Supplementary Material to Accompany:

Molecular Mechanism for $PreQ_1$-II Riboswitch Function Revealed by Molecular Dynamics

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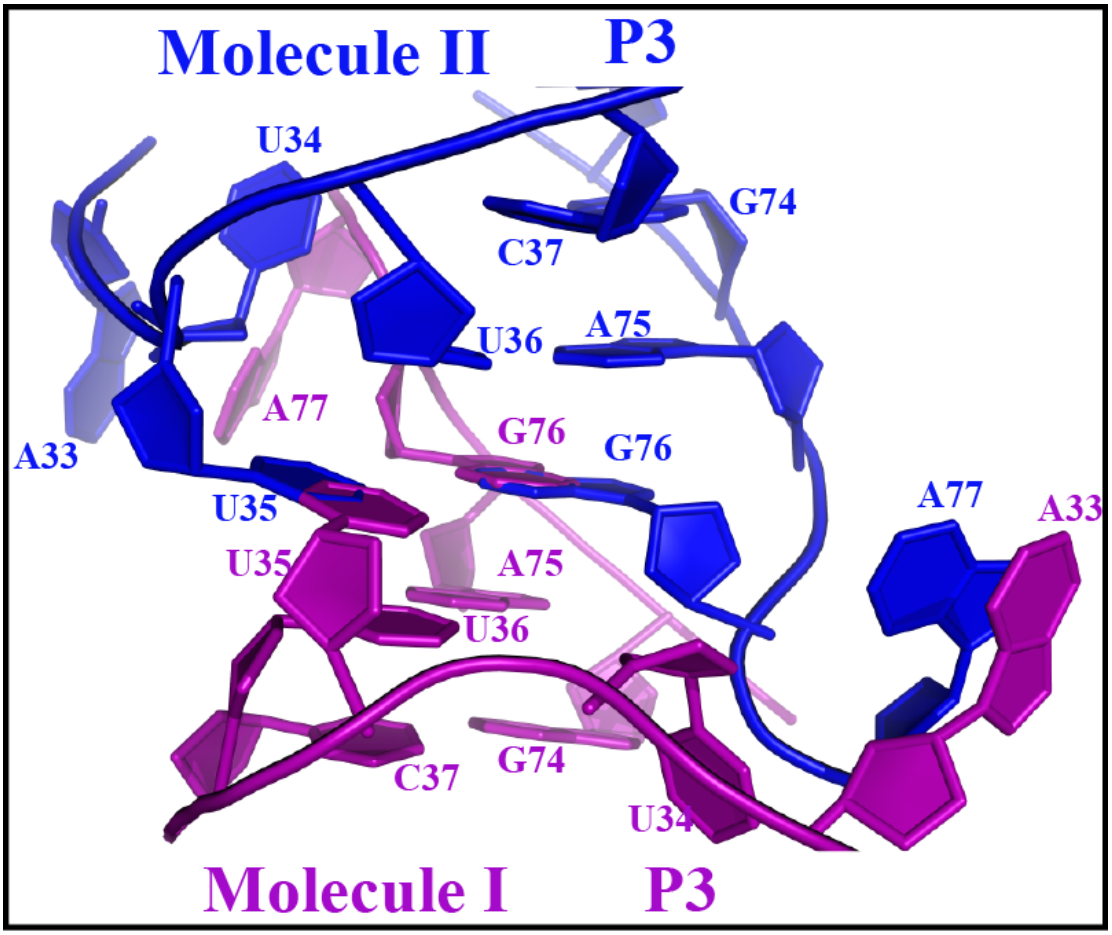

Figure S1. Symmetry-related crystal contacts in the crystal lattice. Molecule I is illustrated in purple and molecule II (symmetry) is illustrated in blue. Nucleotides G76 and U35 were modeled with half-occupancy, and thus appear as spatially overlapping conformations (Liberman et al. 2013). The other conformer of each nucleotide appears to be conformationally disordered. These P3-P3 contacts possibly explain why the crystal structure did not show the expected canonical base pairs between G76 and U35 and also between A77 and U34. A77 from one molecule stacks on A33 from the adjacent molecule, sequestering it from the pair with U34. The partial occupancies and disorder in G76 and U35 suggest that the contacts are altering the

structure compared to that expected in solution.

