BIOGRAPHICAL SKETCH

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NAME: Florian David Schubot

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POSITION TITLE: Associate Professor of Biological Sciences

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
Technische Universität Berlin, Berlin, Germany	B.S.	04/1992	Chemistry
University of Oklahoma, Norman, OK	M.S.	12/1994	Biochemistry
University of Georgia, Athens, GA	Ph.D.	12/2000	Biochemistry & Mol. Biol.
University of Georgia, Athens, GA	Postdoc	12/2002	Structural Biology
NCI-Frederick, MD	Postdoc	12/2005	Structural Biology

A. Personal Statement

During my graduate studies under Bi-Cheng Wang at the University of Georgia, I received extensive training in all aspects of protein crystallization and structure determination. My post-doctoral training at the National Cancer Institute in Frederick focused on the structural characterization of components of the *Yersinia pestis* type three secretion system. At that time, I was also introduced to the unique pathways that regulate virulence factor expression in the related pathogen *Pseudomonas aeruginosa*. These mechanisms became a central research theme when I formed my own group in 2006.

Our current work focuses on the intra- and intermolecular signaling mechanisms underlying multikinase signaling networks that regulate key virulence mechanisms in bacterial pathogens. To address our research questions, we build expertise in the expression and purification of membrane proteins and in the construction of chimeric receptors. While this impacted our overall productivity, we were still able to publish important papers in *Structure*, the *Biochemical Journal*, and most recently, in the *Journal of Biological Chemistry*.

The current application seeks to determine the mechanism of transmembrane signaling in GacS, test the novel idea that GacS signaling might be bidirectional, and determine the role of a newly discovered pseudo-receiver domain in regulating GacS function.

In addition to working on *P.aeruginosa* virulence regulation, we also lend our expertise in structural biology to contribute to several other exciting research projects. Current collaborations include structural studies of methyl-accepting chemotaxis proteins aimed at defining their signal specificity and the structure determination of unique terpene synthase enzymes in an effort to retrace the evolution of specific activity. The former project has produced one publication on the structure of McpX and a second manuscript reporting the novel McpZ receptor structure has just

been accepted by *Proteins: Structure, Function, and Bioinformatics.* Here, I am the sole corresponding author. For terpene synthase project, I am co-authors on a manuscript that has just been accepted for publication with *Protein Science*. A second manuscript describing a novel terpene synthase ligand complex is in preparation.

Ongoing Research Support

NSF
Dorothea Tholl (PI) 7/1/2019-6/30/2024
Collaborative Research: Emergence of terpene cyclization in animals
We support this multi-PI project by providing experimental structural data for various Terpene synthase enzymes, that serve as starting points for directed evolution studies.
Role: Co-PI.

Completed Research Support

NSF Birgit Scharf (PI) 8/1/2018-7/31/2022
Specificity of chemotaxis-driven motility in *Sinorhizobium meliloti* host interaction
The Schubot lab performs crystallographic studies to determine the structural basis for ligand specificity of the chemosensory domains of various *Sinorhizobium meliloti* MCPs.
Role: Co-PI.

NIH-NIAID
Schubot (PI) 05/01/2017-04/30/2021
Molecular basis for the reciprocal regulation of *P. aeruginosa* virulence factors by the signaling kinase RetS.
This R21 type grant has two aims. First we seek to understand the molecular mechanism whereby RetS-kinase inhibits the pivotal GacS/GacA signaling system in *P.aeruginosa.* The second aim sought to identify the molecular ligand of RetS. There is no overlap between the work in this application and the current application.
Role: PI

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2012- Associate Professor, Department of Biological Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA.
2006-2012 Assistant Professor, Department of Biological Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA.
1995 Research Associate, Department of Crystallography, University of Pittsburg, PA

Other Experience and Professional Memberships

Member, American Chemical Society
Member, American Crystallographic Association
Member, American Society for Microbiology

C. Contributions to Science

My graduate work in the group of Dr. Bi-Cheng Wang focused on the characterization of the mitochondrial transcription factor Sc-mtTB. Mitochondrial RNA polymerase is evolutionary related to viral RNA polymerases. Yet, unlike the latter enzyme the mtRNAP requires at least

one transcription factor for transcription initiation. At the time the accepted model assumed that Sc-mtTFB was structurally and functionally related to bacterial sigma factors. However my structural studies clearly demonstrated that Sc-mtTFB is actually closely related to methyltransferases and functionally completely distinct from bacterial sigma factors. These findings had broad implications because mtTFB is conserved across all eukaryotic organisms including humans.

Schubot, F. D., Chen, C. J., Rose, J. P., Dailey, T. A., Dailey, H. A., and Wang, B. C. (2001) The crystal structure of the transcription factor sc-mtTFB offers insights into mitochondrial transcription. *Protein Science* 10: 1980-1988.

