Supplementary Information

Title

Contributions and competition of Mg^{2+} and K^{+} in folding and stabilization of the Twister Ribozyme.

Authors

Abhishek A. Kognole[1] and Alexander D. MacKerell, Jr.[1]*

[1.] Department of Pharmaceutical Sciences, School of Pharmacy, University of Maryland Baltimore.

* Corresponding author. Email – alex@outerbanks.umaryland.edu

Running Title

Variations in Ionic Atmosphere as RNA folds

This file includes:

I. Supplementary Tables S1 to S4

II. Supplementary Figures S1 to S7

Table S1. Number of ions and their concentrations in the studied systems. Ion numbers were designed to obtain approximate concentrations of 100 mM in the 100 mM MgCl2 system. The number of ions took into account the total charge of Twister (-53).

System	Number of Ions			Concentration of Ions		
	Mg^{2+}	K^{+}	Cl^{-}	Mg^{2+}	K^{+}	Cl^{-}
0 mM $MgCl_2$	0	95	42	0.0	216.4	95.7
10 mM $MgCl_2$	5	85	42	11.4	193.6	95.7
20 mM $MgCl_2$	8	79	42	18.2	179.9	95.7
100 mM $MgCl_2$	40	42	69	91.1	95.7	157.1

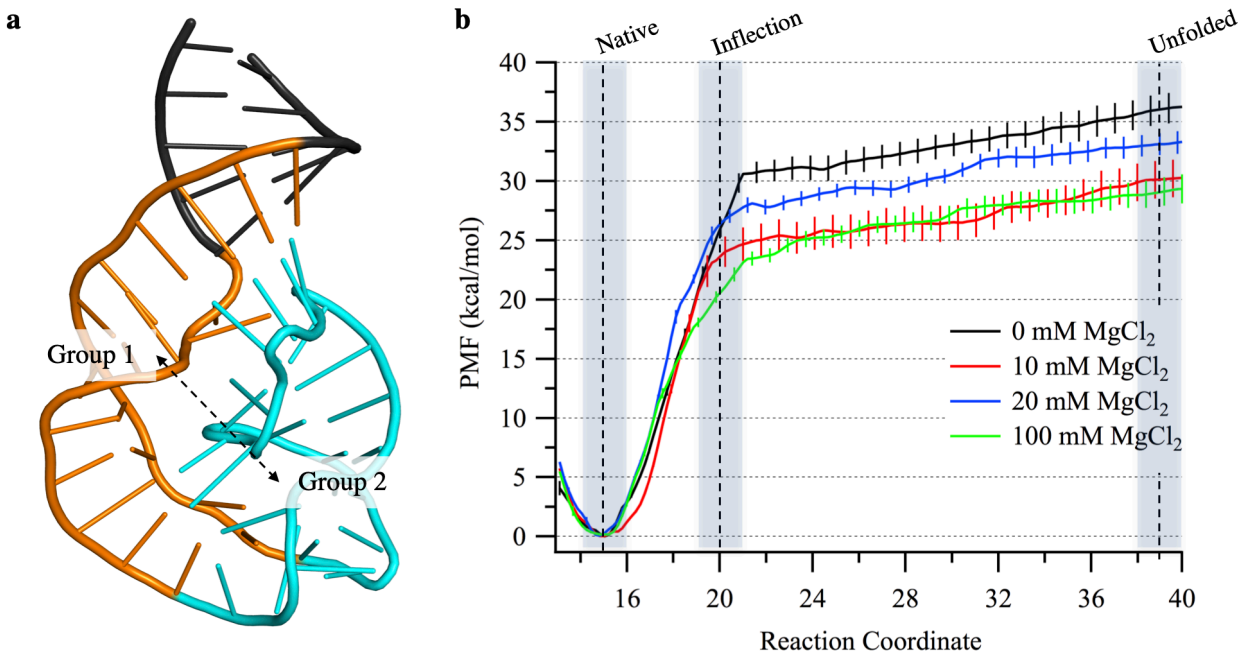

Figure S1. a) Reaction coordinate, Å, is the distance between the centers of mass of the non-hydrogen atoms in group1 (orange cartoon, nucleotides 6:15 + 40:49) and group 2 (cyan cartoon, residues 16:39). b) Potential of mean force (PMF) of Twister ribozyme at different concentrations of $MgCl_2$ based on the GCMC/MD simulations. PMFs were calculated on RC windows at 1 Å intervals from 13 to 40 Å with 60 ns of sampling per window with error bars based on the standard error from five 12-ns samples extracted from the full 60 ns of sampling. Unless otherwise defined, the native state includes RC windows 14 -16, the inflection region includes RC windows 19 – 21 and the partially unfolded state includes RC windows 38 – 40. As discussed in the previous manuscript the umbrella sampling did not sample unfolded states in which the secondary structures was lost, such that the most unfolded state along the reaction coordinate is referred to as a partially unfolded state.

