

DNA Conformational Transition

Questions

- Helix to coil transition;
- B to Z transition;
- B to A transition;
- Protein-induced DNA conformational changes.

DNA conformational transitions

- Find a signal for the transition (CD, UV abs, Heat, Chemical and enzymatic probing, etc);
- Find a physical or chemical way to drive the transition (Temp., Salt, pH, Organic Solvent (alcohol));
- Construct transition curve;
- Model-Analysis.

Methods to Study DNA conformational transition

- UV Absorbance;
- CD (circular Dichroism);
- Calorimetry;
- NMR;
- Others (linear dichroism (LD), Infrared and Raman Scattering, etc).

UV Spectroscopy

- Fundamentals:

Three concepts: Extinction Coefficient, Wavelength of maximum absorption, and hypochromicity;

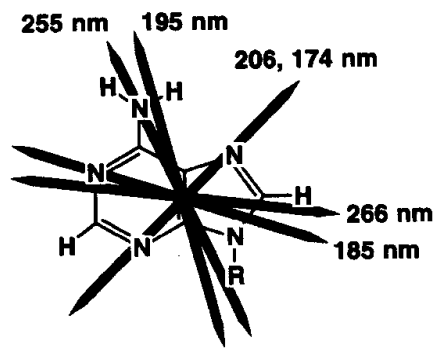
- The Beer-Lambert law:

$$I = I_0 10^{-\epsilon c l} \text{ or } \text{Abs} = \epsilon l c \text{ (Abs} = \log (I_0/I))$$

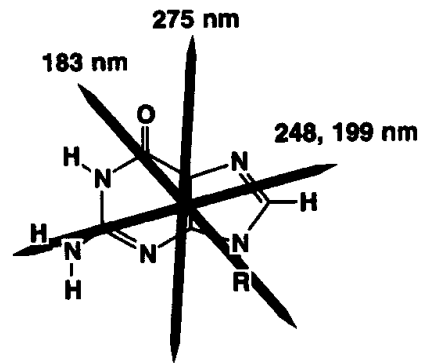
UV Spectra

- A major application of UV absorbance is to determine concentration of nucleic acids;
- For a typical ds DNA with an extinction coefficient per nucleotide of $7000 \text{ M}^{-1}\text{cm}^{-1}$, 1 OD unit is about $0.15 \text{ } \mu\text{mol}$ of nucleotides, which is approximately $50 \text{ } \mu\text{g/ml}$ (ssDNA, $1 \text{ OD}_{260} = 33 \text{ } \mu\text{g/ml}$; ssRNA, $1 \text{ OD}_{260} = 40 \text{ } \mu\text{g/ml}$);
- Hypochromicity (the decrease of absorbance when forming DNA duplex from two single stranded DNA);
- Hyperchromicity (the increase of absorbance when a double-stranded nucleic acid is dissociated into single strands).

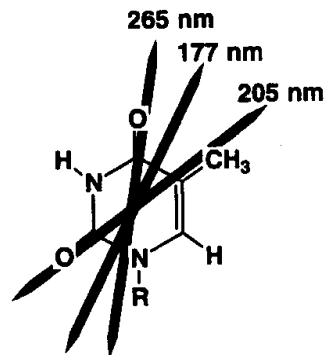
Electron transitions of nucleotide bases



