MBG MINI SYMPOSIUM

hosted by Ditlev E. Brodersen

Tuesday 11 June 2024 at 10:15-11.45
1870-816 Faculty Club

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FeFe-hydrogenase active site assembly

Transition metals are essential to all living organisms because they open chemistries that would not be possible when using only the 22 amino acids that constitute enzymes. Therefore, transition metals are often found at the heart of important reactions, notably in energy metabolism. For instance, transition metals play a key role in the metabolism of gases (H₂, CO₂, N₂) by microorganisms. The organometallic cofactors, which constitute the active sites of the corresponding metalloenzymes (hydrogenase, CODH or nitrogenase) are nowadays inserted into the apo-enzymes by dedicated multiprotein machineries.

In this presentation, I will focus on the assembly of the [2Fe]H center of the FeFe-hydrogenase. This center contains two irons, both bound to cyanide and carbon monoxide ligands and an azadithiolate bridging molecule. Notably we will discuss the role of the two radical S-adenosyl-L-methionine (SAM) enzymes HydG and HydE in this process and will focus on the structure-function relationships of the latter.

Dr. Vincent Chaptal
Molecular Microbiology and Structural Biochemistry, CNRS, Lyon, France

3D protein structures in 4D: R6G narrows BmrA conformational spectrum for a more efficient use of ATP

Multidrug ABC transporters harness the energy of ATP binding and hydrolysis to change conformation and thereby translocate substrates out of the cell to detoxify them. While this general access mechanism scheme is well accepted, molecular details of this interplay is still elusive. Rhodamine6G binding on a catalytic mutant of the homodimeric multidrug ABC transporter BmrA triggers a cooperative binding of ATP on the two identical nucleotide-binding-sites, otherwise Michaelian. We investigated this asymmetric behavior via a structural-enzymology approach, solving cryoEM structure of BmrA at defined ATP ratio along the enzymatic transition, highlighting the plasticity of BmrA as it undergoes the transition from inward to outward facing conformations. Analysis of continuous heterogeneity within cryoEM data and structural dynamics, revealed that Rhodamine6G narrows the conformational spectrum explored by the nucleotide-binding-domains, describing the allosteric effect of drug binding that optimizes the ATP-dependent conversion of the transporter to the outward-facing state. Following on these findings, the effect of drug-binding showed an ATPase stimulation and a maximal transport activity of the wild-type protein at the concentration-range where the allosteric transition occurs. Drug diffusion rate is the likely rate-limiting step of the reaction, while drug transport and ATPase activities are in effect uncoupled.