FA1-MS14-O1

Elucidation of the Phosphate Binding Mode of Ding Proteins Revealed by Sub-Ångstrom X-Ray Crystallography. Dorothee Liebschner[a], Mikael Elias[b], Sebastien Moniot[a], Bertrand Fournier[a], Ken Scott[c], Benoit Guillot[a], Claude Lecomte[a], Eric Chabriere[b]. *[a]Nancy Universite, CRM2, Nancy, France. [b]CNRS-Université de la Mediterranee, AFMB, Marseille, France. [c]Auckland University, Auckland, New Zealand.*
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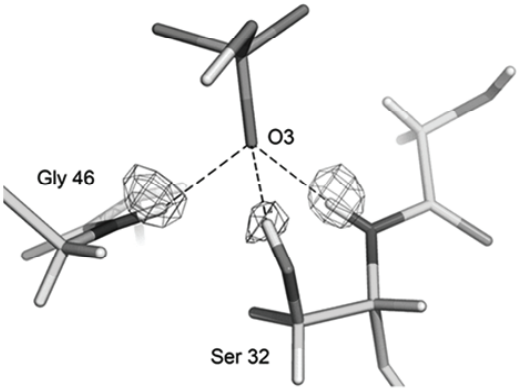
PfluDING is a bacterial protein isolated from *Pseudomonas fluorescens* that belongs to the DING protein family, which is ubiquitous in eukaryotes and extends to prokaryotes. DING proteins and PfluDING have very similar topologies to phosphate Solute Binding Proteins (SBP). The three-dimensional structure of PfluDING was obtained at sub-Ångstrom resolution (0.88Å and 0.98Å) at two different pH (4.5 and 8.5), allowing us to discuss the hydrogen bond network that sequesters the phosphate ion in the binding site. From this high resolution data, we experimentally elucidated the molecular basis of phosphate binding in phosphate SBPs. In particular, contrary to previous theories on phosphate SBPs, accurate electrostatic potential calculations shows that the binding cleft is positively charged. PfluDING structures reveal that only dibasic phosphate binds to the protein both at acidic and basic phosphate, suggesting that the protein binding site environment stabilizes the $HPO4^{2-}$ form of phosphate.



Phosphate binding mode in phosphate high affinity proteins

Keywords: sub-Ångstrom X-ray crystallography; phosphate binding mechanism; electrostatic potential calculation

FA1-MS14-O2

Ultra-High Resolution Protein Structures-Himalayas of Protein Crystallography. Zbigniew Dauter[a], Miroslawa Dauter[b]. *[a]Macromolecular Crystallography Laboratory, NCI, ANL, Argonne, IL, USA. [b]Basic Research Program SAIC Frederick, ANL, Argonne, IL, USA.*
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Whereas the average resolution among almost 50,000 macromolecular X-ray crystal structures in the PDB is about 2.0 Å, some protein crystals are able to diffract to a resolution higher than 0.8 Å, which is the usual limit of Cu-radiation applied to crystals of small organic compounds. Such ultra-high resolution analyses provide a wealth of information about details of stereochemical and electronic structures. The knowledge of such details helps to form the libraries of accurate geometrical targets but also may suggest which exceptions from the standard protein stereochemistry are possible. The intimate knowledge of structural and electronic details is important for understanding of (bio) chemical reactions and interactions among macromolecules and for faithful modeling of their complexes with various ligands. Similarly as the Himalayan mountains higher than 8,000 metres, the crystal structures at resolutions higher than 0.8 Å are there to be conquered – both provide very far-reaching perspectives.

Keywords: ultra-high resolution; stereochemistry; accuracy

FA1-MS14-O3

Protonation State Determination in Proteins Using High Resolution Protein X-Ray Crystallography: Effects of Resolution and Completeness. Stuart J. Fisher[a,b], Matthew Blakeley[a], Sean McSweeney[c], John R. Helliwell[b]. *[a]Institut Laue Langevin, 6 rue Jules Horowitz, Grenoble, 38042, France. [b]The University of Manchester, Department of Chemistry, Brunswick Street, Manchester, M13 9PL. [c]Macromolecular Crystallography Group, European Synchrotron Radiation Facility, 6 rue Jules Horowitz, 38043, Grenoble, France.*
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The advancement of synchrotron X-ray facilities, and the consequent increase in flux currently available to users at such facilities, in combination with advancements in protein crystallisation, means it is becoming increasingly possible to collect protein crystallographic data sets at, or within range of, atomic resolution. These data sets are often highly complete with large multiplicities making them excellent candidates for protonation state determination of amino acid groups using bond length analysis [1, 2]. By carefully considering the differences in bond lengths between, for example a C=O and C-OH bond, and the associated standard uncertainties, it is possible to determine whether these residues are in fact protonated to within a statistically significant level [1]. Here we consider the protonation state determinations in a muscle protein; myosin binding protein c [2] (MyBP-C) at 1.3Å (PDB Code 3CX2, 82% complete), trypsin [3] at 1.2Å (97% complete), and lysozyme [4] at 0.65Å (PDB code 2VB1, 98% complete). The estimated coordinate errors obtained using the Cruickshank DPI [5] (Diffraction component Precision Index), and those determined from the SHELX full matrix inversion have been determined and compared in order to gauge the effect of both resolution and completeness on those values. We find