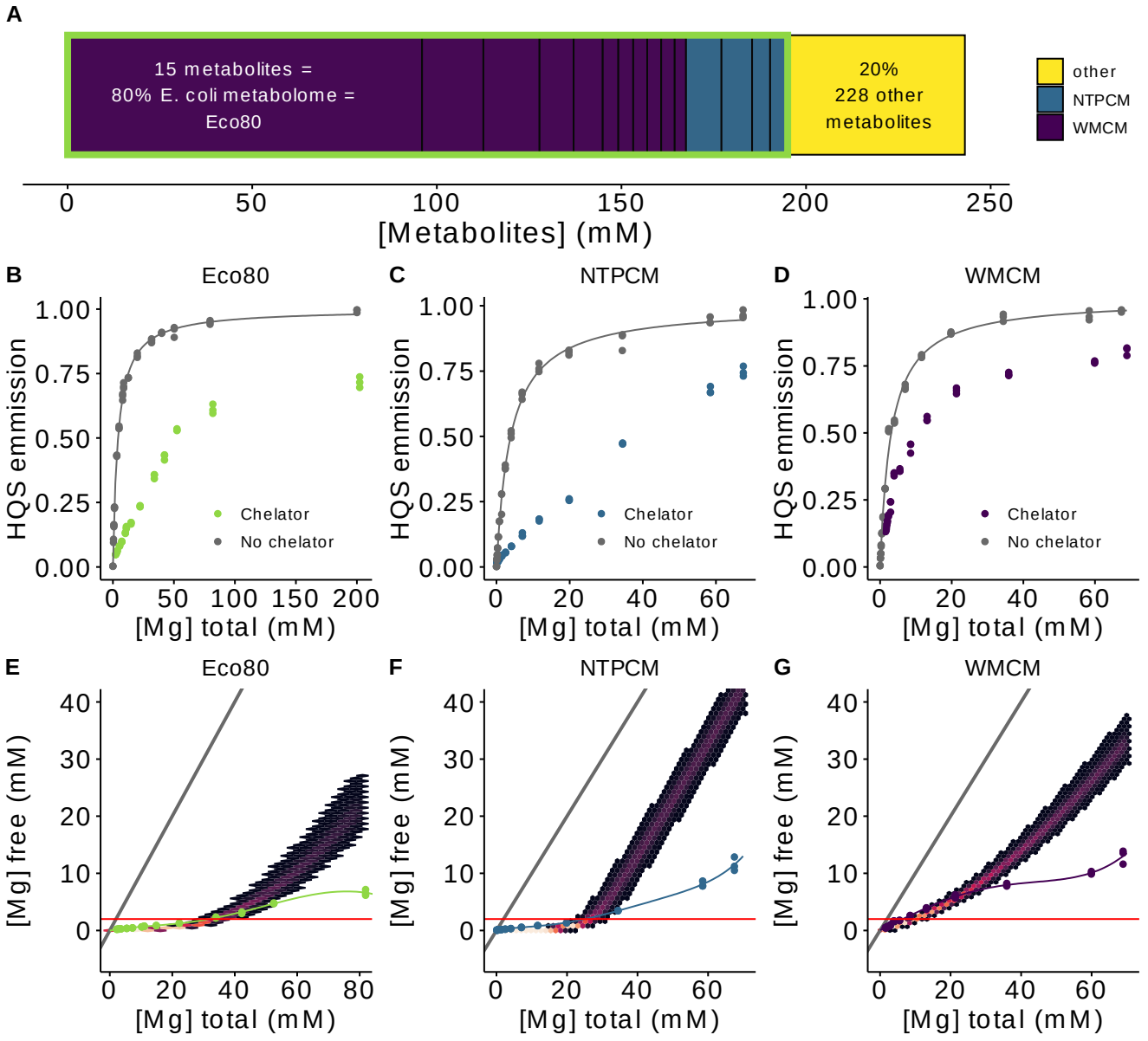


Figure 1 Analysis of Mg^{2+} speciation in *E. coli* metabolite mixtures. Eco80 contains **(A)** E. coli metabolome molar composition. Eco80 contains the 15 most abundant metabolites that compose 80% of the *E. coli* metabolome. NTPCM contains fourt strong Mg^{2+} chelating NTPs, and WMCM contains 11 other weak $Mg^{2+\ binding}$ metabolites **(B-D)** Effect of Mg^{2+} on 8-hydroxyquinoline-5-sulphonic acid (HQS) emission with and without mixtures of metabolites that chelate Mg^{2+}. Grey lines represent fits to determine the binding constant for Mg^{2+} and HQS. **(E-G)** Effect of the total Mg^{2+} concentration on the free Mg^{2+} concentration with mixtures of metabolites that chelate Mg^{2+}. Free Mg^{2+} was calculated using HQS emission and the binding constant for Mg^{2+} and HQS. Grey lines represent the free Mg^{2+} concentration in the absence of chelators (free Mg^{2+} = total Mg^{2+}). Hex bins represent a statistical simulation of 1000 virtual artificial cytoplasms based on experimental errors in K_D determination, experimental errors in reagent concentrations, and single site binding.

