BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
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NAME: Daniel E. Richman

eRA COMMONS USER NAME (credential, e.g., agency login): drichma6

POSITION TITLE: Research Associate

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
University of Rochester, Rochester, NY	BS, BA	05/2008	BS Physics; BA Music, Mathematics
Johns Hopkins University, Baltimore, MD	PhD	05/2015	Physics/Biophysics
Georgia Institute of Technology, Atlanta, GA	Postdoc	06/2016	Chemistry
Johns Hopkins School of Medicine, Baltimore, MD	Postdoc	06/2022	Structural Biology

A. Personal Statement

My long-term research interests involve understanding how conformational change underpins function in proteins and macromolecular assemblies. I aim to use this insight to explain the molecular basis for genetic disorders and provide foundational data for efforts to develop therapeutics. I am also committed to providing technical expertise and training in my career, expanding training procedures I developed for colleagues in cryo-electron microscopy as a postdoc into formal programs and areas of pedagogical advancement.

As a graduate student, I gained a distinctive perspective on science by training in physics, biology, and biophysics departments. I published two first-author papers on how electrostatic effects can lead to protein conformational changes that impact structure and function, and I contributed robust experimental findings to the field that are now used by others to improve electrostatic calculations involving proteins. I independently mastered the theory and practice of NMR spectroscopy, and in doing so, introduced new NMR approaches to a lab that had previously never used the techniques. For postdoctoral training, I first chose to study aspects of protein chemistry that underpin protein evolution, seeking deeper insights into protein folding and energetics. Sensing that I had reached a plateau in the training that this postdoctoral position would afford and before seeking a second postdoctoral position, I sought to use my experience communicating across disciplines to work at the American Association for the Advancement of Science for 6 months to help launch a program aimed at improving collaboration in research centers.

In my main phase of postdoctoral training, I redirected my research toward using hybrid structural and biochemical approaches to dig further into understanding the mechanisms of proteins and their complexes. Returning to Baltimore (as opposed to another venue) for family reasons, I chose to work with Dr. James Berger, an expert in applying such approaches to the study of protein/nucleic-acid systems and transactions. Here I have expanded my knowledge base to encompass new methods (especially single particle electron microscopy, biochemical reconstitution of complexes, enzymology) and new systems (protein-DNA interactions, topoisomerases). My training has built significantly upon my academic and research background and enabled me to learn new concepts and skills necessary for me to succeed as an independent investigator.

A segment of my postdoctoral training was supported by an NIH Postdoctoral Fellowship:
NIH F32 GM128269 (Role: PI) 09/03/2019 - 09/02/2020
Title: *Molecular basis of regulation of DNA engagement and cleavage in topoisomerase VI and meiotic homologs*

B. Positions, Scientific Appointments, and Honors

Positions

09/2008 – 05/2015	Graduate Research Assistant, Johns Hopkins University
08/2015 – 06/2016	Postdoctoral Fellow, Georgia Tech
06/2016 – 12/2016	Program Assistant, American Association for the Advancement of Science
04/2017 – 06/2022	Postdoctoral Research Fellow, Johns Hopkins School of Medicine
07/2022 – present	Research Associate, Johns Hopkins School of Medicine

Honors

2019-20	NIH Kirschstein NRSA Postdoctoral Fellowship
2018-19	Postdoctoral Traineeship with Johns Hopkins School of Public Health Biochemistry and Molecular Biology Dept NIH T32 Program
2009-13	NSF IGERT Fellowship with Johns Hopkins Institute for NanoBioTechnology
2008	*Magna cum laude*, University of Rochester
2007	Dept of Energy National Undergraduate Fellowship in Plasma Physics and Fusion Energy Sciences, MIT Plasma Science and Fusion Center
2006	NSF Research Experience for Undergraduates, University of Rochester

C. Contributions to Science

The accurate determination of electrostatic effects in proteins is essential to connect protein structure to biological function. The two papers I published explain how changes in the charge state of ionizable groups in proteins are coupled to structural reorganization, an effect that is difficult to represent accurately in electrostatic calculations. My NMR spectroscopy studies, which focused a model protein, *Staphylococcal* nuclease, determined the locations, time scales, and physical extent of proton binding-coupled structural fluctuations and subglobal unfolding events. These insights are now being used in the protein electrostatics field to improve sampling of alternative states, including partially unfolded states, making the modeling of energies in proteins more accurate to improve our molecular understanding of biological energy transduction, catalysis, and ligand binding.

