# Methods to understand the role of ordered waters and disordered residues in enzyme catalysis using macromolecular crystallography at physiological temperatures

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X-ray crystallography at cryogenic temperatures (100 K, Cryo-MX), has played an important role in the structural characterization of proteins, such as enzymes, channels and receptors. Roughly 90 % of all crystal structures deposited in the Protein Data Bank (PDB) have been recorded using deep-frozen protein crystals. Although highly successful with regard to solving structures free of radiation damage, it has gradually become clear that the protein conformations observed by Cryo-MX may not necessarily be physiologically relevant. Rapid cooling of the protein crystals can populate non-physiological conformations of proteins because enthalpically stabilized states dominate over high entropy states.[1] The rapid cooling process does not allow full equzillibration, with the result that the observed structures may represent artificial conformations - neither biologically relevant nor thermodynamically favored. [2] Because polar interactions are temperature sensitive, the precise arrangements of protein residues, ligands and solvent molecules in the active sites of enzymes, channels and receptors are prone to significant change upon cryo-cooling.

These changes can lead to false conclusions. This project aims to implement room temperature (RT) crystallography methods to determine enzyme structures that are physiologically more relevant that those emerging from standard measurements at cryogenic temperatures.

[1] D. A. Keedy et al., “An expanded allosteric network in PTP1B by multitemperature crystallography,

fragment screening, and covalent tethering,” Elife, vol. 7, 2018.

[2] M. Fischer, B. K. Shoichet, and J. S. Fraser, “One Crystal, Two Temperatures: Cryocooling Penalties

Alter Ligand Binding to Transient Protein Sites,” ChemBioChem, vol. 16, no. 11, pp. 1560–1564, Jul. 2015