SUPPORTING INFORMATION

Cryo neutron crystallography demonstrates influence of RNA 2′-OH orientation on conformation, sugar pucker and water structure

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Table S1. Neutron data statistics by resolution bin.

d_max	d_min	#obs	#uniq	mult.	%comp	<I>	<I/sI>	r_mrg	r_meas	r_pim	cc1/2
13.80	4.68	1215	322	3.77	94.15	443.9	18.8	0.264	0.309	0.154	0.958
4.68	3.74	1599	329	4.86	97.92	228.0	19.0	0.392	0.437	0.188	0.628
3.74	3.28	1718	322	5.34	96.70	246.7	20.6	0.375	0.412	0.165	0.803
3.28	2.98	1755	317	5.54	94.63	152.5	15.9	0.444	0.481	0.178	0.652
2.98	2.77	1505	321	4.69	94.97	76.4	9.4	0.395	0.433	0.170	0.542
2.77	2.61	1310	301	4.35	90.12	50.4	6.5	0.298	0.329	0.133	0.715
2.61	2.48	1011	283	3.57	88.99	40.3	4.9	0.268	0.302	0.132	0.739
2.48	2.37	930	303	3.07	85.11	35.0	4.0	0.269	0.311	0.149	0.415
2.37	2.28	659	260	2.53	83.87	32.5	3.3	0.241	0.288	0.153	0.285
2.28	2.20	668	287	2.33	83.43	32.2	3.1	0.249	0.297	0.157	0.370
13.80	2.20	12370	3045	4.06	90.95	139.6	10.9	0.362	0.402	0.168	0.918

Table S2. X-ray data statistics by resolution bin.

Resol. [Å]	#Data	#Theory	%Compl.	Redundancy	Mean I	Mean I/s	Rmerge	Rsigma
29.49- 4.04	531	565	94.0	14.42	136.73	54.53	0.0316	0.0220
4.04 - 2.71	1240	1260	98.4	15.84	127.48	55.85	0.0338	0.0205
2.71 - 2.15	1791	1791	100.0	15.75	36.00	51.81	0.0415	0.0179
2.15 - 1.88	1761	1763	99.9	15.77	22.68	47.75	0.0483	0.0186
1.88 - 1.70	1871	1873	99.9	15.64	15.21	43.85	0.0556	0.0197
1.70 - 1.58	1760	1760	100.0	15.46	11.25	38.68	0.0650	0.0215
1.58 - 1.49	1715	1715	100.0	14.95	6.09	30.43	0.0816	0.0271
1.49 - 1.41	1920	1920	100.0	15.22	4.18	25.24	0.0986	0.0326
1.41 - 1.35	1736	1736	100.0	15.18	3.34	21.73	0.1155	0.0386
1.35 - 1.30	1734	1734	100.0	14.96	2.90	19.01	0.1342	0.0444
1.30 - 1.25	2000	2000	100.0	14.70	2.78	17.41	0.1446	0.0485
1.25 - 1.21	1861	1861	100.0	14.18	2.59	15.44	0.1667	0.0546
1.21 - 1.18	1493	1493	100.0	13.91	2.15	13.11	0.1916	0.0653
1.18 - 1.15	1763	1763	100.0	12.47	1.80	10.64	0.2112	0.0832
1.15 - 1.12	1906	1906	100.0	12.57	1.46	9.23	0.2370	0.0995
1.12 - 1.09	2110	2110	100.0	12.24	1.16	7.45	0.2712	0.1251
1.09 - 1.07	1591	1591	100.0	11.97	0.93	6.02	0.3261	0.1574
1.07 - 1.05	1609	1609	100.0	11.85	0.80	5.27	0.3750	0.1819
1.05 - 1.03	1824	1824	100.0	11.42	0.61	4.07	0.4535	0.2407
1.03 - 1.01	1956	1956	100.0	10.92	0.51	3.37	0.5327	0.2948
1.01 - 1.00	1209	1269	95.3	7.34	0.43	2.28	0.6190	0.5141
1.10 - 1.00	8929	8989	99.3	10.96	0.69	4.45	0.4119	0.2284
29.49- 1.00	35381	35498	99.7	13.68	12.44	21.66	0.0546	0.0264

