BIOGRAPHICAL SKETCH

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NAME: Kumar, Pramod

eRA COMMONS USER NAME (credential, e.g., agency login): PRAMOD_KUMAR

POSITION TITLE: Post Doctoral Research Associate

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE (if applicable)	END DATE MM/YYYY	FIELD OF STUDY
Magadh University	BS	07/2003	Zoology (Honours), Botany and Chemistry
Guru Nanak Dev University	MS	07/2006	Biotechnology
IIT-Roorkee, India	PHD	08/2014	Biophysics

A. Personal Statement

My long-term goal is to develop a comprehensive understanding of the relationship between structure and function in membrane proteins with a special emphasis on the effect of the lipid bilayer on the embedded proteins. Among membrane proteins, I have lately become interested in ion channels because their function can be studied in high detail using the tools of single-molecule electrophysiology. My Bachelor in science with zoology honors, and subsequent Masters in Biotechnology have provided me with an excellent background for conducting work at the interface between biochemistry, biophysics, and molecular biology. As a PhD student (IIT-Roorkee), my most significant research outcome was the determination of the structure of the complex formed by plant hormone auxin and 11S globulin by X-ray crystallography at 1.7 Å resolution (1).As a postdoctoral researcher, I entered the membrane-protein field researcher working at Purdue University with Dr. Dinesh Yernool (who was trained by Prof. Eric Gouaux). In this lab, I purified a full-length histidine kinase for the very first time (manuscript under review). Currently, I am working with Prof. Claudio Grosman at the University of Illinois at Urbana Champaign. In the Grosman lab, I have crystallized and determined the structure of a number if ion channels, but the caveats associated with the solubilization in detergent and the forces exerted on the protein by the crystal lattice prevented me from answering the questions we are asking. To circumvent these hurdles, I have decided to apply cryo-EM to ion channels incorporated in phospholipid nanodiscs. We have successfully collaborated with the NYSBC and are writing a manuscript detailing our results. However, it would be highly beneficial for me to get a deeper understanding of cryo-EM map generation and microscope usage. The Grosman lab has a solid record of publications addressing the functional aspects of neurotransmitter-gated ion channels, and seems to be an optimum place to interpret structural information in a sensible manner. We are convinced that a thorough understanding of how structure gives rise to function in these ion channels will bring ambitious goals such as rational-drug design closer to reality.

1. Kumar P, Kesari P, Dhindwal S, Choudhary AK, Katiki M, Neetu, Verma A, Ambatipudi K, Tomar S, Sharma AK, Mishra G, Kumar P. A novel function for globulin in sequestering plant hormone: Crystal structure of Wrightia tinctoria 11S globulin in complex with auxin. Sci Rep. 2017 Jul 5;7(1):4705. PubMed PMID: 28680092; PubMed Central PMCID: PMC5498579.
2. Sharma A, Kumar P, Kesari P, Neetu, Katiki M, Mishra M, Singh PK, Gurjar BR, Sharma AK, Tomar S, Kumar P. Purification and Characterization of 2S Albumin from Seeds of Wrightia tinctoria Exhibiting Antibacterial and DNase Activity. Protein Pept Lett. 2017;24(4):368-378. PubMed PMID: 28128054.
3. Kumar P, Patil DN, Chaudhary A, Tomar S, Yernool D, Singh N, Dasauni P, Kundu S, Kumar P. Purification and biophysical characterization of an 11S globulin from Wrightia tinctoria exhibiting hemagglutinating activity. Protein Pept Lett. 2013 May;20(5):499-509. PubMed PMID: 22973842.

B. Positions and Honors

Positions and Employment

2006 - 2007	Junior Research Fellow, Indian Institute of Technology Kanpur
2014 - 2015	Research Associate, Indian Institute of Technology Roorkee
2015 - 2016	Post Doctoral Research Associate, PURDUE UNIVERSITY
2016 -	Post Doctoral Research Associate, UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN

Other Experience and Professional Memberships

2009 -	Member, Indian Crystallographic Association

Honors

2006	Graduate Aptitude Test in Engineering, Indian Institute of Technology Kharagpur
2006	National Eligibility Test in Life Sciences, Council of Scientific and Industrial Research
2007	Junior Research Fellowship, University Grant Commision
2009	Junior Research Fellowship, Council of Scientific and Industrial Research
2011	Senior Research Fellowship, Council of Scientific and Industrial Research

