BIOGRAPHICAL SKETCH

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NAME: Rakhi Rajan

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

POSITION TITLE: Associate Professor of Chemistry and Biochemistry

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Kerala Agricultural University (KAU), India	B.S.	06/1998	Agriculture
Tamil Nadu Agricultural University (TNAU), India	M.S.	10/2000	Biotechnology
The Ohio State University, Columbus, OH	Ph.D.	08/2007	Biophysics
Northwestern University, Evanston, IL	Postdoc	03/2013	Biochemistry and Structural Biology

A. Personal Statement

My research focuses on biological processes mediated by protein-nucleic acid complexes. The current focus of my group at the University of Oklahoma is the structural and functional characterization of the bacterial and archaeal immune system, CRISPR-Cas, that comprises protein-RNA-DNA complexes. The specific questions that we address are: *(i)* RNA-mediated activation mechanisms of Cas9 and Cas12a proteins; and *(ii)* development of high fidelity Cas proteins for gene editing applications by modulating protein-RNA-DNA interactions; *(iii)* characterizing the protein-nucleic acid interactions essential for CRISPR adaptation, a process to develop immunity against new intruding genetic materials; and *(iv)* the mechanisms by which CRISPR-Cas systems perform non-canonical functions such as catalysis of guide RNA-independent DNA cleavage.

During my graduate studies (under Dr. Charles Bell) and postdoctoral research (under Dr. Alfonso Mondragón), I used biochemistry, biophysics, and structural biology tools to characterize Recombinase A (RecA) and Topoisomerase V (Topo V). I was introduced to CRISPR-Cas systems during the last couple of years of my postdoctoral research to perform biochemical characterization of *Neisseria meningitidis* Cas9 for Dr. Erik Sontheimer's lab (Zhang *et al*., 2020). Since starting my independent research, we have been using a multi-dimensional approach to understand the enzymatic mechanisms of Cas proteins and CRISPR adaptation machinery. Our research has identified a previously unknown RNA-independent DNA cleavage activity by Cas proteins that can potentially impact the safety of CRISPR-Cas mediated gene editing applications (Sundaresan *et al*., 2017). We have reported on methods to develop high-specificity Cas9 variants by engineering bridge helix, a helix that is conserved in several Cas enzymes commonly used for biotechnology applications (Babu *et al.*, 2019). Our contribution to the field of CRISPR adaptation, a mechanism by which bacteria develop CRISPR-based immunity, included distinct mechanisms for type II-A CRISPR adaptation with unique sequence specificities (Van Orden *et al*., 2020). We collaborate with other scientists to enable a comprehensive understanding of our research questions [e.g., Dr. Peter Qin at the University of Southern California for the use of Electron Paramagnetic Resonance (EPR) spectroscopy; Dr. Jin Liu at the University of North Texas Health Science Center for Molecular Dynamics (MD) simulations; Dr. Yihan Shao in my department for computational studies to characterize catalytic mechanisms of Cas9 and Cas12a. The Biochemistry and Structural Biology groups in the Department of Chemistry and Biochemistry at the University of Oklahoma provides an excellent research environment with several core facilities to help our research goals.

This proposal is seeking user time through the National Center for CryoEM Access and Training (NCCAT) at the New York Structural Biology Center to advance our ongoing cryo-EM studies with Cas9, Cas12a, and Cas1-Cas2 nucleic acid complexes. We are requesting NCCAT GUP3 access to screen and identify good

quality grids for our approved Block Allocation Access (BAG) for Krios data collection. Since the University of Oklahoma currently lacks electron microscope infrastructure needed for cryo-grid screening, GUP3 access will be critical for the effective use of our BAG proposal.

