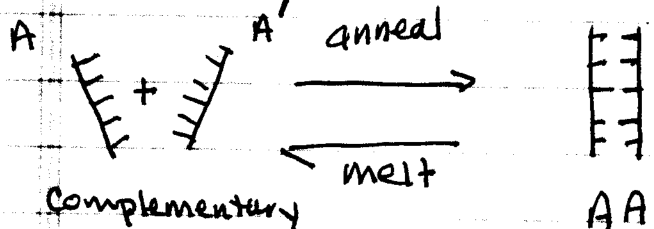
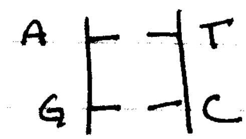
11/30/05

Cooperative Transitions I: DNA hybridization



Complementary oligomers

base pairs



$$K = \frac{[AA']}{[A][A']} \equiv \underbrace{\frac{c_{dim}}{c_A c_{A'}}}_{\text{useful notation}}$$

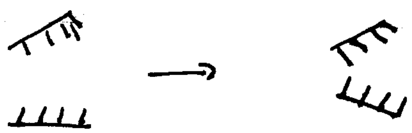
<u>Example</u>

5'-ATAGCA-3' + 5'-TGCTAT-3'

How do we predict T_m? At melting T (T_m), ½ oligomers are hybridized

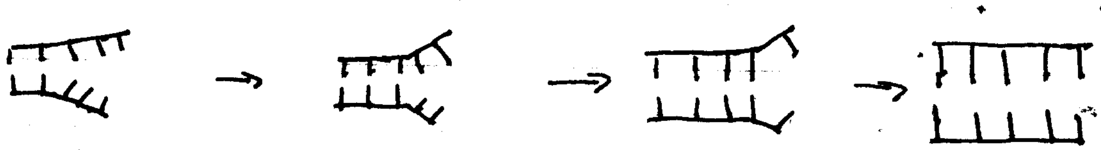
Know from experiments (and logic) that process can be broken into 2 steps:

1. initiation – formation of 1st bond



ΔG°_{init}

2. pairing (sequentially) of remaining bonds



ΔG°_1 ΔG°_2 ΔG°_3 ΔG°_4

How to Calculate ΔG° (and thus obtain "K")?

presume that

$$\underbrace{\Delta G^\circ = \Delta G^\circ_{init}}_{\text{step 1}} + \underbrace{\sum \Delta G^\circ_{bonds}}_{\text{sum of each bond in step 2}}$$

Since we know all possible base pairs, A–T, G–C can we make a table of ΔG° values for each pair?

Possible Ways to parse $\sum \Delta G^\circ_{bonds}$

1. Each pair has a ΔG° value independent of environment

```
A T A G C
| | | | |
| | | | |
T A T C G
```

$$\Delta G^\circ = \Delta G^\circ_{init} + 2\Delta G^\circ_{A-T} + 2\Delta G^\circ_{C-G}$$

* experimentally validated

2. ΔG° for a pair depends on the "nearest neighbor" pair formed just before it

```
A T A G C
| | | | |
| | | | |
T A T C G
```

$\boxed{\text{see handout with values for the 10 possible pairs}}$

$$\Delta G^\circ = \Delta G^\circ_{init} + \Delta G^\circ_{\substack{A-T\\T-A}} + \Delta G^\circ_{\substack{T-A\\A-T}} + \Delta G^\circ_{\substack{A-G\\T-C}} + \Delta G^\circ_{\substack{G-C\\C-G}}$$

at 37°C, 1 M NaCl

$$\Delta G^\circ = 8.1 - 3.7 - 2.4 - 5.4 - 9.3 - 6.0 = -18.7\ \frac{kJ}{mol}$$

From each $\Delta G°$, can calculate $\Delta S°$, $\Delta H°$ (see Table)

