Supporting Information

Molecular Crowding Favors Reactivity of a Human Ribozyme Under Physiological Ionic Conditions

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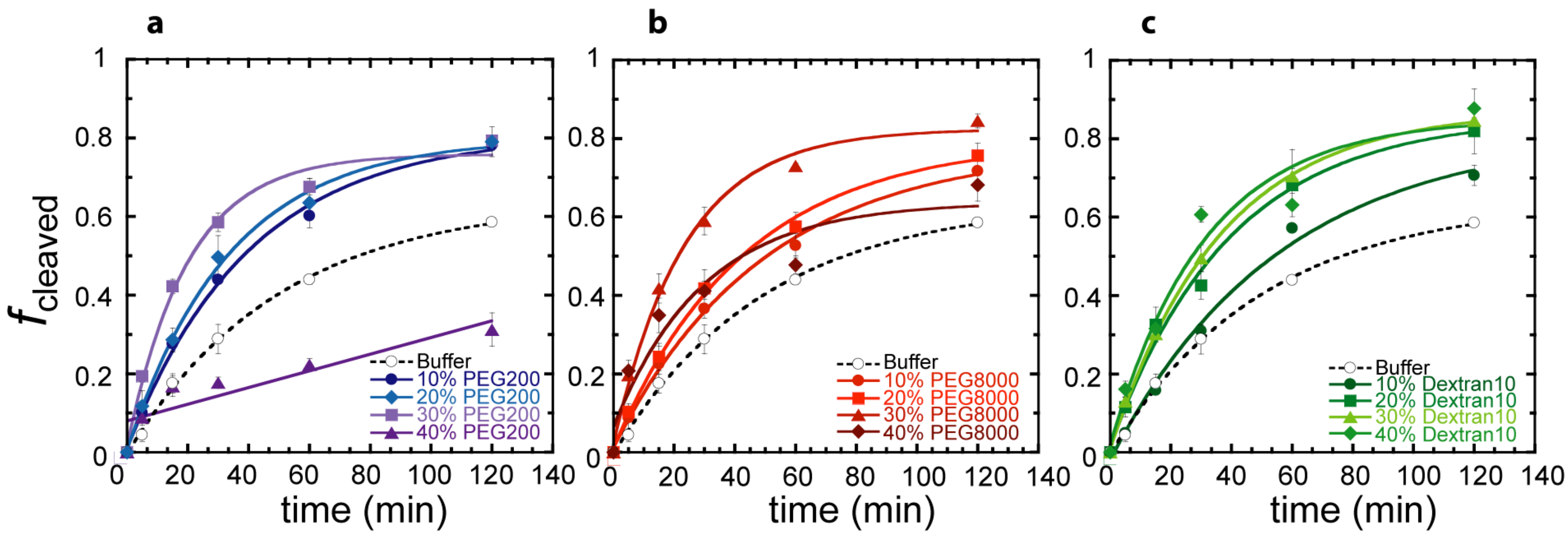
Supporting Methods

