Supplemental Information

MptpA kinetics enhanced by allosteric control of an active conformation

Authors

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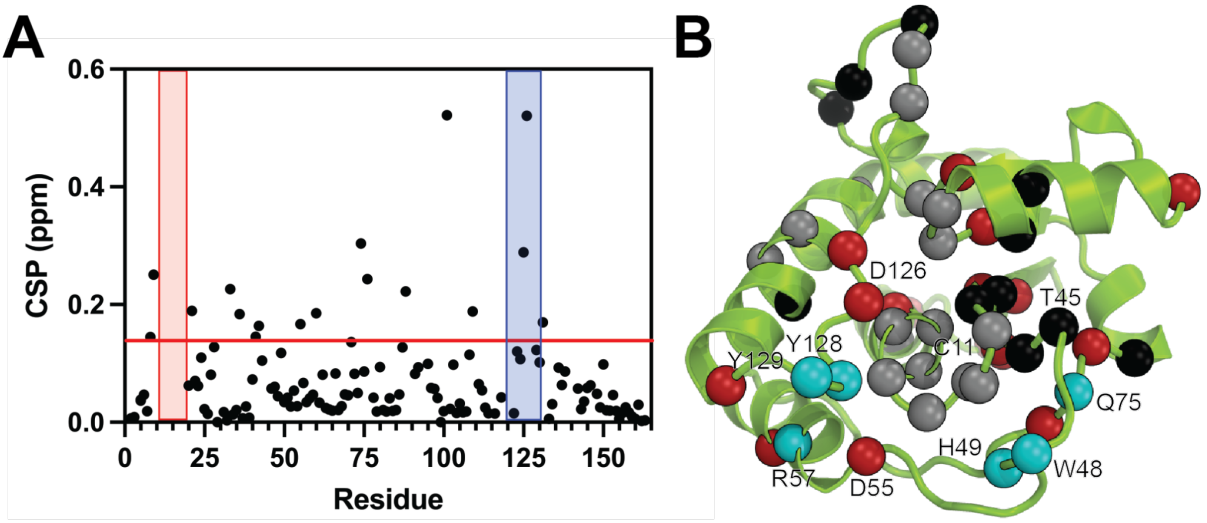


Figure S1. Vanadate induced chemical shift perturbations (CSP).

(A) CSP of combined 1HN, 15NH, and 13Cα are plotted using equation 1 versus residue. Values > 1.5 times the standard deviation of the 10% trimmed mean are indicated as data points above the red horizontal line. The P-loop and acid loop are indicated with red and blue shaded bars respectively. For the P-loop, residues are either exchange broadened or not assigned in either or both the apo and vanadate bound spectra. (B) Residues with significant chemical shift perturbations are shown as red spheres. Residues with shifts that are missing in either the apo or bound spectra are shown in black and unassigned residues are shown in gray. Additional key residues specified in the main text are shown in teal.

Residues with significant CSP values	Unassigned residues	Residues with ambiguous assignments in apo or vanadate bound states
8, 9, 21,33, 36, 41, 42, 55, 60, 74, 76, 88, 101, 109, 125, 126, and 131	12-19, 89, 90, 110, 120, 121, 140, 141, 147	1, 10, 11, 30, 44, 45, 77, 93, 94, 115, 117, 119, 139

Table S1. Vanadate induced CSPs.

A CSP is considered significant if the value is greater than 1.5 times the standard deviation of the 10% trimmed mean. An unassigned residue is defined as any residue whose respective resonance could not be found in the acquired spectra. A residue is considered ambiguous if the resonance was only identified in the apo or vanadate bound spectra. This could be a result of resonance appearing or broadening once ligand binds.