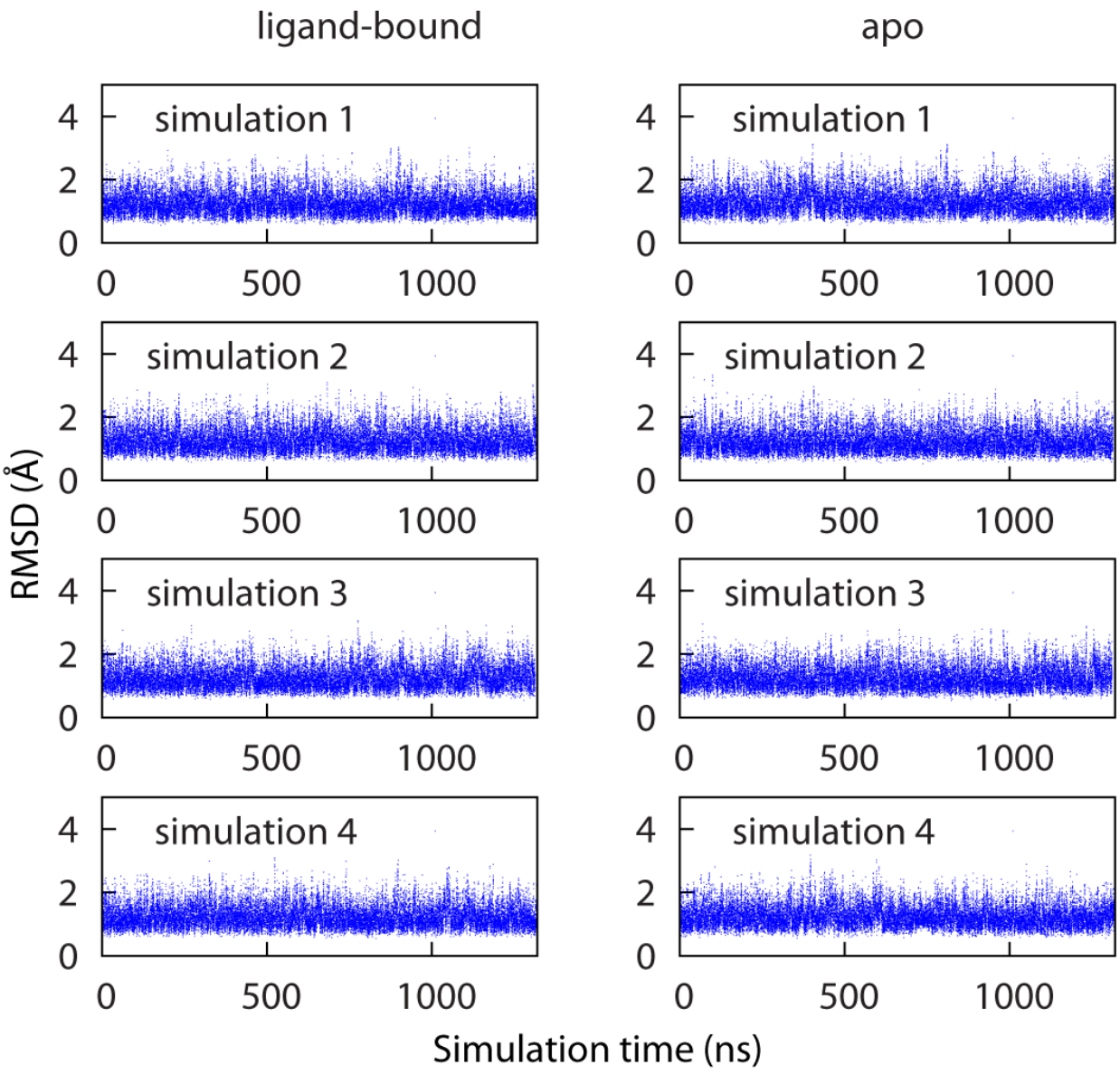

Figure S2. P1 helix mass weighted, heavy-atom RMSD to starting structure as a function of time. The left column shows ligand-bound simulations and the right column shows apo simulations. The location of P1 is illustrated in Figure 1.

