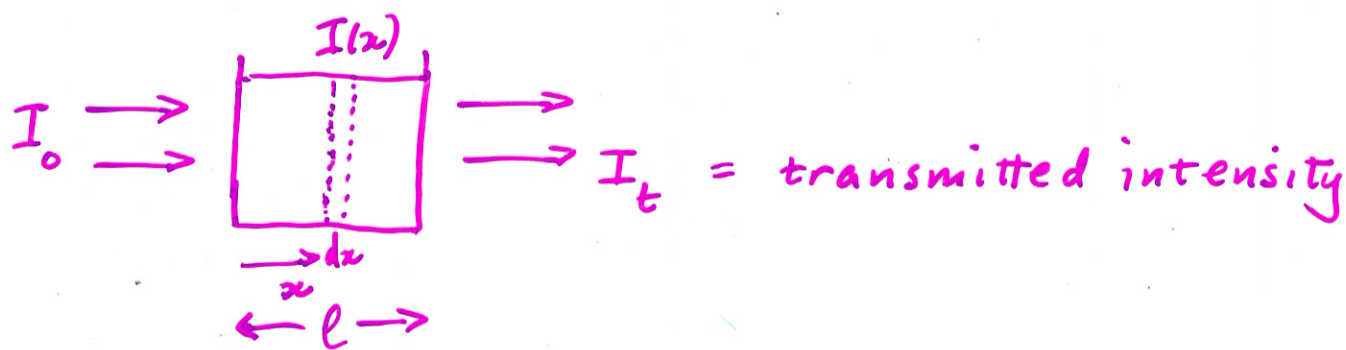


# UV Absorption Spectroscopy

H1



$$\frac{dI}{dx} = -\alpha c I$$

$$I(x) = I_0 e^{-\alpha c x}$$

$$I_t = I_0 e^{-\alpha c l}$$

Absorbance  $A = \log_{10} \frac{I_0}{I_t} = \log_{10} e^{\alpha c l}$

$$= \log_{10} e \cdot \alpha c l$$

$$= \underline{\underline{\epsilon c l}}$$

$\epsilon$  called molar absorptivity (units  $M^{-1} \text{cm}^{-1}$ )

$A$  is dimensionless

Proteins and Nucleic Acids absorb in the UV range 200 - 300 nm

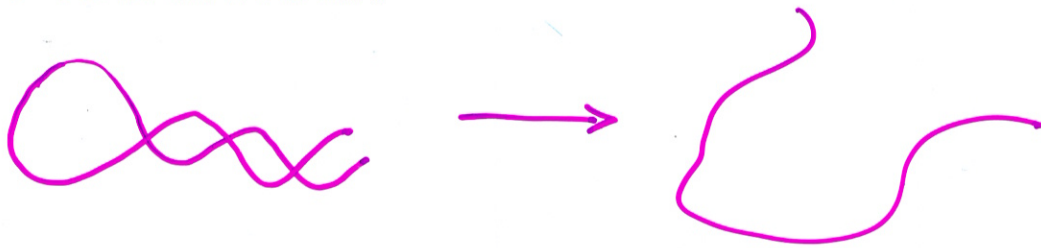
Interaction with electronic and vibrational energy levels.



