Supplemental Material

to

Structure and thermodynamics of a UGG motif interacting with Ba^{2+} and other metal ions: accommodating changes in the RNA structure and the presence of a G(*syn*)–G(*syn*) pair.

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Table S1. X-ray data and refinement statistics.

	RNA-Ba(1)	RNA-Ba(2)	RNA unliganded	RNA-Cs	RNA-Sr	RNA-Cd	RNA-Ca
Data collection							
Space group	$P6_522$	$P4_32_12$	R3	$P2_1$	$P2_1$	$P2_1$	$P2_1$
Cell parameters (Å, °)	a=b=84.0 c=139.0	a=b=34.1 c= 81.3	a=b=49.2 c=69.8	a=28.1 b=91.3 c=139.5 β=91.4	a=28.2 b=90.6 c=139.6 β=91.4	a=28.1 b=91.1 c=138.1 β=91.5	a=28.1 b=90.3 c=138.2 β=91.4
Resolution (Å)	2.26	1.79	2.02	2.86	2.46	2.75	2.85
R_{merge}	0.062 (0.702)	0.090 (0.816)	0.061 (0.722)	0.080 (1.580)	0.044 (0.893)	0.045(0.703)	0.074 (0.893)
I/σ	14.73 (1.93)	11.49(1.89)	16.78(1.88)	8.51 (0.80)	14.36 (1.27)	16.31 (1.63)	14.48 (1.95)
$CC_{1/2}$	99.9 (61.9)	99.8 (72.5)	99.9 (73.7)	99.9 (52.7)	99.9 (59.1)	99.9 (73.1)	99.9 (76.6)
Completeness (%)	99.8 (99.9)	99.7(98.6)	99.8 (99.5)	96.4 (90.3)	99.1.0(99.0)	98.6 (97.4)	99.5 (99.5)
Redundancy	3.9 (3.9)	4.6 (4.6)	5.6 (4.3)	3.5 (3.6)	3.5 (3.6)	3.8 (3.7)	6.9 (7.0)
No. of unique reflections	25605 (unmerged)	8634 (unmerged)	4105	30808 (unmerged)	48753 (unmerged)	18088	16139
Refinement							
Overall mean B-factor ($Å^2$)	47.65	27.47	44.3	93.02	96.65	91.89	90.26
Number of reflections: work/test	24317/1274	8201/428	3686/410	28808/1828	44744/3910 (unmerged)	16240/1789	15145/962
R_{work}/R_{free}	19.09/24.04	18.25/23.61	19.7/24.3	22.1/26.9	19.92/24.78	21.85/26.66	20.77/26.60
RNA atoms	2326	403	380	5343	5366	5320	5320
Water molecules	66	43	21	none	12	1	3
No. ligands	12 Ba^{2+} ions	2 Ba^{2+} ions and 1 Na^+ ion	1 acetate and 4 $SO4^{2-}$ ions	28 of Cs^+ ions	22 Sr^{2+} ions	3 Cd^{2+} ions	none
RMSD in bonds (Å)	0.006	0.005	0.007	0.002	0.006	0.002	0.001
RMSD in angles (°)	1.062	0.93	0.92	0.465	1.085	0.479	0.460
PDB code	8AMG	8AMI	8AMJ	8AMM	8AMN	8AML	8AMK
DOI of raw imiges	10.18150/ TGVHHI	10.18150/ LRK0NW	10.18150/4UIWHU	10.18150/ YJBZDE	10.18150/ FO5TNS	10.18150/ VRMIWO	10.18150/ FOPWWU

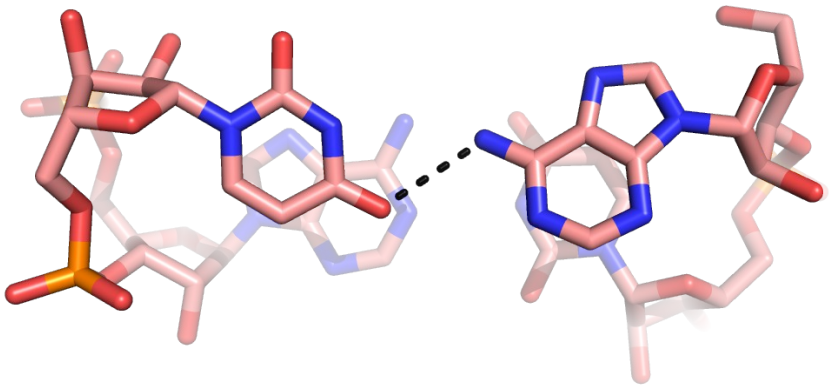


Figure S1. The noncanonical U•A pair of CD duplex in the RNA-Ba(1) structure. The 9U nucleotide is flipped in a way that the sugar moiety is rotated half a turn and the direction of the main chain is reversed. The uracyl ring still stacks with the neighboring 8A residue and interacts *via* the O6 carbonyl with the *exo*-amino group of 1A of chain C (H bond marked in black). According to Leontis and Westhof nomenclature the U-A pair forms *trans* WC-WC pair (10.1017/s1355838201002515).

