CERC3 Young Chemists' Workshop "Biocatalysis"

March 24 - March 27, 2004
Institut für Organische Chemie, Henkestr. 42, 91054 Erlangen, Germany

Program

WEDNESDAY, March 24, 2004

15.00 – 19.00	Registration
19.00 – …	Welcome mixer in the foyer of the great lecture hall of the Institute of Organic Chemistry

THURSDAY, March 25, 2004

09.00 – 09.10	Opening Remarks
	Chair: Markus Reiher
09.10 – 09.45	**Mahdi Abu-Omar** (Purdue University, West Lafayette) *"Mechanistic studies of phenylalanine hydroxylase and a novel example of enzyme self-repair within the second coordination sphere of catalytic iron"*
09.45 – 10.20	**Tatyana Polenova** (University of Delaware, Newark) *"Solid-State NMR Spectroscopy for Studies of Geometry and Electrostatic Environment in Vanadium Haloperoxidases"*
10.20 – 10.55	**Roderich Süssmuth** (Universität Tübingen) *"Abyssomicins, Novel Inhibitors of the para-Aminobenzoic Acid Biosynthesis from the Marine Verrucosispora-Strain AB-18-032"*
10.55 – 11.20	Coffee break
11.20 – 11.55	**Isabel Arends** (Delft University of Technology) *"Laccase Catalysed Aerobic Oxidation of Alcohols"*
11.55 – 12.30	**Catherine Hemmert** (CNRS Toulouse) *"Asymmetric epoxidation with biomimetic catalysts"*
12.30 – 13.05	**Benjamin Davis** (University of Oxford) *"Novel Methods in Carbohydrate-associated Biocatalysis"*
13.05 – 14.30	Lunch break
	Chair: Norbert Jux
14.30 – 15.05	**Markus Reiher** (Universität Bonn) *"The bottom-up approach in theoretical bioinorganic chemistry"*
15.05 – 15.40	**Craig Grapperhaus** (University of Louisville) *"Modeling Oxygenated Cysteine Thiolates at the Active Site of Iron-Containing Nitrile Hydratase with a Thiolate/Thioether Donor Set"*
15.40 – 16.15	**Grit Straganz** (Technische Universität Graz) *"Oxygenases of the cupin-superfamily - insights into the mechanism of the non-heme iron dependent dioxygenase Dke1"*

16.15 – 16.45	Coffee break
16.45 – 17.20	**Paul Dalby** (University College London) *"Expanding the synthetic repertoire of transketolase"*
17.20 – 17.55	**Michael Müller** (Forschungszentrum Jülich) *"Diversity-oriented synthesis: new concepts derived from nature"*
17.55 – 18.30	**Brian Gibney** (Columbia University, New York) *"Heme Protein Biocatalysis: Lessons from the Protein Data Bank and De Novo Heme Protein Design"*
19.00 – …	Franconian dinner buffet in the Institute of Organic Chemistry

FRIDAY, March 26, 2004

	Chair: Michael Müller
09.00 – 10.00	Plenary Lecture **Bernhard Lippert** (Universität Dortmund) *"Where Bioinorganic Chemistry Meets Molecular Architecture: Modelling Metal-Nucleic Acids Interactions"*
10.00 – 10.35	**Frank Bordusa** (MPI für Enzymologie der Proteinfaltung, Halle) *"Proteases: Old Enzymes with new synthetic properties"*
10.35 – 11.00	Coffee break
11.00 – 11.35	**Sandrine Ollagnier de Choudens** (Université Joseph Fourier, Grenoble) *"Biotin synthase: a radical strategy for sulphur insertion into non-activated C-H bonds"*
11.35 – 12.10	**Gideon Grogan** (University of York) *"Structure, Mechanism and Application of an unusual Carbon-Carbon Bond Hydrolase"*
12.10 – 12.45	**Andrea Zocchi** (Université de Neuchâtel) *"Transgenic proteins as hosts for enantioselective catalysis"*
12.45 – 14.15	Lunch break
	Chair: Roderich Süssmuth
14.15 – 14.50	**Thibaud Coradin** (Université Pierre et Marie Curie, Paris) *"Biocatalysis in biomineralization Processes?"*
14.50 – 15.25	**Michaela Kreiner** (University of Glasgow) *"Protein-coated micro-crystals: Applications in biocatalysis"*
15.25 – 16.00	**Cameron Neylon** (University of Southampton) *"Labeling and immobilizing proteins via intein mediated ligation"*
16.00 – 16.30	Coffee break
16.30 – 17.05	**Cecilia Tommos** (Stockholm University) *"De Novo Design as a Tool to Investigate Protein Chemistry"*
17.05 – 17.40	**Virgil Hélaine** (Université Blaise Pascal, Aubiere) *"Directed evolution on transketolase for obtaining new monosaccharides"*
17.40 – 18.15	**Andreas Marx** (Universität Konstanz) *"Taming Giants: Insights into DNA Polymerase Function"*
18.15 –	Poster Session in the foyer of the great lecture hall of the Institute of Organic Chemistry, in between Italian dinner buffet

SATURDAY, March 27, 2004

	Chair: Andreas Marx
09.00 – 09.35	**Rita Pacheco** (Universidade de Lisboa) *"Synthesis of Hydroxamic Acid in TTAB Reversed Micelles using Amidase from Pseudomonas sp."*
09.35 – 10.10	**Erwan Galardon** (Université René Descartes, Paris) *"Modelling the active site of Nitrile Hydratase: oxidation of (N_2S_2)-Fe and (N_2S_2)-Co complexes"*
10.10 – 10.40	**Clotilde Policar** (Université Paris IX) *"Mn complexes as SOD synzymes. Methodology for the evaluation of the SOD-activity: from anhydrous experiments to transient kinetics in aqueous solution"*
10.45 – 11.15	Coffee break
11.15 – 11.50	**Gerard Roelfes** (University of Groningen) *"From Enzyme Models to Synthetic Proteins: a Chemist's Approach to the Study of Enzymes"*
11.50 – 12.25	**Jose Palomo** (CSIC, Instituto de Catalisis, Madrid) *"Modulation of Chirality of Lipases via immobilization techniques"*
12.25 – 12.30	Closing remarks
12.30 – 14.00	Lunch buffet in the Institute of Organic Chemistry
14.00 – 18.30	Excursion to Bamberg
19.00 –	Dinner buffet in the Institute of Organic Chemistry

Lecture Abstracts

(only main authors)

O1 **Mahdi Abu-Omar**
"Mechanistic studies of phenylalanine hydroxylase and a novel example of enzyme self-repair within the second coordination sphere of catalytic iron"

O2 **Tatyana Polenova**
"Solid-State NMR Spectroscopy for Studies of Geometry and Electrostatic Environment in Vanadium Haloperoxidases"

O3 **Roderich Süssmuth**
"Abyssomicins, Novel Inhibitors of the para-Aminobenzoic Acid Biosynthesis from the Marine Verrucosispora-Strain AB-18-032"

O4 **Isabel Arends**
"Laccase Catalysed Aerobic Oxidation of Alcohols"

O5 **Catherine Hemmert**
"Asymmetric epoxidation with biomimetic catalysts"

O6 **Benjamin Davis**
"Novel Methods in Carbohydrate-associated Biocatalysis"

O7 **Markus Reiher**
"The bottom-up approach in theoretical bioinorganic chemistry"

O8 **Craig Grapperhaus**
"Modeling Oxygenated Cysteine Thiolates at the Active Site of Iron-Containing Nitrile Hydratase with a Thiolate/Thioether Donor Set"

O9 **Grit Straganz**
"Oxygenases of the cupin-superfamily - insights into the mechanism of the non-heme iron dependent dioxygenase Dke1"

O10 **Paul Dalby**
"Expanding the synthetic repertoire of transketolase"

O11 **Michael Müller**
"Diversity-oriented synthesis: new concepts derived from nature"

O12 **Brian Gibney**
"Heme Protein Biocatalysis: Lessons from the Protein Data Bank and De Novo Heme Protein Design"

O13 **Frank Bordusa**
"Proteases: Old Enzymes with new synthetic properties"

O14 **Sandrine Ollagnier de Choudens**
"Biotin synthase: a radical strategy for sulphur insertion into non-activated C-H bonds"

O15 **Gideon Grogan**
"Structure, Mechanism and Application of an Unusual Carbon-Carbon bond Hydrolase"

O16 **Andrea Zocchi**
"Transgenic proteins as hosts for enantioselective catalysis"

O17 **Thibaud Coradin**
"Biocatalysis in biomineralization processes?"