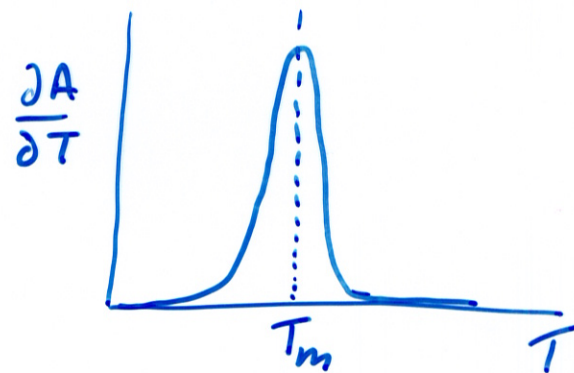
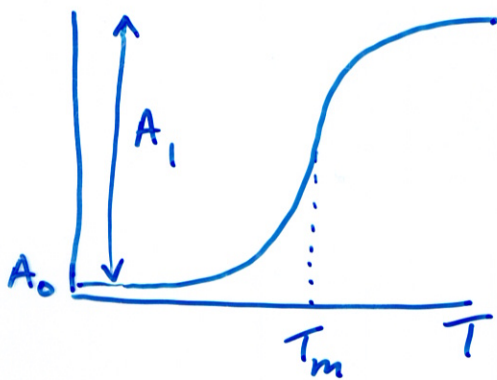
# Observing Helix Coil Transitions

H2

UV In DNA, stacked bases in double helix absorb less per base than partially stacked bases in single strands, which absorb less than mononucleotides.



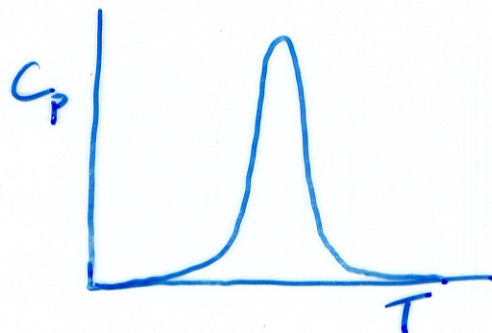
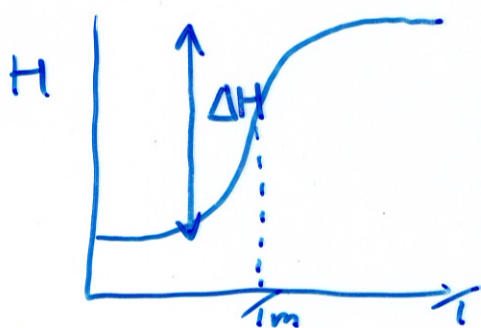
Melting of helical structure leads to an increase in Absorbance — hyperchromicity.



DSC = Differential Scanning Calorimetry

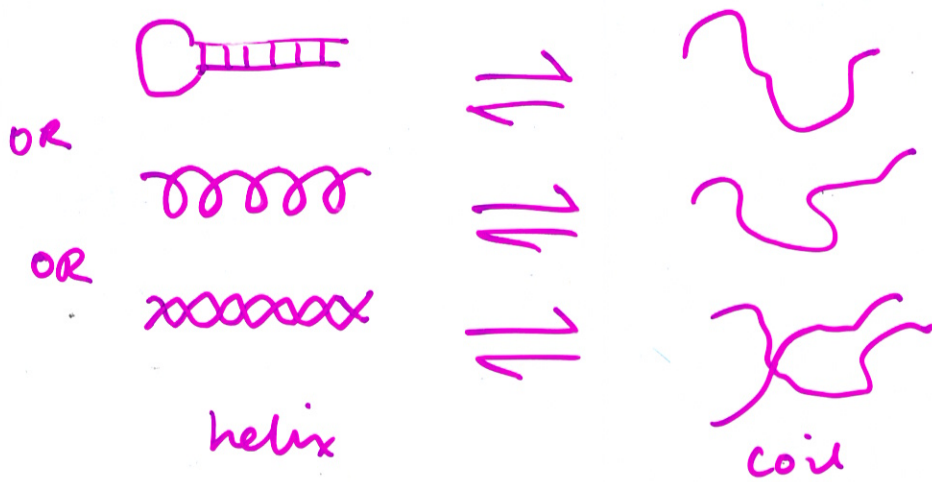
Heat Capacity  $C_p = \frac{\partial H}{\partial T}$

Measures incremental heat energy  $\delta H$  needed to increase temperature by  $\delta T$



## Two-state transition

H3



Free energy change on melting helix

$$\Delta G = \Delta H - T \Delta S$$

enthalpy of  
stacking interactions,  
hydrogen bonds etc

additional entropy of  
helix with respect to coil.

