

**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: Dipali G. Sashital

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

POSITION TITLE: Associate 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<br>(if applicable) | Completion Date<br>MM/YYYY | FIELD OF STUDY         |
|------------------------------------|---------------------------|----------------------------|------------------------|
| University of Michigan, Ann Arbor  | B.S.                      | 5/01                       | Chemistry/Biochemistry |
| University of Wisconsin, Madison   | Ph.D.                     | 12/06                      | Biochemistry           |
| University of Wisconsin, Madison   | Postdoctoral              | 1/07-3/08                  | Biochemistry           |
| University of California, Berkeley | Postdoctoral              | 4/08-12/11                 | Biochemistry           |
| The Scripps Research Institute     | Postdoctoral              | 1/12-12/13                 | Biochemistry           |

**A. Personal Statement**

I am the PI on this proposal for Krios time at NCCAT. In 7 years since starting my lab, I have established a leading research program in the field of CRISPR-Cas biology. My lab has made several major discoveries defining the roles of poorly characterized Cas proteins and uncovering new mechanisms of well-studied systems. My lab employs a wide range of techniques, including structural biology and biochemistry, bacterial and phage genetics, high-throughput screening approaches, bulk and single-molecule FRET, and, through collaboration, *in vivo* studies in plants and animals. This versatility has allowed us to tackle a wide range of important questions related to CRISPR-Cas systems, described below. With the early success of my lab, we are now poised to answer long-standing questions in the field and to explore new avenues of investigation. I have recently spearheaded efforts to secure funding and establish a cryo-EM facility at Iowa State University. This effort included serving as search committee chair to recruit an expert in cryo-EM, Dr. Puneet Juneja, to direct our cryo-EM facility.

I have worked in the CRISPR-Cas field for 10 years and have extensive expertise as a nucleic acid biochemist and structural biologist, with formal training in a variety of structural biology techniques (NMR, X-ray crystallography and cryo-EM). My expertise in these areas has allowed me to contribute extensive professional service as a reviewer of manuscripts (~25 papers per year on average) and as an editorial board member of *The CRISPR Journal* and advisory board member of *Molecular Cell*. I have served on several grant review panels for NSF and NIH over the past few years (listed below). I have served as co-organizer for the International Conference on CRISPR Technologies, (December 2017) and the Loomis & CBC Symposium: Genome Editing: Putting Together the Pieces (May, 2018). I am committed to being a good citizen of the scientific community, both through my lab's research contributions and also through professional service.

The research done in my lab is solely the work of trainees, making research and education inextricable. I have trained nine graduate students, ten undergraduate students, one postdoctoral researcher and two high school students. My goal is to train to develop projects, formulate hypotheses, design experiments, perform technical aspects of research, interpret data, communicate in writing, in figures and in oral presentations, and critically evaluate the literature. I meet regularly with students one-on-one and in group meetings, and I have an open door (or open slack) policy so students can reach me nearly any time. I am proud to have graduated four PhD students who have gone on to successful careers as faculty members (at Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences) or in industry (at Corteva Agriscience, CJ CheilJedang and Integrated DNA Technologies). All five of the undergraduate students who have graduated from my lab have gone onto graduate school (at UCSF, Vanderbilt University, Heinrich Heine University Düsseldorf, and University of Iowa).

- a. Lee, H., Dhingra, Y. & Sashital, D.G. (2019) The Cas4-Cas1-Cas2 complex mediates precise prespacer processing during CRISPR adaptation. *eLife*. e44248. PMCID: PMC6519985
- b. Lee, H., Zhou, Y., Taylor, D.W. & Sashital, D.G. (2018) Cas4-dependent prespacer processing ensures high-fidelity programming of CRISPR arrays. *Mol Cell*. 70, 48-59. PMCID: PMC5889325
- c. Xue, C., Zhu, Y., Zhang, X., Shin, Y.K., & Sashital, D.G. (2017) Real-time observation of target search by the CRISPR surveillance complex Cascade. *Cell Rep*. 21, 3717-3727. PMCID: PMC5753800
- d. Xue, C., Whitis, N.R., & Sashital, D.G. (2016) Conformational control of Cascade interference and priming activities in CRISPR immunity. *Mol Cell*. 64, 826-834. PMCID: PMC5561731

