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Comparative structural analysis of the oligomeric state of bacterial glutamate dehydrogenases

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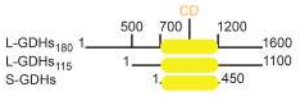
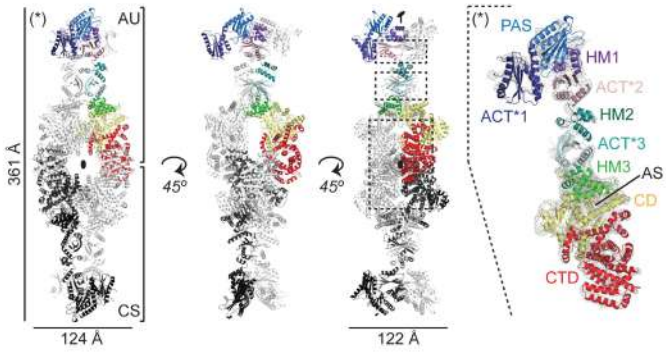
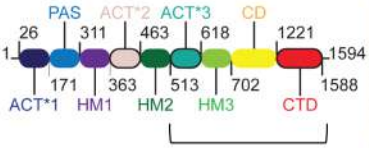
INTRODUCTION

Glutamate dehydrogenases (GDHs) are ubiquitous oligomeric enzymes that are classified into the subfamilies of small GDHs (S-GDHs), which are hexamers composed of 50 kDa monomers, and large tetrameric GDHs (L-GDHs) with 115 or 180 kDa subunits [1]. S-GDHs have been the subject of diverse biochemical and structural studies whereas L-GDHs have been less studied.

The first experimental structure of a bacterial L-GDH$_{180}$ (from *M. smegmatis*) was recently obtained by our group [2]. The mycobacterial L-GDH$_{180}$ (mL-GDH$_{180}$) consists of monomers that contrast with those of S-GDHs by containing long N- and C-terminal extensions flanking the catalytic domain. Such regions are modular and provide the surfaces for oligomerization. Here we report three experimental models of mL-GDH$_{180}$ in different conformations that reveal transitions in the quaternary structure of the enzyme.



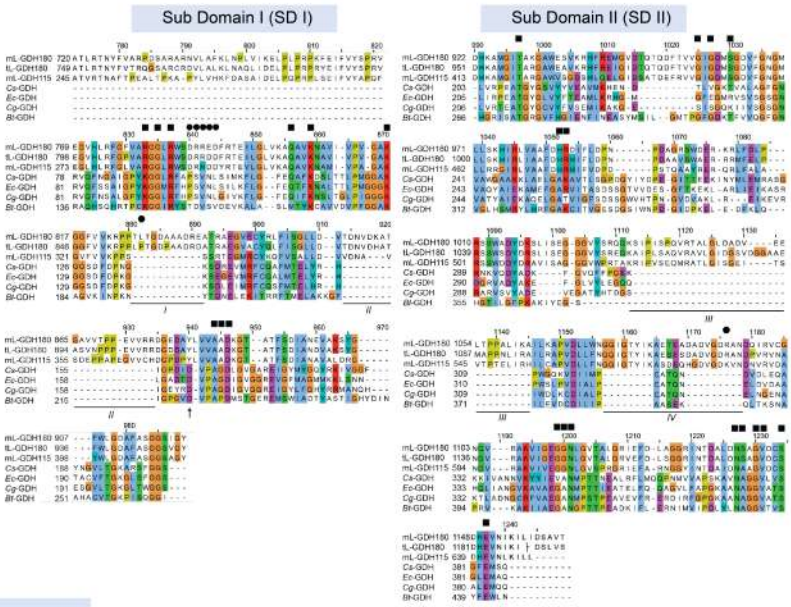




First structural strcuture of a L-GDH$_{180}$(from *M. smegmatis*) Up: the monomers adopt a tetrameric arrangement with D2 symmetry. A protein subunit is shown (model and crystallographic electron density map at 6 Å); the domains are highlighted in different colors, replicated in the scheme in the left. ACT and PAS domains are described in (1); HM = helical motif; CTD = C-terminal domain; AS = active site.

