

Linear Baseline Interpolation of the $\Delta U177$ Pseudoknot and p2b Hairpin dA/dT Derivatives. Before application of the sequential unfolding nonlinear least squares parameter curve-fitting algorithm to dA/dT curves, raw dA/dT curves were subjected to a linear baseline interpolation. Low and high temperature regions where dA/dT achieved constant values were identified and a linear baseline interpolation between these regions was determined using Origin or T-Melt. Figure 1 in Supporting Information gives examples of linear baselines determined for raw dA/dT curves for the $\Delta U177$ pseudoknot in 40 mM NaCl, with no cosolute, 2 M glycine betaine, and 2 M urea at both 260 and 280 nm. In Figure 1, with no added cosolute, the lower temperature region of the dA/dT curve just achieved a constant value and was subjected to a linear baseline interpolation between the low and high temperature regions where dA/dT was constant. At high concentrations of glycine betaine (1-2 M), the dA/dT curve sharpened significantly due to glycine betaine's isostabilizing ability so that the low and high temperature dA/dT values were nearly identical. At high concentrations of urea (1-2 M), the unfolding transitions were shifted to low enough temperatures so that the low temperature region of the dA/dT curve did not achieve a constant value. To establish a baseline at the low temperature region of the dA/dT curve at high urea concentrations, we used the average baseline value for urea-free solutions, compensated for the temperature shift of the unfolding transitions with urea. Application of a linear baseline interpolation to dA/dT curves with 135 mM NaCl followed a similar analysis. However, unlike dA/dT curves at 40 mM NaCl, dA/dT curves in urea solutions at 135 mM NaCl generally achieved constant or nearly constant dA/dT values at low temperature; unfolding transitions occurred at higher temperature in 135 mM NaCl than in 40 mM NaCl. We found this method achieved very consistent

melting temperatures and enthalpies for each of the transitions. A similar method was used for the p2b hairpin.

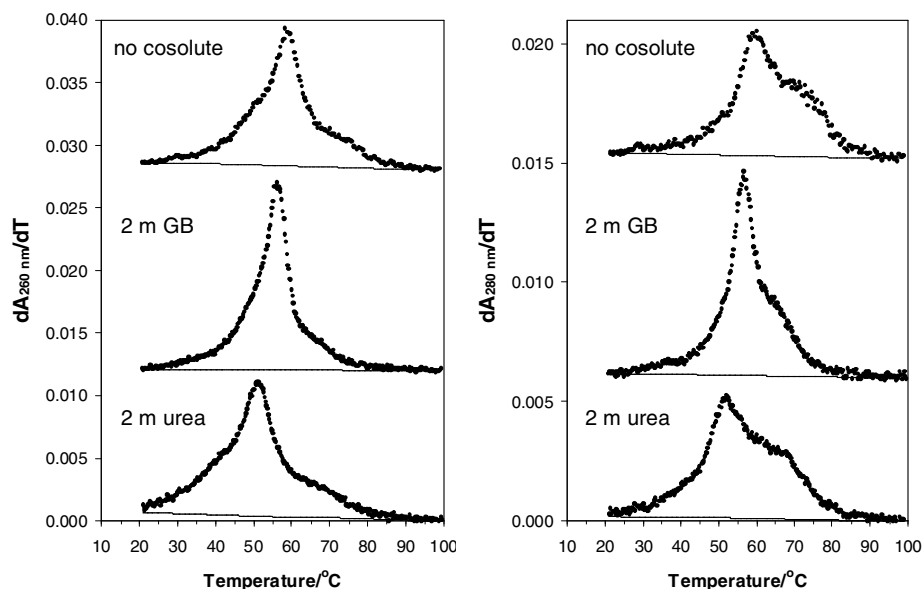


Figure 1

FIGURE 1: Raw absorbance first-derivative melting profiles (dA/dT) at 260 and 280 nm for the $\Delta U177$ hTR pseudoknot in 40 mM NaCl solutions containing either glycine betaine (GB) or urea. Calculated linear baselines (continuous lines) are superimposed on the raw experimental data. Curves are offset on the y-axes to illustrate trends in linear baselines and peak position with added cosolute.