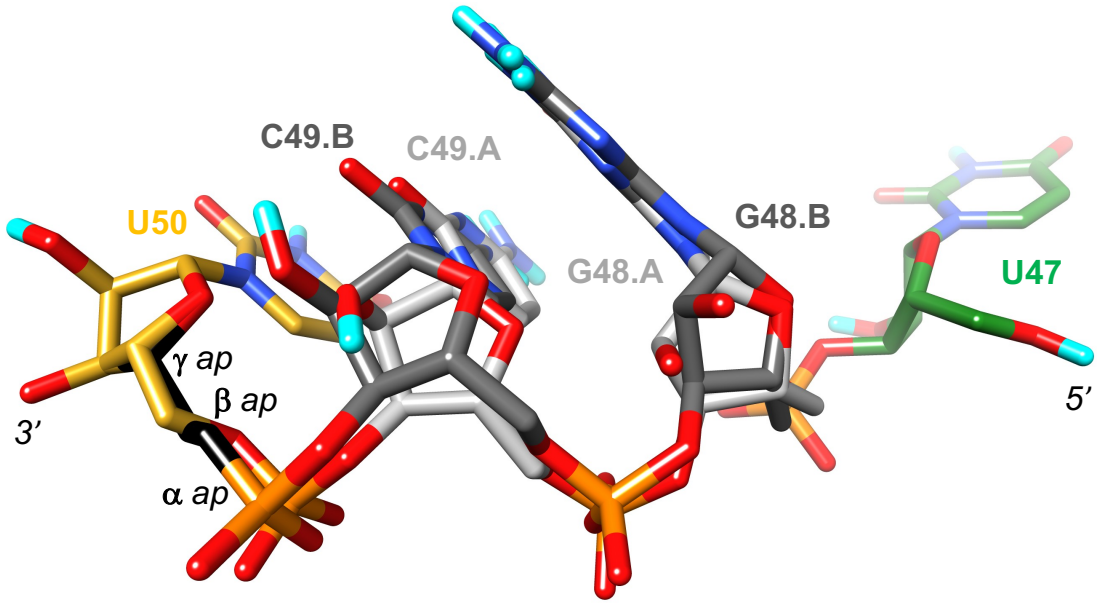
