BIOGRAPHICAL SKETCH

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NAME: Hibbs, Ryan E.

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

POSITION TITLE: Associate Professor, Departments of Neuroscience and Biophysics, Effie Marie Cain Scholar in Medical Research, UT Southwestern Medical Center

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Whitman College, Walla Walla, WA	B.A.	05/00	Chemistry-Biochemistry
University of California, San Diego, CA	Ph.D.	12/06	Neuropharmacology
Vollum Institute, Portland, OR	Post-doc	n/a	Neuroscience/Structural Biology/Biophysics

A. Personal Statement

My lab is pursuing atomic-scale mechanisms of ligand-gated ion channel function. We focused initially on nicotinic acetylcholine receptors, then expanded to include $GABA_A$ receptors, which are members of the same superfamily of pentameric channels. I am fascinated by how these complex proteins respond to binding of a small chemical agonist by triggering the opening of an intrinsic ion conduction pathway >50 Å away. My lab further seeks to probe mechanisms of ion selectivity and allosteric modulation, with a long-term goal of better informing rational therapeutic design for diverse health problems ranging from addiction to neurological disorders, to inflammation. We employ a multidisciplinary approach encompassing molecular biology, protein biochemistry, pharmacology, electrophysiology, x-ray crystallography and cryo-electron microscopy. Long term goals include biophysical analysis of large synaptic signaling complexes and structure-guided drug design relevant to addiction, mental illness, autoimmune diseases, and neurodegeneration. Accomplishments most relevant to the current proposal include our having determined the first high-resolution structures of several nicotinic receptor subtypes as well as that of the synaptic $GABA_A$ receptor. Please see the third and fourth Contributions to Science below for more detail on our structure-function studies of these receptors. A major sticking point in the Cys-loop receptor structural biology field is the paucity of so-called activated or open channel states of the receptors. These ligand-gated ion channels evolved to close (or desensitize) in the sustained presence of agonist, making it very challenging to visualize what a conducting pore looks like. The fast agonist application while plunge freezing approach, using the Chameleon, is thus very appealing; we explain in more detail in the proposal how the kinetics of channel desensitization and rate of sample application and lag time before freezing match nearly ideally for our receptors.

Ongoing research projects that I would like to highlight include:

R01 DA 047325 (PI: Hibbs) 2/1/2019-12/31/2023
NIH/NIDA
Structure and function of GABA-A receptors
This grant supports structural efforts on understanding synaptic $GABA_A$ receptor pharmacology using cryo-EM, lipid bilayer reconstitution and electrophysiological approaches. Work supported by this grant includes in-depth structural pharmacology of a synaptic $GABA_A$ receptor (Kim et al. 2020 *Nature*). Our ability to produce this

heteromeric receptor in amounts and biochemical quality sufficient for high resolution cryo-EM studies is an essential foundation of the proposed research to trap an open/activated channel state of this receptor.

R01 NS120496 (PI: Hibbs) 1/1/2021 – 12/31/2025
NIH/NINDS
Structural basis of nicotinic acetylcholine receptor gating and toxin inhibition.
This proposal focuses on structural biology, electrophysiology, and pharmacology of the human α7 nicotinic receptor and the muscle-type nicotinic receptor. Work in this project provides an important foundation for the current proposal because muscle-type receptor is the target of the current proposal. Two particularly relevant papers related to this nicotinic receptor grant are our 2020 and 2022 papers in *Neuron* and *NSMB*, respectively (both Rahman et al). These studies demonstrate we can reliably determine 2.5-3A resolution structures of the receptor targeted in Aim 2 of this Chameleon application.

B. Positions and Honors

Employment

2019-present	Associate Professor and Effie Marie Cain Scholar in Medical Research, Departments of Neuroscience (primary) and Biophysics (secondary), University of Texas Southwestern Medical Center, Dallas, TX.
2012-2019	Assistant Professor and Effie Marie Cain Scholar in Medical Research, Departments of Neuroscience (primary) and Biophysics (secondary), University of Texas Southwestern Medical Center, Dallas, TX.
2007-2012	Postdoctoral Fellow. Vollum Institute, Oregon Health & Science University, Portland, OR. Advisor: Dr. Eric Gouaux
2001-2006	Graduate Student. Biomedical Sciences, Department of Pharmacology, UCSD, San Diego, CA. Advisor: Dr. Palmer Taylor
2000-2001	Research Assistant in Enzymology. Diversa Corp., San Diego, CA.