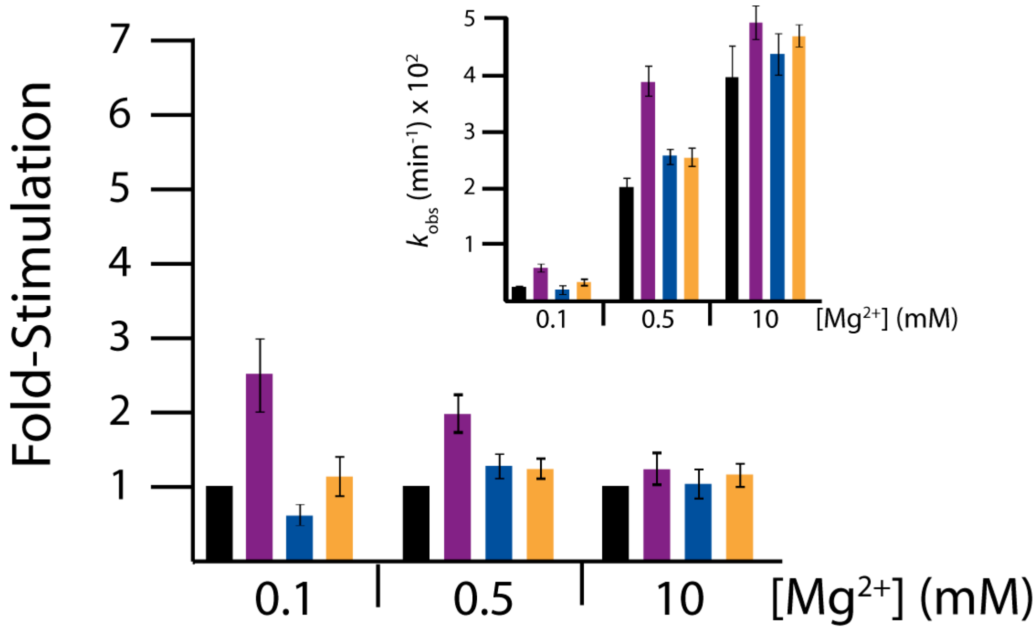
Vapor-Pressure Osmometry. Stock solutions of PEG200, PEG8000, and Dextran10 were prepared at a concentration of 30% wt./vol. A 0.1 *m* $MgCl_2$ stock solution and separate 7 *m* urea stock solution were prepared gravimetrically. A Wescor 5520 vapor-pressure osmometer was used to measure osmolalities at ambient temperature (~23 °C). Before each use of the osmometer, the thermocouple head was cleaned with ammonium hydroxide and then rinsed with DI water. Osmolality standards (Wescor) 100 mOsm, 290 mOsm, and 1000 mOsm were used to calibrate the instrument before measuring samples. The instrument was re-calibrated throughout the procedure when necessary. Data points were measured at least three times on independent days with different samples. Experiments with PEG200 and urea were not reported as the vapor-pressure readings were out of the range of linearity for the instrument. The mOsm reading was converted to activity of water, a_W, using the relationship mOsm= $-(10^6 \ln a_W)/M_1$, where M_1 is the molecular weight of water.[1]

Native Gel Electrophoresis. To probe for the formation of RNA aggregates at the concentrations of RNA used during the SAXS experiments, PAGE was performed under native conditions with different amounts of RNA. Cleaved 1/68 RNA (prepared as described above for SAXS experiments) was doped with cleaved 1/68 RNA [γ-^{32}P] 5'-end labeled RNA (prepared as described above for kinetics experiments). (A small amount of uncleaved –8/68 was left in the sample to serve as a marker.) Unlabeled RNA was present at 0.2, 0.4, or 0.6 mg/mL, while labeled RNA was present only at trace amounts for both 0.5 and 10 mM Mg^{2+}. RNA was renatured at 55 °C for 3 min and allowed to cool to room temperature for 10 min for both unlabeled and labeled RNA. Each sample contained 15% glycerol to help sink samples on the gel. For RNA samples in 0.5 mM Mg^{2+}, RNA was loaded on a 10% native gel containing 25 mM HEPES (pH 8)/100 mM KCl/0.5 mM $MgCl_2$ that was prerun 4 W for ~30 min at ~10 °C in 25 mM HEPES (pH 8)/100 mM KCl/0.5 mM $MgCl_2$ running buffer. For RNA samples in 10 mM Mg^{2+}, RNA was loaded on a 10% native gel containing 25 mM HEPES (pH 8)/100 mM KCl/10 mM $MgCl_2$ that was prerun 4 W for ~30 min at ~10 °C in 25 mM HEPES (pH 8)/100 mM KCl/10 mM $MgCl_2$ running buffer. The gel was run for 8.5 h at ~10 °C. Every 30 min, 100 mL of buffer was cycled from the lower well to the top well. The gel was dried and visualized using a PhosphorImager.

