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Thursday, March 19, Session II

L6

MODULATED PROTEIN CRYSTAL STRUCTURE WITH 28 MOLECULES IN THE ASYMMETRIC UNIT

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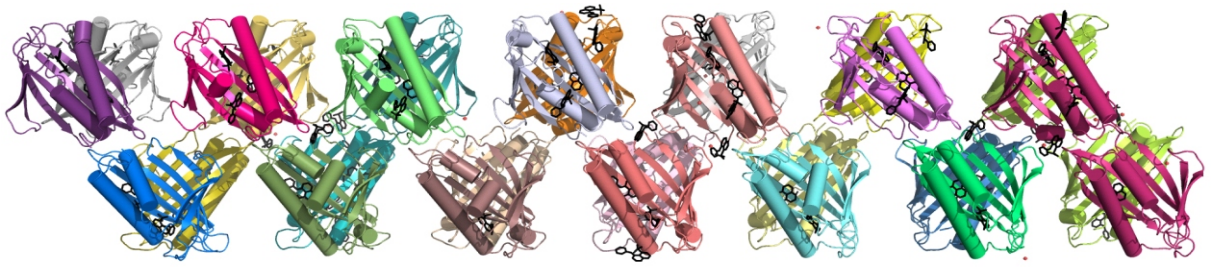
In modulated crystals short-range translational order is lost and the atomic structure cannot be defined by the contents of a single small unit cell. The wave of disorder is described by a modulation function, which restores long-range periodicity. If the modulation period divided by the unit cell translation is a rational number, then the modulation is commensurate, and can be described in an expanded unit cell. Otherwise it is incommensurate. The diffraction pattern of a modulated structure contains strong main reflections from the basic unit cell, surrounded by weaker satellites from the modulation wave. Modulated structures are rare in protein crystallography.

Stress factors induce in plants the expression of Pathogenesis-Related (PR) proteins, divided into 17 classes. PR proteins of class 10 (PR-10) are well studied structurally but their biological function is unclear with an implication in phytohormone binding. PR-10/hormone complexes are studied using fluorescent probes such as ANS (8-anilino-1-naphthalene sulfonate). We crystallized Hyp-1, a PR-10 protein from St John's wort, in complex with ANS. Solution of the apparent $P4_122$ crystal structure was impossible by standard molecular replacement because of evident tetartohedral twinning and a bizarre modulation of reflection intensities with l periodicity of 7. The structure was solved using Phaser and data expanded to $P1$ symmetry. Ultimately, the structure turned out to have $C2$ symmetry with 28 independent protein molecules, arranged in dimers around a non-crystallographic (NCS) 2_1 screw along c with a pitch of ~1/7. The seven-fold repetition along c is indicative of a commensurate modulated structure: the NCS copies are similar but not identical. For instance, the consecutive Hyp-1 molecules bind a varying number (0-3) of the ligand molecules. The structure has been successfully refined to R=22.2% using conventional methods, i.e. with unit cell expanded to encompass the entire commensurate modulation period.



L7

CC* - LINKING CRYSTALLOGRAPHIC MODEL AND DATA QUALITY

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Historically, R_{merge} (also called R_{sym}) has been considered as an indicator of X-ray data quality, and e.g. decisions about exposure time, total rotation range, and suitability of datasets for refinement were based on it. Recently it has been pointed out [1] that the high-resolution value of R_{merge} is not related to R_{work}/R_{free} of a crystallographic model, and is therefore unsuitable to define a resolution cutoff. A more meaningful indicator ($CC_{1/2}$) has been suggested and a derived quantity (CC*) was shown to be limiting for CC_{work}/CC_{free} of a crystallographic model. As a consequence, crystallographers are now in a position to better understand the properties of their data, which may be used to obtain more accurate crystallographic models.

Karplus P. A. and Diederichs K., *Science*, 2012, **336**, 1030-1033.