[*indicates corresponding author]

1. Zhang, Y.†, **Rajan, R.**†, Seifert, H.S., Mondragón, A., Sontheimer, E.J.***** (2015). DNase H activity of *Neisseria meningitidis* Cas9. *Mol Cell*, **60** (2): 242-255. PMCID: PMC4609032. (†equal contributors).
2. Sundaresan, R, Parameshwaran, H.P., Yogesha, S.D., Keilbarth, M.W., and **Rajan, R.*** (2017). RNA-independent DNA cleavage activities of Cas9 and Cas12a. *Cell Rep*, 21: 3728-3739. PMCID: PMC5760271.
3. Babu, K., Amrani, N., Jiang, W., Yogesha, S.D., Nguyen, R., Qin, P.Z., **Rajan, R.*** (2019) Bridge helix of Cas9 modulates target DNA cleavage and mismatch tolerance. *Biochemistry,* 58(24): 1905-1917. PMCID: PMC6496953.
4. Van Orden M.J., Newsom S., **Rajan R.*** (2020). CRISPR type II-A subgroups exhibit phylogenetically distinct mechanisms for prespacer insertion. *J Biol Chem.* 295(32):10956-10968. PMCID: PMC7415960.

Ongoing and recently completed projects that I would like to highlight include:

U.S. Department of Defense, Congressionally Directed Medical Research Progs, DOD-CDMR (HT94252310256)
Rajan, R. (PI); co-PIs: Park, J. H., Ibberson, C.
06/01/2023 to 05/31/2025
Identifying protein motifs in Cas9 essential for bacterial virulence

NIH R03, 1R03AI175981-01A1
Rajan, R. (PI); collaborators: Wu, S., Shao, Y.
12/12/2023-10/31/2025
Characterizing mechanisms of immune protection by the bacterial type II-A CRISPR systems

NIH R21, 1R21GM144860-01
Liu, J. (PI), Rajan, R. (collaborator)
01/01/2022-12/31/2024 (one year no-cost extension)
De novo development of small CRISPR-Cas proteins using artificial intelligence

University of Oklahoma Collaborative Research Faculty Fellowship
Rajan, R. (PI), co-PI: Wilhelm, S.
06/01/2022-05/31/2024
Combining Cas enzyme platform and inductively coupled plasma mass spectrometry (ICP-MS) for early-stage disease diagnosis

University of Oklahoma, Vice President for Research and Partnerships (VPRP)
Rajan, R. (PI), co-PIs: Pan, C., Shao, Y., Agbaga, M-P., Wilhelm, S., Jung, H
03/01/2024-02/28/2026
Developing error-proof CRISPR-Cas9 for treating genetic disorders

NIH Oklahoma Center of Biomedical Research Excellence in Structural Biology (OCSB) P30GM145423
West, A.H. (PI), Shao, Y. (Pilot project leader), Rajan R. (co-PI)
08/15/2022 to 05/31/2024
A combined computational and experimental study of CRISPR Cas9/12a Enzyme reaction mechanisms

NSF MCB-1716423
Rajan, R. (PI), Qin, P.Z., (Co-PI, USC)
09/15/2017-08/31/2023 (2-year no cost extension)
Collaborative Research: Mechanisms of RNA-directed activation of a Cas9 nuclease competent for DNA interrogation

Oklahoma Center for the Advancement of Science and Technology (OCAST), HR20-103
Rajan, R. (PI)
10/01/2020-12/31/2023

Protein engineering to develop stringent CRISPR-Cas tools

University of Oklahoma Faculty Investment Program
Rajan, R. (PI)
04/15/2022-08/31/2023
Atomic-resolution structures of Cas protein complexes by cryo-Electron Microscopy

NIH Centers of Biomedical Research Excellence (COBRE) in Structural Biology, P20GM103640
West, A.H. (PI), Rajan R. project leader
09/06/2017-12/31/2019
Mechanistic characterization of CRISPR-Cas complexes that mediate pathogenicity in the bacterium *Francisella tularensis novicida* (Phase II)

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

07/2020– present	Associate Professor, Department of Chemistry and Biochemistry, University of Oklahoma (OU), Norman, OK
08/2014–06/2020	Assistant Professor, Department of Chemistry and Biochemistry, University of Oklahoma (OU), Norman, OK
04/2013–07/2014	Research Associate with Dr. Alfonso Mondragón, Department of Molecular Biosciences, Northwestern University, Evanston, IL
10/2007–03/2013	Postdoctoral fellow with Dr. Alfonso Mondragón, Department of Molecular Biosciences, Northwestern University, Evanston, IL
06/2002–08/2007	Graduate student with Dr. Charles Bell, Department of Biological Chemistry and Pharmacology, The Ohio State University, Columbus, OH