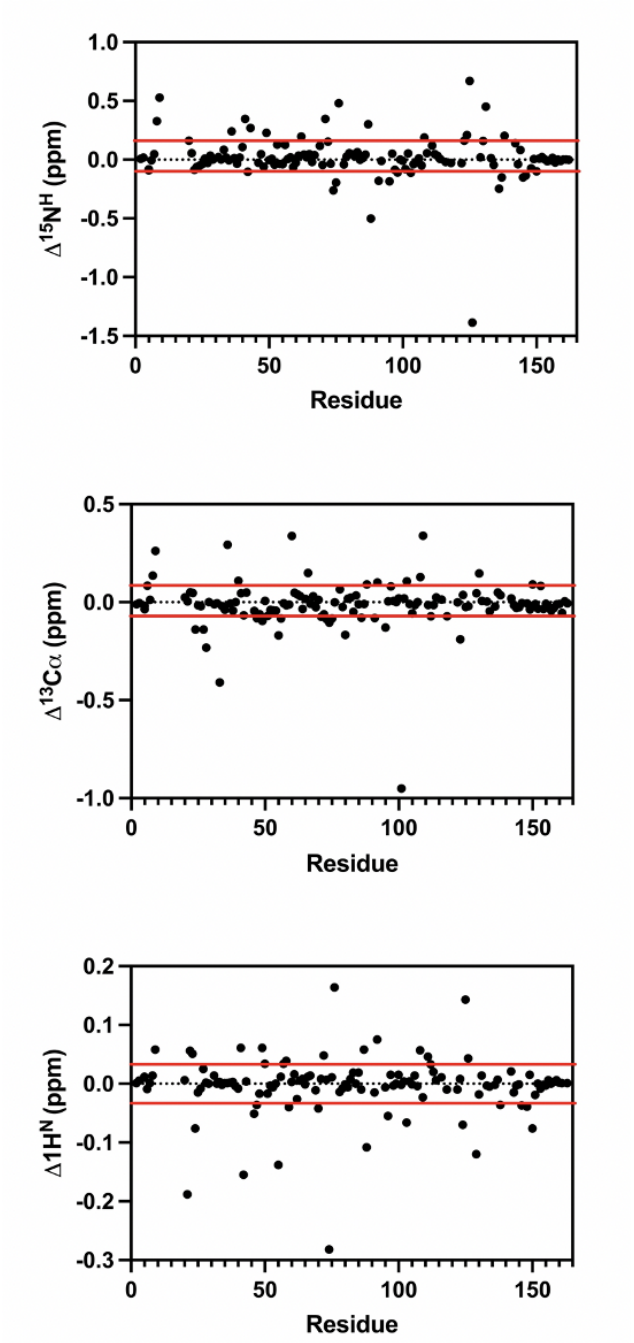


Figure S2. Vanadate induced chemical shift perturbations.

Chemical shift changes (apo-vanadate) are plotted versus residue. The red lines mark the upper and lower cutoffs for CSP values > 1.5x STD. Dev of the 10% trimmed mean.

Experiment	**Residues with significant CSP values**	**Unassigned residues**	**Residues with ambiguous assignments in WT or Q75L spectra**
HNCA	11, 43, 44, 46, 48, 49, 50, 72, 74, 75, 77, 89, and 118	1, 2, 12-19, 78, 119-121, 128, 132, and 135	45 and 47
HNCO	43, 48, 49, 50, 72, 73, 74, 75, 76, 80, 81, and 90	1, 2, 11-19, 78, 119-121, 128, 132, 135	36, 45, 47, 105, and 140

Table S2. Q75L mutation induced CSPs.

A CSP is considered significant if the value is greater than 1.5 times the standard deviation of the 10% trimmed mean. An unassigned residue is defined as any residue whose respective resonance could not be found in the acquired spectra. A residue is considered ambiguous if the resonance was only identified in the apo or mutant spectra. This can occur due to resonance broadening upon introduction of the mutation.