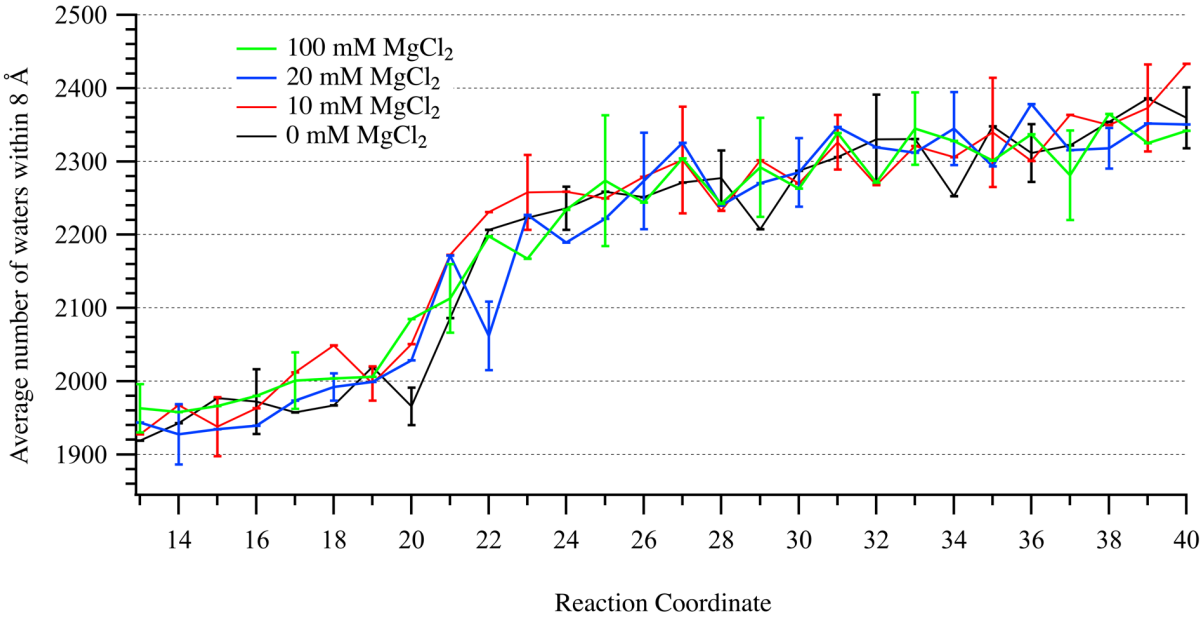

Figure S2. Average number or water within 8 Å of the RNA based on the water O to RNA non-hydrogen atom distance along the reaction coordinate. The results indicate that the volume as defined by the 8 Å cutoff used to define counterion condensation is increasing as unfolding of the RNA occurs. Error bars indicate one standard deviation and are added at intervals for clarity.

