BIOGRAPHICAL SKETCH

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NAME: Harsh Bansia

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

POSITION TITLE: Postdoctoral 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)*	Start Date (MM/YYYY)	Completion Date MM/YYYY	FIELD OF STUDY
Jiwaji University, Gwalior, India	B.Sc.	07/2006	06/2009	Biotechnology
Jiwaji University, Gwalior, India	M.Sc.	07/2009	10/2011	Biotechnology
Indian Institute of Science (IISc), Bengaluru, India	Ph.D.	01/2012	12/2018	Structural and computational Biology
Indian Institute of Science (IISc), Bengaluru, India	Research Associate	03/2019	12/2019	Structural and Computational Biology
CUNY ASRC, New York, USA	Postdoctoral Research Associate	03/2020	-	Structural and Computational Biology

A. Personal Statement

My goal is to investigate the **structure-dynamics-function** correlation in transmembrane receptors such as Ryanodine receptors (RyRs) and G-protein coupled receptors (GPCRs) whose malfunction leads to cardiovascular and neurodegenerative and muscle disorders. To this end, I am using my training in structural and computational techniques including X-ray crystallography, cryo-electron microscopy (cryo-EM), molecular dynamics (MD) simulations and machine learning based image analysis, to experimentally determine molecular movies depicting these proteins in action at unprecedented timescales and details. My aim is to use this information to better understand the working mechanisms of these medically important proteins and provide me with a platform to develop therapeutics to treat the challenging medical conditions associated with their malfunction.

Towards a long-term goal of building a career in structural biology to study proteins relevant in fundamental cellular processes and diseases, I joined Prof. Ramakumar's protein crystallography laboratory at Indian Institute of Science for my PhD. There, I developed a strong background in X-ray crystallography (**solved 7 crystal structures of 4 different proteins**), computational modeling and molecular dynamics simulations while working on various projects sharing a common theme of occurrence of cryptic elements in protein structures. I demonstrated the use of small glycols as probe molecules to identify cryptic sites in proteins by X-ray crystallography (**PDB IDs: 5XC0, 5XC1**) and MD simulations to help drug-design against difficult protein targets **(1).** I determined crystal structures of a ribosome inactivating protein (RIP) (**PDB IDs: 5Z37, 5Z3I, 5Z3J**) and studied its mechanism of ribosome recognition and catalysis and solved crystal structure of fragment crystallizable (Fc) region of antibody to elucidate structural basis for neutralization of RIPs by their

monoclonal antibodies **(2).** I performed crystal structure analysis on a methyltransferase (MTase) from *M. tuberculosis* (**Mtb**) (**PDB ID: 6ID6**), annotated it as carboxyl MTase and studied its role in host-pathogen interactions. I also performed MD and modeling simulations of Mtb proteins - topo I and CtpF providing insights into their mode of action and regulatory roles**.** These projects gave me a foundational background on many important aspects of structural biology and protein dynamics.

While working on CtpF**,** a Ca^{2+} transporting P2A ATPase involved in maintaining Ca^{2+} homeostasis in cells and any disturbance of which is deleterious and causes diseases, I became interested in structural studies of medically relevant membrane proteins and to use my PhD training in discovering cryptic and allosteric sites to help in drug-design efforts. Membrane proteins are in general not easily accessible to X-ray crystallography-based studies. To benefit from the resolution revolution brought by cryo-EM and to leverage its capabilities in studying membrane proteins, I joined Prof. Amedee des Georges' laboratory for my postdoctoral training. Dr. des Georges studies membrane proteins involved in cardiovascular and neurodegenerative disorders such as Ryanodine receptors (RyRs) and G-protein coupled receptors (GPCRs).

Since joining the des Georges laboratory, I have focused my attention on deciphering the function of membrane proteins that are critical for cardiovascular function. My main focus is on the study of the ion channel RyR1 which is critical components of excitation/contraction coupling. Better understanding their function and regulation would be key to the development of drugs to treat muscle disorders and cryo-EM is ideally suited to help with that quest. In particular, I am using physical variables such as temperature and cryo-EM's ability to study dynamics to probe highly regulated RyR protein complex **(3).** Further, I am structurally characterizing RyR1 interactome *in situ*. I have also solved cryo-EM structure of human transferrin receptor 1 bound to DNA aptamer (**PDB ID: 7ZQS, EMDB: EMD-14874**) with applications in cancer therapies **(4).** These projects give me unique opportunity to get the best training in the study of protein dynamics by cryo-EM, while leveraging my expertise in model building, MD simulations and drug-design to further analyze the interactions at play in the dynamics observed and leverage that to develop therapeutics to treat cardiovascular and neurodegenerative disorders.