For this example

$$\Delta H° = \underset{\substack{\uparrow \\ \text{initiation}}}{0.8} - 30.2 - 30.2 - 32.7 - 41 - 35.6 = -168.9 \frac{\text{KJ}}{\text{mol}}$$

$$\Delta S° = \underset{\substack{\uparrow \\ \text{initiation}}}{-23.4} - 85.4 - 89.2 - 87.9 - 102.2 - 95 = -483 \frac{\text{J}}{\text{mol-K}}$$

Note $\Delta S° \equiv$ J/mol K NOT KJ/mol-K

How does the equilibrium depend on T?
It is reasonable (and backed up by experiment) to assume that $\Delta H°$ and $\Delta S°$ are independent of T $\Rightarrow$ Can use values from Table for $T \neq 37C$.

Then $\Delta G°_T \equiv \Delta G°$ at T of interest

$$\Delta G°_T = \Delta H° - T\Delta S° = -RT \ln K = -RT \ln \frac{C_{dim}}{C_A C_{A'}}$$

What we usually want is to know the fraction dimerized as function of T (melting curve).

Some notation

$C_{A,o}$ = initial concentration of A

$C_{A',o}$ = initial concentration of A'

If $C_{Ao} \neq C_{A',o}$, designate $C_{A,o}$ as "limiting" (ie $C_{Ao} < C_{A',o}$)

Then maximum number of dimers, $C_{dim,max}$, is

$C_{dim,max} = C_{Ao}$

call the fraction of total possible dimers f, ie

$$f = \frac{C_{dim}}{C_{dim,max}} = \frac{C_{dim}}{C_{A,0}}$$

and note $C_A = C_{A0} - C_{dim}$ $\quad C_{A'} = C_{A',0} - C_{dim}$

We can thus write

$$K = \frac{C_{dim}}{[C_{A0} - C_{dim}][C_{A',0} - C_{dim}]} = \frac{C_{dim}}{C_{A0}^2 \left[1 - \frac{C_{dim}}{C_{A0}}\right]\left[\frac{C_{A',0}}{C_{A,0}} - \frac{C_{dim}}{C_{A0}}\right]}$$

$$\boxed{K = \frac{f}{C_{A0}[1-f]\left[\frac{C_{A',0}}{C_{A,0}} - f\right]}}$$

Special but common case

$C_{A0} = C_{A'0}$ $\Rightarrow$ equal oligos at start

More notation (used in the literature)

$C_T = C_{A0} + C_{A',0} = 2C_{A0}$

$C_{dim,max} = \frac{1}{2} C_T$

Then $\quad K = \dfrac{f/2}{C_T\left(\frac{1}{2} - \frac{f}{2}\right)^2}$

and

$$\Delta G^\circ_T = \Delta H^\circ - T\Delta S^\circ = -RT \ln\left(\frac{f/2}{C_T\left(\frac{1}{2} - \frac{f}{2}\right)^2}\right)$$

We can solve to get $T(f)$, Temp at which a given f occurs.

$$T_f = \frac{\Delta H^\circ}{\Delta S^\circ + R\ln\left(\frac{C_T\left(\frac{1}{2} - \frac{f}{2}\right)^2}{f/2}\right)} = \frac{\Delta H^\circ}{\Delta S^\circ + R\ln C_T + R\ln\left(\frac{\left(\frac{1}{2} - f/2\right)^2}{f/2}\right)}$$

Define T_m as T where $f = 0.5$

Analysis of $T(f)$ for our example compared to oligomers twice as long shows that longer oligomers have higher, sharper T_m

Oligomer		$\Delta H°$ kJ/mol	$\Delta S°$ kJ/mol-K
ATAGC TATCG	"short"	−168.9	−0.483
ATAGCATAGC TATCGTATGC	"long"	−336.2	−0.919