## B. Positions and Honors

### Positions and Employment

|           |  |
|-----------|--|
| 2014-2019 | Assistant Professor, Roy J. Carver Department of Biochemistry, Biophysics & Molecular Biology, Iowa State University |
| 2019-     | Associate Professor, Roy J. Carver Department of Biochemistry, Biophysics & Molecular Biology, Iowa State University |

### Other Experience and Professional Memberships

|           |   |
|-----------|---|
| 2010-     | Member, RNA Society   |
| 2016-     | Member, American Society for Biochemistry and Molecular Biology               |
| 2017-     | Member, Biophysical Society   |
| 2017-     | Editorial Board Member, CRISPR Journal  |
| 2020-     | Advisory Board Member, Molecular Cell   |
| Mar 2017  | NSF Synthetic Biology Panel, member   |
| 2019-     | Advisory Council Member, Crop Bioengineering Center, Iowa State University    |
| 2019      | NIH SBIR/STTR Cell and Molecular Biology Study Section, member (Mar, Jul)     |
| 2019      | NIH NIGMS Pathways to Independence Award Study Section, ad hoc reviewer (Nov) |
| 2020-2021 | NIH NIGMS Pathways to Independence Award Study Section, member (Jul, Mar)     |

### Honors and Awards

|           |  |
|-----------|--|
| 2019      | Camille Dreyfus Teacher-Scholar Award, Camille and Henry Dreyfus Foundation            |
| 2019      | LAS Award for Mid-Career Achievement in Research, Iowa State University                |
| 2017-2022 | NSF CAREER Award   |
| 2012      | Scaringe Young Scientist Award, RNA Society  |
| 2008-2011 | Damon Runyon Cancer Research Foundation Postdoctoral Fellowship                        |
| 2005-2006 | Wharton Predoctoral Fellowship, University of Wisconsin, Madison                       |
| 2005      | Department of Biochemistry Graduate Mentorship Award, University of Wisconsin, Madison |
| 2004-2005 | Wisconsin Distinguished Graduate Fellowship, University of Wisconsin, Madison          |
| 2001-2004 | NIH Molecular Biosciences Training Grant, University of Wisconsin, Madison             |

## C. Contributions to Science

### **Complete List of Published Work in MyBibliography:**

<https://www.ncbi.nlm.nih.gov/myncbi/1JlxcdpVaRo5m/bibliography/public/>

#### **1. Discovery of Cas4 activity during CRISPR-Cas adaptation mechanism.**

Cas4 is an abundant and conserved CRISPR-associated protein. However, despite being one of the first *cas* genes discovered in 2002, the role of Cas4 remained mysterious for over 15 years. It has long been speculated that Cas4 participates in “adaptation”, the stage of CRISPR immunity when the Cas1-Cas2 complex immunizes a host cell through the acquisition of short fragments of DNA as “spacers” in a CRISPR array. Through NIGMS-funded work, we discovered that Cas4 is an essential component of a higher-order adaptation complex composed of Cas4, Cas1 and Cas2. Using single-particle electron microscopy (EM), we showed that within this complex, Cas4 binds in close proximity to the Cas1 integrase active site, providing clues to the function of Cas4 during spacer capture and integration. Indeed, our biochemical studies showed that Cas4 is required for efficient prespacer processing prior to Cas1-Cas2-mediated integration. Upon activation by Cas1-Cas2, Cas4 processes

single-stranded DNA through endonucleolytic cleavage. Cas4 cleaves precisely upstream of PAM sequences, short DNA motifs that are required for downstream function of the spacer sequence. Prior to cleavage, Cas4 blocks the Cas1 active site, preventing integration of unprocessed prespacers. Thus, Cas4 acts as a gatekeeper to ensure that only functional spacers will be integrated into the CRISPR array. Overall, our results reveal the critical role of Cas4 in maintaining fidelity during CRISPR adaptation, providing a structural and mechanistic model for prespacer processing and integration. **In the current proposal, we will fully define the mechanism of Cas4-mediated prespacer processing by solving high-resolution cryo-EM structures of Cas4-Cas1-Cas2 bound to a variety of substrates and products.**