O18 **Michaela Kreiner**
"Protein-coated micro-crystals: Applications in biocatalysis"

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"Labeling and immobilizing proteins via intein mediated ligation"

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"Mn complexes as SOD synzymes. Methodology for the evaluation of the SOD-activity: from anhydrous experiments to transient kinetics in aqueous solution"

O26 **Gerard Roelfes**
"From Enzyme Models to Synthetic Proteins: a Chemist's Approach to the Study of Enzymes"

O27 **Jose Palomo**
"Modulation of Chirality of Lipases via immobilization techniques"

PL **Bernhard Lippert**
"Where Bioinorganic Chemistry Meets Molecular Architecture: Modelling Metal-Nucleic Acids Interactions"

Mechanistic studies of phenylalanine hydroxylase and a novel example of enzyme self-repair within the second coordination sphere of catalytic iron

Mahdi M. Abu-Omar

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Phenylalanine hydroxylase, a mononuclear non-heme iron enzyme, catalyzes the hydroxylation of phenylalanine to tyrosine in the presence of oxygen and reduced pterin cofactor. X-ray structural studies have established the coordination around the iron metal center and point to significant interactions within the second coordination sphere. One such interaction involves Tyr325 in human phenylalanine hydroxylase (hPAH), which forms a hydrogen-bonding network with an aqua ligand on iron and the pterin cofactor. The full-length tetramer (1-452) and truncated dimer (117-424) Tyr325Phe hPAH mutant enzymes showed similar kinetics, thermal stabilities, and oligomerization profiles as their corresponding wild-type proteins. The possibility of in vivo posttranslational hydroxylation that would restore the activity of hPAH was examined by mass spectrometry on trypsin digested full-length (1-452) hPAH Tyr325Phe point mutant. The amino acid tags obtained by ESI-MS/MS confirmed the presence of a Phe325 in the peptide corresponding to the doubly charged precursor ion at *m/z* 916.4 (L A T I **F** W F T V E F G L C K), and its hydroxylated counterpart in the peptide corresponding to *m/z* 924.4 (L A T I **F-OH** W F T V E F G L C K) by product ion series comprising the fragments y_5-y_{12}. Furthermore, the point mutation Tyr325Ala resulted in an enzyme that was totally inactive, and did not display any evidence of hydroxylation. These results demonstrate the importance of Tyr325 for proper conformation of the active site, substrate binding, and catalysis. The rescue of the Tyr325Phe mutant in hPAH via self-hydroxylation presents a novel example of oxidative repair on the molecular level.

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Solid-State NMR Spectroscopy for Studies of Geometry and Electrostatic Environment in Vanadium Haloperoxidases

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Vanadium haloperoxidases catalyze a two-electron oxidation of halides to hypohalous acid in the presence of hydrogen peroxide; the native enzymes require diamagnetic V(V) for their activity. Vanadium haloperoxidases are the most efficient halide oxidants known to date, and have attracted significant attention due to their potential applications in industrial-scale catalytic bioconversions. However, the mechanism of substrate specificity of these enzymes is poorly understood. The factors governing substrate specificity remain unclear, especially in the context of the recent site-directed mutagenesis results. We employ solid-state NMR spectroscopy to probe the geometry and electrostatic environment of the vanadium center, as well as the ionization states of the active site amino acids. In our initial study, we have addressed a series of novel oxovanadium (V) complexes mimicking the haloperoxidase active site and a series of vanadium-substituted oxoanionic solids, using a combination of ^{51}V solid-state Magic Angle Spinning NMR and quantum mechanical calculations with Density Functional Theory. In the vanadium-substituted ionic oxotungstates, the fine structure constants are determined by the nature and geometry of counter cations as well as the vanadium substitution. The NMR spectra provide an additional measure of sample morphology and positional disorder with respect to vanadium atoms, which cannot be inferred from the X-ray crystallographic data. The experimental results revealed that in the haloperoxidase mimics, variations of the ligand coordination geometry and electronic structure beyond the first coordination sphere have profound effect on the NMR fine structure constants. Moreover, for crystallographically characterized compounds density functional theory- calculations with two different basis sets predict quadrupolar and CSA tensors, which are in close agreement with the experimental NMR values.

These findings are of potential importance for understanding differences in activities due to subtle variations in the active centers of vanadate-dependent haloperoxidases from different microorganisms. Solid-state NMR spectroscopy of vanadium haloperoxidases is under way.

This work is the first step toward correlating the chemical reactivity of the vanadium site in the vanadium (V) coordination and ionic compounds with their molecular and electronic structure, which could have further implications in design of oxovanadium catalysts with tunable properties.

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4. Wenlin Huang, Louis Todaro, Lynn C. Francesconi, Tatyana Polenova (the 2003). ^{51}V Magic Angle Spinning NMR Spectroscopy of Six-Coordinate Lindqvist Oxoanions: A Sensitive Probe For Electronic Environment in Vanadium Containing Polyoxometalates. I. Counterions Dictate ^{51}V Fine Structure Constants in Polyoxometalate Solids, *J. Am. Chem. Soc.* **125**, pp. 5928-5938

Abyssomicins, Novel Inhibitors of the para-Aminobenzoic Acid Biosynthesis from the Marine Verrucosispora-Strain AB-18-032

Bojan Bister[a], Julia Riedlinger[b], Andreas Reicke[b], Daniel Bischoff[a], Hans Zähner, Alan T. Bull, Louis A. Maldonado, Alan C. Ward, Michael Goodfellow, Hans-Peter Fiedler[b], Roderich D. Süssmuth[a]

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Organisms of marine origin are considered as promising source for drug finding. Rare actinomycetes from the deep sea-plain were screened for inhibitors of the *p*-aminobenzoate (*p*ABA) biosynthesis being part of the tetrahydrofolate biosynthesis pathway. Among the few but prominent synthetic inhibitors of the latter are sulphonamides and trimethoprim.

A simple agar-diffusion assay was employed for screening.[1a] Three compounds named abyssomicins were isolated from *Verrucosispora* strain AB 18-032 collected from a sediment sample in the Japanese Sea. The inhibitory effects of abyssomicin C as the major metabolite could be depleted upon addition of *p*ABA. Structure elucidation was performed by ESI-FTICR-MS, 2D-NMR and X-ray crystallography.[1b] Besides similarities to tetrocarcin-type antibiotics, the oxabicyclo-partial structure of abyssomicin C resembles the solution conformation of chorismic acid[2] as well as synthetic chorismate mutase inhibitors.[3] Furthermore, the Michael-system (C7-C9) of abyssomicin C lacking in other antibiotically inactive abyssomicins is supposed to be involved in an irreversible enzyme trapping-mechanism.

