match' method for using modified GFPs as crystallization partners while avoiding the disorder potentially associated with single linker approaches.

Keywords: GFP scaffold, complex formation, solubility

MS.57.4

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Immutable glycine based perfect alignment of protein families

William L. Duax,[ab] Robert Huether,[b] David Dziak,[a] Courtney McEachon,[a] [a]*Hauptman-Woodward Institute, Buffalo NY. (USA).* [b]*Department of Structural Biology, University at Buffalo. Buffalo, NY (USA). E-mail: duax@hwi.buffalo.edu*

The search vector X(0,24)X(21)RX(5)[PNHFLSEQ]X(3)[GSDRENA]X(6)XG[KRNQMHSIY]X(6) [LIVMP] X(4)[LIVMCT][GSDFYNA]X(2)[LFI][GASR][DEA][FYME]X(2)[STP]X(5)[HKFYMT]X(0,25) retrieves and aligns the S19 ribosomal proteins (RibPros) of all species for which full genomes are in SwissPro/TrEMBL(3983) with no false hits.

The alignment is achieved for S19 by identifying two amino acids that are 100% conserved in all sequences (a Gly and an Arg) and noting that only one insertion or deletion site of limited size and precise location has arisen in S19 over the course of its evolution. The position immediately before the immutable Gly is occupied by only one of two amino acids (Asp or Asn). An Asp in this position isolates all Gram positive (G+) bacteria and an Asn isolates all Gram negative (G-) bacteria. Similar alignments of a dozen other RibPros have been achieved by identifying sequence sites in which 100% conserved Gly(G), Ala(A), Arg(R), and Pro(P) residues have been used as markers for perfect alignment and precise location of indels. It is possible to identify a few positions in each RibPro that separate G+ from G- bacteria and can be used to parse phylum, class, order, and genus to create a consistent rooted phylogenic trees unaffected by horizontal gene transfer. The perfect alignments reveal homology between cyanobacteria (G-) and chloroplasts and between actinobacteria (G+) and eukaryotes. When the residues that are fully conserved in all members of a RibPro family and those that are associated with G+ to G- transition and divergent speciation are mapped onto the 3D structures in the ribosome, conservations and co-evolutionary patterns offer insight into the details of evolution of the ribosome. Gly size and achirality account for its conservation in Ribpros and other extended families of proteins. The primordial role of GARP residues in protein folding may be related to there physical and stereochemical properties and to the fact that the only GC-rich codons encode two of each of G, A, R, and P. We have used GARP based techniques to isolate and align heat shock proteins, beta barrels, and 40,000 short chain oxidoreductase (SCOR) enzymes. Perfect alignment of the SCORs permits unambiguous identification of residues defining cofactor and substrate specificity, species distribution and function. GARP maximization within these families suggests that the last universal common ancestor at the root of the species tree is a primordial actinobacteria. Four genus of this order make no use of 24 of the codons ending in G or C and little use of the other 8 codons ending in G or C in the encoding of the 20 RibPros of the Small subunit. These four genus do not have tRNAs specific to most of the codons ending in G or C. These and other characteristics of these genus suggest that they arose before the entire genetic code was defined. Support in part by: Mr Roy Carver, Stafford Graduate Fellowship, Caerus Forum Fund, The East Hill Foundation and the generous help of a number of High School students from the Buffalo NY area.

Keywords: evolution, ribosomal protein, alignment.

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Differential scanning fluorimetry at the ESFRI-Instruct Core Centre Frankfurt

Yvonne Thielmann, Martin Kohlstädt, Iris von der Hocht, Jürgen Köpke, Hartmut Michel, *Department for Molecular Membrane Biology, Max-Planck-Institute of Biophysics, Frankfurt am Main (Germany).* E-mail: yvonne.thielmann@biophys.mpg.de

Within ESFRI (European Strategy Forum on Research Infrastructures) and INSTRUCT (Integrated Structural Biology Infrastructure for Europe) we operate the Core Centre Frankfurt, which is devoted to membrane proteins.