C. Contribution to Science

1. 1. My doctoral studies were focused on the structure of globulins found in plant seeds. I have successfully isolated and purified 11S and 2S globulins (a, b). They constitute two major classes of storage protein. The 11S class has given nice diffracting crystals and formed a complex with the plant hormone auxin. This was the first structural study of a plant hormone binding to a seed protein to best of our knowledge. This observation will allow further studies of the effect of hormones on plant seeds. On the basis of the structural and biochemical findings, it was proposed that 11S globulin has the ability to sequester the plant hormone auxin, which may act as a possible source of hormone at the onset of germination (c).
   a. Kumar P, Kesari P, Dhindwal S, Choudhary AK, Katiki M, Neetu, Verma A, Ambatipudi K, Tomar S, Sharma AK, Mishra G, Kumar P. A novel function for globulin in sequestering plant hormone: Crystal structure of Wrightia tinctoria 11S globulin in complex with auxin. Sci Rep. 2017 Jul 5;7(1):4705. PubMed PMID: 28680092; PubMed Central PMCID: PMC5498579.
   b. Sharma A, Kumar P, Kesari P, Neetu, Katiki M, Mishra M, Singh PK, Gurjar BR, Sharma AK, Tomar S, Kumar P. Purification and Characterization of 2S Albumin from Seeds of Wrightia tinctoria Exhibiting Antibacterial and DNase Activity. Protein Pept Lett. 2017;24(4):368-378. PubMed PMID: 28128054.
   c. Kumar P, Patil DN, Chaudhary A, Tomar S, Yernool D, Singh N, Dasauni P, Kundu S, Kumar P. Purification and biophysical characterization of an 11S globulin from Wrightia tinctoria exhibiting hemagglutinating activity. Protein Pept Lett. 2013 May;20(5):499-509. PubMed PMID: 22973842.
2. 1. In my post-doctoral studies at Purdue University, I have expressed and purified the full-length membrane-spanning histidine kinase from the potassium-regulating bacterial KdpD-kdpE two-component system. This study characterized the influence of the lipid environment on the kinase–phosphatase switching (manuscript under review).

D. Additional Information: Research Support and/or Scholastic Performance

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
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NAME: Grosman, Claudio F.

eRA COMMONS USER NAME: GROSMAN

POSITION TITLE: Professor of Molecular and Integrative Physiology

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Buenos Aires (Argentina)	B.S/M.S.	03/1991	Biochemistry
University of Buenos Aires (Argentina)	Ph.D.	12/1996	Biophysics
State University of New York at Buffalo	Postdoc.	07/2002	Biophysics

A. Personal Statement.

I have been studying ion channels for 25 years, and the pentameric ligand-gated ion channels (pLGICs)—that is, the muscle nicotinic acetylcholine receptor (AChR) and its "cousins"—for almost 20 years. In the nervous system, the pLGICs act either post-synaptically to mediate fast synaptic transmission or pre-synaptically to modulate the release of neurotransmitter. pLGICs are the target of therapeutic and recreational drugs, and mutations to these channels are often associated with disease.

My doctoral training took place in a Department of Physical and Analytical Chemistry. It was in this environment that I developed an interest in applying the tools of physical chemistry to unravel the secrets of biology. At the time, it seemed to me that the field of ion channels was a most fertile ground to combine biomedical relevance with highly accurate measurements and quantitative rigor; 25 years later, it seems to me that I was right. As a graduate student, I studied the ion channels present in different types of syncytial epithelium (more specifically, the human placenta and the tegument of some pathogenic tapeworms) using the planar lipid-bilayer reconstitution technique and single-channel recordings. The unparalleled level of detail attainable through such single-molecule observations captivated me, then, and continues to amaze me every time I see a single-channel trace on an oscilloscope. To deepen my understanding of ion-channel function and sharpen my recording and analysis skills, I decided to join Prof. Anthony Auerbach (SUNY–Buffalo) for postdoctoral work, in 1997. I changed poorly characterized channels for the muscle AChR; the reconstitution technique for patch clamp; and simple software for the most sophisticated computer programs. Indeed, QuB software was "born" in Buffalo while I was there, and I was responsible for testing the practical performance of the program until I left.

In 2002, I started my independent career at the University of Illinois (Urbana-Champaign). While continuing our detailed work at the single-channel level, I first incorporated macroscopic electrophysiology using ultra-fast (100–150 μs) ligand-application methods—a set of tools without which no detailed ensemble-level information about the kinetics of a ligand-gated channel can be obtained. Years later, I incorporated X-ray crystallography of ion channels, which is now conducted entirely in my lab. More recently, I added computational tools: we routinely run molecular dynamics (MD) and Brownian dynamics (BD) simulations of ion permeation, and MD simulations of ion-channel conformational changes. Most recently, we have incorporated negative-stain and cryo- electron microscopy using a combination of UIUC transmission electron microscopes and those in the Structural Biology Facility of Northwestern University (Evanston, IL).