Melting temp is where  $\Delta G = 0$  :  $T_m = \frac{\Delta H}{\Delta S}$

equil const.  $K = \frac{[\text{coil}]}{[\text{helix}]} = e^{-\Delta G/kT}$

partition function  $Z = 1 + K$

helix fraction  $\theta = \frac{1}{1+K}$

coil fraction  $1 - \theta = \frac{K}{1+K}$

$$A = A_0 + A_1 \frac{K}{1+K}$$

$$K = e^{-\frac{1}{kT}(\Delta H - T\Delta S)}$$

$$= e^{\frac{\Delta H}{k} \left( \frac{1}{T_m} - \frac{1}{T} \right)}$$

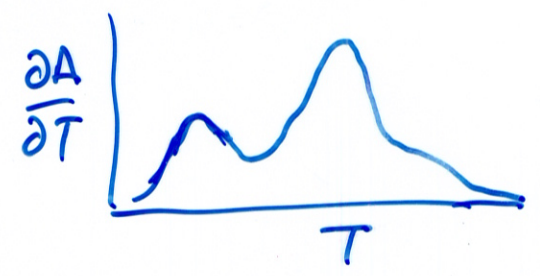
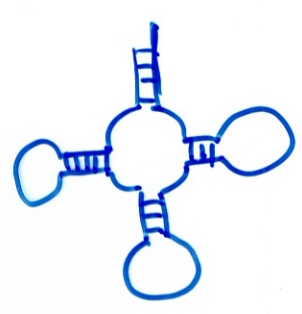
$$\frac{\partial A}{\partial T} = A_1 \frac{\partial}{\partial T} \left( \frac{K}{1+K} \right)$$

$$= A_1 \frac{d}{dK} \left( \frac{K}{1+K} \right) \frac{\partial K}{\partial T}$$

$$= \underline{\underline{A_1 \frac{1}{(1+K)^2} \cdot K \frac{\Delta H}{kT^2}}}$$

$$\frac{\partial K}{\partial T} = K \cdot \frac{\Delta H}{kT^2}$$

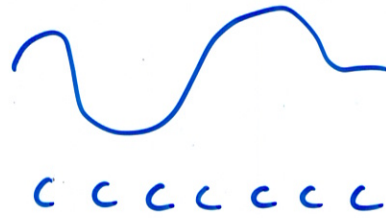
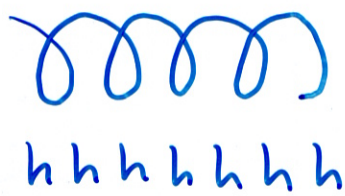
at  $T = T_m$   
 $K = 1$        $\frac{\partial A}{\partial T} \Big|_{T=T_m} = \frac{A_1}{4} \frac{\Delta H}{kT_m^2} = \text{peak height}$



RNA structures composed of many short helices. Melting can sometimes be described by a series of two-state transitions

See Exercise on Theimer + Liedroc paper

# Length dependence of 2-state transition #5



helix composed of  $N$  units (residues, basepairs)

free energy per residue  $\Delta G_r = \Delta H_r - T\Delta S_r$

free energy per helix

$$\Delta G_{tot} = N(\Delta H_r - T\Delta S_r) - \Delta G_{ends}$$

could be missing bonds at end of helix, or entropic penalty of hairpin loop

Define statistical weight of coil state = 1

$$\text{Weight of helix state} = e^{\frac{N\Delta G_r - \Delta G_{ends}}{kT}}$$

$$= \sigma s^N$$

where  $s = e^{\frac{\Delta G_r}{kT}} \begin{cases} > 1 \text{ at low } T \\ < 1 \text{ at high } T \end{cases}$

