Supplementary data for:

# The *Mycobacterium tuberculosis* protein tyrosine phosphatase MptpA features a pH dependent activity overlapping the bacterium sensitivity to acidic conditions

Michael Kovermann[a], Alessandra Stefan[b,c], Chiara Palazzetti[b], Fabian Immler[a], Fabrizio Dal Piaz[d], Luca Bernardi[e], Valentina Cimone[b], Maria Laura Bellone[f], Alejandro Hochkoeppler[b,c]*

[a]Department of Chemistry, University of Konstanz, Universitätstraße 10, 78464 Konstanz (Germany)

[b]Department of Pharmacy and Biotechnology, University of Bologna, Viale Risorgimento 4, 40136 Bologna (Italy)

[c]CSGI, University of Firenze, Via della Lastruccia 3, 50019 Sesto Fiorentino (Italy)

[d]Department of Medicine, University of Salerno, Via S. Allende, 84082 Baronissi (Italy)

[e]Department of Industrial Chemistry "Toson Montanaro", University of Bologna, Viale Risorgimento 4, 40136 Bologna (Italy)

[f]Department of Pharmacy, University of Salerno, Via Giovanni Paolo II 132, 84084 Fisciano (Italy)

*Corresponding Author. e-mail: a.hochkoeppler@unibo.it

## Figure S1

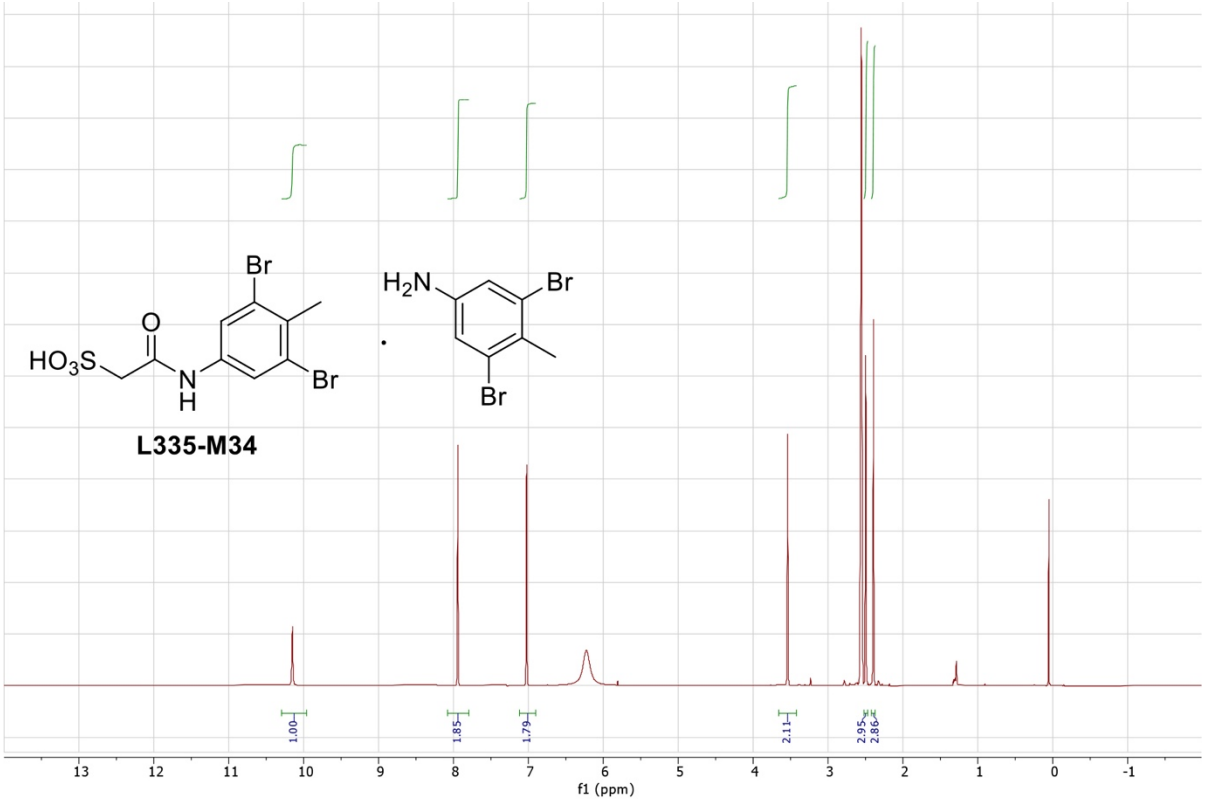


**Figure S1. $^{1}$H NMR spectrum (300 MHz, DMSO-$d_6$) of L335-M34 salified with 3,5-dibromo-4-methyl aniline (white solid).**

## Figure S2

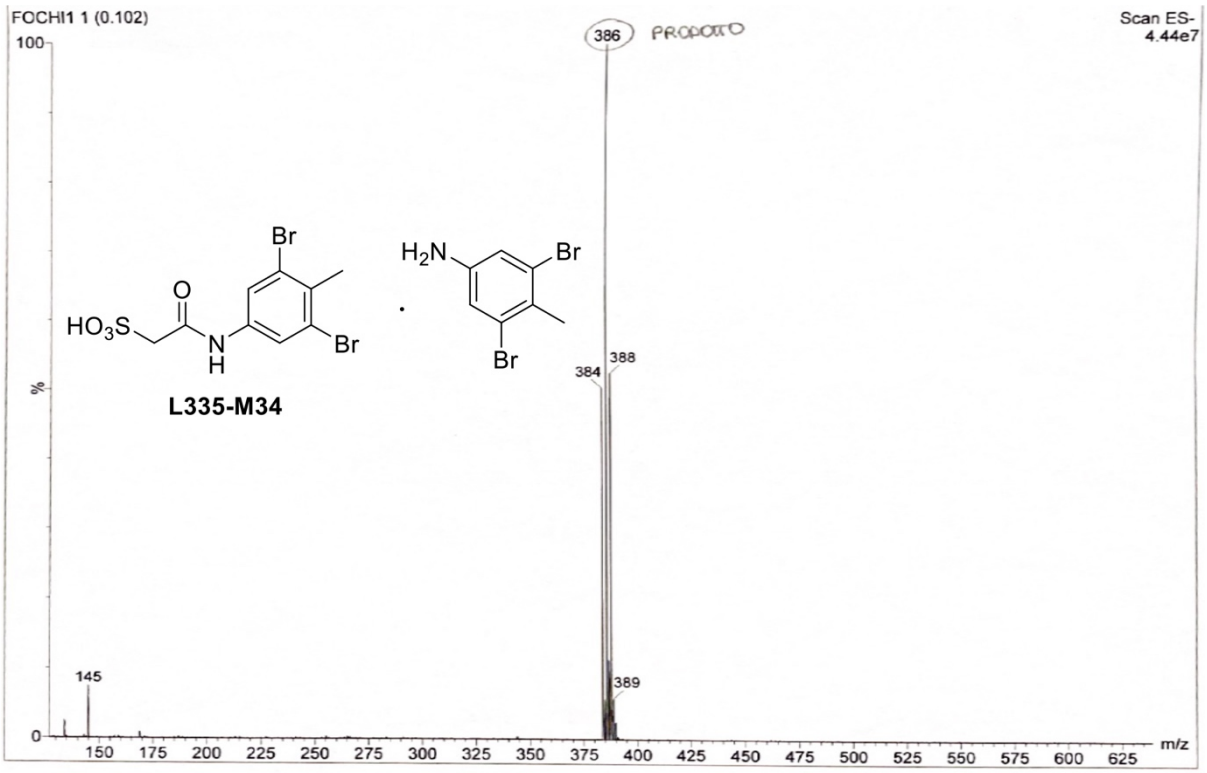


**Figure S2. ESI-MS spectrum of L335-M34 salified with 3,5-dibromo-4-methyl aniline (white solid).**

The expected molar mass of L335-M34 (ESI-MS for $[C_9H_9Br_2NO_4S - H^+]^-$) accounts to 383.8541 Da.

