REPORT – 2nd Ph.D. Year

Davide Odino (XXXV Cycle)

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RESEARCH ACTIVITY

My PhD project focuses on applications of scanning probe microscopy and spectroscopy to the structural and mechanical characterization of biological systems and interfaces for the study of protein aggregation diseases, including the effects of drugs against aggregation.

During the first year of my PhD, I investigated the effects of trodusquemine, an aminosterol that has been proposed as a drug candidate against Parkinson's and Alzheimer's diseases, on the morphological and mechanical properties of supported lipid bilayers mimicking the neuronal membrane.

In the second year of my PhD, I continued the trodusquemine study carrying out AFM force spectroscopy measurements on supported bilayer membranes to determine the bilayer elastic modulus at different trodusquemine concentrations (1-15 μM). In the presence of trodusquemine, I observed an increase in Young's modulus, corresponding to an increase in the bilayer mechanical strength. This result is in agreement with the increase of the breakthrough force, which is the maximum force that the bilayer can withstand before rupture, that I had measured last year. The elastic modulus was obtained by fitting the Sneddon model to the force curves. However, this model is valid only for an infinite sample thickness, in which the rigid substrate is too far from the indentation region to affect the nanomechanical properties of the sample. In supported lipid bilayers, whose typical thickness is about 5 nm, the contribution to the Young's Modulus of the underlying rigid substrate is not negligible. Therefore, I applied a fourth-order correction formula, a function of the ratio between the contact radius and the thickness of the indented membrane, for determining the correct Young's Modulus [1].

In the second half of the second year, I studied the effect of alpha-synuclein, the protein involved in Parkinson's disease, on the mechanical properties of microtubules. The latter are components of the cytoskeleton, which is involved in various cellular activities and provides shape and stiffness to the cell [2]. Microtubules result from the polymerization of tubulin protein, forming a hollow tube about 25 nm in diameter. Since microtubules are very fragile, and are destroyed when imaged in tapping mode, I exploited the Quantitative Imaging technique which is now accessible with the new Nanowizard IV AFM recently acquired by the Physics Department. Using this approach I analyzed the mechanical properties and morphological characteristics of microtubules polymerized in the absence and in the presence of alpha-synuclein at different molar ratios (tubulin:alpha-synuclein 1:1, 1:4, 1:8). The results showed that increasing alpha-synuclein, an increase in microtubule stiffness is observed.

When studying protein aggregation, fluorescently labelled proteins are often employed. An important methodological issue is to establish whether the labelling process affects the aggregate structure and properties.

This goal can be achieved through STED-AFM measurements Within this framework, I started a third activity concerning the preparation of insulin fibres labelled with two fluorescent markers: Atto 647 and Atto 594. I have observed the fibers with the STED microscope, obtaining a confocal image and a STED image for each marker. STED microscopy produces super-resolution images by selective deactivation of the fluorophores. I am currently carrying out the colocalization analysis to determine whether a spatial correlation exists between the two dyes. We plan to acquire simultaneous STED and AFM images to check whether the markers are uniformly distributed in the aggregates or unlabeled aggregates are also present.

As a side activity, I participated in a study of the interaction between ataxin, a protein with polyglutamine (poly Q) stretches that aggregates and forms neuronal inclusions, and AQAMAN, a novel inhibitor that prevents polyQ protein aggregation. I acquired tapping mode AFM images showing that the inhibitor has an immediate effect on the structure of the ataxin aggregates and it is also able to disrupt pre-formed aggregates.

Finally, as a second side activity I performed breakthrough force measurements to characterize the mechanical properties of supported lipid bilayers with varying cholesterol content. The results have been included in a paper recently published in the Journal of Physical Chemistry Letters.

[1] Chiodini S. et al. Bottom Effect in Atomic Force Microscopy Nanomechanics. Small 2020, 2000269. DOI: 10.1002/smll.202000269

[2] Iwan A. T. Schaap et al. Elastic Response, Buckling, and Instability of Microtubules under Radial Indentation. Biophysical Journal, Volume 91, August 2006, 1521–1531. DOI: 10.1529/biophysj.105.077826

PUBLICATIONS

Errico S.; Lucchesi G.; Odino D.; Muscat S.; Capitini C.; Bugelli C; Canale C.; Ferrando R.; Grasso G.; Barbut D.; Calamai M.; Danani A.; Zasloff M.; Relini A.; Caminati G.; Vendruscolo M.; Chiti F. Making biological membrane resistant to the toxicity of misfolded protein oligomers: a lesson from trodusquemine. Nanoscale (2020), 12, 22596-22614. DOI: 10.1039/D0NR05285J

Canepa E.; Bochicchio D.; Gasbarri M.; Odino D.; Canale C.; Ferrando R.; Canepa F.; Stellacci F.; Rossi G.; Dante S. and Relini A. Cholesterol Hinders the Passive Uptake of Amphiphilic Nanoparticles into Fluid Lipid Membranes. *J. Phys. Chem. Lett.* 2021, 12, 35, 8583–8590. DOI: doi.org/10.1021/acs.jpclett.1c02077

EXAMS

“Nanophotonics and nanofabrication”- prof M. Giordano (exam expected to be given by October)

CONFERENCES

- 13th European Biophysics Congress (EBSA), July 24-28, 2021, Vienna, Austria. Odino D., Errico S., Canale C., Ferrando R., Chiti F., Relini A. Trodusquemine increases the mechanical stability of lipid membranes (**poster**). *Eur Biophys J*, **50** (Suppl 1): S136. DOI: https://doi.org/10.1007/s00249-021-01558-w
- Biophysical Society 65th Annual Meeting (Virtual). February 22-26, 2021. Odino D., Errico S., Canale C., Ferrando R., Chiti F., Relini A. Interaction between Biomimetic Lipid Membranes and Trodusquemine: An Atomic Force Microscopy Study. (**poster**). DOI: https://doi.org/10.1016/j.bpj.2020.11.2052