

Supplementary Material for:

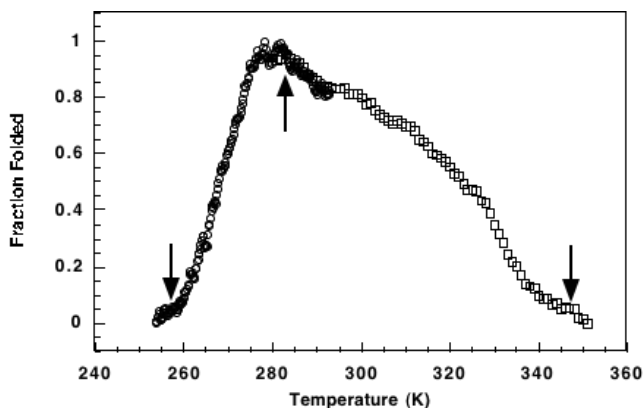
**Heat Capacity Changes in RNA Folding: Application of Perturbation
Theory to Hammerhead Ribozyme Cold Denaturation**

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Figure S1.

A.



B.

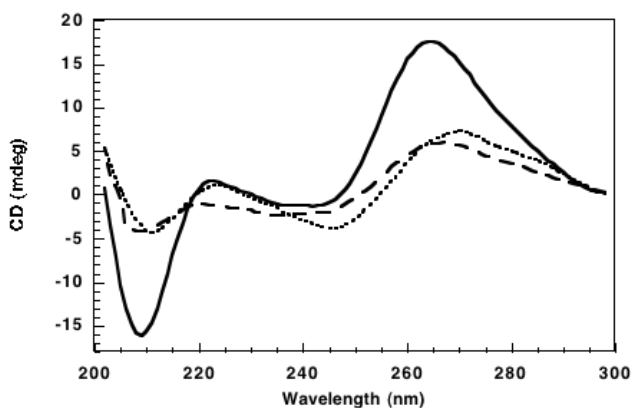


Figure S1. Melting curves for hot- and cold denaturation of the same sample of HH16, and CD spectra of folded and denatured states. (A) Melting curves for cold refolding (circles) and hot unfolding (squares) of the same sample of HH16, collected by CD at 265 nm. Datasets overlap in the region of 278-293 K. Arrows indicate temperatures at which wavelength spectra were taken of the sample. (B) CD spectra of cold-denatured (dotted line), folded (solid line), and heat-denatured (dashed line) states of HH16, collected at 257 K, 283 K, and 347 K, respectively.

HH16 ($A_{260} = 0.5$) in 40% methanol, 500 mM NaCl, 50 mM sodium cacodylate pH 6.6 was cold-denatured by slow cooling to 253 K, ramping at 8 min per degree within a jacketed cell attached to a circulating bath of 90% methanol. The sample was then refolded by ramping up the temperature to 293 K, again at 8 min per degree, monitoring the CD signal at 265 nm. The sample was transferred to a temperature-controlled Peltier cell, and allowed to equilibrate at 278 K. Then, the sample was heat-denatured by ramping at 0.5 K per min up to 353 K, monitoring the CD signal at 265 nm.