# FIGURE S3

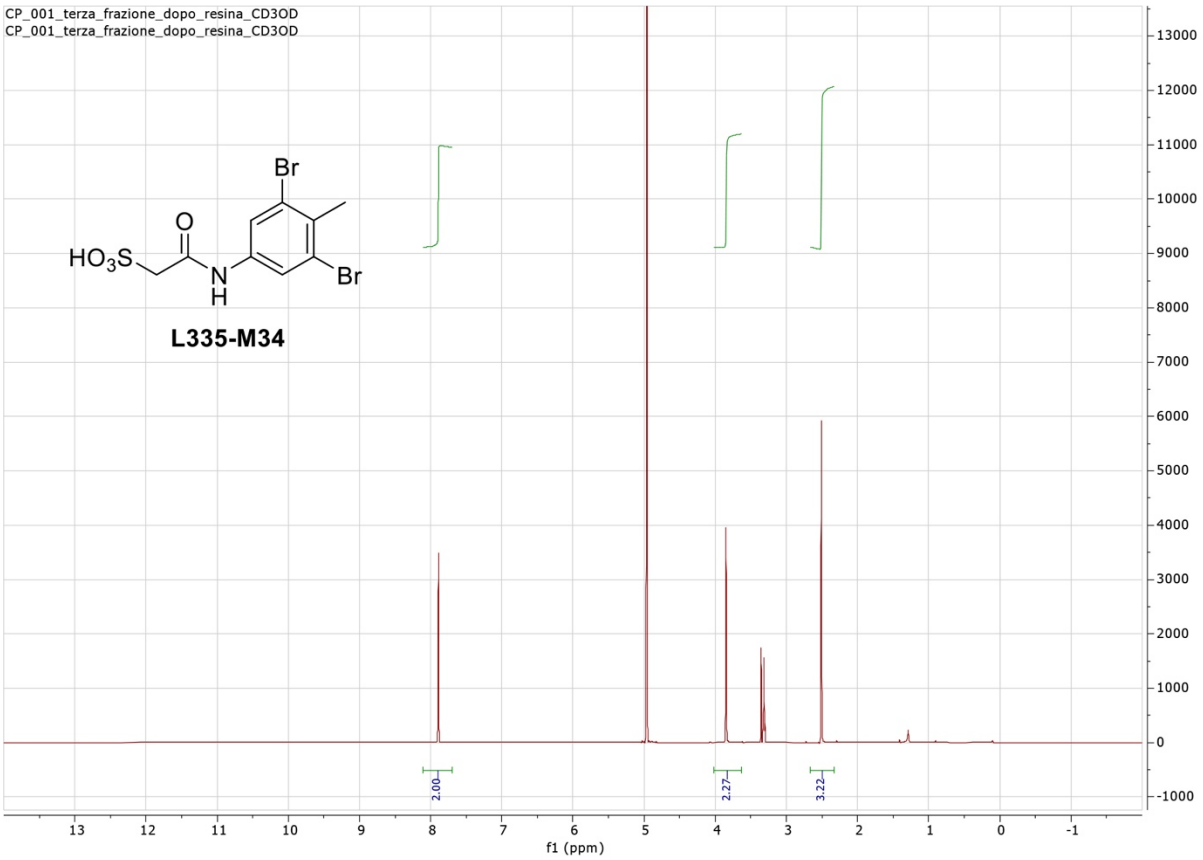


**Figure S3. $^{1}$H NMR spectrum (300 MHz, $CD_3OD$) of L335-M34.**

## FIGURE S4

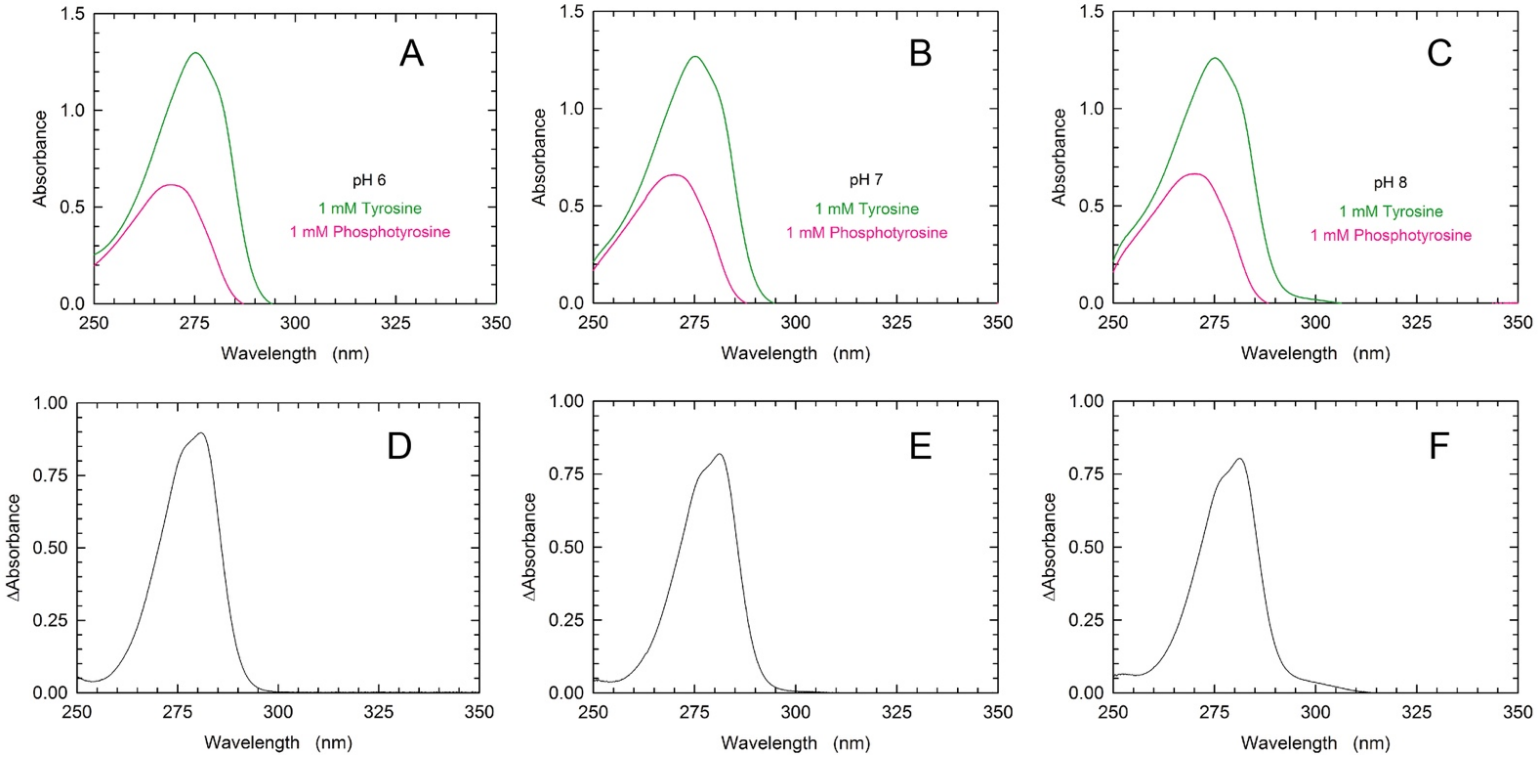


**Figure S4. UV absorbance spectra of tyrosine and phosphotyrosine over the 6-8 pH interval.** (**A-C**) Absorbance spectra of 1 mM tyrosine (green lines) and 1 mM phosphotyrosine (magenta lines) at pH 6, 7, and 8 (panels A, B, and C, respectively). (**D-F**) Difference spectra (tyrosine minus phosphotyrosine) determined at pH 6 (panel D), 7 (panel E), and 8 (panel F). The difference spectra were calculated using the data reported in panels A, B, and C. The data reported in panels D, E, and F were used to calculate the $\Delta\varepsilon_{Tyr\text{-}pTyr}$ at $\lambda$ = 290 nm, the value of which was estimated as equal to 0.14±0.02 $mM^{-1}cm^{-1}$.

