and the serine protease domain positioned on top. The structure of the individual apple domains is represented by a novel topological motif. The FXI structure combined with our previous structural analysis of the Glycoprotein Ib receptor domain[1] allows us to construct a model of the activating ternary complex formed with thrombin.

[1] Uff et al., *J. Biol. Chem*.,2002, **277**, 35657 - 35663.

Keywords: receptor binding, coagulation, protease

MS43.27.6

Acta Cryst. (2005). A**61**, C59

Structural Studies of the CLD from Aggrecan

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Aggrecan is an important protein in the extracellular matrix (ECM) in the cartilage and its function is to organize the forming of the hyaluronan-lectican complexes in the ECM. Aggrecan consists of three globular domains and a central region of an elongated glycosaminoglycans-carrying region. The N-terminal domain (G1) binds to hyaluronan and the C-terminal domain (G3) has been shown to be involved in the binding to two types of ligands: sulphated glycolipids on the cell surface and dimeric/multimeric ECM proteins, e.g. fibulin-2 and tenascin-R. The CLD within the G3 domain of aggrecan has been observed to make a tight protein-protein interaction (K_D 12nM) with fibronectin type III repeats (FnIII) 3-5 from tenascin-R (TN3-5). Interestingly it has been shown that, though the complex is totally dependent on Ca^{2+}, as would be expected of a CLD, the interaction does not depend on carbohydrate. This is one of only a few direct protein-protein interactions of CLDs involving Ca^{2+}.

The structural studies will give us a first insight how lecticans use the CLD domain to interact with different ECM proteins. This is the first structure showing a calcium-dependent protein-protein interaction involving a C-type lectin domain that is not mediated by a carbohydrate. We have also solved the CLD of aggrecan in a unbound state, giving clues of the importance of the calcium ions.

Keywords: protein-protein interaction, calcium-binding protein, complex structure

MS44 NUCLEIC ACIDS - TRANSCRIPTION, TRANSLATION AND REPAIR

Chairpersons: Stephen Neidle, Eric Westhof

MS44.27.1

Acta Cryst. (2005). A**61**, C59

Eukaryotic Translesion Synthesis DNA Polymerases: Structure and Function

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Cellular DNA is continually damaged by external and internal agents, and both eukaryotes and prokaryotes possess DNA polymerases that can replicate through DNA lesions. Humans have four such (Y-family) polymerases – Polκ, Polι, Polη, and Rev1 – each with a unique DNA damage bypass and fidelity profile. Polη, for example, is unique in its ability to replicate through UV-induced cyclobutane pyrimidine dimers (CPDs), while Polκ is inefficient at replicating through a T-T dimer but can readily extend from mispaired termini. Polι is perhaps the most unusual with varied efficiencies and fidelities opposite different template bases. I will present our structural work on these eukaryotic DNA repair polymerases, with an emphasis on the basis of their specialization in lesion bypass.

Keywords: DNA-polymerase, DNA-repair, replication

MS44.27.2

Acta Cryst. (2005). A**61**, C59

Mechanism for *de novo* RNA Synthesis by T7 RNA Polymerase

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DNA-directed RNA polymerases (RNAP) are distinguished by their ability to initiate *de novo* synthesis of polymer, RNA, from a promoter without the requirement for a 3'OH primer-terminus. The RNAP from bacteriophage T7 requires no accessory factors for RNA synthesis during the initiation phase of transcription. T7 RNAP is unique, however, in its requirement for *de novo* synthesis to start with the incorporation of two GTPs at the beginning of transcription.

