target for the first anti-HIV drugs, it still has potential for development of new drugs including the targeting of as yet unexploited regions such as the RNaseH active site and tRNA primer binding.

Keywords: HIV reverse transcriptases, inhibitor binding, drug resistance

MS36.26.5

Acta Cryst. (2005). A**61**, C51

Structure-based Vaccine Design of Human Rhinovirus: HIV Chimeras as Candidate AIDS Vaccines

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Our laboratory team has developed a system for generating combinatorial libraries of cold-causing human rhinoviruses (HRVs) that effectively display immunogenic peptide segments from a variety of pathogens. We have used this system to generate chimeric HRV-HIV-1 viruses displaying regions of the HIV-1 membrane-spanning protein gp41 that are part of the conserved and critical viral fusion machinery. We have generated chimeric HRVs displaying the so-called ELDKWA epitope of this region of gp41 that elicit immune responses able to broadly and potently cross-neutralize HIV-1 primary isolates, the first neutralizing responses reported for any ELDKWA-based immunogens. Ultimately, such immunogens might serve as valuable constituents in an AIDS vaccine.

Structural considerations for this vaccine engineering system will be discussed. We have obtained diffraction data at CHESS and BNL for several HRV:HIV-1 chimeras; structure determination is in progress. We are also investigating structures of chimeric virus complexed with anti-HIV neutralizing antibodies or Fab fragments. An important long-term goal is to identify three-dimensional correlates of immunogenicity and apply the knowledge to facilitate vaccine design and development using a structure-based approach.

Keywords: virus structure, virus engineering, immunology

MS37 INTRACELLULAR TRAFFICKING OF BIOMOLECULES

Chairpersons: Yoshiro Yoneda, David Owen

MS37.26.1

Acta Cryst. (2005). A**61**, C51

Transport out of the Nucleus and Beyond: Molecular Mechanisms

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The movement of proteins and RNAs between the nucleus and cytoplasm of eukaryotic cells is mediated by nucleo-cytoplasmic transport receptors. Most receptors belong to the karyopherin β family of protein, which are also known as importins or exportins according to whether they import or export cargo into/from the nucleus. The directionality of import and export processes depends on the small GTPase, Ran. In contrast to most proteins/RNAs, mRNAs are transported out of the nucleus by a transport factor unrelated to the karyopherin family. mRNA export is linked to quality control mechanisms that make sure that only correctly transcribed and processed mRNAs are exported and translated. A ubiquitous quality control mechanism is nonsense-mediated mRNA decay (NMD). NMD is a surveillance pathway that detects mRNAs containing premature translation termination codons (PTCs) and degrades them before they give rise to truncated protein products. In humans, detection and degradation of PTC-containing mRNAs is dependent on splicing. The splicing-dependence is correlated to the exon junction complex (EJC), a multiprotein assembly that is deposited on mRNAs at the end of splicing upstream of exon junctions. EJC components mark aberrant mRNAs for detection by the NMD machinery and deliver the targeted mRNA to degrading enzymes such as the exosome.

X-ray structures of components of the mRNA export/surveillance machinery give insights on the molecular mechanisms with which they function.

Keywords: protein-RNA interactions, macromolecular assemblies, intracellular trafficking

MS37.26.2

Acta Cryst. (2005). A**61**, C51

Cse1: the Structure of an Exportin in its Closed, Cytosolic State

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Cse1 mediates nuclear export of importin-alpha, the nuclear localization signal (NLS) import adaptor. We report the 3.1Å resolution structure of cargo-free Cse1, representing this HEAT-repeat protein in its cytosolic state. Cse1 is compact, consisting of N- and C-terminal arches that interact to form a ring. Comparison with the structure of cargo-bound Cse1 shows a major conformational change leading to opening of the structure upon cargo binding.

The largest structural changes occur within a hinge region centered at HEAT repeat 8. This repeat contains a conserved insertion that connects the RanGTP and importin-alpha contact sites and that is essential for binding. In the cargo-free state, the RanGTP binding sites are occluded and the importin-alpha sites are distorted. Mutations that destabilize the N- to C-terminal interaction uncouple importin-alpha and Ran binding, suggesting that the closed conformation prevents association with importin-alpha.

