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

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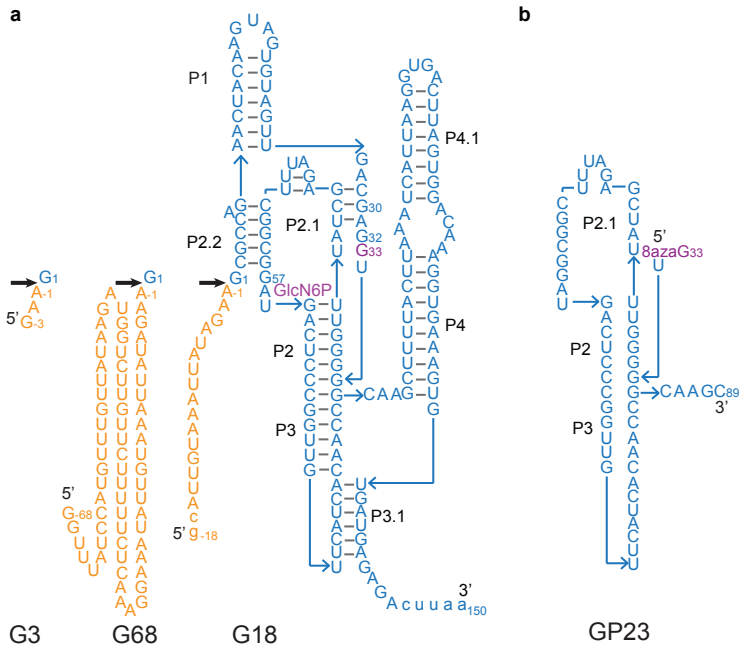
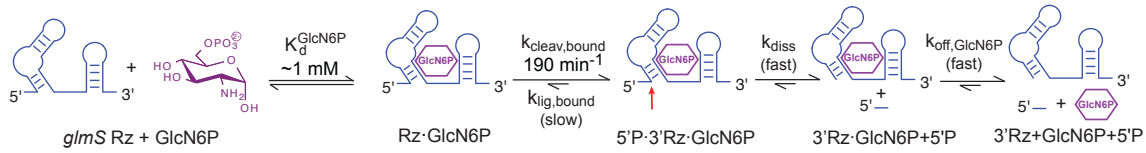
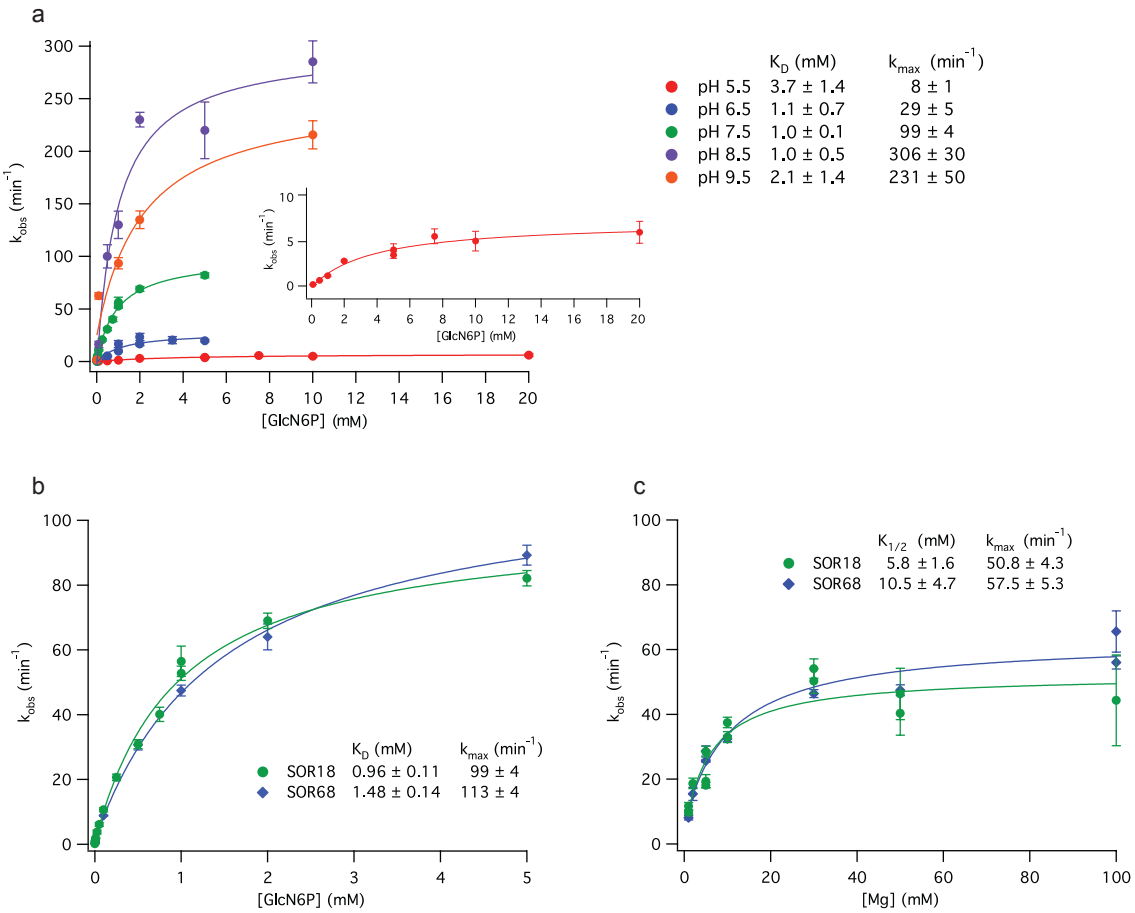
SUPPLEMENTARY FIGURES



