SUPPORTING INFORMATION – Schaffner-Barbero et al.

TABLE S1 Compounds reported to inhibit FtsZ

Compound	References
5,5'-Bis-(8-anilino-1-naphtalenesulfonate)	(*1*)
3-methoxybenzamide (3-MBA)	(*2*)
SRI-3072 & SRI-7614	(*3*)
Viriditoxin	(*4*)
Zantrins Z1-Z5	(*5, 6*)
Ruthenium red	(*7*)
Peptides FtsZp1-3	(*8*)
C8-substituted GTP analogues	(*9, 10*)
Sanguinarine	(*11*)
PC170942 & PC58538	(*12*)
Dichamanetin	(*13*)
2'"-hydroxy-5"-benzylisouvarinol-B	(*13*)
4-Aminofurazan derivative - A189	(*14*)
Taxane derivative (non cytotoxic)	(*15*)
Guanine derivatives (combinatorial)	(*16*)
Totarol	(*17, 18*)
Cinnamaldehyde	(*19*)
DAPI	(*20*)
N-benzyl-3-sulfonamidopyrrolidines	(*21*)
Curcumin	(*22, 23*)
Berberine sulphate	(*24*)
MciZ (peptide)	(*25*)
PC190723 and related difluoromethoxybenzamide derivatives	(*26-29*)
OTBA	(*30*)
Edeine B	(*31*)
Chrysophaentins A-H	(*32*)

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Table S2. MM-GBSA averaged energies along the MD trajectory and the standard deviations over the last 500 ps for each period of 10 ns for 2VAP---GDP complex. The entropy[a] was estimated at t = ∞. All the units are in kcal/mol.

2VAP---GDP	**X-ray**	**MIN**	**10ns**	**20ns**	**30ns**	**40ns**	**50ns**
$\Delta H_{MM\text{-}GBSA}$	-9.03	-50.50	-27.29	-14.85	-16.57	-8.84	-14.15
σ	0.00	0.00	7.00	4.59	3.87	5.21	5.07
$-T\Delta S$ (t = ∞)[a]							-2.56
$\Delta G = \Delta H - T\Delta S$							**-16.71**

[a]The calculated entropies (*S*) are dependent on the length (*t*) of the trajectory that is analyzed, but clearly tend to a limit (S_∞) as the width of the sampling window is increased, as previously described [Harris,S.A., Gavathiotis, E., Searle, M.S., Orozco, M., Laughton, C.A. Cooperativity in drug-DNA recognition: A molecular dynamics study. J. Am. Chem. Soc. 123, 12658–12663 (2001).]. The entropies calculated for a range of window widths are fitted using the empirical relationship:

$$S(t) = S_\infty - \frac{\alpha}{t^n}$$

where α and n are the fitting parameters. This procedure allows for an estimate of S_∞ that is independent of the length of the MD simulation. The fittings were performed with gnuplot version 4.0 program.

Table S3. MM-GBSA averaged energies along the MD trajectory and the standard deviations over the last 500 ps for each period of 10 ns for 1W5A---GTP complex. The entropy was estimated at t = ∞ (see footnote in Table S2). All the units are in kcal/mol. (ca), chain A; (cb), chain b.

1W5A---GTP	**X-ray**	**MIN**	**10ns**	**20ns**	**30ns**	**40ns**	**50ns(ca)**	**50ns(cb)**
$\Delta H_{MM\text{-}GBSA}$	-28.02	-165.76	-105.36	-104.93	-101.91	-99.27	-93.61	-106.95
σ	0.00	0.00	7.96	5.71	5.40	5.60	5.77	5.83
$-T\Delta S$ (t = ∞)[a]							5.30	2.98
$\Delta G = \Delta H - T\Delta S$							**-88.31**	**-103.97**

Table S4. MM-GBSA energies for individual FtsZ residues contributing to GDP binding along the MD trajectory averaged over the last 0.5 ns for each period of 10 ns.Units, kcal/mol.

