



Project Proposal for Ph.D.

Project Details	
Project Title	Molecular Dynamics Studies on the Mechanism of Ligand Binding, Gating and Allosteric Communication in Acetylcholine Ion Channels
Project Summary (Minimum 500 and maximum 2000 characters)	Nicotinic acetylcholine receptors (nAChRs) are members of a large super family of pentameric ligand-gated ion channel (LGICs) receptors. They are implicated in cognition, mood disorders, nicotine addiction, sudden infant death syndrome (SIDS), analgesia, and are targets for the treatment of Alzheimer’s, depression, epilepsy, Parkinson’s and schizophrenia^{1,2}. These allosteric proteins exist in at least two alternative conformational states: resting (R) and active (R*) which differ in their functional properties. In the absence of agonists, AChRs open rarely, but with a finite open probability. Agonist binds with greater affinity to R* vs R and drives R→R* conformational switch that leads to opening of the channel. Different agonists render different amounts of energies by virtue of their chemical interaction with the ligand binding domain (LBD). How receptors sense and transduce the chemical energy of a ligand is still not clearly understood. AChRs have at least 2 ligand binding sites located in the extracellular domain of the receptor. The ‘gate’ that obstructs ion flow is located ~50 Å away from the LBD, in the transmembrane domain. How energy of ligand-binding is transmitted from the LBD to the gate by long-distance energy transfer is still a mystery. The gate of the receptor is located at the narrowest region in the channel pore. But the gate is not narrow enough to obstruct ion flow through it. It would be interesting to understand how the gate regulates ion flow through the ion channel. We aim to investigate ligand binding, gating and allosteric communication in this physiologically important class of ion channel proteins using neuromuscular junction AChRs as the model system. The proposal will be studied under the following broad aims: Aim 1: Investigate the mechanism of cholinergic ligand binding to the LBD. To this end, the kinetic rate constants (k), binding and gating free energy changes (ΔG) and structural dynamics of ligand-bound neuromuscular nAChRs will be determined by using single channel patch-clamp electrophysiology, protein engineering, thermodynamics and molecular dynamics simulations. Aim 2: Elucidate allosteric communication from LBD to gate. Long-range interaction between the LBD and gate will be explored by experimental mutant-cycle analyses and by performing principal component analyses (PCA) to identify the modes of vibrations in the receptor. Further, we will perform Phi (ϕ)-value analyses by mutating key residues in different subunits to gain insight about the transition states of the receptor in the R-R* gating pathway. Aim 3: Characterize the nature of the channel gate and the mechanism of ion flux. We will dissect the role of the gate in regulating ion flow through the channel by engineering strategic mutagenesis, measuring the gating energies by single channel patch-clamp and correlating with the free energies of transfer (ΔG_{trans}) for the residues. The nature of the gating barrier will be investigated by potential of mean force calculations using MD simulations. The expected outcome of this proposal can be generalized for the broader class of heteropentameric LGICs, such as nAChRs, GABAR and GlyR. References: 1. Lindstrom, J. Nicotinic acetylcholine receptors in health and disease. Molecular neurobiology 15, 193-222, doi:10.1007/BF02740634 (1997). 2. Kasianowicz, J. J. Introduction to ion channels and disease. Chem Rev 112, 6215-6217, doi:10.1021/cr300444k (2012).

Ph.D. Supervisors			
Role	Name of Faculty	Academic Unit in IITD/Institute/University	Email ID (Official)
Supervisor 1	Prof. Hemant Kumar Kashyap	Chemistry, IITD	hkashyap@chemistry.iitd.ac.in
Supervisor 2	Prof. Tapan Kumar Nayak	KSBS, IITD	tapannayak@iitd.ac.in

Project requirements (Student qualifications, experience required, etc) *The candidate will be shortlisted based on common shortlisting criteria decided by ScRC (SIRe)
• M.Sc./M.Tech. in any branch of chemical or biological sciences with good academic record

Source of fellowship/funding (CSIR/UGC/DBT/ICMR/ICAR/NEET-PG/DST-INSPIRE/IRD/FITT Project details, if any)
Candidate with his/her own fellowship /institute assistantship

Role of Faculty Members involved:	
Supervisor-1	Prof. Hemant K. Kashyap will supervise on the in-silico studies on ligand binding, gating and the transition state of the receptor by using MD simulations.
Supervisor-2	Prof. Tapan Kumar Nayak expertise will help in validation of the simulation results with experimental data.