Keywords: Cse1, exportin, nuclear transport

MS37.26.3

Acta Cryst. (2005). A**61**, C51

Cracking of the Targeting Signal Embedded in Mitochondrial Presequences

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Most mitochondrial proteins are synthesized in the cytosol as precursor proteins with a cleavable N-terminal presequences and are imported into mitochondria. Protein import into mitochondria is mediated by protein assemblies in the mitochondrial membranes. A subunit, Tom20, functions as a general protein import receptor by recognizing presequences of preproteins. Although no consensus sequence is found, Tom20 recognizes a wide variety of presequences.

To understand the structural basis of the presequence recognition, we determined the NMR and crystal structures of Tom20 in a complex with a presequence peptide. Note that the presequence was fixed to Tom20 via a designed intermolecular disulfide bond to obtain crystals. The bound presequence forms an amphiphilic a-helix. NMR titration experiments indicated the presence of a unique presequence binding site in Tom20, and defined a common five-residue pattern in different presequences. To refine this pattern, we introduced a new peptide library approach using the formation of an intermolecular disulfide bond. We propose that a presequence is regarded as a collective entity of short amino acid sequences that are recognized by several proteins including Tom20. The organization (position, order, and overlapping) of these binding segments is unique for each presequence. This view explains why no consensus sequences are found by simple sequence comparisons.

Keywords: protein transport, molecular recognition, crystallographic and NMR solution state structures

MS37.26.4

Acta Cryst. (2005). A**61**, C51-C52

Vps29: a Phosphoesterase Fold that acts as an Interaction Scaffold in the Assembly of Retromer

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Membrane sorting between secretory and endocytic organelles is predominantly controlled by small carrier vesicles or tubules that are layered on their cytoplasmic faces by specific protein coats. Recently we have begun studies of a novel putative membrane coat complex termed retromer. Retromer contains five subunits, Vps35, Vps26, Vps29, Snx1 and Snx2 and is responsible for tubule-based retrieval of proteins from the endosomal system to the Golgi, for example recycling mannose-6-phosphate receptors that traffic lysosomal hydrolases from the TGN to endosomes. We have determined the crystal structure of the mammalian retromer subunit Vps29, showing that it has structural similarity to divalent metal-containing phosphoesterases. However, although Vps29 can coordinate metals in a similar manner it has no detectable phosphatase activity *in vitro*, suggesting a novel specificity or function. We show that Vps29 and Vps26 bind directly to distinct regions of Vps35 and together form a high affinity heterotrimeric sub-complex. Mutagenesis reveals the structural basis for interaction of Vps29 with Vps35 and subsequent membrane association of Vps29 *in vivo*. Furthermore, we demonstrate that a conserved hydrophobic surface distinct from the primary Vps35 binding site can mediate assembly of the Vps29p-Vps26p-Vps35p sub-complex with sorting nexins in yeast, and mutation of either site results in a defect in retromer-dependant membrane trafficking.

Keywords: membrane trafficking, protein phosphatases, X-ray structure

MS37.26.5

Acta Cryst. (2005). A**61**, C52

Crystallization of Molybdate-Binding Protein of Xanthomonas citri

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We report the crystallization and prelliminary data of the periplasmic molybdate-binding protein (ModA) of the plant pathogen *Xanthomonas citri*, responsible for the cancer disease affecting citrus plants. Structures of molybdate transporters have been solved in other species including *Escherichia coli* and *Azotobacter vinelandii* [1, 2], however, no ortholog derived from plant-associated bacteria have been reported so far. The 26 kDa protein has been overproduced in *E. coli*, purified, and crystallized in complex with Na_2MoO_4. The crystallization of ModA using the sitting-drop vapour-diffusion method with PEG 4000 as precipitant is described. Crystals belong to the orthorhombic space group $P222_1$, with unit-cell parameters a = 68,16, b = 172,21, c = 112,05. A X-ray diffraction data were collected to a maximum resolution of 1,7 Å using a synchrotron-radiation source. Structure refinement is in progress. The ongoing biochemical characterization in combination with the structural analysis, will assist the elucidation of the structure-activity relationship in regulating the uptake of molybdate in *Xanthomonas*.