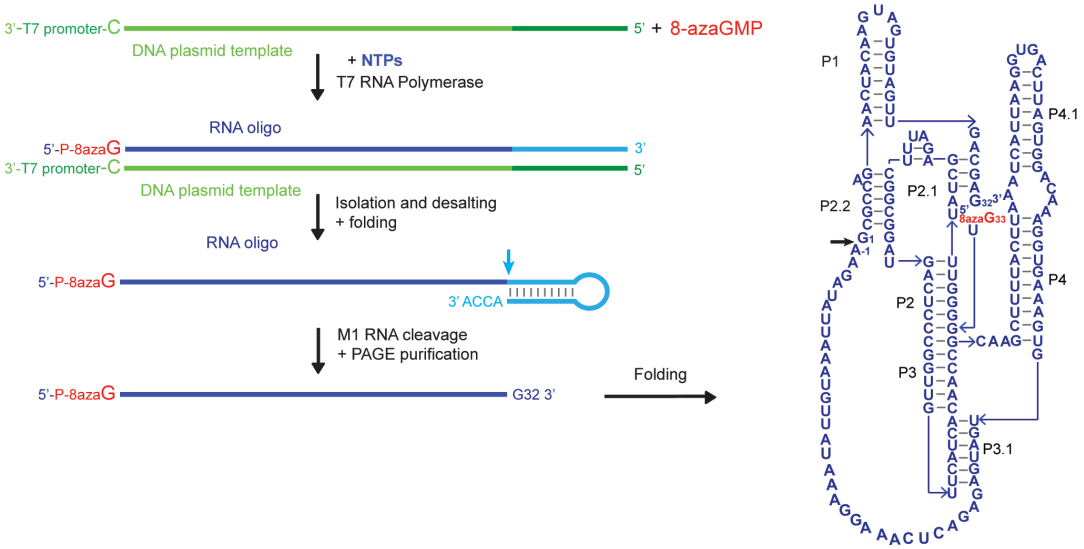
Supplementary Figure S1. RNAs used for kinetic and fluorescence experiments. **a)** G18 ribozyme used for kinetic studies, G68 ribozyme used to investigate ligation activity and G3 ribozyme used to evaluate product dissociation kinetics. **b)** GP23 is a 57 nt unstructured RNA used as a control for fluorescence studies.



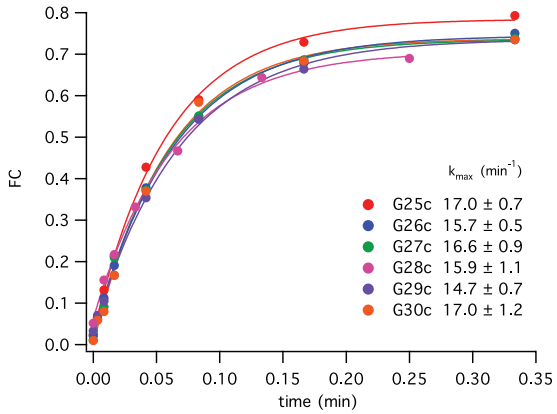
Supplementary Figure S2. Minimal kinetic mechanism of the *glmS* ribozyme reaction that includes binding of the cofactor GlcN6P, self-cleavage and product dissociation.



