BIOGRAPHICAL SKETCH

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NAME	POSITION TITLE
Schmeing, T. Martin	Professor of Biochemistry
eRA COMMONS USER NAME (credential, e.g., agency login)	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
McGill University, Montreal, Canada	B.Sc.	05/98	Biochemistry
Yale University, New Haven, Connecticut	M.Sc. Ph.D.	05/02 05/04	Molecular Biophysics and Biochemistry
Laboratory of Molecular Biology, Cambridge, UK	Postdoc CDF	12/09 06/10	Biochemistry

A. Personal Statement

The overarching focus of my research career is to study, visualize and understand nature's synthetic megaenzymes, and the mechanisms they employ to synthesize molecules important for human health. I approach this topic with a combination of molecular biology, biochemistry, chemical biology and structural biology. I trained using these techniques while addressing questions about the function of the ribosome (the essential complex that makes all proteins), and now lead a group using these techniques to study nonribosomal peptide synthetases (macromolecular machines that produce a vast variety of small molecule therapeutics).

We are interested in expanding our knowledge of nonribosomal peptide synthetases, polyketide synthases, and their hybrids. We pursue understanding of the structure and the mechanisms they employ to synthesize bio-active molecules. We ask questions on a large range of scales, from elucidating the precise catalytic details of how amino acids and other building blocks are chemically assembled into the products, to explaining the large-scale rearrangements, transient domain-domain interactions, and intermodular communication these enzymes need to perform in their assemble-line style synthetic process.

We find megaenzymes intrinsically interesting because they are among the largest and most complicated molecular machines in nature. In addition, the wide-ranging activities of their small peptide products, and the unfulfilled potential to engineer them for production of new bio-active molecules, make megaenzymes a tantalizing source of novel therapeutics and chemicals.

1. Pistofidis A, Ma P, Li Z, Munro KA, Houk KN, **Schmeing TM**. Structures and mechanism of condensation in nonribosomal peptide synthesis. ***Nature*** 2024 advanced online: doi.org/10.1038/s41586-024-08417-6
2. Patteson JB*, Fortinez CM*, Putz AT, Rodriguez-Rivas J, Bryant LH, Adhikari K, Weigt M, **Schmeing TM**§, Li B§. Structure and function of a dehydrating condensation domain in nonribosomal peptide synthesis. ***Journal of the American Chemical Society*** 2022 Aug 10;144(31):14057-14070.
3. Sharon I, Pinus S, Grogg M, Moitessier N, Hilvert D, **Schmeing TM**. A cryptic third active site in cyanophycin synthetase creates primers for polymerization. ***Nature Comm*** 2022 Jul 7; 13:3923.
4. Reimer JM*, Eivaskhani M*, Harb I, Guarné A, Weigt M, **Schmeing TM**. Structures and bioengineering of a dimodular nonribosomal peptide synthetase. ***Science*** 2019 Nov 8; 366(6466).
5. Huguenin-Dezot N*, Alonzo DA*, Heberlig GW, Mahesh M, Nguyen DP, Dornan MH, Boddy CN, **Schmeing TM**§, Chin J§. Trapping biosynthetic acyl-enzyme intermediates with encoded 2,3-diaminopropionic acid. ***Nature*** 2019 Jan 3; 565(7737):112-117

B. Positions and Honors

Positions and Employment

2010 – 2016	Assistant Professor, McGill University, Montreal, Canada
2016 – 2021	Associate Professor, McGill University, Montreal, Canada
2021 – present	Professor, McGill University, Montreal, Canada
2014 – 2018	Associate Director, Centre for Structural Biology, McGill University, Montreal, Canada
2018 – present	Associate Director, Centre for Structural Biology, McGill University, Montreal, Canada
2018 – 2020	Associate Director, Facility for Electron Microscopy Research, McGill University

