UV Absorption Spectroscopy

\( \frac{\text{d}I}{\text{d}x} = -\alpha c I \)

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

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

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

\[ = \log_{10} e^{\alpha c l} \]

\[ = \epsilon c l \]

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

A is dimensionless

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

Interaction with electronic and vibrational energy levels.

\( A \) spectrum specific to molecule
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.

\[ \text{DSC} = \text{Differential Scanning Calorimetry} \]

Heat Capacity \[ C_p = \frac{\Delta H}{\Delta T} \]

Measures incremental heat energy \( \Delta H \) needed to increase temperature by \( \Delta T \)
Two-state transition

or

or

helix

coil

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 \( 1 - \Theta = \frac{K}{1+K} \)
\[ A = A_0 + A_1 \frac{K}{1+K} \]

\[ \frac{\partial A}{\partial T} = A_1 \frac{\partial}{\partial T} \left( \frac{K}{1+K} \right) = A_1 \frac{d}{dK} \left( \frac{K}{1+K} \right) \frac{\partial K}{\partial T} = A_1 \frac{1}{(1+K)^2} \cdot \frac{K \Delta H}{kT^2} \]

\[ K = e^{-\frac{1}{kT} (\Delta H - T \Delta S)} = e^{\frac{\Delta H}{k} \left( \frac{1}{T_m} - \frac{1}{T} \right)} \]

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

At \( T = T_m \) \( \left. \frac{\partial A}{\partial T} \right|_{T=T_m} = \frac{A_1 \Delta H}{4 k T_m^2} \) = 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 and Giedroc paper
Length dependence of 2-state transition

\[ \begin{align*}
\text{helix composed of } N \text{ units (residues, basepairs)} & \\
\text{free energy per residue } & \Delta G_r = \Delta H_r - T \Delta S_r \\
\text{free energy per helix } & \Delta G_{\text{tot}} = N (\Delta H_r - T \Delta S_r) - \Delta G_{\text{ends}} \\
\text{could be missing bonds at end of helix, or entropic penalty of hairpin loop} & \\
\text{Define statistical weight of coil state } & = 1 \\
\text{Weight of helix state } & = e^{\frac{N \Delta G_r - \Delta G_{\text{ends}}}{kT}} \\
& = \sigma S^N \\
\text{where } & S = e^{\frac{\Delta G_r}{kT}} > 1 \text{ at low } T \\
& \sigma = e^{\frac{-\Delta G_{\text{ends}}}{kT}} < 1 \text{ at high } T \\
& \text{always } \ll 1\end{align*} \]
\[ Z = 1 + \sigma s^N \]
\[ \Theta = \frac{\sigma s^N}{1 + \sigma s^N} \] (helix fraction)

Consider case where \( \Delta G_{\text{ends}} = \Delta H_r \) (i.e., missing bond)

\[ \Delta G_{\text{tot}} = (N-1) \Delta H_r - N T \Delta S_r \]
\[ T_m = \frac{\Delta H_r}{\Delta S_r} \left( 1 - \frac{1}{N} \right) \]

\[ \Theta \] increasing \( N \)

sharper transition for larger \( N \)

Unrealistic for large \( N \)

peak height \( \sim N \)

peak width \( \sim \frac{1}{N} \)
owest temperatures transition is about energy. At higher apparent activation difference in rates I and III are quite activation energies xation kinetics, the es well below the activation energy obly dominant above 1. Because form II actions within II are has lost some of the has not gained any de too slow for the croseconds. Therefore, the large activation ary structure alone, al change involves thalpy changes. A loss of all tertiary from the energetics be at least stable that this must be a base interactions be broken for the III is a hairpinlike electrostatic free it could be more low salt concentra- observed by equilibrium. Three distinct ding to this transition overlapping tem- e results shown in rational changes. al relaxation steps derable additional iniques.

Figure 24-22
Schematic of thermal melting of E. coli tRNA<sub>434</sub>. 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).]
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

允许解卷自两端。

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

\[ \Omega_n = N - n + 1 \]

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

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

可以用这个来证明

\[ \sum_{n=1}^{N} s^n = \frac{s^{N+1} - s}{s - 1} \]

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

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

检查这个！

为家庭作业
Prob of having helix of length $n$ is

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

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

Exercise: evaluate $\Theta$ from this sum.

Alternative means

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

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

$$s \frac{\partial Z}{\partial s} = \sum_{n} n \Omega_n \sigma s^{n} = N Z \Theta$$

$$\Theta = \frac{s}{N Z} \frac{\partial Z}{\partial s}$$

Another way to write as

$$\Theta = \frac{s}{N} \int \frac{d\ln Z}{\partial s}$$

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

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

In practice, there is a limiting curve for very long chains.
\[ \theta = \frac{\sigma_s}{(s-1)^2} \left( \frac{rs^{n+2} - (n+2)s^{n+1} + (n+3)s - n}{n(1 + \sigma_s(s-1)^2)[s^{n+1} + n - (n+1)s]} \right) \]  

(20-37)

It is possible to show that \( \theta = 0 \) when \( s = 0 \), and that \( \theta \to 1 \) 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](Image)

Distributions of helical lengths at various values of the average helical length.