- e. Lee, H., Dhingra, Y. & Sashital, D.G. (2019) The Cas4-Cas1-Cas2 complex mediates precise prespacer processing during CRISPR adaptation. *eLife*. e44248. PMCID: PMC6519985
- f. Lee, H., Zhou, Y., Taylor, D.W. & Sashital, D.G. (2018) Cas4-dependent prespacer processing ensures high-fidelity programming of CRISPR arrays. *Mol Cell*. 70, 48-59. PMCID: PMC5889325

## 2. Discoveries on the activation and tunability of CRISPR-Cas immune response.

In NIGMS funded work, my lab has made several important discoveries that reveal how CRISPR-Cas systems initiate immunity and tune their activity to respond to different threats from infectious invaders. In *Escherichia coli*, the CRISPR (cr)RNA-guided surveillance complex Cascade recognizes invader DNA through complementary base pairing, leading to nucleolytic destruction of the invader through a process called CRISPR interference. When target mutations block interference, Cascade binding instead triggers a rapid adaptation response that “primes” the bacteria for increased immunity. We have developed multiple high-throughput screening and biophysical tools for investigating the effects of target mutations on interference and priming. Early work in my lab showed that mutations in the target DNA can have variable effects on interference activity, and that some crRNA sequences are highly tolerant of target mutations. Using a FRET system developed in our lab, we went on to discover that target mutations induce a conformational change in Cascade that attenuates interference and increases priming. This conformational control enables precise regulation of each activity, allowing Cascade to direct the appropriate immune response depending on the type of target it encounters. We have also addressed an important activation step that occurs prior to interference and priming. Before initiating either process, Cascade must locate the complementary invader sequence among the vast amount of DNA in the cell. Using single-molecule FRET, we found that Cascade uses multiple protein motifs to sample DNA through two alternative modes en route to locating the target. Overall, our work on the *E. coli* CRISPR-Cas system has revealed important mechanisms for how immunity is activated and tuned following infection.

- a. Phan, P.T., Schelling, M., Xue, C., & Sashital, D.G. (2019) Fluorescence-based methods for measuring target interference by CRISPR-Cas systems. *Methods Enzymol*. 616, 61-85. PMCID: PMC6637736
- b. Xue, C., Zhu, Y., Zhang, X., Shin, Y.K., & Sashital, D.G. (2017) Real-time observation of target search by the CRISPR surveillance complex Cascade. *Cell Rep*. 21, 3717-3727. PMCID: PMC5753800
- c. Xue, C., Whitis, N.R., & Sashital, D.G. (2016) Conformational control of Cascade interference and priming activities in CRISPR immunity. *Mol Cell*. 64, 826-834. PMCID: PMC5561731
- d. Xue, C., Seetharam, A.S., Musharova, O., Brouns, S.J.J., Severinov, K., Severin, A.J., & Sashital, D.G. (2015) CRISPR interference and priming varies with individual spacer sequences. *Nuc Acids Res*. 43, 10831-47. PMCID: PMC4678831

## 3. Defining specificity of Class 2 Cas endonucleases.

Cas9 and Cas12a have become enormously popular tools for genome editing. However, Cas effectors often display low specificity. While relaxed specificity is beneficial for immunity by protecting against viral escape, it can lead to off-target effects during genome editing. Surprisingly, off-target analyses suggest that Cas12a is more specific than Cas9, making it more attractive for safely editing the human genome. These studies raise the question of how Cas12a can provide effective immunity against rapidly evolving phage. To understand this paradox, my lab has developed high-throughput methods to explore the specificity of Cas9 and Cas12a. We have compared the native specificities of Class 2 endonucleases outside of the context of eukaryotic DNA, which can mask cleavage activities of these bacterial proteins. We have found that several Cas9 and Cas12a variants can bind and nick targets with extensive mutations; however, these events do not result in double-strand breaks, reducing the number of off-target edits that occur during genome editing. Surprisingly, we also discovered that once Cas12a binds to a target, it displays unexpected nickase activity against non-specific dsDNA that may be beneficial for immune response but detrimental during genome editing. Overall, our results highlight the

importance of thorough investigations of Cas effector mechanisms prior to widespread use for human genome editing.

