Supplementary data

Selecting a starting model for an MD study to simulate the T to R transition in LmPYK

Our paper that hypothesised a ' rock and lock' allosteric mechanism for LmPYK (Morgan et al (2010) J.Biol.Chem. **285:**12892-12898) presented the following X-ray structures:

3HQN = LmPYK	(T-state	2.0 Å)
3HQQ = LmPYK + F26BP	(R'-state	5.1 Å)
3HQP = LmPYK + F26BP + ATP + Oxalate	(R-state .	2.7Å)

Figure S1 shows how the tetramer structures of these three different liganded states are related to each other by concerted rigid body rotations of the core A-C domains by between 1.5^0 and 8^0 . The RMS fit for the individual chains (summarised in Table S1) are however very close and have values between 0.6Å and 0.3Å showing that the conformations of the A and C domains are essentially the same in each of the structures.

The effector-only structure (3HQQ) has very large cell dimensions of a= 243Å. b = 254Å and c = 892 Å and despite diffracting to better than 2.7 Å, the closeness of Bragg spots only allowed data to be processed to 5 Å resolution. However there are six tetramers in the asymmetric unit and rigid-body refinement of the AC domains for each of the 24 crystallographically independent chains was used to provide strong experimental evidence that the tetramers adopted a conformation close to the fully-liganded R-state structure (3HQP).

The purpose of the MD simulations was to test the 'rock and lock' hypothesis and investigate whether F26BP effector binding alone (i.e. without substrate ligands in the active site) could stabilise an R-state tetramer conformation. In selecting 3HQQ as the starting model for MD simulations we argued that the structures of the individual chains in the tetramer are reliable as they have an RMSfit of ~0.3Å to the relatively high resolution structure 3HQP. However, the small rigid body differences between the tetramers in 3HQQ , 3HQP and 3HQN are likely to provide important information about the T to R conformational transition (governed by binding of the F26BP effector molecule). Another reason for using the 3HQQ structure with the bound F26BP ligand is that it provides a straightforward way of generating a pseudo *apo* structure for a parallel effector-free MD simulation. The alternative starting points for the MD simulation of an effector-only bound tetramer would be to remove all the tightly-bound ligands (ATP, oxalate and ions) from the active site of the R-state structure (3HQP). Alternatively starting with the T-state structure would necessitate modelling-in the F26BP into the effector site which is disordered in the T-state 3HQN structure.

Table S1

	3HQN (T-state) 3HQP (R state)	**3HQQ (FBP-bound) 3HQN (T-state)**	**3HQQ (FBP-bound) 3HQP (R-state)**
Tetramer RMS fit	3.6Å	2.5Å	2.5Å
AC core RMS fit	0.62Å	0.62Å	0.26Å
Relative rotation of AC core	5.2°	8°	1.4°

