PD. Dr. 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

2008–2015 **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:H_2 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 (H_2), NADH-dependent FeFe-hydrogenase, CO-dehydrogenase (CO), formate-dehydrogenase (CO_2) and nitrogenase (N_2). Elucidating the biosynthesis and catalytic mechanism of these enzymes at the molecular level (bio-catalysis).

One of the major focus of my current work is understanding how the complex cofactors of [NiFe]-hydrogenases is assembled. Our biochemical-genetic strategy involves isolation of the maturation proteins, then following the stepwise synthesis and assembly of cofactors using a broad range of methodologies. This includes manipulation of genes, overexpression, anaerobic purification of the maturation protein complexes and intermediates in preparative amounts, then *in vitro* reconstitution of the pathway for cofactor assembly. 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, N_2, CO_2 and H_2/O_2 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
- Organizer of weekly lab meetings, seminars and thesis defenses of students (Institute of Microbiology at Martin Luther University, 2008–2015)
- Responsible for the hygiene & safety instruction; and for the proper disposal of biological/ chemical waste of the microbiology laboratories (UC-Berkeley and Martin Luther University)
- 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, 1995 and 2004)

6. TEACHING

Lectures and lab courses

Biophysik für Biochemiker*innen	lecture & lab course	Freie Univ. Berlin	2016–2019
Forschungsgruppenpraktika (MSc)		Martin Luther Univ.	2008–2015
Project Modul Mikrobiologie (MSc)		Martin Luther Univ.	2008–2014
Vertiefungspraktikum Mikrobiologie	lecture & lab course	Martin Luther Univ.	2008–2015
Mikrobiologie für Biochemiker	lecture & lab course	Martin Luther Univ.	2008–2015
Project module Biochemistry	lab course	UC Berkeley	2005–2007
Grundpraktikum Mikrobiologie	lecture & lab course	Marburg Univ.	2001–2004

Supervision of Students

Daily supervision of 9 MSc- and 7 B.Sc. students at MPI-Marburg, Martin Luther University and FU-Berlin. Co-supervision of 6 PhD students at Martin Luther University and Freie Universität Berlin.

7. EXTERNAL FUNDING

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

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

8. PUBLICATIONS

1- Stripp S.T., Oltmanns J., Müller C., Ehrenberg D., Schlesinger R., Heberle J., Adrian L., Schünemann V., Pierik A., and **Soboh B*** (2021) Electron Inventory of the Iron-Sulfur Scaffold Complex HypCD Essential in [NiFe]-Hydrogenase Cofactor Assembly. ChemRxiv https://doi.org/10.26434/chemrxiv.13736632.v1

2- Senger M., Laun K., **Soboh B.** and Stripp S.T. (2018) Infrared Characterization of the Periplasmatic O_2-sensitive [NiFe]-hydrogenase from E. coli. Catalysts, (8), 530

3- 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.

4- 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.

5- 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.

6- 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.

7- 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.

8- 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.

9- 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

10- Trchounian K., **Soboh B**., Sawers RG. and Trchounian A. (2013) Contribution of hydrogenase 2 to stationary phase H_2 production by *Escherichia coli* during fermentation of glycerol. Cell. Biochem. Biophys. 66-(1)103-108.

11- 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

12- 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

13- 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

14- 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

15- 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. BMCMicrobiol.11:173

16- 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

17- 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. FEBSLett. 584(8),1487-92

18- 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

19- 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

20- 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

21- 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

22- 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 ΔnifHDK* strain. J. Biol. Chem., 281, 36701-36709cofactor of nitrogenase. Mol. Microbiol.,63 (1),177-92

23- 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

24. 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

25. Soboh B., Linder D. and Hedderich R. (2002) Purification and catalytic properties of a CO-oxidizing:H_2 evolving enzyme complex from *Carboxydothermus hydrogenoformans* Eur.J. Biochem. 269, 5712-21.