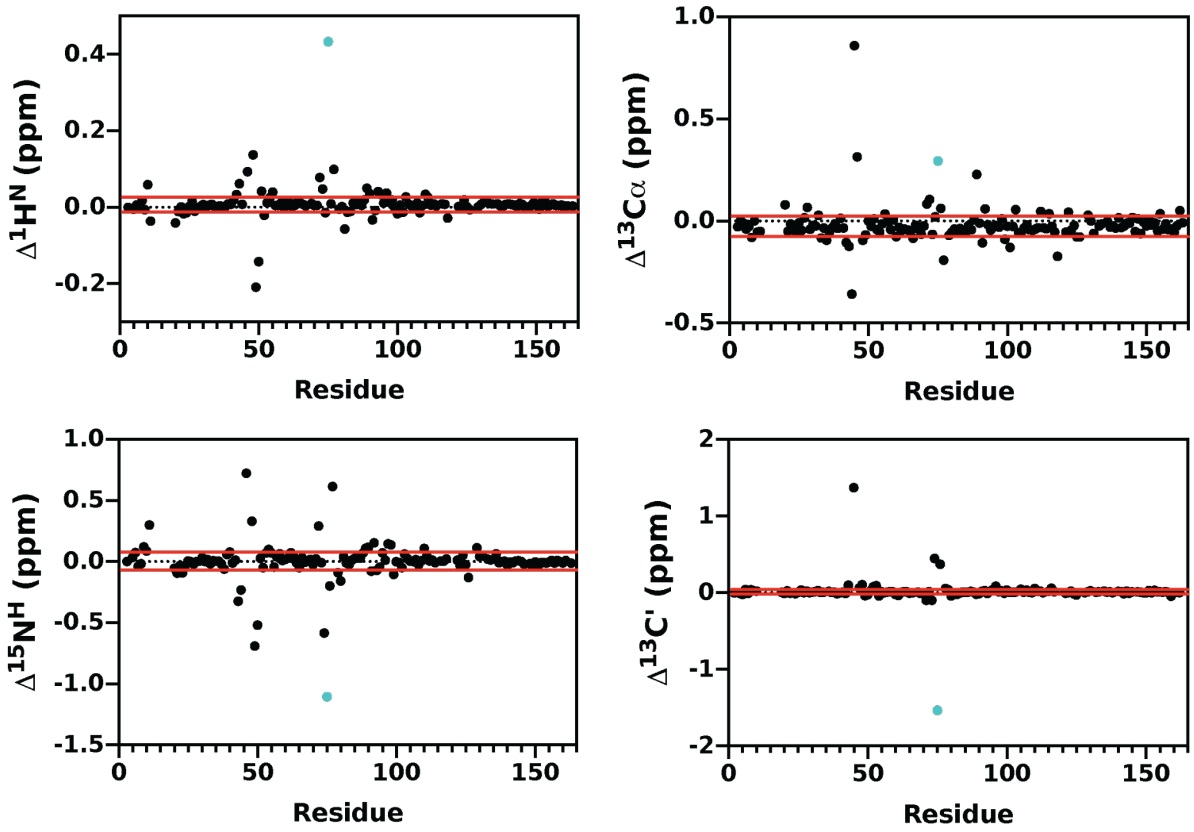
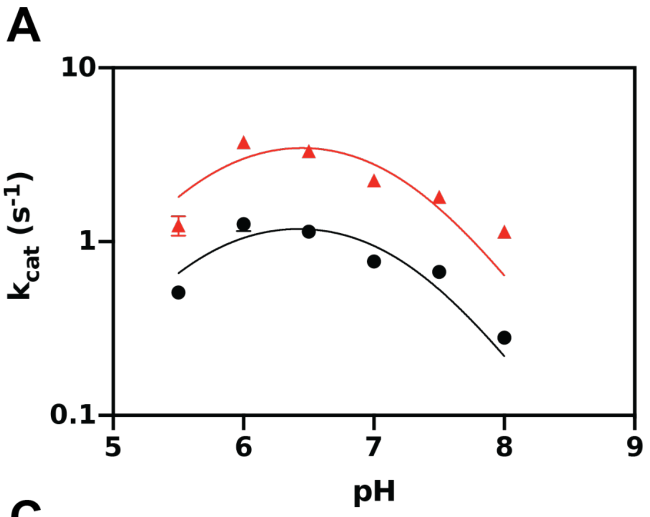


Figure S3. Q75L mutation induced CSPs.

Chemical shift changes (*wt* – Q75L) are plotted versus residue. The red lines mark the upper and lower cutoffs for CSP values >1.5X Std. Dev of the 10% trimmed mean. The teal circle is the site of the mutation.



B

pH	WT k_{cat} (s^{-1})	Q75L k_{cat} (s^{-1})
5.5	0.51 ± 0.04	1.24 ± 0.16
6	1.26 ± 0.11	3.74 ± 0.19
6.5	1.14 ± 0.05	3.32 ± 0.14
7	0.77 ± 0.02	2.25 ± 0.07
7.5	0.67 ± 0.05	1.81 ± 0.06
8	0.28 ± 0.01	1.14 ± 0.03

C

pH	5.5	6.0	6.5	7.0	7.5	8.0
Q75L/WT (k_{cat})	2.4 ± 0.4	3.0 ± 0.3	2.9 ± 0.2	2.9 ± 0.1	2.7 ± 0.2	4.0 ± 0.2

Figure S4. Steady State Kinetics over a pH Range for *wt* and Q75L.
(a) The k_{cat} values obtained from a Michaelis-Menten plot for *wt* (Black) and Q75L (Red) were plotted versus pH. Reactions were performed at 21°C in triplicate. The data was fit to $k_{cat} = k_{cat}^{max}/(1+10^{-pH}/10^{-pKa1})+10^{-pKa2}/10^{-pH})$ and are shown as solid lines. Values obtained for pKa1 and pKa2 were nearly identical for *wt* (pKa1: 5.64 ± 0.37 and pKa2: 7.21 ± 0.32) and Q75L (pKa1: 5.70 ± 0.43 and pKa2: 7.20 ± 0.39). (b) Table listing kcat values obtained for *wt* and Q75L at varying pH values. (c) Table listing the k_{cat} ratio of Q75L to *wt* at varying pH values.

