Enzyme structural flexibility at extreme temperatures

Integrative structure of a hyperthemophilic PEP-synthase

netherlands proteomics centre

Pascal Albanese[1,2], Wenfei Song[3], Siri van Keulen[4], Sem Tamara[1,2], Jeroen Koendjbiharie[5], Serve Kengen[5], Alexandre Bonvin[4], Friedrich Förster[3], Albert J.R. Heck[1,2], Richard Scheltema[1,2]

[1] Biomolecular Mass Spectrometry and Proteomics Group, [3] Cryo-Electron Microscopy Group and [4] Utrecht NMR Group @ Bijvoet Center for Biomolecular Research & Utrecht Institute for Pharmaceutical Sciences, Utrecht University, 3584 CH Utrecht, The Netherlands
[2] Netherlands Proteomics Center, Utrecht University, Utrecht, The Netherlands
[5] Laboratory of Microbiology, Wageningen University, 6708 WE Wageningen, The Netherlands

OVERVIEW

All living organisms rely on a combination of 3 basics compounds for carbon metabolism: pyruvate, phosphoenolpyruvate and oxaloacetate [1]. The enzymes that catalyze the interconversion between these metabolic intermediates however diverge in different organisms, according to their ecological niche. Here, we combined various Mass Spectrometry (MS) - based approaches and cryo-electron microscopy (EM) with structural modelling and phylogenetic reconstruction to describe the dynamic structure of a hyperthermostable phosphoenolpyruvate synthase (PPSA) purified from *Pyrococcus furiosus*. This anaerobic hyperthermophile Archaeon thrive at temperatures around 95-99^C, unimaginable conditions to maintain a proper protein folding and exchange metabolites. Yet, it possess an extremely sophisticated molecular machine allowing to carry out cellular processes in extreme environments, and which deeper understanding can help improving the stability of e.g. economically relevant enzymes and protein nanoparticles for biomedical applications.

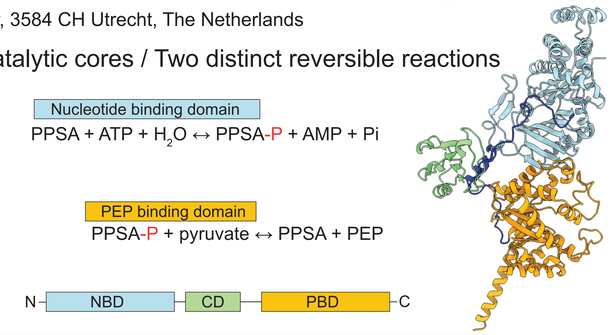
Two catalytic cores / Two distinct reversible reactions

Nucleotide binding domain

$PPSA + ATP + H_2O \leftrightarrow PPSA\text{-}P + AMP + Pi$

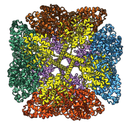
PEP binding domain

$PPSA\text{-}P + pyruvate \leftrightarrow PPSA + PEP$

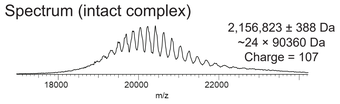


Native & Top Down-MS

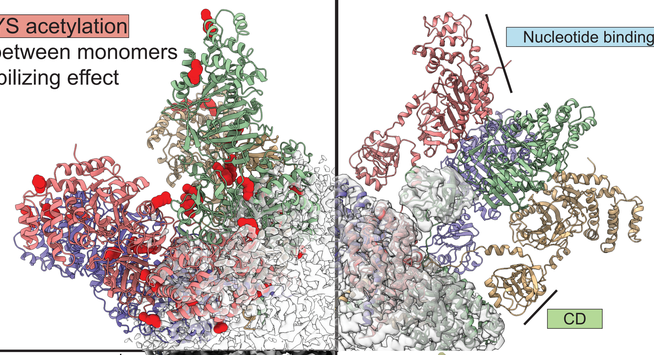
The functional oligomer is a **~2.15 MDa complex** (Native-MS) composed of **24 subunits of ~90 kDa**, for which multiple proteoforms were identified (Top-Down and Bottom-Up proteomics).



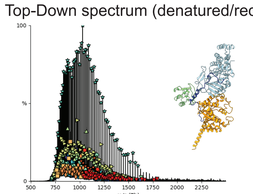
Native Spectrum (intact complex)



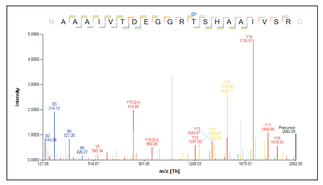
Extensive LYS acetylation at the interfaces between monomers have a stabilizing effect



Top-Down spectrum (denatured/reduced)



