BIOGRAPHICAL SKETCH

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NAME: Edwin Antony

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

POSITION TITLE: 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	Completion Date	FIELD OF STUDY
Loyola College, Tamil Nadu, India	B.Sc.	05/1998	Zoology & Chemistry
St. Joseph's College, Tamil Nadu, India	M.Sc.	05/2000	Biochemistry
Wesleyan University, Middletown, CT	Ph.D.	05/2005	Mol. Biol. - Biochemistry
Johns Hopkins University, Baltimore, MD	Postdoc	04/2006	Mol. Biophysics
Washington University in St. Louis, MO	Postdoc	03/2010	X-ray Crystallography & Enzymology

A. Personal Statement

Research: My laboratory focuses on understanding the fundamental mechanisms of action of multi-domain and multi-subunit enzyme complexes. We utilize pre-steady state kinetics, single molecule fluorescence, biophysical, and structural tools to identify the mechanistic steps that drive enzymatic activity and functional specificity. Currently, there are four major areas of research emphasis in my laboratory. First is the identification and mechanistic exploration of proteins involved in DNA repair and recombination. The second investigates the mechanism of ATP-dependent electron transfer in oxidoreductases such as nitrogenase and nitrogenase-like enzymes. The third emphasis is on the origins of substrate binding specificity within multi-domain RNA-binding proteins. Finally, we focus extensively on method development using non-canonical amino acids to generate site-specifically labeled and post-translationally modified proteins for biophysical studies. Over the past decade my group has made significant discoveries on the mechanism of action of many enzymes as described in our publications.

My formal research training started with rapid-kinetic approaches to study DNA mismatch repair proteins in graduate school with Prof. Manju Hingorani (Wesleyan Univ.), followed by postdoctoral training in live cell fluorescence imaging with Prof. David Yue (Johns Hopkins Univs). I shifted to structural and mechanistic studies of DNA repair proteins with Prof. Tom Ellenberger and Prof. Timothy Lohman (Washington Univ. in St. Louis) and all these research endeavors have collectively shaped my independent research program since 2012. My research group is now well established and broadly focuses on enzyme mechanisms. My expertise and track record as a highly collaborative PI and eagerness to adopt new technologies to investigate enzyme mechanisms align well with the efforts outlined in this proposal. Our research strengths are in deciphering how large multi-domain and multi-protein complexes work together to accomplish complex biological processes. This is highlighted in the mechanisms we have uncovered for Replication Protein A (Chadda *et. al.* NAR, 2024; Roshan *et. al.* Nat. Comm. 2023; Kuppa *et. al.* Nat. Comm. 2022; Dhingra *et. al.* PNAS. 2021; Pokhrel *et. al.* Nat. St. Mol. Biol. 2019; Yates *et. al.* Nat. Comm. 2018, etc.), nitrogenase & nitrogenase-like oxidoreductases (Kashyap *et. al.* BioRxiv 2024; Corless *et. al.* JBC. 2021; Corless *et. al.* JBC. 2020; Danyal *et. al.* PNAS. 2016; Duval *et. al.* PNAS. 2013; etc.) and now Rad52 (Deveryshetty *et. al.* BioRxiv 2024; and Deveryshetty *et. al.* Nat. Comm. 2023). In the case of RPA, we showed that existing models for how the multiple DNA binding and protein interaction domains are utilized are incorrect/incomplete. Since RPA coordinates almost all DNA metabolic processes, our findings helped redefine how DNA is presented during repair, replication, and recombination. Similarly, for more than three decades electron transfer events in nitrogenase have been considered identical in both halves of the enzyme complex. My group uncovered that electron transfer is asymmetric in nitrogenase and

