Insights into the substrate uptake mechanism of Mycobacterium tuberculosis ribose 5-phosphate isomerase and perspectives on drug development

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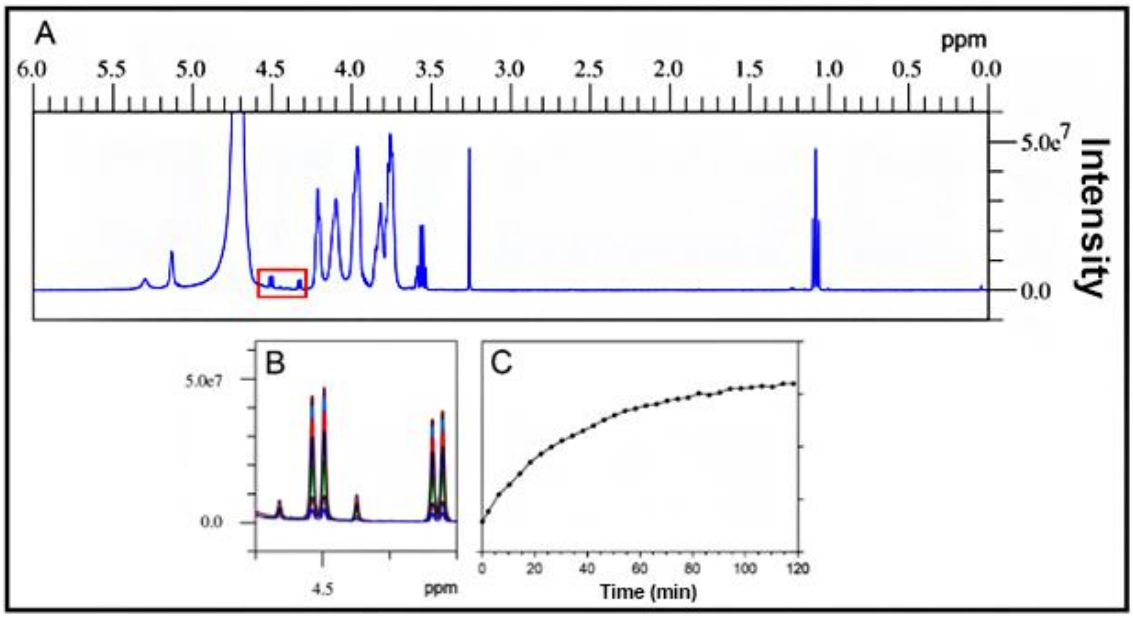


Figure S1. Enzymatic activity of MtRpiB by 1D ^{1}H NMR. (A) Ribose-5-phosphate (R5P) spectrum. The data was acquired with 10 mM of the substrate at 25 °C in 20 mM Tris-HCl pH 7.4. The red box highlights the presence of traces of ribulose-5-phosphate in the reagent. (B) Superposition of the first 6 spectra acquired after the addition of 12.5 nM of the MtRpiB in the NMR tube containing R5P. Each spectrum was acquired for 4 minutes. (C) MtRpiB protein progress curve following the Ru5P peak at 4.2 ppm. The result shows that the recombinant enzyme is active.

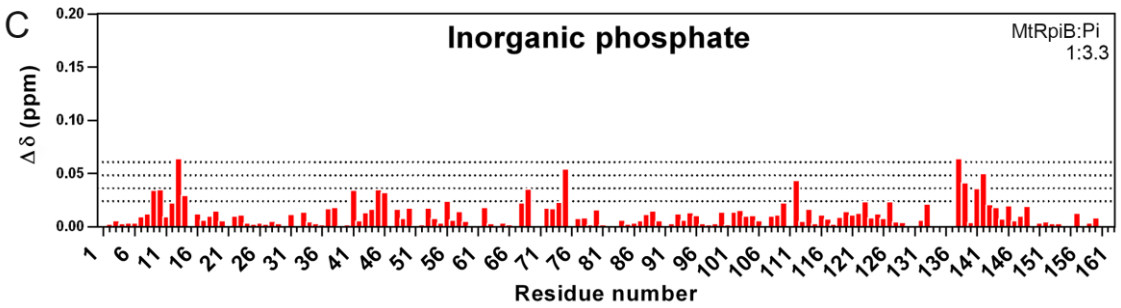


Figure S2. Chemical shift perturbation (CSP) of MtRpiB upon binding to the inorganic phosphate with 3.3 x excess. The ligand-induced CSP (Δδ) was plotted for each amino acid measuring 2D [^{1}H, ^{15}N] TROSY chemical shifts from free and bound protein. The tested compounds were (A) ribose 5-phosphate, (B) ribose, and (C) inorganic phosphate (Pi). The dotted lines represent one, two, three, and four standard deviations above the averaged chemical shift changes of residues.

