'*In crystallo*' substrate binding triggers major domain movements and reveals magnesium as a co-activator of *Trypanosoma brucei* pyruvate kinase

Supplementary Material

Table S1. Pairwise protein sequence comparisons of trypanosomatid, yeast, human M2 and *E.coli* PYKs

	*Tb*PYK	*Tc*PYK	*Lm*PYK	*Hs*M2PYK	*Sc*PYK	*E.coli* PYK
*Tb*PYK	100	81	74	48	48	42
*Tc*PYK		100	76	47	48	42
*Lm*PYK			100	47	48	42
*Hs*M2PYK				100	49	44
*Sc*PYK					100	43
E.coli PYK						100

The pairwise sequence analysis was obtained from EMBL-EBI web server (EMBOSS Stretcher): http://www.ebi.ac.uk/Tools/psa/emboss_stretcher/

Values are overall percent sequence identities.

Hs, Homo sapiens; Sc, Saccharomyces cerevisiae

Table S2. Angles of AC-core rigid body rotation from T- to R-state of *Tb*PYK

T-state [a]	R-state	Rotation Angle [b]
apo *Lm*PYK	*Tb*PYK/F26BP/Mg	$8.3^{o}\pm0.2^{o}$
apo *Lm*PYK	*Tb*PYK/F26BP/PEP/Mg	$8.3^{o}\pm0.2^{o}$

[a] The PDB ID for apo *Lm*PYK is 3hqn.

[b] Calculated rotation angles with standard deviation

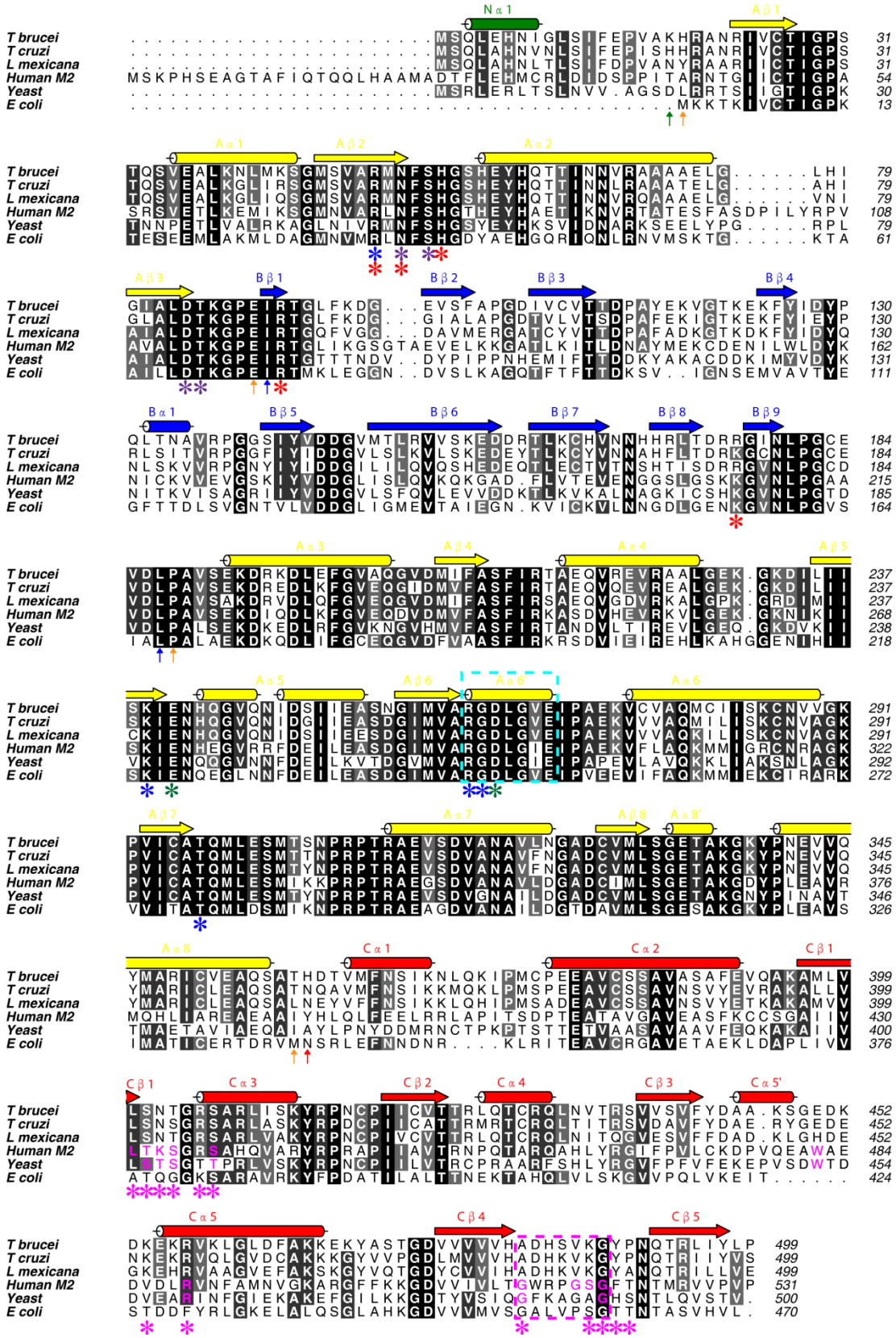
T brucei
T cruzi
L mexicana
Human M2
Yeast
E coli

