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Structural and functional characterization of the Protein-O-MannosylTransferase of Mycobacterium tuberculosis

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RIVIÈRE C., GERAUD N., FALCOU C., FABRE E., RIVIÈRE M.

Institute of Pharmacology and Structural Biology (IPBS), Centre National de la Recherche Scientifique (CNRS), UFTMIP-UPS
Department of Tuberculosis and Infection Biology, 205 Route de Narbonne, 31077 Toulouse, France

Introduction

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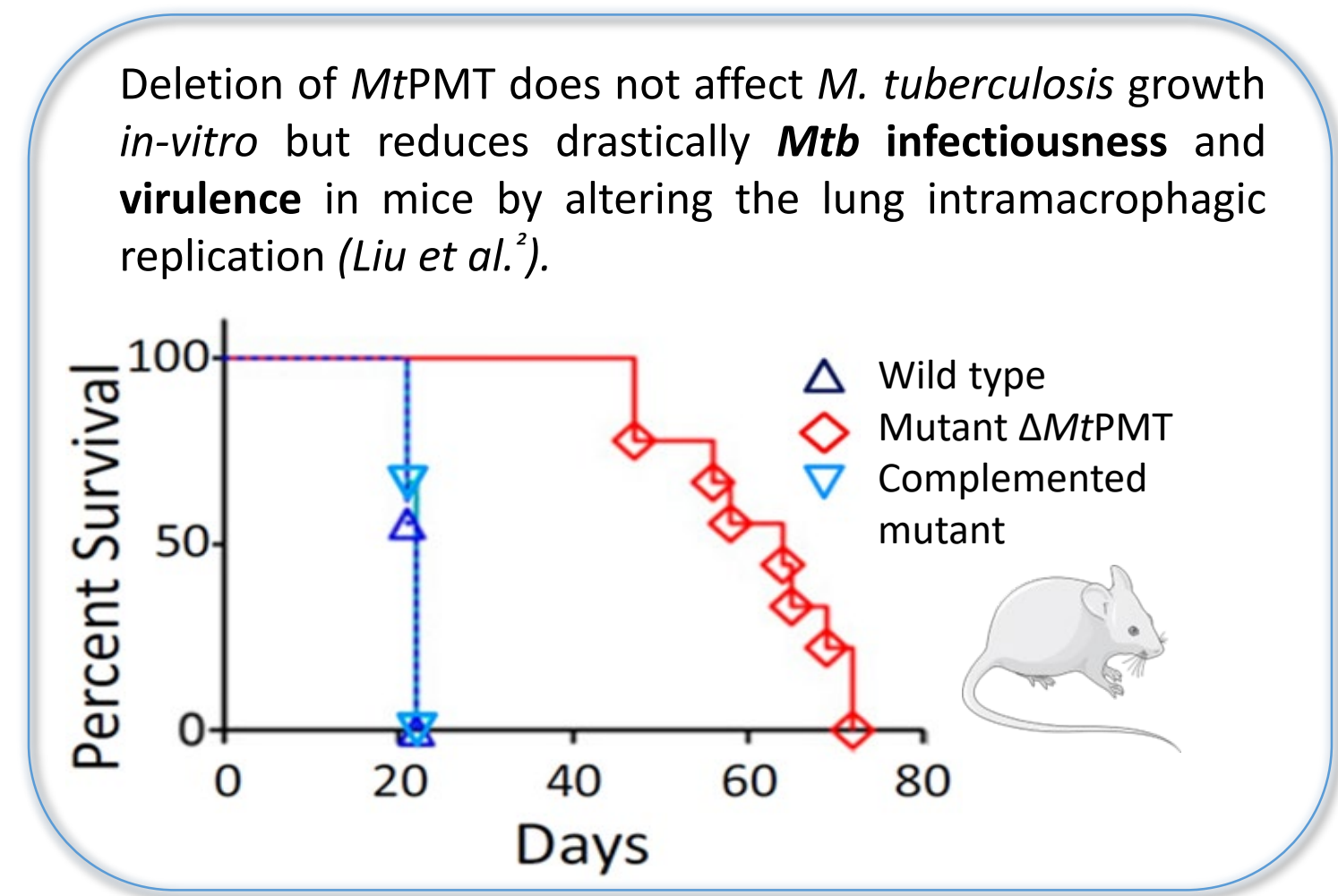
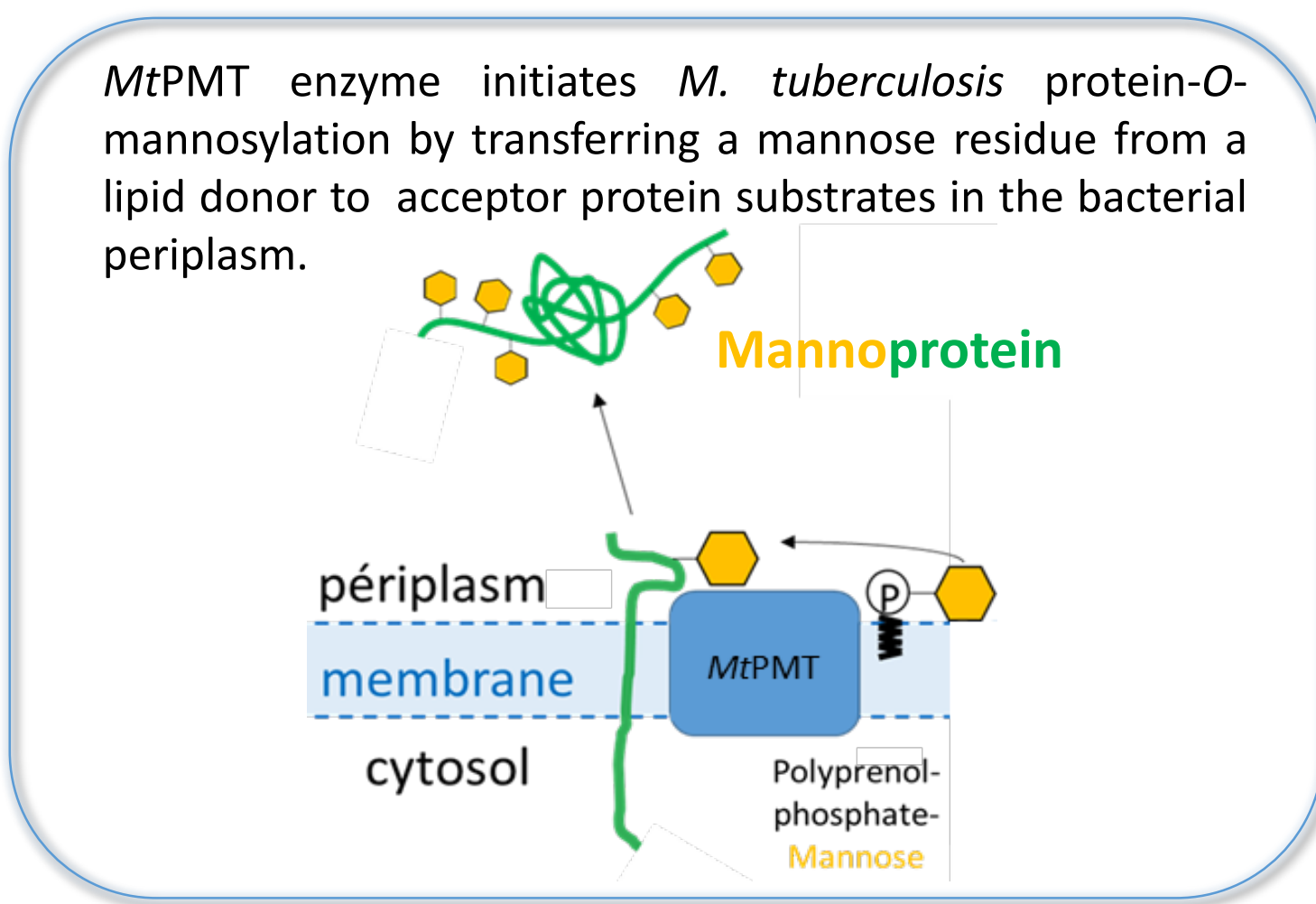
Mycobacterium tuberculosis (Mtb)

- The etiologic agent of the human Tuberculosis (TB)
- 1,5 millions of deaths in 2020 (World Health Organization)
- Multi and extensively drug-resistant (MDR and XDR) strains emergence and spread
 - Urgent need for innovative drug development¹

Anti-virulence strategy is a promising approach for innovative therapeutics

- Targets non-essential virulence factor
- Less likely to cause resistance (doesn't affect pathogen's vital metabolic pathways)
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Bacterial protein-O-mannosylation process as a new virulence factor of Mtb



➢ MtPMT : Potential target to fight Tuberculosis

Major advantage: its pleiotropic effect prevents the emergence of drug-resistance³

Aim

Search for specific inhibitors of the *M. tuberculosis* MtPMT with potential therapeutic value (anti-virulence therapy).

