Abstract:

Powering F_{420}-dependent biocatalysts with molecular hydrogen

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Co-supervisor 2: Maria Andrea Mroginski, TU Berlin (UniSysCat)

Biocatalysts, which rely on the F_{420} cofactor, allow the asymmetric reduction of enoates, imines and ketones at ambient conditions and with high selectivity. The stochiometric supply of reducing equivalent in form of $F_{420}H_2$ is too expensive for industrial applications. H_2-driven cofactor recycling through hydrogenases is 100% atom efficient and causes no by-products or greenhouse gases (Fig. 1). The known F_{420}-reducing hydrogenases are highly O_2 sensitive and thus unattractive for applied biocatalysis. This highly interdisciplinary project aims to design an O_2-tolerant, F_{420}-dependent hydrogenase that provides $F_{420}H_2$ to a F_{420}-dependent and biotechnologically relevant biocatalyst (e.g. for fine chemicals production). While molecular docking studies will be performed in the group of Prof. Dr. Mroginski, the molecular biology and biocatalysis will be conducted in the team of Dr. Lauterbach. Our project will provide valuable insights into the engineering of enzymatic cofactor binding sites and coupling of enzymes.

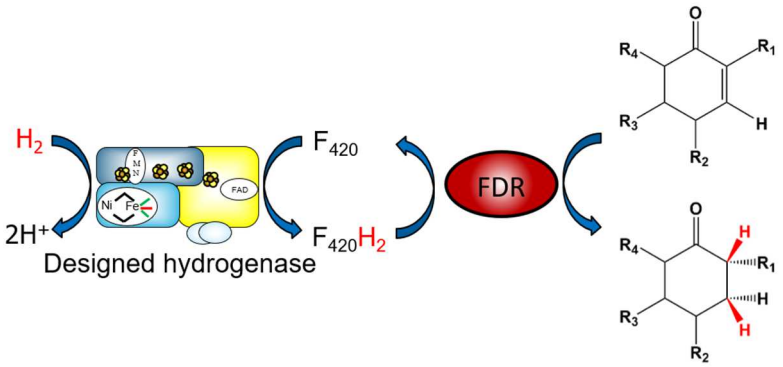

Fig. 1: Design of a F_{420}-dependent hydrogenase for H_2-driven enoate reduction via a F_{420}-dependent reductase (FDR). Yellow and brown spheres represent Fe-S clusters (hydrogenase, left side), green lines represent the CN ligands and the red line represents the CO ligand.

Extended description version of the project:

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1. Overall goal of the project

The overall goal of the project is to power F_{420}-dependent biocatalysts with molecular hydrogen (H_2). The deazaflavin cofactor F_{420} has a low redox potential that makes it suitable as an alternative to nicotinamide cofactors for use in asymmetric reduction reactions by unique F_{420}-dependent biocatalysts (see below). The recycling of the F_{420} cofactor, however, is one of the major limiting steps for applying F_{420}-dependent biocatalysts. In this project, we will design an O_2-tolerant hydrogenase that reduces F_{420} and couples the resulting hybrid enzyme with F_{420}-dependent oxidoreductases for fine chemicals synthesis. To obtain insights into reaction kinetics and electron transfer, we will study the hybrid enzyme by means of biochemical, spectroscopic, and computational techniques. Based on results and the improvement of the hydrogenase through enzyme engineering approaches, the complexity of the H_2-driven biotransformation will be progressively increased. The ultimate goal is the creation of an optimized whole cell for F_{420}-mediated biocatalysis with H_2 as the fuel.

2. State of the art

The application of biocatalysts enables a "greener" chemistry and is an important step towards environmentally friendly industrial production, which saves energy and reduces pollution. Over 20% of industrial relevant biocatalysts require reduced cofactors to perform regio-, stereo- and substrate-specific conversions.

