Impact

1. Provide the field with in *vivo-like* MCM conditions to help understand how RNA folds *in vivo*
2. Show changes in global RNA secondary structure stability

Experiments

1. Model metabolome/metalome
2. Use ITC to determine exact Kds at pH 7 and relevant ionic strength - Temperature
3. Use Draper dye (8-hydroxyquinoline-5-sulfonic acid) to confirm free and chelated Mg concentrations
4. Use fluorescence Tms to show changes in global RNA secondary structure stability + configurational stability

Side notes for discussion

1. Rouskin Nat. Commun. ~2014 How much energy would the helicase secondary structure weakening cost?
2. Swap single stranded and double stranded state in fluorescence layout
3. Write abstract – Wait on the intro
4. Martin Grueblee JACS communications
5. Mathew's JACS communication
6. Figure 1 Change A to C and Show MCM
7. Add a cut off line Table 1
8. Commas SE, sig figs, MCM into Table 1
9. Intro, Pilak and coworkers begin with cell extracts. However, we will take a bottom up approach to complexity, with the intention of understanding speciation and molecular contribution.

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The Bacterial Metabolome and Metalome Weakens Global RNA Secondary Structure Stability

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KEYWORDS

Magnesium ion, Chelated magnesium, RNA folding, secondary structure, metabolites

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ABSTRACT: Herein, we examine the complicated network of interactions amoung RNA, the metabolome, and the metalome in *E. coli*. First, we examine the effects of temperature on the binding affinity of the top 14 *E. coli* metabolites, comprising 80% of the total metabolome, at physiological pH and monovalent ion concentrations using ITC and HQS fluorescence titrations. Then, we use this information to inform creation of artificial cytoplasms that mimic *in vivo* E. coli conditions. We then examine the effects of the these *in vivo-like* conditions on RNA thermodynamic stability and RNA chemical stability. We find that these *in vivo-like* conditions lead to opposing effects, wherein thermodynamic stability is weakened but chemical stability is strengthened.

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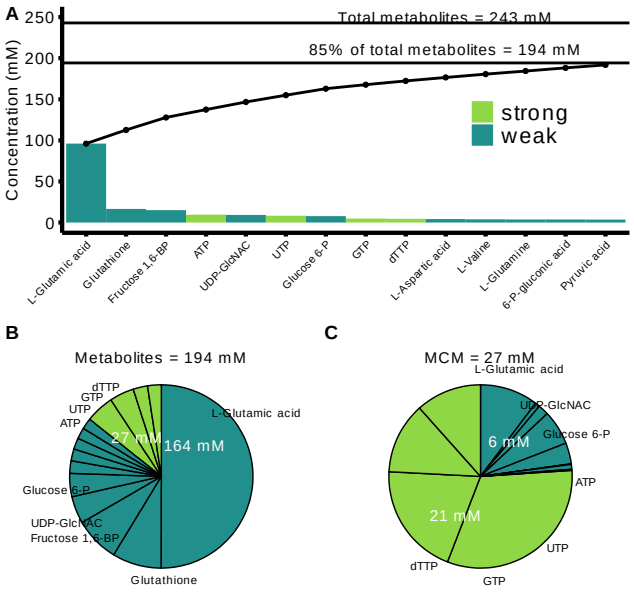

Figure 1. Cellular conditions of metabolite chelated magnesium (MCM). Metabolites that bind Mg^{2+} with a K_D of less than 2 mM are considered strong magnesium chelators. (A) 14 abundant metabolites compose 80% of the *E. coli* metabolome. (B) The top 14 most abundant metabolites organized by Mg^{2+} bind-

ing strength. (C) Total MCM for the top 14 most abundant metabolites organized by Mg^{2+} binding strength.

Table 1. The top 14 most abundant metabolites that comprise 80% of the *E. coli* metabolome.

Metabolite	Concentration (mM)	K'_D (mM)	Mg^{2+} chelation strength[c]
ATP	9.63	0.28 (±0.01)[a]	strong
UTP	8.29	0.238 (±0.004)[a]	strong
GTP	4.87	0.200 (±0.009)[a]	strong
dTTP	4.62	0.163 (±0.003)	strong
L-Glutamic acid	96	67[b]	weak
Glutathione	16.6	147[b]	weak
Fructose 1,6-BP	15.2		weak
UDP-Glc-NAC	9.24		weak
Glucose 6-P	7.88		weak
L-Aspartic acid	4.23	285[b]	weak
L-Valine	4.02	26487[b]	weak
L-Glutamine	3.81	130315[b]	weak
6-P-gluconic acid	3.77		weak
Pyruvic acid	3.66	134[b]	weak

[a]Determined at 37 °C with Isothermal titration calorimetry. Error is the propagated standard error in the fit parameter.
[b]Determined by correcting absolute binding constants from the liturature for pH and ionic strength.
[c]metabolites with K_Ds for Mg^{2+} less than 2 mM are considered strong Mg^{2+} chelators.

