Shubhi Pandey

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Indian Institute of Technology Kanpur
Kanpur - 208016
Uttar Pradesh

About Me

Graduate Student

A passionate researcher currently studying the various aspects of G protein coupled receptors (GPCRs). I have been actively involved in studies aimed at understanding the structure and functions of GPCRs, using various biophysical and biochemical approaches. In the upcoming years, I aim to expand my research horizon in the current field, which will focus on exploring the intricacies of GPCR signaling pathways involved in the manifestation of human diseases and disorders.

Education

Doctor of Philosophy, Biochemistry, and Structural Biology — **July 2016-Present**
Indian Institute of Technology, Kanpur
Uttar Pradesh.
CPI: 7.7/10

Masters of Science (M.Sc), Biochemistry — **Completed, June 2015**
School of Life Sciences, University of Hyderabad, Hyderabad
Telangana.
CPI: 7.94/10

Bachelor of Science, Zoology and Chemistry — **Completed, July 2013**
University of Lucknow, Lucknow
Uttar Pradesh.
Marks: 65.1%

Work Experience

July 2016 - Current
Graduate student
Indian Institute of Technology Kanpur
Kanpur, Uttar Pradesh

Thesis advisor: Dr. Arun K. Shukla

Ph.D. Thesis: *"Novel insights into the signaling and functions of complement C5a receptors"*

November 2014-April 2015
M.Sc Dissertation
University of Hyderabad
Hyderabad, Telangana

Thesis advisor: Dr. Naresh Babu Sepuri

M.Sc Thesis: *"Identification of substrates for Methionine sulfoxide reductases"*

Awards and Achievements

All India Rank 31 in Lectureship-CSIR NET (Council of Scientific and Industrial Research- National Eligibility Test), 2016 (Life Sciences)

All India Rank 410 in GATE (Graduate Aptitude Test in Engineering), 2013 (Life Sciences)

Awarded Certificate of Merit by CBSE (Central Board of Secondary Education) for being among top 0.1% of successful candidates in AISSE (All India Secondary School Examination), 2007

Conference Proceedings

December 21-23, 2018, Oral Presentation entitled "Partial ligand-receptor interaction yields signaling and trafficking bias at the human complement receptor, C5aR1" at XLII AISCB (All India Society of Cell Biology) Conference held in BITS Pilani, Goa Campus.

Skills

Mammalian cell culture: Maintenance of adherent *HEK293* cell lines and suspension THP1 cell lines, development of receptor expressing stable cell lines, generation of lentivirus-based knockdown cell lines, development of receptor-arrestin knockdown dual stable cell lines, Chemical based transfection in adherent *HEK293* cell lines.

Insect cell culture: Maintenance of suspension *sf9* cells, Baculovirus based transfection in *sf9* cells.

Protein expression: *E.coli* based BL21, shuffle and M55244 strains, insect-based sf9 cells.

Protein purification: GST-tag, FLAG-tag, His-tag, Strep-tag, and Protein-L based affinity purification.

Chromatography: Affinity chromatography on manually packed resins and FPLC based ion exchange and gel filtration chromatography.

Molecular biology: Cloning, Regular PCR

Protein chemistry: Protein cross-linking using chemical crosslinkers, site-specific labeling.

Biochemical assays: Co-immunoprecipitation, ELISA, Enzyme complementation based reporter assays.

General: UV-Vis spectroscopy, Agarose gel electrophoresis, SDS-PAGE, Western Blotting.

Computer proficiency and software: MS Windows, Prism GraphPad, ImageJ, Image Lab, Biorender, Adobe Illustrator, PyMOL, Microsoft office.

Career Highlights

Established Complement C5a receptor system in lab for understanding its signaling and structure.

Successfully designed strategy for making stable lines expressing the receptor(C5aR1) on βarrestin knockdown background.

Successfully optimized the purification of complement C5a receptors in the lab from insect-based cell line.

