BIOGRAPHICAL SKETCH

NAME: Justin Finley Acheson

eRA COMMONS USER NAME: JFA9UNIH

POSITION TITLE: Postdoctoral Fellow

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	Start Date	Completion Date	FIELD OF STUDY
Indiana University East	-	08/2004	-	Biology
Indiana University Purdue University - Indianapolis	B.S.	08/2007	05/2009	Biology
Indiana University Purdue University - Indianapolis	B.A.	08/2007	05/2009	Chemistry
University of Wisconsin - Madison	Ph.D.	08/2009	04/2015	Biochemistry
University of Tokyo (JAPAN)	n/a	06/2013	08/2013	Enzymology
University of Virginia (postdoc)	n/a	04/2015	10/2017	Membrane Biophysics
University of Virginia (postdoc fellow)	n/a	11/2017	present	Membrane Biophysics

A. Personal Statement

My long-term research goals are to understand multi-membrane spanning secretion systems that contribute to bacterial pathogenesis and/or antibiotic resistance. To get to this point I took several unexpected turns. As an undergraduate I studied disordered proteins under Dr. A. Keith Dunker and Dr. Vladimir (Volodya) Uversky. Through their direction, I learned of the world of molten globules and the importance of disorder for function. Surprisingly, when discussing my intention to go to graduate school Dr. Dunker suggested I train as a crystallographer. Taking that advice, I rotated in structural biology labs upon entering the Integrated Program in Biochemistry at the University of Wisconsin – Madison. I joined Dr. Brian Fox's lab, because I respected his hands-off approach that gave me freedom to pursue the overarching research goals. While Dr. Fox was not a crystallographer, I was fortunate for the access to Dr. George Phillip's and his lab personnel/equipment, as well as Dr. Ivan Rayment. In Dr. Fox's lab, I learned the remarkable catalytic power of O2 dependent metalloenzymes focusing on diiron enzymes. I first focused mainly on necessary protein-protein interactions and then shifted to investigating catalysis *in crystallo*. To expand my research capabilities, I received a National Science Foundation Eastern Asia and Pacific Summer Institutes Fellowship to research in Dr. Hideaki Nojiri's lab at the University of Tokyo, Japan. Dr. Hideaki investigates a similar monoiron enzymes and I spent the summer learning isothermal calorimetry titration in redox dependent enzymes. The results of my binding and crystallographic experiments revealed how protein-protein interactions independently influence the reaction mechanism. At the molecular level, I was also able to trap intermediates by pushing the reaction *in crystallo* by tightly controlling the redox state of the enzymes. As I continued throughout my graduate research I became more and more enticed the work of membrane structural biologists. While I am a champion of basic research, I wanted to expand my work into meaningful research that could have a positive impact on human health.

The projects I have been involved with throughout my career have allowed me to develop many skills in structural biology including X-Ray crystallography, enzymology, protein-protein interactions, electron-transfer, bioinformatics, and more. As a postdoc, I have gained skills in membrane protein expression and purification,

however I still lack techniques and skills that would allow me to become an independent investigator. Joining Dr. Zimmer's lab has been a great in gaining these new skills, and his excitement for research and approach is something to be emulated. The proposed research will broaden my expertise by combining structural techniques emphasizing cryo-EM. Dr. Zimmer has an impressive publication record with experience directing several high impact projects. His exceptional work will be invaluable for me to gain experience thinking as an independent researcher.

