BIOGRAPHICAL SKETCH

NAME: Sungsoo Michael Yoo

eRA COMMONS USER NAME (credential, e.g., agency login): smy003

POSITION TITLE: Postdoctoral Fellow

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Yonsei University	B.S.	02/2004	Chemistry Biochemistry
Cornell University	Ph.D.	08/2012	Chemistry
Cornell University	Post-doc	10/2013	Cell Biology
Thomas Jefferson University	Post-doc	present	Biochemistry

A. Personal Statement

As a scientist, the potential of my scientific work leading to improvements in medicine, has been a great motivation for me. In that respect, I am especially excited to be involved in GPCR research, where GPCR is a major target for small molecule drugs, and I hope contribute by becoming an independent investigator and explore the diverse mode of GPCR's signaling especially through arrestin, first by studying the structure and dynamics of the complex. With the training in cell biology during my PhD program and the training in biochemistry that is in progress, I believe I am on a great track to realize such goal.

With my interest in health science, I chose to be trained under Dr. Richard Cerione, a biochemist and cell biologist, instead of chemists investigating more traditional chemistry topics. Under his guidance, I was trained to study the signaling networks in cancer cells to figure out how the protein of interest contributed to the transformation of cancer cells. This involved cellular assays and techniques that probed the transformed phenotype of cells, such as, soft agar assays, migration assays, apoptosis assays, and general cell biological techniques such as immunofluorescence and cell-line development using viral infection. Through these experimental tools I was able to implicate the genes that are involved in eliciting oncogenic phenotype of a cell. (Apparent productivity loss at this time is partly due to the fact that my first project involving phophatases was cancelled after several years.) At the same time, it led me to appreciate that, in order to fully describe the molecular mechanism of a protein function, biochemistry is an essential tool. With this in mind, I joined Dr. Jeffrey Benovic's laboratory to seek for an opportunity to work on biochemical problems. Here, I was fortunate enough to participate in an exciting project attempting to characterize the interaction between two important proteins, namely β2AR and β-arrestin1 in molecular detail. Here, I have been trained in protein purification & biochemical analyses. I plan to continue to learn various biophysical tools that will be used to further elucidate the nature of the interaction between the two proteins. These will include single-particle electron microscopy with cryoEM and spectroscopic methods such as FRET.

In addition, I will be involved in diverse activities that this lab and the institute provide to enable me to mature as an independent researcher. This involves further training in grant writing, public presentation in conferences, attending courses in science and in ethics, and mentoring students.

I will refer the two publications that represent the evolution of my scientific interests

Yoo SM, Antonyak MA, Cerione RA. The adaptor protein and Arf GTPase-activating protein Cat-1/Git-1 is

required for cellular transformation. 2012 J Biol Chem. 287: 31462-70.

Carr, R. III, Song, J., Carter, R. L., Du, Y., Yoo, S. M., Kobilka, B. K., Cheung, J. Y., Tilley, D. G., and Benovic, J. L. b-arrestin-biased signaling through the b_2-adrenergic receptor promotes cardiomyocyte contraction. Proc. Natl. Acad. Sci. U.S.A. 113(28): E4107-4116

B. Positions and Honors

1. Positions

Activity/Occupation	Starting Date (mm/yy)	Ending Date (mm/yy)	Field	Institution/Company	Supervisor/Employer
Soldier/Non-comissioned officer	08/98	10/00	Military	Republic	Capt. Baker/Republic of Korea Army
Volunteer	07/01	07/02	Volunteer	Holy Welfare Hospital	Sister Lee
Teaching Assistant	07/04	07/06	Education	Cornell University	Department of Chemistry and Chemical Biology
Post-Doctoral Fellow	09/12	10/13	Research	Cornell University	Richard Cerione/Veterinary School of Medicine
Post-Doctoral Fellow	11/13	present	Research	Thomas Jefferson University	Jeff Benovic/Thomas Jefferson University

2. Academic and Professional Honor

2001-2002 University Designated Academic Scholarship

2001 High Honors

2004 Graduated with Highest GPA in Chemistry, and 2nd Highest in the School of Science

2006-2008 Chemistry and Biology Interface Training Grant

C. Contributions to Science

1. **Participation in understanding the role of beta-pix/Cool-1 phosphorylation in Src-promoted cell migration.**
   - Historical Background: beta-pix/Cool-1 phosphorylation by Src kinase has already been shown to be important in Src-promoted oncogenic phenotypes, especially in cell growth and anchorage-independent growth. We sought other potential roles of the phosphorylation of beta- pix/Cool-1 in Src-mediated phenotypes, such as migration.
   - Influence on Science: This study showed that beta-pix/Cool-1 phosphorylation by Src is also important for enhanced migration of Src-transformed cells.
   - Contribution: I performed experiments that resulted in a figure.

- Publication:

1. Feng Q, Baird D, Yoo S, Antonyak M, Cerione RA. Phosphorylation of the cool-1/beta- Pix protein serves as a regulatory signal for the migration and invasive activity of Src- transformed cells. 2010 J Biol Chem. 285, 18806-16.

2. Finding of a novel role for ArfGTPase Activating Protein (ArfGAP) Git-1/Cat-1 in cellular transformation.

- Historical Background: ArfGAP protein Git-1/Cat-1 is a major binding partner for beta-pix/Cool-1, and its function had been mainly characterized within the context of cell migration or cell spreading. Since Cerione lab had found that beta-pix/Cool-1 had an important role in cellular transformation, we investigated Git-1/Cati-1's potential function in cellular transformation.

