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Supplementary materials to:

The Substrate Versatility of Δ¹-Pyrroline-5-carboxylate Reductase (ProC) from *Escherichia coli*

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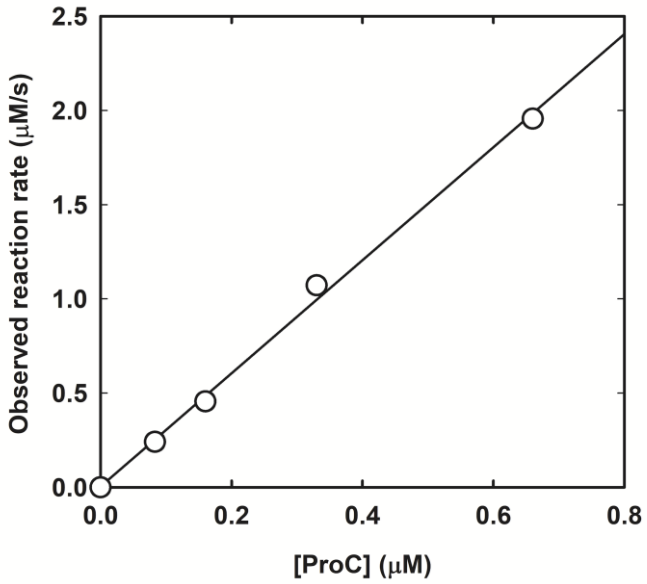


Figure S1. Linear dependence of the L-Pro oxidation rate as a function of ProC concentration. Conditions: L-Pro 20 mM, $NADP^+$ 1.5 mM, 50 mM Bis-tris propane buffer, pH 10, 25°C The enzyme concentration refers to the monomer.

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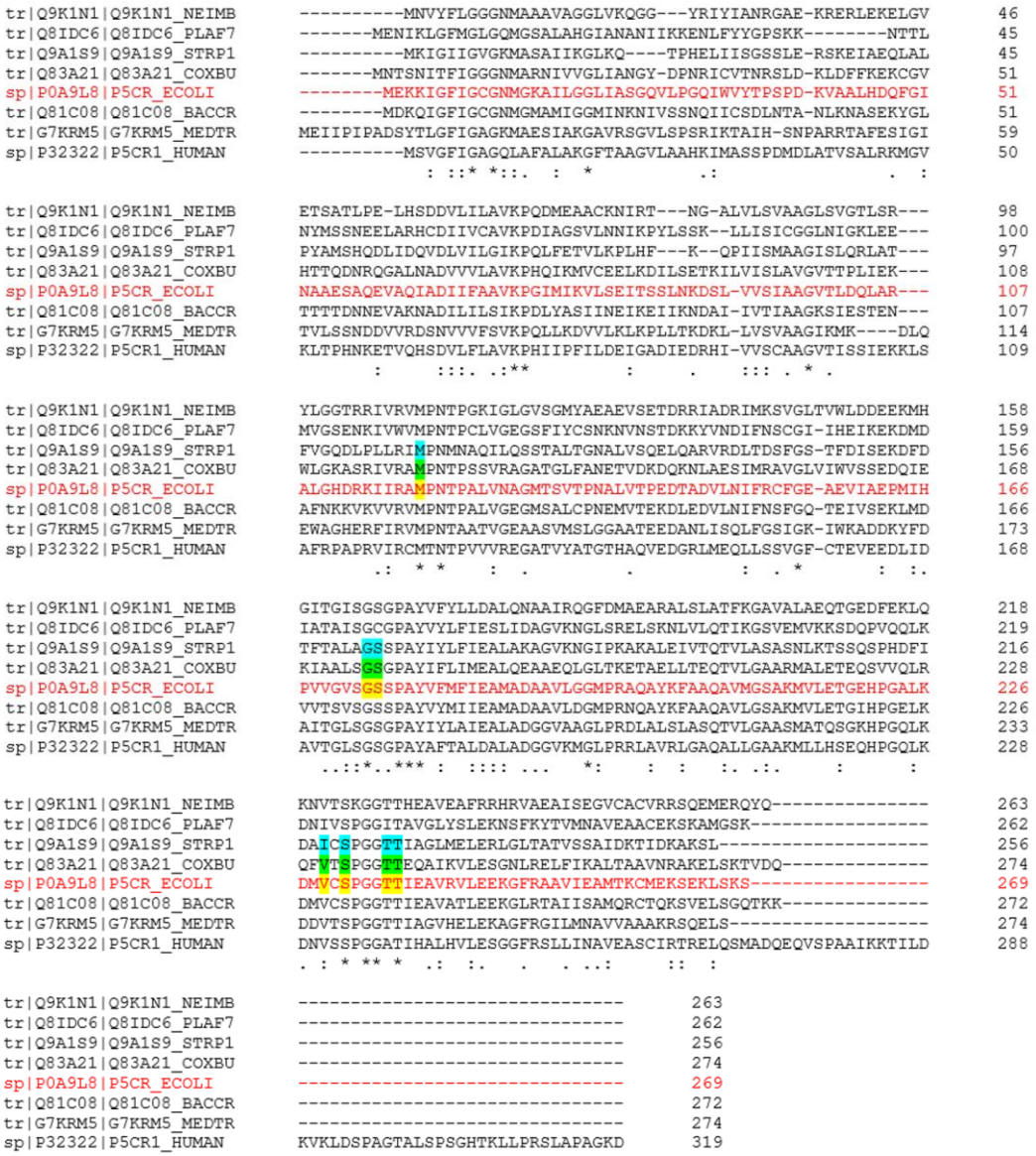