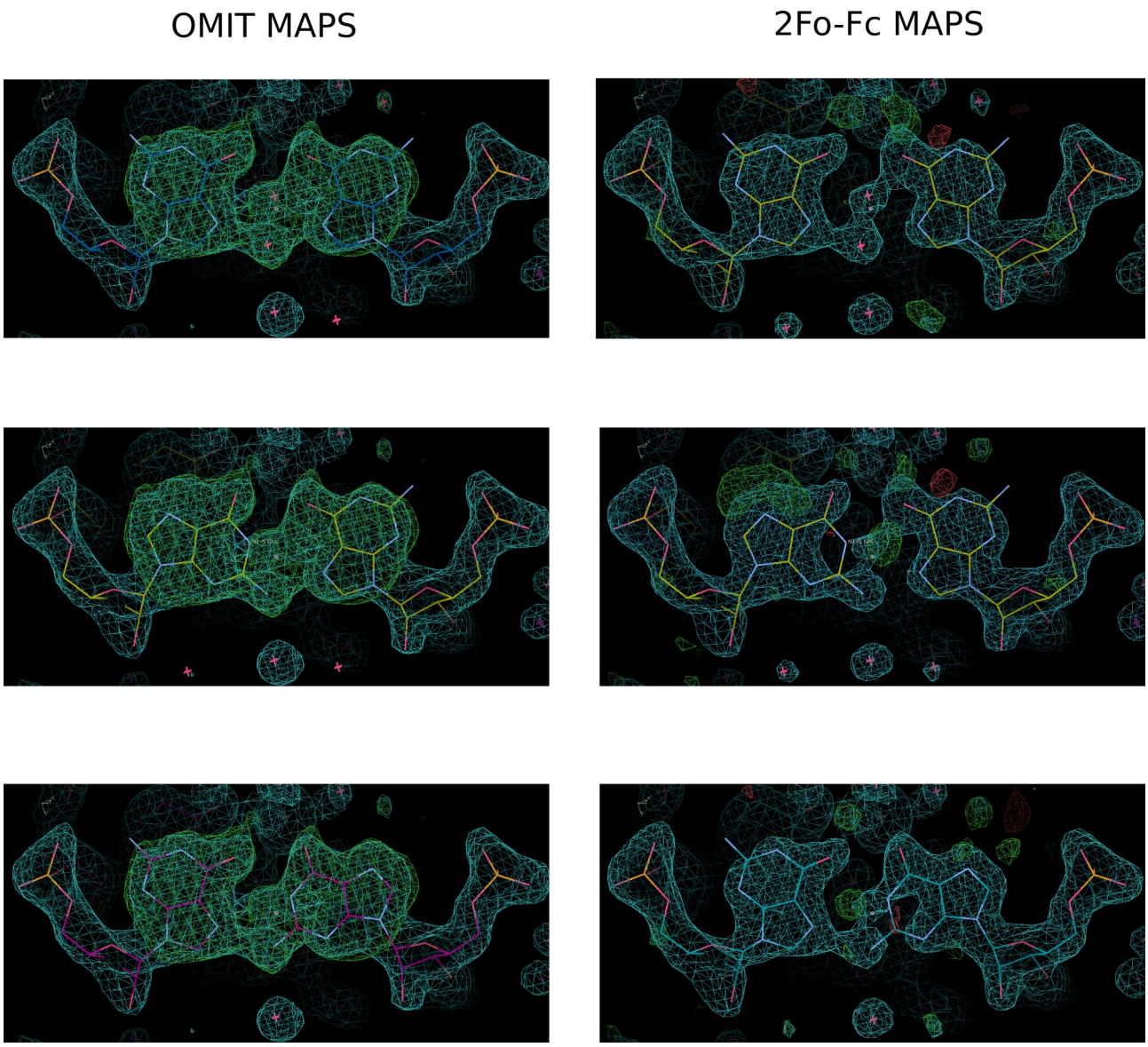


Figure S2. The electron density map analysis of G(syn)-G(syn) pair observed in the structure of RNA-Ba(2). On the left panel the guanosine residues are modeled into omit map (green) in three different arrangements: G(*syn*)-G(*syn*), G(*anti*)-G(*syn*), G(*syn*)-G(*anti*). The right panel represents the calculated $2F_o–F_c$ electron density maps for each G-G arrangement. The omit maps were contoured at 3σ level while the $2F_o–F_c$ electron density maps (blue) were contoured at 1σ level.