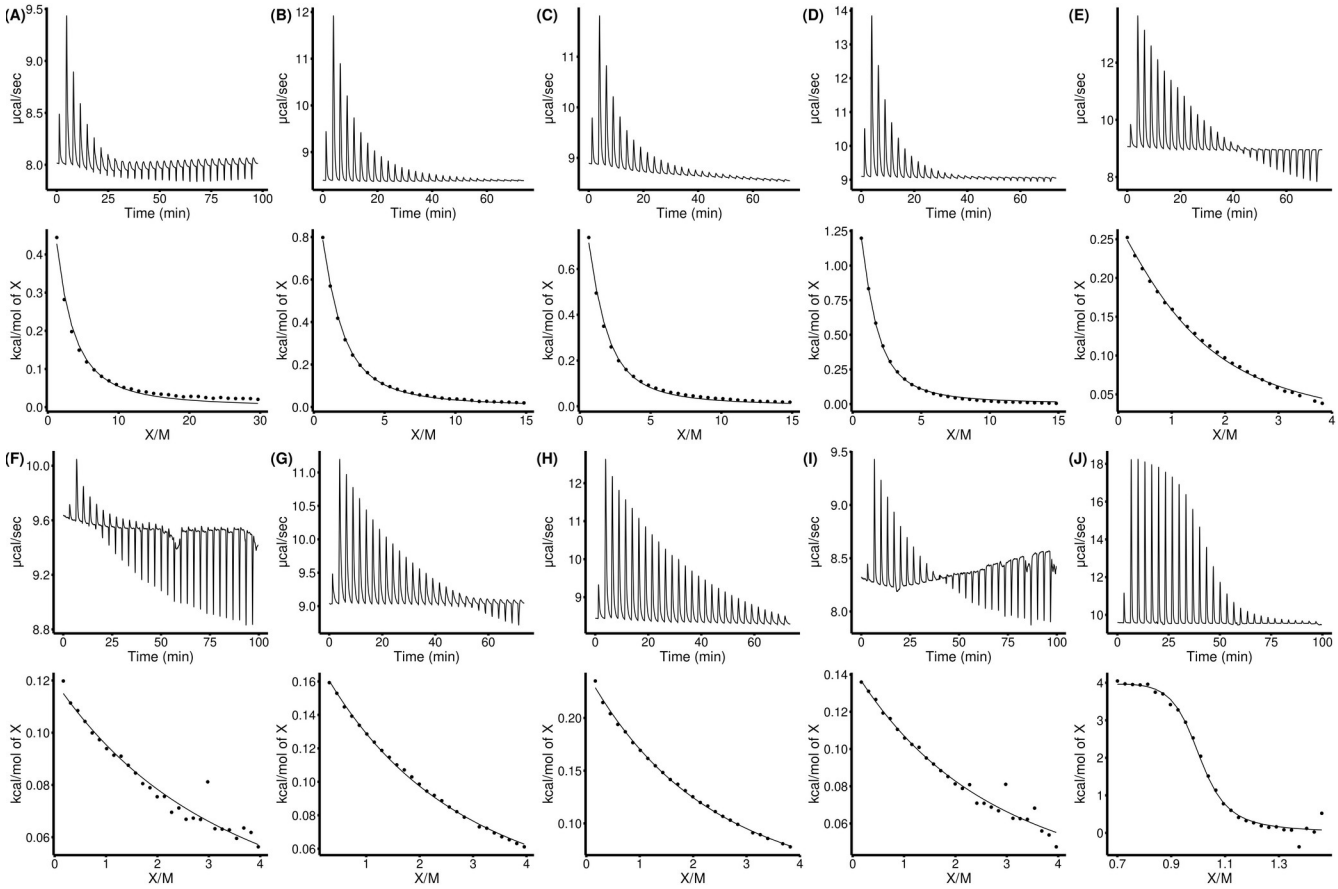
Table 1. Eco80: the top 15 most abundant metabolites that comprise 80% of the *E. coli* metabolome.

Metabolite	Conc. (mM)	K_D (mM)	Chelation stength
ATP	9.63	0.28 (0.01)[a]	NTPCM (Strong[d])
UTP	8.29	0.248 (0.004)[a]	NTPCM (Strong[d])
GTP	4.87	0.201 (0.007)[a]	NTPCM (Strong[d])
dTTP	4.62	0.160 (0.003)[a]	NTPCM (Strong[d])
L-Glutamic acid	96	520 (50)[b]	WMCM (Weak[d])
Glutathione	16.6	NA[c]	WMCM (Weak[d])
Fructose 1,6-bisphosphate	15.2	5.9 (0.1)[a]	WMCM (Weak[d])
UDP-N-acytylglucosamine	9.24	29 (2)[a]	WMCM (Weak[d])
Glucose 6-phosphate	7.88	17.3 (0.2)[a]	WMCM (Weak[d])
L-Aspartic acid	4.23	465 (12)[b]	WMCM (Weak[d])
L-Valine	4.02	NA[c]	WMCM (Weak[d])
L-Glutamine	3.81	NA[c]	WMCM (Weak[d])
6-Phospho-gluconic acid	3.77	14.4 (0.2)[a]	WMCM (Weak[d]
Pyruvic acid	3.66	3.6 (0.9)[b]	WMCM (Weak[d])
Dihydroxyacetone phosphate	3.06	20 (1)[a]	WMCM (Weak[d])

[a]Determined at 37 °C with Isothermal titration calorimetry. Error is the propagated standard error in the fit parameters.
[b]Determined at 37 °C with HQS emission Error is the propagated standard error in the fit parameters.
[c]No binding observed as per SI Figure 2

[d]Metabolites with KDs for Mg^{2+} less than 2 mM are considered strong Mg^{2+} chelators.