Fig. S1. Sequence alignment of pyruvate kinases from *T. brucei, T. cruzi, L. mexicana, Homo sapiens* M2, *S. cerevisiae* and *E. coli*. The sequence alignment was performed using the program Clustal Omega at the European Bioinformatics Institute (Goujon *et al*., 2010; Sievers *et al*., 2011). Secondary structural elements defined in *Tb*PYK/F26BP/Mg by DSSP (Kabsch & Sander, 1983; Joosten *et al*., 2011) are shown above the sequences (only α-helices and β-strands are shown). Secondary structural elements are labelled in different colours corresponding to their domain regions: N-terminal domain (green), A-domain (yellow), B-domain (blue) and C-domain (red). Domain boundaries are indicated by vertical arrows in domain-specific colours. The conservation of the residues is indicated by shading from black (identical in five or six sequences) to grey (conserved in four) to white (low or no conservation). Residue numbers corresponding to each PYK are listed after the sequences. In *Tb*PYK, the amino acids involved in divalent metal binding (PEP-coordinating metal, Mg-1 site) (*), potassium metal ion binding (*), substrate PEP binding (*) and effector F26BP binding (*) are indicated by asterisks. The red asterisks (*) indicate product ATP binding residues in *Lm*PYK. Residues 263-269 of the small α-helix Aα6´ which are involved in allosteric regulation and in binding divalent metal and the substrate PEP are indicated by a dashed box (cyan). The effector loop residues are indicated by a pink dashed box. The amino acids involved in effector F16BP binding for human M2PYK and yeast PYK are coloured pink. The figure was generated using the program Aline (Bond & Schüttelkopf, 2009).