The stoichiometric addition of reduced cofactors is too expensive for industrial large-scale applications. Hydrogenases are enzymes that catalyze the oxidation and reduction of H_2. The use of hydrogenases for cofactor regeneration provides an efficient H_2-driven synthesis of fine chemicals with 100 % atom efficiency without formation of by-products or greenhouse gases.[1] In contrast to most other hydrogenases, the oxygen-tolerant NAD^+-reducing hydrogenase from *Ralstonia eutropha* ("soluble hydrogenase", *Re*SH) is capable of catalyzing H_2 cleavage even in the presence of O_2.[2] H_2 conversion takes place at the NiFe center within the hydrogenase module, which is connected to the NAD binding site within the reductase module through a chain of Fe-S clusters and flavins.[3] Recently, our group extended the substrate spectrum of the *Re*SH towards NADPH and also flavins.[4] We have already demonstrated H_2-driven production of various amines, (S)-1-phenylethanol, 1-octanol and a set of nitrogen heterocycles with NAD, NADP, FMN and FAD as redox mediators. [1, 4a, 5]

However, many biotechnologically relevant enzymes with unique stereo-, regio- and substrate-specificity rely on the F_{420} cofactor.[6] For example, the F_{420}-dependent reductases (FDR) reduce α,β-unsaturated ketones and aldehydes in an enantio- and regioselective fashion. Interestingly, the opposite enantiomer is produced by FDR in contrast to the class of old yellow enzymes (Fig. 1).[6]

The F_{420} cofactor and F_{420}-dependent oxidoreductases are exclusively found in actinobacteria and methanogens. All natural F_{420}-reducing hydrogenases known so far are highly O_2-sensitive, which makes production and purifying F_{420}-dependet hydrogenases challenging and H_2-driven F_{420} recycling for O_2-dependent redox catalysts not feasible.[7]

For the rational design of a hybrid hydrogenase with new catalytic properties, molecular modelling approaches will be used, including docking experiments, classical molecular dynamics simulations and quantum chemical calculations.[8] The outcome of the modeling approaches will guide the molecular biological and biochemical experiments.

3. Specific aims and how they may be reached:

WP 1: *In silico* design of an O_2-tolerant hydrogenase with F_{420}-reducing properties.

In WP1, we will design a hydrogenase for H_2-driven F_{420} reduction in the presence of O_2. In this project, we pursue this goal *via* the so-called design-build-test-learn (DBTL) cycles. Thereby we will focus on the O_2-tolerant NAD^+-reducing *Re*SH[1] and the O_2-sensitive F_{420}-reducing hydrogenase from *Methanosarcina barke*ri (*Mb*SH).[7] Based on the molecular modeling, we will design hybrid enzymes that display both O_2 tolerance *AND* F_{420} reduction (DBTL cycle, design) by following two strategies: First only the NAD(H)-binding site of the *Re*SH will be remodeled to accommodate the F_{420} cofactor. In the second approach the entire F_{420}-reducing protein module of *Mb*SH will be docked to the hydrogenase module of the *Re*SH. The integrity and stability of the

resulting hybrids will be evaluated through classical molecular dynamics techniques, substrate binding will be investigated via flexible docking approaches, and the hydride transferring properties of the F_{420} binding site will be further studied through QM/MM approaches.

WP 2: Generation, purification and characterization of hydrogenase hybrids that reduce F_{420} with H_2

In WP 2, we will build a small library of hybrid hydrogenases (DBTL cycle, $build_0$) based on the *in silico* design performed in WP1. A strain for facilitated genetic engineering to design modified metalloenzymes is already available in the Lauterbach team.[4a] We will employ an *R. eutropha* strain that is susceptible to electroporation. UV/Vis photometry will be used to test functional hybrid enzymes (DBTL cycle, test). Hybrids showing H_2-driven F_{420} reduction activity will be investigated with in-house biochemical and analytical techniques available in the **Lenz** group. Spectroscopic studies of promising hybrid enzymes will be conducted in collaboration with UniSysCat partners, including ICP-OES (**S. Leimkühler**, University Potsdam), FTIR, SEIRA and EPR spectroscopy (**I. Zebger**, TU Berlin; **M. Horch**, **C. Teutloff**, **R. Bittl**, FU Berlin) and Mössbauer spectroscopy (**C. Limberg**, HU Berlin). Expertise in *Mb*SH biocatalysis and crystallisation will be provided by **H. Dobbek**, HU Berlin. We will get insights into biocatalysis of selected hybrids through electrochemical techniques in cooperation with **K. Vincent. (**University of Oxford, Clara-Immerwahr awardee of UniSysCat). In a final "learning" step, we will use our gained knowledge about the function of hybrids (electron transfer, kinetic parameters, stability) and a new target-specific DBTL cycle will be started for improvements of biocatalytic properties by rational design.

