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Supporting information for article:

Neutron crystallographic analysis of the nucleotide-binding domain of Hsp72 in complex with ADP

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Table S1 Hydrogen bond distances of water cluster 2.

Internal water cluster			Water cluster 2 – Hsp72		
Acceptor	Donor	Distance (Å)	Acceptor	Donor	Distance (Å)
W12−O	W14−D2	1.93	L11−mainchain O	W12−D2	2.06
W13−O	W14−D2	1.90	G12−mainchain O	W15−D1	2.07
W15−O	W14-D1	1.97	T13−mainchain O	W17−D2	1.88
W15−O	W16-D2	1.95	S40−mainchain O	W18−D2	2.02
W17−O	W15−D2	1.89	T145−mainchain O	W12−D1	1.82
W18−O	W16-D1	1.88	T158−O$^{\gamma}$	W13−D2	2.03
W18−O	W17-D1	2.02	W14−O	S120−D$^{\gamma}$	1.98
			W16−O	S120−D$^{\gamma}$	1.98
			W17−O	K71−mainchain D	2.27
Average		1.93	Average		2.03

"Hydrogen bond distances" are between the hydrogen atom and the acceptor atom.

Table S2 RMSD values (Å) of C^{α} distances between the superposed NBD of Hsp72.

RMSD (Å)	WT-ADP neutron @RT (PDB code 7F4X)	WT-AMPPnP @cryo (PDB code 2E8A)	WT-ADP @cryo (PDB code 3ATU)	Y149A-AMPPnP @cryo (PDB code 7F50)	Y149A-ADP @cryo (PDB code 7F4Z)
WT-ADP neutron @RT (PDB code 7F4X)	-	0.39	0.28	0.38	0.38
WT-AMPPnP @cryo (PDB code 2E8A)		-	0.37	0.34	0.35
WT-ADP @cryo (PDB code 3ATU)			-	0.33	0.28
Y149A-AMPPnP @cryo (PDB code 7F50)				-	0.25

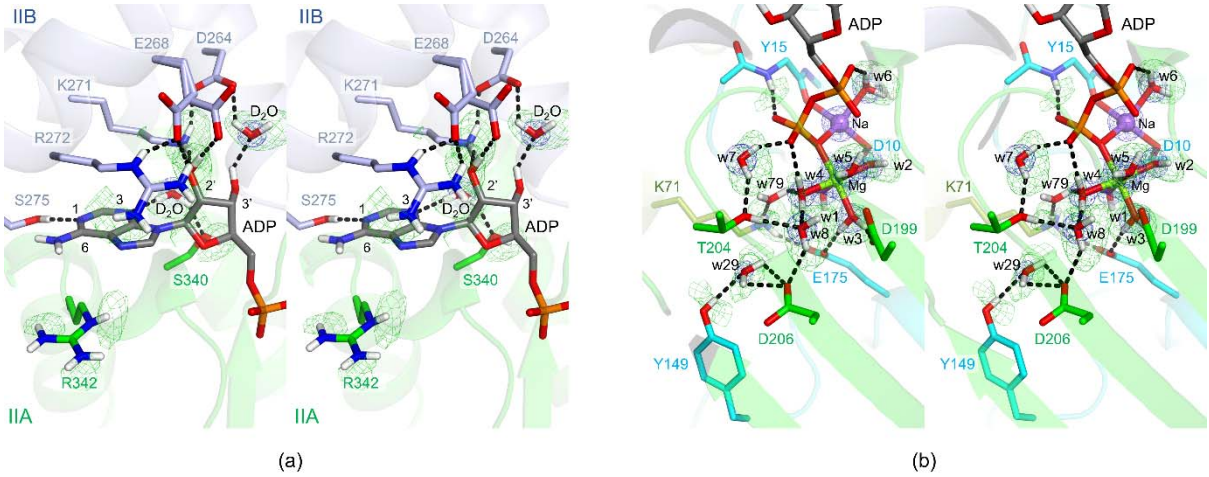


Figure S1 Neutron and X-ray maps around the adenyl group and ribose moiety (a), and the phosphate moiety (b) (PDB code 7F4X). The $|F_o|$-$|F_c|$ neutron scattering length density map omitting deuterium atoms is contoured at +4σ, and $2|F_o|$-$|F_c|$ electron density map omitting water molecules and sodium ions is contoured at 2σ. The neutron scattering density maps and X-ray electron density maps are indicated as green and blue mesh, respectively.