SI Table 1. Recipe for the Eco80 artificial cytoplasm (Add in later in word when I am formatting the tables)



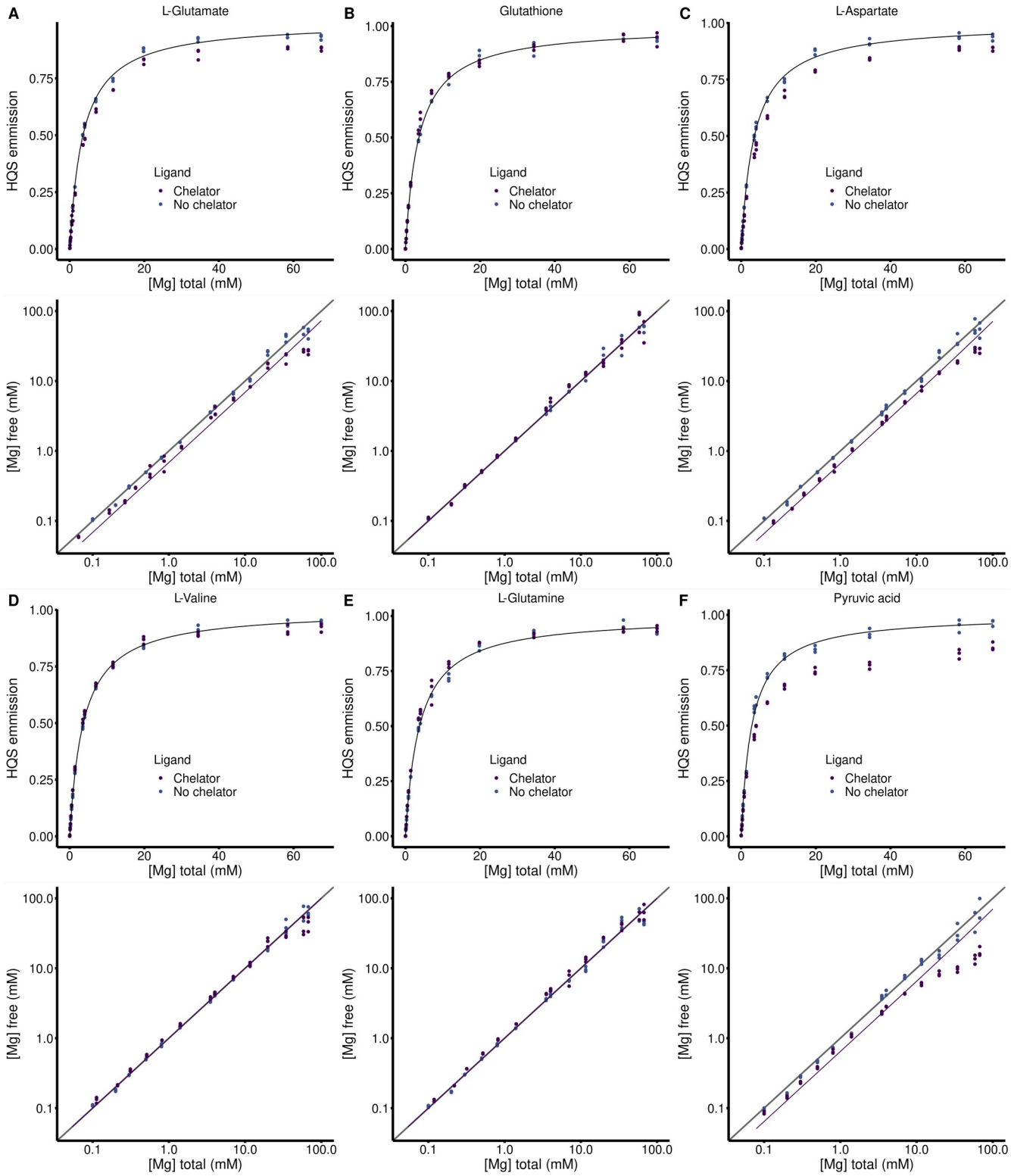
SI Figure 1 Isothermal titration calorimetry (ITC) analysis of Mg^{2+} binding to metabolites in 240 mM NaCl 140 mM KCl 10 mM HEPES pH 7.0 at 37 °C. $MgCl_2$ was titrated into metabolites and the power was monitored over time (Top panel). Heat of the injection was calculated by integrating the raw power curve, and the background heat of $MgCl_2$ diltution, collected on buffer containing no metabolite, was subtracted to produce the isotherms in the bottom panels. Lines in bottom pannels represent fits to the Weismann isotherm equation to determine apparent association constants. **(A)** Adenosine triphosphate (ATP). **(B)** Uridine triphosphosphate (UTP). **(C)** Guanosine triphsophate (GTP). **(D)** deoythymidine triphosphate (dTTP). **(E)** Fructose 1,6-bisphosphate. **(F)** Uridine diphosphate (UDP)-N-acytylglucosamine. **(G)** Glucose 6-phosphate. **(G)** 6-phosphogluconic acid. **(H)** phosphoenol pyruvate. **(G)** Ethylene diamine-tetracetic acid (EDTA).