Nonlinear Least Squares Parameters for the Sequential Transitions Unfolding Model. For each salt and cosolute concentration, initial applications of the sequential transition curve-fitting algorithm used several sets of initial guesses for the curve-fitting parameters. For the $\Delta U177$ pseudoknot, the melting temperature of stem 2 (the middle transition) was essentially independent of the initial guesses. However, for some initial

guesses, the amplitude A_2 of the stem 2 transition dropped to near zero, although the transition temperature $T_{m,2}$ was the same as with other initial guesses with a nonzero A_2 . In the case where A_2 was set to zero, the tertiary transition temperature (first transition) increased while the transition temperature of stem 1 (last transition) decreased, as compared to those initial parameter trials that led to a nonzero A_2 . To prevent the curve-fitting algorithm from setting A_2 to zero, A_2 was constrained to a minimum value for a given data set based on a nonzero A_2 value generated from an initial data set at the same salt and cosolute concentration that used initial parameter guesses that generated the nonzero A_2 value.

Since the p2b hairpin transitions were well separated in temperature, no amplitude constraints were used in the fitting procedure.

Table 1: Thermodynamic Parameters for Unfolding the Δ U177 hTR Pseudoknot and p2b hTR Hairpin Secondary and Tertiary Structures as Functions of Glycine Betaine (GB) and Urea Molality^{a,b}

