

## CHAPTER 6

### BIOPHYSICS

#### Doctoral Theses

01. KAUSHIK (Vineeta)  
**Insights into the Structure, Function and Evolution of Archaeal Cyclophilins.**  
Supervisor : Dr. Manisha Goel  
Th 24784

*Abstract*  
(Not Verified)

Chaperones are a class of proteins that interact with nascent polypeptide chains and assist them to fold in a correct manner. A variety of chaperones are known to be ubiquitously distributed in all the three domains of life i.e. bacteria, archaea and eukaryotes. From the evolutionary analysis, it is well established that the molecular machinery of archaea is a simpler version of the eukaryotic cellular machinery. Therefore, archaeal systems can be used as a models for understanding the working of the eukaryotic protein folding machinery and thereby help address the various protein folding diseases. In the process of protein folding, cis-trans isomerization of peptide-prolyl bonds is a vital step. Various PPIase (Peptidyl-prolyl cis-trans isomerases) play a key role in accelerating this cis-trans isomerization reaction around the peptidyl-prolyl imide bond. Three PPIase superfamilies are currently known i.e. cyclophilins, FKBP (FK506 binding proteins) and parvulins. Cyclophilins are known to play a vital role in various cellular processes like protein trafficking, receptor complex stabilization, apoptosis, receptor signalling, and various immune responses. Although few cyclophilins from eukaryotes and prokaryotes have been characterized, very limited information is available for the role of archaeal cyclophilins in cellular proteostasis. Therefore, we aimed to functionally characterize cyclophilins from different archaeal organisms. We worked on two archaeal cyclophilins, Methanobrevibacter ruminantium (MrCyp) from phylum euryarchaeota and Nitrosopumilus maritimus (NmCyp) from phylum thaumarchaeota. MrCyp exhibits both PPIase and chaperone-like activity but only the PPIase activity could be determined for the NmCyp protein due its poor stability. The thermodynamic parameters for MrCyp and NmCyp suggest that MrCyp is more stable than NmCyp. The final objective of our work was to identify the regions responsible for differences in the structural and functional properties of these archaeal cyclophilins. To address this, molecular dynamic simulations were performed under different environment conditions for both cyclophilins.

#### *Contents*

1. Introduction and objectives 2. Review of literature 3. Methodology 4. In-silico analysis of archaeal cyclophilins 5. Biophysical and biochemical characterization of archaeal cyclophilin from methanobrevibacter ruminantium (MrCyp) 6. Biophysical and biochemical characterization of archaeal cyclophilin from nitrosopumilus maritimus (NmCyp) 7. Comparative study of MrCyp and NmCyp using molecular dynamics simulation 8. Conclusions and future prospects. References. Annexure. Publications.

02. SHRIVASTAVA (Rajan)  
**Collective Behaviour of ION Channels and its Role in Neuronal Action Potential.**  
Supervisor : Prof. Subhendu Ghosh  
Th 24783

*Abstract*  
(*Verified*)

Ion channels are the key component responsible for exchange of ions and metabolites through the cellular and organelle membranes. Various kinds of ion channels like voltage-gated Na<sup>+</sup> channels, voltage-gated K<sup>+</sup> channels, voltage-dependent anion channel (VDAC), etc. are present as clusters on cell membranes. Cluster organization facilitates physical proximity of ion channels which can regulate electrical properties of individual channels. To test this hypothesis we performed single-channel and multi-channel bilayer electrophysiology recording of VDACs and S6 peptides. VDAC is most abundant outer mitochondrial membrane (OMM) protein responsible for mitochondrial ATP supply and Ca<sup>2+</sup> homeostasis. It was observed by us that VDAC gating dynamics can be modulated by ubiquitous Ca<sup>2+</sup> regulated protein Calmodulin. S6 is a channel forming peptide usually present as one of the transmembrane domains of voltage-gated potassium channel (KvAP) from *Aeropyrum pernix*. Electrophysiological data analysis proves the existence of collective behaviour in VDACs and S6 peptides cluster. The observed unitary conductances (conductance of one ion channel in cluster) were different as compared to single channel conductances. On the other hand, open channel noise analysis of VDAC and S6 peptide shows deviation in noise profile due to presence of neighbouring channels. We concluded that cluster organization modulated gating properties of individual channel confirming presence of channel-channel interactions. In order to investigate how exactly neighbouring ion channels affect gating (opening and closing) properties of individual channel, a Zimm-Bragg type model of ion channel collective behaviour was developed. The model successfully described the multichannel gating behaviour of VDAC and self-organization of cluster with respect to its neighbouring ion channels. At the end we investigated the role of collective behaviour in modulation or self-regulation of neuronal function. Our studies through modification in classical Hodgkin-Huxely model demonstrate that collective behaviour of ion channels can regulate action potential onset dynamics along with its firing frequency.

*Contents*

1. Introduction 2. Investigation of collective behavior of Ion channels using electrophysiology 31 3. Ion channel collective behavior and noise analysis 4. Modelling collective behavior of Ion channels 5. Role of Ion channel collective behavior in generation of action potential 6. Summary and future prospects 7. References