My career goal is to establish an independent research program aimed towards using structural biology to study pharmaceutically relevant proteins and putting to use the obtained knowledge to treat challenging cardiovascular and neurodegenerative disorders.

1. **Bansia H**[$]**,** Mahanta P, Yennawar NH and Ramakumar S[$] (**2021**) Small Glycols Discover Cryptic Pockets on Proteins for Fragment-based Approaches[#]. ***J Chem Inf Model,*** 61, 3:1322-1333, (**[$]: corresponding authors**) [#]Publication highlighted in the **Practical Fragments** blog. http://practicalfragments.blogspot.com/2021/02/antifreeze-opens-cryptic-pockets.html
2. **Bansia H**, Bagaria S, Karande AA and Ramakumar S **(2018)** Structural basis for neutralization of cytotoxic abrin by monoclonal antibody D6F10. ***FEBS J.*** 286(5):1003-1029
3. **Bansia H**, Catalano C, Melville Z, Guo Y, Marks AR and des Georges A **(2021)** Investigating gating mechanisms of ion channels using temperature-resolved cryoEM. ***Microscopy and Microanalysis*** 27 (S1), 1690-1694, doi:10.1017/S1431927621006206
4. Cheng EL*, Cardle II*, Kacherovsky N*, **Bansia H***, Wang T*, Zhou Y, Raman J, Yen A, Gutierrez D, Salipante SJ, des Georges A, Jensen MC, Pun SH **(2022)**. Discovery of a Transferrin Receptor 1-Binding Aptamer and Its Application in Cancer Cell Depletion for Adoptive T-Cell Therapy Manufacturing. ***J Am Chem Soc***, 144, 30:13851-13864. (***: equal contributions***)

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments:

02/2020 – present	Postdoctoral Research Associate, Prof. Des Georges lab, Structural Biology Initiative, Advanced Science Research Center, City University of New York, NY, USA
03/2019 – 12/2019	Research Associate, Prof. V. Nagaraja lab, Department of Microbiology and Cell Biology, Indian Institute of Science (IISc), Bengaluru, India
01/2012 – 12/2018	PhD research scholar, Prof. S. Ramakumar lab, Department of Physics, Indian Institute of Science (IISc), Bengaluru, India

Other Experience and Professional Memberships

10/2022 – 12/2022	I-Corps Entrepreneurial Lead, New York City Regional Innovation Node
09/2021 – present	Member, New York Academy of Sciences (**NYAS**)
11/2020 – present	Professional partner, American Heart Association (**AHA)**
2020 – present	Member, New York Structural Biology Discussion Group
2015 – 2016	Founding member (Secretary) of **IISc-SIAM** (Society for Industrial and Applied Mathematics) student chapter at IISc (Indian Institute of Science)

Honors and Awards:

2023	CUNY Postdoctoral Travel Award for 3DEM GRC
2014	Senior Research Fellow, Council for Scientific and Industrial Research (**CSIR)**
2012	Junior Research Fellow, Council for Scientific and Industrial Research (**CSIR**)
2011	**GATE** (Graduate Aptitude Test in Engineering) PhD fellowship
2007	Prize**,** paper presentation on Magnetically Levitated Trains, inter college competition

Conferences and workshops:

09/2022-05/2023	ASRC-NSF Postdoctoral Research Training Program
2023	3DEM GRC and GRS, Newry, ME, USA (**Poster**)
2022	2nd COMPPÅ Symposium, New York, NY, USA (**Poster**)
2021	Microscopy and Microanalysis**,** Virtual Meeting, New York, NY, USA **(Poster)**
2019	Biological Transactions: From molecules to organisms**,** Bengaluru**,** India **(Poster)**
2017	24th Congress and General Assembly of the International Union of Crystallography, Hyderabad, India (**Poster**)
2013	Recent Advances in Computational Drug Design (RACDD), Schrödinger, India
2012	Workshop on Molecular Phylogenetics, Bengaluru, India