- a. Murugan, K., Seetharam, A.S., Severin, A.J. & D.G. Sashital. (2020) High-throughput in vitro specificity profiling of natural and high-fidelity CRISPR-Cas9 variants. *bioRxiv* doi.org/10.1101/2020.05.12.091991
- b. Murugan, K., Seetharam, A.S., Severin, A.J. & D.G. Sashital. (2020) CRISPR-Cas12a has widespread off-target and dsDNA-nicking effects. *J Biol Chem*, 295, 5538-5553. PMID: PMC7186167
- c. Banakar, R., Eggenberger, A.L., Lee, K., Wright, D.A., Murugan, K., Zarecor, S., Lawrence-Dill, C.J., Sashital, D.G. & Wang, K. (2019) High-frequency random DNA insertions upon co-delivery of CRISPR-Cas9 ribonucleoprotein and selectable marker plasmid in rice. *Sci Rep*, 9, 19902. PMID: PMC6934568
- d. Mekler, V., Kuznedelov, K., Murugan, K., Sashital, D.G., & Severinov, K. (2019) CRISPR-Cas molecular beacons as tools for studies of assembly of CRISPR-Cas effector complexes and their interactions with DNA. *Methods Enzymol*, 16, 337-363. PMID: PMC6930961

#### 4. Cas protein activity in RNA processing and DNA recognition.

As a postdoc in Jennifer Doudna's lab, my work provided foundational information on CRISPR RNA-guided immunity by *E. coli* Cascade. Using X-ray crystallography and biochemistry, I established the mechanism of crRNA biogenesis by the RNA endonuclease Cas6e (formerly Cse3). I solved structures of Cas6e in complex with substrate RNAs and discovered a conformational change in the enzyme that is required for RNA cleavage and has important implications in Cascade assembly. In addition, my work on the Cas8e (formerly Cse1) subunit demonstrated for the first time that Cas8e participates in double-stranded DNA target binding by Cascade by recognizing the PAM. This study established the model for Cascade target search through PAM recognition, which my lab recently defined more thoroughly using smFRET (Xue et al. *Cell Rep*, 2017) and will continue to study in the proposed research. Together, these studies provided some of the first high-resolution structural information on the *E. coli* Cascade complex, and several important insights into the mechanism of Cascade assembly and target recognition.

- a. Sashital, D.G., Wiedenheft, B., & Doudna, J.A. (2012) Mechanism of foreign DNA selection in a bacterial adaptive immune system. *Mol Cell*. 46, 606-15. PMID: PMC3397241
- b. Sashital, D.G., Jinek, M., & Doudna, J.A. (2011) An RNA-induced conformational change required for CRISPR RNA cleavage by the endoribonuclease Cse3. *Nat Struct Mol Biol*. 18, 680-7. PMID: 21572442

#### 5. Structural investigations of large RNA-protein complexes.

As a postdoc in Jamie Williamson's lab, I used single-particle negative-stain and cryo-EM to determine the structures of 30S ribosomal subunit assembly intermediates from crude *E. coli* lysates, allowing for the direct visualization of this complex biogenesis pathway. Combined with quantitative mass spectrometry, we determined both the conformation and composition of early 30S assembly intermediates and those that accumulated upon deletion of assembly co-factors. Overall, this study illuminated the complex pathway of in vivo ribosome assembly and established an important set of tools for dissecting the composition of heterogeneous samples. The expertise in cryo-EM that I gained through this project has enabled structural studies of CRISPR-Cas complexes in my own lab.

My Ph.D. thesis work in the lab of Samuel Butcher focused on understanding the architecture of small nuclear RNAs at the active center of the spliceosome. The active site of the spliceosome is composed of two small nuclear RNAs (snRNA), U2 and U6, which are responsible for the chemistry of splicing. During my graduate career in the Butcher lab, I used nuclear magnetic resonance (NMR) to probe the structure of the U2-U6 snRNA complex, providing important insights into the conformational dynamics of this spliceosomal active center. Together, these studies demonstrate my technical expertise in multiple structural biology techniques that will be critical to the success of my ongoing research program.