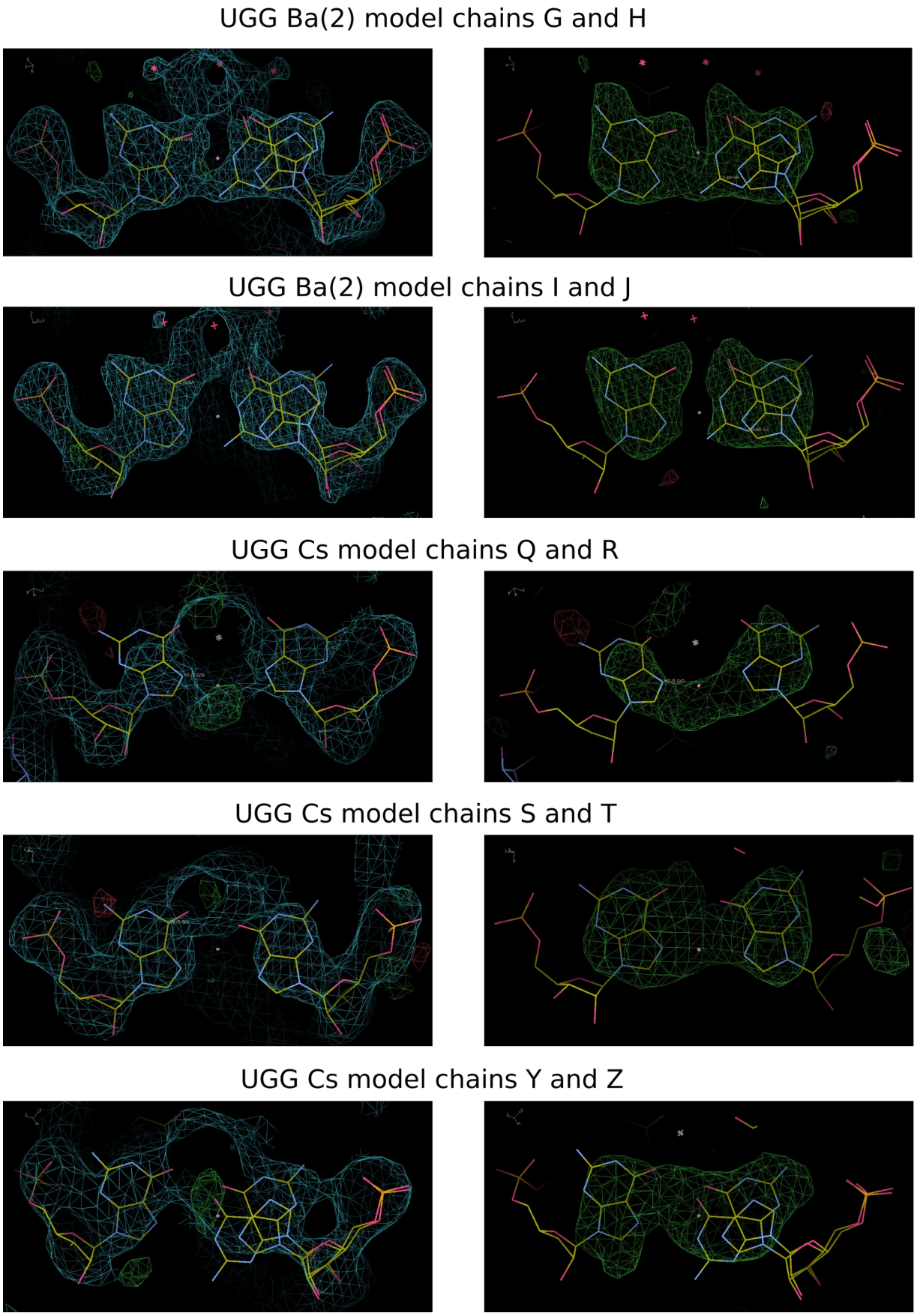


Figure S3. The $2F_o–F_c$ (left panel; blue) and omits maps (right panel; green) calculated for G-G pairs. The omit maps were contoured at 3σ level while the $2F_o–F_c$ electron density maps were contoured at 1σ level.

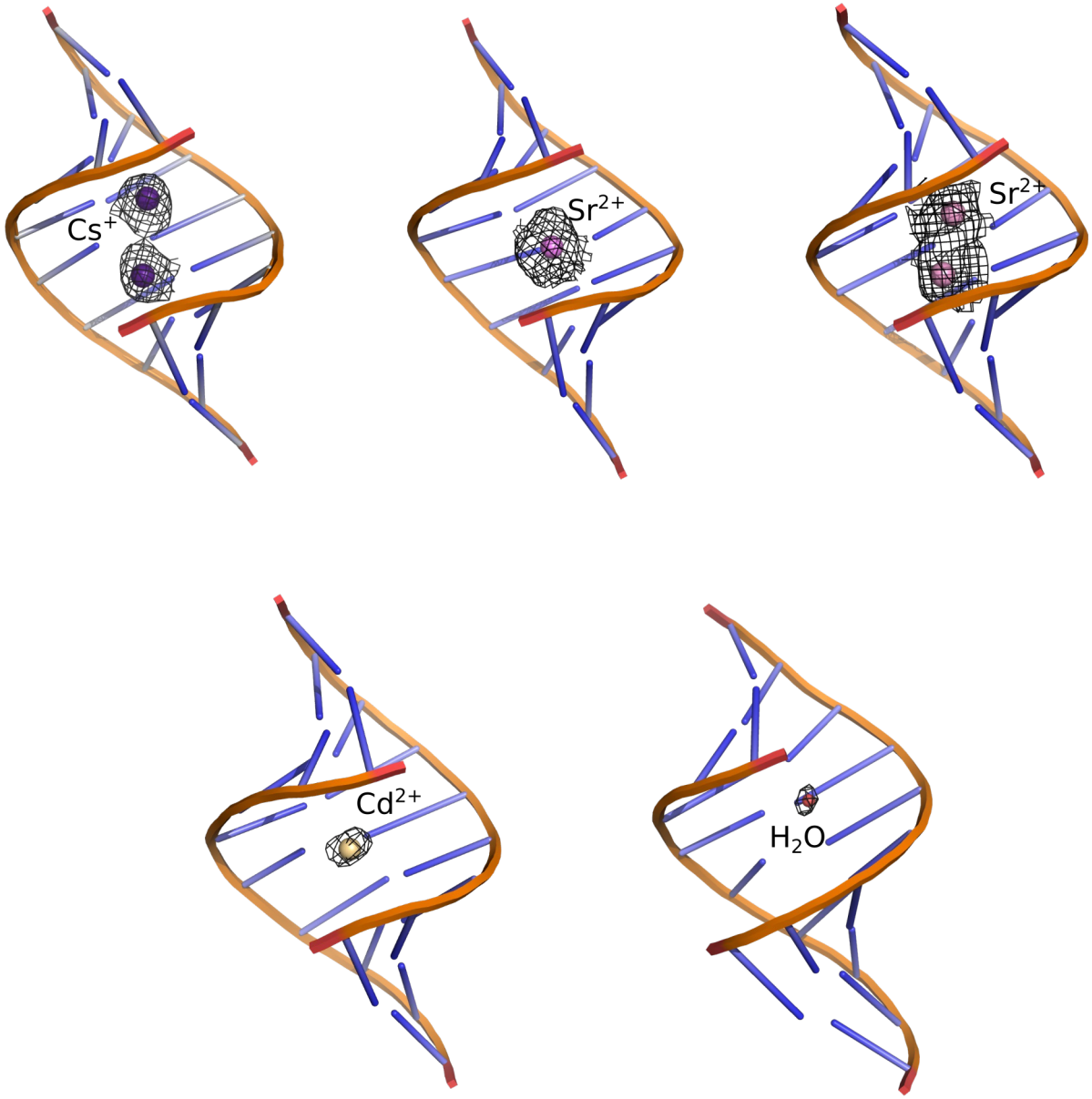


Figure S4. Cartoon representation of AUGUGGCAU models with bound metal ions in the major groove. The $2F_o–F_c$ electron density map (gray) was contoured at 1σ level. Sphere represents metal ions. The structure with water molecule was obtained in the presence of Ca^{2+} ions. The relatively low electron density for Cd^{2+} probably indicates its partial occupancy or disorder.