Schubot, F. D., Chen, C. J., Rose, J. P., and Wang, B. C. (2000) Crystallization and preliminary X-ray diffraction analysis of the mitochondrial transcription factor sc-mtTFB from *Saccharomyces cerevisiae*. *Acta Crystallographica D: Biological Crystallography* 56: 902-903.

After completion of my doctoral work, I remained with the Wang group for a couple more years to assist with building the pipeline for the newly-funded structural genomics initiative. At the time, a major question related to the development of efficient salvage pathways for proteins that failed to crystallize in the first screen. Classic tools such a limited proteolysis and surface entropy reduction through site-directed mutagenesis appeared too labor intensive. At that time, I came across earlier work from Ivan Raymont utilizing reductive methylation to improve the diffraction of myosin crystals. This method offered an opportunity to reuse already purified proteins. Although, I started working on this in the Wang laboratory, I departed for my post-doc before completing the work. However, I subsequently applied this method to the *de novo* crystallization of a ternary YopN-SycN-YscB complex, a regulatory plug of the *Y.pestis* type three secretion system. In addition to the biological insights this case study proved three important things: (1) The chemical methylation reaction can be applied to protein complexes. (2) The methylation reaction may be carried out with selenomethionine-derivatized protein. (3) The modification is significant enough to cause *de novo* crystallization of a previously recalcitrant protein.

Schubot, F. D., Jackson, M. W., Penrose, K. J., Cherry, S., Tropea, J. E., Plano, G. V., and Waugh, D. S. (2005) Three-dimensional structure of a macromolecular assembly that regulates type III secretion in *Yersinia pestis*. *J. Mol. Biol.*, 346(4), 1147-61.

Schubot, F. D. and Waugh D. S. (2004) A pivotal role for reductive methylation in the *de novo* crystallization of a ternary complex composed of *Yersinia pestis* virulence factors YopN, SycN and YscB. *Acta Crystallographica D: Biological Crystallography* 60: 1981-86.

Ferracci, F., **Schubot, F. D**., Waugh, D. S., and Plano, G. V. (2005) Selection and Characterization of *Yersinia pestis* YopN Mutants that Constitutively Block Yop Secretion. *Molecular Microbiology,* **57(4),** 970-87.

Starting my own group in 2006, I decided to focus on mechanisms of transcriptional regulation of virulence factors in *Pseudomonas aeruginosa*. At the time, the four-protein ExsACDE signaling cascade had emerged as a novel regulatory mechanism tying upregulation of the type three secretion system to host cell contact. There were several completely novel aspects to the signaling process. Our group structurally characterized the entire cascade. We provided the molecular basis for the ExsC-ExsE, the ExsC-ExsD interactions, and gained some insights into the interactions between the transcriptional activator ExsA and its inhibitor ExsD.

The second major focal point of our work, also subject of this application, have been the molecular interactions between the non-canonical signal histidine kinases RetS and GacS that control the expression of more than 400 genes in *Pseudomonas aeruginosa*, and thus

orchestrate the transition of the bacterium between planktonic and biofilm lifestyles. We contributed three crystal structures and biochemical studies, which offered insight into the highly unconventional interplay between these two enzymes.

Through collaborations we have also become more broadly interested in transmembrane signaling. From this work we published two papers on structural studies of the sensory domains of bacterial chemoreceptors and a third is in preparation.

Salar, S. *et al.* (2023) The structural analysis of the periplasmic domain of Sinorhizobium meliloti chemoreceptor McpZ reveals a novel fold and suggests a complex mechanism of transmembrane signaling. *Proteins* **91**, 1394-1406,doi.org:10.1002/prot.26510

Ryan Kaler, K. M., Nix, J. C. & **Schubot, F. D.** (2021) RetS inhibits Pseudomonas aeruginosa biofilm formation by disrupting the canonical histidine kinase dimerization interface of GacS. *J Biol Chem*, 101193, doi:10.1016/j.jbc.2021.101193.

Shrestha M., Bernhards R.C., Fu Y, Ryan K, **Schubot F. D.** (2020) Backbone Interactions Between Transcriptional Activator ExsA and Anti-Activator ExsD Facilitate Regulation of the Type III Secretion System in *Pseudomonas aeruginosa*. *Scientific Reports* 10(1): 9881.

Mancl, J. M., Ray, W. K., Helm, R. F. & Schubot, F. D. (2019) Helix Cracking Regulates the Critical Interaction between RetS and GacS in Pseudomonas aeruginosa. *Structure* **27**, 785-793 e785, doi:10.1016/j.str.2019.02.006.

Shrestha, M. *et al.* (2018) Structure of the sensory domain of McpX from Sinorhizobium meliloti, the first known bacterial chemotactic sensor for quaternary ammonium compounds. *Biochem J* 475, 3949-3962 (2018), doi.org:10.1042/Bcj20180769

The full publication record may be found here: **https://orcid.org/0000-0002-7403-4735**