Thermodynamics of DNA hybridization

A combination of spectroscopy and calorimetry was used to determine the free energies of melting of short oligo-nucleotides. Based on these measurements the free energy of a helix can be determined based on10 sets of nearest-neighbor pairs shown on the next slide.

In addition to these values we need to know the free energy of the initiation (i.e. the first base pair). The overall free energy is then calculated from:

$$\Delta G^o = \Delta G^o \text{ (initiation)} + \Sigma\, \Delta G^o \text{ (nearest neighbors)}$$

			ΔG°	ΔH°	ΔS°
A 5′ – *A* 3′ – *T* *T*	→	5′ – *AA* 3′ – *TT*	– 7.9	– 38.1	– 101.3
T 5′ – *A* 3′ – *T* *A*	→	5′ – *AT* 3′ – *TA*	– 6.3	– 36.0	– 99.7
A 5′ – *T* 3′ – *A* *T*	→	5′ – *TA* 3′ – *AT*	– 3.8	– 25.1	– 71.5
C 5′ – *A* 3′ – *T* *G*	→	5′ – *AC* 3′ – *TG*	– 5.4	– 27.2	– 73.2
A 5′ – *C* 3′ – *G* *T*	→	5′ – *CA* 3′ – *GT*	– 7.9	– 24.3	– 55.0

Breslauer et al. PNAS 93 3746 (1986)

			ΔG°	ΔH°	ΔS°
G 5′ – *A* 3′ – *T* *C*	→	5′ – *AG* 3′ – *TC*	– 6.7	– 32.6	– 86.9
A 5′ – *G* 3′ – *C* *T*	→	5′ – *GA* 3′ – *CT*	– 6.7	– 23.4	– 56.0
G 5′ – *C* 3′ – *G* *C*	→	5′ – *CG* 3′ – *GC*	– 15.1	– 49.8	– 116.4
C 5′ – *G* 3′ – *C* *G*	→	5′ – *GC* 3′ – *CG*	– 13.0	– 46.4	– 112.1
C 5′ – *C* 3′ – *G* *G*	→	5′ – *CA* 3′ – *GT*	– 13.0	– 46.0	– 110.7

Breslauer et al. PNAS 93 3746 (1986)

Sample problem

Determine the melt temperature for the oligonucleotide

$$\begin{matrix} \text{5'-ATAGCA-3'} \\ + \\ \text{3'-TATCGT-5'} \end{matrix} \rightleftharpoons \begin{matrix} \text{5'-ATAGCA-3'} \\ \text{3'-TATCGT-5'} \end{matrix}$$

Solution:

$\Delta G^o = \Delta G^o$ (initiation) + $\Sigma\ \Delta G^o$ (nearest neighbors)

$$= \Delta G^o\ \{initial) + \Delta G^o\begin{pmatrix} AT \\ TA \end{pmatrix} + \Delta G^o\begin{pmatrix} TA \\ AT \end{pmatrix} + \Delta G^o\begin{pmatrix} AG \\ TC \end{pmatrix} + \Delta G^o\begin{pmatrix} GC \\ CG \end{pmatrix} + \Delta G^o\begin{pmatrix} CA \\ GT \end{pmatrix}$$

= 20.9 - 6.3 - 3.8 - 6.7 - 13.0 - 6.9

= -16.8 kJ

Initiation can be treated as a single value required to start the hybridization. It is unfavorable, but then all other interactions are favorable.

Sample problem

Determine the melt temperature for the oligonucleotide

$$\begin{matrix} \text{5'-ATAGCA-3'} \\ + \\ \text{3'-TATCGT-5'} \end{matrix} \rightleftharpoons \begin{matrix} \text{5'-ATAGCA-3'} \\ \text{3'-TATCGT-5'} \end{matrix}$$

Solution (cont;d):

$\Delta G^o = -16.8$ kJ

Notice that the the free energy of initiation is positive. Initiation is unfavorable because of the entropy that must be overcome to bring the chains together. To calculate the melt temperature we need the enthalpy of reaction as well.

$$\Delta H^o = \Delta H^o\begin{pmatrix} AT \\ TA \end{pmatrix} + \Delta H^o\begin{pmatrix} TA \\ AT \end{pmatrix} + \Delta H^o\begin{pmatrix} AG \\ TC \end{pmatrix} + \Delta H^o\begin{pmatrix} GC \\ CG \end{pmatrix} + \Delta H^o\begin{pmatrix} CA \\ GT \end{pmatrix}$$

$$= -36.0 \quad -25.1 \quad -32.6 \quad -46.4 \quad -24.3$$

$$= -164.4 \text{ kJ}$$

Sample problem

Given that ΔG^o = -16.8 kJ/mol and ΔH^o = -164.4 kJ/mol for the hexamer, determine the melt temperature.

