Supporting Information

An Active Site Guanine Participates in *glmS* Ribozyme Catalysis in its Protonated State

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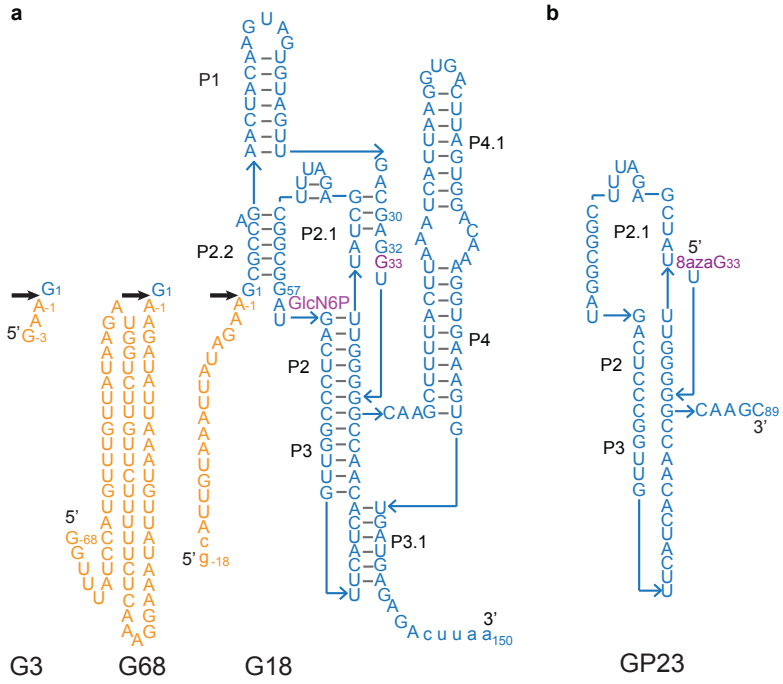
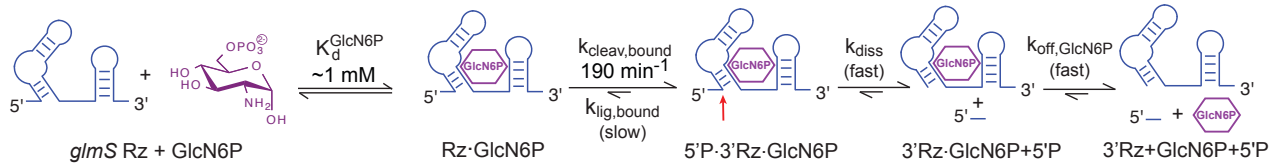
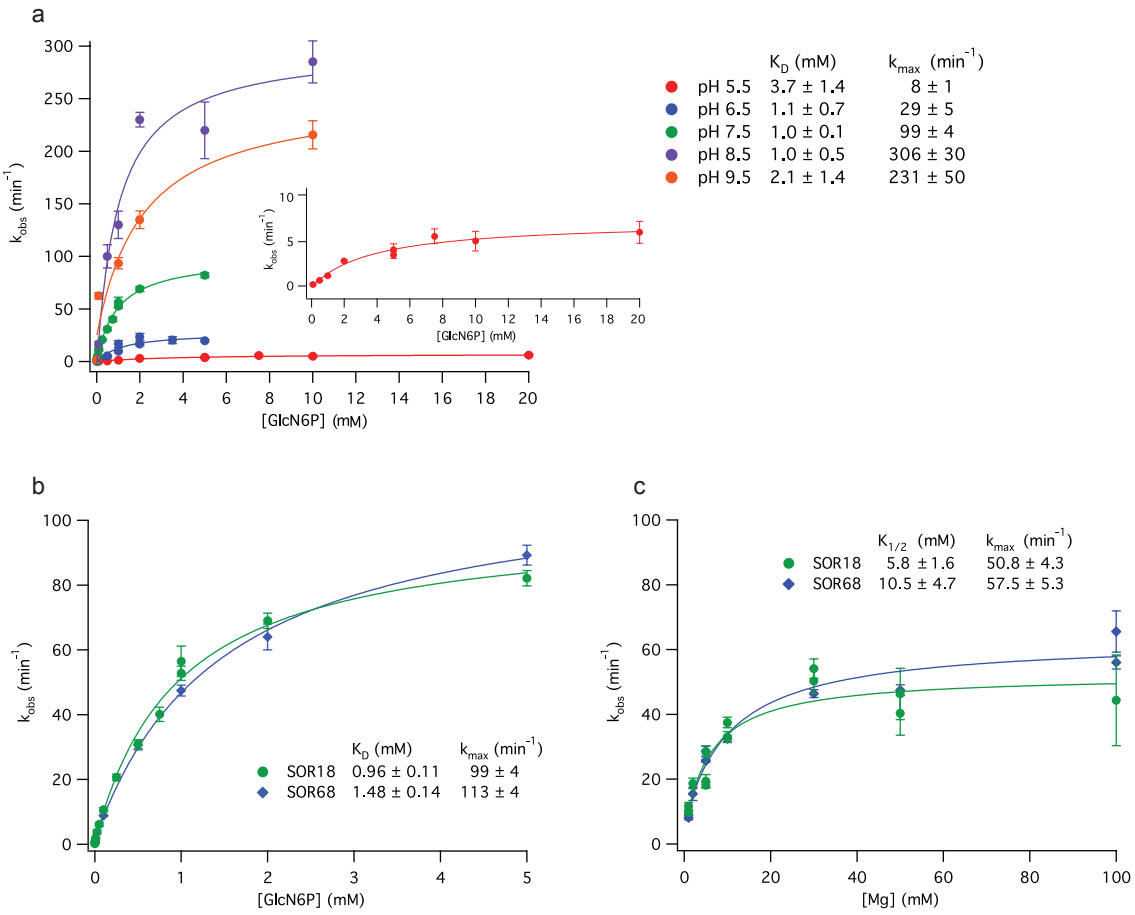
SUPPLEMENTARY FIGURES



