Supporting Information

Carma J. Nelson, Michael J. LaConte, Bruce E. Bowler
Direct Detection of Heat and Cold Denaturation for Partial Unfolding of a Protein

1. Conversion of raw absorbance data at 695 nm to ΔG_{obs}.

The method for accomplishing this conversion is shown graphically in Figure S1.

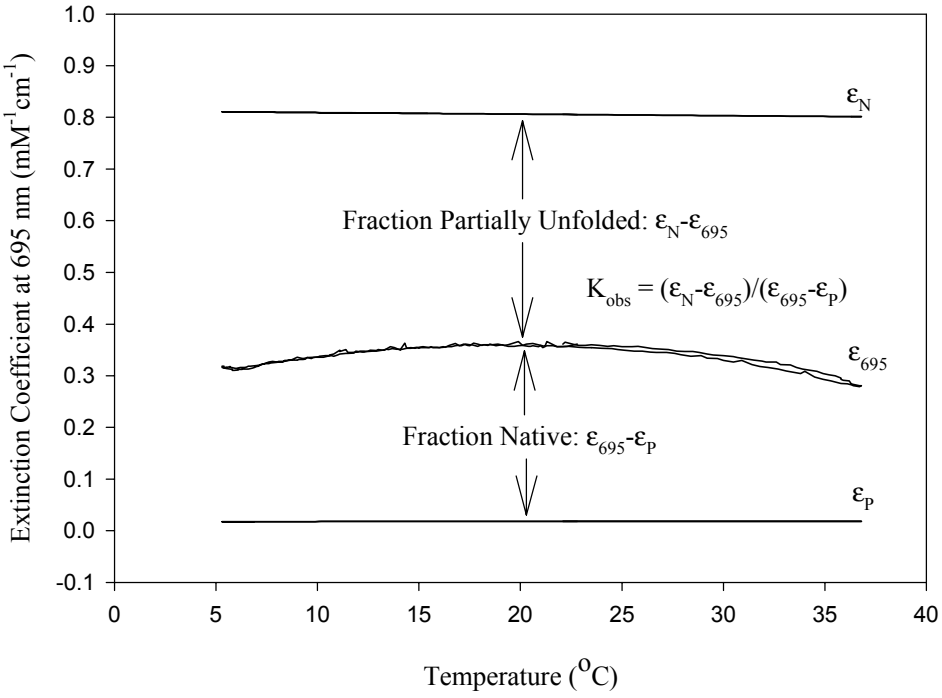

Figure S1. Graphical illustration of the method used to determine the equilibrium constants from plotting extinction coefficient data at 0.4 M gdnHCl versus temperature (^{o}C). K_{obs} is the observed equilibrium constant for the native to partially unfolded state transition at each temperature determined from the partially unfolded fraction (native baseline minus the sample value) divided by the native fraction (sample value minus the partially unfolded state baseline). Data were collected in the presence of 20 mM Tris, 40 mM NaCl at pH 7.5 (25 ^{o}C).

Absorbance data for the wild type iso-1-cytochrome *c* (contains cysteine 102 to serine mutation to prevent intermolecular disulfide dimer formation, as does the His 73 iso-1-cytochrome *c*) which does not form the partially unfolded state were collected at 695 nm as a

function of temperature and guanidine hydrochloride (gdnHCl) concentration (0, 0.1, 0.15, and 0.2 M gdnHCl). The absorbance at 695 nm, A_{695}, was fit to a straight line by least squares methods to provide native state baselines (A_{695} with heme-Met 80 ligation).

A_{695} data for the wild type iso-1-cytochrome *c* were collected as a function of temperature in 2, 2.5 and 3.0 M gdnHCl to obtain the behavior of A_{695} in the absence of heme-Met 80 ligation (partially unfolded and fully unfolded state behavior of this band are assumed to be the same since both states lack heme-Met 80 ligation and thus lack significant absorbance at this wavelength). The native baseline and partially unfolded state baseline intercepts at 0 ^{o}C as a function of [gdnHCl] were determined:

Eqn. (S1):

$$\varepsilon_{N(0\,^{o}C)} = \varepsilon_{695,N0} + m_{g,N}[\text{gdnHCl}]$$

Eqn. (S2):

$$\varepsilon_{P\,(0\,^{o}C)} = \varepsilon_{695,P0} + m_{g,P}[\text{gdnHCl}]$$

where $\varepsilon_{695,N0}$ and $\varepsilon_{695,P0}$ are the absorbance values at 0 ^{o}C for the native baseline and the intermediate state baseline in 0 M gdnHCl, $m_{g,N}$ and $m_{g,P}$ are the [gdnHCl] dependence of native and partially unfolded state baselines at 0 ^{o}C (see Table S1). The values from this equation were used to calculated the temperature dependence of the native and intermediate state baselines at different [gdnHCl]:

Eqn. (S3):

$$\varepsilon_{N}(T) = \varepsilon_{N(0\,^{o}C)} + m_{T,N}T$$

Eqn. (S4):

$$\varepsilon_{P}(T) = \varepsilon_{P(0\,^{o}C)} + m_{T,P}T$$

where $m_{T,N}$ and $m_{T,P}$ are the slopes of the native and partially unfolded state baselines as a function of temperature, T, respectively (see Table S1). Within error the values of $m_{T,N}$ and $m_{T,P}$ were independent of [gdnHCl]. The values of $\varepsilon_{N(0^oC)}$ and $\varepsilon_{P(0^oC)}$ were calculated at the appropriate [gdnHCl] for each data set.

Table S1. Parameters used to generate native and partially unfolded state baselines.

Parameters	Values	
$\varepsilon_{695,N0}$	0.747	$(mM^{-1}cm^{-1})$
$\varepsilon_{695,I0}$	0.0198	$(mM^{-1}cm^{-1})$
$m_{g,N}$	0.163	$(mM^{-1}cm^{-1}M^{-1})$
$m_{g,p}$	$-5.16x10^{-3}$	$(mM^{-1}cm^{-1}M^{-1})$
$m_{T,N}$	$-3.0x10^{-4}$	$(mM^{-1}cm^{-1}{}^oC^{-1})$
$m_{T,I}$	$1.2x10^{-5}$	$(mM^{-1}cm^{-1}{}^oC^{-1})$

2. Methodology for assessing the temperature dependence of the (pK_a- pH) term in equation 1.

Previous data on the His 73 variant of iso-1-cytochrome *c* show that the pK_a for the histidine driving the conformational transition is 6.7 ± 0.1.[1] Position 73 in the iso-1-cytochrome *c* sequence is highly solvent exposed,[2] so we used data for the temperature dependence of the pK_a of His 105 of ribonuclease, which is solvent exposed and has a similar pK_a near 25 ^oC.[3] The data for His 105 of ribonuclease are linear over the range studied (10-41 ^oC), giving dpK_a/dT =

[1] Nelson, C. J.; Bowler, B. E. *Biochemistry* **2000**, *39*, 13584-13594.
[2] Bowler, B. E.; May, K.; Zaragoza, T.; York, P.; Dong, A.; Caughey, W. S. *Biochemistry* **1993**, *32*, 183-190.
[3] Roberts, G. K. C.; Meadows, D. H.; Jardetzky, O. *Biochemistry* **1969**, *8*, 2053-2056.

-0.0204 (I = 0.2). Thus the equation used for the temperature dependence of the pK_a in equation 1 is:

Eqn. (S5):

$pK_a(T) = 6.7 - 0.0204*(T-298.15)$, where T is in degrees Kelvin.

Similar, more extensive data for imidazole[4] is linear over the range 0-50 ^oC and gives very similar values of dpK_a/dT of -0.0219 (I = 0) and -0.0222 (I = 0.1).

Our Tris buffer is adjusted to pH 7.5 at 25 ^oC. The pK_a versus temperature data for Tris deviate slightly from linearity,[5] so we selected data for pK_a versus T over the range 5 ^oC to 35 ^oC where our data were collected. In this temperature range, pK_a versus T for Tris buffer is linear with a correlation coefficient $R^2 = 0.9986$ and gives $dpK_a/dT = -0.0292$. Therefore the temperature dependence of the pH in equation 1 was represented by equation S6:

Eqn. (S6):

$pH = 7.5 - 0.292*(T-298.15)$, where T is in degrees Kelvin

Thus, the term (pK_a - pH) in equation 1 is given by equation S7:

Eqn. (S7):

(pK_a - pH) = Eqn. (S5) - Eqn. (S6) = $-3.43 + 0.0088*T$

Since the pK_a values of histidine and Tris have similar temperature dependences, the maximal correction due to the $RT\mathit{ln}(1 + 10^{(pKa - pH)})$ term in equation 1 is small, about 0.02 kcal/mol at low T (~ 5 ^oC) and about -0.02 kcal/mol at high T (~ 37 ^oC).

[4] Datta, S. P.; Grzybowski, A. K. *J. Chem. Soc. (B)* **1966**, 136-140.
[5] Datta, S. P.; Grzybowski, A. K.; Weston, B. A. *J. Chem. Soc.* **1963**, 792-796.