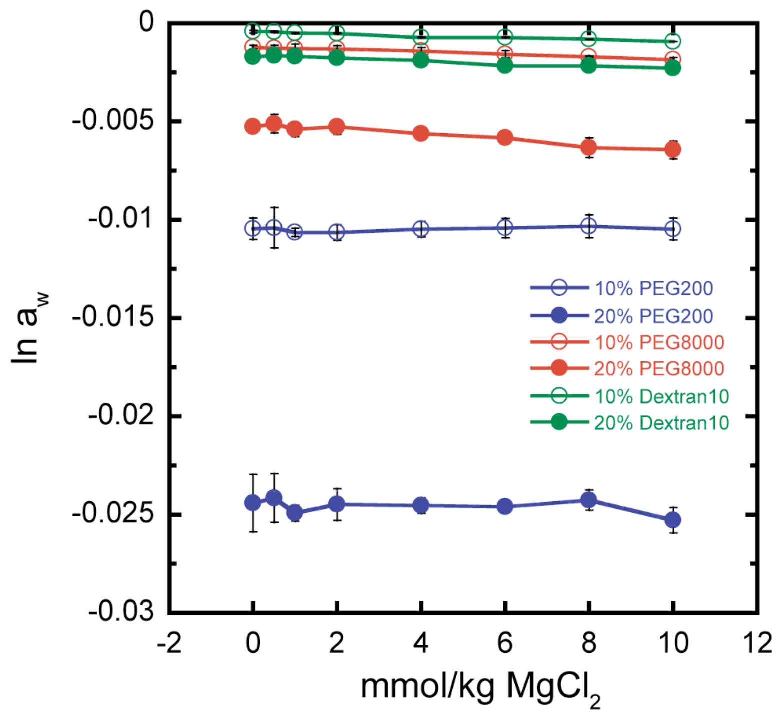
2) Supporting Figures



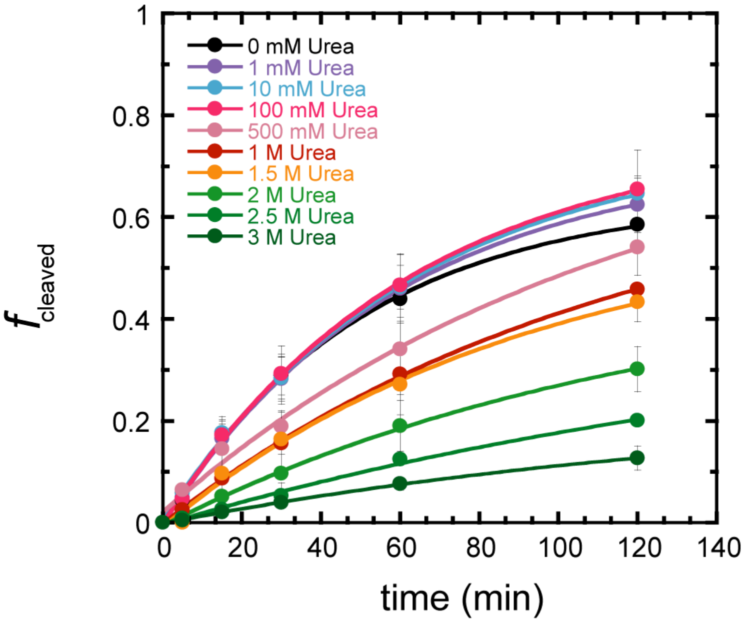
Supporting Figure S1. Concentration effects of crowding and cosolute agents on self-cleavage of *CPEB3* ribozyme in 0.5 mM Mg^{2+}. *CPEB3* ribozyme kinetics in 0.5 mM Mg^{2+} with **(a)** PEG200, **(b)** PEG8000, and **(c)** Dextran10 at various concentrations (from 0% to 40% w/v). Increase in catalytic rate was observed with increasing amount of cosolute to 30% for PEG200 and PEG8000; inhibition was found at 40%, especially for PEG200. The rate in the presence of Dextran10 increased with all amounts of the additive. Optimal values of crowding and cosolute agents were selected for Mg^{2+}-dependence for urea experiments, which were based upon largest enhancement in rate and biological relevance.



