Supporting Information for

Time-Resolved RNA SHAPE Chemistry

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Benzoyl cyanide 2′-*O*-adduct formation and hydrolysis. Adduct formation between [^{32}P]-labeled 3′-phosphoethyl-5′-adenosine (pAp-ethyl)[1] and benzoyl cyanide (BzCN, Sigma-Aldrich) was followed by adding 10% (vol/vol) BzCN (60 mM final, in DMSO; 35.2 μL) to 1.1× reaction buffer [235.4 μL, 11.1 mM $MgCl_2$, 111 mM NaCl, 111 mM Hepes (pH 8.0)] using a chemical quench-flow instrument[2] (KinTek, Model RQF-3; drive syringes were 1.0 and 10 mL for the BzCN reagent and buffer reaction loops, respectively) and quenched with 1 vol 250 mM dithiothreitol [at the following reaction times (in sec): 0.02, 0.07, 0.15, 0.25, 0.40, 0.60, 0.80, 1.0, 1.3, 1.7, 2.5]. Each quenched reaction was precipitated by addition of 10 vol isobutanol, resolved by gel electrophoresis (30% polyacrylamide; 29:1 acrylamide:bisacrylamide; 0.4 mm × 28.5 cm × 23 cm; 30 W, 45 min), and quantified by phosphorimaging. We observed a single adduct band under all conditions, consistent with a mechanism in which BzCN reacts at the 2′-OH group and not significantly with other positions in the ribose or base moieties.

Rate constants for BzCN hydrolysis were obtained using an equation that accounts for parallel reaction of BzCN by 2′-*O*-adduct formation (k_{adduct}) and hydrolysis ($k_{hydrolysis}$):[3]

$$\text{Fraction product} = 1 - exp[(k_{adduct}/k_{hydrolysis})(e^{-k_{hydrolysis}t} - 1)]$$

(see Figure 3 in main text). The term $k_{adduct}/k_{hydrolysis}$ was obtained from the fraction adduct formed at long time points, where (as $t \rightarrow \infty$) $k_{adduct}/k_{hydrolysis} = -\ln(1 - \text{fraction product}) = 0.02$ for all experiments.

Structure-Selective RNA Modification. The RNase P specificity domain RNA[4] was synthesized as described.[1] RNA (60 pmol) in 60 μL sterile water was heated at 95 °C for 2 min, cooled on ice, treated with 36 μL of 3× no-Mg^{2+} folding buffer [333 mM NaCl, 333 mM Hepes (pH 8.0)], and incubated at 37 °C for 5 min. Tertiary structure folding was initiated by adding 12 μL of 10× $MgCl_2$ (100 mM). After mixing, 9 μL of this solution was removed (at 5, 10, 15, 20, 30, 60, 120, 180, 300, 600, 900, and 1200 s) and added directly to 1 μL 10× BzCN (600 mM in DMSO). No-reagent control reactions were added to 1 μL neat DMSO. Modified RNA was recovered by ethanol precipitation [90 μL sterile water, 5 μL NaCl (5 M), 1 μL glycogen (20 mg/mL), 400 μL ethanol; 30 min at –80 °C] and resuspended in 10 μL of TE [10 mM Tris (pH 8.0), 1 mM EDTA].

Primer Extension. The general procedure was that outlined previously.[1,5,6] Briefly, a fluorescently labeled DNA primer (5′ VIC- or NED-labeled GAA CCG GAC CGA AGC CCG; 3 μL, 0.3 μM) was annealed to the RNA (10 μL, from the previous step) by heating at 65 °C (6

min) and 35 °C (5 min). Reverse transcription buffer and Superscript III were added and the reactions incubated at 52 °C for 30 min. Primer extension reactions were quenched by adding an equal volume of a mixture of sodium acetate (1.5 M, pH 5.2) and EDTA (40 mM, pH 8.0) and the resulting cDNAs were recovered by ethanol precipitation, washed twice with 70% ethanol, dried by vacuum for 10 min, and resuspended in 10 μL de-ionized formamide. Dideoxy sequencing markers were generated using unmodified RNA and primers labeled with unique fluorophores (6-FAM or PET, 0.6 μM), and by adding 1 μL of 2′,3′-dideoxycytosine (10 mM) or 2′,3′-dideoxyguanosine (0.25 mM) triphosphate after addition of reverse transcription buffer. cDNA extension products were separated by capillary electrophoresis using an Applied Biosystems 3130 DNA sequencing instrument.

Data Analysis. Raw traces from the ABI 3130 were processed using ShapeFinder.[7] Data sets were normalized by excluding the 2% most reactive nucleotides (3 total) and dividing by the average intensity of the next 8% most reactive nucleotides (12 total). After data normalization, time-dependent changes in reactivity were judged to be significant if they were 0.2 SHAPE units or greater. Sixteen nucleotides exhibited significant reactivity changes by this criterion; the average change in SHAPE reactivity was 0.4. Folding rates for individual nucleotides were obtained by normalizing intensities (I) to the first time point and fitting to either a single

$$I = A + (1 - A)e^{-k_2 t}$$

or double

$$I = A + (1 - A - B)e^{-k_1 t} + Be^{-k_2 t}$$

exponential. Nucleotides 130, 175, 176, 177, 178, 179, 180, 206 and 208 were fit as a single exponential and nucleotides 189, 190, 195, 196, 217, 220, and 229 were fit to the double exponential (Table S1). Secondary structure for the RNase P specificity domain was adapted from Lescoute and Westhof.[8]

References

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Nucleotide	fast k_1 (s^{-1})	slow k_2 (s^{-1})	Folding domain
A130	—	0.006 ± 0.002	
U175	—	0.006 ± 0.001	T-loop
G176	—	0.004 ± 0.002	T-loop
A177	—	0.002 ± 0.0005	T-loop
A178	—	0.004 ± 0.0008	T-loop
A179	—	0.004 ± 0.001	T-loop
G180	—	0.0030 ± 0.001	T-loop
U189	0.06 ± 0.03	0.002 ± 0.003	J11/12
G190	0.07 ± 0.03	0.004 ± 0.004	J11/12
A195	0.05 ± 0.04	0.002 ± 0.006	J11/12
G196	0.04 ± 0.01	0.003 ± 0.003	J11/12
A206	—	0.002 ± 0.001	GAAA Tetraloop-Receptor
A208	—	0.004 ± 0.0009	GAAA Tetraloop-Receptor
G217	0.05 ± 0.02	0.003 ± 0.002	J12/11
A221	0.08 ± 0.07	0.005 ± 0.005	J12/11
A229	0.02 ± 0.04	0.001 ± 0.001	

Table S1. Folding rate constants for nucleotides in the RNase P specificity domain involved in forming tertiary interactions.