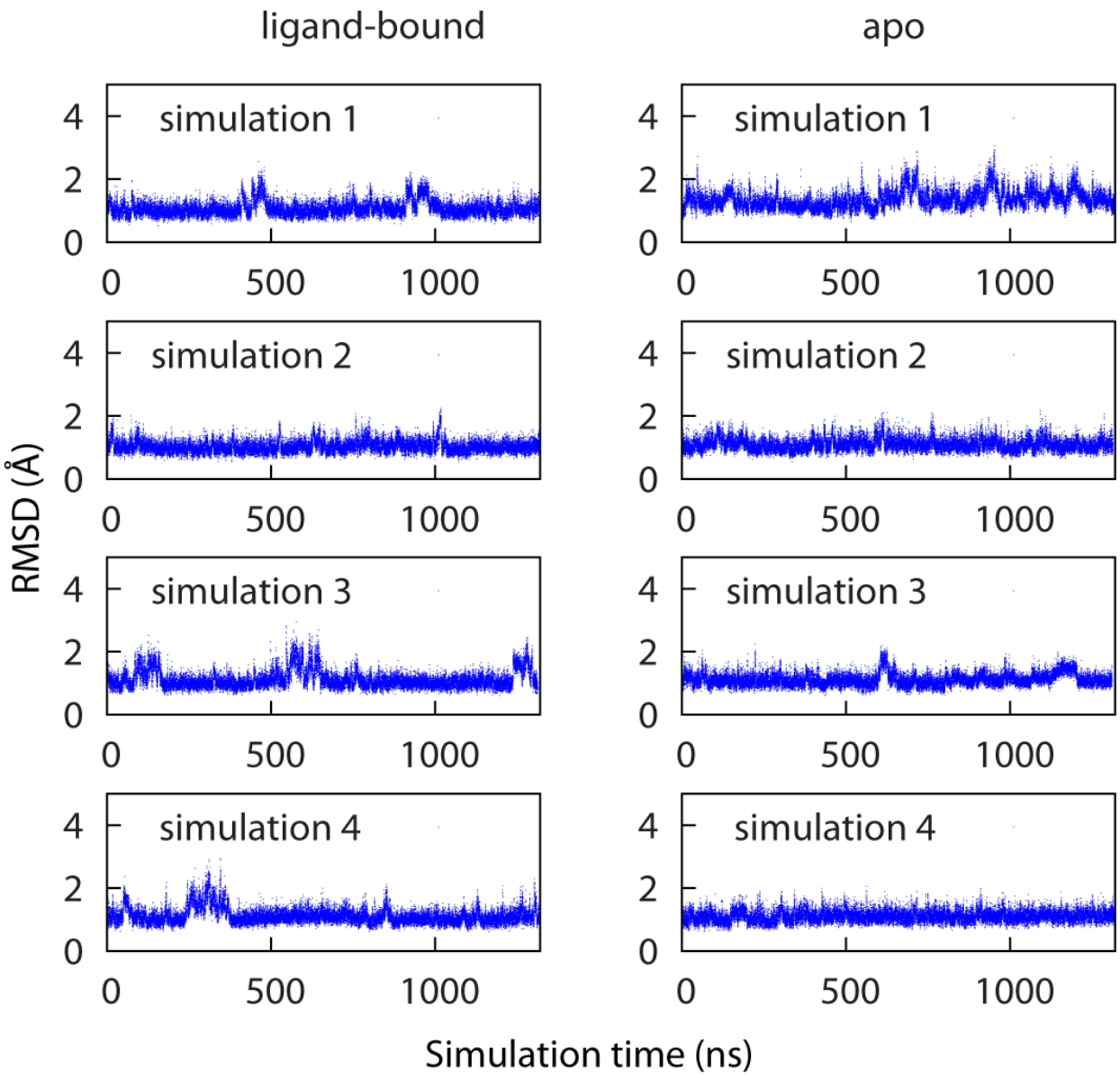

Figure S3. P2 helix mass-weighted, heavy-atom RMSD to starting structure as a function of time. The left column shows ligand-bound simulations and the right column shows apo simulations. The location of helix P2 is illustrated in Figure 1.