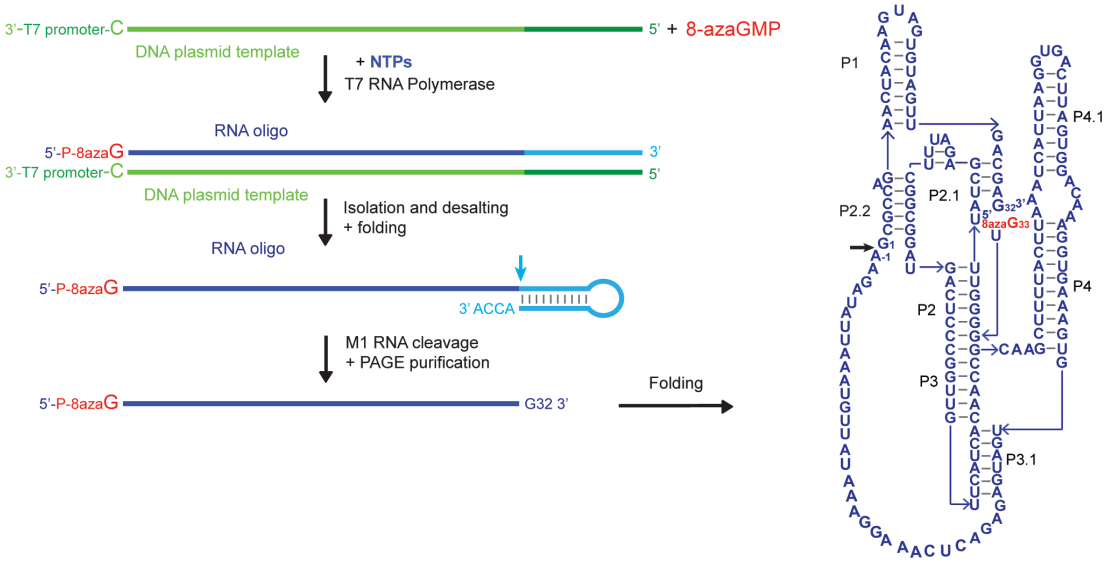
Supplementary Figure S1. RNAs used for kinetic and fluorescence experiments. **a)** G18 ribozyme used for kinetic studies, G68 ribozyme used to investigate ligation activity, and G3 ribozyme used to evaluate product dissociation kinetics. Lowercase indicates residues not present in the natural sequence from *Bacillus anthracis*. **b)** GP23 is a 57 nt unstructured RNA used as a control for fluorescence studies.