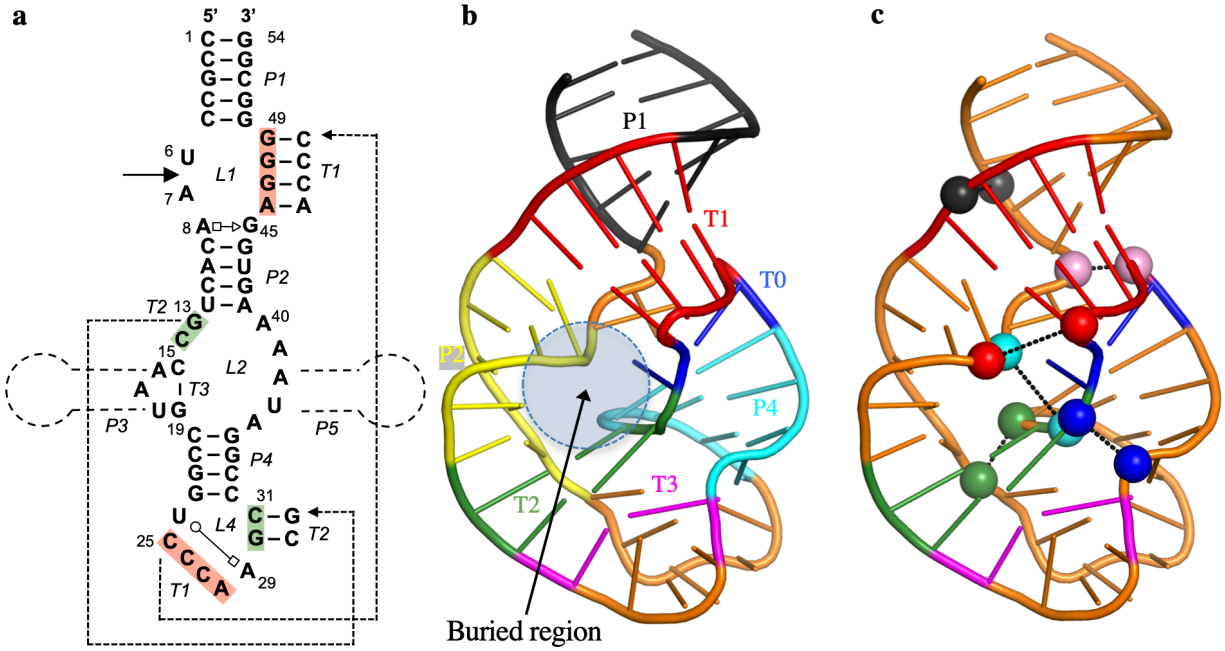

Figure S3. Structure of Twister ribozyme and spatially adjacent phosphate pairs. a) Twister Ribozyme secondary and tertiary contacts. The scissile bond is indicated with a solid arrow and the omitted P3 and P5 loops are indicated. b) Contacts are illustrated as cartoons colored as follows P1 – black, P2 – yellow, P4 – cyan, T0 – blue, T1 – red, T2 – green, T3 – magenta, rest of the RNA – orange. c) Twister ribozyme in its native state with spatially adjacent phosphate pairs that are present in the folded, tertiary structure (same-colored spheres connected with dashed line; 4-46 – black, 9-28 – red, 6-25 – pink, 8-31 – cyan, 20-30 – blue, 32-41 – green). This figure has been reprinted with permission from Kognole and MacKerell (2020) Biophysical Journal 118(6), 1424-1437.

Table S2. Number of Mg^{2+} and K^+ ions around the phosphate backbone of Twister in 0, 10 and 20 $MgCl_2$ systems. The 3 Å cutoff corresponds to direct contact of ions with non-bridging phosphate oxygens (NBPOs), 3 – 5.5 Å range represents outer-shell contacts, 5.5 – 8 Å range represents diffuse (non-dehydrated) contacts. The 8 Å cutoff defines the full condensed-ion atmosphere. The errors represent standard error of mean calculated over five 12 ns trajectories.

	Mg^{2+} within	0 – 3 Å	3 – 5.5 Å	5.5 – 8 Å	0 – 8 Å	beyond 8 Å
10 mM	RC_15-18	1.11 ± 0.10	2.77 ± 0.14	0.17 ± 0.14	4.05 ± 0.10	0.95 ± 0.10
	RC_19-22	0.46 ± 0.12	3.45 ± 0.22	0.21 ± 0.24	4.12 ± 0.16	0.88 ± 0.16
	RC_23-26	0.72 ± 0.20	3.13 ± 0.24	0.21 ± 0.18	4.06 ± 0.11	0.94 ± 0.11
	RC_27-30	0.82 ± 0.24	2.91 ± 0.27	0.20 ± 0.17	3.93 ± 0.12	1.07 ± 0.12
	RC_31-34	0.31 ± 0.11	3.06 ± 0.21	0.24 ± 0.26	3.61 ± 0.19	1.39 ± 0.19
	RC_35-40	1.02 ± 0.23	2.57 ± 0.30	0.22 ± 0.28	3.81 ± 0.19	1.19 ± 0.19
20 mM	RC_15-18	0.78 ± 0.16	4.50 ± 0.31	0.36 ± 0.37	5.64 ± 0.26	2.36 ± 0.26
	RC_19-22	0.81 ± 0.12	4.44 ± 0.21	0.35 ± 0.25	5.60 ± 0.19	2.40 ± 0.19
	RC_23-26	0.48 ± 0.05	4.79 ± 0.19	0.37 ± 0.25	5.64 ± 0.17	2.36 ± 0.17
	RC_27-30	0.28 ± 0.11	4.98 ± 0.25	0.34 ± 0.30	5.60 ± 0.20	2.40 ± 0.20
	RC_31-34	0.86 ± 0.19	4.32 ± 0.24	0.37 ± 0.19	5.55 ± 0.13	2.45 ± 0.13
	RC_35-40	0.35 ± 0.14	4.95 ± 0.19	0.37 ± 0.18	5.67 ± 0.12	2.33 ± 0.12
	K^+ within	0 – 3 Å	3 – 5.5 Å	5.5 – 8 Å	0 – 8 Å	beyond 8 Å
0 mM	RC_15-18	6.57 ± 0.20	23.73 ± 0.28	4.04 ± 0.29	34.34 ± 0.21	60.66 ± 0.21
	RC_19-22	5.99 ± 0.10	23.49 ± 0.18	4.01 ± 0.19	33.49 ± 0.12	61.51 ± 0.12
	RC_23-26	5.08 ± 0.14	23.23 ± 0.24	3.76 ± 0.23	32.07 ± 0.13	62.93 ± 0.13
	RC_27-30	4.82 ± 0.26	22.90 ± 0.37	3.75 ± 0.36	31.47 ± 0.25	63.53 ± 0.25
	RC_31-34	5.27 ± 0.20	22.23 ± 0.28	3.74 ± 0.24	31.24 ± 0.15	63.76 ± 0.15
	RC_35-40	4.85 ± 0.15	22.43 ± 0.21	3.63 ± 0.24	30.91 ± 0.19	64.09 ± 0.19

