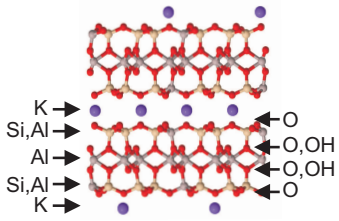

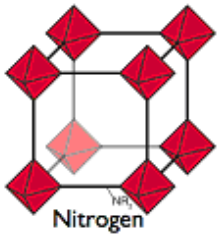

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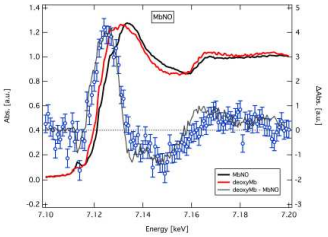

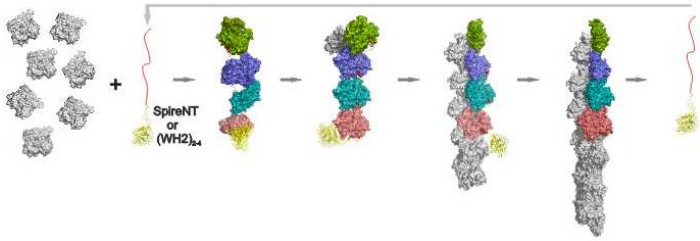

SLS Symposium on

Measurements in Solution

Tuesday, October 11, 2011

10:00 to 12:15, WBGB/019

10:00 Time-resolved pump-probe XAS on ligated Myoglobin in physiological media
F. Lima, C. Milne, M.H. Rittmann-Frank, R. van der Veen, M. Reinhard, M. Benfatto and M. Chergui

10:30 Metal-Organic Frameworks: from Synthetic to Catalytic Challenges Towards Enzyme-Like catalysis
M. Ranocchiari and J. A. van Bokhoven

11:00 Coffee

11:15 Molecular architecture of the Spire-actin nucleus and its implication for actin filament assembly
T. Sitar, J. Gallinger, A.M. Ducka, T.P. Ikonen, P. Joel, K.M. Trybusd, M. Schleicher, A.A. Noegel, R. Huber, T.A. Holak

11:45 Density profile of water confined in nanoslit
S. Chodankar, E. Perret, K. Nygård, O. Bunk, D.K. Sataphathy, R.M. Espinosa Marzal, T.E. Balmer, M. Heuberger and J. Friso van der Veen

Time-resolved pump-probe XAS on ligated Myoglobin in physiological media

F. Lima[1], C. Milne[1], M.H. Rittmann-Frank[1], R. van der Veen[1] M. Reinhard[1], M. Benfatto[2] and M. Chergui[1]
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Heme proteins, such as *Hemoglobin (Hb)* and *Myoglobin (Mb)* are crucial in the process of respiration, cellular signalling and muscle activity. In addition to binding O_2, the ferrous iron in Mb also binds other diatomic ligands such as CO and NO[1]. Despite the wealth of information available from numerous experimental and theoretical studies, a microscopic description of ligand detachment and rebinding remains elusive, and fundamental questions are still to be answered. Of particular interest is the study of NO rebinding to Mb. The recombination of the NO molecule to Myoglobin is thought to have two geminate phases near room temperature[2]. Following ultrafast photodissociation in the aqueous phase near room temperature, the NO molecule geminately recombines within $100 - 200$ picoseconds, which is much faster than in the case of CO, and suggests that it has an effective barrier much smaller than the latter. In addition, the time scale for NO recombination, is comparable to that of heme/protein relaxation.

