BIOGRAPHICAL SKETCH

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NAME: Joseph H. Davis

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

POSITION TITLE: Whitehead Career Development Assistant Professor of Biology

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	Completion Date	FIELD OF STUDY
University of California, Berkeley	B.A.	12/2003	Biological Engineering (Dr. Michael A. Marletta). *Nitric Oxide sensing in prokaryotes*
University of California, Berkeley	B.S.	12/2003	Computer Science (Dr. Richard Karp) *Computational folding of RNAs*
Massachusetts Institute of Technology	Ph.D.	04/2010	Biology (Dr. Robert T. Sauer). *Understanding and harnessing energy-dependent proteolysis*
The Scripps Research Institute	Post-doctoral	07/2017	Biochemistry (Dr. James R. Williamson). *Modular assembly of a bacterial ribosome*
The Sanford Burnham Prebys Institute	Visiting scientist	07/2017	Biology of aging and autophagy (Dr. Malene Hansen).

A. Personal Statement

Interests: I have always been interested in how large cellular complexes are built, how these molecular machines are regulated and directed at their targets, and how they are eventually recycled and degraded. These interests have been complimented by my longstanding interests in developing methods to understand such problems. In graduate school, I designed and engineered bacterial protease complexes to understand how they worked and revealed a simple, yet fundamental tethering mechanism was sufficient for substrate delivery. Additionally, I developed methods to immobilize the bacterial protease ClpXP on a microscope slide to monitor the enzyme's activity with single-molecule resolution. This approach has since been used widely to study these machines.

After graduate school, I helped start a metabolic engineering company (Ginkgo BioWorks) as the first employee. There, we engineered protein scaffolds to help assemble complexes of metabolic enzymes to produce specialty and fine chemicals in microbes. After our initial goals were met, I decided to return to Academia to pursue longer-term goals by developing tools and techniques to understand macromolecular complex assembly. As a post-doc, I developed quantitative mass spectrometry and cryo-EM analysis methods to monitor ribosome assembly kinetics in vivo, to analyze the structures of heterogeneous assembly intermediates, and to use this information to probe the assembly process. During this time, I addressed several fundamental questions about ribosome assembly including: (i) How rapid is assembly under ideal or sub-optimal conditions? (ii) What role do assembly factors play? (iii) Is assembly sequential or parallel? (iv) How does cooperative protein binding and rRNA folding drive assembly, and (iv) How common are assembly errors and how are they fixed?

As an independent investigator, my lab has developed a series of cryoEM analysis tools, including cryoDRGN, MAVEn, and tomoDRGN, and we are actively applying these to a variety of macromolecular complexes to better understand how structural heterogeneity is linked to function.

My Training: I hold undergraduate degrees in Computer Science and Biological Engineering, which has helped immensely in my lab's software development efforts. Additionally, over ~20 years spent as a researcher, I have learned a wide array of biochemical, biophysical, and genetic techniques that have allowed me to address biological questions mechanistically. Finally, my significant managerial experience at a small, growing startup company has been critical in establishing my independent lab and in recruiting and training my extremely talented and intellectually diverse team, which currently consists of nine Ph.D. students and two post-docs with backgrounds in Mathematics, Computer Science, Biology, Chemistry, and Chemical Engineering. Given the size

and composition of my lab, much of my focus in dedicated to mentoring this incredible group of scientists, and I will work directly with trainees supported by this grant to foster their scientific and professional development.

Training record: My lab is still relatively new, with students first joining my lab in 2018. Thus, only a single Ph.D. student has graduated from the group, however her Ph.D. was extremely successful, and she secured a tenure track faculty position at Princeton University immediately after graduating from my lab. Four PhD students are slated to graduate this academic year, with one pursuing an independent Fellows position, and the others currently arranging post-doctoral studies. Two post-docs who trained in my group are now working a Scientists I (1) and Principal Scientist (1) at local biotech companies. A current post-doc in my group is currently on the job market for a tenure-track faculty position and has a number of interviews scheduled for this Fall.

B. Positions, Scientific Appointments, and Honors

ACTIVITY/ OCCUPATION	START DATE	END DATE	FIELD	INSTITUTION/ COMPANY	SUPERVISOR/ EMPLOYER
Associate Professor	08/01/23	-	Biology	Mass. Inst. Tech. (MIT)	-
Associate Member	04/01/21	-	Biology	The Broad Institute	-
Assistant Professor	08/01/17	08/01/23	Biology	Mass. Inst. Tech. (MIT)	-
Visiting Scientist	08/01/15	08/01/17	Genetics	Sanford Burnham Inst.	Dr. Malene Hansen
Post-doc. Fellow	01/01/12	07/01/17	Structural Bio.	Scripps Research Inst.	Dr. James Williamson
1st employee	04/01/10	01/01/12	Synthetic Bio.	Ginkgo BioWorks, Inc.	Dr. Tom Knight
Technician	06/01/02	11/01/04	Biochemistry	UC Berkeley	Dr. Michael A. Marletta

Honors

2021	Alfred P. Sloan Foundation Fellow in Computation and Evolutionary Molecular Biology
2021	National Science Foundation CAREER Award
2020	James H. Ferry Faculty Research Award, MIT.
2019	Whitehead career development chair, MIT.
2018	Charles E. Reed Faculty Initiative Award, MIT.
2012-2015	Jane Coffin Childs Post-doctoral Fellowship.
2008-2010	Vertex Research Fellowship.
2000-2004	Regent's Scholarship, UC Berkeley.