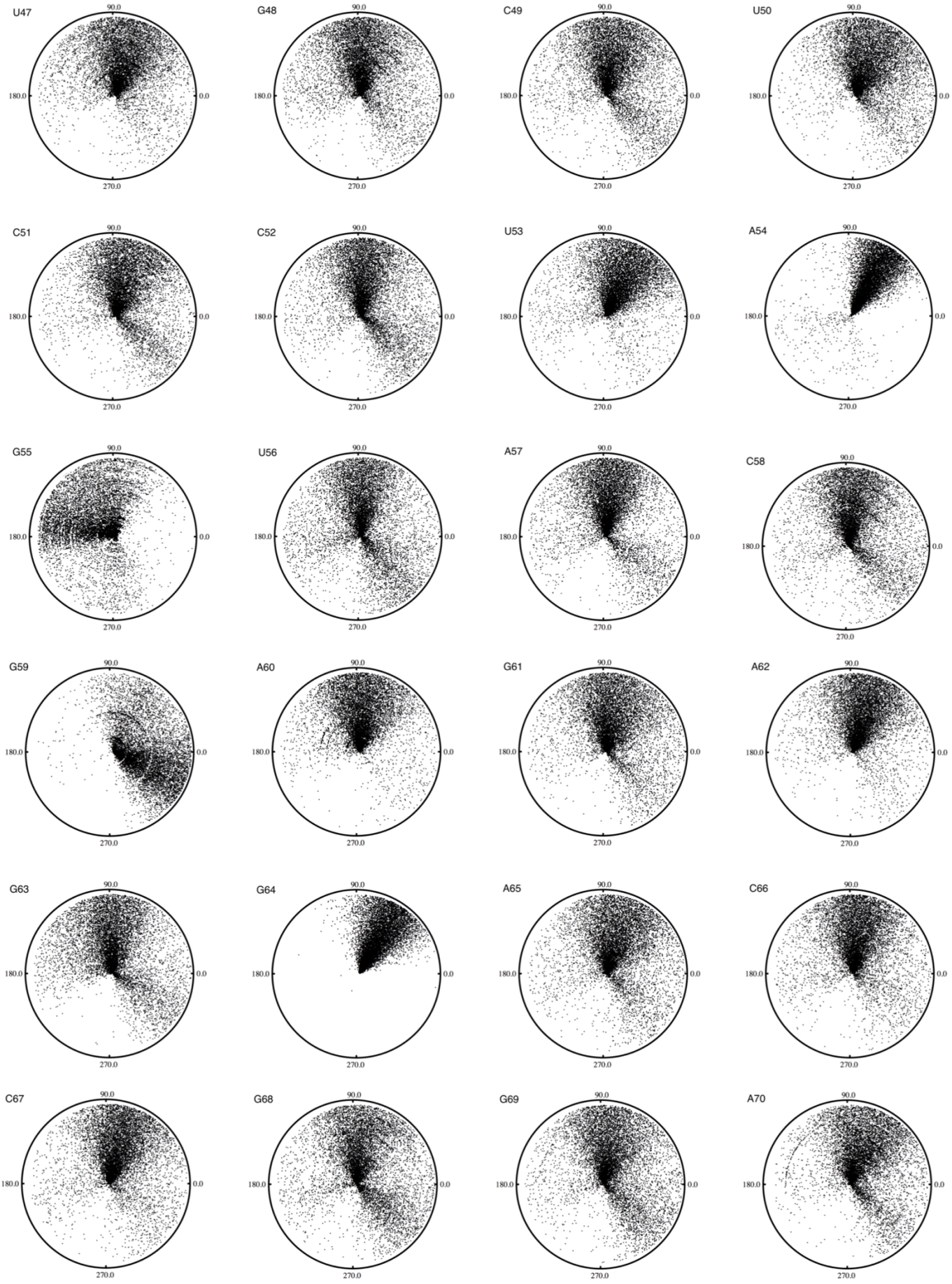


