## BIOGRAPHICAL SKETCH

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NAME: Benovic, Jeffrey L.

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

POSITION TITLE: Thomas Eakins Endowed 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 is focused on understanding the molecular and cellular basis of G protein-coupled receptor (GPCR) signaling. Our primary focus is on the biochemistry and cell biology of GPCR kinases (GRKs) and arrestins and understanding how dysregulation of GPCRs contributes to the development of disease. We have significant experience studying arrestin-mediated regulation of GPCRs, such as the $\beta_2$-adrenergic receptor ($\beta_2$AR) and the chemokine receptor CXCR4, and how CXCR4 contributes to the development of cancer. This work has involved extensive characterization of the mechanisms involved in receptor phosphorylation and arrestin binding, the structural basis for GRK and arrestin interaction with GPCRs, and how these processes regulate signaling and trafficking. We have also developed strategies to bias GPCR signaling with our initial efforts focused on the use of palmitoylated peptides and small molecules to mediate biased signaling. We utilize a variety of strategies in our work including biophysical approaches, biochemical and genetic analysis, molecular and cellular biology, and animal models.

On the administrative side, I have served as director of the Molecular Pharmacology and Structural Biology PhD program at Jefferson from 1998-2008, on the Jefferson College of Graduate Studies Advisory Committee for Postdoctoral Affairs from 2004-2008, on the MD/PhD program steering committee from 1992-2014, and as Associate Director of Education at the Sidney Kimmel Cancer Center since 2015. I have also served as PI of NIH training grants for medical students (T35 HL07845), pre- and postdoctoral trainees (T32 DK07705), and pre-doctoral trainees in our Biochemistry and Molecular Pharmacology PhD program (T32 GM100836). I have received awards from the Jefferson College of Graduate Studies (2008) and the Jefferson Postdoctoral Association (2009) for service and mentoring to pre- and postdoctoral fellows as well as the Yun and Sophie Yen Faculty Award for Distinguished Training in Translational Research from the Jefferson College of Biomedical Sciences in 2016. I also received the 2014 Julius Axelrod Award in Pharmacology from the American Society for Pharmacology and Experimental Therapeutics for research and mentoring and was chosen as the keynote speaker for the Molecular Pharmacology Division Postdoctoral Award presentations at the 2015 Experimental Biology meeting. Since starting my independent research program, I have trained 27 postdoctoral fellows (11 are currently faculty, 14 are in industry, 1 is a physician and 1 is in nursing) and 17 pre-doctoral fellows (6 are currently faculty, 5 are in industry, 1 is a physician, 1 is at NIH, 1 is a patent attorney, 1 is teaching high school, and 2 remain in training). I currently have 4 pre-doctoral fellows, 2 postdoctoral fellows, 2 Research Associates and a Research Assistant Professor training in my lab.

### B. Positions and Honors

**Positions:**

| | |
|---|---|
| 7/89-6/91 | Assistant Professor, The Fels Institute for Cancer Research, Temple University School of Medicine, Philadelphia, PA |
| 7/91-6/95 | Associate Professor, Department of Pharmacology, Thomas Jefferson University, Philadelphia, PA |
| 7/95-6/05 | Professor, Department of Biochemistry and Molecular Pharmacology, Thomas Jefferson University, Philadelphia, PA |
| 1/97-6/05 | Professor and Vice Chair, Department of Microbiology and Immunology, Thomas Jefferson University, Philadelphia, PA |
| 1/93-8/13 | Leader, Kimmel Cancer Center Program in Cancer Cell Biology and Signaling |
| 1/98-6/08 | Director, Molecular Pharmacology and Structural Biology Graduate Program |
| 1/05-12/05 | Interim Deputy Director, Kimmel Cancer Center |
| 7/05-1/17 | Professor and Chair, Department of Biochemistry and Molecular Biology, Thomas Jefferson University, Philadelphia, PA |
| 7/13-Present | Thomas Eakins Endowed Professor, Department of Biochemistry and Molecular Biology, Thomas Jefferson University |
| 9/15-Present | Associate Director of Education, Sidney Kimmel Cancer Center |

