

Dr. rer. nat. habil. Basem Soboh

Microbiology, Biochemistry and Genetic Biophysics

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1. PERSONAL INFORMATION

Born 1974 in Gaza, Palestine. Married, two children (*2009 and *2012)

2. PROFESSIONAL POSITIONS

2016–present

Freie Universität Berlin

Group leader at the Department of Physics, Genetic Biophysics

Topic: *In vitro* biosynthesis of [NiFe]-hydrogenases

2008–2015

Martin Luther University

Group leader at the Department of Biology, Institute of Microbiology

Topic: *In vitro* biosynthesis of complex Fe-S cofactors

2005–2008

University of California, Berkeley

Postdoctoral Scholar at the Department of Plant and Microbial Biology (with Prof. Paul W. Ludden)

Topic: Biosynthesis of the iron-molybdenum cofactor of nitrogenase

2004–2005

MPI for Terrestrial Microbiology

Postdoctoral Scholar, Department of Biochemistry (with Prof. Rolf Thauer)

Topic: Characterization of energy-conserving [NiFe]-hydrogenases and CO-dehydrogenases

3. EDUCATION

2009–2016

Martin Luther University

Habilitation in Microbiology, Institute of Microbiology (with Prof. Dr. Gary Sawers)

Topic: *In vitro* biosynthesis of complex Fe-S cofactors of nitrogenase and [NiFe]-hydrogenase

2001–2004

Philipps-University Marburg

Dissertation in Biology, Department of Biochemistry at the MPI Marburg (with Prof. Rolf Thauer)

Topic: A multisubunit membrane-bound energy conserving [NiFe] hydrogenase and a NADH-dependent Fe-only hydrogenase. Degree: Dr. rer. nat. (*summa cum laude*)

1998–2001

Philipps-University Marburg

Diploma in Biology, subject combination: Microbiology, Biochemistry, Genetics and Virology

Topic: Purification and catalytic properties of a CO-oxidizing:H₂ evolving enzyme complex.

Supervision: Prof. Rolf Thauer. Degree: diploma (*sehr gut*)

1997–1998

Philipps-University Marburg

Intensive German course

1992–1996

Al-azhar University Gaza

Bachelor of Science, studies of Chemistry and Microbiology

Degree: B.Sc. (*excellent*)

4. HONOR / AWARDS

- 2003–2005 “Max Planck Society Fellowship”, Marburg, Germany
1996–2003 “Excellence Scholarship WUS-Award”, World University Service, Germany
1993–1996 “First Prize-Scholarship for Bachelor Excellence”; Al-azhar-University, Gaza

5. RESEARCH PROFILE

Main Research Interests

Understanding microbial metabolism and energy conversion with respect to gas-processing enzymes e.g. energy converting membrane bound [NiFe] hydrogenases (H₂), NADH-dependent Fe-hydrogenase, CO-dehydrogenase (CO), formate-dehydrogenase (CO₂) and nitrogenase (N₂). Elucidating the biosynthesis and catalytic mechanism of these enzymes at the molecular level (bio-catalysis)

A major focus of my current work is understanding how the cofactor of [NiFe]-hydrogenases is assembled. Our biochemical-genetic strategy involves isolation of the maturation proteins, then following the stepwise biosynthesis and assembly of cofactors in real time. We are using a broad range of methodologies. This includes manipulation of genes, overexpression and the anaerobic purification of maturation protein complexes that required for the *in vitro* reconstitution of the pathway for cofactor biosynthesis. The analytical methods include anoxic enzyme kinetics, FPLC, metabolite analysis (HPLC, GC), functional protein-protein interaction (thermophoresis), metal detection (ICP-MS), and native gel electrophoresis. Spectroscopic methods include UV/Vis-, electron paramagnetic resonance (EPR)-, Mössbauer-, resonance Raman-, and Fourier-transform infrared (FTIR) spectroscopy. Furthermore, we apply anaerobic crystallization, electrophysiology experiments using planar lipid bilayers and protein film electrochemistry in order to record the catalytic currents of enzyme complexes. Large size membrane proteins and enzyme complexes will be determined using cryo electron microscopy.

