Ice Formation and Solvent Nanoconfinement in Protein Crystals

Robert E. Thorne[a], David Moreau[a], and Hakan Atakisi[a]

[a]Physics Department, Cornell University, Ithaca, NY, 14853, USA, ret6@cornell.edu

Ice formation on and within protein crystals is a major obstacle to cryocrystallographic study of protein structure. Roughly 20% of PDB entries exhibit evidence of ice contamination [1,2]. Ice formation is particularly problematic when studying crystals with large solvent contents and large solvent cavities, and has also limited studies of how a protein's structural ensemble evolves with temperature in the biophysically interesting range from ~260 K to the protein-solvent glass transition near 200 K.

Using protein crystals having solvent cavities as large as ~70 Å, we use time-resolved X-ray diffraction to study the response of the protein and internal solvent during rapid cooling [3]. Solvent nanoconfinement suppresses freezing temperatures and ice nucleation rates so that ice-free, low-mosaicity diffraction data can be reliably collected down to 200 K without the use of cryoprotectants. Solvent flows during cooling associated with differential contraction of the solvent cavity volume and solvent volume are estimated. A dramatic crystal reordering after cooling, associated with unit cell expansion at *constant* cold temperatures temperatures of 220 and 240 K, provides direct evidence for these solvent flows and their role in creating mosaic disorder [4].

Hexagonal ice (I_h) forms in external solvent, but internal crystal solvent forms stacking disordered ice (I_{sd}) with a near random stacking of cubic and hexagonal planes. Analysis of powder diffraction from internal ice and single crystal diffraction from the protein lattice shows that the maximum crystallisable solvent fraction decreases with decreasing crystal solvent cavity size, and that a ~6 Å thick layer of solvent adjacent to the protein surface cannot crystallize [3].

These results establish protein crystals as excellent model systems for study of nanoconfined solvent. By combining fast cooling, intense X-ray beams, and fast X-ray detectors, complete structural data sets for high-value targets including membrane proteins and large complexes may be collected at ~200-260 K that have lower mosaicities, comparable B factors, and that may allow more confident identification of ligand binding than in current cryocrystallographic practice.

References

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