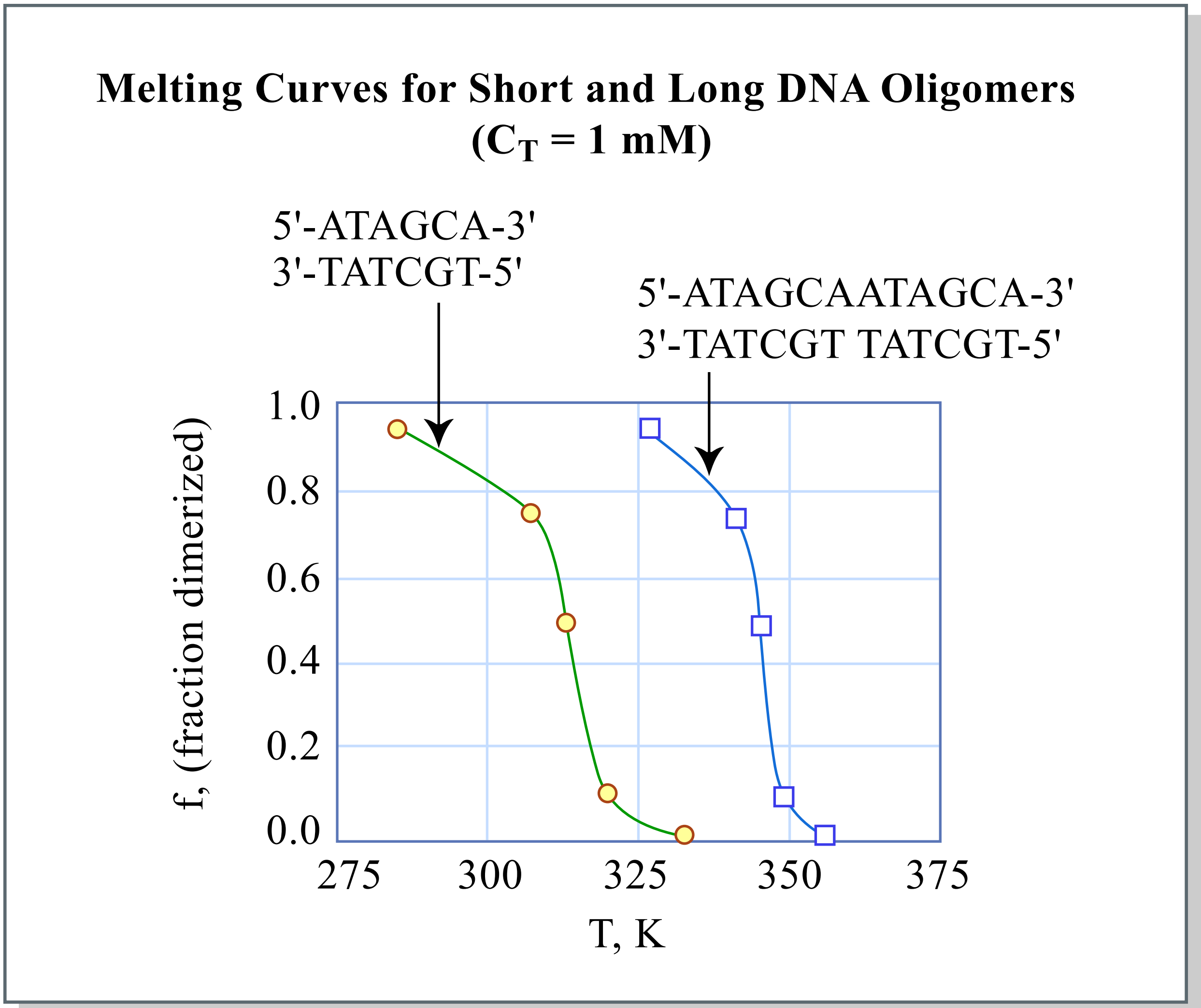