## FIGURE S5

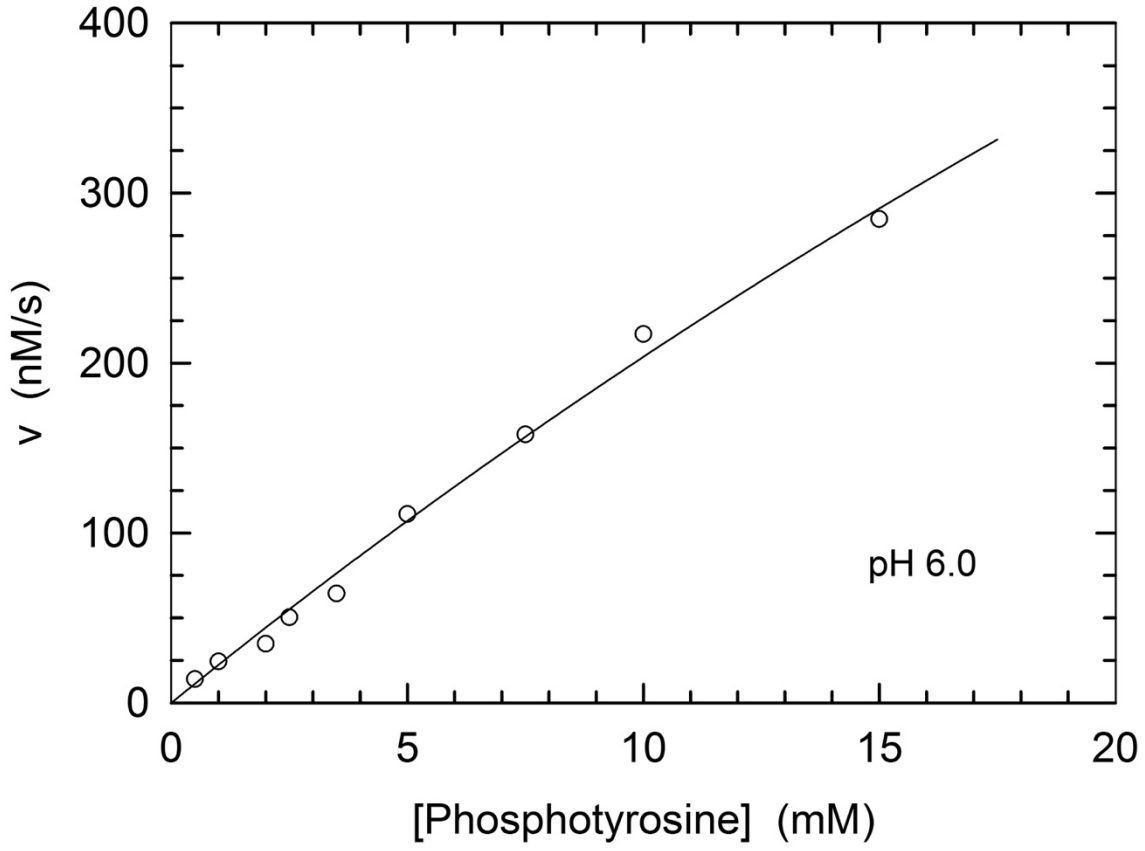


**Figure S5. Dependence on substrate concentration of the MptpA$_{sW48}$ catalytic action exerted at pH 6.**

The enzyme activity was determined in the presence of 420 nM enzyme, 2 mM EDTA, and a universal buffer consisting of Tris-HCl and Bis-Tris, 25 mM each. The continuous line represents the best fit of the Michaelis-Menten equation to the experimental observations.

