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Tracking Individual Water Molecules Bound to Proteins. Helmut Durchschlag[a], Peter Zipper[b]. [a]*Institute of Biophysics and Physical Biochemistry, University of Regensburg, Germany.* [b]*Physical Chemistry, Institute of Chemistry, University of Graz, Austria.*
E-mail:helmut.durchschlag@biologie.uni-regensburg.de

High-resolution crystallographic or NMR techniques provide information on the precise 3D structure of proteins and bound water molecules. Knowledge of hydration sites of proteins is essential for many reasons, including understanding manifold interactions as crucial prerequisites for flexibility, dynamics and functionality, and the construction of tailor-made proteins, e.g. in context with drug-design projects.
A critical inspection of anhydrous and hydrated protein models obtained by crystallography with models derived from other techniques and calculation approaches allows comparing the quality of the models under analysis. Among the outstanding problems identified by a critical assessment are amount and position of the water molecules. A variety of techniques were examined and both models and molecular parameters were analyzed: Conventional and ab initio modeling approaches signify satisfactory agreement between crystal- and SAXS-based protein models, provided hydration contributions and other precautions are taken into account [1]. Recourse to crystallographic data also allows hydrodynamic modeling; in the case of multibead assemblages novel modeling refinements (efficient bead reductions) have to be adopted [2, 3]. The creation of hydrated models from cryo-electron microscopy data necessitates qualified assumptions regarding hydration [4]. Combining the exact surface topography (molecular dot surface; derived from atomic coordinates of proteins) and our recent hydration algorithms (programs HYDCRYST and HYDMODEL) allows the prediction of individual water molecules preferentially bound to certain amino acid residues [5-9]; a critical comparison of the water sites on the surface, in crevices or channels proves far-reaching identity of crystallographic data and predictions.
The good agreement of the results found for hydrated models by crystallography and other techniques offers the possibility to complement different techniques and to predict details such as the localization of potential water sites - even in those cases where no crystallographic waters or water channels have been identified. Examples presented include proteins ranging from simple proteins to complex, multisubunit, liganded proteins in the MDa range, and water-channel proteins as well.

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Keywords: hydration of proteins; individual water molecules; water channels

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Structural Studies of the WXG-100 Proteins and the Complementary Analysis Using FRET. Young-Hwa Song[a], Christian Poulsen[a], Matthias Wilmanns[a]. [a]*EMBL-Hamburg, Notkestr. 85, 22549 Hamburg, Germany.*
E-mail: song@embl-hamburg.de

The potential virulence factor, CFP-10/ESAT-6 complex of *Mycobacterium tuberculosis* belonging to the WXG-100 protein family has been studied extensively in the last decades. Although the solution structure of the complex has been published the function of this complex still remains unclear. To study the function of the complex we have at first expressed and purified the complex in milligrams of quantity in its native form using *M. smegmatis* expression system. Our strategy was applied to orthologous and paralogous pairs from virulent and a-virulent mycobacteria. We could express and purify many different CFP-10/ESAT-6 like pairs from *M. tuberculosis, M. leprae, M. smegmatis.* In contrast to using heterologous expression system of *E.coli, M.smegmatis* produces equi-molar ratio of CFP-10 and ESAT-6, as well as the reported post-tranlational modification of N-terminal acetylation of ESAT-6 could be observed. Due to the wide presents of CFP-10/ESAT-6 like pairs in virulent as well as a-virulent mycobacterium, it is key importance to understand the functional and structural differences among the proteins of this family. As starting point we have crystallized and determined the atomic structure of the CFP-10/ESAT-6 complex produced under native condition to 2.1A. We further determined a structure of the protein complex belonging to the WXG-100 family from *S. agalatiae*, which forms a four-helical bundle similar to the structure of the CFP-10/ESAT-6, however formed by a homodimer. Using FRET we could characterize that CFP-10 and ESAT-6 indeed can form only a heterodimer and not a homodimer. We could also confirm that the WXG-100 protein form *S. agalatiae* can only form a homodimer. Based on our study we could get some insight about the key residues for formation of a homo- vs heterodimer complex-formation of the proteins of the WXG100 family.

Keywords: ESAT-6; WXG-100; FRET

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Structural Insight into the Motor Protein A_1A_O ATP Synthase and Implications in Coupling Events. Gerhard Grüber[a], M. Sony Subramanian Manimekalai[a], Anil Kumar[a], Asha M. Balakrishna[a], Shovanlal Gayen[a]. [a]*Nanyang Technological University, School of Biological Sciences, 60 Nanyang Drive, Singapore 637551, Republic of Singapore.*
E-mail: ggrueber@ntu.edu.sg

The molecule ATP (adenosine triphosphate) is the common energy currency of cells. Pro- and eukaryotic F_1F_O ATP synthases and archaeal A_1A_O ATPsynthases are responsible for most of its synthesis. Archaea are rooted close to the origin in the tree of life. Therefore, these microorganisms are the excellent model systems to study the first energy converters, the A_1A_O ATP synthase. The enzyme is composed of an A_3B_3 headpiece, a central and two peripheral stalks, an ion-translocating part A_O, and a collar-like structure, formed by subunit E. The central stalk is made of subunits C-F, whereby the peripheral stalks are formed by the subunits H and *a*, respectively. ATP is synthesized on the A_3:B_3 headpiece and the energy provided for that process is transfered to the membrane-bound A_O domain [1]. The energy coupling between the two active domains occurs via the stalk part(s). We solved the crystal structures for the nucleotide-binding subunits A (61 kDa) and B (54 kDa) of the A-ATP synthase in the presence and absence of ATP, ADP and Pi, which together with fluoresecence correlation spectroscopy data, provide information on the nucleotide-binding and catalytic sites [2,3]. We will show intermediate positions of the nucleotide which could be trapped at high resolution, showing the ATP molecule on its way to the actual binding pocket. Significant insight into the central stalk and collar-domain came from our NMR solution structures of subunits E, F and H. Finally, the dynamic interaction of F with subunit B will be described [4].

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