Academy and University Center Nové Hrady

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Oponentský posudek doktorské disertační práce

Štěpan Timr: Molecules in Cell Membranes.

The thesis handed in by Štěpan Timr deals with molecules in cell membranes and represents a theoretical study using computational simulations in close collaboration with experimentalists. Hereby the thesis focuses on two categories of sensor molecules, membrane-embedded synthetic fluorescent probes and peripherally associated calcium-sensing proteins, aiming to characterize these molecules in the membrane environment. Štěpan Timr closely collaborates with experimentalists of the Lazar group in the Institute of Microbiology that developes two-photon polarization microscopy methods and microscopes and relies on such atomistic insights for the development of reliable molecular probes, methodology improvements and the proper mechanistic understanding of physiological processes in living cells.
The thesis is written in good english with a minimum of typos and mistakes. The thesis is divided into 7 chapters, a brief introduction into the general topic which is followed by a chapter "Modeling of membrane systems" discussing computational models of biological membranes and the simulation methods employed in the presented work. The subsequent chapters then introduce in detail the topics "Membrane Fluorescent Probes", "Membrane Binding of Proteins", "Mechanism of the Myristoyl Switch on Recoverin", "Cholesterol Oxidation" and "Modeling of Transmenbrane Voltage". Hereby each of these chapters includes the methodology applied ("Simulation approach") and the results relevant for these topics. The thesis then is closed by a conclusion chapter summing up the main findings, nicely demonstrating that classical MD coupled with *ab initio* electronic structure calculations "have the ability to elucidate atomistic details of various membrane-related processes and that this way simulations can also assist in the development of experimental techniques."
The thesis finally gives a list of four publications with the candidate being the first author on two of them, however a closer look into WOS shows that the candidate actually has published already 6 publications, one being a Nature Methods paper on 2PPM, and was cited by August 30 already 50 times (47 times without auto-citations), which is a remarkable high number for a PhD student. Štěpan Timr thus without any doubt does not only fulfill all formal requirements for a successful defense but has also proven the scientific relevance and recognition of his work within the international scientific community.

Finally, for the purpose of discussion, I have a few remarks/questions to the candidate:

1. The atomistic model of a HDL particle used in the cholesterol binding study seems to be created from scratch and not based on a X-ray or cryo-EM structure. Is this correct? How do you rate your model in terms of realistically representing HDL? How large is the variation in HDL in general (composition, structure)? From the figures is looks as if the apoAI-apolioproteins do only interact with the lipids and not mutually, or at least not specifically. What do you think is their role in HDL? Would the particles be stable without apoAI-apolioproteins? And finally, the figs give the impression that the binding of cholesterol is rather unspecific and mostly entropy driven, which would be in line with the fact that the oxidation state does not play a role in binding. Do you have an estimate about the entropy and enthalpy contribution of the binding?

2. My second question, as somebody studying allostery in proteins for a long time, is targeted to the calcium binding to recoverin. In understand that so far no free energies of binding were calculated and thus one can

hardly quantify the positive cooperativity resulting from the first Ca^{2+} binding event that is to EF3. However, what strikes me is the fact that you propose that the protein after the first binding event stays in the T-state (or a T-I equilibrium) and only the second binding event shifts the equilibrium to the R-state. This contradicts to a certain degree the MWC (or concerted) model proposed by Jaques Monod (and described on the example of hemoglobin) where the first binding event shifts the structural equilibrium of the whole system to the R-state and subsequent ligand binding occurs to the R-state. In your case the binding-competent conformation for the second state is described as an I-state that you assign with T. On which grounds? Couldn't it be that your so called "I-state" be also just a binding competent conformation of the R-state? If you simulate the R-state with 2 Ca^{2+}, do you ever observe the I-state at least for a few ps?

For all what is said above it is my pleasure to state that I can fully recommend Štěpan Timr for being awarded the PhD degree.

(Český doplneˇk: Štěpan Timr jásně prokázal tvůrčí schopností, prácé bez sebemenších pochybů splňuje požadavky kládené na disertační přáce)

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