Supplemental Information for 'Effects of Trimethylamine N-Oxide (TMAO) and Crowding Agents on the Stability of RNA Hairpins.'

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Tables 3 and 4 give the distance values at which the potentials (Eqs. (1) and (2)) are less than 0.001 k_BT. The radial distribution function between all heavy atoms involving TMAO and the 5GP nucleotide are given in Figs. 10-12. The free energy profiles F(Q) as a function of Q, used to determine T_m, are given in Fig. 13.

Tables

Table 3: The Soft-Sphere Potential of Eq. (1) was force-shifted such that the potential and force go to zero at the distances indicated below.

Interaction Type	2.7 Å osmolytes r_{cut}/Å	7.0 Å osmolytes r_{cut}/Å
O{S,P,B}	8.3	16.8
OO	10.2	27.0

Table 4: The Lennard-Jones Potential of Eq. (2) was force-shifted such that the potential and force go to zero at the distances indicated below.

Interaction Type	2.7 Å osmolytes r_{cut}/Å	7.0 Å osmolytes r_{cut}/Å
OB	18.1	N/A
OO	21.4	N/A

Table 5: Using the supplementary material from the Lambert and Draper (LD) study,[1] we compute the shift in melting temperature of the 16-nt hairpin used in that study in the presence of various osmolytes (all at 1 molal concentration). The equation determining T_m in the presence of the osmolytes is $\Delta\Delta G^0 = \Delta H^0 T_m(0)(1/T_m(\Phi) - 1/T_m(0))$, where $T_m(0) = 348.3$ K and $\Delta H^0 = 55.6$ kcal/mol are the T_m and enthalpy of a transition at $\Phi = 0.0$. $\Delta T_m \equiv T_m(\Phi)$ - $T_m(0)$.

Osmolyte	$\Delta\Delta G^0$ (kcal/mol)	ΔT_m (K)
Urea	0.38	-2.4
Proline	0.57	-3.5
Betaine	0.24	-1.5
Sorbitol	0.26	-1.6
Sucrose	0.28	-1.7
Glycerol	0.18	-1.1
Ethylene Glycol	0.24	-1.5
Methanol	0.18	-1.1
TMAO	0.08	-0.5

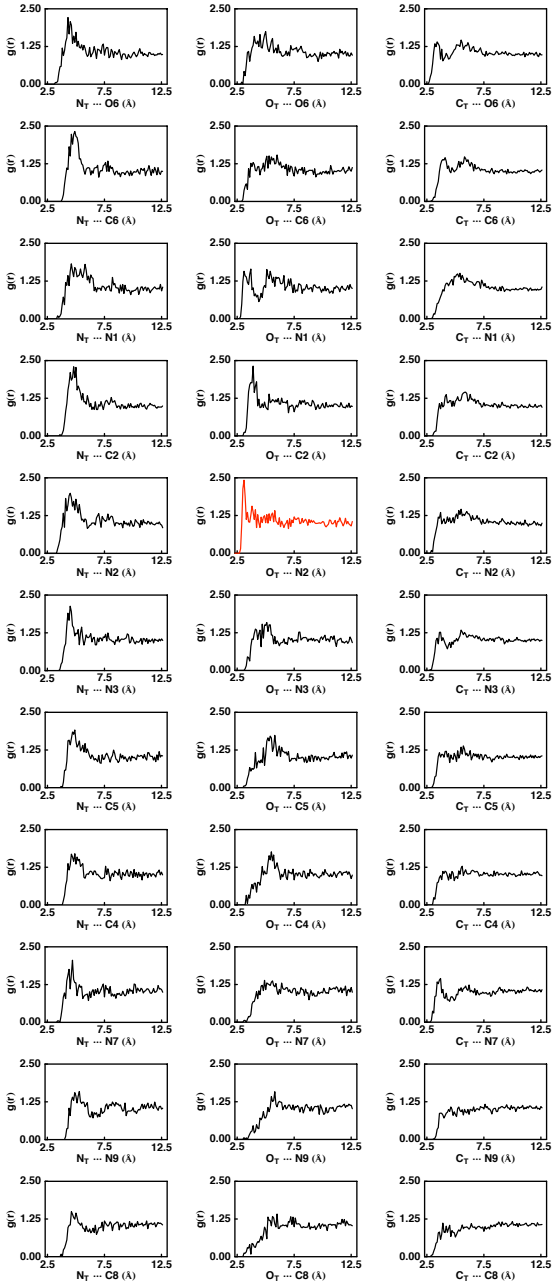

Figure 10: All possible radial distribution functions between TMAO and heavy atoms of the 5GP base. N_T, O_T, and C_T correspond to TMAO nitrogen, oxygen, and carbon atoms respectively. 5GP atoms are labelled as in figure 1**C** of the main text. A peak of 2.42 in g(r) associated with $O_T \cdots$ N2 separation occurs at r = 3.1 Å(red curve), and is consistent with hydrogen bonding between the pair.