	<u>ΔU177 tertiary</u>		<u>ΔU177 stem 2</u>		<u>ΔU177 stem 1</u>		<u>p2b uridine helix-pentaloop</u>		<u>p2b stem 1</u>		
	$T_m/^\circ\text{C}$	$\Delta H_{T_m}^\circ / \text{kcal}\cdot\text{mol}^{-1}$	$T_m/^\circ\text{C}$	$\Delta H_{T_m}^\circ / \text{kcal}\cdot\text{mol}^{-1}$	$T_m/^\circ\text{C}$	$\Delta H_{T_m}^\circ / \text{kcal}\cdot\text{mol}^{-1}$	$T_m/^\circ\text{C}$	$\Delta H_{T_m}^\circ / \text{kcal}\cdot\text{mol}^{-1}$	$T_m/^\circ\text{C}$	$\Delta H_{T_m}^\circ / \text{kcal}\cdot\text{mol}^{-1}$	
	40 mM NaCl										
[GB]/m											
0	52.6 ± 0.7	34.8 ± 0.9	59.3 ± 0.1	84.0 ± 0.7	71.0 ± 0.1	52.5 ± 0.7	47.4 ± 0.1	36.0 ± 0.2	64.5 ± 0.1	48.4 ± 0.3	
0.5	52.1 ± 0.7	35.5 ± 0.8	58.5 ± 0.1	91.9 ± 0.7	69.5 ± 0.1	54.7 ± 0.8	47.2 ± 0.2	35.0 ± 0.3	62.8 ± 0.1	47.0 ± 0.4	
1.0	51.8 ± 0.7	37.1 ± 1.1	57.6 ± 0.1	98.6 ± 1.0	67.8 ± 0.1	59.9 ± 1.3	47.1 ± 0.3	35.6 ± 0.4	61.2 ± 0.1	49.3 ± 0.5	
1.5	51.5 ± 0.5	39.7 ± 0.9	56.9 ± 0.1	108.8 ± 0.8	66.1 ± 0.1	62.9 ± 1.1	46.3 ± 0.6	38.6 ± 0.7	59.4 ± 0.2	46.8 ± 0.7	
2.0	51.5 ± 0.3	42.5 ± 0.9	56.3 ± 0.1	114.5 ± 0.9	64.6 ± 0.2	60.6 ± 1.5	46.1 ± 0.4	34.9 ± 0.4	57.9 ± 0.2	47.2 ± 0.6	
[urea]/m											
0.5	49.7 ± 0.7	35.2 ± 1.0	57.3 ± 0.1	79.4 ± 1.0	69.7 ± 0.2	51.7 ± 0.8	45.0 ± 0.1	32.3 ± 0.3	63.2 ± 0.1	48.4 ± 0.3	
1.0	47.9 ± 0.9	32.2 ± 1.2	55.0 ± 0.1	79.9 ± 0.8	67.1 ± 0.2	45.2 ± 0.7	42.5 ± 0.2	31.4 ± 0.3	61.0 ± 0.1	47.1 ± 0.2	
1.5	46.0 ± 0.4	28.8 ± 1.5	53.1 ± 0.1	68.5 ± 0.8	66.7 ± 0.1	49.4 ± 0.5	40.6 ± 0.3	31.9 ± 0.4	59.7 ± 0.1	46.4 ± 0.4	
2.0	43.8 ± 0.3	31.3 ± 1.3	51.6 ± 0.1	68.1 ± 0.7	65.4 ± 0.1	47.1 ± 0.5	38.1 ± 0.1	32.8 ± 0.3	58.5 ± 0.1	47.0 ± 0.3	
	135 mM NaCl										
[GB]/m											
0	60.5 ± 0.8	34.2 ± 1.0	67.7 ± 0.1	86.3 ± 0.8	79.9 ± 0.1	52.7 ± 1.2	50.6 ± 0.1	33.3 ± 0.2	71.3 ± 0.1	53.1 ± 0.3	
0.5	60.5 ± 1.3	35.1 ± 1.0	67.3 ± 0.1	94.9 ± 1.1	78.8 ± 0.2	53.0 ± 1.6	50.4 ± 0.1	30.8 ± 0.2	70.2 ± 0.1	52.8 ± 0.4	
1.0	60.6 ± 1.0	37.3 ± 0.9	66.9 ± 0.1	101.1 ± 1.1	77.6 ± 0.2	57.1 ± 2.2	49.6 ± 0.2	29.9 ± 0.2	68.8 ± 0.1	53.0 ± 0.3	
1.5	60.9 ± 0.8	35.3 ± 0.7	65.9 ± 0.1	107.6 ± 0.8	75.6 ± 0.2	60.8 ± 2.0	49.7 ± 0.1	30.2 ± 0.2	67.3 ± 0.1	53.6 ± 0.4	
2.0	61.0 ± 0.7	34.6 ± 0.6	65.4 ± 0.1	113.9 ± 0.8	74.5 ± 0.2	62.3 ± 1.8	49.5 ± 0.2	30.6 ± 0.2	66.1 ± 0.1	53.6 ± 0.4	
[urea]/m											
0.5	58.8 ± 1.2	30.2 ± 1.2	65.6 ± 0.1	80.7 ± 1.0	78.3 ± 0.2	51.0 ± 1.1	48.5 ± 0.1	34.1 ± 0.2	69.7 ± 0.1	50.8 ± 0.4	
1.0	56.2 ± 0.9	28.7 ± 1.0	63.7 ± 0.1	77.6 ± 0.8	76.6 ± 0.2	47.3 ± 1.0	46.4 ± 0.1	36.0 ± 0.2	68.2 ± 0.1	50.6 ± 0.2	
1.5	54.4 ± 0.9	32.5 ± 0.8	62.2 ± 0.1	78.0 ± 0.9	75.4 ± 0.2	47.6 ± 0.9	44.5 ± 0.1	35.1 ± 0.3	66.7 ± 0.1	49.0 ± 0.4	
2.0	52.6 ± 0.6	31.4 ± 0.7	60.5 ± 0.1	79.0 ± 0.8	73.4 ± 0.2	42.1 ± 0.7	41.9 ± 0.2	31.6 ± 0.5	65.2 ± 0.1	47.0 ± 0.4	

^aAll RNA-cosolute solutions prepared in a 10 mM sodium phosphate, pH 7.0 buffer.

^bAverage of a minimum of two independent experiments with standard errors from the sequential transitions fitting algorithm.

Table 2: Sequential Two-State Unfolding A_i Fitting Parameters at 260 and 280 nm for 2.25 μ M Δ U177 hTR Pseudoknot as Functions of Glycine Betaine (GB) and Urea Molality^{a,b,c}

