Supporting Information

Crowders perturb the entropy of RNA energy landscapes to favor folding

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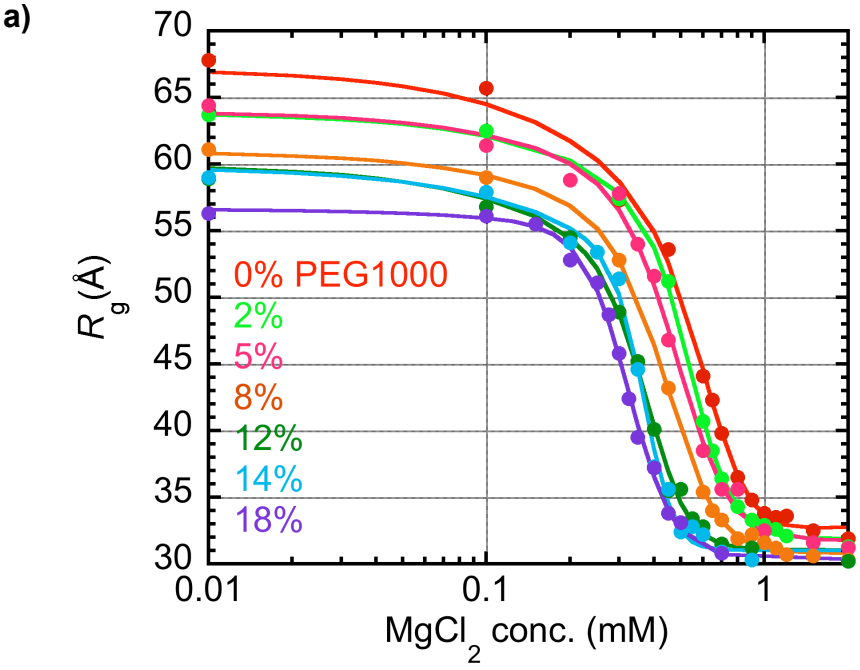
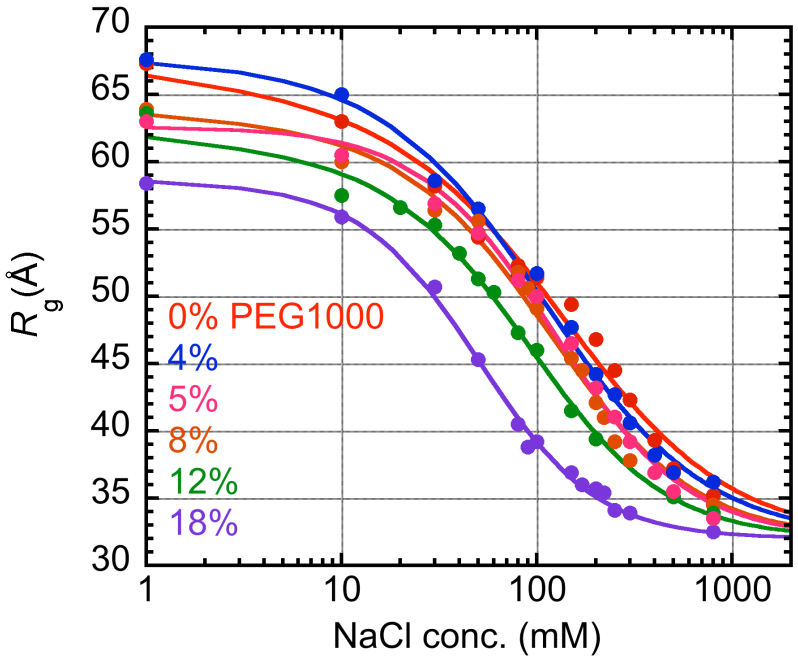
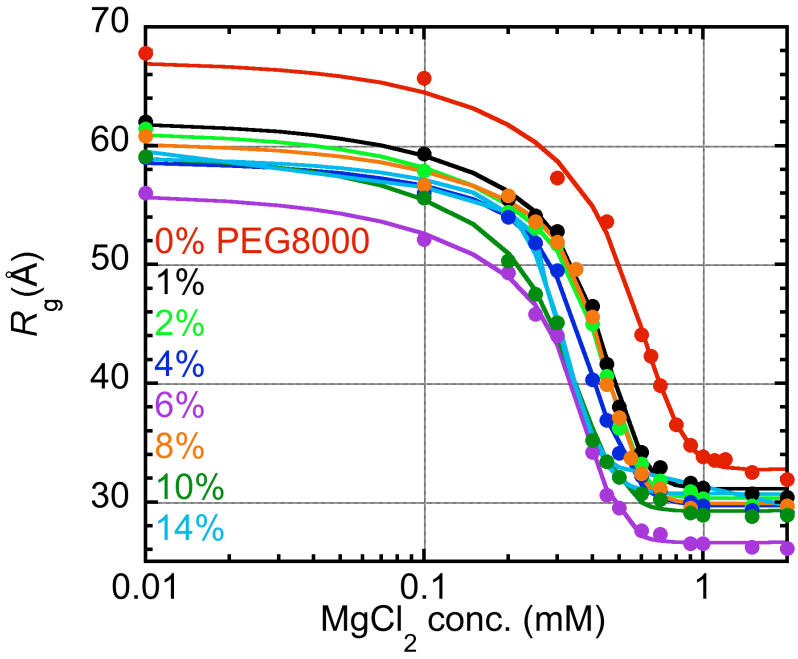
FIGURE S1