we established the correct order of events in this important enzyme. We have since uncovered conservation of such asymmetry and allostery in photosynthetic nitrogenase-like enzymes (DPOR). We have solved several high resolution cryoEM structures of DPOR under substrate turnover conditions and mapped out how long-range electron transfer occurs over 100Å. Similarly, in our most recent work on Rad52, we finally uncovered how the two halves of the protein work together to promote Rad51 filament formation. The work also revealed how the functionally homologous (but structurally divergent) BRCA2 complex shares features similar to Rad52. We consider this an extremely important discovery in the field of homologous recombination! While these protein complexes are dynamic, have multiple domains and disordered regions, we overcome these challenges with a combinatorial toolkit of sophisticated biophysical and structural methodologies. We relish the opportunity to collaborate with other teams to tackle the mechanisms of action of large enzyme complexes. This strategy has been extremely successful and teaches trainees to rigorously attack a problem through open collaborations. Our work on RPA is supported by a grant from the NIGMS (R35 GM133967). A grant from the DOE (DE-SC0017866) supports our research on oxidoreductases. *Neither grant has any overlap with the proposed research on RNA-binding proteins which has been supported thus far by PI's start-up funding*.

Service. My group serves the community through mentoring and research training for high school and undergraduate students. More than a dozen high school students and over 50 undergraduate students have been trained in my group. Almost all of them have gone onto professional careers in science including Ph.D., MD, MD-Ph.D., and industry research positions. Many of the undergraduate students have received grants to support their summer research and over the past two years students have been supported by supplements from the NIH. Recent accolades for my students include a Goldwater fellowship and a F99/K00 fellowship. My graduate students have proceeded to postdoctoral work at NEB, MIT, Columbia, and directly to biotech companies. I will strive to ensure that my laboratory provides a stimulating and safe environment for trainees, accommodate special needs, and improve diversity. I have also served as a reviewer for numerous scientific journals, served on more than dozen grant review panels, and raised funds for scientific conferences. I have helped organize scientific meetings and conferences and will continue serving in the next phase of my career.

Research Team. My research team is currently composed of one senior scientist, one postdoctoral fellow, two graduate students, and two well-trained undergraduate students. Dr. Rajnandani Kashyap is a post-doc fellow who works on electron transfer enzymes and has extensive expertise in structural biology and performs all the cryoEM experiments. Dr. Mohamed Ghoneim is a senior scientist and talented single molecule spectroscopist and carries out all the single molecule TIRF, C-Trap, and ensemble FRET measurements. Ayush Mistry and Tania Sultana are graduate students in the group. Three new direct-entry graduate students are scheduled to join the group in Fall 2025

Collaborations. The structural and functional complexity of multi-domain proteins requires a broad experimental toolkit to uncover the nuances of the mechanism of action. Over the past decade, we have established a network of collaborators who have both provided unique expertise and taken advantage of our biophysical approaches and FEncAA tools. For the proposed work, the protein translation, *in vivo*, cellular, and cancer-biology part of the work are carried out in Dr. Sofia Origanti's group (Co-PI; St. Louis University). Structural-MS experiments are performed in collaboration with Dr. Brian Bothner (Montana State Univ.). NMR experiments are performed in collaboration with Dr. Haribabu Arthanari (Dana Farbar). EPR and SAXS experiments are performed in collaboration with Dr. Ritimukta Sarangi (SLAC beamline). Finally, CryoEM grid preparation and data collection are performed by our team members at WUCCI (Washington Univ. in St. Louis). A manuscript describing a collaborative study on the phosphorylation-induced changes in IMP1/IMP3 by the Antony, Origanti, Bothner, and Arthanari groups is under preparation: (Kaushik V, Mattice J, Toerner R, Chadda R, Vayyeti A, Bothner B, Arthanari H, Origanti S and Antony E. mTORC2 phosphorylation of IMP1 and IMP3 drives mRNA recognition through differential configurational rearrangements of RNA-binding domains and disordered linkers).