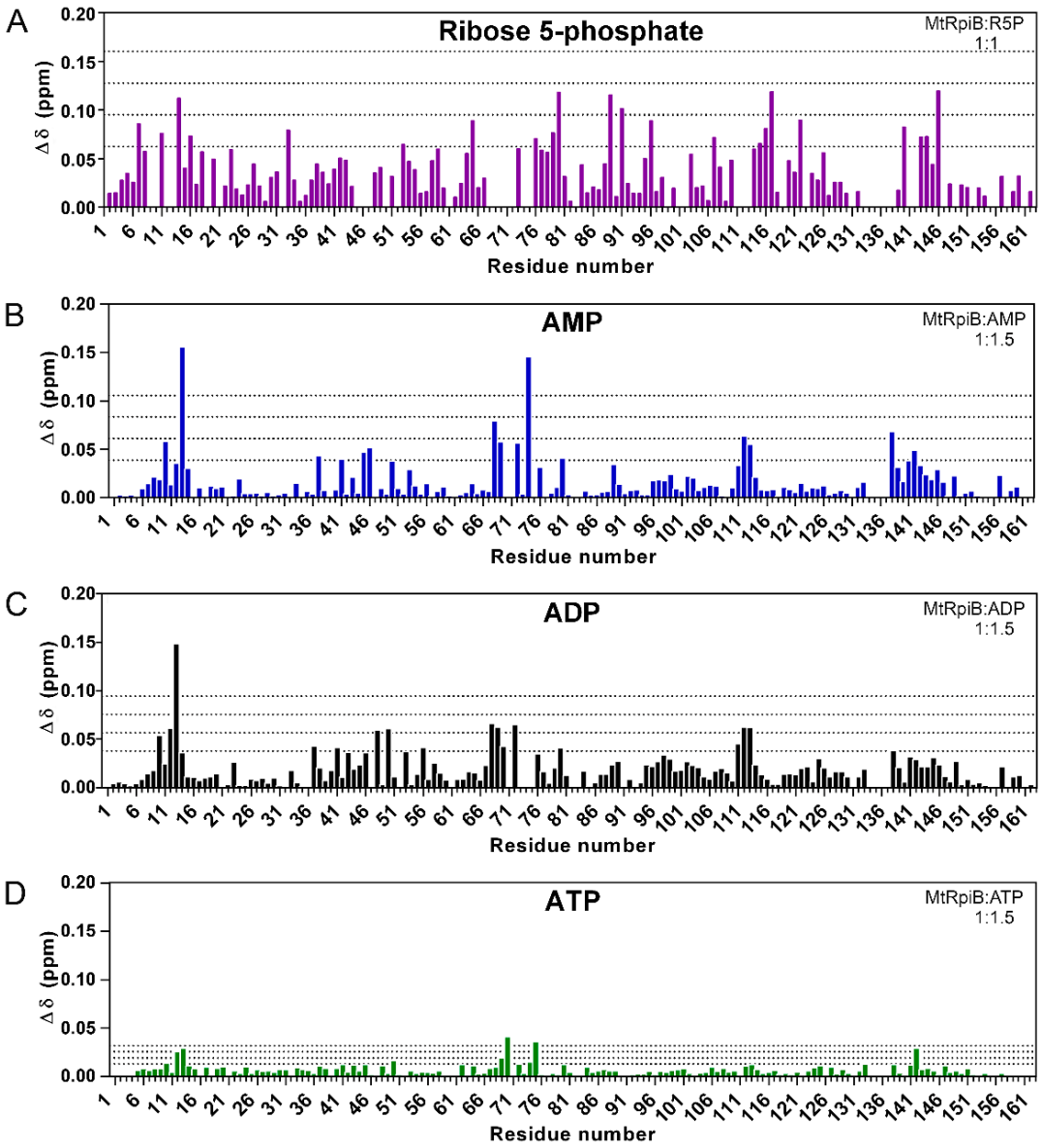
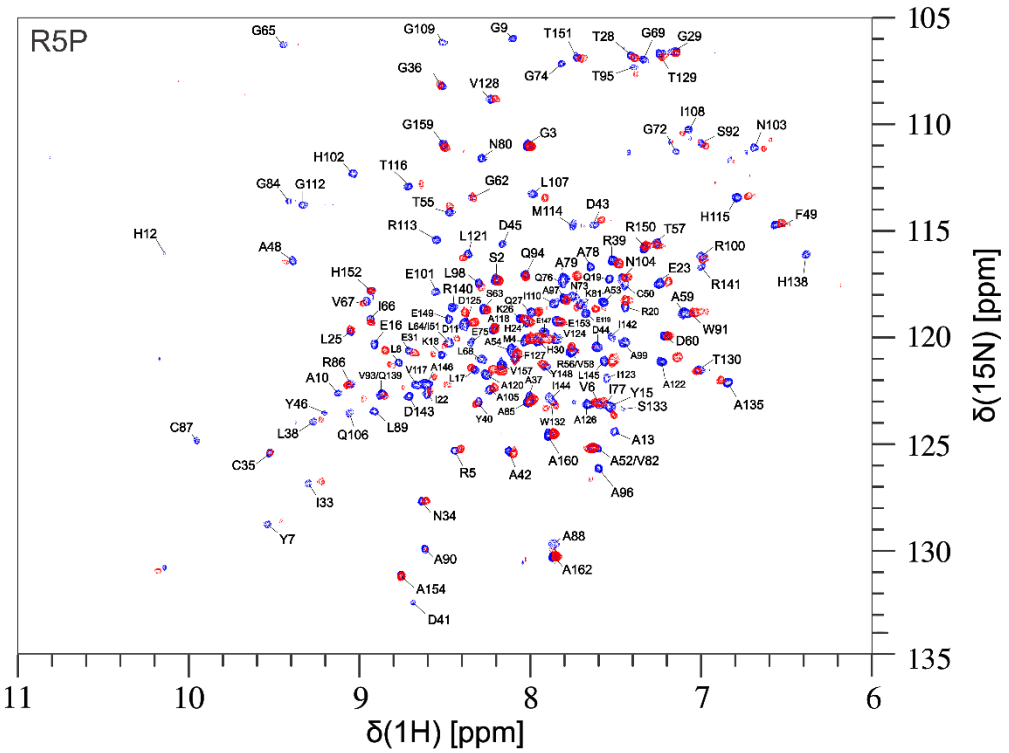
