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

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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-