**Honors:**

NIH Training grant award (1981-1984); Howard Hughes Medical Institute Research Fellow (1987-1989); Winter Conference on Brain Research Fellowship (1991); American Heart Association Established Investigator (1994-1999); Seven Transmembrane Domain Receptor Club of Quebec, Keynote Speaker (1999); ISI Highly Cited Researcher in Biology and Biochemistry; IBRO School of Neuroscience, Plenary Speaker (2004); S. G. Ferguson Memorial Seminar (2005); NIH MERIT Award (2006); Jefferson College of Graduate Studies Alumni Board Lifetime Membership Award (2008); Jefferson Postdoctoral Association Distinguished Mentor Award (2009); Thomas Eakins Endowed Professorship (2013); National Academy of Inventors member (2013); ASPET Julius Axelrod Award (2014); ASPET Molecular Pharmacology Division Postdoctoral Awards, Keynote Speaker (2015); The Yun and Sophie Yen Faculty Award for Distinguished Training in Translational Research from the Jefferson College of Biomedical Sciences (2016); Temple Translational Science Symposium, Keynote Speaker (2017); Case Western Reserve Department of Pharmacology retreat, Keynote Speaker (2017); Argentine Biomedical Societies meeting, Plenary Speaker (2017); Sidney Kimmel Cancer Center Achievement in Basic Research Award (2018), Jeffrey L. Benovic Award and Lectureship (2018), Sidney Kimmel Cancer Center Impact Award (2019)

**Service:**

Southeastern Pennsylvania American Heart Association Review Committee Member (1991-1996), NIH Pharmacology Study Section Member (1996-2000), G Protein Signaling Workshop (Organizing Committee, 1998-, Organizer, 2006, 2010, 2014), Gordon Conference on Second Messengers and Protein Phosphorylation (Co-Vice Chair, 2004, Co-Chair, 2005), NIH Subcommittee A - Cancer Centers (2007, 2009, 2011, 2015), NIH MIST Study Section (2007, 2008, 2011, 2019-2023), NIH Cell Biology SEP ZRG1 CB-N (2008), NIH MNPS Study Section (2008, 2009, 2013, 2016, 2017), NIH Pathway to Independence Awards ZGM1 BRT-9 (2008-2010, 2012), NIH EUREKA Awards SEP ZGM1 GDB-7 (2010), NIH College of CSR Reviewers (2010-2012), NIH Training and Workforce Development (TWD-A) (2014-2018), ASBMB Publications Committee (2011-2015, Chair 2013-2014), ASBMB Finance Committee (2013-2014), ASBMB Council (2013-2014), FASEB Conference on GRKs and Arrestins (Co-Organizer, 2017)

**Editorial Boards:**

Journal of Receptors and Signal Transduction (1991-2012), Journal of Biological Chemistry (1995-2000), Molecular Pharmacology (1998-present), Molecular Endocrinology (2002-2003), Journal of Cell Biology (2004-2016), Cell (2009-present), Associate Editor, Biochemistry (2001-2015), Faculty of 1000, Neuronal Signaling Mechanisms Section (2009-2011)

**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 $\beta_2$-adrenergic receptor ($\beta_2$AR). This led to the discovery of the $\beta$-adrenergic receptor kinase (now called GRK2), an enzyme that phosphorylates the agonist-occupied form of the $\beta_2$AR. The initial manuscript on this work was published in 1986 and while rhodopsin kinase (GRK1) had been previously identified, these studies provided the first evidence that there was a family

of such kinases and that this family would likely play a broad role in regulating GPCRs. Additional key discoveries of our work included cloning the cDNA for GRK2 (published in 1989), identifying additional members of the GRK family (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 $\alpha$-helix, finding that GRKs are phospholipid-dependent enzymes and contain an RGS homology domain that mediates interaction with G$\alpha$ subunits, demonstrating that GRKs also phosphorylate and/or interact with a wide variety of additional proteins, linking GRKs with various biological processes including cell cycle regulation, and using *C. elegans* as a model system to understand GRK function. Our recent efforts have focused on the use of biophysical approaches such as X-ray crystallography, electron microscopy, and hydrogen deuterium exchange mass spectrometry to understand the structures of GRKs alone and in complex with other proteins such as GPCRs and calmodulin.

a. So, C. H., Michal, A., Komolov, K., Luo, J., and **Benovic, J. L**. G protein-coupled receptor kinase 2 (GRK2) is localized to centrosomes and mediates epidermal growth factor-promoted centrosomal separation. *Mol. Biol. Cell* 24: 2795-2806, 2013. PMCID: PMC3771943.

*b.* 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.

c. 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.

d. 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 $\beta_2$-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.

**2. Role of arrestins in G protein-coupled receptor regulation.** My early research on arrestins was also initiated while I was a trainee in the Lefkowitz lab and provided the first evidence linking arrestins with the regulation of hormonal signaling in 1987 and the first cloning of a non-visual arrestin ($\beta$-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 $\beta$-arrestins bind to clathrin and serve an essential role in $\beta_2$AR endocytosis and included mapping the binding interface between these proteins and ultimately co-crystallizing a complex of $\beta$-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 $\beta$-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**. $\beta$-arrestin acts as a clathrin adaptor in endocytosis of the $\beta_2$-adrenergic receptor. *Nature* 383: 447-450, 1996.

b. 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.

c. 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

d. Tian, X., Irannejad, R., Bowman, S. L., Du, Y., Puthenveedu, M. A., von Zastrow, M., and **Benovic, J. L**. The $\alpha$-arrestin ARRDC3 regulates the endosomal residence time and intracellular signalling of the $\beta_2$-adrenergic receptor. *J. Biol. Chem.* 291: 14510-14525, 2016. PMCID: PMC4938174.

