Supporting Information

Table S1: Partial atomic charges of the TMAO and TMAOP models

Atom	TMAO	TMAOP
O	-0.70	-0.17
HO	--	0.50
N	-0.83	-0.53
C1	-0.35	-0.35
C2	-0.35	-0.35
C3	-0.35	-0.35
H11	0.25	0.25
H12	0.25	0.25
H13	0.25	0.25
H21	0.25	0.25
H22	0.25	0.25
H23	0.25	0.25
H31	0.25	0.25
H32	0.25	0.25
H33	0.25	0.25

Table S2: The average RMS difference over the last 60 ns of the simulation and the error analysis is based on random block analysis. Results are presented for non-hydrogen atoms excluding the terminal nucleotides and were aligned to the respective atom selections used for the RMS difference calculation.

Region	**0 M TMAO**	**0.4 M TMAO**	**2.0 M TMAO**	**0.4 M TMAO/TMAOP**	**2.0 M TMAO/TMAOP**
WC	2.21 ± 1.01	3.02 ± 0.45	3.09 ± 0.64	2.35 ± 0.31	2.41 ± 0.52
All (excluding termini)	3.26 ± 1.02	4.35 ± 0.43	4.89 ± 0.73	3.68 ± 0.32	2.60 ± 0.43

Table S3: The crystallographic native tertiary contacts as defined by Klein *et. al*.[12] in their supplemental materials excluding terminal nucleotides.

Tertiary Contacts	Type of Contact
G11 N2 – A16 N1 G11 N3 – A16 N6	Hoogsteen
A14 N1 – U32 O2' A14 N6 – U32 O2	Hoogsteen
A23 N1 – G2 O2' A23 N6 – G2 N3	Trans sugar edge
U24 O4 – G2 N2	Single H-bond
A25 N1 – C21 O2'	Single H-bond
A25 N6 – A3 N3 A25 N7 – A3 O2'	Hoogsteen
A26 N1 – U20 O2' A26 N6 – U20 O2	Hoogsteen
A26 N6 – G4 N3 A26 N7 – G4 O2'	Hoogsteen
A27 N1 – C19 O2' A27 N6 – C19 O2	Hoogsteen

Table S4: The average number of water molecules per nucleotide with the oxygen atom within 3.5 Å of the selected atoms of the $PreQ_1$ RNA molecule. Results are presented for RNA in the presence of 2.0 M TMAO where 100% of the TMAO is neutral (Neutral TMAO) and where 10% of TMAO is protonated (10% TMAOP). The average values are based on an average over the last 60 ns for each simulation and the error analysis is based on random block analysis over six blocks differing by 10 ns increments.

Selected atoms	All		Difference
	Neutral TMAO	10% TMAOP	
2'-hydroxyl (O2')	0.8 ± 0.3	0.7 ± 0.1	-0.1
Ribose (O4')	0.3 ± 0.2	0.3 ± 0.1	-0.0
Phosphate (O1P & O2P)	3.2 ± 0.2	2.6 ± 0.2	-0.6
Minor groove (N3, O2 & N2)	0.7 ± 0.1	0.4 ± 0.1	-0.3
Major groove (N7, N4, O4, O6, & N6)	0.9 ± 0.2	0.5 ± 0.2	-0.4
Total	4.3 ± 0.3	3.8 ± 0.3	-0.5

Selected atoms	Non-WC		Difference
	Neutral TMAO	10% TMAOP	
2'-hydroxyl (O2')	0.8 ± 0.2	0.8 ± 0.2	-0.0
Ribose (O4')	0.4 ± 0.2	0.3 ± 0.2	-0.1
Phosphate (O1P & O2P)	3.3 ± 0.2	3.1 ± 0.2	-0.2
Minor groove (N3, O2 & N2)	0.7 ± 0.1	0.6 ± 0.2	-0.1
Major groove (N7, N4, O4, O6, & N6)	1.1 ± 0.3	0.6 ± 0.3	-0.5
Total	4.4 ± 0.3	4.0 ± 0.2	-0.4

Selected atoms	WC		Difference
	Neutral TMAO	10% TMAOP	
2'-hydroxyl (O2')	0.8 ± 0.1	0.6 ± 0.1	-0.2
Ribose (O4')	0.2 ± 0.2	0.2 ± 0.1	-0.0
Phosphate (O1P & O2P)	3.2 ± 0.3	2.3 ± 0.2	-0.9
Minor groove (N3, O2 & N2)	1.0 ± 0.2	0.5 ± 0.1	-0.5
Major groove (N7, N4, O4, O6, & N6)	0.9 ± 0.1	0.7 ± 0.2	-0.2
Total	4.3 ± 0.4	3.5 ± 0.4	-0.8

Table S5: The average number of Mg^{2+} per nucleotide within 3.5 Å of the selected atoms of the $PreQ_1$ RNA molecule. The average values are based on an average over the last 60 ns for each simulation and the error analysis is based on random block analysis over six blocks differing by 10 ns increments.