Honors

2022	Norman Hackerman Award in Chemical Research (Welch Foundation)
2019	UC San Diego Outstanding Alumnus Award
2019	Welch Foundation Research Award
2016	Welch Foundation Research Award
2014	McKnight Scholar Award
2014	Klingenstein-Simons Award
2014	Friends of the Alzheimer's Disease Center Research Award
2013	Welch Foundation Research Award
2012	NIH/NINDS K99/R00 Pathway to Independence Award
2008	NIH/NINDS F32 Postdoctoral NRSA Grant
2006	Roland Robbins Pharmacology Dissertation Award for 2006 doctoral thesis
2004	PhRMA Foundation Pre-Doctoral Fellowship

Other Experience and Professional Membership

2022-2024	NIH Study Section Biochemistry and Biophysics of Membranes (BBM) standing member
2021-present	Search Committee member for Vice President and Chief Diversity Officer, UTSW
2021-present	Vice Chair for Diversity, Equity and Inclusion, Department of Neuroscience, UTSW
2020-present	Founding member, Neuroscience Working Group on Diversity & Equity
2020-present	Founding director, Beyond the Lab – Career development program for postdocs in Neuroscience
2019-present	Cryo-EM proposal reviewer, Pacific Northwest National Lab.
2019-2021	Editorial Advisory Board for the Journal of General Physiology
2018-2021	NIH Study Section Biophysics of Neural Systems (BPNS) standing member
2014, 2016	Ad hoc reviewer in NIH BPNS study section
2012-present	Member, Biophysical Society
2005-present	Member, Society for Neuroscience

C. Contributions to Science

Dynamics and structure of pentameric neurotransmitter ("Cys-loop") receptor ligand binding domain

Fast chemical neurotransmission is essential for all aspects of nervous system function. This signaling event depends upon binding of neurotransmitter to ligand-gated channels that then open an ion permeation pathway through the membrane, converting a chemical signal into an electrical one. I have been fascinated by this process—neurotransmitter recognition and channel gating—since the start of my PhD research in 2001. In that year the first high resolution structural information was just coming out for the nicotinic family of neurotransmitter receptors, which were the first to be cloned, characterized, purified and imaged. A Dutch group had discovered a naturally occurring soluble molluscan protein that is homologous to the human receptor's extracellular ligand binding domain. They determined the X-ray crystal structure of this protein and provided the field, at long last, with a structural basis for understanding functional mechanisms.

My work in this field began then, using this new snail protein and its X-ray structure as a starting point for studies of protein dynamics in solution. To study protein dynamics, I used a combination of cysteine mutagenesis, fluorophore labeling, steady-state as well as time-resolved fluorescence measurements and analytical ultracentrifugation. In publications a-c below, I mapped binding sites on the protein for agonists and antagonists and determined the effects of ligand binding on the dynamics of protein substructures. It was clear to me however that the real utility of structural and dynamic information comes from their combination. Thus, at the end of my PhD work I determined crystal structures of the soluble ligand-binding domain in complex with partial agonists (publication (d) below) to illustrate, both from dynamic and atomic-resolution structural perspectives, how partial agonists work.