Other Professional Experiences

2010 – present	Reviewer, *Cell, Mol. Cell, Cell Rep., Nature, Nature Structure & Mol. Biol., Nature Comm., Nature Chem. Biol., EMBO, PNAS, eLife, RNA, J. Mol. Bio., J. Proteomics Res.,*
2022	Session Chair for ML in CryoEM*, American Crystallographic Association*

C. Contributions to Science

i. Sensing of nitric oxide by bacterial heme-binding proteins. As an undergraduate student at UC Berkeley (2000-2003), I gained extensive independent research experience in the laboratory of Dr. Michael A. Marletta. There, I learned a variety of biochemical techniques to express, purify, and characterize a family of gas-sensing hemeproteins, revealing conserved sequence motifs that allows these proteins to discriminate between chemically similar gasses - O_2 and NO·.

1. Karow, D.S., Pan, D., Davis, J.H., Behrends, S., Mathies, R.A., & Marletta, M.A. Characterization of functional heme domains from soluble guanylate cyclase. *Biochemistry*, **2005**.
2. Boon, E.M., Davis, J.H., Tran, R., Karow, D.S., Huang, S.H., Pan, D., Miazgowicz, M.M., Mathies, R.A., & Marletta, M.A. Nitric oxide binding to prokaryotic homologs of the soluble guanylate cyclase beta1 H-NOX domain. *The Journal of Biological Chemistry*, **2006**.

ii. Assembly and function of bacterial proteases. The bacterial ClpXP protease system includes unfoldase, protease, and adaptor proteins that act in concert to target, deliver, unfold and degrade substrate proteins. I determined the minimal set of structural elements required for adaptor-mediated substrate delivery by

engineering synthetic protease and adaptor chimeras. This work revealed that simple tethering of the adaptor was sufficient for substrate delivery and demonstrated that the adaptor/protease link must be dynamic and of relatively low affinity. The resulting system, which rapidly and specifically degrades tagged substrates, provided a new tool to probe the function of essential genes and highlighted how the design of synthetic macromolecular complexes could be improved using a detailed understanding of natural complexes. In related work, I studied the kinetics of substrate delivery and degradation both in bulk and in single-molecule experiments, improving our understanding of how these machines function. I have now continued this work by applying cryoEM and our related analysis tools to better understand the operating principles of ClpXP.

3. Davis JH, Baker TA, Sauer RT. Engineering synthetic adaptors and substrates for controlled ClpXP degradation. *Journal of Biological Chemistry* **2009**.
4. Shin Y*, Davis JH*, Brau RR, Martin A, Kenniston JA, Baker TA, Sauer RT, Lang MJ. Single-molecule denaturation and degradation of proteins by the AAA+ ClpXP protease. *Proceedings of the National Academy of Science U.S.A.* **2009**.
5. Davis JH, Baker TA, Sauer RT. Small-molecule control of protein degradation using split adaptors. *ACS Chemical Biology* **2011**.
6. Ghanbarpour A, Cohen SE, Fei X, Bell TA, Baker TA, Davis JH*, Sauer RT*. A closed translocation channel in the substrate-free AAA+ ClpXP protease diminishes rouge degradation. *bioRxiv; in press at Nature Communication* **2023.**
7. Ghanbarpour A, Fei X, Baker TA, Davis JH*, Sauer RT*. The SspB adaptor drives structural changes in the AAA+ ClpXP protease during ssrA-tagged substrate delivery. *Proceedings of the National Academy of Science U.S.A*. **2023**.

iii. High-throughput, combinatorial DNA assembly. I was hired as the first employee at Ginkgo BioWorks, a synthetic biology start-up company. There I was instrumental in designing the lab, hiring and managing incoming scientists, and securing both public and private funds; this experience has been directly portable to my independent group. At Ginkgo, my team developed a novel DNA assembly scheme that allows for high-throughput, combinatorial cloning of large (>10 component) plasmids for expression/regulation of entire metabolic pathways. We also used a series of insulated promoters I had previously designed to tightly regulate production of various metabolic enzymes, and regulated proteolysis tags from my graduate studies. This work resulted three engineered strains that produced the desired small molecule, and a series of patents protecting the underlying technology.

8. Kelly JR, Rubin AJ, Davis JH, *et al.* Measuring the activity of BioBrick promoters using an in vivo reference standard. *J. Biol. Eng.* **2009**.
9. Davis JH, Rubin AJ, Sauer RT. Design, construction and characterization of a set of insulted bacterial promoters. *Nucleic Acids Research* **2011**.