	A_1^{260}	A_2^{260}	A_3^{260}	ΣA_i^{260} ^d	A_1^{280}	A_2^{280}	A_3^{280}	ΣA_i^{280} ^d
	40 mM NaCl							
[GB]/m								
0	0.100 ± 0.009	0.055 ± 0.007	0.049 ± 0.008	0.205 ± 0.014	0.030 ± 0.002	0.030 ± 0.003	0.051 ± 0.002	0.111 ± 0.004
0.5	0.098 ± 0.014	0.048 ± 0.003	0.041 ± 0.003	0.188 ± 0.015	0.032 ± 0.006	0.033 ± 0.002	0.050 ± 0.003	0.114 ± 0.007
1.0	0.095 ± 0.009	0.049 ± 0.005	0.035 ± 0.007	0.179 ± 0.012	0.031 ± 0.002	0.037 ± 0.001	0.045 ± 0.002	0.113 ± 0.003
1.5	0.099 ± 0.027	0.050 ± 0.015	0.035 ± 0.006	0.183 ± 0.032	0.037 ± 0.004	0.036 ± 0.003	0.044 ± 0.003	0.116 ± 0.006
2.0	0.099 ± 0.014	0.052 ± 0.011	0.037 ± 0.008	0.188 ± 0.020	0.038 ± 0.001	0.034 ± 0.001	0.045 ± 0.001	0.116 ± 0.002
[urea]/m								
0.5	0.095 ± 0.007	0.059 ± 0.005	0.044 ± 0.001	0.198 ± 0.009	0.030 ± 0.002	0.035 ± 0.003	0.053 ± 0.001	0.119 ± 0.004
1.0	0.094 ± 0.007	0.048 ± 0.005	0.053 ± 0.010	0.195 ± 0.013	0.032 ± 0.001	0.025 ± 0.002	0.058 ± 0.006	0.115 ± 0.006
1.5	0.106 ± 0.001	0.060 ± 0.002	0.047 ± 0.003	0.214 ± 0.004	0.031 ± 0.001	0.035 ± 0.004	0.048 ± 0.002	0.114 ± 0.005
2.0	0.095 ± 0.015	0.059 ± 0.004	0.048 ± 0.005	0.209 ± 0.016	0.028 ± 0.005	0.038 ± 0.005	0.055 ± 0.002	0.122 ± 0.008
	135 mM NaCl							
[GB]/m								
0	0.100 ± 0.008	0.049 ± 0.005	0.036 ± 0.004	0.185 ± 0.010	0.034 ± 0.003	0.031 ± 0.001	0.059 ± 0.001	0.124 ± 0.004
0.5	0.101 ± 0.001	0.054 ± 0.001	0.045 ± 0.002	0.200 ± 0.003	0.032 ± 0.001	0.027 ± 0.001	0.053 ± 0.003	0.112 ± 0.003
1.0	0.101 ± 0.010	0.049 ± 0.004	0.037 ± 0.004	0.188 ± 0.011	0.036 ± 0.002	0.035 ± 0.001	0.057 ± 0.001	0.128 ± 0.002
1.5	0.097 ± 0.003	0.049 ± 0.003	0.030 ± 0.005	0.176 ± 0.007	0.035 ± 0.001	0.037 ± 0.001	0.041 ± 0.003	0.112 ± 0.003
2.0	0.104 ± 0.001	0.054 ± 0.001	0.029 ± 0.001	0.187 ± 0.001	0.032 ± 0.001	0.033 ± 0.001	0.035 ± 0.002	0.100 ± 0.002
[urea]/m								
0.5	0.101 ± 0.003	0.055 ± 0.001	0.042 ± 0.003	0.199 ± 0.004	0.030 ± 0.002	0.029 ± 0.001	0.054 ± 0.001	0.112 ± 0.002
1.0	0.100 ± 0.003	0.051 ± 0.003	0.041 ± 0.003	0.192 ± 0.005	0.034 ± 0.002	0.031 ± 0.002	0.062 ± 0.004	0.127 ± 0.005
1.5	0.093 ± 0.009	0.047 ± 0.008	0.041 ± 0.009	0.181 ± 0.015	0.033 ± 0.001	0.033 ± 0.001	0.064 ± 0.007	0.131 ± 0.007
2.0	0.095 ± 0.006	0.047 ± 0.002	0.049 ± 0.005	0.190 ± 0.008	0.031 ± 0.001	0.022 ± 0.001	0.060 ± 0.001	0.113 ± 0.001

^aAll RNA-cosolute solutions prepared in a 10 mM sodium phosphate, pH 7.0 buffer.

^bAverage of a minimum of two independent experiments.

^c A_1 represents the absorbance change for the Δ U177 tertiary unfold, A_2 is the absorbance change for the Δ U177 stem 2 unfold, and A_3 is the absorbance change for the Δ U177 stem 1 unfold.

^dThe sum ΣA_i represents the total change in absorbance for unfolding the Δ U177 pseudoknot.

