Supporting Material

A ribokinase family conserved monovalent cation binding site enhances the MgATP-induced inhibition in *E. coli* phosphofructokinase-2

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Table 1 Full data collection and refinement statistics

Full data collection and refinement statistics	PFK2 KCl	PFK2 CsCl
Data Collection		
Space group	$P222_1$	$P222_1$
Cell dimensions (Å) a, b, c.	43.81, 88.84, 176.13	43.86, 88.91, 176.41
Detector	PILATUS 6M	PILATUS 6M
X-ray source	ESRF ID29	ESRF ID29
Wavelength (Å)	0.976	0.976
Resolution range (Å)	50.0 – 1.70 (1.80 – 1.70)	50.0 – 1.85 (1.96 – 1.85)
Redundancy	4.33 (4.42)	2.42 (2.39)
Rmeans (%)*	5.8 (66.8)	6.7 (58.5)
Completeness(%)	98.8 (98.1)	96.4 (96.2)
Total reflections	330515 (53412)	267935 (42414)
Unique reflections	76204 (12301)	109841 (17693)
$I / \sigma(I)$	15.06 (2.04)	10.48 (2.07)
Refinement parameters		
Reflections used for refinement	76250	109841
R (%)**	18.30	17.40
R_{Free}(%)**	20.80	20.90
Overall averaged B-factor ($Å^2$)	25.50	29.03
Ligand averaged B-factor($Å^2$)	25.64	33.27
No. of protein atoms	4560	4558
No. of water molecules	604	544
No. of ligand atoms	192	141
Ramachandran Plot		
Favoured region (%)	99.19	99.18
Residues in disallowed regions (%)	0.00	0.00
RMSD from ideal geometry		
r.m.s. bond lengths (Å)	0.007	0.007
r.m.s. bond angles (°)	1.157	1.144

The numbers in parentheses are from the highest resolution shell .

* Rmerge = $\Sigma hkl \Sigma\Sigma i \mid Ii(hkl) - \langle I(hkl)\rangle \mid / \Sigma hkl \Sigma i\, Ii(hkl)$, where Ii (hkl) is the observed intensity of measured reflection, and $\langle I(hkl)\rangle$ is the averaged intensity over equivalent reflections from different measurements.

**R is conventional crystallographic R factor, $\Sigma| \mid Fobs \mid – \mid Fcalc| \mid / \Sigma| Fobs |$, where Fobs and Fcalc are the observed and calculated structure factors, respectively. Five percent of the reflections that were excluded from the refinement were used in the Rfree calculation.

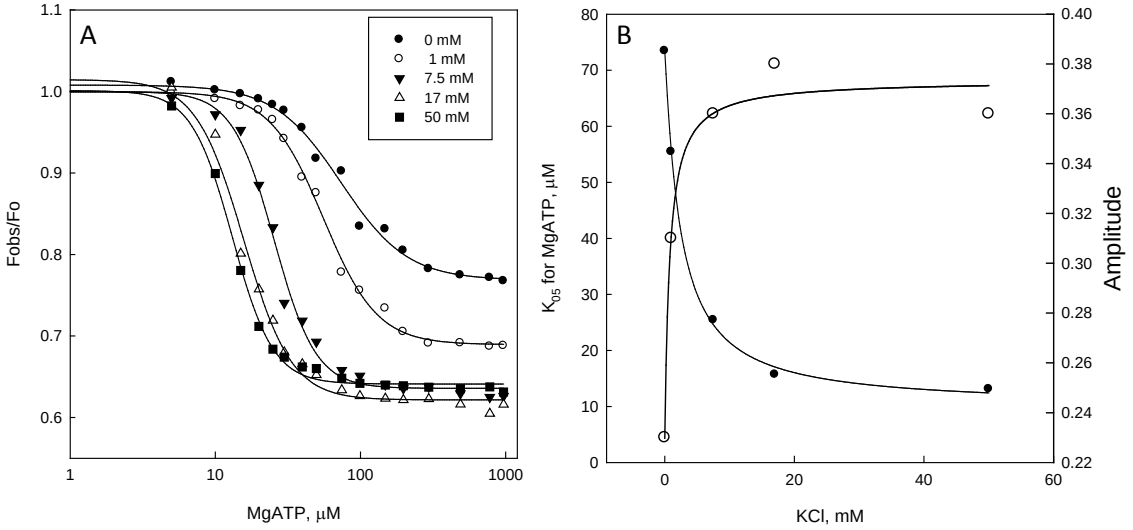

Table 2. Effect of KCl on the binding parameters for MgATP followed by intrinsic fluorescence.

KCl (mM)	[a]$K_{0.5}$	nH	Amplitude
0	73 ± 4.5	1.9 ± 0.2	0.33 ± 0.06
1	55 ± 1.7	2.3 ± 0.14	0.31 ± 0.04
7.5	25 ± 1	3.0 ± 0.3	0.36 ± 0.05
17	15 ± 0.7	2.6 ± 0.25	0.38 ± 0.05
50	13 ± 0.29	3.0 ± 0.17	0.36 ± 0.02

[a]Values in μM. Errors obtained from the fitting procedure.