Limitations

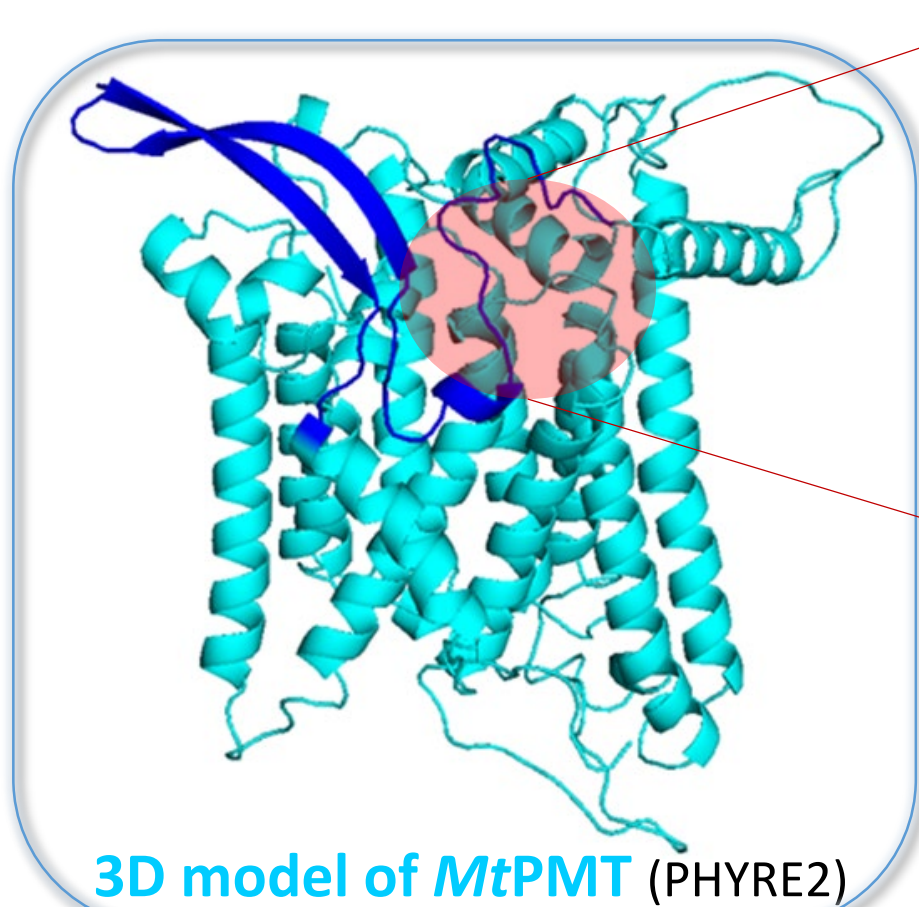
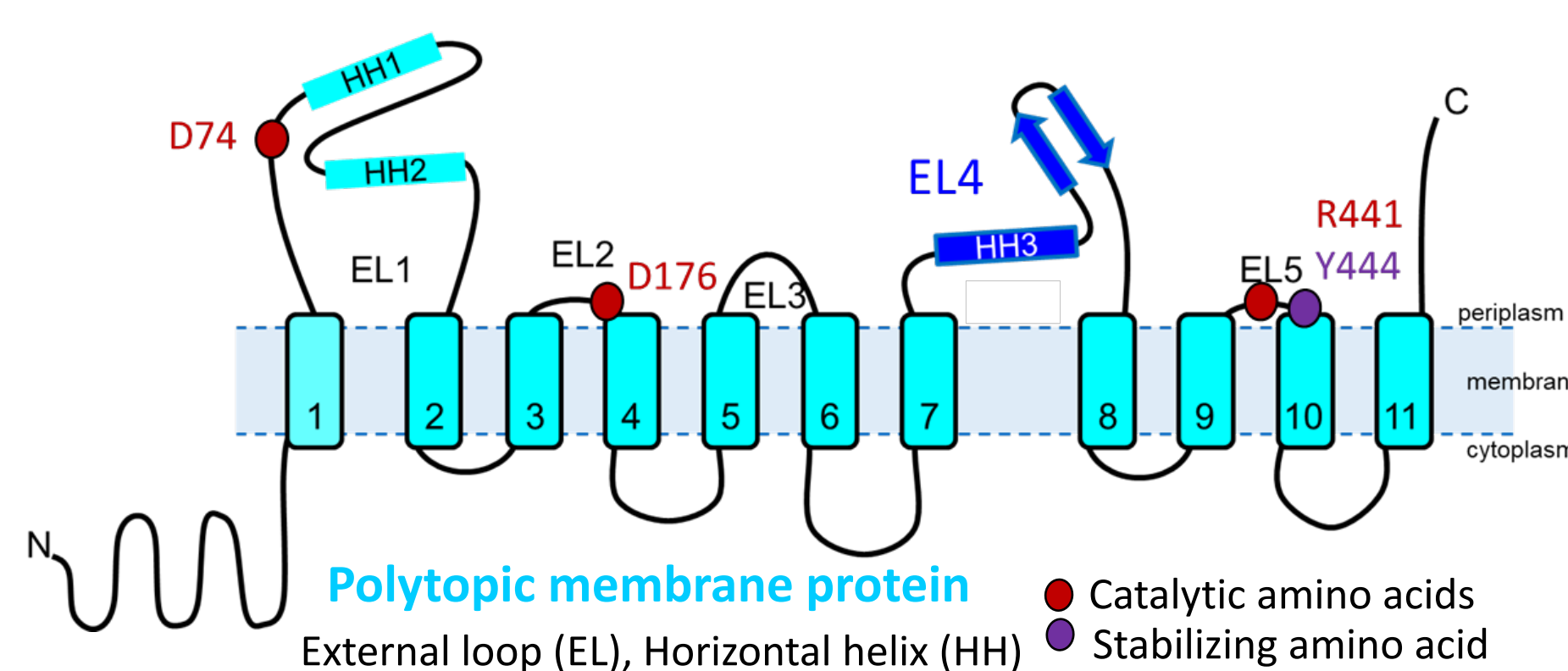
- MtPMT is an integral membrane protein
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- Protein-O-MannosylTransferase (PMT) activity detected in Actinobacteria, yeasts and animals
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- Human PMTs are essential (their mutations lead to congenital muscular dystrophies)

Approach

Deciphering of the MtPMT structure-function relationship by mutational analyses to identify specific features distinguishing the MtPMT from the eukaryote PMTs and that could be specifically targeted for *in-vivo* activity inhibition.

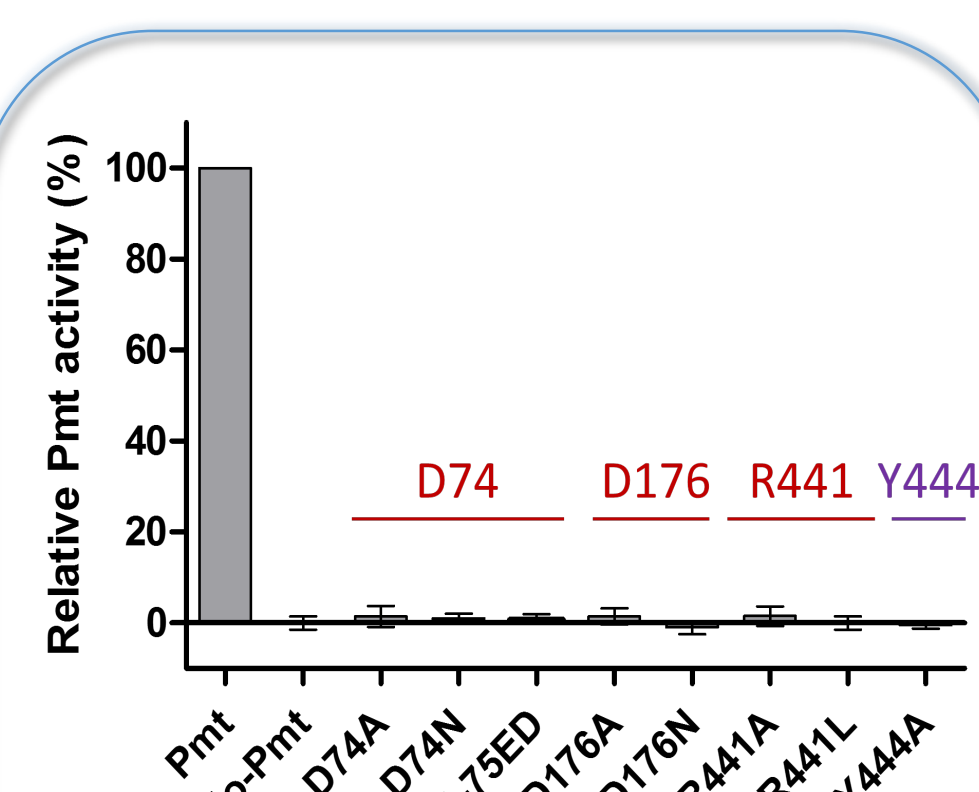
Result I: Understanding the molecular mechanism of MtPMT

MtPMT belongs to the C-Type GlycosylTransferase (GT-C) family. Sequence alignments of MtPMT with 6 GT-Cs (including 2 PMTs) with resolved 3D structures^{4,5,6,7,8} enabled the construction of a 3D model. MtPMT is predicted as a large polytopic membrane protein with 11 transmembrane helices (TMH), 5 cytosolic loops and 5 external loops (EL) associated to the mannosyl transferase activity in the periplasm.

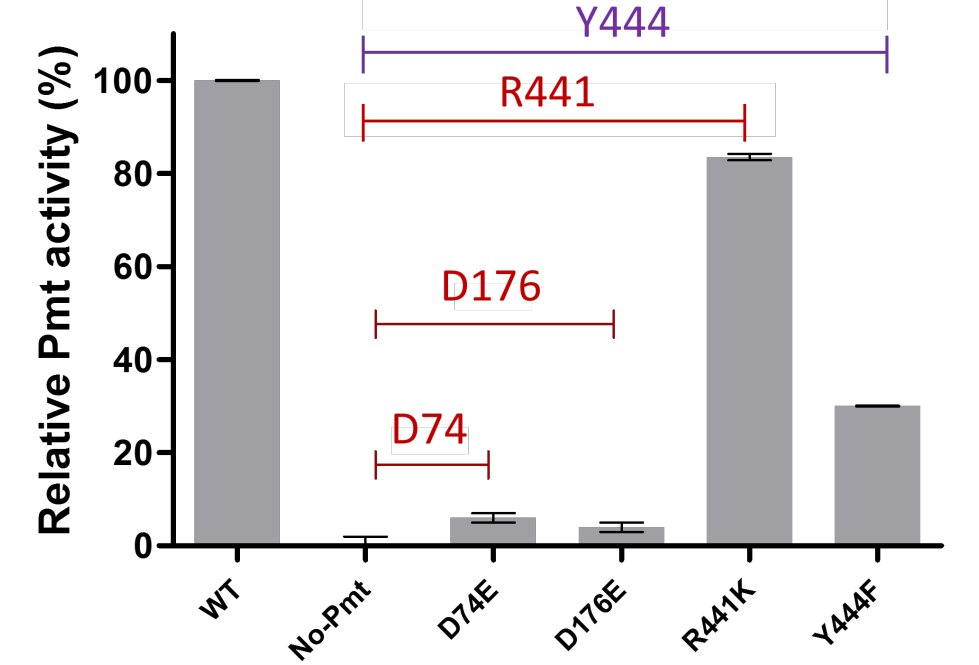


Catalytic triad of MtPMT : D74, D176 and R441

Residues described as catalytic in the GT-Cs are conserved within MtPMT sequence and appear to form a catalytic pocket in the 3D model.