C. Contributions to Science

I. Structural studies on membrane proteins and receptors.

Since joining the des Georges laboratory, I have been working on membrane proteins and receptors implicated in cardiovascular and neurological disorders. Ryanodine receptors (RyRs), calcium release channels, are key elements in skeletal and cardiac muscle excitation-contraction coupling (E-C coupling). Abnormal RyR gating is responsible for muscle disorders including cardiac arrhythmias, heart failure, central core disease and malignant hyperthermia. I am using temperature as a thermodynamic variable in conjunction with cryo-EM to probe the gating mechanism of RyR channels **(a)** and leverage the obtained knowledge as a platform for the development of allosteric drugs that can restore normal RyR function. β-arrestins1 and 2 can promiscuously couple though unknown mechanisms to active states of >800 distinct GPCRs. In order to shed light on the molecular mechanism underscoring the promiscuous coupling I have interpreted cryo-EM structures of a prototypical GPCR, the β2-adrenoceptor (β2AR) bound to β-arrestin1 in core conformation by model building and extensive computational analysis. I have also solved cryo-EM structure of human transferrin receptor 1 bound to DNA aptamer with applications in cancer therapies **(b,c).** Several molecular architectures target Transferrin receptor 1 to deliver therapeutic payloads across blood-brain barrier (BBB) in targeting neurological disorders.

a. **Bansia H**, Catalano C, Melville Z, Guo Y, Marks AR and des Georges A **(2021)** Investigating gating mechanisms of ion channels using temperature-resolved cryoEM. ***Microscopy and Microanalysis*** 27 (S1), 1690-1694, doi:10.1017/S1431927621006206
b. Cheng EL*, Cardle II*, Kacherovsky N*, **Bansia H***, Wang T*, Zhou Y, Raman J, Yen A, Gutierrez D, Salipante SJ, des Georges A, Jensen MC, Pun SH **(2022)**. Discovery of a Transferrin Receptor 1-Binding Aptamer and Its Application in Cancer Cell Depletion for Adoptive T-Cell Therapy Manufacturing. ***J Am Chem Soc***, 144, 30:13851-13864. **(*: equal contributions***)
c. Protein Data Bank (**PDB**) and **EMDB** depositions**:**
7ZQS. Bansia H., Wang T., Gutierrez D., des Georges A.
EMD-14874. Wang T., **Bansia H.,** Gutierrez D., des Georges A.

II. Use of small glycols to identify cryptic sites on proteins: implications in drug-design.

Cryptic sites are visible in ligand-bound protein structures but are occluded in inbound structures. Targeting cryptic sites for drug-design provides an attractive option for proteins not tractable by classical binding sites. For years, efforts to develop inhibitors against K-RAS, an oncogene mutated in human cancers, were unsuccessful until a new cryptic site was found leading to successful targeting of K-RAS. However, owing to their hidden nature, it is difficult to identify cryptic sites. In this work, I have demonstrated the use of small glycols as probe molecules to identify cryptic sites on a diverse set of proteins (RBSX, IL-2, NPC-2, Bcl-xL, G-actin, Myosin, Glutamate receptor 2) for fragment-based approaches **(a, b).** To that end I have used crystallography experiments **(c)**, explicit-solvent and cosolvent molecular dynamics (MD) simulations, database construction and analysis **(a, b).**

a. **Bansia H*,** Mahanta P, Yennawar NH and Ramakumar S* (**2021**) Small Glycols Discover Cryptic Pockets on Proteins for Fragment-based Approaches[#]. ***J Chem Inf Model,*** 61, 3:1322-1333,
(*: corresponding authors) [#]Publication highlighted in the **Practical Fragments** blog
http://practicalfragments.blogspot.com/2021/02/antifreeze-opens-cryptic-pockets.html
b. **Bansia H,** Mahanta P and Ramakumar S **(2017)** Certain small glycols as cryptic pocket finders in proteins. ***Acta Cryst***. A73, C263.
c. Protein Data Bank (**PDB**) depositions**:**
5XC0 and **5XC1. Bansia H,** Mahanta P and Ramakumar S.

III. Structural studies on ribosome-inactivating proteins and their neutralization by antibodies

Abrin is an extremely cytotoxic ribosome inactivating protein (RIP). Abrin A-chain (ABA) is a rRNA N Glycosidase that arrests protein synthesis leading to cell death. The *kcat* for glycosidase activity is unusually high (~2000 ribosomes min^{-1}) precluding experimental structure for the RIP-Ribosome complex. Further, the identity of the catalytic water molecule implicated in the glycosidase reaction mechanism is debatable. I solved high-resolution crystal structures of apo-ABA and ABA complexed with substrate analogs and identified the catalytic water molecule thereby shedding light on ABA's substrate binding and catalytic mechanism (a,**b**). I used constraints derived from the structures of ABA-substrate analogs in an experiment-guided computational protocol to construct ABA-Ribosome complex, providing structural information on the experimentally elusive RIP-Ribosome complex (**a**). Monoclonal antibody (mAb) D6F10 is the only known antibody that neutralizes the toxicity of abrin. However, the neutralization mechanism is poorly understood. I translated experimental information as constraints in Rosetta Antibody and SnugDock algorithms to generate a 3D homology model of variable region (Fv) **(c)** of D6F10 and docked it with apo-ABA structure to obtain computational model of ABA-D6F10 Fv complex (**a**). Comparison of RIP-Ribosome and RIP-Antibody complexes revealed steric hindrance as the primary mechanism underlying neutralization of RIPs by their mAbs (**a**). Additionally, I determined the crystal structure of fragment crystallizable (Fc) region of the D6F10 (**b**).

