Abstract

Zinc-dependent hydrolases are a class of metalloenzymes that require zinc ions to catalyse hydrolytic reactions. Structural studies of these enzymes shall provide detailed information about the processing of their natural substrates, domain organization, and overall structural fold. This thesis describes the structural properties of two different metallohydrolases 1) human histone deacetylase 6 (HDAC6) and 2) glutamate carboxypeptidase II (GCPII) by utilizing a different set of biophysical techniques.

HDAC6 is a structurally unique multidomain enzyme comprised of unstructured and globular domains. It regulates the plethora of cellular processes by removing an acetyl group from lysine side chains of target proteins. It has been known to deacetylate non-histone substrates such as tubulin, Hsp90, cortactin, and peroxiredoxins. Given its structural complexity, complete structural information of full-length HDAC6 is missing and available information is limited to its globular domains only. Hence, the integrative approach was employed in combining experimental data from several orthogonal biophysical techniques to build an in-solution structural model of HDAC6. The study reports that HDAC6 adopts multiple conformations due to its unstructured regions and exists as an ensemble of conformers in solution. The model also explains the oligomerization tendency of HDAC6 that is mediated by its N-terminal microtubule-binding domain (MBD).

Glutamate carboxypeptidase II (GCPII) is a membrane-bound metallopeptidase harbouring two zinc ions in the active site. Overexpression of GCPII in different tissues is associated with various neurological disorders and prostate cancer. Hence, researchers have a significant interest in designing inhibitors targeting GCPII. GCPII-specific inhibitors usually contain a functional zinc binding group (ZBG) engaging zinc ions in the active site of GCPII. In this thesis, we report a structural comparison between sulfonamide and phosphorus-based inhibitors, where both functional groups serve as ZBGs. Previous studies reported that sulfonamide-based compounds

had 1000-fold weaker inhibitory potency as compared to phosphorus-based inhibitors, however, detailed mechanistic explanation was not provided up to date. To understand the enzyme-inhibitor interaction pattern in detail, we solved a high-resolution structure of a sulfonamide-based inhibitor in a complex with GCPII. Structural comparison between sulfonamide- and phosphorus-based inhibitors revealed an atypical binding pattern of the former, where one oxygen atom coordinates the catalytic zinc atom while the second oxygen interacts with a water molecule in the active site. Phosphorus-based compounds directly coordinate the zinc ions in the active site, replacing the water molecule, thus mimicking the transition state of the hydrolytic reaction. Our study confirms that the sulfonamide moiety is a weaker ZBG compared to phosphorus-based moieties, and this fact is reflected in its lower potency towards GCPII.