Selected atoms	3.5 Å All	5.5 Å All
2'-hydroxyl (O2')	0.01 ± 0.03	0.10 ± 0.06
Ribose (O4')	0.01 ± 0.01	0.05 ± 0.03
Phosphate (O1P & O2P)	0.16 ± 0.05	0.36 ± 0.05
Minor groove (N3, O2 & N2)	0.01 ± 0.02	0.04 ± 0.03
Major groove (N7, N4, O4, O6, & N6)	0.01 ± 0.01	0.09 ± 0.08
Total	0.18 ± 0.07	0.27 ± 0.07

Selected atoms	Non-WC	Non-WC
2'-hydroxyl (O2')	0.01 ± 0.04	0.07 ± 0.06
Ribose (O4')	0.01 ± 0.01	0.05 ± 0.05
Phosphate (O1P & O2P)	0.06 ± 0.07	0.24 ± 0.07
Minor groove (N3, O2 & N2)	0.01 ± 0.02	0.04 ± 0.06
Major groove (N7, N4, O4, O6, & N6)	0.01 ± 0.01	0.02 ± 0.02
Total	0.10 ± 0.07	0.22 ± 0.07

Selected atoms	WC	WC
2'-hydroxyl (O2')	0.01 ± 0.03	0.07 ± 0.06
Ribose (O4')	0.01 ± 0.01	0.01 ± 0.03
Phosphate (O1P & O2P)	0.29 ± 0.05	0.55 ± 0.05
Minor groove (N3, O2 & N2)	0.01 ± 0.02	0.01 ± 0.02
Major groove (N7, N4, O4, O6, & N6)	0.01 ± 0.02	0.18 ± 0.09
Total	0.28 ± 0.08	0.47 ± 0.07

Figure S1: Probability distribution of radius of gyration of the riboswitch. [Blue: No TMAO, Red: TMAO 0.4 M (10% protonated), Black: TMAO 2.0 M (10% protonated), Green: TMAO 0.4 M REX-CpHMD, Magenta: TMAO 2.0 M REX-CpHMD]

