SUPPLEMENTARY INFORMATION

Pistol Ribozyme Adopts a Pseudoknot Fold Facilitating Site-specific In-line Cleavage

Aiming Ren[1,2,4], Nikola Vušurović[3,4], Jennifer Gebetsberger[3], Pu Gao[2], Michael Juen[3], Christoph Kreutz[3], Ronald Micura[3] and Dinshaw J. Patel[2]

[1]Life Sciences Institute, Zhejiang University,
Hangzhou, 310058, China

[2]Structural Biology Program
Memorial Sloan-Kettering Cancer
New York, NY, 10065

[3]Institute of Organic Chemistry
Leopold-Franzens University and
Center of Molecular Biosciences CMBI
Innsbruck A-6020, Austria

[4]These authors made equal contributions

*Corresponding authors: pateld@mskcc.org (DJP) and ronald.micura@uibk.ac.at (RM)

SUPPLEMENTARY RESULTS

Supplementary Table 1. Crystallographic statistics for *env25* pistol ribozyme

Crystal	**Native**	**$[Ir(NH_3)_6]^{3+}$ soak**	**Mn^{2+} soak**
Data collection	24-ID-C	24-ID-C	24-ID-C
Space group	$P322_1$	$P322_1$	
Cell dimensions			
a, *b*, *c* (Å)	56.2, 56.2, 89.3	56.5, 56.5, 88.3	56.0, 56.0, 89.5
α, β, γ (º)	90, 90, 120	90, 90, 120	90, 90, 120
		Peak	
Wavelength (Å)	0.9792	1.1052	1.7712
Resolution (Å)	89.3-2.73 (2.86-2.73)*	88.3-2.68 (2.81-2.68)	89.5-3.27 (3.53-3.27)
R_{pim}	0.037(0.727)	0.038(0.618)	0.052 (0.589)
$I/\sigma I$	12.8 (0.9)	14.9 (1.2)	9.4 (1.1)
Completeness (%)	99.8 (100)	99.8 (100)	95.7 (98.5)
Redundancy	6.2 (6.7)	6.2 (6.4)	3.1 (3.2)
Refinement			
Resolution (Å)	48.7-2.73	49.0-2.68	48.5-3.27
No. reflections	4612	8819	3743
R_{work}/R_{free}	0.20/0.25	0.19/0.24	0.24/0.29
No. atoms			
RNA	1237	1237	1237
Cations	3	7	2
Water	1	15	2
B-factors			
RNA	112.7	103.8	129.2
Cations	94.9	107.8	112.2
Water	103.4	85.1	83.1
R.m.s deviations			
Bond lengths (Å)	0.017	0.009	0.004
Bond angles (º)	1.45	1.04	1.07

*Values for the highest-resolution shell are in parentheses.

SUPPLEMENTARY FIGURE CAPTIONS

Supplementary Figure 1. Long-range pairing alignments in the structure of the env25 pistol ribozyme. a, Representation of the continuous stacking alignment involving stem segments (starting from the bottom) P3, non-canonical paired segment, PK and P1. **b**, Hydrogen-bond network involving the U17-A18-A19-A20-A21-(U20)-A23 segment and the minor groove of stem P1. **c**, The minor groove triple involving the minor groove edge of G24 and the Watson-Crick G5-C12 pair. G5 is a highly conserved residue in the pistol ribozyme. **d**, Mg^{2+} binding site close to, but not directly coordinated to, the catalytic cleavage G53-U54 site in the pistol ribozyme. **e**, An expended view of the bound Mn^{2+} cation-binding site at the G53-U54 cleavage site in the pistol ribozyme, with the Mn^{2+} anomalous electron density map contoured at σ level. Mn^{2+} cation is shown as a purple sphere.