B. Positions and Honors

Positions and Employment

ACTIVITY/ OCCUPATION	START DATE MM/YYYY	END DATE MM/YYYY	FIELD	INSTITUTION/ COMPANY	SUPERVISOR/ EMPLOYER
Visiting Researcher	06/2013	08/2013	Enzymology	University of Tokyo	Hideaki Nojiri
Postdoc	04/2015	-	Membrane structural biology		Jochen Zimmer

B. Positions and Honors

Positions and Employment

2015 - Postdoctoral Researcher, University of Virginia
2017 - NIH Ruth Kirstein NRSA F32 Postdoctoral Fellow

Other Experiences and Professional Memberships

2010 American Crystallographic Society
2013 US-JSPS Alumni Association
2014 Molecular Biology Society of Japan
2017 Biophysical Society

Academic and Professional Honors

Vilas Conference Travel Award	2014
Steenbock Research Travel Award	2014
Thomsen Distinguished Graduate Research Fellowship	2012 – 2013
NSF/JSPS EAPSI Summer Fellow (PI) - University of Tokyo, Prof. Hideaki Nojiri	Summer 2013
NIH Ruth Kirstein NRSA F32 Postdoctoral Fellow	2017-

C. Contributions to Science

Peer-reviewed Publications

Acheson JF, Bailey LJ, Brunold TC, Fox BG. In-crystal reaction of toluene bound diiron hydroxylase. *Nature*. (2017)

Morgan JL, **Acheson JF**, Zimmer J. Structure of a Type-1 secretion system ABC transporter. *Structure*. **25**(3), 522-529 (2017)

Acheson JF, Moseson H, Fox BG. Structure of T4moF, the Toluene 4-monooxygenase ferredoxin oxidoreductase *Biochemistry* **54**, 5980-8 (2015)

Acheson JF, Bailey LJ, Elsen NL, and Fox BG. Structural basis for biomolecular recognition in overlapping binding sites in a diiron enzyme system *Nat. Commun.* **5**:5009 (2014).

Takasuka TE, **Acheson JF,** Bianchetti CM, Prom BM, Bergeman LF, Book AJ, Currie CR, Fox BG. Biochemical properties and atomic resolution structure of a proteolytically processed beta-mannanase from cellulolytic Streptomyces sp. SirexAA-E. *PLoS One* **9**, e94166 (2014).

Bailey LJ*, **Acheson JF***, McCoy JG, Elsen NL, Phillips Jr. GN, Fox BG. Crystallographic analysis of active site contributions to regiospecificity in the diiron enzyme toluene 4-monooxygenase. *Biochemistry* **51**, 1101-1113 (2012).

Yamada J, Phillips JL, Patel S, Goldfein G, Caestagne-Morelli A, Huang H, Reza R, **Acheson J**, Krishnan VV, Newsman S, Gopinathan A, Lau EY, Colvin ME, Uversky VN, and Reaxch MF. A bimodal distribution of two distinct categories of intrinsically disordered structures with separate functions in FG nucleoporins. *Mol Cell Proteomics* **9**, (2010).

Presentations

Structural definition of electron-transfer complexes in two distinct ferredoxin-dependent diiron enzymes. Molecular Biology Society of Japan, Yokohama, Japan. **Acheson JF**, Bailey LJ, Elsen, NL, & Fox BG. (poster, 2014)

From global to local, creating a functional electron-transfer complex. UW – Madison X-Ray Super-group **Acheson JF** (presentation, 2014)

Structure of the diiron hydroxylase-Reiske ferredoxin electron-transfer complex. 32nd Midwest Enzyme Chemistry Conference **Acheson JF** & Fox BG. (poster, 2012)

Structural characterization of outer-sphere mutations in toluene 4-monooxygenase. American Crystallographic Association Bailey LJ*, **Acheson JF***, Fox BG. (poster, 2010)
*Authors contributed equally

Protein Data Bank Entries

Structure of the toluene 4-monooxygenase NADH oxidoreductase T4moF, K270S K271S variant (PDB 4GWM)

Crystal Structure of the toluene 4-monoxygenase hydroxylase-ferredoxin C7S E16C C84A C85A variant electron-transfer complex. (PDB 4P1B)

Crystal Structure of the toluene 4-monoxygenase hydroxylase-ferredoxin C7S C84A C85A variant electron-transfer complex. (PDB 4P1C)

High resolution structure of the catalytic domain of mannanase SActE_2347 from *Streptomyces* sp. SirexAA-E (PDB 4FK9)