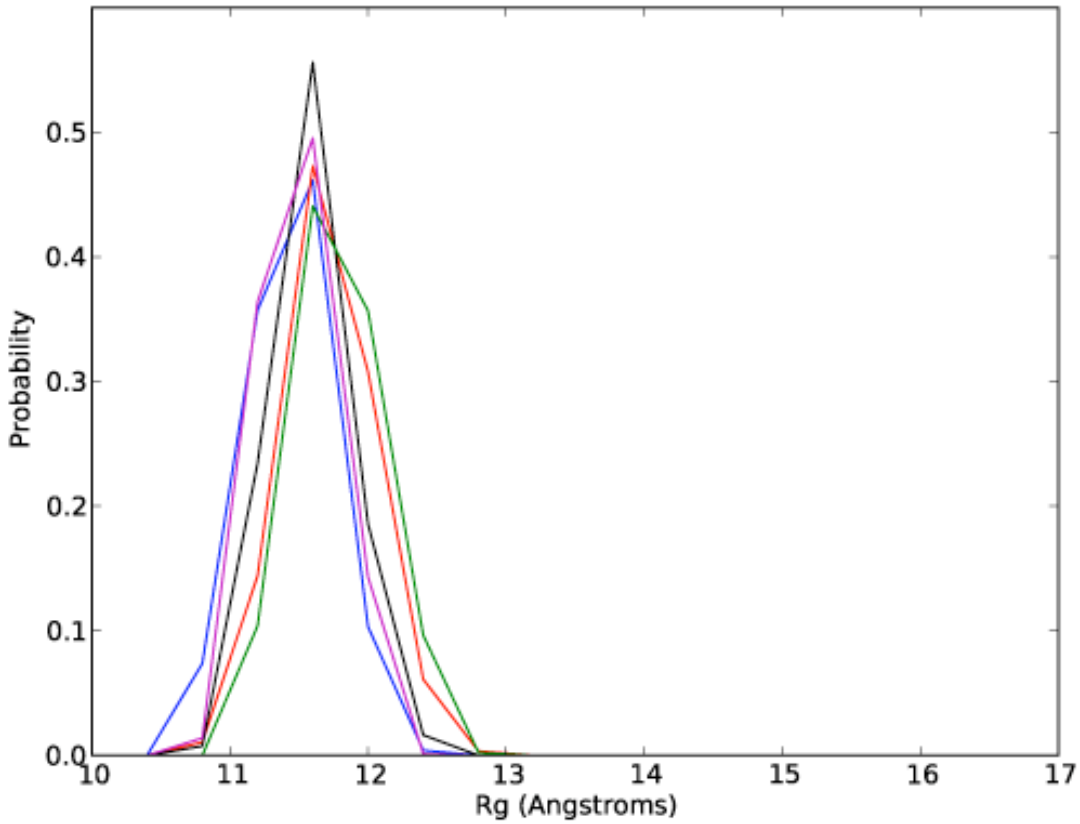
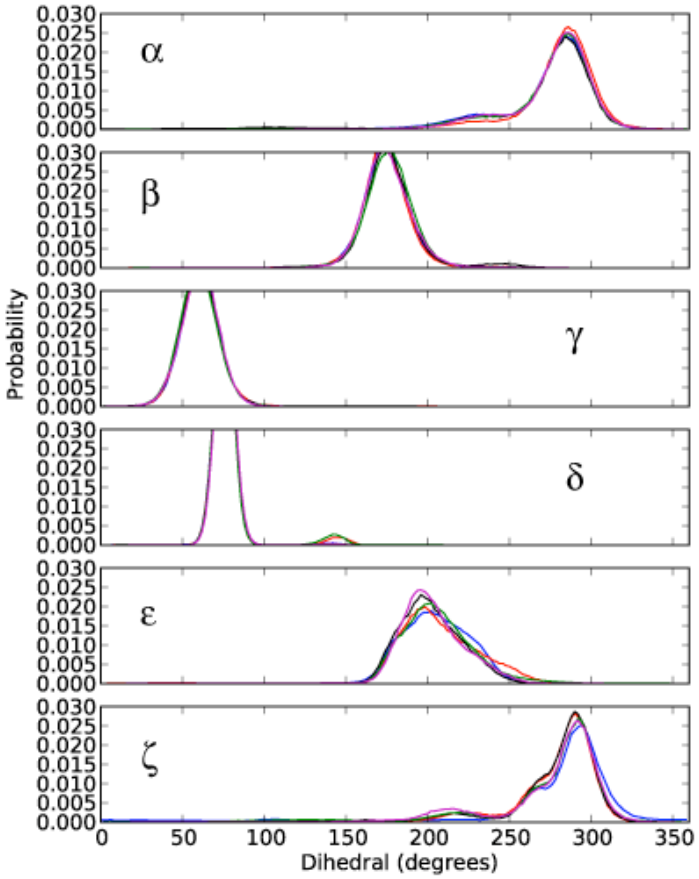


Figure S2: Probability distributions for the phosphodiester backbone and chi torsion angles of the WC-region of the RNA. [Blue: No TMAO, Red: TMAO 0.4 M, Black: TMAO 2.0 M, Green: TMAO 0.4 M (10% protonated), Magenta: TMAO 2.0 M (10% protonated),]



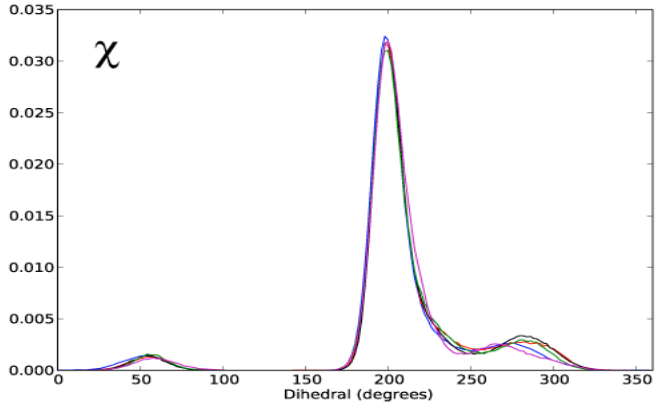


Figure S3: Probability distribution of the WC base-pairings. [Blue: No TMAO, Red: TMAO 0.4 M (10% protonated), Black: TMAO 2.0 M (10% protonated), Green: TMAO 0.4 M REX-CpHMD, Magenta: TMAO 2.0 M REX-CpHMD]