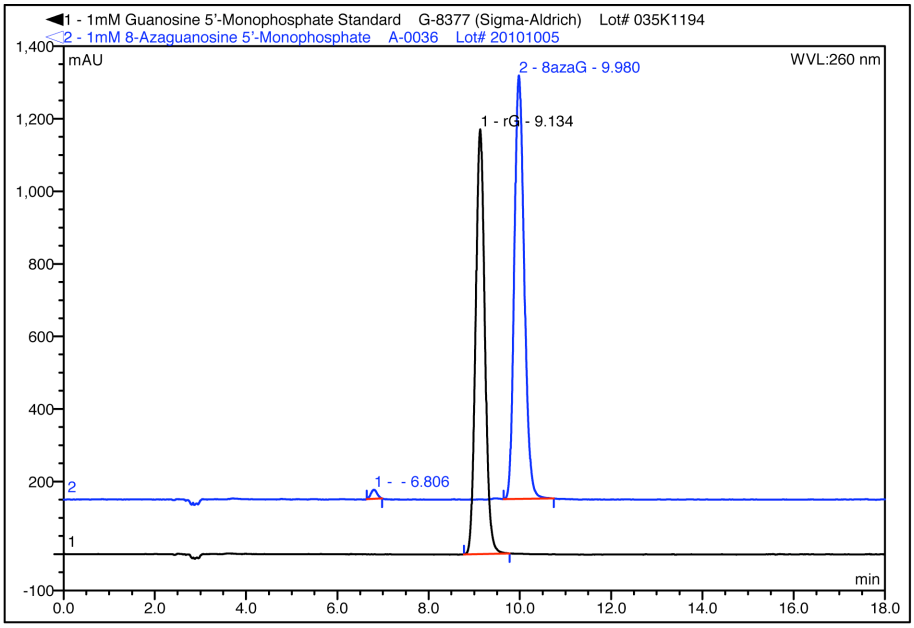
Supplementary Figure S3. *glmS* ribozyme affinity for GlcN6P and Mg^{2+}. **a)** GlcN6P concentration dependence of G18 binding at different pH values (50 mM buffer, 0.1 mM EDTA, 50 mM $MgCl_2$, 25 ˚C). **b)** Comparison of GlcN6P binding curves for G18 and G68 (50 mM HEPES pH 7.5, 0.1 mM EDTA, 50 mM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



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) for the *glmS* circular permutes with different chain lengths.

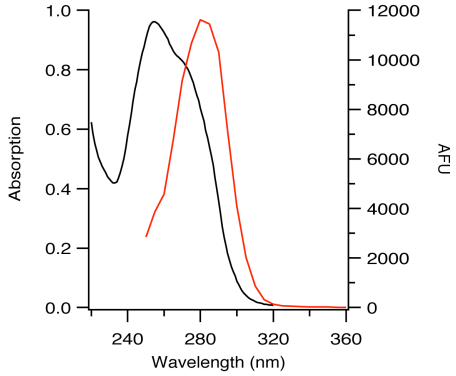


No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	6.81		22.058	3.556	1.34	n.a.	BMB*^
2	9.98	8azaG	1037.775	261.834	98.66	n.a.	BMB^
Total:			1059.833	265.389	100.00	0.000	

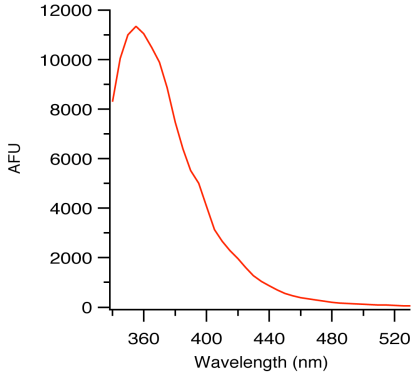
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	9.13	rG	1170.297	275.165	100.00	n.a.	BMB^
Total:			1170.297	275.165	100.00	0.000	

Supplementary Figure S6. HPLC analysis of 8-Azaguanosine 5'-Monophosphate. Quality control reversed phase HPLC chromatogram. 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 °C.[1]