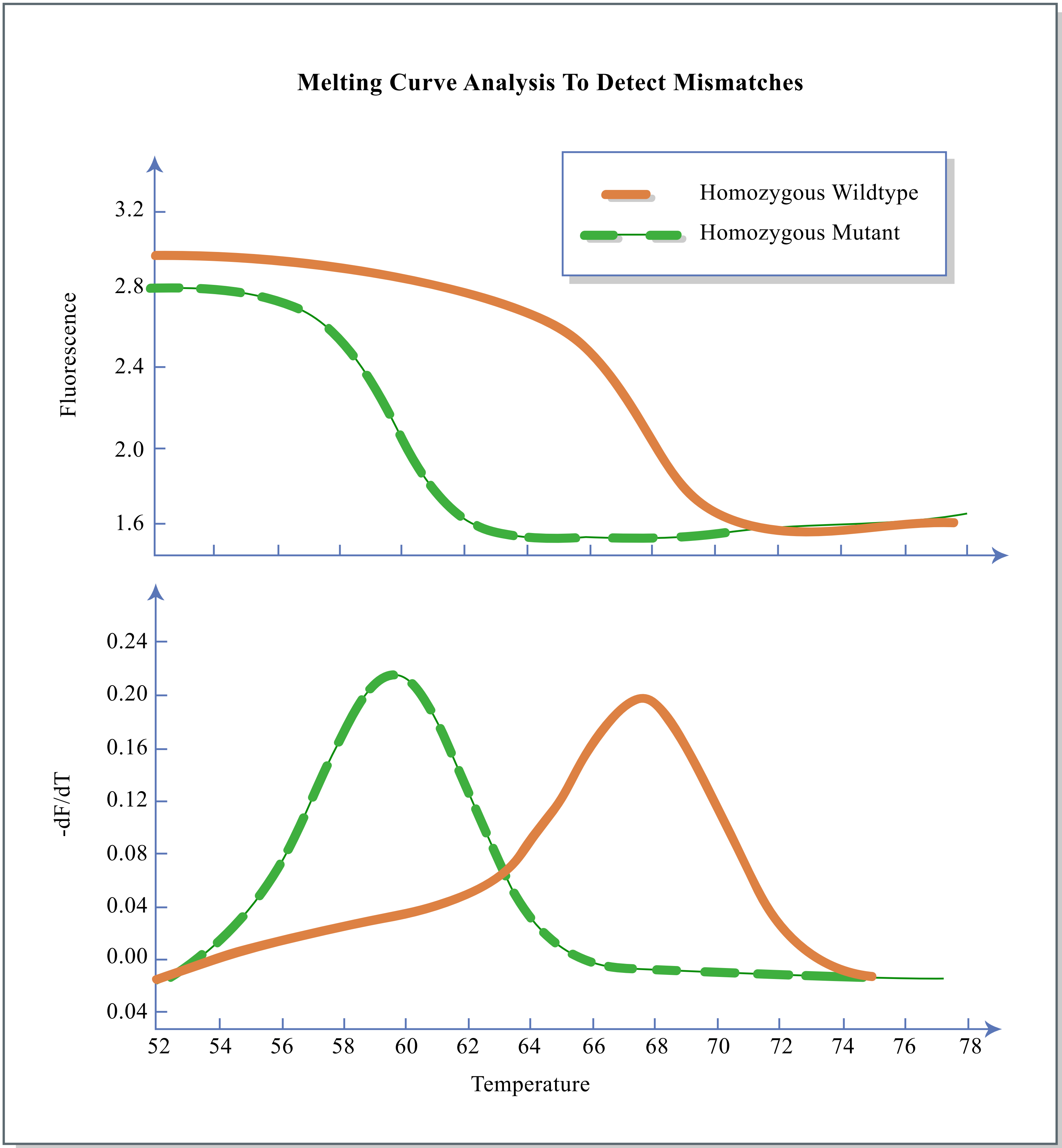