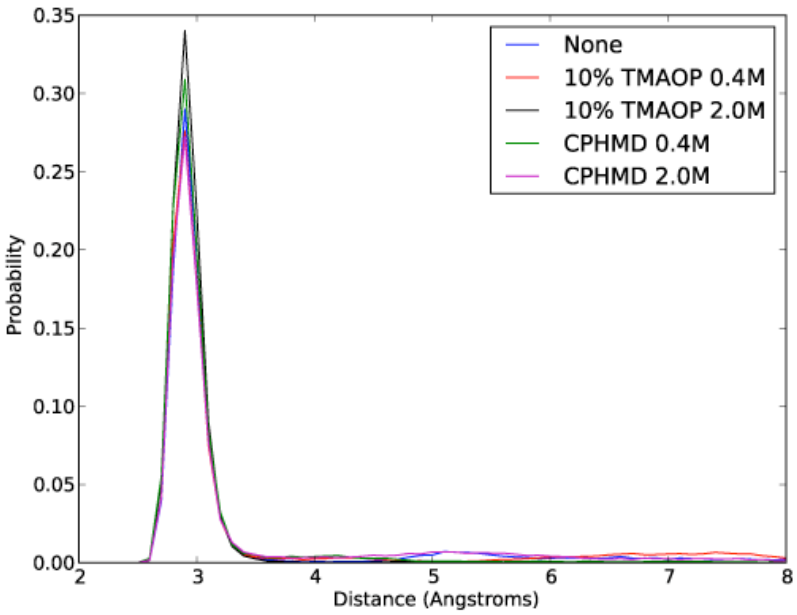


Figure S4: Radial distribution functions of the water oxygen atom around the canonical (WC) and non-canonical (Non-WC) regions of the RNA structure. Results are presented for the (A) 2'-hydroxyl, (B) sugar O4', (C) phosphate, (D) minor-groove side of the bases and (E) major-groove side of the bases [Black: No TMAO, Blue: TMAO 0.4 M (10% protonated), Red: TMAO 2.0 M (10% protonated)]. The minor and major grooves were defined based on the atoms O2, N2, and N3 for the minor groove and N7, N6, O6, N4, and O4 for the major groove. Normalization was based on number of trajectory frames and bulk density.