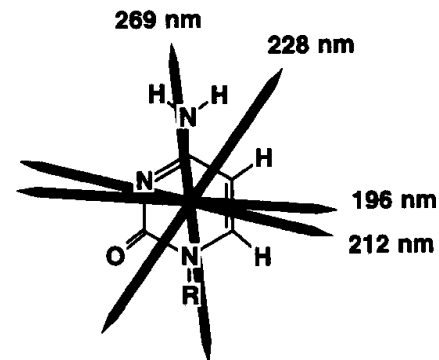
Adenine



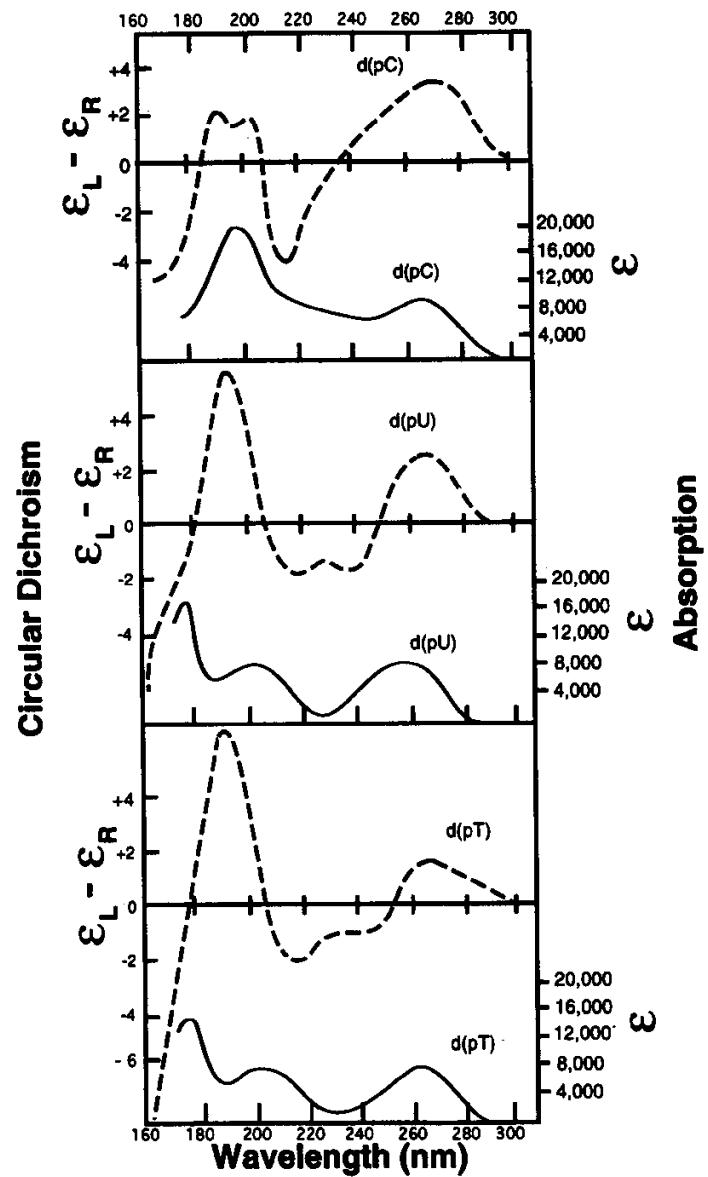
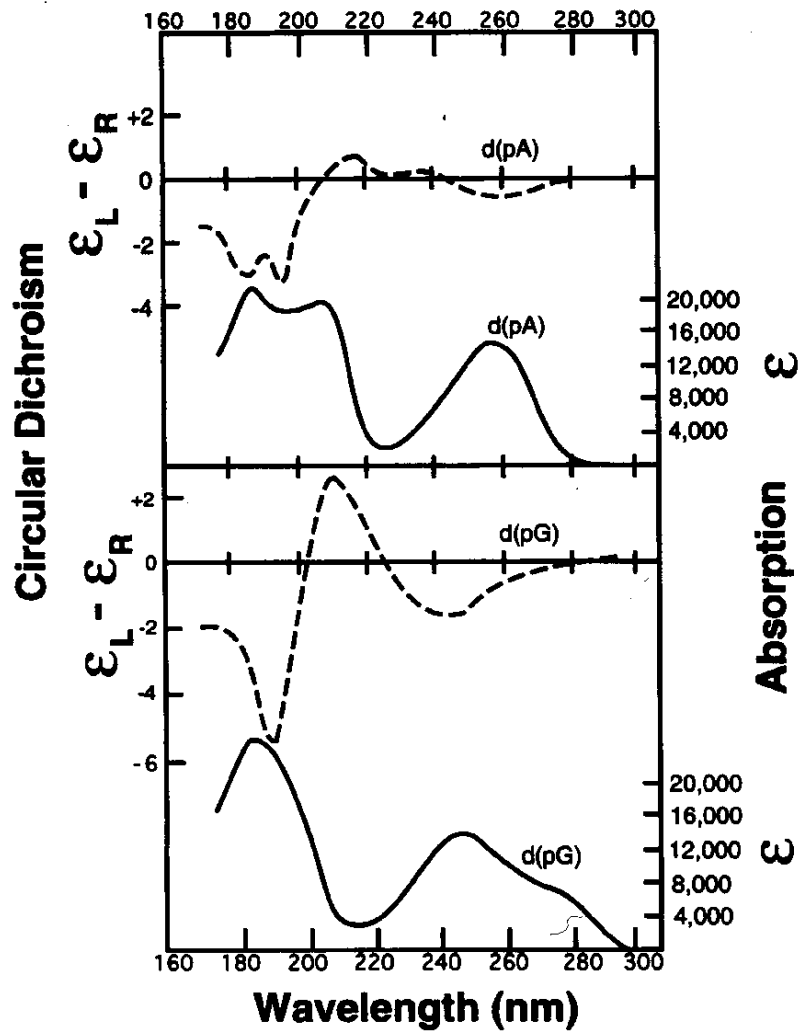
Guanine



