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Opponent's review of the Doctoral Thesis by Mgr. Kseniya Ustinova entitled

„Substrate specificity of histone deacetylases “

The doctoral thesis presented by Kseniya Ustinova focuses on the investigation of the molecular basis of tubulin recognition by histone deacetylase 6 (HDAC6). HDAC6 is involved in many cellular processes and is considered as a promising target for the treatment of various pathologies such as cancer and neurodegenerative diseases. However, our understanding of interactions between HDAC6 and its substrates is still far from being complete. Therefore the aim of this doctoral thesis is important and timely.

The results section of the thesis is divided into three parts. In the first one, Kseniya Ustinova biochemically characterized the deacetylation activity of HDAC6 and also investigated HDAC6s interaction with microtubules using total internal reflection fluorescence (TIRF) microscopy. These experiments revealed that the free tubulin dimers represent the preferred HDAC6 substrate.

The second part of the results section deals with the investigation of the N-terminal domain of HDAC6. The results of this section indicate that the disordered N-terminal domain of HDAC6 is a microtubule-binding domain critical for efficient tubulin deacetylation, thus suggesting that the recognition of substrates by HDAC6 is more complex than previously appreciated and that regions outside the tandem catalytic domains are essential for proficient substrate deacetylation.

The last part of the results section focuses on the rational design of selective HDAC6 inhibitors as potential therapeutic agents for the treatment of various neurological disorders. This study describes a series of HDAC6 inhibitors which were designed, synthesized and evaluated for their inhibitory activity. An addition, one of the inhibitors (compound SS-208 which exhibits the antitumor activity) was crystallized with the second catalytic domain of *D. rerio* HDAC6. The solved structure enabled detailed description of interaction between the compound and the catalytic site of HDAC6.

The presented doctoral thesis is written in a shortened version and its formal structure follows the standard division into three main parts. The first one consists of a theoretical background in which the basic facts concerning histone deacetylases and especially HDAC6 including its structure, function, role in pathologies and targeted inhibition are described. The second part summarizes main results and includes a description of author's contribution to the obtained results. The last part consists of a summary of obtained results and their discussion. Both the formal and the graphical quality of the presented thesis are fully satisfactory. The occurrence of typing errors is minimal.

For the sake of discussion during the defense of the thesis, I have following questions related to presented results:

1. How does the genetic ablation of HDAC6 affect cellular/tissue functions?
2. Is HDAC6 the only histone deacetylase known to deacetylate tubulin?
3. It has been shown that the subcellular localization of HDAC4 is controlled through interaction with the 14-3-3 protein. Are there any indications that HDAC6 is also a binding partner of 14-3-3?
4. Although HDAC6 possesses two catalytic domains, only the second deacetylase domain is responsible for the tubulin deacetylase activity. What is the biological function of the first (N-terminal) catalytic domain? Furthermore, is it known why the addition or deletion of 5 amino acid residues within the linker between the catalytic domains has so significant impact on HDAC6 activity?
5. On which evidence is based the claim that the N-terminal domain of HDAC6 is an intrinsically disordered protein?
6. Why just HDAC1 was used to test the selectivity of the HDAC6 inhibitor SS-208?

In conclusion, the doctoral thesis presented by Kseniya Ustinova represents a significant contribution to the characterization of HDAC6 and its function. The thesis is written in intelligible language and obtained results were carefully analyzed and discussed and all were already published in respected international journals with IF.

In conclusion, the presented thesis clearly demonstrates that Kseniya Ustinova is able of independent scientific work. Since the thesis presented by Kseniya Ustinova satisfies all requirements for the doctoral thesis **I fully recommend its acceptance.**

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