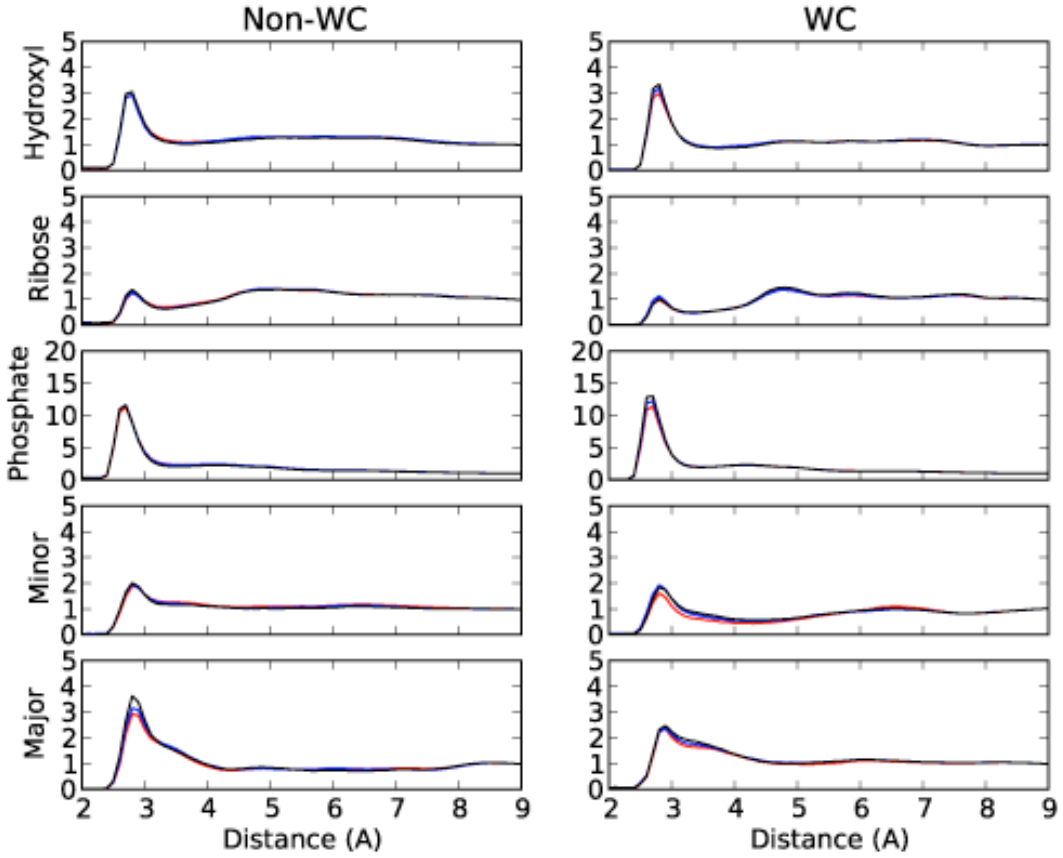


Figure S5: Two-dimensional histogram of the minimum anionic phosphate-TMAOP distances versus the minimum major groove-TMAOP distances. The phosphate and major grooves were defined based on the atoms O1P and O2P for the phosphate and N7, N6, O6, N4, and O4 for the major groove. Normalization was based on number of trajectory frames.

