

Keywords: water structure, metal coordination complexes, weak interactions

## MS.07.4

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### Diffused scattering and dynamic disorder observed nucleotide hydrates

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Nucleotides are the basic components of nucleic acids. Nucleotides crystallize as hydrates. Layered structures made of molecular layers of nucleotides and inorganic layers of counter ions and crystal water molecules are constructed. In some cases, diffused scatterings attribute to dynamic disorder of inorganic layers were observed, and phase transitions occurred around 200 K. Freezing of hydration water around biomolecules causes attention in connection to the glass transition of proteins. Disodium uridine 5'-monophosphate heptahydrate ( $\text{Na}_2\text{UMP}\cdot 7\text{H}_2\text{O}$ ) was one of the representative cases. Fundamental diffractions streak along the  $c^*$  axis and weak diffused diffractions due to a super-lattice were observed at 300 K (Fig. 1). Phase transition occurred around 220 K, and diffused diffractions changed to spots. There were 42 water molecules and 12 sodium ions in an asymmetric unit of the averaged structure at 300 K. In the low temperature phase, the numbers became four-fold, *i.e.*, 168 water molecules and 48 sodium ions in an asymmetric unit. The hydrogen bonding and sodium coordination networks in nucleotide hydrates were analyzed, and the origin of the dynamic disorder will be discussed.

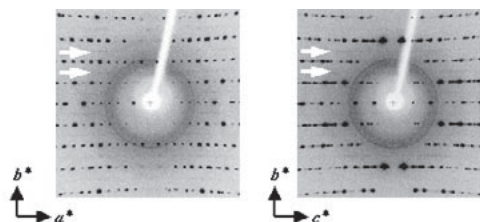


Figure 1 Oscillation photographs of  $\text{Na}_2\text{UMP}\cdot 7\text{H}_2\text{O}$  at 300K

Keywords: nucleotide, disorder of hydrogen-bonding network, hydrates

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### Incompatible host-guest strategy to enclathrate water clusters into polyoxometalate crystals

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Many compounds crystallize as hydrated forms when precipitated from aqueous or water-containing solutions. Water plays an important role in constructing such crystals. One extreme example of them is the clathrates of hydrophobic gaseous molecules. Another extreme example may be water molecules enclathrated in hydrophobic environments. Recently, we have discovered

discrete water clusters enclathrated in fairly hydrophobic voids constructed by the tetraphenylphosphonium cations, which may be regarded as examples of water clusters in hydrophobic environments. The tetraphenylphosphonium cations self-assemble by the C-H $\cdots\pi$  interactions to form three-dimensional host framework with periodical voids of about 1 nm in diameter, to which guest polyoxometalate anions (e.g.  $\text{V}_{10}\text{O}_{28}^{6-}$  and  $\text{PV}_{14}\text{O}_{42}^{11-}$ ) are incorporated. However, the sizes and charges of the voids and the anions are incompatible and thus some of the voids remain unoccupied. These voids lead to the formation of discrete water clusters. Temperature dependent single crystal X-ray diffraction illustrated the melting behavior of the water cluster. In one of these examples, the distribution of the clusters become ordered or disordered depending on the crystallization conditions, resulting in the disappearance/appearance of the diffuse scattering recorded on its single crystal diffraction images.

Keywords: water structure, hydrogen bonds, polyoxometalates

## MS.08.1

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### Stop-action movie of UvrD helicase unwinding DNA

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Molecular mechano-chemical coupling is a fundamental process in biology. To capture molecular locomotion by X-ray crystallography, we determined a dozen crystal structures of UvrD-DNA complexes in the absence or presence of ATP hydrolysis analogs. After combining multiple structures representing each functional state and accounting for crystal lattice effects, we obtained a stop-action movie of UvrD helicase unwinding DNA one base pair per ATP hydrolyzed. For the first time, we show that each ATP-hydrolysis cycle delivers a power stroke in two parts. Binding of ATP is coupled with unwinding of one base pair, and release of ADP and Pi is coupled with translocation of the newly unpaired single base. Combining our new mutagenesis, structural and kinetic studies with published data, we have put forward the model of dual active states of UvrD for its dual functions (dsDNA unwinding and RecA removal from ssDNA) in DNA replication and repair.

Keywords: mechano-chemical coupling, motor protein, helicase

## MS.08.2

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### Stop codon recoding mechanism revealed by the suppressor tRNA<sup>Pyl</sup>/PylS complex structure