- a. Sashital, D.G.\*, Greeman, C.A.\*, Lyumkis, D., Potter, C.S., Carragher, B., & Williamson, J.R. (2014) A combined quantitative mass spectrometry and electron microscopy analysis of ribosomal 30S subunit assembly in *E. coli*. *eLife*. 3:e04491. PMID: PMC4371863 \*Equal Contribution
- b. Sashital, D.G., Venditti, V., Angers, C.G., Cornilescu, G., & Butcher, S.E. (2007) Structure and thermodynamics of a conserved U2 snRNA domain from yeast and human. *RNA*, 13, 328-38. PMID: PMC1800520

- c. Sashital, D.G., Cornilescu, G., McManus, C.J., Brow, D.A. & Butcher, S.E. (2004) U2-U6 RNA folding reveals a group II intron-like domain and a four-helix junction. *Nat Struct Mol Biol*, 12, 1237-42. PMID: 17242306
- d. Sashital, D.G., Allmann, A.M., Van Doren, S.R., & Butcher, S.E. (2003) Structural basis for a lethal mutation in U6 RNA. *Biochemistry*, 42, 1470-7. PMCID: PMC3124365

#### D. Additional Information: Research Support

## Ongoing Research Support

|   |               |                       |
|---|---------------|-----------------------|
| Camille Dreyfus Teacher-Scholar Award   | Sashital (PI) | 05/15/2019-05/14/2024 |
| The Camille Dreyfus Teacher-Scholar Award provides an unrestricted research grant and is not associated with a particular project in the lab. Foundation approval is not required for budgetary changes after an award is made. |               |                       |

NIH-NIGMS R01 GM115874-01A1 Sashital (PI) 09/02/16-8/31/21

## Defining CRISPR Adaptation and Interference Mechanisms in *E. coli*

The goal of this study is to determine the molecular mechanisms of CRISPR immune response by defining the effects of Cas protein, crRNA and target sequence on Cascade function, conformation and dynamics.

|                              |               |                   |
|------------------------------|---------------|-------------------|
| NSF-MCB CAREER Award 1652661 | Sashital (PI) | 02/01/17-01/31/22 |
|------------------------------|---------------|-------------------|

**CAREER:** Defining and improving Class 2 CRISPR-Cas endonuclease sequence specificity

The goal of the proposal is to define and improve the specificity of Class 2 CRISPR–Cas endonucleases.

|                            |                                   |                 |
|----------------------------|-----------------------------------|-----------------|
| NSF Standard Grant 1715411 | Underbakke (PI), Sashital (co-PI) | 8/15/17-8/14/22 |
|----------------------------|-----------------------------------|-----------------|

## Defining the architecture of the Pyk2 activation complex

The goal of the proposal is to determine interactions between the kinase Pyk2 and scaffolding proteins in the post-synaptic density using multiple techniques, including single-particle EM.

### Completed Research Support (past 5 years)

|   |               |                       |
|---|---------------|-----------------------|
| Iowa State Crop Bioengineering Consortium | Sashital (PI) | 07/01/2015-06/30/2016 |
|---|---------------|-----------------------|

## Enhancing Cas9 target-binding specificity using modified sgRNA

## Methods development to use chemically modified guide RNAs for RNA-guided genome editing endonucleases.

USDA-NIFA IOW05480 Wang (PI), Sashital (co-PI) 09/01/16-8/31/19

# A Data-Driven Approach to CRISPR Design for Reduced Off-Target Activity in Plant Genome Editing

The goal of this study is to determine how genome editing specificity in major crop plants is affected by high-fidelity Cas9 nucleases, and to optimize platforms for prediction of Cas9 off-target effects.

NIH-NIGMS R21 R21AI140101-01 Andreotti (PI), Sashital (co-PI) 5/10/18-4/30/20

## Multimerization of the B cell kinase BTK; a preorganized signaling unit

The goal of the proposal is to determine the structure of multimerized forms of the tyrosine kinase BTK using single-particle EM.

