REPORT – 1st Ph.D. Year

Davide Odino (XXXV Cycle)

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RESEARCH ACTIVITY

My PhD project is focused on two main research topics: 1) the structural and mechanical characterization of lipid membranes on a solid support and the analysis of the possible modifications in their properties induced by external agents such as pathological proteins and potential drugs; 2) the measure of calcium influx in cells induced by mechanical stimuli in the absence and in the presence of pathological proteins. The common goal of these two areas is to identify the molecular mechanisms that drive neurodegeneration in Parkinson's and Alzheimer's diseases and to get insight, at molecular level, into the action of therapeutic approaches.

My first year of PhD research activity has concerned the study of the structural characteristics and nanomechanical properties of biomimetic membranes and the analysis of their possible modifications induced by trodusquemine, a promising drug candidate for neurodegenerative diseases. I studied biomimetic membranes consisting in a lipid bilayer on a solid support. I prepared supported lipid bilayers by depositing a suspension of large unilamellar vesicles onto a freshly cleaved mica substrate in the presence of $CaCl_2$ to favor vesicle fusion. I considered a lipid mixture that mimics the neuronal membrane, giving rise to a phase separation between a fluid matrix (L_α phase), more disordered at the molecular level, and ordered domains (L_β phase) with larger thickness, these types of platforms mimic lipid rafts which are interaction sites involved in vivo in a host of physiological processes. Different molar concentrations of trodusquemine were added to these phase-separated membranes. In particular, I analyzed the effects of low concentrations (1-15 μM) which are more interesting for therapeutic applications.

Initially, I experimentally investigated the morphology of membranes with and without trodusquemine using the atomic force microscope. The analysis of the AFM images provided different quantitative parameters, such as the L_β domain height, measured respect to the L_α phase; domain circularity; the mean area per domain; the total surface area of L_β domains. In the range of trodusquemine concentrations under study, the coexistence of the ordered and the disordered phases was preserved. This suggests that the typical trodusquemine concentrations found to be pharmacologically effective do not alter the membrane morphology essential for its biological function. However, at 5 μM trodusquemine the mean area per domain decreases strongly and the total surface area of L_β domains increases. Therefore, 5 μM of trodusquemine represents a particularly favourable condition for the formation of ordered domains. Furthermore, I performed force spectroscopy measurements on the supported lipid membranes, again using AFM. In AFM force measurements, the deflection of the cantilever and piezo displacement are recorded and converted into force versus distance curves. The analysis of force-distance curves allowed us to obtain the bilayer

breakthrough force, which is the maximum force that the bilayer can withstand before rupture. The L_β domains exhibited no or few breakthrough events, so the majority of the measured breakthrough forces corresponded to L_α regions. Compared to the absence of trodusquemine, in the presence of 5 μM trodusquemine an increase of the breakthrough force is observed, corresponding to an increase in the mechanical strength of the bilayer. This growth in resistance to indentation could contribute to increased resistance of the membranes to the toxic action of misfolded protein oligomers. These results have been included in a paper which is at present under review.

I am currently carrying out force spectroscopy measurements on supported bilayer membranes using AFM, to determine the bilayer elastic modulus. The latter can be obtained by fitting the Sneddon model to the force curves. This model considers a semi-infinite sample contacted by a spherical-shaped tip. The goal is to provide a more complete physical description of the lipid membrane, characterizing the elasticity of ordered domains and fluid matrix in the absence and presence of trodusquemine.

Finally, as a side activity, I also contributed to the study of the interaction of phase separated supported lipid bilayers and amphiphilic gold nanoparticles having negatively charged surface functionalization, using AFM for the characterization of bilayer morphology before and after interaction with nanoparticles. These results have been published recently in Nanoscale.

PUBLICATIONS

Canepa E.; Salassi S.; de Marco A.; Lambruschini C.; Odino D.; Bochicchio D.; Canepa F.; Canale C.; Dante S.; Brescia R.; Stellacci F.; Rossi G.; Relini A. Amphiphilic Gold Nanoparticles Perturb Phase Separation in Multidomain Lipid Membranes. Nanoscale. (2020) DOI: 10.1039/d0nr05366j

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. Under review

EXAMS

- “Fisica della material soffice” - prof A. Relini (passed exam)
- “Biosensing” - prof O. Cavalleri, prof E. Angeli (passed exam)
- “Atomic Force Spectroscopy” – prof A. Relini (passed exam)

CONFERENCES

Partecipation 106° Italian Physical Society Congress

Educational assistance for student DiFi stage 2020