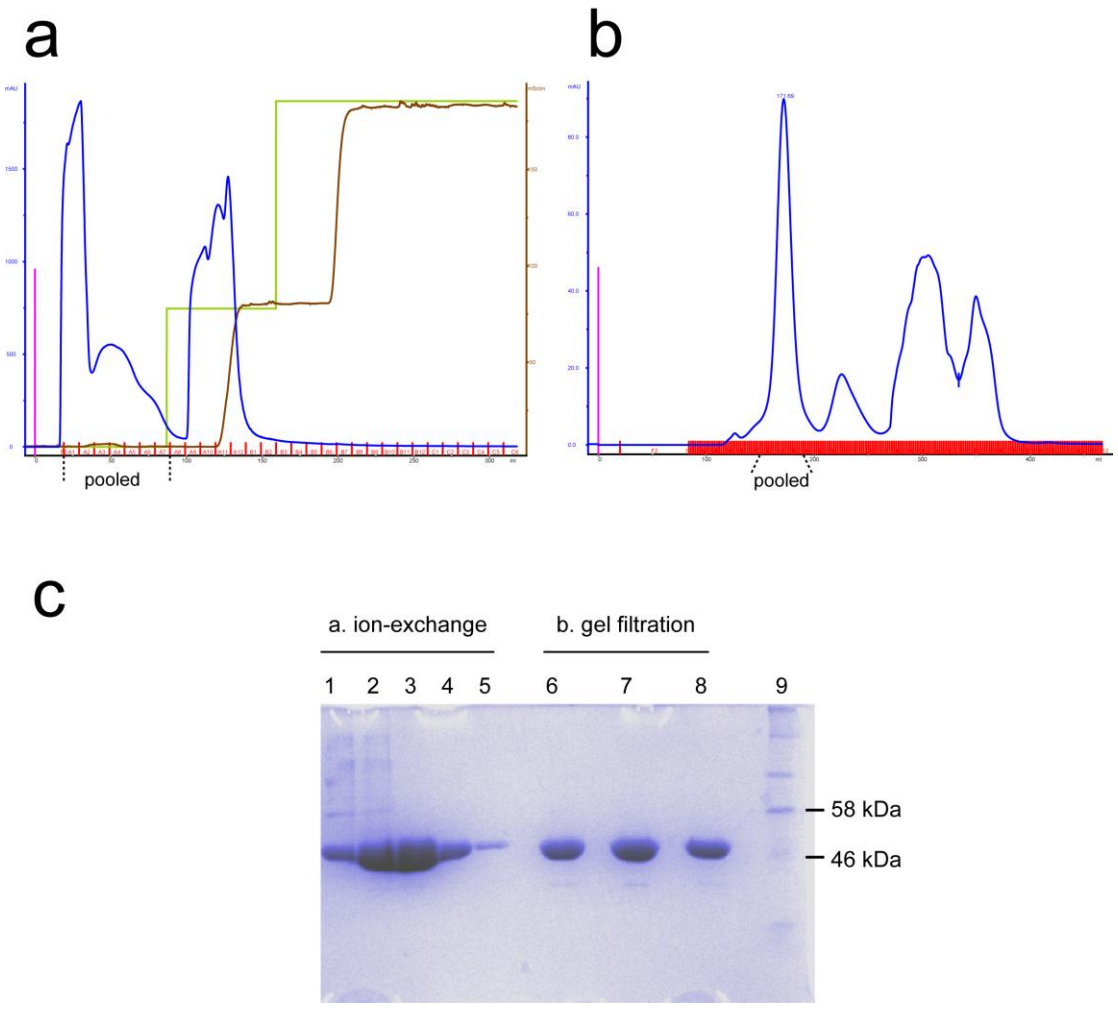

Fig. S2. Purification profiles for untagged *Tb*PYK**.** (a) Step 1: Ion exchange - elution profile from tandem ion-exchange columns (Hiprep DEAE FF 16/10 and Hiprep SP FF 16/10). The blue, green, brown and red curves represent the UV trace, percentage of salt concentration, buffer conductivity, and eluted fraction, respectively. Fractions which were pooled and concentrated for the next step are indicated. (b) Step 2: Gel filtration - elution profile from a Superdex 200pg XK 26/60 gel filtration column (319 ml); the elution peak of target protein *Tb*PYK is indicated at 171.69 ml retention volume. Fractions which were pooled and concentrated for storage are indicated. (c) SDS-PAGE analysis of protein purity for purification steps. Gel lanes 1-5 represent the flow through fractions A1-A5 from the first step of purification (ion exchange); gel lanes 6-8 represent the *Tb*PYK elution peak corresponding to the retention volume of ~171 ml; gel lane 9 has the protein molecular weight markers.