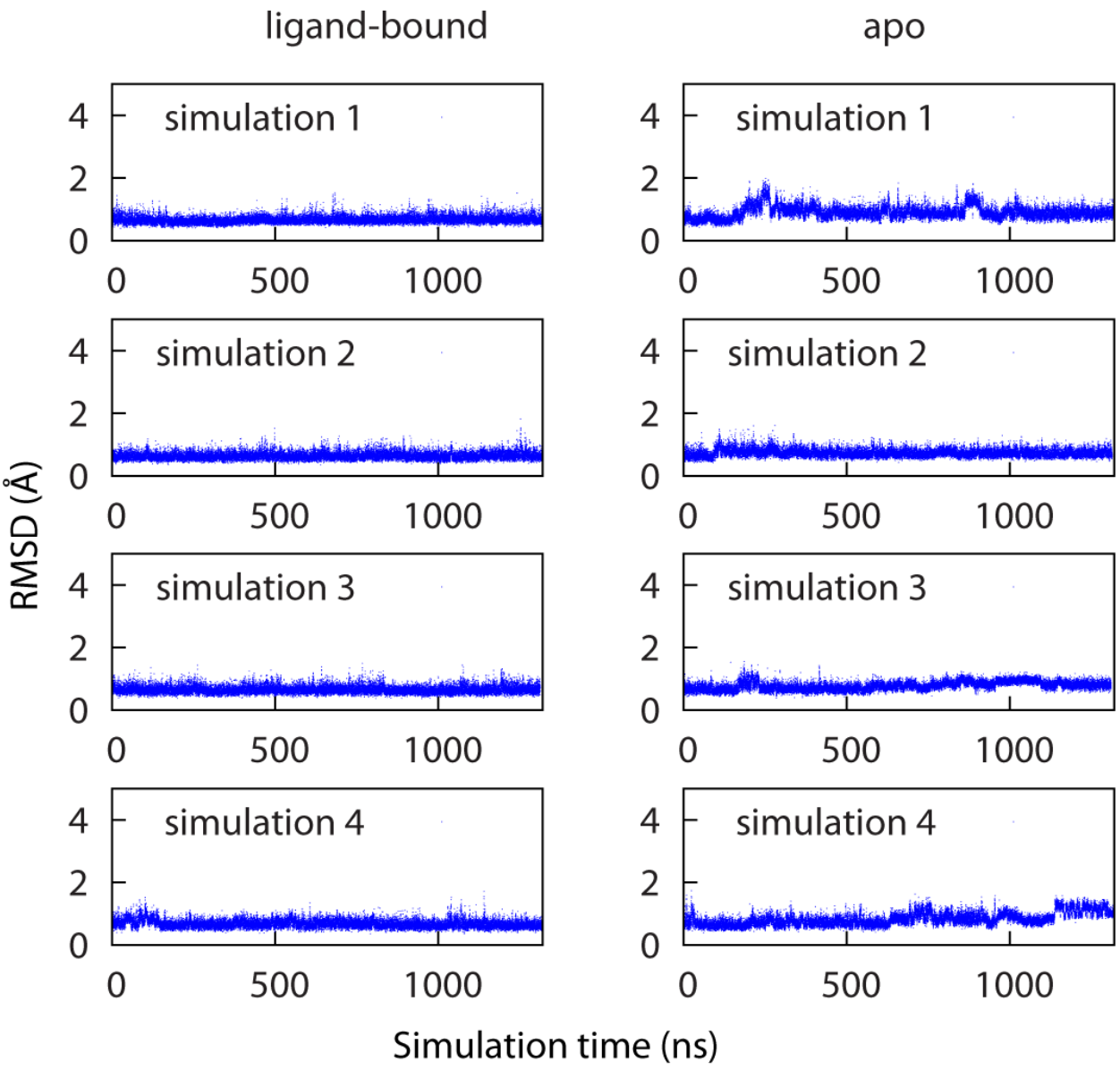

Figure S4. P3 helix mass-weighted, heavy-atom RMSD to starting structure as function of time. The left column shows ligand-bound simulations and the right column shows apo simulations. Helix P3 is defined as nucleotides C37, C38, U39, U40, A71, A72, G73 and G74 (Figure 1).