Here we try to understand membrane proteins based on an accurately known structure. Our work emphasizes on the structure determination of proteins and protein complexes integrated into the cellular membranes of living organisms. The importance for life of membrane proteins can be deduced from a huge variety of duties, for the communication of the cell with its environment, like signal exchange, as well as energy transduction and specific uptake and/or release of substrates of all kind.

Within the Core Centre four labs for protein and crystal handling were established: Verification of the protein by mass spectrometry, thermal stability tests with a calorimetric approach or differential scanning fluorimetry (DSF), fully automated crystallization and analysis of the crystals by X-ray diffraction.

DSF is a quick method to determine the melting temperature (T_m) of a protein. It requires a smaller sample amount compared to calorimetric methods and uses fluorimetric readout. We use the realtime PCR machine Rotor-Gene Q from Qiagen to determine melting temperatures of membrane proteins with different dyes. This is a demanding task because interaction of the dye with detergent micelles causes scattering of the incident beam, which may totally overlap the fluorescence signal of interest. Therefore various dyes as e.g. SyproOrange®, CPM, ANS or Nile Red have been tested for fluorescence readout with Lysozyme and compatibility with detergents as DDM, OG, C12E8, LDAO and Fos12. This information is used to choose an optimal dye and detergent combination. For several membrane proteins T_m values have been determined for different pH, salt concentration, detergent mixture or mutations. The difference in T_m can be used to rank buffer conditions, additives and mutants according to their enhancement in protein stability. This allows to use the most promising targets with suitable buffer and ligand conditions directly in the crystallization trials. Promising candidates have been taken to crystallization trials.

Keywords: membrane_protein, stability, crystallization

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Ensemble refinement of protein crystal structures in PHENIX

Tom Burnley,[a] Pavel Afonine,[b] Paul Adams, [b] Piet Gros, [a] [a]*Department of Crystal and Structural Chemistry, Bijvoet Center, Utrecht University (The Netherlands).* [b]*Lawrence Berkeley National Laboratory (USA).* Email: b.t.burnley@uu.nl

Despite recent advances in data collection and processing in protein crystallography, there remains a significant discrepancy between measurement and model error for macromolecular crystal structures. This has been attributed, at least in part, to the incomplete modelling of atomic disorder. Here we present an alternative refinement method which simultaneously includes anisotropic and anharmonic disorder. This ensemble refinement uses a molecular dynamics approach

augmented with time-averaged x-ray restraints [1] to produce a series of Boltzmann-weighted structures that represents the conformational space sampled during a simulation. The resulting ensemble typically contains 100-250 structures and is shown to significantly improve the model error (as judged by Rfree), in comparison with traditional methods. This new method is suitable for diffraction data with upper resolution limits in the range of 1-3Å d-spacing. This method does not require excessive computation time and can be run on a standard desktop machine.

Ensemble refinement was developed, and is available, within the PHENIX software suite [2]. It utilises a maximum-likelihood target function in conjunction with a dual explicit- and bulk-solvent model and can be used with any heterogeneous atom or group.

In addition to the improved global statistics, ensemble refinement reveals highly-resolved local disorder features which are demonstrated to reflect important functional details for a number of test cases.

[1] P. Gros, W.F. van Gunsteren,W.G.J. Hol, *Science, **1990**, 249*, 1149-1152. [2] P.D. Adams, P.V. Afonine, G. Bunkóczi, V.B. Chen, I.W. Davis, N. Echols, J.J. Headd, L.-W. Hung, G.J. Kapral, R.W. Grosse-Kunstleve, A.J. McCoy, N.W. Moriarty, R. Oeffner, R.J. Read, D.C. Richardson, J.S. Richardson, T.C. Terwilliger, P.H. Zwart. *Acta Crystallographica Section D,* **2010,** *66*, 213-221.

Keywords: macromolecular, refinement, disorder

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Low resolution refinement in the program – REFMAC

Garib N Murshudov, *YSBL*, *Chemistry Department, University of York, York, (UK) YO10 5YW.* E-mail: garib@ysbl.york.ac.uk

Despite rapid advances in Macromolecular X-ray Crystallographic (MX) methods, derivation of reliable atomic models from low resolution diffraction data still poses many challenges. The main reason for this is that the number of observations relative to the number of adjustable parameters is small and furthermore signal to noise ratio in the experimental data is very low. As a consequence derivation of biologically meaningful information from such data is challenging. Intrinsic mobility of macromolecules means that in many cases growing crystals diffracting to higher resolution is not possible and low resolution data must be used to derive some useful structural information.