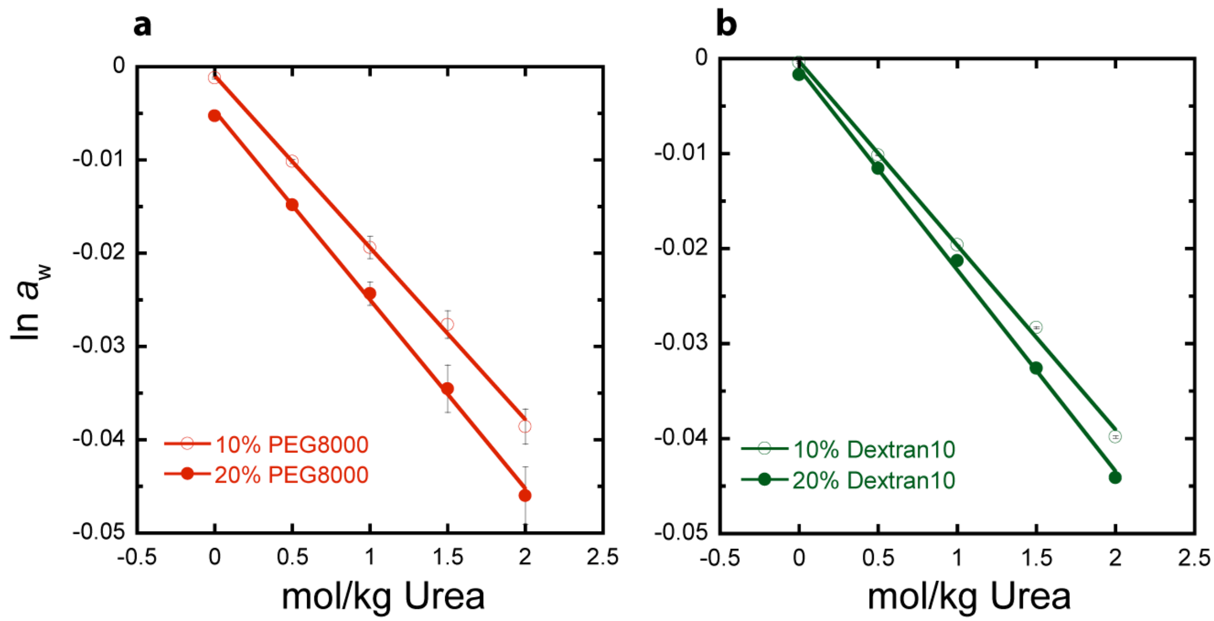
Supporting Figure S2. WT *CPEB3* ribozyme kinetics in the presence of larger molecular weight crowding agents in various magnesium concentrations. Main plot displays relative rate enhancement reported as "fold-stimulation" by comparing rate of condition with additive relative to rate of condition with buffer only (black) for each magnesium concentration, where Dextran40 (purple), Dextran70 (light blue), and Ficoll70 (orange) were present at a concentration of 30% w/v. Inset: Raw self-cleavage rates of the WT *CPEB3* ribozyme for the above-described conditions.