- Influence on Science: This finding was the first demonstration of Git-1/Cat-1 having a role in cellular transformation, specifically in cervical carcinoma. This function of Git-1/Cat-1 was dependent on the ability of binding paxillin. Given that Git-1/Cat-1 is a GAP for Arf small GTPases, the study has added to the recent appreciation that Arf small GTPases are not only involved in intracellular trafficking, but can also contribute to cancerous growth characteristics such as cell survival and anchorage-independent growth. In fact, a novel model of Git-1/Cat-1 and paxillin interaction regulating Arf1 activation, which, in turn, regulated S6Kinase and Akt activation was suggested.

- Contribution: Research design, experiments, writing.

- Publication:

1. Yoo SM, Antonyak MA, Cerione RA. The adaptor protein and Arf GTPase-activating protein Cat-1/Git-1 is required for cellular transformation. 2012 J Biol Chem. 287: 31462-70.

2. Yoo SM, Latifkar A, Cerione RA, Antonyak MA. Cool-associated Tyrosine-phosphorylated Protein 1 Is Required for the Anchorage-independent Growth of Cervical Carcinoma Cells by Binding Paxillin and Promoting AKT Activation. 2017 J Biol Chem. 292: 3947-57

3. Yoo SM, Cerione RA, Antonyak MA.The Arf-GAP and protein scaffold Cat1/Git1 as a multifaceted regulator of cancer progression. 2017 Small GTPases. DOI: 10.1080/21541248.2017.1362496

- Abstracts:

1. Yoo, Sungsoo. A Novel Role For The Adaptor Protein And ArfGtpase-Activating Protein Cat-1/Git-1 In Cellular Transformation. Thesis, Cornell University. 2012

2. Yoo SM, Antonyak MA, Cerione RA. Unexpected role of Cat-1/Git-1 in cellular transformation. FASEB SRC Protein Kinases and Protein Phosphorylation, CO, July 2009 Yoo SM, Antonyak MA, Cerione RA.

3. Unexpected role of Cat-1/Git-1 in cellular transformation through the regulation of Arf GTPases and binding paxillin. FASEB SRC Protein Kinases and Protein Phosphorylation, CO, July 2011

3. Participation in an effort to study the structure and dynamics of β2AR/β-arrestin interaction and understand arrestin-mediated β2AR signaling.

- Historical Background: β2AR is a prototypical GPCR that is regulated by arrestins and the interaction has been characterized in various systems. The detail structural information of how the complex forms, however, is yet not available and we plan to apply conditions we have identified to

structural approaches such as cryoEM and obtain high-resolution structure of the complex. There has also been great interest in biased signaling of GPCRs and pepducins are seen as candidates to elicit specific GPCR signaling. Pepducins are lipidated peptides derived from the intracellular loops of a G protein-coupled receptor (GPCR) that can stimulate or inhibit downstream signaling processes of their cognate receptor. Previously, the Benovic lab has identified and characterized G_S-biased pepducins for β2AR. Here pepducins that can promote arrestin-biased signaling is researched.

- Influence on Science: Here, the study identifies and characterizes arrestin-biased pepducins and their potential as a next generation therapeutic for heart failure are examined.

- Contribution: Provided purified arrestins for characterization of biased signaling.

- Publication:

    1. Carr, R. III, Song, J., Carter, R. L., Du, Y., Yoo, S. M., Kobilka, B. K., Cheung, J. Y., Tilley, D. G., and Benovic, J. L. b-arrestin-biased signaling through the b_2-adrenergic receptor promotes cardiomyocyte contraction. Proc. Natl. Acad. Sci. U.S.A. 113(28): E4107-4116

- Abstract:

    1. Biochemical and biophysical characterization of β-arrestin1 interaction with the β2-adrenergic receptor. G-protein Signaling Workshop, PA, June 2018.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Benovic, Jeffrey L.

eRA COMMONS USER NAME (credential, e.g., agency login): jlb104

POSITION TITLE: Thomas Eakins Professor of Biochemistry and Molecular Biology

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)*	Completion Date MM/YYYY	FIELD OF STUDY
Pennsylvania State University, University Park, PA	B.S.	05/1976	Biochemistry
Duke University, Durham, NC	Ph.D.	06/1986	Biochemistry
Duke University, Durham, NC	Postdoctoral	06/1989	Biochemistry

A. Personal Statement

My laboratory studies the molecular and cellular basis of G protein-coupled receptor (GPCR) signaling with a focus on understanding how dysregulation of GPCRs and interacting proteins such as heterotrimeric G proteins, GPCR kinases (GRKs) and arrestins contributes to the development of disease. We have characterized the mechanisms involved in receptor phosphorylation and arrestin binding, the structural basis for GRK and arrestin interaction with GPCRs, and how these processes regulate receptor signaling and trafficking. We have also developed strategies to bias GPCR signaling with our initial efforts focused on the use of peptides and small molecules to mediate biased signaling. In addition, we study G_q signaling in airway disease and uveal melanoma and have identified strategies to inhibit wild type and mutant G_q function. The present application is focused on developing strategies to study β-arrestin complexes with GPCRs.