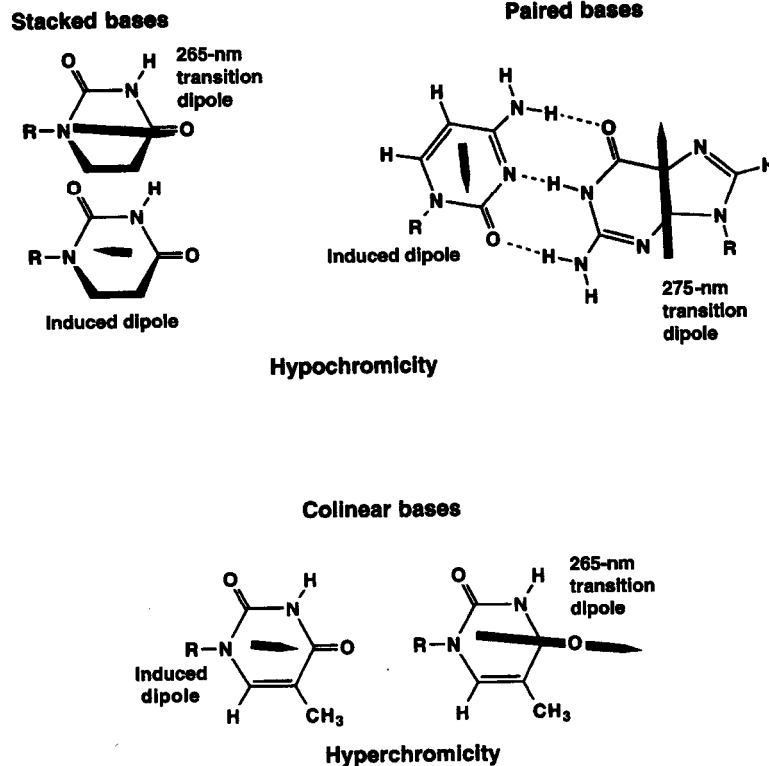
Thymine



Cytosine



Effects of base-base interaction on polynucleotide extinction coefficient



The absorption of a nucleic acid depends on the sum of the absorption plus an effect due to their interactions. Thus the extinction coefficient at any wavelength depends on its sequence and conformation.

Effects of base-base interaction on polynucleotide extinction coefficient

- The interaction between the transition dipoles are Coulombic. As there is no net change in charge for a transition, the interaction can be approximated by dipole-dipole interactions; they are of order r^{-3} with r the distance between bases. Thus the nearest neighbor approximation can be used to estimate the extinction coefficient of a single strand oligonucleotide. For 5'-ApCpGpUp...ApG-3':

$$\varepsilon = 2[\varepsilon(\text{ApC}) + \varepsilon(\text{CpG}) + \varepsilon(\text{GpU}) + \dots + \varepsilon(\text{ApG})] - [\varepsilon(\text{Cp}) + \varepsilon(\text{Gp}) + \varepsilon(\text{Up}) + \dots + \varepsilon(\text{Ap})]$$

$\varepsilon(\text{ApCpGpUp} \dots \text{ApG})$ is the extinction coefficient per mole of strand. The parameters $\varepsilon(\text{ApC})$, $\varepsilon(\text{CpG})$, and so on, are the extinction coefficients of the component dinucleoside phosphates per mole of nucleotide; that is why they are multiplied by 2. The parameters $\varepsilon(\text{pC})$, $\varepsilon(\text{pG})$, and so on, are the extinction coefficients of the nucleotides. The dinucleoside phosphates count each nucleotide twice, except for the two end ones, which is the reason the extinction coefficients of the nucleotides (except the two end ones) must be subtracted.