1. Richman, D. E., Majumdar, A. & García-Moreno E, B. pH dependence of conformational fluctuations of the protein backbone. *Proteins* 82, 3132–3143 (2014).
2. Richman, D. E., Majumdar, A. & García-Moreno E., B. Conformational Reorganization Coupled to the Ionization of Internal Lys Residues in Proteins. *Biochemistry* 54, 5888–5897 (2015).

BIOGRAPHICAL SKETCH

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NAME: James M. Berger

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

POSITION TITLE: Professor of Biophysics and Biophysical Chemistry

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
University of Utah, Salt Lake City, UT	BS	05/1990	Biochemistry
Harvard University, Cambridge, MA	PhD	9/1995	Structural Biology
Whitehead Institute, MIT, Cambridge, MA	Fellow	6/1998	Structural Biology

A. Personal Statement

Research in my laboratory focuses on understanding the mechanisms and functions of multisubunit assemblies that control the organization, preservation, and flow of genetic information in cells. We are particularly interested in developing kinetically-accurate, atomic-level models that explain how biological macromolecules transduce chemical energy into force and motion, and how dynamic assemblies support DNA replication, gene expression, chromosome superstructure, and other essential nucleic-acid transactions.

My group uses a blend of structural, biochemical, biophysical, and *in vivo* methods to define the architecture, function, evolution, and regulation of protein/nucleic acid complexes. X-ray crystallography and biochemistry initially formed the core of our approach; however, we now use these methods in conjunction with other tools such as small-angle X-ray scattering, single-molecule approaches, and electron microscopy. Since the inception of the group in 1995, we have biochemically and structurally defined the range and nature of key functional intermediates and structural transitions for a variety of nucleotide-dependent “molecular machines,” including topoisomerases, helicases, primases, SMC-family proteins, and replication initiation complexes. Our efforts have allowed us to define how biological systems use these factors to organize, transport, and reshape target nucleic-acid substrates at a physical level, and how their actions are controlled by both protein-protein interactions and small-molecule agents. My lab has a consistent track record of bringing new concepts and fundamentally important discoveries to the field, and in innovating new approaches and technologies to studying multi-protein and protein/nucleic-acid assemblies in general.

I have maintained a strong commitment to training throughout my career. While at Berkeley, I served as Head Graduate Advisor for the Department of Molecular and Cell Biology and I oversaw the construction and implementation of a new graduate course curriculum. At Johns Hopkins, I have served as the Director of our department’s first-year medical student course and have created and directed two new graduate courses. I have mentored a total of 28 doctoral students and 26 post-doctoral fellows to date. Of those who have moved on from the lab, the majority (>95%) have gone on to productive careers in academia (20), biotechnology/pharma (14), law (4), and medicine (1). The remainder are employed in activities such as teaching and consulting.

Ongoing projects:

NIH R37 GM071747 (Role: PI) 06/01/2022 - 05/31/2027
Title: *Mechanistic Studies of Replication Initiation in Prokaryotes*

NIH R01 CA077373 (Role: PI) 03/01/19– 02/28/23
Title: *Structural and Biochemical Analyses of Type II DNA Topoisomerases*

NIH R01 GM141045 (Role: PI) 04/01/2021 - 03/31/2026
Title: *Studies to Explore DNA Replication Proteins in Functional Assemblies through Intrinsically Disordered Domains*