## FIGURE S6

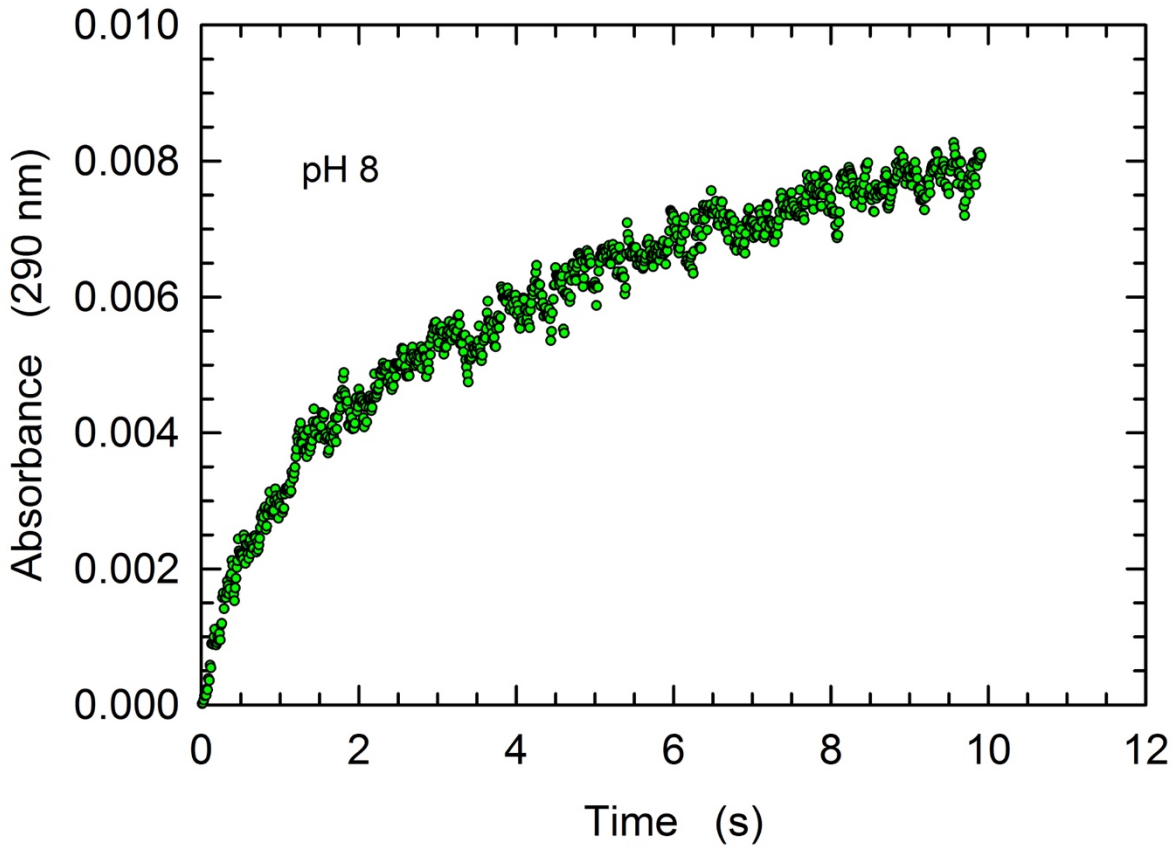


**Figure S6. Stopped-flow assay at pH 8 of the pre-steady-state activity of MptpA$_{sW48}$ at the expense of phosphotyrosine.**

Changes in Absorbance (green dots) induced by mixing 15 µM MptpA$_{sW48}$ with 10 mM phosphotyrosine, at pH 6.