a. **Hibbs, R.E.**, Talley, T.T. and Taylor, P. "Acrylodan conjugated cysteine side chains reveal conformational state and ligand site locations of the acetylcholine binding protein." ***Journal of Biological Chemistry*** 2004: 279(27)28483-91. PMID: 15117947

b. **Hibbs, R.E.**, Johnson, D.A., Shi, J., Hansen, S.B., Taylor, P. "Structural dynamics of the α-neurotoxin-acetylcholine binding protein complex: hydrodynamic and fluorescence anisotropy decay analyses." ***Biochemistry*** 2005 (44)16602-11. PMID: 16342951

c. **Hibbs, R.E.**, Radic, Z., Taylor, P., Johnson, D.A. "Influence of agonists and antagonists on the segmental motion of residues near the agonist binding pocket of the acetylcholine-binding protein." ***Journal of Biological Chemistry*** 2006: 281(51)39708-18. PMID: 17068341

d. **Hibbs, R.E.***, Sulzenbacher, G.*, Shi, J., Talley, T.T., Conrod, S., Kem, W.R., Taylor, P., Marchot, P., Bourne, Y. "Structural determinants for interaction of partial agonists with acetylcholine binding protein and neuronal alpha7 nicotinic acetylcholine receptor." ***EMBO Journal*** 2009: 28(19):3040-51. *Equal contribution. PMID: 19696737.

Structural biology of invertebrate Cys-loop receptors

When I started my post-doctoral work there was still no reliable, high-resolution structural information for an intact (transmembrane) Cys-loop receptor. This absence of structural information left decades of functional studies without a solid framework for interpretation. That then was my goal—to get the structure and use it to understand basic receptor mechanisms of ligand recognition and ion permeation. I began by screening a panel of Cys-loop receptors from different subfamilies and different species and found one, a glutamate-gated chloride channel from *C. elegans*, that behaved well biochemically. I raised monoclonal antibodies (with the help of a core facility) and made Fab fragments to aid in crystallization of the receptor. Through two years of crystal optimization I was able to obtain a high resolution structure of this receptor in complex with different ligands and ions (publication (a) below). Then to improve diffraction of different apo crystal forms of this chloride channel I worked with another postdoc in the lab to develop a method for screening lipid additives as stabilizing agents for crystallization (publication (b), below). Using this method I determined structures of this same receptor in two new conformations, one stabilized by a lipid (publication (c) below).

a. **Hibbs, R.E.** and Gouaux, E. "Principles of activation and permeation in an anion-selective Cys-loop receptor." ***Nature*** 2011: 474(7349):54-60. PMID: 21572436

b. Hattori, M.*, **Hibbs, R.E.*** and Gouaux, E. "A fluorescence-detection size-exclusion chromatography-based thermostability assay to identify membrane protein expression and crystallization conditions." ***Structure*** 2012: 20(8):1293-9. PMID: 22884106. *****Equal contribution.

c. Althoff, T.*, **Hibbs, R.E.***, Banerjee, S. and Gouaux E. “X-ray structures of GluCl in apo states illuminate gating mechanism of Cys-loop receptors.” ***Nature*** 2014: 512(7514)333-7. PMID: 25143115. ***Equal contribution

Nicotinic acetylcholine receptor structure and function

I started my independent laboratory in 2012 with a focus on structural principles underlying function of nicotinic acetylcholine receptors. As most nicotinic receptors are obligate heteromers, we began by devising a biochemical assay to monitor subunit stoichiometry in a population (Morales-Perez et al. 2016 *Structure*). We have now applied this assay to nicotinic and $GABA_A$ receptor targets, which has allowed us to manipulate subunit stoichiometry and produce defined assemblies. We were successful in determining the x-ray structure of the α4β2 subtype of the nicotinic receptor, which was the first high-resolution structure for any nicotinic receptor, and the first of a heteromeric Cys-loop receptor (see publication (a) below). We then leveraged developments in cryo-EM to obtain structures of two subunit assemblies of this α4β2 nicotinic receptor from one sample, revealing principles of assembly, ligand recognition, and the basis of different single channel permeabilities between the two subunit assemblies (publication (b)). Following work on addiction mechanisms with the α3β4 receptor subtype (Gharpure et al. 2019 *Neuron*), we developed a new approach for isolating and reconstituting the *Torpedo* ray muscle-type nicotinic receptor and obtained its structure at 2.7 Å with α-bungarotoxin bound (publication (c)). Our most recent published study is on the α7 nicotinic receptor and structurally mapping its gating cycle, as mentioned in the personal statement (publication (d)). We further just published (at NSMB, Rahman et al.) on how the plant toxin curare antagonizes the muscle nicotinic receptor through stabilizing a desensitized state.