Select recent publications from the group:

1. Deveryshetty J., Chadda R., Mattice J., Karunakaran S., Rau M.J., Basore K., Pokhrel N., Englander N., Fitzpatrick J.A.J., Bothner B., **Antony E**. Yeast Rad52 is a homodecamer and possesses BRCA2-like bipartite Rad51 binding modes. *Nature Communications*. 2023. 14: 6215. PMID: 37798272.
2. Roshan P., Kuppa S., Mattice J.R., Kaushik V., Chadda R., Pokhrel N., Tumala B.R., Bothner B., **Antony E*.**, and Origanti S*. An Aurora B-RPA signaling axis secures chromosome segregation fidelity. *Nature Communications*. 2023. 14(1):3008. PMID: 37230964. *co-corresponding authors.
3. Kuppa S., Deveryshetty J., Chadda R., Mattice J., Pokhrel N., Patterson A., Dhingra N., Pangeni S., Sadauskas M.K., Shiekh S., Ha T., Zhao X., Bothner B., and **Antony E.** Rtt105 configurationally staples

RPA and blocks facilitated exchange and interactions with RPA-interacting proteins. *Nature Communications*. 2022 13(1):5152. PMID: 36056028

4. Pokhrel N. C., Caldwell C.C., Corless E.I., Tillison E.A., Tibbs J. Joic, N., Taibei, A., Wold, M.S., Spies M., **Antony E**. Dynamics and Selective Remodeling of the DNA Binding Domains of RPA. *Nature Structural and Molecular Biology*. 2019. 26(2):129-136. PMID: 30723327

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2022- Professor, Biochemistry and Molecular Biology, St. Louis Univ. School of Medicine, St. Louis, MO.
2019-2022 Associate Professor, Biochemistry and Molecular Biology, St. Louis Univ. School of Medicine, St. Louis, MO.
2019 Associate Professor, Biological Sciences, Marquette University, Milwaukee, WI.
2015-2019 Assistant Professor, Biological Sciences, Marquette University, Milwaukee, WI.
2012-2015 Gaurth R. Hansen Assistant Professor, Department of Chemistry and Biochemistry, Utah State University, Logan, UT.
2010-2012 Research Instructor, Department of Biochemistry and Molecular Biophysics, Washington University School of Medicine, St. Louis, MO. (Mentor: **Dr. Timothy M. Lohman**).
2006-2010 Postdoctoral Fellow, Department of Biochemistry and Molecular Biophysics, Washington University School of Medicine, St. Louis, MO. (Mentor: **Dr. Tom Ellenberger**).
2005-2006 Postdoctoral Fellow, Department of Biomedical Engineering, The Johns Hopkins University, Baltimore, MD. (Mentor: **Dr. David T. Yue**).
2000-2005 Graduate Student, Department of Molecular Biology and Biochemistry, Wesleyan University, Middletown, CT. (Mentor: **Dr. Manju M. Hingorani**).

Other experience and professional affiliations

2024 Grant Panel reviewer, National Science Foundation, Genetic Mechanisms
2024 Grant Panel reviewer, National Institutes of Health (MSFA)
2024 Grant Panel reviewer, National Institutes of Health NSRA F08 Panel
2024 Grant Panel reviewer, National Science Foundation, Chemistry of Life Processes
2023 Grant Panel reviewer, NIH NRSA Grant Panel
2025, 2023, 2022, 2021, 2020, 2018 Grant Panel reviewer, Department of Energy, Basic Energy Sciences
2021 Grant Panel reviewer, NIH Program grant study section, Program Grant Panel
2020 Grant Panel reviewer, National Institutes of Health (MSFC)
2019 Grant Panel reviewer, National Science Foundation, Chemistry of Life Processes
2016- International Reviewer, French National Research Agency.
2016 Grant Panel reviewer, National Institutes of Health (MSFA)
2014- International Reviewer, Canada Foundation for Innovation
2014- International Reviewer, Netherlands Organization for Scientific Research
2009- Associate Member, American Cancer Society
2009- Member, Biophysical Society
2006- Member, American Society for Biochemistry and Molecular Biology