Figure S1. A. Effect of KCl on the MgATP binding measured by intrinsic fluorescence. **A**. MgATP binding measured using several concentration of KCl (0 mM (•), 1 mM (o), 7.5 mM (▼), 17 mM (△) and 50 mM (■). Changes in the intrinsic fluorescence are expressed as a fraction of the observed fluorescence (Fobs) for each concentration and the fluorescence intensity at zero MgATP concentration (Fo). The continuous lines correspond to a Hill fit. Table **2** shows the constants for MgATP binding obtained for each concentration of KCl. **B**. The $K_{0.5}$ and amplitudes calculated from the MgATP binding curves are plotted as function of the concentration of KCl. From the variation of the $K_{0.5}$ for MgATP an apparent K_d of 2.6 mM was obtained for potassium binding.

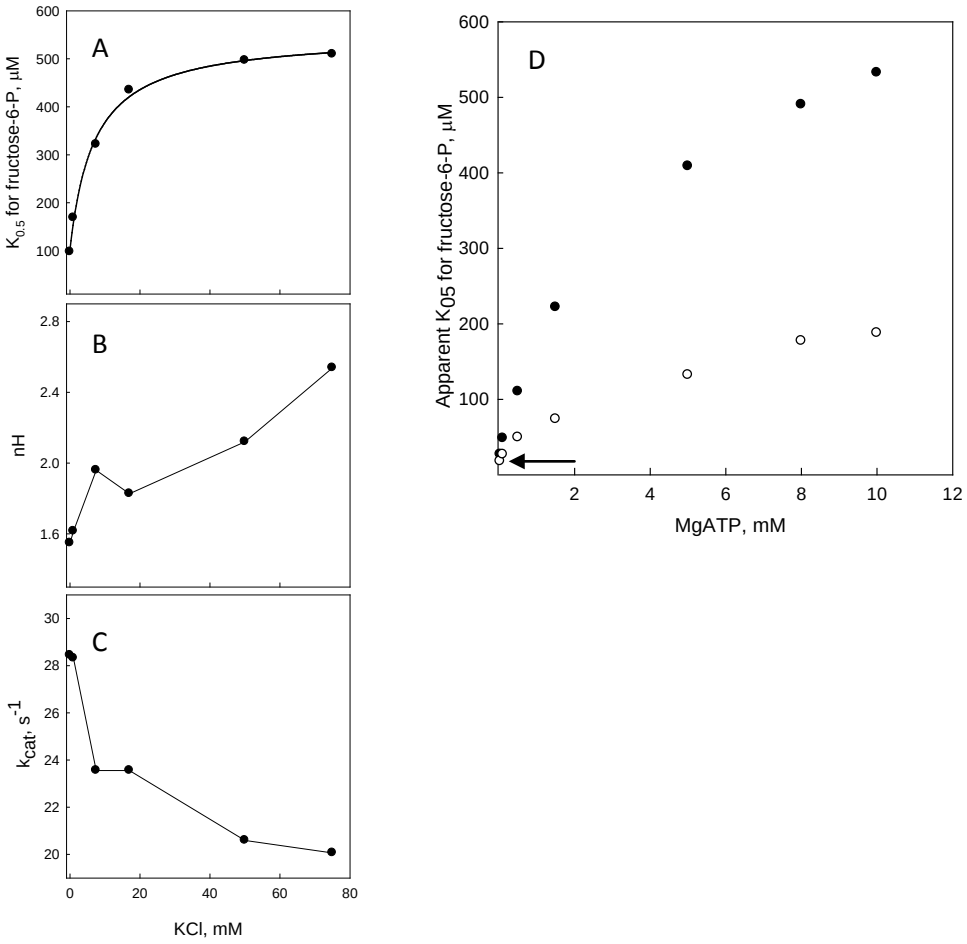

Figure S2. **Effect of KCl on the apparent kinetic parameters for fructose-6-P**. The left panel shows the variation of **A**, k_{cat}; **B**, $K_{0.5}$.; **C**, Hill coefficient obtained from the saturation curves for fructose-6-P measured at several KCl concentrations. Measurements were performed at 1 mM MgATP. The constants for each saturation curve were obtained using the Hill equation and plotted against the concentration of KCl. **D**. Variation of $K_{0.5}$ for fructose-6-P as a function of the MgATP concentration in the absence (o) or presence (•) of 50 mM KCl. The arrow indicates the K_d value for fructose-6-P obtained in the absence of MgATP using intrinsic fluorescence measurements.

Table 3. Contact distances calculated for Cs^+ and K^+.

	S250	V252	A286	N289	G291	R293
K^+	3.09	3.03	2.94	2.78	2.84	2.93
Cs^+	3.31	2.97	2.99	2.96	3.01	3.05

Distances (in Å) are given between cesium or potassium and oxygen atoms of the ion-binding site.