Figure S1. Residues G48 and C49 exhibit dual conformations termed A and B, with carbon atoms colored in light gray and dark gray, respectively. Backbone and glycosidic torsion angles fall into the same ranges in the two conformers (Table 3, main text), but the 2′-OH of C49.A has a phosphate orientation and the 2′-OH of C49.B has a base orientation. In U50, backbone torsion angles α to γ associated with the A conformer fall into the standard ranges (*sc*-, *ap*, *sc*+). The backbone of the B conformer in that region adopts an extended orientation with all three angles exhibiting an *ap* conformation (highlighted with black bonds and labeled). Conformational disorder near strand termini, e.g. fraying of base pairs, is not uncommon in crystal structures of DNA and RNA. The extended backbone variant seen here in conformer B is a common feature in DNA and RNA A-form duplexes. It is possible that the orientation of the C49 2′-OH group is somehow involved in flipping the α and γ backbone angles in U50 from sc-/sc+ to *ap*/*ap*. However, this single observation adjacent to a backbone region with twofold conformational disorder is not sufficient to draw a conclusion either way.


U47
G48
C49
U50
C51
C52
U53
A54
G55
U56
A57
C58
G59
A60
G61
A62
G63
G64
A65
C66
C67
G68
G69
A70
90.0
180.0
0.0
270.0

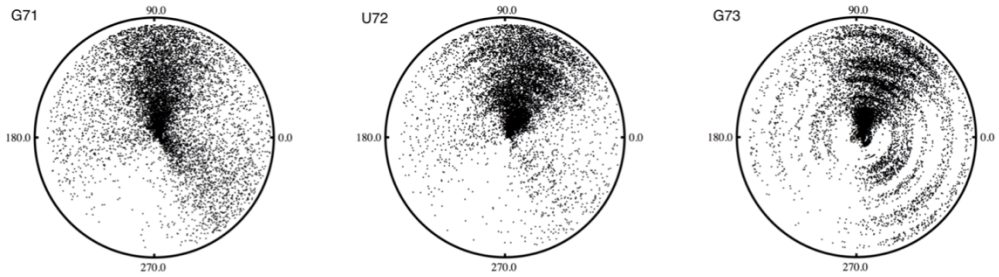
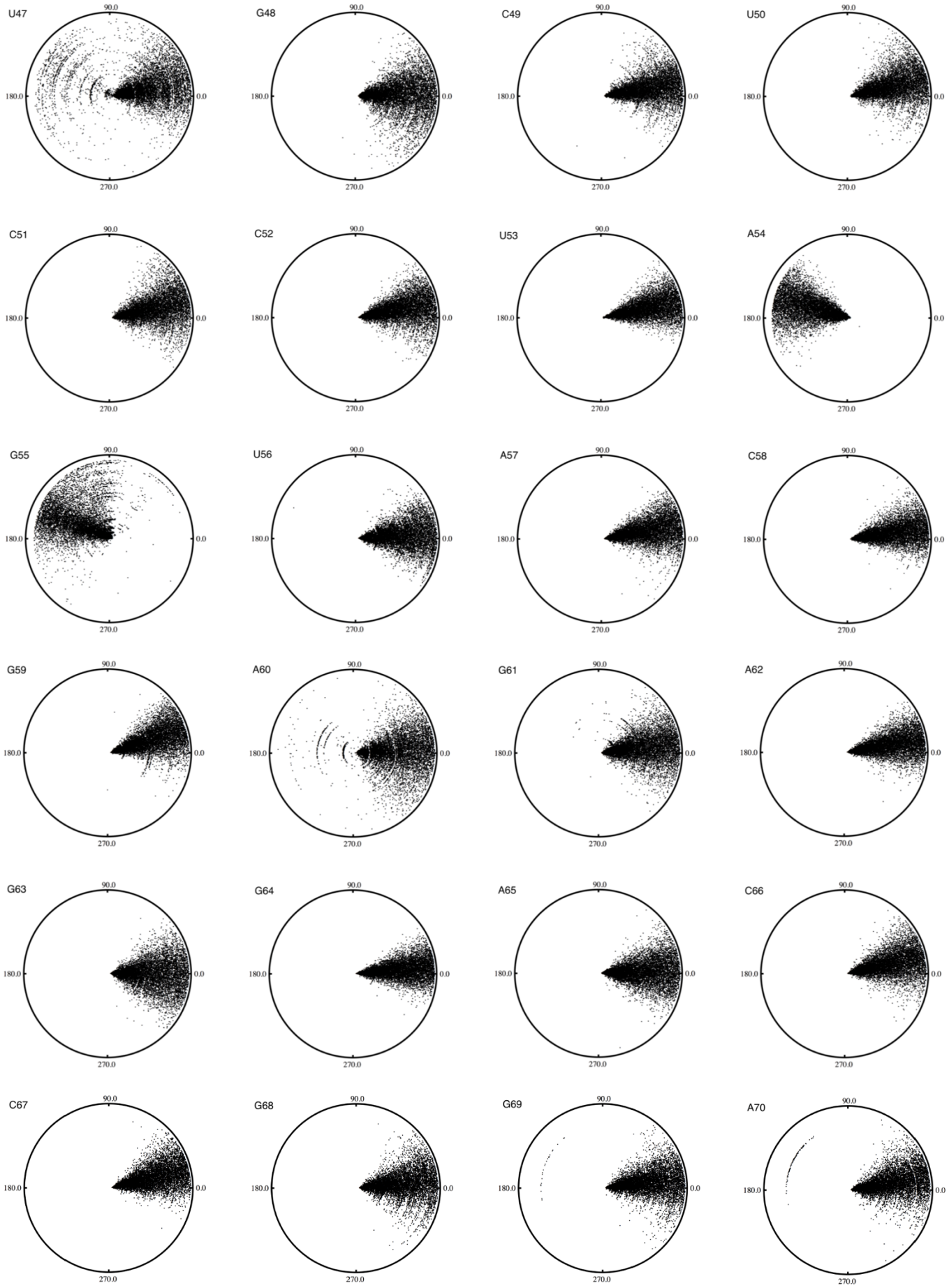