Academic and professional honors

2024 Senior & Junior non-book scholarly works award, St. Louis University.
2024 Vice-chair, Midwest Enzymes Mechanisms Conference
2024 Session chair, 28th Enzyme Mechanisms Conference, Naples, FL
2022 Research Institute Fellow, St. Louis University.
2021 Chair, Biophysical Society Annual Meeting, Macromolecular Machines Subgroup.
2020 Vice-chair, Biophysical Society Annual Meeting, Macromolecular Machines Subgroup.
2018 Way-Klingler Young Scholar Award, Marquette University
2017 Session Chair: 25th Enzyme Mechanisms Conference, St. Pete's Beach, FL
2013 Selected for the Cottrell Scholars Collaborative New Faculty Workshop, DC
2005 Peterson Fellowship (outstanding graduate research), Wesleyan University, CT
2005 Barry Kiefer Prize (best graduate thesis), Wesleyan University, CT
2004 American Society of Microbiology Travel Award, DNA repair meeting, Bermuda
2000 AMM Charity National Scholarship (National award for graduate education), Chennai, India
2000 University Academic First Rank, St. Joseph's College, India

C. Contributions to Science

C1. The mechanism of action of Replication Protein A (RPA). RPA is an essential single-strand DNA binding protein that interacts with over three dozen proteins and coordinates almost all DNA metabolic processes including replication, repair, and recombination. RPA is composed of a series of DNA binding domains (DBDs A-E) and protein-interaction domains (PIDs). For over three decades DBDs A & B have been considered high-affinity binders for RPA function. We developed FEncAA (fluorescence enhancement through non-canonical amino acids; Pokhrel *et. al.* NAR.2017) which enabled us to capture the binding, dissociation, and remodeling of select domains within full-length RPA using rapid-kinetic and single-molecule methods. This breakthrough revealed that DBDs-A & B are dynamic whereas DBDs-C, D and E stably interact with DNA (Yates *et. al.* Nat. Comm. 2018 & Pokhrel *et. al.* NSMB. 2019), and we established that RPA functions as 'dynamic' & 'less-dynamic' halves (Ahmad *et. al.* NAR. 2021). These discoveries challenge current models for the mechanism of RPA function and have led to more interesting unknown mechanistic questions about RPA. We have now uncovered how RPA is regulated, and spurious protein interactions are inhibited by the Rtt105 chaperone (Kuppa *et. al.* Nat. Comm. 2022). Another exciting new discovery is the AurkB-RPA signaling axis during mitosis (Roshan *et. al.* Nat. Comm. 2023).

1. Roshan P., Kuppa S., Mattice J.R., Kaushik V., Chadda R., Pokhrel N., Tumala B.R., Bothner B., **Antony E*.**, and Origanti S*. An Aurora B-RPA signaling axis secures chromosome segregation fidelity. *Nature Communications*. 2023. 14(1):3008. PMID: 37230964. *co-corresponding authors.
2. Kuppa S., Deveryshetty J., Chadda R., Mattice J., Pokhrel N., Patterson A., Dhingra N., Pangeni S., Sadauskas M.K., Shiekh S., Ha T., Zhao X., Bothner B., and **Antony E.** Rtt105 configurationally staples RPA and blocks facilitated exchange and interactions with RPA-interacting proteins. *Nature Communications.* 2022 13(1):5152. PMID: 36056028
3. Pokhrel N. C., Caldwell C.C., Corless E.I., Tillison E.A., Tibbs J. Joic, N., Taibei, A., Wold, M.S., Spies M., **Antony E**. Dynamics and Selective Remodeling of the DNA Binding Domains of RPA. *Nature Structural and Molecular Biology*. 2019. 26(2):129-136. PMID: 30723327
4. Yates L.A., Aramayo R.J., Pokhrel N., Caldwell C.C., Kaplan J.A., Perera R.L., Spies M., **Antony E.,** and Zhang X. A structural and dynamic model for the assembly of Replication Protein A on single-stranded DNA. *Nature Communications.* 2018. 9(1):5447. PMID: 30575763

