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Centre of Excellence
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Ref: Application of B.Sc. Aparna Malisetty for attendance of the BioExcel Summer School on Biomolecular Simulations: 4 June (Introduction) and and 7-11 2021

Letter of recommendation for Aparna Malisetty

As the main supervisor of the Master's thesis, I strongly recommend Aparna Malisetty as a participant in the BioExcel Summer School on Biomolecular Simulations, which would be of great importance to her and also for me and my entire research group. Thank you for your great course offer, which we are excited to apply for.

I have known Aparna since mid-2019, when she was highly recommended by Angelika Lampert (Professor of Physiology, RWTH Aachen University) due to her excellent performance in the Biomedical Engineering M.Sc. programme at RWTH Aachen University. Due to her excellent computer skills (which are also evident from her CV), I was happy to offer Aparna a paid position as a student assistant. Her task was to use an existing high-resolution X-ray crystal structure to identify positions in the human P2X3 receptor (a ligand-gated ion channel) that would allow insertion of the Myc-9E10 antibody epitope (10-residue sequence: EQKLISEEDL) into the human P2X3 polypeptide chains at positions that were as functionally silent as possible. Using the software Modeller, Aparna identified six potentially suitable positions without external help. All six corresponding mutants were generated by Aparna as plasmids using molecular biology methods she learned in my lab. All six mutants proved to be well expressed in *Xenopus laevis* oocytes, homotrimerization competent and functional, but not necessarily silent for reasons that we would like to figure out in the near future.

After an internship at LifeTec Group, Eindhoven, The Netherlands, as part of the M.Sc. programme, Aparna returned to write her Master's thesis in my research group. The aim of her master thesis is to structurally interpret data available to us by our previous extensive biochemical and functional alanine scanning mutagenesis study on the P2X1 receptor. Aparna's initial analyses made evident to her that deeper insights into the available data require molecular dynamics simulations. This is because the alanine scanning mutations (in blocks of 4-5 residues each) not only influence the potential contact sites between the P2X1 subunits (as I had expected), but also the local folding of the polypeptide chain. As members of RWTH Aachen University, we are in principle entitled to computing time on a supercomputer (JUWELS) at the computing centre there. We are therefore confident that we can carry much more extensive calculations, but a comprehensive introduction to the basic knowledge required for this seems essential to us.

The enormous importance of this computer-based structure analysis in general for all areas of molecular medicine is beyond question. With the recent revolution in cryo-electron microscopic structural elucidation of membrane proteins, I believe we are only at the very beginning of fascinating new insights into the biophysics of ion channels to fully exploit their enormous therapeutic potential.

TFor Aparna, the course would come at just the right time, having just familiarized herself with relevant, academically accessible programmes through her cleverness and perseverance. An exciting 1-ns molecular dynamics simulation, which she realized completely on her own on her desktop computer, shows a misfolding of the first transmembrane domain of the P2X1 receptor due to a 5-alanine residue substitution. This may explain the previously for us unexplainable biochemical observation of an aggregation of the P2X1 polypeptide in the absence of a physically relevant contact site (all assembly-relevant subunit-subunit contact sites appear to reside in the large ectodomain in the P2X receptors). However, so far Aparna was not able to include the lipid bilayer in her calculations. We hope that by taking part in the course, Aparna will gain insights into how to take the lipid bilayer into account in her calculations.

I am convinced that participation in this course will give Aparna the decisive step forward to be able to make scientifically sound structural predictions of membrane proteins using state-of-the-art approaches.

With best regards

Günther Schmalzing

University Professor of Pharmacology

RWTH Aachen University

I would be happy to provide you with further information if required