Other Experience and Professional Memberships

2022/15– present	Secretary & Board of Trustees, Canadian Foundation for Development of Microscopy
2023 – present	Mentor, Canadian Organization for Undergraduate Health Research
2012 – present	Canadian "Node", International Network of Protein Engineering Centres (INPEC)
2022 – present	Member, Canadian Society of Microbiologists
2015 – present	Member, The Protein Society
2017 – present	Member, American Crystallographic Association (ACA)
2018 – present	Member, McGill Interdisciplinary Initiative in Infection and Immunity (MI4)
2023 – present	Member, McGill Anti-Microbial Resistance Centre
2019 – present	Member, The Canadian Anti-infective Innovation Network (CAIN)
2011 – present	Member, Réseau Québécois de recherche sur les médicaments
2015 – present	Member, Canadian Society for Molecular Biosciences
2018 – present	Member, Microscopical Society of Canada / Société de microscopie du Canada
2024 – present	Member, American Peptide Society
2022 – present	Member, Canadian Society for Chemistry

Honors

2010	Career Development Award, Human Frontier Science Program Organization
2010 – 2013	Bhagirath Singh Early Career Award in Infection and Immunity
2011	New Investigator Award, Canadian Institutes of Health Research
2011 – 2016	Tier II Canada Research Chair in Structural Biology in Macromolecular Machines
2016	Joe Doupe Young Investigator Award, Canadian Society for Clinical Investigation
2016 – 2021	Tier II Canada Research Chair in Structural Biology in Macromolecular Machines
2017	Warwick Structural Biology Lecturer
2017	New Investigator Award, Canadian Society for Molecular Biosciences
2021	Early Promotion to Full Professor
2022 – 2029	James McGill Professorship

C. Contributions to Science

1. **Architecture and synthetic cycle of NRPSs:** Nonribosomal peptide synthetases (NRPSs) are microbial megaenzymes that synthesize natural product therapeutics and green chemicals. NRPSs are organized in repeating sets of domains called modules; each module adds one building block substrate to the nascent peptide. We solved 4 structures of the initiation module of the NRPS that synthesizes linear gramicidin. These included the first structure of an initiation module and the first structure of a module containing a tailoring domain. They reveal how the formylation domain is incorporated into the NRPS assembly-line architecture and how it adapts to work with partner domains. Our complementary study showed how the pre-gene transfer formyltransferase protein was well suited for incorporation.

   It had been debated for decades how NRPS modules are arranged in 3D, which is of fundamental importance for NRPS function. Many favoured regular super-helical or sheet models. We determined a series of structures that show novel biologically relevant states, reveal one of the largest conformation changes observed in any enzyme (>200 Å), and achieve the goal of visualizing multi-modular NRPSs. Importantly, massive conformational differences between structures resolve the long-standing question of the large-scale architecture of NRPSs: They are incredibly dynamic and clearly do not have a single, regular, higher-order structure.

a. Reimer JM, Aloise MN, Harrison PM, **Schmeing TM**. Synthetic cycle of the initiation module of a formylating nonribosomal peptide synthetase. ***Nature*** 2016 Jan 14; 529(7585).
b. Tarry MJ*, Haque SA*, Bui KH, **Schmeing TM**. X-ray crystallography and electron microscopy of cross- and multi-module nonribosomal peptide synthetase proteins reveal a flexible architecture. ***Structure***, 2017 May 2; 25(5).
c. Reimer JM*, Harb I*, Ovchinnikova OG*, Jiang J, Whitfield C§, **Schmeing TM**§. Structural insight into a novel formyltransferase and evolution to a nonribosomal peptide synthetase tailoring domain. ***ACS Chemical Biology*** 2018 Nov 16;13(11):3161-3172.
d. Reimer JM*, Eivaskhani M*, Harb I, Guarné A, Weigt M, **Schmeing TM**. Structures and bioengineering of a dimodular nonribosomal peptide synthetase. ***Science*** 2019 Nov 8; 366(6466).
e. Fortinez CM, Bloudoff K, Sharon I, Harrigan C, Strauss M, **Schmeing TM**. Structures and function of a nonribosomal peptide synthetase module – tailoring oxidase complex. ***Nature Communications*** 2022 Jan 27; 13:548.