$\sigma = e^{-\frac{\Delta G_{ends}}{kT}}$  always  $\ll 1$

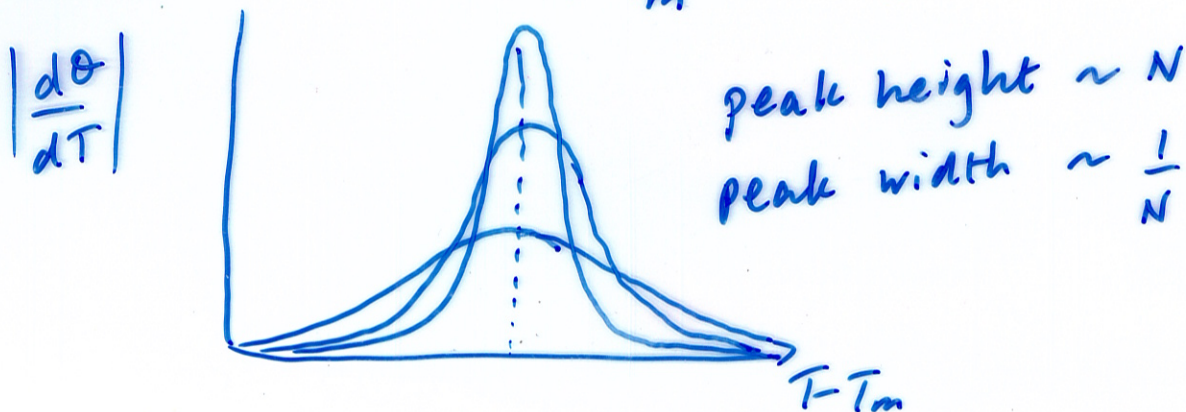
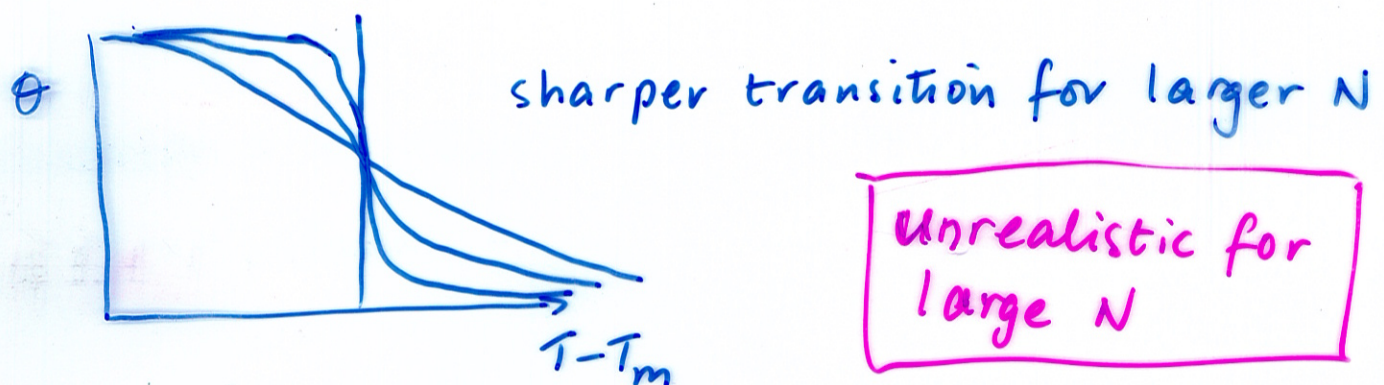
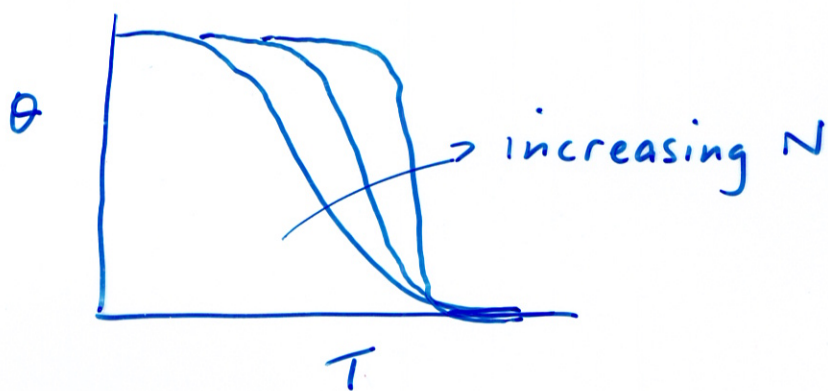
$$Z = 1 + \sigma s^N$$