Figure S2. Multiple alignment of the sequences obtained by BLASTing the *E. coli* ProC sequence (in red) against the PDB database. The active site residues are highlighted in the ProC sequence of *E. coli* (yellow), *Coxiella burnetii* (green, corresponding to the 3TRI structure [1]) and *Streptococcus pyogenes* (cyan, corresponding to the 2AMF structure [2]).

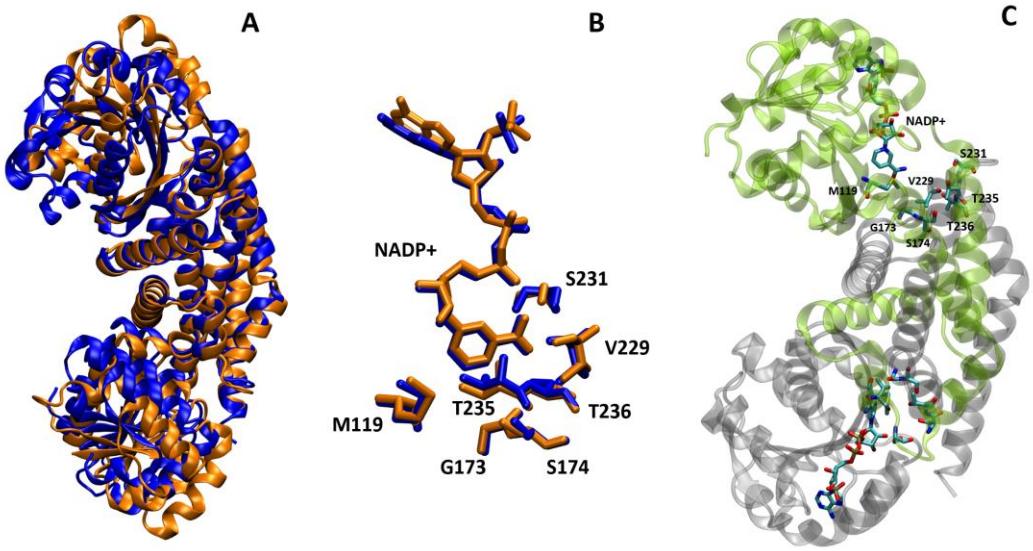


Figure S3. Computed structures of the *E. coli* ProC produced by the Swiss model server Panel A: structural alignment of the model based on the 3TRI template (blue) with the one based on the 2AMF template (orange). For the latter, only the dimeric functional unit is shown for comparison. Panel B: structural alignment of the active site alone (same color code as in panel A). Panel C: domain swapping of the dimeric unit of the 3TRI based model (in gray chain A and in green chain B), with the two «swapped» active sites in licorice, colored by atom type.