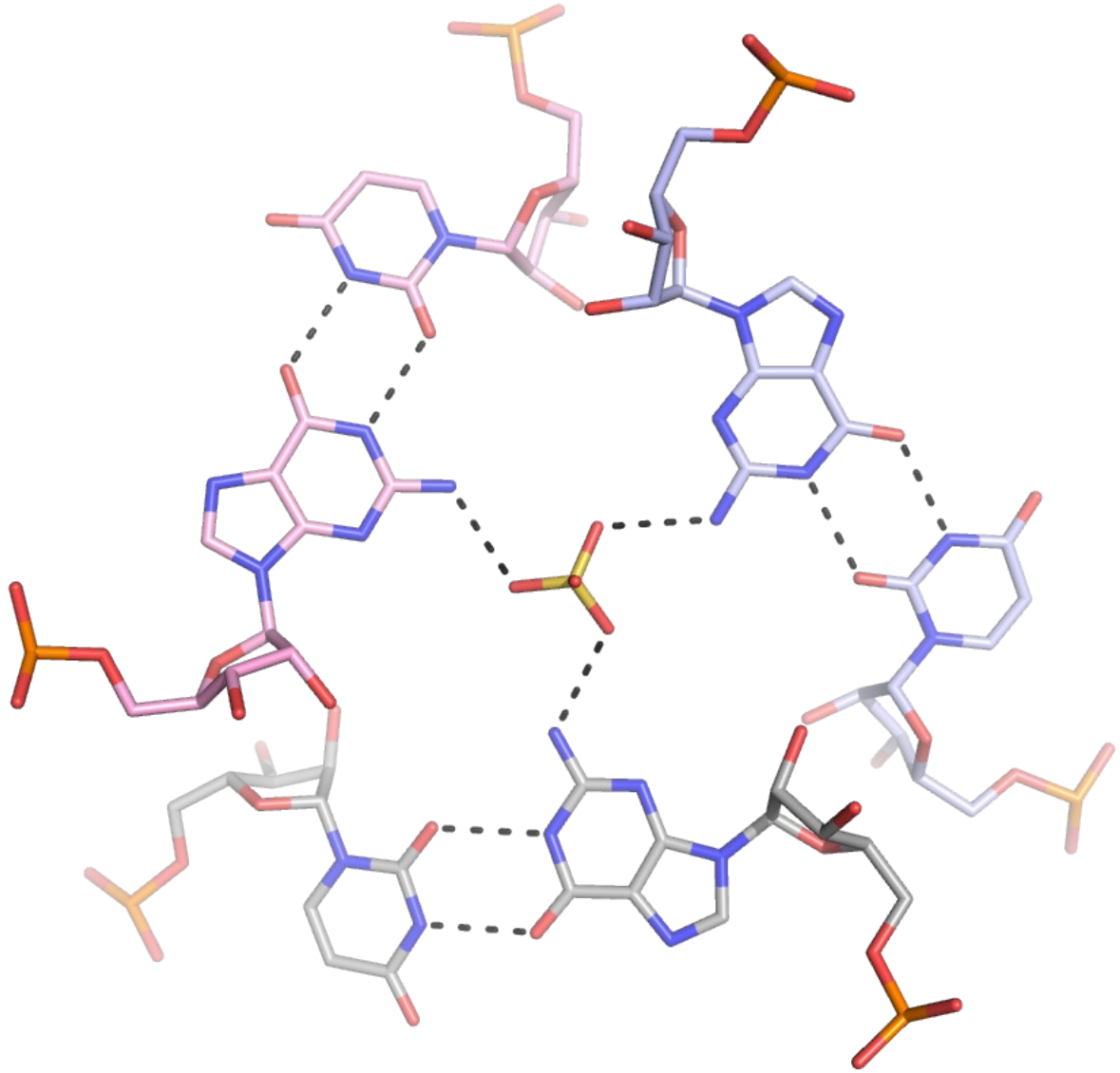


Figure S5. Interactions of sulfate ion with *exo*-amino groups of guanosine residues associated with U·G pairs. The sulfate ion is located on the three-fold axis and interacts with three symmetry-related RNA chains. The hydrogen bonds are dash-lines.

Table S2. Shear (in Å) calculated using 3DNA.

Shear parameter based on RC8--YC6 vectors

	structure	RNA-Ba(1)						RNA-Ba(2)	unliganded	RNA-Sr						
	chain ID	AB	CD	EF	GH	IJ	KL	AB	AB	AB	CD	EF	GH	IJ	KL	MN
No. base pair																
1	A-U	-0.03	-3.12	-0.33	-0.26	0.11	-0.21	-0.05	-0.32	-0.47	-0.09	-0.38	-0.92	-0.16	-0.28	0.24
2	U-A	0.08	-0.09	0.24	0.02	0.41	0.35	-0.03	0.11	-0.25	-0.20	0.12	0.66	0.12	-0.21	-0.30
3	G-C	-0.05	-0.39	-0.06	-0.08	0.23	-0.08	-0.24	-0.33	-0.04	-0.07	-0.20	-0.09	-0.68	-0.33	0.20
4	U-G	2.31	2.62	2.23	2.22	2.01	2.25	2.16	2.47	2.07	2.19	2.60	2.36	2.09	2.16	2.32
5	G-G	-2.88	-2.62	-2.83	-2.52	-0.00	-2.32	-0.01	-2.61	-2.96	-2.47	-2.55	-2.57	-2.32	-2.48	-2.90
6	G-U	-1.97	-2.74	-2.13	-2.45	-2.19	-2.25	-2.13	-2.26	-2.05	-2.32	-2.44	-2.19	-2.29	-2.48	-2.20
7	C-G	0.16	-0.10	-0.03	0.25	0.24	-0.49	0.23	0.36	0.27	0.24	0.09	0.18	0.34	-0.10	0.07
8	A-U	0.52	-0.27	-0.50	-0.41	-0.45	-0.01	0.09	0.15	0.11	0.67	0.24	0.11	0.63	0.28	-0.22
9	U-A	-0.14	-0.03	0.21	-0.04	0.38	0.04	0.25	0.56	0.43	0.10	-0.08	-0.19	0.38	0.24	0.52
	average value	**-0.22**	**-0.75**	**-0.36**	**-0.36**	**0.08**	**-0.30**	**0.03**	**-0.21**	**-0.32**	**-0.05**	**-0.29**	**-0.29**	**-0.21**	**-0.36**	**-0.25**
	s.d.	**1.48**	**1.81**	**1.45**	**1.43**	**1.09**	**1.38**	**1.08**	**1.51**	**1.45**	**0.85**	**1.53**	**1.48**	**1.40**	**1.42**	**1.52**