2. **Understanding peptide bond formation by NRPSs:** The key catalytic event in NRPS synthesis is the peptide bond forming condensation reaction. Despite many dozens of studies, comprehension of this reaction was lacking, and there has been lively disagreement as to the mechanism. Studies designed to provide insight into C domain catalysis are challenging because although the aminoacyl- and peptidyl-condensation substrates are covalently linked to dedicated transport domains in the NRPS megaenzyme, these linkages are transient and NRPSs are necessarily extremely flexible. Previously, we developed a chemical biology approach to capture complexes of substrate analogs bound to the condensation (C) domain for the first time (Cell Chem Biol 2016) and characterized conformational changes (JMB 2013). Now (Nature, under review), we have used a novel combination of chemical biology approaches to trap and determine structures of pre- and post-condensation states of an 1800+ residue megaenzyme. These structures, which reveal the active conformation only when both substrates are bound, allow quantum mechanistic simulations of condensation. They reveal that the catalytic power comes from electrostatic forces, not from a general base, as is often assumed.
    a. Pistofidis A, Ma P, Li Z, Munro KA, Houk KN, **Schmeing TM**. Structures and mechanism of condensation in nonribosomal peptide synthesis. ***Nature*** 2024 online ahead of print
    b. Bloudoff K, Fage CD, Marahiel MA, **Schmeing TM**. Structural and mutational analysis of the nonribosomal peptide synthetase heterocyclization domain provides insight into catalysis. ***Proceedings of the National Academy of Sciences USA*** 2017 Jan 3; 114(1).
    c. Bloudoff K, Alonzo DA, **Schmeing TM**. Chemical probes allow structural insight into the condensation reaction of nonribosomal peptide synthetases. ***Cell Chemical Biology*** 2016 Mar 17;23(3).
    d. Bloudoff K, Rodionov D, **Schmeing TM**. Crystal structures of the first condensation domain of the CDA synthetase suggest conformational changes during the synthetic cycle of nonribosomal peptide synthetases. ***Journal of Molecular Biology***, 2013 Sept 9;425(17).

3. **Structures and mechanisms of depsipeptide synthetases:** Depsipeptides are made up of both amino acids and hydroxy acids. The potent K+ ionophores cereulide and valinomycin are cyclic depsipeptides consisting of alternating amino and hydroxy acids and made by similar non-canonical NRPSs. There are two peculiar aspects of depsipeptide synthetases. First, they have an embedded ketoreductase (KR) domain in every second module. These modules select α-keto acids and stereospecifically reduce them to α-hydroxy acids for incorporation into the nascent depsipeptide. We characterized cereulide synthetase in vitro, then determined the structure of a depsipeptide synthetase initiation module, including the KR domain and adenylation domain. This structure shows a surprising domain configuration and pseudo-duplication that provides insight into the gene transfer and evolution of the parental depsipeptide synthetase and reveals necessary transitions in the module's synthetic cycle. The second peculiarity is that instead of using each module only once, these synthetases iteratively make 3 tetradepsipeptide intermediates, which the terminal thioesterase (TE) domain processively oligomerizes and then cyclizes to release a dodecapeptide product. With Jason Chin, we developed a novel unnatural amino acid substitution to elucidate the biosynthetic pathway of these TE domains. We trapped and crystallized the first and last depsipeptidyl-TE intermediates in the catalytic cycle to show how conformational changes in the TE domains control the switch from oligomerization to cyclization.

a. Alonzo DA, Magarvey NA, **Schmeing TM**. Characterization of cereulide synthetase, a toxin-producing macromolecular machine. ***PLOS ONE***, 2015 Jun 4;10(6).
b. Huguenin-Dezot N*, Alonzo DA*, Heberlig GW, Mahesh M, Nguyen DP, Dornan MH, Boddy CN, **Schmeing TM**§, Chin J§. Trapping biosynthetic acyl-enzyme intermediates with encoded 2,3-diaminopropionic acid. ***Nature*** 2019 Jan 3; 565(7737):112-117.
c. Alonzo DA*, Chiche-Lapierre C*, Tarry MJ, Wang J, **Schmeing TM**. Structural basis of keto acid utilization in nonribosomal depsipeptide synthesis. ***Nature Chemical Biology*** 2020 May;16(5):493-496.