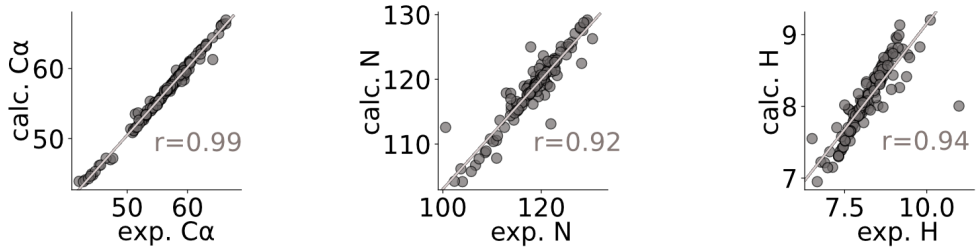


Figure S5. Computed vs. Experimental Shifts. Correlation of NMR $^{1}H^{N}$, $^{15}N^{H}$, and $^{13}C^{\alpha}$ experimental chemical shifts vs. computed shifts (using ShiftX2[43]), averaged along the trajectory of *wt*MptpA. *r* is the Spearman correlation coefficient computed between the experimental and calculated values.

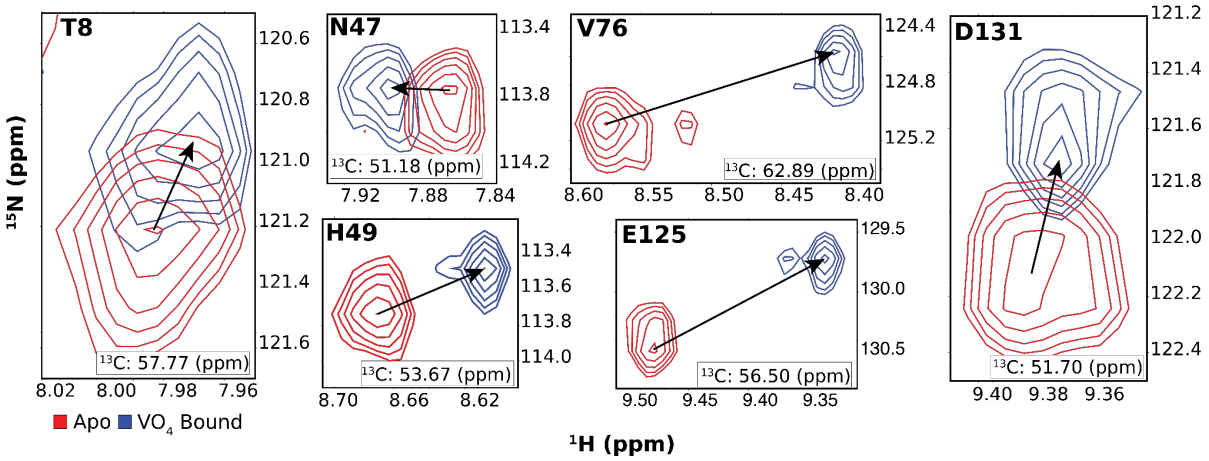


Figure S6. CSP *wt*-to-vanadate bound MptpA. 1H15N CSPs of residues with NMR centrality values over 1.0 STDs of the 10% trimmed mean (T8, N47, H49, E125, and D131) or in the Q loop proximal to Q75 (V76)

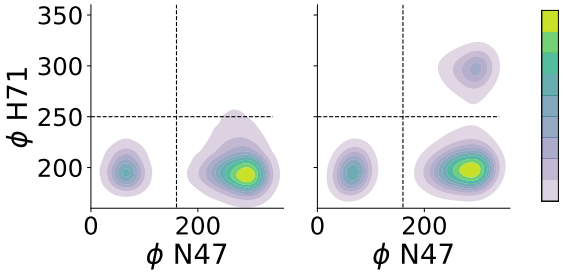


Figure S7. Conformational landscape obtained from the ϕ dihedral angle of N47 and H71. Unlike in *wt*MptpA, the absence of the Q75-N47 hydrogen bond allows for a change in the relative orientation of the W-loop and Q-loop, resulting in the rotation of H71.