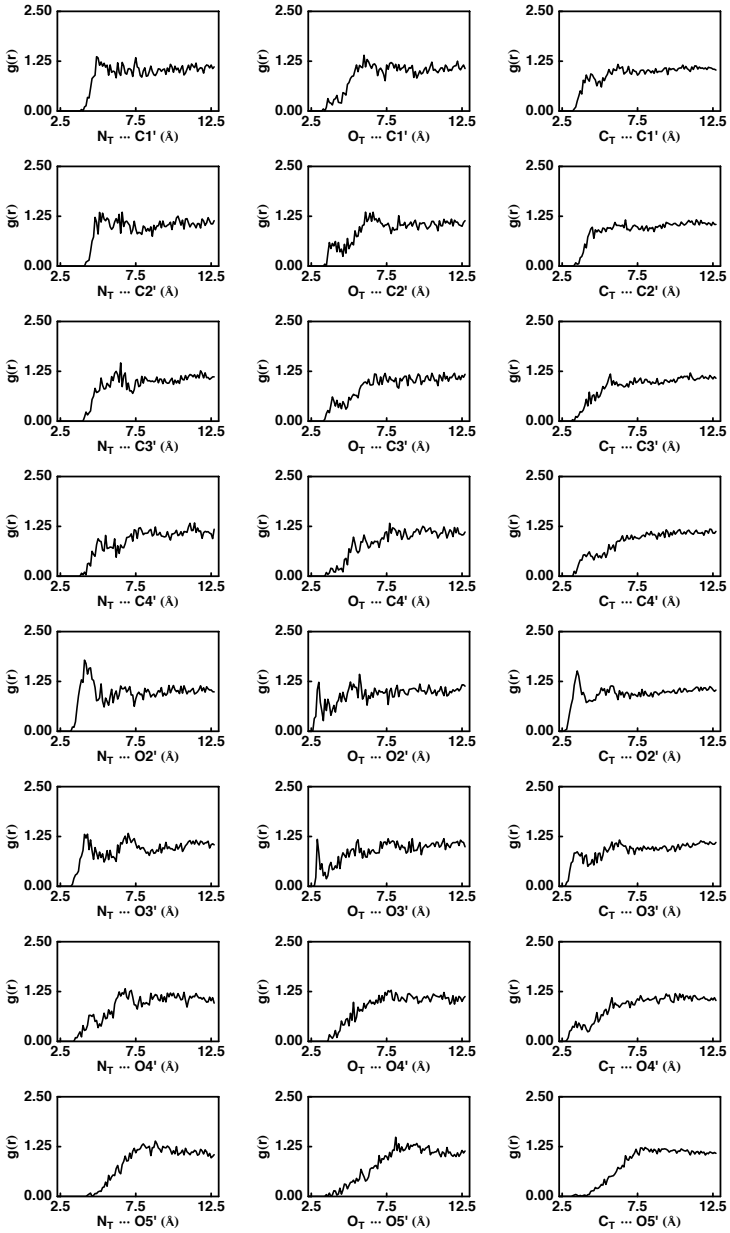

Figure 11: All possible radial distribution functions between TMAO and heavy atoms of the 5GP sugar. N_T, O_T, and C_T correspond to TMAO nitrogen, oxygen, and carbon atoms respectively. 5GP atoms are labeled according to PDB convention (e.g., see residue 5GP in PDB entry 1iyb). Although the distance of approach reveals a potential for hydrogen-bonding between O_T and the O2′ and O3′ hydroxyl groups, the excess density is very small.

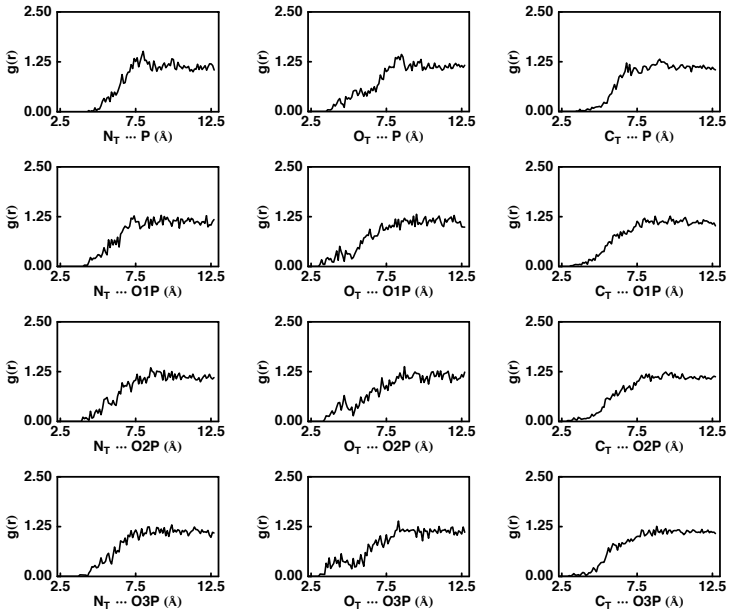

Figure 12: All possible radial distribution functions between TMAO and heavy atoms of the 5GP phosphate. N_T, O_T, and C_T correspond to TMAO nitrogen, oxygen, and carbon atoms respectively. 5GP atoms are labeled according to PDB convention (e.g., see residue 5GP in PDB entry 1iyb). TMAO is depleted from all heavy atoms of the phosphate group.