2VAP---GDP	X-ray	MIN	10ns	20ns	30ns	40ns	50ns
A48	-8.78	-8.69	-2.55	-3.06	-3.20	-3.16	-3.62
T159	-0.60	-0.66	-3.35	-0.57	-0.77	-0.56	-0.66
F162	-0.48	-0.23	-1.43	-2.49	-1.16	-2.45	-1.44
F208	-7.13	-7.23	-2.96	-5.80	-5.54	-4.22	-4.89
A211	-0.24	-0.65	-2.72	-0.32	-0.25	-0.18	-0.40
D212	-10.07	-11.48	-3.61	-0.74	-0.25	-0.49	-1.01
G130	-2.84	-2.87	-2.43	-2.55	-2.40	-1.83	-1.82
L131	-1.48	-1.76	-2.99	-3.09	-2.93	-1.22	-2.42
G132	-2.23	-3.75	-3.22	-1.85	-1.75	-1.88	-1.54
P161	-2.28	-1.51	-2.07	-1.97	-1.80	-1.52	-1.26
R169	-7.67	-28.79	-15.46	-15.88	-16.31	-12.52	-16.52
G133	-6.83	-10.10	-7.80	-7.63	-7.57	-7.12	-7.59
G46	-7.80	-9.99	-1.70	-2.25	-2.33	-1.86	-2.17
G47	-8.31	-9.10	-1.48	-1.93	-2.10	-2.08	-2.19
G99	-1.02	-0.67	-1.70	-0.07	-0.03	-0.01	-0.02
G134	-6.76	-7.31	-8.85	-5.28	-8.29	-9.11	-8.21
T135	-8.59	-12.13	-12.84	-14.81	-15.29	-14.86	-15.57
G136	-6.05	-6.58	-4.38	-4.51	-4.21	-4.16	-4.13

Table S5. MM-GBSA energies for individual FtsZ residues (ca and cb stand for chain a or chain b within PDB 1W5A) contributing to GTP binding along the MD trajectory averaged the last 0.5 ns for each period of 10 ns. Units, kcal/mol.

1W5A---GTP	X-ray	MIN	10ns	20ns	30ns	40ns	50ns (ca)	50ns (cb)
A48	-9.18	-4.33	-5.31	-7.53	-8.47	-8.17	-7.32	-2.95
N51	-0.45	0.64	-1.14	-0.90	-1.55	-0.97	-0.71	-0.09
F208	-6.25	-6.69	-1.20	-0.18	-0.09	-0.21	-0.15	-5.10
K209	-0.64	-0.77	-6.01	-5.76	-4.84	-4.44	-4.45	-0.87
D212	-8.18	-11.77	-1.54	-7.77	-7.85	-8.25	-8.37	-1.98
G130	-2.39	-2.31	-2.09	-2.75	-2.34	-2.46	-2.78	-2.08
L131	-1.59	-1.56	-1.16	-1.61	-1.37	-1.42	-1.22	-2.11
G132	-2.50	-3.58	-2.73	-3.26	-2.95	-2.62	-2.81	-3.46
F162	-0.38	-0.28	-1.33	-0.62	-0.14	-1.28	-1.19	-0.57
E165	0.81	-9.21	-5.61	0.30	0.98	-5.92	-1.47	-7.46
R169	-8.36	-24.2	-2.10	-6.08	-5.96	-0.93	-2.40	-20.12
G47	-7.75	-8.40	-7.65	-9.66	-9.40	-9.80	-8.69	-5.10
G133	-7.78	-12.04	-8.79	-8.50	-8.20	-8.26	-7.70	-8.72
C45	-1.42	-2.16	-1.12	-1.05	-1.19	-1.15	-0.99	-0.42
G46	-8.29	-8.19	-6.32	-7.37	-8.63	-8.08	-6.77	-4.66
N70	-1.04	-2.59	-1.61	-1.38	-2.01	-1.40	-0.12	-1.63
G134	-9.84	-15.11	-10.41	-9.95	-9.76	-10.13	-9.35	-9.30
T135	-4.56	-23.85	-19.81	-18.90	-19.58	-19.65	-17.97	-17.75
G136	-5.38	-6.16	-4.62	-4.72	-5.16	-5.06	-4.85	-4.50
G96	-5.09	-5.99	-6.28	-1.33	-0.09	-0.09	-0.10	-0.97
A97	-7.80	-8.71	-7.44	-1.79	-0.06	-0.06	-0.07	-0.89
G98	-2.58	-9.14	-4.01	-0.22	-0.01	-0.06	0.08	-1.25
G99	-5.13	-8.14	-5.27	-0.77	-0.33	-0.45	-0.10	-1.62
C129	-1.94	-1.24	-0.51	-1.76	-1.88	-1.92	-1.84	-1.63
T159	-0.48	-0.42	-1.09	-3.44	-1.44	-2.37	-2.92	-2.08

Table S6. Individual interactions energies (MM-GBSA) assigned to the different parts of the GDP molecule averaged along the MD trajectory over the last 0.5 ns for each period of 10 ns. All the values are in % of total free energy change.