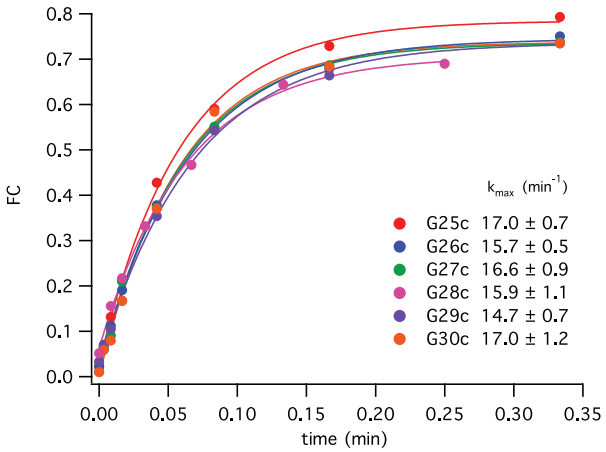
Supplementary Figure S2. Minimal kinetic mechanism of the *glmS* ribozyme reaction that includes binding of the cofactor GlcN6P, self-cleavage, and ligand and product dissociation.



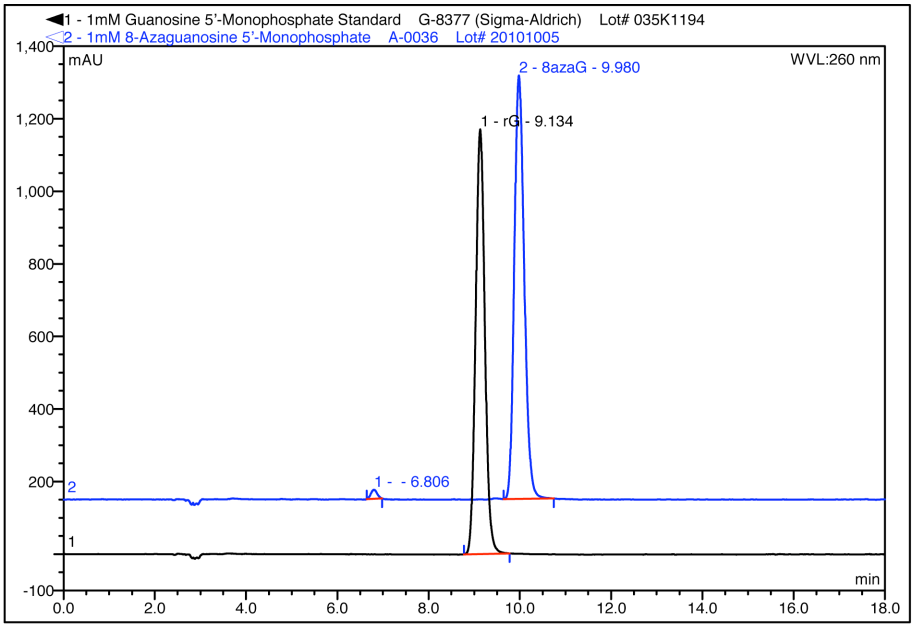
Supplementary Figure S3. *glmS* ribozyme affinity for GlcN6P and Mg^{2+}. **a)** GlcN6P concentration dependence of G18 binding at different pH values (50 mM buffer, 0.1 mM EDTA, 50 mM $MgCl_2$, 25 ˚C). **b)** Comparison of GlcN6P binding curves for G18 and G68 (50 mM HEPES pH 7.5, 0.1 mM EDTA, 50 mM $MgCl_2$, 25 ˚C). **c)** Comparison of the Mg^{2+} dependence of G18 and G68 cleavage kinetics (50 mM HEPES pH 7.5, 0.1 mM EDTA, 25 ˚C, 1 mM GlcN6P, 25 ˚C).