a. **Bansia H**, Bagaria S, Karande AA and Ramakumar S **(2018)** Structural basis for neutralization of cytotoxic abrin by monoclonal antibody D6F10. ***FEBS J.*** 286(5):1003-1029
b. Protein Data Bank **(PDB)** depositions**:**
5Z37, 5Z3I, 5Z3J and 6ID7. **Bansia H**, Karande AA and Ramakumar S.
c. **Bansia, H***., Ramakumar, S*. **(2023).** Homology Modeling of Antibody Variable Regions: Methods and Applications. In: Filipek, S. (eds) Homology Modeling. Methods in Molecular Biology, vol 2627. Humana, New York, NY. https://doi.org/10.1007/978-1-0716-2974-1_16.
(*: corresponding authors)

IV. Structural and computational studies of proteins from pathogenic *Mycobacterium tuberculosis*

The topological problems associated with RNA transactions uses topoisomerase activities however the mechanisms are not properly elucidated. Using modeling simulations of DNA topoisomerase I (topo I) from mycobacteria, I proposed the reaction mechanism of topo I on RNA wherein, in contrast to DNA, vicinal 2`OH of RNA acts as a possible proton donor/acceptor during first/second transesterification reactions **(a).** The proposed mechanism pointed towards the set of topo I residues involved in the RNA topoisomerase activity and helped my colleague Phoolwanti Rani with her topo I mutation, activity assay and further downstream experiments **(a).** The cation specificity of cation transporting P-type ATPase F (CtpF) from *Mycobacterium*

tuberculosis (Mtb) was unknown. I generated a homology model of CtpF and used it in structural bioinformatic analysis along with multiple sequence alignment to map the possible cation binding sites of CtpF helping my colleague Rajni Garg to experimentally characterize CtpF as a Ca2+-transporting P2A ATPase **(b).** Rv0731c is a putative methyltransferase from human pathogen Mtb and is annotated as SAM-dependent methyltransferase. However, its annotation with respect to the methyl-accepting substrate was lacking. I solved apo and SAM-bound crystal structures of this methyltransferase (**c**). I used the crystal structures in structural bioinformatic analyses to annotate it as a carboxyl methyltransferase and studied its role in host-pathogen interactions.

a. Rani P, Kalladi S, **Bansia H**, Rao S, Jha RK, Jain P, Bhaduri T and Nagaraja V **(2020)** A Type IA DNA/RNA topoisomerase with RNA hydrolysis activity participates in ribosomal RNA processing. ***Journal of Molecular Biology***. doi:10.1016/j.jmb.2020.08.012
b. Garg R, Borbora SM, **Bansia H**, Rao S, Singh P, Verma R, Balaji KN and Nagaraja V. **(2020)** Mycobacterium tuberculosis calcium pump CtpF modulates the autophagosome in an mTOR-dependent manner. ***Front. Cell. Infect. Microbiol***. doi:10.3389/fcimb.2020.00461
c. Protein Data Bank **(PDB)** depositions**:**
6ID6: **Bansia H**, Kalladi SM, Nagaraja V and Ramakumar.