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Laccase Catalysed Aerobic Oxidation of Alcohols

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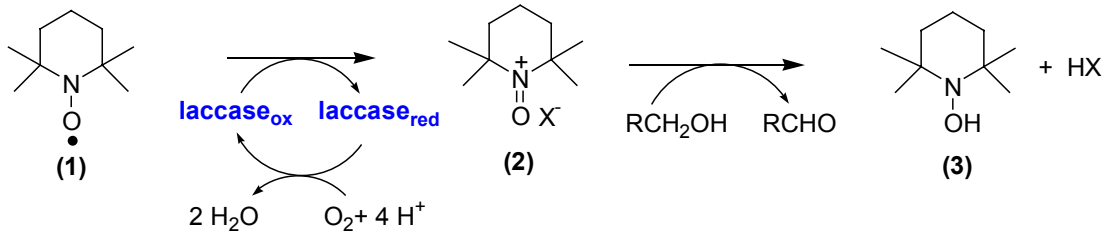
The selective oxidation of alcohols is a pivotal reaction in organic synthesis, which is often hampered by the use of stoichiometric reagents and toxic byproducts. The development of green, biocatalytic methods for the selective oxidation of alcohols is therefore an important goal. Copper enzymes are known to catalyse the aerobic oxidation of alcohols and a well-known example thereof is provided by galactose oxidase. Another group of copper-dependant oxidases comprises the laccases (EC 1.10.3.2). These isoenzymes generally contain four copper centers per protein molecule and catalyse the oxidation of electron rich aromatic substrates, usually phenols or aromatic amines, via four single electron oxidation steps concomitant with the four electron reduction of O_2 to H_2O [1].

In the delignification of lignocellulose, the action of laccase is enforced by so-called mediators: low molecular weight electron transfer agents, that shuttle electrons from the lignin to the enzyme [2]. In our project we use TEMPO as a mediator to extend the action of laccase towards the oxidation of a range of alcohols. The following sequence is proposed:



One-electron oxidation of TEMPO (**1**) affords the oxoammonium cation (**2**) which oxidises the alcohol via a heterolytic patway, giving the carbonyl product and the hydroxylamine (**3**). Compound (**3**) can either be reoxidised by dioxygen, laccase or the oxoammonium cation (**2**). Typically the laccase/TEMPO catalysed aerobic oxidation of alcohols requires high loadings of TEMPO and laccase, which hampers the commercial application [3]. We have undertaken a detailed kinetic and mechanistic study in order to develop a more effective system and to extend the scope to a broad range of alcohol substrates.

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Asymmetric epoxidation with biomimetic catalysts

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Optically active epoxides are key intermediates in organic chemistry because they can undergo stereospecific ring-opening reactions, giving rise to a wide variety of biologically and pharmaceutically compounds.[1] There still continues to be great interest in the design of catalysts for asymmytric alkene epoxidation. The most notable successes were the chiral manganese salen complexes developed by Jacobsen[2] et al. and Katsuki[3] et al. at about the same time, which were the first catalysts able to perform the asymmetric epoxidation of unfunctionalized olefins in high enantiomeric excess.

In the present work, we have developed a new strategy to synthesize macrocyclic chiral manganese salen complexes. The macrocyclisation of the Schiff bases is expected to increase the stability of the corresponding homogeneous catalysts for recycling. Moreover, the synthetic strategy allows the modulation of each building block of the molecules (bulky groups, chiral dimiime and the junction arm for the macrocyclisation).

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Novel Methods in Carbohydrate-Associated Biocatalysis

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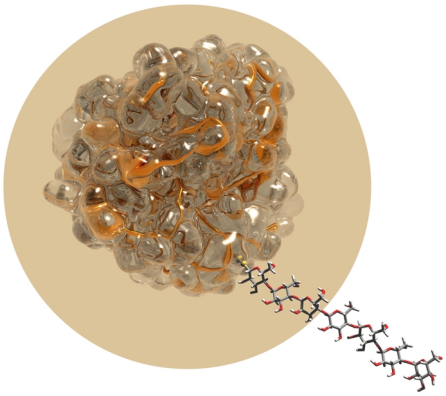
Sugars are critical biological markers that modulate the properties of proteins. Our work studies the interplay of proteins and sugars. This lecture will discuss recent developments in biocatalysis in our laboratory in two areas: (i) glycoenzyme synthesis – the use of protein glycosylation to modulate enzyme function; and (ii) carbohydrate-processing enzyme mechanism – the engineering and study of glycosidases & glycosyltransferases and the synthesis of probes of their mechanism.



(i) Glycoenzyme synthesis: Glycoproteins occur naturally in mixtures within which each component has different properties but they are difficult to obtain pure. To explore the key properties of these glycosylated proteins, there is a pressing need for methods that will not only allow the preparation of pure glycosylated proteins, but will also allow the preparation of non-natural variants for the determination of structure-activity relationships (SARs).[1] We have described the first examples of an answer to this problem which allows site- and sugar- specific glycosylation of proteins using a novel combined site-directed mutagenesis (SDM) and chemical modification strategy.[2] This has allowed the effects of protein glycosylation to be explored precisely for the first time.[3] Precisely glycosylated enzymes can be used in • preparative biocatalysis[4] • drug delivery[5] and • selective protein degradation[6]. A new class of glycoconjugate, the *glycodendriprotein*, has also been developed[7] that acts as a powerful nanomolar inhibitor of bacterial interactions. Furthermore, 2nd generation glycosylating reagents[8] allow the incorporation of the largest known carbohydrates in site selective glycosylations yet require only very small amounts of sugar.

(ii) Carbohydrate-Processing Enzymes: Glycosidases are powerful tools for use in glycoside synthesis and potential targets for therapeutic intervention. Many elegant studies have made significant advances towards understanding the reactive catalytic mechanisms of these enzymes. However, the mechanism by which substrate specificity is determined is still largely unclear. We have begun to explore the underlying basis of substrate selectivity through mutagenesis to tailor the substrate tolerance of glycosidase catalysts and enhance their synthetic utility.[9] In addition, we have developed methods[10] for the ready construction of arrays of inhibitors as probes of carbohydrate-processing enzymes and which have allowed the identification of novel inhibitors.[10] These include novel stereodynamic aza-sugar strategies that have allowed the first synthesis of the naturally occurring hydrophobically-modified aza sugar, Adenophorine.[12] In addition, through the use of a novel high throughput mass spectrometric screening system we have begun to evaluate the specificity and activity of the entire 107 strong *A thaliana* glycosyltransferase family GT-1. Initial results reveal some unusual activities and highlight some striking functional similarities with some bacterial family GT-1 enzymes.

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The bottom-up approach in theoretical bioinorganic catalysis

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For arriving at a detailed microscopic understanding of reactions taking place in metallo-enzymes, theoretical methods are urgently needed in order to supplement experimental data. Prominent examples, where experimental and theoretical methods compete and yield complementary results, are the yet unknown mechanisms of nitrogenase and NiFe hydro-genase.

While theoretical methods allow for a rigorous control of errors (at least in principle), methods of density functional theory (DFT) still suffer of serious failures. Because of the fact that the activity of metalloenzymes is largely determined by the transition metal atom or cluster in the active center, it is inevitable to establish accurate and efficient quantum chemical methods for reliable calculations of such electronic structures. We set out to study failures and benefits of current DFT methods on experimentally well characterized biomimetic model systems and models of active sites in metalloenzymes in order to improve on the presently available methodologies.

The talk starts with a discussion of structures and energetics of Sellmann-type nitrogenase model complexes, which will reveal serious deficiencies of standard density functionals with respect to the calculation of spin state energetics (with consequences for the calculation of reaction energies).

Furthermore, understanding electronic structures requires tools for qualitative analyses of electronic wavefunctions. Here, we will discuss a novel semi-quantitative method for the fast estimation of intramolecular hydrogen bond energies as well as an implementation of Davidson's local spin definition applied to the FeMo-cofactor of nitrogenase.

Next, the calculation of molecular properties in a rigorous quantum mechanical framework requires smart algorithms, which do not calculate all but only the chemically relevant properties. The talk demonstrates how this can be achieved for molecular vibrations, for which we have established the so-called Mode-Tracking methodology.