Research Projects

Project 1: Identification and functional characterization of a synthetic peptide ligand for human C5aR1 for understanding signaling and trafficking bias.
Duration: December 2017-June 2019
Project summary

- Identified a high affinity synthetic peptide as an agonist for C5aR1.
- Performed luminescence based cAMP assays and coimmunoprecipitation to assess G protein and βarrestin coupling in response to peptide agonist.
- Measured C5aR1 mediated signaling and cellular responses induced by the synthetic peptide.
- Successfully demonstrated the biased response of the synthetic peptide in G protein vs. βarr coupling, ERK activation vs. endocytosis, and cytokine release vs. chemotaxis.

Project 2: Exploring the intrinsic bias at non-canonical, βarrestin coupled seven transmembrane receptors.
Duration: December 2017- July 2021
Project summary

- This study focussed on comprehensive characterization of canonical and non-canonical seven transmembrane receptors(7TMRs) in terms of transducer coupling, GRK engagement, and downstream signaling.
- Assessed G protein coupling and activation using second messenger and enzyme complementation assays.
- Established conformational divergence of βarrs for distinct non-canonical 7TMRs using enzyme complementation and BRET based assays.
- Successfully demonstrated the downstream signaling for non-canonical 7TMRs using a phospho antibody-based array.
- This study successfully established C5aR2 and D6R as exclusively arrestin coupled receptors.

Project 3: Understanding distinct contributions of βarrestin isoforms in regulating complement C5a receptor functions.
Duration: April 2018- March 2021
Project summary

- Successfully performed knockdown of βarr1 and βarr2 on C5aR1 expressing stable cell lines.
- Assessed the role of βarr1 and 2 in various signaling and trafficking events upon C5aR1 activation, viz. ERK1/2 phosphorylation, receptor internalization, and cAMP second messenger response.
- We demonstrated that βarr1 and 2 performed distinct and overlapping functions in regulation of ERK activation and receptor internalization, respectively.

Project 4: Structural characterization of a non-canonical C5aR2 receptor-βarr1 complex.
Duration: December 2019- Present
Project summary

- Designed a chimeric C5aR2 receptor construct for expression in insect cell-based protein expression system.
- Optimized the expression and purification of chimeric C5aR2 from Sf9 cells.
- Devised different strategies for reconstitution of a complex of C5aR2-βarr1.
- Size exclusion chromatography displayed an excellent monodisperse profile for C5aR2-βarr1 complex.
- Visualization of the protein complex by single-particle negative stain electron microscopy displayed densities for receptor, βarr1, and Fab fragment.