a. Morales-Perez, C.L., Noviello, C.M. and **Hibbs, R.E.** “X-ray structure of the human α4β2 nicotinic acetylcholine receptor.” ***Nature*** 2016: 538(7625):411-415. PMID: 27698419.

b. Walsh, R.M. Jr.#, Roh S.H.#, Gharpure, A., Morales-Perez, C.L., Teng, J. and **Hibbs, R.E**. “Structural principles of distinct assemblies of the human α4β2 nicotinic receptor.” ***Nature*** 2018: 557(7704):261-265. PMID: 29720657. #Equal contributions

c. Rahman, M.M., Teng, J., Worrell, B.T., Noviello, C.M., Lee, M., Karlin, A., Stowell, M.H.B.# and **Hibbs, R.E.#** “Structure of the native muscle-type nicotinic receptor and inhibition by snake venom toxins.” ***Neuron*** 2020 106(6):952-962. PMID: 32275860. #Co-corresponding authors

d. Noviello, C.M., Gharpure, A., Mukhtasimova, N., Cabuco, R., Baxter, L., Borek, D., Sine, S.M. and **Hibbs, R.E.** “Structure and gating mechanism of the α7 nicotinic acetylcholine receptor.” ***Cell*** 2021 184(8):2121-2134. PMID: 33735609.

$GABA_A$ receptor structure and mechanism

$GABA_A$ receptors assemble as pentamers like nicotinic receptors, but form anion selective channels, mediate fast neuronal inhibition, and are found at 30-40% of the hundreds of trillions of synapses in the brain. We worked out how to recombinantly produce and purify the predominant synaptic $GABA_A$ receptor isoform, which comprises α, β and γ subunits, and is sensitive to benzodiazepines, anesthetics, barbiturates and neurosteroids. We used cryo-EM to determine the structure of this assembly in 2018- the first structure of a physiological $GABA_A$ receptor- in complex with both GABA and flumazenil. Flumazenil is a benzodiazepine-site antagonist that is used to reverse benzodiazepine-induced anesthesia and is the principal antidote for benzodiazepine overdose. We followed this work, last year, with a second paper on the $GABA_A$ receptor, presenting 8 structures in complex with antagonists, benzodiazepines and general anesthetics, complemented by MD simulations and electrophysiology to understand diverse mechanisms of potentiation and inhibition.

Taken together, my career scientific contributions have been focused on protein structure and function pertaining to the pentameric family of neurotransmitter receptors. My work both as a graduate student and as a postdoctoral researcher produced dynamic insights into receptor function as well as the first x-ray structures of a eukaryotic member of this receptor superfamily. My independent lab has had recent success in human receptors, and in moving from homomers into more physiologically relevant heteromers as well as receptors from native tissue. These studies have elucidated structure-based mechanisms of ligand recognition, ion selectivity, allosteric modulation, channel activation, conformational modulation by lipids and ligand-induced changes in structural dynamics.

a. Zhu, S., Noviello, C.M., Teng, J., Walsh, R.M. Jr., Kim, J.J. and **Hibbs, R.E.** “Structure of a human synaptic $GABA_A$ receptor.” ***Nature*** 2018: 559(7712):67-72. PMID: 29950725.

b. Kim, J.J., Gharpure, A., Teng, J., Zhuang, Y., Howard, R.J., Zhu, S., Noviello, C.M., Walsh, R.M. Jr., Lindahl, E., **Hibbs, R.E.** "Shared structural mechanisms of general anesthetics and benzodiazepines." ***Nature*** 2020 585(7824):303-308. PMID: 32879488.
c. Kim, J.J. and **Hibbs, R.E.** "Direct structural insights into $GABA_A$ receptor pharmacology." ***TiBS*** 2021 46(6):502-517. PMID: 33674151 (Review)

Complete List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/myncbi/ryan.hibbs.1/bibliography/public/

