Molecular-Dynamics Simulations of the ATP/apo State of a Multidrug ATP-Binding Cassette Transporter Provide a Structural and Mechanistic Basis for the Asymmetric Occluded State

Supporting Material

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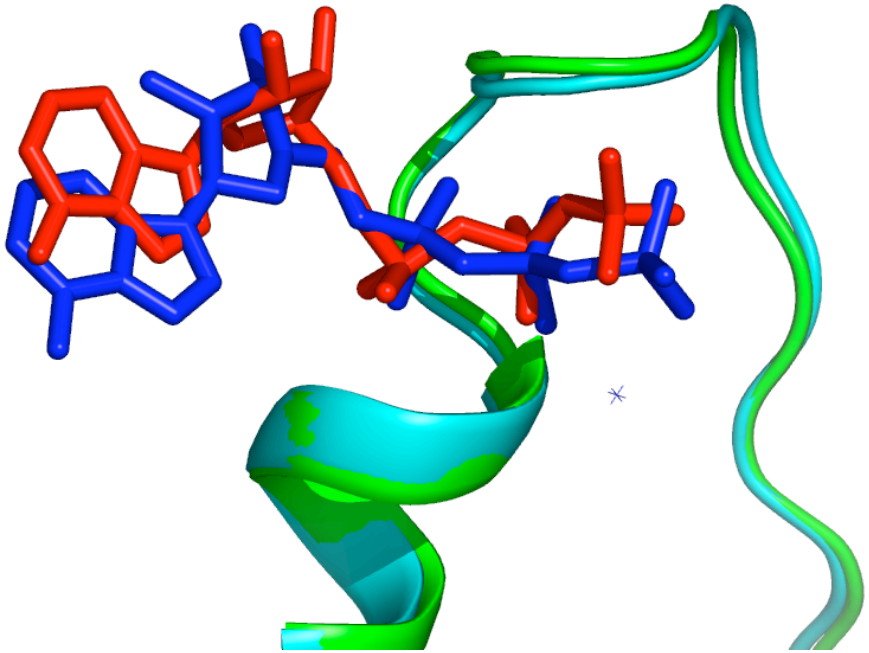
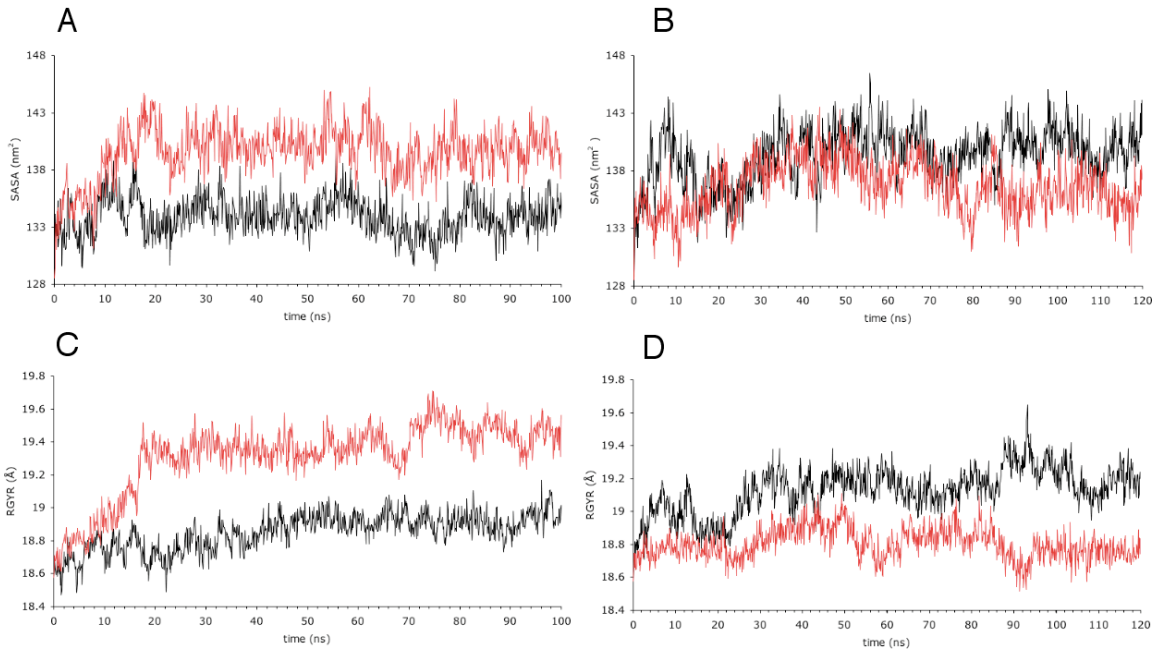
Suppl. Figure 1