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

INSTITUTION	YEAR	COURSE TITLE	GRADE
Eight Point Scale Grading System: Grade/Qualitative Assessment/Point value of Grade **S**/Outstanding/8; **A**/Excellent/7; **B**/Very Good/6; **C**/Good/5; **D**/Satisfactory (Pass grade)/4; **F**/Fail/0			
Indian Institute of Science	2012-2013	Macromolecular Crystallography	S
Indian Institute of Science	2012-2013	Proteomics	A
Indian Institute of Science	2011-2012	Bioinformatics	A
Indian Institute of Science	2011-2012	Conformational and Structural Aspects of Biopolymers	A
Indian Institute of Science	2011-2012	Introduction to X-ray Crystallography	S
Grading System: **1 – 50** scale; scale required for passing - **18**			
Jiwaji University	2009-2011	Microbiology	42
Jiwaji University	2009-2011	Biostatistics & Computer Applications	42
Jiwaji University	2009-2011	Molecular & Immunodiagnostics	38
Jiwaji University	2009-2011	Immunotechnology	44
Jiwaji University	2009-2011	Genetic Engineering	36
Jiwaji University	2009-2011	Basic Enzymology and Environmental Biotechnology	42
Jiwaji University	2009-2011	Bioprocess Engineering & Technology	35
Jiwaji University	2009-2011	Microbial and Animal Biotechnology	43
Jiwaji University	2009-2011	Biomolecules & Bio-Techniques	38
Jiwaji University	2009-2011	Entrepreneurship Biotechnology & IPR	47

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
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NAME: des Georges, Amédée

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

POSITION TITLE: Assistant professor

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
Universite Pierre and Marie Curie, Paris	BS	09/2003	Biochemistry
Universite Pierre and Marie Curie, Paris	MS	07/2004	Biochemistry
University of Cambridge, Laboratory of Molecular Biology, Cambridge, Cambridgeshire	PHD	10/2008	Molecular Biology
Howard Hughes Medical Institute - Columbia University, New York, New York	Postdoctoral Fellow	08/2015	Structural biology

A. Personal Statement

The goal of my research program is to understand the allosteric regulation of membrane proteins by small molecules and protein effectors. Those mechanisms are much less understood in membrane proteins compared to their soluble counterparts due to the challenges specific to the membrane environment. I use structural and computational techniques to study these dynamic processes, such as cryo-electron microscopy, machine learning image analysis methods and molecular dynamics simulation, with the aim to better understand important regulatory processes and uncover new targets for pharmacological drug development.

During my PhD at the Laboratory of Molecular Biology in Cambridge, UK, I applied biochemistry and cryo-electron microscopy to studying the regulation of microtubule dynamics. As a postdoctoral associate in Dr. Joachim Frank's lab, I laid the groundwork for the structural study of translation initiation— a major regulatory checkpoint of protein translation — using cryo-electron microscopy. As the cryo-EM resolution revolution came about, I thought to leverage its new capabilities by applying the technique to membrane proteins. I initiated a collaboration with the laboratories of Wayne Hendrickson and Andrew Marks to solve the structure of the ryanodine receptor using cryo-EM, which met with success in 2014. As one of the first membrane protein structures solved by single-particle cryo-EM, the structure of this 2.3 MDa membrane protein complex was a tremendous advance for both structural biology in showing the possibilities offered by cryo-EM.

In 2015, I started my research group at the CUNY Advanced Science Research Center, an interdisciplinary research center located approximately 150 m away from the New York Structural Biology Center. My interest is in deciphering the regulatory mechanisms controlling membrane proteins function using cryo-EM. I strive to use and develop methods to open new possibilities in our quest to understand allosteric mechanisms at the molecular level: new methods to sort images of heterogeneous samples, to model dynamic processes, and to extract proteins from their membranes, focused on ion channels and G protein-coupled receptors. I am also interested in the molecular mechanisms at play during pathogen-host interaction and the dynamic events leading to virus and toxin entry into hosts, and the methods my group develops are very relevant to the current proposal.

Our ultimate aim is to increase significantly our molecular understanding of the allosteric modulation of membrane proteins both in vitro and in the context of the cells where they are expressed. Progress towards such knowledge has the potential to open the way for the design of small molecule allosteric modulators with very well controlled effects on their targets, aiding the development of drugs with limited side effects.

Ongoing and recently completed projects that I would like to highlight include:

Ongoing support:
5 R35 GM 133598-03
Des Georges (PI)
8/1/2019-6/30/2024
Understanding membrane proteins' allosteric modulation with cryo-EM

CT0060521- Institut de Recherches Servier contract (foreign sponsor)
Des Georges (PI)
11/1/2017-10/31/2024
Develop methods to image small proteins by cryo-EM

CT0079525- Institut de Recherches Servier contract (foreign sponsor)
Des Georges (PI)
10/2019-3/9/2024
Cryo-electron microscopy structural experiments of protein complexes

Past support:
1 R56 AI 152397-01A1
Weber (PI)
8/6/2021-7/30/2022
Structure-based targeting of the C. difficile toxin (CDT) from hypervirulent bacterial strains