SI Table 2 Apparent binding constants determined with Isothermal titration calorimetry (ITC).

Metabolite	Syringe (mM)	Cell (mM)	dH (kcal/mol)	K' (M^{-1})	K_D' (mM^{-1})
ATP	15 mM $MgCl_2$[a]	0.1 mM ATP[a]	1.83 (0.04)	3600 (200)	0.28 (0.01)
UTP	15 mM $MgCl_2$[a]	0.2 mM UTP[a]	1.70 (0.01)	4200 (70)	0.248 (0.004)
GTP	15 mM $MgCl_2$[a]	0.2 mM GTP[a]	1.43 (0.02)	5000 (200)	0.201 (0.007)
dTTP	15 mM $MgCl_2$[a]	0.2 mM dTTP[a]	2.19 (0.02)	6300 (300)	0.160 (0.003)
Fructose 1,6-BP	100 mM $MgCl_2$[a]	5.0 mM Fructose 1,6-BP[a]	0.414 (0.004)	169 (4)	5.9 (0.1)
UDP-GlcNAC	100 mM $MgCl_2$[a]	5.0 mM UDP-GlcNAC	0.57 (0.02)	34 (2)	29 (2)
Glucose 6-P	100 mM $MgCl_2$[a]	5.0 mM Glucose 6-P[a]	0.555 (0.003)	57.9 (0.7)	17.3 (0.2)
6-P-gluconic acid	100 mM $MgCl_2$[a]	5.0 mM 6-P-gluconic acid[a]	0.662 (0.005)	70 (1)	14.4 (0.2)
Dihydroxyacetone phosphate	100 mM $MgCl_2$[a]	5.0 mM dihydroxy-acetone phosphate[a]	0.50 (0.01)	51 (3)	20 (1)
EDTA	6 mM $MgCl_2$[a]	1.5 mM EDTA 1.0 mM $MgCl_2$[a,b]	2.85 (0.04)	220,000 (30,000)	0.0045 (0.0006)

[a]240 mM NaCl 140 mM KCl 10 mM HEPES pH 7.0 at 37 °C

[b]Mg^{2+} and EDTA were incorporated into the cell in order to sequester trace tight binding metal ions and thereby negate their contribution to ITC signal.



SI Figure 2 HQS analysis of Mg2+ binding to metabolites in 240 mM NaCl 140 mM KCl 20 mM MOPS 0.01 mM EDTA 0.001% SDS pH 7.0. (Top panels) Dependence of HQS emission on the total concentration of $MgCl_2$ in the presence and absence of a metabolite chelators. Black lines represent a fit to SI equation 1 to determine the F_{max}, F_{min}, and K_{HQS}. (Bottom panels) Dependence of the free Mg^{2+} concentration on the total concentration of $MgCl_2$ in the presence and absence of a metabolite chelators. Grey lines represent where the free Mg^{2+} concentration equals the total concentration of $MgCl_2$, Purple lines represent a fit to SI equation 4 to determine the association constant between HQS and a chelator. **(A)**

240 mM L-glutamate. **(B)** 194 Glutathione. **(C)** 240 mM L-aspartate. **(D)** 240 mM L-valine. **(E)** 240 mM L-glutamine. **(F)** 5 mM pyruvic acid.

SI Table 3 Apparent binding constants determined with HQS emission. F_{max}, F_{min}, and K_{HQS} are determined by fitting HQS emission in the absence of chelators and used to calculate the free Mg^{2+} concentration in each sample. Metabolite binding constants, K' and K_D'. are determined by fitting the relationship between the free Mg^{2+} concentration and the total $MgCl_2$ concentration using SI Equation 4.

Metabolite	F_{max}	F_{min}	K_{HQS} (mM^{-1})	K' (mM^{-1})	K_D' (mM^{-1})
L-Glutamic acid	187,000 (1000)	0 (810)	0.281 (0.008)	0.0019 (0.0002)	520 (50)
Glutathione	182,000 (1000)	592 (750)	0.279 (0.007)	NA[c]	NA[c]
L-Aspartic acid	196,000 (1000)	0 (820)	0.283 (0.007)	0.0021 (0.0001)	465 (12)
L-Valine	188,200 (800)	495 (580)	0.274 (0.005)	NA[c]	NA[c]
L-Glutamine	190,000 (1400)	516 (110)	0.27 (0.01)	NA[c]	NA[c]
Pyruvic acid	188,000 (1500)	0 (1300)	0.35 (0.01)	0.28 (0.07)	3.6 (0.9)

[c]No binding observed as per SI Figure 2

SI Table 3. HQS fits in the absence of chelators, used to determine free Mg2+ concentrations. F_{max}, F_{min}, and K_{HQS} are determined by fitting HQS emission in the absence of chelators and used to calculate the free Mg^{2+} concentration in each sample.