Disruption, by site-directed mutagenesis, of biochemical functions of D74, D176, R441 and Y444 residues prevents totally MtPMT catalysis in bacteria.



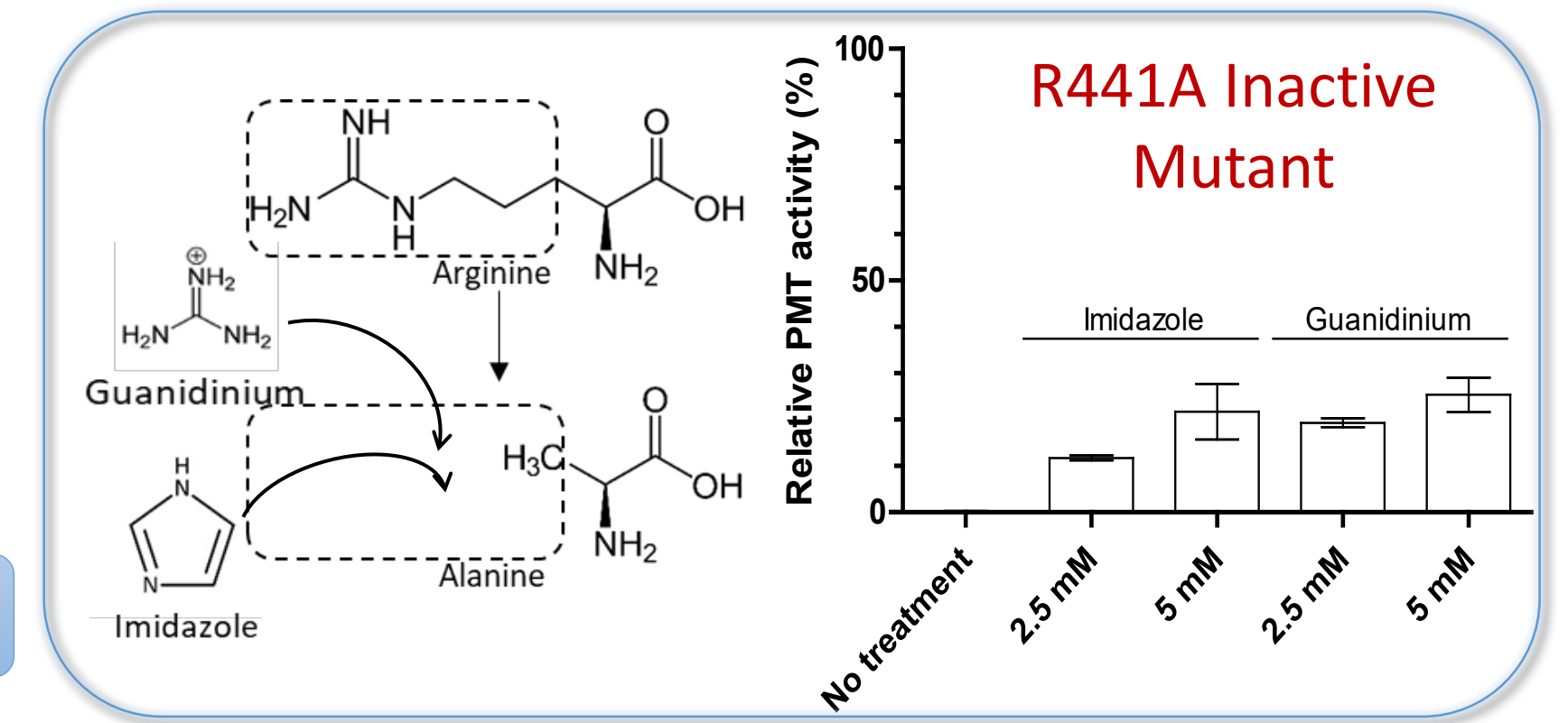
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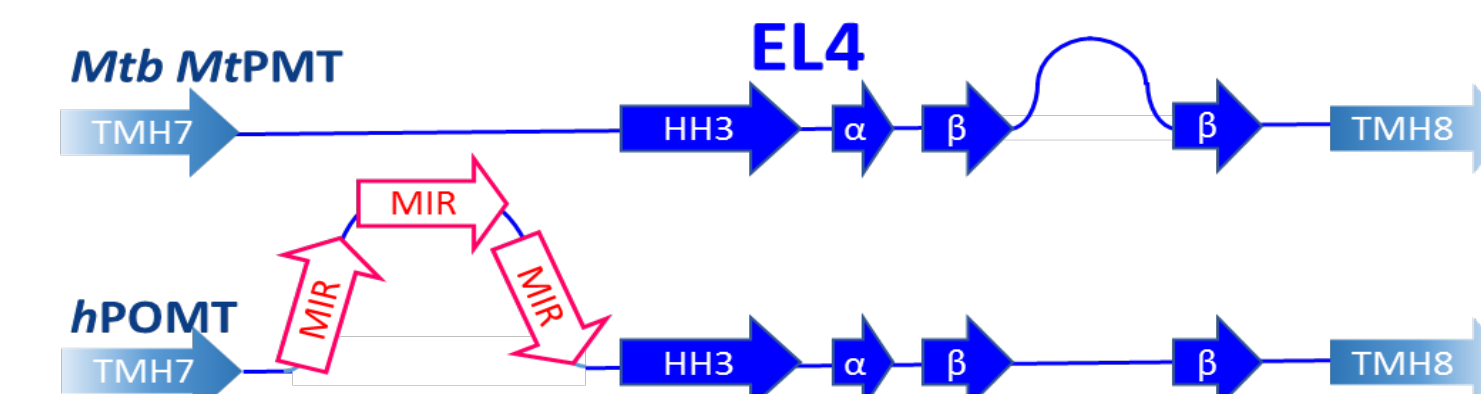
Partial chemical rescue of the lack of activity of the MtPMT R441A mutant by treatment of living bacteria with basic exogenous compounds (imidazole or guanidinium) mimicking the missing side chain of arginine.

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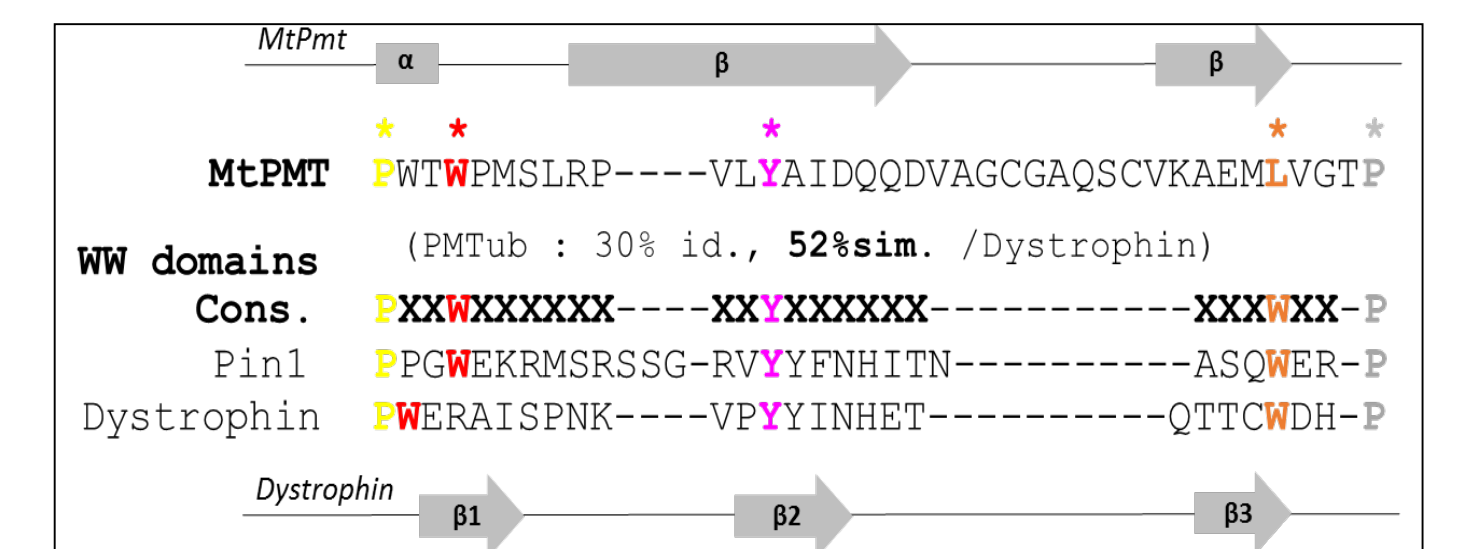
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In order to identify targetable functional motifs specific to MtPMT, the structural differences with GT-Cs and with eukaryotic PMTs were explored revealing the external loop 4 (EL4) as the most divergent structural domain between bacterial and human PMTs.