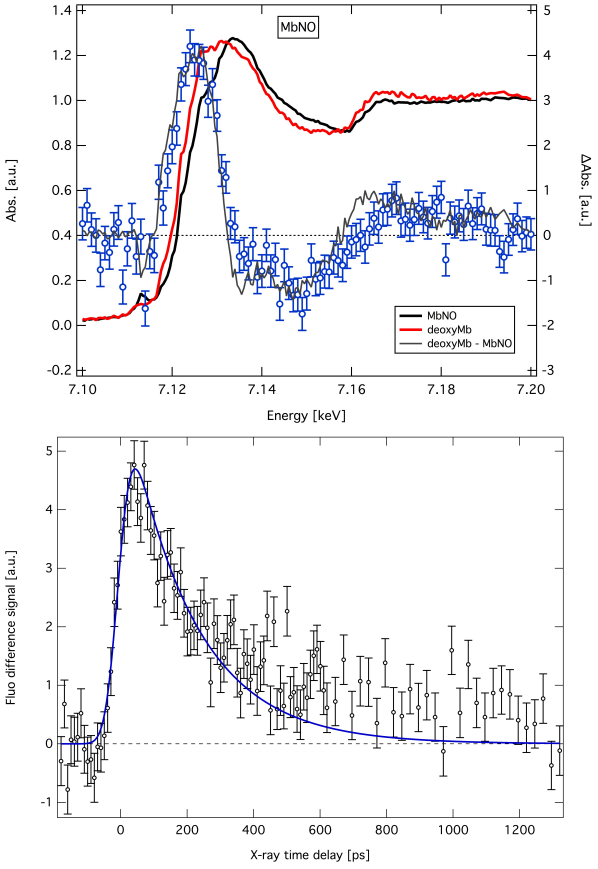

Figure 1: (*top*)Time resolved XAS around the Iron edge of 4mM MbNO at 50 ps time delay. (*bottom*) Time-scan of MbNO showing a decay of 200 ± 20 ps.

X-ray Absorption Spectroscopy (XAS) is a suitable experimental technique to probe local and electronic structures in disordered media - in which most of the relevant chemical and biological process occur. The low-energy part of the XAS is of great interest for biochemical studies since it is extremely sensitive to the geometrical and electronic structure of the absorbing site. Extending the XAS technique to the time domain allowes the study of ultrafast photoinduced processes like intramolecular electron transfer, high-to-low spin change and chemical bond formation[3].

The main scientific issue explored by this technique is the first report of time-resolved XAS of ligand photodissociation in Mb[3] (MbCO and MbNO) under phisiological conditions - buffer solution, 4 mM concentration, room temperature and ambient pressure - with 50 ps time resolution. A time-delay scan shows a decay of ca. 200 ± 20 ps, which is consistent with the optical-only experiements[1].

We used the MXAN code[4] to perform fits on the XAS spectra of different forms of ligated Myoglobin in physiological media, both before and after photoexcitation. The fits were done by changing the structural parameters of the site around the iron atom, *i.e.*, the heme, the ligand and the distal and proximal Histidines. Our results are in good agreement with the structures determined by x-ray crystallography, showing the power of the technique in determining local structures of proteins in physiological conditions. Further applications of the high-repetition rate ultrafast x-ray experiments will be discussed.

References

[1] E. Antonini and M. Brunori, *Hemoglobin and Myoglobin in Their Reactions With Ligands*, **1971**.
[2] D. Ionascu, *et al.*, *JACS*, **2005**, *127*(48) 16921.
[3] F. Lima, *et al.*, *Rev. Sci. Instrum.*, **2011**, *82* 063111.
[4] M. Benfatto, *et al.*, *J. Synch. Rad.*, **2001**, *8* 1087.

Metal-Organic Frameworks: from Synthetic to Catalytic Challenges Towards Enzyme-Like catalysis

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Metal-organic frameworks (MOFs), materials constituted by multidentate organic building blocks connected by inorganic units, have recently emerged as a novel class of crystalline porous polymers with unique properties.[1] MOFs have great potential in catalysis due to the possible production of single-atom active sites, which can be synthesized by combining the concepts of isoreticularity and post-synthetic modification (PSM).[2] The design of single atom catalysts that try to apply concepts derived from homogeneous catalysis present however more than one challenge.

Our strategy to heterogenize known homogeneous catalysts is to synthesize MOFs of various pore size and containing nitrogen and phosphorus anchor points to be functionalized with chiral Rh complexes. These functionalized frameworks are and will be employed as catalysts for the asymmetric hydrogenation and isomerization of olefins (Figure 1).