References:

1. Please, visit our web pages at www.thch.uni-bonn.de/tc/groups/reiher for detailed information and references

Modeling Oxygenated Cysteine Thiolates at the Active Site of Iron-Containing Nitrile Hydratase with a Thiolate/Thioether Donor Set

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Nitrile hydratase (NHase), which catalyzes the hydration of nitriles to amides, is an intriguing enzyme not only based on the utility of its function, but also the uniqueness of its active site.[1] The enzyme incorporates iron, normally associated with redox active enzymes, in a non-redox role. Secondly, the N_2S_3 donor environment about the iron contains two modified cysteine residues resulting in a mixed thiolato (RS^-), sulfenato ($RS(O)^-$), sulfinato (RSO_2^-) donor set. Such sulfur oxygenation is unprecedented in biological systems and its consequences remain undetermined. A series of iron complexes based on the pentadentate ligand 4,7-bis(2'-methyl-2'-mercaptopropyl)-1-thia-4,7-diazacyclononane), $(bmmp\text{-}TASN)^{2-}$, have been synthesized and characterized as models of iron-containing NHase.[2] The ligand binds iron(III) with a single, variable additional ligand, X (X = Cl, NO, CN, OFeL). As demonstrated by EPR and NMR, the nature of X, and not the presence of the two π-donating thiolate donors, determines the spin-state of the complex. Comparison of the metric data within the series highlights a key difference between high-spin and low-spin iron-thiolate bonding.[2,3] Whereas the iron-thiolate and iron-thioether bond distances are quite different for high-spin iron(III) they are indistinguishable for low-spin iron. The relevance of the spectroscopic and structural results to nitrile hydratase is described and the similarities and differences of thiolates, thioethers, and S-oxygenates as donors to low-spin iron are presented. Density functional theory (DFT) calculations of the iron-nitrosyl complex are consistent with our description of iron-sulfur bonding in this system and reveals the HOMO region is dominated by Fe-S bonding.

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Oxygenases of the cupin-superfamily - insights into the mechanism of the non-heme iron dependent dioxygenase Dke1

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The cleavage of C-C bonds via molecular oxygen by non-heme metal dependent dioxygenases plays an essential role in numerous biochemical pathways. Although ubiquitous in nature, this reaction class does not have a direct chemical counterpart. The requirements for the active site of a natural or biomimetic metal-complex to trigger oxygenative C-C bond cleavage are under active investigation. While especially the C-C bond cleavage of intradiol and extradiol cleaving catechol dioxygenases have been subject of intense study, reports on oxygenases of the cupin family are limited (1). The latter are very diverse regarding their metal cofactor as well as their substrate acceptance. The diketone cleaving dioxygenase Dke1 [EC 1.13.11.50] is one exponent of the cupin-super family (2,3). Its relaxed substrate spectrum towards basic β-dicarbonyl structures (4) offers interesting new possibilities for the investigation of the enzyme mechanism – giving us the possibility to directly study electronic effects on various steps of the enzme mechanism, such as the oxygenative C-C bond cleavage. Our findings offer intriguing insights into the principle nature of oxygen activation and C-C bond cleavage in non-heme metal dependent dioxygenases.

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Expanding the synthetic repertoire of transketolase

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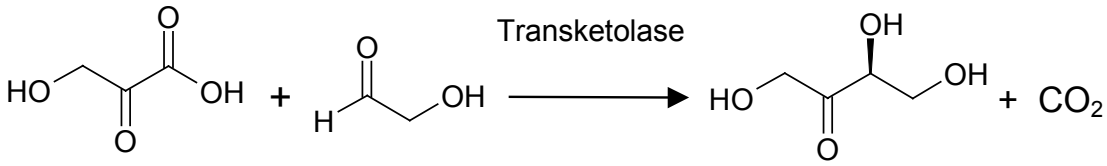
Transketolase catalyses the transfer of a two-carbon ketol unit from xylulose 5-phosphate to an aldehyde acceptor such as erythrose 4-phosphate, producing a new C-C bond and chiral centre with high enantioselectivity. An alternative ketol donor, beta-hydroxypyruvate, can be used to ensure that the reaction is irreversible, producing CO_2 as a byproduct, and hence cost effective at large scale.



To widen the appeal of using enzymes as catalysts for organic synthesis it is desirable to improve their properties in terms of catalyst stability in non-physiological conditions and acceptance of a broader range of substrates. Our efforts to address both of these issues will be presented.

Firstly, we aim to improve enzyme stability by directed evolution and thus require a screening method that directly measures enzyme stability. A screening approach will be presented in which enzyme unfolding can be measured directly in an automatable microwell system, thus avoiding the need to use indirect screens such as thermoinactivation assays.

Secondly, we are exploring the use of bioinformatics combined with genetic engineering to obtain a broad-range transketolase that could be used more generally in organic synthesis, but also as a valuable starting point for directed evolution in the development of large-scale biocatalytic routes. The methodology for identifying potential broad-range enzymes will be presented and the results of preliminary kinetic characterisations will be rationalised in terms of structural considerations.

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Diversity-oriented synthesis: 'new' concepts derived from nature

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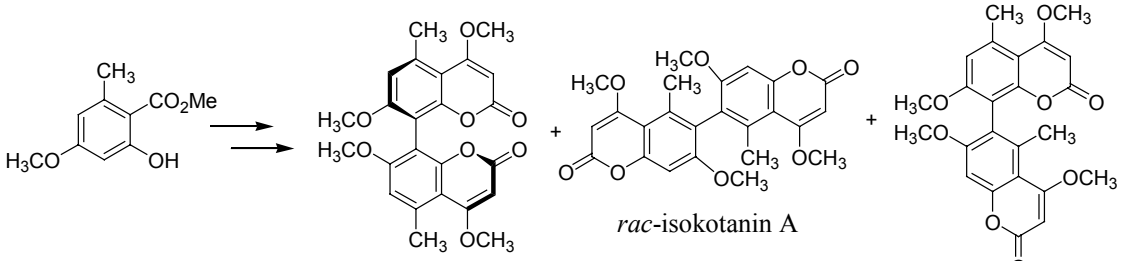
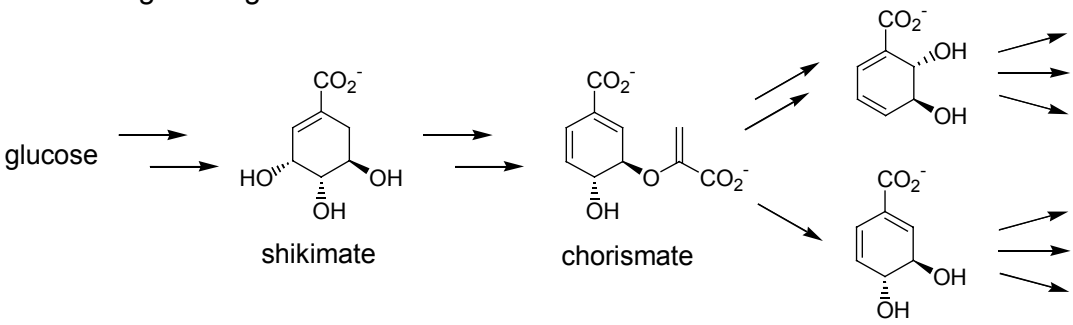
Target-oriented synthesis has been the major task of synthetic organic chemistry. Diversity-oriented synthesis became a major point of interest within the last decade. Aspects of both strategies are used by nature for the biosynthesis of highly specific molecules as well as a broad diversity of many compounds with unknown biological function.[1] In our own work we utilize diversity-oriented aspects according to bio-synthesis in (target-oriented) organic synthesis. This has been applied e.g. in the synthesis of regioisomeric natural products,[2] for the development of different ThDP-dependent enzyme-catalyzed reactions,[3] and for the microbial production of 'unnatural' metabolites.[4]

Natural product synthesis:



Metabolic engineering:



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Heme Protein Biocatalysis: Lessons from the Protein Data Bank and De Novo Heme Protein Design

