Cold Denaturation of the Hammerhead Ribozyme

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Supporting Information.

Derivation of the fitting equations

Optical thermal melt data were fit to the double-baseline equation:

$$\Delta\varepsilon_R = (m_i T + b_i)(1-\alpha) + (m_f T + b_f)\alpha$$

where $\Delta\varepsilon_R = \theta$ / (32,980 · [HH16] · 0.1 cm · 55 nucleotides), *m* and *b* are the initial and final baseline slopes and intercepts, respectively, and α is the fraction of folded HH16.

α was derived as follows, where T_m is the melting temperature, C_T is the total strand concentration of HH16, *K* is the equilibrium constant for the unfolding reaction, and *R* is the gas constant. Boxed equations are from Turner, D. In *Nucleic Acids: Structures, Properties and Functions*; Bloomfield, V. A.; Crothers, D. M.; Tinoco, I., Jr., Eds.; University Science Books: Sausalito, CA, 2000; pp. 272-273.

$$\Delta G^\circ = -RT\ln K = \Delta H^\circ - T\Delta S^\circ$$

$$-RT\ln K = \Delta H^\circ - T\Delta H^\circ\left(\frac{\Delta S^\circ}{\Delta H^\circ}\right)$$

and

$$\boxed{\frac{1}{T_m} = \frac{R\ln\left(\frac{C_T}{4}\right)}{\Delta H^\circ} + \frac{\Delta S^\circ}{\Delta H^\circ}}$$

therefore

$$-RT\ln K = \Delta H^\circ - T\Delta H^\circ\left(\frac{1}{T_m} - \frac{R}{\Delta H^\circ}\left(\ln\left(\frac{C_T}{4}\right)\right)\right)$$

$$-RT\ln K = \Delta H^\circ - \frac{T\Delta H^\circ}{T_m} + RT\ln\left(\frac{C_T}{4}\right)$$

$$\ln K = \frac{\Delta H^\circ}{R}\left(\frac{1}{T_m}\right) - \frac{\Delta H^\circ}{RT} - \ln\left(\frac{C_T}{4}\right)$$

$$\ln K + \ln\left(\frac{C_T}{4}\right) = \frac{\Delta H^\circ}{R}\left(\frac{1}{T_m} - \frac{1}{T}\right)$$

$$\ln\left(K \cdot \frac{C_T}{4}\right) = \frac{\Delta H^\circ}{R}\left(\frac{1}{T_m} - \frac{1}{T}\right)$$

$$K \cdot \frac{C_T}{4} = \exp\left(\frac{\Delta H^\circ}{R}\left(\frac{1}{T_m} - \frac{1}{T}\right)\right)$$

and

$$\boxed{K = \frac{2\alpha}{(1-\alpha)^2 \cdot C_T}}$$

therefore

$$\alpha = \left(\frac{1+4K \pm \sqrt{8K+1}}{4K}\right)$$

Figure S1

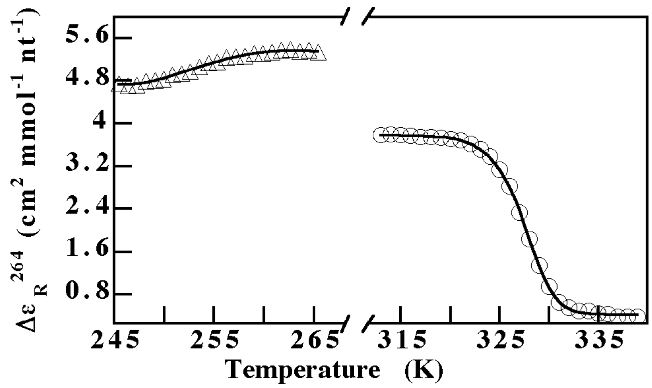

Figure S1. Comparison of hot (O) and cold (Δ) unfolding transitions of 10 μM HH16 in 50 mM acetate pH 5.0, 10 mM Mg^{2+}, and 40% MeOH (cold transition only). Excessive hydrolysis precluded analysis of high-temperature melting data of the Mg^{2+}-containing sample in the presence of MeOH. Solid lines are two-state fits of the CD data at 264 nm.