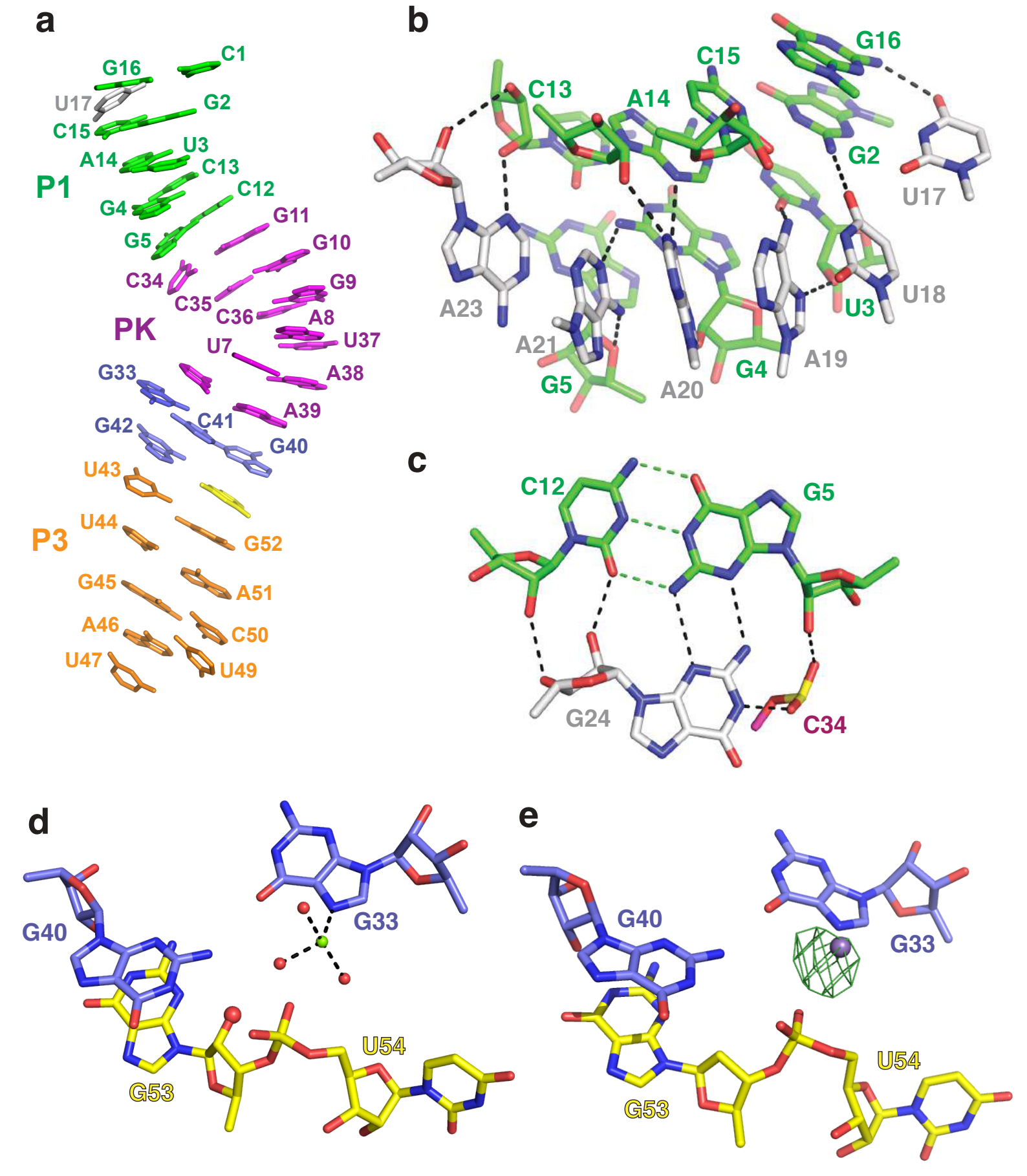
Supplementary Figure 2. Self-cleavage of the *env25* pistol ribozyme. a, Cartoon presentation of the cleavage assay. **b**, HPLC trace of wild type (WT), and mutants **c**, A32U, and **d**, A32G. Cleavage analyzed at 55 μM RNA each strand; 2 mM $MgCl_2$, 100 mM KCl, 30 mM HEPES, pH 7.5, 23 °C. R 47-nt ribozyme, S 11-nt substrate; C1 and C2, 6-nt and 5-nt cleavage products. HPLC conditions: Dionex DNAPac colum (4 x 250 mm), 80 °C, 1 mL min^{-1}, 0–60% buffer B in 45 min. Buffer A: Tris–HCl (25 mM), urea (6 M), pH 8.0. Buffer B: Tris–HCl (25 mM), urea (6 M), $NaClO_4$ (0.5 M), pH 8.0.

