SUPPORTING INFORMATION

A Local Allosteric Network in Heat Shock Protein 70 (Hsp70) Links Inhibitor Binding to Enzyme Activity and Distal Protein-Protein Interactions

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Supplemental Figure S1. Time evolution of the RMSD (Å) values (backbone atoms) from the 3C7N X-ray for ADP-only, ATP-only, MKT-ADP and MKT-ATP systems (three replicas each).

Supplemental Figure S2. Analysis of axes spanning lobe I and II on representative cluster conformation for ATPonly (left) and MKTATP (right) NBD. The values calculated on MKT-ADP and ADPonly structures are reported.

Supplemental Figure S3. Cluster analysis (on backbone atoms) performed on a unique meta-trajectory resulting from the concatenation of the ADPonly, ATPonly, MKTADP, and MKTATP MD runs (sampling = 200 ps).

Supplemental Figure S4. Interactions and conformational analyses of the allosteric switches (loop12, loop147, loop200, loop225 and sub69 in red, blue, purple, yellow and green respectively) on the most representative structure of the ADPonly, ATPonly and MKTATP state.

Supplemental Figure S5. Additional literature examples of the importance of loop222 and the benzyl position of JG-98. (A) Anti-proliferative activity of previously reported JG-98 analogs (adapted from Shao et al. 2018 J. Med. Chem. 61:6163). Note that ortho-substitution enhances activity on both cancer cell lines. (B) Binding of JG-98 analogs to mutants of Hsc70 (the JG-294 results are adapted from Shao et al. 2018 J. Med. Chem. 61:6163). Binding was measured as described in the text for JG-231 and its analogs.

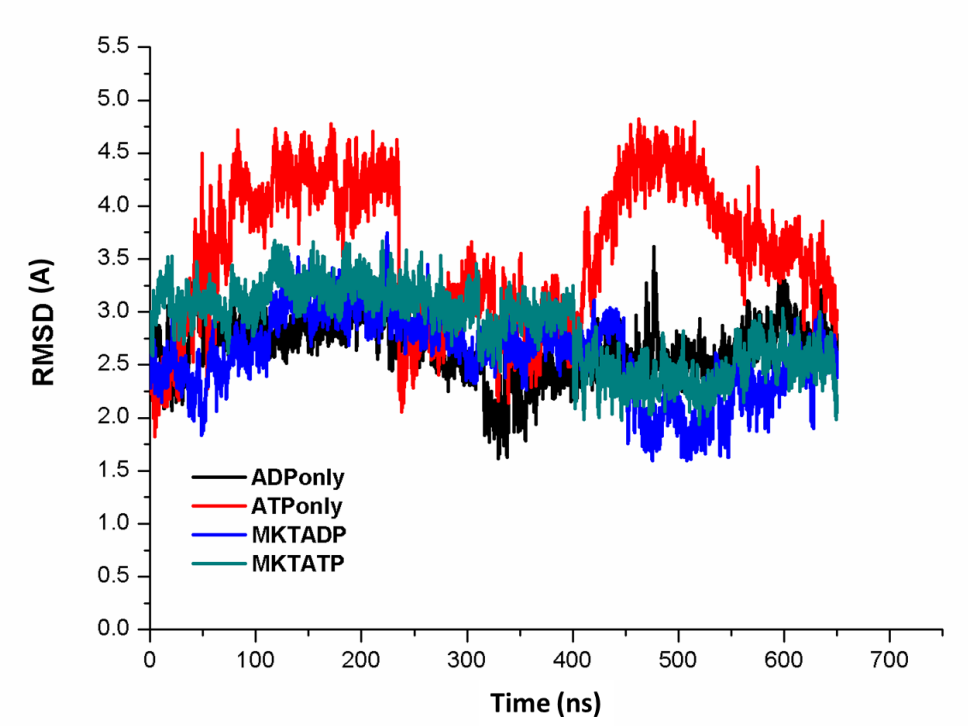

Figure S1. Time evolution of the RMSD (Å) values (backbone atoms) from the 3C7N X-ray for ADP-only, ATP-only, MKT-ADP and MKT-ATP systems (three replicas each).