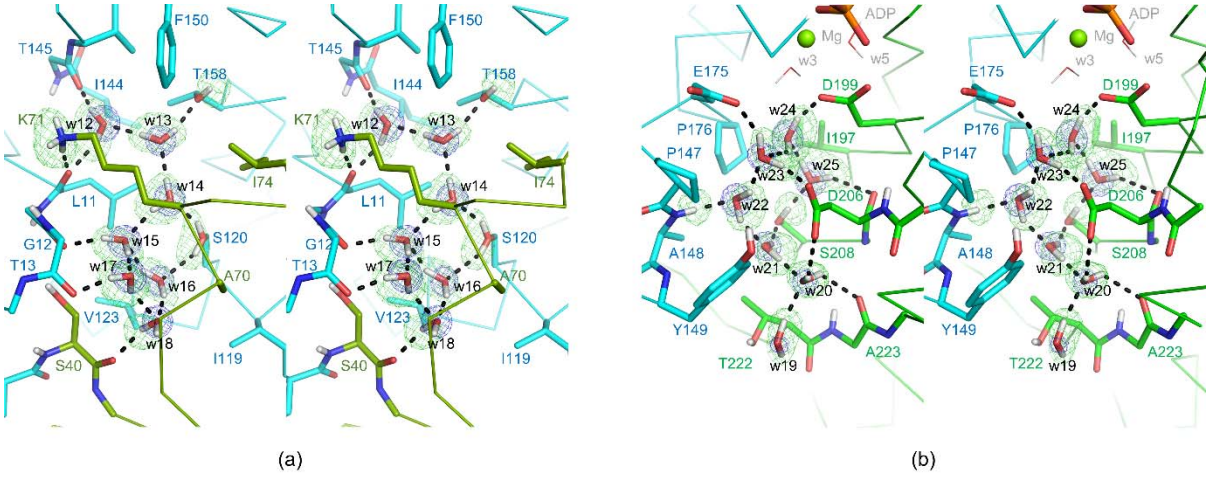
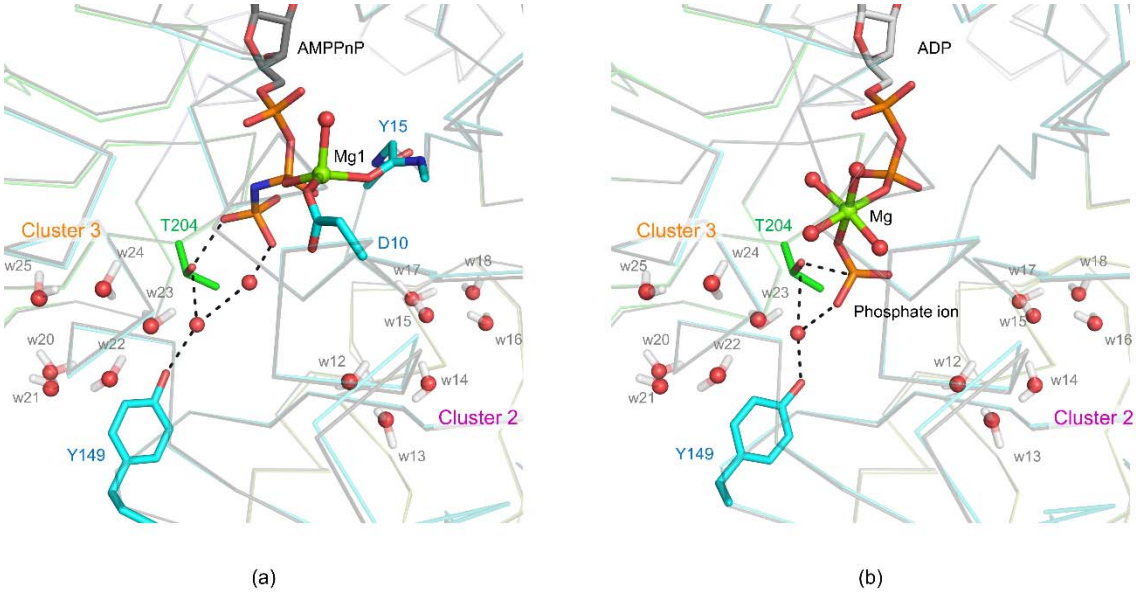
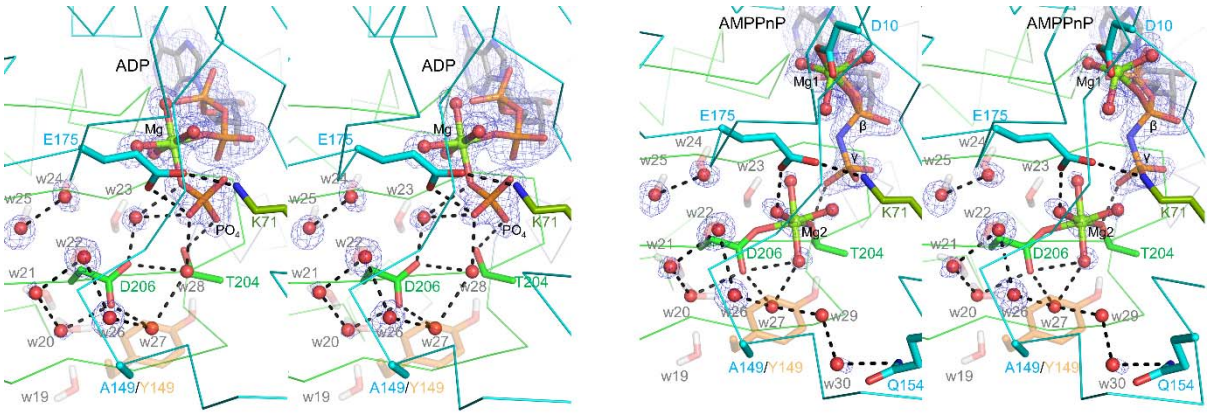