Metabolite	F_{max}	F_{min}	K_{HQS} (mM^{-1})
Eco80	185,100 (800)	124 (1000)	0.239 (0.005)
NTPCM	187,000 (1500)	436 (1000)	0.26 (0.01)
WMCM	179,000 (1600)	0 (1400)	0.32 (0.01)

Table 2. Total Mg^{2+} concentrations used to obtain 2 mM free Mg^{2+} in artificial cytoplasms.

Condition	Total [Mg^{2+}] (mM)	Chelated [Mg^{2+}] (mM)	Free [Mg^{2+}] (mM)
Eco80	31.6	29.6	2.0
NTPCM	25.0	23	2.0
WMCM	6.4	4.5	2.0

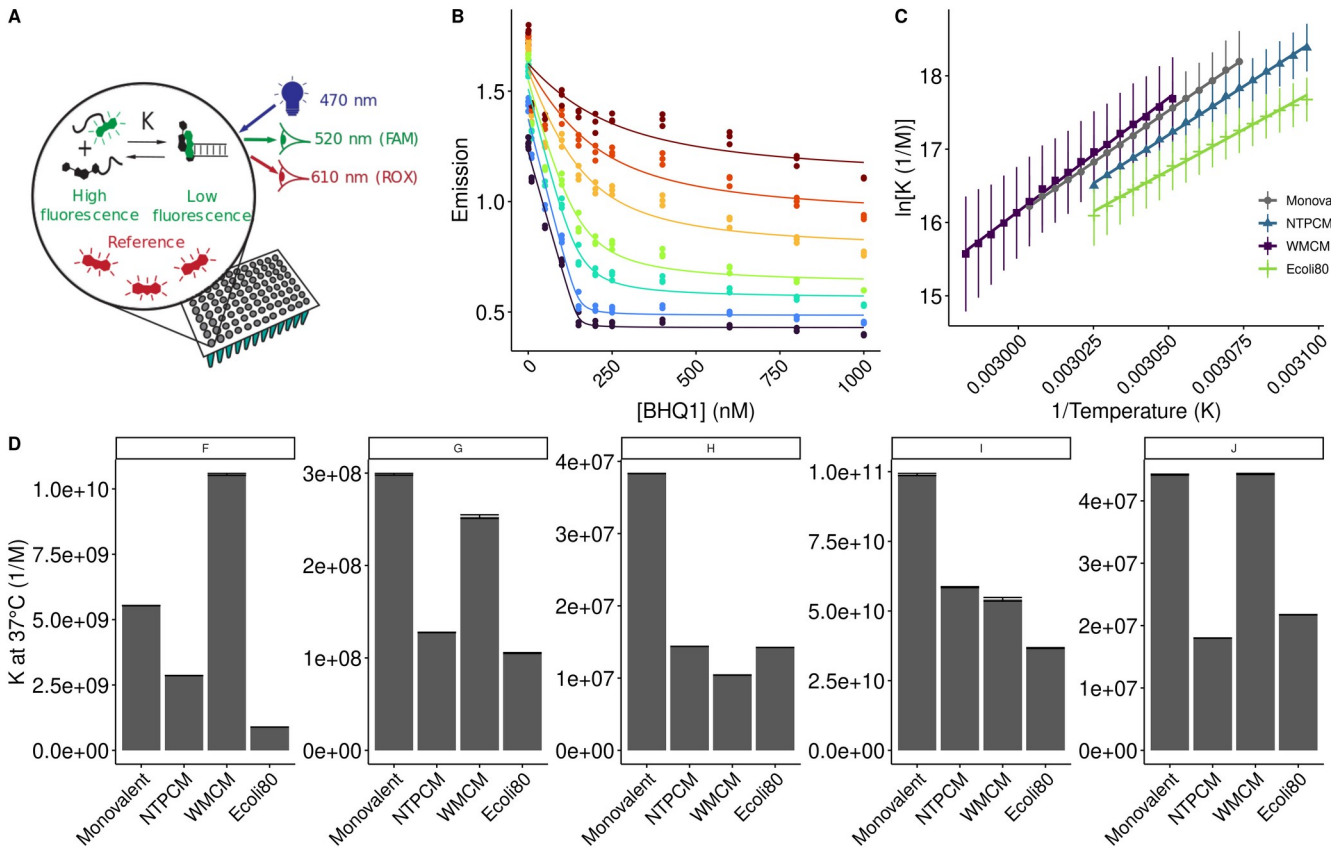
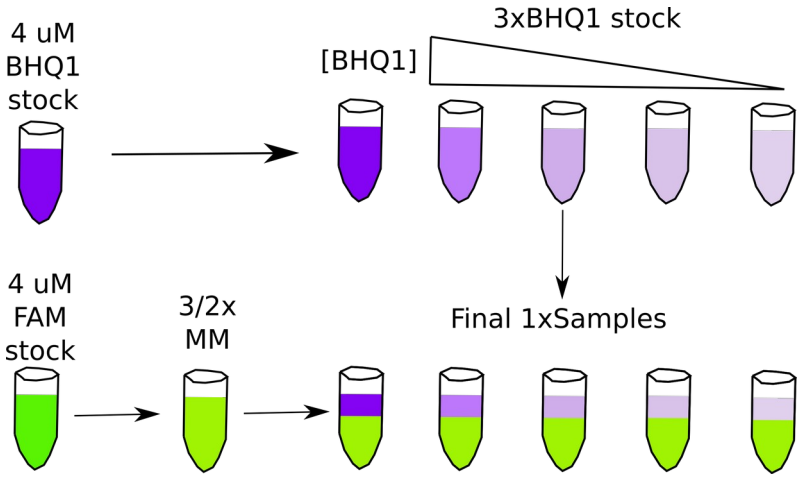
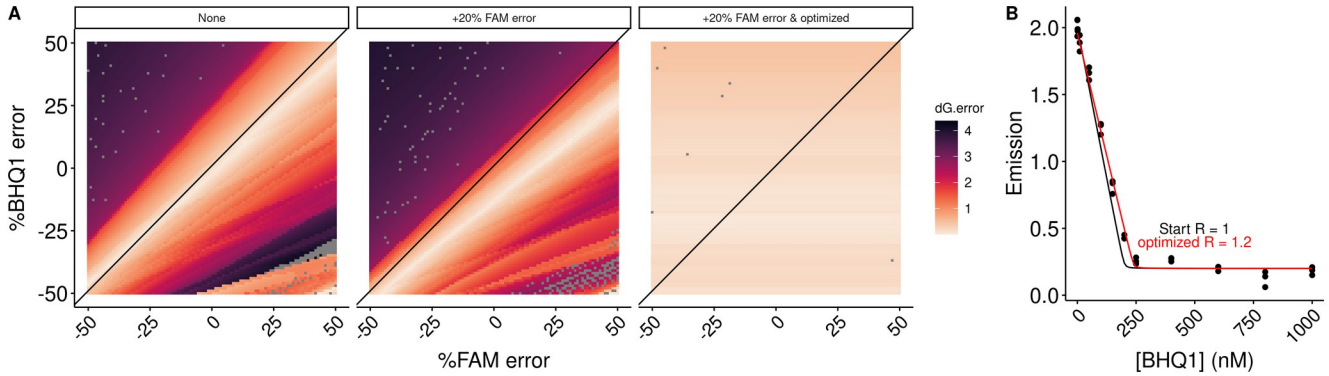