Figure S1

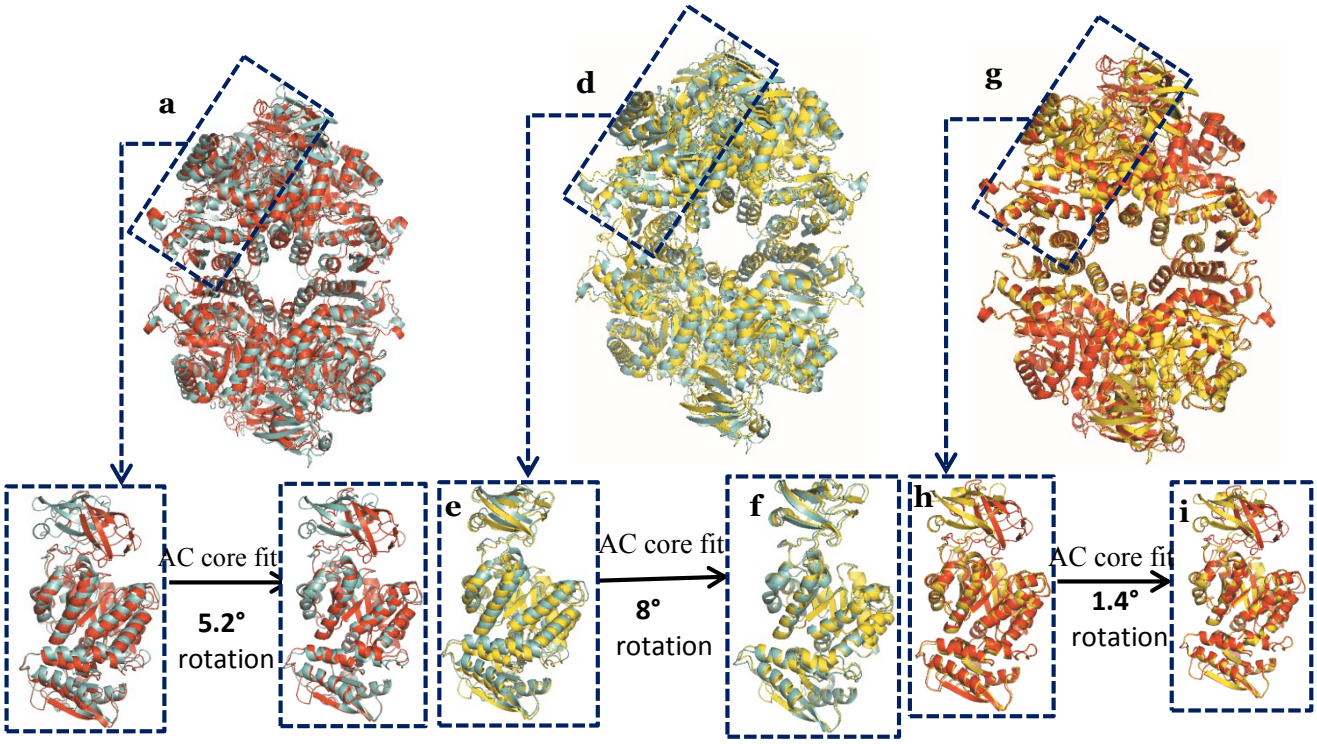

Figure S1

Structural overlays of the T and R state leishmania mexicana pyruvate kinase crystal structure with 3HQQ (FBP-LmPYK)

a) superposition of 498 Cα atoms of all 4 tetramer chains of 3HQN (T state, cyan) and 3HQP (R state, red) gives an rms fit of 3.6Å
b) detail from a) showing superposition of one of the tetramer chains
c) superposition of 18-88 and 187-498 Cα atoms for the A and C domains of one chain (RMS fit 0.6Å) achieved by a rigid body rotation of 5.2^0

d) superposition of 498 Cα atoms of all 4 tetramer chains of 3HQQ (simulation structure, yellow) and 3HQN (T state, cyan) give an RMS fit of 2.5Å
e) detail from d) showing superposition of one of the tetramer chains
f) superposition of Cα 18-88 and 187-498 atoms for the A and C domains of one chain (RMS fit 0.6Å) achieved by a rigid body rotation of 8^0

g) superposition of 498 Cα atoms of all 4 tetramer chains of 3HQQ (simulation structure, yellow) and 3HQP (R state, red) give an RMS fit of 2.5Å
h) detail from g) showing superposition of one of the tetramer chains
i) superposition of 18-88 and 187-498 Cα atoms for the A and C domains of one chain (RMS fit 0.26Å) achieved by a rigid body rotation of 1.4^0

Figure S2 Root Mean Square Deviation (RMSD) calculated for the Apo Tetramer (ligand free tetramer of LmPYK) with respect to the starting structure. The graph is calculated over the length of the simulation (80 nanoseconds). The MD simulation was calculated using a time step of 5fs. The mean RMSD was 2.5 Å. Abscissa denotes the time in picoseconds while the ordinate represents the RMSD in Å units.