Toluene 4-monooygenase HD complex with inhibitor bromobenzoate (PDB 3Q3M)

Toluene 4-monooygenase HD complex with p-nitrophenol (PDB 3Q3N)

Toluene 4-monooygenase HD complex with phenol (PDB 3Q3O)

Toluene 4-monooygenase HD complex with p-cresol (PDB 3Q14)

Toluene 4-monooygenase HD complex with inhibitor p-aminobenzoate (PDB 3Q2A)

Toluene bound in the resting active site of toluene 4-monooxygenase (T4moH) (PDB 5TDS)

Oxygenated toluene intermediate in toluene 4-monooxygenase (T4moHD) after reaction in the crystal (PDB 5TDT)

Toluene 4-monooxygenase (T4moHD) bound to product after turnover in crystal (PDB 5TDU)

Intermediate O2 diiron complex in the Q228A variant of Toluene 4-moonoxygenase (T4moHD) (PDB 5TDV)

D. Additional Information: Research Support and/or Scholastic Performance

YEAR	COURSE TITLE	GRADE
	IU EAST	
2004	Principle of Chemistry I	A
2004	Principle of Chemistry II	A
2005	Calculus I	B+
2006	Microbiology	A
2006	Organic Chemistry I	A
2006	Genetics	A
2006	Protein Purification	A
2007	General Physics II	A
2007	Organic Chemistry II	A
2007	Biological Chemistry	A
	IUPUI	
2007	Immunology with Lab	A
2007	Analytical Chemistry	A
2007	Independent Research	A
2007	Ecology and Evolution	A-
2008	Cell Biology	A
2008	Embryology	B+
2008	Intro Instrumental Analysis	A-
2008	Cellular Biochemistry	B+
2008	Integral Calculus and Analytical Geometry 2	B-
2009	Biosynthesis and Physiology	B
2009	Physical Chemistry	B-
2009	Senior Research Thesis	A
	UW – MADISON	
2009	Protein & Enzyme Structure & Function	AB

YEAR	COURSE TITLE	GRADE
2009	Methods in Biochemistry	AB
2009	Professional Responsibility (Ethics)	A
2010	Coenzymes & Cofactors	A
2010	Macromolecular Crystallography	A
2010	Biochemical Functions of Macromolecules	B
2010	Methods of Enzyme Action	S
2011	Chemical Crystallography	B
2011	Advanced Microbial Physiology	B

BIOGRAPHICAL SKETCH

NAME: Jochen Zimmer, D. Phil.

eRA COMMONS USER NAME: JOCHEN

POSITION TITLE: Associate Professor

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	Completion Date	FIELD OF STUDY
University of Bayreuth, Bayreuth/D		9/95-7/97	Undergraduate degree
Swiss Federal Institute of Technology, Zurich/CH	Diploma	9/97-3/00	Graduate degree
University of Oxford, Oxford/UK	D.Phil.	9/00-9/03	Molecular biophysics
Harvard Medical School, Boston/USA	Post-doctoral	1/04-9/09	Biochemistry and protein crystallography

A. Personal Statement

I have always been interested in how cells communicate with their environments, particularly how hydrophilic solutes and biopolymers cross the hydrophobic lipid membrane that surrounds every cell. My training in molecular and structural biology in the laboratories of Prof. Dame Louise Johnson and Dr. Declan Doyle at the University of Oxford/UK and Prof. Tom Rapoport at the Harvard Medical School/USA has been inspiring and encouraged me to establish an equally creative and curiosity-driven environment in my own group.

My laboratory uses a broad spectrum of structural and molecular biology techniques to describe biological processes on a molecular level. Our current focus is studying the synthesis and membrane translocation of extracellular complex carbohydrates, such as alginate, cellulose and hyaluronan. The approach is multi-pronged: we aim to functionally reconstitute complex biological processes *in vitro* from purified components and to determine the 3-dimensional structures of the key factors accounting for the observed activities.