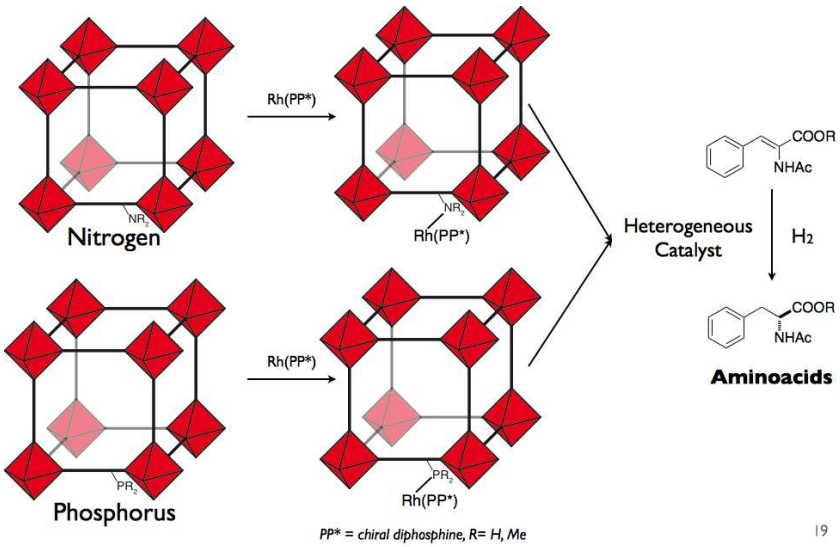

Figure. 1: General view of our approach to asymmetric catalysis by MOFs.

This challenging multidisciplinary project starts with the synthesis of organic precursors to synthesize P-MOFs, which are not described in the literature. In this contribution, we describe how we solved synthetic problems to synthesize phosphines in high yield with one or more carboxylic acids by a novel green process that uses water as solvents and Pd/C as catalyst. We also present how N-MOFs have been utilized to perform asymmetric hydrogenation of olefins to achieve activity similar to that of the corresponding homogeneous catalysts, but with higher enantioselectivity. We show a novel method to achieve high conversion of PSM by vapor diffusion (VP-PSM) to demonstrate how we can produce good material with homogeneous distribution of active sites. Finally, we analyze the possibility of performing enzyme-like catalysis and we explain how a bridge between synthesis and analysis at the synchrotron facility will make easier the development and the fundamental studies of this exciting topic.

[1] Ranocchiari M, van Bokhoven J. A. (2011) Phys Chem Chem Phys 13:6388.

[2] Kristine K. Tanabe, Seth M. Cohen (2011) Chem Soc Rev 13:498.

Molecular architecture of the Spire-actin nucleus and its implication for actin filament assembly

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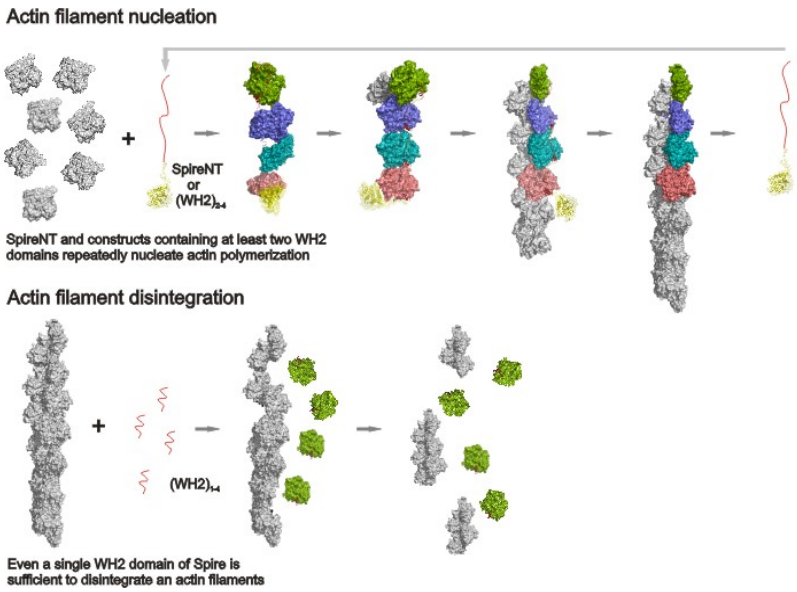
Many cellular processes, including cell motility, cell adhesion, endo/exocytosis, intracellular and membrane trafficking, maintenance of cell shape and polarity depend on rapid and dynamic remodelling of the actin cytoskeleton. Actin monomers (G-actin) from the large pool of cytoplasmic actin bind to monomer-sequestering proteins and assemble to filaments (F-actin). The first step in the assembly of actin filaments is nucleation. Instability of the actin multimers is the rate-limiting step in actin filament assembly. To overcome this kinetic barrier, as well as the activity of sequestering proteins, cells have developed diverse mechanisms of nucleation driven by sets of actin nucleating proteins.