Figure 2 *E. coli* metabolite and Mg^{2+} mixtures destabilize RNA secondary structure.



SI figure 3 *E. coli* metabolite and Mg^{2+} mixtures destabilize RNA secondary structure.



SI figure 4 *E. coli* metabolite and Mg^{2+} mixtures destabilize RNA secondary structure.

Table 3. Stability of RNA helices in *E. coli* metabolite mixtures.

Helix	Sequence (5'-FAM/ BHQ1-3')	AU content	Condition	dH (kcal/mol)	dS (cal/mol/K)	dG (kcal/mol)	ddG (kcal/mol)
F	CGCAUCCU/ AGGAUGCG	0.38	2 mM free	-55.9 (0.2)	-136.0 (0.7)	-13.82 (0.01)	
			NTPCM	-52.2 (0.4)	-125 (1)	-13.41 (0.02)	0.41 (0.02)
			WMCM	-61.4 (0.8)	-152 (2)	-14.22 (0.05)	-0.40 (0.05)
			Ecoli80	-44.5 (0.7)	-102 (2)	-12.70 (0.04)	1.13 (0.04)
G	CCAUAUCA/ UGAUAUGG	0.63	2 mM free	-53.4 (1.0)	-133 (3)	-12.02 (0.04)	
			NTPCM	-42.9 (0.5)	-101 (1)	-11.50 (0.02)	0.52 (0.04)
			WMCM	-53 (2)	-132 (7)	-11.9 (0.1)	0.10 (0.01)
			Ecoli80	-57 (2)	-146 (5)	-11.38 (0.05)	0.64 (0.06)
H	CCAUAUUA/ UAAUAUGG	0.75	2 mM free	-53.5 (0.4)	-137 (1)	-10.76 (0.01)	
			NTPCM	-45.0 (0.2)	-112.5 (0.5)	-10.158 (0.002)	0.60 (0.01)
			WMCM	-43 (2)	-107 (5)	-9.94 (0.02)	0.80 (0.02)
			Ecoli80	-41.3 (0.2)	-100.4 (0.7)	-10.15 (0.01)	0.61 (0.01)
I	CGGAUGGC/ GCCAUCCG	0.25	2 mM free	-71.1 (0.8)	-179 (2)	-15.6 (0.06)	
			NTPCM	-70.4 (0.6)	-177 (2)	-15.28 (0.05)	0.32 (0.08)
			WMCM	-65.5 (2)	-162 (7)	-15.2 (0.2)	0.4 (0.2)
			Ecoli80	-69.7 (0.8)	-176 (3)	-14.0 (0.1)	0.61 (0.08)
J	CGUAUGUA/ UACAUACG	0.63	2 mM free	-63.2 (0.9)	-169 (3)	-10.85 (0.02)	
			NTPCM	-59 (1)	-157 (4)	-10.30 (0.01)	0.55 (0.02)
			WMCM	-67 (1)	-180 (3)	-10.85 (0.02)	0.00 (0.03)
			Ecoli80	-61 (1)	-164 (3)	-10.41 (0.01)	0.44 (0.02)