Table 6.1
 Extinction Coefficients per Nucleotide ($M^{-1} \text{ cm}^{-1} \times 10^{-3}$) at
 260 nm, 25°C, 0.1 Ionic Strength, pH 7 for Nucleotides and
 Dinucleoside Phosphates to Calculate Extinction Coefficients of
 Single Strands^a

	RNA	DNA		RNA	DNA
Ap	15.34	15.34	CpG	9.39	9.39
Cp	7.60	7.60	CpU (CpT)	8.37	7.66
Gp	12.16	12.16	GpA	12.92	12.92
Up (Tp)	10.21	8.70	GpC	9.19	9.19
ApA	13.65	13.65	GpG	11.43	11.43
ApC	10.67	10.67	GpU (GpT)	10.96	10.22
ApG	12.79	12.79	UpA (TpA)	12.52	11.78
ApU (ApT)	12.14	11.42	UpC (TpC)	8.90	8.15
CpA	10.67	10.67	UpG (TpG)	10.40	9.70
CpC	7.52	7.52	UpU (TpT)	10.11	8.61

^aTable I from Gray et al., 1995. The extinction coefficients are estimated to be accurate to ± 0.10 for the monomers and $\pm 4\%$ for the dimers.

By using parameters from synthetic polynucleotides, the following equations can be used to estimate the extinction coefficients for dsDNA and dsRNA:

$$\varepsilon_{260} = 7585 - 1285f_{\text{AT}} + 1685f_{\text{AT}}(f_{\text{AT}} - 1)$$

$$\varepsilon_{260} = 7010 - 530f_{\text{AU}} - 130f_{\text{AU}}(f_{\text{AU}} - 1)$$

f_{AT} or f_{AU} is AT or AU base pair composition fraction,

Detection of conformational changes by UV spectroscopy

- Helix-Helix transitions:
B to A or B to Z transition;
- Helix-coil transitions: melting curves

