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Biophysical Chemistry

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Abstract: Biophysical chemistry at the Department of Chemistry, University of Basel, covers the NMR analysis of protein–protein interaction using paramagnetic tags and sophisticated microscopy techniques investigating the dynamics of biological matter.

Keywords: Cyclenes · Fibrins · Lanthanides · Matrix proteins · Microfluids · Protein–protein interactions · Self-assemblies

Lanthanide Chelating Tags for Pseudo-contact Shift NMR Spectroscopy

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Determination of the three-dimensional structure of proteins in solution is a stronghold of modern bio-molecular NMR spectroscopy. Even more important for understanding processes in the living cell is the characterization of interaction sites and surfaces of protein–protein and protein–ligand complexes. NMR can provide not only structural but also dynamic information on this subject. Pseudo contact shift (PCS) NMR spectroscopy has a unique property, as it is a long-range method that can monitor distances of more than 50 Å, in combination with precise angle information.[1–3]

In order to induce PCS in a protein, a paramagnetic metal ion with an anisotropic magnetic susceptibility tensor has to be in permanent contact with the protein. This can be achieved by site-specific tagging of a surface-exposed cysteine side-chain with an appropriate lanthanide chelating tag. The difference between the chemical shifts of the protein nuclei in samples where the tag is loaded with either a diamagnetic lanthanide like lutetium or a strongly paramagnetic lanthanide like dysprosium is caused by the PCS.

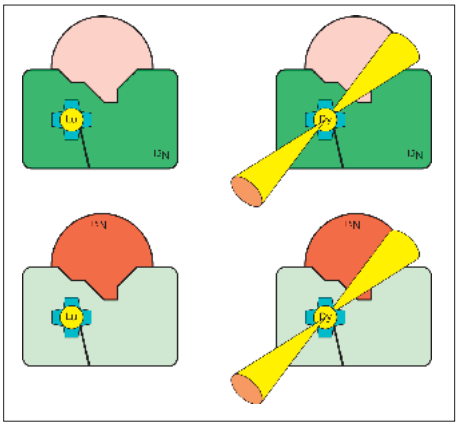


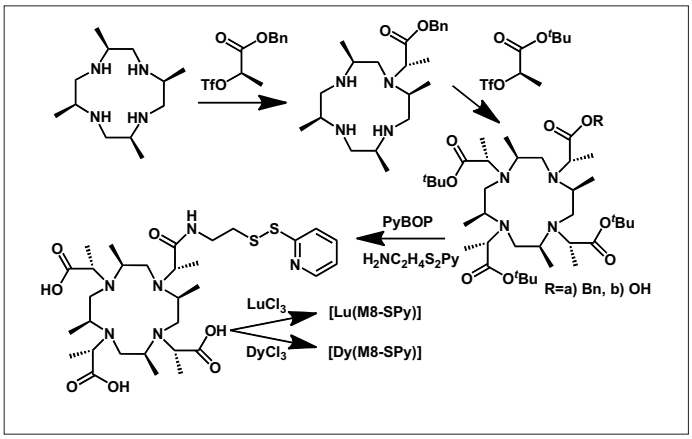
Fig. 1. Determination of orientation and contact interface in a protein complex by PCS.

Even very small PCS in the ppb range can be measured accurately using very sensitive two-dimensional ^{1}H-^{15}N correlation-NMR experiments (*e.g.* HSQC experiments) that can be performed even on larger proteins, where advanced three- and four-dimensional NMR experiments are not applicable due to sensitivity reasons.