19IPLOI34760706- American Heart Association Innovative Project Award
Des Georges (PI)
07/01/2019–06/30/2022
Deciphering GPCR signaling by allosteric and biased ligands using cryo-EM

G-2018-11286, City University of New York
des Georges (PI)
04/01/20-03/31/21
Junior Faculty Research Award in Science and Engineering

1. Bansia H, Catalano C, Melville Z, Guo Y, Marks AR, des Georges A[$]. Investigating gating mechanisms of ion channels using temperature-resolved cryo-EM. Microscopy and Microanalysis 2021 27 (S1), 1690-1694, doi:10.1017/S1431927621006206 (*[$]: co-corresponding author*)
2. Xu X[#], Godoy-Ruiz R, Adipietro KA, Peralta C, Ben-Hail D, Varney KM, Cook ME, Roth BM, Wilder PT, Cleveland T, Grishaev A, Neu HM, Michel SLJ, Yu W, Beckett D, Rustandi RR, Lancaster C, Loughney JW, Kristopeit A, Christanti S, Olson JW, MacKerell AD, des Georges A[$], Pozharski E[$], Weber DJ[$]. Structure of the cell-binding component of the *Clostridium difficile* binary toxin reveals a di-heptamer macromolecular assembly. Proc Natl Acad Sci U S A. 2020 Jan 14;117(2):1049-1058. PMCID: PMC6969506. (*[#]: CUNY PhD student*; *[$]: co-corresponding authors*)
3. Dashti A, Mashayekhi G, Shekhar M, Ben Hail D, Salah S, Schwander P, des Georges A[$], Singharoy A[$], Frank J[$], Ourmazd A[$]. Retrieving functional pathways of biomolecules from single-particle snapshots. Nat Commun. 2020;11(1):4734. PMCID: PMC7501871. (*[$]: co-corresponding authors*)
4. Nguyen AH, Thomsen ARB, Cahill TJ 3rd, Huang R, Huang LY, Marcink T, Clarke OB, Heissel S, Masoudi A, Ben-Hail D, Samaan F, Dandey VP, Tan YZ, Hong C, Mahoney JP, Triest S, Little J 4th, Chen X, Sunahara R, Steyaert J, Molina H, Yu Z, des Georges A[$], Lefkowitz RJ[$]. Structure of an endosomal signaling GPCR-G protein-β-arrestin megacomplex. Nat Struct Mol Biol. 2019 Dec;26(12):1123-1131. PMCID: PMC7108872. (*[$]: co-corresponding authors*)

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2015 -	Assistant Professor, CUNY Advanced Science Research Center, Structural Biology Initiative, NY, NY
2015 -	Assistant Professor, The City College of New York, Department of Chemistry and Biochemistry, New York, NY
2009 - 2015	Postdoctoral fellow, HHMI - Columbia University, Biochemistry and Molecular Biophysics, New York, NY

Honors and Awards

2020 Junior Faculty Research Award in Science and Engineering, City University of New York.

C. Contribution to Science

1. **Structure and dynamics of transmembrane receptors**. Cryo-EM is ideally suited to solve a number of challenging structures of membrane proteins. This motivated me to initiate a collaboration with the laboratories of Andy Marks and Wayne Hendrickson with the objective of using cryo-EM to obtain the structure of the ryanodine receptor, a structure they had failed to solve by X-ray crystallography despite 9 years of effort. Together, we obtained a 5Å resolution structure of this important pharmacological target for heart and muscular diseases (d). Further improvements in data acquisition and processing applied to different states of the channel allowed us to further improve the resolution to 3.2Å and to elucidate its mechanism of activation by calcium, ATP and caffeine (c). To gain further insight into the allosteric control of this 2MDa, we obtained the free-energy landscape of the channel in multiple ligand states using geometric machine learning methods developed by the group of Abbas Ourmazd. This gave us complex molecular movies of the channel as it transitions between ligand states (a). We apply these methods to other membrane receptors of important pharmacological relevance, such as G protein-coupled receptors and their complexes (b), with the aim of better understanding their allosteric modulation and help design drugs with greater specificity and efficacy.

