APPLICANT BIOGRAPHICAL SKETCH

NAME OF APPLICANT: KELLOGG, ELIZABETH

eRA COMMONS USER NAME: LIZKELLOGG

POSITION TITLE: Assistant Professor

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	COMPLETION DATE MM/YYYY	FIELD OF STUDY
University of California, Berkeley, CA	B.S.	05/2006	Bioengineering
University of Washington, Seattle, WA	Ph.D.	12/2012	Biochemistry
University of California, Berkeley, CA	Postdoctoral Fellow	10/2018	Molecular and Cellular Biology

A. Personal Statement

My career as a structural biologist and biophysicist is defined by a deep interest in the molecular mechanisms that shape protein evolution, structure, function, and dynamics. My training has focused on developing and structurally characterizing dynamic macromolecular complexes. During my time at Berkeley, I became fascinated by the exquisite precision with which protein structures interact with nucleic acid in order to manipulate and safeguard the genome. Clearly, we have only begun to scratch the surface on our basic understanding regarding how protein-nucleic interactions drive faithful transmission of genetic information as well as regulate access to the genome. Recent structural examples, including my recent structure on the P element transposase, highlight how DNA distortions can be energetically harnessed by macromolecular complexes to carry out crucial biological functions (such as DNA transposition). However, these important complexes are often dynamic and therefore refractory to crystallography and often too large for NMR. Though Cryo-EM is becoming the tool of choice in structural biology, image-processing algorithms are currently optimized to produce high-resolution structures of rigid complexes, completely failing to capture meaningful structural information for dynamic systems. Ultimately, the goal of my lab is to expand on the strategies I utilized in my postdoctoral work to create a generalized framework for studying dynamic nucleo-protein complexes to characterize how protein and nucleic acid interactions ultimately lead to genomic regulation. This framework will utilize an inter-disciplinary approach involving cryo-EM, atomic modeling, and protein biochemistry. We have already obtained preliminary data for CRISPR-Transposon chimeras and endogenously purified transcriptional complexes and are currently working to refine the cryo-EM images and build atomic models. In addition, we are working to push the boundaries of cryo-EM with the goal of interpreting cryo-EM maps whose limited-resolution stems from structural heterogeneity.

Since my lab opened in 2018 at Cornell University, we have pursued a research program that focuses on revealing the details of dynamic and challenging nucleo-protein complexes using a combination of high-resolution cryo-EM coupled with atomic modeling. My new lab is already productive; I have an experienced technician, a graduate student and enthusiastic undergraduate students. My first paper as co-corresponding author on an unusual DNA transposase structure has recently been accepted for publication at *Nature Structure and Molecular Biology* (August 2019). Cornell is a vibrant, inter-disciplinary environment for my growing lab, and we maintain close ties across departments: Chemistry, Applied Physics, Molecular Medicine, and Microbiology. In addition to the numerous core facilities including sequencing and mass spectrometry, we have ample access to the Cornell Center for Materials Research (CCMR) which provides all the necessary equipment for cryo-EM sample preparation and imaging. Our primary tool is the 200 kV Arctica/K3 microscope which we use for cryo-screening and preliminary data acquisition.

B. Positions and Honors

2018 - present Assistant Professor, Molecular Biology and Genetics, Cornell University, Ithaca NY

Professional Memberships

2013 – present Member, Biophysical society
2016 - 2017 Member, American Society of Cellular Biology (ASCB)

Honors

2004 Guidant Fellowship award winner
2006 UC Berkeley Bioengineering Department Citation Winner
highest honor bestowed by department
2006 Summa Cum Laude in College of Engineering- Bioengineering
2007 NSF Graduate Research Fellowship, Honorable Mention
2017 Burroughs Wellcome Fund Collaborative Travel Grant Award
2018 K99/R00 Pathway to Independence Career Development Award
2020 Biophysical Society *Cryo-EM Subgroup* Program Co-Chair
2020 American Crystallographic Society, *Protein Dynamics* Co-Chair

C. Contributions to Science

1. Development of new models to describe protein free-energies and thermodynamics

I was driven during my Ph.D. to develop more accurate computational models to describe protein structure in the lab of Dr. David Baker at the University of Washington in Seattle, WA. I first led the development of a novel method to assess the thermodynamic impact of mutagenesis on protein stability. This approach's novelty results from introduction of mainchain flexibility to account for protein structural changes upon mutation. The method is capable of accurately accounting for non-conservative mutations, which are most likely to disrupt protein structure. In addition, this method is generally useful for protein engineering applications and has been useful to many labs (cited ~300 times). One of the first applications of mutagenesis deep-sequencing relied on this algorithm to interpret *in vitro* selection data.