Figure S2 Neutron and X-ray maps around water cluster 2 (w12-w18) (a) and water cluster 3 (w19-w25) (b) (PDB code 7F4X). The $|F_o|$-$|F_c|$ neutron scattering length density map omitting deuterium atoms is contoured at +4σ, and the $2|F_o|$-$|F_c|$ electron density map omitting water molecules is contoured at 2σ. The neutron scattering density maps and X-ray electron density maps are indicated as green and blue mesh, respectively.



(a) (b)

Figure S3 X-ray crystal structure of Hsp72-AMPPnP (PDB code 2E8A) (a) and Hsp72-ADP (PDB code 3ATU) (b) superimposed on the neutron crystal structure of Hsp72-ADP (PDB code 7F4X). The nucleotide molecules, Y204 and Y149 of the X-ray structure are shown in stick model. The water molecules of the X-ray structure are shown in red sphere model. The water molecules of the overlaid joint XN structure are shown in semi-transparent stick model.



(a) (b)

Figure S4 Omit difference Fourier maps for Y149A-Hsp72 in complex with ADP (PDB code 7F4Z) (a) and Y149A-Hsp72 in complex with AMPPnP (PDB code 7F50) (b). The electron density maps are contoured at 4σ. The side chain of Y149 and water cluster 3 of the superposed joint XN structure (PDB code 7F4X) are shown in semi-transparent stick model. The neutron scattering density maps and X-ray electron density maps are indicated as green and blue mesh, respectively.