10 mM	**RC_15-18**	4.69 ± 0.23	19.66 ± 0.35	2.97 ± 0.29	27.32 ± 0.28	57.68 ± 0.28
	RC_19-22	4.45 ± 0.12	18.37 ± 0.47	3.15 ± 0.19	25.97 ± 0.45	59.03 ± 0.45
	RC_23-26	4.07 ± 0.19	18.70 ± 0.33	2.91 ± 0.23	25.68 ± 0.23	59.32 ± 0.23
	RC_27-30	4.02 ± 0.13	18.13 ± 0.31	3.06 ± 0.36	25.21 ± 0.31	59.79 ± 0.31
	RC_31-34	4.10 ± 0.27	18.23 ± 0.41	3.17 ± 0.24	25.50 ± 0.41	59.50 ± 0.41
	RC_35-40	3.49 ± 0.17	18.15 ± 0.30	3.15 ± 0.24	24.79 ± 0.26	60.21 ± 0.26
20 mM	**RC_15-18**	4.37 ± 0.20	17.74 ± 0.36	3.02 ± 0.55	25.13 ± 0.42	53.87 ± 0.42
	RC_19-22	3.83 ± 0.10	16.99 ± 0.38	3.06 ± 0.39	23.88 ± 0.25	55.12 ± 0.25
	RC_23-26	3.45 ± 0.14	17.44 ± 0.36	2.66 ± 0.40	23.55 ± 0.27	55.45 ± 0.27
	RC_27-30	3.49 ± 0.26	16.39 ± 0.49	2.73 ± 0.62	22.61 ± 0.42	55.39 ± 0.42
	RC_31-34	3.72 ± 0.20	16.84 ± 0.51	2.73 ± 0.60	23.29 ± 0.41	55.71 ± 0.41
	RC_35-40	3.06 ± 0.15	16.75 ± 0.34	2.65 ± 0.45	22.46 ± 0.33	56.54 ± 0.33

Table S3. Average number of K^+ displaced per Mg^{2+} ion for 0 – 8 Å cutoff at different stages of the reaction coordinate for the 100, 20 and 10 mM $MgCl_2$ systems compared to the 0 mM $MgCl_2$ system.

	100 mM	**20 mM**	**10 mM**
RC_15-18	1.39	1.63	1.73
RC_19-22	1.36	1.72	1.83
RC_23-26	1.32	1.51	1.57
RC_27-30	1.32	1.58	1.59
RC_31-34	1.28	1.43	1.59
RC_35-40	1.28	1.49	1.61
Avg	**1.32 ± 0.04**	**1.56 ± 0.10**	**1.65 ± 0.10**

Table S4. Number of K^+ ions within 3.5 Å of the N7/O2/O4/O6 atoms of nucleobases. The analysis was carried out for nucleobases in helical regions and in non-helical regions.

	Helical			Non-helical		
	Native	**Inflection**	**Unfolded**	**Native**	**Inflection**	**Unfolded**
100 mM $MgCl_2$	2.15 ± 0.20	2.25 ± 0.25	1.40 ± 0.09	2.16 ± 0.24	2.06 ± 0.25	0.92 ± 0.16
20 mM $MgCl_2$	3.88 ± 0.18	3.48 ± 0.18	3.37 ± 0.17	4.44 ± 0.15	4.03± 0.21	2.93 ± 0.20
10 mM $MgCl_2$	3.93 ± 0.22	3.76 ± 0.18	3.63 ± 0.11	4.93 ± 0.22	4.39 ± 0.18	3.81 ± 0.11
0 mM $MgCl_2$	4.69 ± 0.09	4.62 ± 0.09	4.42 ± 0.14	6.30 ± 0.20	5.64 ± 0.07	4.71 ± 0.15

The helical region is comprised of 26 nucleotides and the non-helical region is comprised of 28 nucleotides.