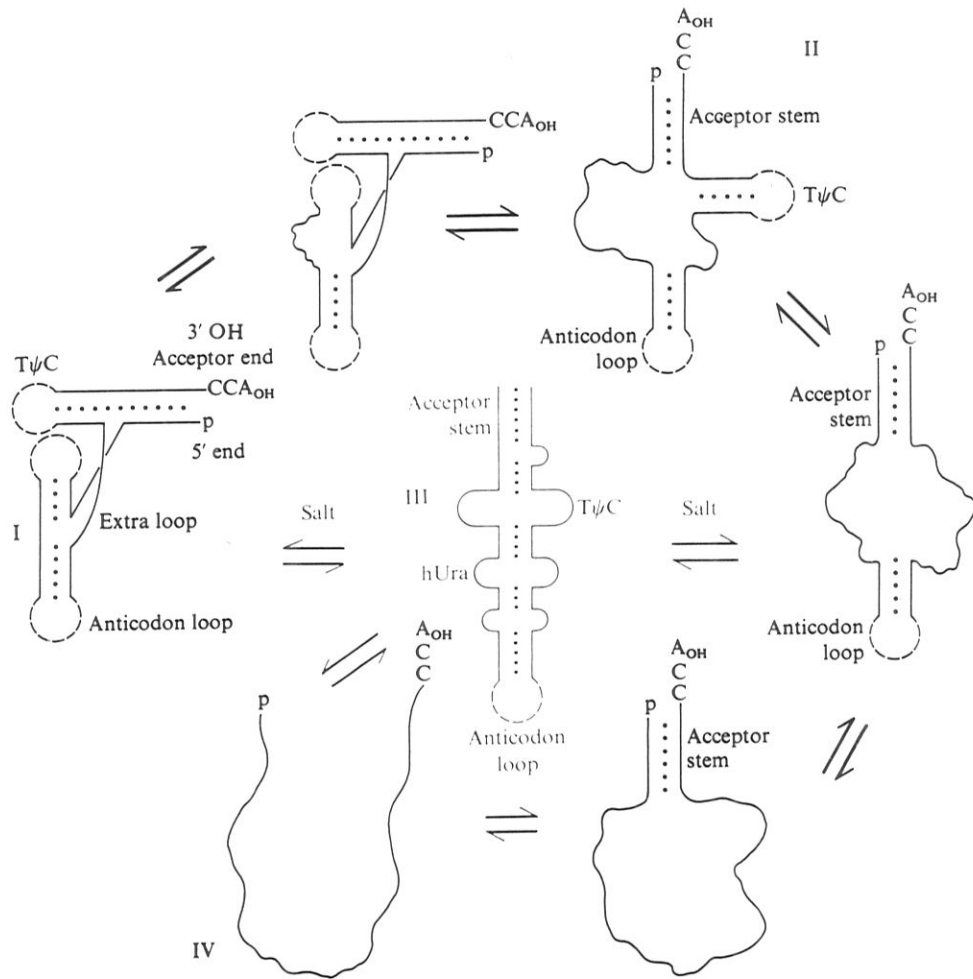
$$\theta = \frac{\sigma s^N}{1 + \sigma s^N} \quad (\text{helix fraction})$$