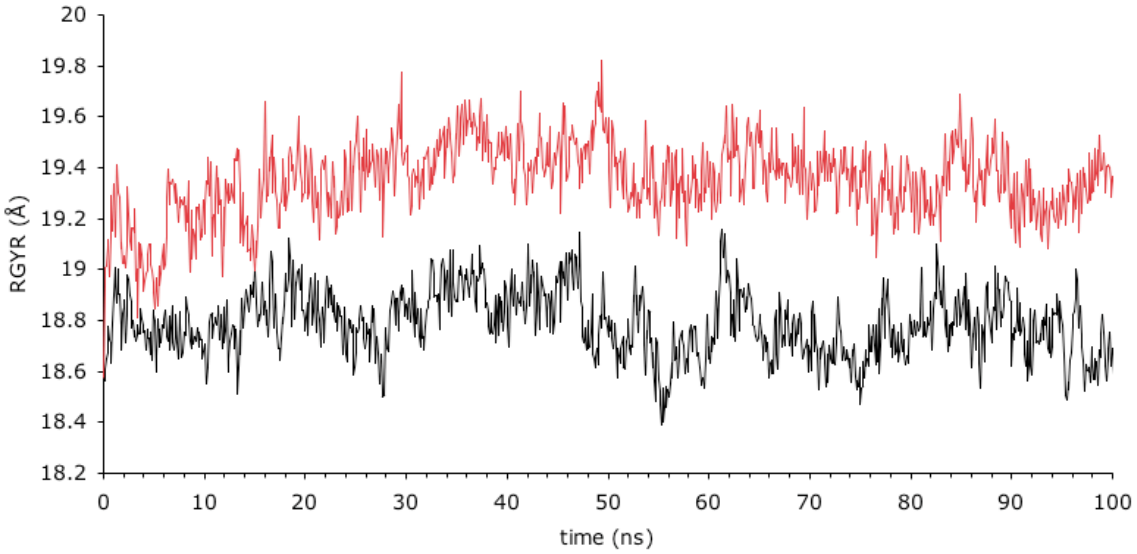
Suppl. Figure 1. Comparison of the binding mode of the nucleotide in the Sav1866 and MJ0796 ABC structures.
Structural overlay using Cα atom coordinates of the P-loop region of Sav1866 (cyan) and MJ0796 (green). The nucleotides are shown in stick form with the ATP molecule from the MJ0796 structure (red) and the AMPPNP molecule from the Sav1866 structure (blue).

Suppl. Figure 2



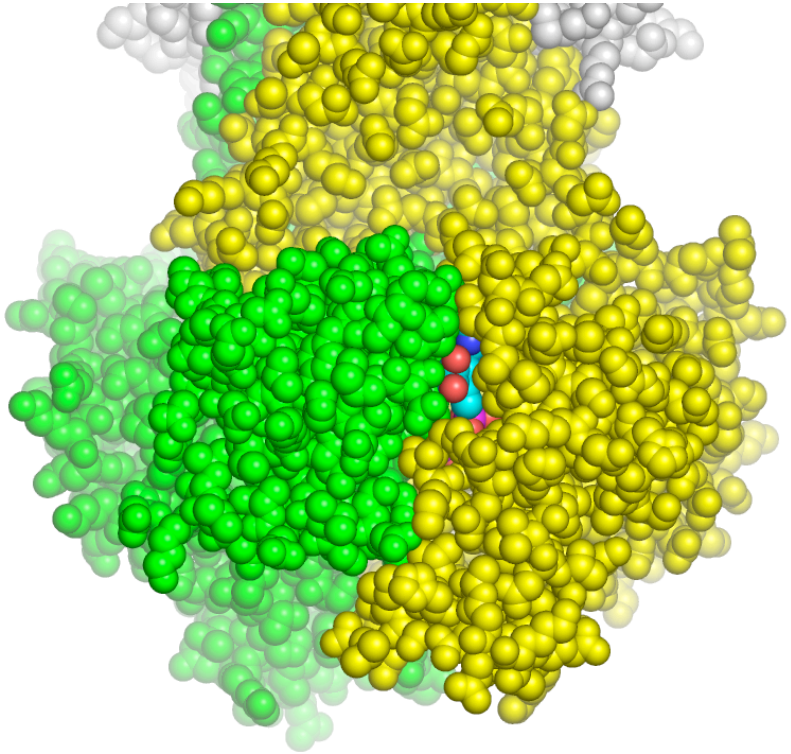
Suppl. Figure 2. Opening of the apo NBD in the ATP/apo simulations.
Time course of the solvent accessible surface area (SASA) and radius of gyration (RGYR) of each NBD (residues 338-578) in the ATP/apo simulations. SASA calculated with a probe radius of 1.4 Å. NBDA black, NBDB red. (*A*) SASA simulation 2A. (*B*) SASA simulation 2B. (*C*) RGYR simulation 2A. (*D*) RGYR simulation 2B.

