Supplementary Material

Analysis of melting curve data through fitting of a sigmoid

T_ms and energy parameters in the paper have been determined assuming that the maximum of the absorbance derivative corresponds approximately to the midpoint of transition. To check the validity of this approach, we determined the T_m alternatively by fitting a nonlinear curve to the experimental data.

First, a baseline was fitted to and subtracted from the linear part at lower temperatures. Next, a data window is chosen that only includes the transition of kissing complex to hairpin. The selected data window was normalized according to

$$f(T) = (f_{max} - f_{min}) * \frac{A260(T) - A\,260_{min}}{A260_{max} - A260_{min}} + f_{min},$$

where $A260_{min}$ and $A260_{max}$ are the upper and lower limits of the experimental data, f(T) is the fraction of hairpin, and f_{min} and f_{max} are fitting parameters for the upper and lower limits of the normalized data window. The normalized plot is fitted with a sigmoidal function

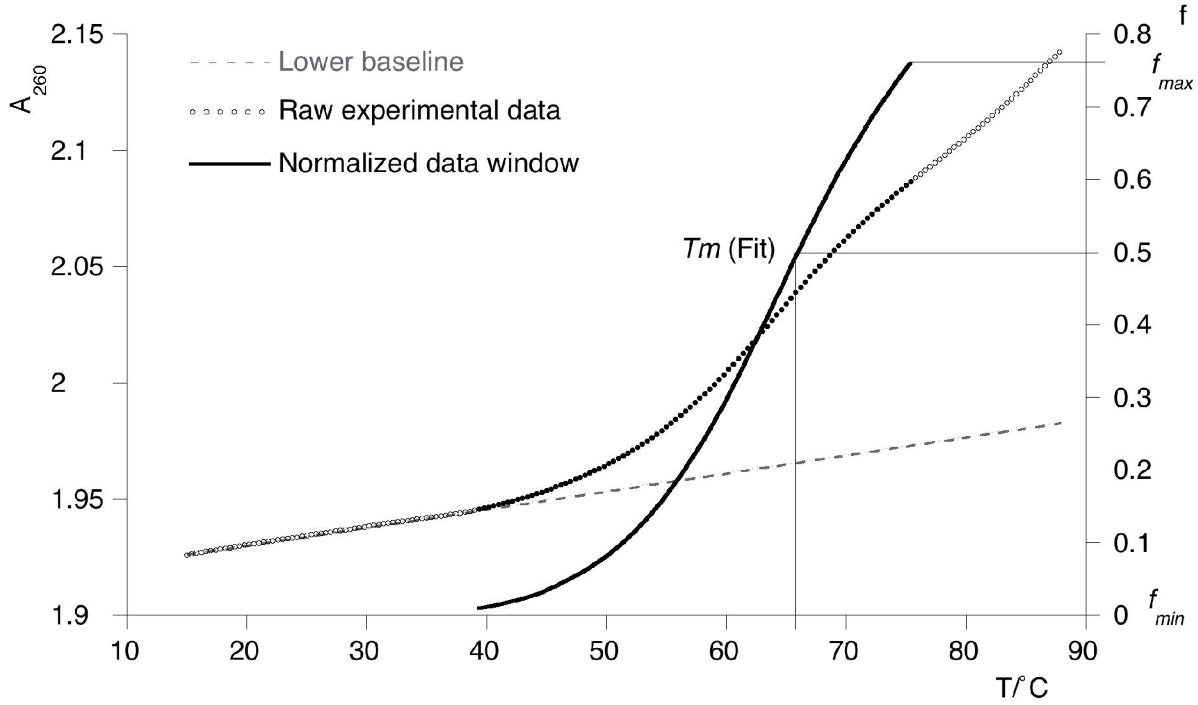
$$f(T) = \frac{\left(\frac{T}{Tm}\right)^n}{1+\left(\frac{T}{Tm}\right)^n}.$$

