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Review of doctoral thesis

Molecules in Cell Membranes

by **Mr. Stepan Timr**.

In his cumulative doctoral thesis, Stepan Timr studies the reversible binding of recoverin to membranes, and the molecular mechanisms behind the calcium-induced myristoyl switch, as well as the non-linear optical properties of membrane-embedded fluorescent dyes.

In the first part (Chapter 3, attached paper I), Stepan Timr addressed the optical properties of dyes embedded in biomembrane mimetics, heading for a proper interpretation of polarization microscopy experiments in terms of the molecular geometry underlying a measured anisotropy in light absorption. Knowledge about the orientation of biomolecules in cell membranes is of utmost importance for an improved understanding of membrane organization and the tightly coupled question of biomembrane functions. Fluorescence experiments on specific dyes were analyzed using atomistic molecular dynamics (MD) simulations. Here, Stepan proved by comparison of transition dipole moments from experiment and simulations that the two-photon absorption may efficiently be described by the transition dipole moment vector for the studied membrane-embedded dyes. The results will likely have a strong impact on a number of experimental studies investigating the orientation of membrane compoments. Noteworthy, the study required a tight interaction between experiment and theory, and both classical state-of-the-art MD simulations as well as calculations from time-dependent density functional theory were employed.
Accordingly, the work was published in J. Phys. Chem. B in 2015 with Stepan as first author, a second earlier study (not part of this thesis) focused on one dye only and was published as well in J. Phys. Chem. B (2014), stressing Stepan's continuous and successful efforts in the interpretation of fluorescence experiments.

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The second part of Stepan Timr's thesis analyzes in detail the binding of recoverin to membranes using both classical all-atom and coarse-grained MD simulations. As part of a calcium-dependent feedback loop, recoverin may inhibit the rhodopsin kinase which regulates or deactivates via phosphorylation the light-sensitive rhodopsin. The focus is put here on (a) the binding of recoverin at high calcium concentrations with its exposed myristoyl moiety to phospholipid bilayers (Chapter 4), and (b) on the involved conformational transitions in recoverin upon calcium binding or calcium loss (Chapter 5). In a very clearly presented study, Stepan was able to confirm the important role of the myristoyl group in membrane anchoring of recoverin occurring on a time scale of less than 10 ns, with a large binding free energy of 10 kcal mol^{-1}. In a clever application of the advantages of coarse-grained simulations, the membrane insertion and recoverin orientation to different lipid bilayers was investigated. This work marks a significant step towards an improved understanding of the action of recoverin. Therefore, and due to the biological importance of recoverin and a number of other related myristoyl switches, the submitted work will without doubt be published in a very good journal. In a sophisticated follow-up work, Stepan Timr was able to shed light on the calcium-dependent conformational transition of recoverin. Using microsecond atomistic siumulations as well as replica exchange MD, he could pinpoint the EF3 loop of recoverin as the main motif taking action upon addition of calcium.

The specific interaction of ions with biomolecules in general attracted large interest in the last decades, it may not only determine conformational transitions like here for recoverin, but as well influence our understanding e.g. of ion channels. Therefore, the question of ion charge scaling is of utmost importance for the simulation community. Stepan Timr not only makes use of such a reported scaling, but in addition clearly shows the necessity for a rescaling of amino acid charges at least on highly charged moieties, by combining in a clever way neutron diffraction experiments with classical MD simulations. This study is not concluded here, but will likely mark a cornerstone for a number of follow-up studies heading for correspondingly refined biomolecular force fields.

The thesis is clearly structured. The main introduction provides an adequate framework to the thesis, and the methods chapter a concise introduction into the simulation methodology, the challenges in MD simulations, potential of mean force calculations and different replica exchange

methods. The literature work of Stepan Timr is excellent; He shows an excellent overview of literature and dissects the seminal contributions to the field. Methodologically, the thesis covers an exceptional scale, from *ab initio* calculations, over atomistic MD to coarse-grained modeling, additionally applying modern free energy approaches as well as enhanced sampling techniques.

In summary, I strongly support the acceptance of the doctoral thesis of Stepan Timr by the Faculty.

Erlangen, August 15, 2017

Rainer Böckmann