Statistically sensible analysis of low resolution diffraction data requires tackling of two related but distinct problems i) stabilisation of ill-posedness of refinement procedures - reduction the effective number of parameters without sacrificing completeness of atomic models ii) calculation of maximal signal/minimal noise electron density that would not suffer from bias towards model errors. Solving the first problem is necessary to derive reliable atomic model and the second problem to calculate interpretable electron density that is used in model (re)building.

1) The first problem is usually tackled using additional restraints based on structural information. Available structural information are a) known similar three-dimensional structures b) secondary structures; c) NCS if present; d) in addition it is also possible to exploit the fact that during refinement inter-atomic distances should not change dramatically. It has already been shown that using these restraints improves reliability of the derived models. As a result of model improvement errors in the derived atomic models are reduced, and it means that calculated phases have less error hence reducing noise in the electron density related to the model errors.

2) Sharpening of an electron density while increasing signal amplifies noise masking out “true” signal. There are several approaches to such problems. These include: a) regularisation using Tikhonov-Sobolev method; b) Wiener filters and c) Bayesian filters. These techniques attempt to answer to one common question: how to enhance signal without noise amplification? Another problem in map sharpening is that it assumes that all atoms have the same B values. It is in general not true and there is a distribution of B values – inverse gamma distribution. Moreover individual atoms’ oscillation depends on its position in the asymmetric unit. These facts need to be accounted for if accurate map sharpening tools to be designed. In this presentation some approaches to these problems will also be discussed.

Keywords: refinement, macromolecule, restrained

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Better ligand representation in BUSTER protein-complex structure determination

Oliver S. Smart, Thomas O. Womack, Claus Flensburg, Peter Keller, Wlodek Paciorek, Andrew Sharff, Clemens Vonrhein, Gérard Bricogne, *Global Phasing Ltd., Sheraton House, Cambridge CB3 0AX, (UK).* E-mail: osmart@globalphasing.com

The generation of reliable restraints for novel small molecule ligands in protein complexes is of great importance for both model placement into density and subsequent refinement. We have recently released GRADE [1], a procedure whose main source of restraint information is the Cambridge Small Molecule Database (CSD), queried using the MOGUL program [2], developed by the CCDC. Where small-molecule information is lacking, grade uses quantum chemical procedures to set restraint values. GRADE automatically produces restraints that are compatible with the Engh and Huber EH99 restraints used for the protein during building and refinement. Particular care has to be taken when interpreting CSD data in order to produce restraints for torsion angles. This is likely to be because small molecule crystal structures are often less strained than those found in protein complexes.

An alternative to conventional stereochemical restraint functions is provided by the direct use of quantum mechanics to compute the potential energy of the ligand. This involves invoking a quantum chemical program to provide the potential energy and its gradients for the ligand conformation in each cycle of BUSTER refinement. It will be shown how the results of the direct use of QM for ligands in refinement complement the use of CSD data.

[1] BUSTER package http://www.globalphasing.com/buster/. [2] I.J. Bruno et al, *J. Chem. Inf. Comput. Sci.* **2004***, 44,* 2133-2144.

Keywords: refinement, quantum_chemistry, database

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Structure solution by molecular replacement using *ab initio* protein models

Daniel Rigden, Jaclyn Bibby, Olga Mayans, Ronan Keegan Martyn Winn, *University of Liverpool; STFC*, *(UK).* Email: drigden@liv.ac.uk

Molecular Replacement uses a known search model to solve the unknown crystal structure of a related protein, but is dependent on the availability of a model having sufficient structural similarity. *Ab initio* modelling has developed to the extent that its results can sometimes be used to successfully phase diffraction data. Thus, *ab initio* models can be tried as search models where structural homologues are not available and experimental phasing is difficult [1].