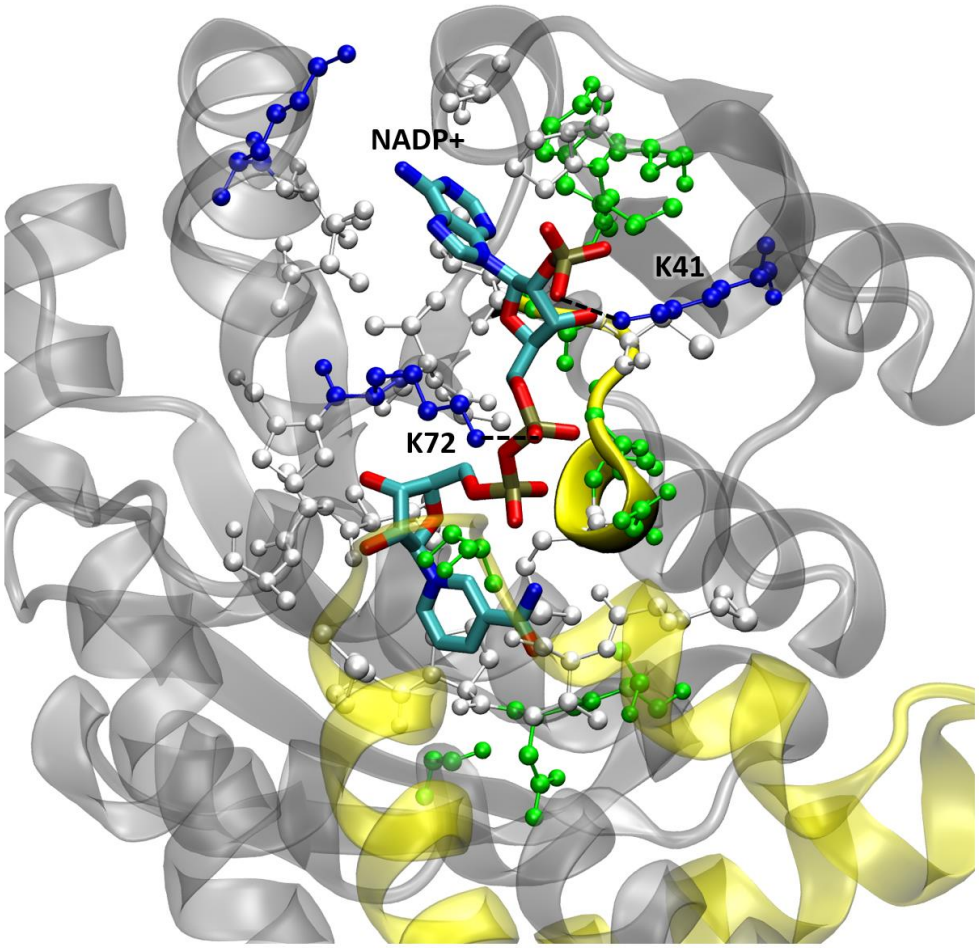


Figure S4. Positioning and interactions of NADP at the ProC active site. $NADP^+$ is located inside a groove between the N-terminal domain and the jointed dimerization domains (in transparent gray and yellow the two swapped domains), with the nicotinamide ring forming a wall of the active center pocket. The typical dinucleotide-binding motif GXGXXA/G (residues 9-14, in opaque yellow), well conserved throughout the ProC enzyme family [2], is present. It forms a loop between strand 1 and helix 1 in the N-terminal domain and helps to maintain the correct position of the central part of $NADP^+$. The coenzyme is held in place mainly by hydrophobic interactions (white ball-and-sticks residues) and by two salt bridges between the $NADP^+$ phosphate groups and Lys41 and Lys72 in the ProC N-terminal domain. Interestingly, in the 3TRI template both these residues are conserved, whilst in 2AMF the first Lys is substituted with an Arg and the second one, although present, is far away from the $NADP^+$ phosphate. Only a few H-bonds are formed by $NADP^+$ with protein backbone atoms. It is worth noting that in the ProC crystal structures several water bridges mediate the interaction between protein and coenzyme, to stabilize the position of the latter.