Supplementary Figure 3. NMR spectroscopic demonstration of stem P2 formation of the *env25* pistol ribozyme in solution. a, Chemical structures of

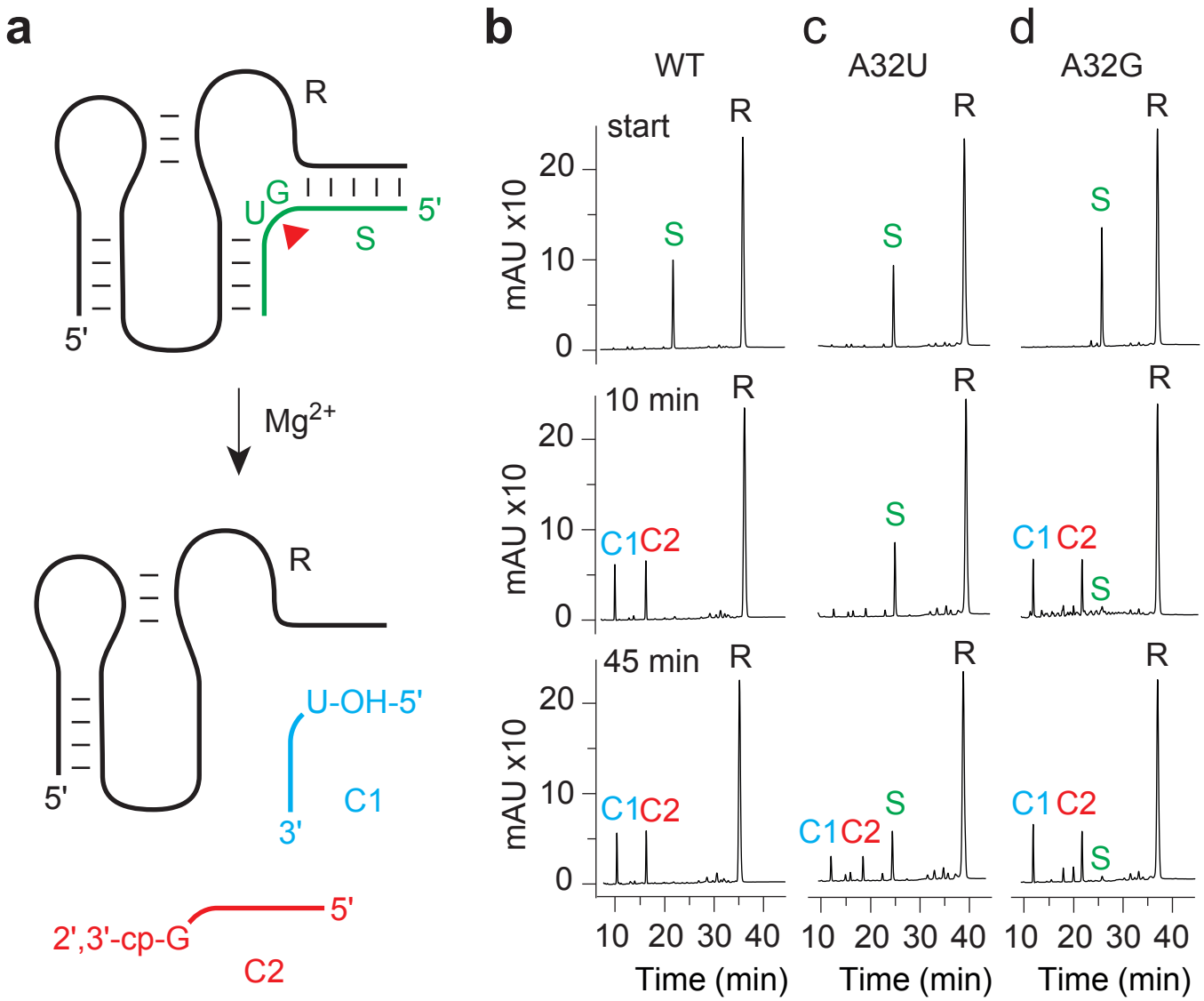
^{15}N1/3-labeled U26 used. **b**, $^{1}J(^{1}H,^{15}N)$ HSQC spectra of the ^{15}N-U26 pistol complex showing the appearance of a correlation corresponding to substrate binding.