Table S3. Shift (in Å) calculated using 3DNA.

Shift parameter based on consecutive C1'-C1' vectors

	structure	RNA-Ba(1)						RNA-Ba(2)	unliganded	RNA-Sr						
	chain ID	AB	CD	EF	GH	IJ	KL	AB	AB	AB	CD	EF	GH	IJ	KL	MN
No. base pair step																
1	AU/AU	-0.30	-0.77	-0.29	-0.63	-0.75	-0.28	-0.30	0.21	-0.74	-0.33	0.25	0.14	0.24	-0.34	-0.69
2	UG/CA	0.44	0.83	0.78	0.70	0.50	1.10	0.64	-0.60	0.37	0.11	-0.08	-0.35	-0.01	0.19	0.72
3	GU/GC	0.18	0.49	0.15	0.26	0.55	-0.01	0.08	-0.09	0.33	0.36	-0.17	0.10	0.16	-0.00	0.05
4	UG/GG	1.17	1.70	1.82	1.44	2.45	2.24	2.61	0.71	1.66	1.16	1.27	1.76	1.48	2.02	1.35
5	GG/UG	-1.95	-1.37	-1.27	-1.97	-2.60	-1.63	-2.60	-2.96	-2.22	-1.86	-2.39	-1.57	-1.46	-1.19	-1.84
6	GC/GU	-0.01	-0.37	-0.11	0.26	-0.18	-0.34	0.02	-0.29	-0.21	-0.03	-0.33	-0.25	0.03	-0.31	-0.14
7	CA/UG	-0.54	-0.52	-0.41	-0.41	-0.72	-0.46	0.06	0.39	-0.52	-0.07	-0.16	-0.11	-0.23	-0.21	-0.07
8	AU/AU	0.94	-0.04	0.34	0.12	0.39	0.27	-0.10	0.07	-0.24	0.25	-0.06	-0.26	-0.34	0.02	-0.23
	average value	**-0.01**	**-0.01**	**0.13**	**-0.03**	**-0.04**	**0.11**	**0.05**	**-0.32**	**-0.20**	**-0.05**	**-0.21**	**-0.07**	**-0.02**	**0.02**	**-0.11**
	s.d.	**0.98**	**0.98**	**0.91**	**1.01**	**1.44**	**1.15**	**1.42**	**1.14**	**1.10**	**0.85**	**1.01**	**0.91**	**0.81**	**0.91**	**0.94**

Table S4. Helical twist (in degree) calculated using 3DNA.

Twist parameter based on consecutive C1'-C1' vectors

	structure	RNA-Ba(1)						RNA-Ba(2)	unliganded	RNA-Sr						
	chain ID	AB	CD	EF	GH	IJ	KL	AB	AB	AB	CD	EF	GH	IJ	KL	MN
No. base pair step																
1	AU/AU	34.7	0.85	30.45	33.38	34.7	34.21	33.92	33.64	31.44	33.37	31.68	33.73	33.59	35.51	32.36
2	UG/CA	37.55	34.51	33.75	34.02	33.52	35.8	35.4	32.85	34.18	36	35.74	33.67	35.27	34.85	36.22
3	GU/GC	31.72	33.76	34.93	32.22	38.09	33.85	34.81	25.59	30.25	30.35	26.57	32.1	28.95	29,12	26.89
4	UG/GG	34.15	32.28	32.82	42.28	34.07	32.96	35.56	38.5	35.55	40.77	33.77	36.76	37.37	34.57	39.87
5	GG/UG	32.46	38.26	40.57	29.26	36.03	38.16	36.82	31.59	34	33.62	36.11	33.61	30.33	40.72	35.18
6	GC/GU	34.62	29.98	31.41	33.2	34.2	36.3	33.33	29.49	29.35	31.45	28.36	30.86	28.22	28.37	30.13
7	CA/UG	33.28	35.36	37.21	36.35	37.34	36.39	32.06	32.99	33.76	35.57	33.21	33.73	33.66	32.23	31,07
8	AU/AU	33.91	34.08	33.32	34.56	31.92	35.37	34.88	32.8	32.6	34.09	32.78	32.19	32	33.71	36.75
	average value	**34.05**	**34.03**	**34.31**	**34.41**	**34.98**	**35.38**	**34.60**	**32.18**	**32.64**	**34.40**	**32.28**	**33.33**	**32.42**	**34.28**	**33.91**
	s.d.	**1.76**	**2.56**	**3.27**	**3.77**	**2.05**	**1.66**	**1.47**	**3.68**	**2.14**	**3.19**	**3.34**	**1.74**	**3.16**	**3.72**	**4.41**

Table S5. Propeller (in degree) calculated using 3DNA.