OMB No. 0925-0001 and 0925-0002 (Rev. 03/2020 Approved Through 02/28/2023)

BIOGRAPHICAL SKETCH

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NAME: Chojnacka, Weronika

eRA COMMONS USER NAME (credential, e.g., agency login): S184079

POSITION TITLE: Graduate Student

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*)*	START DATE MM/YYYY	END DATE MM/YYYY	FIELD OF STUDY
Lodz University of Technology, Poland	B. of Engineering	10/2013	04/2017	Biotechnology
University of Warsaw, Poland	M.S.	10/2017	06/2020	Biotechnology
UT Southwestern Medical Center, Dallas, TX	Ph.D.	08/2020	present	Biophysics

A. Personal Statement

I am a Graduate Student at University of Texas Southwestern Medical Center in the Department of Neuroscience and in the program of Molecular Biophysics. I joined Dr. Ryan Hibbs's lab in March 2021. Here, I am working on unraveling structural mechanisms of pharmacology and physiology of $GABA_A$ receptors.

$GABA_A$ receptors ($GABA_A$Rs) belong to the Cys-loop superfamily of ligand gated ion channels and are the major inhibitory neurotransmitter receptors in the brain. Dysfunction of $GABA_A$Rs leads to neurological and mental illnesses including insomnia, anxiety, amnesia and epilepsy. $GABA_A$Rs are divided into synaptic and extrasynaptic receptors that are responsible for different types of ion conductance. Synaptic $GABA_A$Rs generate a transient, rhythmic neuronal ion conductance, called phasic inhibition. Extrasynaptic $GABA_A$Rs are responsible for slower neurotransmission, called tonic inhibition. There are many subunits of this protein that assemble into a variety of pentamers. The $\alpha_1\beta_2\gamma_2$ $GABA_A$R subtype is the most abundant form in the human brain and is the focus of my NCCAT Chameleon proposal.

$GABA_A$Rs have a rich pharmacology, being targeted by many therapeutics and recreational drugs such as barbiturates, benzodiazepines, anticonvulsants, neurosteroids, anesthetics, ethanol and others. Structural mechanisms of action of many of these have been studied. The infamous drug methaqualone, widely known as Quaalude, also targets and positively modulates $GABA_A$Rs, yet its structural mechanism is unknown. Methaqualone is a central nervous system depressant that was used clinically as an anxiolytic and sedative, and due to its euphoric properties quickly became a recreational drug in the 1960s-70s. It is now popular as a recreational drug in South Africa, and many of its synthetic derivatives can be found around the world. Its restricted accessibility as a schedule I drug in the United States has led to it being understudied. During my PhD training I am focusing on structural, mutagenesis and electrophysiology studies of $GABA_A$R interactions with methaqualone and its potent derivative PPTQ, with the overall goal of understanding how Quaaludes allosterically modulate receptor activity.

Investigating Quaalude's mechanism of action may pave the way for their chemical modification toward improved therapeutics. So far, I have been able to obtain two high-resolution (2.6, 2.9 Å) cryo-EM structures of the synaptic $\alpha_1\beta_2\gamma_2$ $GABA_A$ receptor in complex with GABA and methaqualone as well as GABA and PPTQ. These success in sample preparation and structure determination provide the foundation for this NCCAT Chameleon proposal to trap an open-activated channel state of the $GABA_A$ receptor.

$GABA_A$Rs, like all its superfamily members, mediate ion conductance through changes in structural conformation that is called a gating cycle. The gating cycle constitutes of three major physiological (and conformational) states: resting, activated and desensitized. To date, the activated state structure with an open channel has been resolved for glycine, nicotinic (in my lab) and serotonin receptors, however, the $GABA_A$R activated state structure is absent and is a major goal in the field. To fully understand the gating mechanism of $GABA_A$Rs, studying what structural changes occur during transition among these states is essential. Within the last several years many structures of $GABA_A$Rs in complex with a variety of its targets have been solved, from my lab and others. However, most of these are in a desensitized state and few are in a resting state. My goal is to address the missing open-activated state and analyze structural changes occurring throughout the gating cycle.

