

**CHARACTERIZATION OF THE PROTEIN CRYSTAL GROWTH APPARATUS FOR MICROGRAVITY ABOARD THE SPACE STATION**

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Protein Crystal Growth Apparatus for Microgravity (PCAM) trays have been used in Shuttle missions to crystallize proteins in a microgravity environment. The crystallization experiments are 'sitting drops' similar to that in Cryschem<sup>TM</sup> trays, but the reservoir solution is soaked in a wick. From early 2001, crystallization experiments are conducted on the International Space Station using mission durations of months rather than two weeks on previous shuttle missions. Experiments were set up in April 2001 on Flight 6A to characterize the time crystallization experiments will take to reach equilibrium in a microgravity environment using salts, polyethylene glycols and an organic solvent as precipitants. The experiments were set up to gather data for a series of days of activation with different droplet volumes and precipitants. The experimental set up on ISS and results of this study will be presented. These results will help future users of PCAM to choose precipitants to optimize crystallization conditions for their target macromolecules for a particular mission with known mission duration.

Changes in crystal morphology and size between the ground and space grown crystals of a protein and a protein DNA complex flown on the same mission will also be presented.

**Keywords: MICROGRAVITY EQUILIBRIUM KINETICS INTERNATIONAL SPACE STATION**

**EVIDENCE OF STRUCTURE ADJUSTING FROM DISORDER TO SMECTIC ORDERING IN INTERFACE BOUNDARY LAYERS**

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During crystal growth of NH<sub>4</sub>, K and (H,D)<sub>2</sub>PO<sub>4</sub> (ADP, KDP and DKDP) from supersaturated solution, the molecular structure of crystals, interface boundary layers and the bulk solutions have been investigated in-situ by using laser Raman microprobe and ab initio molecular orbital calculation. Within the stable boundary layers, all O-P-O angle vibration bands of phosphate groups are found to appear significant changes. The significant Raman bands of 1200 cm<sup>-1</sup> P-O-H in plane deformations and 1120, 1150 cm<sup>-1</sup> PO<sub>2</sub> asymmetric stretch bands of H<sub>2</sub>PO<sub>4</sub><sup>-</sup>, which are similar to those of the crystals appear respectively. The bands of P-O-H out plane deformations and the PO<sub>2</sub> symmetric stretch bands of [H<sub>2</sub>PO<sub>4</sub>]<sub>n</sub><sup>n-</sup> (n=1,2,3) appear also respectively. These changes in ADP and KDP show evidently difference. This difference should be attributed to the species of P-O bonded cations. The experimental Raman spectra are satisfactorily reproduced by the present calculation frequencies. The evidences are presented for the cation effects and the readjustment of geometry of anionic phosphate groups in the stable interface boundary layers. The most significant effects of cations are to cause the changes of O-P-O bond angle, the electronic density re-distribution of the phosphate groups and the adjusting of P-O-H bond orientation, thus lead to the readjustment of the geometry of phosphate groups and desolvation. The trend of the readjustment is close to the geometry of crystal structure unit and the forming of the smectic ordering structure of the anions-cations at the proximate interface.

**ZINC ACTIVATION OF THE NUCLEOPHILE IN TETRAMERIC CYTIDINE DEAMINASE**

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Cytidine deaminases (CDAs) catalyze the formation of uridine and deoxyuridine from cytidine and deoxycytidine, respectively. There are two different classes of this enzyme: a homodimeric form (D-CDA) with two zinc ions per dimer and a homotetrameric form (T-CDA) with four zinc ions per tetramer.

We have determined the first structure of T-CDA from *Bacillus subtilis* to 2.0 Å resolution [1]. We also study T-CDA from the extremophiles *Bacillus caldolyticus* (Topt 72°C) and *Bacillus psychrophilus* (Topt 10°C). The crystal structure of *B. caldolyticus* T-CDA has been determined to 1.46 Å resolution and the *B. psychrophilus* T-CDA has recently been crystallized.

An intriguing difference between T-CDA and D-CDA is the zinc coordinating residues: three cysteine residues in the T-CDA and two cysteine residues and one histidine residue in D-CDA. The role of the zinc ion is to activate a water molecule and thereby generate a hydroxide ion. How the zinc ion in T-CDA surrounded by three negatively charged residues can create a similar catalytic activity of T-CDA compared to D-CDA has been an enigma. The structure of T-CDA reveals that the negative charge caused by the three ligands is partly neutralized by an arginine residue (R56) hydrogen-bonded to two of the cysteine residues and the dipoles of two  $\alpha$ -helices.

R56 has been mutated to a glutamine residue and an alanine residue. Enzyme kinetics and the crystal structures of these mutants confirm the importance of R56 for efficient catalysis by T-CDA.

Reference

[1] Johansson, E., Mejlhede, N., Neuhard, J. & Larsen, S. (2002) *Biochemistry* 41, 2563-2570.

**Keywords: CYTIDINE DEAMINASE, ZINC ENZYME, PYRIMIDINE SALVAGE PATHWAYS**

**THERMAL VIBRATIONS IN COMPLEX ANTIFLUORITES FROM LATTICE DYNAMICS AND "LOCAL NORMAL MODE" MODEL.**

H.-B. Bürgi, N. Rangavittal and D. Chernyshov

A lattice dynamical model based on a combined Rigid-Ion and Valence Force Field has been developed for the complex antifluorites K<sub>2</sub>PtCl<sub>6</sub>, K<sub>2</sub>ReCl<sub>6</sub> and K<sub>2</sub>OsCl<sub>6</sub>. It reproduces a range of experimental quantities: phonon dispersions, Raman and IR frequencies, elastic constants, heat capacity and the temperature dependences of Anisotropic Displacement Parameters (ADPs).

ADPs of K<sub>2</sub>PtCl<sub>6</sub> measured and calculated in the temperature range 8-380K have been analyzed with a "local normal mode" model including one independent, triply-degenerate translational mode for K and one for PtCl<sub>6</sub>, a librational mode for PtCl<sub>6</sub> and temperature independent contributions for high frequency PtCl<sub>6</sub>-deformations [1]. It is found that the resulting line spectrum is a reasonable approximation to partial phonon densities-of-states for K and PtCl<sub>6</sub>, respectively. The line spectrum reproduces the temperature dependence of ADPs with an accuracy exceeding the experimental standard uncertainties by about an order of magnitude. The specific heat CV(T) calculated from this approximation agrees with lattice dynamical simulations in general features but deviates in some details. Local normal mode analysis may be improved by introducing a correction for the dispersion of acoustic modes and explicit mixing of local normal modes belonging to different sub-lattices, e.g. K and PtCl<sub>6</sub>.

[1] H.B. Bürgi and S. Capelli, *Acta Cryst.*, (2000), A56, 403

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