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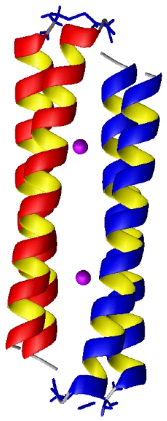

X-ray structure of an apo maquette.[2]

Our approach to the study of metalloproteins critical to biocatalysis is to engineer and fabricate peptide structures that incorporate metal cofactors toward the goal of generating molecular *maquettes*, simplified functional versions of complex native enzymes. Herein, we have analyzed all structurally characterized natural heme proteins[1] to design novel synthetic heme protein maquettes for the investigation of the fundamental design principles natural heme proteins. The designed four helix bundle protein, shown at right, is elaborated with histidine ligands to bind heme, Fe(protoporphyrin IX). Using heme protein maquettes, we have delineated the environmental factors which alter the heme reduction potential, a fundamental chemical property of natural cytochromes. The type of porphyrin macrocycle, the local electrostatic environment, the burial of the heme and the influence of pH all contribute to the modulation of the heme reduction potential. In designed heme proteins, we can modulate the heme reduction potential by 435 mV - *nearly half the range observed for natural heme proteins*. Evaluation of the Fe(III) and Fe(III) heme binding constants and the resultant electrochemistry provides insight into the absolute (de)stabilization of these states by the protein environment.[3] We have expanded the repertoire of ligands available for heme protein design by using nonnatural amino acids containing pyridine, triazole and tetrazole sidechains.[4] Altering the axial ligands leads to significant changes in heme spectroscopy (electron paramagnetic resonance, magnetic circular dichroism, resonance Raman), reduction potential (+286 mV) and Fe(III) and Fe(II) stability constants (150,000-fold). These results will be compared with those from the set of structurally characterized natural heme proteins.

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Proteases: Old enzymes with new synthetic properties

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Forced by successful enzyme, medium and substrate engineering methods, proteases have gained in importance as regio- and stereospecific catalysts in organic synthesis.[1] Especially for applications that are based on their native hydrolysis activity, such as regiospecific ester hydrolysis or the kinetic resolution of racemates, proteases are now generally recognized as normal bench reagents. In principle, these engineering methods allow also for the reduction of competitive acyl donor hydrolysis, the alteration of the enzyme specificity and the minimization of undesired proteolytic cleavages, which are the main drawbacks when proteases are used as catalysts for reverse proteolysis. However, even the most impressive examples published so far are handicapped by the intrinsic drawbacks of the protease catalyst that are mainly: i) the restricted specificities and selectivities of the available proteases and ii) the permanent risk of proteolytic side reactions of both the starting compounds and the products formed. Summarizing these characteristics, proteases appear to be far away from being perfect catalysts for universal and flexible peptide synthesis. Inevitably, this conclusion holds true for classical approaches, but is wrong when considered as a general rule. In fact, the combined use of substrate mimetics,[2] genetically optimized peptide ligases[1] and solid phase peptide synthesis approaches[1] have broaden the synthetic scope and flexibility of the enzymatic method. This allows for the coupling and modification of cleavage-sensitive peptide fragments and the selective labeling of polypeptides at the *N*-terminus in an irreversible manner. Besides the coupling of coded amino acid moieties a broad spectrum of noncoded once, such as D-amino acids,[3] carbohydrate moieties[4] or nonpeptidic carboxylic acids,[5] undergo the coupling approach and further broaden its scope for organic synthesis. Selected original examples including the synthesis of longer polypeptides, peptide isosteres, structural diverse *N*-linked peptidoglycans, and the selective coupling of non-amino acid derived carboxylic acids to peptides and proteins will be presented. Particular attention will be paid to the synthetic utility of this powerful chemoenzymatic approach and to its unique degree of flexibility.

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Biotin synthase: a radical strategy for sulphur insertion into non activated C-H bonds

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Biotin synthase catalyzes the last step of the biotin biosynthesis pathway. The reaction consists in the introduction of a sulfur atom into dethiobiotin, thus requiring activation of C-H bonds. Radical activation of the dethiobiotin substrate is performed by the association of two biotin synthase cofactors, the iron-sulfur cluster and S-adenosylmethionine. Recently we have identified a new enzymatic activity of biotin synthase, a cysteine desulfurase activity, which may provide the sulfur introduced into biotin. This activity is dependent on pyridoxal-5-phosphate and proceeds through a protein bound persulfide. By site-directed mutagenesis experiments two conserved cysteines residues were shown to be critical for this activity and are good candidates as a site for persulfide. A new mechanism for sulfur insertion into dethiobiotin, in which persulfide play a key role, is proposed.

Structure, Mechanism and Application of an Unusual Carbon-Carbon Bond Hydrolyase

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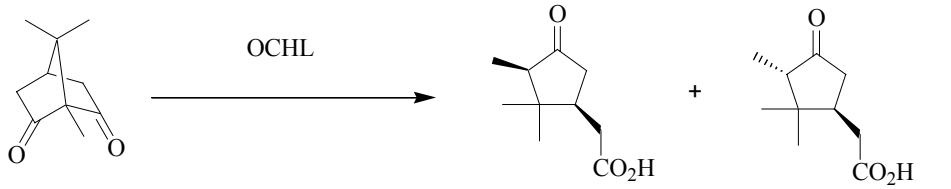
6-oxocamphor hydrolyase (OCHL) is a cofactor independent enzyme that catalyses the desymmetrisation of bicyclic *β*-diketones to yield cyclic keto acids of high optical purity *via* an enzymatic *retro*-Claisen reaction (**Figure**) [1,2].



6-oxo camphor, **1**

(2*R*-4*S*)-α-campholinic acid, **2**, 6:1 ratio

(2*S*-4*S*)-α-campholinic acid, **3**

The structure of the enzyme has been solved to 2Å resolution [3], the active site located, and a number of site-directed mutants prepared in an effort to shed light on the mechanism of the enzyme. The low k_{cat} plus low K_M mutant His122Ala crystallised in complex with the minor diastereomer of the natural product, (2*S*, 4*S*)-α-campholinic acid. This ligand complex reveals many of the molecular determinants of prochiral selectivity in the enzyme, provides clues to mechanism and also provides suggestions for engineering isozymes of opposite selectivity.

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Transgenic proteins as hosts for enantioselective catalysis

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Biochemical and physiological processes utilize preferentially one enantiomer over its mirror-image. Infact, small mirror-image impurities may cause severe pharmacological and toxicological side effects. Thus, a major goal in chemistry is to generate enantiomerically pure compounds. We produce artificial metalloenzymes introducing stereoselectivity in catalyses that normally generate a racemate. The principle of our metalloenzymes lies on the incorporation of active metal catalyst precursors in a well-defined chiral protein environment, by the biotin-(strept)avidin technology. This technology ensures the precise localization of a biotinylated moiety without the need of a chemical coupling step with the host protein.

Expression systems. Avidin is a homotetrameric glycoprotein found in avian, reptilian and amphibian egg white. Each monomer can bind a (+)-biotin (vitamin H) molecule with an extraordinary affinity (K_a= ~10^{14} M^{-1}). We have overexpressed an artificial avidin gene in *Pichia pastoris*. The recombinant protein is secreted in the extracellular medium with a maximal yield of 400 mg/L. The purification only requires the basification of the culture medium prior to affinity chromatography.

Streptavidin is the prokaryotic counterpart of avidin, with the same tridimensional eight-stranded β-barrel homotetrameric structure, but only 32% protein sequence homology. It is produced by *Streptomyces avidinii*. Each of subunit can bind a (+)-biotin molecule with almost the same affinity as avidin. We have overexpressed a streptavidin gene in *E. coli*. The recombinant protein is targeted into the cytoplasm. The multi-step purification involves lysis of the cells, enzymatic digestion of nucleic acids and a denaturing-renaturing step prior to affinity chromatography. In these conditions, we obtain up to 160 mg of streptavidin per L of culture. Streptavidin is also overexpressed in *P. pastoris* with the same yield as avidin.