My lab is a small-operation endeavor that favors quality over quantity. It is, perhaps, because of this particular approach to Science that my record of publications may be deemed modest as far as numbers are concerned, but robust if the quality of the journals—and, especially, the amount of data per paper—are taken into consideration. Throughout my career, it has been my personal choice to write papers that contain

extensive sets of results and analysis. This is the type of papers that I enjoy reading, and thus, the type of papers I like generating; I don't like dividing my papers in small installments. I am fortunate to have been accompanied by senior scientists who also prefer this particular approach to Science, and who help me instill this style on the junior members of the group. Combining quantitative electrophysiology, mechanistic thinking, and structural and computational biology, we are poised to push the limits of what is known about neurotransmitter-gated ion channels.

B. Positions and Honors

Positions and Employment

1997–2000	Postdoctoral Fellow, Dept. Physiology and Biophysics, SUNY at Buffalo (Mentor: Anthony Auerbach)
2000–2002	Research Assistant Professor; Dept. Physiology and Biophysics, SUNY at Buffalo
2002–2008	Assistant Professor; Dept. Molecular and Integrative Physiology, University of Illinois at Urbana-Champaign
2008–2013	Associate Professor; Dept. Molecular and Integrative Physiology, University of Illinois at Urbana-Champaign
2013–present	Professor; Dept. Molecular and Integrative Physiology, University of Illinois at Urbana-Champaign
2015–present	Affiliated faculty of the Computational Science and Engineering Group, College of Engineering, University of Illinois at Urbana-Champaign
2017–present	Head; Dept. Molecular and Integrative Physiology, University of Illinois at Urbana-Champaign

Other Experience and Professional Memberships

1995–present	Member of the Biophysical Society
2001–present	Member of the Society of General Physiologists
2006	Temporary (Ad-Hoc) member of the NIH Biochemistry and Biophysics of Membranes (BBM) Study Section.
2008	Temporary (Ad-Hoc) member of the NIH Biophysics of Neural Systems (BPNS) Study Section.
2010–present	Member of the Society of Latin American Biophysicists
2010–2014	Director of An Institutional NRSA in Molecular Biophysics
2011–2017	Member of the NIH Biophysics of Neural Systems (BPNS) Study Section
2014–present	Member of the Editorial Advisory Board of *The Journal of General Physiology*
2015	Temporary (Ad-Hoc) reviewer of grant proposals submitted to the National Science Centre of Poland

Honors

2012	James E. Heath Award for Excellence in Teaching Physiology, School of Molecular and Cellular Biology University of Illinois at Urbana-Champaign
2012	Outstanding Advisor Award, Medical Scholar Program, College of Medicine, University of Illinois at Urbana-Champaign.
2014–2017	Richard and Margaret Romano Professorial Scholar
2015	Faculty Excellence Award, School of Molecular and Cellular Biology, University of Illinois at Urbana-Champaign.

C. Contribution to Science

1. Channel gating as a "wave" of conformational change. As a postdoc in Tony Auerbach's lab, I "borrowed" the concept of linear free-energy relationships—developed by physical organic chemists to probe the transition state of chemical reactions—and applied it to the closed ⇌ open conformational change of ion

channels using single-channel recordings. My background in Physical Chemistry certainly came in handy, here. This was the first application of this concept to the study of the conformational change of a protein; it had previously been used to study the unfolded ⇌ folded reaction of soluble proteins, mainly, by Alan Fersht (MRC, Cambridge, UK). From this work (first published in 2000), the concept of ion-channel gating proceeding as a propagating "wave" of conformational change emerged for the muscle AChR. We inferred that, as the channel opens, the low-affinity → high-affinity conformational change of the transmitter-binding sites happens first, whereas the non-conductive → conductive conformational change of the pore happens last, with the opposite "wave" taking place as the open channel closes. Remarkably, these papers set the foundation of what was going to become the main focus of the Auerbach lab for the following 15+ years. Gratifyingly, these ideas and methods have recently started being applied to probe the gating mechanism of other ion channels, as well, including voltage-dependent K^+ channels and CFTR.

a. Grosman, C., Zhou, M., and Auerbach, A. 2000. Mapping the conformational wave of acetylcholine receptor channel gating. *Nature* 403:773-776. PMID: 10693806.

b. Cymes, G. D., **Grosman, C.**, and Auerbach, A. 2002. Structure of the transition state of gating in the acetylcholine-receptor channel pore. A Phi-value analysis. *Biochemistry* 41:5548-5555. PMID: 11969415.

c. Grosman, C. 2002. Linear free-energy relationships and the dynamics of gating in the acetylcholine receptor channel. A Phi-value analysis of an allosteric transition at the single-molecule level. *Journal of Biological Physics* 28:267-277.

d. Grosman, C. 2003. Free-energy landscapes of ion-channel gating are malleable: changes in the number of bound ligands are accompanied by changes in the location of the transition state in acetylcholine-receptor channels. *Biochemistry* 42:14977-14987. PMCID: PMC1463891.