B. Positions and Honors

Positions and Employment

2020 – present
Graduate studies at University of Texas Southwestern Medical Center, Molecular Biophysics Program, Laboratory of Dr. Ryan Hibbs

2017 – 2020
Master's Degree studies in Biotechnology, specialization of Molecular Biotechnology at University of Warsaw, Faculty of Biology, Department of Systems Biology, Poland

Jul 2018 – Jun 2019
Visiting Junior Researcher at University of Texas Southwestern Medical Center, Laboratory of Dr. Yunsun Nam

May 2017 – Jul 2017
Intern at Austrian Centre of Industrial Biotechnology (acib) GmbH Graz, Austria, Laboratory of Dr. Margit Winkler

2013 – 2017
Bachelor of Engineering Degree in Biotechnology, specialization of Molecular Biotechnology and Technical Biochemistry at Lodz University of Technology, International Faculty of Engineering, Faculty of Biotechnology and Food Sciences, Institute of Technical Biochemistry, Poland

Feb 2016 – Jun 2016
Student exchange organized by Erasmus+ Program in Institut National des Sciences Appliquees (INSA) de Lyon, France

C. Contribution to Science

Undergraduate Research:

In 2016 I spent six months working on my Bachelor's Thesis at Lodz University of Technology under the supervision of Prof. Grzegorz Bujacz. The premise of the research was the determination of enantioselectivity of lipase B derived from Candida antarctica (CALB) using crystallography. CALB is an industrially important biocatalyst that catalyzes both the hydrolysis and esterification of carboxylic acid esters. We intended to uncover what mechanism is responsible for the preferential binding of one enantiomeric form of organophosphorus alcohol over another in the esterification reaction. We attempted to answer this question by performing co-crystallization of CALB with both enantiomers of those ligands. The protein crystallized and gave good resolution data in experiments with both ligands, however, after solving the structure we observed that protein crystallized without the ligands bound. We confirmed the low binding affinity by microscale thermophoresis. These results led us to the conclusion that organophosphorus alcohols we used, are secondary substrates and their sole binding to the protein is insufficient to create a stable complex. At this point, crystallographic methods would not be optimal to answer our question, as both ligands must form a static complex with the protein which is very difficult to obtain. However, the model of CALB obtained in this work would be useful in molecular dynamics study.

Graduate Research:

In 2018 I was selected to work as Visiting Junior Researcher in the laboratory of Dr. Yunsun Nam at the University of Texas Southwestern Medical Center in Dallas, for a year. Among multiple projects that I was responsible for, the one I was occupied with the most was focused on structural and biochemical studies of the newly discovered human N6-methyladenosine methyltransferase - ZCCHC4. ZCCHC4 has been shown to target 28S rRNA and to influence the development of hepatocellular carcinoma. It exhibits a significantly distinctive methyltransferase domain sequence when compared to other identified N6-methyladenosine RNA methyltransferases, yet its structure remains unknown. A substantial part of my work was devoted to ZCCHC4 structure solving, however, I have also performed biochemical studies on its interactions with RNA substrates. Within a year I made significant progress in the structure search. To accomplish that, I designed protein constructs and optimized crystallization conditions so that the grown crystals diffracted to the resolution starting from 8 Å, improving up to 4 Å. Due to the uniqueness of ZCCHC4, the structure solving must be done ab initio, which requires the resolution of the "phase problem". This can be achieved by the introduction of a heavy atom that is either intrinsic to the protein or is bound to it. The substitution of intrinsic methionine with selenomethionine is widely used, however, in my case did not bring satisfactory results. I attempted to solve this problem by establishing a new protocol in the laboratory which involves soaking the crystals in halide salts. My protocol resulted in crystals diffracting to 4 Å resolution.