Suppl. Figure 3



Suppl. Figure 3. More compact state of the ATP-bound NBD in the monomer simulations.
Time course of the radius of gyration (RGYR) of each the NBD (residues 338-578) in the ATP-bound (black) and apo (red) monomer simulations.

Suppl. Figure 4



Suppl. Figure 4. Occluded active sites in the Sav1866 structure.
Space filling representation of the Sav1866 structure illustrating the sequestered state of the bound nucleotides. The view is parallel to the plane of the membrane, with regions used in the simulations coloured green (monomer A) and yellow (monomer B). The nucleotides are shown in space filling representation with oxygen red, nitrogen blue, carbon cyan, phosphorous gold. Regions not included in the simulated structure are coloured grey.

Suppl. Table 1. Interactions between protein atoms and the bound nucleotide in crystal structures of ABC transporter NBDs.[a]

PDB[b]	Protein name	ligand	OA 9S	OA 9N	OB 4N[c]	OB 5N	OB 6N	OB 7N	OB 8N	OB 7S	OB MG	MG 8S	WB 8S	WB W1	MG W2	OG 7S	OG MG	Q MG[d]	OG SS2	OG SS4
1G6H	MJ1267	ADP	✓	✓	✓[e]	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	-	-	-	-	-
1F3O	MJ0796	ADP	✓	✓	✓[e]	✓	✓	✓[e]	✓	✓	✓	✓	✓	✓	✓	-	-	-	-	-
1JJ7	TAP1	ADP	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	-	-	-	-	-
1Q3HA	CFTR	AMPPNP	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✖	✖	✓	✓	✓	-	-
1Q3HB	CFTR	AMPPNP	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✖	✖	✓	✓	✓	-	-
1Q3HC	CFTR	AMPPNP	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	-	-
1Q3HD	CFTR	AMPPNP	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	-	-
1R10A	CFTR	ATP	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✖	✖	✖	✓	✓	-	-
1R10B	CFTR	ATP	✓	✓	✓	✓	✓	✓	✓	✖	✓	✓	✓	✖	✖	✖	✓	✓	-	-
1R0XA	CFTR	ATP	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	-	-
1R0XB	CFTR	ATP	✓	✓	✓	✓	✓[e]	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	-	-
1R0XC	CFTR	ATP	✓	✓	✓	✓	3.39	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	-	-
1R0XD	CFTR	ATP	✓	✓	✓	✓	✓[e]	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	-	-
1OXVA	GLCV	AMPPNP	✓	✓	✓	3.35	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	-	-
1OXVB	GLCV	AMPPNP	✓	✓	✓	3.37	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	-	-
1OXVC	GLCV	AMPPNP	✓	✓	✓	3.39	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	-	-
1OXUA	GLCV	ADP	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	-	-	-	-	-
1OXUB	GLCV	ADP	✓	✓	✓	3.42	✓	✓	✓	✓	✓	✓	✓	✓	✓	-	-	-	-	-
1OXUC	GLCV	ADP	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	-	-	-	-	-
2CBZ	MRP1	ATP	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	-	-
1XEF(4)	HlyB	ATP	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✖	✓	✓
2IXE(2)	TAP1	ATP	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✖	✓	✓
2IXF(4)	TAP1	ATP	✓	✓	✓	✓[e]	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✖	✓	✓
3FVQ(2)	FPBC	ATP	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
1L2T(2)	MJ0796	ATP	✓	✓	✓	✓	✓	✓	✓	✖	✓	✓	✓	✓	✓	✓	✓	✖	✓	✓
3C41B	ARTP	AMPPNP	✓	✓	✓	3.48	✖	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✖	-	-
2ONJ(2)	Sav186	AMPPNP	✖[g]	✖[g]	✖	✓	✖	✖	✓	✓	✖	✓	✓	✖	✖	✖	✖	✓	✖	✖