Publications

- **Shubhi Pandey**, Punita Kumari, Mithu Baidya, Ryoji Kise, Yubo Cao, Hemlata Dwivedi-Agnihotri, Ramanuj Banerjee, Xaria X. Li, Cedric S. Cui, John D. Lee, Kouki Kawakami, Jagannath Maharana, Ashutosh Ranjan, Madhu Chaturvedi, Gagan Deep Jhingan, Stéphane A. Laporte, Trent M. Woodruff, Asuka Inoue and Arun K. Shukla*. *Intrinsic bias at non-canonical,* β-*arrestin-coupled seven transmembrane receptors.* **Molecular cell** (in press).
- **Shubhi Pandey,** Xaria X Li, Ashish Srivastava, Mithu Baidya, Punita Kumari, Hemlata Dwivedi, Madhu Chaturvedi, Eshan Ghosh, Trent M Woodruff and Arun K Shukla*. *Partial ligand-receptor engagement yields functional bias at the human complement receptor, C5aR1*. **Journal of Biological Chemistry, 2019,** 294(24), 9416-2429.
- **Shubhi Pandey,** Shirsha Saha, and Arun K Shukla*. *Transmitting the Signal: Structure of the* β*1-Adrenergic Receptor-Gs Protein Complex*. **Molecular Cell, 2020,** 80(1), 3-5.
- **Shubhi Pandey**, Jagannath Maharana, Xaria X Li, Trent M Woodruff, and Arun K Shukla*. *Emerging Insights into the Structure and Function of Complement C5a Receptors*. **Trends in Biochemical Sciences, 2020,** 45(8), 693-705.
- **Shubhi Pandey**, Jagannath Maharana and Arun K Shukla*. *The Gut Feeling: GPCRs Enlighten the Way*. **Cell Host and Microbe, 2019,** 26(2), 160-162.
- **Shubhi Pandey,** Debarati Roy and Arun K Shukla*. *Measuring surface expression and endocytosis of GPCRs using whole-cell ELISA*. **Methods in Cell Biology, 2019,** 149, 131-140**.**
- Mithu Baidya, Punita Kumari, Hemlata Dwivedi-Agnihotri, **Shubhi Pandey**, Madhu Chaturvedi, Tomasz Maciej Stepniewski, Kouki Kawakami, Yubo Cao, Stéphane A Laporte, Jana Selent, Asuka Inoue, and Arun K Shukla*. *Key phosphorylation sites in GPCR s orchestrate the contribution of* β*-Arrestin 1 in ERK 1/2 activation*. **EMBO Reports**, **2020**, 21(9), e49886.
- Hemlata Dwivedi-Agnihotri, Madhu Chaturvedi, Mithu Baidya, Tomasz Maciej Stepniewski, **Shubhi Pandey**, Jagannath Maharana, Ashish Srivastava, Natarin Caengprasath, Aylin C Hanyaloglu, Jana Selent, and Arun K Shukla*. *Distinct phosphorylation sites in a prototypical GPCR differently orchestrate* β*-arrestin interaction, trafficking, and signaling.* **Science advances, 2020,** 6(37), eabb8368.
- Yang Lee, Tony Warne, Rony Nehmé, **Shubhi Pandey**, Hemlata Dwivedi-Agnihotri, Madhu Chaturvedi, Patricia C Edwards, Javier García-Nafría, Andrew GW Leslie, Arun K Shukla, and Christopher G Tate*. *Molecular basis of* β*-arrestin coupling to formoterol-bound* β*1-adrenoceptor*. **Nature, 2020,** 583 (7818), 862-866.
- Mithu Baidya, Punita Kumari, Hemlata Dwivedi-Agnihotri, **Shubhi Pandey**, Badr Sokrat, Silvia Sposini, Madhu Chaturvedi, Ashish Srivastava, Debarati Roy, Aylin C Hanyaloglu, Michel Bouvier, and Arun K Shukla*. *Genetically encoded intrabody sensors report the interaction and trafficking of* β*-arrestin 1 upon activation of G protein–coupled receptors.* **Journal of Biological Chemistry, 2020,** 295(30), 10153-10167.

- Eshan Ghosh, Hemlata Dwivedi, Mithu Baidya, Ashish Srivastava, Punita Kumari, Tomek Stepniewski, Hee Ryung Kim, Mi-Hye Lee, Jaana van Gastel, Madhu Chaturvedi, Debarati Roy, **Shubhi Pandey**, Jagannath Maharana, Ramon Guixà-Gonzàlez, Louis M Luttrell, Ka Young Chung, Somnath Dutta, Jana Selent and Arun K Shukla*. *Conformational sensors and domain-swapping reveal structural and functional differences between β-arrestin isoforms.* **Cell Reports, 2019,** 28(13), 3287-3299.e6.
- Haris Ahsan Safdari, **Shubhi Pandey,** Arun K Shukla and Somnath Dutta. *Illuminating GPCR signaling by cryo-EM.* **Trends in Cell Biology, 2018,** 28 (8), 591-594.
- Ravi Ranjan, **Shubhi Pandey** and Arun K Shukla*. *Biased opioid receptor ligands: Gain without Pain.* **Trends in Endocrinology and Metabolism, 2017,** 28(4), 247-249.

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References

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I, Shubhi Pandey, hereby declare that the information contained herein is true and correct to the best of my knowledge and belief.

Shubhi Pandey