After a year spent at University of Texas, I came back to Poland to finish my Master's thesis under the supervision of Dr. Maciej Kotlinski at the University of Warsaw. My research focused on the reconstitution of a human nucleosome in vitro with the further attachment of post-translationally modified variants of H1 histone. The strength of H1 binding to the nucleosome is thought to function as a major regulator of chromatin structure and function. H1 acquires many post-translational modifications that influence the binding affinity. The premise of my research was to examine binding affinities of histone H1 to the nucleosome core using isothermal titration calorimetry (ITC) as well as study structural changes in H1 orientation within the chromatosome with structural biology methods. We were interested in comparing binding affinities of the globular domain of H1 that possesses post-translational modifications with the intact one. More interestingly, we decided to create the domain using solid-phase protein synthesis. This approach would allow us to add post-translational modifications to the domain in a controlled manner.

BIOGRAPHICAL SKETCH

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NAME: Goswami, Umang

eRA COMMONS USER NAME (credential, e.g., agency login): S430101

POSITION TITLE: Graduate Student

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*)*	START DATE MM/YYYY	END DATE MM/YYYY	FIELD OF STUDY
Kurukshetra University, India	B.Tech.	08/2009	05/2013	Biotechnology
Texas A&M University, College Station, TX	M.S.	08/2016	05/2018	Biotechnology
UT Southwestern Medical Center, Dallas, TX	Ph.D.	08/2018	present	Biophysics

A. Personal Statement

I am currently a PhD student at UT Southwestern Medical Center in Dallas, TX, with Dr. Ryan Hibbs. Here I am pursuing structure-function studies of a prototypical neurotransmitter receptor. More specifically, I have been using cryo-EM and electrophysiology to understand how the nicotinic acetylcholine receptor at the neuromuscular junction opens and closes in response to diverse ligands. To this end, I have successfully purified the muscle-type nicotinic receptor from the electric organ of the *Torpedo* ray, a tractable model system, reconstituted the receptor into lipid nanodiscs, and prepared cryo-EM grids for data collection at the PNCC and on our local microscopes (Krios, Glacios, Arctica). I have been able to reconstruct several ligand complexes to 2.7-3.3 Å overall resolution and I am currently working on building and refining the atomic models into the EM density maps. I have also been able to heterologously express and purify neuronal nicotinic receptors in HEK cells. I am currently supported by the American Heart Association's pre-doctoral fellowship for my work on the muscle nicotinic receptor.

A major outstanding question in the nicotinic receptor field relates to the open channel, activated state of the receptor. I and others in our lab have determined structures in the two other major physiological states in the gating cycle (resting, and desensitized). There is no robust structural information for an open channel state of this prototypical nicotinic receptor. As such, we cannot rigorously probe state transitions underlying activation and desensitization, at a structural level. I propose to resolve this major problem in the field here.

I have successfully purified the protein with different ligands and processed the cryo-EM data, which resulted in high-resolution 3D reconstructions of the receptor. Currently our lab has cryo-EM 3D reconstructions of the muscle nicotinic receptor in resting (apo), anesthetic bound and desensitized states at <3Å resolution. The short-lived open-activated state structure is impossible to obtain through standard blot-plunge freeze grid preparation technique that we use in the lab, because in the sustained presence agonist, the channels desensitize too rapidly. I will utilize my time on Chameleon to obtain an

open state structure of the muscle nicotinic receptor. The idea is to add the endogenous agonist (acetylcholine) to the apo/resting receptor right before freezing, with a difference of milliseconds between agonist application and plunge freezing. This is the part where Chameleon's ability to mix and freeze samples within 54 ms will be essential. The receptor undergoes desensitization on a similar time scale of tens of milliseconds (~50% are desensitized after 50 ms); hence my hope is to obtain a large enough fraction of the particles in the open state from cryo-EM imaging. 50% should be ample. The open state protein particles can then be selected from desensitized or apo state particles using 3D classification. We have been able to identify multiple conformations of the nicotinic receptor in a single sample using masked particle subtraction followed by 3D classification with different ligands. I am confident that we can use a similar approach to identify particles in the open state from the sample prepared by the Chameleon.