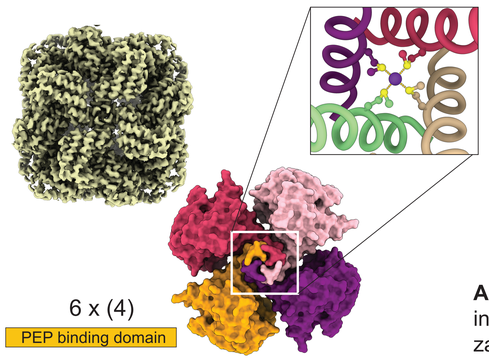
Multiple proteoforms were detected

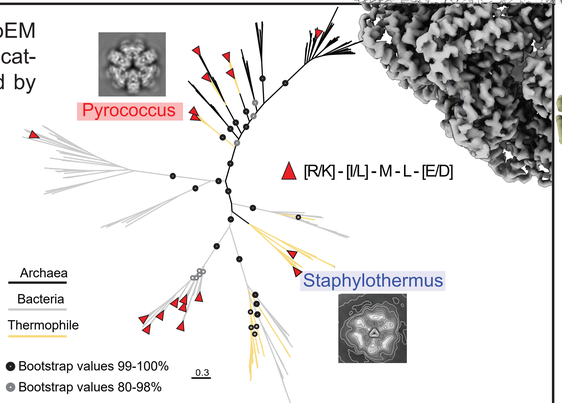


THR 440 is the key phosphorylated residue of the CD domain

Cryo-EM & ICP-MS

Stable core (without disulphide bonds!): From the CryoEM structure it emerged that the core, containing one of the two catalytic centers: the PEP-binding domain (PBD), is stabilized by Fe(II) planar coordination (ICP-MS) by Methionine-799.

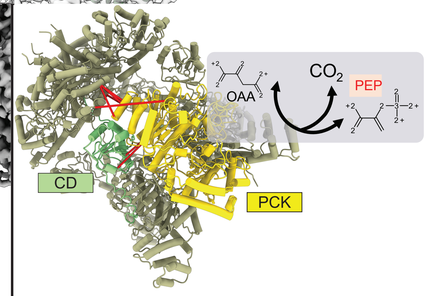




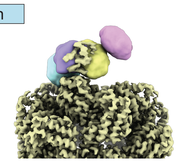
Ancestral origin: The motif containing the MET 799 is widespread in thermophile Archaea species, some sharing the same oligomerization state [3], and is likely realted to enhanced thermal stability.

Stable core mediated by MET 799 - Fe(II) coordination

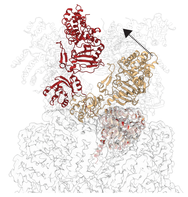
Structural dynamics of the N-terminal domain and coupled enzymes



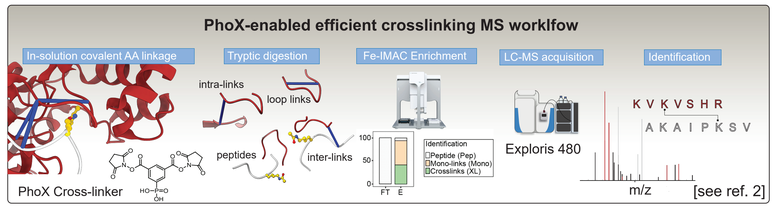
PEP carboxykinase (PCK) reproducibly interacts with PPSA. Using distance restraints from XL-MS and coevolution analysis PCK was docked to the main complex unraveling its pivotal position whereby it hinders the CD domain trajectory.

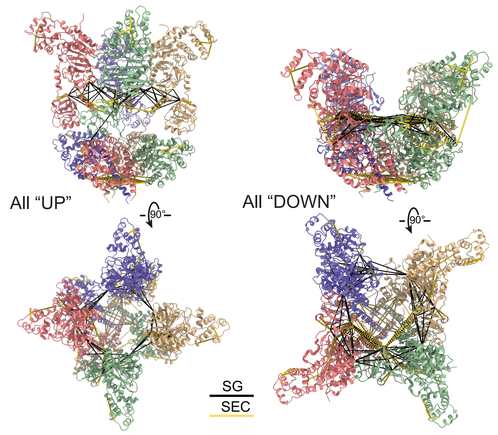


Multiple position of the CD domain were potentially found → The whole N-terminus is higly flexible

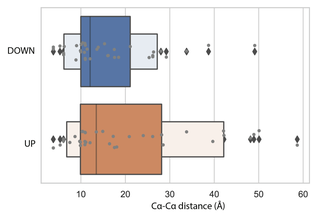


Extreme flexibility: The central domain (CD) is shuttling the phosphate between the two catalytic cores (tens of Å apart!), and is connected to both by long connecting loops. The structure and position was partially modelled according to an additional low resolution density in the cryoEM map.





145 (PPSA purified via Sucrose gradient) and 115 (PPSA purified via SEC) highly reproducble crosslinks can be mapped on the 4 predicted confromations mixed in various combinations wihin an acceptable distance cut-off of ~30 Å.



Crosslinkig MS and Structural Modelling

OUTLOOK

The *P. furiosus* PPSA complex may represent an ancestral metabolic enzyme configuration that exhibits sophisticated mechanisms to cope with extreme environmental conditions (elevated temperature and pressure). The combination of cryoEM and structural modelling with in-depth structural proteomics profiling allowed the identification of conformational states, functional interactors and fine details on functional PTMs. The very stable, yet intrinsically flexible structure, represent a unique combination of features that will pave the path for enanched temperature stability in mesophilic organisms.

References

[1] Koendjbiharie et al., FEMS Microbiol. Rev., 2020.
[2] Steigenberger et al., ACS Cent. Sci.,2019.
[3] Harauz et al., J. Struct. Biol., 1996.

scheltemalab.com

NWO

ThermoFisher SCIENTIFIC