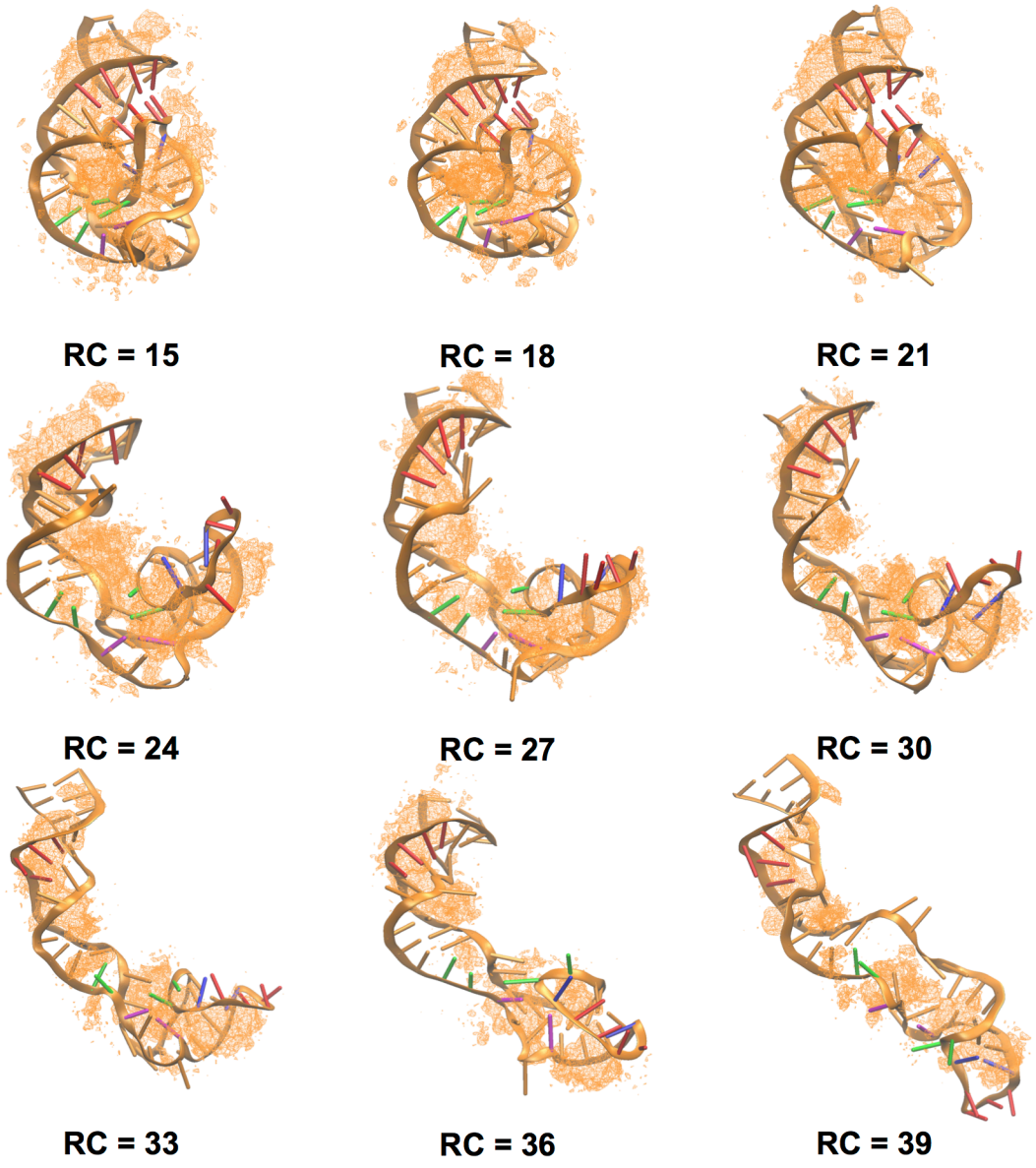

Figure S4. GFE maps of the distribution of K^+ (orange) ions around the Twister ribozyme at various stages along the umbrella sampling reaction coordinate in 0 mM $MgCl_2$. Twister is shown as orange cartoon with T0 bases in blue, T1 bases in red, T2 bases in green and T3 bases in magenta cartoon. GFE level is -2.0 kcal/mol.