Beckman Foundation (Role: PI) 07/01/17 - 06/30/24
Title: *Establishment of a High-Resolution Cryo-Electron Microscopy Center at Johns Hopkins University*

Recently completed projects:

NIH R01 CA077373 (Role: PI) 04/01/14 - 02/28/19
Title: *Structural and Biochemical Analyses of Type II DNA Topoisomerases*

NIH R01 CA030490-35 (Role: Subcontract; PI – Michael Botchan, UC Berkeley) 04/07/16 - 03/31/21
Title: *The Structure, Function and Regulation of Eukaryotic DNA Replication Initiator Complexes*

Mathers Foundation (Role: PI) 12/01/17 - 11/30/20
Title: *Imaging the Un-Imageable: A New Approach to Revealing the Molecular Form and Function of Dynamic Biological Nano-Machines*

Selected citations using electron microscopy spanning a range of time periods for the lab:

1. Arias-Palomo E*, Puri N, O'Shea Murray VL, Yan Q, **Berger JM*** (*co-corresponding authors). Physical Basis for the Loading of a Bacterial Replicative Helicase onto DNA. Mol Cell. 2019 Apr 4;74(1):173-184. PMCID: *in progress*

2. Bleichert F, Leitner A, Aebersold R, Botchan MR*, **Berger JM*** (*co-corresponding authors). Conformational control and DNA-binding mechanism of the metazoan origin recognition complex. Proc Natl Acad Sci U S A. 2018;115(26):E5906-E5915. PMCID: PMC6042147

3. Arias-Palomo E, **Berger JM**. An Atypical AAA+ ATPase Assembly Controls Efficient Transposition through DNA Remodeling and Transposase Recruitment. Cell. 2015 Aug 13;162(4):860-71. (Accompanying *Preview*, Cell, 2015) PMCID: PMC4537775

4. Costa A, Ilves I, Tamberg N, Petojevic T, Nogales E, Botchan MR*, **Berger JM*** (*co-corresponding authors). "The structural basis for MCM2-7 helicase activation by GINS and Cdc45," Nat Struct Mol Biol, **18(4):**471-7, 2011. (*Editor's choice*, Science, **332:**14, 2011)

B. Positions, Scientific Appointments, and Honors

Positions Held:

9/90-8/95	Graduate student, Department of Biochemical, Molecular, Cellular, and Developmental Biology, Harvard University (Mentors: Profs. James C. Wang and Stephen C. Harrison).
8/95-6/98	Whitehead Fellow, Whitehead Institute for Biomedical Research, Massachusetts Institute of Technology.
7/98-6/03	Assistant Professor of Biochemistry and Molecular Biology, Dept. of Molecular and Cell Biology, University of California, Berkeley.
9/99-present	Staff Member, Physical Biosciences Division, Lawrence Berkeley National Laboratory.
7/03-6/05	Associate Professor of Biochemistry and Molecular Biology, Dept. of Molecular and Cell Biology, University of California, Berkeley.
7/05-7/13	Professor of Biochemistry and Molecular Biology, University of California, Berkeley
6/06-7/13	Director, QB3 Macrolab, University of California, Berkeley
7/13-present	Professor, Biophysics and Biophysical Chemistry, Johns Hopkins University School of Medicine
9/14-present	Professor of Oncology, Johns Hopkins University School of Medicine (secondary appointment)
9/14-present	Co-Director, Cancer Chemical and Structural Biology Program of the Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University School of Medicine
7/17-present	Professor of Pharmacology, Johns Hopkins University School of Medicine (secondary appointment)
10/17-7/18	Deputy Director, Institute for Basic Biomedical Sciences, Johns Hopkins University School of Medicine

8/18-present Director, Institute for Basic Biomedical Sciences, Johns Hopkins University School of Medicine

Awards and Honors:

2018 Elected, National Academy of Medicine
2017 Michael and Ann Hankin and Partners of Brown Advisory Professor in Scientific Innovation, Johns Hopkins School of Medicine
2016 Royal Society of Chemistry Michael J. Gait Lectureship Award
2013 Elected, National Academy of Sciences
2012 Elected, American Academy of Arts & Sciences
2011 Recipient, National Academy of Sciences Award in Molecular Biology
2008-2013 Walter and Ruth Schubert Family Chair in Biochemistry and Molecular Biology, UC Berkeley
2008 David A. Shirley Award for Outstanding Scientific Achievement at the Advanced Light Source, Lawrence Berkeley National Laboratory
2006 American Chemical Society Pfizer Award in Enzyme Chemistry
2006 ASBMB Schering Plough Research Institute Scientific Achievement Award
1999-2004 Fellow of the David and Lucille Packard Science and Engineering Research Foundation.
1999 Faculty Research Award for the Biological Sciences (UC Berkeley).
1995-1998 Whitehead Fellow, Whitehead Institute for Biomedical Research, Massachusetts Institute of Technology.
1990-1993 National Science Foundation Graduate Studies Fellowship.
1990 *Summa cum Laude*, University of Utah.

Professional Activities (NIH):

2016 (Feb) *Ad hoc* reviewer, MGA study section
2015 (Oct) *Ad hoc* reviewer, MSFC study section
2014 (Oct) *Ad hoc* reviewer, MGA study section
2014 (July) *Ad hoc* reviewer, NIH SEP
2013-2014 Member, NIGMS Future of Structural Biology Committee
2012 (Nov) Co-chair, NIH SEP
2012 (Jun) *Ad hoc* member, NIH MSFC study section
2012 (Apr) Co-chair, NIH SEP
2006-2010 Permanent Member, NIH MGA study section
2005 (Jun) *Ad hoc* reviewer, NIH MGA study section
2004 (Jun) *Ad hoc* reviewer, NIH MBC2 study section
2002-2003 Study section member, P01 review panel

Professional Activities (Other):

2020 Co-chair, 2021 Nucleic Acids Gordon Research Conference
2020 Co-chair, 2020 FASEB 'Machines on Genes' Research Conference (postponed to 2021 due to Covid-19)
2019 Vice-chair, 2019 Nucleic Acids Gordon Research Conference
2018 Vice-chair, 2018 FASEB 'Machines on Genes' Research Conference
2016 Advisory Committee Member, 2017 Gordon Research Conference on DNA Topoisomerases in Biology and Medicine
2015-2016 Co-organizer, DNA replication, repair and recombination session, 2016 ASBMB Annual Meeting
2015-2016 Advisory Committee Member, 2016 EMBO Conference on DNA Topoisomerases and Topology
2013-2014 Advisory Committee Member, 2014 Gordon Research Conference on DNA Topoisomerases in Biology and Medicine
2013 Chair/Organizer, Keystone Symposium on DNA Replication and Repair
2012-present Editor, *Journal of Molecular Biology*
2011 Advisory Committee member for Topo 2011 Conference, Academia Sinica, Teipei, Taiwan
2004-present Editorial board member, *Structure*
2002-present Section Editor (DNA replication and repair) for Faculty of 1000
2006-2011 Academic Editor, *PLOS*

2003-2010	Member, Berkeley Center for Structural Biology Steering Committee, Advanced Light Source, Lawrence Berkeley National Laboratory
2010	Co-organizer, 24th Annual Meeting of the Protein Society, San Diego, CA
2008	Member, Advisory Committee for Topo 2008: DNA Topoisomerases in Biology and Medicine, John Innes Centre, Norwich, UK
2007	Co-Chair/Organizer, FASEB Conference on "Helicases and Nucleic Acid Enzymes"
2006	Co-editor for Current Opinion in Structural Biology, Protein/nucleic-acid interactions section
2000-2004	Member, Scientific Advisory Committee, Advanced Light Source (ALS) at Lawrence Berkeley National Laboratory