Patents / patent applications:

10. Way JC, Davis JH. Methods and molecules for yield improvement involving metabolic engineering. PCT International Application Serial No. PCT/US2010/036902
11. Davis JH, Shetty RP. Genetically-encoded volatile reporters of cell state. USPTO Patent Application # 61/423,569. Priority Date 12/15/2010.

iv. Modular assembly of the bacterial ribosome, and the role of assembly factors. Ribosome biogenesis is a multistep process requiring coordinated folding and binding of three large ribosomal RNAs and ~50 ribosomal proteins, and it is guided by a series of assembly factors. In collaboration with the Ortega and Britton labs, I developed a hybrid genetic/qMS/cryo-EM approach to understand the role of various assembly factors (AFs). These methods allowed us to stall assembly and thus accumulate assembly intermediates. I developed a method using pulse-labeling and mass spectrometry to determine if the accumulated intermediates were competent for maturation and to measure their protein composition. Additionally, I employed single particle cryo-EM to understand the structural consequences of AF depletion and to relate various assembly intermediates to one another. Using this approach, we found that: *(i)* a series of AFs are critical for assembly of an entire block of the large subunit; *(ii)* the ribosomal functional centers mature last, suggesting an evolutionary advantage to ensuring that subunits are not able to enter the translational cycle until they have fully matured; (*iii*) the order of r-protein association is highly flexible and can be 're-routed', (*iv*) limitation of a single r-protein has long-range structural effects, (*v*) subunit assembly is initiated on the

solvent face of the particle and can proceed along three parallel pathways, and (*vi*) RNA misfolding events are common.

11. Jomaa A*, Jain N*, Davis JH*. Williamson JR, Britton RA, Ortega J, Functional domains of the 50S subunit mature late in the assembly process. *Nucleic Acids Research* **2014**.
12. Stokes JM, Davis JH, Mangat CS, Williamson JR, Brown ED. Discovery of a small molecule that inhibits bacterial ribosome biogenesis. *Elife* **2014**.
13. Davis JH*, Tan YZ*, Carragher B, Potter CS, Lyumkis D, Williamson JR. Modular assembly of the bacterial large ribosomal subunit. *Cell* **2016**.
14. Razi A, Davis JH, Hao Y, Jahagirdar D, Thurlow B, Basu K, Jain N, Gomez-Blanco J, Britton RA, Vargas J, Guarne A, Woodson SA, Williamson JR, Ortega J. Role of Era in assembly and homeostasis of the ribosomal small subunit. *Nucleic Acids Research* **2019**.
15. Sun J*, Kinman LK*, Jahagirdar J, Ortega J, Davis JH. KsgA facilitates ribosomal small subunit maturation by proofreading a key structural lesion. *Nature Structural and Molecular Biology* **2023**.

v. **Methodological advances in single particle Cryo-EM.** Motivated by a desire to determine structures of highly heterogeneous ribosome assembly intermediates, I helped develop three methodological advances that have seen widespread adoption. First, when attempting to image very immature ribosome assembly intermediates, I found that pathologically preferred orientation limited my ability to perform 3D reconstructions. In response, I helped develop a tilted-stage collection strategy that has since been widely adapted. Second, I developed a computational approach to rapidly compare 10s-100s of structures of related assembly intermediates given an atomic model. This work allowed me to readily interpret the large number of assembly intermediates I had identified in [14] through rounds of 3D classification. Finally, as an independent investigator, my group has developed a completely novel 3D reconstruction method that allows for the reconstruction of continuously heterogeneous structures. This method has generated great enthusiasm within the structural biology community, and we are actively collaborating with ~6 groups to apply our method to their most challenging datasets.

16. Tan YZ, Baldwin PR, Davis JH, Williamson JR, Potter CS, Carragher B, Lyumkis D. Addressing preferred specimen orientation in single-particle cryo-EM through tilting. *Nature Methods* **2017**.
17. Zhong E, Bepler T, Berger B, Davis JH. CryoDRGN: Reconstruction of heterogeneous cryo-EM structures using neural networks. *Nature Methods* **2021**.
18. Zhong E, Lerer A, Davis JH, Berger B. CryoDRGN2: Ab initio neural reconstruction of 3D protein structures from real cryo-EM images. *Proceedings of the IEEE/CVF ICCV* **2021**.
19. Kinman L, Powell B, Zhong E, Berger B, Davis JH. Uncovering structural ensembles from single particle cryo-EM data using cryoDRGN. *Nature Protocols* **2022**.
20. Powel B, Davis JH. Learning structural heterogeneity from cryo-electron sub-tomograms with tomoDRGN. *bioRxiv; in revision at Nature Methods* **2023**.

Complete list of Published Work in My Bibliography:
https://www.ncbi.nlm.nih.gov/myncbi/browse/collection/52788705/