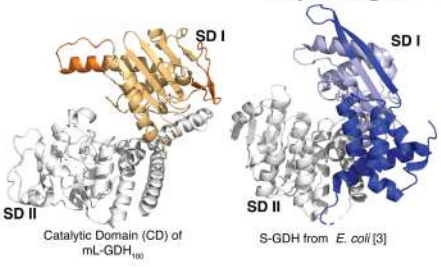
MATERIALS AND METHODS

To accomplish the intended objective, protein sequences of selected GDHs (4 S-GDHs and 3 L-GDHs) were aligned using T-Coffee and Clustal.
Using the tool PyMol, experimental structures of S-GDHs were 3D-aligned with the structure of the catalytic domain of the L-GDH from *M. smegmatis*.



RESULTS AND CONCLUSIONS

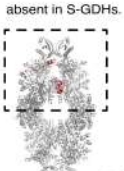
Exploring mL-GDH$_{180}$ architecture



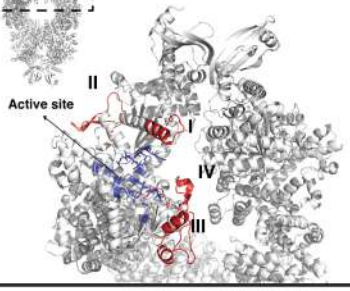
Regions involved in the oligomerization of S-GDHs (dark blue) are absent in mL-GDH180, suggesting that the CD from L-GDHs may not be involved in the stabilization of the cuarternary structure of the enzyme,

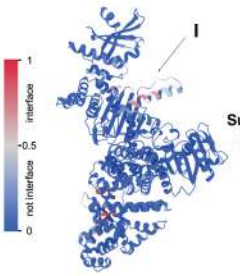
Using PeSTo, we identified a motif in the catalytic domain of the enzyme, which matches the region I mentioned before and that is likely to participate in protein-protein interactions.

Comparing sequences and structures of different S-GDHs and mL-GDH$_{180}$, we found four conserved motifs in the catalytic domain of mL-GDH$_{180}$ that are absent in S-GDHs.



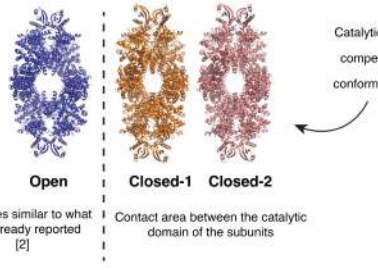
They are located near the active site and any of their interactions may modify the enzyme activity,

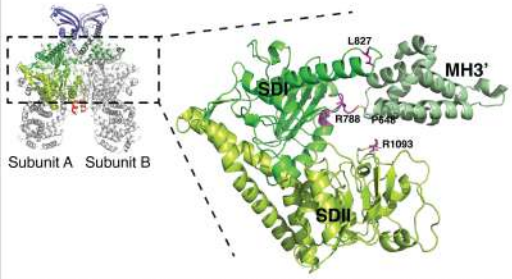




PeSTo [4] is an AI that predicts protein-protein interactions. The novelty of this algorithm is that it has a low computational cost so it allows to run big protein structures such as ours.

Analysis of new CryoEM models obtained in the presence of NAD$^+$





Novel interface interactions observed involving the catalytic domain of the closed conformations of mL-GDH$_{180}$ [2].

The residues in magenta are part the motifs I and IV mentionded before. This suggests that these regions could be hot spots for regulation.

REFERENCES

[1] Minambres et al. (2001)
[2] Lazaro et al. (2021)
[3] Sharkey et al. (2013)
[4] Krapp et al. (2023)