Experimental Expertise

Protein biochemistry

- Anaerobic purification (FPLC) of metalloproteins, enzymes and native membrane protein complexes in preparative amounts for biochemical and spectroscopic investigations
- *In vitro* reconstitution of pathways for the assembly of Fe-S containing proteins, e.g., [NiFe] hydrogenase and FeMo nitrogenase. Protein refolding and reconstitution of [Fe-S] clusters
- Functional protein-protein interaction under native and anoxic conditions (Microscale thermophoresis, isothermal titration calorimetry)
- Characterization of electron transfer reactions in metalloenzymes and redox cofactors.
- Detailed enzyme kinetics, e.g., UV-vis, anaerobic activity assays, native PAGE with activity staining and analytic methods like liquid and gas chromatography (HPLC, GC)
- Setting up crystallization experiments of oxygen-labile metalloproteins and optimizing hits for diffraction experiments

Microbiology

- Large scale anaerobic cultivation of wild type-thermophilic and recombinant bacteria
- Gas handling for cultivation with CO, N₂, CO₂ and H₂/O₂ mixtures
- Microbial physiology of FeMo nitrogenase (*A. vinelandii*) and [NiFe] hydrogenases (*E. coli*, *C. hydrogenoformans* and *T. tengcongensis*)

Molecular genetics

- Manipulation of genes (site directed mutagenesis, generation of chromosomal mutants, transduction of foreign genes, cloning, overexpression, etc.)
- Bioinformatics analysis, real-time PCR and generation of knock-outs in *E. coli*

Miscellaneous

- Part of the DFG priority program 1927 “Iron-Sulfur for Life” ironsulfurforlife.de (2016–present)
- Working at the Department of Physics since 2016 increased my direct experience of spectroscopic techniques e.g. ATR-FTIR, SEIRAS, EPR-, Raman-, Mössbauer- and EXAFS spectroscopy. This enables me to design and implement complex interdisciplinary research
- Experienced laboratory with biological safety level 2 for growth, storage and manipulation of bacteria classified as S2. (UC-Berkeley and Martin Luther University)
- Intensive training in medical microbiology and infectious diseases (Microbiology department at Al Shifa hospital in Gaza).

6. TEACHING

Lectures and lab courses

Grundpraktikum Mikrobiologie	lecture & lab course	Marburg Univ.	2001–2004
Vertiefungspraktikum Mikrobiologie	lecture & lab course	Marburg Univ.	2001–2004
Project module Biochemistry	lab course	UC Berkeley	2005–2007
Mikrobiologie für Biochemiker	lecture & lab course	Martin Luther Univ.	2008–2015
Mikrobiologie für Biologen	lecture & lab course	Martin Luther Univ.	2008–2015
Project Modul Mikrobiologie (MSc)		Martin Luther Univ.	2008–2014
Forschungsgruppenpraktika (MSc)		Martin Luther Univ.	2008–2015
Molekulare Mikrobiologie	lecture	Martin Luther Univ.	2015–2016
Biophysik für Biochemiker	lecture & lab course	Freie Univ. Berlin	2017–present
Protein-biochemie für Biophysiker	lecture	Freie Univ. Berlin	2017–present
Microscale thermophoresis	lecture	Freie Univ. Berlin	2018–present

Direct supervision of students

Permanent teaching, reviewing students' theses, overseeing daily activities and quality control in the laboratory, supervising laboratory personnel and students, collecting, analyzing, and interpreting lab results. Furthermore, I am responsible for the organization of the weekly lab meetings, for hygiene and safety instruction, as well as for the proper disposal of biological and chemical waste of the microbiology laboratories.

7. EXTERNAL FUNDING

DFG Grant No. SO1325/5-2	345.550 €	2019–2022
DFG Grant No. SO1325/5-1	335.550 €	2016–2019

Funding and financial support from the Deutsche Forschungsgemeinschaft (DFG) via the SPP 1927 priority program Iron-Sulfur for Life for “development of an *in vitro* reconstitution system for the analysis of [NiFe]-hydrogenase maturation”.

8. Selected invited talks & Conference participation

GRC Metallocofactors (2022, Newport USA), **12th International Hydrogenase Conference** (2019, Lisbon PRT), **A Passion for Biochemistry and Microorganisms** (2019, MPI, Marburg), **GRC Cell Biology of Metals** (2017, Mount Snow USA), **11th international Hydrogenase Conference** (2016, Marseilles FR), **10th International Hydrogenase Conference** (2013, Szeged, HUN), **BioH₂Workshop** (2008, Marburg, Germany), **GRC Iron-Sulfur Enzymes** (2006, New London, USA), **7th International Hydrogenase Conference** (2004, Reading, U.K.).

