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

Immutable glycine based perfect alignment of protein families

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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.

MS.57.5

Differential scanning fluorimetry at the ESFRI-Instruct Core Centre Frankfurt

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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

MS.58.1

Ensemble refinement of protein crystal structures in PHENIX

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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