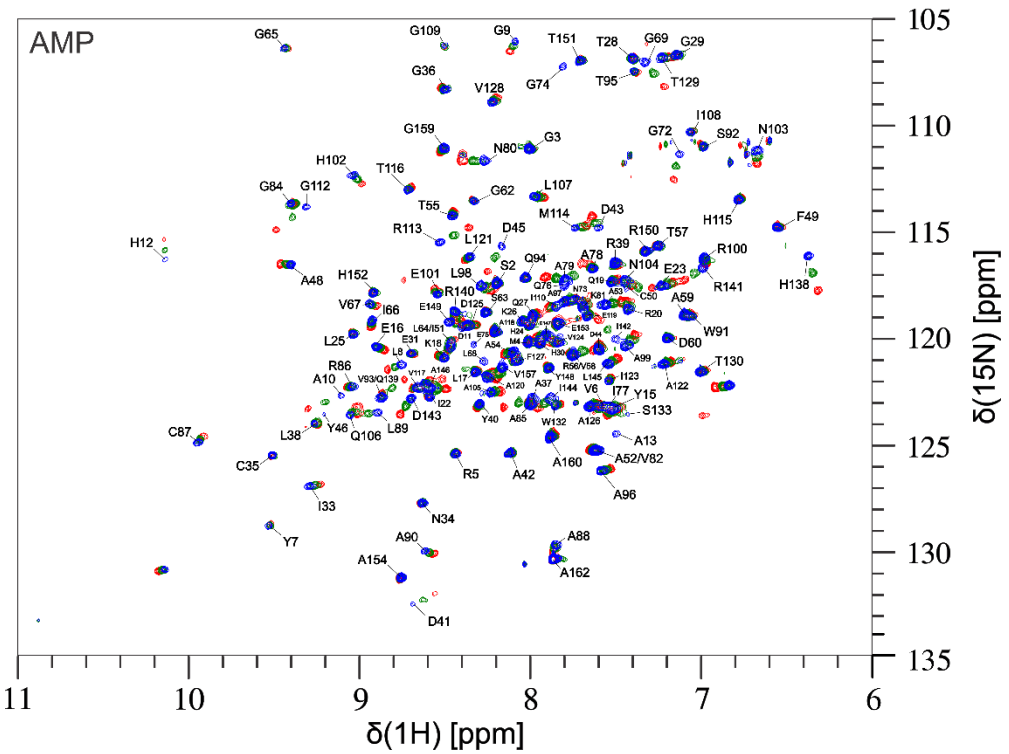