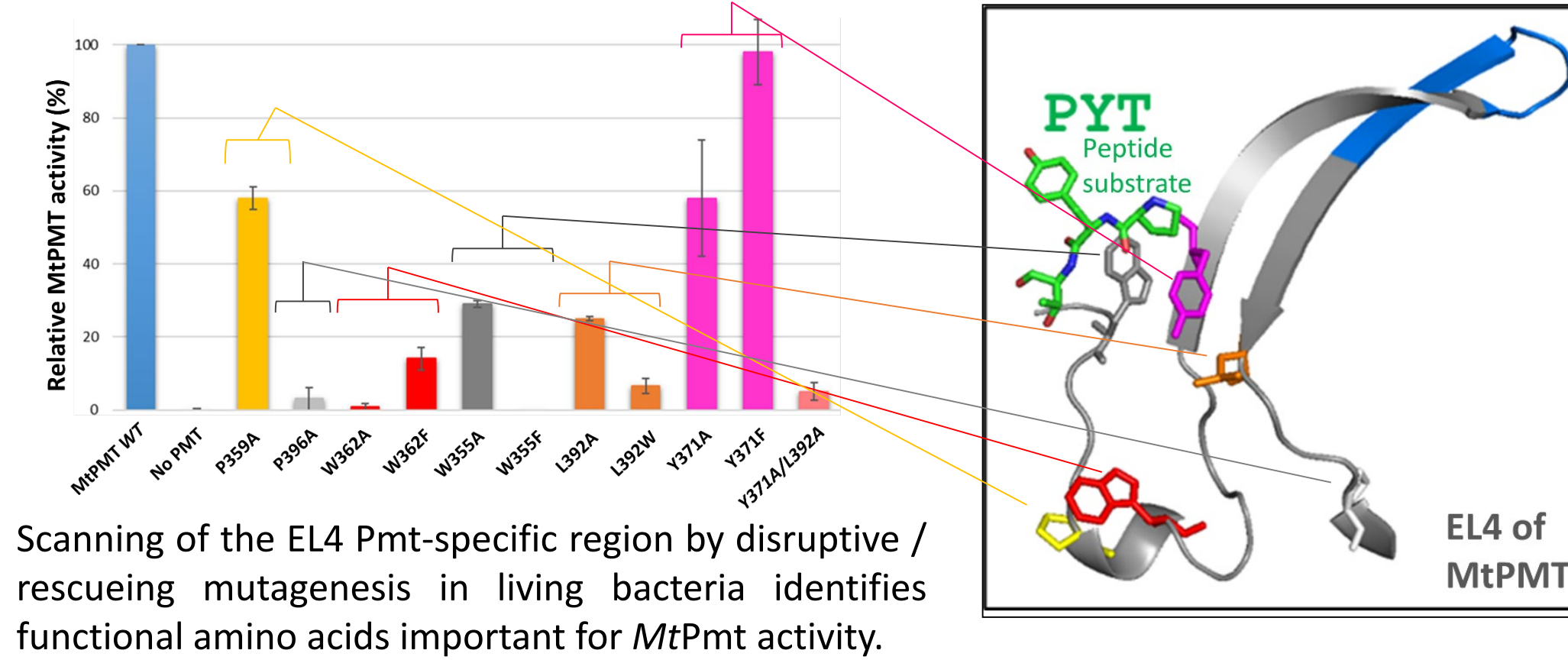
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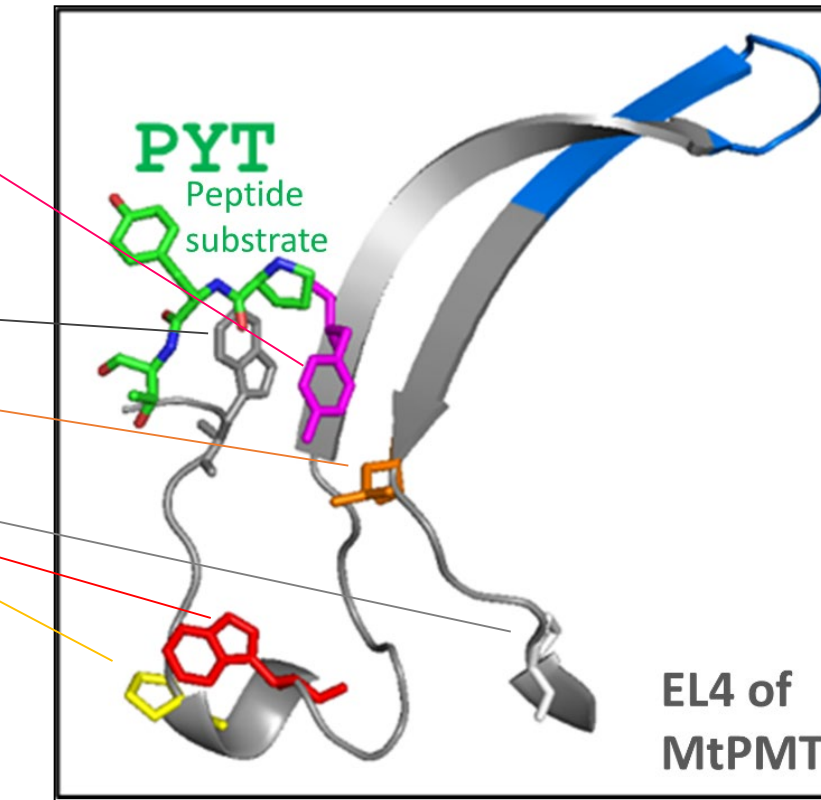


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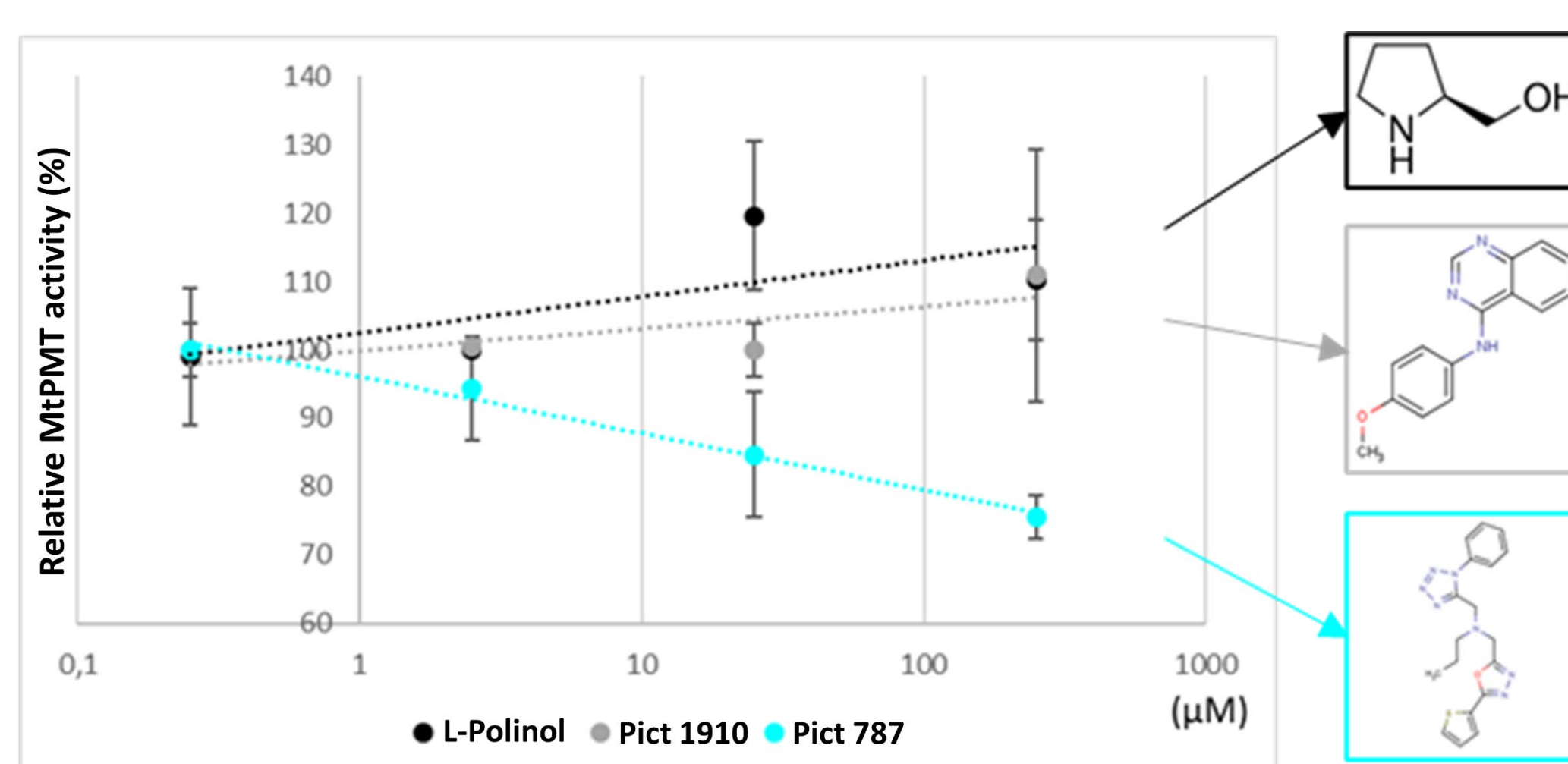


MtPMT EL4 3D model. Colored residues correspond to the consensus amino acids conserved in the WW domains. The blue region corresponds to an insert of 10 mycobacteria-specific residues. Docking simulation of the ScPmt2⁹ PYT peptide substrate (in green) in the MtPMT EL4 homologue domain model.

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Result IV: *In-vivo* targeting protein O-mannosylation with proline analogues



Chemical treatment of living bacteria with the compound Pict787, an analogue of proline-containing peptide, inhibits the WT-MtPMT activity up to 25% with a dose-effect without affecting bacterial growth.