Other Experience and Professional Memberships

Proposal Reviewer, National Science Foundation (NSF)- Molecular and Cellular Biosciences, Molecular Biophysics (ad-hoc reviewer and panelist); Combined NSF and National Institutes of Health panel; The Wellcome Trust-DBT India Alliance Fellowship, Dutch Research Council (NWO) Talent Programme – Veni Domain Science, Swiss National Science Foundation
Member, American Chemical Society (2018-present)
Member, American Society for Biochemistry and Molecular Biology (2017, 2020)
Member, Biophysical Society (2004–07, 2019)
Member, American Heart Association (2012, 2015, 2017)
Member, American Association for the Advancement of Science (2006–07, 2010-13)
Member, American Crystallographic Association (2010–11)
Manuscript Reviewer for *Nucleic Acids Research, Molecular Therapy*, *Viruses, Cell Reports, PLOS One*, *Scientific Reports, ACS Synthetic Biology, Frontiers in Genome Editing Tools and Mechanisms, Current Opinion in Structural Biology, Journal of Biomolecular Structure & Dynamics, Microbial Cell Factories, Microbial Biotechnology, RNA Biology, Foodborne Pathogens and Disease*
Associate Editor: *Frontiers in Cellular and Infection Microbiology, Specialty section:* Molecular Bacterial Pathogenesis (2018–current)

Honors

2021	Senior Faculty Fellowship Award, University of Oklahoma, College of Arts and Sciences, Norman, OK
2016	Junior Faculty Fellowship Award, University of Oklahoma, Research Council, Norman, OK
2016	Poster abstract selected for the late-breaker poster at the American Society for Microbiology Microbe 2016 meeting
2015	Junior Faculty Fellowship Award, University of Oklahoma, Research Council, Norman, OK
2012	Best poster award, Northwestern University Biophysics Symposium
2010–13	American Heart Association Postdoctoral Fellowship, Northwestern University
2006	Outstanding Student Research Achievement Award, The Ohio State University Biophysics program

2004 Best poster award, The Ohio State University Molecular and Cellular Biochemistry annual retreat
2000–02 University Grants Commission Junior Research Fellow National Eligibility Test Scholarship for pursuing a Ph.D. degree in India
1998–2000 Jawaharlal Nehru University (M.S.) Scholarship from the Department of Biotechnology (India)
1996–97 Chinnamma Thomas Memorial Endowment for highest GPA (B.S., 3rd yr., KAU, India)

C. Contributions to Science
[*indicates corresponding author]

1. Conformational and mechanistic characterizations of DNA cleavage by Cas9.
One of the research interests of my laboratory is to characterize the mechanisms by which Cas9 targets and cleaves DNA. An in-depth mechanistic characterization will enable increasing stringency with which Cas proteins carry out DNA cleavage, leading to the development of protein variants with high fidelity for gene editing and gene therapy applications. Our lab reported for the first time, an unprecedented, RNA-independent DNA cleavage by Cas proteins, and protein engineering approaches to remove such promiscuous DNA cleavage (Sundaresan *et al.*, 2017, Newsom *et al.*, 2023). Our lab also reported for the first time that manipulating the interactions of a helix, called the bridge helix, that is highly conserved across several Cas9s can serve as a method to develop stringent protein variants with low off-target DNA cleavage for gene editing (Babu *et al*., 2019, Babu *et al*., 2021).