To elucidate the interaction surface and the relative orientation of two interacting proteins A (green shape in Fig. 1) and B (red shape), a series of HSQC experiments needs to be measured on up to four different samples with alternating isotope and metal labelling. The two diamagnetic Lu-samples yield the reference shifts of proteins A and B in the complex. From the paramagnetic Dy-tagged sample with ^{15}N-labelled protein A, the metal position, the size and the relative orientation of the five-dimensional magnetic susceptibility tensor χ can be extracted. The PCS of the Dy-sample where protein B is labelled with ^{15}N, yields the distance and the orientation of protein B relative to A.[3]

It is a surprisingly difficult task to design a chelating tag for this purpose, as a number of criteria have to be accomplished. The chelator has to bind lanthanides with very high affinity and in an entirely rigid mode as any flexibility within the ligand or with respect to the protein will lead to dramatically reduced PCS by motional averaging.[4,5]

Häussinger has recently presented a new lanthanide chelating tag 'M8', based on a sterically overcrowded DOTA framework.[6] Scheme 1 shows the synthesis of metal complexes of M8, starting from tetramethylcyclen.[7]



Scheme 1. Syntheses of lanthanide complexes of M8-SPy.

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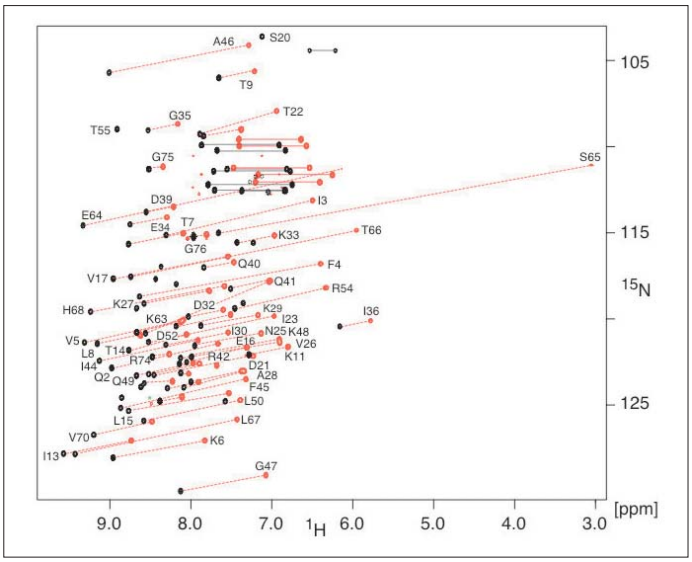

Fig. 2. ^{1}H-^{15}N-HSQC spectrum of Ubi-S57C tagged with DyM8 (red) or LuM8 (black).

The steric bulk in M8 serves two purposes. It efficiently suppresses conformational exchange between two stereoisomers that was observed in the non-methylated parent DOTA-compound.[8] In addition, it reduces the mobility of the chelate on the protein surface.

As a result, DyM8 yields very large PCS when linked to ubiquitin. In fact, every single amide resonance in the HSQC of DyM8-tagged Ubiquitin S57C (Fig. 2) is shifted, which is unprecedented for a single attachment point lanthanide chelating tag.

Having achieved the proof of principle, current research of Häussinger's group aims at further improving the properties of the new ligand and applying this novel technique to challenging protein-protein interactions.[9]

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In situ Dynamics and Structural Evolution of Biological Matter

Thomas Pfohl

Micro- and nanofluidics offer a variety of tools for the *in situ* creation and characterization of soft materials, complex fluids, and biological self-assemblies. Prominent advantages of these tools are the small length scales, compatible with those of the systems in question, and their suitability for a wide range of scientific explorations. Therefore, these methods have a significant impact on biophysics, material sciences, and biotechnology.[1–3]

Combining microfluidic tools with microscopy techniques, such as fluorescence microscopy, polarization microscopy, confocal Raman microscopy, or small angle X-ray scattering enables novel studies on dynamics of biological matter and of self-assembly processes in confined geometries and microflow.[4–7]

Frequently used microfluidic devices consist of two crossed channels with three inlets and one outlet. As shown in Fig. 1, such an arrangement allows the hydrodynamic focusing of a solution stream by two side streams. Such devices can be used for diffusive mixing and the examination of any reactions if the solution in the inlet channel and the side channels contain different reactants. Within the outflow channel, reactants are mixed due to the molecular diffusion, whereby the reaction is initiated. Steady-state concentration gradients, which are generated in the microchannel device, give rise to different concentration ratios along the main stream. Thus, during one measurement different concentration ratio dependent states of the reaction are accessible by varying the observation positions in the channel (*e.g.* c_1, c_2, c_3 in Fig. 1a).[7] Reducing the width of the intermixing zone at the confluence of the streams by the rate of the flow velocities or by geometric constraints, fast complete mixing down to microseconds can be achieved. Therefore, the time evolution of reactions can be studied, since different spatial positions along the outflow channel correspond to different reaction times at a constant concentration (*e.g.* t_1, t_2, t_3 in Fig. 1b).[8]

