and bioconjugation strategies, quantitative multi-parameter microscopies, and the judicious combination of optical spectroscopy techniques with optical microscopy or scanning probe techniques. In essence, what is required is microspectroscopy with high spatial, temporal, and spectroscopic resolution.

Fibrillar aggregation of proteins is a self-assembly process of significance to biological function, to specific industrial applications, and increasingly, to disease. Thus, the polymerization of actin is essential to biological function, the fibrillization of specific food proteins provides texture to food, while aggregating proteins forming amyloid are implicated in the pathogenesis of many human diseases. Aggregation is often a consequence of partial or full denaturation of proteins, and provides insights into the mysteries of protein folding and misfolding.

To better address the chemical biology and biophysics of protein aggregation we have developed several hybrid microscopies combining different imaging modes to provide additional insights into biomolecular interactions.

Self-assembly of the human alpha-synuclein protein resulting in protein aggregates of diverse morphology is a feature of Parkinson's Disease and other neurodegenerative disorders known as synucleinopathies. This aggregation process is representative of the interconversion of an unfolded protein into nanostructures with typical amyloid features. The morphologies of the nanostructures formed and the kinetics of the aggregation are modulated, among other factors, by solution conditions, mutations in the protein, and the effect of the support surfaces. We have applied a wide repertoire of biophysical techniques to continuously monitor and visualize at the molecular level the self-assembly of wild-type

alpha-synuclein and various mutants. Using a combination of hybrid optical and scanning probe techniques and ensemble and single molecule fluorescence spectroscopy we are seeking to gain direct insight into different modes of alpha-synuclein self-assembly and to identify key factors modulating the aggregation process, contributing further to our understanding of the molecular biophysical bases of disease-related conformational changes of proteins.

The technologies developed for these experiments have broader applications to the study of biological molecules on surfaces.

REFERENCES:

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