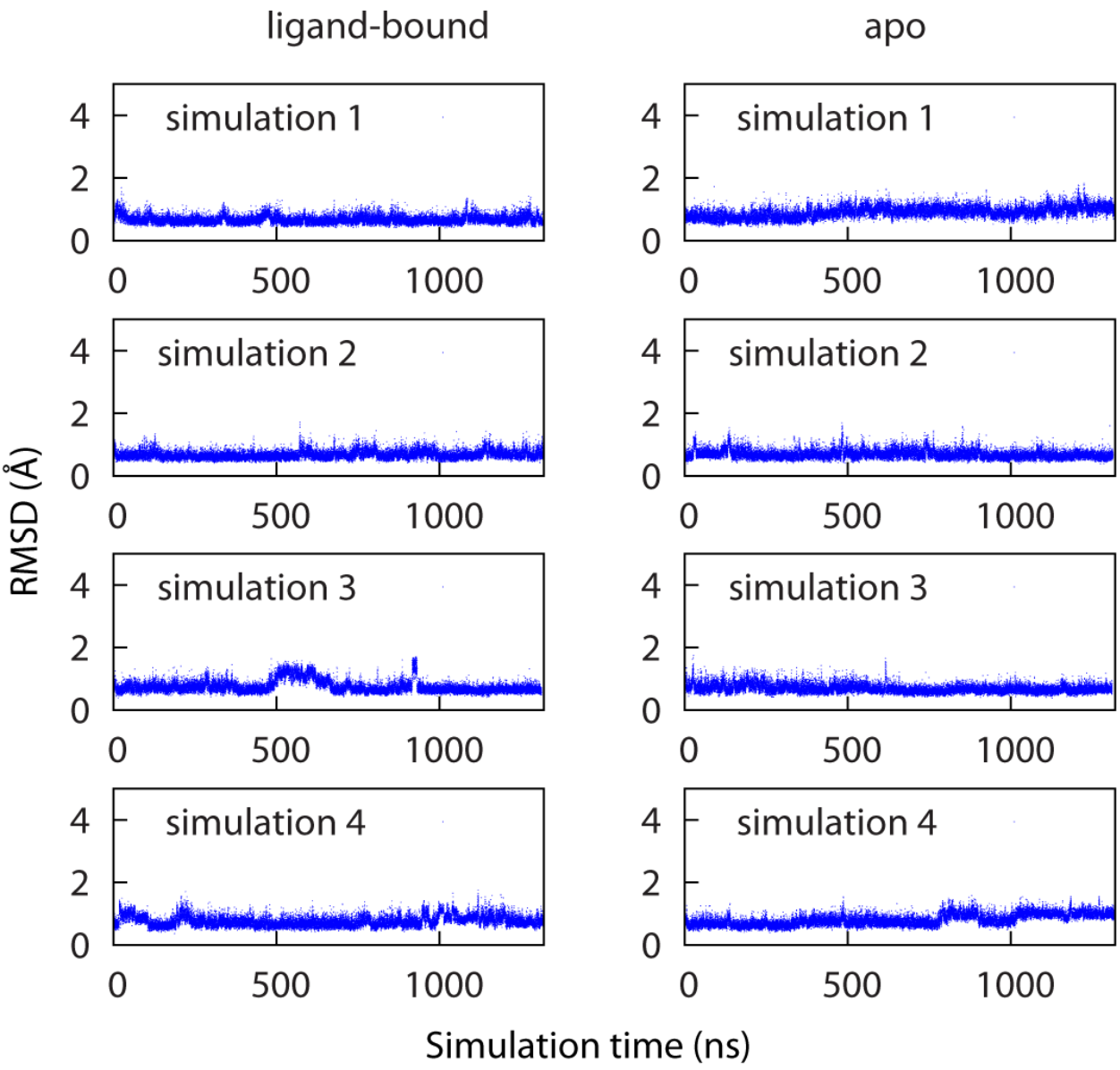

Figure S5. P4 helix mass-weighted, heavy-atom RMSD to starting structure as a function of time. The left column shows ligand-bound simulations and the right column shows apo simulations. The location of helix P4 is illustrated in Figure 1.