## FIGURE S7

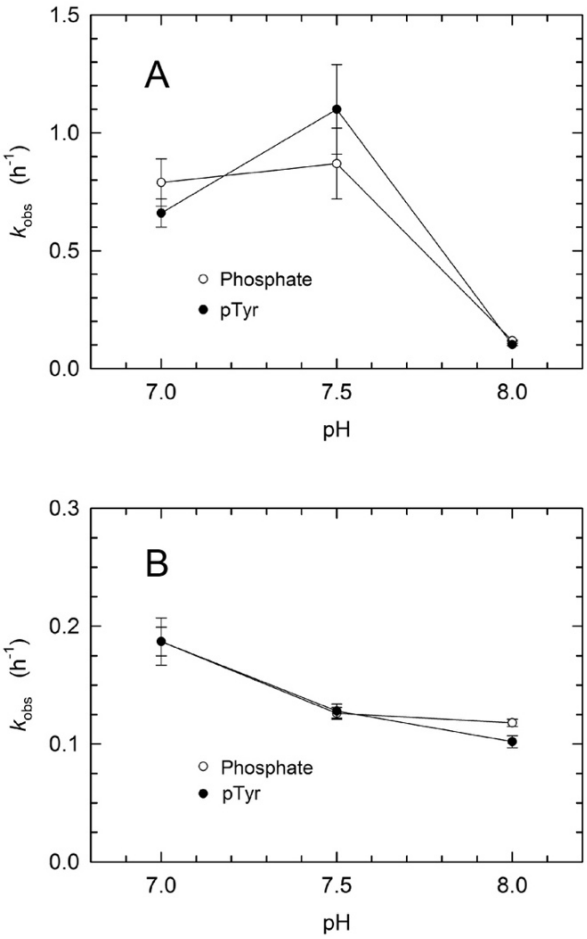


**Figure S7. Kinetic rate constants relative to substrate consumption and to product generation determined as a function of pH by NMR spectroscopy for the hydrolysis of pTyr catalysed by MptpA$_{sW48}$.**

(**A-B**) Dependence on pH conditions of the kinetic rate constants for phosphate generation and pTyr consumption (empty and filled circles, respectively) evaluated by observing with NMR spectroscopy the pTyr hydrolysis catalysed by MptpA$_{sW48}$. The kinetic rate constants determined for the fast (panel A) and the slow (panel B) phase of each reaction are shown. At pH 8 only a single (slow) phase was detected, and the corresponding $k_{obs}$ is therefore reported in both panels A and B.