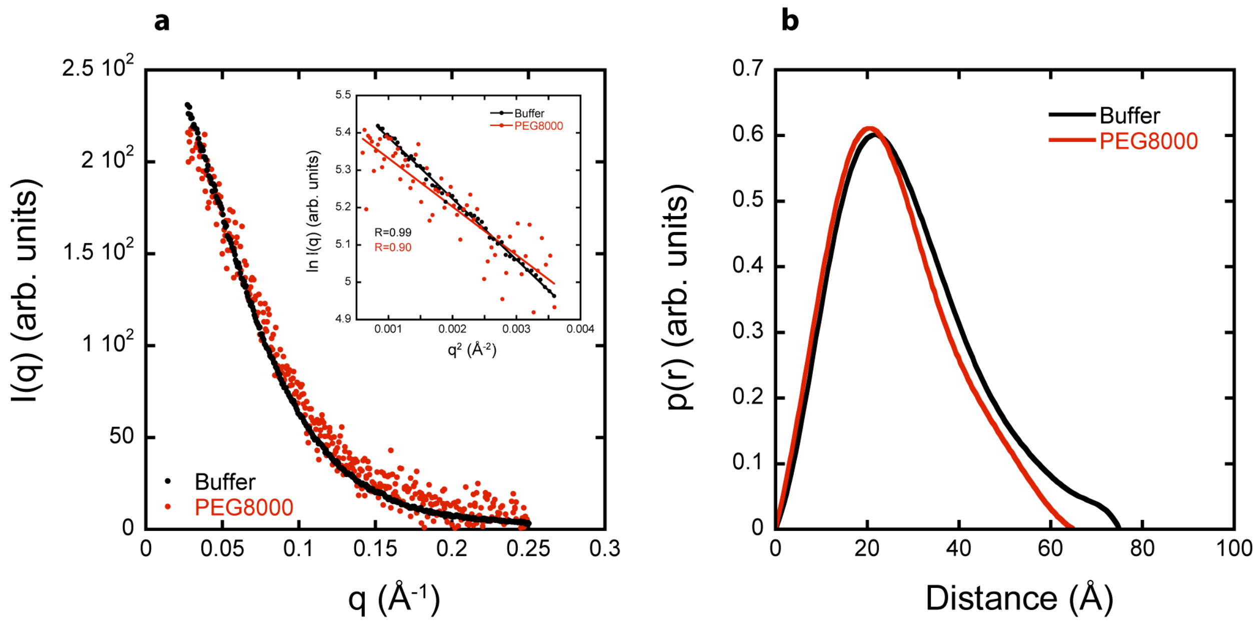
Supporting Figure S3. Water activity in additive/magnesium solutions measured by vapor-pressure osmometry. Water activity was determined in the background of 10% and 20% w/v PEG200, PEG8000, and Dextran10 with 0-10 mmol/kg Mg^{2+} present. The equal distance between the lines for each additive indicates that the additive and magnesium impact the activity of water independently.



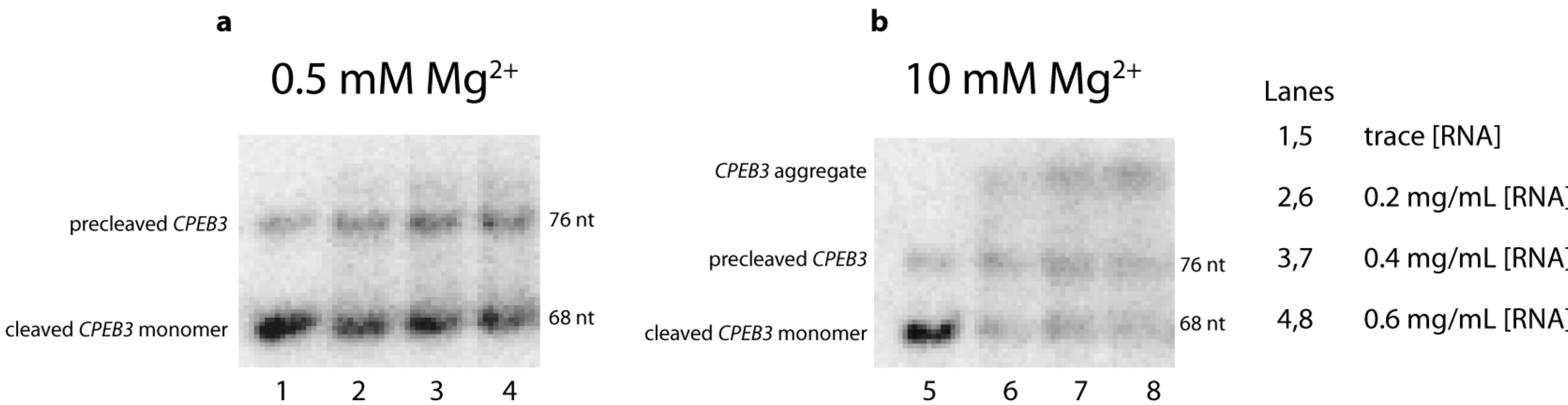
Supporting Figure S4. Observed cleavage rate for *CPEB3* ribozyme in 0.5 mM Mg^{2+} and various concentrations of urea. All reactions contained 0.5 mM $MgCl_2$, and were carried out at 37 °C. Reactions were initiated by addition of Mg^{2+}. Reaction rate is slowed as the concentration of urea is increased.