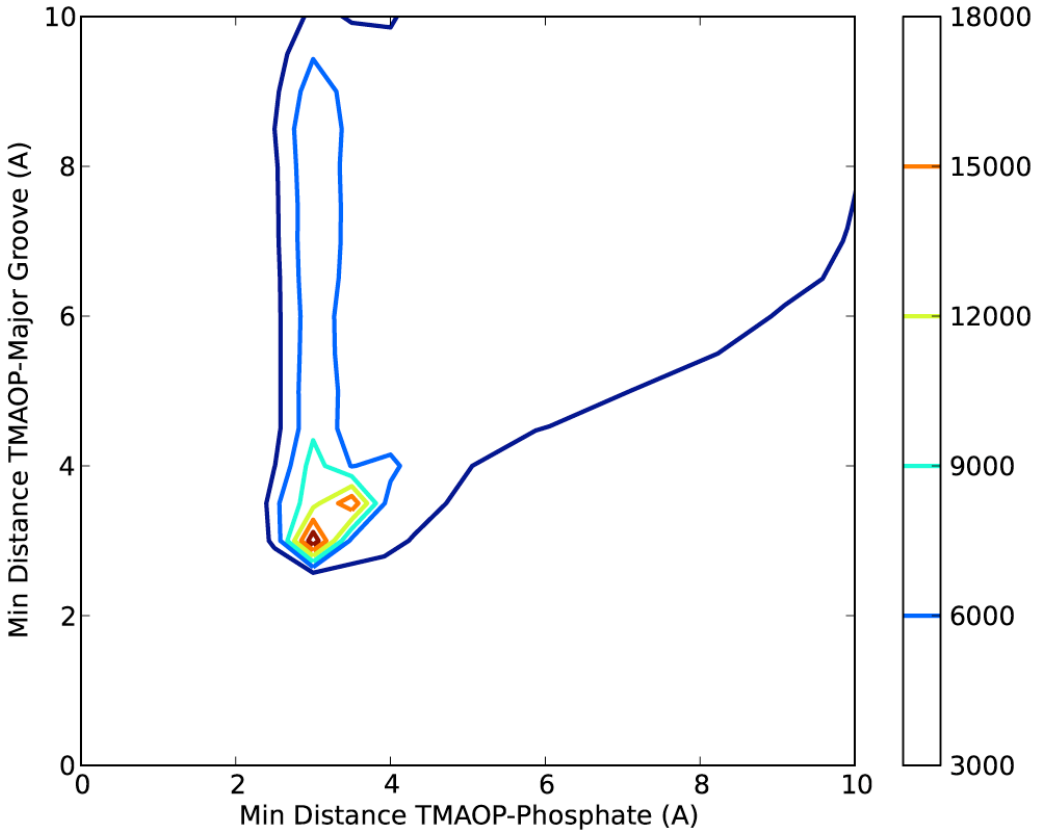


Figure S6: Radial distribution functions of the TMAO-oxygen versus the TMAOP-oxygen around the canonical (i.e. - those involved in WC base pairs) and non-canonical regions of the RNA structure at 2.0 M concentration using 10% explicit TMAOP and REX-CpHMD. ((A) 2'-hydroxyl region (B) sugar region (C) phosphate region (D) minor-groove side of the base region (E) major-groove side of the base region [Red: protonated TMAO in the non-canonical region, Black: neutral TMAO in the non-canonical region, Green: protonated TMAO in the canonical region, Blue: neutral TMAO in the canonical region]. The minor and major grooves were defined based on the atoms O2, N2, and N3 for the minor groove and N7, N6, O6, N4, and O4 for the major groove. Normalization was based on number of trajectory frames and bulk density.

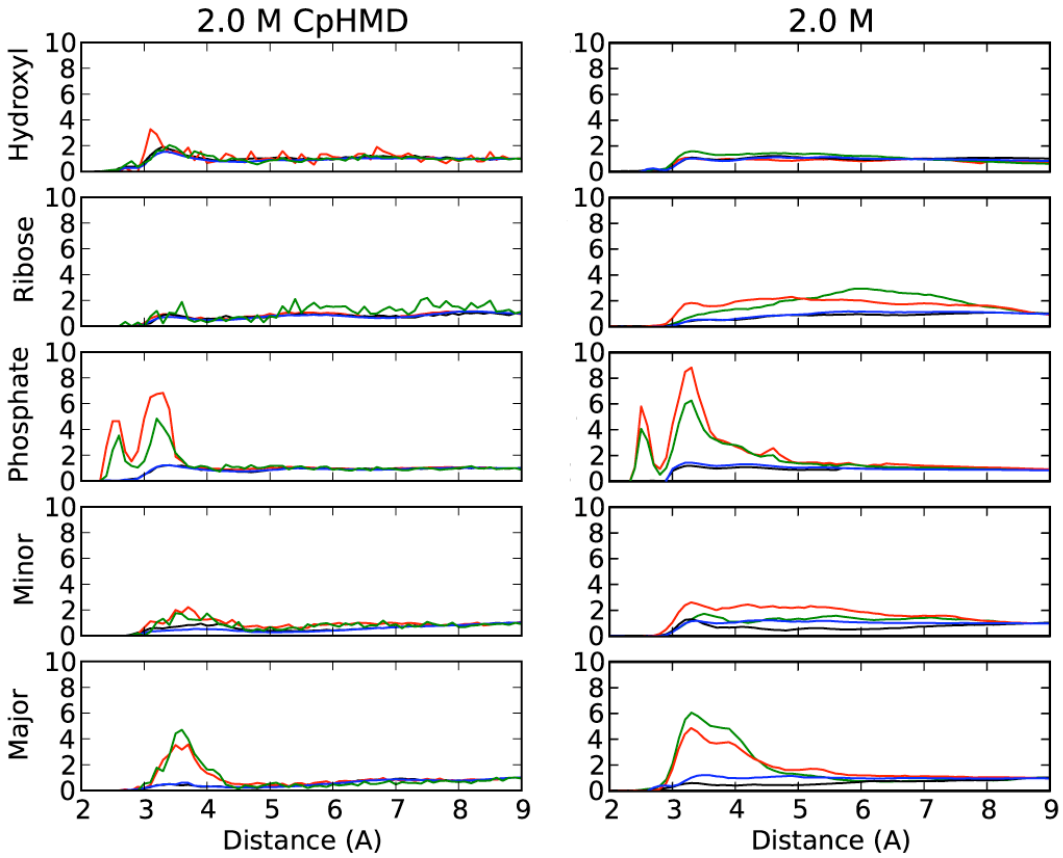


Figure S7: Comparison of the 0 TMAO, 20 mM $MgCl_2$ and 2.0 M 10% TMAOP MD simulations. Upper) RMSD versus time with the R_g probability distribution over the final 60 ns as the inset and Lower) native contact analysis versus simulation time along with the probability distribution as the inset. These results may be compared with the results presented in Figures 2 and 4 of the main manuscript.