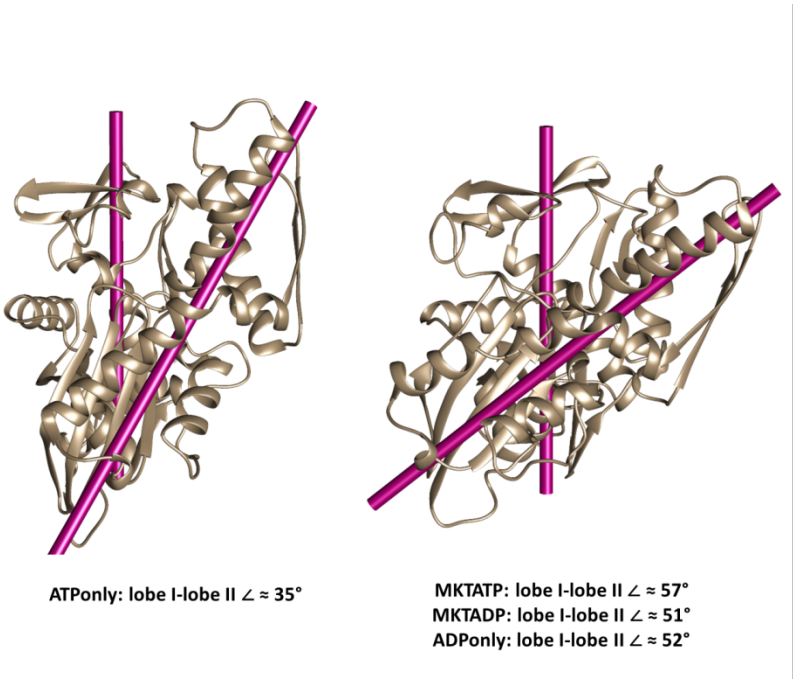

Figure S2. Analysis of axes spanning lobe I and II on representative cluster conformation for ATPonly (left) and MKTATP (right) NBD. The values calculated on MKT-ADP and ADPonly structures are reported.

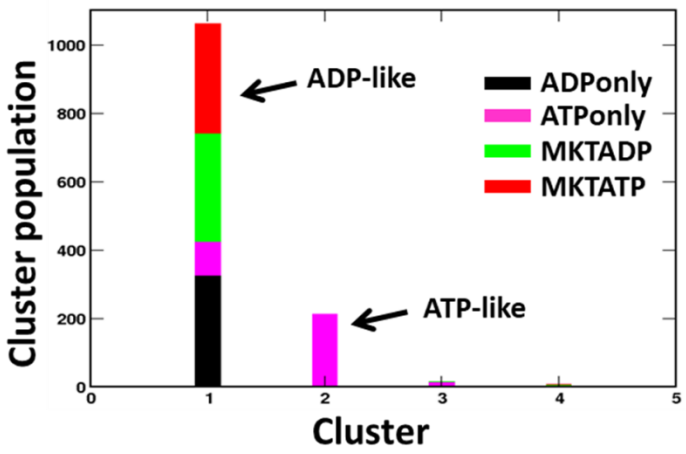

Figure S3. Cluster analysis (on backbone atoms) performed on a unique meta-trajectory resulting from the concatenation of the ADPonly, ATPonly, MKTADP, and MKTATP MD runs (sampling = 200 ps). The outcome is not a real MD time-evolution, but a meta-trajectory whose frames are obtained by joining all the runs computed for each system.