We examined the structural and kinetic basis for *de novo* synthesis of RNA by using a series of novel DNA constructs which varied the template DNA initiation sequence and the incoming NTP analogues. Nine structures of T7 RNAP with promoter DNA and/or an incoming pair of NTPs were determined to 2.2 to 3.2 Å resolution and are *the first structural examples of de novo RNA synthesis by an RNA polymerase*. Different promoter-template DNA constructs are bound to the enzyme in virtually identical conformations. The two initiating NTPs are accommodated in the enzyme by changes to both the geometry of the active site and a novel bend in template DNA. The incoming NTPs are recognized by novel enzyme and template DNA interactions. Active site residues make a large contribution to the recognition of the initiating NTPs in addition to the specific DNA template base-pair interactions. Pre-steady state kinetic measurements support the idea that discrimination of initiating nucleotide by the enzyme plays a greater role than template specification of nucleotide selection for *de novo* synthesis of RNA by T7 RNA polymerase.

Keywords: transcription initiation, polymerase, nucleotide selection

MS44.27.3

Acta Cryst. (2005). A**61**, C59

Structural Basis for RNA-regulated Gene Expression

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RNA molecules have been discovered at the heart of several central aspects of gene expression, from protein biosynthesis by the ribosome to the targeting of new proteins to the correct intracellular locale to RNA interference (RNAi). Understanding how these RNA-mediated processes work will illuminate central aspects of modern cell biology and also provide important clues to the possibly fundamental role of RNA in the evolution of life. I will describe our efforts to understand the structural basis for RNA function, highlighting recent discoveries about the recognition and cleavage of double-stranded RNA in the early steps of the RNAi pathway.

Keywords: RNA structure, RNA, gene expression

MS44.27.4

Acta Cryst. (2005). A**61**, C59-C60

Structural Basis for Specific Recognition of the UsnRNP m_3G-cap by Snurportin1

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The small nuclear ribonucleoprotein particles (snRNPs) are the major components of the splicing machinery that removes introns from pre-mRNA. In metazoans, the snRNP biogenesis is an ordered process requiring both nuclear and cytoplasmic phases. After transcription, the snRNAs U1, U2, U4, and U5 are exported into the cytoplasm, where the assembly with seven Sm proteins occurs and the snRNA 5′-cap nucleotide is modified from a 7-methyl-guanosine (m^7G-) to a 2,2,7-trimethyl-guanosine (m_3G-) cap. The hyper-methylated m_3G-cap represents one of the two nuclear localisation

signals of the snRNPs. As an import adaptor snurportin1 bridges the interaction between the m_3G-cap bearing snRNPs and the nuclear import receptor importin-β, which mediates the interaction with and translocation through the nuclear pore complex. Snurportin1 contains a N-terminal importin-β-binding (IBB) domain and a m_3G-cap-binding region, which shows no similarity to other known nuclear import factors. We have solved the crystal structure of the m_3G-cap binding domain of snurportin1 by means of MIRAS, and the structure was refined at 2.4 Å resolution. The crystal structure reveals an unexpected binding mode for the m_3G-cap, that significantly differs from other cap-binding proteins such as eIF4E and CBP20. The structural basis for the discrimination of m^7G-cap bearing RNAs by snurportin1 will be discussed.

Keywords: RNA-protein interactions, nuclear transport, MIRAS

MS44.27.5

Acta Cryst. (2005). A**61**, C60

Structural Basis for Antigene and Antisense Duplexes with Modified Nucleotides

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Oligonucleotides containing polyamines are currently being evaluated as potential antigene and antisense compounds. Those with 5-(*N*-aminohexyl)carbamoyl-2'-deoxyuridine (^{N}U) and its 2'-*O*-methyl derivative (^{N}U$_m$) exhibit improved nuclease resistance. Furthermore, these nucleotides stabilize duplex formation of the modified DNA and its target DNA or RNA strand. X-ray structures of these duplexes have shown good correlation between the conformational changes and the observed chemotherapeutic properties.

The amide groups of the modified uracil bases form six-membered rings through the intramolecular NH---O4 hydrogen bonds, so that the aminohexyl chains protrude into the major grooves. Some of the terminal ammonium groups are involved in intra-duplex interactions with phosphate oxygen anions, whereas the others interact with those of the adjacent duplex. Such interactions contribute to the stability of duplex formation. The 2'-*O*-methyl modification in ^{N}U$_m$ shifts the ribose ring toward the C3'-*endo* conformation and influences duplex stability. Observed changes in the dimensions of the minor grooves and in the hydration structures are well correlated to nuclease resistance.