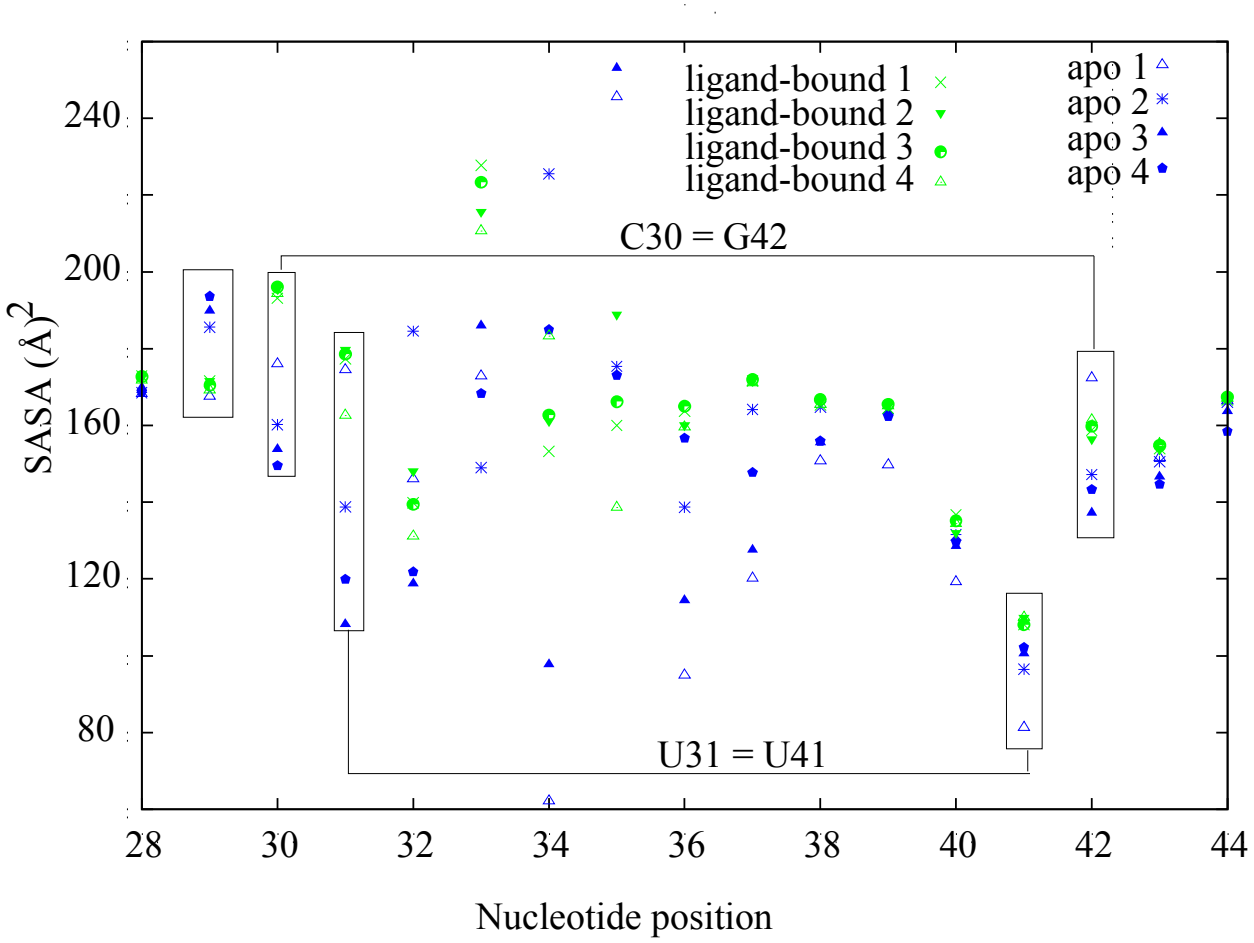

Figure S6. Solvent accessible surface area (SASA) per nucleotide, averaged over the last 1.2 μs of production simulation time, shown for each simulation.

Table S1. RMSDs of individual helices. STD, MAX, and MIN are the standard deviation, maximum, and minimum RMSD, respectively. Statistics are calculated for the last 1.2 μs of the simulation time.