Mutagenesis. Site directed mutagenesis and Site saturation mutagenesis are utilized for modification of the loops connecting β-sheets around the biotin-binding pocket. In most cases, the original amino acid was exchanged with a glycine, to confer more flexibility to the loop. Synthesis of an artificial gene is utilized to optimize the expression of streptavidin or avidin in a given expression system and to create chimerical proteins. These three methods are used to elucidate the factors responsible for enantioselection.

Main results. Streptavidin proved to be a better chiral inducer than avidin in the vast majority of experiments. For example, the best enantiomeric excess (*ee*) for the reduction of acetamidoacrylic acid with a mutant streptavidin as host protein affords acetamidoalanine with 96% *ee* (R). The best *ee* with mutant avidin for the same substrate was only 77% (S). Preliminary results also indicate that a chimerical avidin bearing one loop of streptavidin behaves more like streptavidin.

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Biocatalysis in Biomineralization Processes?

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When considering the role of inorganic species in biochemical processes, only isolated ions or metalloprotein active sites are often taken into account. However, biominerals, i.e. solid phases deposited by living organisms, are widespread examples of inorganic species present in a condensed state. The question arises whether some specific biomolecules are involved in the formation of these biogenic materials. This presentation will try to give an overview of the actual knowledge in this field, especially focusing on silica-based biominerals.[1]

In the case of calcium salts like phosphate in bones or carbonate in mollusk shells, some proteins and poly-saccharides have been shown to favour precipitation as well as to control the morphology and crystal structure of the deposited solid. However, no real catalytic activity of these biomolecules have been demonstrated.

In contrast, for silica present in several plants, algae or sponges, the possibility for proteins to interact specifically with silica molecular precursors has been suggested. Some of these biomolecules were isolated and their activity towards silica formation was studied. In parallel, model systems were used to get a better understanding of the interactions that may arise between proteins and silica precursors.

These results suggest that although different biomolecules can activate silica formation, a true catalytic process has not yet been unambigously identified at this time. This can be partially attributed to the fact that the silica precursors used by the organisms, as well as their reactivity within the cells, are still poorly known. Nevertheless, in addition to their fundamental implications, these studies have already been fruitful in designing new silica-based “bio-inspired” materials.[2]

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Protein-coated microcrystals: Applications in biocatalysis

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Enzymes frequently exhibit poor activity in organic media when compared to their respective activities in aqueous solution.[1] To overcome this problem, many strategies aimed at optimising enzyme activity, such as manipulation of the micro-environment and a range of immobilisation strategies have been studied. Whilst studying how different methods of protein dehydration affected enzyme activity a new mode of self-assembly was discovered. This new mode of self-assembly leads to the formation of well-organised protein layers on the surface of micron-sized crystals of water-soluble excipients *e.g.* salts, sugars or amino acids. The crystals are produced in a one-step process: a saturated aqueous solution of the excipient and protein is added drop-wise to a suitable water miscible organic solvent. Instantaneous co-precipitation occurs and the crystal lattice energy forces the protein molecules to migrate to the surface of the excipient crystals.[2] We termed this novel system: protein coated micro-crystals (PCMC).

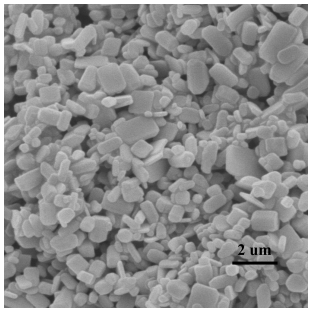

Figure 1. Scanning Electron Microscopy image of lipase coated micro-crystals.

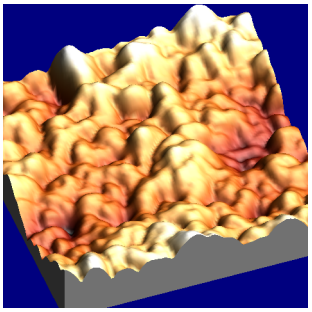

Figure 2. 3D Topographical AFM image of a single protein coated micro-crystal. Scan size: 400nm x 400nm

To date, we have studied this system using (i) proteases and (ii) lipases. Catalytic activity measurements showed that (a) the protein-coated crystals exhibited significantly higher activity than their freeze-dried counterparts when assayed under the same conditions in the organic solvent and (b) the crystals re-dissolved instantly in aqueous buffer without loss of the original aqueous activity. This rapid dehydration method leads to good retention of native structure (as shown by active site titration) and *Tapping Mode* Atomic Force Microscopy (AFM) images have confirmed that the protein layer is located at the surface of the crystal and is easily accessible to the titrant/substrate. Extending the choice of crystalline core material allows fine-tuning of the biocatalyst system. For example, use of solid-state buffers as crystalline matrix increases the activity of proteases and simultaneously provides buffering capacity.[3]

This precipitation method leads to the formation of a fine suspension (typically 0.1-5 μm for K_2SO_4 as carrier) with the protein-coated micro-crystals homogenously dispersed in the organic solvent.[2] Such PCMC suspensions are easy to handle and can be dispensed accurately as a suspension. This together with their high storage stability at room temperature and high activity makes them favourable candidates for biocatalyst screening programmes.

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Labeling and immobilizing proteins via intein mediated ligation

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Intein-mediated ligation provides a site-specific method for the attachment of molecular probes to proteins. The method is inherently flexible with regard to either the protein sequence or the attached probe or solid support, but practical difficulties have limited the widespread use of this valuable labeling system for the attachment of small- to medium-sized molecules. We report studies to improve the efficiency and practical application of these reactions, including the assembly of plasmids for the expression of target-intein fusion proteins and the analysis of their reaction with a fluorescent cysteine derivative under a range of conditions. Optimal ligation of the fluorophore to the target protein is critically dependent on the degree of oxidation of the fluorescent cysteine derivative. Efficient ligation has been achieved with freshly prepared fluorescent cysteine derivative under rigorously anaerobic conditions. Similar ligation yields have also been achieved using more practically convenient conditions including anaerobic reaction with addition of thiophenol, or aerobic reaction with the further addition of tricarboxyethylphosphine.

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De Novo Design as a Tool to Investigate Protein Chemistry

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The biological functions of proteins are multifaceted and range from providing cellular building material to catalyzing chemical reactions to acting as gatekeepers to control the flux of ions and other molecules across the cell membrane. Today we are limited in our knowledge of connecting structural information to the functional properties of a protein, thus stimulating increased research in this area. Ideally, one day it will be possible to look at the amino-acid sequence of a protein and from this information alone predict its three-dimensional structure and deduce its function.

Varies approaches are being taken to address the protein “structure-to-function” problem. The most common method is to investigate structural and functional relationships of natural proteins by, for example, site-directed mutagenesis studies. An alternative approach has emerged in recent years involving the construction of proteins from scratch, so called *de novo* protein design. The goal with the *de novo* protein approach is to derive design rules on how to create new, man-made proteins with specific catalytic and biological functions.

We have developed two α-helical model proteins denoted α_3W and α_4W. α_3W has been characterized in some detail while the development of α_4W is more recent. To illustrate the versatility of *de novo* design in protein research, three different studies of the α_3W system will be described. In the first study we use a combination of experimental and theoretical methods to investigate the redox properties of α_3W. The second project involves using α_3W to examine the interaction energy of π-cation pairs. π -cation interactions between aromatic (Phe, Tyr, Trp) and basic (His, Lys, Arg) residues are common in natural proteins, although the strength of these interactions has thus far been poorly characterized experimentally. We have used high pressure to determine the energy of a Trp/Lys π -cation interaction in α_3W. With this technique we avoid the uncertainties associated with introducing mutations. Finally, a method will be described in which protein folding is driven by increasing the energy of the unfolded state rather than lowering the energy of the folded state.