b)


70
65
60
55
50
45
40
35
30
R_g (Å)
0% PEG1000
4%
5%
8%
12%
18%
1
10
100
1000
NaCl conc. (mM)

c)


70
60
50
40
30
R_g (Å)
0% PEG8000
1%
2%
4%
6%
8%
10%
14%
0.01
0.1
1
$MgCl_2$ conc. (mM)

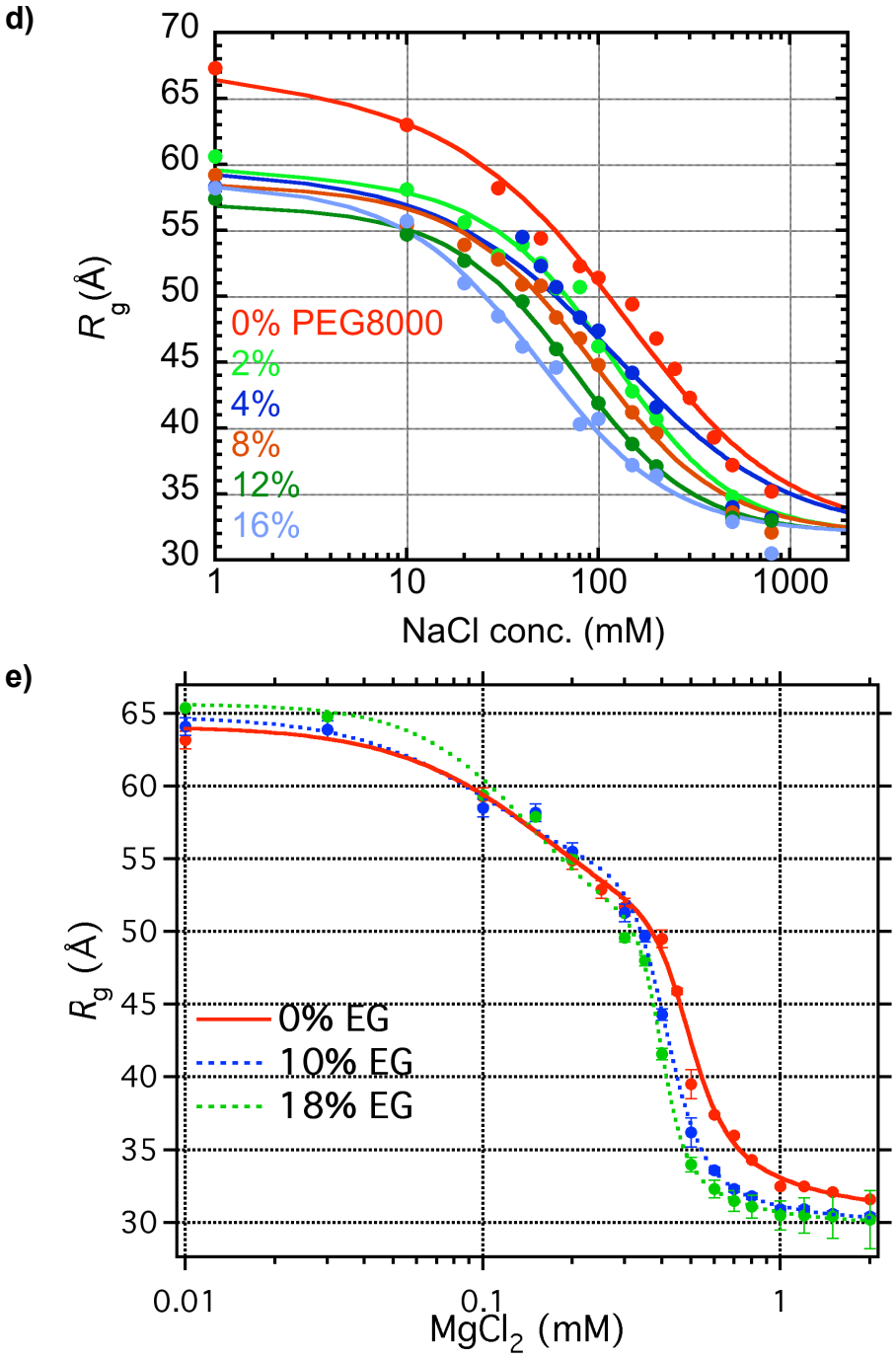
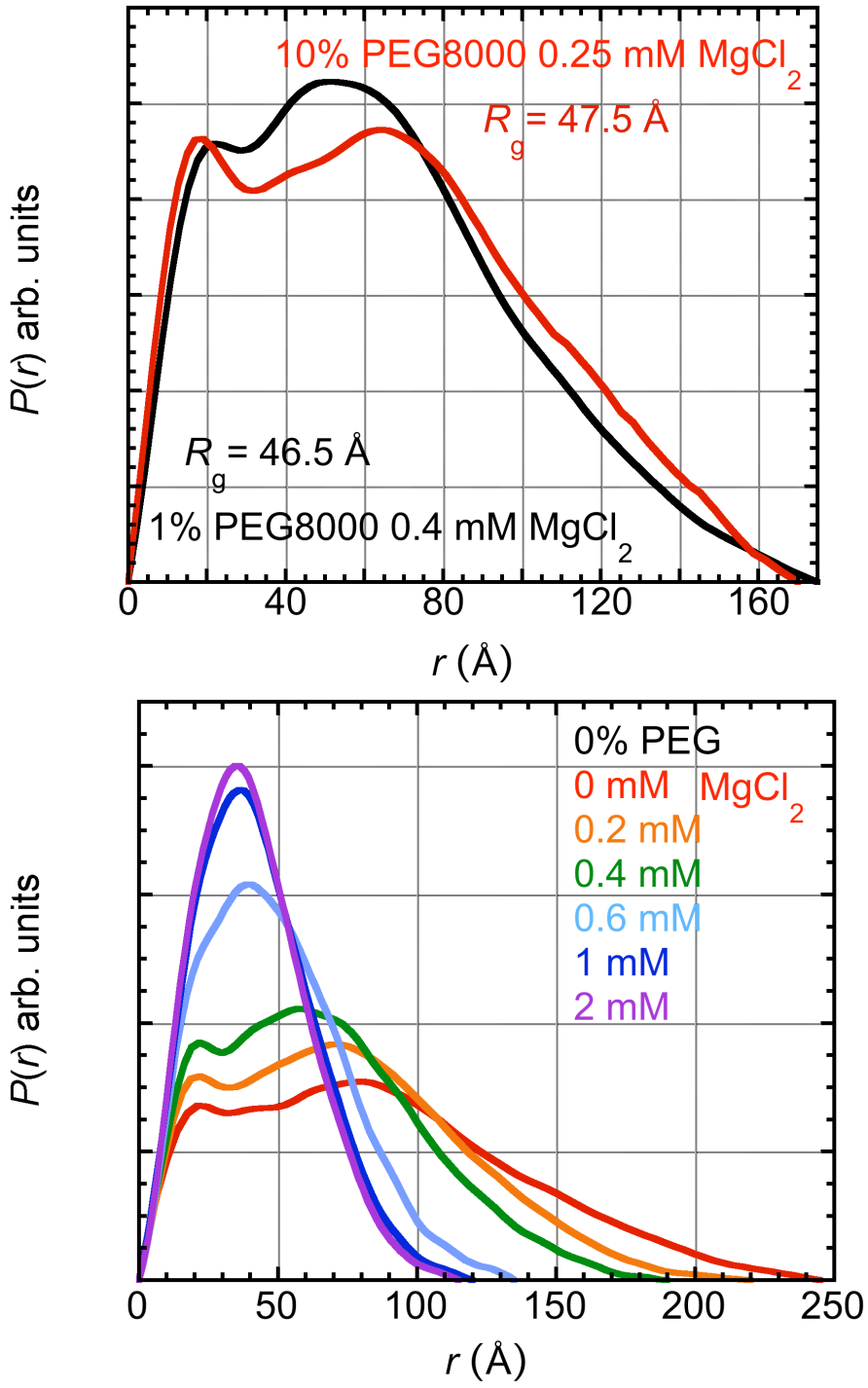