**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: Juneja Puneet

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

POSITION TITLE: Scientist IV, Cryo-Electron Microscopy Facility, Office of Biotechnology

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<br>(if<br>applicable) | Completion<br>Date<br>MM/YYYY | FIELD OF STUDY     |
|--|------------------------------|-------------------------------|--------------------|
| Guru Nanak Dev University, India                         | B.Sc.                        | 06/2005                       | Biotechnology      |
| Himachal Pradesh University, India                       | M.Sc.                        | 06/2007                       | Biotechnology      |
| University of Konstanz, Germany                          | Ph.D.                        | 04/2014                       | Structural Biology |
| University of Konstanz, Germany                          | Postdoctoral                 | 11/2014                       | Structural Biology |
| Max Planck Institute of Molecular Physiology,<br>Germany | Postdoctoral                 | 05/2017                       | Structural biology |
| Oak Ridge National Lab, Oak Ridge                        | Postdoctoral                 | 06/2018                       | Structural biology |

**A. Personal Statement**

I am key personnel on this proposal for Krios time at NCCAT. I am a Ph.D. scientist with 11 years' experience in Structural Biology and Biophysics using Cryo Electron Microscopy, X-ray Crystallography and Biochemistry. I have PhD and postdoctoral training in Cryo EM and X-ray Crystallography working on biochemistry and structures of protein ligand complexes membrane proteins and large assemblies. Subsequent experience in leading fast paced Core facility, providing training and consultation have made me comfortable in leading multiple projects and teams in Structural biology.

**B. Positions and Honors**

2020-                 Scientist IV/Cryo-EM facility Manager, Cryo Electron Microscopy Facility, Office of Biotechnology, Iowa state University, Ames.

2018-2020         Associate Scientist/Core Director, Robert P. Apkarian Integrated Electron Microscopy Core, Bioconnector, Emory University, Atlanta.

**C. Contributions to Science**

1. Wang F, Gnewou O, Modlin C, Beltran LC, Xu C, Su Z, **Juneja P**, Grigoryan G, Egelman EH, Conticello VP. [Structural analysis of cross  \$\alpha\$ -helical nanotubes provides insight into the designability of](#)