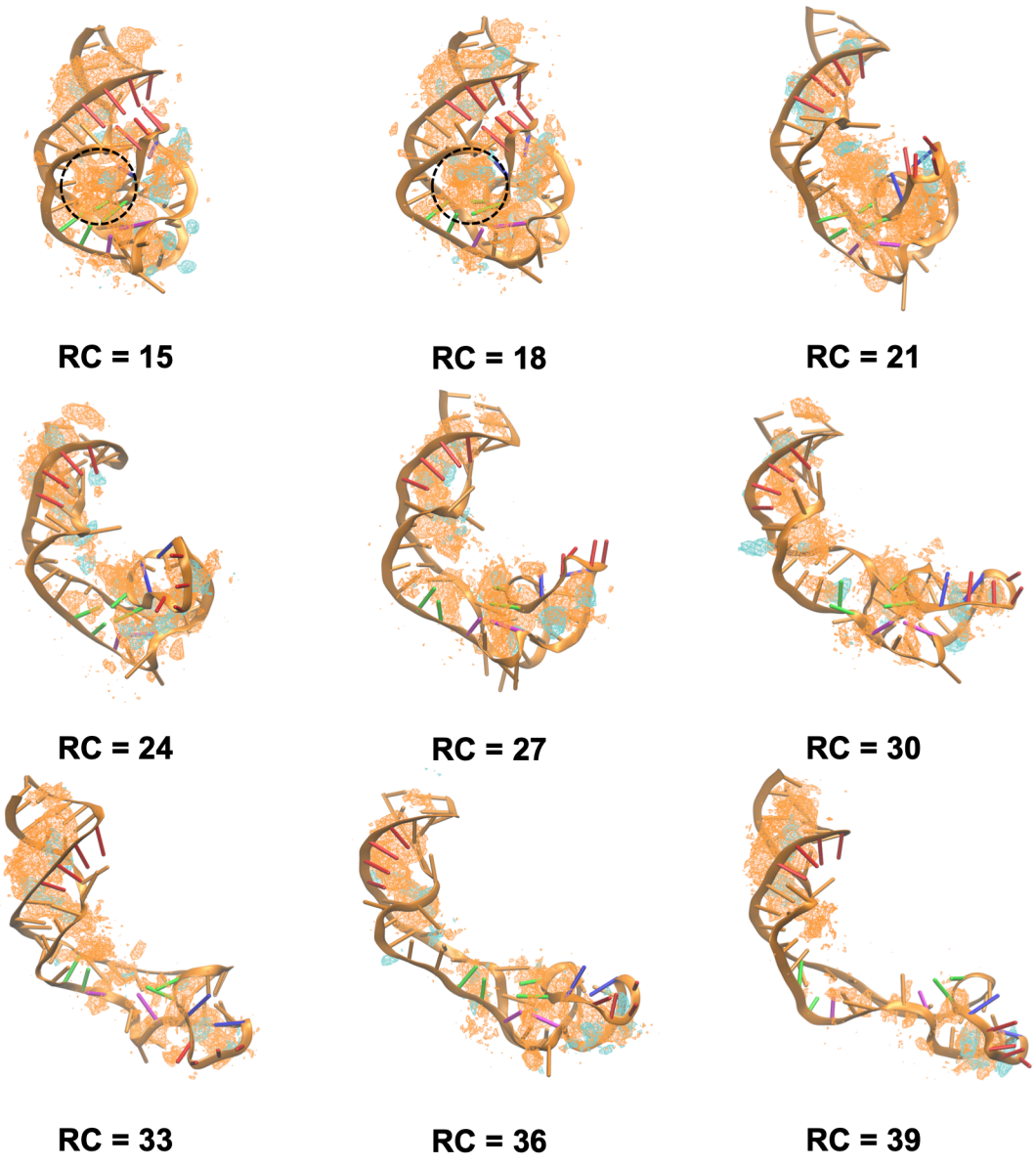

Figure S5. GFE maps of the distribution of K^+ (orange) and Mg^{2+} (cyan) ions around the Twister ribozyme at various stages along the umbrella sampling reaction coordinate in 10 mM $MgCl_2$. Twister is shown as orange cartoon with T0 bases in blue, T1 bases in red, T2 bases in green and T3 bases in magenta cartoon. GFE level is -2.0 kcal/mol. Buried binding sites at RC = 15 & 18 are indicated with dashed circle.

