**Name / Affiliation**

Tatsuro Nishikino

Assistant Professor, Department of Life Science and Applied Chemistry, Nagoya Institute of Technology, Japan.

**Education, Experience**

2017–2020 JSPS Research Fellow (DC1), Nagoya University

Ph.D. in Molecular Biology, Nagoya University (2020)

2020–2023 JSPS Research Fellow (PD), Osaka University

2023–2024 Specially Appointed Assistant Professor, Nagoya Institute of Technology

2024–Present Assistant Professor, Nagoya Institute of Technology

**Research Interests**

Bacterial flagellar motor, Microbial rhodopsins, Protein structure and biophysics

**Seminar title**

Molecular mechanisms of energy conversion in the bacterial flagellar motor of marine *Vibrio*.

**Abstract**

The bacterial flagellar motor is a model system for elucidating the energy-conversion mechanism of ion motive force in membrane-embedded biological nanomachines [1]. The flagellum, one of the motility organelles of bacteria, is a supramolecular complex composed of more than twenty proteins. Flagellar motility enables bacteria to swim toward environments favorable for their survival. Counterclockwise (CCW) rotation of the flagellar motor generates smooth, straight swimming, whereas clockwise (CW) rotation induces tumbling, allowing cells to reorient their swimming direction. The flagellum consists of three major components: the filament, which functions as a helical propeller; the motor, a bidirectional rotating ring-shaped structure embedded in the membrane; and the hook, which connects the motor to the filament. Flagellar motility is also closely associated with bacterial pathogenicity. Pathogenic bacteria such as *Escherichia coli*, *Salmonella*, and *Vibrio cholerae* utilize flagellar motility for effective colonization of host tissues. Thus, understanding the structure and function of the flagellar motor is essential not only for elucidating the fundamental mechanisms of biological energy conversion but also for gaining insight into bacterial infection and pathogenesis.

The motor consists of two major functional elements, the rotor and the stator, which are not only essential for the torque generation but also regulate the rotational direction of the motor. Using cryo-electron tomography, we identified the conformational differences between CCW and CW rotational states of the rotor [2]. The stator functions as an ion channel and is composed of two membrane proteins, A and B. Rotation of the stator, driven by the coupled flow of coupling ion, transmits force to the rotor to generate motor torque. However, the ion-flux pathway and the underlying energy-conversion mechanism have remained unclear. To address these questions, we determined the structure of the sodium-driven stator complex, PomAPomB from marine *Vibrio*, using

single-particle cryo-electron microscopy. Together with the structure of the inhibitor-bound state, we identified the sodium-binding site and gained insight into how sodium ion flow drives conformational changes within the stator [3].

In this presentation, I will discuss the energy-conversion mechanism of the bacterial flagellar motor based on these structural findings.

**References**

[1] M. Homma, T. Nishikino, S. Kojima, *Microbiol Immunol*., **2022**, 66, 75-95.

[2] B.L. Carroll, T. Nishikino, W. Guo, S. Zhu, S. Kojima, M. Homma, J. Liu., *Elife*. **2020**, 9, e61446.

[3] T. Nishikino, N. Takekawa, J. Kishikawa, M. Hirose, S. Kojima, M. Homma, T. Kato, K. Imada. *Proc Natl Acad Sci U S A*. **2025**, 122, e2415713122.

**ABSTRACTS FOR THE 5 SHORT TALKS**

**Short talk #1**

**Speaker: Nanako Hattori[1]**

[1]Department of Life Science and Applied Chemistry, Nagoya Institute of Technology, 466-8555, Japan

**Title:** Unique Proton Transport Dynamics of the Novel Inward Proton Pump GhXeR

Microbial rhodopsins exhibit various functions, such as ion transport and sensor for signal transduction, through structural changes induced by retinal photoisomerization. We found a microbial rhodopsin from a marine bacterium *Guptibacillus hwajinpoensis*, which is a homolog of an inward proton-pumping xenorhodopsin (GhXeR). Its amino acid sequences of the motif in the third transmembrane helix contain aspartic acid, serine and serine (DSS). Here we measured the ion transport activity in Escherichia coli cells, and confirmed inward proton pump activity. We applied various spectroscopic analysis to GhXeR, including transient absorption and FTIR spectroscopy [1]. We would like to discuss the molecular mechanism of GhXeR.