4. **Cyanophycin metabolism**: Cyanophycin is a peptide polymer consisting of a long poly-L-Asp backbone with L-Arg residues attached to each of the Asp β-carboxylate side chains through isopeptide bonds. We have recently provided leaps forward in understanding of cyanophycin metabolism. First, our structural and functional experiments to reveal how CphA1 is configured and how, in general, it synthesizes cyanophycin. Structure of CphA1 enzymes from different species revealed two distinct tetrameric architectures. Cryo-EM structures of one CphA1 with a series of substrate analogs explain some of the steps in which nascent cyanophycin polymer is elongated at each of the active sites, and showed that a domain of hitherto unknown structure and function (the N domain) loosely anchors cyanophycin to enable processive polymerization. Then we turned to the outstanding question of primer. CphA1 enzymes were almost always described as primer-dependent activity, requiring existing cyanophycin chains to extend. Remarkably, we discovered a cryptic metallopeptidase-like active site in the N domain of some CphA1s, and established that this active site provides primer independence. Although CphA1 enzymes had been studied for decades, it was completely unexpected that they harbor a third active site, and extraordinary that it would catalyze hydrolysis of peptide bonds, where the two active sites of known function catalyze (iso)peptide bond formation. The N domain slowly cleaves a small proportion of cyanophycin to (β-Asp-Arg)4 peptides, which are ideal primers for biosynthesis.

   After elucidating how nature has devolved CphA2 into a β-Asp-Arg dipeptide polymerase, we turned to cyanophycin catabolism. We first solved a covalent acyl-enzyme complex of cyanophycinase and cyanophycin and see a shallow, surface-exposed substrate binding pocket. This is very different from the substrate pockets of typical proteases and peptidases, explaining the mutually exclusivity of these enzymes. Finally, we discovered a novel family of dedicated cyanophycin dipeptide hydrolases, CphZ, and their specificity for cyanophycin-derived β-dipeptides with biochemical and structural experiments. Remarkably, ~10,000 sequences of CphZ family enzymes are present in databases. Accompanying bioinformatics show cyanophycin metabolism genes to be very common: ~11% of complete bacterial genomes encode at least one gene for cyanophycin metabolism, compared to the ~38% of genomes which encode at least one gene for glycogen metabolism. These numbers highlight that it is quite common for bacteria to be cyanophycin producers or scavengers, and that cyanophycin is a much more important polypeptide than currently appreciated.
   a. Markus LMD*, Sharon I*, Munro K, Grogg M, Hilvert D, Strauss M§, **Schmeing TM**§. Structure and function of a hexameric cyanophycin synthetase 2. ***Protein Science*** 2023 July; 32(7):e4685.
   b. Sharon I, **Schmeing TM**. Bioinformatics of cyanophycin metabolism genes and characterization of promiscuous isoaspartyl dipeptidases that catalyze the final step of cyanophycin degradation. ***Scientific Reports*** 2023 13:8314.
   c. Sharon I*, McKay G*, Nguyen D§, **Schmeing TM**§. Discovery of cyanophycin dipeptide hydrolase enzymes suggests widespread utility of the natural biopolymer cyanophycin. ***Proceedings of the National Academy of Sciences USA*** 2023 120(8), e2216547120.
   d. Sharon I, Pinus S, Grogg M, Moitessier N, Hilvert D, **Schmeing TM**. A cryptic third active site in cyanophycin synthetase creates primers for polymerization. ***Nature Communications*** 2022 Jul 7; 13:3923.
   e. Sharon I, Grogg M, Hilvert D, **Schmeing TM**. Structure and function of the β-Asp-Arg polymerase cyanophycin synthetase 2. ***ACS Chemical Biology*** 2022, 17, 3, 680-700.
   f. Sharon I, Haque AH, Grogg M, Lahiri I, Seebach D, Leschziner AE, Hilvert D, **Schmeing TM**. Structures and function of the amino acid polymerase cyanophycin synthetase. ***Nature Chemical Biology*** 2021 Oct; 17, 1101–1110.