WP 3: Hydrogenase hybrids that power F_{420}-dependent biocatalysts

In WP 3, promising F_{420}-reducing hydrogenase hybrids obtained in WP2 will be coupled to a well described F_{420}- FDR.[6] Based on the conversion of the model compound menadione, we will determine the biocatalytic properties and interplay of enzymes by GC-FID. For NMR and GC-MS based product analysis, we can rely on the infrastructure of the institute of chemistry at the TU Berlin. In collaboration with **K. Vincent** (University of Oxford), we will immobilize hybrids and FDR in a continuous flow reactor for rapid heterogeneous biocatalysis.

WP 4: Design of a third artificial redox pool in *R. eutropha* for F_{420}-mediated biocatalysis

In WP 4, we will design an artificial $F_{420}H_2/F_{420}$ pool in *R. eutropha* to power recombinant FDRs with H_2. The advantage of an artificial $F_{420}H_2/F_{420}$ pool is that it is not shared by other anabolic pathways. Recently, an *E. coli* strain has been described that carried a recombinant biosynthesis for the cofactor F_{420} and produced F_{420} levels comparable those of actinobacteria.[9] We will construct an analogous plasmid for F_{420} synthesis in *R. eutropha* and will analyse the recombinantly produced enzymes and F_{420} by immunoblots and GC-MS, respectively. Co-synthesizing a hydrogenase hybrid (WP 2) and FDR (WP 3) will allow whole-cell catalysis fuelled by H_2. The reduction of menadione will serve as the model compound and as a read-out for efficient H_2-driven biotransformation. For H_2- and electro-driven whole cell biocatalysis, the PhD student will collaborate with PhD students from the Marie-Curie network **ConCO2rde** and the **SPP 2240**.

References:

[1] (a) **Lauterbach, L.; Lenz, O**., *Curr Opin Chem Biol* 2019, *49*, 91-96, DOI: 10.1016/j.cbpa.2018.11.020; (b) **Lauterbach, L.; Lenz, O.; Vincent, K. A**., *FEBS J* 2013, *280* (13), 3058-68, DOI: 10.1111/febs.12245

[2] **Lauterbach, L.; Lenz, O.**, *J Am Chem Soc* 2013, *135* (47), 17897-905, DOI: 10.1021/ja408420d

[3] Shomura, Y.; … Higuchi, Y., *Science* 2017, *357* (6354), 928-932, DOI: 10.1126/science.aan4497

[4] (a) Preissler, J.; Reeve, H. A.; Zhu, T.; Nicholson, J.; Urata, K.; **Lauterbach, L.**; Wong, L. L.; **Vincent, K. A.**; **Lenz, O.**, *ChemCatChem* 2020, *12* (19), 4853-4861, DOI: 10.1002/cctc.202000763; (b) Al-Shameri, A.; Willot, S. J. P.; Paul, C. E.; Hollmann, F.; **Lauterbach, L.**, *Chem Comm*2020, *56* (67), 9667-9670, DOI: 10.1039/D0CC03229H

[5] Al-Shameri, A.; Petrich, M. C.; Junge Puring, K.; Apfel, U. P.; Nestl, B. M.; **Lauterbach, L.**, *Angew Chem Int Ed Engl* 2020, DOI: 10.1002/anie.202001302

[6] Shah, M. V.; …; Scott, C., *Catalysts* 2019, *9* (10), 868, DOI: doi.org/10.3390/catal9100868

[7] Ilina, Y.; Lorent, C.; Katz, S.; Jeoung, J.-H.; Shima, S.; Horch, M.; **Zebger, I.**; **Dobbek, H.**, *Angew Chem Int Ed Engl* 2019, *58* (51), 18710-18714, DOI: 10.1002/anie.201908258

[8] Damborsky, J.; Brezovsky, J., *Curr Opin Chem Biol* 2014, *19*, 8-16, DOI: 10.1016/j.cbpa.2013.12.003

[9] Bashiri, G.; ….; Baker, E. N.; Jackson, C. J., *Nat Comm* 2019, *10* (1), 1558, DOI: 10.1038/s41467-019-09534-x