Propeller parameter based on RC8--YC6 vectors

	structure	RNA-Ba(1)						RNA-Ba(2)	unliganded	RNA-Sr						
	chain ID	AB	CD	EF	GH	IJ	KL	AB	AB	AB	CD	EF	GH	IJ	KL	MN
No. base pair																
1	A-U	-13.91	-26.05	-13.34	-10.88	-21.36	-22.39	-9.76	-9.70	-0.52	-12.53	-14.02	-7.96	-12.00	-11.54	-8.11
2	U-A	-16.81	-13.58	-19.82	-14.46	-19.65	-21.05	-16.23	-15.49	-14.03	-16.63	-17.66	-12.90	-19.68	-22.43	-20.75
3	G-C	-18.79	-22.28	-19.40	-17.11	-18.91	-34.17	-23.50	-15.59	-19.73	-20.38	-13.44	-17.20	-22.39	-23.32	-18.50
4	U-G	-14.88	-18.12	-15.61	-17.52	-7.61	-32.53	-15.20	-10.06	-14.20	-14.38	-14.88	-16.57	-16.32	-16.06	-13.97
5	G-G	-17.15	-22.22	-21.18	-20.39	-20.30	-25.02	-21.40	-11.77	-13.07	-16.71	-23.85	-15.75	-14.87	-18.25	-17.83
6	G-U	-18.44	-19.86	-15.63	-16.39	-14.32	-15.40	-13.78	-13.73	-12.87	-13.81	-12.12	-14.04	-14.43	-13.69	-15.37
7	C-G	-23.65	-20.15	-23.71	-19.20	-22.15	-20.57	-21.39	-21.43	-18.76	-17.95	-17.09	-22.53	-19.25	-18.38	-15.55
8	A-U	-29.95	-6.73	-17.69	-20.61	-15.85	-22.20	-18.68	-16.94	-25.52	-13.55	-18.63	-14.18	-20.01	-17.21	-19.12
9	U-A	-11.39	-14.96	-18.75	-15.28	-17.88	-13.86	-12.34	-11.38	-13.83	-5.98	-9.19	-12.00	-5.72	-10.20	-15.40
	average value	**-18.33**	**-18.22**	**-18.35**	**-16.87**	**-17.56**	**-23.02**	**-16.92**	**-14.01**	**-14.73**	**-14.66**	**-15.65**	**-14.79**	**-16.07**	**-16.79**	**-16.07**
	s.d.	**5.55**	**5.75**	**3.17**	**3.10**	**4.49**	**6.82**	**4.64**	**3.78**	**6.78**	**4.09**	**4.25**	**4.01**	**5.08**	**4.48**	**3.69**