- [filamentous peptide nanomaterials](#). Nat Commun. 2021 Jan 18;12(1):407. doi: 10.1038/s41467-020-20689-w. PubMed PMID: 33462223; PubMed Central PMCID: PMC7814010.
2. Tillman MC, Imai N, Li Y, Khadka M, Okafor CD, **Juneja P**, Adhiyaman A, Hagen SJ, Cohen DE, Ortlund EA. [Allosteric regulation of thioesterase superfamily member 1 by lipid sensor domain binding fatty acids and lysophosphatidylcholine](#). Proc Natl Acad Sci U S A. 2020 Sep 8;117(36):22080-22089. doi: 10.1073/pnas.2003877117. Epub 2020 Aug 20. PubMed PMID: 32820071; PubMed Central PMCID: PMC7486800.
  3. Sherekar M, Han SW, Ghirlando R, Messing S, Drew M, Rabara D, Waybright T, **Juneja P**, O'Neill H, Stanley CB, Bhowmik D, Ramanathan A, Subramaniam S, Nissley DV, Gillette W, McCormick F, Esposito D. [Biochemical and structural analyses reveal that the tumor suppressor neurofibromin \(NF1\) forms a high-affinity dimer](#). J Biol Chem. 2020 Jan 24;295(4):1105-1119. doi: 10.1074/jbc.RA119.010934. Epub 2019 Dec 13. PubMed PMID: 31836666; PubMed Central PMCID: PMC6983858.
  4. Cao D, Gao Y, Roesler C, Rice S, D'Cunha P, Zhuang L, Slack J, Domke M, Antonova A, Romanelli S, Keating S, Forero G, **Juneja P**, Liang B. [Cryo-EM structure of the respiratory syncytial virus RNA polymerase](#). Nat Commun. 2020 Jan 17;11(1):368. doi: 10.1038/s41467-019-14246-3. PubMed PMID: 31953395; PubMed Central PMCID: PMC6969064.
  5. Shrestha UR, **Juneja P**, Zhang Q, Gurumoorthy V, Borreguero JM, Urban V, Cheng X, Pingali SV, Smith JC, O'Neill HM, Petridis L. [Generation of the configurational ensemble of an intrinsically disordered protein from unbiased molecular dynamics simulation](#). Proc Natl Acad Sci U S A. 2019 Oct 8;116(41):20446-20452. doi: 10.1073/pnas.1907251116. Epub 2019 Sep 23. PubMed PMID: 31548393; PubMed Central PMCID: PMC6789927.
  6. Klink BU, Zent E, **Juneja P**, Kuhlee A, Raunser S, Wittinghofer A. [A recombinant BBSome core complex and how it interacts with ciliary cargo](#). Elife. 2017 Nov 15;6. doi: 10.7554/eLife.27434. PubMed PMID: 29168691; PubMed Central PMCID: PMC5700813.
  7. Hubrich F, **Juneja P**, Müller M, Diederichs K, Welte W, Andexer JN. [Chorismatase Mechanisms Reveal Fundamentally Different Types of Reaction in a Single Conserved Protein Fold](#). J Am Chem Soc. 2015 Sep 2;137(34):11032-7. doi: 10.1021/jacs.5b05559. Epub 2015 Aug 19. PubMed PMID: 26247872.
  8. **Juneja P**, Horlacher R, Bertrand D, Krause R, Marger F, Welte W. [An internally modulated, thermostable, pH-sensitive Cys loop receptor from the hydrothermal vent worm Alvinella pompejana](#). J Biol Chem. 2014 May 23;289(21):15130-40. doi: 10.1074/jbc.M113.525576. Epub 2014 Apr 9. PubMed PMID: 24719323; PubMed Central PMCID: PMC4031562.
  9. **Juneja P**, Rao A, Cölfen H, Diederichs K, Welte W. [Crystallization and preliminary X-ray analysis of the C-type lectin domain of the spicule matrix protein SM50 from Strongylocentrotus purpuratus](#). Acta Crystallogr F Struct Biol Commun. 2014 Feb;70(Pt 2):260-2. doi: 10.1107/S2053230X14000880. Epub 2014 Jan 22. PubMed PMID: 24637770; PubMed Central PMCID: PMC3936458.
  10. **Juneja P**, Hubrich F, Diederichs K, Welte W, Andexer JN. [Mechanistic implications for the chorismatase FkbO based on the crystal structure](#). J Mol Biol. 2014 Jan 9;426(1):105-15. doi: 10.1016/j.jmb.2013.09.006. Epub 2013 Sep 13. PubMed PMID: 24036425.

#### Complete List of Published work in my Bibliography (8 Total)

<https://www.ncbi.nlm.nih.gov/myncbi/puneet.juneja.1/bibliography/public/>

#### Manuscripts in Preparations

- a. Williams, E., Jenkins, M., Zhao, H., **Juneja, P**, Lutz, S. Cryo-EM structure of engineered variant of the Encapsulin from *Thermotoga maritima* (TmE).

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

##### Conference Talks/Posters

1. Structure and putative mechanism of putative FkBO-chorismatase. (Sept-2013), 27th Rhine-Knee Regional Meeting on Biocrystallography, Schluchsee, Germany.
2. Structure insights into active site of Chorismatase. (March-2013), Department of Pharmaceutical Science, Freiburg. Germany.
3. A pH sensitive Cys loop receptor from a thermophilic worm, Immobilization of Torpedo nAChR and oligomerization behaviour. (June 2012), Annual Neurocypres Meeting. Vienna, Austria.

4. Purification and crystallization strategies for GABAA  $\beta$ 3 receptor and Torpedo nAChR. (May 2011), Annual Neurocypres Meeting. Bergamo, Italy.
5. Expression and Purification and GABAA  $\beta$ 3 receptor and Alpha 7 nAChR. (May 2010), Annual Neurocypres Meeting. Athens, Greece.

#### POSTERS

1. Presented Poster at 3rd International Workshop on Expression, Structure and Function of Membrane Proteins, (Sept 2012) Florence, Italy.
2. Presented Poster at Bilbao Advance Course on Biophysics- Expression, Purification and Crystallization of Membrane Protein, (July 2012) Bilbao, Spain.
3. Presented Poster at Gordon research Conference -Mechanism of Membrane Transport, (June 2011) Biddeford, USA.