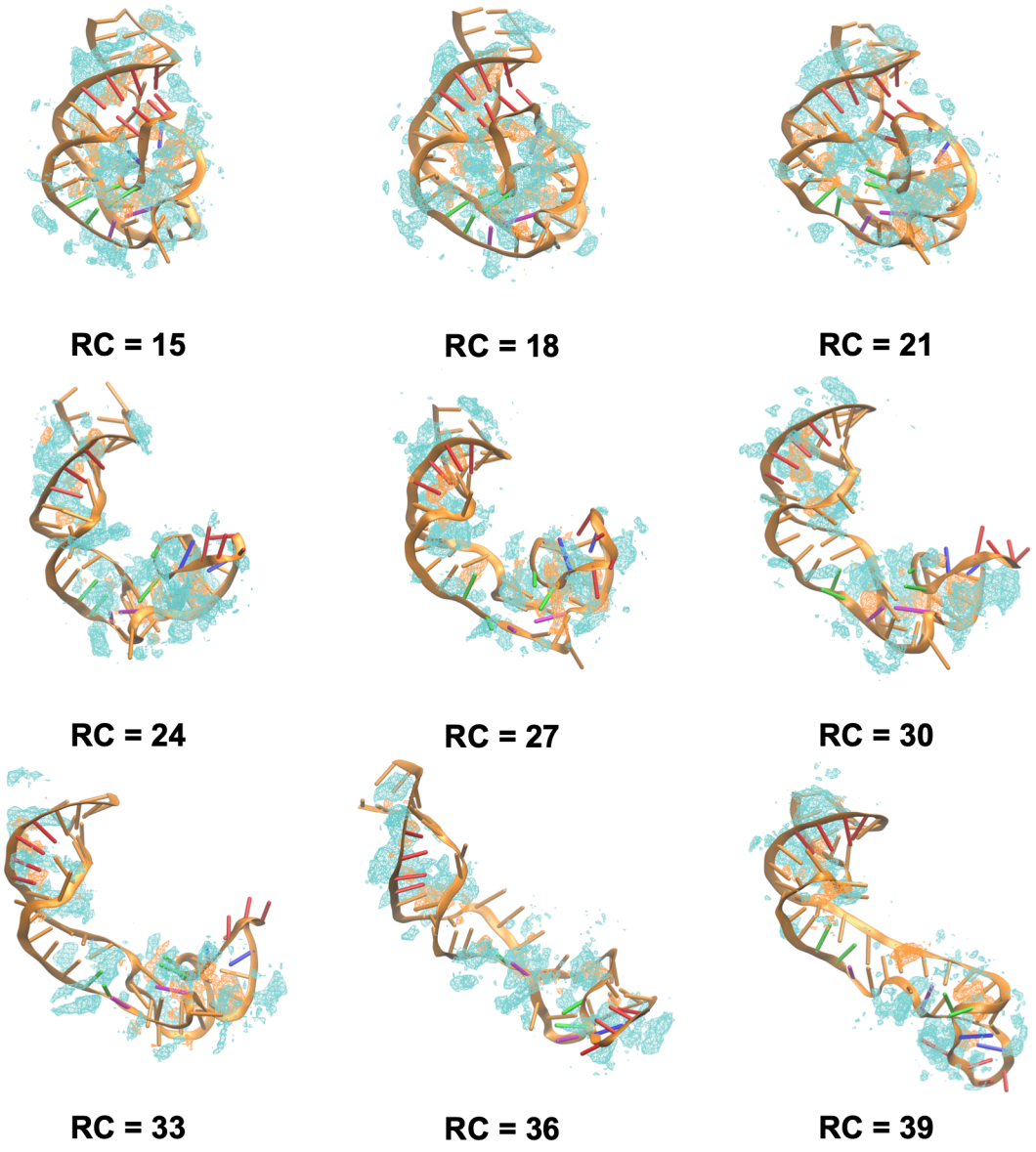

Figure S6. GFE maps of distribution of K^+ (orange) and Mg^{2+} (cyan) ions around the Twister ribozyme at various stages along the umbrella sampling reaction coordinate in 100 mM $MgCl_2$. Twister is shown as orange cartoon with T0 bases in blue, T1 bases in red, T2 bases in green and T3 bases in magenta cartoon. GFE level is -2.0 kcal/mol.

