## MS5-O4 Crystal structure of HDAC6: insights into molecular assembly, selective inhibition and microtubule deacetylation

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Histone deacetylases (HDACs) form a large family of enzymes catalyzing the removal of ε-N acetyl groups from acetylated lysines on target proteins. HDACs are categorized into four classes with class I, II, and IV containing zinc-dependent enzymes (HDAC 1-11) and class III proteins using nicotine adenine dinucleotide as cofactor (SIRT 1-7) [1]. HDAC6 is a unique class II member as it is the only histone deacetylase featuring two catalytic domains and a C-terminal ubiquitin binding domain. In addition, while most HDACs are located in the nucleus acting on acetylated histone peptides, HDAC6 is mainly found in the cytosol where it regulates acetylation states of a diverse set of proteins such as tubulin, cortactin, HSP90, and many more. HDAC6 is a major regulator of the aggresome pathway, influences microtubule dynamics and the function of regulatory T-cells, and plays a role in influenza virus infection [2,3,4]. It has been shown to be involved in several cancers, neurodegenerative diseases and inflammatory processes and is actively pursued as promising drug target by pharmaceutical companies and academic groups [5]. Although several HDAC6 specific inhibitors have recently been developed by combinatorial chemistry approaches, the lack of structural information prevented further structure-based drug design and understanding of selective inhibition over other HDAC family members. Here, we present high resolution crystal structures of HDAC6 inhibitor complexes which give insight into selective inhibition and which might have the potential to enlarge the chemical inhibitor space as well as making use of completely new $Zn^{2+}$ binding groups. Additional structures reveal for the first time the interdomain assembly of the two catalytic domains and the positioning of the connecting linker, while functional analyses shed light onto the role of the two catalytic domains in microtubule deacetylation and catalysis.

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## MS5-O5 Probing nucleotide-induced conformational changes and interaction studies of the GTPase EngA

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Microorganisms resistant to antibiotics and the geographic areas affected by drug resistance have been drastically increasing. Antimicrobial resistance has become one of the major concerns on public health and the return to the investment on new antibiotics is of major importance. One of the strategies has been to identify genes critical to the survival of bacteria as enzymes encoded by these genes represent potential targets for antibiotic design.

EngA is a GTPase conserved in bacteria and involved in ribosome biogenesis. While essential in bacteria, EngA does not have any human ortholog and can thus be targeted to selectively act on bacteria eradication. Our work aims at understanding how EngA interacts with the ribosome and to identify inhibitors for its function.

We have used a multitechnique approach to investigate ligand-induced conformational changes in EngA and unveil its role in ribosome binding. EngA has the unique feature among GTPases of bearing two G-domains (1).

We have probed conformational changes by SAXS and limited proteolysis and have observed a change in protein structure and a higher rate of proteolysis induced by GTP. The conformation adopted in solution in the presence of GTP does not relate with any of the crystal structures of EngA. Attempts to crystallize EngA in the GTP-bound form have resulted so far in 4 structures in different crystal form, but adopting the conformation observed for the GDP-bound state, despite the presence of GTP in one G-domain and some changes in switch regions. Some of the regions sensitive to proteolysis display different kinetics in the apo- and GTP-bound states. Analysis of these fragments may give us insights into which regions become more or less accessible.

Interactions studies confirmed better binding of EngA to the ribosome in the presence of GTP, suggesting the new conformation is more prone to bind the ribosome. Ongoing analysis of the complex by cryo-EM will allow us to visualise the EngA conformation when bound to the ribosome and will possibly help us characterize the interface.

In parallel, an ELISA assay is being set up to screen inhibitors in order to identify molecules able to block the interaction between EngA and the ribosome.

Our latest results will be presented at the conference.

1. Foucher AE, Reiser JB, Ebel C, Housset D, Jault JM. Potassium acts as a GTPase-activating element on each nucleotide-binding domain of the essential Bacillus subtilis EngA. PloS One. 2012;7(10):e46795

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