C. Contributions to Science

C.1 – Replication initiation mechanisms. In all cells, the onset of DNA replication is controlled by dedicated ATPases that assist with origin recognition, helicase loading, and at times the melting of parental template DNA strands. How these "initiator" factors coordinate interactions with appropriate nucleic acid substrates and each other to promote replisome assembly is not understood at a molecular level. We determined that all cellular replication initiators are predicated on a common AAA+ ATPase fold, but that bacterial, archaeal and eukaryotic initiator homologs form markedly different oligomeric complexes that interact with client DNA substrates in highly distinct manners. Our work has helped resolve both recent and long-standing problems, from how certain mutations implicated in primordial dwarfism disorders impact the stability of the eukaryotic Origin Recognition Complex (ORC) to how bacterial DnaA recognizes and melts replication origins.

1. Parker MW, Bell M, Mir M, Kao JA, Darzacq X, Botchan MR*, **Berger JM*** (*co-corresponding authors). A new class of disordered elements controls DNA replication through initiator self-assembly. Elife. 2019 Sep 27;8. pii: e48562. (Accompanying *Insight* - Elife, 2019; *Recommended*, F1000). PMCID: PMC6764820
2. Hood IV, **Berger JM**. Viral hijacking of a replicative helicase loader and its implications for helicase loading control and phage replication. Elife. 2016 May 31;5. pii: e14158. PMCID: PMC4887207
3. Bleichert F, Botchan MR*, **Berger JM*** (*co-corresponding authors). Crystal structure of the eukaryotic origin recognition complex. Nature. 2015 519:321-6. (Recommended, F1000) PMCID: PMC4368505
4. Duderstadt KE, Chuang K, **Berger JM**. "DNA stretching by bacterial initiators promotes replication origin opening." Nature. **478(7368):**209-13, 2011. PMCID: PMC3192921

C.2 – Molecular control of DNA superstructure. The appropriate management of higher-order chromosome packaging and superstructure depends on the action of enzymes that modulate DNA supercoiling, looping, and topology. How these organizing factors interact with target DNA segments to disentangle intertwined strands and actively control DNA twist and writhe is a long-standing question in the field. We have helped establish how type IIA topoisomerases bind and cleave duplex DNA and how ATP binding and hydrolysis coordinate the passage of a second DNA segment through this break. We have also helped to define the evolution of different type II topoisomerase families, connecting one branch of this group to meiotic recombination processes, and have begun to establish how type II topoisomerases are regulated by partner protein interactions.

1. Hobson MJ, Bryant Z, **Berger JM**. Modulated control of DNA supercoiling balance by the DNA-wrapping domain of bacterial gyrase. Nucleic Acids Res. 2020 Feb 28;48(4):2035-2049. PMCID: in progress
2. Wendorff TJ, **Berger JM**. Topoisomerase VI senses and exploits both DNA crossings and bends to facilitate strand passage. Elife. 2018 Mar 29;7. pii: e31724. PMCID: PMC5922973
3. Vos SM, Lyubimov AY, Hershey DM, Schoeffler AJ, Sengupta S, Nagaraja V, **Berger JM**. Direct control of type IIA topoisomerase activity by a chromosomally encoded regulatory protein. Genes Dev. 2014 Jul 1;28(13):1485-97. PMCID: PMC4083091
4. Schmidt BH, Osheroff N, **Berger JM**. Structure of a topoisomerase II-DNA-nucleotide complex reveals a new control mechanism for ATPase activity. Nat Struct Mol Biol. 2012 Nov;19(11):1147-54. PMCID: PMC3492516