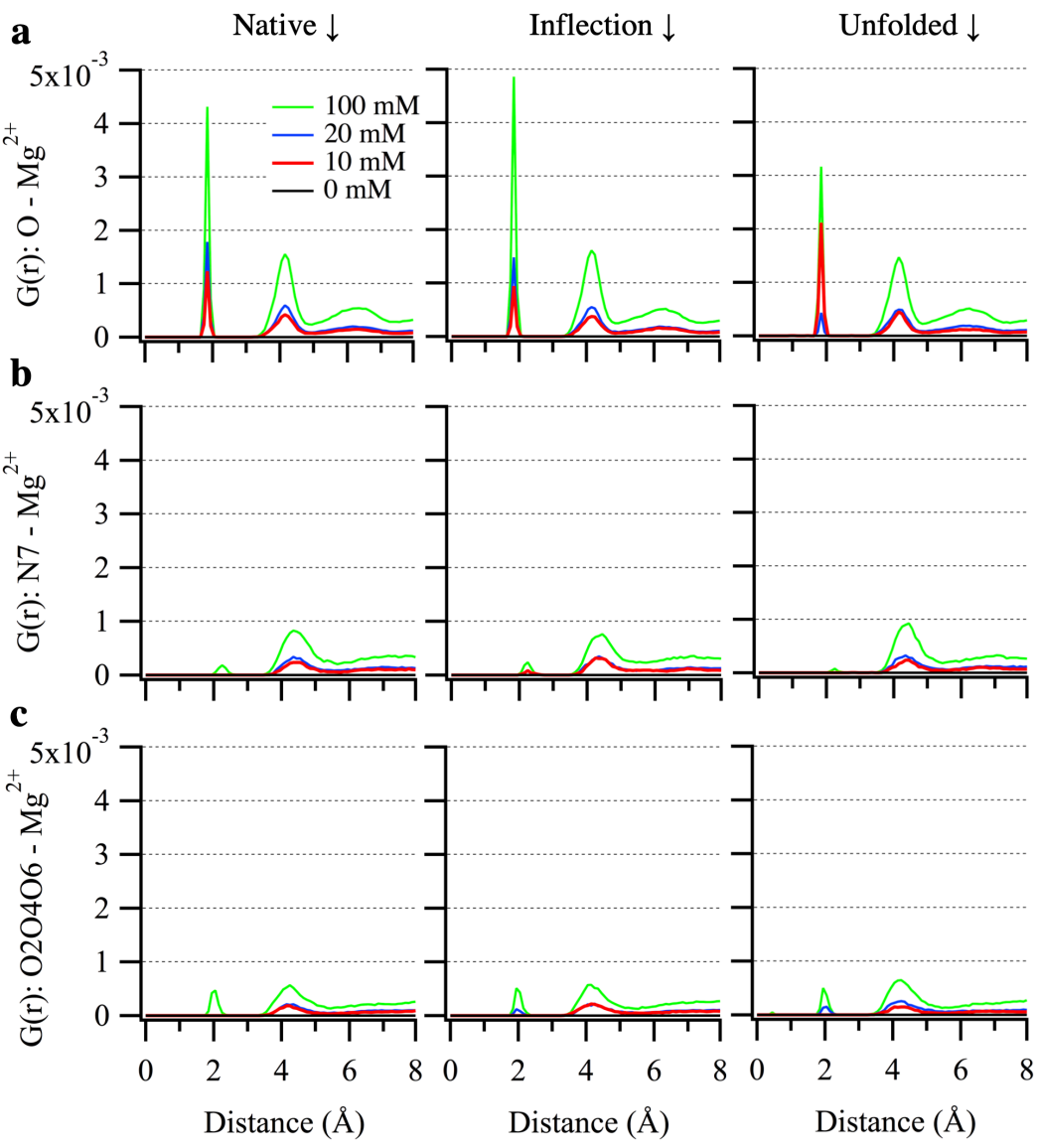

Figure S7. Radial distribution functions of Mg^{2+} around a) the non-bridging phosphate oxygens (NBPO), b) the Gua-N7/Ade-N7 atoms, and c) O2/O4/O6 atoms of the nucleobases at native state (RC=14-16), inflection point (RC=19-21) and unfolded state (RC=38-40).

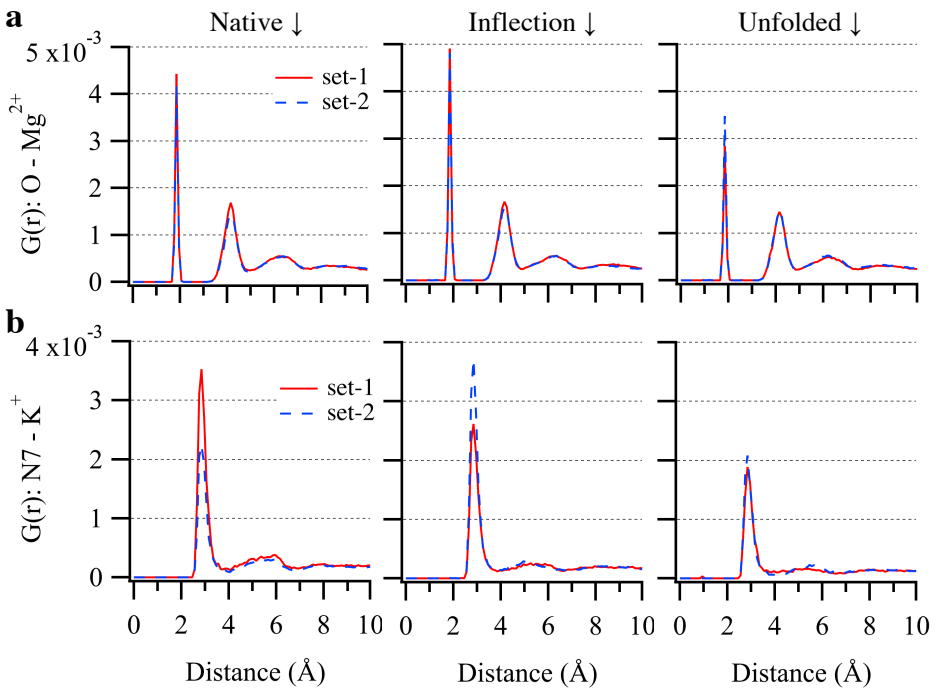

Figure S8. Radial distribution functions of (Upper) Mg^{2+} and the non-bridging phosphate oxygens (NBPO) and K^+ and Guanine N7 atoms from the Set 1 (red line) and Set 2 (blue dashed line) portions of the GCMC/Umbrella sampling trajectories at the native state, inflection region and unfolded state. The similarity of the overall pattern of the two curves indicates that the extend of convergence of ion sampling is adequate for the conclusions being made in the study.