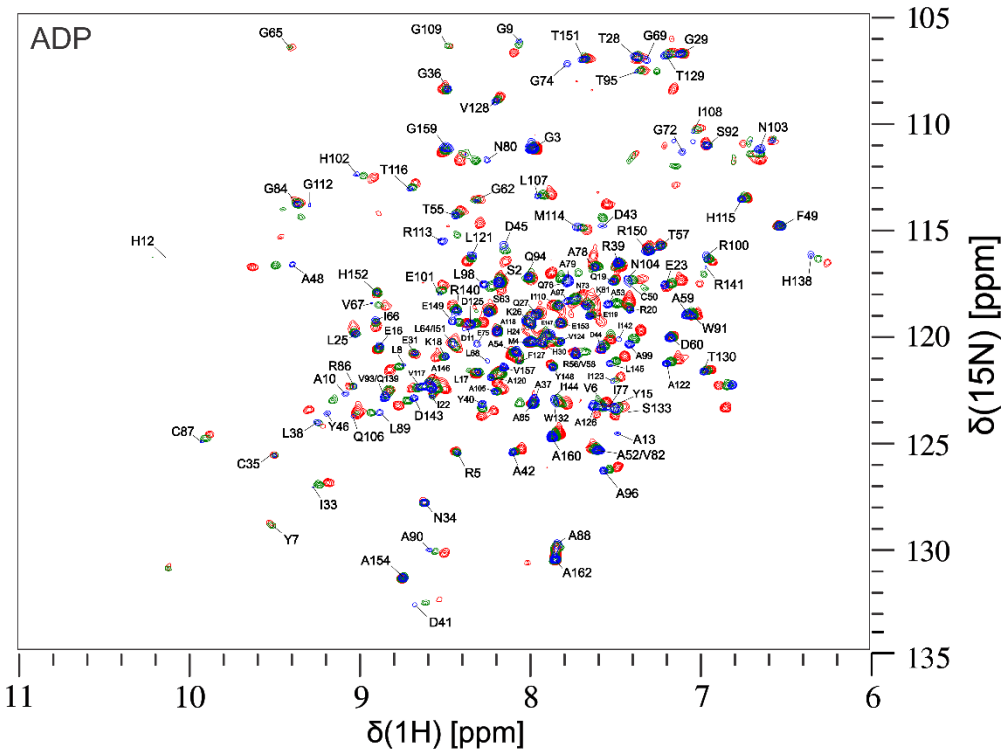
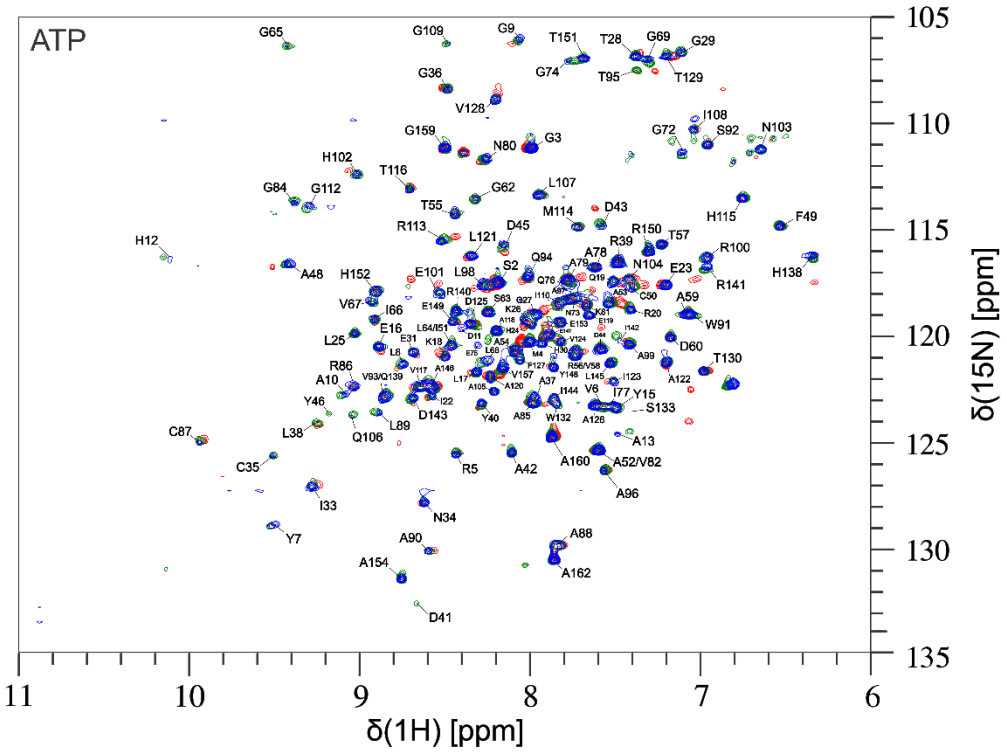