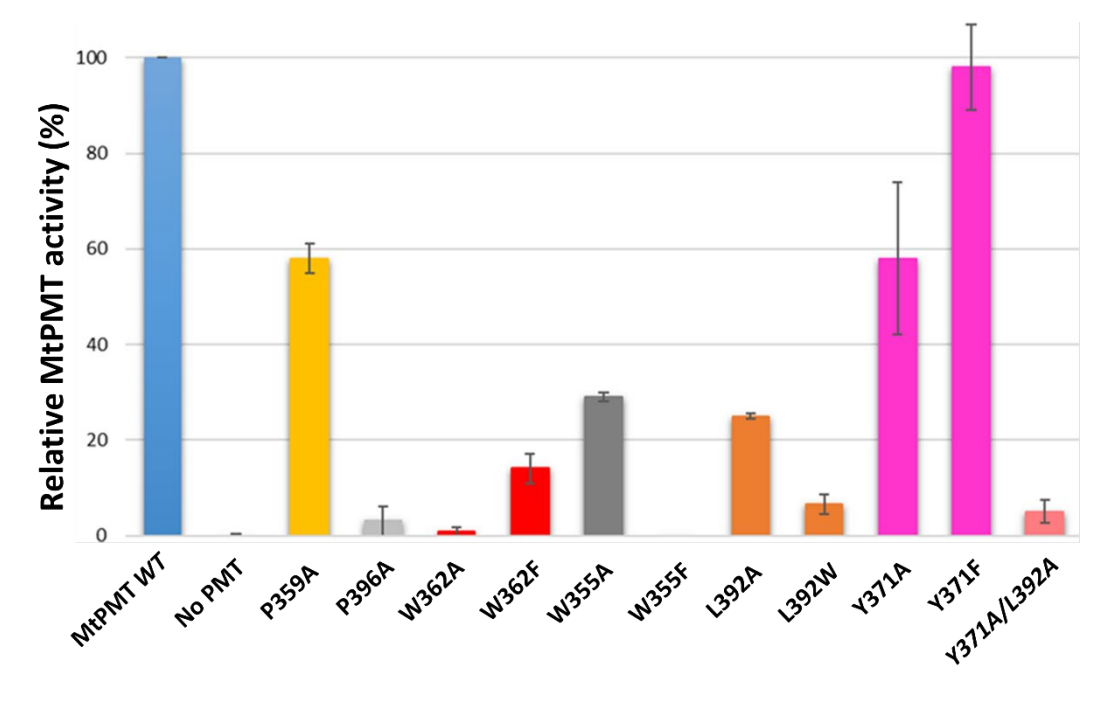
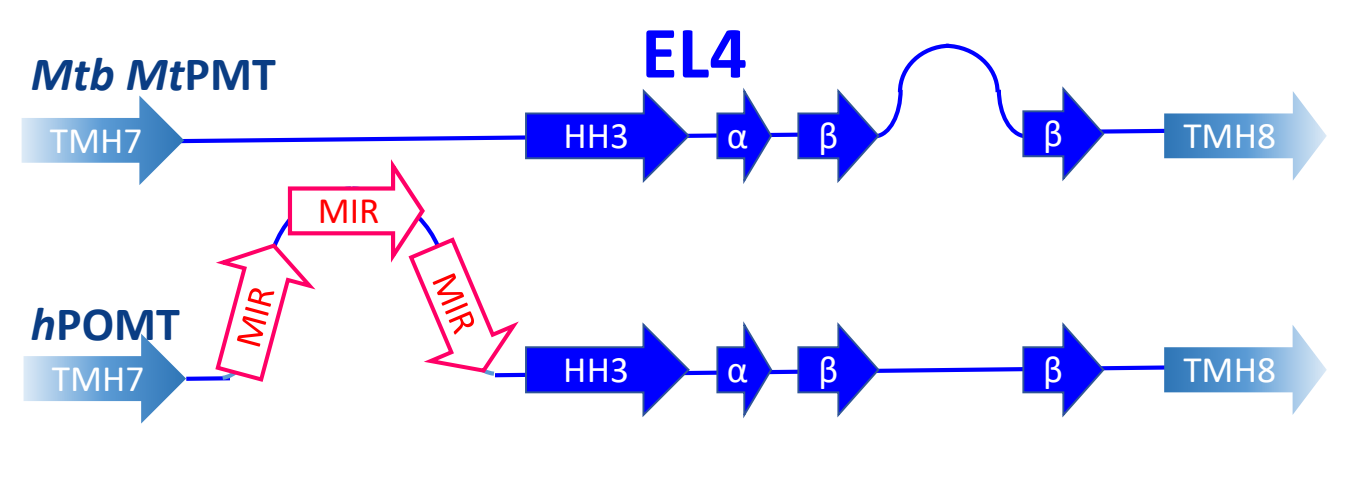
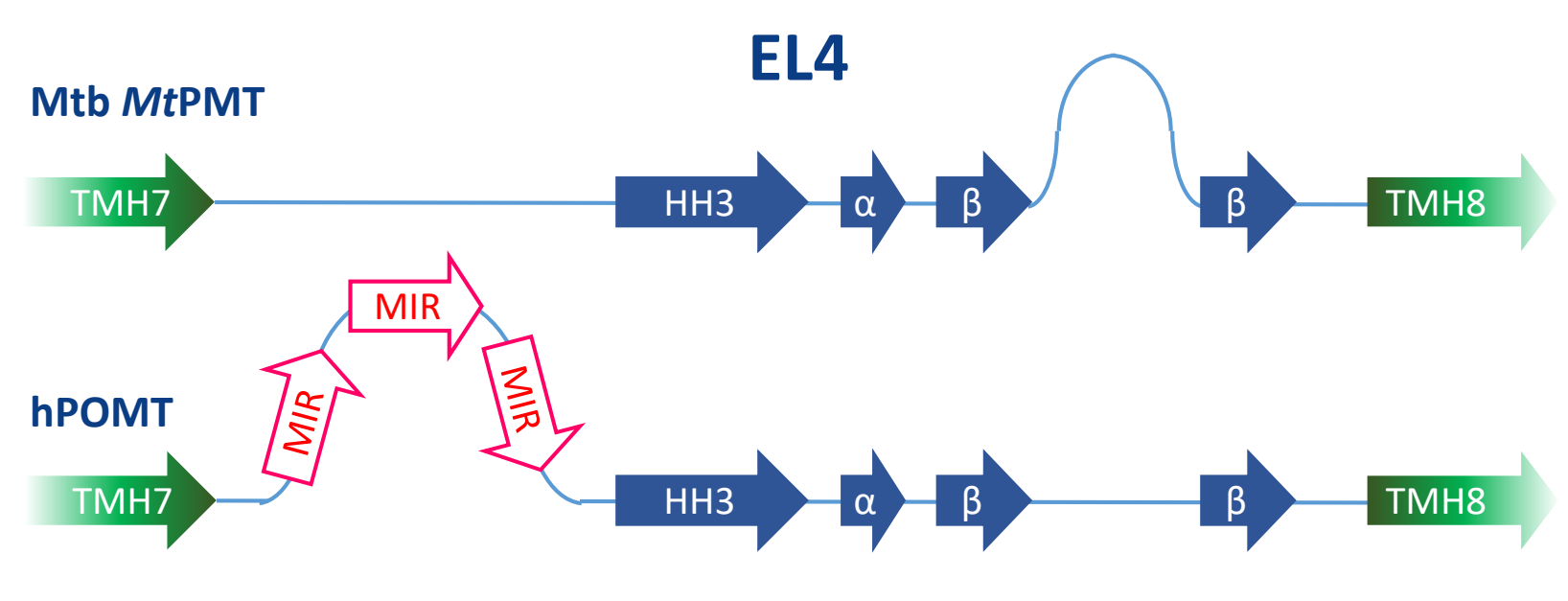
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- Conformational analysis in solution of EL4 isolated domain : WW domains are autonomous modules able to interact with their substrates when isolated products in solution¹⁰. We will explore whether the primary sequence homology of MtPMT EL4 with WW domains induces similar behavior.
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MtPMT : a potential target of innovative therapeutic inhibitors

