Fitting of Excess Heat Capacity Curves.

Experimental excess heat capacity curves were fit to the following model originally described by Wyman and Gill for n independent, two-state transitions. [1,2]

$$Cp(T) = \sum_{i=1}^{n} Cp(T)_i + \frac{\tau^2}{R} * \sum_{i=1}^{n} \frac{\left(\Delta H(T)_i^2\right) * \exp\left[-\Delta H(T)_i * (\tau - \tau_{mi})/R\right]}{\left\{1 + \exp\left[-\Delta H(T)_i * (\tau - \tau_{mi})/R\right]\right\}^2} \qquad \textbf{eq. (1)}$$

Equation 1 is modified from equation (5.36) given in Wyman and Gill to account for the temperature dependence of the enthalpy ($\Delta H(T)_i$) and to take account of the contributions of the differing native and denatured heat capacity of each of the sub-transitions. The impact of strand dissociation is not considered in this model, because concentration dependent denaturation studies show the denaturation process for our Ω-DNA's to behave in a pseudo-monomolecular manner. Nevertheless, we note, that strand separation may slightly impact the shape of the melting curves at high temperature.

In equation 1, $\tau = \frac{1}{T}$ is the inverse temperature at any point along the curve and $\tau_{mi} = \frac{1}{Tm_i}$ is the inverse melting temperature of the i th component; $\Delta H(T)_i$ is the enthalpy change associated with unfolding of the ith component at temperature T determined from the enthalpy change at T_m according to the standard relations:

$$\Delta H(T)_i = \Delta H(Tm)_i - \Delta Cp_i (Tm_i - T) \qquad \textbf{eq. (2a)}$$

and

$$\Delta Cp_i = Cp_i^D - Cp_i^N \qquad \textbf{eq. (2b)}$$

where D and N indicate denatured and native respectively.

We assume that each transition's contribution to the overall heat capacity change is proportional to it's contribution to the overall enthalpy change; specifically

$$\Delta Cp_i = \frac{\Delta H(T_m)_i}{\sum_{i=1}^{n} \Delta H(T_m)_i} \Delta Cp(T^*)$$

where $\Delta Cp(T^*)$ is the heat capacity change for the overall denaturation process at the weighted average transition temperature $T^* = \frac{\sum_{i=1}^{n} T_{mi}\Delta H_i}{\sum_{i=1}^{n} \Delta H_i}$.

The native and denatured state heat capacities of the i th component are assumed to change linearly with temperature and are described by equation 3:

$$Cp(T)_i^N = m^N T + Cp(0)^N$$ **eq. (3a)**

The heat capacity of the denatured state differs from that of the native state by ΔCp_i at T^*, that is $Cp(T^*)_i^D = Cp(T^*)_i^N + \Delta Cp_i$ so

$$Cp(T)_i^D = m^D (T - T^*) + Cp(T^*)^N + \Delta Cp_i$$ **eq. (3b)**

The imposition of linear models for the temperature dependences of the native and denatured heat capacities with the pre- or post-transition baselines of each transition sharing a common slope m^N or m^D may appear somewhat arbitrary, however, we note that the overall experimental excess heat capacity curves outside the melting domain are best described by linear changes in native and denatured heat capacities (**see figure S1**)

From the temperature dependence of the native and denatured state heat capacities the contribution of the i th component to the heat capacity baseline, $Cp(T)_i$ is calculated according to the following relation:

$$Cp(T)_i = \alpha(T)_i Cp(T)_i^N + (1 - \alpha(T)_i)Cp(T)_i^D$$ **eq. (4)**

where $\alpha(T)_i$ represents the fraction of the i th component that remains native at temperature T and is calculated according to equation 5.

$$\alpha(T)_i = \frac{\Delta H(T)_i - \left\langle \frac{\{\Delta H(T)_i * \exp[-\Delta H(T)_i * (\tau - \tau_{mi})/R]\}}{\{1 + \exp[-\Delta H(T)_i * (\tau - \tau_{mi})/R]\}} \right\rangle}{\Delta H(T)_i}$$ **eq. (5)**

Heat capacity curves were fit using the model described above and the Solver function in Microsoft Excel. The 2n+4 adjustable parameters in the fits were Tm_i; $\Delta H(Tm)_i$, $\Delta Cp(T^*)$, $Cp(0)^N$, m^D and m^N.

We fit our experimental data to this model for n =1, n=2 and n=3. Although all but one of our DSC melting curves show only a single near symmetrical denaturation peak giving the appearance of a single two state transition, we find that we cannot obtain satisfactory fits to our experimental data for n=1 (see **figure S1**). By contrast we obtained good agreement between the experimental curves and the fitted curves when n=2 for all Ω-DNA constructs. n=3 does not give a statistically significant improvement in fit of the experimental parameters. The parameters obtained from our curve fitting exercise are listed in table 1S (Supplementary Material).

(1) Gill, S. J.; Richey, B.; Bishop, G.; Wyman, J. *Biophys Chem* **1985**, *21*, 1-14.
(2) Wyman, J.; Gill, S. J. *Binding and Linkage. Functional Chemistry of Biological Macromolecules*; University Science Books: Mill Valley, CA, 1990.

TABLE 1S: Thermodynamic parameters derived by fitting experimental DSC curves to 2 independent two-state transitions (Eric- revised)

Ω-DNA Complex	**Transition**	**Tm [ºC]**	**ΔH_{deconv} [kcal mol^{-1}]**	**ΔCp_{deconv} [cal mol^{-1} K^{-1}]**
CAG	**T***	**63.0**	**173.7**	**1490**
	1	**60.9**	**90.9**	
	2	**65.5**	**79.7**	
CAG-F1	**T***	**62.6**	**164.0**	**1635**
	1	**59.3**	**81.7**	
	2	**66.0**	**82.3**	
CAG-F3	**T***	**62.7**	**169.4**	**1143**
	1	**60.5**	**91.7**	
	2	**65.2**	**77.7**	
CAG-F5	**T***	**62.2**	**147.7**	**165.6**
	1	**58.1**	**69.4**	
	2	**65.8**	**78.0**	
CAG-FStem	**T***	**52.5**	**143.1**	**1635**
	1	**49.1**	**64.5**	
	2	**55.2**	**78.5**	
CTG-FStem	**T***	**50.5**	**117.1**	**2160**
	1	**34.8**	**57.0**	
	2	**65.3**	**60.5**	

Figure S1 (Supplementary material) : Fitting of excess heat capacity curve of CAG Ω-DNA by one (A) two (B) or three (C) two state transitions as described above. Note that there is no statistical improvement in fit for n=3 versus n=2.