Figure S3. Chemical shift perturbation (CSP) of MtRpiB upon binding to R5P and AMP derivatives. The ligand-induced chemical shift perturbation (Dd) was plotted for each amino acid measuring 2D [^{1}H, ^{15}N] TROSY chemical shifts from free and bound protein. The tested compounds were **(A)** ribose 5-phosphate, **(B)** adenosine monophosphate (AMP), **(C)** adenosine diphosphate (ADP), and **(D)** adenosine triphosphate (ATP). The dotted lines represent one, two, three, and four standard deviations above the averaged chemical shift changes of residues.


R5P
δ(1H) [ppm]
δ(15N) [ppm]
11
10
9
8
7
6
105
110
115
120
125
130
135


AMP
δ(1H) [ppm]
δ(15N) [ppm]
11
10
9
8
7
6
105
110
115
120
125
130
135


ADP
δ(1H) [ppm]
δ(15N) [ppm]
11
10
9
8
7
6
105
110
115
120
125
130
135


ATP
δ(1H) [ppm]
δ(15N) [ppm]
11
10
9
8
7
6
105
110
115
120
125
130
135

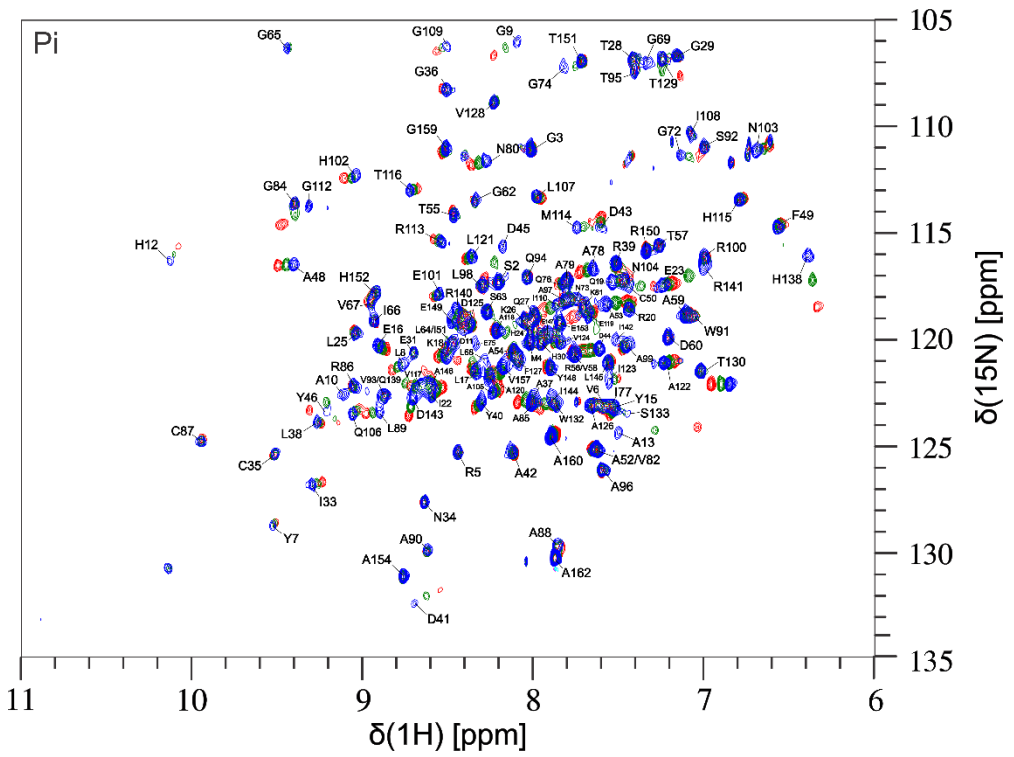


Figure S4. Raw data of Chemical Shift Perturbation (CSP) experiments of MtRpiB in the presence of R5P, AMP, ADP, ATP, and Pi. The chemical shift data acquired in the ^{1}H-^{15}N HSQCs were used for the