Our current model systems include microbial, vertebrate, and plant systems that form at least two groups. One represents membrane-integrated processive glycosyltransferases that couple polysaccharide synthesis with the secretion of polymer; the other includes transporter-mediated translocation, where polysaccharides are synthesized in the cytosol and secreted via dedicated ABC transporters. Examples include cell surface polysaccharides such as cellulose, hyaluronan and alginate (group 1) and microbial O antigens and capsular polysaccharides (group 2).

We use all techniques of molecular biology, biochemistry and glycobiology to isolate, functionally reconstitute, and characterize the proteins under study. For structural analyses, we employ X-ray crystallography and, increasingly, cryo-electron microscopy to gain mechanistic insights into the translocation processes.

B. Positions and Honors

01/2004-2/2009 Post-doctoral research fellow, Harvard Medical School, Department of Cell Biology, Boston/USA

10/2009-3/2015 Assistant Professor, University of Virginia School of Medicine, Department of Molecular Physiology and Biological Physics, Charlottesville/USA

04/2015 - Associate Professor, University of Virginia School of Medicine, Department of Molecular Physiology and Biological Physics, Charlottesville/USA

Honors

2000: Boehringer Ingelheim Research Fellow
2004: Hulme Continuation Grant, Brasenose College, University of Oxford
2015: International Bruce Stone Award for excellence in plant biochemistry
2017: Pinn Scholar Award, University of Virginia School of Medicine

C. Contribution to science

1. Structural and functional characterization of post-translational protein translocation

As a postdoctoral researcher in Tom Rapoport's laboratory at the Harvard Medical School, I performed *in vitro* protein translocation assays to study the mechanism by which the bacterial ATPase SecA moves unfolded polypeptides through the membrane-embedded protein translocation channel (SecY channel). I also determined the crystal structure of the SecA ATPase in complex with the SecY channel. This structure reveals how SecA interacts with the SecY channel and provides a mechanistic model for SecA-mediated protein translocation. Based on this structure, we designed biochemical disulfide cross-linking experiments that allowed us to identify the domains of SecA that contact and move the translocating polypeptide chain. Taken together, this work was perceived as one of the research highlights of 2008 published in Nature.

- **Zimmer, J**., Li, W. & Rapoport T. A., A novel dimer interface and conformational transitions revealed by an X-ray structure of B. subtilis SecA, J. Mol. Biol. 2006, 364(3), 259-65.
- Erlandson, K., Miller, S., Nam, Y., Osborne, A. R., **Zimmer, J**. & Rapoport T. A., A role for the two-helix finger of the SecA ATPase in protein translocation, Nature 2008, 455, 984-987, (HHMI funded).
- **Zimmer, J**., Nam, Y. & Rapoport, T., A. Structure of a complex of the ATPase SecA and the protein translocation channel. Nature 2008, 455, 936-943, (HHMI funded).
- **Zimmer, J**., Rapoport, T.A., Conformational flexibility and peptide interaction of the translocation ATPase SecA, J. Mol. Biol. 2009, 394(4), 606-612, PMC2832196.

2. Functional analyses of hyaluronan biosynthesis

Hyaluronan (HA) is one of the most abundant glycosaminoglycans in the human body where it forms a major component of the extracellular matrix, particularly in soft connective tissues. HA is a hetero-polysaccharide consisting of alternating glucuronic acid and N-acetylglucosamine units, formed by a membrane-embedded glycosyltransferase, the HA synthase (HAS). By establishing a chemically defined *in vitro* HA biosynthesis assay, my laboratory demonstrated that HAS not only synthesizes HA from UDP-activated substrates, but also translocates the nascent HA polymer across the membrane through a pore formed by its own transmembrane region. Further, we compared HA biosynthesis in bacteria and viruses and established that the model systems use fundamentally different reaction mechanisms. While bacterial HAS function as an obligate dimer and elongates the 'reducing end' of the HA polymer, the viral enzyme (homologous to vertebrate HAS) is monomeric and elongates HA's opposite end. Thus, bacterial HASs may be a result of convergent evolution.