Ongoing and Recently Completed Research Support

R35 GM122541 (PI-Benovic) 08/01/17 - 07/31/22
NIH
Regulation of G protein-coupled receptor signaling and trafficking

R01 HL142310 (PI-Benovic) 04/01/18 - 03/31/22
NIH
Structural and dynamic analysis of GRK interaction with G protein-coupled receptors

R01 HL136219 (MPI-Benovic/Tilley) 01/09/17 - 12/31/21 (NCE)
NIH
Characterization of β-arrestin biased β_2-adrenergic receptor signaling in cardiovascular function

P01 HL114471 (PI-Panettieri) 07/15/13 - 07/31/24
NIH
Selective targeting of GPCR signaling in airway disease
Role: Project 3 and Discovery Core Leader

Falk Medical Research Trust Transformational Award (PI-Aplin) 11/30/17 - 08/29/20
Falk Medical Research Trust
Targeted inhibitor strategies in uveal melanoma
Role: co-investigator

B. Positions, Scientific Appointments, and Honors

Positions

2013-present	Thomas Eakins Professor, Department of Biochemistry and Molecular Biology, Thomas Jefferson University, Philadelphia, PA
2015-2019	Associate Director of Education, Sidney Kimmel Cancer Center
2005-2017	Professor and Chair, Department of Biochemistry and Molecular Biology, Thomas Jefferson University, Philadelphia, PA
2005	Interim Deputy Director, Kimmel Cancer Center
1998-2008	Director, Molecular Pharmacology and Structural Biology Graduate Program
1997-2005	Professor and Vice Chair, Department of Microbiology and Immunology, Thomas Jefferson University, Philadelphia, PA
1995-2005	Professor, Department of Biochemistry and Molecular Pharmacology, Thomas Jefferson University, Philadelphia, PA
1993-2013	Leader, Kimmel Cancer Center Program in Cancer Cell Biology and Signaling
1991-1995	Associate Professor, Department of Pharmacology, Thomas Jefferson University, Philadelphia, PA
1989-1991	Assistant Professor, The Fels Institute for Cancer Research, Temple University School of Medicine, Philadelphia, PA

Scientific Appointments

2021-present	Advisory Board, Trends in Pharmacological Sciences
2019-present	NIH Molecular and Integrative Signal Transduction (MIST) Study Section Member
2017	FASEB Conference on GRKs and Arrestins (Co-Organizer)
2014-2018	NIH Training and Workforce Development (TWD-A) Study Section Member
2013-2014	ASBMB Finance Committee
2013-2014	ASBMB Council
2011-2015	ASBMB Publications Committee (Chair 2013-2014)
2010	NIH EUREKA Awards SEP ZGM1 GDB-7
2009-2020	Editorial Board, Cell
2009-2011	Faculty of 1000, Neuronal Signaling Mechanisms Section
2008	NIH Cell Biology SEP ZRG1 CB-N
2008-2017	NIH Molecular Neuropharmacology and Signaling (MNPS) Study Section (ad hoc)
2008-2012	NIH Pathway to Independence Awards ZGM1 BRT-9
2007-2015	NIH Subcommittee A - Cancer Centers (ad hoc)
2007-2011	NIH Molecular and Integrative Signal Transduction (MIST) Study Section (ad hoc)
2005	Gordon Conference on Second Messengers and Protein Phosphorylation (Co-Chair)
2004	Gordon Conference on Second Messengers and Protein Phosphorylation (Co-Vice Chair)
2004-2016	Editorial Board, Journal of Cell Biology
2002-2003	Editorial Board, Molecular Endocrinology
2001-2015	Associate Editor, Biochemistry
1998-present	Editorial Board, Molecular Pharmacology
1998-present	G Protein Signaling Workshop (Organizing Committee, Organizer, 2006, 2010, 2014, 2018)
1996-2000	NIH Pharmacology Study Section Member
1995-2000	Editorial Board, Journal of Biological Chemistry
1994-1999	American Heart Association Established Investigator
1991-1996	Southeastern Pennsylvania American Heart Association Review Committee Member
1991-2012	Editorial Board, Journal of Receptors and Signal Transduction

Honors

2019	Sidney Kimmel Cancer Center Impact Award
2019	Sidney Kimmel Medical College Research Career Achievement Award
2019	Theodore M. Brody Distinguished Lecturer, Department of Pharmacology & Toxicology, Michigan State University
2018	Sidney Kimmel Cancer Center Achievement in Basic Research Award
2018	Jeffrey L. Benovic Endowed Award & Lectureship (established 2018)
2017-2022	NIH Outstanding Investigator Award

2017	Temple Translational Science Symposium, Keynote Speaker
2017	Case Western Reserve Department of Pharmacology retreat, Keynote Speaker
2017	Argentine Biomedical Societies meeting, Plenary Speaker
2016	The Yun and Sophie Yen Faculty Award for Distinguished Training in Translational Research, Jefferson College of Biomedical Sciences
2015	ASPET Molecular Pharmacology Division Postdoctoral Awards, Keynote Speaker
2014	ASPET Julius Axelrod Award
2013	Thomas Eakins Endowed Professorship
2013	National Academy of Inventors member
2009	Jefferson Postdoctoral Association Distinguished Mentor Award
2008	Jefferson College of Graduate Studies Alumni Board Lifetime Membership Award
2006-2016	NIH MERIT Award
2005	S. G. Ferguson Memorial Seminar
2004	IBRO School of Neuroscience, Plenary Speaker
2000	ISI Highly Cited Researcher in Biology and Biochemistry
1999	Seven Transmembrane Domain Receptor Club of Quebec, Keynote Speaker
1994-1999	American Heart Association Established Investigator
1991	Winter Conference on Brain Research Fellowship
1987-1989	Howard Hughes Medical Institute Research Fellow
1981-1984	NIH Training grant award

