Habilitation Thesis

Domain: Physics

Investigation of biomembrane processes at uni - molecular level, with relevance in the evolution of current pathologies

Loredana - Cristina MEREUTA, Ph. D., Associate Professor
'Alexandru Ioan Cuza' University of Iasi, Faculty of Physics,

Iași – 2022

Summary of Habilitation Thesis

The Habilitation theses intitled ‘*Investigation of biomembrane processes at uni - molecular level, with relevance in the evolution of current pathologies*’, comprise two main sections dedicated to scientific research and teaching activities, which are considered by the author the base of a fulfilled academic profession, and at the end the Bibliography.

One of the main goals of biophysics is to determine molecular functions using well defined concepts and methods from physics in order to understand and modulate biological effects of complex systems and elucidate how these biological systems work in order to prevent disease and discover new cures.

The first section of these theses details new paradigms approached in our projects which bring novel insights towards a better understanding of phenomena occurring at the cellular membrane level, between several peptides and lipid-protein systems.

In chapter I from the first section, the author describes one of the main areas of her postdoctoral research which concerns a class of molecules named ***antimicrobial peptides (AMPs)***, ***considered the new class of future antibiotics***. With the current increase in resistance to conventional antibiotics, the high degree of selectivity possessed by AMPs makes them promising candidates for new generations of drugs to fight antibiotic - resistant strains of pathogens. Specifical, one aim of our work was to elucidate different aspects regarding the influence of electrical and mechanical properties of the lipid bilayer upon the activity of selected antimicrobial peptides and we proposed a new paradigm according to which asymmetric changes in the monolayer dipole and surface potential extend their effects spatially by altering the intramembrane potential, whose gradient is sensed by distantly located peptides. Another study of ours concerning AMPs, aimed at exploring the role of primary structure distribution and topology of interracially localized aromatic amino acids in peptide–membrane interactions and underlying peptide activity. This approach provides a complementary tool for studying the topological transitions underlying peptide activity in lipid membranes, and may prove useful in prediction methods for the activity

of de novo engineered, W- or F-rich membrane active peptides. The social and scientific impact of these studies is outlined by the ability to design and develop new therapeutic agents with improved quality and specificity towards their targets.

In the second chapter of the first section the author describes some of the most significant and representative scientific achievements in ***the field of nanopore unimolecular exploration***, devoted to the fallow main topics:

(i) *Probing the interactions between metal cations and truncated amyloid peptides - Aβ*, which are at the core of Alzheimer's disease pathology. Our methodology able to detect and recognize ions or other small molecules in bulk, e.g., peptide inhibitors of Aβ oligomerization, with protein nanopores, may complement existing spectroscopic techniques to study more effectively structure−function relationships of various Aβ oligomerization and misfolding inhibitors, and screen drugs counteracting the Aβ toxicity.

(ii) *Identifying and controlling different sub-states on the translocating pathway of peptides through a protein nanopore*. Generally, any information about peptides moving along the different structural compartments of protein nanopores, and/or description of conformational changes of such analytes within nanopores, was difficult to detect and investigate because analyte translocation is too fast relative to the time resolution of single-molecule current recording instrumentation. By employing the electro-osmotic force as a movement-retarding force, opposed to the electrophoretic one, and working in conjunction with nanopore sensors able to achieve temporal resolution of microseconds, our approach considerably slows down the passage of analogues of the cecropin A–magainin antimicrobial chimera peptide through the nanopore. This in turn provides an unique opportunity to visualize sequentially the peptide translocation process, and shows that different sub-states on the translocating pathway can be identified, controlled and kinetically quantified. Through the analysis of single molecule descriptors such as current blockage induced by peptide within the protein pore and residence time in various domains of the diffusing pathway, our data suggest that the barrier to peptide capture can be easily controlled by modulating the flow through the α-hemolysin β-barrel, via changes in the pH alone. By controlling, via pH buffer changes, the electrophoresis and electro-osmosis processes that modulate the peptide

translocation through distinct regions within the a-HL pore, we demonstrated that different sub-states on the translocating pathway can be kinetically quantified.

(iii) *Hybridization-based detection and validation of ssDNA fragments, with a protein nanopore.* We proposed a new strategy devoted to detecting the selective hybridization between various ssDNAs and either cationic polypeptide-functionalized, or nonfunctionalized peptide nucleic acids (PNAs), with a single α-HL nanopore isolated in a bilayer lipid membrane. In one endeavor, we monitored the change in the aqueous concentration of cationic polypeptide-functionalized PNAs as is occurs due to the hybridization with target, anionic ssDNAs. As an alternative route, single-nanopore current recordings were used to selectively probe the presence of aqueous ssDNAs, through monitoring in terms of blockades amplitudes and kinetic features the topological changes of target ssDNAs inside the vestibule of the α-HL nanopore, following hybridization with nonfunctionalized PNA fragments. In both approaches, the sensitivity of the nanopore sensor to reveal kinetics details describing the α-HL - PNA-DNA duplexes reversible interaction at uni-molecular level, demonstrated the potential to identify single-base mismatches in individual ssDNA strands, precluding the need for chemical modification, tagging or amplification.

The last chapter of the first section of these theses comprises our latest research results which may offer new perspectives in understanding the ***pathogenesis of the SARS-CoV-2 infection, its treatment, and real-time detection.*** Through electrophysiology and fluorescence spectroscopy experiments, we show that even in the absence of a specific receptor protein (the angiotensin-converting enzyme 2 receptor), the region-binding domain of S1 subunit from SARS-CoV-2 spike protein attaches to and permeabilizes neutral phospholipid membranes. Once more we demonstrate the capability of an α-hemolysin (α-HL) protein nanopore as molecular sensor to detect in real time the immunological reaction between SARS-CoV-2 spike S1 protein’s RBD and a specific antibody, which may be useful for diagnosing the positive presence of regions of the viral proteomic material. Additionally, as an alternative method for a portable biosensing platform, we propose and implement a effective direct detection and recognition of the S1 protein, based on its specific interaction with a designed monoclonal antibody and the ACE2 receptor, through the

response elicited by such complexes on the membrane potentials of a reconstituted planar lipid membrane.

The second section of these theses comprise the professional activity regarding the implementation of scientific expertise in teaching activities and the future plans for career development which is based on to two main pillars: ***scientific research*** and ***teaching activity***. Regarding to this, our research group seek to create an innovative and productive work environment in which to engage and initiate young researchers in the fascinating world of science, continuously trying to find answers to relevant and current issues in the field of biophysics.