Next, I became interested in expanding thermodynamic models to account for the entire free-energy landscape, not just the folded (i.e. native) state. Such a 'map' of the free-energy would be powerful; it could predict protein-folding pathways, their probabilities, and also the existence of long-lived (i.e. transiently stable) unfolded structures. I quickly discovered that simulation data cannot be readily converted into easily interpretable free-energy maps, therefore I developed a novel method to 'translate' kinetic states into free-energy maps. I also showed that this new technique could identify additional kinetic traps that were undetected by conventional methods of constructing free-energy landscapes.

The beginning of my own lab poses the opportunity to repurpose these techniques in order to characterize protein conformational change in the context of cryo-EM density maps. The vitrified protein samples contain a thermodynamic equilibrium of states at room temperature, therefore containing all the data needed to reconstruct a free-energy landscape. While current cryo-EM image processing algorithms focus on identifying the most populated, discrete states (equivalent to energetic basins), we ultimately aim to characterize the thermodynamic equilibria. This would give us information on the long-lived (stable) states, the short-lived (transiently structured) states, and the kinetics of interconverting between these states.

Kellogg EH, Leaver-Fay A., Baker D. "Role of Conformational Sampling in Computing Mutation-Induced Changes in Protein Structure and Stability" *Proteins: Structure, Function, Bioinformatics*. 29(3):830-8, March 2011, PMC3760476

Fowler DM., Araya CL., Fleishman SJ., **Kellogg EH.**, Stephany JJ., Baker D., Fields S. "High-Resolution Mapping of Protein Sequence-Function Relationships" *Nature Methods.* 7(9):741-6, September 2010

Liu Y., **Kellogg EH**, Liang H. "Canonical and Micro-canonical Analysis of Folding of Trpzip2: An All-atom Replica Exchange Monte-carlo Simulation Study" *Journal of Chemical Physics.* 137(4):045103, July 2012

Kellogg EH, Lange OF., Baker D., "Evaluation and optimization of discrete state models of protein folding" *Journal of Physical Chemistry B*. 116(37):11405-13, September 2012

2. **High resolution cryo-EM studies of heterogeneous cytoskeletal structures**

My interest in both protein structure and dynamics drew me to cryo-EM for my postdoctoral work in the lab of Dr. Eva Nogales. I was initially drawn to cryo-EM because functionally relevant conformations of protein structures are preserved *in solution*. During my postdoctoral training, the cryo-EM projects I became interested in consisted almost entirely of challenging cases of heterogeneous and dynamic complexes; these systems represented the limit of current cryo-EM techniques and presented opportunities to capitalize on my modeling expertise. One crucial cellular system whose dynamics are intrinsic to its function is the microtubule, a cytoskeletal filament that cannot be crystallized in its physiologically relevant (filamentous) form. I first interpreted the medium-resolution (4.5 – 5.5 Å) cryo-EM maps (obtained using film) of microtubules in different nucleotide-bound states. Nucleotide-hydrolysis results in microtubule depolymerization. While we could quantify the subtle structural changes in the lattice by monitoring the long-range helical parameters, the resolution of the maps was too poor for manual model-building techniques. My structural interpretations (obtained using Rosetta) helped to inform future structural studies carried out at higher resolution using the direct electron detector.

Strikingly, the Taxol-stabilized microtubules were the least well-resolved and the most puzzling from a structural standpoint. Therefore, I set out to characterize the molecular mechanism of Taxol-stabilization using the new K2 camera. Using new 3D image sorting techniques, I was able to identify the source of heterogeneity in Taxol-stabilized microtubules was via flexibility within the microtubule walls. Interestingly, this was not isolated to Taxol but was a feature of any small molecule that bound to the Taxol-binding pocket. Even more surprising, I identified distinct 'modes' of microtubule stabilization. Whereas Taxol-binding induced heterogeneity within the structure of the microtubule, a different anti-cancer drug (peloruside) had an altogether separate effect: it seemed to stabilize microtubules by regularizing the microtubule walls. The results of these studies were published in two first author papers in *Cell* and *JMB*.

The capstone of my postdoctoral work focused on revealing the atomic structure of the physiologically relevant, microtubule-bound form of tau. Using a combination of computational protein modeling, cryo-EM, and protein engineering, we identified the atomic interactions between tau and tubulin that account for tau's ability to nucleate and stabilize microtubules *in vivo*. The implications of this structural study are far-reaching. First, I was able to make specific biological conclusions regarding tau's native function and correlate this with biochemical data that had puzzled the tau field for decades. More importantly, this study is the first demonstration of the power of modeling combined with cryo-EM. As one of the most extreme examples of a highly dynamic protein structures, tau is a protein that has no regular secondary structure or globular fold. The results described in this paper reveals the power of this approach to study the structure of even the most dynamic (and transiently structured) protein complexes.