Figure S2. Polar coordinate plots of SRL κ angles in the MD simulations. The torsion angle value in degrees is displayed on the circle perimeter and the time axis is displayed along the circle radius, with t = 0 at the origin and t = 10 microseconds at the circle perimeter.


U47
G48
C49
U50
C51
C52
U53
A54
G55
U56
A57
C58
G59
A60
G61
A62
G63
G64
A65
C66
C67
G68
G69
A70
90.0
180.0
0.0
270.0

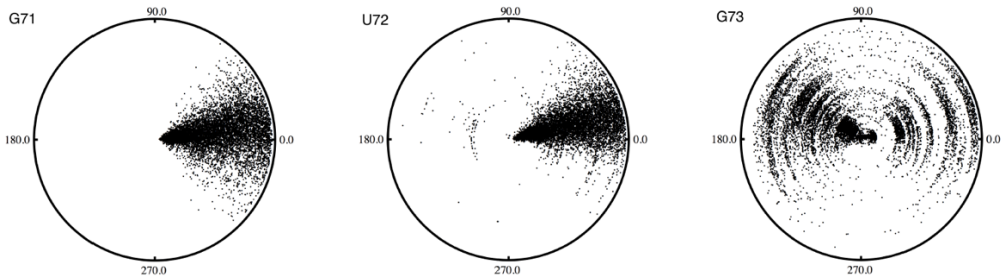


Figure S3. Polar coordinate plots of SRL sugar puckers (pseudorotation phase angle *P*) in the MD simulations. The *P* angle value in degrees is displayed on the circle perimeter and the time axis is displayed along the radius, with t = 0 at the origin and t = 10 microseconds at the circle perimeter.