- Hubbard, C., Patel, M.S. & **Zimmer, J**., The hyaluronan synthase catalyzes the synthesis and membrane translocation of hyaluronan, J. Mol. Biol. 2012, 418(1-2), 21-31, (start-up funds)
- Blackburn, M., Hubbard, C., Kiessling, V., Kloss, B., Tamm, L., **Zimmer, J.**, Distinct mechanisms of hyalurnan biosynthesis in different kingdoms of life, Glycobiology 2018 Feb 1;28(2):108-121 PMC6192386

3. Mechanism of cellulose biosynthesis

Cellulose is the most abundant biopolymer on earth and is primarily formed by vascular plants as a major component of the cell wall. Cellulose is also a common biofilm component where it is synthesized and secreted via an inner and, in Gram-negative bacteria, outer membrane-spanning complex. Cellulose is a linear polymer of glucose molecules synthesized inside the cell by the membrane-integrated cellulose synthase. During polymer synthesis, the nascent chain is secreted through a channel formed by cellulose synthase. To unravel the mechanism of cellulose biosynthesis, my laboratory first established an in vitro cellulose biosynthesis assay and solved several crystal structures of the bacterial cellulose synthase complex containing the catalytically active BcsA and associated BcsB subunits. Structures of 5 different BcsA-B conformations at

intermediate states during cellulose synthesis and membrane translocation combined with biochemical analyses provided unparalleled insights into the enzyme's reaction and translocation mechanism.
- Morgan, JLW., Strumillo, J., **Zimmer, J**., Crystallographic snapshot of cellulose synthesis and membrane translocation, Nature 2013, 493, 181-6, PMC3542415
- Omadjela, O., Narahari, A., Strumillo, J., Mélida, H., Mazur, O., Bulone, V., **Zimmer, J**., BcsA and BcsB form the catalytically active core of bacterial cellulose synthase sufficient for in vitro cellulose synthesis, PNAS 2013, Oct 29;110(44):17856-61, PMC3816479
- Morgan, JLW., McNamara, JT., **Zimmer, J**., Mechanism of activation of bacterial cellulose synthase by cyclic-di-GMP, Nat. Struct. Mol. Biol., 2014 May;21(5):489-96, PMC4013215
- Morgan, JLW., McNamara, JT., **Zimmer, J**., Observing cellulose biosynthesis and membrane translocation in crystallo, Nature 2016, 531, 329-334, PMC4843519

4. Functional reconstitution of and in vitro fiber formation by plant cellulose synthases

The mechanism of cellulose biosynthesis is most likely evolutionarily conserved. However, while most bacteria produce so-called amorphous cellulose as a biofilm component, plants organize the individual cellulose polymers into cable-like structures that are wrapped around the cell to form the load-bearing component of the cell wall. Despite decade-long efforts, plant cellulose biosynthesis had not been reconstituted from purified components, preventing insights into the minimal machinery required for cellulose microfibril formation.
We overcame these challenges by establishing cellulose microfibril formation *in vitro* by functionally reconstituting heterologously expressed and purified poplar cellulose synthase-8. Our studies revealed that microfibril formation is an intrinsic property of plant cellulose synthase, with no other plant-derived components required for glucan bundling.
- Pallinti, P., Cho, SH., Diaz-Moreno, SM., Kumar, M., Nixon, BT., Bulone, V., **Zimmer, J.**, A single heterologously expressed plant cellulose synthase isoform is sufficient for cellulose microfibril formation in vitro, PNAS 2016, 113(40), 11360-11365, (DOE funded)

