BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Ingeborg Schmidt-Krey

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

POSITION TITLE: Associate Professor of Biological Sciences, Associate Professor of Chemistry & Biochemistry

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
Mississippi State University, Starkville	B.Sc.	05/1991	Biochemistry
Karolinska Institute, Stockholm	Ph.D.	05/1999	Biophysics and Structural Biology
Harvard Medical School, Boston	Post-doc	07/2000	Structural Biology
Max-Planck-Institute of Biophysics, Frankfurt	Post-doc	07/2003	Structural Biology
Max-Planck-Institute of Biophysics, Frankfurt	DFG Young Investigator	07/2006	Structural Biology

A. Personal Statement

The main research focus of my laboratory is on structural studies of eukaryotic membrane proteins by cryo-EM. In addition, my laboratory has investigated under which conditions and how optimal 2D crystallization of membrane proteins, that is crucial to electron crystallography, occurs. With the breakthroughs in recent years in cryo-EM, we are applying a change of strategy to an increasing number of our membrane protein projects by using single particle cryo-EM. The membrane protein purification, solubilization and reconstitution required for electron crystallography have provided my laboratory with important tools and skills for single particle cryo-EM of membrane proteins that are either detergent-solubilized or reconstituted into nanodiscs. I have extensive experience in collecting and processing high-resolution cryo-EM data from spending nearly one year of my PhD at Yoshinori Fujiyoshi's laboratories in Nara and Kyoto, six years at the Max-Planck-Institute of Biophysics in Werner Kühlbrandt's department, and visiting Ken Taylor's Titan Krios facility at Florida State University for cryo-EM data collection. Since fall 2017 my laboratory has regular access to Ken Taylor's Titan Krios facility via an NIH U24 cryo-EM consortium grant, which is instrumental in providing us with cryo-EM time for single-particle high-resolution data collection. I serve as a steering committee member for the U24 cryo-EM consortium.

B. Positions and Honors

Positions

1999-2000	Postdoctoral fellow, Department of Cell Biology, Harvard Medical School, Boston, MA.
2000-2002	EMBO postdoctoral fellow, Department of Structural Biology, Max-Planck-Institute of Biophysics, Frankfurt, Germany.
2003-2006	German Research Foundation (DFG) young investigator, Department of Structural Biology, Max-Planck-Institute of Biophysics, Frankfurt, Germany.
2006 – 2012	Assistant Professor, School of Biology, Georgia Institute of Technology, Atlanta, GA.
2011 – 2012	Assistant Professor, School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA.

2012 – current	Associate Professor, School of Biology/Biological Sciences, School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA.

Honors/Awards

1996	SCANDEM travel grant (Aarhus, Denmark)
1998	Swedish Structural Biology Network travel grant (Kyoto, Japan)
1997 – 1999	Sodertorn PhD fellowship
2000	Jane Coffin Child fellowship, declined
2001 – 2002	EMBO long-term fellowship
2003 – 2006	German Research Foundation Young Investigator Grant
2007 – current	EMBO/EMBL/CERN Women in Science Ambassador
2008 – 2009	Class of 1969 Teaching Fellow. Georgia Institute of Technology, Atlanta, GA, U.S.A.
2016 – current	NIH U24 Consortium Steering Committee Member

Other Experience and Professional Memberships

2002-06	European Union Sixth Framework Programme Expert (2002-2006)
2007-13	European Union Seventh Framework Programme Evaluator/Review Expert (2007-2013)
2007-13	European Union Seventh Framework Programme Monitor Expert (2007-2013)
2007 – current	Member, Biophysical Society
2007 – current	Member, Southeastern Microscopy Society
2008 – current	Member, Microscopy Society of America
2009 – current	Reviewer: NIH ((F30/F31/F32/F33, P01, R00/K99, R01, R03, R15, R25, R25, U24, X02)); NSF; Italian Ministry of Health, Directorate for Health and Technologies Research; Italian Ministry of Labour, Health and Social Policies, Department of Innovation reviewer; Israel Science Foundation; Lise Meitner Programme, Austria; Netherlands Organization for Scientific Research (NWO), Chemical Sciences, domain Exact and Natural Sciences

C. Contributions to Science

1. Structural studies of rat liver microsomal glutathione transferase 1 by electron crystallography: 2D crystallization and structure determination

My PhD project in Hans Hebert's laboratory was the 2D crystallization and structure determination by electron crystallography of microsomal glutathione transferase 1 (MGST1). When I induced the first highly ordered crystals of MGST1, less than a handful of membrane protein structures had been solved. Thus, MGST1 was one of the first eukaryotic membrane proteins to result in highly ordered crystals, and initially with Werner Kühlbrandt's and Da-Neng Wang's generous help, we obtained a 4Å resolution projection map. This was followed by projection maps of two different crystal forms at 3Å resolution via a collaboration with Yoshinori Fujiyoshi's laboratory. From early 1997 to early 1999 I spent nearly one year in Prof. Fujiyoshi's laboratory to collect and process high-resolution data (image and electron diffraction), resulting in a 3D structure at 6Å resolution), which with further data collection by a subsequent PhD student resulted in an atomic model. At the time of my graduate studies, little was known about the secondary structure of membrane proteins, and even less so about eukaryotic membrane protein structure. Consequently, the 3D structure at 6Å resolution was not only important for gaining insights into MGST1 structure and function, but it also contributed to our general understanding of membrane protein structure.

