Functional studies and spatial structure of new retinal-binding proteins

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Structural and functional characteristics of new unexplored microbial rhodopsins, as well as high-resolution data of the structure of these membrane proteins and their mutant variants, are the necessary input information for the development of concepts of the relationship between the structure and function of retinal-containing proteins, using molecular modeling techniques and improving bioengineering designs of photosensitive proteins for solving problems optogenetics and biophotonics.
To define the function and to obtain high-resolution structural information for new microbial rhodopsins particularly found by knowledge-based prediction bioinformatics search of microbial rhodopsins [1], we use the well-developed pipeline of methods of heterologous expression, isolation and purification, functional characterization of previously unexplored microbial rhodopsins, determination of their kinetic characteristics, and determination of their spatial structure of atomic resolution by X-ray diffraction. Under the study are unexplored rhodopsins of *Sphingomonas paucimobilis* and *Pantoea anthophila*, also various mutant forms of rhodopsin from Gram-positive bacteria *Exiguobacterium sibiricum* (ESR), previously obtained and characterized by the authors of the project [2], *Exiguobacterium sp.* AT1b and *Exiguobacterium* 7-3 from permafrost. Molecular modeling methods [3] were used to predict the structures and dynamic properties for amino acid substitutions, general stability, and thermal stability of unexplored rhodopsins. Based on the proteins' structures, spectral properties were calculated by QM/MM methods.
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1. Ushakov, A., et al. Knowledge-based prediction model for characterization of microbial rhodopsins for optogenetics. *FEBS Journal* (2016). Volume 283, P-03.03.2-014
2. Petrovskaya, L., et al. ESR—a retinal protein with unusual properties from Exiguobacterium sibiricum. *Biochemistry (Moscow)* (2015), 80, 688-700.
3. Nekrasova, O., et al. Complexes of peptide blockers with Kv1.6 pore domain: molecular modeling and studies with KcsA-Kv1.6. *J. Neuroimmune Pharmacol.* (2016), 12, 260-276

Probing the assembly dynamics and structure of styrene–maleic acid lipid particles by coarse-grained simulations

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Amphiphilic maleic acid-containing alternating copolymers composed of alternating maleic acid and styrene (SMA) account for a major recent methodical breakthrough in the study of membrane proteins. They were found to directly solubilize phospholipids and membrane proteins both from artificial and natural bilayers yielding discoidal SMA/lipid particles (SMALPs) [1]. SMA-encased nanolipoparticles are comprised of lipid or lipid/protein cores surrounded by a polymer belt and have, depending on the preparation routine, diameters of 10–30 nm. Within particular preparation, the size of particles is uniform, which renders them suitable for diverse experimental techniques, e.g., for cryo-electron microscopy and EPR measurements [2, 3].
Although many empirical studies indicate the great potency of SMA copolymers for membrane research, the mechanisms of their action remain obscure. It is unknown what factors account for the very assembly of SMA-encased lipid particles and why they have a uniform size.
We have developed a coarse-grained (CG) molecular model of SMA-polymers within the framework of the popular MARTINI CG force field. The obtained model was used to probe the behavior of SMA polymers with varying composition/charge/concentration in solution as well as their interaction with lipid membranes.
The results of the simulations indicate that SMA polymers with styrene/maleic acid ratios of 2:1 and 3:1 form SMALPs by different mechanisms: either via poration or via a mechanism similar to the microvesicle release. Also, we found that the SMA polymers tend to aggregate in solution into clusters of the specific size what might account for the uniform size of the SMALPs.

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1. Dörr, Jonas M., et al. The styrene–maleic acid copolymer: a versatile tool in membrane research. European Biophysics Journal 45.1 (2016). https://doi.org/10.1007/s00249-015-1093-y
2. Parmar, Mayuriben, et al. Using a SMALP platform to determine a sub-nm single particle cryo-EM membrane protein structure. BBA-Biomembranes 1860.2 (2018). https://doi.org/10.1016/j.bbamem.2017.10.005
3. Voskoboynikova, N., et al. Characterization of an archaeal photoreceptor/transducer complex from Natronomonas pharaonis assembled within styrene–maleic acid lipid particles. RSC Advances 7.81 (2017). https://doi.org/10.1039/C7RA10756K

The role of OsO_4 fixation in the contrast formation of cellular membrane structures

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For the investigation of intracellular structures with the aid of atomic force microscopy, traditional preparation methods for transmission electron microscopy are often used [1]. One of the drawbacks of this methods is a low contrast on AFM topography and phase images. So, for successful AFM study of cellular membrane structures, these protocols should be optimized. In the present work, the role of osmium tetroxide in AFM images contrast formation was investigated. OsO_4 acts as a lipid structures fixative and greatly affects cell mechanical properties. For purposes of the study, monolayers of fibroblasts cultures were fixed in glutaraldehyde and half of the samples were additionally fixed with OsO_4. After that, all of the samples were dehydrated in graded ethanols, embedded in Epon 812 epoxy resin, and cut on a microtome for subsequent AFM study.
On the samples fixed with OsO_4, a low contrast was observed both on topographical and phase images. The interior of cells was quite homogeneous. The only detectable ultrastructure features were cytoplasmic membranes and nuclear laminas. Samples prepared without OsO_4 showed noticeably higher contrast. Many intracellular organelles appeared such as endoplasmic reticulum, mitochondria, and cristae of an individual mitochondrion.