Keywords: antigene, antisense, crystal structure

MS45 PACKING OF ORGANIC MOLECULAR COMPOUNDS

Chairpersons: Jonathan W. Steed, Carolyn Brock

MS45.27.1

Acta Cryst. (2005). A**61**, C60

Porosity in Molecular Crystals

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Crystals composed of purely organic compounds have largely been ignored as gas sorption substrates since such molecules usually pack with efficiencies in the narrow range of 60 to 67%. Consequently, void spaces larger than 25Å^3 are seldom encountered in organic solids. The host lattices of solvated inclusion compounds are often described as possessing zero-, one-, two- or three-dimensional solvent-accessible voids if the guest molecules are located in isolated cavities, channels, layers or networks of channels, respectively. It is therefore attractive to envision facile removal of the solvent molecules from these materials to yield highly porous host lattices analogous to those of zeolites. In reality, the process of desolvation is almost always accompanied by reassembly of the host molecules in the solid state to form one or more so-called apohost phases, where the pure compound is once again efficiently packed. However, a few exceptions to this phenomenon are known to exist.

We are interested in using the principles of crystal engineering to design and construct new solids for applications such as gas sorption. Although the availability of vacant lattice voids is essential, these solids are apparently not required to be "porous" in the classical sense when considering the van der Waals surfaces of the constituent host molecules. This contribution will focus very generally on the concept of porosity in molecular crystals, and on the phenomenon of guest transport within a solid host framework.

Keywords: porous materials, self assembly supramolecular chemistry, crystal engineering

MS45.27.2

Acta Cryst. (2005). A**61**, C60

Exploring Structures and Structural Phenomena: The Derived Crystal Packing Model

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Improvements in the prediction and the design of molecular crystals have been dramatically enhanced the last decades. However, several problems during crystallization such as polymorphism or two-dimensional defects can lead to difficulties in interpreting the success of a theoretical study.

In this context, we developed the Derived Crystal Packing (DCP) model [1]. This two-step procedure allows to generate crystal structures (daughter phases) starting from periodic fragments retrieved from a known mother phase. The study of many examples has shown that concomitant polymorphism, twinning and epitaxies can be a direct consequence of the structural and energetical similarities between the mother and the daughter phases.

Theses issues will be illustrated by the case of (±) Modafinil, a pharmaceutical compound known to crystallize in several polymorphic forms and solvates [2].

[1] Gervais C., Coquerel G., *Acta Cryst. B*, 2002, **58**, 662. [2] Pauchet M., Gervais C., Courvoisier L., Coquerel G., *Cryst. Growth. Des.*, 2004, **4**, 1143-1151.

Keywords: crystal structure prediction, twinning, polymorphism

MS45.27.3

Acta Cryst. (2005). A**61**, C60-C61

Crystal Structure Analysis and Solid Form Selection in the Pharmaceutical Industry

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The selection of the solid form is an important milestone in the development of new drug product. The aim of the process is to select the solid form with the most desirable properties including aqueous solubility, chemical and physical stability and suitable drug product processing attributes for formulation e.g. mechanical properties. The selected form may be either the free base or acid of the active pharmaceutical ingredient (API) or a salt.

It is vital to ensure the most thermodynamically stable polymorphic form has been selected. Different polymorphs have unique physical properties resulting in different solubilities, chemical and physical stabilities and different bioavailabilities. Metastable polymorphs may convert to more stable forms on processing and examples of this have been reported [1]. The characterization of all solid forms is important and can provide many intellectual property opportunities [2].

Crystal structure analysis, taking a molecular perspective of the crystalline state, can be combined with both manual analytical techniques (e.g. PXRD, thermal analysis, microscopy) and automated high throughput solid form screening techniques to ensure the optimum solid form is selected.

[1] Bauer J.F., Spanton S., Henry R., Quick J., Dziki W., Porter W., Morris J.,