**3. Biased signaling.** While the concept of biased or functionally selective signaling has only recently been appreciated in the GPCR field, we had initially tested this concept for the $\beta_2$AR many years ago. This involved comparing the ability of various full and partial agonists to stimulate cAMP production 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 $\beta_2$AR. For CXCR4, we worked with the Bouvier laboratory and found that a specific CXCR4 lipidated peptide (called a pepducin) could function in a biased manner to selectively promote CXCR4 coupling to $G_i$. For the $\beta_2$AR, we screened a library of pepducins and found several that had a striking

bias towards promoting $\beta_2$AR interaction with either $G_s$ or β-arrestin. These are currently being pursued in the context of airway disease (for $G_s$ bias) and heart failure (for β-arrestin bias). 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 $\beta_2$AR. These molecules provide an opportunity to yield insight on the structures and dynamics that control selective protein interactions with GPCRs.

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 Gs-biased allosteric agonists. *J. Biol. Chem.* **289**: 35668-35684, 2014. PMCID: PMC4276837. Selected as Paper of the Week; Highlighted by a report on National Public Radio.

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

c. 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 $\beta_2$-adrenergic receptor promotes cardiomyocyte contraction. *Proc. Natl. Acad. Sci. U.S.A*. **113**: E4107-4116, 2016. PMCID: PMC4948363.

d. 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 $\beta_2$-adrenergic receptor-specific signaling. *Theranostics* **8**: 4664-4678, 2018. PMCID: PMC6160776.

**4. Regulation of CXCR4 function.** While much of our work has focused on using the $\beta_2$AR as a model, 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 in the C-terminal tail of the receptor 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. Our current efforts are focused on identification of biased ligands to regulate CXCR4 function in cancer.

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. PMCID: PMC5438129.

**Summary of Published Work** (>285 total publications; *h*-index=110; >37,000 citations)

https://www.ncbi.nlm.nih.gov/sites/myncbi/jeffrey.benovic.1/bibliography/40763704/public/?sort=date&direction=ascending

**D. Research Support**

**Ongoing Research Support**

1 R35 GM122541-02 (Benovic) 08/01/17 - 07/31/22
NIH
Regulation of G protein-coupled receptor signaling and trafficking
Main Goals: Characterize the role of GRKs and arrestins in regulating the function of G protein-coupled receptors.

1 R01 HL142310-01 (Benovic) 04/01/18 - 03/31/22
NIH
Structural and dynamic analysis of GRK interaction with G protein-coupled receptors
Main Goals: Characterize the structure and dynamics of GRK interaction with G protein-coupled receptors using GRK5 and the $\beta_2$-adrenergic receptor as a model.

1 R01 HL136219-02 (Benovic/Tilley, MPI) 01/09/17 - 12/31/20
NIH
Characterization of $\beta$-arrestin biased $\beta_2$-adrenergic receptor signaling in cardiovascular function
Main Goals: 1) Dissect the mechanism of $\beta$-arrestin-biased $\beta_2$AR signaling; 2) perform high throughput screening to identify compounds that promote $\beta$-arrestin-biased $\beta_2$AR signaling; 3) determine the mechanism of $\beta$-arrestin-biased $\beta_2$AR-mediated cardiomyocyte contractility *in vivo*; and 4) evaluate the effects of $\beta$-arrestin-biased $\beta_2$AR signaling on cardiomyocyte survival *in vitro* and in response to acute cardiac injury *in vivo*.

5 P01 HL114471-05 (Panettieri) 07/15/13 - 06/30/19
Project 3 and Discovery Core Leader (Benovic)
NIH
Selective targeting of GPCR signaling in airway disease
Main Goals of project 3: 1) Develop $G_s$-biased pepducins for the $\beta_2$AR; 2) define the GRKs and $\beta$-arrestins that regulate $\beta_2$AR function in human airway smooth muscle cells; and 3) develop strategies to broadly inhibit $G\alpha_q$- and $G\beta\gamma$-signaling pathways that play an important role in mediating airway smooth muscle contraction.
Main Goals of Discovery Core: Supports shRNA, small molecule and virtual screening efforts on the $\beta_2$AR, OGR1 and TAS2R signaling pathways.

Commonwealth of Pennsylvania (Taraschi) 01/01/16 - 12/31/19
Characterization of chemokine receptor function in cancer (Benovic)
Main Goals: Identify compounds to modulate chemokine receptor function in cancer.