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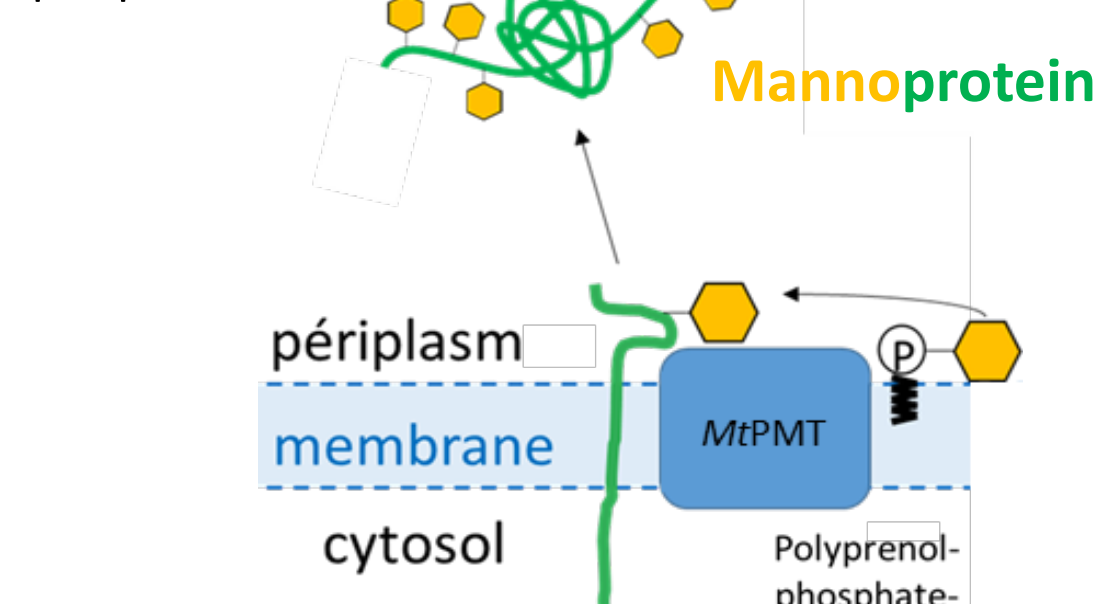
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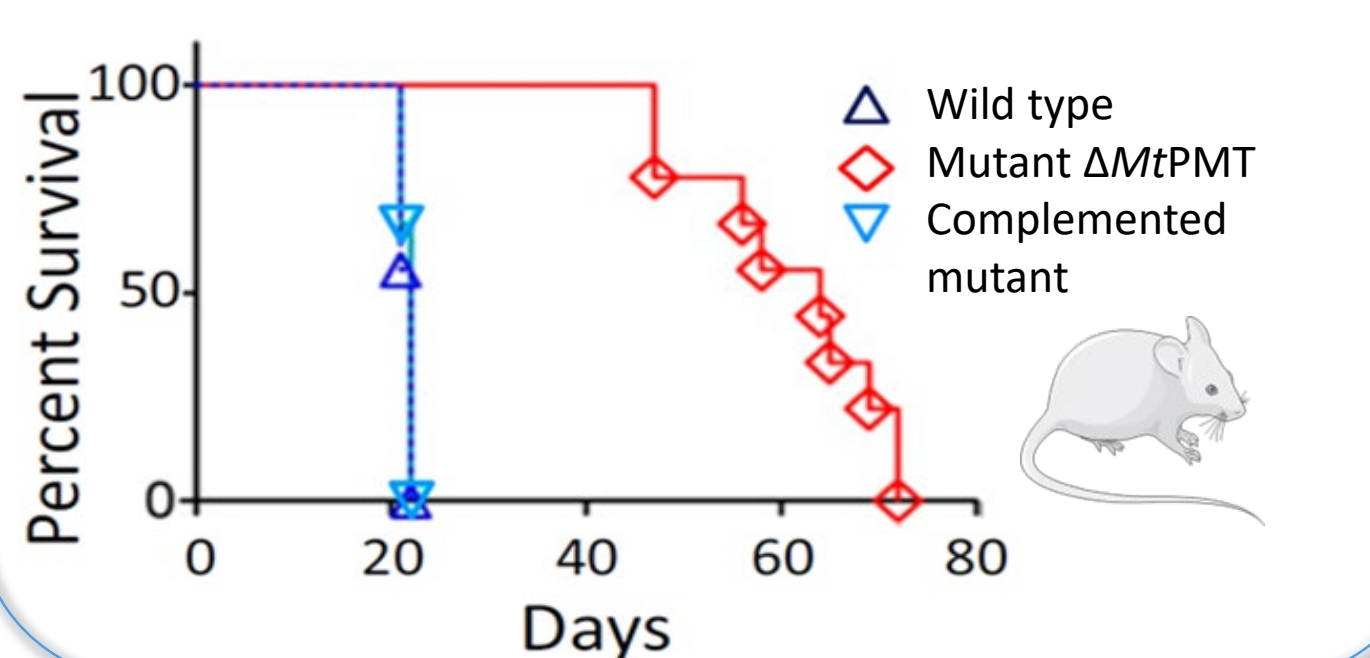
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Bacterial protein-O-mannosylation process as a new virulence factor of Mtb

MtPMT enzyme initiates *M. tuberculosis* protein-O-mannosylation by transferring a mannose residue from a lipid donor to acceptor protein substrates in the bacterial periplasm.