2VAP---GDP	X-ray	MIN	10ns	20ns	30ns	40ns	50ns
Guanine ring[a]	30.21	23.19	18.42	15.99	13.81	14.52	15.00
Ribose ring[b]	13.18	16.02	21.41	21.08	19.65	16.80	17.56
α-phosphate[c]	9.57	19.63	17.43	19.26	19.29	17.20	19.62
β-phosphate[d]	47.04	41.16	42.74	43.67	47.30	51.48	47.82

a $$E(Guanine) = E(\mathrm{D212}) + E(\mathrm{A211}) + E(\mathrm{F208}) + \\ + E(\mathrm{A48}) + \frac{2}{3}E(\mathrm{T159}) + \frac{2}{3}E(\mathrm{F162})$$

b $$E(Ribose) = \frac{1}{3}E(\mathrm{T159}) + \frac{1}{3}E(\mathrm{F162}) + E(\mathrm{P161}) + \\ + E(\mathrm{G130}) + E(\mathrm{L131}) + E(\mathrm{G132}) + \\ + \frac{1}{3}E(\mathrm{R169})$$

c $$E(\alpha - phosphate) = \frac{2}{3}E(\mathrm{R169}) + \frac{1}{2}E(\mathrm{G133})$$

d $$E(\beta - phosphate) = \frac{1}{2}E(\mathrm{G133}) + E(\mathrm{G46}) + E(\mathrm{G47}) + \\ + E(\mathrm{C99}) + E(\mathrm{G134}) + E(\mathrm{T135}) + \\ + E(\mathrm{G136})$$

Table S7. Individual interactions energies (MM-GBSA) assigned to the different parts of the GTP molecule (ca and cb stand for chain a or chain b within PDB 1W5A) averaged along the MD trajectory over the last 0.5 ns for each period of 10 ns. All the values are in % of total free energy change (hyd and elec stand for hydrophobic and electrostatics contributions respectively).

1W5A---GTP	X-ray	MIN	10ns	20ns	30ns	40ns	50ns (ca)	50ns (cb)
Guanine ring[a]	17.90	12.41	10.11	19.00	17.60	18.78	20.74	12.11
Ribose ring [b]	15.06	24.55	15.75	15.67	14.50	16.00	14.70	35.00
α-phosphate[c]	9.01	3.83	5.44	8.36	9.40	8.87	9.00	4.21
β-phosphate[d]	33.78	16.72	29.25	33.74	37.00	35.75	34.29	25.54
γ-phosphate [e]	24.26	33.85	39.45	23.23	21.50	20.61	21.27	23.14

a $$E(Guanine) = \frac{1}{2}E_{hyd}(\mathrm{A48}) + E(\mathrm{N51}) + E(\mathrm{F208}) + \\ + \left[\frac{1}{2}E_{elec} + E_{hyd}\right](\mathrm{K209}) + E(\mathrm{D212}) + E(\mathrm{C129}) + \\ + E(\mathrm{T159})$$

b $$E(Ribose) = \frac{1}{2}E_{hyd}(\mathrm{A48}) + E(G130) + E(\mathrm{L131}) + \\ + E(\mathrm{G132}) + E(\mathrm{F162}) + E(\mathrm{E165}) + \\ + E(\mathrm{R169}) + \frac{1}{2}E_{elec}(\mathrm{K209})$$

c $$E(\alpha - phosphate) = E_{hyd}(\mathrm{G47}) + E_{elec}(\mathrm{A48}) + \frac{1}{3}E(\mathrm{G133})$$

d $$E(\beta - phosphate) = E(\mathrm{C45}) + E(\mathrm{G46}) + E_{elec}(\mathrm{G47}) + \\ + E(\mathrm{N70}) + \frac{1}{3}E(\mathrm{G133}) + E(\mathrm{G134}) + \\ + E_{hyd}(\mathrm{T135}) + E(\mathrm{G136})$$

e $$E(\gamma - phosphate) = E(\mathrm{G96}) + E(\mathrm{A97}) + E(\mathrm{G98}) + \\ + E(\mathrm{G99}) + \frac{1}{3}E(\mathrm{G133}) + E_{elec}(\mathrm{T135})$$

TABLE S8: Compounds from virtual screening the ChemBridge libary assayed for possible binding to the FtsZ nucleotide site, their MM-GBSA binding energies (ΔG; Materials and Methods). The ZINC and SMILES codes and be used to retrieve their chemical structures.