Figure S1. Salt titration curves of R_g values at a range of PEG concentrations. (a) $MgCl_2$ is the co-solute being titrated in solutions with PEG1000. Solid lines are fits to an adjusted Hill equation (Eq 1) Parameters of the fits are given in Table S1. (b) NaCl is the co-solute being titrated in solutions with PEG1000. Solid lines are fits to Eq 2. Parameters of the fits are given in Table S2. (c) $MgCl_2$ is the co-solute being titrated in solutions with PEG8000. Solid lines are fits Eq 1. Parameters of the fits are given in Table S1. (d) NaCl is the co-solute being titrated in solutions with PEG8000. Solid lines are fits Eq 2. Parameters of the fits are given in Table S2. e) $MgCl_2$ is the co-solute being titrated in solutions with ethylene glycol (EG). These measurements

in EG were made from a different stock of RNA and we believe that the differences between R_gs in this titration (unfolded RNA in 0% EG, R_g = 65.1 Å; 0% PEG from Fig S1a) R_g = 67.8) are due to these differences.

FIGURE S2



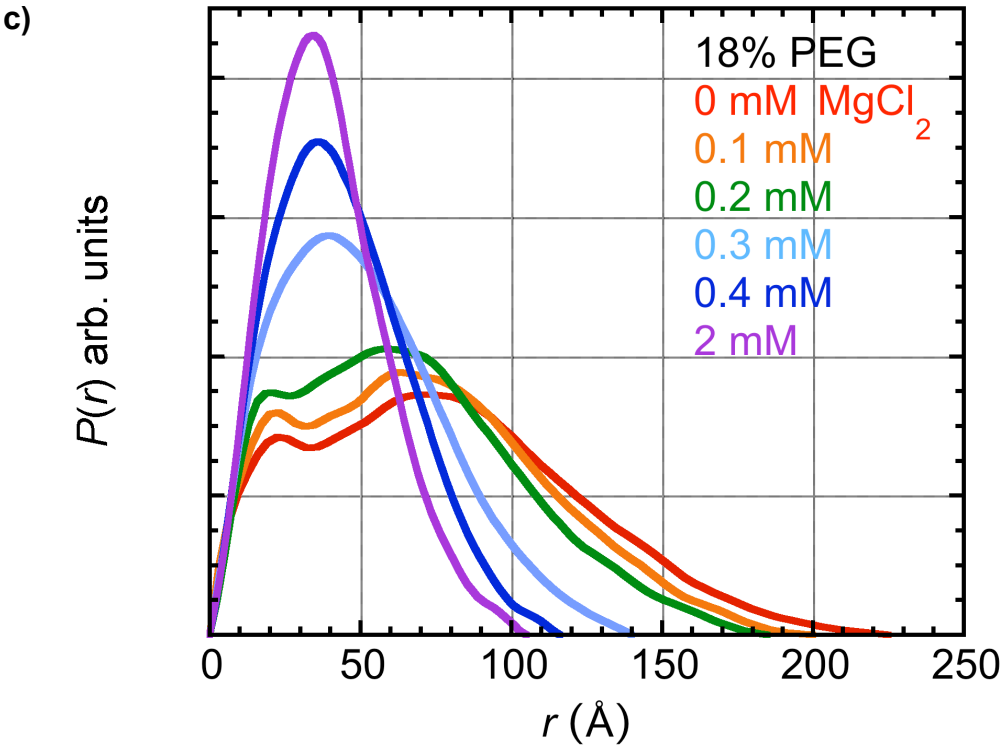
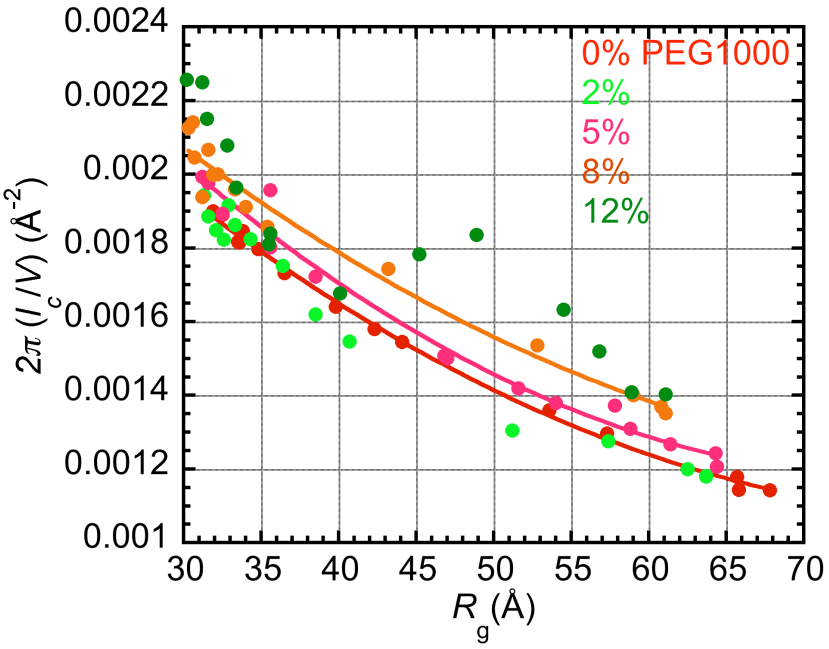


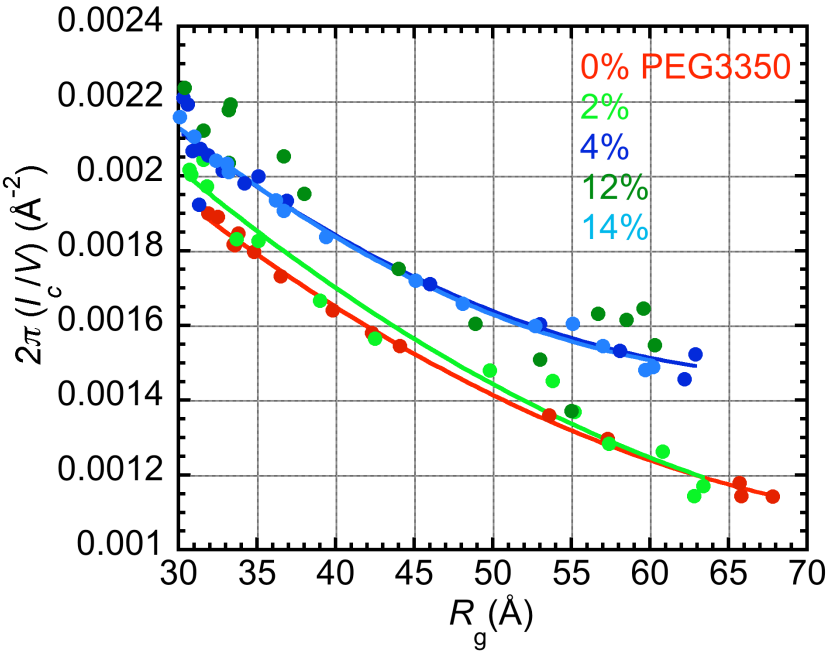
Figure S2. The RNA distance distribution functions calculated using GNOM (Svergun, D. *J.Appl.Cryst*. **1992**, *25*, 495). a) Calculated from the scattering functions in Figure 4a). b) and c) are the distance distribution functions for a selected number of scattering functions in the 0% and 18% PEG1000 titrations.