Consider case where  $\Delta G_{\text{ends}} = \Delta H_r$  (ie missing bond)

$$\Delta G_{\text{tot}} = (N-1) \Delta H_r - NT \Delta S_r$$

$$T_m = \frac{\Delta H_r}{\Delta S_r} \left(1 - \frac{1}{N}\right)$$





**Figure 24-22**

Schematic of thermal melting of *E. coli* tRNA<sup>Met</sup>. The order in which various regions of structure are disrupted was established from the NMR and kinetic studies shown in Figure 24-23. Also indicated are the four states (I through IV) believed to correspond to regions of the phase diagram for this tRNA shown in Figure 24-21. The form of tRNA stable only at low salt and low temperature (III) is shown in color. [After D. M. Crothers et al., *J. Mol. Biol.* 87:63 (1974).]

ng that 50 bases is  
melting.

melting. His results  
ons of highest A-T  
favorable to disrupt  
ing G-C content.  
ansitions of natural  
gh. This is a com-  
energy of the melted  
ed to meet at their  
f separated strands  
uclei.

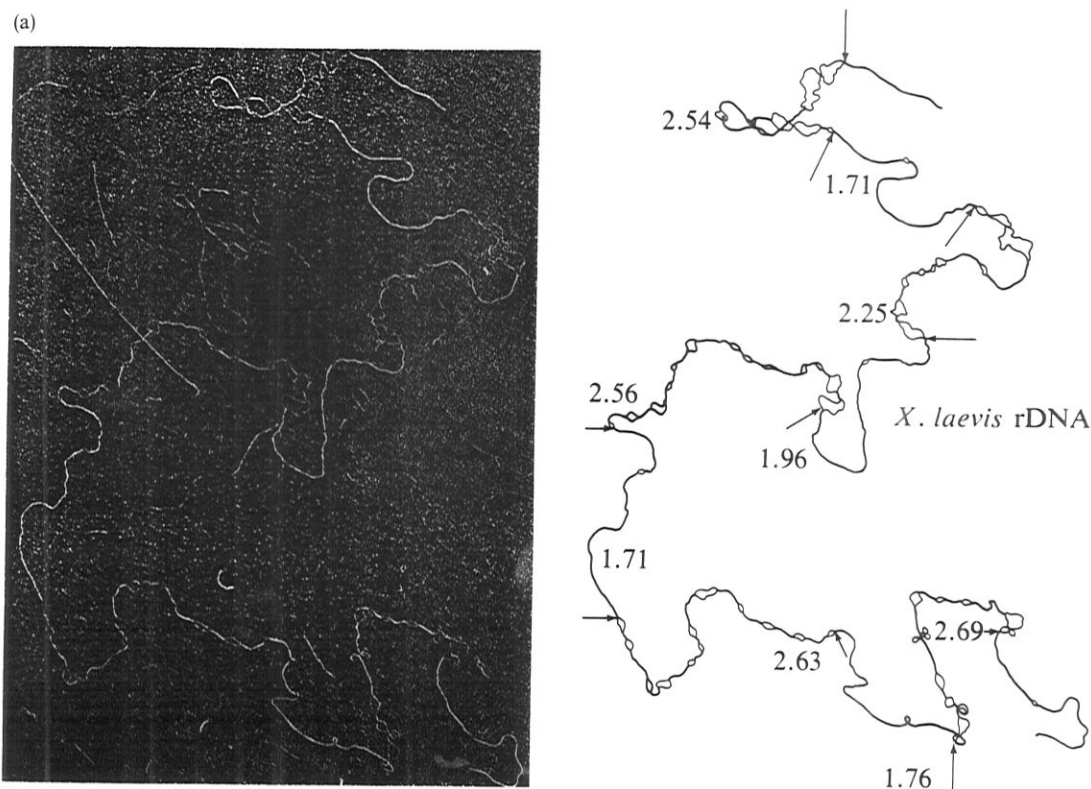
should be possible  
cking renaturation.  
he electron micro-  
e of DNA is avail-  
ular fraction of a  
is partially melted  
owed to react with  
amino groups of  
ons when the DNA