L8

STRUCTURAL STUDIES OF HUMAN PARECHOVIRUS-1

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Human parechoviruses (HpEVs) are small icosahedral viruses, which cause a spectrum of debilitating illnesses in infants including cardiomyopathy and encephalitis. A basic building block of their virion is composed of three proteins referred to as VP-1, VP-3, and VP-0, which assemble into pentameric assemblies, where by 12 pentamers make up the virion. For decades, HpEVs were considered to be closely related to human enteroviruses due to similarities in the disease etiology they cause. We solved a crystal structure of HpEV-1, to 3.1 Å resolution, which demonstrates that HpEVs are different from enteroviruses. HpEV-1 lacks a capsid surface structural feature known as the canyon, which is a site of receptor binding in many enteroviruses. Moreover, a cavity of VP1, which is normally occupied by a pocket factor in enteroviruses is completely filled with bulky hydrophobic residues in HpEVs. Furthermore, the N-terminus of V-P0 of HpEV-1 appears to have undergone 3D dimensional domain swapping, which results in inter-pentamer connectivity not seen in enteroviruses. HpEVs virion is smaller than any enteroviruses characterized to date. Internal surface of HpEV-1 is negatively charged around the five-fold axis of the pentamer and this is where a stretch of icosahedrally-ordered RNA was found to reside. RNA bases were found to stack against a highly conserved tryptophan-21 residue of VP-3 protein. Mapping sequence differences of various parechovirus serotypes onto the structure of HpEV-1, demonstrated that serotype-specific differences tend to reside on the surface of the virion. Fitting the crystal structure of the virion into the previously determined tomographic reconstruction of HpEV-1-receptor complex revealed that receptor binding occurs close to the 5-fold symmetry axis. Unexpectedly, the structure also demonstrated that the major antigenic-site of HpEV-1 is found on the inside of the virion. Structure-based phylogenetic analysis established that HpEVs are closer related to human hepatitis A virus than to any known enterovirus. In summary, our work provides structural framework for further understanding the life cycle of human parechoviruses and for the development of novel anti-viral therapeutics against this class of human pathogens.

L9

STRUCTURE AND GENOME RELEASE MECHANISM OF HUMAN CARDIOVIRUS SAFFOLD VIRUS-3 (SAFV-3)

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Saffold virus (SAFV) is the human *Cardiovirus* closely related to the Theiler murine encephalomyelitis virus (TMEV), of the family *picornaviridae* (1). It was reported that, SAFV might cause respiratory, gastrointestinal, and central nervous system infections (1,2). To date 11 genotypes of SAFV have been identified (1, 3). In the present study, the three-dimensional structure of SAFV-3 has been determined at 2.5 Å resolution. Although the architecture of the major capsid proteins VP1, VP2 and VP3 of SAFV-3 is similar to other cardioviruses, there are some differences on the surface loops. The presence of disulphide bond on the surface of VP3, surprisingly diminish the stability and infectivity of SAFV-3. Several capsid-binding and replication inhibitors of other picornaviruses fail to have any effect on SAFV-3. It was also shown that SAFV-3 dissociates in to pentameric subunits upon the genome release.

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Thursday, March 19, Session III

L10

STRUCTURE OF LLT1, A LIGAND FOR HUMAN NKR-P1, AND ITS VARIABILITY UNDER VARIOUS CONDITIONS

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Natural killer cells (NK cells) are large granular lymphocytes – a type of white blood cells. They are able to kill virally infected, stressed or tumor cells. Unlike T-cells, the activity of NK cells is innate, they do not need to have previous experience with a tumor – they are natural killers.

NKR-P1 (CD161) is a receptor on a surface of human NK cells. LLT1 is a ligand for NKR-P1 receptor, expressed primarily on activated lymphocytes and antigen presenting cells. The interaction of the ligand with the receptor inhibits NK cell cytotoxicity; however, it may have also activation effects in some cases. Extracellular domains of both binding partners, NKR-P1 and LLT1, have C-type lectin like (CTL) fold.

Using X-ray diffraction, we determined four structures of LLT1 [1] from protein produced in HEK293S GnTI- cells. The protein with $GlcNAc_2Man_5$ glycosylation packs into hexamers (consisting of three dimers) in crystals. The protein deglycosylated after the first N-acetylglucosamine was found in our crystal structures in forms of dimers (in pH 7.0) and monomers (in pH 3.5).

The LLT1 structures (Figure 1) show that LLT1 follows the "classical" mode of dimerization known from other structures with the same fold (CD69 [2], Clr-g [3]). The series of the LLT1 structures bring insight into variability of the dimerization interface, flexibility of the outer long loop of the CTL domain and influence of glycosylation on the structure.

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