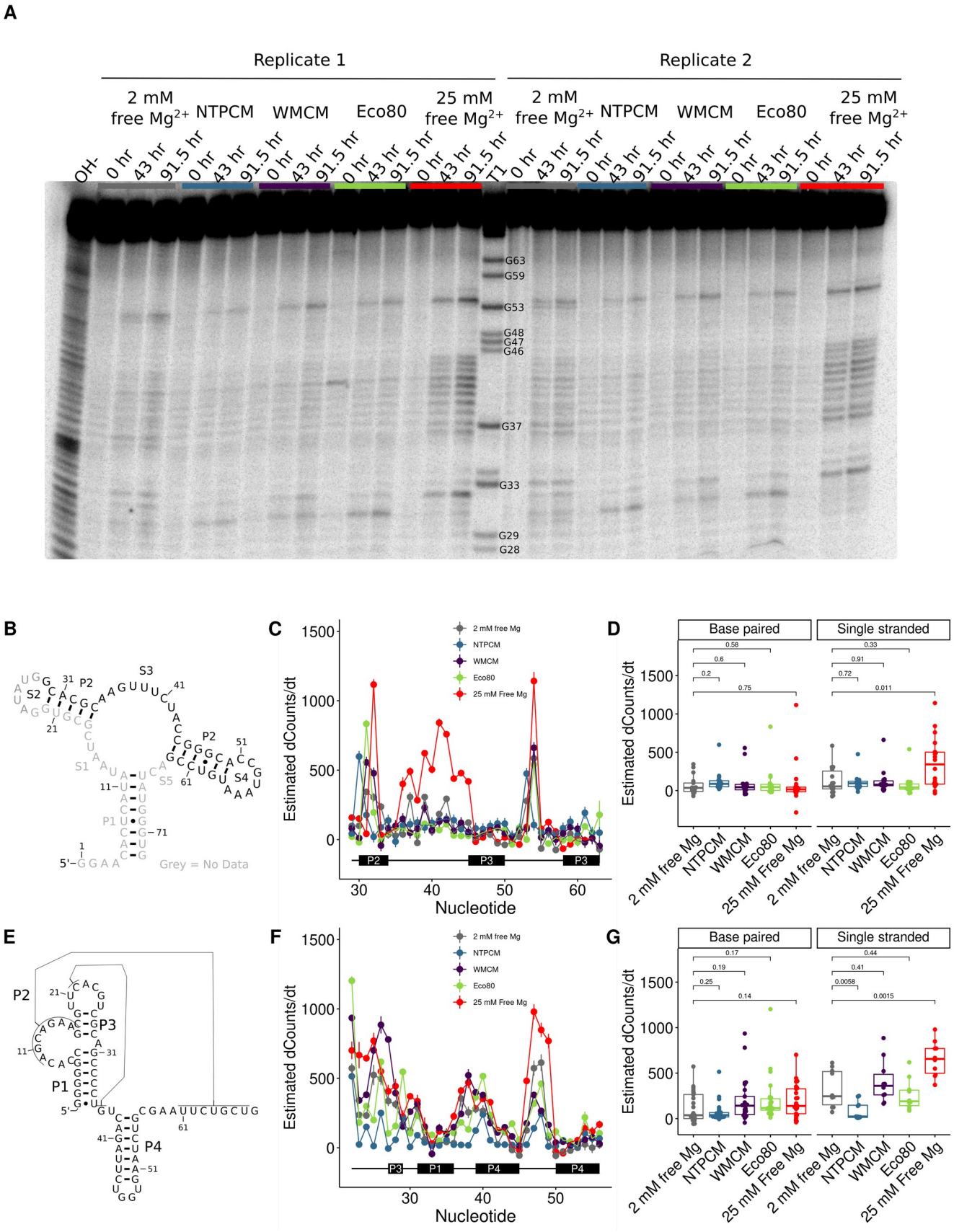

A
Replicate 1
Replicate 2
2 mM free Mg2+
NTPCM
WMCM
Eco80
25 mM free Mg2+
OH-
0 hr
43 hr
91.5 hr
T1
G63
G59
G53
G48
G47
G46
G37
G33
G29
G28
B
S3
P2
S2
S1
S4
S5
P1
P3
P4
Grey = No Data
C
Estimated dCounts/dt
Nucleotide
2 mM free Mg
NTPCM
WMCM
Eco80
25 mM Free Mg
P2
P3
D
Base paired
Single stranded
Estimated dCounts/dt
0.58
0.6
0.2
0.75
0.33
0.91
0.72
0.011
E
F
G
0.17
0.19
0.25
0.14
0.44
0.41
0.0058
0.0015