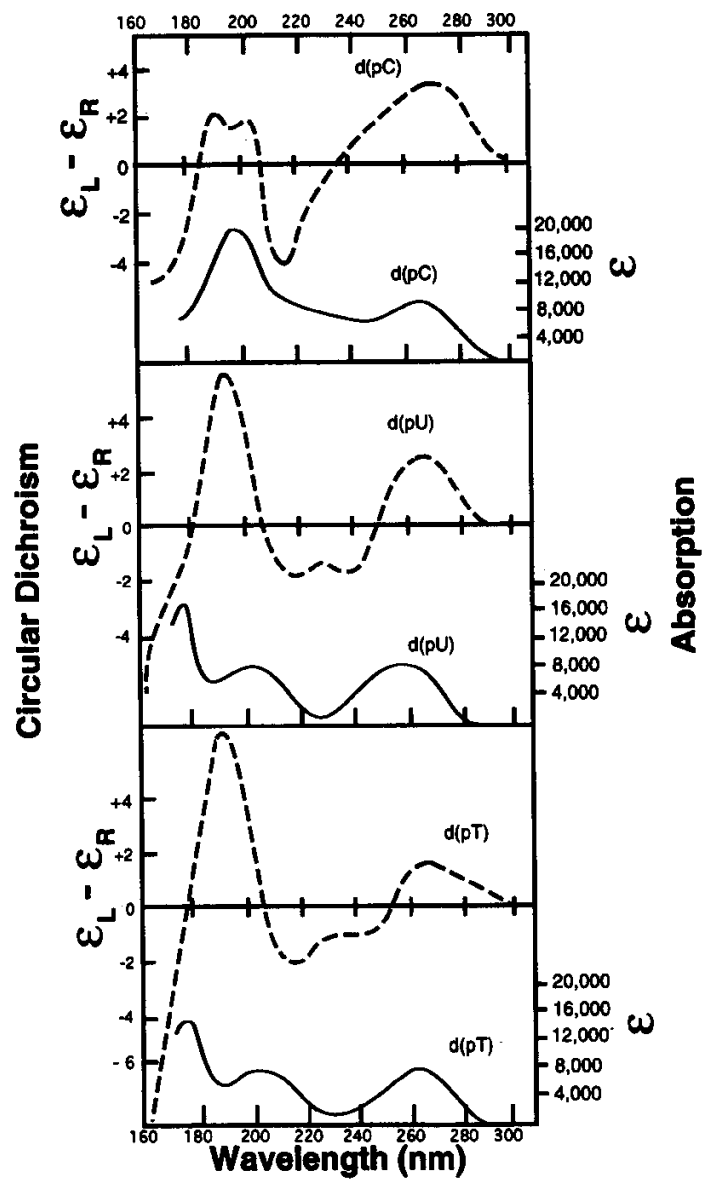
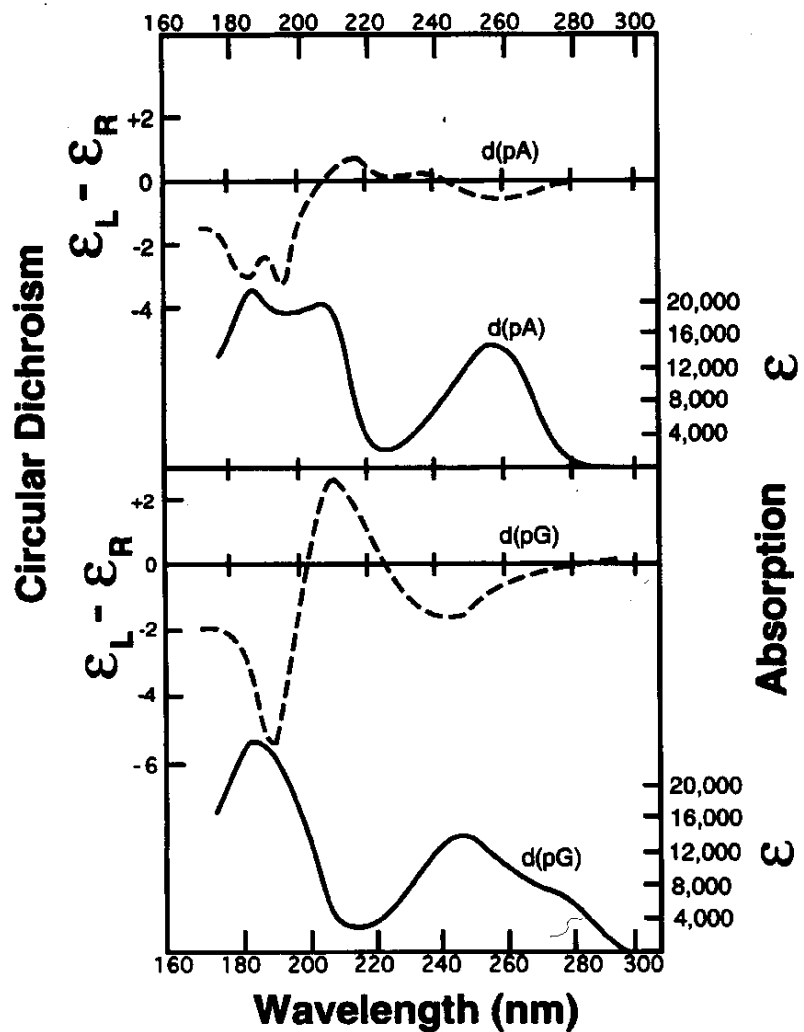
Circular Dichroism

- **Circular dichroism is the difference in light absorption for incident left and right circularly polarized light. Only molecules that are not superimposable on their mirror images chiral molecules can have CD. Thus, bases are not circularly dichroic; but nucleosides, nucleotides, and polynucleotides are. Molar CD is:**

$$\epsilon_L - \epsilon_R = (A_L - A_R)/lc$$

The subscripts L and R refer to left and right circularly polarized light.

