where several transitions have been reported and related to a wide range of diverse driving forces. In this talk, a comprehensive review of those phenomena will be presented.

Concerning the instrumentation, a special attention will be paid to microscopic techniques able to identify the role of heterogeneities at the surface phase transitions. It is manifest that the elevated flux of the third generations synchrotron radiation sources are essentially profited in the area of the X-ray microscopy. Now a day, those light sources are able to provide high brightness at micrometric or even nanometric beam sizes. In such context, photoemission microscopes will be introduced as tools capable to fill up the existing emptiness between the STM spectroscopy and the low-spatially resolved traditional ARPES and NEXAFS in the area of Surface Phase transitions.

Keywords: phase transition, microspectroscopy, surface

MS.57.1

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An attempt to prepare membrane proteins using the wheat cell-free protein production system

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The cell-free protein production system we developed from wheat embryos has the significant advantage of producing eukaryotic multidomain proteins in a folded state. In this paper, I will briefly introduce the characteristics and capability of this system together with some results of our ongoing work on the biochemical drawing of protein networks and examples of sample preparation including multiprotein complexes for structural analysis. I will then focus on our recent attempt to develop a versatile methodology for preparing membrane proteins using the wheat cell-free system. Performing translation in the presence of liposomes, we could produce all of the 30 membrane proteins chosen for the test, which included GPCRs and ion channels, each in the form of a protein-liposome complex. These complexes were isolated by brief centrifugation without any purification tags, and were solubilized with a detergent solution. Each protein was separated by Superdex200 gel-filtration column using a buffer solution containing fos choline-14. The purified membrane proteins all exhibited mono-disperse peaks in chromatogram. Three proteins HRH2, DRD1, and HTR3A, were selected for ligand binding assay. The Kd values determined by Biacore using respective defective mutants as a reference confirmed specific binding activity retained in each of the three purified proteins. Although the binding affinity of proteins were not high enough at the moment, further optimization of the solubilization conditions may bring the protocol as a useful HT-methodology for preparing membrane protein samples.

Keywords: preparation of proteins, cell-free, difficult protein

MS.57.2

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Structural biology of G protein-coupled receptors

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G protein-coupled receptors (GPCR) constitute the largest family of integral membrane proteins that transmit signals inside cells in response to a variety of extracellular stimuli. Despite the great significance of GPCRs in cell physiology and human health, structural information for this family of receptors is limited, and many essential details related to the mechanism of signal transduction and ligand specificity and selectivity are just beginning to emerge.

Recent breakthroughs in GPCR structural biology have been made possible by the progress in protein engineering, as well as the development and automation of crystallization technologies using lipidic matrices. Structures of 7 different GPCRs have been solved to date, 3 of which were captured in both inactive and active states.

Here we present recently determined structures of the human CXCR4 chemokine G protein-coupled receptor bound to a small molecule It1t and a cyclic peptide antagonist CVX-15 [1]; the structure of the human dopamine D3 receptor in complex with the antagonist eticlopride [2]; and the structure of the human adenosine A_{2A} receptor bound to an agonist UK-432097 [3]. The CXCR4 structures reveal a consistent set of receptor homodimers and provide insights into chemokine signaling and HIV-1 recognition. Structural details of the dopamine D3 receptor help us to better understand the pharmacological specificity between the dopamine D2 and D3 receptors. Comparison of A_{2A} structures bound to antagonist and agonist sheds light on the mechanism of GPCR activation.

The GPCR Network has been established to work with the GPCR community scientists interested in obtaining structural data on different receptors. Interested researchers should visit http://gpcr.scripps.edu for more information.

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Keywords: receptor, signal, transduction

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Application of split fluorescent proteins to challenges in crystallography: present and future

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The "GFP ToolBox" developed at LANL for the Protein Structure Initiative (PSI) includes spontaneously assembling engineered fragments of fluorescent proteins that can be used to tag and label proteins in living cells and cell extracts. These tools can be used to address challenges in protein expression, screening for complex formation, and crystallization. (1) Using actual examples from challenging multi-domain proteins, we show how this technology can be used in library screens to find soluble protein modules that are well-suited for crystallographic study. (2) We show how the technology can be used to screen for stable protein complexes that can be co-purified. (3) Finally we describe recent experiments and show a preliminary structure in which a small beta hairpin fragment of the GFP scaffold has been inserted into loops and turns of a target protein. The remainder of the GFP scaffold is added, binding to the displayed fragment and reconstituting the GFP barrel. This paves the way to a 'mix-and-

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

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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.

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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

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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