## ALLOSTERIC REGULATION OF GTP CYCLOHYDROLASE I

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GTP Cyclohydrolase I (GCH1) is a homodecameric protein complex of approximately 250kDa molecular weight. GCH1 catalyses the conversion of guanosine triphosphate (GTP) to dihydroneopterin triphosphate (H2NTP). The proposed complex reaction involves the hydrolytic opening of the imidazole ring of GTP and the formation of a formamido-pyrimidine intermediate. Release of formate, Amadori rearrangement of the ribose, and closure of the dihydropyrazine ring affords the product. This reaction is the initiating step in the biosynthesis of tetrahydrobiopterin (BH4) [1].

BH4 functions as co-factor in neurotransmitter biosynthesis. The BH4 biosynthetic pathway and GCH1 have been identified as promising targets to treat pain disorders in patients [2].

The function of mammalian GCH1s is regulated by a metabolic sensing mechanism involving a regulator protein, GCH1 feedback regulatory protein (GFRP). GFRP is a pentamer of 9.5kDa subunits, which binds to GCH1 to form inhibited or activated complexes dependent on availability of co-factor ligands, BH4 and phenylalanine, respectively [3].

We determined high resolution structures of human GCH1-GFRP complexes by cryoEM and X-ray crystallography. cryoEM revealed structural flexibility of specific and relevant surface lining loops, which were unresolved by X-ray crystallography due to crystal packing. The overall resolution of the cryoEM structures of 2.9 and 3.0Å allows for visualization of ligand binding to functional sites of the regulated complexes. Further, we studied allosteric regulation of isolated GCH1 by X-ray crystallography. Using the combined structural information we are able to obtain a comprehensive picture of the mechanism of allosteric regulation.

Local rearrangements in the allosteric pocket upon BH4 binding result in drastic changes in the quaternary structure of the enzyme leading to a more compact, tense form of the inhibited protein and translocate to the active site, leading to an open, more flexible structure of its surroundings. Inhibition of the enzymatic activity is not a result of hindrance of substrate binding, but rather a consequence of accelerated substrate binding kinetics as shown by STD-NMR and site-directed mutagenesis [4].

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