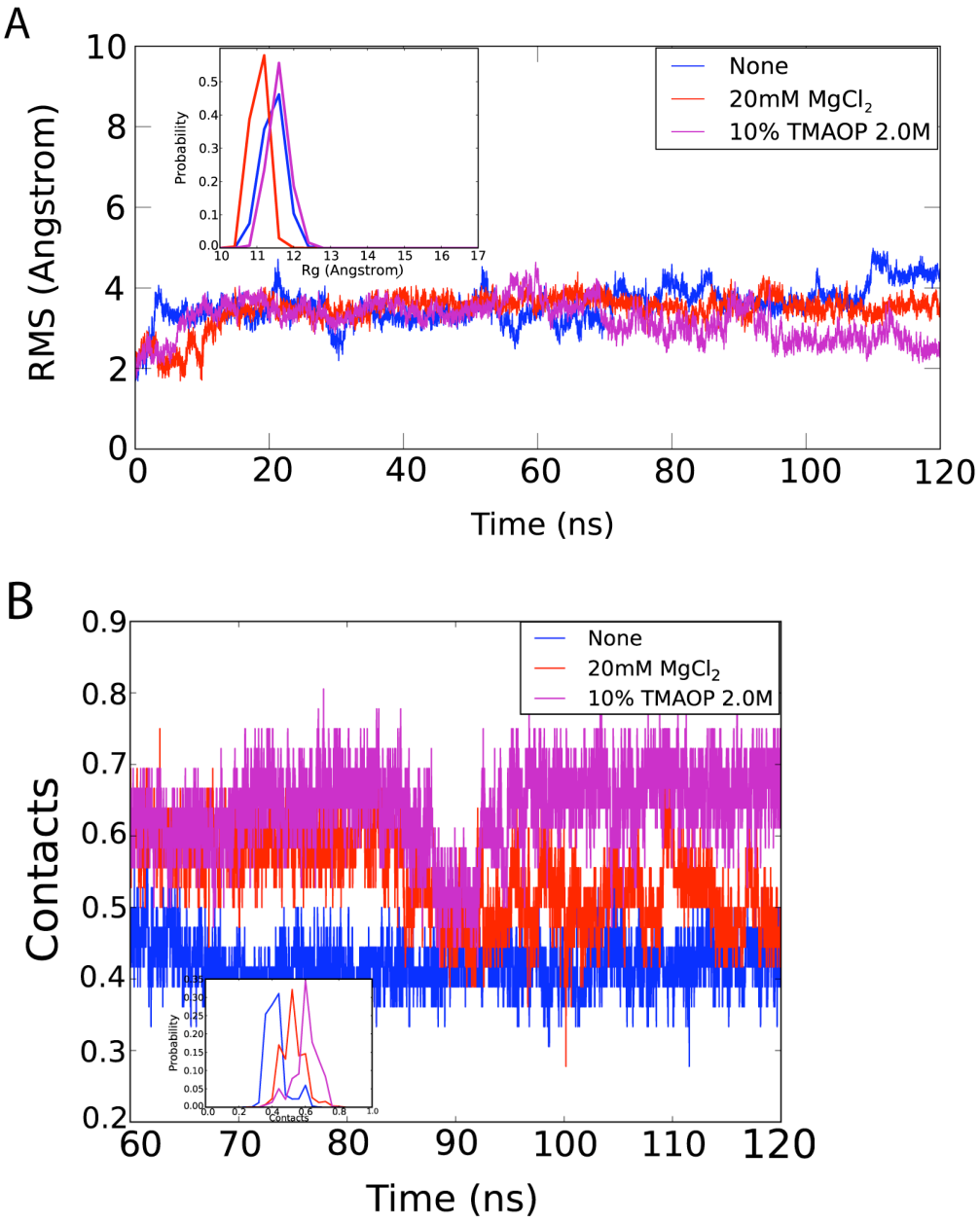


Figure S8: Radial distribution functions of Mg^{2+} or TMAOP around the non-canonical (Non-WC) and canonical (WC) regions of the RNA molecule for the 20 mM $MgCl_2$ and 2.0 M 10% TMAOP systems, respectively. All TMAOP non-hydrogen atoms were included in the analysis. Results are presented for the **(A)** 2'-hydroxyl O2', **(B)** sugar O4', **(C)** phosphate, **(D)** minor-groove side of the bases, and **(E)** major-groove side of the bases. [Red: protonated Mg^{2+}, Black: protonated TMAO]. The minor and major grooves were defined based on the atoms O2, N2, and N3 for the minor groove and N7, N6, O6, N4, and O4 for the major groove. Normalization was based on number of trajectory frames and the bulk density.