A. 42 ^{o}C

B. 48 ^{o}C

C. 52 ^{o}C

D. 58 ^{o}C

Sample problem

Given that ΔG^o = -16.8 kJ/mol and ΔH^o = -164.4 kJ/mol for the hexamer, determine the melt temperature.

A. 42 ^oC

B. 48 ^oC

C. 52 ^oC

D. 58 ^oC

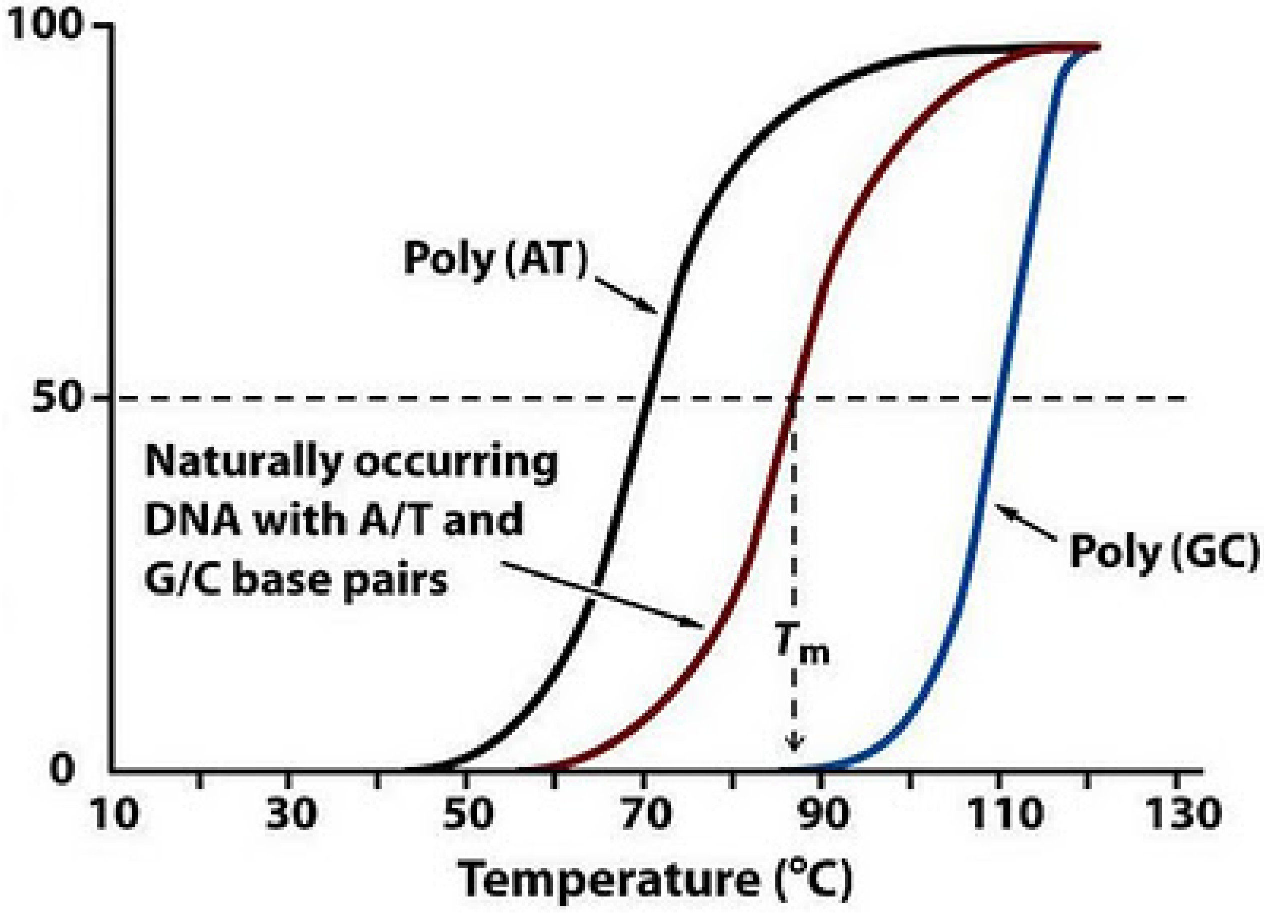
$\Delta S^o = (\Delta H^o - \Delta G^o)/T = (-164.4+16.8)/298 \times 1000$

$= -495.3$ J/mol-K

The melt temperature occurs when $\Delta G^o = 0$.

$T = \Delta H^o/\Delta S^o = -164{,}400/(-495.3) = 331\ K = 58\ ^oC$

DNA hyperchromicity



DNA hyperchromicity
Observed in reverse by fluorescence