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 nicotinic 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 [3]. It has been shown to be involved in several cancers, neurodegenerative diseases and inflammatory processes and is actively pursued as promising drug target.

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.


Keywords: Histone deacetylase, HDAC6, crystal structure, enzyme inhibition, drug design

Keywords: GTPase, ribosome, protein-protein interaction, conformational changes, X-ray crystallography, SAXS, cryo-EM,
The sarcomere is the minimal contractile unit in the cardiac and skeletal muscle, where actin and myosin filaments slide past each other to generate tension. This molecular machinery is supported by a subset of highly organised cytoskeletal proteins that fulfil architectural, mechanical and signalling functions, including the giant proteins titin, obscurin and nebulin as well as the cross-linking proteins α-actinin and myomesin (1).

The cross-linking of actin and myosin at the boundaries of their filamentous structures is essential for the muscle integrity and function. In the Z-disks – the lateral boundaries of the sarcomere machinery – the protein α-actinin-2 cross-links antiparallel actin filaments from adjacent sarcomeres, and additionally serves as a binding platform for a number of other Z-disk proteins. Among them is FATZ, also known as calsarcin and myozenin, which appears in the early stages of myofibrillogenesis together with α-actinin-2 [1]. α-Actinin is an antiparallel dimer, where each subunit is composed of an N-terminal actin binding domain, which is connected by a neck region to the four spectrin-like repeats (rod), and a C-terminal calmodulin-like domain. FATZ is composed of conserved N- and C-terminal regions connected by an intrinsically disordered segment. FATZ is believed to act as an adaptor protein linking α-actinin to other Z-disk proteins, but the structural information at molecular level on FATZ and complexes with any of its interaction partners still remains unknown.

We employed a combination of structural and biophysical approaches to elucidate the three-dimensional structure and dynamics of human α-actinin-FATZ complex. Circular dichroism, NMR and small angle X-ray scattering data of FATZ alone and in complex with α-actinin showed that FATZ is an IDP in solution, and that it does not fold upon binding. The non-bound portion displays pronounced structural plasticity and dynamics, characteristic of fuzzy complexes. Furthermore, the crystal structure shows that two stretches of FATZ interact in mostly extended conformation with the rod domain of α-actinin, and that FATZ binding might displace the N-terminal lobe of the calmodulin-like domain from the position found in the structure of full length α-actinin-2 [2], suggesting a specific role in the...