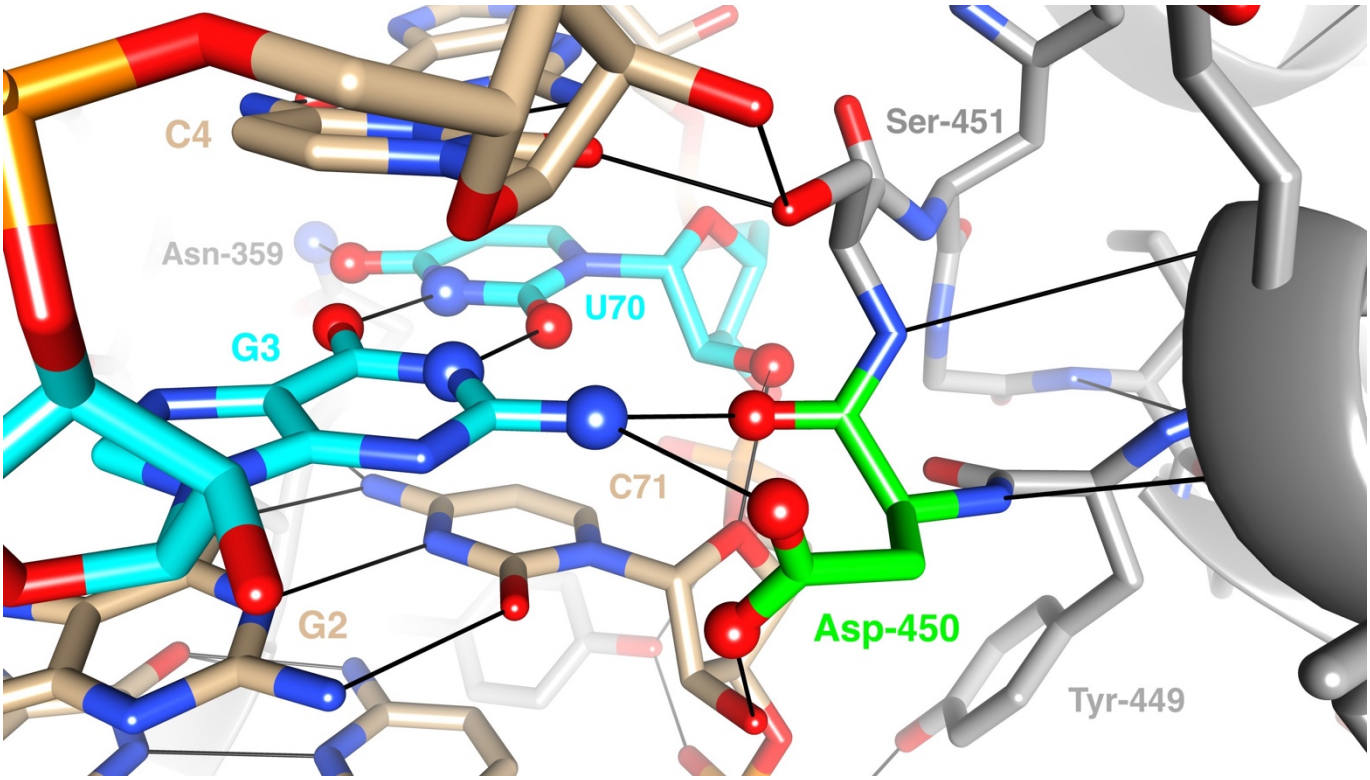


Figure S4. Close-up view of the G3•U70 pair (cyan carbon atoms) in the $tRNA^{Ala}$ acceptor stem contacted by the cognate alanyl-tRNA synthetase in the crystal structure of the complex from *Archaeoglobus fulgidus* (PDB ID 3WQY) (1). The water molecule bridging O2′(U) and N2(G) seen in the SRL crystal structure and MD simulations is displaced by the keto oxygen of Asp-450 (green carbon atoms) that also establishes a contact to the exocyclic amino group of G3 via its side chain. In the major groove Asn-359 uses its amide moiety to form an H-bond to O4 of U70 (background, left).