    a. Dashti A, Mashayekhi G, Shekhar M, Ben Hail D, Salah S, Schwander P, des Georges A[$], Singharoy A[$], Frank J[$], Ourmazd A[$]. Retrieving functional pathways of biomolecules from single-particle snapshots. Nat Commun. 2020;11(1):4734. PMCID: PMC7501871. (*[$]: co-corresponding authors*)

    b. Nguyen AH, Thomsen ARB, Cahill TJ 3rd, Huang R, Huang LY, Marcink T, Clarke OB, Heissel S, Masoudi A, Ben-Hail D, Samaan F, Dandey VP, Tan YZ, Hong C, Mahoney JP, Triest S, Little J 4th, Chen X, Sunahara R, Steyaert J, Molina H, Yu Z, des Georges A[$], Lefkowitz RJ[$]. Structure of an endosomal signaling GPCR-G protein-β-arrestin megacomplex. Nat Struct Mol Biol. 2019 Dec;26(12):1123-1131. PMCID: PMC7108872. (*[$]: co-corresponding authors*)

    c. des Georges A*, Clarke OB*, Zalk R*, Yuan Q, Condon KJ, Grassucci RA, Hendrickson WA, Marks AR, Frank J. Structural Basis for Gating and Activation of RyR1. Cell. 2016 Sep 22;167(1):145-157.e17. PMCID: PMC5142848. (**: co-first authors*)

    d. Zalk R*, Clarke OB*, des Georges A*, Grassucci RA, Reiken S, Mancia F, Hendrickson WA, Frank J, Marks AR. Structure of a mammalian ryanodine receptor. Nature. 2015 Jan 1;517(7532):44-9. PMCID: PMC4300236. (**: co-first authors*)

2. **Structural study of pathogen-host interactions**. We are making strides towards a better understanding of pathogen-host interactions using state-of-the art single-particle and tomographic cryo-EM. The entry of pathogens or pathogen toxins into their hosts are dynamic processes that cryo-EM is ideally suited to tackle. We have recently obtained the structure of the Clostridium difficile binary toxin by single-particle cryo-EM in collaboration with David Weber at University of Maryland (d), a critical first step towards understanding the pathogenicity of this hypervirulent bacterial strain. We have an active and productive collaboration with the laboratories of Anne Moscona and Matteo Porotto at Columbia University to decipher the steps of entry of the respiratory paramyxovirus into their host using a combination of molecular and structural biology tools including cryo-electron tomography and sub-tomogram averaging (a,b,c).

Knowledge gained and tools developed will be applicable to deciphering the mechanism of host entry of other enveloped viruses, including COVID-19 (a).

a. Marcink, T.C., Kicmal, T., Armbruster, E., Zhang, Z., Zipursky, G., Golub, K.L., Idris, M., Khao, J., Drew-Bear, J., McGill, G. and Gallagher, T., Porotto M, des Georges A, Moscona A. Intermediates in SARS-CoV-2 spike–mediated cell entry. *Science Advances*. 2022 Aug 19; 8(33), p.eabo3153. PMCID: PMC9390989
b. Marcink TC, Wang T, des Georges A, Porotto M, Moscona A. Human parainfluenza virus fusion complex glycoproteins imaged in action on authentic viral surfaces. PLoS Pathog. 2020 Sep;16(9):e1008883. PMCID: PMC7529294.
c. Marcink TC, Yariv E, Rybkina K, Más V, Bovier FT, des Georges A, Greninger AL, Alabi CA, Porotto M, Ben-Tal N, Moscona A. Hijacking the Fusion Complex of Human Parainfluenza Virus as an Antiviral Strategy. mBio. 2020 Feb 11;11(1) PMCID: PMC7018645.
d. Xu X[#], Godoy-Ruiz R, Adipietro KA, Peralta C, Ben-Hail D, Varney KM, Cook ME, Roth BM, Wilder PT, Cleveland T, Grishaev A, Neu HM, Michel SLJ, Yu W, Beckett D, Rustandi RR, Lancaster C, Loughney JW, Kristopeit A, Christanti S, Olson JW, MacKerell AD, des Georges A[$], Pozharski E[$], Weber DJ[$]. Structure of the cell-binding component of the *Clostridium difficile* binary toxin reveals a di-heptamer macromolecular assembly. Proc Natl Acad Sci U S A. 2020 Jan 14;117(2):1049-1058. PMCID: PMC6969506. ([#]: *CUNY PhD student*; [$]*: co-corresponding authors*)