9. Selected PUBLICATIONS

- 1- **Soboh, B***, Adrian, L. and Stripp, S. T. (2022) An *in vitro* reconstitution system to monitor iron transfer to the active site during the maturation of [NiFe]-hydrogenase, Journal of Biological Chemistry, doi: <https://doi.org/10.1016/j.jbc.2022.102291>.
- 2- Stripp, S. T., Oltmanns, J., Muller, C. S., Ehrenberg, D., Schlesinger, R., Heberle, J., Adrian, L., Schunemann, V., Pierik, A. J., and **Soboh, B***. (2021) Electron inventory of the iron-sulfur scaffold complex HypCD essential in [NiFe]-hydrogenase cofactor assembly. Biochemical journal 478, 3281-3295
- 3- Senger M., Laun K., **Soboh B.** and Stripp S.T. (2018) Infrared Characterization of the Periplasmatic O₂-sensitive [NiFe]-hydrogenase from E. coli. Catalysts, (8), 530
- 4- Senger M., Stripp S.T. and **Soboh B***. (2017) Proteolytic Cleavage Orchestrates Cofactor Insertion and Protein Assembly in [NiFe]-hydrogenase Biosynthesis. J Biol Chem. (28):11670-11681.
- 5- Stripp ST., Lindenstrauss U., Sawers RG. and **Soboh B***. (2015) Identification of an Isothiocyanate on the HypEF Complex Suggests a Route for Efficient Cyanyl-Group Channeling during [NiFe]-Hydrogenase Cofactor Generation. PLoS One e0133118. doi: 10.1371/journal.pone.0133118.
- 6- Stripp ST., Lindenstrauss U., Granich C., Sawers RG. and **Soboh B***. (2014) The influence of oxygen on [NiFe]-hydrogenase cofactor biosynthesis and how ligation of carbon monoxide precedes cyanation. Plos ONE 9 e107488. doi: 10.1371/journal.pone.0107488.
- 7- **Soboh B***, Lindenstrauss U., Granich C., Javaid M., Herzberg M., Claudia T. and Stripp ST. (2014) [NiFe]-hydrogenase maturation *in vitro*: analysis of the roles of the HybG and HypD accessory proteins. Biochemical journal. 1;464(2):169-77.
- 8- **Soboh B.**, Stripp ST., Bielak C., Lindenstrauss U., Braussemann M., Javaid M., Hallensleben M., Granich C., Herzberg M., Heberle J. and Sawers RG. (2013) The [NiFe]-hydrogenase accessory chaperones HypC and HybG of *Escherichia coli* are iron- and carbon dioxide-binding proteins. FEBS Lett. 19;587(16):2512-6.
- 9- **Soboh B.** and Sawers RG. (2013) [NiFe]-hydrogenase cofactor assembly. In: Encyclopedia of Inorganic and Bioorganic Chemistry - Metals in Cells, Chapter eibc2154 ISBN: 9781119951438. doi: 10.1002/9781119951438.eibc2154.
- 10- Stripp ST., **Soboh B.**, Lindenstrauss U., Braussemann M., Herzberg M., Nies DH. , Sawers RG. and Heberle J. (2013) HypD is the Scaffold Protein for Fe-(CN)₂CO Cofactor Assembly in [NiFe]-Hydrogenase Maturation. Biochemistry, 52 (19), 3289–32962
- 11- Trchounian K., **Soboh B.**, Sawers RG. and Trchounian A. (2013) Contribution of hydrogenase 2 to stationary phase H₂ production by *Escherichia coli* during fermentation of glycerol. Cell. Biochem. Biophys. 66-(1)103-108.
- 12- **Soboh B.**, Stripp ST., Muhr E., Granich C., Braussemann M., Herzberg M., Heberle J. and Sawers RG. (2012) [NiFe]-hydrogenase maturation: isolation of a HypC-HypD complex carrying diatomic CO and CN- ligands. FEBS Lett., 586(21) 3882-3887
- 13- **Soboh B.**, Kuhns M., Braussemann M., Waclawek M., Muhr E., Pierik AJ. and Sawers RG. (2012) Evidence for an oxygen-sensitive iron-sulfur cluster in an immature large subunit species of *Escherichia coli* [NiFe]-hydrogenase 2. Biochem Biophys Res Commun. 424(1),158-163
- 14- Petkun S., Shi R., Li Y., Asinas A., Munger C., Zhang L., Waclawek M., **Soboh B.**, Sawers RG. and Cygler M. (2011) Structure of Hydrogenase Maturation Protein HypF with Reaction Intermediates Shows Two Active Sites. Structure 19 (12), 1773–1783