[a] Only structures containing an ion located analogously to the catalytic magnesium in P-loop proteins were examined. Walker A residues G-X-X-G-X-G-K-S/T-S/T are numbered 1-9 with "N" indicating the backbone amide nitrogen atom and "S" the appropriate sidechain atom. Oxygen atoms of the nucleotide pyrophosphate moiety are denoted OA, OB, and OG corresponding to oxygen atoms of the α-, β-, and γ-phosphates, respectively. The proximal Walker B aspartate carboxyl oxygen atom is indicated by "WB", the catalytic metal ion by "MG" and the conserved glutamine sidechain oxygen atom by "Q". The coordinating water molecules of the magnesium ion are denoted "W1" and "W2", with W1 indicating the water that also binds to the Walker B aspartate. The hydroxyl oxygen of the proximal LSGGQ signature sequence serine is indicated by "SS2" and the backbone nitrogen of the second glycine by "SS4". Hydrogen bonds were determined using the application SwissPDBViewer and are indicated by a tick where present and cross where not. Where a hydrogen bond was not detected, but the distance between the atoms was less than 3.5Å, the distance is given. For interactions with the catalytic magnesium, a cutoff of 2.8Å was used.

[b] Where there are differences between NBD monomers in the crystallographic asymmetric unit, the values are given for each monomer, with the PDB code followed by the respective chain identifier given in the PDB entry. Where all monomers are the same, the number of monomers in the crystallographic asymmetric unit is given in brackets when greater than 1.

[c] Refers to the phosphoester oxygen of the β-phosphate.

[d] This interaction is not observed in any crystal structures where Mg^{++}ADP is the bound nucleotide.

[e] Weaker hydrogen bond indicated by SwissPDBViewer.

[f] Walker A residue 3 does not have a sidechain that can form a hydrogen bond.

[g] Although a hydrogen bond is detected between an a-phosphate oxygen and the Walker A atom, it is not the equivalent α-phosphate oxygen to that observed the other crystal structures.

Suppl. Table 2. Distances between protein atoms and the bound nucleotide in the MD simulations.[a]

Run[b]	OA 9S	OA 9N	OB 4N	OB 5N	OB 6N	OB 7N	OB 8N	OB 7S	OB MG	MG 8S	WB 8S	WB W1	MG W2	Q MG	OG 3S	OG 7S	OG MG	OG SS2	OG SS4
1A	2.83	3.01	2.93	3.14	3.09	2.95	3.07	2.81	2.07	2.17	2.6	2.73	2.11	2.1	2.77	2.71	2.02	2.88	3.02
1B	2.86	3	2.95	3.15	3.1	2.96	3.04	2.79	2.08	2.16	2.62	2.62	2.11	2.1	2.73	2.69	2.01	2.85	3.04
2A	2.84	2.96	2.94	3.13	3.04	2.94	3.08	2.79	2.08	2.19	2.65	2.71	2.13	2.12	2.72	2.69	2.01	3.02	2.95
2B	2.85	3.03	2.98	3.12	3.12	2.99	3.05	2.78	2.08	2.16	2.63	2.66	2.12	2.1	2.74	2.72	2.01	3.03	2.98
3	2.81	2.98	3.03	3.09	3.12	3.04	3.01	2.8	2.09	2.15	2.62	2.65	2.12	2.09	3.15	2.71	2.02	-	-

[a] Distances are in angstroms (Å), measured at 10ps intervals. Standard deviations for all protein-nucleotide atom distances were < 0.2Å and for distances to the magnesium ion < 0.1Å. Atoms indicated as in Table S1.
[b] The letter refers to the ATP bound to the P-loop in monomer A or monomer B.

Suppl. Table 3. Overlap of ANM mode 1 with residue fluctuations in the simulations.

simulation	complex (ATP/apo)		complex (apo/ATP)		monomer	
monomer (nucleotide)	A (ATP)	B (apo)	A (apo)	B (ATP)	apo	ATP
overlap all [a]	11.0	12.9	17.8	11.5	16.6	14.2
correlation ANM1/PC1 [b]	0.451 (18.5)	0.812 (30.4)	0.964 (24.4)	0.786 (24.3)	0.908 (22.5)	0.558 (20.8)

[a] percent overlap between ANM mode 1 and all Cα atom fluctuations.
[b] Correlation coefficient between the projections of the simulation trajectory frames onto the ANM mode 1 and PCA mode 1. Percentage contribution of PC1 to total Cα atom fluctuations given in parentheses.