FIGURE S3

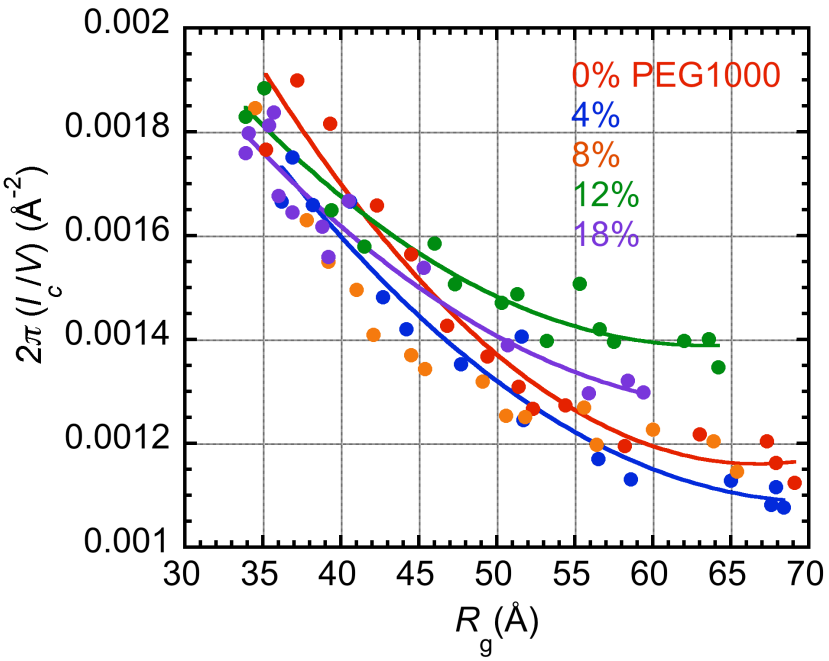
a)


0% PEG1000
2%
5%
8%
12%
$2\pi (I_c/V)$ (Å^{-2})
R_g (Å)
0.0024
0.0022
0.002
0.0018
0.0016
0.0014
0.0012
0.001
30 35 40 45 50 55 60 65 70

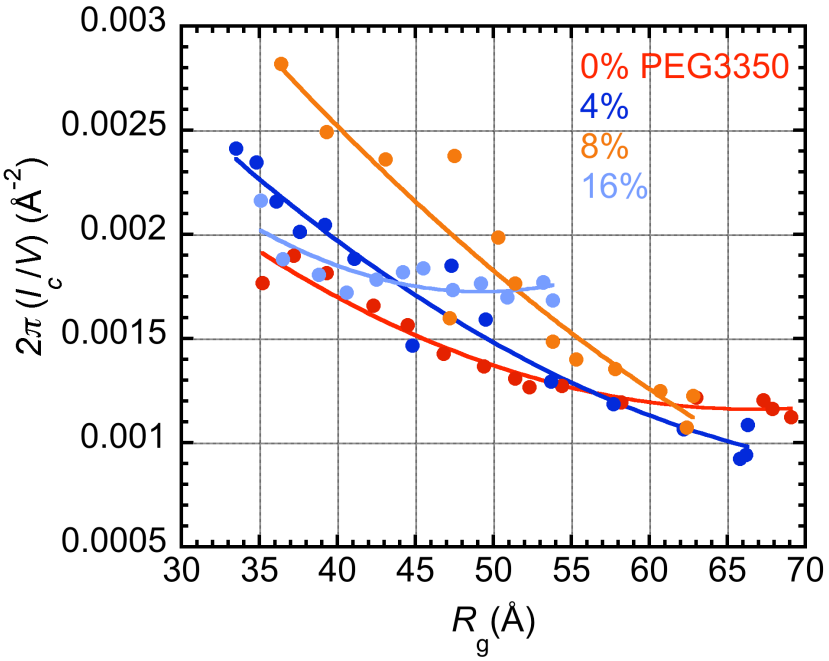
b)