Figure by MIT OCW.



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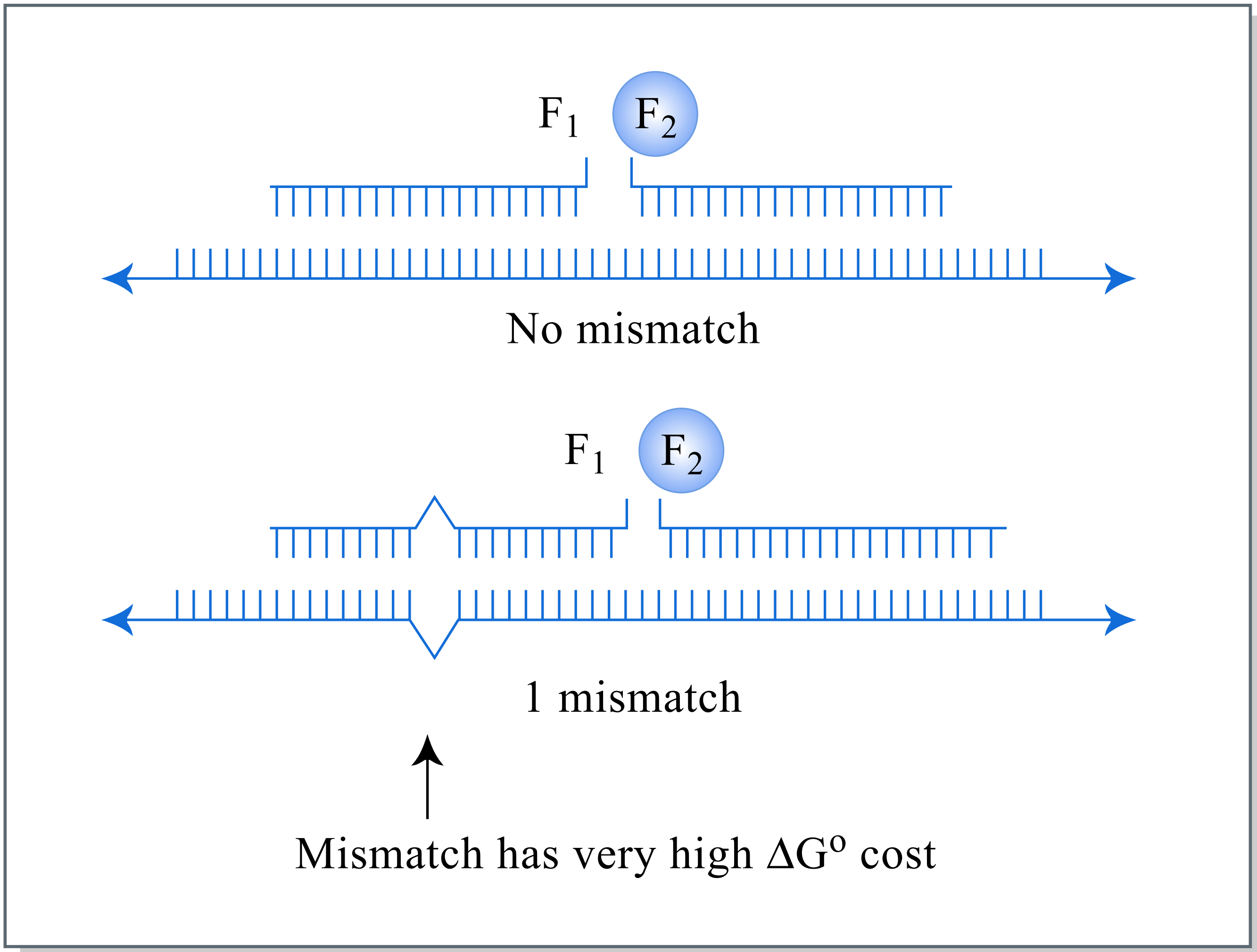

Figure by MIT OCW.