- Schmidt-Krey I, Mitsuoka K, Hirai T, Murata K, Cheng Y, Fujiyoshi Y, Morgenstern R, Hebert H. The three-dimensional map of microsomal glutathione transferase 1 at 6 Å resolution. EMBO J. 2000 Dec 1;19(23):6311-6. PubMed PMID: 11101503; PMCID: PMC305867.
- Schmidt-Krey I, Murata K, Hirai T, Mitsuoka K, Cheng Y, Morgenstern R, Fujiyoshi Y, Hebert H. The projection structure of the membrane protein microsomal glutathione transferase at 3 Å resolution as determined from two-dimensional hexagonal crystals. J Mol Biol. 1999 Apr 30;288(2):243-53. PMID: 10329140.
- Schmidt-Krey I, Lundqvist G, Morgenstern R, Hebert H. Parameters for the two-dimensional crystallization of the membrane protein microsomal glutathione transferase. J Struct Biol. 1998 Oct;123(2):87-96. PMID: 9843664.

- Hebert H, Schmidt-Krey I, Morgenstern R. The projection structure of microsomal glutathione transferase. EMBO J. 1995 Aug 15;14(16):3864-9. PMID: 7664727; PMCID: PMC394465.

2. Structural studies of human leukotriene C_4 synthase and human vitamin K-dependent γ-glutamyl carboxylase by electron crystallography

Human leukotriene C_4 synthase (LTC_4S) is a membrane protein that plays a considerable role in inflammatory diseases and several cancers. I induced LTC_4S to crystallize to reproducibly form highly ordered 2D arrays. From this work, resulting in a 4.5Å resolution projection map, we determined the number of subunits as well as transmembrane α-helices, and gained critical information on 2D crystal formation of integral membrane proteins. These crystals could be improved beyond 4Å resolution. Much controversy existed about the oligomeric arrangement, and I could show for the first time that LTC_4S is a trimer within the membrane and not a dimer. Human vitamin K-dependent γ-glutamyl carboxylase (GGCX) is also of medical importance in that it catalyzes the post-translational modification of seven proteins in the blood coagulation cascade. Thus, it is central to hemostasis and a large number of blood-coagulation diseases. We could induce human GGCX to form 2D crystals showing GGCX to be a monomer.

- Schmidt-Krey I, Kanaoka Y, Mills DJ, Irikura D, Haase W, Lam BK, Austen KF, Kühlbrandt W. Human leukotriene C_4 synthase at 4.5 Å resolution in projection. Structure. 2004 12(11):2009-14. PMID: 15530365.
- Zhao G, Johnson MC, Schnell JR, Kanaoka Y, Haase W, Irikura D, Lam BK, Schmidt-Krey I. Two-dimensional crystallization conditions of human leukotriene C_4 synthase requiring adjustment of a particularly large combination of specific parameters. J Struct Biol. 2010 169(3):450-4. PubMed PMID: 19903529; NIHMSID: NIHMS164517; PMCID: PMC2826519.
- Schmidt-Krey I, Haase W, Mutucumarana V, Stafford DW, Kühlbrandt W. Two-dimensional crystallization of human vitamin K-dependent gamma-glutamyl carboxylase. J Struct Biol. 2007 Feb;157(2):437-42. PMID: 16979907.

3. Towards guidelines for two-dimensional crystallization of eukaryotic membrane proteins

Once a membrane protein can be purified in sufficient quantities, the major bottleneck in the structure determination by electron crystallography is 2D crystallization, including identification of the first ordered arrays. We systematically investigated 2D crystallization conditions for several membrane proteins and determined guidelines for optimizing the crystallization process, which lead to improved 2D crystallization often within weeks rather than months or years. While 2D crystallization is the major bottleneck in electron crystallography, screening for 2D crystals is not a trivial task as samples need to be evaluated for morphology and order, and initial success often involves the challenging identification of small crystals. We have outlined strategies how first lattices with even minimal dimensions of small membrane proteins can be identified.

- Johnson, M C., Uddin, Y. M., Neselu, K., Strickland, K.M., Schmidt-Krey, I. (2019) 2D crystallography of membrane protein single-, double- and multi-layered ordered arrays. *Meth Mol Biol*, in press.
- Uddin, Y.M., Schmidt-Krey. I. (2015) Inducing two-dimensional crystallization of membrane proteins by dialysis for electron crystallography. Methods Enzymol. 557:351-62. PMID: 25950973.
- Zhao G, Johnson MC, Schnell JR, Kanaoka Y, Haase W, Irikura D, Lam BK, Schmidt-Krey I. Two-dimensional crystallization conditions of human leukotriene C_4 synthase requiring adjustment of a particularly large combination of specific parameters. J Struct Biol. 2010 Mar;169(3):450-4. PMID: 19903529; NIHMSID: NIHMS164517; PMCID: PMC2826519.
- Schmidt-Krey I. Electron crystallography of membrane proteins: two-dimensional crystallization and screening by electron microscopy. Methods. 2007 Apr;41(4):417-26. PMID: 17367714.