Table 3: Sequential Two-State Unfolding A_i Fitting Parameters at 260 and 280 nm for 2.25 μ M p2b hTR Hairpin as Functions of Glycine Betaine (GB) and Urea Molality^{a,b,c}

	A_1^{260}	A_2^{260}	ΣA_i^{260} ^d	A_1^{280}	A_2^{280}	ΣA_i^{280} ^d
	40 mm NaCl					
[GB]/m						
0	0.063 \pm 0.011	0.038 \pm 0.004	0.101 \pm 0.011	0.033 \pm 0.006	0.051 \pm 0.004	0.084 \pm 0.007
0.5	0.058 \pm 0.008	0.036 \pm 0.001	0.094 \pm 0.008	0.034 \pm 0.005	0.048 \pm 0.001	0.082 \pm 0.005
1.0	0.056 \pm 0.004	0.033 \pm 0.004	0.089 \pm 0.005	0.036 \pm 0.009	0.048 \pm 0.003	0.084 \pm 0.010
1.5	0.051 \pm 0.007	0.037 \pm 0.003	0.088 \pm 0.007	0.027 \pm 0.006	0.050 \pm 0.003	0.077 \pm 0.007
2.0	0.060 \pm 0.004	0.032 \pm 0.005	0.092 \pm 0.006	0.032 \pm 0.005	0.050 \pm 0.007	0.081 \pm 0.008
[urea]/m						
0.5	0.071 \pm 0.013	0.036 \pm 0.001	0.107 \pm 0.013	0.036 \pm 0.004	0.045 \pm 0.007	0.081 \pm 0.008
1.0	0.067 \pm 0.008	0.036 \pm 0.009	0.103 \pm 0.012	0.038 \pm 0.011	0.049 \pm 0.006	0.087 \pm 0.013
1.5	0.064 \pm 0.009	0.036 \pm 0.008	0.101 \pm 0.012	0.035 \pm 0.007	0.052 \pm 0.008	0.086 \pm 0.011
2.0	0.059 \pm 0.005	0.040 \pm 0.015	0.099 \pm 0.016	0.031 \pm 0.006	0.049 \pm 0.011	0.080 \pm 0.012
	135 mm NaCl					
[GB]/m						
0	0.096 \pm 0.016	0.045 \pm 0.009	0.141 \pm 0.018	0.053 \pm 0.009	0.064 \pm 0.012	0.118 \pm 0.015
0.5	0.088 \pm 0.007	0.038 \pm 0.001	0.125 \pm 0.007	0.050 \pm 0.006	0.055 \pm 0.011	0.104 \pm 0.006
1.0	0.098 \pm 0.006	0.036 \pm 0.004	0.134 \pm 0.007	0.055 \pm 0.005	0.056 \pm 0.007	0.111 \pm 0.005
1.5	0.089 \pm 0.004	0.032 \pm 0.001	0.121 \pm 0.004	0.049 \pm 0.014	0.053 \pm 0.001	0.102 \pm 0.001
2.0	0.094 \pm 0.018	0.035 \pm 0.002	0.128 \pm 0.018	0.050 \pm 0.010	0.055 \pm 0.003	0.105 \pm 0.011
[urea]/m						
0.5	0.064 \pm 0.006	0.033 \pm 0.002	0.097 \pm 0.007	0.033 \pm 0.003	0.046 \pm 0.001	0.078 \pm 0.003
1.0	0.059 \pm 0.001	0.035 \pm 0.002	0.093 \pm 0.002	0.029 \pm 0.001	0.046 \pm 0.001	0.075 \pm 0.001
1.5	0.055 \pm 0.001	0.034 \pm 0.002	0.089 \pm 0.002	0.029 \pm 0.001	0.046 \pm 0.003	0.075 \pm 0.003
2.0	0.063 \pm 0.012	0.035 \pm 0.005	0.098 \pm 0.013	0.037 \pm 0.009	0.049 \pm 0.002	0.086 \pm 0.009

^aAll RNA-cosolute solutions prepared in a 10 mM sodium phosphate, pH 7.0 buffer.

^bAverage of a minimum of two independent experiments.

^c A_1 represents the absorbance change for the p2b uridine helix-pentaloop unfold and A_2 is the absorbance change for the p2b stem 1 unfold.

^dThe sum ΣA_i represents the total change in absorbance for unfolding the p2b hairpin.