Probing Ligand-Binding Of Endothiapepsin By "Temperature-Resolved" Macromolecular Crystallography

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Continuous developments of cryogenic X-ray crystallography have provided most of our knowledge in 3D protein structures, which has been further augmented by the revolutionary advances in cryoEM recently. However, a single structure conformation identified at cryogenic temperatures may introduce a fictitious structure as a result of cryogenic cooling artifacts, limiting the overview of inherent protein physiological dynamics, which play a critical role in protein biological functions. Here, we developed an ambient temperature X-ray crystallography method using temperature as a trigger to record movie-like structure snapshots. The method has been used to show how TL00150, a 175.15 Da fragment, undergoes structural changes in endothiapepsin (EP). We observed a surprising fragment binding discrepancy between the cryocooled and the physiological temperature structures, and captured multiple binding poses and their interplay with DMSO. The observations here open up new promising prospects for structure determination and interpretation at physiological temperatures, and also their implication in structure-based drug discovery.

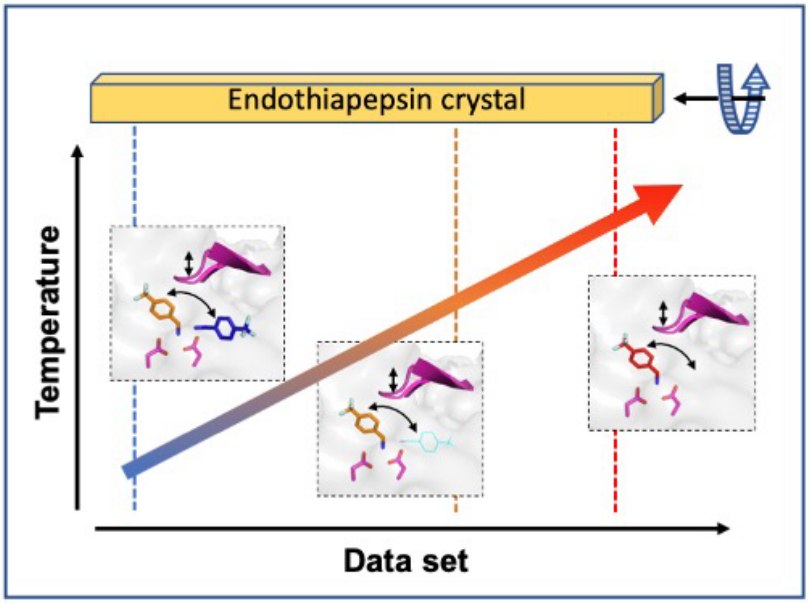

Figure 1