a. Sundaresan, R, Parameshwaran, H.P., Yogesha, S.D., Keilbarth, M.W., and **Rajan, R.*** (2017). RNA-independent DNA cleavage activities of Cas9 and Cas12a. *Cell Rep*, 21: 3728-3739. PMCID: PMC5760271.
b. Babu, K., Amrani, N., Jiang, W., Yogesha, S.D., Nguyen, R., Qin, P.Z., **Rajan, R.*** (2019) Bridge helix of Cas9 modulates target DNA cleavage and mismatch tolerance. *Biochemistry,* 58(24): 1905-1917. PMCID: PMC6496953.
c. Babu, K., Kathiresan, V., Kumari, P., Newsom, S., Parameshwaran, H.P., Chen, X., Liu, J., Qin, P.Z., **Rajan, R**.* (2021). Coordinated actions of Cas9 HNH and RuvC nuclease domains are regulated by the bridge helix and the target DNA sequence. *Biochemistry*. 60(49):3783-3800. PMCID: PMC8675354.
d. Newsom, S.N., Wang, D.S., Rostami, S., Schuster, I., Parameshwaran, H.P., Joseph, Y.G., Qin, P.Z., Liu, J.,* **Rajan R.*** (2023). Differential divalent metal binding by SpyCas9's RuvC active site contributes to nonspecific DNA cleavage. CRISPR J. 2023 Dec;6(6):527-542. PMCID: PMC10753984.

2. Conformational and mechanistic characterizations of DNA cleavage by Cas12a.
Research in the CRISPR field has revealed similarities and differences between Cas9 and Cas12a, the two Cas protein families that are widely used for genome applications. We take a parallel route by comparing and contrasting DNA cleavage mechanisms of Cas9 and Cas12a. We showed that Cas12a also possesses the unprecedented, RNA-independent DNA cleavage and that it can use diverse DNA substrates for this activity compared to Cas9 (Sundaresan *et al.*, 2017). We established that the method of modulating the interactions of bridge helix with RNA and DNA to improve DNA cleavage stringency is conserved in Cas12a (Parameshwaran *et al.,* 2021, Martin *et al.*, 2023). We also collaborate with other CRISPR experts to understand the mechanisms of guide-RNA-mediated DNA cleavage of Cas12a (Jiang *et al*., 2019).

a. Sundaresan, R, Parameshwaran, H.P., Yogesha, S.D., Keilbarth, M.W., and **Rajan, R.*** (2017). RNA-independent DNA cleavage activities of Cas9 and Cas12a. *Cell Rep*, 21: 3728-3739. PMCID: PMC5760271.
b. Jiang, W., Singh, J., Allen, A., Li, Y., Kathiresan, V., Qureshi, Q., Tangprasertchai, N., Zhang, X., Parameshwaran, H.P., **Rajan, R.**, Qin, P. Z.* (2019), "CRISPR-Cas12a Nucleases Bind Flexible DNA Duplexes without RNA-DNA Complementarity." *ACS Omega*, **4**, 17140-17147. PMCID: PMC6811856.
c. Parameshwaran, H.P., Babu, K., Tran, C., Guan, K., Allen, A., Kathiresan, V., Qin, P.Z., and **Rajan, R.*** (2021). The bridge helix of Cas12a imparts selectivity in *cis*-DNA cleavage and regulates *trans*-DNA cleavage. *FEBS Lett*, 595(7):892-912. PMCID: PMC8044059.
d. Martin, L., Rostami, S., and **Rajan, R.*** (2023). Optimized protocols for the characterization of Cas12a activities. *Methods Enzymol*, 679:97-129. PMID: 36682874.