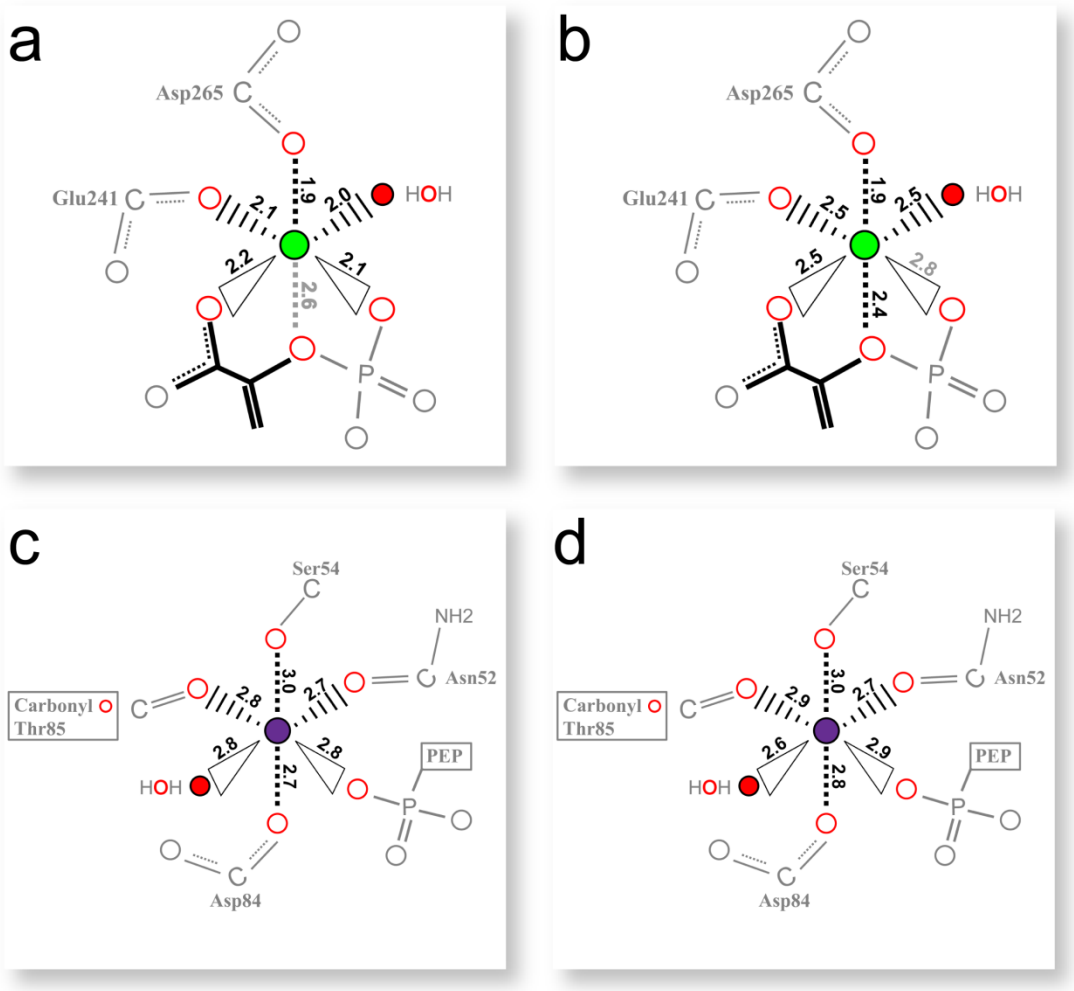

Fig. S3. Schematic representations of metal ion coordination at the active site of *Tb*PYK/F26BP/PEP/Mg. (a, b) Mg^{2+} (green spheres) coordination in chain A and chain B, respectively. The interatomic distances for the interactions are given in Ångstroms. The two Mg^{2+} coordination spheres have slight differences which may be related to the conformation of the B-domain and the side-chain orientation of Phe213. (c, d) K^{+} (purple spheres) coordination in chain A and chain B, respectively (distances are in Ångstroms).