Alushin GM.*, Lander GC.*, **Kellogg EH***, Zhang R., Baker D., Nogales E. "High-resolution microtubule structures reveal the structural transitions in $\alpha\beta$-tubulin upon GTP hydrolysis" *Cell* 157(5):1117-29, May 2014
*equal contribution

Kellogg EH., Hejab NMA, Howes S., Northcote P., Miller JH., Diaz FJ., Downing KH. and Nogales E. "Insights into the distinct mechanisms of action of taxane and non-taxane microtubule stabilizers from cryo-EM structures" *Journal of Molecular Biology* 429 (5):633-646, March 2017

Howes SC., Geyer E., LaFrance BJ., Zhang R., **Kellogg EH**., Westermann S., Rice L., and Nogales E., "Structural and functional differences between yeast and mammalian microtubules revealed by cryo-EM" *Journal of Cell Biology* DOI: 10.1083/jcb.201612195, June 2017

Kellogg EH*, Hejab NMA*, Poepsel S., Downing KH., DiMaio F, Nogales E. "Near-atomic model of microtubule-tau interactions" *Science* 360(6394):1242-6, May 2018, PMID29748322
*equal contribution

3. **A novel structure of a unique DNA transposase: cryo-EM structure of P-element transposase**

Transposons, or mobile genetic elements, are selfish genes that propagate throughout host genomes. Though the encoded protein products, called transposases, share similarities at the fold level, the lack of sequence similarities among diverse transposon superfamilies hinders both a comprehensive understanding of the molecular requirements of transposition and an evolutionary history of these ancient macromolecular machines. One such superfamily, called P-element transposons, are widely found throughout *Drosophila* populations and have unusual and unique features, including both the requirement of GTP as a co-factor for transposition as well as the generation of long 17-bp single-stranded DNA overhangs upon excision from the genome. In collaboration with Donald Rio's lab at UC Berkeley, I obtained a near-atomic (3.6 Å) resolution cryo-EM structure of the P-element transposase strand-transfer (i.e. integration) complex. Surprisingly, the structure revealed a highly unusual DNA structure, consisting of a register-shifted DNA duplex, that is stabilized by GTP. This model is predictive and accurate, as it is consistent with biochemical data that tests the importance of this register shift (using mutagenesis) and reveals the atomic interactions that necessitates the use of GTP rather than other abundantly available nucleotides such as ATP. All in all, this structure constitutes the first step that describes the unique transposition mechanism of the P-element transposase and explains how this evolutionarily distinct and divergent family is related to other 'cut-and-paste' DNA transposase superfamilies.

Kellogg EH. "Towards a high-resolution cryo-EM structure of the P-element transposase" *speaker*, Keystone meeting for mobile genetic elements, Santa Fe, Mexico, March 2017.

Ghanim G.*, **Kellogg EH*≠**, Nogales E., Rio DC≠. "Molecular mechanism of the P-element transposase" *accepted at Nature Structural and Molecular Biology*
*equal contribution
≠co-corresponding author

Complete List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/sites/myncbi/elizabeth.kellogg.2/bibliography/55165175/public/?sort=date&direction=ascending

D. Research Support

Ongoing Research Support
R00 GM124463 Kellogg, Elizabeth (PI) 01/01/2019 – 01/01/2021
"Molecular Basis of Genome Organization and Integrity using Cryo-EM"
The goal of this research project is to structurally characterize important nucleoprotein complexes that play crucial roles in genome organization and integrity using cryo-EM
Role: PI

Completed Research Support
K99 GM124463 Kellogg, Elizabeth (PI) 09/01/2017 – 09/30/2018
"Towards an understanding of telomere end protection: Cryo-EM studies of shelterin structure and function"
The goal of this research project is to structurally characterize the shelterin complex on model telomere (DNA) substrates using cryo-EM
Role: PI

Current
R00 GM124463 Kellogg, Elizabeth (PI) 01/01/2019 – 01/01/2021
"Molecular Basis of Genome Organization and Integrity using Cryo-EM"
The goal of this research project is to structurally characterize important nucleoprotein complexes that play crucial roles in genome organization and integrity using cryo-EM
Role: PI

Pending

RM1 Kellogg, Elizabeth (collaborator)
"Structure, Function, and Dynamics of Macro-molecular Complexes that Execute and Regulate Genome Function"

Beckman Foundation Kellogg, Elizabeth (PI)
"Unleashing the Full Potential of Cryo-EM: From Static Portraits to Dynamic Movies"