4. Fibrilization of the glaucoma-associated olfactomedin domain of myocilin

My graduate student Yaunhee Kim and I collaborated with the Lieberman laboratory at Georgia Tech (Chemistry & Biochemistry) to identify amyloid fibril formation of the olfactomedin domain of myocilin under conditions linked to glaucoma. Our TEM studies were critical in visualizing the fibrilization for the first time.

- Orwig SD, Perry CW, Kim LY, Turnage KC, Zhang R, Vollrath D, Schmidt-Krey I, Lieberman RL (2012) Amyloid fibril formation by the glaucoma-associated olfactomedin domain of myocilin. J Mol Biol 421(2-3):242-55. PMID: 22197377.

5. Imaging and/or electron tomography of nanoparticles and nanotubes

Collaborations with chemistry/engineering colleagues allowed my students and myself to gain experience with nanoparticles and nanotubes. One example is fast detection of specific pathogens allowing for highly efficient treatment. Non-covalent conjugates of Au-nanoparticles (Au-NPs) and poly-paraphenylene-ethynylene (PPE) were developed by the Bunz and El-Sayed laboratories to rapidly identify bacterial pathogens. The conjugates are employed to identify bacteria after initial fluorescence-quenching. This occurs with the conjugation of cationic Au-NPs and anionic PPE. Upon contact with negatively charged bacterial surfaces, PPE is released and its fluorescence thereby restored, providing for identification within minutes. In collaboration with Prof. Bunz and colleagues we were able for the first time to show that the Au-NPs are internalized by bacteria in hot-spot areas rather than interacting with the surface (Hayden *et al.*). In collaboration with the Xia laboratory, my PhD student Matt Johnson and I collected electron tomography data to visualize bimetallic nanocrystals (Zhu *et al.*), while a collaboration with the Nair laboratory aimed at characterizing the formation and growth of metal oxide nanotubes (Yucelen *et al.*). In a collaboration with the Schuster and Williams laboratories, my PhD student Matt Johnson and I have helped to visualize DNA-Au-nanoparticle arrays.

- Hayden SC, Zhao G, Saha K, Phillips RL, Li X, Miranda OR, Rotello VM, El-Sayed MA, Schmidt-Krey I, Bunz UH. Aggregation and interaction of cationic nanoparticles on bacterial surfaces. J Am Chem Soc. 2012 Apr 25;134(16):6920-3. PMID: 22489570.
- Zhu C, Zeng J, Tao J, Johnson MC, Schmidt-Krey I, Blubaugh L, Zhu Y, Gu Z, Xia Y. Kinetically controlled overgrowth of Ag or Au on Pd nanocrystal seeds: from hybrid dimers to nonconcentric and concentric bimetallic nanocrystals. J Am Chem Soc. 2012 Sep 26;134(38):15822-31. PMID: 22947077.
- Yucelen GI, Kang DY, Schmidt-Krey I, Beckham HW, Nair S. A Generalized Kinetic Model for the Formation and Growth of Single-Walled Metal Oxide Nanotubes. Chem. Eng. Sci.. 2013; 90:200-212.
- Ma, Z., Chen, W., Johnson, M.C., Schmidt-Krey, I., Williams, L., Schuster, G. (2014). Modular-DNA Programmed Molecular Construction of "Fixed" 2D and 3D-Au Nanoparticle Arrays. *Chemistry of Materials.* 26 (19), 5499 – 5505.

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

<u>Ongoing Research Support</u>

Cystic Fibrosis Foundation MCCART18G0 co-PI Schmidt-Krey 11/01/2018 – 10/31/2020
CFTR Structure and Function: Dependence Upon Membrane Lipids

NIH 3R01GM049245-23S1 co-PI Schmidt-Krey 08/01/2016 – 07/31/2020
DNA Methylation: Structures, Functions, and Regulation

NIH U24-GM116788 MPI Schmidt-Krey 07/01/2016 – 6/30/2021
The Southeastern Consortium for Microscopy of MacroMolecular Machines.

<u>Pending Research Support</u>

DOE, Office of Science 2019 - 2022
Electron cryo-microscopy and single particle analysis as structure-function probe in plant photosynthesis
Role: Co-PI

<u>Completed Research Support</u>

NIH 3 U24 GM116788-02S1 MPI Schmidt-Krey 07/01/2017 – 06/30/2018
Funds to replace a Tridem Energy Filter/CCD camera with a BioQuantum/K3 and to obtain

a Volta phase plate.

NIH R01-EY021205 Lieberman (PI) 03/2011 – 2016
Characterization of purified myocilin: Glaucoma as a protein misfolding disease.
The goal of this study is to use structural and biophysical techniques to characterize the folded and misfolded states of myocilin and its olfactomedin domain. The Schmidt-Krey laboratory visualized the fibrilization of the olfactomedin domain by TEM.
Role: significant collaborator