0% PEG3350
2%
4%
12%
14%
$2\pi (I_c/V)$ (Å^{-2})
R_g (Å)
0.0024
0.0022
0.002
0.0018
0.0016
0.0014
0.0012
0.001
30 35 40 45 50 55 60 65 70

c)


0% PEG1000
4%
8%
12%
18%
0.002
0.0018
0.0016
0.0014
0.0012
0.001
30 35 40 45 50 55 60 65 70
$2\pi (I_c/V)$ (Å^{-2})
R_g (Å)

d)


0% PEG3350
4%
8%
16%
0.003
0.0025
0.002
0.0015
0.001
0.0005
30 35 40 45 50 55 60 65 70
$2\pi (I_c/V)$ (Å^{-2})
R_g (Å)

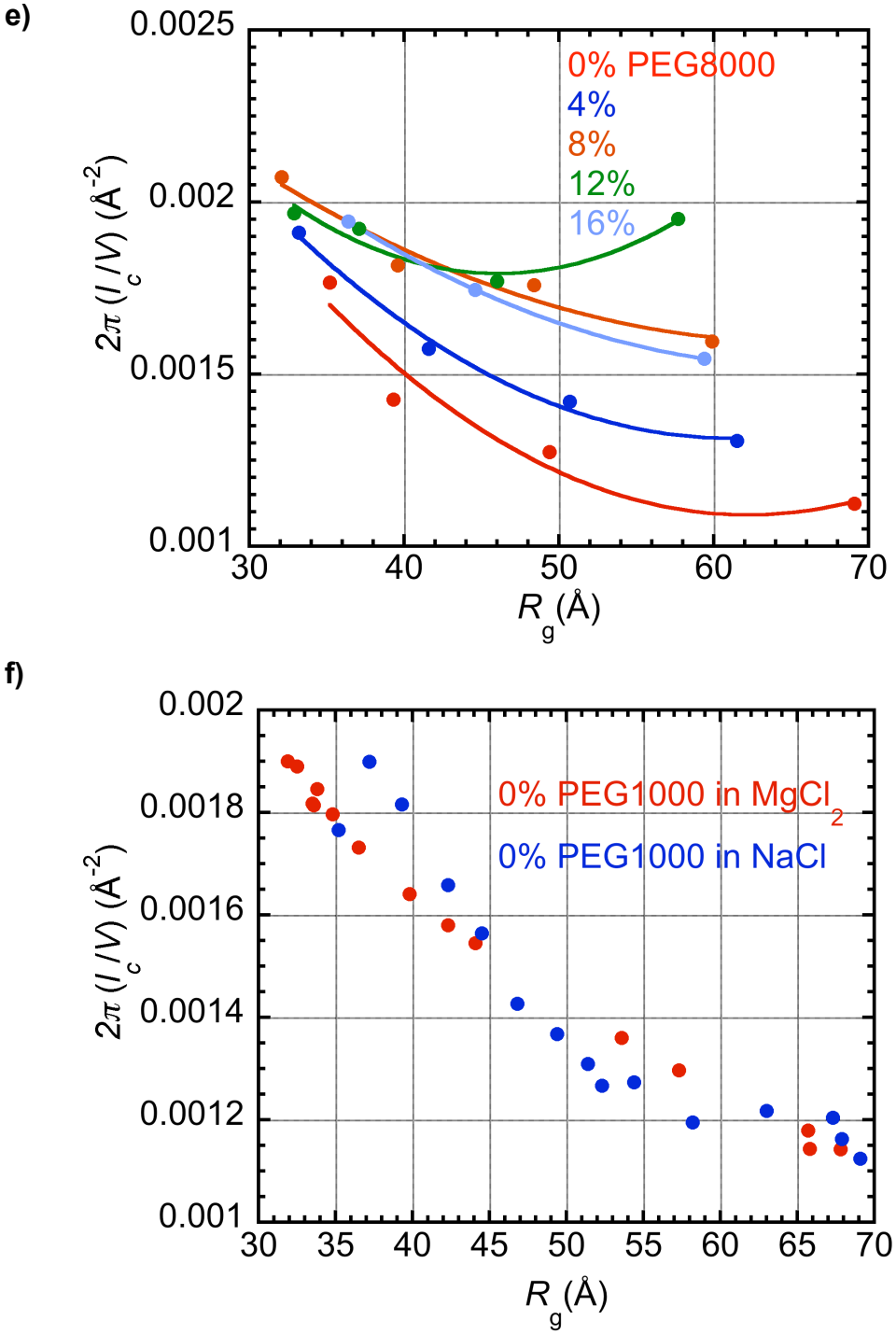


Figure S3. The correlation length per scattering volume, l_c/V, of *Azoarcus* ribozyme. Plotted as a function of R_g for RNA particles stabilized in solutions containing (a) PEG1000 as a macromolecular crowder and $MgCl_2$ as the stabilizing co-solute. (b) PEG3350 as a macromolecular crowder and $MgCl_2$ as the stabilizing co-solute. (c) PEG1000 as a macromolecular crowder and NaCl as the stabilizing co-solute. (d) PEG3350 as a macromolecular crowder and NaCl as the stabilizing co-solute. (e) PEG8000 as a

macromolecular crowder and NaCl as the stabilizing co-solute. (f) No crowders present, comparing data with $MgCl_2$ and NaCl as the folding co-ion. The data from some PEG concentrations were omitted due to excess scatter.

Table S1. Fitting parameters for Hill equation (Eqn 1) for titrations of R_g with $MgCl_2$. Plots are shown in figures s1a) PEG1000, 5a) PEG3350, and s1c) PEG8000.