## FIGURE S8

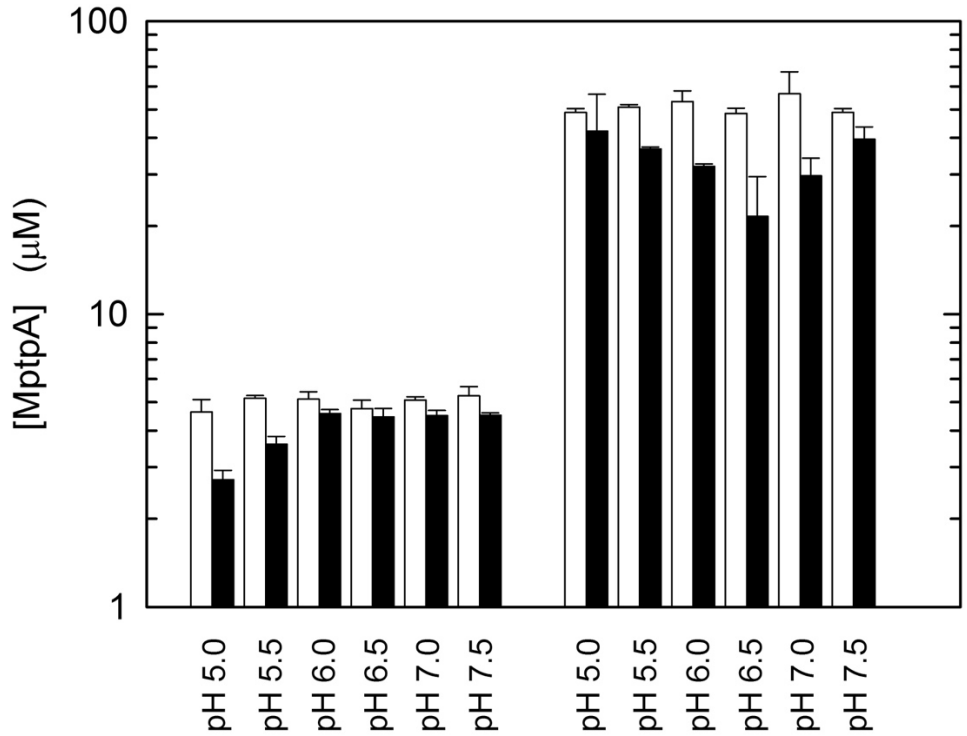


**Figure S8. Solubility of $MptpA_{sW48}$ as a function of pH and protein concentration.**

Two series of $MptpA_{sW48}$ samples were prepared to contain 5 or 50 μM enzyme at the indicated pH values (white bars). After 24 hours of incubation at room temperature all samples were centrifuged, and the residual $MptpA_{sW48}$ concentration of the supernatants accordingly obtained was determined (black bars). The error bars represent standard deviation.

## FIGURE S9

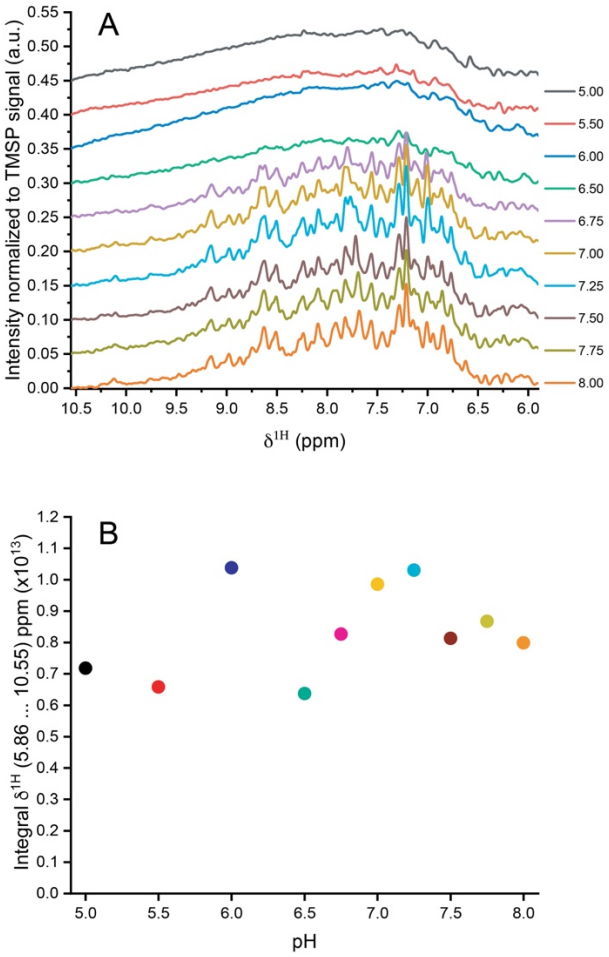


**Figure S9. One-dimensional $^{1}$H NMR spectra reporting on aromatic and amide protons of MptpA$_{sW48}$ observed at different pH values.**

(**A**) NMR spectra of 60 µM MptpA$_{sW48}$ in Tris-HCl/Bis-Tris (50 mM each), 150 mM NaCl and 1 mM EDTA, acquired at 298 K at pH 5.0, 5.5, 6.0, 6.5, 6.75, 7.0, 7.25, 7.5, 7.75, and 8.0 (black, red, blue, green, magenta, light brown, cyan, brown, ochre, and orange lines, respectively). (**B**) Numerical values for the integral of the signals obtained in 1D $^{1}$H NMR spectra of MptpA$_{sW48}$ over the range 5.86-10.55 ppm. Colour coding as in panel A.