C. Contribution to Science

1. G protein-coupled receptor kinases. I started my career in the laboratory of Dr. Robert Lefkowitz with the goal of elucidating the mechanisms that regulate desensitization of the β_2-adrenergic receptor (β_2AR). This led to the discovery of the β-adrenergic receptor kinase (now called GRK2), an enzyme that phosphorylates the agonist-occupied form of the β_2AR. This work was published in 1986 and provided the first evidence that there was a family of such kinases. Additional key discoveries of our work included cloning the cDNA for GRK2 (1989), identifying additional GRKs (GRK3 in 1991 and GRK5 and GRK6 in 1993), demonstrating that GRKs are activated by GPCR binding and that this is regulated by an N-terminal amphipathic α-helix, finding that GRKs are phospholipid-dependent enzymes and contain an RGS homology domain that mediates interaction with Gα subunits, demonstrating that GRKs also phosphorylate and/or interact with a wide variety of additional proteins, and linking GRKs with various biological processes including cell cycle regulation. Our recent efforts have focused on the use of biophysical approaches to understand the structures of GRKs in complex with other proteins such as GPCRs and calmodulin as well as the role of GRKs in insulin processing and secretion.

a. Komolov, K. E., Bhardwaj, A.*, and **Benovic, J. L.*** Atomic structure of GRK5 reveals distinct structural features novel for G protein-coupled receptor kinases. *J. Biol. Chem*. **290**: 20629-20647, 2015. PMCID: PMC4543624. *Co-corresponding author. Selected as Paper of the Week; Podcast; Cover article; featured in ASBMB TODAY.

b. Wang, J., Luo, J., Aryal, D. K., Wetsel, W. C., Nass, R., and **Benovic, J. L**. G protein-coupled receptor kinase-2 (GRK-2) regulates serotonin metabolism through the monoamine oxidase AMX-2 in *Caenorhabditis elegans*. *J. Biol. Chem*. **292**: 5943-5956, 2017. PMCID: PMC5392585.

c. Komolov, K. E., Du, Y., Duc, N. M., Betz, R. M., Rodrigues, J. P. G. L. M., Leib, R.D., Patra, D., Skiniotis, G., Adams, C. M., Dror, R.O., Chung, K. Y., Kobilka, B. K.*, and **Benovic, J. L.*** Structural and functional analysis of a β_2-adrenergic receptor complex with GRK5. *Cell* **169**: 407-421, 2017. PMCID: PMC4543624. *Co-corresponding author. Featured article with Paperclip; recommended by the Faculty of 1000 as Exceptional.

d. Komolov, K. E.*, *Sulon, S. M*.*, Bhardwaj, A., van Keulen, S. C., Duc, N. M., Laurinavichyute, D. K., Lou, H. J., Turk, B. E., Chung, K. Y., Dror, R. O., and **Benovic, J. L.** Structure of a GRK5-calmodulin complex reveals molecular mechanism of GRK activation and substrate targeting. *Mol. Cell* **81**: 321-339, 2021. PMCID: PMC7855534. *co-first author.

2. Role of arrestins in G protein-coupled receptor regulation. My early research on arrestins was also initiated while I was a trainee and provided the first evidence linking arrestins with the regulation of hormonal signaling in 1987 and the first cloning of a non-visual arrestin (β-arrestin) in 1990. My lab went on to develop a binding assay that enabled dissection of the molecular basis for arrestin interaction with GPCRs. Additional efforts revealed that β-arrestins bind to clathrin and serve an essential role in β_2AR endocytosis and included

mapping the binding interface between these proteins and ultimately co-crystallizing a complex of β-arrestin with clathrin. Our current efforts are focused on: 1) elucidating the role of a family of arrestin domain containing (ARRDC) proteins in GPCR trafficking and signaling and 2) using biophysical approaches to study the structure and dynamics of β-arrestin interaction with GPCRs.

a. *Goodman, O. B., Jr*., *Krupnick, J. G*., Santini, F., Gurevich, V. V., Penn, R. B., Gagnon, A. W., Keen, J. H., and **Benovic, J. L**. β-arrestin acts as a clathrin adaptor in endocytosis of the β_2-adrenergic receptor. *Nature* **383**: 447-450, 1996.

b. Kang, D. S., *Kern, R. C*., Puthenveedu, M. A., von Zastrow, M., Williams, J. C.*, and **Benovic, J. L**.* Structure of an arrestin-2/clathrin complex reveals a novel clathrin binding domain that modulates receptor trafficking. *J. Biol. Chem.* **284**: 29860-29872, 2009. PMCID: PMC2785616. *Co-corresponding author

c. *Michal, A. M*., Tran, T. H., Ryder, A., Liu, C., Rimm, D. L., Rui, H., and **Benovic, J. L.** Differential expression of arrestins is a predictor of breast cancer progression and survival. *Breast Cancer Res. Treat*. **130**: 791-807, 2011. PMCID: PMC3156829.

d. *Tian, X*., Irannejad, R., *Bowman, S. L*., Du, Y., Puthenveedu, M. A., von Zastrow, M., and **Benovic, J. L**. The α-arrestin ARRDC3 regulates the endosomal residence time and intracellular signalling of the β_2-adrenergic receptor. *J. Biol. Chem.* **291**: 14510-14525, 2016. PMCID: PMC4938174. Selected for a special virtual issue on "Signaling through space and time".