Supporting Figure 4. **NMR spectroscopic analysis of the *env25* pistol ribozyme in solution – reference experiments.** **a**, Chemical structure of the ^{13}C2-labeled adenosine used. **b**, $^{1}J(^{1}H, ^{13}C)$ HSQC spectra of a short RNA duplex with labeled A in a Watson-Crick base pair at varying pH values; the protonated form is in slow exchange; estimated pK_a value as indicated. **c**, Exemplary $^{1}J(^{1}H,^{13}C)$ HSQC spectra of pH-dependent NMR experiments of a ^{13}C2-A labeled single-stranded RNA (left), chemical shift changes of ^{13}C2-A, with changes in the pH value derived from $^{1}J(^{1}H,^{13}C)$ HSQC spectra (right); the line represents the least-square fit (also see reference[44]) estimated pK_a values as indicated. Conditions: *c*(RNA) = 80 to 120 µM; 25 mM NaCl, 2 mM $MgCl_2$, 15 mM sodium phosphate, H_2O/D_2O 9:1, 298 K.

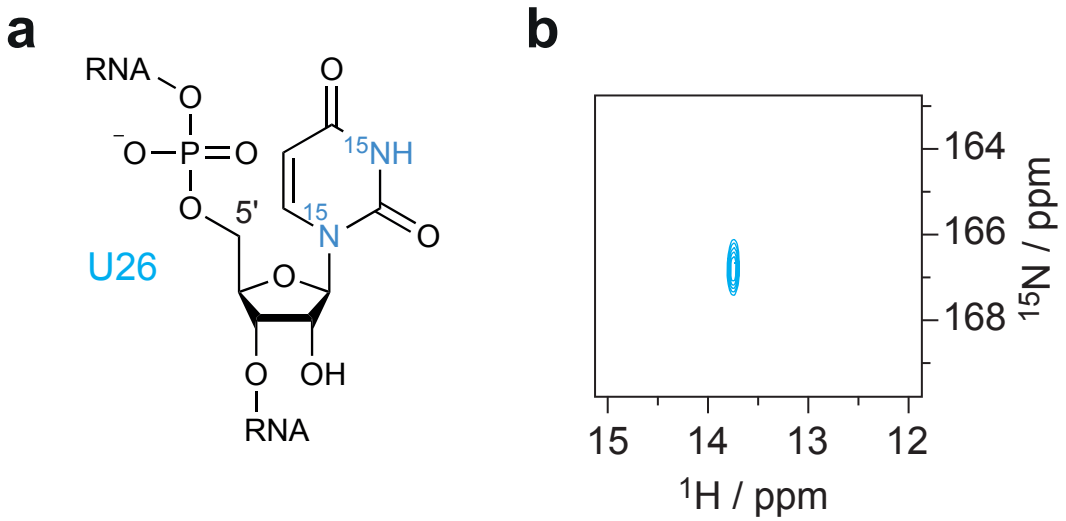
Supplementary Figure 5. Temperature dependence of the cleavage kinetics of the *env25* pistol ribozyme. **a**, Chemical structure of Ap nucleoside used. **b**, Quantitative assay for rate determination at 20 °C. Exemplary fluorescence time course recorded at two different temperatures can be compared as shown in panel b (20 °C) and Fig. 6c (15 °C).