2. Ion channels as allosteric proteins and MWC-type mechanisms. Also as a postdoc in the Auerbach lab, I pioneered the use of low-efficacy agonists (such as choline, for the AChR) for the study of gain-of-function mutations (GOF). Until that time, the study of GOF mutants was very difficult because the efficacies of typical agonists on the wild-type channel are so high that the response of GOF mutants saturates. Thus, the use of low-efficacy or "partial" agonists facilitated the study of these mutations (most naturally occurring, disease-causing mutations are of the GOF type) and paved the way for the studies of the transition state of gating described above. Also, this approach allowed us—for the first time—to characterize channel gating in terms of the elementary reaction steps of a thermodynamic cycle, namely, the closed ⇌ open equilibrium constants of unliganded and liganded channels as well as the affinities of the channel–ligand complex in the closed- and open-channel conformations.

a. Grosman, C., and Auerbach, A. 2000. Kinetic, mechanistic and structural aspects of unliganded gating of acetylcholine receptor channels. A single-channel study of M2 12' mutants. *Journal of General Physiology* 115:621-635. PMCID: PMC2217228.

b. Grosman, C., and Auerbach, A. 2000. Asymmetric and independent contribution of M2 12' residues to diliganded gating of acetylcholine receptor channels. A single-channel study with choline as the agonist. *Journal of General Physiology* 115:637-651. PMCID: PMC2217223.

c. Grosman, C., Salamone, F. N., Sine, S. M, and Auerbach, A. 2000. The extracellular linker of muscle acetylcholine receptor channels is a gating control element. *Journal of General Physiology* 116:327-339. PMCID: PMC2233691.

d. Grosman, C. and Auerbach, A. 2001. The dissociation of acetylcholine from open nicotinic receptor channels. *Proceedings of the National Academy of Sciences* 98:14102-14107. PMCID: PMC61175.

3. Engineered ionizable side chains and the impact of rotamer side chains on ion permeation. As an independent investigator, my group has kept introducing new applications of quantitative electrophysiology. X-ray crystallography and (in most cases) cryo-EM require the solubilization of the protein of interest in detergents, and it may be argued that the consequences of this process have not been estimated carefully, yet. Aware of this important limitation of "classical" structural-biology approaches, in 2005, we developed a single-channel methodology that reports on structural aspects of the open-channel conformation with single

side-chain resolution, at room temperature, and in the context of the plasma membrane of a living cell. We applied this methodology to the muscle AChR, and we learned not only about the structure of the AChR in the open state, but also, about the effect of the protein environment on the kinetics and equilibrium properties of proton binding to ionizable side chains.

One of the key advantages of working with a heteromeric pLGIC like the muscle AChR is that its nearly invariant subunit composition ($\alpha_2\beta\delta\varepsilon$) allows us to engineer single-subunit mutations most easily. Using single-channel recordings, and taking advantage of the knowledge gained through our engineering of ionizable residues throughout the AChR's pore domain, we dissected the role of the ring of four glutamates and one glutamine of this channel's charge-selectivity filter. We concluded that the four glutamates adopt two sets of rotamers, one that contributes and one that does not contribute to set the rate of ion conduction. We complemented this electrophysiology work with computer simulations of a muscle-AChR structural model using molecular dynamics and Brownian dynamics simulations. Most recently, we extended the notion of the central role played by side-chain conformation in the phenomenon of ion permeation to include not only conductance, but also, selectivity, not only the acidic side chains of the charge-selectivity filter, but also the adjacent arginines, and not only the muscle AChR, but also, all other members of the pLGIC superfamily.

a. Cymes, G. D., Ni, Y., and **Grosman, C.** 2005. Probing ion-channel pores one proton at a time. *Nature* 438:975-980. PMCID: PMC1384014.

b. Cymes, G. D. and **Grosman, C.** 2008. Pore-opening mechanism of the nicotinic acetylcholine receptor evinced by proton transfer. *Nature Structural and Molecular Biology* 15:389-396. PMCID: PMC2596065.

c. Cymes, G. D. and **Grosman, C.** 2011*a*. Tunable pK_a values and the basis of opposite charge selectivities in nicotinic-type receptors. *Nature* 474:526–530. PMCID: PMC3121909.

d. Cymes, G. D. and **Grosman, C.** 2012. The unanticipated complexity of the selectivity-filter glutamates of nicotinic receptors. *Nature Chemical Biology* 8:975–981. PMCID: PMC3508336.