Hydrodynamic focusing devices with steady-state concentration gradients are ideal tools to study the evolution and non-equilibrium dynamics of self-assembly processes of biomaterials. In several experiments the compaction of DNA by oppositely charged molecules of different sizes and charges has been analyzed by means of small angle X-ray scattering and diffraction.[6,9] Artificial compaction molecules (*e.g.* polyamine dendrimers) show

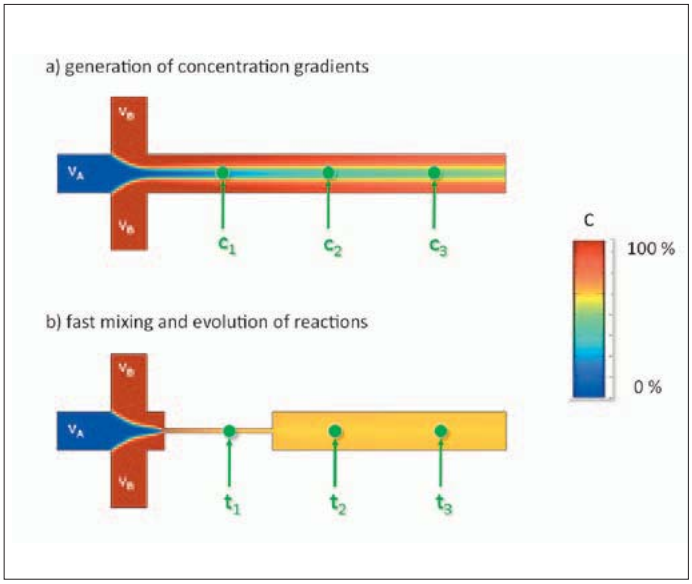

Fig. 1. 2D finite element method (FEM) simulations of steady-state concentration distributions in representative microfluidic devices for a) generation of concentration gradients and for b) fast mixing for temporal observations of chemical and biophysical reactions.

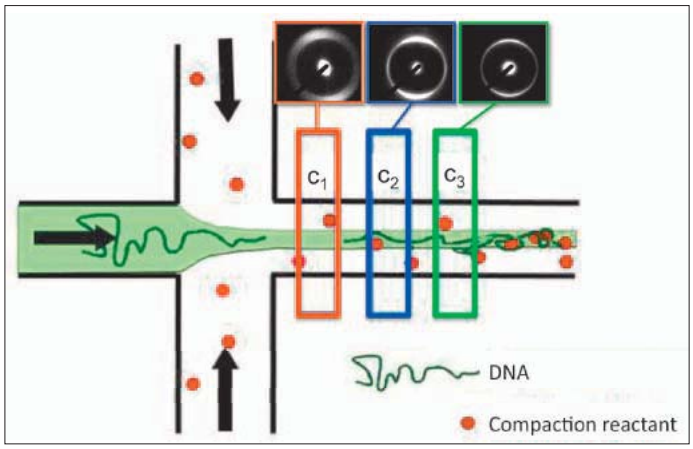

Fig. 2. Dynamics of DNA compaction processes in a hydrodynamic focusing device. Small angle X-ray diffraction patterns at different local position corresponding to different concentration ratios.