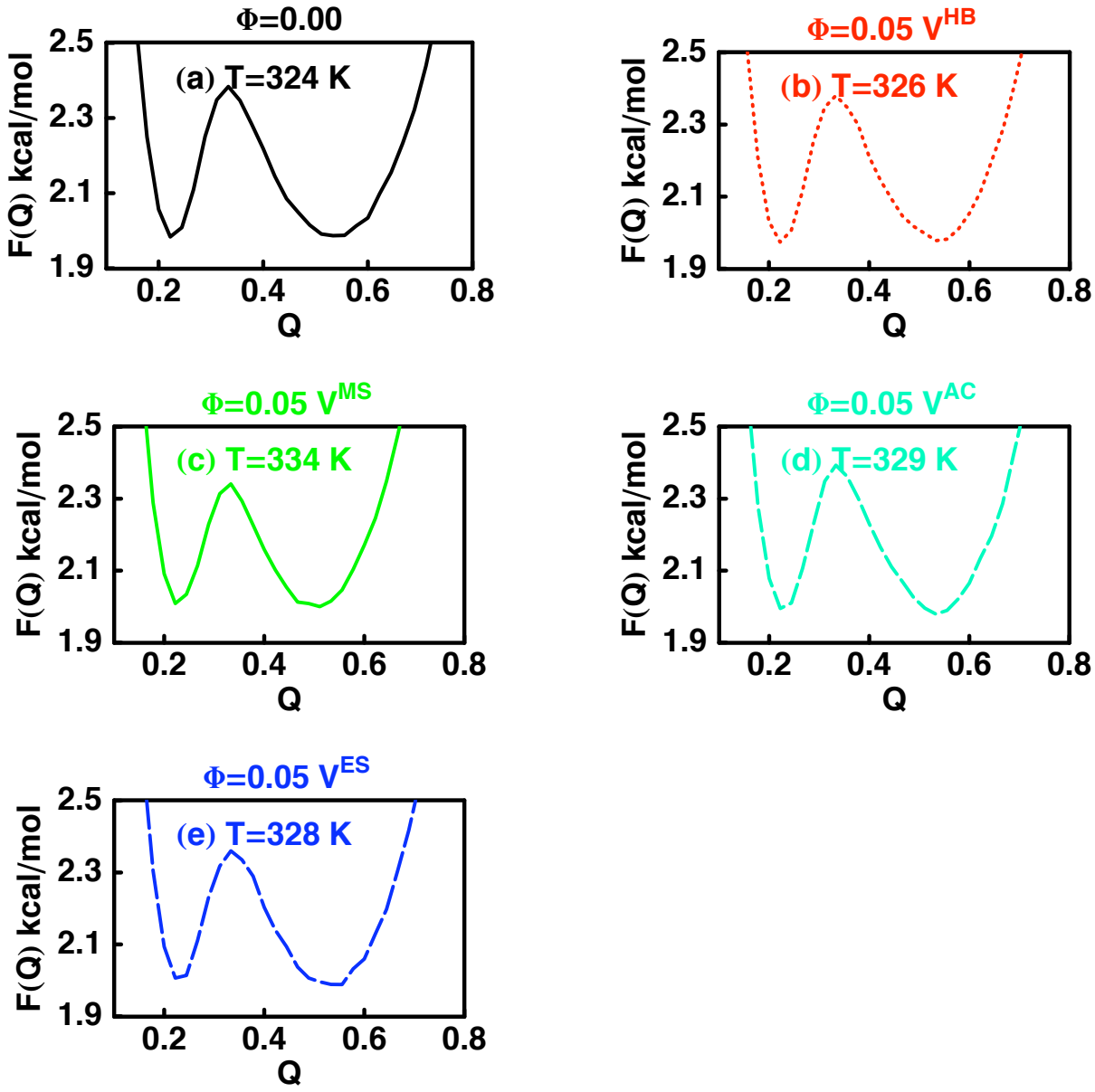

Figure 13: Melting temperatures (T_ms) for different models and conditions are calculated by determining the temperature at which free energy profiles have basins of equal depth. The order parameter used here is the fraction of native (base-base) contacts. In the absence of osmolyte, the hairpin's T_m = 324 K (solid black curve). In the entropic limit (either blue dashed curve), the hairpin's T_m increases by 4-5 K. The maximum increase in T_m (10 K) occurs for osmolytes capable of simultaneous attractive interaction with multiple base sites (green solid curve). Finally, the red dotted curve reveals that osmolyte/base hydrogen-bonding results in a minimal increase in T_m of only 2 K.

References and Notes

1. Lambert, D.; Draper, D. E. *J. Mol. Biol.* **2007,** *370,* 993-1005.