C.3 – Ring ATPase mechanism. A myriad number of essential cellular processes, ranging from DNA replication and chromatin remodeling to vesicle trafficking and proteolytic degradation, rely on oligomeric, ring-shaped ATPases. How a common class of ATPase folds can actively support such a broad number of systems and physiological roles is a wide-ranging basic research question. My group has focused on understanding how certain hexameric members of the RecA and AAA+ ATPase superfamilies act as DNA and RNA motor and remodeling proteins. Our efforts have helped to define the organization of higher-order

hexameric helicase assemblies and how accessory factors assist in controlling motor function and mechanism. We also have revealed the structural basis of distinct ring ATPase-opening and -assembly mechanisms that permit the loading of helicases such as the Rho transcription termination factor, the papilloma virus E1 protein, the MCM2-7 complex, and the DnaB replicative helicase and onto target nucleic acid substrates. Our efforts have generally helped establish how ATP binding and hydrolysis can be coupled to nucleic acid movement through a hexameric motor, and we determined why RecA and AAA+-type hexameric helicases move DNA or RNA with the opposing (5'-3' vs. 3'-5') polarities.

1. Lawson MR, Ma W, Bellecourt MJ, Artsimovitch I, Martin A, Landick R, Schulten K, **Berger JM**. Mechanism for the Regulated Control of Bacterial Transcription Termination by a Universal Adaptor Protein. Mol Cell. 2018. PMCID: PMC6151137
2. Lawson MR, Dyer K, **Berger JM**. Ligand-induced and small-molecule control of substrate loading in a hexameric helicase. Proc Natl Acad Sci U S A. 2016 Nov 29;113(48):13714-13719. PMCID: PMC5137764
3. Strycharska MS, Arias-Palomo E, Lyubimov AY, Erzberger JP, O'Shea VL, Bustamante CJ, **Berger JM**. "Nucleotide and partner-protein control of bacterial replicative helicase structure and function." Mol Cell. 2013 Dec 26;52(6):844-54. (*F1000 Recommended*) PMCID: PMC3929961
4. Thomsen ND and **Berger JM**, "Running in reverse: the structural basis for translocation polarity in hexameric helicases" Cell, **139:**523-534, 2009 (Accompanying Cell *Preview*, **139:**458-459; Nature *News&Views*, **462:**581-584; Rated "Exceptional", Faculty of 1000; LBNL Advanced Light Source *Science Highlight*). PMCID: PMC2772833

C.4 – Applied and Translational Research. Although a majority of my group's research has focused on answering fundamental questions concerning the connection between macromolecular structure/function relationships and biology, we also have an established track record in developing innovative solutions to technical problems and in addressing practical issues. For example, we helped co-develop (with Prof. Steven Quake, at Stanford) the first microfluidic device for crystallizing nano-volume solutions of proteins and/or nucleic acids by free-interface diffusion. We created a high-throughput screening approach to trapping protein/DNA complexes through disulfide bond formation, and we developed a novel fluorescent reporter for monitoring DNA supercoiling status in real time. Finally, we have determined co-structures of certain nucleic acid-dependent motors bound to clinically used therapeutics. We anticipate continuing with these types of efforts as the need and opportunity arise.

1. Blower TR, Bandak A, Lee ASY, Austin CA, Nitiss JL, **Berger JM**. A complex suite of loci and elements in eukaryotic type II topoisomerases determine selective sensitivity to distinct poisoning agents. Nucleic Acids Res. 2019 Sep 5;47(15):8163-8179. PMCID: PMC6735899
2. Lee JH, Wendorff TJ, **Berger JM**. Resveratrol: a novel type of topoisomerase II inhibitor. J Biol Chem. 2017 Dec 22;292(51):21011-21022. PMCID: PMC5743075
3. Hood IV, **Berger JM**. Viral hijacking of a replicative helicase loader and its implications for helicase loading control and phage replication. Elife. 2016 May 31;5. pii: e14158. PMCID: PMC4887207
4. Blower TR, Williamson BH, Kerns RJ, **Berger JM**. Crystal structure and stability of gyrase-fluoroquinolone cleaved complexes from *Mycobacterium tuberculosis*. Proc Natl Acad Sci U S A. 2016 Feb 16;113(7):1706-13. PMCID: PMC4763791

A complete list of publications, excluding chapters and articles not accessed by PubMed, can be found at: https://www.ncbi.nlm.nih.gov/myncbi/james.berger.1/bibliography/public/