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Assessment of the doctoral thesis by Shivam Shukla "Structural studies of metal-dependent hydrolases: Histone Deacetylase 6 and Glutamate Carboxypeptidase II"

The doctoral thesis of Mr. Shivam Shukla focusses on structural studies of metal dependent hydrolases HDAC6 and GCPII. The thesis is built around a published first-author paper of Mr. Shukla on the in-solution structure and oligomerization of human HDAC6 and on a co-authored manuscript describing the structural characterization of sulfonamide inhibitor binding to GCII. The thesis is organized into a standard format broadly including chapters on the introduction, aims of the thesis, materials and methods used, results obtained and their discussion. The author succinctly introduces metal dependent hydrolases, HDACs in general, GCPII structure, mechanism, inhibitors and its biological roles, and summarizes approaches of integrated structural biology to tackle HDAC6.

The core of the results consists in integrated structural analysis of HDAC6, published in the FEBS Journal. The author has used a variety of relevant biophysical techniques such as SAXS, native MS, cross-linking-MS, analytical ultracentrifugation, HD exchange and molecular modelling. The use of all methods is justified logically and critically, and interpretation of the partial results and overall conclusions is careful and adequate. The main findings are identification of unstructured regions, and finding that HCAD6 can dimerize mainly via its MBD domain and ionic interactions. Full in-solution structural ensemble of HDAC6 was modelled using ensemble optimization method. Finally, possible biological significance of HDAC6 oligomerization is discussed, mainly in the formation of stress granules or aggresomes.

The second part, focusing on GCPII, produced a co-crystal structure of a sulfonamide inhibitor bound to GCPII. The structure has explained the observation that sulfonamides are poor inhibitors of GCPII (whereas that may be efficient inhibitors of other metalloenzymes and metal dependent hydrolases). Specifically, the structure shows that oxygen atoms of the sulfonamide do not efficiently coordinate zinc ions in the active site of GCPII. Has this manuscript been submitted or is it now under review?

The thesis has overall good quality, and it meets the formal requirements of the study board. Mr. Shukla has authored also another paper from the advisor's lab, on the expression and purification of recombinant human protoporphyrinogen oxidase IX, which is exempt from the thesis. The thesis is written in a succinct, brief way, and typos of formal problems are infrequent. I would however appreciate a well visible section where contributions of the author to each of the projects and papers would be clearly defined.

Overall, I recommend the thesis for public defense, and have two questions or question sets for the candidate to stimulate discussion.

1. The text asserts that the ZnF domain binds ubiquitin and is required to recruit misfolded proteins to the aggresome via HDAC6. Does the author expect that the ZnF domain prefers mono or polyubiquitin? Does it exhibit selectivity for different polyubiquitin linkage chains? How could this be analyzed?

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2. The author shows that ubiquitin binding to HDAC6 does not influence its oligomerization. How can this be put into context with the proposed role of HDAC6 in misfolded protein recruitment into aggresomes? Has the author tested whether any oligo ubiquitin chains could influence oligomerization? This seems mechanistically more likely.

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