

The vaccinia virus A46 protein comprises 240 a.a. and according to structure predictions has a single globular domain with a short hypothetical unstructured C-terminus. Both A46 alone and a fusion with maltose binding protein (MBP) expression vectors were constructed. Only the fused protein construct was expressed at the sufficient level for further studies. Using TEV proteolysis, we could cleave A46 protein from the MBP-A46 construct. Both MBP-A46 and cleaved A46 protein were purified to homogeneity. The MBP-A46 construct behaved as a dimer where as A46 alone behaved as a tetramer on size exclusion chromatography. A limited proteolysis experiment confirmed the presence of one domain in A46. We set up a number of crystallization screens with A46 protein but failed to obtain any reproducible crystals.

The cellular target of A46 protein is the adaptor protein MyD88, which participates in numerous signal transduction cascades during both innate and acquired immune responses. The MyD88 protein is composed of two domains, including the C-terminal TIR (Toll/IL1-like) domain. According to bioinformatic predictions, the viral A46 protein should contain a TIR fold, similar to MyD88. Thus, the TIR domain in MyD88 is believed to be the site of the interaction between A46 and its target. To test this hypothesis, the C-terminal part of the murine MyD88 protein, comprising the TIR domain (146-296 a.a.), was expressed with a GST-tag and purified. We will now study the A46 and MyD88 interactions via pull-down assays and isothermal titration calorimetry, as well as crystallization of the purified complex.

Keywords: vaccinia, A46, MyD88

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Reorganization of the shell protein during the maturation of bacteriophage T7

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Maturation of dsDNA bacteriophages involves the assembly of the virus prohead from a limited set of structural components followed by rearrangements required to acquire the stability that is necessary for infecting a host under challenging environmental conditions.

Bacteriophage T7 infects *Escherichia coli* and belongs to the Podoviridae family. It has a 40kb dsDNA, an icosahedral capsid with a triangulation number T=7, and a non-contractile tail [1]. The virus shell is made of 415 copies of protein gp10A. The prohead also contains a scaffolding protein (gp9) and a dodecameric connector (gp8) that attaches a core complex to one of the 12 5-fold vertices. The structure of the T7 prohead and mature head have been solved by three-dimensional cryo-electron microscopy [2], and quasi-atomic models for the procapsid and the mature capsid shells have also been obtained [3, 4], revealing that the main protein gp10A has an HK97-like fold.

The comparison of both structures reveals the molecular basis of T7 shell maturation [4]. The mature capsid presents an expanded and thinner shell than the prohead, with a drastic rearrangement of the protein monomers that increases their interacting surfaces, in turn resulting in a new bonding lattice. The rearrangements include tilting, in-plane rotation, and radial expansion of the subunits, as well as a relative bending of the A- and P-domains of each subunit.

Our results suggest that T7 might represent one of the simplest

primordial capsid maturation mechanisms.

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Single crystal X-ray diffuse scattering from supra-molecular assemblies

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Unlike Bragg scattering which only contains single body information, diffuse scattering contains two-body information and this enables the local structure and dynamics to be probed. In some instances static (occupational) disorder may feature in these studies but in others the disorder is of thermal origin and the resulting structured *thermal diffuse scattering* (TDS) is used to obtain details of the correlations that occur between both the inter- and intra-molecular displacements. Due to the recent advancements in computing power, methods that involve the construction of models of disordered crystals from which the diffuse scattering is calculated have become more and more effective.

Original methods for the evaluation of harmonic potentials used in Monte Carlo simulations for modeling the diffuse scattering from molecular crystals has been superseded by implementing an empirical formula that effectively simplifies this procedure [1]. The Method works so well that now not only is implementing disorder models (<50 atoms per molecule) more rapid (as applied to previous studies of problems associated with polymorphism in pharmaceuticals), we can also begin to accurately model the diffuse scattering features from larger supra-molecular systems. We have applied these novel techniques to problems involving supra-molecular host guest assemblies by performing simulations to model the diffuse scattering from single crystals of 1,11-undecadioic acid/urea and t-butylcalix-4-arene.

The urea inclusion compound (UIC) for 1,11-Undecadioic acid has a well resolved structure in which the guest is disordered over three positions with respect to the lattice repeat of the urea channels. A result of this disorder is strong layers of diffuse scattering perpendicular to the channel axis at one-third increments between the average 'Bragg' Layer lines. Our methods use Ising-type short-range order models for the site occupations of the guests to show that the direction for the correlation of the guest molecules between channels is actually trigonal, even though the average structure is orthorhombic. A good fit to the diffuse features is established from applying the correct correlation energies in the model.

t-Butylcalix-4-arene if crystallized with different mixtures of Pyridine-N-oxide (PNO) or Nitrobenzene (PhNO₂) can form what we know to be two isomorphous crystalline phases 'beta' or 'delta'. This is reflected by the apparent differences in cell dimensions and major guest occupation in the 'calix' cavity. Each of the crystals can have varying amounts of PNO or PhNO₂ depending on the growth

conditions but there exists a narrow phase boundary where for the beta form the major component is always PhNO₂ and in the delta form the major component is always PNO. Even though the packing for the structures is very similar the observed diffuse scattering for these two phases is very different and different phase transitions are observed upon cooling these crystals. Our methods are used to attempt to model the diffuse scattering to explain the ordering of the guests and their dynamical behavior in the host lattice.