on of the preferred  
l regions still have  
with formaldehyde  
examined in the  
ed loops (or loops  
h double-stranded  
NA from *Xenopus*  
s are recorded by  
epeated for a large  
inguish one end of  
atures usually are  
l of the denatured  
by inspection (Fig.  
d it is useful for a

secondary-structure  
udies of RNA, but  
an focusing on the  
st regions to melt.  
ong the otherwise  
f RNA secondary

structure should correspond to the most stable regions of the DNA duplex that codes for this RNA. Temperature is not a good environmental variable for secondary-structure mapping, because of the cooperativity of the final stretches of thermal melting and because formaldehyde reactivity at temperatures above  $T_m$  probably is too vigorous. Organic solvent denaturants have proven to be more useful, and most work has been done with aqueous formamide solutions.

Figure 22-24c shows an example for ribosomal DNA and RNAs from *Xenopus laevis*. By comparing RNA and DNA results, one can assign the region of the DNA coding for the 40S rRNA precursor, and also can assign regions within it that code for the mature 18S and 28S rRNA.



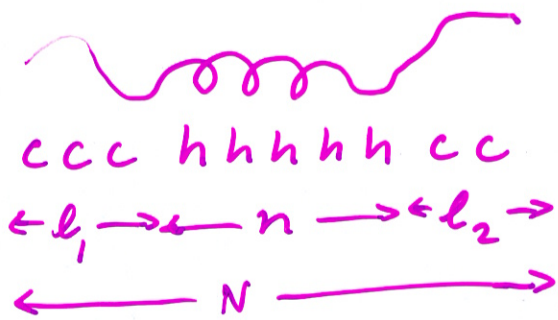
**Figure 22-24**

*Use of electron microscopy in sequence analysis of ribosomal DNA and RNA from *Xenopus laevis*.*

(a) Denaturation map of a single rDNA molecule. Alkali treatment was used to cause partial denaturation. After formaldehyde fixation, the molecule is viewed by the Kleinschmidt technique. The tracing clearly shows a repeating pattern of easily melted and stable duplex regions.



# Zipper model



allow unwinding  
from ends.

$$Z = 1 + \sum_{n=1}^N \Omega_n \sigma s^n$$

↑  
number of states with a  
helix of length ~~n~~ n

$$\Omega_n = N - n + 1$$

$$Z = 1 + \sum_{n=1}^N (N - n + 1) \sigma s^n$$

$$= 1 + \frac{\sigma s^2}{(s-1)^2} \left( s^N + \frac{N}{s} - N - 1 \right)$$

Can prove this using  $\sum_{n=1}^N s^n = \frac{s^{N+1} - s}{s-1}$

$$\sum_{n=1}^N n s^n = s \frac{d}{ds} \sum_{n=1}^N s^n$$

$$= \frac{s}{(s-1)^2} \left( N s^{N+1} - (N+1) s^N + 1 \right)$$

check this!  
for homework

Prob of having helix of length  $n$  is

$$p(n) = \frac{\Omega_n \sigma s^n}{Z}$$

$$\text{Helix fraction } \theta = \sum_{n=1}^N \frac{n}{N} p(n)$$

Exercise : evaluate  $\theta$  from this sum.

Alternative means

$$Z = 1 + \sum_n \Omega_n \sigma s^n$$

$$\frac{\partial Z}{\partial s} = \sum_n n \Omega_n \sigma s^{n-1}$$

$$s \frac{\partial Z}{\partial s} = \sum_n n \Omega_n \sigma s^n = NZ\theta$$

$$\theta = \frac{s}{NZ} \frac{\partial Z}{\partial s}$$

— can also write as

$$\theta = \frac{s}{N} \frac{\partial \ln Z}{\partial s}$$

Exercise : evaluate  $\theta$  this way and check they are the same

Zipper model again predicts transition gets sharper as  $N \rightarrow \infty$ .