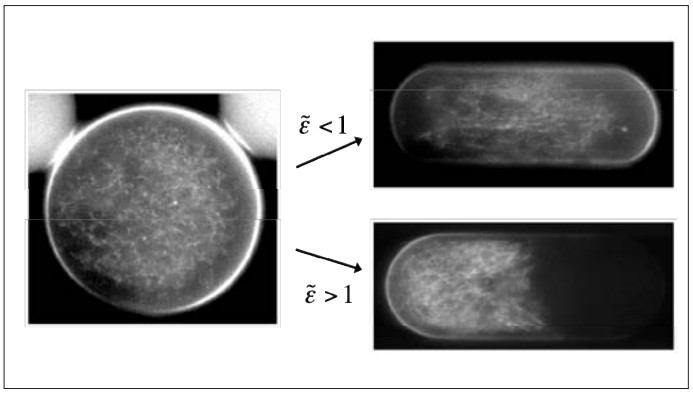

Fig. 3. Mechanical deformation of fibrin networks encapsulated in picoliter droplet. The fibrin networks are mechanical deformed by squeezing microdroplets through narrow channels; for deformation rates $\tilde{\varepsilon} < 1$ the deformation is reversible, for $\tilde{\varepsilon} > 1$ irreversible and a densification of the network is observed.

analogous behavior to natural compaction reagents, such as histone proteins in mammalian cells.[10] Studies on the compaction as well as decompaction of DNA have an enormous impact on our understanding of the physical and chemical phenomena that are important in chromatin organization and in gene expression regulation.

Small angle X-ray diffraction of DNA compaction processes at various positions within microfluidic hydrodynamic focusing devices corresponding to different compositions render the dynamics of condensing processes on molecular scale (Fig. 2).

For these experiments, synchrotron X-ray beams are focused down to a few micrometers by Bragg-Fresnel-lenses or compound refractive lenses in order to scan within the reaction channel of the microdevices.[11] A concurrent alignment of DNA assemblies owing to hydrodynamic focusing improves the characterization of these liquid-crystalline structures and the continuous flow setup avoids radiation damage of the material.[9]

In experiments on the fibrous extracellular matrix protein collagen I, microfluidics in combination with polarization microscopy, computational fluid dynamics, and scanning small angle X-ray scattering give insights into the dynamic evolution of the hierarchical organization of native collagen fibrils.[5,12] In a stable pH-gradient the *in situ* formation of collagen fibrils can be observed. Slight modifications of the microfluidic device design enable the formation of collagen fibers and bundles.

Experiments on cytoskeletal and other matrix proteins follow a bottom-up approach to understand the fundamental mechanisms of bundling and network formation with increasing hierarchy and complexity. Using microfluidics as a tool ***Pfohl***'s group can investigate not only the evolution of filament growth, bundling, and cross-linking within protein networks but also probe the influence of external stimuli and hydrodynamic stress on these networks. Apart from their fundamental relevance, the unique phenomena of soft biological objects under microflow conditions, such as cross-streamline migration and tumbling, may have a great impact in biotechnical applications, such as analyzing and sorting individual molecules or organisms based on of their mechanical properties. Dynamics of single actin filaments, in geometries of dimensions and mesh sizes of the cytoskeleton are studied by means of fluorescence microscopy. The microfluidic channels are used to mimic the confining potential of the cytoskeleton.[13,14] Owing to comprehensive image analyses, the filaments can be analyzed regarding polymer properties, such as tangent-correlations, end-to-end distributions, as well as segment distributions.[14,15] Motility studies of actin-filaments in parabolic channel flow reveal the interplay between stochastic and deterministic motion of semi-flexible polymers in hydrodynamic flow. Using the extensional flow velocity profile within a hydrodynamic focusing device, the elasticity and relaxation of individual filaments can be probed.[4]

Robust three-dimensional fibrin protein networks, which are essential in blood clotting, are formed and encapsulate in microdroplets.[16] Here, microfluidic devices have been tailored to strategically generate and study these biological networks by confinement in picoliter volumes.[17] The fibrin networks are mechanical deformed by squeezing the droplets through narrow channels (Fig. 3). The deformation can be controlled by the geometry and the flow rate. Analyzing these deformations the Young modulus of the encapsulated network can be determined.

Potential directions for future microfluidic-based studies involve mimicking the complexity of eukaryotic chromatin and the cytoskeletal networks as well as emulating the complex flow conditions of unicellular parasites in mammalian blood streams.[18]

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