Compound	ΔG	ZINC code	SMILES Code
1	-54.220	ZINC01013671	COc1ccccc1N2CCN(CC2)C(=O)c3c(c4nc(cc(n4n3)C(F)(F)F)c5cccs5)Cl
2	-51.190	ZINC02835510	c1ccc(c(c1)C(=O)Nc2ccc(cc2O)Cc3ccc(c(c3)NC(=O)c4ccccc4F)O)F
3	-46.240	ZINC04754551	Cc1ccc(c(c1)C)Nc2cc(c3c(c2[N+](=O)[O-])non3)N4CCOCC4
4	-42.870	ZINC01123094	c1ccc2c(c1)C3c4ccccc4C2[C@H]5[C@H]3C(=O)N(C5=O)CC(=O)N6CCOCC6
5	-51.510	ZINC02877880	CCc1c2c3cc(c(cc3c(nc2n(n1)c4ccccc4)c5cc(ccc5OC)OC)OC)OC
6	-52.620	ZINC02896368	c1ccc(cc1)C2=C(c3ccccc3C2=O)Nc4ccc(cc4)NC5=C(C(=O)c6c5cccc6)c7ccccc7
7	-45.940	ZINC01210791	c1ccc(cc1)Sc2ccc(o2)/C=c/3\c(=O)n4c5ccccc5nc4s3
8	-47.640	ZINC02905790	c1cc2cccnc2c(c1)OC(=O)c3cccc(c3)S(=O)(=O)Oc4cccc5c4nccc5
9	-40.040	ZINC04361168	c1ccc2c(c1)[C@@H]3CC(=NN3[C@@H](O2)c4cc5c(cc4Cl)OCO5)c6cccs6
10	-39.220	ZINC02325357	CC(C)(C)c1ccc(cc1)c2nnc(n2/N=C/c3cccc(c3)OC)S
11	-38.150	ZINC04498679	COc1ccccc1N2CC[NH+](CC2)Cc3cc(c(c4c3cccn4)[O-])[N+](=O)[O-]
12	-38.870	ZINC01202593	CC(C)c1ccc(cc1)N2C(=O)C(=Cc3ccc(o3)I)C(=O)NC2=O
13	-43.690	ZINC01206280	CCc1ccccc1NC(=O)c2ccccc2N(Cc3ccccc3)S(=O)(=O)c4ccc(cc4)OC
14	-37.250	ZINC04622072	COc1ccccc1Nc2cc(c3c(c2[N+](=O)[O-])non3)N4CCCCCC4
15	-32.120	ZINC04707049	Cc1ccc(c(c1)Nc2cc(c3c(c2[N+](=O)[O-])non3)N4CCOCC4)C
16	-33.170	ZINC02952617	c1cc(cc(c1)[N+](=O)[O-])C(=O)c2cccc(c2)S(=O)(=O)c3cccc(c3)[N+](=O)[O-]
17	-35.920	ZINC01187206	CCOc1ccccc1NC(=O)c2ccccc2N(Cc3ccccc3)S(=O)(=O)c4ccc(cc4)OC

TABLE S9: Compounds from virtual screening the IBScreen libary assayed for possible binding to the FtsZ nucleotide site, their MM-GBSA binding energies (ΔG; Materials and Methods), ZINC and SMILES codes. Compounds 5 and 8 were provided as a racemic mixture.