C2. The discovery of asymmetry in ATP-driven electron transfer enzymes. Nitrogenase functions to reduce dinitrogen to ammonia and several nitrogenase-like enzymes function in the biosynthesis of bacteriochlorophyll. These enzymes are oxidoreductases that catalyze multiple rounds of electron transfer coupled to ATP binding and hydrolysis. These enzymes are also interesting because they catalyze elemental chemical reactions dating back to the origins of life on earth and have complex metal centers and structural features that are conserved to date in many human enzymes. These enzymes function as large complexes with two identical catalytic halves and we established the order of catalytic events in these proteins, a 4-decade old unresolved mystery (Duval *et. al.* PNAS. 2013). Subsequently, we uncovered that the two halves functioned in an asymmetric manner with electron transfer in one half allosterically controlling events in the other (Danyal *et. al.* PNAS. 2016. Corless *et. al.* JBC. 2020). We also discovered a [4Fe-4S] cluster protection mechanism through a disordered region (Corless *et. al.* JBC. 2020). Current work in the group focuses on single molecule and cryoEM studies to capture the transition states during their substrate reduction reactions.

1. Corless E., Imran S.M., Watkins B., Bacik J., Mattice J., Patterson A., Danyal K., Soffe M., Kitelinger R., Seefeldt L., Origanti S., Bennett B., Bothner B., Ando N. and **Antony E.** The flexible N-terminus of BchL autoinhibits activity through interaction with its [4Fe-4S] cluster and relieved upon ATP binding. *J. Biol. Chem.* 2020. 296: 100107. PMID: 33219127
2. Corless E., Bennett B., and **Antony E.** Substrate recognition induces sequential electron transfer across subunits in the nitrogenase-like DPOR complex. *J. Biol. Chem*. 2020. 295(39):13630-13639. PMID: 32737200
3. Danyal K., Shaw S., Page T., Duval S., Fielding A.J., Horitani M., Marts A.R., Lukoyanov D., Dean D.R., Raugei S., Hoffman B.M., Seefeldt L.C. and **Antony E.** Negative cooperativity in the nitrogenase Fe protein electron delivery cycle. *Proc. Nat. Acad. Sci.* 2016. 113(40):E5783-E5791. PMID: 27698129
4. Duval S., Danyal K., Shaw S., Dean D.R., Hoffman B.M., **Antony E***., and Seefeldt L.C.* Establishing the order of electron transfer and ATP hydrolysis in Nitrogenase. *Proc. Nat. Acad. Sci*. 2013. 110:16414-16419. (*Co-Corresponding Authors). PMID: 24062462

C3. Mechanism of action of pro- and anti-HR mediators. Homologous recombination (HR) processes in the cell, especially pre-synaptic Rad51 filament formation, are tightly regulated by pro- and anti-HR mediators. Pro-HR mediators such as Rad52 and BRCA2 function to stabilize the Rad51 filament. Anti-HR mediators such as Srs2

and HelQ are motor proteins that translocate on DNA to disassembly Rad51 filaments. We have uncovered many aspects of how Srs2 functions, developed assays to assess Rad51 filament dynamics, and more recently focused on how Rad52 functions. My expertise in pre-steady state analysis of studying ATPases is fundamental to designing new experiments to capture these helicases in action. We are now focused on developing C-trap experiments to obtain high-resolution information on how these enzymes move on the DNA.

1. Dhingra N., Kuppa S., Wei L., Pokhrel N., Baburyan S., Meng X., **Antony E.,** and Zhao X. The Srs2 helicase dampens DNA damage checkpoint by recycling RPA from chromatin. *Proc. Nat. Acad. Sci.* 2021. 118(8): e2020185118. PMID: 33602817
2. Hormeno S, Wilkinson O.J., Aicart-Ramos C., Kuppa S., **Antony E.** Dillingham M.S., and Moreno-Herrero F. Human HELB is a processive motor protein which catalyses RPA clearance from single-stranded DNA. Ms. under review. *Proc. Nat. Acad. Sci.* 2022. 119(15):e2112376119. PMID: 35385349
3. **Antony E.**, Tomko E.J., Xiao Q., Krejci L., Lohman T.M., and Ellenberger T.E. Srs2 dismantles Rad51 filaments by a protein-protein interaction triggering ATP turnover and dissociation of Rad51 from DNA. *Molecular Cell.* 2009. 35:105-115. PMID: 19595720
4. *Yupeng Q.,* **Antony E.,** Doganay S., Koh H.R., Lohman T.M., and Myong S. Srs2 prevents Rad51 filament formation by repetitive motion on DNA. *Nature Communications*. 2013. 4:2281. PMID: 23939144

