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Supporting information for article:

Structure of a double-domain phosphagen kinase reveals an asymmetric arrangement of the tandem domains

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Table S1 The melting temperature (T_m) of ddAK from *Anthopleura japonicas* in different buffers.

Buffer [a]	**pH**	T_m **(°C)**
Sodium acetate	4.5	37.5
Sodium acetate	5.0	42.7
MES	5.5	44.9
BIS-TRIS	5.5	45.0
MES	6.0	46.2
MES	6.5	47.3
BIS-TRIS	6.5	47.4
HEPES	7.0	48.3
Tris-HCl	7.5	48.0
Tris-HCl	8.0	48.3
Tris-HCl	8.5	47.8
CHES	9.0	47.4
CHES	9.5	44.0

[a] Buffers were prepared at a concentration of 50 mM in the presence of 20 mM sodium chloride.

Table S2 The melting temperature (*T*m) of ddAK from *Anthopleura japonicas* in the presence of different ligands.

Ligands [a]	**Concentration**	T_m **(°C)**
Control	N/A	48.4
ADP	10	49.7
ATPγS	1	49.5
AMPPNP	1.5	50.2
Arginine	10	53.0
ADP/Arginine	5 / 5	53.6
ATPγS/Arginine	1/1	50.2
AMPPNP/Arginine	1.5 / 5	53.4

[a] Ligands were solved in a buffer containing 50 mM HEPES (pH 7.0) and 20 mM sodium chloride.

Table S3 The specific activity of ddAK from *Anthopleura japonicas*

Mutants	Specific activity (μM/min/mg) [a]	Relative activity (%) [b]
Wild type	279.87 ± 10.90	100
R122A	166.49 ± 25.70	59.49 ± 9.18
R122K	193.14 ± 8.37	69.01 ± 2.99
D408A	200.30 ± 32.29	71.57 ± 11.54
D408E	169.21 ± 8.38	60.46 ± 2.30
R501A	159.02 ± 16.96	56.82 ± 6.06
R122D/D408R	167.66 ± 18.78	59.91 ± 6.71
R122Y/D408H	286.54 ± 19.03	102.38 ± 6.80
ΔTGE1	180.74 ± 19.26	64.58 ± 6.88
ΔTGE2	156.00 ± 8.73	55.74 ± 3.12
ΔTGE1/2	92.65 ± 8.91	33.10 ± 3.18

a The specific activities are the mean ± SD of triplicate determinations.

b The relative activity of the wild type is defined as 100.00%.

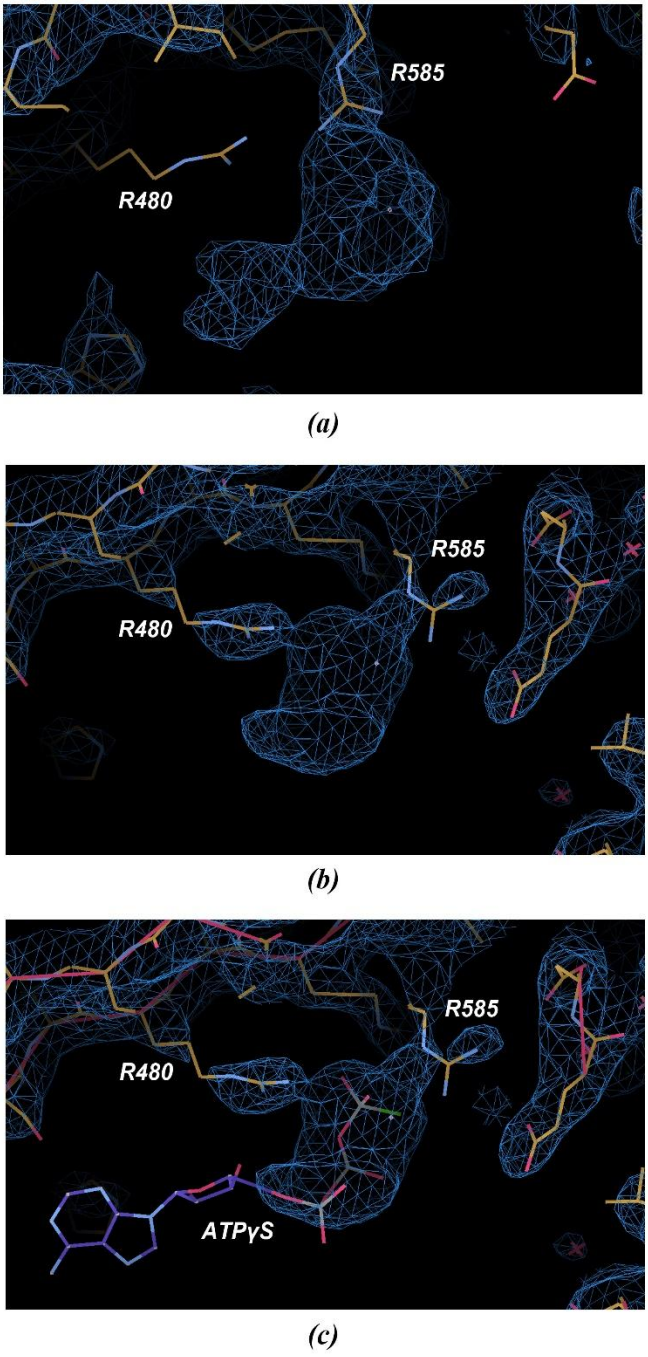

Figure S1 Uninterpretable electron density maps around the active site of ddAK-D2 in the arginine/ATPγS-bound complex structure. The omit map (at 1.0 contour level) depicted in blue meshes was calculated by using PHENIX.CalculateMaps (Afonine, Grosse-Kunstleve et al. 2012) with a protein model without any ligands to provide phases. Figures were prepared using COOT (Emsley and Cowtan 2004). (*a*) The uninterpretable map around the active site of ddAK-D2 in chain A. (*b*) The uninterpretable map around the active site of ddAK-D2 in chain B. (*c*) The same map in (*b*) overlapped to a refined model with ATPγS at the active site. A

reasonable explanation to this bulk of electron density map could be the negatively charged phosphoryl groups of ATPγS, of which the nucleoside group cannot be observed due to its extremely high flexibility. In the crystallization solution of this complex, there is no more small molecules than ATPγS that can provide such a strong electron density, but other possibilities still remain. Therefore, we decided to leave these uninterpretable maps empty with no small molecules to fit in.