4. Quantitative macroscopic electrophysiology. The study of ligand-gated ion channels at the ensemble level and under non-stationary conditions is challenging because the alternate application and washout of ligand needs to be controlled precisely, and this is not easy to achieve in practice. In my lab, we have successfully applied an ultra-fast ligand-perfusion method that allows us to mimic the kinetics of receptor–neurotransmitter interaction in fast chemical synapses. Using this tightly controlled ligand-perfusion system, we have characterized the response of several pLGICs, AMPA and P2X receptors to "trains" of high-frequency stimulation. These trains mimic the repetitive exposure to neurotransmitter that would take place in a synapse in the absence of synaptic-vesicle depletion.

a. Elenes, S., Ni, Y., Cymes, G. D., and **Grosman, C.** 2006. Desensitization contributes to the synaptic response of gain-of-function mutants of the muscle nicotinic receptor. *Journal of General Physiology* 128:615-627. PMCID: PMC2151585.

b. Elenes, S., Decker, M., Cymes, G. D., and **Grosman, C.** 2009. Decremental response to high-frequency trains of acetylcholine pulses but unaltered fractional Ca^{2+} currents in a panel of 'slow-channel syndrome' nicotinic-receptor mutants. *Journal of General Physiology* 133:151-169. PMCID: PMC2638206.

c. Papke, D., Gonzalez-Gutierrez, G., and **Grosman, C.** 2011. Desensitization of neurotransmitter-gated ion channels during high-frequency stimulation: A comparative study of Cys-loop, AMPA and purinergic receptors. *Journal of Physiology* 589:1571–1585. PMCID: PMC3099016.

d. Papke, D., and **Grosman, C.** 2014. The role of intracellular linkers in gating and desensitization of human pentameric ligand-gated ion channels. *Journal of Neuroscience* 34:7238–7252. PMCID: PMC4028499.

5. Structure-function studies of bacterial pLGICs: X-ray crystallography and patch-clamp electrophysiology. Because of their biochemical tractability, the bacterial members of the pLGIC superfamily have become a subject of intense study. We have published a number of papers in which we highlighted the difficulties in assigning functional states to protein structures. We have also described functional differences between these bacterial channels and their counterparts from animals.

a. Gonzalez-Gutierrez, G. and **Grosman, C.** 2010. Bridging the gap between structural models of nicotinic receptor superfamily ion channels and their corresponding functional states. *Journal of Molecular Biology* 403:693–705. PMCID: PMC2966540.

b. Gonzalez-Gutierrez, G., Lukk, T., Agarwal, V., Papke, D., Nair S. K., and **Grosman, C.** 2012. Mutations that stabilize the open state of the *Erwinia chrisanthemi* ligand-gated ion channel fail to change the conformation of the pore domain in crystals. *Proceedings of the National Academy of Sciences* 109:6331–6336. PMCID: PMC3341056.

c. Gonzalez-Gutierrez, G., Cuello, L. G., Nair, S. K., and **Grosman, C.** 2013. Gating of the proton-gated ion channel from *Gloeobacter violaceus* at pH 4 as revealed by X-ray crystallography. *Proceedings of the National Academy of Sciences* 110:18716–18721. PMCID: PMC3832033.

d. Gonzalez-Gutierrez, G., and **Grosman, C.** 2015. The atypical cation-conduction and gating properties of ELIC underscore the marked functional versatility of the pentameric ligand-gated ion-channel fold. *Journal of General Physiology* 146:15–36. PMCID: PMC4485021.

Complete List of Published Work in My Bibliography:

http://www.ncbi.nlm.nih.gov/sites/myncbi/claudio.grosman.1/bibliography/40353096/public/?sort=date&direction=ascending..

D. Research Support.

ONGOING

R01 NS042169 Grosman (PI); Period: 06/01/2003–05/31/2023

NIH/NINDS

“Mechanisms of Neurotransmitter-gated Ion Channels”. The overall goal of this project is to understand the properties of the members of the pentameric ligand-gated ion-channel superfamily in terms of structure and function.

Role: PI.

COMPLETED

U54 GM087519 Eduardo Perozo (PI); Period: 09/24/2015-08/31/2016

NIH/NIGMS

“Membrane Protein Structural Dynamics Consortium”. The goal of this sub-award was to develop conformation-specific synthetic antigen binders of pentameric ligand-gated ion channels.

Role: Consortium-PI.