[1] N. Hattori, Y. Ito, Y. Furutani, T. Nishikino, H. Kandori, *J Phys Chem B*. **2026**, 130, 5495-5506.

**Short talk #2**

**Speaker: Mako Ooka[1]**

[1]Department of Life Science and Applied Chemistry, Nagoya Institute of Technology, 466-8555, Japan.

**Title:** Residue-Specific Assignment of Peptide Backbone Vibrations in Rhodopsin by Lysine Isotope Labeling

Light-induced difference FTIR spectroscopy is a powerful technique to investigate structural changes of photoreceptive proteins through vibrational signals. In the case of light-induced structural changes of rhodopsins, FTIR spectra exhibit highly complex signals originating not only from retinal chromophore, but also from peptide backbone, side chains, lipid membranes, and water molecules. To disentangle these overlapping signals, difference spectra of isotopically labelled samples have been extensively analyzed. For example, isotopic substitution of water (O vs $^{18}$O) enables identification of hydrogen-bonding interactions of internal water molecules, and isotopic labelling of the lysine side chain (N vs $^{15}$N) reveals changes in hydrogen-bonding strength of the retinal Schiff base. In contrast, signal changes originating from protein backbone, such as those in the Amide-I, -II, -III and -A regions, have remained largely unexplored due to the extensive overlap of numerous vibrational modes, making their spectral assignment highly challenging. In this study, we attempted residue-specific assignment of peptide backbone vibrations in light-induced difference FTIR spectra of KR2, an outward $Na^+$-pumping rhodopsin.

**Short talk #3**

**Speaker: Denisa Andronic**[1]

[1]University of Biophysics, Faculty of Physics

**Title:** Peptide-Lipid Interactions in Molecular Dynamics Simulations of Membrane Nanopores

Denisa-Sonia Andronic[1], Shalmali Kharche[2], Hrushikesh Malshikare[2], Durba Sengupta[2], Kalina Hristova[3], William C. Wimley[4], Ana-Nicoleta Bondar[1,5,6,7]

[1]University of Bucharest, Faculty of Physics, Str. Atomistilor 405, Magurele, Romania; [2]National Chemistry Laboratory Pune, India; [3]Johns Hopkins University, Department of Materials Science and Engineering, Whiting School of Engineering and Institute for NanoBio Technology, Baltimore, Maryland 21218, USA; [4]Tulane University School of Medicine, Department of Biochemistry and Molecular Biology, New Orleans, Louisiana 70112, USA; [5]Research Institute of the University of Bucharest, ICUB, University of Bucharest, Panduri Street 90, Bucharest 050663, Romania; [6]University of Bucharest, Faculty of Interdisciplinary Sciences, Str. Transilvaniei nr. 6, Bucharest; [7]Forschngszentrum Jülich, Computational Biomedicine, Wilhelm-Johnen Straße, Jülich 5428, Germany

Peptide nanopores could facilitate the direct delivery of macromolecular therapeutics, such as proteins, enzymes or antibodies, across the cellular membrane. Two families of membrane-active peptides possessing such pore-forming capabilities are the synthetically evolved pH-dependent delivery (pHD) peptides and the related pH-independent macrolittins. Since traditional structural biology methods cannot provide three-dimensional structures of these pores, experiments must be combined with computational studies to understand the molecular interactions that govern the pore-formation process. Here we present results from atomistic molecular dynamics (MD) simulations on model systems with peptide nanopores of different sizes embedded in hydrated lipid bilayers. The MD simulations reveal that the lipids associated with the water-filled pores sample highly unusual conformations, with their headgroups buried deep into the membrane plane, and their acyl chains either splayed or aligned parallel to the membrane surface. Our current efforts are aimed at quantifying the role of the lipid molecules in peptide dynamics, bundle formation and pore stabilization. This work was supported in part by the National Institutes of Health NIH R01 GM 151326 and by computing time on the supercomputer JURECA at the Forschungszentrum Jülich under grants no. TOXINTOCURE and PHDPORES. D-SA is the recipient of a Doctoral Scholarship from the Doctoral School of the Faculty of Physics, University of Bucharest

**Short talk #4**

**Speaker: Ioana Dumitru**

**Title: Molecular Mechanism of lactate transport via the MCT1-Basigin complex**

Ioana-Daniela Dumitru,[1] Maike Menzel[2], Eric Beitz[2] and Ana-Nicoleta Bondar[1, 3, 4]

[1]Faculty of Physics, University of Bucharest, Str. Atomiştilor 405, 077125 Bucharest-Măgurele, Romania; [2]Dept. of Pharmaceutical and Medicinal Chemistry, Pharmaceutical Institute, Christian-Albrechts University of Kiel, Gutenbergstr. 76, 24118 Kiel, Germany; [3]Forschungszentrum Jülich, Institute of Computational Biomedicine, IAS-5/INM-9, Jülich, Germany; [4]Faculty for Interdisciplinary Sciences, University of Bucharest, Str. Transilvaniei 6, Bucharest 010798, Romania.

