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 HYDMODELL) 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, multissubunit, liganded proteins in the MDa range, and water channels.

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Keywords: hydration of proteins; individual water molecules; water channels
The molecule ATP (adenosine triphosphate) is the common energy currency of cells. Pro- and eukaryotic $F_1$F$_0$, ATP synthases and archaenal $A_1$A$_0$, ATP synthases 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 $A_1$A$_0$, 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 $A_3B_3$ 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 fluorescence 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].


**Keywords:** crystal structure; NMR spectroscopy; ATP synthase