5. **Structure and function of the ribosome.** I contributed significantly our knowledge of the ribosome. First, in Tom Steitz's lab, I solved structures of the large ribosomal subunit with substrates, intermediates and

products. This at last revealed a reaction pathway for the key chemical step of protein synthesis, which had been sought for >50 years: After substrate binding causes induced fit, the reaction proceeds with a substrate-assisted proton shuttle. No proteins are required, so the ribosome is a ribozyme. My studies were called the "jewel in the crown" of Steitz's Nobel research by the committee. Then, with Venki Ramakrishnan, I solved structures of the ribosome with EF-Tu and aminoacyl-tRNA, which revealed how proper codon-anticodon match sensing maintains the fidelity of protein synthesis and how activation of GTP hydrolysis occurs for translation factors.

a. **Schmeing, T.M.**, Seila, A.C., Hansen, J.L., Freeborn, B., Soukup, J.K., Scaringe, S.A., Strobel, S.A., Moore, P.B., Steitz, T.A. A pre-translocational intermediate in protein synthesis observed in crystals of enzymatically active 50S subunits. *Nat. Struct. Biol.* 9,225 (2002).
b. **Schmeing, T.M.**, Huang, K.S., Kitchen, D.E., Strobel, S.A., Steitz, T.A. Structural insights into the roles of the 2' hydroxyl of the peptidyl-tRNA and water in the peptidyl transferase reaction. *Mol. Cell* 20,437 (2005).
c. **Schmeing, T.M.**, Huang, K.S., Strobel, S.A., Steitz, T.A. An induced fit mechanism to promote peptide bond formation and exclude hydrolysis of peptidyl-tRNA. *Nature* 438, 520 (2005).
d. **Schmeing, T.M.**, Voorhees, R.M., Kelley, A.C., Gao, Y.G., Murphy, F.V., Weir, J.R., Ramakrishnan, V. The crystal structure of the ribosome bound to EF-Tu and tRNA. *Science* **326**, 688 (2009).
e. Voorhees, R.M., **Schmeing, T.M.**, Kelley, A.C., Ramakrishnan, V. The mechanism for activation of GTP hydrolysis on the ribosome. *Science* **330**, 835 (2010).
f. **Schmeing, T.M.**, Voorhees, R.M., Kelley, A.C., Ramakrishnan, V. How mutations in tRNA distant from the anticodon affect the fidelity of decoding. *Nat Struct Mol Biol.* **18**, 432 (2011).

Link to List of Published Works

http://www.ncbi.nlm.nih.gov/pubmed?Db=pubmed&Cmd=DetailsSearch&Term=schmeing+tm[Author]+OR+schmeing+m[Author]

D. Research Support

Ongoing support

3. FRQS Centres — Programme de subvention des centres et instituts de recherche, Fonds de recherche du Québec – Santé (FRQS)
"Centre de recherche en biologie structurale"
Directeur and Principal Applicant: Schmeing; Co-applicants: 45 Centre members
April 2024 – March 2030, $4,200,000

2. CIHR — Project Grant, Canadian Institutes of Health Research
"Structures and functions of nonribosomal peptide synthetases" PJT-169142
October 2021 – September 2026, $1,063,350

1. CIHR — Project Grant, Canadian Institutes of Health Research
"Hybrids and commonalities of thiotemplate enzymes" PJT-178084
April 2020 – March 2025, $860,000