CSP presented in Figures 2 and 3. In the HSQCs the free protein peaks are colored in blue. The peaks of the protein in the presence of an intermediary concentration of ligand are colored in green and the peaks of the protein with a higher ligand concentration are colored in red. The peaks colored in green were acquired with 0.4 mM of AMP, ADP, ATP, and 2 mM of Pi, while the peaks colored in red were acquired with 0.15 mM of R5P, 2 mM of AMP, 12 mM of ADP, 12.8 mM of ATP, and 16 mM of Pi.

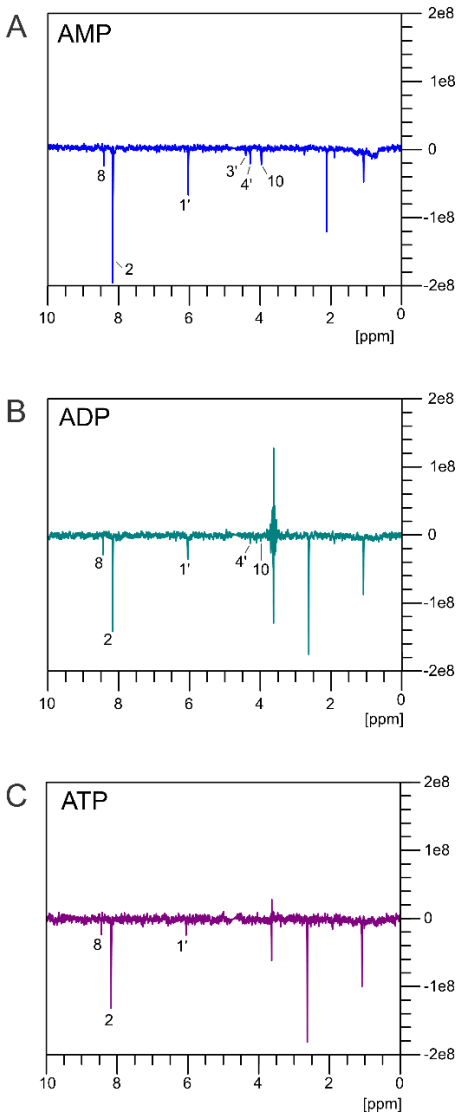


Figure S5: Saturation transfer difference (STD) NMR of MtRpiB interaction with adenosine monophosphate (AMP), adenosine diphosphate (ADP), and adenosine triphosphate (ATP). (A) AMP, (B) ADP, and (C) ATP. The saturation frequency used was 520 Hz (0.65 ppm) and the saturation time was 2 seconds. The peaks resonances were assigned and the interacting hydrogens are annotated following the figure 6B nomenclature

Figure S6-Video S1. **Molecular dynamics (MD) simulation of the structural model for the molecular complex of MtRpiB with adenosine diphosphate (ADP)**