MonoCarboxylate Transporters (MCTs) couple protonation dynamics with lactate transport across the membrane. Among MCTs, MCT1 is widely expressed in a variety of tissues, such as the heart, skeletal muscle, brain and red blood cells. MCT1 has the highest affinity for the lactate molecule and is of particular interest as potential target to develop cancer therapies. Three-dimensional structures of open-state MCT1 bound to the Basigin chaperone revealed that lactate binds at a polar site where it interacts with K38, a residue thought to donate a proton to the lactate molecule. We use atomic-level computations to characterize the reaction mechanism of the MCT1-Basigin complex. Our simulations and graph-based analyses indicate that water molecules help establish a dynamic hydrogen-bond network that connects the lactate molecule to remote sites at the intracellular side of the membrane. We hypothesize that deprotonation of K38 could involve protein conformational changes associated with altered content and dynamics of the internal water molecules. MD simulations were performed on JURECA-DC at the Forschungszentrum Jülich, project DYNAMICNETWORKS. I-DD is supported by a doctoral scholarship from the University of Bucharest, Faculty of Physics.

**Short talk #5**

**Speaker: Alina Halaicu**

**Title: Lipid-dependent dynamic of FGFR3 transmembrane domain**

Alina-Maria Halaicu,[1] Kalina Hristova,[2] and Ana-Nicoleta Bondar[1,3,4]

[1]University of Bucharest, Faculty of Physics, Str. Atomistilor 405, Magurele, Romania; [2]Johns Hopkins University, Department of Materials Science and Engineering, Whiting School of Engineering and Institute for NanoBio Technology, Baltimore, Maryland 21218, USA; [3]Research Institute of the University of Bucharest, ICUB, University of Bucharest, Panduri Street 90, Bucharest 050663, Romania; [4]Forschngszentrum Jülich, Computational Biomedicine, Wilhelm-Johnen Straße, Jülich 5428, Germany

Receptor tyrosine kinases (RTKs) regulate essential cellular processes, including cell growth, proliferation, and differentiation, and their dysregulation is associated with numerous neurological and oncological pathologies. RTKs are type I single-pass proteins that transmit extracellular signals across the plasma membrane, typically through ligand-induced receptor dimerization, followed by activation of intracellular kinase domain. Kinase domain activation further sets into motion a series of intracellular protein-protein interactions that regulate gene transcription. The mechanism by which signals are transmitted across the membrane, and how the membrane composition influences RTKs activation, remain unclear.

The subject of this study is to determine and understand how lipid membrane composition influences the conformational dynamics of RTK's domains, using Fibroblast Growth Factor Receptor 3 (FGFR3) as a model system. We performed atomistic molecular dynamics simulations of FGFR3 transmembrane dimers in different lipid bilayers: phosphatidylcholine (POPC and DOPC), phosphatidylethanolamine (DOPE), and phosphatidylserine (DOPS). Several distinct initial dimer conformations were simulated to evaluate lipid-dependent effects.

Our results reveal lipid-dependent differences in the conformational dynamics and organization of the FGFR3 transmembrane dimer. Spontaneous lipid intercalation between the transmembrane helices is observed across the simulations, together with changes in interhelical distances and helix-crossing geometries. Protein–lipid hydrogen-bond analysis further reveals interaction networks at both membrane interfaces, with the intracellular residues R397 and R399 displaying particularly prominent interactions with the surrounding lipids. Overall, our findings indicate that the lipid environment contributes to shaping the conformational ensemble of the FGFR3 transmembrane dimer through a combination of lipid intercalation and specific protein–lipid interactions. The authors gratefully acknowledge computing time on the supercomputer JURECA at the Forschungszentrum Jülich under grants no. DYNAMICNETWORKS and PHDPORES.