Figure 3 *E. coli* metabolite and Mg^{2+} mixtures stabilize the chemical structure of RNA. **(A)** Raw degradation assay gel image for the Guanine riboswitch aptamer incubated in artificial cytoplasms at 37 °C and pH 7. The OH- lane contains a hydrolysis ladder which cleaves after every nucleotide and T1 contains the RNA treated with T1 ribonuclease which cleaves after every G. Enough Mg^{2+} was added to each artificial cytoplasm to have 2 mM Mg^{2+} as determined in Figure 1. **(B)** Secondary structure of the guanine riboswitch aptamer. **(C)** Estimated increase in counts as a function of time at each residue in different solution conditions as a function of location in the RNA. **(D)** Estimated increase in counts as a function of time in different conditions grouped by paired and unpaired bases. Significance was determined using a student's t-test. **(E)** Secondary structure of the cleaved human CPEB3 HDV ribozyme. **(C)** Estimated increase in counts as a function of time at each residue in different solution conditions as a function of location in the RNA. **(D)** Estimated increase in counts as a function of time in different conditions grouped by paired and unpaired bases. Significance was determined using a student's t-test.

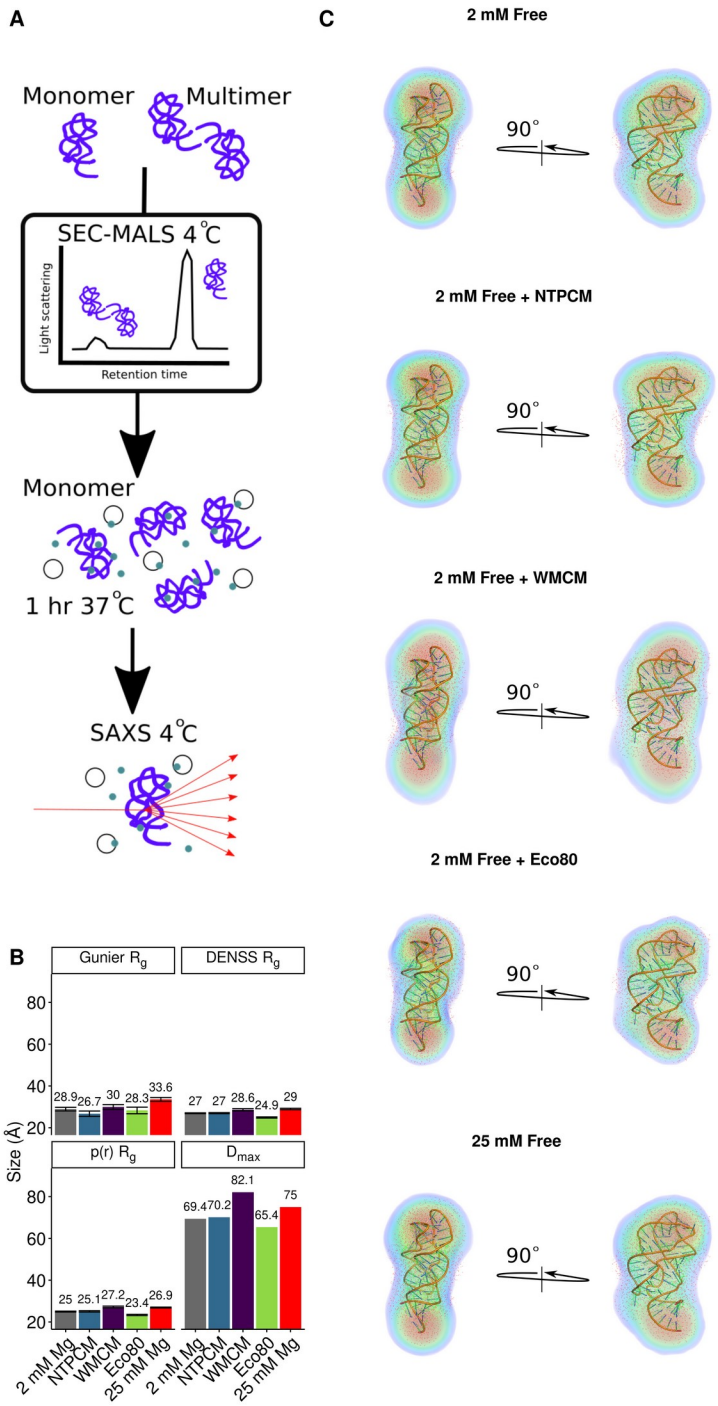


Figure 4 *E. coli* metabolite and Mg^{2+} mixtures increase functional RNA compactness.