Table 2. RNA helices tested in this study.

Helix	Sequence (5'-FAM/BHQ1-3')	Length	AU content
A	CGAAAGGU/ACCUUUCG	8	0.5
B	CGAACUCU/AGAGUUCG	8	0.5
C	CUGAGUC/GACUCAG	7	0.43
D	CGUUGC/GCAACG	6	0.33
E	CGACGC/GCGUCG	6	0.18
F	CGCAUCCU/AGGAUGCG	8	0.38
G	CCAUAUCA/UGAUAUGG	8	0.63
H	CCAUAUUA/UAAUAUGG	8	0.75
I	CGGAUGGC/GCCAUCCG	8	0.25
J	CGGAUGGC/GCCAUCCG	8	0.63

Figure 3. Effects of cellular concentrations of *E. coli* metabolites on RNA helix thermostability.

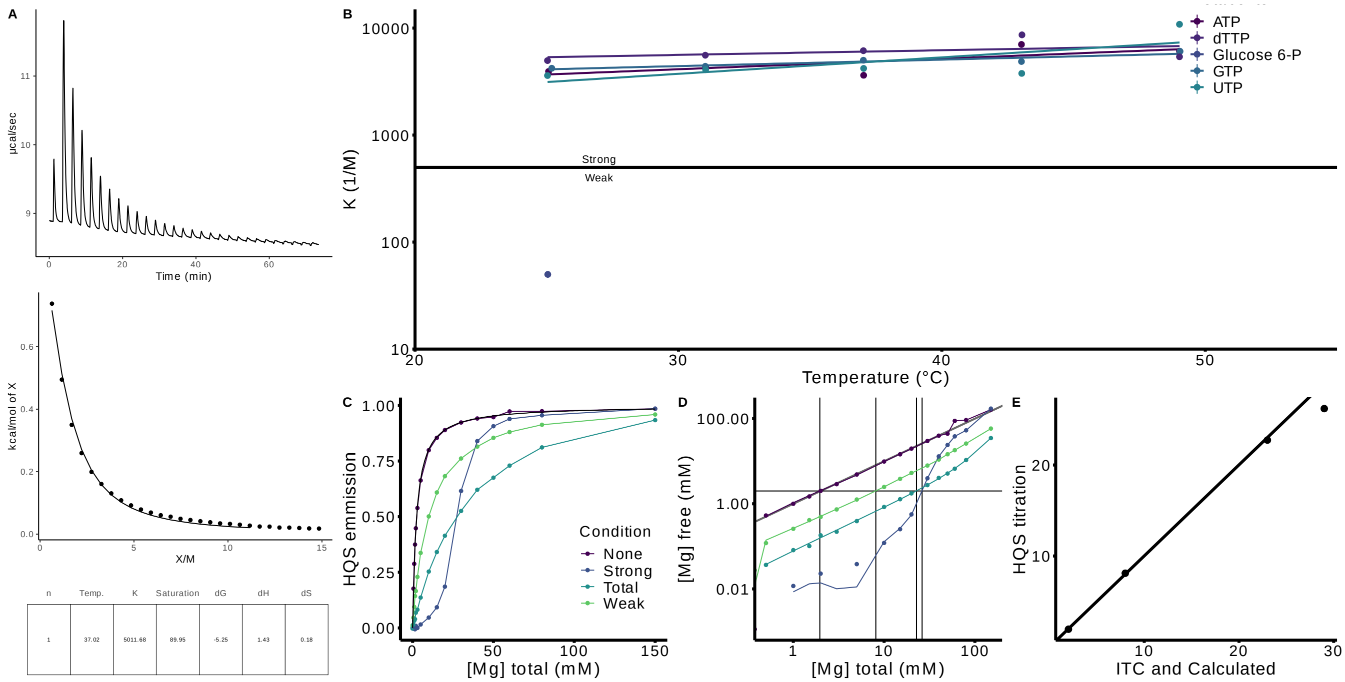

Figure 4. The temperature dependence of metabolites binding Mg at cellular pH and ionic strength.

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Figure 5. Cartoon depiction of the three way network of RNA, metabolite, and Mg2+ interactions.

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ABBREVIATIONS

CCR2, CC chemokine receptor 2; CCL2, CC chemokine ligand 2; CCR5, CC chemokine receptor 5; TLC, thin layer chromatography.

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