Falk Medical Research Trust (Aplin) 11/30/17 - 11/29/19
Co-Investigator (Benovic)
Falk Medical Research Trust Transformational Award
Targeted Inhibitor Strategies in Uveal Melanoma
Main Goals: Develop and characterize inhibitors to block mutant $G\alpha_{q/11}$ effects in uveal melanoma.

OPP1195023 (Margolskee) 07/01/18 - 06/30/20
Co-Investigator (Benovic/Scott)
Bill & Melinda Gates Foundation
Development of a universal bitter taste blocker
Main Goals: Develop peptides that antagonize interaction of taste receptors with gustducin.

5 P30 CA056036-18 (Knudsen) 06/22/95 - 05/31/23
NIH
Translational research in cancer
Institutional Cancer Center Core Grant
Associate Director of Education (Benovic)

**Completed Research Support**

5 R37 GM047417-22 (Benovic) 01/01/94 - 02/28/18
NIH
Role of arrestins in G protein-coupled receptor regulation
Main Goals: To use cell biology and X-ray crystallography to define the role of arrestins and arrestin domain containing proteins in regulating G protein-coupled receptor signaling and trafficking.

4 R01 GM044944-25 (Benovic) 07/01/91 - 04/30/18
NIH
G protein-coupled receptor kinases
Main Goals: To define the role of GRKs in regulating G protein-coupled receptor signaling using *C. elegans* as a model system.

## 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<br>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 b2-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.

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

1. Undergraduate Work: Yonsei University in Seoul, Korea.

| Year | Major-Related Courses | Grade | Year | Non Major-Related Science Courses | Grade |
|---|---|---|---|---|---|
| 1998 | Organic Chemistry 1 | B | 1996 | Calculus 1 | A |
| 2001 | General Chemistry & Experiments 1 | A | 1996 | General Physics & Laboratory 2 | B |
| 2001 | Analytical Chemistry 1 | A | 1997 | Modern Physics 1 | B |
| 2001 | Inorganic Chemistry 1 | A | 1998 | Linear Algebra 1 | A |
| 2001 | Physical Chemistry 1 | A | 2001 | Advanced Calculus 1 | A |
| 2001 | General Biology and Laboratory 1 | A | 2001 | Advanced Calculus 2 | A |
| 2001 | General Biology and Laboratory 2 | A | 2001 | Computer 1 | A |
| 2001 | Physical Chemistry 2 | A | 2001 | Linear Algebra 2 | B |
| 2001 | Chemistry Experiment 1 | B | 2002 | Real Analysis 1 | A |
| 2001 | Organic Chemistry 2 | A | 2002 | Modern Algebra1 | A |
| 2001 | Analytical Chemistry | A | 2002 | Differential Geometry 1 | A |
| 2002 | Advanced Chemistry & Experiment 1 | A | 2002 | Calculus 2 | A |
| 2002 | Chemistry Experiment 2 | A | 2002 | Computer 2 | A |
| 2002 | General Chemistry & Experiment 2 | A | 2002 | Modern Physics 2 | A |
| 2002 | Chemistry Experiment 3 | A | 2002 | Statistics | A |
| 2002 | Inorganic Chemistry 2 | A | 2003 | General Physics and Laboratory 1 | A |
| 2002 | Advanced Chemistry & Experiment 2 | A | | | |
| 2003 | Biochemistry 1 | A | | | |
| 2003 | Biochemistry Laboratory 2 | B | | | |
| 2003 | Enzymology | A | | | |
| 2003 | Biophysical Chemistry | B | | | |
| 2003 | Biochemistry Laboratory 1 | A | | | |
| 2003 | Biochemistry of Nucleic Acid | A | | | |
| 2003 | Biochemistry 2 | A | | | |
| 2003 | Molecular Physiology | A | | | |

## 2. Graduate Work: Cornell University in Ithaca, NY.

| Year | Courses | Grade |
|---|---|---|
| 2004 | Advanced Inorganic Chemistry 1 | A |
| 2004 | Advanced Organic Chemistry | B+ |
| 2004 | Chemical Aspects of Biological Process | B+ |
| 2004 | Advanced Chemistry & Experiment 2 | A |
| 2004 | Cellular & Molecular Pharmacology | V |
| 2004 | Survey of Cell Biology | A- |
| 2004 | Functional Origin of Eukaryotic Cells | B+ |
| 2005 | Molecular Basis of Human Disease | S |
| 2006 | Current Topics in Biochemistry | SX |
| 2007 | Ethical Issues & Professional Responsiblity | SX |
| 2008 | Chemistry of Signal Transduction | A+ |

## 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**