Deletion of MtPMT does not affect *M. tuberculosis* growth *in-vitro* but reduces drastically Mtb infectiousness and virulence in mice by altering the lung intramacrophagic replication (Liu et al.²).



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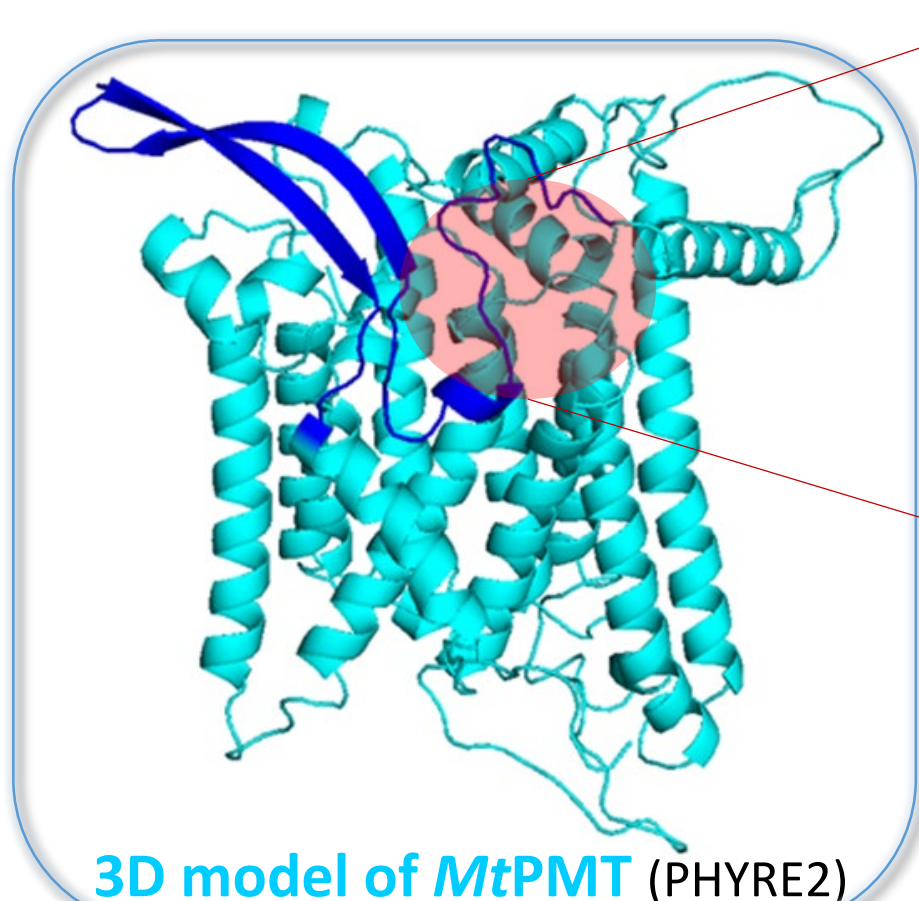
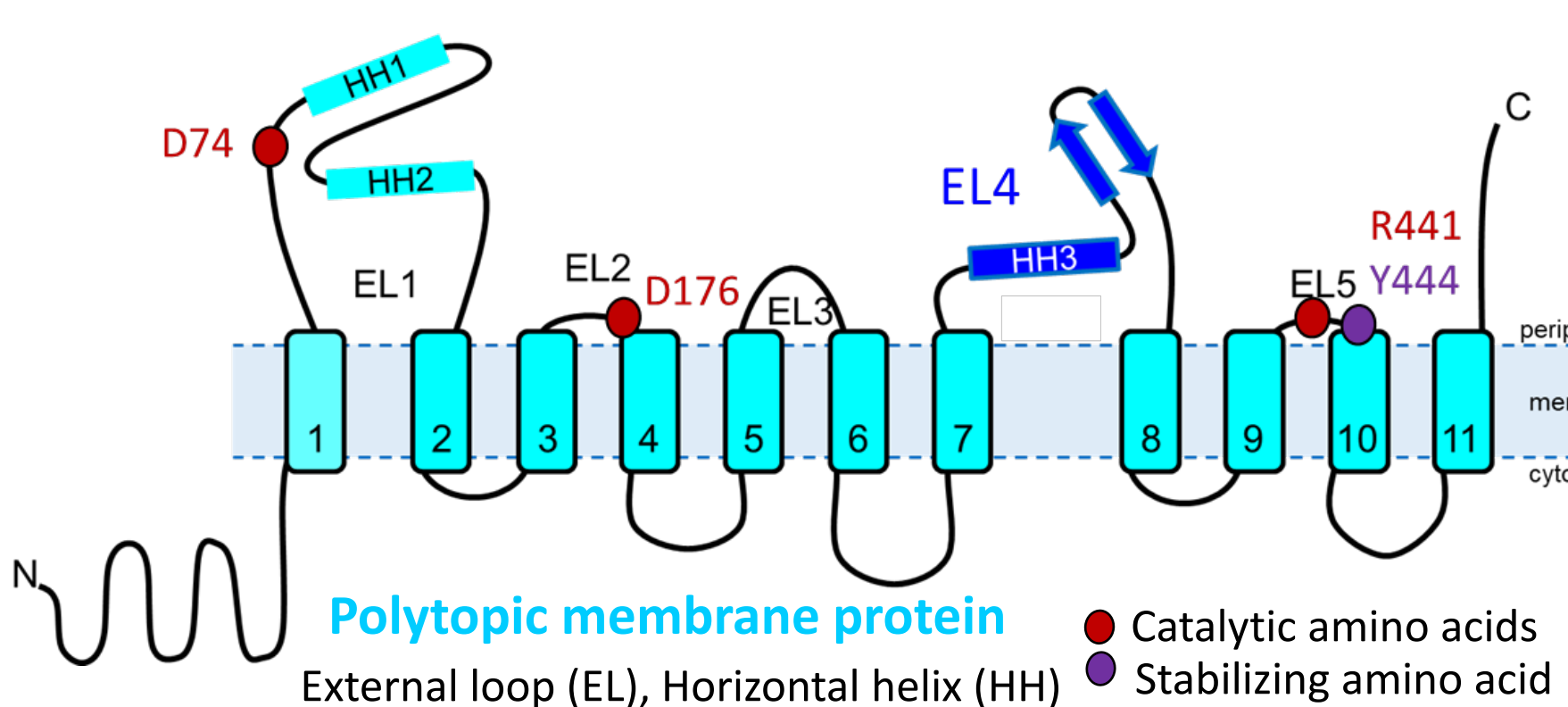
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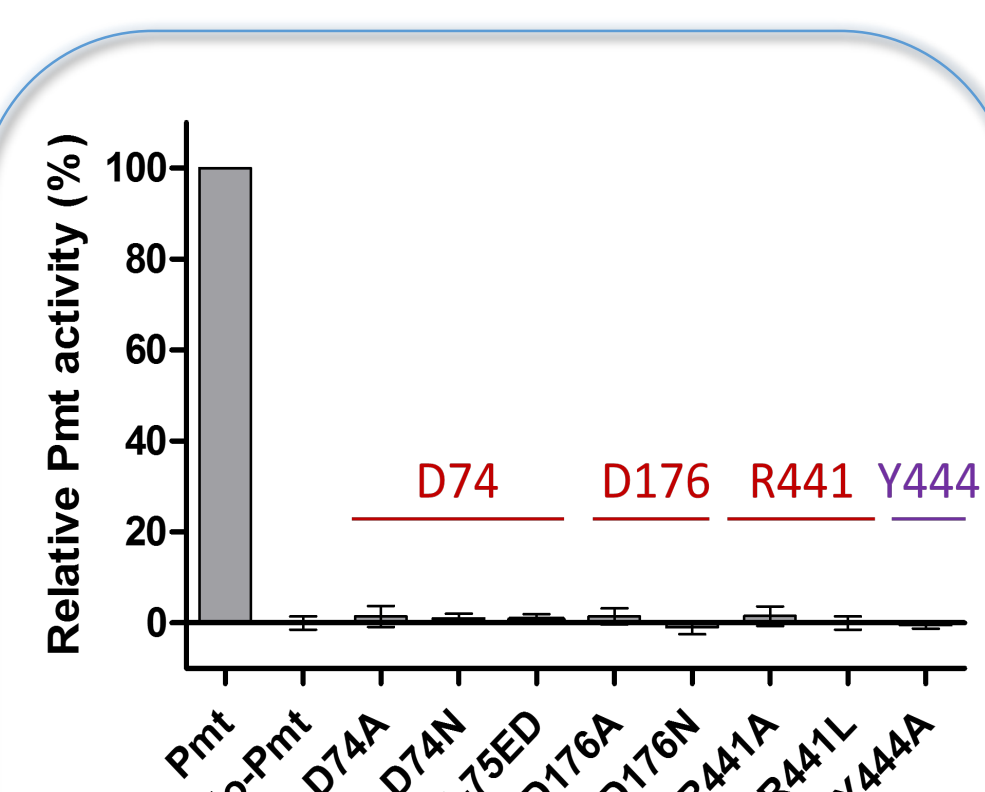
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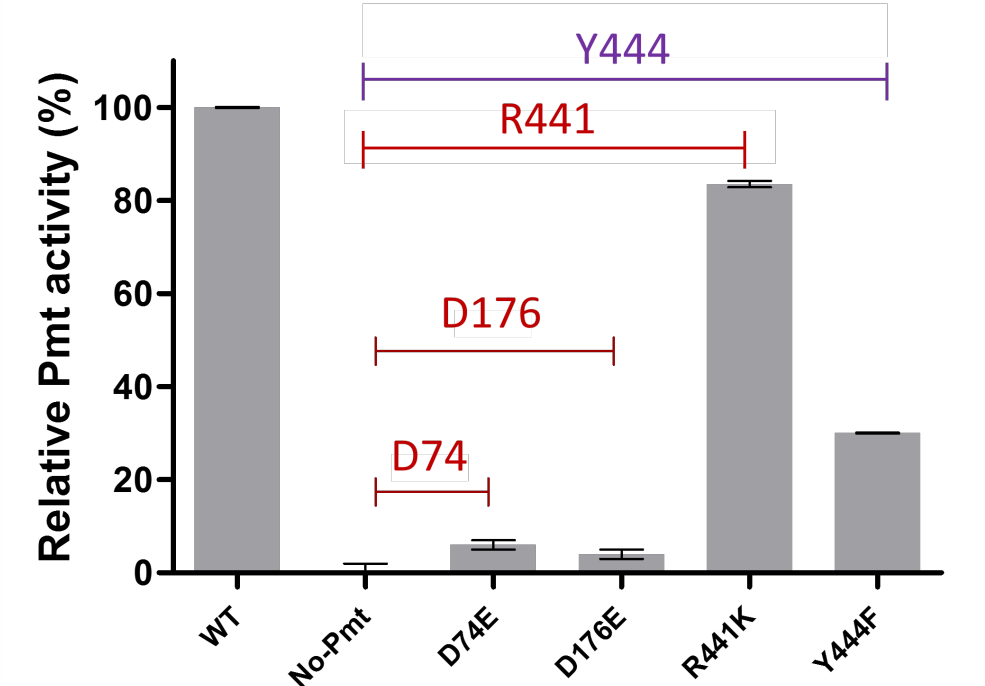