Compound	ΔG	ZINC code	SMILES Code
1	-56.01	ZINC01862699	Cn1c2c(c(=O)[nH]c1=O)n(c(n2)N3CCCC3)CCCSc4nnnn4c5ccccc5
2	-51.17	ZINC02222202	c1ccc(cc1)c2c[n+](c3n2CCC3)CC(=O)Nc4ccc(cc4)Oc5ccccc5
3	-48.41	ZINC02205956	C[C@@H](c1nc2c3c4c(sc3ncn2n1)CCCCC4)N5C(=O)c6ccccc6C5=O
4	-48.34	ZINC04135366	c1ccnc(c1)[C@@H]2CCCCC2=NNc3ccc(cc3[N+](=O)[O-])[N+](=O)[O-]
5	-47.61	ZINC02345916	CCOc1cc(cc(c1[O-])[N+](=O)[O-])[C@H]2Nc3c(c4c(s3)C[NH+](CC4)C)C(=O)N2
6	-45.59	ZINC02196523	COC(=O)c1ccc(cc1)OCCN2c3ccccc3N4C2=[NH+]CC4
7	-44.04	ZINC05350455	C[C@@H]1CCc2c(sc3c2c4nc(nn4cn3)CN5C(=O)c6ccccc6C5=O)C1
8	-43.82	ZINC02345919	CCOc1cc(cc(c1[O-])[N+](=O)[O-])[C@@H]2Nc3c(c4c(s3)C[NH+](CC4)C)C(=O)N2
9	-43.24	ZINC04414377	Cn1c2ccc(cc2n(c1=O)C)NC(=O)Cn3cnc4c3cccc4
10	-43.12	ZINC01117923	c1ccc(cc1)n2c(nnn2)Oc3ccccc3C(=O)N4CCOCC4
11	-43.05	ZINC02206216	CC[NH+](CC)CCCNC(=O)c1nc2c3c4c(sc3ncn2n1)CCCC4
12	-42.48	ZINC05461887	COc1cccc(c1c2nn3c(nnc3s2)c4c5c([nH]n4)CCC5)OC
13	-42.37	ZINC02355147	c1ccc2c(c1)C(=O)N(C2=O)Cc3nc4c5c6c(sc5ncn4n3)CCCC6
14	-40.34	ZINC01270429	COc1cccc(c1)c2nn3c(nnc3s2)c4cc([nH]n4)c5ccccc5
15	-40.10	ZINC04772681	c1ccc2c(c1)cnn(c2=O)Cc3nc4c5c6c(sc5ncn4n3)CCCCC6
16	-40.01	ZINC00856854	CC1(C[C@H](CCO1)n2c(=O)c3c(n4c2nnc4)-c5ccccc5CC36CCCCC6)C
17	-39.87	ZINC04844048	c1ccc(cc1)C[NH+]2CCc3c(sc4c3c(=O)n(c5n4nnn5)c6ccccc6)C2
18	-39.53	ZINC04017739	CCCO[P@](=O)(c1c(cc(cc1[N+](=O)[O-])C(F)(F)F)[N+](=O)[O-])[O-]
19	-37.38	ZINC04116739	c1ccc(c(c1)Nc2cc(c3c(c2[N+](=O)[O-])non3)N4CCOCC4)O

FIGURE LEGENDS

FIGURE S1. A. Titration of apoFtsZ with *mant*-GTP employing anisotropy. B. Titration of *mant*-GDP (50 nM) with apoFtsZ. The lines correspond to the best fits to the binding data.

FIGURE S2. Evolution of the interaction free energy values (according to MM-GBSA method in kcal/mol) along the MD trajectories for GDP in 2VAP (A) and GTP in 1W5A (B).

FIGURE S3. A- Minimized average structures corresponding to the last 500 ps of MD taken in periods of 10 ns superimposed onto the X-ray one for GDP (2VAP). B- Minimized average structures corresponding to the last 500 ps taken in periods of 10 ns superimposed onto the X-ray one for GTP (1W5A).