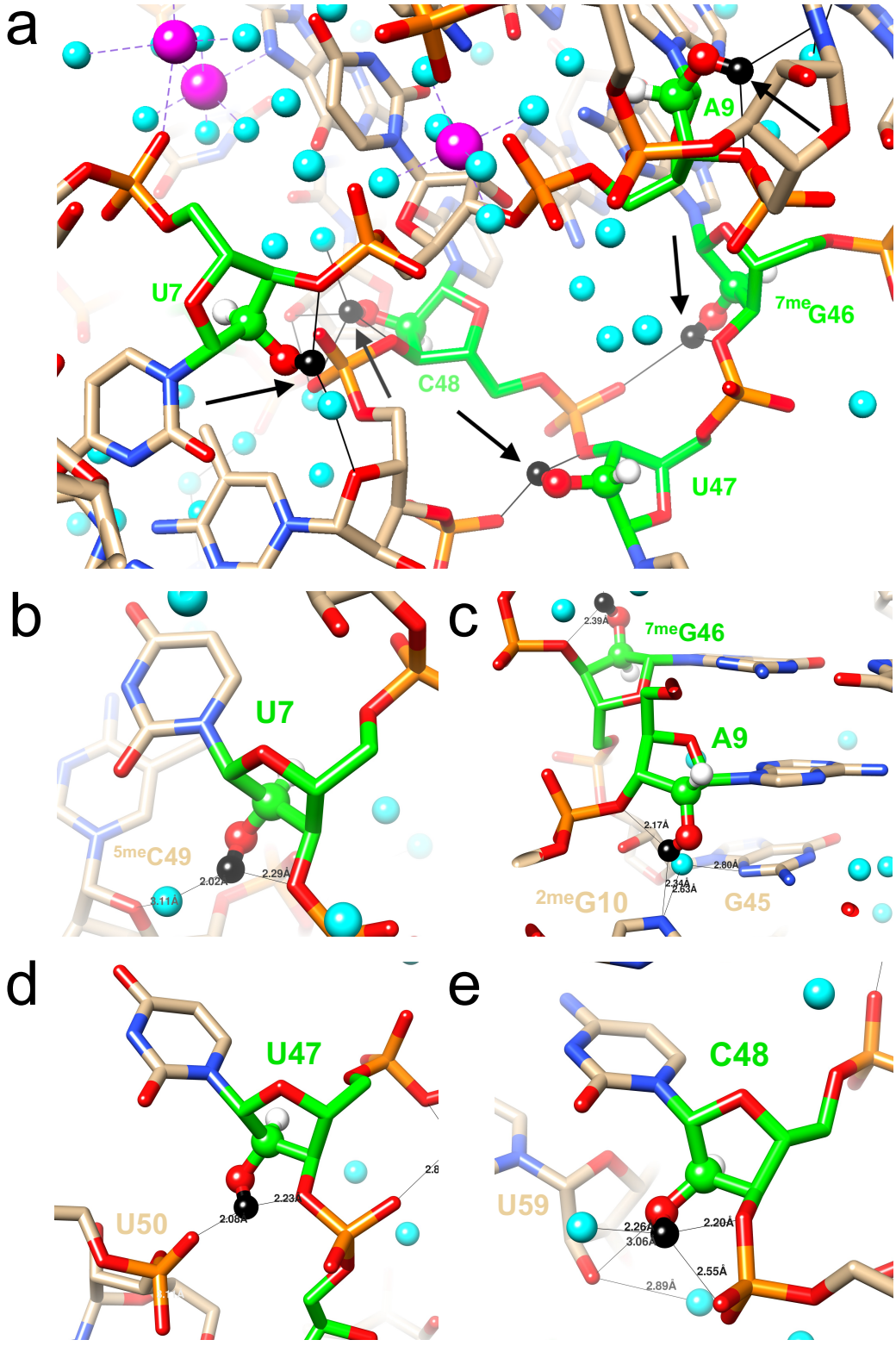


Figure S5. H-bonding interactions by 2′-hydroxyl groups of five ribonucleotides that adopt an unusual C2′-*endo* pucker (labeled and ribose carbon atoms highlighted in green) in the crystal structure of $tRNA^{Phe}$ at 1.92 Å resolution (PDB ID 1EHZ) (2). (a) Overall view and close-up views of (b) U7, (c) A9 and ^{7me}G46, (d) U47, and (e) C48. The positions of H2′ and the HO2′ hydrogen atoms were calculated in each case and

the κ angle H2′-C2′-O2′-HO2′ (atoms shown in ball-and-stick mode and colored in white, green, red and black, respectively) was adjusted to +160°. In each case the 2′-OH donates in two or more H-bonds to neighboring oxygen and/or nitrogen acceptors (arrows). Water molecules and Mg^{2+} ions are shown as spheres colored in cyan and magenta, respectively, the coordination sphere of metal ions is indicated by dashed lines, and H-bonds are drawn with thin solid lines with distances in Å (panels b-e).