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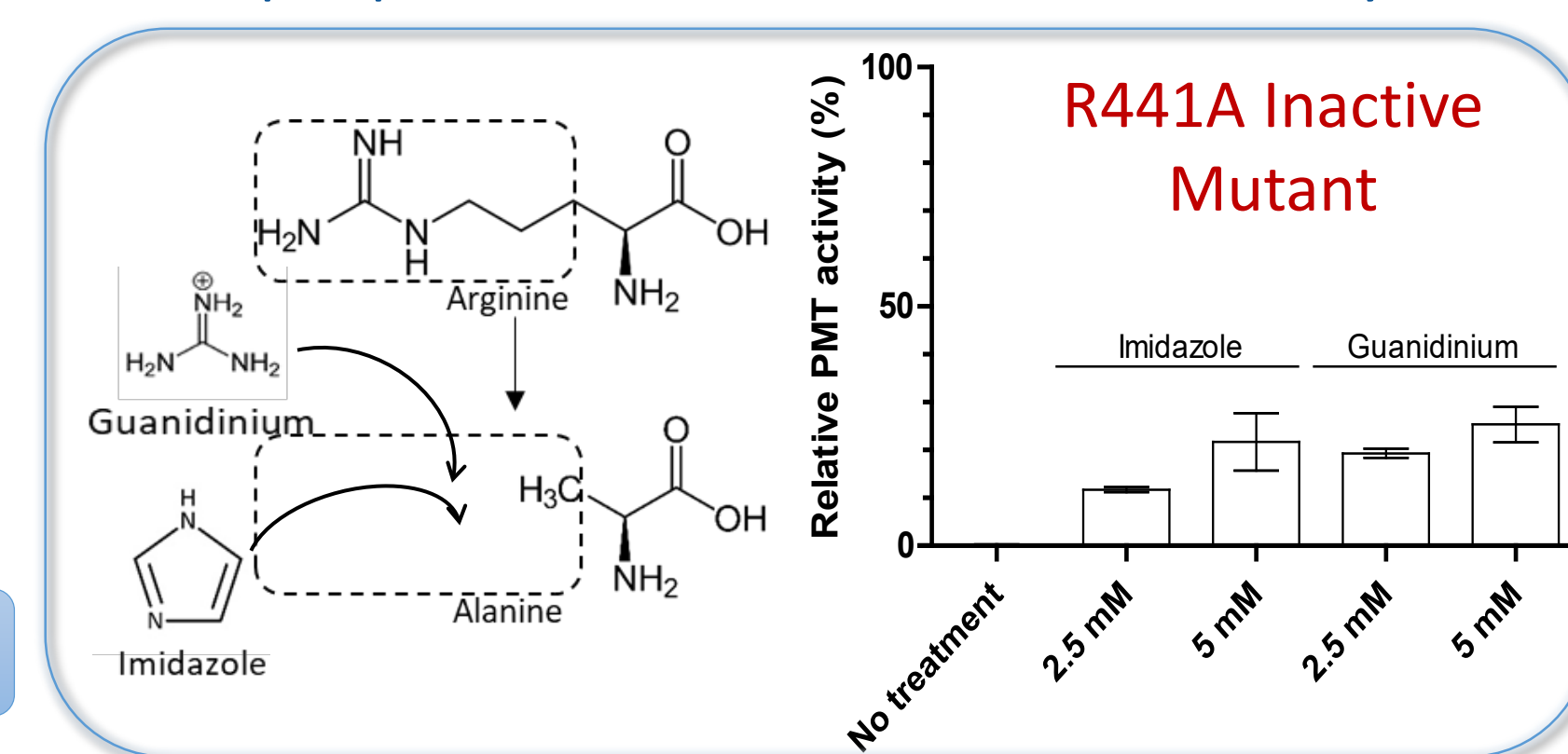
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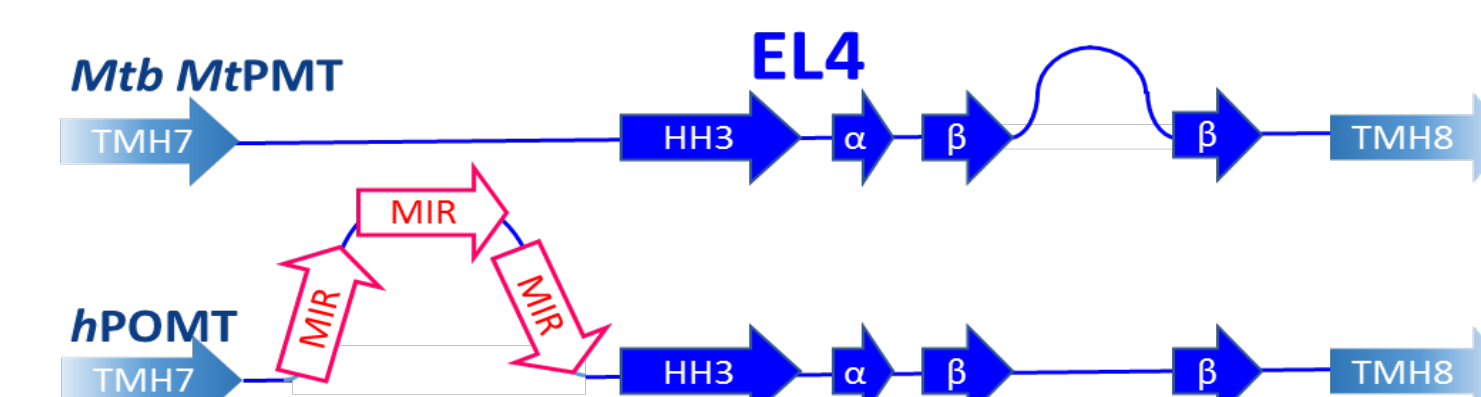
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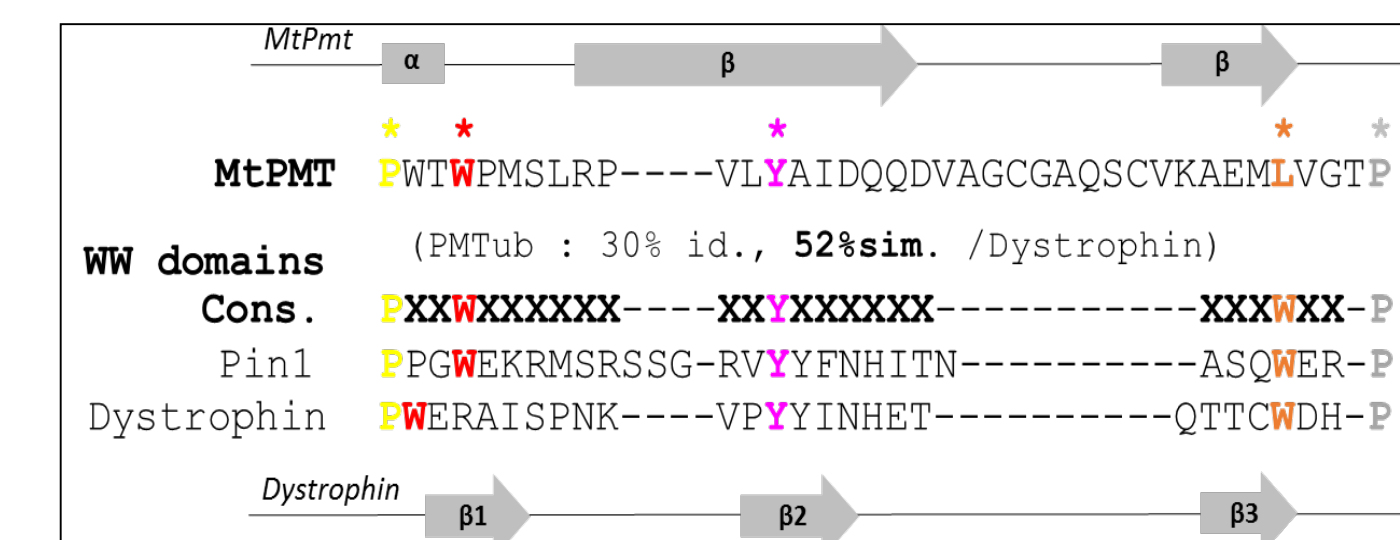
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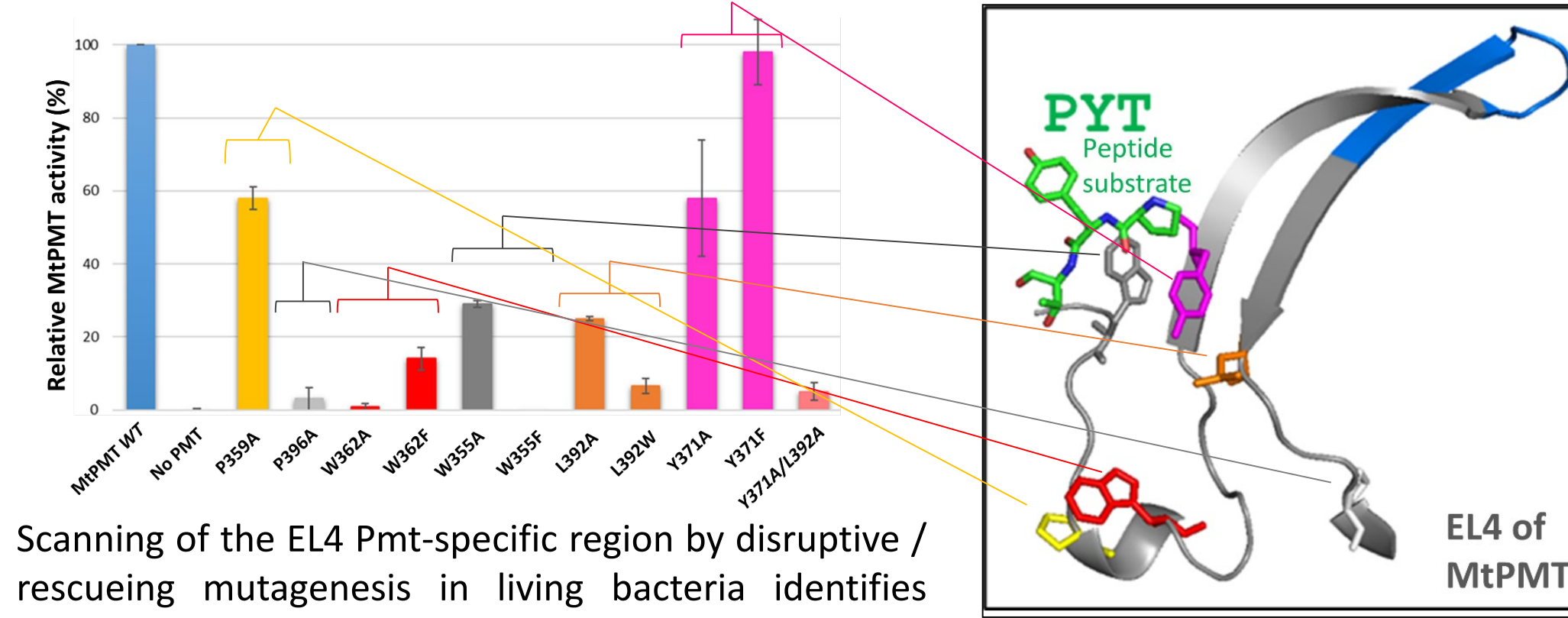
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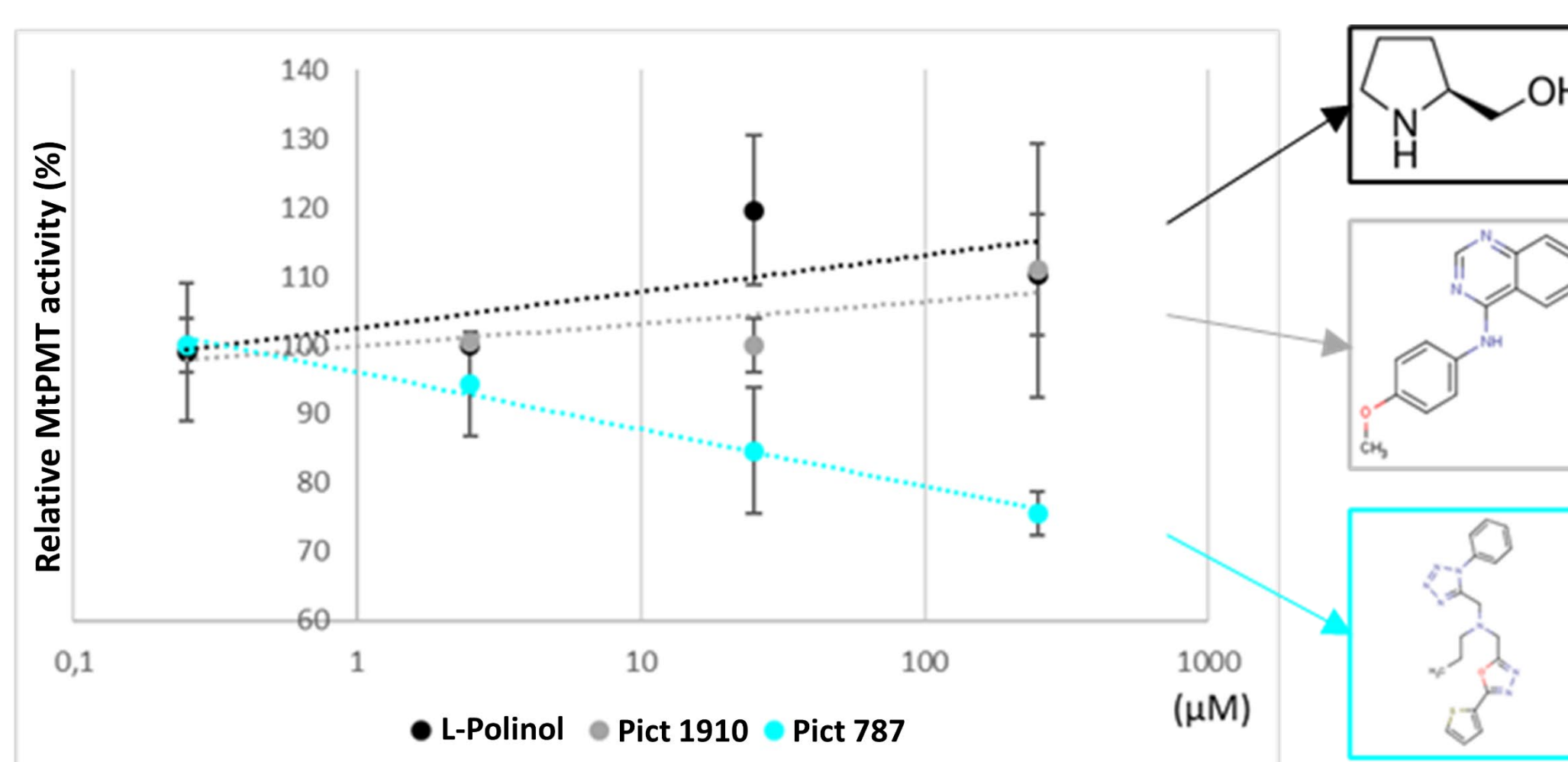


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