3. Biased signaling. While the concept of biased or functionally selective signaling has recently been appreciated in the GPCR field, we had initially tested this concept for the β_2AR many years ago. This involved comparing the ability of various full and partial agonists to stimulate cAMP production through G_s and promote GRK2 phosphorylation and revealed a close correlation between these pathways. More recently, we revisited this issue with a focus on CXCR4 and the β_2AR. For the β_2AR, we screened a library of lipidated peptides (pepducins) and found several that had a striking bias towards promoting β_2AR interaction with either G_s or β-arrestin. In addition, our high throughput screening efforts have identified a number of small molecules that can function as either biased agonists or biased allosteric modulators of the β_2AR. These molecules provide an opportunity to yield insight on the structures and dynamics that control selective protein interactions with GPCRs and are currently being pursued in the context of airway disease (for G_s bias) and heart failure (for β-arrestin bias).

a. *Carr, R. III*, Du, Y., Quoyer, J., Panettieri, R. A. Jr., Janz, J. M., Bouvier, M., Kobilka, B. K., and **Benovic, J. L**. Development and characterization of pepducins as G_s-biased allosteric agonists. *J. Biol. Chem.* **289**: 35668-35684, 2014. PMCID: PMC4276837. Selected as Paper of the Week.

b. *Carr, R. III*, Schilling, J. Song, J., Carter, R. L., Du, Y., Yoo, S. M., Traynham, C. J., Koch, W. J., Cheung, J. Y., Tilley, D. G., and **Benovic, J. L**. β-arrestin-biased signaling through the β_2-adrenergic receptor promotes cardiomyocyte contraction. *Proc. Natl. Acad. Sci. U.S.A*. **113**: E4107-4116, 2016. PMCID: PMC4948363.

c. Grisanti, L. A., de Lucia, C., Thomas, T. P., Carter, R. L., Gao, E., Koch, W. J., **Benovic, J. L**., and Tilley, D. G. Pepducin-mediated cardioprotection via β-arrestin-biased β_2-adrenergic receptor-specific signaling. *Theranostics* **8**: 4664-4678, 2018. PMCID: PMC6160776.

d. *Ippolito, M*. and **Benovic, J. L.** Biased agonism at β-adrenergic receptors. *Cell. Signal*. **80**: 109905, 2021. PMCID: PMC7878421.

4. Regulation of CXCR4 function. We have also extensively studied CXCR4, a GPCR that has been linked with several diseases including WHIM Syndrome, AIDS and cancer. Our work on CXCR4 began in the late 90s and initially identified a potential role for GRKs and arrestins in CXCR4 regulation. Additional efforts revealed that agonist-dependent degradation of CXCR4 is linked with ubiquitination of specific lysines and served as the first mammalian GPCR where agonist-dependent ubiquitination was shown to mediate receptor sorting to lysosomes. We were also the first to identify a role for a specific E3 ubiquitin ligase (AIP4) in ubiquitination and sorting of a mammalian GPCR. Additionally, we used mass spectrometry and phospho-specific antibodies to dissect the phosphorylation sites, kinases and functional role of CXCR4 phosphorylation. CXCR4 has served as an important model for understanding the mechanisms linking GPCR phosphorylation, signaling and sorting.

a. Marchese, A. and **Benovic, J. L**. Agonist-promoted ubiquitination of the G-protein-coupled receptor CXCR4 mediates lysosomal sorting. *J. Biol. Chem*. **276**: 45509-45512, 2001. Recommended by the Faculty of 1000.

b. Marchese, A., Raiborg, C., Santini, F., Keen, J. H., Stenmark, H., and **Benovic, J**. **L**. The E3 ubiquitin ligase AIP4 mediates ubiquitination and sorting of the G protein-coupled receptor CXCR4. *Dev. Cell* **5**: 709-722, 2003.

c. *Busillo, J. M*., Armando, S., Sengupta, R., Meucci, O., Bouvier, M., and **Benovic, J**. **L**. Site-specific phosphorylation of CXCR4 is dynamically regulated by multiple kinases and results in differential modulation of CXCR4 signaling. *J. Biol. Chem*. **285**: 7805-7817, 2010. PMCID: PMC2844224. Recommended by the Faculty of 1000.

d. *Luo, J., **Busillo, J. M*., Stumm, R., and **Benovic, J**. **L**. G protein-coupled receptor kinase 3 and protein kinase C phosphorylate the distal C-terminal tail of the chemokine receptor CXCR4 and mediate recruitment of β-arrestin. *Mol. Pharm*. **91**: 554-566, 2017. *co-first author. PMCID: PMC5438129.