Supporting Figure S5. Water activity in additive/urea solutions measured by vapor-pressure osmometry. Water activity was determined in the background of 10% and 20% w/v, PEG8000, and Dextran10 with 0-2 mol/kg urea present. There is a slight deviation between the slopes of the lines indicating that the additives and urea are not entirely impacting the activity of water independently. However, the extent of this effect is minimal and does not account for the rescue of activity by additives in high concentrations of urea.



Supporting Figure S6. SAXS scattering profiles and p(r) plots in 10 mM Mg^{2+}. For both panels, plots are for RNA in buffer (black) and buffer with 20% PEG8000 (red). **(a)** Scattering profile. RNA scattering profile in the presence of PEG8000 is normalized to RNA scattering profiles in buffer alone to account for differences in contrast. R_g was determined from data in the Guinier regime (qR_g<1.3) (inset), and R_g values are provided in Table 1. Linear behavior extending to low q supports absence of aggregation. **(b)** p(r) plots. For PEG8000, the distribution is narrower and the peak is at smaller distance which is reflected in a smaller R_g. Data in 10 mM Mg^{2+} were subjected to SEC immediately prior to collecting SAXS data, which was performed at the SIBYLS beamline.



Supporting Figure S7. Native gel analysis of cleaved 1/68 *CPEB3* ribozyme in conditions used for SAXS. Native gels were performed in both Mg^{2+} concentrations used in SAXS, 0.5 and 10 mM Mg^{2+}, to look for aggregation of SAXS samples. These data were used to assist in the interpretation of the SAXS envelopes. A small amount of precleaved -8/68 ribozyme was included as a marker (see Materials and Methods). **(a)** In 0.5 mM Mg^{2+}, virtually no aggregation of RNA was observed with increasing concentration of RNA; however, **(b)** in 10 mM Mg^{2+}, aggregation of RNA was observed at all concentrations used in SAXS experiments. The percent aggregation, defined as the upper band divided by the sum of the upper and lower bands, was 4%, 45%, 52%, and 54%, for lanes 5-8, respectively. 'Trace' RNA refers to ~nM concentrations and shows no evidence of aggregation at either 0.5 or 10 mM Mg^{2+}.

3) Supporting Table

Supporting Table S1. Observed rates of *CPEB3* WT and C-2A ribozymes with crowding and cosolute agents in 0.5 and 10 mM Mg^{2+} and urea.