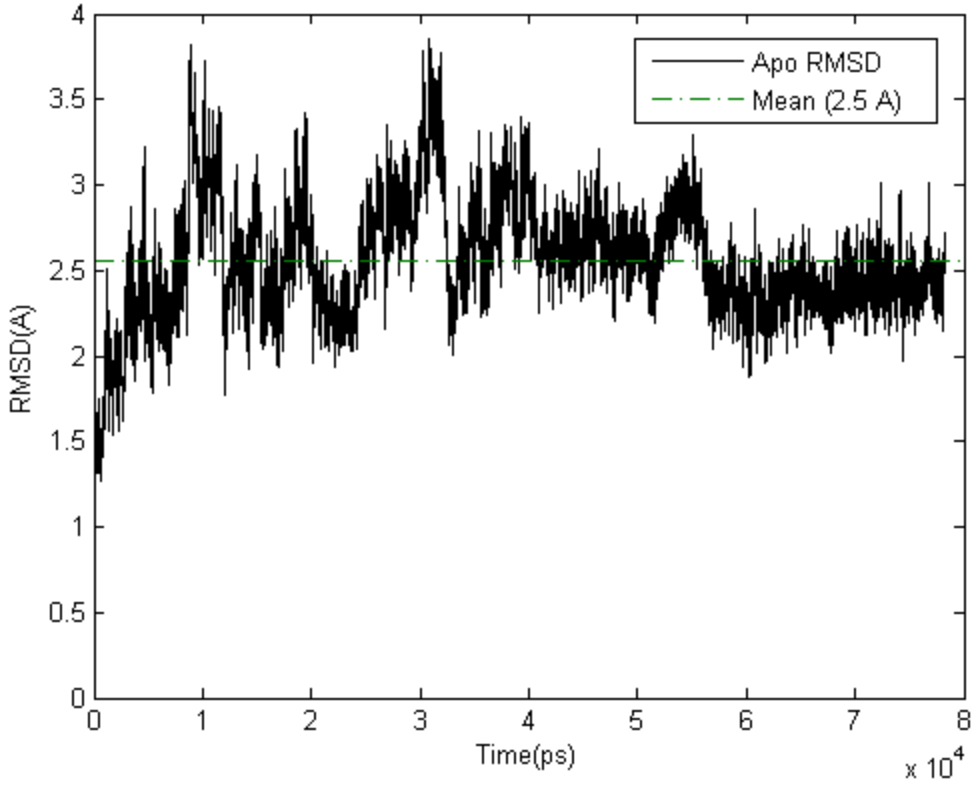

Figure S3 Root Mean Square Deviation calculated for the Holo Tetramer (LmPYK tetramer with allosteric activator, fructose-2,6-bisphosphate) with respect to the starting structure. The graph is calculated over the entire 50 nanoseconds long simulation. The MD simulation was calculated using a time step of 3fs. The mean RMSD as observed was 3.7 Å. Abscissa denotes the time in picoseconds while the ordinate represents the RMSD in Å units.