5. ABC transporter-mediated polymer secretion

ABC transporters are expressed in all kingdoms of life where they perform extremely important functions from nutrient uptake to drug extrusion and lipid flipping. Classical ABC importers and exporters function by alternating access whereby the substrate-binding site is either exposed to the intra- or extracellular environment. The underlying conformational changes are coupled to ATP binding to and hydrolysis by the transporter's cytosolic nucleotide binding domains. ABC transporters involved in biopolymer-translocation constitute an important, yet poorly characterized transporter family that likely do not function by 'alternating access'. Examples include Type-1 secretion systems for polypeptide as well as O antigen and capsular polysaccharide ABC transporters for complex carbohydrate translocation. Our group provided the first two examples of biopolymer-translocating ABC transporters by solving the structures of the Type-1 secretion system PrtD and the O antigen Wzm/Wzt ABC transporters, allowing unique insights into their translocation mechanisms.
- Morgan, JLW., Acheson, J., **Zimmer, J.**, Structure of a Type-1 Secretion System ABC Transporter, Structure 2017 Mar 7;25(3):522-529, (start-up funds)
- Bi. Y., Mann, E., Whitfield, C., **Zimmer, J**., Structure of a channel forming O antigen polysaccharide ABC transporter, Nature 2018, Jan 18;553(7688):361-365, PMC5978415

D. Research Support

Ongoing Research Support

CLSF-EFRC (Zimmer, co-PI) 08/01/2018 – 7/31/2022
Department of Energy
Renewed funding for the functional and structural characterization of cellulose microfibril-forming plant cellulose synthases. The major goal of the CLSF-supported research is to unravel how cellulose microfibrils are formed in plant cell walls. Our research aims at determining how cellulose synthase supra-molecular complexes are formed in biological membranes.

1R01GM110143-01A (Zimmer, PI) 01/01/2015 – 12/01/2018
NIH General Medicine
Molecular Biology of Hyaluronan Biosynthesis
The major goal of the grant is to determine the mechanism by which processive hyaluronan synthases synthesize and secrete hyaluronan.

1R01GM101001-06A1 (Zimmer) 01/01/2018 – 11/30/2021
NIH General Medicine
Mechanism of bacterial cellulose synthesis and transport across biological membranes
The major goal of this research is to unravel how cellulose is synthesized and secreted in Gram-negative bacteria to aid in biofilm formation.

R21AI137697-01 (Zimmer) 02/02/2018 – 1/31/2020
NIH Allergy and Infectious Diseases
Mechanisms of microbial toxin and polysaccharide secretion
The major goals of this research are to isolate Type 1 Secretion System translocation intermediates for structural analyses as well as to develop an *in vitro* system for generating O antigen substrates to be used in future functional and structural analyses of O antigen secretion.

R01GM12966601 (Zimmer) 09/01/18 – 8/31/22
NIH General Medicine
Molecular Mechanisms of Microbial Complex Carbohydrate Secretion
The main goals of this research are to determine the molecular basis for O antigen polysaccharide secretion in Gram-negative bacteria.

Completed Research Support

Ref. 160029 (Zimmer) 04/01/2016 – 03/31/2017
Mizutani Foundation for Glycoscience
Structural and Functional Biology Reveal the Mechanism of Hyaluronan Synthesis
The major goal of this grant is to support a postdoctoral fellow in the field of glycoscience. This grant mechanism does not allow PI salary.

1R01GM101001 (Zimmer, PI) 05/01/2012 – 02/28/2017
NIH General Medicine
Mechanism of cellulose synthesis and transport across biological membranes.
The major goal of this proposal is to determine the mechanism by which cellulose synthase synthesizes and transports cellulose across bacterial membranes and to elucidate the function of the cellulose synthase BcsB subunit.

Jeffress Memorial Trust (Zimmer, PI) 01/01/2012 – 12/31/2012
In vitro reconstitution of bacterial cellulose synthesis
The Jeffress Memorial Trust provides one-time funding for pilot studies including support for laboratory personnel, equipment and supplies for early stage investigators in the Commonwealth of Virginia.

UVa R&D (Zimmer, PI) 01/01/2012 – 12/31/2012

Determining the mechanism of synthesis and membrane translocation of hyaluronan

The UVa Research and Development Grant is provided by the University of Virginia School of Medicine. It provides one-time funding for pilot projects. The major goal of this proposal is to map the path of hyaluronan through the hyaluronan synthase by chemical cross-linking.