Supplementary Figure S4. Schematic representation of the strategy used to prepare the circular permute G28c with GMP or 8azaGMP at the 5'-end and uniform 3'-terminus through M1 RNA processing.



Supplementary Figure S5. Cleavage kinetics at pH 7.5 (50 mM HEPES, 0.1 mM EDTA, 50 mM $MgCl_2$, 10 mM GlcN6P, 25 ˚C) for the *glmS* circular permutes with different chain lengths. G25c-G30c have 29 to 34 nt linkers, respectively.

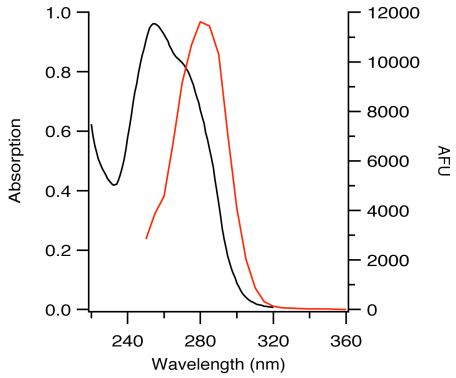


No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	6.81		22.058	3.556	1.34	n.a.	BMB*^
2	9.98	8azaG	1037.775	261.834	98.66	n.a.	BMB^
Total:			1059.833	265.389	100.00	0.000	

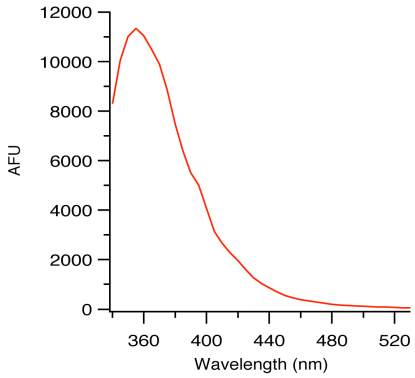
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	9.13	rG	1170.297	275.165	100.00	n.a.	BMB^
Total:			1170.297	275.165	100.00	0.000	

Supplementary Figure S6. Reversed phase HPLC analysis of 8-azaguanosine 5'-monophosphate. Column: Higgins HAISIL 100 C18. Conditions: 83.3 mM triethylammonium phosphate, 10% methanol (v/v), pH 6.0 (w/ phosphoric acid). Isocratic, 30 min. UV detection at 260 nm. T = 20 ºC.[1]