		BOUND				APO			
		1	2	3	4	1	2	3	4
P1	Average	1.33	1.27	1.28	1.27	1.27	1.3	1.27	1.26
	STD	0.37	0.35	0.35	0.34	0.34	0.36	0.35	0.35
	MAX	3.38	3.47	3.2	3.26	3.07	3.2	3.04	3.18
	MIN	0.5	0.52	0	0.49	0.52	0.47	0	0.54
P2	Average	1.37	1.11	1.12	1.11	1.08	1.04	1.13	1.13
	STD	0.29	0.19	0.2	0.18	0.23	0.19	0.28	0.25
	MAX	3.21	2.23	2.39	2.13	2.55	2.26	3.19	3.03
	MIN	0.65	0.54	0	0.57	0.51	0.55	0	0.58
P3	Average	1.05	0.77	0.83	0.89	0.72	0.69	0.7	0.73
	STD	0.19	0.12	0.13	0.22	0.11	0.11	0.12	0.13
	MAX	2.36	1.65	1.85	1.78	1.57	1.73	1.52	1.74
	MIN	0.44	0.35	0	0.4	0.39	0.38	0	0.39
P4	Average	0.91	0.69	0.69	0.81	0.68	0.68	0.75	0.77
	STD	0.18	0.13	0.13	0.18	0.13	0.12	0.2	0.17
	MAX	2.02	2.02	1.79	1.66	1.7	1.71	1.8	1.87
	MIN	0.39	0.38	0	0.37	0.38	0.37	0	0.36

Force field parameters and charges used for the ligand during molecular dynamic simulations:

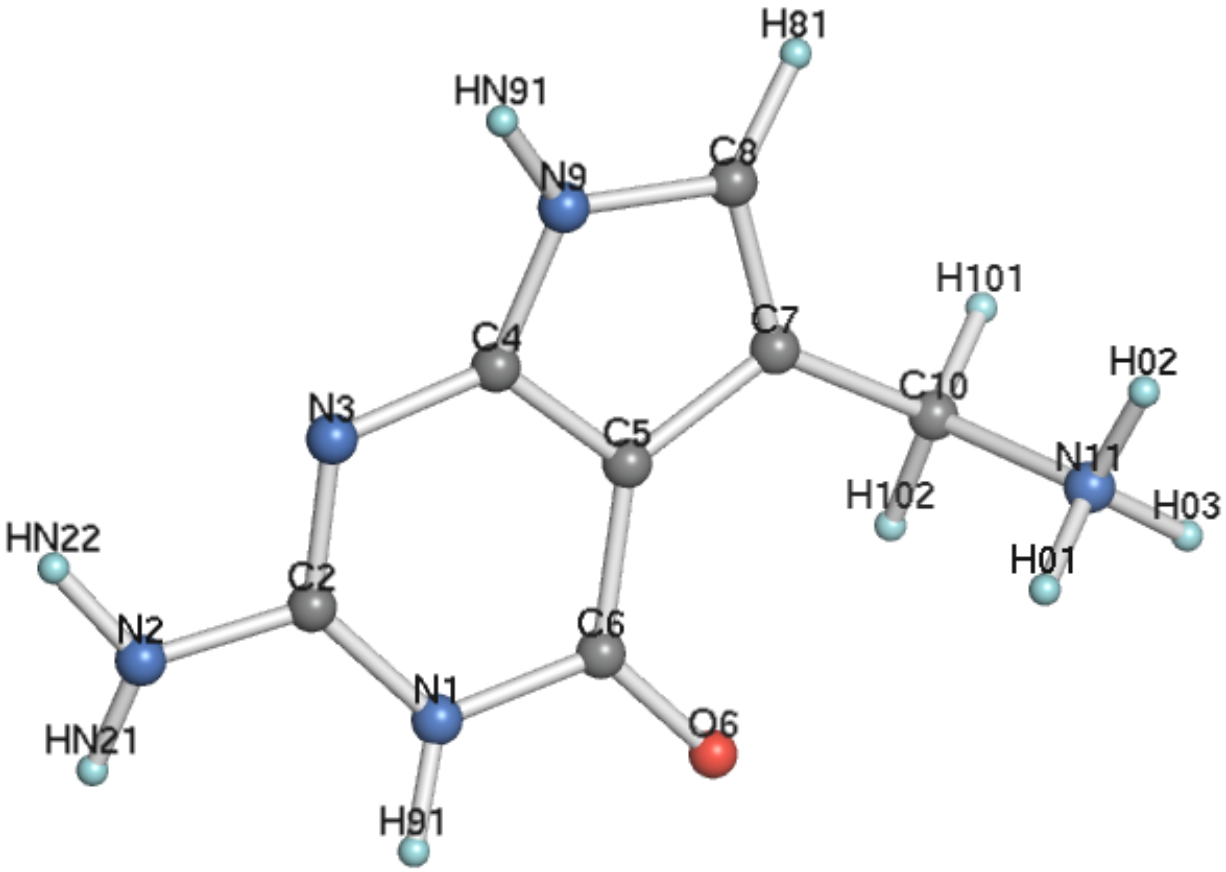

Figure S7. $PreQ_1$ ball-stick representation. This Figure shows the $preQ_1$ ligand geometry-optimized structure using RED RESP server (Dupradeau et al. 2010) and with hydrogens added using 'reduce' from AmberTools (Case et al. 2005). The Figure shows the atom names as used in the partial charge table (Table S2).

Table S2. RESP charges for $preQ_1$ ligand. Atom name is labeled on Supplement Figure S7. Atom types were taken from GAFF (Case et al. 2005).

Atom name	GAFF atom type	RESP Charge
N1	n	-0.406
H91	hn	0.3456
C2	cc	0.7342
N2	nh	-0.9421
HN21	hn	0.4428
HN22	hn	0.4428
N3	nd	-0.603
C4	cd	0.3178
C5	cc	-0.0718
C6	c	0.4592
O6	o	-0.58
C7	cc	-0.1324
C8	cd	-0.2198
H81	h4	0.2409
N9	na	-0.2645
HN91	hn	0.3654
C10	c3	0.0084
H101	hx	0.1183
H102	hx	0.1183
N11	n4	-0.2222
H01	hn	0.2827
H02	hn	0.2827
H03	hn	0.2827

Table S3. Additional force field parameters for $PreQ_1$. Force field parameters were generated with 'antechamber' from AmberTools (Case et al. 2005) with the GAFF force field model and used together with the standard force field parameters by sourcing leaprc.garff. All nonbonded, bonded, mass, dihedral and angle parameters were taken from the gaff force field. The missing improper dihedrals were generated with parmchk (Case et al. 2005) from AmberTools, and these parameters are provided below.

IMPROPER				
c -cc-n -hn	1.1	180.0	2.0	General improper torsional angle (2 general atom types)
n -nd-cc-nh	1.1	180.0	2.0	Using default value
cc-hn-nh-hn	1.1	180.0	2.0	Using default value
cc-na-cd-nd	1.1	180.0	2.0	Using default value
c -cc-cc-cd	1.1	180.0	2.0	Using default value
cc-n -c -o	10.5	180.0	2.0	General improper torsional angle (2 general atom types)
c3-cc-cc-cd	1.1	180.0	2.0	Using default value
cc-h4-cd-na	1.1	180.0	2.0	Using default value
cd-cd-na-hn	1.1	180.0	2.0	General improper torsional angle (2 general atom types)

References:

Case DA, Cheatham TE, Darden T, Gohlke H, Luo R, Merz KM, Onufriev A, Simmerling C, Wang B, Woods RJ. 2005. The Amber biomolecular simulation programs. *J Comput Chem* **26**: 1668-1688.

Dupradeau F-Y, Pigache A, Zaffran T, Savineau C, Lelong R, Grivel N, Lelong D, Rosanski W, Cieplak P. 2010. The R.E.D. tools: advances in RESP and ESP charge derivation and force field library building. *Phys Chem Chem Phys* **12**: 7821-7839.

Liberman JA, Salim M, Krucinska J, Wedekind JE. 2013. Structure of a class II preQ1 riboswitch reveals ligand recognition by a new fold. *Nat Chem Biol* **9**: 353-355.