3. **High-resolution structure determination of asymmetric particles by single-particle cryo-EM**. When I joined the laboratory of Joachim Frank in 2008, the highest-resolution structure of an asymmetrical molecule by single-particle cryo-EM was 6.7Å. The highest resolution structure of a eukaryotic ribosome was 10Å. I optimized the data collection strategy and data processing methods implemented in the SPIDER data processing software package and obtained a map of the Trypanosoma brucei ribosome at 4.9Å resolution. At the time of publication, this was the highest resolution structure of an asymmetrical macromolecule obtained by cryo-EM (c,d). This included implementing an unbiased resolution estimation, which was very uncommon prior to the implementation of "gold-standard" resolution estimation procedures (c). The quality of the map obtained allowed my coworker Yaser Hashem to model de-novo a number of peculiar features of this ribosome, which could serve as basis for the development of more specific anti-parasitic drugs. With recent groundbreaking technical developments, I strive to disseminate the technique and train collaborators in obtaining structures of other medically relevant biological macromolecules (a,b).

a. Montemiglio LC, Testi C, Ceci P, Falvo E, Pitea M, Savino C, Arcovito A, Peruzzi G, Baiocco P, Mancia F, Boffi A, des Georges A[$], Vallone B[$]. Cryo-EM structure of the human ferritin-transferrin receptor 1 complex. Nat Commun. 2019;10(1):1121. PMCID: PMC6408514. ([$]*: co-corresponding authors*)
b. Chase J, Catalano A, Noble AJ, Eng ET, Olinares PD, Molloy K, Pakotiprapha D, Samuels M, Chait B, des Georges A[$], Jeruzalmi D[$]. Mechanisms of opening and closing of the bacterial replicative helicase. Elife. 2018;7 PMCID: PMC6391071. ([$]*: co-corresponding authors*)
c. des Georges A, Hashem Y, Buss SN, Jossinet F, Zhang Q, Liao H, Fu J, Jobe A, Grassucci RA, Langlois R, Bajaj C, Westhof E, Madison-Antenucci S, Frank J. Computational Methods for Three-Dimensional Microscopy Reconstruction. Herman GT, Frank J, editors. New York, NY: Springer New York; 2014. p.97-132.
d. Hashem Y*, des Georges A*, Fu J, Buss SN, Jossinet F, Jobe A, Zhang Q, Liao HY, Grassucci RA, Bajaj C, Westhof E, Madison-Antenucci S, Frank J. High-resolution cryo-electron microscopy structure of the Trypanosoma brucei ribosome. Nature. 2013;494(7437):385-9. PMCID: PMC3659406. (*: *co-first authors*)

4. **Structures of translation complexes at important regulatory checkpoints**. The advent of more powerful algorithms for classifying electron microscopy images allowed me to study more heterogeneous and challenging samples involved in translation regulation. Together with my coworker Yaser Hashem, I obtained the first structure of a eukaryotic translation initiation complex, the 43S pre-initiation complex. It was the first time that key eukaryotic initiation factors were observed bound to the ribosome in a cryo-EM structure. I obtained the structure of this complex from the only 5% of particles having all factors bound in the dataset. This study represented the first report of in-silico purification of a heterogenous complex using

the Bayesian image classification implemented in RELION (d). With a direct electron detector, I later improved the resolution of that structure to 6Å and from that map, my coworker Yaser Hashem built a polyalanine model of the multisubunit initiation factor eIF3 (a). Using the same strategies, we also obtained structures of the HCV IRES mRNA bound to the 40S ribosome showing how it displaces the initiation factor eIF3 (c) and the first sub-nanometer structure of a eukaryotic translation termination complex (b).

a. des Georges A, Dhote V, Kuhn L, Hellen CU, Pestova TV, Frank J, Hashem Y. Structure of mammalian eIF3 in the context of the 43S preinitiation complex. Nature. 2015 Sep 24;525(7570):491-5. PMCID: PMC4719162.
b. des Georges A, Hashem Y, Unbehaun A, Grassucci RA, Taylor D, Hellen CU, Pestova TV, Frank J. Structure of the mammalian ribosomal pre-termination complex associated with eRF1.eRF3.GDPNP. Nucleic Acids Res. 2014 Mar;42(5):3409-18. PMCID: PMC3950680.
c. Hashem Y*, des Georges A*, Dhote V, Langlois R, Liao HY, Grassucci RA, Pestova TV, Hellen CU, Frank J. Hepatitis-C-virus-like internal ribosome entry sites displace eIF3 to gain access to the 40S subunit. Nature. 2013 Nov 28;503(7477):539-43. PMCID: PMC4106463. (**: co-first authors*)
d. Hashem Y*, des Georges A*, Dhote V, Langlois R, Liao HY, Grassucci RA, Hellen CU, Pestova TV, Frank J. Structure of the mammalian ribosomal 43S preinitiation complex bound to the scanning factor DHX29. Cell. 2013 May 23;153(5):1108-19. PMCID: PMC3730827. (**: co-first authors*)

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