Abstracts

– Protein Structure and Function –

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Structure and dynamics of the human iron-binding protein Lactoferrin in solution

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Lactoferrin is an iron-binding protein with antimicrobial activity as part of the innate immune system. It consists of two domains, each with a iron(III)-binding site located in a cleft. The reversible iron-binding is supposedly connected with a conformational change of the clefts [1]. Our study aimed to elucidate the link between iron-binding, conformational change and domain dynamics in solution. A combined approach of small angle neutron scattering (SANS) for structural characterization and neutron spin echo spectroscopy (NSE) to elucidate the dynamic properties of different binding states was undertaken. The data of the SANS experiments were compared with calculations from 3D structures (crystallography and homology). The result proves that the binding site clefts are closed when occupied and open otherwise. The evaluation of the NSE data was based on the methodology by Biehl et al [2] involving the normal modes of deformation. It was found that the dominant internal dynamics are independent of the conformation, with relaxation times on the 50ns scale and displacements of <0.7nm. The prevalent motions are overdamped relative movements of the main domains like stretching and twisting. [1] Anderson et al., Nature, 1990 [2] Biehl et al., Soft Matter, 2011

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Efficient assembly of nanoliter crystallization screens with handheld motorized pipette

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High-throughput crystallization screening in multiwell format revolutionized the field of X-ray crystallography. However, reliable assembly of nanoliter drops has required the use of robotics. We have developed a protocol for the reproducible manual assembly of nanoliter-sized protein vapour-diffusion crystallization trials in a 96/192-drop format. The protocol exploits the repetitive pipetting mode of motorized handheld pipettes and needs no additional expensive instrumentation. Determination of precision of pipetting crystallization solutions with different viscosity proved that the handheld pipetting is comparable to robotics. We have tested the reproducibility and robustness of the protocol by repeated crystallization of an antibody Fab fragment in sitting drop on 96-well plate. We believe that the method could be useful especially for small crystallographic groups since it needs no additional expensive instrumentation [1]. We envisage that the use of motorized handheld pipettes would confer similar advantages also in the screening of crystallization conditions for organic molecules.
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[1] Skrabana, R., Cehlar, O. & Novak, M. (2012). J. Appl. Cryst. 45, 1061–1065.

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Investigation of the dynamics of ion translocation subunits from respiratory complex I

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Complex I is part of the respiratory chain and, therefore, a key protein in energy production. The high resolution structure of the entire complex was recently determined, but its catalytic mechanism is still poorly understood. Respiratory Complex I is a L-shaped protein, formed by a hydrophilic and a membrane domains. The charge translocation machinery is incorporated in the membrane domain and includes three large homologous antiporter-like subunits: NuoL, M and N. NuoL has 14 conserved transmembrane (TM) helices, with two antiparallel repeats of 5 TM each (half-channels), which are typical of secondary transporters. It has been accepted that the coupling ion in Complex I is the proton. Recently we observed also Na^+ transport in Complex I from some bacteria, in a Na^+/H^+ antiporter manner. This raised new questions regarding the transport by the subunits from Complex I. Our goal is to investigate the dynamics of H^+ and Na^+ transport in the NuoL subunit.
In this work, we study the structural antiparallel repeats of NuoL. Different constructs, carrying the two repeats together or separately, were expressed in *E.coli* and different biophysical techniques are used to understand the dynamics of that antiporter-like subunit.

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Structural determinants of superoxide reduction

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Superoxide radical $O_2^{\cdot -}$ is the univalent reduction product of molecular oxygen, known to be involved in a variety of cell toxicity mechanisms. While aerobes contain several antioxidant defence systems, such as superoxide dismutases, anaerobes and microaerophiles may depend only on the recently discovered superoxide reductases to keep oxygen toxic species below poisonous thresholds. Superoxide reductases are 14 kDa mononuclear iron proteins and are classified according to the number of iron centres, as 1Fe-SOR (neelaredoxins-Nlrs) or 2Fe-SOR (desulfoferrodoxins). SORs have a catalytic non-heme iron centre coordinated in a square-pyramidal geometry to four histidines in the equatorial plane, and a fifth axial position occupied by a cysteine. In the SOR resting state (oxidized form) a glutamate residue is present as sixth ligand, completing an octahedral geometry. The active center of SORs is present in a domain common to both 1Fe- and 2Fe-SORs. Our research focuses on the molecular mechanism of 1Fe-SORs, and aims to understand the role of the conserved key glutamate and lysine residues, proposed to return the enzyme to its oxidized resting state and to direct superoxide to the active site, respectively. Herein we present the crystal structures of two wild-type SORs.