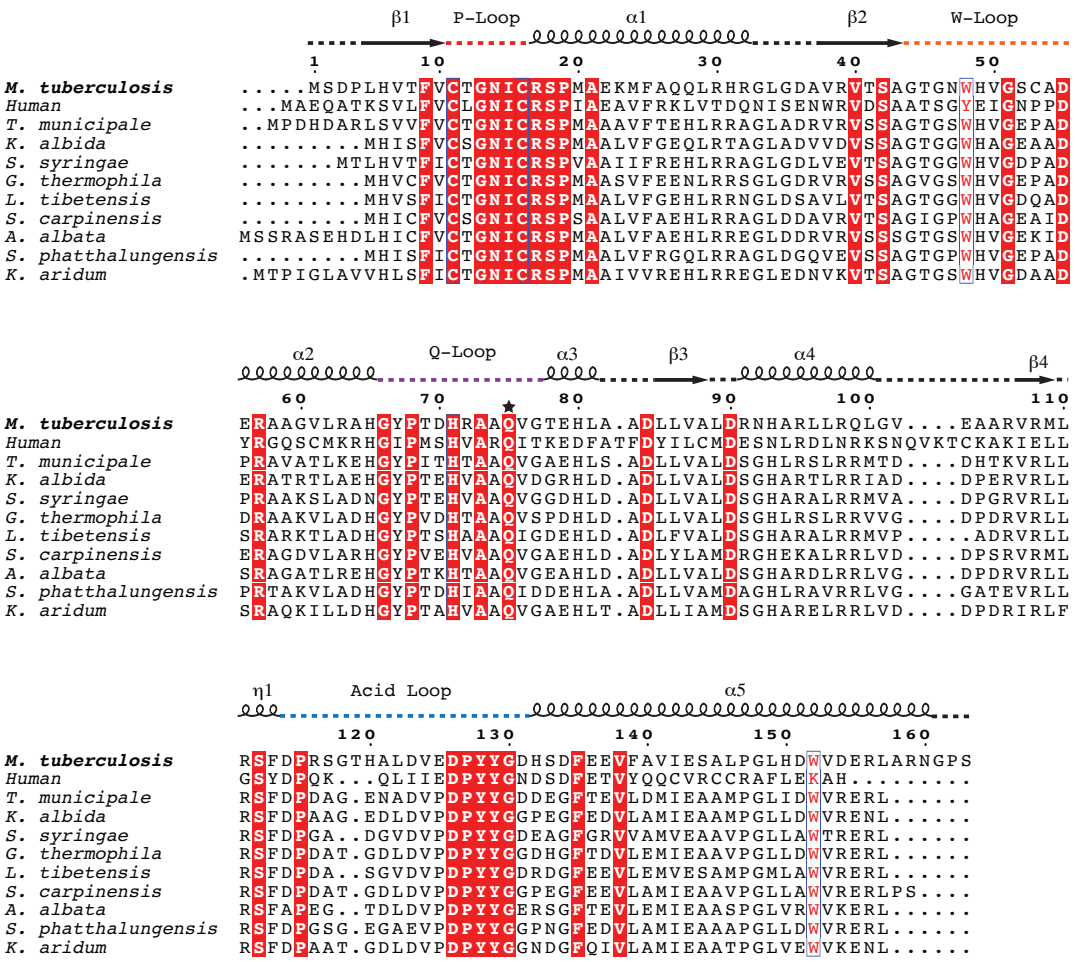


Figure S8. Sequence Alignment. The sequence for mPTPA from *M. Tuberculosis* was aligned against ten proteins identified using BLAST (except for the human LMWPTP) and aligned using Clustal Omega followed by ESPript 3.0. Residues in red boxes are considered strictly conserved and red residues are those with similarity scores above the SimilarityGlobalScore (0.7) using a BLOSUM62 scoring matrix. The secondary structures have been included (PDB: 2LUO) with the P-loop (red), W-loop (orange), Q-loop (purple), and acid loop (blue) specified. Residue Q75 is indicated by the black star.

References:

1. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol. 1990 Oct 5;215(3):403-10. doi: 10.1016/S0022-2836(05)80360-2. PMID: 2231712.

2. Madeira F, Park YM, Lee J, et al. The EMBL-EBI search and sequence analysis tools APIs in 2019. Nucleic Acids Research. 2019 Jul;47(W1):W636-W641. DOI: 10.1093/nar/gkz268. PMID: 30976793; PMCID: PMC6602479.

3. Xavier Robert, Patrice Gouet, Deciphering key features in protein structures with the new ENDscript server, Nucleic Acids Research, Volume 42, Issue W1, 1 July 2014, Pages W320–W324, https://doi.org/10.1093/nar/gku316.