3. **Characterization of CRISPR adaptation mechanisms.**
   Adaptation is the process by which bacteria and archaea develop immunity against a new phage infecting them. This involves excising short DNA fragments and inserting into the CRISPR locus precisely, to avoid self-targeting of the genome and disruption of essential genes during the insertion process. We conducted bioinformatic studies to locate DNA motifs that may contribute to site-specific insertions of phage DNA into bacterial genome. We identified conserved 3′-leader motifs in type II-A CRISPR systems (Van Orden *et al*., 2017). Following this bioinformatic study, we characterized the role of DNA sequences in sub-group specific DNA insertion that revealed distinct mechanisms for DNA insertion in the seemingly related CRISPR type II-A subtypes (Van Orden *et al*., 2020).
   a. Van Orden, M., J., Klein, P., Babu, K., Najar, F.Z., **Rajan, R.*** (2017). Conserved DNA motifs in the type II-A CRISPR leader region. *PeerJ,* 5:e3161. https://peerj.com/articles/3161/. PMCID: PMC5382924.
   b. Van Orden M.J., Newsom S., **Rajan R.*** (2020). CRISPR type II-A subgroups exhibit phylogenetically distinct mechanisms for prespacer insertion. *J Biol Chem* 295(32):10956-10968. PMCID: PMC7415960.
   c. Flusche, T., **Rajan, R.** Molecular details of DNA integration by CRISPR-associated proteins during adaptation in bacteria and archaea. (2023). *Adv. Exp. Med. Biol.* 1414:27-43. PMID: 35852729.

4. **The structure and mechanism of Topoisomerase V (Topo V).**
   Topoisomerases enable supercoiling and relaxing DNA. Topo V present in *Methanopyrus kandleri*, a hyperthermophilic archaeon, is unique because it has both DNA relaxation and DNA repair activities in the same polypeptide. I determined the structures of several fragments of Topo V encompassing different active sites: structure of an N-terminal 44 kDa fragment of Topo V that showed conformational changes essential for binding DNA (Rajan *et al*., 2010); biochemical characterization of the topoisomerization reaction, (Rajan *et al*., 2014); crystal structures of N-terminal 78 kDa and 94 kDa fragments of Topo V that illustrated the architecture of the AP lyase sites (Rajan *et al*., 2013, Rajan *et al*., 2016).
   a. **Rajan, R.**, Taneja, B., and Mondragón, A.***** (2010). Structures of minimal catalytic fragments of topoisomerase V reveals conformational changes relevant for DNA binding. *Structure*, **18** (7): 829-838. PMCID: PMC2907367.
   b. **Rajan, R.**, Prasad, R., Taneja, B., Wilson, S.H., and Mondragón, A.***** (2013). Identification of one of the apurinic/apyrimidinic lyase active sites of topoisomerase V by structural and functional studies. *Nucleic Acids Res,* **41** (1): 657-666. PMCID: PMC3592480.
   c. **Rajan, R.**, Osterman, A.K., Gast, A.T., Mondragón, A.***** (2014). Biochemical characterization of the topoisomerase domain of *Methanopyrus kandleri* topoisomerase V. *J Biol Chem,* **289** (42): 28898-28909. PMCID: PMC4200249.
   d. **Rajan, R.**, Osterman, A., Mondragón, A.***** (2016). *Methanopyrus kandleri* topoisomerase V contains three distinct AP lyase active sites in addition to the topoisomerase active site. *Nucleic Acids Res,* **44** (7): 3464-3474. PMCID: PMC4838376.

5. **Structural and functional characterization of RecA.**
   RecA protein catalyzes the strand exchange reaction in bacterial homologous recombination. *Deinococcus radiodurans* (Dr) is an extremophilic bacterium resistant to ionizing radiations, and the extreme resistance is attributed to its efficient DNA repair mechanism catalyzed in part by RecA. During my graduate study in Dr. Bell's laboratory at The Ohio State University, I determined the first structure of Dr RecA by X-ray crystallography (Rajan *et al*., 2004). The Dr RecA structure showed features that supported the "inverse strand exchange pathway" in Dr. In addition, I used a set of biochemical assays to better understand the DNA sequence preferences exhibited by Ec RecA (Rajan *et al*., 2006).
   a. **Rajan, R.**, and Bell, C.E.***** (2004). Crystal structure of RecA from *Deinococcus radiodurans*: insights into the structural basis of extreme radioresistance. *J Mol Biol,* **344** (4): 951-963. PMID: 15544805.
   b. **Rajan, R.**, Wisler, J.W., and Bell, C.E.***** (2006). Probing the DNA sequence specificity of *Escherichia coli* RECA protein. *Nucleic Acids Res,* **34** (8): 2463-2471. PMCID: PMC1459065.

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