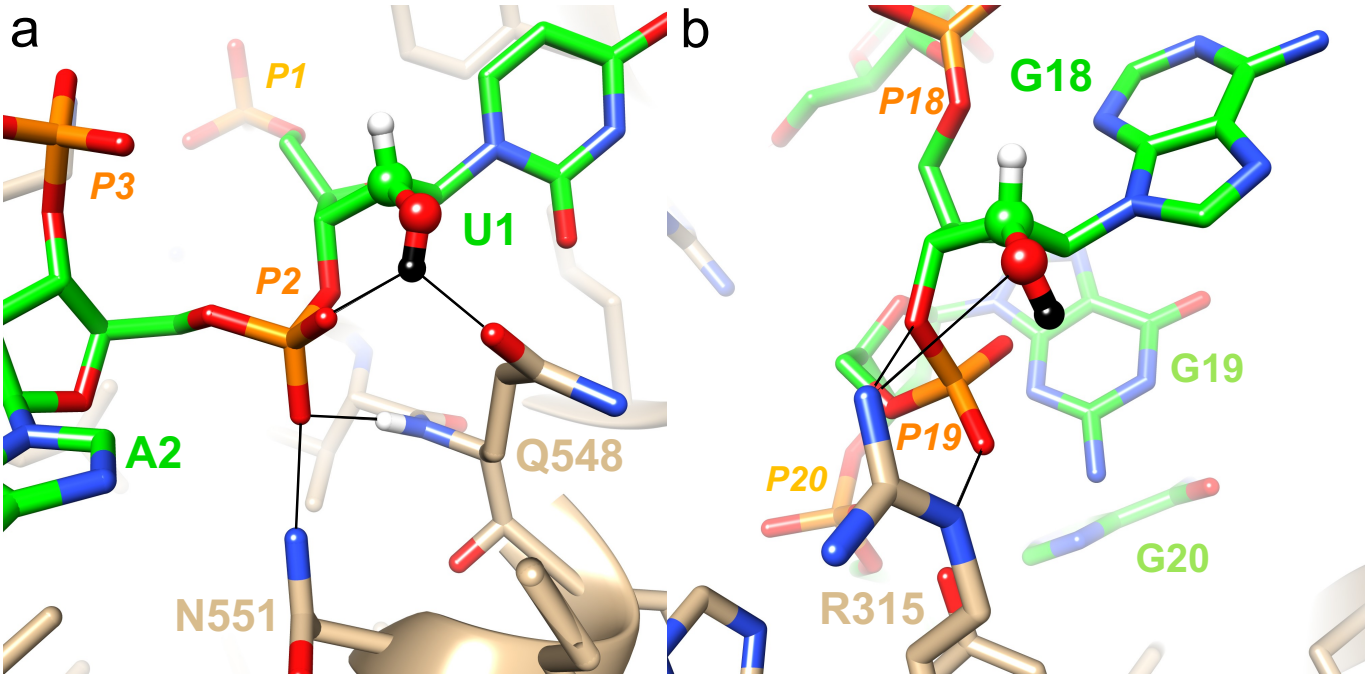


Figure S6. Nucleotides (a) U1 and (b) G18 adopt a C2′-*endo* pucker in the crystal structure of the Ago2:miR-20a complex at 2.25 Å resolution (PDB ID 4F3T) (3). The positions of H2′ and the HO2′ hydrogen atoms were calculated in each case and the κ angle H2′-C2′-O2′-HO2′ (atoms shown in ball-and-stick mode and colored in white, green, red and black, respectively) was adjusted to +160°. Selected residues and phosphate groups are labeled and H-bonds are drawn with thin solid lines.

References

1. Naganuma, M., Sekine, S., Chong, Y. E., Guo, M., Yang, X. L., Gamper, H., Hou, Y. M., Schimmel, P. and Yokoyama, S. (2014) The selective tRNA aminoacylation mechanism based on a single G.U pair. *Nature*, **510**: 507-511.
2. Shi, H. and Moore, P. B. (2000) The crystal structure of yeast phenylalanine tRNA at 1.93 A resolution: a classic structure revisited. *RNA*, **6**, 1091-1105.
3. Elkayam, E., Kuhn, C. D., Tocilj, A., Haase, A. D., Greene, E. M., Hannon, G. J. and Joshua-Tor, L. (2012) The structure of human argonaute-2 in complex with miR-20a. *Cell*, **150**, 100-110.