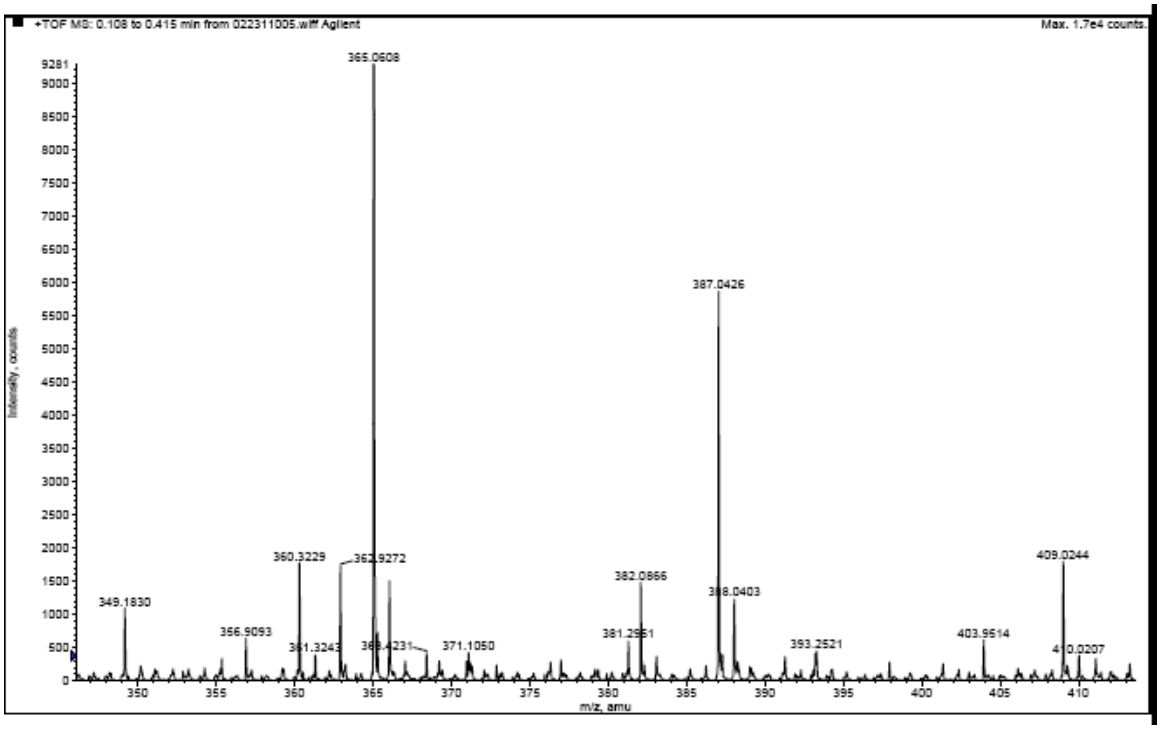
a



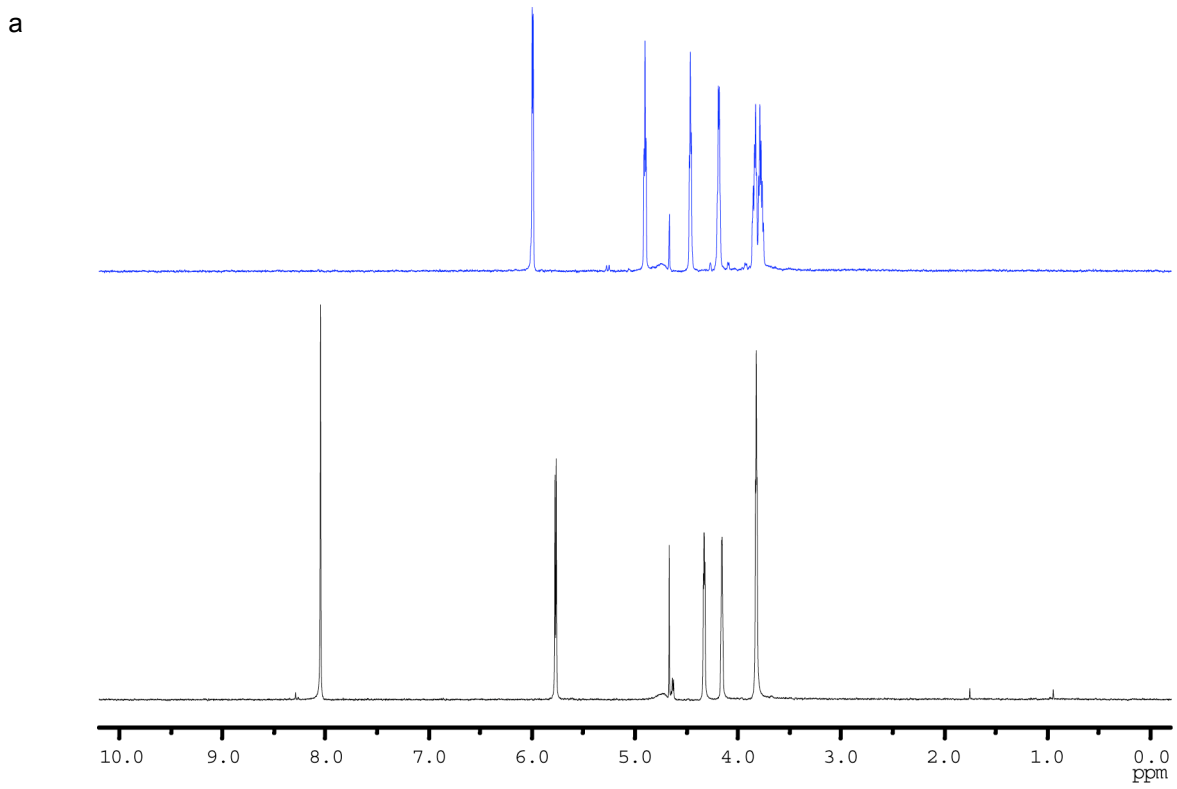
b

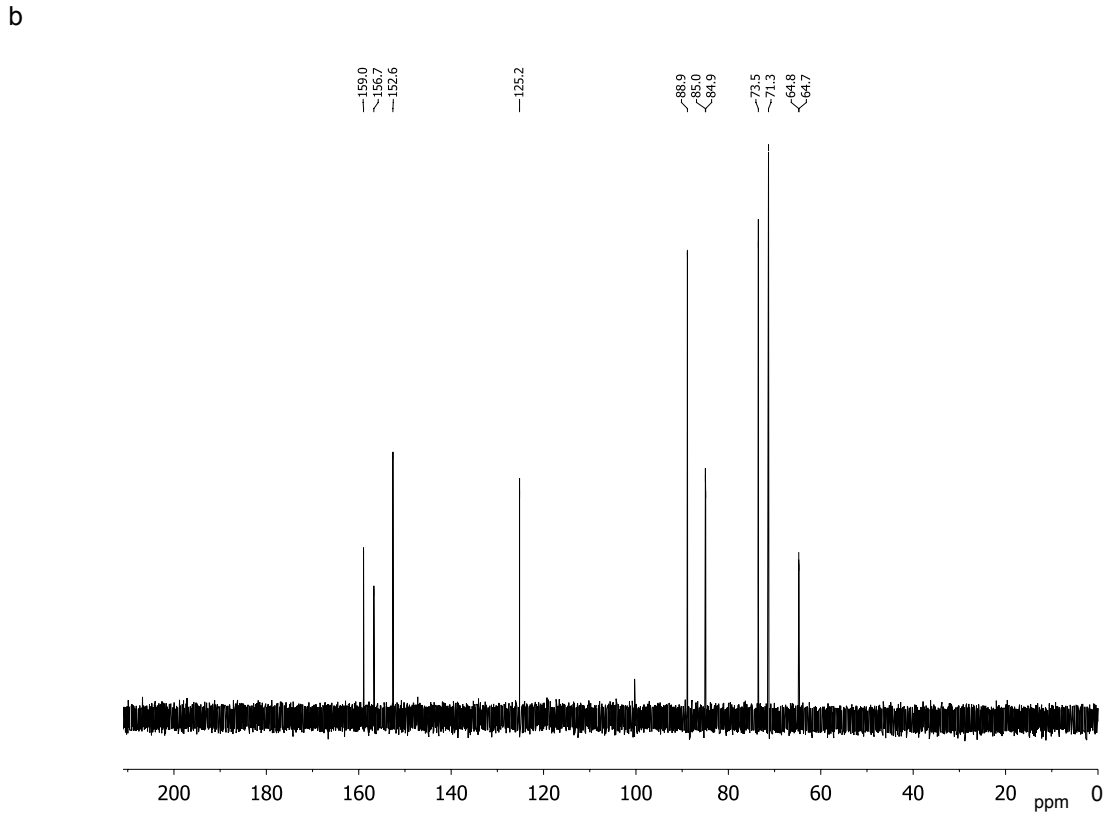


c



Supplementary Figure S7. UV and MS analysis of 8-azaguanosine 5'-monophosphate (8azaGMP). **a)** 1mM 8azaGMP pH 7.6 UV spectrum (black) and excitation spectrum (red). **b)** 1mM 8azaGMP pH 7.6 emission spectrum. **c)** HRMS (ESI(+)-TOF) of 8azaGMP showing $[M+H]^+$ (365.0608) and $[M+Na]^+$ (387.0426).





Supplementary Figure S8. NMR characterization of 8-azaguanosine 5'-monophosphate. **a)** ^{1}H NMR spectra (500 MHz, D_2O) of 5 mM 8azaGMP (top) and guanosine 5′-monophosphate (bottom). **b)** ^{13}C NMR spectrum (150 MHz, D_2O) of 10 mM 8azaGMP.

SUPPLEMENTARY REFERENCES

(1) Batey, R. T.; Battiste, J. L.; Williamson, J. R. In *Methods in Enzymology*; Thomas, L. J., Ed.; Academic Press: 1995; Vol. Volume 261, p 300.