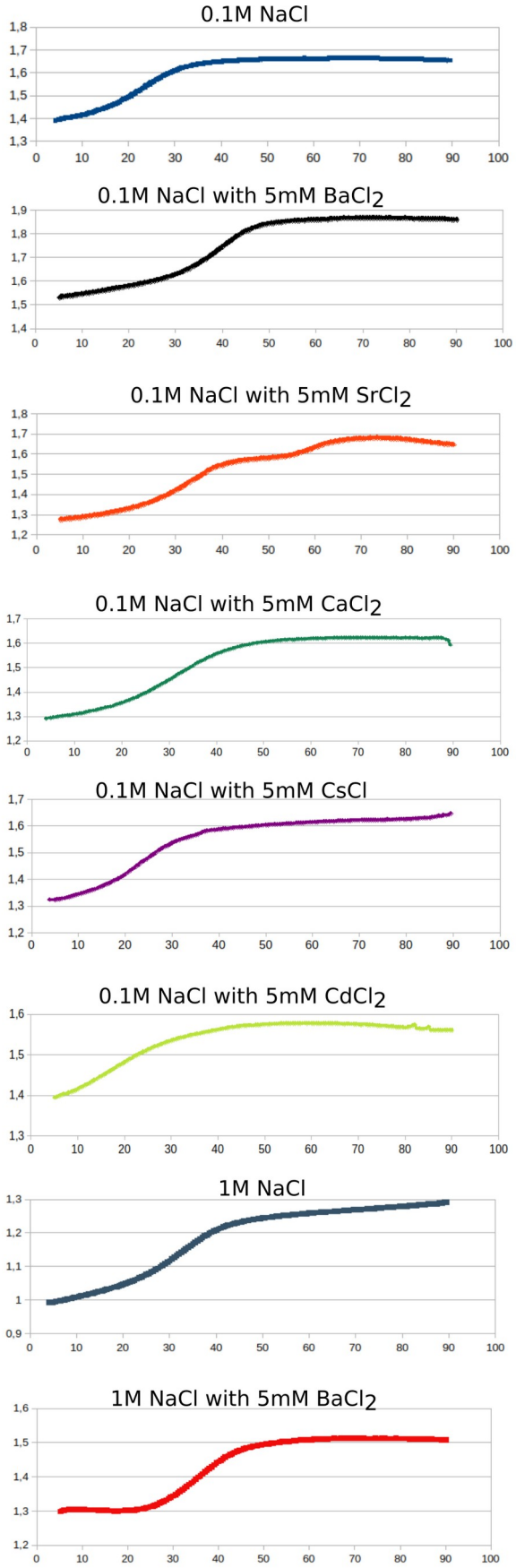
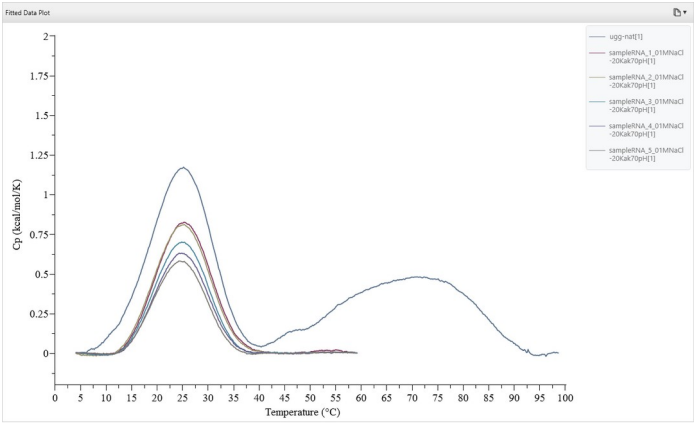
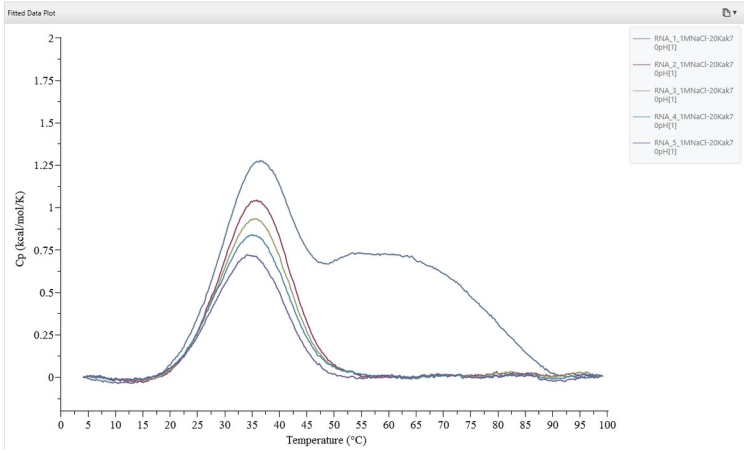
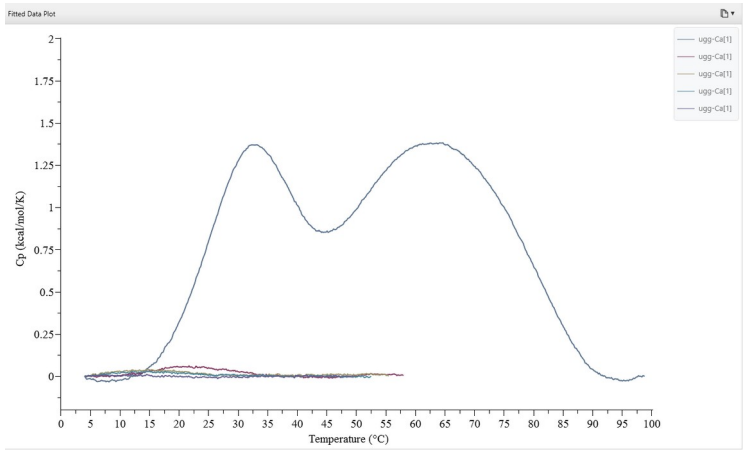
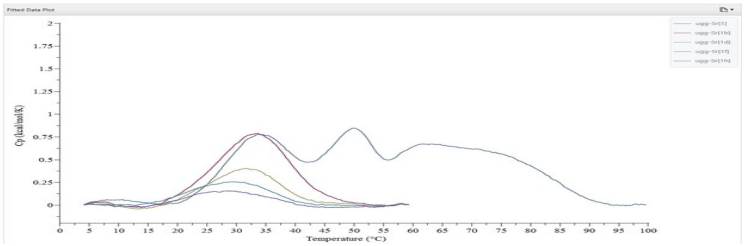
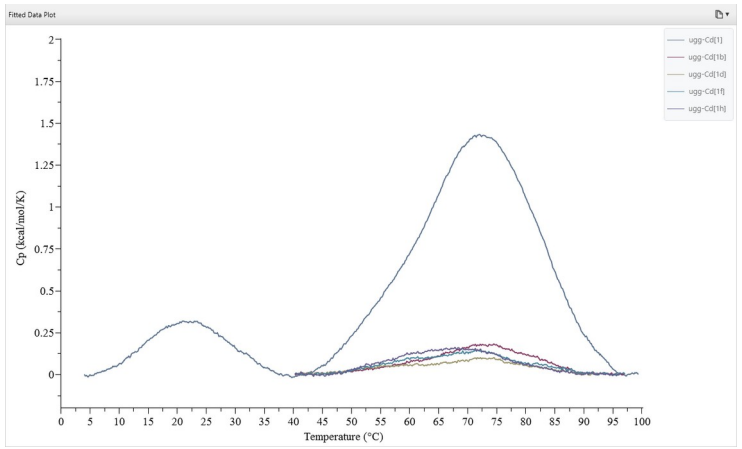
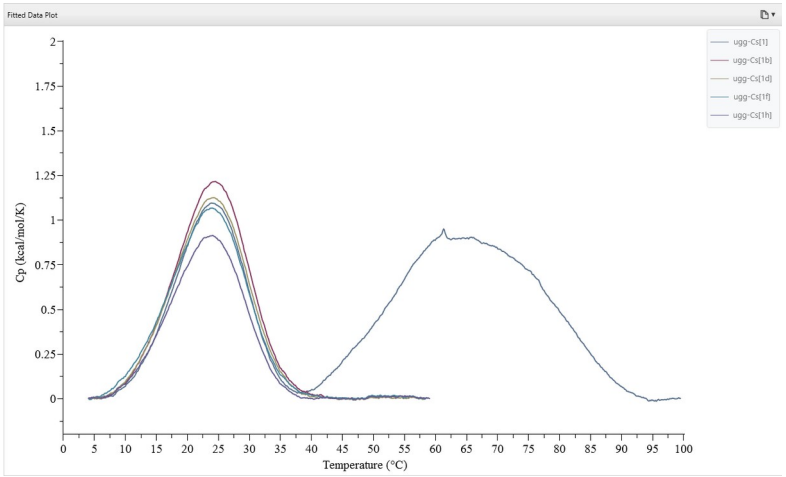


Figure S6. The representative absorbance curves, as a function of temperature (°C), for the AUGUGGCAU oligomer measured in different buffers.

Table S6. The melting temperature Tm of the AUGUGGCAU oligomer calculated for each cycle of DSC measurement and corresponding normalized DSC curves.

