

Title: Ice formation in protein crystals: effects of nanoconfinement

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Abstract: Ice formation within protein crystals is a significant obstacle to cryocrystallographic study of protein structure and has long prevented studies of how a protein's structural ensemble evolves with temperature in the most biophysically interesting range from 260 K to the protein-solvent glass transition near 200 K, where non-harmonic motions cease. Using protein crystals with solvent cavities as large as 68 Å, larger than 98% of deposited structures in the Protein Data Bank, we study ice formation in response to quenches to temperatures between 260 K and 180 K as a function of glycerol concentration using time-resolved X-ray diffraction. Maximum temperatures at which ice formation is observed are consistent with expected melting point suppression due to nanoconfinement. Internal crystalline solvent forms neither pure cubic or hexagonal ice, but a complex mixture where atomic planes of cubic and hexagonal ice stack in a disordered manner. The fraction of cubic planes in this ice increases with the addition of glycerol. Comparison of diffraction intensities from internal ice and the protein lattice allows the maximum ice volume fraction to be determined, and crystallography allows the solvent volume fraction within the unit cell to be determined. Combining these results, we determine the maximum crystallizable solvent fraction within three different protein crystal systems, and these fractions indicate that roughly a monolayer of water adjacent to the protein surface cannot be crystallized. Of greatest practical importance in crystallography, we show that with temperature steps and fast data collection before ice nucleation, ice free crystallographic data sets can be obtained with high probability from crystals with supercooled cryoprotectant-free liquid solvent at temperatures as low as 200 K. Moreover, this supercooled solvent facilitates post-cooling crystal relaxations from kinetically-favored states toward a new low-temperature equilibrium, and this can lead to substantial increases in crystal order.