5. Targeting G_q signaling. Heterotrimeric G_q proteins regulate a number of signaling pathways including activation of phospholipase Cβ with subsequent increases in diacylglycerol production and intracellular Ca^{2+} release. G_q signaling has been implicated in a number of diseases including in uveal melanoma where activating mutations in $G\alpha_q$ or $G\alpha_{11}$ are oncogenic, and in airway diseases such as asthma and chronic obstructive pulmonary disease (COPD) where inhibition of G_q-coupled receptors serves as a major therapeutic. We have utilized two strategies to inhibit G_q signaling, one that uses a lipidated peptide (pepducin) to target the GPCR-G_q interface and the second to target G_q directly using depsipeptides. We are currently pursuing these approaches as a way of treating airway disease and cancer.

a. *Carr, R. III*, Koziol-White, C., Lam, H., An, S. S., Tall, G. G., Panettieri, R. A. Jr., and **Benovic, J. L**. Interdicting G_q activation in airway disease by receptor-dependent and receptor-independent mechanisms. *Mol. Pharm.* **89**: 94-104, 2016. PMCID: PMC4702101.

b. Chua, V., *Lapadula, D*., *Randolph, C*., **Benovic, J. L**., Wedegaertner, P., and Aplin, A. E. Dysregulated GPCR signaling and therapeutic options in uveal melanoma. *Mol. Cancer Res*. **15**: 501-506, 2017. PMCID: PMC5468095.

c. *Lapadula, D*., Farias, E., *Randolph, C. E*., Purwin, T., McGrath, D., Charpentier, T., Zhang, L., Wu, S., Terai, M., Sato, T., Tall, G. G., Zhou, N., Wedegaertner, P. B., Aplin, A. E., Aguirre-Ghiso, J., and **Benovic, J. L**. Effects of oncogenic $G\alpha_q$ and $G\alpha_{11}$ inhibition by FR900359 in uveal melanoma. *Mol. Cancer Res*. **17**: 963-973, 2019. PMCID: PMC6445713.

Note that PhD students are italicized.

Complete List of Published Work (>300 total publications; *h*-index=117; >44,000 citations)

https://www.ncbi.nlm.nih.gov/myncbi/jeffrey.benovic.1/bibliography/public/

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Bhardwaj, Anshul

eRA COMMONS USER NAME (credential, e.g., agency login): axb050

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)*	Completion Date MM/YYYY	FIELD OF STUDY
Barkatullah University, Bhopal, India	B.S.	07/1999	Biochemistry, Microbiology
Barkatullah University, Bhopal, India	M.S.	07/2001	Biochemistry, Microbiology
Freie University, Berlin, Germany	Ph.D.	01/2006	Biochemistry
SUNY Upstate Medical University, Syracuse, NY	Postdoctoral	06/2009	Structural Biology

A. Personal Statement

My research background and expertise are in the use of structural biology tools and biochemical-biophysical techniques to understand protein structure and function. As a graduate student at the Freie University, Berlin and a post-doctoral fellow at SUNY upstate, I have studied molecular and regulatory mechanisms underlying nucleocytoplasmic transport, viral assembly and viral genome packaging-ejection mechanisms using a hybrid approach of combining x-ray crystallography, SAXS, electron microscopy and various structural, molecular interaction-biochemical techniques. I have determined several novel protein structures, importantly phage Sf6 tail needle knob (pdb 3RWN), importin beta bound to snurportin1 IBB-domain (pdb 3LWW), phage HK620 tail needle (pdb 5BU5, 5BVZ), HS1 knob (pdb 4K6B), gp26_2M (pdb 4LIN), DUSP26 (pdb 4HRF), importin alpha-scramblase 4 NLS complex (pdb 3Q5U), the small terminase subunit of phage P22 (pdb 3P9A), crystal structures of AMP-PNP and sangivamycin bound GPCR kinase 5(pdb 4TND, 4TNB), the crystal structures of asymmetry causing mutants of HIV gp41 subunit (5KA5, 5KA6) and recently GRK5-Calmodulin complex (pdb -pending). The series of successful structural biology-oriented research work allowed me to build a strong foundation in x-ray crystallography and biophysical interaction techniques. This allows me to play an active collaborating role in several research projects of high clinical relevance leveraging on my structural biology expertise.

From 2010 to 2019, I was a Scientific Manager for the X-ray crystallography and molecular interactions shared resource facility, at Thomas Jefferson University. This facility is one of the 6 NCI-supported research facilities at the Sidney Kimmel Cancer Center (SKCC), within Thomas Jefferson University. As a facility manager, I have overseen and trained internal and external users in x-ray crystallographic data collection and structure determination, and in-solution biophysical techniques such as small angle x-ray scattering (SAXS), Circular Dichroism (CD), Analytical Ultracentrifugation (AUC), Surface Plasmon Resonance (SPR), Nano-ITC, and VP-ITC. In summary, I am well qualified to carry out structural biology work specifically the CryoEM studies described in this application and I have the required expertise, leadership and motivation to do so.

B. Positions and Honors

Positions and Employment

2002-2005 Research Scientist, Max-Delbrueck-Centre for Molecular Medicine, Berlin, Germany
2006- 2009 Post-doctoral fellow, SUNY Upstate Medical University, Syracuse, NY
2010- 2019 Facility Manager, SKCC X-ray Crystallography and Molecular Interactions shared resource, Thomas Jefferson University, Philadelphia, PA
2011- 2014 Research Instructor, Department of Biochemistry and Molecular Biology, Thomas Jefferson University, Philadelphia, PA
2015 - Research Assistant Professor, Department of Biochemistry and Molecular Biology, Thomas Jefferson University, Philadelphia, PA

Other Experience and Professional Memberships

2000 - 2001 Research trainee, National Institute for Immunology, New Delhi, India
2001 - 2002 Research Assistant, International Centre for Genetic Engineering and Biotechnology, New Delhi, India
2007- Member, American Association for the Advancement of Science