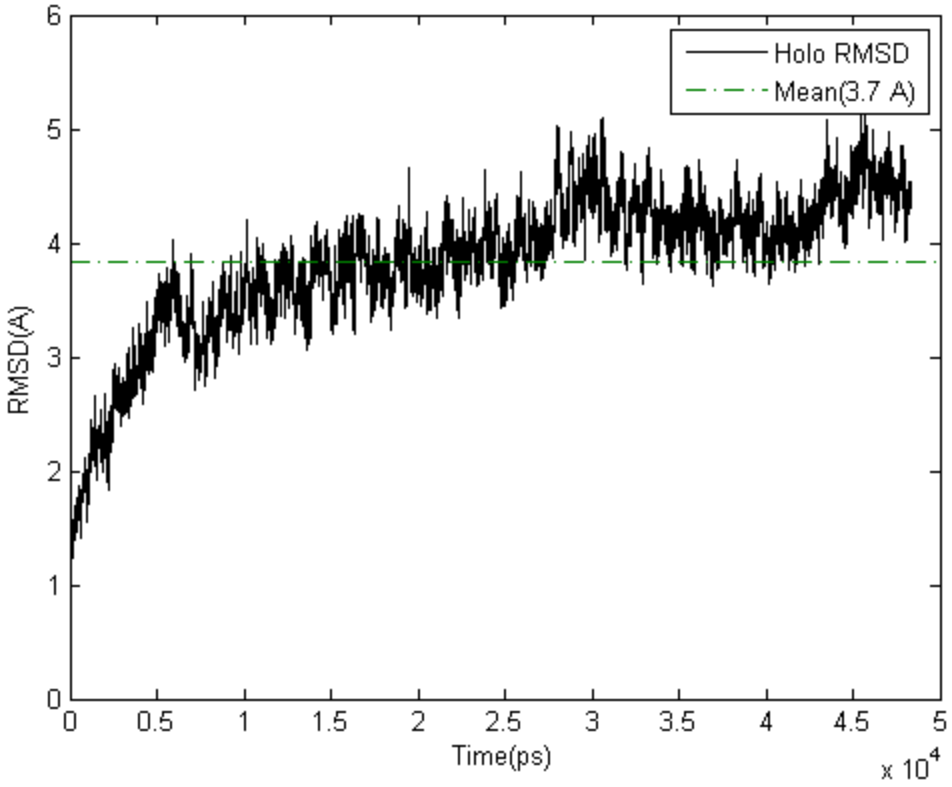

Figure S4 Root Mean Square Deviation calculated for the Apo Monomer (ligand free isolated monomer of LmPYK) with respect to the starting structure. The graph is obtained over the entire 50 nanosecond simulation. The MD simulation was calculated using the time step of 5fs. The mean RMSD as observed was 2.6 Å. Abscissa denotes the time in picoseconds while the ordinate represents the RMSD in Å units.

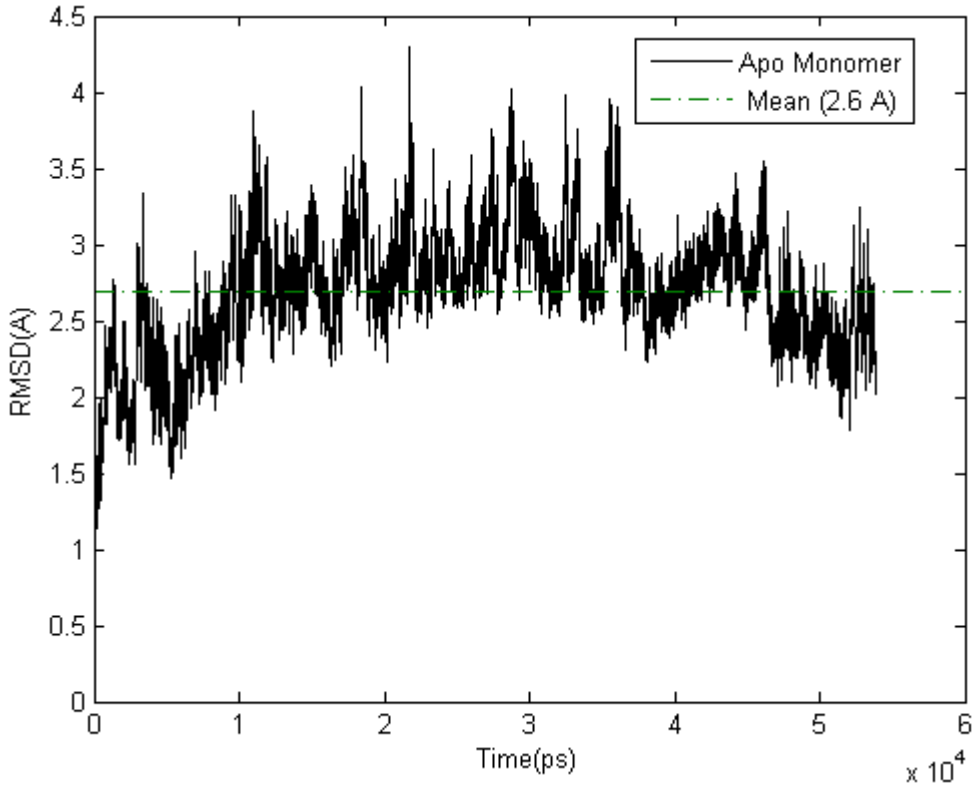

Figure S5 Root Mean Square Deviation calculated for the Holo Monomer (LmPYK isolated monomer with allosteric activator, fructose-2,6-bisphosphate) with respect to the starting structure. The graph is obtained over the entire 65 nanoseconds simulation. The MD simulation was calculated using a time step of 3fs. The mean RMSD as observed was 3.8 Å. Abscissa denotes the time in picoseconds while the ordinate represents the RMSD in Å units.