- 15-** Pinske C., Krüger S., **Soboh B.**, Ihling C., Kuhns M., Braussemann M., Jaroschinsky M., Sauer C., Sargent F., Sinz A. and Sawers RG. (2011) Efficient electron transfer from hydrogen to benzyl viologen by the [NiFe]-hydrogenases of *Escherichia coli* is dependent on the coexpression of the iron-sulphur cluster-containing small subunit. *Arch. Microbiol.* 193(12),893-903
- 16- Soboh B.**, Pinske C., Kuhns M., Waclawek M., Ihling C., Trchounian K., Trchounian A., Sinz A., and Sawers RG. (2011) The respiratory molybdo-selenoprotein formate dehydrogenases of *Escherichia coli* have hydrogen: benzyl viologen oxidoreductase activity. *BMC Microbiol.* 11:173
- 17- Soboh B.**, Krüger S., Kuhns M., Pinske C., Lehmann A. and Sawers RG. (2010) Development of a cell-free system reveals an oxygen-labile step in the maturation of [NiFe]-hydrogenase 2 of *E. coli*. *FEBS Lett.* 584 (18), 4109-4114
- 18- Soboh B.**, Boyd ES., Zhao D, Peters JW. and Rubio LM. (2010) Substrate specificity and evolutionary implications of a NifDK enzyme carrying NifB-co at its active site. *FEBS Lett.* 584(8),1487-92
- 19-** Rubio LM., Hernández JA., **Soboh B.**, Zhao D., Igarashi RY. , Curatti L. and Ludden PW. (2008). The Role of Nif Proteins in Nitrogenase Maturation. *Plant Science and Biotechnology in Agriculture*, Book: Biological Nitrogen Fixation, Volume 42, pp 325-328
- 20-** Curatti L., Hernandez JA., Igarashi RY., **Soboh B.**, Zhao D. and Rubio LM. (2007). *In vitro* synthesis of the iron-molybdenum cofactor of nitrogenase from iron, sulfur, molybdenum and homocitrate using purified proteins. *Proc. Natl. Acad. Sci.* 104 (45), 17626-31
- 21-** George SJ., Igarashi RY., Piamonteze C., **Soboh B.**, Cramer SP. and Rubio LM. (2007) Identification of a Mo-Fe-S cluster on NifEN by Mo K-edge EXAFS. *J. Am. Chem. Soc.*, 129(11),3060-3061
- 22-** Hernández JA., Igarashi RY., **Soboh B.**, Curatti L., Dean DR., Ludden PW. and Rubio, LM. (2006) NifX and NifEN exchange biosynthetic precursors of the iron-molybdenum cofactor of nitrogenase. *Mol. Microbiol.*, 63 (1),177-92
- 23- Soboh B.**, Igarashi RY., Hernandez JA. and Rubio LM. (2006) Purification of a NifEN protein complex that contains bound Mo and a FeMo-co precursor from an *Azotobacter vinelandii* Δ nifHDK strain. *J. Biol. Chem.*, 281, 36701-36709 cofactor of nitrogenase. *Mol. Microbiol.*, 63 (1),177-92
- 24- Soboh B.**, Forzi L., Stojanowic A. and Hedderich R. (2004) Energy-converting [NiFe] hydrogenases from archaea and bacteria: ancestors of complex I. *Biochimica et Biophysica Acta (BBA) – Bioenergetics.* 1658
- 25. Soboh B.**, Linder D. and Hedderich R. (2004) A multisubunit membrane-bound [NiFe] hydrogenase and a NADH-dependent Fe-only hydrogenase in the fermenting bacterium *Thermoanaerobacter tengcongensis* *Microbiology* 150, 2451-2463
- 26. Soboh B.**, Linder D. and Hedderich R. (2002) Purification and catalytic properties of a CO-oxidizing:H₂ evolving enzyme complex from *Carboxydotherrmus hydrogenoformans* *Eur.J. Biochem.* 269, 5712-21.