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Keywords: diffuse scattering, monte-carlo simulations, supra-molecular chemistry.

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Diffuse scattering study of aspirin forms (I) and (II)

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Polymorphism of aspirin crystals has recently become an important issue, following reports of a second polymorph [1], because of the widespread popular use of aspirin as an analgesic. The reported structure appeared to correspond to one of the low-energy structures predicted in energy calculations [2]. Bond et al. [3] at first cast some doubt on the findings and stated ‘form (II) of aspirin as reported . . . may just as easily be derived, to the accuracy and precision reported, . . . from experimental diffraction data collected from . . . a single crystal of the well known form (I)’. Subsequently they investigated a number of different crystals that were supposedly form (II) [4]. In this paper the authors concluded that form (II) crystals consisted of “an intergrowth of two ‘polymorphic’ domains” and that ‘each aspirin crystal is an integral whole in which the domains are intimately connected with each other, with possibly many turnovers of domain within a single crystal’. In order to try to make sense of these findings we have undertaken a diffuse scattering study.

Full three-dimensional diffuse scattering data have been recorded for both polymorphic forms [(I) and (II)] of aspirin and these data have been analysed using Monte Carlo computer modeling [5]. The observed scattering in form (I) is well reproduced by a simple harmonic model of thermally induced displacements. The data for form (II) show, in addition to thermal diffuse scattering (TDS) similar to that in form (I), diffuse streaks originating from stacking fault-like defects as well as other effects that can be attributed to strain induced by these defects. The present study has provided strong evidence that the aspirin form (II) structure is a true polymorph with a structure quite distinct from that of form (I). The diffuse scattering evidence presented shows that crystals of form (II) are essentially composed of large single domains of the form (II) lattice with a relatively small volume fraction of intrinsic planar defects or faults comprising misoriented bilayers of molecular dimers. There is evidence of some local aggregation of these defect bilayers to form small included regions of the form (I) structure. Evidence is also presented that shows that the strain effects arise from the mismatch of molecular packing between the defect region and the surrounding form (II) lattice. This occurs at the edges of the planar defects in the *b* direction only.

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α -“NaLuF₄”: 6 fold twinning with modulation and diffuse scattering

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Hexagonal β -NaLuF₄ (Ln=Y,La-Lu) compounds are a family of up-conversion materials which emit visible light upon IR excitation, e.g. show green and blue emission for Er³⁺, Yb³⁺ and Tm³⁺, Yb³⁺ doping, respectively. Understanding of the properties of these technologically important materials requires a knowledge of their structures at an atomic level. [1] We are presently studying two phases of so-called “cubic α -NaLuF₄” obtained from the melt with a likely composition of Na₅Lu₉F₃₂.

Phase 1 show strong Bragg scattering, commensurate satellite reflections, significant diffuse scattering, while phase 2 shows just strong Bragg reflections with diffuse scattering. The strong, apparently cubic, reflections in reciprocal lattice rows are not collinear and they are split at high angles. This suggests that the crystal is a multiple twin of a structure of lower symmetry with near overlap of reflections. If the satellites are treated as Bragg peaks, an orthorhombic supercell and six fold twinning follow with likely a space group of *Cmmm*.

The apparently cubic main reflections vary in size and position on going from low angle to high angle. To account for this variation an increased mask size was used to integrate the main reflections. To avoid problems arising from the different peak profiles of main and satellite reflections the latter were integrated with an absence condition that eliminates the mains.

Considering only the positions of heavy atoms in the asymmetric unit of the small cell (based on the apparently cubic reflections), the average structure may be described equally well in two different ways for both the phases. The presence of residual electron density in the difference Fourier map of both descriptions was interpreted in terms of disordered fluorine atoms. Their positions are chemically more meaningful for one of the two heavy-atom models.

For the phase 1 structure, the phases of the superstructure reflections were determined by band flipping [2] implemented in the program Superflip. [3] The reconstructed difference electron density map shows two distinct commensurately modulated parallel columns of cations: one with varying Na⁺/Lu³⁺ occupancy and one with positional displacements of the ions from the average structure positions. Interestingly, two different solutions result from the band flipping with equal probability. These two solutions differ only by the details of positional and occupational modulation. The two solutions are distinct and the correct one can be identified by subsequent structure refinements.

