BIOGRAPHICAL SKETCH

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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*)

Complete List of Published Work in My Bibliography:

https://www.ncbi.nlm.nih.gov/myncbi/amedee.des georges.1/bibliography/public/

BIOGRAPHICAL SKETCH

NAME: Jack Mechler

eRA COMMONS USER NAME: JMECHLER

POSITION TITLE: Graduate Student Research Assistant

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
Saint Lawrence University, Canton, NY	BS	09/2014	05/2018	Biochemistry
CUNY Graduate Center, New York, NY	PhD (Candidate)	9/2021	2026 (Expected)	Biochemistry

A. Personal Statement

Ever since a summer research project during my undergraduate career, I have harbored an interest in the structural mechanisms of Alzheimer's disease and amyloid proteins. During that time, I found my passion for structural biology and biochemistry, and I have oriented to pursue that interest ever since. This first research project as an undergraduate was a Summer Research Fellowship, with the goal of characterizing by scanning electron microscopy novel amyloid proteins from soil bacteria. I continued that research into a senior Capstone project, glad to build out my skills in microscopy and protein purification. This research experience made me fascinated by amyloids and their role in neurodegenerative diseases. It also made me realize how much there was to learn about these diseases. This motivated me to pursue a PhD to dive deeper into these topics. I therefore applied to the CUNY Graduate Center Biochemistry PhD program, which I entered successfully. Through this program I hope to hone my technical skills in structural biology to be able to take a wholistic and informed approach to the investigation of Alzheimer's and amyloids. During my rotations I learned a wide variety of biochemical techniques to better understand how best to pursue this interest. I worked in the organic chemistry lab of Dr. Adam Profit to synthesize anti-amyloid aggregation therapeutics. I then worked with spectroscopic techniques, including Infrared (IR) and Circular Dichroism (CD) spectroscopy, in the lab of Dr. Ruel Desamero to probe the kinetics of amyloid aggregation. Finally, I rotated in the lab of Dr. Amedee des Georges primarily using Cryo-Electron Microscopy to investigate induced allostery in membrane proteins by variations in temperature. I went on to join Dr. des Georges' lab and have been wielding what I learned about the technique to study the calcium channel Ryanodine Receptor (RyR), who's mis-regulation is implicated in Alzheimer's disease.

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2021 (Present) Graduate Research Assistant
2020 Substitute Highschool Teacher
2017 Summer Research Fellow, Saint Lawrence University

Honors

2021 (Present) CUNY Science Scholarship
2021 (Present) CUNY Graduate Assistantship
2021 (Present) CUNY Tuition Fellowship
2014 – 2018 Sesquicentennial Scholarship, Presidential Achievement Award
2017 Saint Lawrence University Summer Research fellowship

C. Contributions to Science

1. Undergraduate Research:

Initially interested in the effects of amyloid fiber aggregation in Alzheimer's disease, I was led to research the fibers in a different context. Not in the brain, but in bacteria. I worked to develop methods for isolating novel bacterial amyloid fibers from a *Microbacterium* found in soil during a Summer Research Fellowship with Dr. Nadia Marano. These fibers form a crucial structural component of biofilms, which can complicate infections in implanted medical devices, and play a part in the pathology of many diseases. I hoped that in understanding the building blocks of the fibers, I could do my part in building the base of knowledge on which future therapies would stand. After the summer, I continued my research as a capstone project, culminating at presentations at the regional meeting of the American Chemical Society and the NY6 Undergraduate Research Conference. I left behind detailed methods for the isolation of novel amyloid fibers which future researchers could use to continue pursuing a greater understanding of these proteins.

Bovee H, Mechler J, Koloski J, Berrus H, Korn A, Olendzenski L, Marano N. (2020). Purification of Amyloid Fibers Formed by the Tetracycline Resistant Soil Bacterium, Microbacterium oryzae. The *FASEB Journal*. 34. 1-1. 10.1096/fasebj.2020.34.s1.04449.

2. Graduate Research:

Continuing my interest in Alzheimer's at the CUNY Graduate Center, I rotated in the labs of Dr. Adam Profit and Dr. Desamero, synthesizing peptoids antagonistic to amyloid aggregation, and using CD and IR spectroscopy to quantify the effectiveness of the treatment. Learning these methods gave me insight into an aspect of the system and research I didn't have before and gave me additional research tools with which to approach the subject. In the des Georges lab I moved toward the study of ion channel function, as they are key to neuronal function. I started studying MscS mechanical gating using Cryo-EM and temperature probing during my rotation, and then moved toward the study of RyR, as it has been shown to be involved in Alzheimer's disease. This current project, under the mentorship of Dr. des Georges and Dr. Emily Armbruster, seeks to build a greater understanding of the role of the three isoforms of RyR found in brain tissues. I will use Cryo-electron Tomography to characterize the arrays it forms in neurons and cross-linking mass spectroscopy and Single Particle Cryo-EM to identify and characterize its protein binding partners. This knowledge should be pivotal for future drug discovery research

D. Scholastic Performance

YEAR	COURSE TITLE	GRADE
	Saint Lawrence University	
2014	Lab for General Chemistry	A
2014	Calculus 1	B+
2014	General Biology 1	B+
2014	General Chemistry 1	A+
2014	Lab for General Biology 1	A
2015	General Chemistry 2	A
2015	Lab for General Chemistry 2	A
2015	Calculus 2	B+
2015	Global Pacific: Power	A-
2015	Lab for General Biology 2	B
2015	General Biology 2	B
2015	Genetics	B-
2015	Language and the Human Experience	A-
2015	Organic Chemistry	B-

YEAR	COURSE TITLE	GRADE
2015	University Physics	B
2016	Intro to Cellular Biology	B+
2016	Organic Chemistry 2	B
2016	University Physics 2	B
2016	Icons of Islamic Architecture	A-
2016	Techniques of Fiction	A
2016	Biochemistry	A
2016	Research Methods in Molecular	B
2016	Science and Pseudoscience of Anthropology	A
2017	Research Methods for Florescence and Confocal Microscopy	B-
2017	Advanced Biochemistry	B+
2017	Research Methods in Scanning Electron Microscopy	B+
2017	Politics of Pacific Southeast Asia	A+
2017	Advanced Methods in Fiction	A+
2017	Human Origins	A-
2017	Plagues and Peoples	A-
2017	Social Life of Ancient Things	B
2017	Senior Research Experience (Biochemistry)	A
2018	Research Methods in Biomolecular	C
2018	Intro: Archaeology	A
2018	Ancient Civilizations	A
2018	Senior Research Experience (Anthropology)	B
2018	Biophysical Chemistry	C+
2018	Senior Research Experience (Biochemistry)	A+
	The Graduate Center, CUNY	
2021	Advanced Biochemistry 1	B+
2021	Research Techniques Biochemistry 1	A
2021	Research Techniques Biochemistry 2	A
2021	Basic Seminar in Biochemistry	A
2021	Bioorganic Chemistry	B+
2021	Seminar in Biochemistry	P
2022	Advanced Biochemistry 2	B
2022	Research Techniques Biochemistry 1	A
2022	Research Techniques Biochemistry 2	A
2022	Basic Seminar in Biochemistry 2	A-
2022	Physical Biochemistry	B+
2022	Physical Biochemistry 2	A+
2022	Seminar in Biochemistry	P

Note: Lab courses are graded as a part of the corresponding non-lab course. P is a grade of Pass in a pass/fail course. All other courses are graded A+ to F. Saint Lawrence University courses require a D to pass, while Graduate Center courses require a B to complete.