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The genetic code strictly assigns 64 codon triplets to the 20 canonical amino acids, except for three stop codons, UAG (amber), UGA (opal)

and UAA (ochre). These stop codons are occasionally recoded to an amino acid by specialized transfer RNAs (tRNAs) called suppressor tRNAs. In all organisms, the UGA codon is recoded to selenocysteine (Sec; the 21st amino acid), depending on the downstream enhancer sequence SECIS, through multi-step Sec synthesis on tRNA<sup>Sec</sup> (opal suppressor). In methanogenic archaea and some anaerobic eubacteria, the UAG codon is recoded to pyrrolysine, the 22nd amino acid, through direct acylation of amber suppressor tRNA<sup>Pyl</sup> by pyrrolysyl-tRNA synthetase (PylS). The tRNA<sup>Pyl</sup> suppressor has an unusual structure, lacking most of the consensus features of canonical tRNAs, analogous to mammalian mitochondrial tRNAs. It is thus unclear how such unusual tRNAs function in the ribosome and how PylS discriminates between suppressor and canonical tRNAs. Here we present the crystal structure of the PylS/tRNA<sup>Pyl</sup> complex from *Desulfotobacterium hafniense* at 3.1 Å resolution. The suppressor tRNA<sup>Pyl</sup> has an unusual minimal core structure formed by a non-standard D-loop, a TYC-loop and a variable loop, which are reorganized to mimic the canonical L-shape to function normally in the ribosome. PylS recognizes the unusual compact core structure by steric compatibility, which enables discrimination of the suppressor from the other canonical tRNAs.

Keywords: tRNA, aminoacyl-tRNA synthetase, stop codon

### MS.08.3

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#### DNA transfer machines

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Whatever the route used, horizontal gene transfer, a rapid way for bacterial evolution, requires sophisticated protein machinery to enable DNA to cross the cell envelope barriers. Increased antibiotic resistance among pathogens is a troubling consequence of this microbial capacity. Mechanisms leading to horizontal gene transfer in bacteria are categorized into transduction, transformation and conjugation. Transduction occurs via bacteriophages, which may incorporate portions of the host bacterial DNA and introduce them into newly infected hosts. Natural transformation consists of the uptake of naked DNA from the environment. Finally, conjugation is the unidirectional transfer of ssDNA of conjugative plasmids from a donor to a recipient cell. The conjugation system can be divided into two discrete specialized modules: the relaxosome, which triggers plasmid DNA processing and replication, and a type IV secretion system, which impels protein and single-stranded DNA through the membranes. In addition, a coupling protein, which links these two modules, and a number of ancillary proteins are required. Over recent decades, research efforts in the field have clarified many aspects of the system. In particular, structural biology is providing details of the molecular architecture of several of the components involved. Russi, S., Boer, R. & Coll, M. (2008). Molecular Machinery for DNA Translocation in Bacterial Conjugation. In *Plasmids: Current Research and Future Trends*. Horizon Scientific Press, Londres.

Keywords: protein-DNA complexes, DNA translocation, bacterial conjugation

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#### RNA-protein interactions in the U4 snRNP core domain

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The spliceosome responsible for processing pre-mRNA to mRNA is assembled from RNA-protein particles called snRNPs. The U1, U2, U4 and U5 snRNPs contain in their core a common set of seven Sm proteins (B/B', D1, D2, D3, E, F and G), which assemble on the particle-specific snRNA (small nuclear RNA) by collectively recognizing a conserved U-rich heptad sequence called the Sm site. Addition of particle-specific proteins to the core assembly completes the mature snRNP. The Sm proteins share a conserved sequence motif in two segments, called Sm1 and Sm2, joined by a linker of variable length. We have reconstituted and crystallized the human U4 snRNP core domain comprising the U4 snRNA Sm site with flanking stem-loops and the seven Sm proteins. The crystals belong to space group  $P3_1$  ( $a = 248.0$  Å,  $c = 251.9$  Å) with 12 copies of the core domain in the asymmetric unit. They diffract X-rays to 3.6 Å resolution. The structure was solved by a series of MAD experiments using SeMet-substitution of different groups of Sm proteins, followed by molecular replacement to the native crystal. Our results show that the seven Sm proteins form a closed ring through H-bonding across the Sm1, Sm2 motifs all the way round, and the snRNA is threaded through the pore. The ring has a flat side where the N-terminal helices lie, and a conical side where the linkers protrude. At the rim where the 5' RNA enters the pore from the flat side, the seven Sm proteins bind the Sm site heptad (AUUUUUG) in a one-to-one but asymmetric manner. In addition, the proteins make non-sequence-specific interactions with the 5' and 3' stems. Our findings are applicable to other snRNP cores, because of the conservation of the Sm site and constancy of the Sm proteins.

Keywords: RNA splicing, crystal engineering, noncrystallographic symmetry

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#### Structural basis of lipid biosynthesis regulation in Gram-positive bacteria

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FapR is a global transcriptional repressor that controls the expression of several genes involved in the biosynthesis of fatty acids and phospholipids in many Gram-positive bacteria. The FapR monomer has a helix-turn-helix motif linked to an effector-binding domain that displays a 'hot-dog' fold, similar to that of several thioesterases known to process acyl-CoA substrates but different from other known bacterial transcriptional regulators (Schujman et al, 2006). Binding of the specific inducer, malonyl-CoA, promotes conformational changes in the protein that causes the FapR-DNA complex to dissociate or prevents its formation. Furthermore, site-directed mutations which disrupt the FapR-malonyl-CoA interaction result in a lethal phenotype in *Bacillus subtilis*, suggesting that this homeostatic signaling pathway could be a target for novel chemotherapeutic