FIGURES

Figure S1

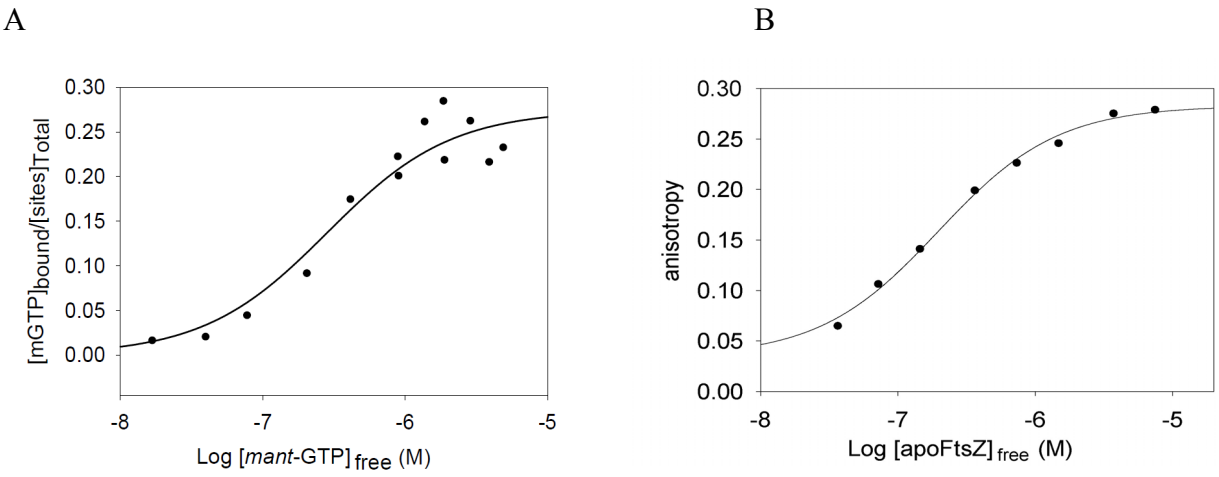


Figure S2

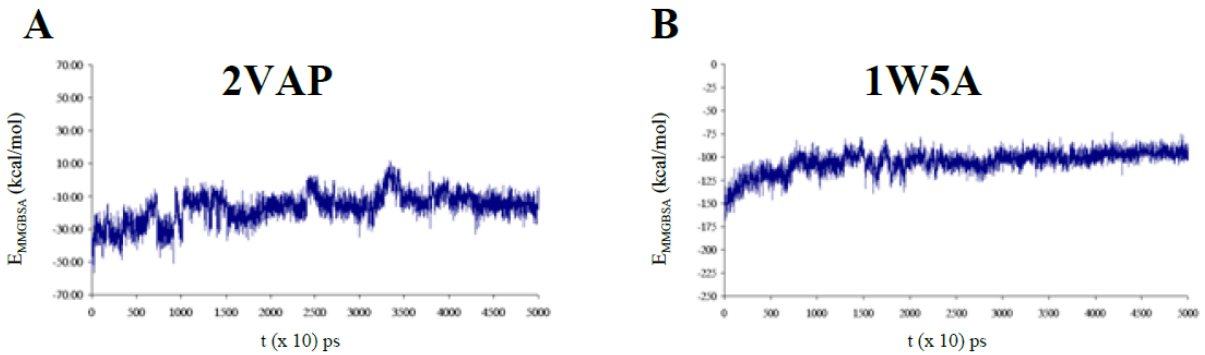
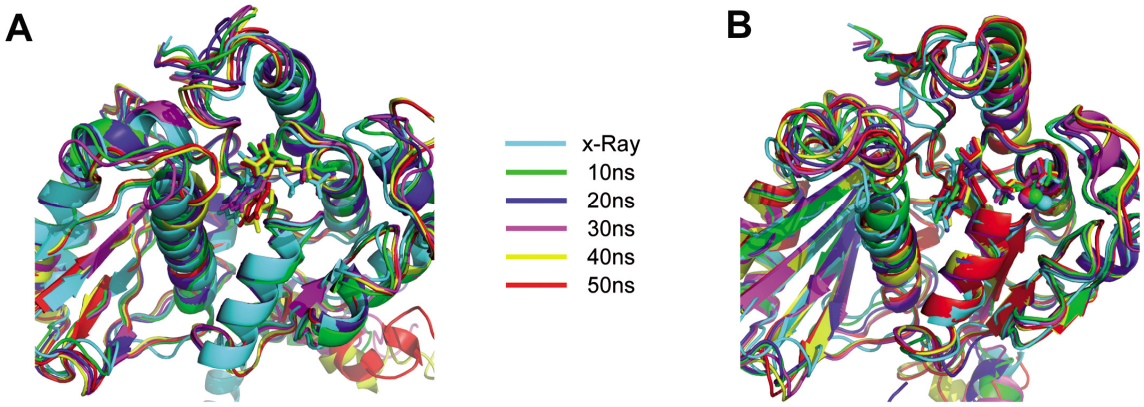


Figure S3



OTHER SUPPORTING INFORMATION

PDB files with the average structures from the molecular dynamics trajectories after 0 and 50 ns: 2vap.t0.pdb, 2vap-t50.pdb, 1w5a.subA.t0.pdb, 1w5a.subA.t50.pdb, 1w5a.subB.t0.pdb, 1w5a.subB.t50.pdb