Honors

1993 Governors scout award, Governor of the state Mr. Qureshi
1994 Presidents scout award, President of the India Dr. Sharma
1996 - 1999 University scholarship from Barkatullah University (earned annually)
1999 Scholarship award for securing top position in B.S.
1999 Scholarship award for securing top position in M.S first semester.
2000 - 2001 University research scholarship award to conduct research training at National Institute of Immunology, New Delhi, India

C. Contributions to Science

1. My early research work on DNA recombination proteins and bacteriophage infectious tail machinery during graduate studies and postdoctoral training heavily utilized biochemical and biophysical techniques. This work involved molecular biology tools together with expression and purification of over dozen isolated proteins and detailed structural characterization to get a better understanding of their physiological roles. Also, importantly shed light on cross-play between various interacting partners supporting mechanisms of action. The fundamental work has a huge relevance to basic biology and to the field.

a. Bhardwaj A, Welfle K, Misselwitz R, Ayora S, Alonso JC, Welfle H. (2006) Conformation and stability of the *Streptococcus pyogenes* pSM19035-encoded site-specific beta recombinase, and identification of a folding intermediate. *Biol Chem.* 2006 May;387(5):525-33. PMID: 16740123
b. Bhardwaj A, Olia AS, Walker-Kopp N, Cingolani G. (2007) Domain organization and polarity of tail needle GP26 in the portal vertex structure of bacteriophage P22. *J Mol Biol.* 2007 Aug 10;371(2):374-87. PMID: 17574574
c. Olia AS, Bhardwaj A, Joss L, Casjens S, Cingolani G. (2007) Role of gene 10 protein in the hierarchical assembly of the bacteriophage P22 portal vertex structure. *Biochemistry*. 2007 Jul 31;46(30):8776-84. PMID: 17620013
d. Bhardwaj A, Walker-Kopp N, Casjens SR, Cingolani G. (2009) An evolutionarily conserved family of virion tail needles related to bacteriophage P22 gp26: correlation between structural stability and length of the alpha-helical trimeric coiled coil. *J Mol Biol.* 2009 Aug 7;391(1):227-45. PMCID: PMC2713385

2. A significant portion of my postdoctoral training in Dr. Cingolani laboratory was dedicated to studying viral genome ejection and packaging mechanisms using phage P22 as a model system employing a hybrid approach of combining structural biology, cell biology, and biochemical-physical techniques. In collaboration with Dr. Sherwood Casjens, we studied various viral tail apparatus components that are involved in genome ejection and host cell penetration. One of the major achievements of this work was determining the first atomic resolution crystal structures of gp26 protein homologs that act as a plug to trap newly packaged genomes in the Podoviridae

virions. Furthermore, I also contributed to structural and biophysical studies of the research work focusing on studying viral genome packaging mechanisms. I resolved the first crystal structure of phage P22 small terminase unit that is part of multi-component viral genome packaging motor. My efforts led to various co-authored publications describing results of the research work.

a. Bhardwaj A, Walker-Kopp N, Wilkens S, Cingolani G. (2008) Foldon-guided self-assembly of ultra-stable protein fibers.*Protein Sci.* 2008 Sep;17(9):1475-85. PMCID: PMC2525528
b. Bhardwaj A, Molineux IJ, Casjens SR, Cingolani G. (2011) Atomic structure of bacteriophage Sf6 tail needle knob. *J Biol Chem.* 2011 Sep 2;286(35):30867-77. PMCID: PMC3162447, RCSB PDB code: 3RWN
c. Roy A, Bhardwaj A, Cingolani G. (2011) Crystallization of the nonameric small terminase subunit of bacteriophage P22.*Acta Crystallogr Sect F Struct Biol Cryst Commun.* 2011 Jan 1;67(Pt 1):104-10. PMCID: PMC3079985
d. Roy A, Bhardwaj A, Datta P, Lander GC, Cingolani G. (2012) Small terminase couples viral DNA binding to genome-packaging ATPase activity.*Structure*. 2012 Aug 8;20(8):1403-13. PMCID: PMC3563279, RCSB PDB code: 3P9A
e. Leavitt JC, Gogokhia L, Gilcrease EB, Bhardwaj A, Cingolani G, Casjens SR. (2013) The tip of the tail needle affects the rate of DNA delivery by bacteriophage P22. *PLoS One.* 2013 Aug 12;8(8):e70936. PMCID: PMC3741392, RCSB PDB code: 4K6B
f. Bhardwaj A, Casjens SR, Cingolani G. (2014) Exploring the atomic structure and conformational flexibility of a 320Å long engineered viral fiber using X-ray crystallography. *Acta Crystallogr D Biol Crystallogr*. 2014 Feb;70(Pt2):342-353. PMCID: PMC3940195, PDB code: 4LIN
g. Bhardwaj A, Olia AS, Cingolani G. (2014) Architecture of viral genome delivery molecular machines. *Curr Opin Struct Biol*. 2014 Apr;25:1-8. PMCID: PMC4040186
h. Bhardwaj A, Sankhala RS, Olia, AS, Brooke D, Casjens SR, Taylor DJ, Prevelige PE Jr, Cingolani G. (2016) Structural plasticity of the protein plug that traps newly packaged genomes in Podoviridae virions. *J Biol Chem.* 2016 Jan 1;291(1):215-26. PMCID: PMC4697157, RCSB PDB codes: 5BVZ, 5BU5, 5BU8, 4ZKP, 4ZKU, 4ZXQ