Spire is an important representative of a group of actin nucleators, containing four 17-27 amino acid long actin-binding motifs called WH2 repeats. Nucleators share the common ability to gather actin monomers into a nucleation complex, but the arrangement of actins might vary significantly among them.

Solution X-ray scattering can be used to bridge the gap between high-resolution static subunit models and functional studies involving large and possibly flexible complexes. We have measured the solution form factors of various native and engineered spire-actin complexes [1] and used them to disambiguate between possible conformations observed in crystals. The data allowed us to construct a model of the full N-terminal spire-actin complex in solution.



Total internal reflection fluorescence (TIRF) microscopy and fluorescence based actin polymerization assay provided insight into the dynamics of the actin nucleation and sequestration activity of spire. The results were combined to form a model of spire-initiated F-actin nucleation and dissociation.

References:

[1] T. Sitar *et al.*, submitted.

Density profile of water confined in nanoslit

S. Chodankar[1], E. Perret[1], K. Nygård[1], O. Bunk[1], D.K. Sataphathy[2], R.M. Espinosa Marzal[3], T.E. Balmer[3], M. Heuberger[4] and J. Friso van der Veen[1,3].

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Two surfaces in contact are found in everyday life. For hydrophilic surfaces in contact, the natural presence of a confined layer of water in the narrow gap between them has important implications for geochemical and biological processes such as swelling of clays and diffusion of water through nanopores. Numerous surface force experiments have been performed on such systems. However, force studies do not provide information on the molecular structure of the confined water. Recently we have adapted a surface force apparatus for specular X-ray reflectivity (XRR) determination of the electron density profile of the confined fluid across the gap. The confinement device features two freshly cleaved, cylindrically curved, mica membranes in crossed geometry, which are brought close together in order to create an atomically flat contact area. We determined by use of XRR the distance between the surfaces and the electron density profile of the naturally present water across the hydrophilic gap at nominal zero humidity. The minimum distance between two flat mica surfaces at nominal zero humidity are separated by average distance of 1.76 nm. Determined electron density profiles of confined water along confinement direction show distinct peaks, indicating molecular layering of water molecules. The surface potassium ions (K^+) exists in two states: (i) retained on the surface and partially hydrated forming inner-sphere complexes and (ii) desorbed in the gap with full hydration existing as outer-sphere complexes. Peak positions of the layers provides a qualitative indication of such desorbed K^+ ions in the gap. The average electron density of confined water is equivalent to that of a bulk water.

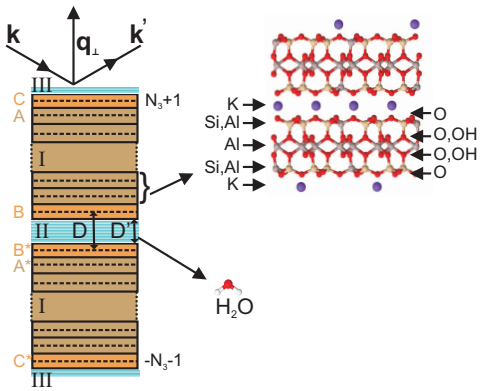

Figure 1: Confinement geometry. Left-hand side: Opposing single-crystal mica membranes (I), and water con¯ned in thegap (II) and adsorbed on the outer mica surfaces (III). Right-hand side: molecular structures of muscovite mica and water.