Directed evolution on transketolase for obtaining new monosaccharides

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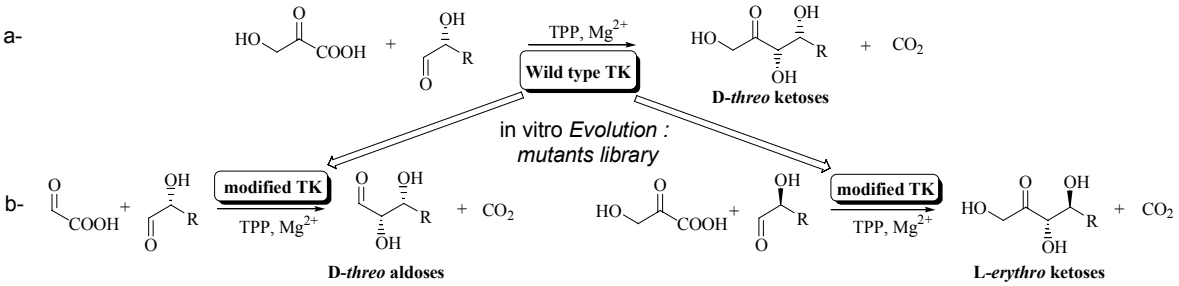
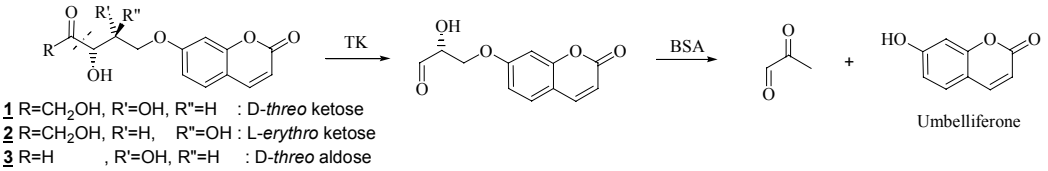
During several years, we used capabilities (enantioselectivity, stereospecificity) of transketolase (TK) for obtaining many D-*threo* ketoses of biological interest (scheme 1a).[1] Nevertheless, like many other enzymes, substrate specificity is limited. We decided to evolve this enzyme in order to obtain D-*threo* aldoses or L-*erythro* ketoses (non natural configuration) (Scheme 1b). Thus, we used molecular biology techniques, especially random mutagenesis, on wild type TK gene. After expression of the protein, we obtained a library of different modified TK that have to be screened to select the most powerful enzyme we are looking for.



Scheme 1

We finalized a qualitative and quantitative screening test on wild type TK using as a substrate a ketose of natural configuration (D-*threo*) bearing umbelliferone (scheme 2).[2] Wild type TK is able to recognize this substrate, then to clive it thus releasing umbelliferone, a highly fluorescent compound.



1 R=CH_2OH, R'=OH, R"=H : D-*threo* ketose
2 R=CH_2OH, R'=H, R"=OH : L-*erythro* ketose
3 R=H , R'=OH, R"=H : D-*threo* aldose

Scheme 2

We are going to use this screening test for searching modified TK able to catalyze synthesis of D-*threo* aldoses or L-*erythro* ketoses. For that purpose we are preparing the corresponding substrates by chemically and/or enzymatically routes.

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Taming Giants: Insights into DNA Polymerase Function

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One essential prerequisite of any organism is to keep its genome intact and to accurately duplicate it before cell division. All cellular DNA synthesis required for DNA repair, recombination, and replication depends on the ability of DNA polymerases to recognize the template and insert the canonical nucleotide. A DNA polymerases is presented with a pool of four structurally similar deoxynucleotide triphosphates (dNTPs) from which it must select the sole correct (i.e., Watson-Crick base paired) substrate for incorporation into the growing DNA strand. This leads to the question: Which properties could enable a DNA polymerase to catalyze nucleotide incorporation with a selectivity far greater than that which is dictated by the thermodynamic differences between base pairs in free solution? Using chemically engineered tools, we were able to gain new insight into these complex enzymatic processes.[1]

Apart from their pivotal role in biological process DNA polymerases are widely applied in numerous molecular biological and biotechnological applications. After the completion of the human genome sequence the discovery of small dissimilarities in the sequence of different individuals is one of the prime tasks today, since genetic differences may influence the variability of patients' response to drugs. Highly selective DNA replication systems should simplify the detection of single nucleotide polymorphisms (SNP) in genes without need for further tedious time- and costs-consuming analytical procedures as applied to date. In the talk chemical and genetic approaches towards achieving DNA polymerase based systems with improved selectivity for efficient genome-typing will be discussed.[2]

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Synthesis of Hydroxamic Acid in TTAB Reversed Micelles using Amidase from *Pseudomonas sp.*

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Hydroxamic acids derivatives have a wide spectrum of application due to their ion metal chelating capacity, mainly their capacity to inhibit metalloproteinases that are involved in human diseases [Fournand, 1997]. These compounds are used in several pharmaceutical drugs. Amidases (E.C. 3.5.1.4) are enzymes that in nature catalyse the hydrolysis of amide bonds in small aliphatic amides but these enzymes also have the capacity to catalyse the acyl group transferase to amines what enlarged the possibility of synthesising several hydroxamic acids derivatives if the acyl acceptor is hydroxylamine. Amidases with this capacity have been isolated mainly from *Pseudomonas* and *Rhodococcus* strains [Brown, 1973 and Fournand 1998]. As the amidase catalysed reaction involves a ping-pong bi-bi mechanism [Fournand, 1998] with the formation of acyl-enzyme complex that can transfer the acyl unit either to an amine or to the water present in the system, it is the kind of reaction to be performed in organic medium in order to diminish the presence of water. Reverse micelles formed in organic medium have been used to encapsulate enzymes in order to catalyse the synthesis of amide bonds with the formation of dipeptides [Serralheiro 1994]. In these systems the enzyme remains soluble, what avoids problems with mass transfer that could slow down the reaction velocity and they can easily be transformed into a continuous reaction system. In this work the amidase from a *Pseudomonas aeruginosa* gene expressed in *E. coli*, was purified and encapsulated in reverse micelles of the cationic surfactant tetradecylltrimethyl ammonium bromide dissolved in heptane/octanol. The effect of several parameters of the system that could influence the enzymatic activity were analysed. It is the results of this study that are to be presented.

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Modelling the active site of Nitrile Hydratase: oxidation of (N_2S_2)-Fe and (N_2S_2)-Co complexes

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Nitrile Hydratases (NHases) are bacterial metalloenzymes which catalyze the hydratation of nitriles to the corresponding amides. Their active site contains a low spin non-heme iron(III) or non-corrin cobalt(III) center. Two carboxamido nitrogens, one cysteine-sulfur, and two oxidized sulfurs (one cysteine-sulfinic and one cysteine-sulfenic acid) constitute the donor set around the metal, in addition to a water/hydroxide molecule in the active form. This unique coordination sphere, which has implications on the mecanism of the hydratation, has pushed several groups[1] to design synthetic models of the catalytic center of iron and cobalt NHases. Results obtained in our group in recent years will be discussed.

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Designing of Mn complexes as SOD synzymes. Evaluation of the SOD-activity: from anhydrous experiments to transient kinetics in aqueous solution.