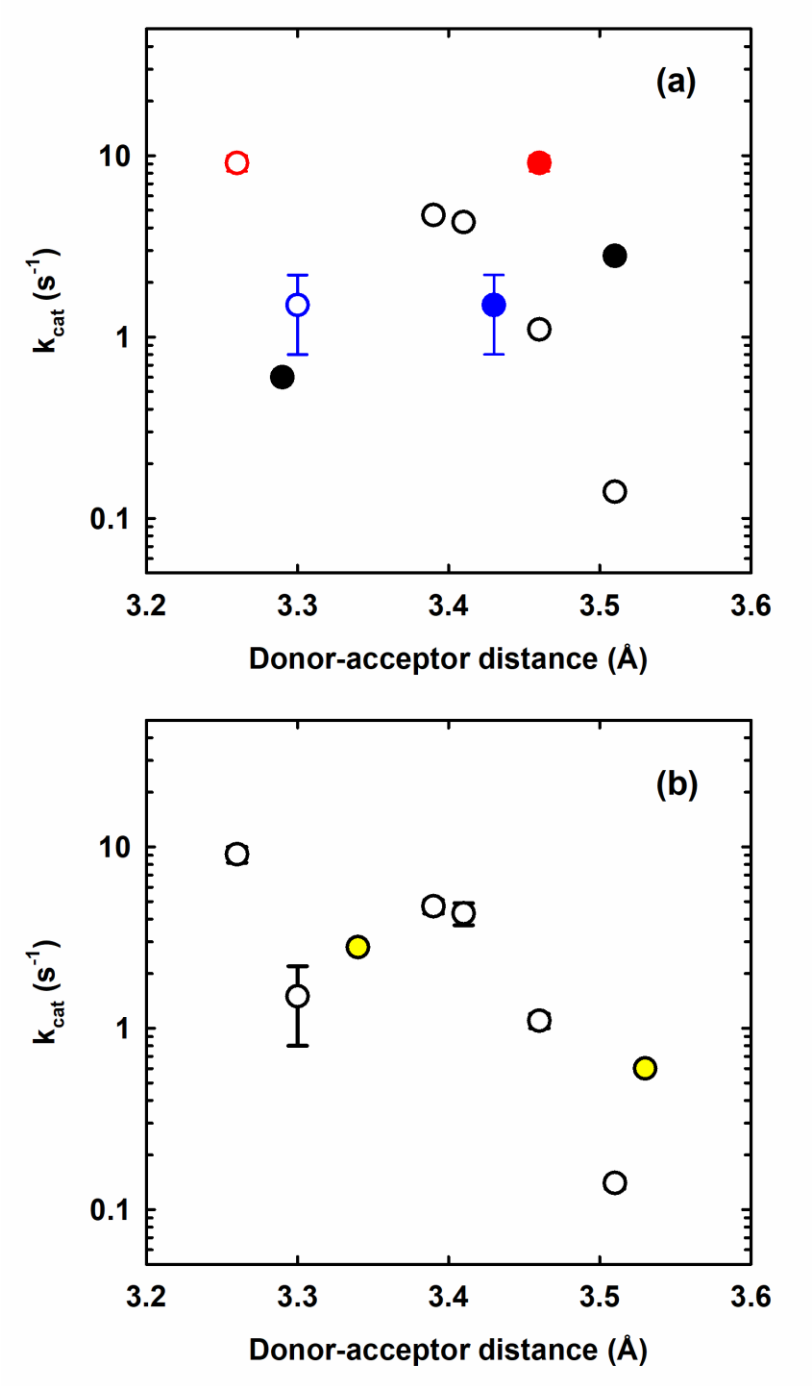


Figure S5. Representation of k_{cat} (values form Table 1 in the main text) as a function of the calculated distance between the hydride donor carbon on the substrate (e.g., C5 in the case of L-Pro) and the acceptor C4 atom on the nicotinamide ring. (a) The distances (measured from the best poses shown in Figures 5 and 6 of the main text) refer to substrates docked with a protonated (filled circles) or neutral (empty circles) amino group. For DHP (red) and cis-4-hydroxy-L-proline (blue) distances calculated for both the protonated and neutral forms are presented. (b) Correlation of k_{cat} with the distance of the best energy pose for compounds docked all with a neutral amino group. White circles correspond to the empty circles in panel a, whereas the yellow circles refer to L-Pro and PIP docked with an unprotonated amino group. With respect to the protonated form, the distance diminished for L-Pro and increased for PIP.

References

1. Franklin, M.C.; Cheung, J.; Rudolph, M.J.; Burshteyn, F.; Cassidy, M.; Gary, E.; Hillerich, B.; Yao, Z.K.; Carlier, P.R.; Totrov, M.; et al. Structural Genomics for Drug Design against the Pathogen *Coxiella Burnetii*. *Proteins: Structure, Function and Bioinformatics* 2015, *83*, 2124–2136.
2. Nocek, B.; Chang, C.; Li, H.; Lezondra, L.; Holzle, D.; Collart, F.; Joachimiak, A. Crystal Structures of Δ1-Pyrroline-5-Carboxylate Reductase from Human Pathogens *Neisseria Meningitides* and *Streptococcus Pyogenes*. *J Mol Biol* 2005, *354*, 91–106.