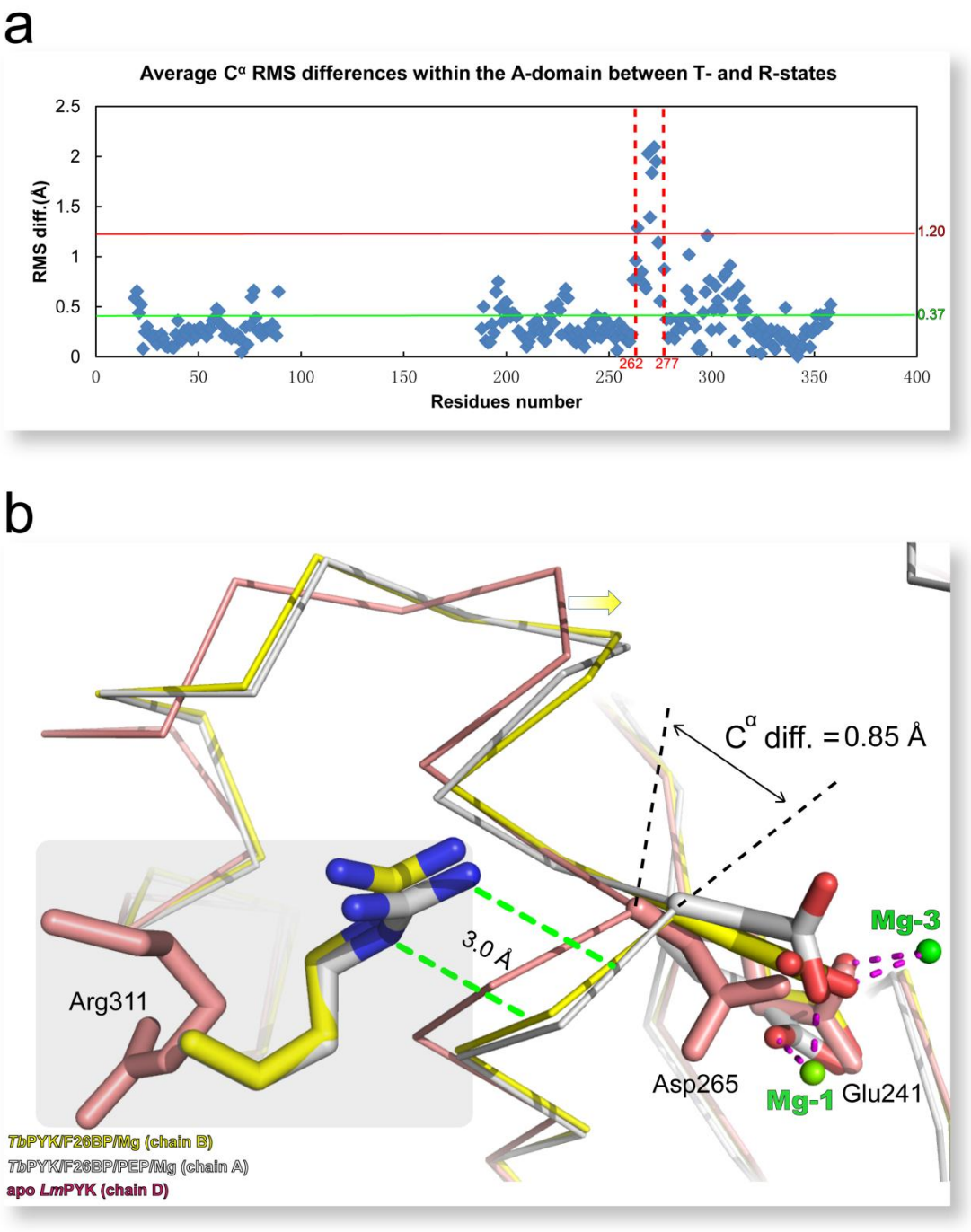

Fig. S4. The C^{α} RMS differences identify a significant shift of a small motif within the A-domain between the T-state of *Lm*PYK and the R-state of *Tb*PYK. (a) The calculation for RMS differences was performed by the superposition of the A-domains (19-89, 188-358) from inactive T-state (apo *Lm*PYK, 3hqn) and active R-state (*Tb*PYK/F26BP/Mg) structures. The RMS differences were plotted as a function of residue numbers. A small motif (residues 262-277) with high RMS differences was identified, and is indicated by red dashed lines. The average C^{α} RMS differences for all residues of this small motif is 1.20 Å as indicated by the continuous red line, compared to 0.37 Å for the average C^{α} RMS differences for all residues of the A-domains (indicated by the green line). A similar motif shift of 1.20 Å between the T- and R-states of *Lm*PYK was also

observed (data not shown). No significant shift of this motif was found between the structures of *Tb*PYK/F26BP/Mg and *Tb*PYK/F26BP/PEP/Mg (with an average C^{α} RMS fit of 0.26 Å for the residues of this motif). (b) The superposed structures are apo *Lm*PYK (pink) in the inactive T-state, and *Tb*PYK/F26BP/Mg (yellow) and *Tb*PYK/F26BP/PEP (white), both in the active R-state. The structures are shown as a ribbon while relevant residues are shown as sticks. The Mg^{2+} ions are shown as spheres in green. The interactions between the protein and Mg^{2+} ions are indicated by pink dashed lines. The shift of the small motif including Aα6′ is indicated by the arrow. The C^{α} atom of residue Asp265 which coordinates the Mg^{2+} ion in *Tb*PYK/F26BP/Mg or *Tb*PYK/F26BP/PEP/Mg has a similar shift of 0.85 Å compared to the T- state structure of apo *Lm*PYK. The interactions between Arg311 (in the neighbouring chain) and the small motif are indicated by green dashed lines and the interaction distance is about 3.0 Å.