In practice there is a limiting curve for very long chains.

$$\theta = \frac{\sigma s}{(s-1)^3} \left( \frac{ns^{n+2} - (n+2)s^{n+1} + (n+2)s - n}{n\{1 + [\sigma s/(s-1)^2][s^{n+1} + n - (n+1)s]\}} \right) \quad (20-37)$$

(20-28)

series:

(20-29)

 $s^n + 1]$ 

(20-30)

Equation 20-26 yields

 $-1]$ 

(20-31)

partition function. The

(20-32)

e term in the partition  
ls. Using this equation,

(20-33)

(20-34)

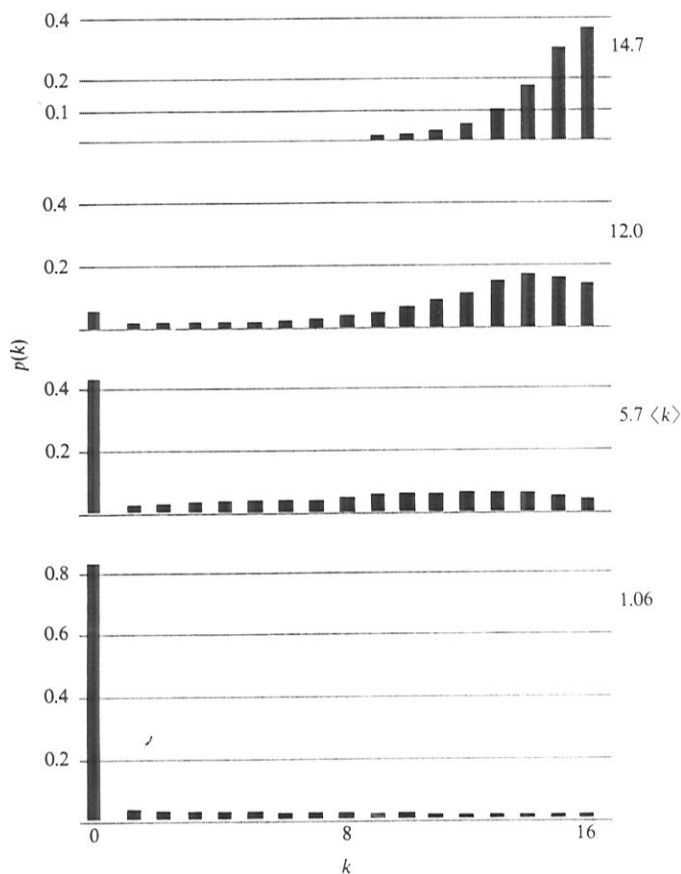
(20-35)

(20-36)

action method; it shows  
as a simple derivativeproach is that certain  
atives of the partition  
yields

It is possible to show that  $\theta = 0$  when  $s = 0$ , and that  $\theta \rightarrow 1$  as  $s$  becomes large. The transition from helix to coil occurs at intermediate values of  $s$ . For long chains, it can be shown that the midpoint of the transition ( $\theta = 0.5$ ) occurs at  $s = 1$ , according to this model. Because  $s$  is an equilibrium constant, its value can easily be varied—for example, by temperature changes. The relationship between the equilibrium constant  $s$  and observed experimental parameters is discussed in Section 20-7.

Equation 20-32 may be used to calculate values of  $p(k)$ , the distribution of helical lengths, as a molecule progresses through the helix-coil transition. Figure 20-10 shows examples of such calculations for a chain of 16 units. Bar graphs of the



**Figure 20-10**

Distributions of helical lengths at various values of the average helical length.  
[After J. A. Schellman, *J. Phys. Chem.* 62:1485 (1958).]