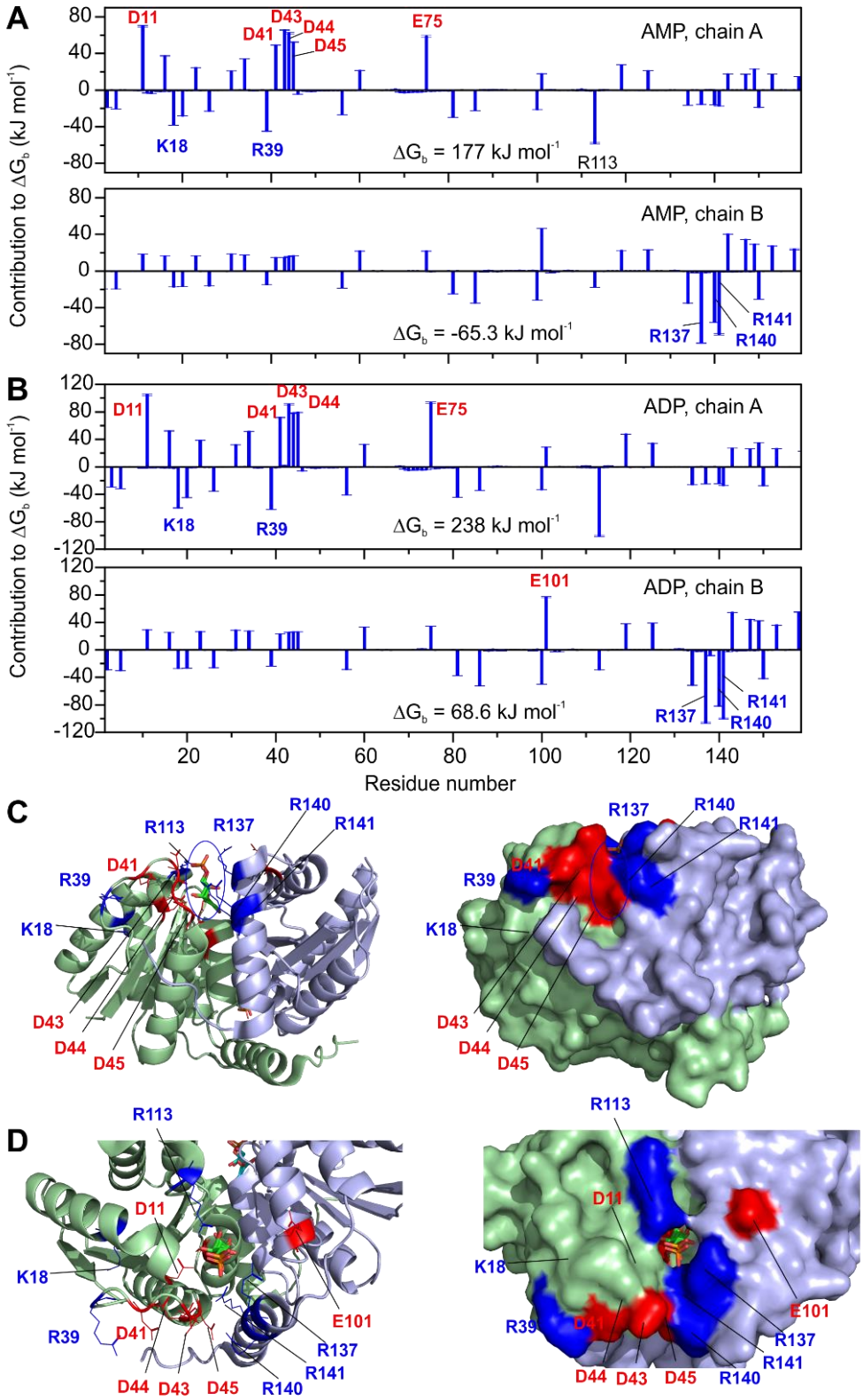


Figure S7. Energy contribution to Gibbs free energy change (ΔG_b) for the binding of the nucleotides with MtRpiB. (A) AMP, chain A and chain B, (B) ADP, chain A and chain B. (C) lateral view of the ribbon (left) and the surface of MtRpiB highlighting in blue the 6 residues with the most favorable contribution to ΔG_b and in red the 6 most unfavorable contribution to ΔG_b. (D)) top view of the ribbon (left) and the surface of mtRpiB highlighting in blue the 6 residues with the most favorable contribution to ΔG_b and in red the 6 most unfavorable contributions to ΔG_b. Chain A is in grey and B is in green. The highlighted residues were depicted in A and B. The residues with the most favorable contributions to ΔG_b are (in decrescent order): R137B, R141B, R113A, R140B, R39A, K18A (AMP) and R137B, R113A, R141B, R140B, R39A, K18A (ADP). The residues with the most unfavorable contributions to ΔG_b are

(in decrescent order): D11A, D44A, D44A, E75A, D45A, D41A (AMP) and D11A, E75A, D42A, D44A, E101B, D41A (ADP). The theoretical ΔG_b is the sum of all individual contributions. We are more concerned with the individual contribution than with the total value, which is highly dependent on the dielectric constant used in the calculation (we used 2).