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Report

Research activity

Peptides and proteins possess an inherent tendency to convert from their native functional states into intractable amyloid aggregates. This phenomenon is associated with a range of increasingly common human disorders, including Alzheimer's and Parkinson's diseases, type II diabetes, and several systemic amyloidosis. Several experimental evidences indicated that amyloid toxicity is associated with the interaction between the aggregates of protein and cell membranes. In particular the lipid composition and organization of the plasma membrane seems to play a important role in neurodegenerative processes.
Atomic Force Microscopy (AFM) offers a powerful biophysical approach for the study of planar supported lipid bilayers (SLB) of variable composition as membrane model systems. I exploited AFM in quantitative imaging mode to study the interaction between amyloid protein and model membrane with physiological relevance.
I studied the interaction between α-synuclein, a peptide involved in Parkinson's disease, and planar supported lipid bilayers (SLBs). SLBs are a well established model of the plasma membrane of eukariotic cells. It is known that Alpha-syn is present in both the inner (cytosol) and extracellular space. Both the endogenous and exogenous components seem to have a role in the neurodegenerative process typical of the disorder. It is also demonstrated that the physiological membrane have an asymmetric structure, i.e., the lipid composition of the inner and outer leaflet are different. As a consequence of this asymmetry, also the organization of the lipid phase in the two leaflet are different. In particular I employed SLBs with two different compositions, mimicking the inner and the outer leaflet of the cell membrane respectively. It is known that the presence of the rigid substrate, that support the membrane, reduces the diffusion of the single lipid molecules within the membrane and induce a mechanical stabilization of the membrane, that increase the resistance of the bilayer toward the action of an external agent. Our group found that in particular the ordered domains are able to overcome the destabilizing effect of molecules/nanoparticles that are thought to damage the cell membrane. In the second year, I proposed a new approach, making the peptides interact with lipid vescicles in solution, creating a planar bilayer after this interaction. In this way, Alpha -syn is in contact with a tridimensional membrane, that is better resembling the properties of the cell membrane. Vesicle's lipids are freely diffusing without the influence of the rigid substrate. These results are complementary to the one obtain with the first method, and the combination of the two approaches leaded to a deeper interpretation of the mechanism under investigation.

results obtained

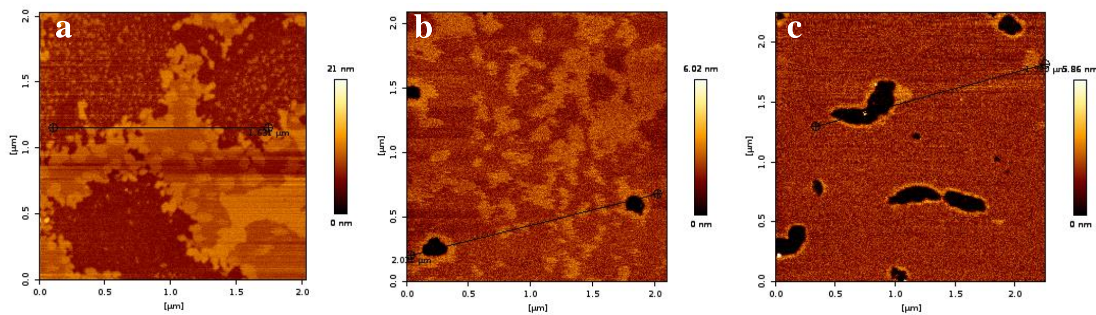

Figure 1. Representative AFM images of mINT +alpha-syn at concentration 1 nM (a), 40 nM (b) and 200 nM (c).

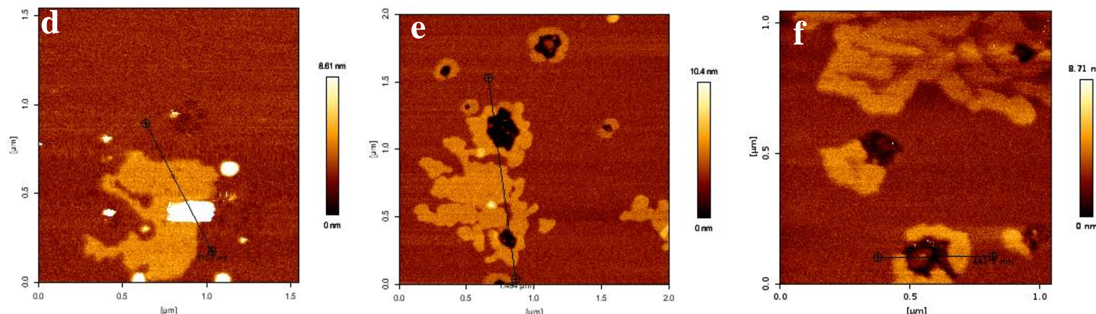

Figure 2. Representative AFM images of mEXT +alpha-syn at concentration 1 nM (d), 40 nM (e) and 200 nM (f)

Future plane

A second part of my project is related to the characterization of the aggregation process of amyloidogenic peptides with advanced correlative techniques. It has been recently demonstrated, and, in particular, I prepared samples of insulin labelled with fluorescent probes, generally employed for optical techniques such as confocal or STED microscopy. I use different dye to protein ratio, i.e. only different fraction of the whole insulin molecules are labeled. I will analyze these samples with a combined setup that couple AFM and STED. After setting the working condition, I will apply the technique to Alpha-syn aggregates. We will use fluorescent labeling method to induce the aggregation and to label the molecules at different dye-to-protein ratios.

Formation activity

I followed and passed these courses:

1) Advanced optical fluorescence microscopy methods

   Dr. Luca Lanzano, Dr. Paolo Bianchini

2) Fluorescence super-resolution microscopy: Basis, applications and perspective

Dr. Giuseppe Vicidomini

Webinars

1) STEDYCON & Huygens: super-resolution scaled up**,** Abberior Instruments GmbH
2) Transmitted Light Microscopy**,** Andor Technology, Oxford Instruments Company
3) The History of Microscopy, Andor Technology, Oxford Instruments Company