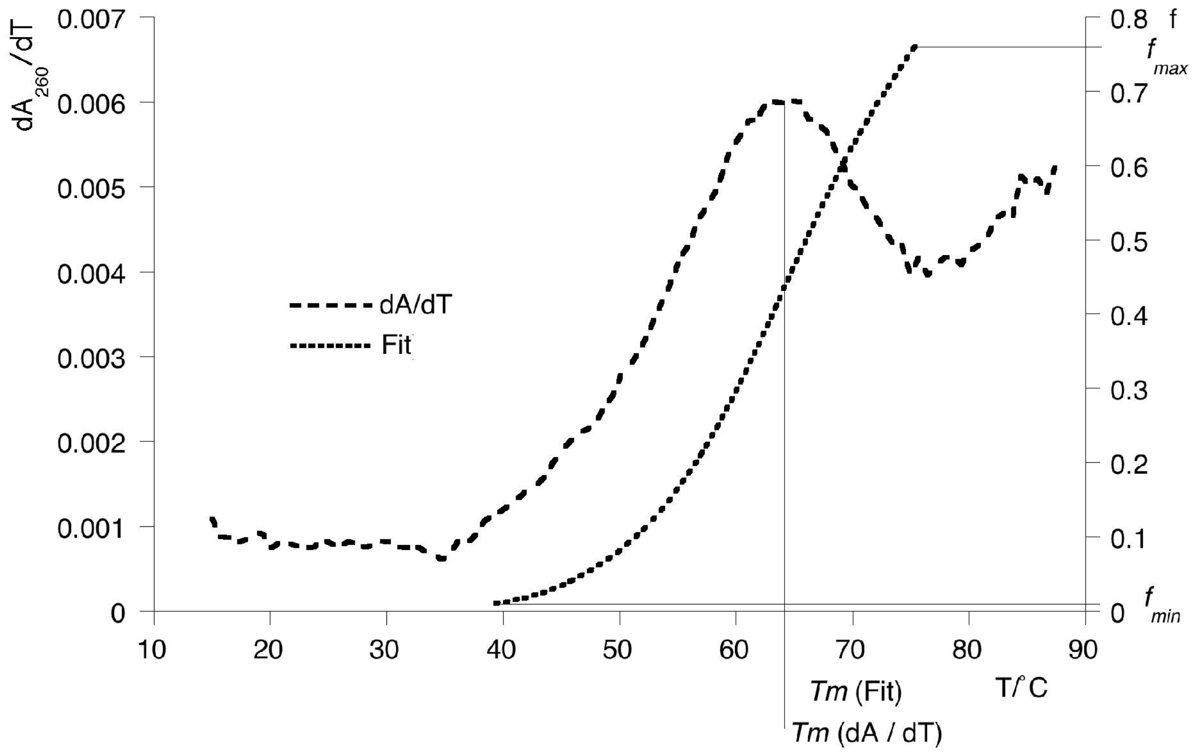
By varying the upper and lower boundaries f_{min} and f_{max}, the normalized curve is recursively adjusted, until an optimum fit is obtained as judged by the value of R. The sigmoidal function then gives a direct estimate of T_m.

Comparing the T_m obtained by both methods, we note a systematic difference of the Tm by about 2°C, as would be expected for an equilibrium involving annealing of two molecules (ref. 25). The resulting energy differences are seemingly small.

Data analysis:	***Derivative***	***Fit***
[CCGACC kissing]/µM	T_m/°C	T_m/°C
0.5	55.1	56.5
1	56.8	59.3
1.5	58.8	60.8
2	59.3	61.5
3	60.8	62.7

4	60.7	62.6
6	62.4	64.4
10	64.2	66.1
ΔH_{app} [kcal mol^{-1}]	**-68**	**-73.4**
ΔS_{app} [cal mol^{-1} K^{-1}]	**-175.8**	**-190.7**
ΔG_{app}^{37} [kcal mol^{-1}]	**-13.48**	**-14.24**
compare: ΔG_{duplex}^{37} [kcal mol^{-1}]	-9.5	-9.5
$\Delta\Delta G^{37}$ [kcal mol^{-1}]	-3.98	-4.74




dA$_{260}$/dT
0.007
0.006
0.005
0.004
0.003
0.002
0.001
0
10
20
30
40
50
60
70
80
90
T/°C
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
f
f$_{max}$
f$_{min}$
dA/dT
Fit
Tm (Fit)
Tm (dA / dT)