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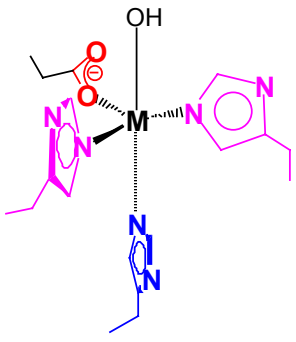
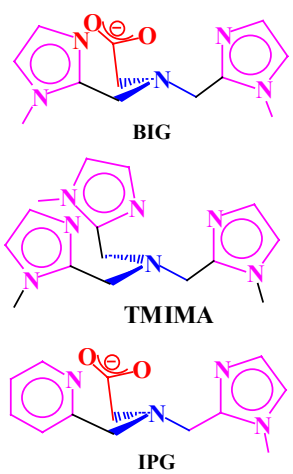
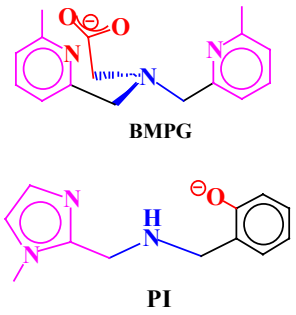
Reactivity of superoxide is of current interest as it plays a key role in oxidative stress. Superoxide dismutases (SODs) are metalloenzymes involved in the protection of the cell against superoxide. Elucidation of reaction mechanisms of superoxide with metal centers, both at the active site of superoxide dismutases and with inorganic complexes is important to design efficient synthetic compounds valuable as pharmaceuticals. A family of Mn(II) and Mn(III) complexes have been synthesised based on N-tripodal ligands (see figure). Their structures have been solved, showing a large variety of structures in the solid state: inorganic polymers with bridging carboxylatos to dimer and monomer.[1,2,3] The reactivity towards superoxide has been studied, both in anhydrous medium and in aqueous buffers.[2] In anhydrous medium, EPR, cyclic voltammetry, low-temperature UV-visible spectroscopy were used to seek for intermediates species or species directly derived. Dimeric species where shown to be produced. In aqueous buffer, the Fridovitch-McCord test showed that this series of complexes display SOD-activity, by comparison to the literature (k_{cat} from 3 10^6 to 7 10^6 $M^{-1}s^{-1}$). Reactivity of two of these complexes has been studied by pulsed radiolysis. It provided kinetics constant in agreement with that from Fridovith-McCord test. Moreover, unambiguous evidences for catalytic activity were obtained. A mechanism has been proposed that is consistent with all experimental data.
This study shows the complementarity of experiments in anhydrous and aqueous buffer.



Fe and Mn-SODs' schematic active site





N-tripodal based ligands

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From Enzyme Models to Synthetic Proteins: a Chemist's Approach to the Study of Enzymes

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In this talk I wish to demonstrate what a chemist can contribute to the understanding of enzymes, in particular by using the power of synthesis.

The first part will deal with the study of synthetic active site models. Enzyme models have the advantage that they are readily available and easier to study than an actual enzyme. The information obtained from these model compounds can help increase our understanding of how enzymes work. Also these models can serve as a starting point for the development of a new generation catalysts with increased activity and selectivity. Our research has been focussed on iron complexes with multi-dentate nitrogen ligands as models for non-heme iron containing oxygenases. In the course of this study several iron-peroxide intermediates have been characterized and their relation to the observed catalytic activity has been established.

A main criticism of enzyme models is that they're models. This means that you can ask the question: how relevant is the information you obtain from a model compounds when you disregard the protein matrix in which the active site is embedded? To address this we make proteins containing non-natural amino acids as mechanistic or spectroscopic probe, so we can get information from the actual protein. Selenium is ideal as a mechanistic probe since it is isosteric to sulphur. So it can be introduced in the form of selenocysteine or selenomethionine and thus cause minimal structural perturbation. In the second part of the talk I will discuss (semi-) synthetic strategies to selenium containing proteins and the application of selenium as a mechanistic and/or NMR probe.

Modulation of Chirality of Lipases *via* immobilization techniques

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Lipases are the enzymes more utilized in the resolution of chiral compounds. To improve the results, the use of many different techniques has been proposed (protein engineering, random mutagenesis with selection pressure, screening in the Nature, etc). However, bearing in mind that enzymes are commonly utilized in the industry in an immobilized form, a simple strategy has been recently reported to permit to greatly improve the results using lipases. The strategy is based in the dramatic conformational changes of lipases during catalysis and the use of a battery of immobilization protocols that may permit to immobilize the lipases via different orientations, with different rigidity or generating different environment. This may be combined with the experimental conditions to greatly alter the results. Thus, very interesting results hass been found using lipases from different sources and different compounds, where the E values have been moved from almost negligible to more than 100 just using this very simple strategy, the so-called “conformational engineering of lipases”.

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Where Bioinorganic Chemistry Meets Molecular Architecture: Modelling Metal-Nucleic Acids Interactions

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Metal ions are natural counter ions of the polyanionic nucleic acids. Depending on the nature of the metal and its environment by nucleobase donor atoms, metal-nucleic acid interactions can range from non-specific to highly specific ones. Although basic metal binding patterns with nucleic acid constituents are reasonably well understood,[1] the role of metal ions in special tertiary interactions, especially with folded RNA structures and multistranded DNAs are still a topic of great interest. Applying non-physiological metal ions, artificial analogues of natural base-metal aggregates can be synthesized and tested for potential uses as chemical probes or novel chemotherapeutic agents[2,3] other than the antitumor agent Cisplatin.[4] Renewed interest in metal-nucleobase interactions stems from the suspected or proven role of metal ions in RNA catalysis.[5] Apart from a *direct* involvement of metals in such reactions, there is also the possibility of an *indirect* role of metal ions by initating acid-base catalysis as a consequence of profound perturbations of the normal pK_a values of nucleobases following metal coordination.[6]

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CERC3 Young Chemists' Workshop "Biocatalysis"

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Poster Titles

(only main authors)

P1 **Mahdi Abu-Omar**
"Investigations of nitric oxide reactions with non-heme phenylalanine"

P2 **Frank Bordusa**
"Old Enzymes with new synthetic properties"

P3 **Thibaud Coradin**
"Bacteria encapsulation in sol-gel silica matrices"

P4 **Paul Dalby**
"Expanding the synthetic repertoire of transketolase"

P5 **Erwan Galardon**
"Modelling the active site of Nitrile Hydratase: oxidation of (N_2S_2)-Fe and (N_2S_2)-Co complexes"

P6 **Brian Gibney**
"Heme Protein Biocatalysis: Lessons from the Protein Data Bank and DeNovo Heme Protein Design"

P7 **Craig Grapperhaus**
"Synthesis and Characterization of N_2S_3X-Fe Models of Iron-Containing Nitrile Hydratase"

P8 **Virgil Hélaine**
"Directed evolution on transketolase for obtaining new monosaccharides"

P9 **Michaela Kreiner**
"Protein-coated micro-crystals"

P10 **Andreas Marx**
"Taming giants: Insights into DNA Polymerase Function"

P11 **Cameron Neylon**
"Labeling and immobilizing proteins via intein mediated ligation"

P12 **Sandrine Ollagnier de Choudens**
"Recent developments on biotin synthase"

P13 **Rita Pacheco**
"Biocatalysis of hydroxamic acid using reverse micelles"

P14 **Jose M. Palomo**
"Modulation of lipases chirality via different immobilization techniques"

P15 **Tatyana Polenova**
"Solid-State NMR Spectroscopy for Studies of Geometry and Electrostatic Environment in Vanadium Haloperoxidases"

P16 **Clotilde Policar**
"Imidazole and imidazolate iron complexes: on the way for tuning 3D-structural characteristics and reactivity. Redox interconversions controled by protonation state"

P17 **Markus Reiher**
"DFT calculations of spin states and spin barriers in transition metal catalysis"

P18 **Gerard Roelfes**
"(Semi-)Synthetic Enzymes"

P19 **Grit Straganz**
"Structure-activity relationships of the non-heme iron dependent dioxygenase Dke1"

P20 **Roderich Süssmuth**
"Abyssomicins – novel inhibitors of the p-aminobenzoic acid/tetrahydrofolate biosynthesis"

P21 **Cecilia Tommos**
"De Novo Design as a Tool to Investigate Protein Chemistry"

P22 **Andrea Zocchi**
"Artificial metalloenzymes for enantioselective catalysis based on biotin-avidin technology"

Authors