Buffer	**Run number**	**RNA concen-tration (µM)**	**Tm1 (ºC)**	**Tm2 (ºC)**	**Tm3 (ºC)**	**DSC curves**
0.1M NaCl	1	**298**	**24.58**	69.19	-	
	2	<298	25.16	-	-	
	3	<<298	24.89	-	-	
	4	<<<298	24.76	-	-	
	5	<<<<298	24.72	-	-	
	6	<<<<<298	24.46	-	-	
1M NaCl	1	**221**	**36.02**	62.23	-	
	2	<221	35.55	-	-	
	3	<<221	35.24	-	-	
	4	<<<221	34.91	-	-	
	5	<<<<221	34.39	-	-	

0.1M NaCl + 5mM $CaCl_2$	1	**211**	**32.72**	63.57	-	
	2	<211	-	-	-	
	3	<<211	-	-	-	
	4	<<<211	-	-	-	
	5	<<<<211	-	-	-	
0.1M NaCl + 5mM $SrCl_2$	1	**291**	**34.06**	49.23	67,78	
	2	<291	32.89	-	-	
	3	<<291	31.30	-	-	
	4	<<<291	28.87	-	-	
	5	<<<<291	28.31	-	-	

0.1M NaCl + 5mM $CdCl_2$	**1**	**160**	**21.80**	71.81	-	
	2	<160	-	-	-	
	3	<<160	-	-	-	
	4	<<<160	-	-	-	
	5	<<<<160	-	-	-	
0.1M NaCl + 5mM CsCl	1	**251**	**23.55**	65.61	-	
	2	<251	23.92	-	-	
	3	<<251	23.68	-	-	
	4	<<<251	23.37	-	-	
	5	<<<<251	23.26	-	-	

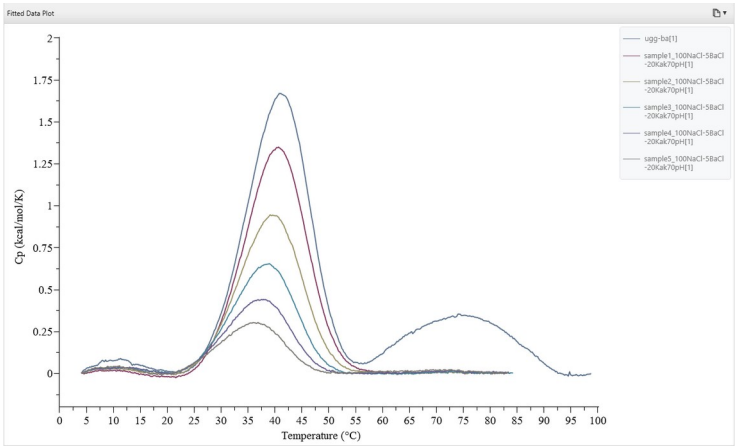
0.1M NaCl + 5mM $BaCl_2$	1	260	40.58	74.66	-	
	2	<260	40.12	-	-	
	3	<<260	39.13	-	-	
	4	<<<260	37.96	-	-	
	5	<<<<260	36.88	-	-	
	6	<<<<<260	36.73	-	-	

Table S7. Crystals structures of models containing G·U pairs with bound Ba^{2+} ions.

PDB code	Primary Citation	DOI
1KD4	The Crystal Structure of r(GGUCACAGCCC)2, Barium form Kacer, V., Scaringe, S.A., Scarsdale, J.N., Rife, J.P. Acta Crystallogr.,Sect.D 59 423-432 (2003)	10.1107/S0907444902021911
6H0R	X-ray structure of SRS2 fragment of Rgs4 3' UTR Heber, S., Gaspar, I., Tants, J.N., Gunther, J., Moya, S.M.F., Janowski, R., Ephrussi, A., Sattler, M., Niessing, D. Nat Commun 10 1659-1659 (2019)	10.1038/s41467-019-09655-3
6CK5	PRPP riboswitch from T. mathranii bound to PRPP Knappenberger, A.J., Reiss, C.W., Strobel, S.A. Elife 7 (2018)	10.7554/eLife.36381
6CB3	Crystal structure of the L.Lactis YkoY riboswitch bound to cadmium Bachas, S.T., Ferre-D'Amare, A.R. Cell Chem Biol 25 962-973.e5 (2018)	10.1016/j.chembiol.2018.04.016
3F2W	Crystal structure of the FMn riboswitch bound to FMN, Ba2+ soak. Serganov, A., Huang, L., Patel, D.J. Nature 458 233-237 (2009)	10.1038/nature07642
3OXJ	Crystal structure of glycine riboswitch, soaked in Ba2+ Huang, L., Serganov, A., Patel, D.J. Mol.Cell 40 774-786 (2010)	10.1016/j.molcel.2010.11.026
4AOB	SAM-I riboswitch containing the T. solenopsae Kt-23 in complex with S-adenosyl methionine Schroeder, K.T., Daldrop, P., Mcphee, S.A., Lilley, D.M.J. RNA 18 (2012)	10.1261/RNA.032409.112
4Y1I	Lactococcus lactis yybP-ykoY Mn riboswitch bound to Mn2+ Price, I.R., Gaballa, A., Ding, F., Helmann, J.D., Ke, A. Mol.Cell 57 1110-1123 (2015)	10.1016/j.molcel.2015.02.016
5FJC	SAM-I riboswitch bearing the H. marismortui Kt-7 variant C-2bU Huang, L., Wang, J., Lilley, D.M.J. Nucleic Acids Res. 44 (2016)	10.1093/NAR/GKW201
7OUO	Crystal structure of RNA duplex [UCGUGCGA]2 in complex with Ba2+ cation Ruszkowska, A., Zheng, Y.Y., Mao, S., Ruszkowski, M., Sheng, J. Front Mol Biosci 8 762786-762786 (2021)	10.3389/fmolb.2021.762786
4B5R	SAM-I riboswitch bearing the H. marismortui K-t-7 Daldrop, P., Lilley, D.M.J. RNA 19 (2013)	10.1261/RNA.036657.112