3. Structural biology of nuclear import machinery and protein trafficking pathways. I contributed my structural biology expertise to study underlying mechanisms of macromolecular nucleocytoplasmic transport. We did a comprehensive analysis of importin beta binding affinity for FG-rich nucleoporins lining the Nuclear Pore Complex. This work involved extensive use of x-ray crystallography and biophysical tools such as Surface Plasmon Resonance and Isothermal titration Calorimeter to accurately measure thermodynamic and kinetic binding parameters of the interaction. More recently, I have also contributed my expertise in studies focusing on defining guiding principles and mechanisms of membrane protein nuclear transport.

a. Bhardwaj A, Cingolani G. (2010) Conformational selection in the recognition of the snurportin importin beta binding domain by importin beta. *Biochemistry*. 2010 Jun 22;49(24):5042-7. PMID: 20476751, RCSB PDB code: 3LWW
b. Lott K, Bhardwaj A, Mitrousis G, Pante N, Cingolani G. (2010) The importin beta binding domain modulates the avidity of importin beta for the nuclear pore complex. *J Biol Chem.* 2010 Apr 30;285(18):13769-80.PMCID: PMC2859540
c. Lott K, Bhardwaj A, Sims PJ, Cingolani G. (2011) A minimal nuclear localization signal (NLS) in human phospholipid scramblase 4 that binds only the minor NLS-binding site of importin alpha1. *J Biol Chem.* 2011 Aug 12;286(32):28160-9. PMCID: PMC3151061, RCSB PDB code: 3Q5U
d. Lokareddy RK, Hapsari RA, van Rheenen M, Pumroy RA, Bhardwaj A, Steen A, Veenhof LM, Cingolani G. (2015) Distinctive properties of the nuclear localization signals of an inner nuclear membrane proteins Heh1 and Heh2. *Structure*. 2015 Jul 7;23(7):1305-16. PMCID: PMC4768490, RCSB PDB codes: 4PVZ, 4XZR

4. Therapeutic protein design and engineering. It's well known that protein fold dictates function. As a trained structural biologist at Jefferson, I have been actively involved in efforts towards the development of engineered proteins that can be utilized therapeutically. One of such collaborative effort is directed toward the development of engineered antibody-based blocker of localized fibrosis with collagen telopeptide. This research work is led by Dr. Andrzej Fertala and of highly collaborative nature that utilizes specialties of many basic sciences researchers and clinicians at Jefferson. I secured independent funding as well to pursue goals of this project.

a. Fertala J, Steplewski A, Kostas J, Beredjiklian P, Williams G, Arnold W, Abboud J, Bhardwaj A, Hou C, Fertala A. (2013) Engineering and characterization of the chimeric antibody that targets the C-terminal telopeptide of the α2 chain of human collagen I: A next step in the quest to reduce localized fibrosis. *Connect Tissue Res.* 2013;54(3):187-96. PMCID: PMC3896972

5. In addition to the recent contributions described above I have been actively involved in G protein-coupled receptor kinase 5 (GRK5) structure-function studies led by Prof. Jeffrey L. Benovic at Jefferson. I have determined an atomic resolution crystal structure of GRK5.AMP-PNP and GRK5.sangivamycin complexes, more recently I have also resolved 2 angstrom structure for GRK5-Calmodulin complex (unpublished work). This work further attests that I have necessary structural biology expertise and training to contribute meaningfully to the goals of the proposed application. I look forward to contributing and expanding these studies to next level.

a. Komolov KE, Bhardwaj A*, Benovic JL*. (2015) Atomic structure of GRK5 reveals distinct structural features novel for G protein-coupled receptor kinases. *J Biol Chem.*Aug 21;290(34):20629-47. PMCID: PMC4543624,RCSB PDB codes: 4TNB, 4TND

*co-corresponding authors

6. Publications from other collaborative efforts –

a. Thangavel C, Boopathi E, Liu Y, Haber A, Ertel A, **Bhardwaj A**, Addya S, Williams N, Ciment SJ, Cotzia P, Dean JL, Snook A, McNair C, Price M, Hernandez JR, Zhao SG, Birbe R, McCarthy JB, Turley EA, Pienta KJ, Feng FY, Dicker AP, Knudsen KE, Den RB.RB loss promotes prostate cancer metastasis. (2017) *Cancer Research*. 77(4):982-995.
b. Khasnis MD, Halkidis K, **Bhardwaj A**, Root MJ. (2016) Receptor activation of HIV-1 Env leads to asymmetric exposure of the gp41 trimer. (2016)*PLoS Pathogens.* 12(12):e1006098
RCSB PDB codes: 5KA5, 5KA6
c. Thangavel C, Boopathi E, Liu Y, McNair C, Haber A, Perepelyuk M, **Bhardwaj A**, Addya S, Ertel A, Shoyele S, Birbe R, Salvino JM, Dicker AP, Knudsen KE, Den RB. Therapeutic Challenge with a CDK 4/6 Inhibitor Induces an RB-Dependent SMAC-Mediated Apoptotic Response in Non-Small Cell Lung Cancer. (2018) Clinical Cancer Research. 24(6):1402-1414.

Complete List of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/myncbi/browse/collection/50041260/?sort=date&direction=descending

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