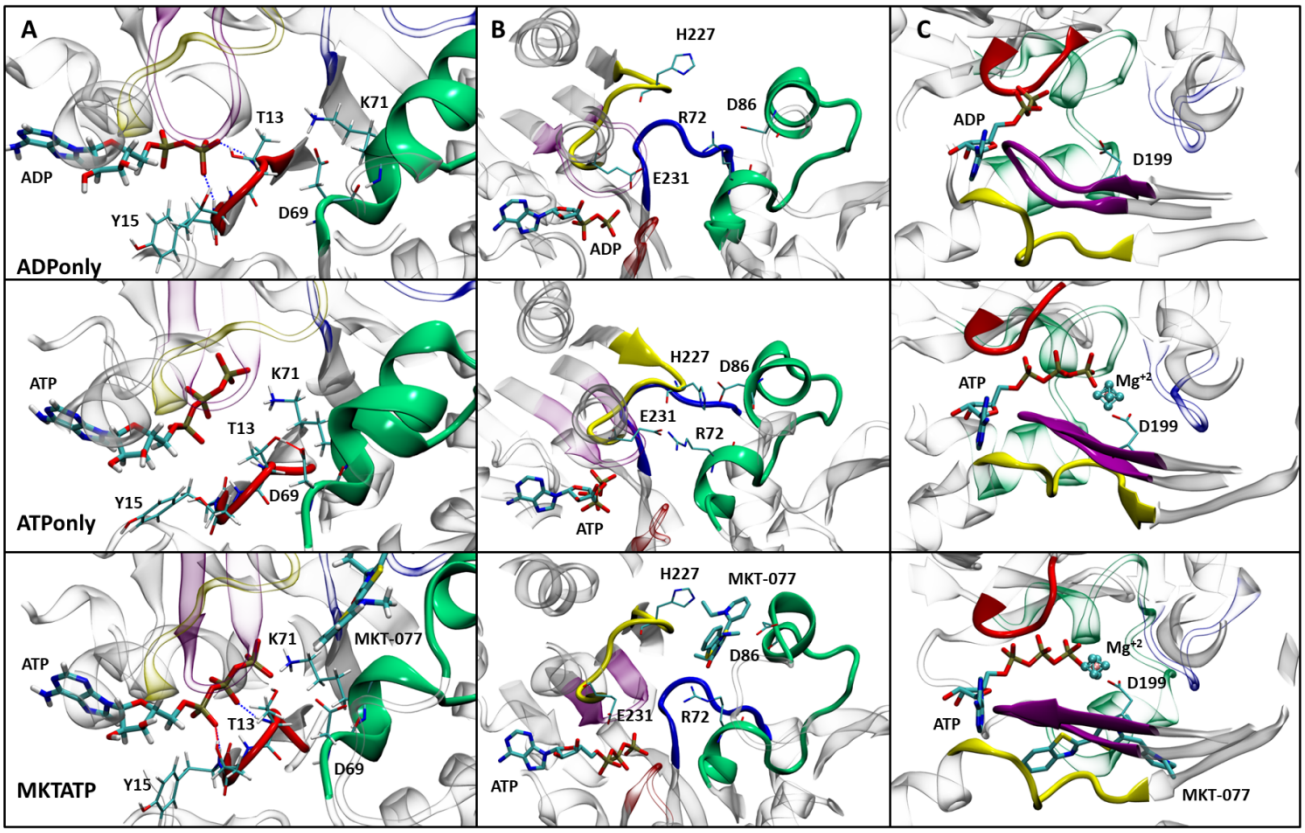

Figure S4. Interactions and conformational analyses of the allosteric switches (loop12, loop147, loop199, loop222 and sub69 in red, blue, purple, yellow and green respectively) on the most representative structure of the ADPonly, ATPonly and MKTATP state.

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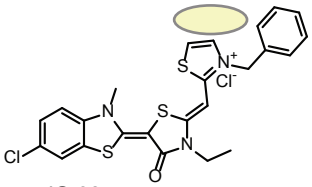
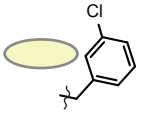
(A) Additional literature examples of the importance of ortho-substitution of the benzyl group in JG-98

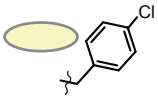


JG-98

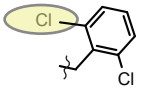
JG-241



JG-271



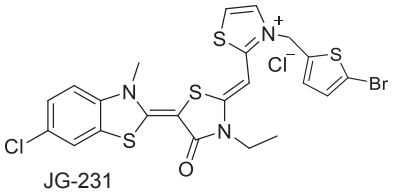
JG-255



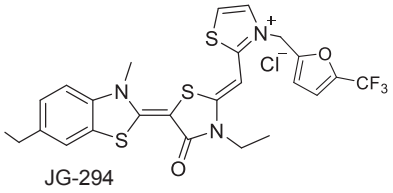
JG-337

	MCF7 (IC_{50} μM)	MDA-MB-231 (IC_{50} μM)
JG-98	0.7	0.4
JG-241	0.19	0.18
JG-271	0.26	0.51
JG-255	1.45	0.41
JG-337	0.16	0.16

(B) Mutations in the MKT-077 binding site weaken the affinity for analogs



JG-231



JG-294

	JG-231 Kd μM	JG-294 Kd μM
$Hsc70_{WT}$	1.0	0.7
Y222A	3.8	13
H227A	3.1	37
L228A	2.7	21
R76A (control)	1.2	0.8

Figure S5. Additional literature examples of the importance of loop222 and the benzyl position of JG-98. (A) Anti-proliferative activity of previously reported JG-98 analogs (adapted from Shao et al. 2018 J. Med. Chem. 61:6163). Note that ortho-substitution enhances activity on both cancer cell lines. (B) Binding of JG-98 analogs to mutants of Hsc70 (the JG-294 results are adapted from Shao et al. 2018 J. Med. Chem. 61:6163). Binding was measured as described in the text for JG-231 and its analogs.