C4. Development of genetic code expansion (GCE) tools to study protein dynamics. To study the dynamics of individual domains/regions within a multi-subunit enzyme, or to obtain a signal from a single enzyme within a milieu of a multi-protein reaction, a fluorescence signal from the single entity is required. Using non-canonical amino acids (ncAA) we site site-specifically position fluorophores such that a direct readout is obtained upon substrate binding. We also use this approach to study the conformational flexibility of enzymes by positioning two site-specific probes within a protein. In addition, we generate phospho-Ser carrying proteins using GCE. We synthesize our own ncAA probes and continue to spearhead application GCE to investigate DNA repair enzymes. Stopped flow and single-molecule fluorescence and FRET assays are used to capture dynamics.

1. Bednar R.M., Jana S., Kuppa S., Franklin R., Beckman J., **Antony E.**, Cooley R.B., Mehl R.A.. Genetic incorporation of two mutually orthogonal bioorthogonal amino acids that enable efficient protein dual-labeling in cells. *ACS Chemical Biology*. 2021. PMID: 34590824
2. Pokhrel, N. C., Caldwell, C.C., Corless, E.I.; Tillison, E.A.; Tibbs, J.; Joic, N.; Taibei, A.; Wold, M.S.; Spies, M.; **Antony, E**. Dynamics and Selective Remodeling of the DNA Binding Domains of RPA. *Nature Structural and Molecular Biology*. 2019. 26(2):129-136. PMID: 30723327
3. Pokhrel H., Origanti S., Davenport E.P., Gandhi D., Kaniecki K., Mehl R.A., Greene E.C., Dockendorff C., and **Antony E.** Monitoring replication protein A (RPA) dynamics in homologous recombination through site-specific incorporation of non-canonical amino acids. *Nucleic Acids Research*. 2017. 45(16):9413-9426. PMID: 28934470
4. Kuppa S., Pokhrel N., Corless E., Origanti S., and **Antony E.** Generation of fluorescent versions of *Saccharomyces cerevisiae* RPA to study the conformational dynamics of its ssDNA-binding domains. *Methods Mol. Biol.* 2021. 2281:151-168. PMID: 33847957

C5. Discovery of how mismatch repair enzymes function. My thesis work uncovered how the DNA mismatch repair protein MutS and Msh2-Msh6 recognized single mismatches in the genome. Pre-steady state kinetic analysis of DNA driven ATP binding and hydrolysis revealed stabilization of an ATP-driven stabilized form of MutS that initiated mismatch recognition and downstream repair events.

1. **Antony E.**, and Hingorani M.M. Mismatch recognition-coupled stabilization of Msh2-Msh6 in an ATp bound state at the initiation of DNA repair. *Biochemistry*. 2003. 42(25):7682. PMID: 12820877
2. **Antony E**., and Hingorani M.M. Asymmetric ATP binding and hydrolysis activity of the *Thermus aquaticus* MutS dimer is key to modulation of its interactions with mismatched DNA. *Biochemistry*. 2004. 43(41):13115. PMID: 15476405
3. **Antony E.**, Khubchandani S., Chen S., and Hingorani M.M. Contribution of Msh2 and Msh6 subunits to the asymmetric ATPase and DNA mismatch binding activities of *Saccharomyces cerevisiae* Msh2-Msh6 mismatch repair protein. *DNA Repair*. 2006. 5(2):153. PMID: 16214425
4. Zito C.R, **Antony E**., Hunt J.F, Oliver D.B., Hingorani M.M. Role of a conserved glutamate residue in the *Escherichia coli* SecA ATPase mechanism. *J Biol Chem*. 2005. 280(15):14611. PMID: 15710614

A complete list of publications from my group can be found at:
https://www.ncbi.nlm.nih.gov/myncbi/10uuWxaRXvLkh/bibliography/public/