[1] Hu Y., Rech S., Gunsalus R.P., Rees D.C., *.Nat.Struct.Biol.*, 1997, **4**, 703-7. [2] Lawson D.M., Williams C.E., Mitchenall L.A., Pau R.N., *Structure.* 1998, **6**, 1529-39.

Keywords: ModA protein, Xanthomonas citri, crystallization

MS38 CONTROLLED BUILDING OF CRYSTALS FROM NON-COVALENT INTERACTIONS

Chairpersons: Christer Aakeroy, Alessia Bacchi

MS38.26.1

Acta Cryst. (2005). A**61**, C52

Understanding and Using Solution Chemistry to Direct Crystal Nucleation

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Our understanding of non-covalent interactions which determine crystal packing in the solid state has progressed enormously over the last years due largely to the explosion in numbers of crystal structure determinations and their availability via the Cambridge Structural Database. In the context of *controlled* building of crystals however, this information is not enough, we also have to consider the interactions which exist in the solution phase at the time of nucleation. Such information can be gleaned from a number of sources: thermodynamic and colligative data (eg solubility, freezing point depression); UV/vis spectroscopy; vibrational spectroscopies; NMR; and neutron scattering.

This paper reports on the use of these techniques in understanding the key interactions in highly concentrated solutions of urea, benzoic acid, tetrolic acid, sulfamerazine and 2,6dihydroxybenzoic acid. In many cases there is a clear link between solvent mediated self assembly and the resulting crystal structures.

Keywords: nucleation, solutions, chemistry

MS38.26.2

Acta Cryst. (2005). A**61**, C52

Molecular Tectonics : from Tectons to Networks

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Molecular crystals are compact and periodic entities. They are defined by the nature of their molecular components and interactions between them in the solid state. Although a crystal is described by translation of the unit cell into three directions of space, one may describe it as a network by considering intermolecular interactions as specific recognition patterns. The approach dealing with such an analysis is called molecular tectonics [1]. The latter is based on tectons which are construction units bearing within their backbone assembling programmes. The design and formation of molecular networks with predefined dimensionality and connectivity may be ensured by the nature and localisation of recognition sites within the structure of tectons.

The strength of molecular tectonics is related to the fact that not only it allows to describe a given crystal in terms of networks but, also and more interestingly, this approach allows to conceive molecular networks through the specific design of tectons [2].

A variety of tecons and molecular networks based on diverse intermolecular interactions will be presented.

[1] Hosseini M. W., *Acc. Chem. Res*.,2005, **38**, *in press*. [2] Hosseini M. W., *Cryst. Eng. Comm*, 2004, **6**, 318.

Keywords: tectons, networks, chemistry

MS38.26.3

Acta Cryst. (2005). A**61**, C52-C53

Testing the Reliability of the Self-complementary Noncovalent Interactions: Supramolecular Implications and Supramolecular Design

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Noncovalent interactions play a special role in supramolecular chemistry, which has been defined by Lehn [1] as “chemistry beyond the molecule”. Noncovalently assisted synthetic procedures are used to assemble various types of supramolecular species. These syntheses rely on the stabilization provided by noncovalent interactions between recognition sites incorporated within precursors. As a recognition motif utilized to guide the synthesis, various types of noncovalent interactions can be used. These are, specifically, hydrogen bonds (H-bonds), stacking interactions, electrostatic interactions, hydrophobic interactions, charge-transfer interactions, and metal coordination [2]. Unconventional polymers composed of covalent and noncovalent bonds differ dramatically from standard, conventional polymers with just covalent bonds. They posses novel physical, optical, electrochemical, photochemical, biological, and catalytic properties. Targeted synthesis of macro- and supramolecular structures of various sizes, shapes, and functionality has now become possible. Supramolecular chemitry offers incredible applications in various