B. Positions and Honors

Positions and Employment

2018 - current	Ph.D. Student, Department of Neuroscience, University of Texas Southwestern, Dallas, TX
2016 - 2018	Graduate Research Assistant, Department of Electrical and Computer Engineering, Texas A&M University, College Station, TX
2014 - 2016	High School Tutor, Aakash Institute, New Delhi, India
2013 - 2014	Research Assistant, CRAMS Technologies, Faridabad, India
2013 - 2014	High School Teacher, Modern Vidya Niketan High School, Palwal, India

Other Experience and Professional Memberships

2020 - present	Member, American Heart Association
2016 - 2018	Member, Biotechnology Society, Texas A&M University
2013 - 2014	English Project Volunteer with NGO Make a Difference
2012	Student member of Souvenir committee for a national conference
2011 - 2012	Organizer of technology and cultural festivals: Genesis, Excelsior and Taknik

Honors

2018	Outstanding May Graduate, MS in Biotechnology
2016 - 2017	Biotechnology Graduate fellowship
2016 - 2017	Vice President, Biotechnology Society, Texas A&M University, College Station, TX
2013	3rd place in student research convention, Anveshan
2012	Won district level science quiz
2013	Graduated B.Tech in first class with distinction
2011-2013	Won University level public speaking competition, Rostrum, Kurukshetra University, India
2008	Certificate of Merit for distinctive performance in Nationwide Science and Technology exam by Central Board of Secondary education

C. Contributions to Science

1. **Undergraduate Research:** I worked with Dr. Swati Dahiya at Kurukshetra University and completed two projects. In my first project I evaluated the effects of medicinal plant extracts on growth of primary chicken embryo fibroblasts and their antimicrobial activity. In the second project, I

isolated malachite green dye degrading bacterium *Burkholderia cepacia* from wastewater samples. The results were compiled into a project report and submitted to the department. I also worked on a project aimed at isolating bacteriophages specific to food borne pathogens. The resulting posters were presented in the departmental conference, NexGen Biotechnology 2012.

a. Partial Phytochemical characterization and evaluation of *in vitro* antimicrobial activity of various extracts of *Camellia sinensis* against *Staphylococcus arlettae.* S. no PB-26 (First author)
b. Partial Phytochemical characterization and evaluation of *in vitro* antimicrobial activity of various extracts of *Terminalia chebula* against *Staphylococcus arlettae.* S. no PB-22 (Second author)
c. Partial Phytochemical characterization and evaluation of *in vitro* antimicrobial activity of various extracts of *Withania somnifera* against *Staphylococcus arlettae.* S. no PB-25 (Third author)
d. Isolation of bacteriophages against food borne pathogens. S. no. IB-1 (Fourth author)

2. **Graduate Research**: During my master's at Texas A&M University, I worked on developing a fluorescent spectroscopy-based method to distinguish live and dead bacteria in solution. I also assisted in the development of a handheld synchronous scan spectrophotometer capable of detecting bacterial pathogens *in situ*. My work resulted in two publications and a patent.
    a. Li R, Goswami U, Walck M, Khan K, Chen J, Cesario TC, Rentzepis PM. Hand-held synchronous scan spectrometer for in situ and immediate detection of live/dead bacteria ratio. Rev Sci Instrum. 2017 Nov;88(11):114301.
    b. Li R, Goswami U, King M, Chen J, Cesario TC, Rentzepis PM. In situ detection of live-to-dead bacteria ratio after inactivation by means of synchronous fluorescence and PCA. Proc Natl Acad Sci U S A. 2018 Jan 23;115(4):668-673.
    c. https://patents.google.com/patent/WO2018039624A1/en

3. **Graduate Research**: In my ongoing predoctoral research I am working on the structural and functional aspects of synaptic proteins with a focus on ligand gated ion channels. Currently I am characterizing the effects of general anesthetics and neuromuscular blockers on the muscle-type nicotinic receptor. Anesthetic regulation of nicotinic receptor can have effects on cardiovascular homeostasis, which in part is modulated by nicotinic receptors through the autonomic nervous system. I have successfully purified the ion channel from *Torpedo* fish and have obtained a high-resolution structure, which clearly shows the effect of general anesthetic etomidate on the muscle type nicotinic receptor.