a
G16
C1
U17
G2
C15
U3
A14
C13
P1
C12
G4
G11
G5
G10
C34
G9
C35
C36
A8
PK
U37
U7
A38
G33
A39
G42
C41
G40
U43
U44
P3
G52
G45
A51
A46
C50
U47
U49
b
C15
G16
C13
A14
G2
U17
A23
U18
U3
A21
A19
G4
G5
A20
c
C12
G5
G24
C34
d
G40
G33
U54
G53
e
G40
G33
U54
G53

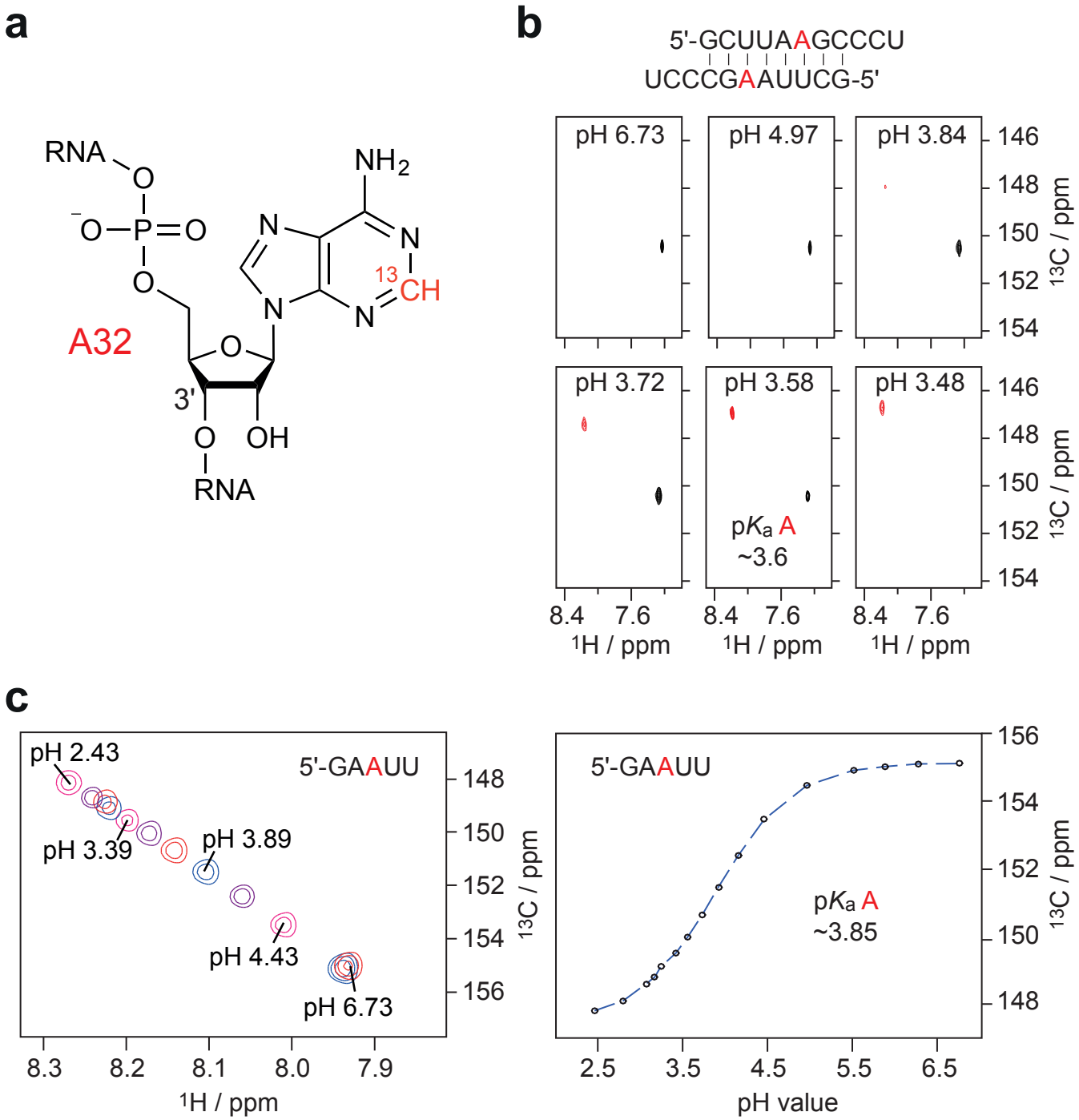
Sup. Fig. 1


a
R
G
U
5'
S
5'
Mg2+
R
U-OH-5'
5'
C1
3'
2',3'-cp-G
5'
C2
b
c
d
WT
A32U
A32G
start
10 min
45 min
R
S
C1
C2
mAU x10
0
10
20
10 20 30 40
Time (min)

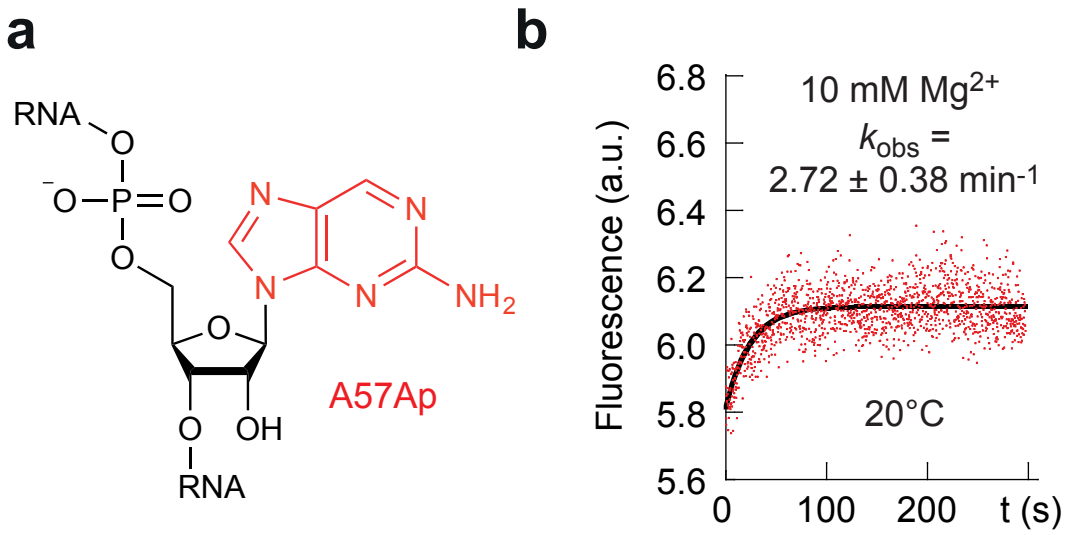
Sup. Fig. 2


a
RNA
O
O
O
P
O
O
5'
U26
O
15NH
15
N
O
O
OH
O
RNA
b
164
166
168
15N / ppm
15
14
13
12
1H / ppm

Sup. Fig. 3


a
RNA
O
O
P
O
O
O
NH2
N
N
N
13CH
N
A32
O
3'
O
OH
RNA
b
5'-GCUUAAGCCCU
UCCCGAAUUCG-5'
pH 6.73
pH 4.97
pH 3.84
pH 3.72
pH 3.58
pH 3.48
pKa A
~3.6
146
148
150
152
154
13C / ppm
8.4
7.6
1H / ppm
c
pH 2.43
5'-GAAUU
pH 3.39
pH 3.89
pH 4.43
pH 6.73
148
150
152
154
156
13C / ppm
8.3
8.2
8.1
8.0
7.9
1H / ppm
5'-GAAUU
pKa A
~3.85
156
154
152
150
148
13C / ppm
2.5
3.5
4.5
5.5
6.5
pH value

Sup. Fig. 4


a
RNA
O
⁻O—P=O
O
N
N
N
N
NH2
O
A57Ap
O
OH
RNA
b
10 mM Mg2+
kobs =
2.72 ± 0.38 min-1
Fluorescence (a.u.)
6.8
6.6
6.4
6.2
6.0
5.8
5.6
20°C
0
100
200
t (s)

Sup. Fig. 5