WT Condition	k_{obs} (min^{-1})	C-2A Condition	k_{obs} (min^{-1})
	0 M Urea		**0 M Urea**
0.5 mM Mg^{2+}	0.019 ± 0.002	0.5 mM Mg^{2+}	0.061 ± 0.004
0.5 mM Mg^{2+} & 30% PEG200	0.038 ± 0.003	0.5 mM Mg^{2+} & 20% PEG200	0.120 ± 0.007
0.5 mM Mg^{2+} & 30% PEG8000	0.043 ± 0.005	0.5 mM Mg^{2+} & 40% PEG8000	0.167 ± 0.012
0.5 mM Mg^{2+} & 30% Dextran10	0.028 ± 0.001	0.5 mM Mg^{2+} & 40% Dextran10	0.167 ± 0.010
10 mM Mg^{2+}	0.040 ± 0.006	10 mM Mg^{2+}	0.256 ± 0.016
10 mM Mg^{2+} & 30% PEG200	0.036 ± 0.006	10 mM Mg^{2+} & 20% PEG200	0.271 ± 0.031
10 mM Mg^{2+} & 30% PEG8000	0.044 ± 0.008	10 mM Mg^{2+} & 40% PEG8000	0.217 ± 0.013
10 mM Mg^{2+} & 30% Dextran10	0.036 ± 0.007	10 mM Mg^{2+} & 40% Dextran10	0.356 ± 0.013
	0.5 M Urea		**0.5 M Urea**
0.5 mM Mg^{2+}	0.009 ± 0.003	0.5 mM Mg^{2+}	0.034 ± 0.001
0.5 mM Mg^{2+} & 30% PEG200	0.039 ± 0.003	0.5 mM Mg^{2+} & 20% PEG200	0.132 ± 0.024
0.5 mM Mg^{2+} & 30% PEG8000	0.029 ± 0.001	0.5 mM Mg^{2+} & 40% PEG8000	0.139 ± 0.006
0.5 mM Mg^{2+} & 30% Dextran10	0.018 ± 0.004	0.5 mM Mg^{2+} & 40% Dextran10	0.112 ± 0.006
10 mM Mg^{2+}	0.028 ± 0.004	10 mM Mg^{2+}	0.459 ± 0.109
10 mM Mg^{2+} & 30% PEG200	0.052 ± 0.009	10 mM Mg^{2+} & 20% PEG200	0.309 ± 0.014
10 mM Mg^{2+} & 30% PEG8000	0.038 ± 0.002	10 mM Mg^{2+} & 40% PEG8000	0.378 ± 0.040
10 mM Mg^{2+} & 30% Dextran10	0.044 ± 0.006	10 mM Mg^{2+} & 40% Dextran10	0.439 ± 0.038
	2.5 M Urea		**3 M Urea**
0.5 mM Mg^{2+}	0.0017 ± 0.0005	0.5 mM Mg^{2+}	0.007 ± 0.002
0.5 mM Mg^{2+} & 30% PEG200	0.018 ± 0.001	0.5 mM Mg^{2+} & 20% PEG200	0.015 ± 0.001
0.5 mM Mg^{2+} & 30% PEG8000	0.020 ± 0.003	0.5 mM Mg^{2+} & 40% PEG8000	0.051 ± 0.005
0.5 mM Mg^{2+} & 30% Dextran10	0.018 ± 0.002	0.5 mM Mg^{2+} & 40% Dextran10	0.029 ± 0.001
10 mM Mg^{2+}	0.028 ± 0.002	10 mM Mg^{2+}	0.131 ± 0.008
10 mM Mg^{2+} & 30% PEG200	0.041 ± 0.008	10 mM Mg^{2+} & 20% PEG200	0.162 ± 0.015
10 mM Mg^{2+} & 30% PEG8000	0.052 ± 0.006	10 mM Mg^{2+} & 40% PEG8000	0.297 ± 0.025
10 mM Mg^{2+} & 30% Dextran10	0.036 ± 0.007	10 mM Mg^{2+} & 40% Dextran10	0.191 ± 0.007

Supporting Reference

(1) Kilburn, D., Roh, J. H., Guo, L., Briber, R. M., and Woodson, S. A. (2010) Molecular crowding stabilizes folded RNA structure by the excluded volume effect. *J. Am. Chem. Soc. 132*, 8690-8696.