PEG M_W	**wt./vol. (%)**	C_m (mM)	±	Hill coeff, n	±	k, dR_g/dC	±
1000	0	0.65	0.05	5.0	1.0	27	4
	2	0.54	0.01	5.6	0.4	18	2
	5	0.49	0.03	4.5	0.4	18	5
	8	0.44	0.02	4.6	0.3	18	3
	12	0.38	0.02	5.6	0.8	25	6
	14	0.37	0.01	8.4	1.4	22	5
	18	0.31	0.01	5.5	0.4	7	5
3350	2	0.51	0.04	4.2	0.5	26	5
	4	0.55	0.04	4.6	0.2	27	1
	8	0.43	0.02	4.8	0.3	13	5
	12	0.40	0.01	6.9	0.7	16	4
	14	0.36	0.02	4.7	0.5	18	6
	18	0.26	0.01	4.9	0.2	10	4
8000	1	0.47	0.02	6.2	0.8	29	3
	2	0.46	0.02	6.6	1.0	31	4
	4	0.39	0.02	5.4	0.5	21	4
	6	0.39	0.02	6.5	0.9	34	4
	8	0.45	0.01	7.1	0.8	25	3
	10	0.37	0.03	4.9	0.7	38	7
	14	0.32	0.01	6.3	0.8	19	8

Table S2. Fitting parameters for Hill equation (Eqn 1) for titrations of R_g with NaCl. Plots are shown in figures s1b) PEG1000 and 5b) PEG3350.

PEG M_W	**wt./vol. (%)**	C_m (mM)	±	Hill coeff, n	±	$k = c\ln(10)dR_g/dC$	±
1000	0	112	9	1.05	0.04	1.7	0.3
	4	82	6	1.09	0.04	0.5	0.3
	5	94	5	1.29	0.07	0	N/A
	8	93	4	1.20	0.10	0.9	0.2
	12	78	5	1.28	0.06	1.3	0.3
	18	42	3	1.49	0.07	0.4	0.3
3350	4	75	6	1.30	0.05	0.9	0.5
	5	99	8	1.55	0.13	0	N/A
	8	58	5	1.50	0.02	1.5	0.2
	12	57	3	1.44	0.07	0.8	0.2
	16	55	2	1.48	0.04	0.5	0.1
8000	2	102	12	1.41	0.14	1	0.5
	4	96	16	1.00	0.97	0.5	0.3
	8	77	5	1.31	0.09	0.5	0.3
	12	63	4	1.40	0.06	0.4	0.2
	16	40	7	1.26	0.13	0.4	0.7