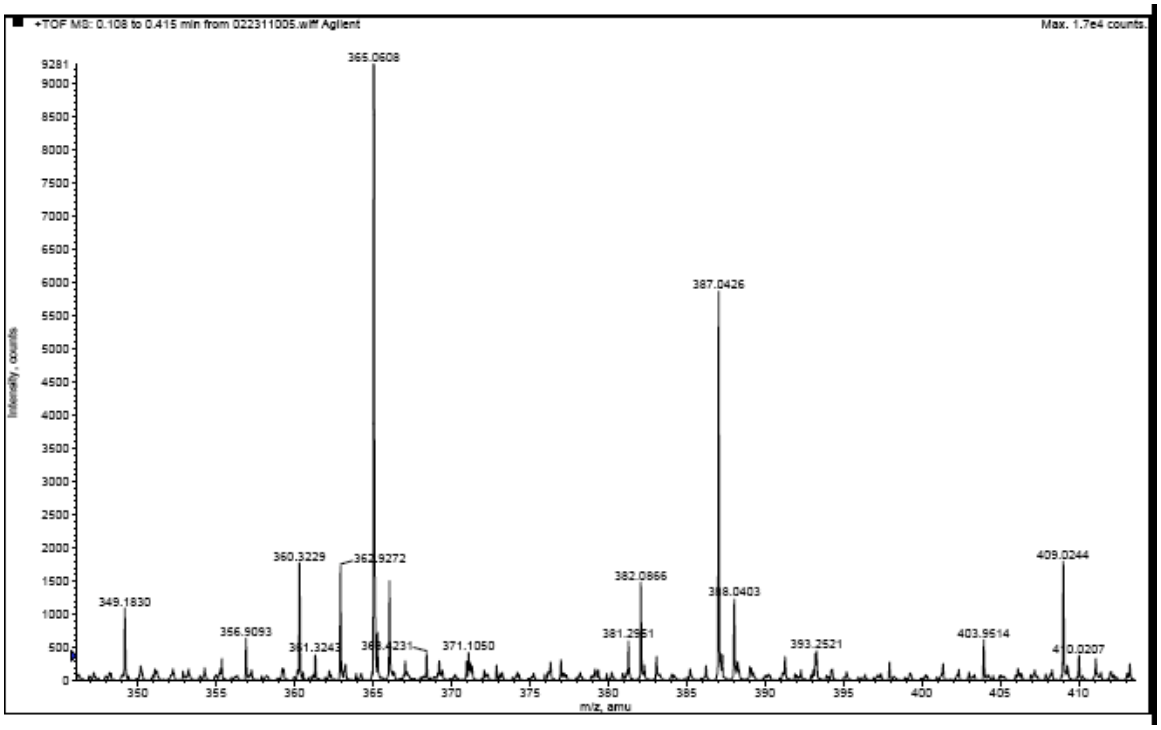
a



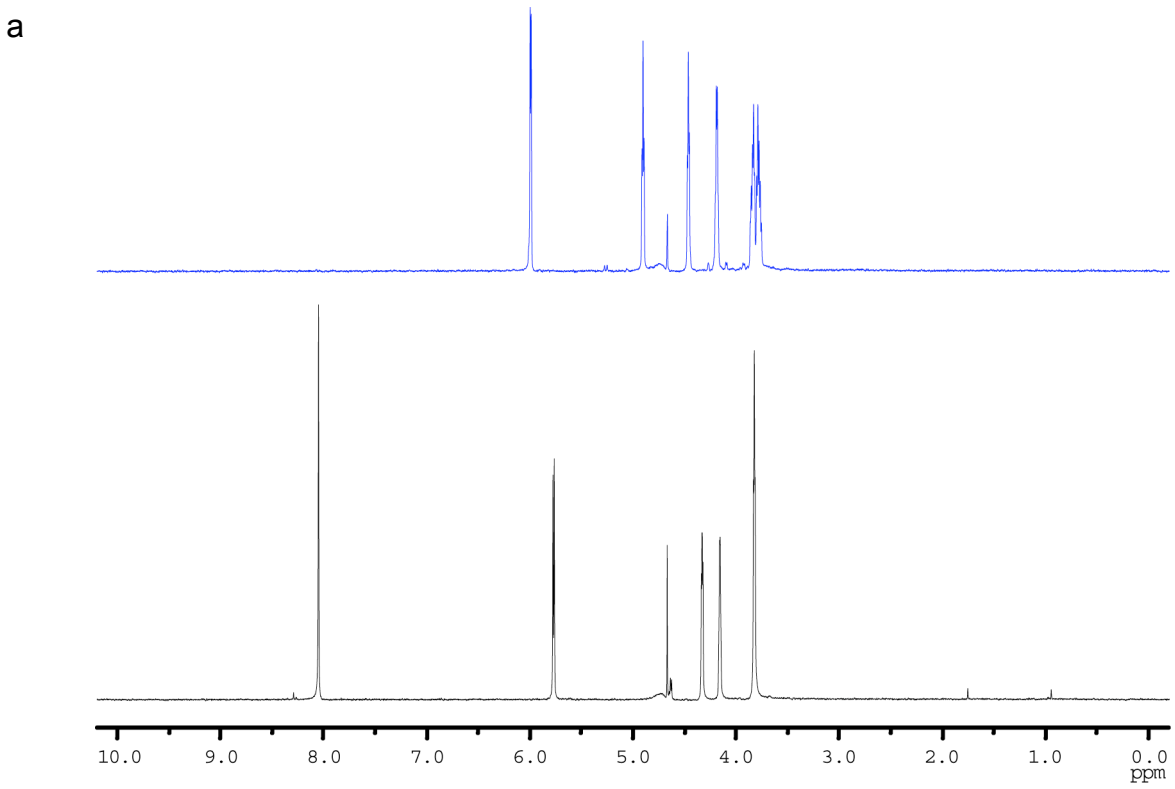
b

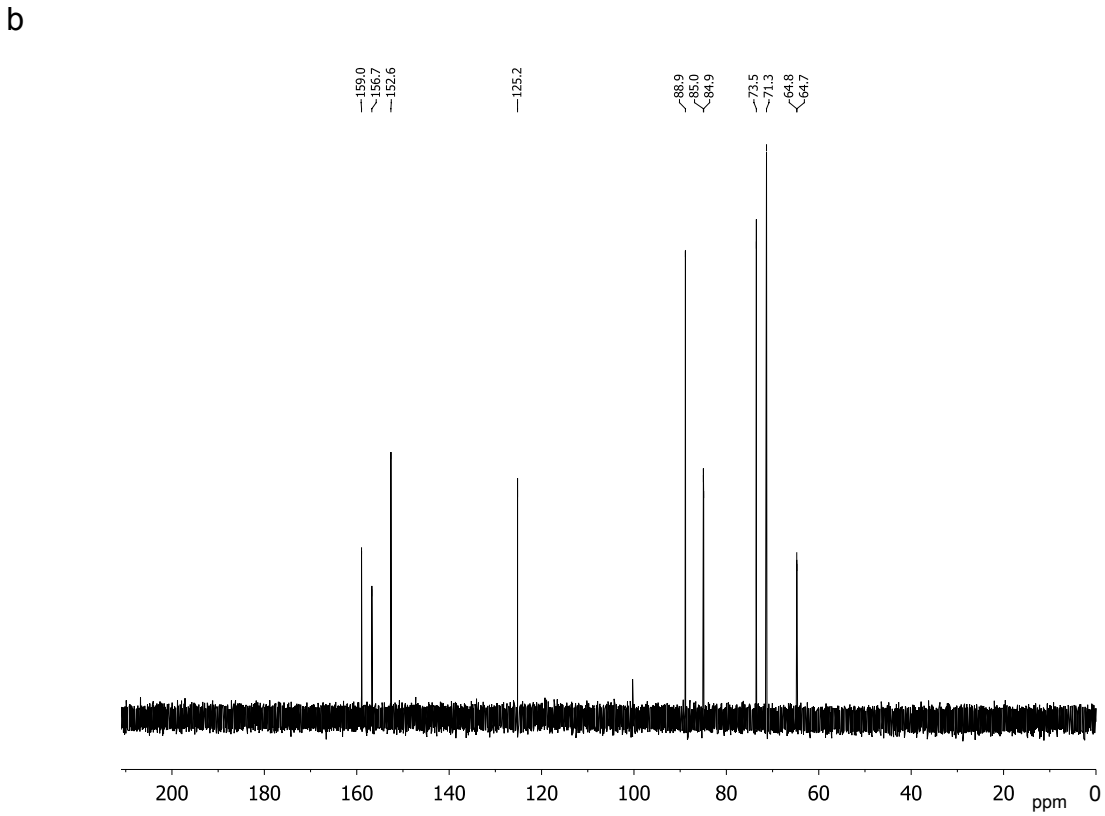


c



Supplementary Figure S7. UV and MS analysis of 8-Azaguanosine 5'-Monophosphate. **a)** 1mM 8azaGMP pH 7.6 UV spectrum (black) and excitation spectrum (red). b) 1mM 8azaGMP pH 7.6 emission spectrum. **b)** 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 (D_2O) of 5 mM 8azaGMP (top) and guanosine 5′-monophosphate (bottom). **b)** ^{13}C NMR spectrum (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.