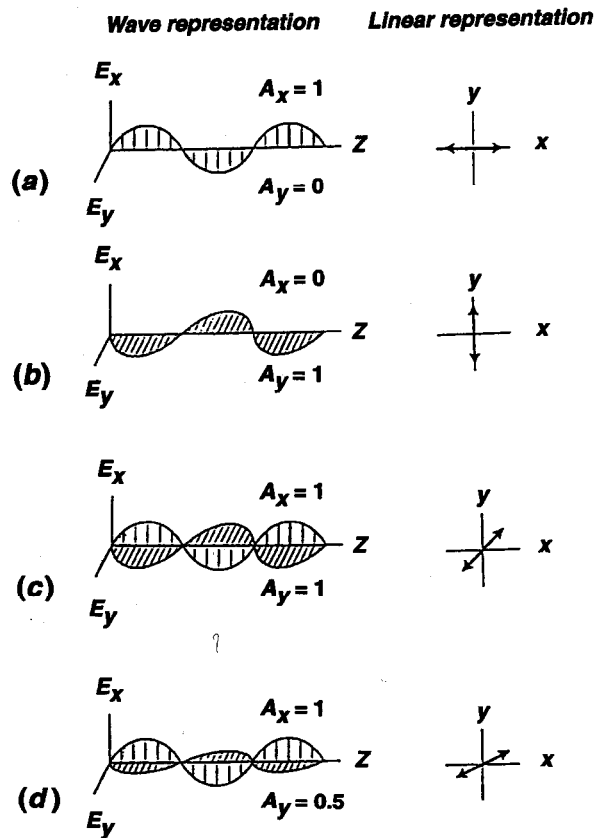
- **The molar extinction coefficient of the nucleotide are at least 1000 times larger than the difference in extinction coefficients for left and right polarized light; however, the sensitivity of CD is as great as that of absorbance.**
- **CD spectra are more sensitive to conformation than absorption spectra, and can be used to distinguish different forms of DNA (A, B, and Z-DNA).**



Circular Dichroism: Fundamentals

- **Light is an oscillating electromagnetic field in which the electric and magnetic fields oscillate in the plane perpendicular to the direction of the propagation of the light;**
- **For linearly polarized light, the electric field oscillates in only one direction;**
- **Circularly polarized light is represented by a vector of constant length that rotates in the x-y plane at the frequency of the light. The tip of the vector traces out a circle; it can move clockwise or counterclockwise to give right- or left-handed circularized light. Circular dichroism is the differential absorption of the right and left circularly polarized beams.**
- **CD depends on not only the magnitude of electron density displacement during transition, but also the shape of the path taken by this transition, thus has handedness.**

Linearly polarized light



Right circularly polarized light

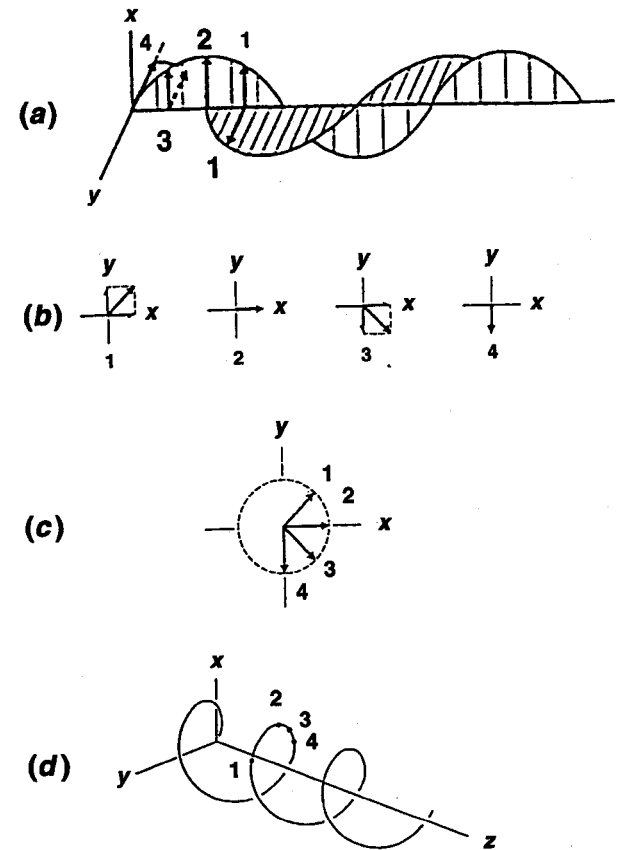
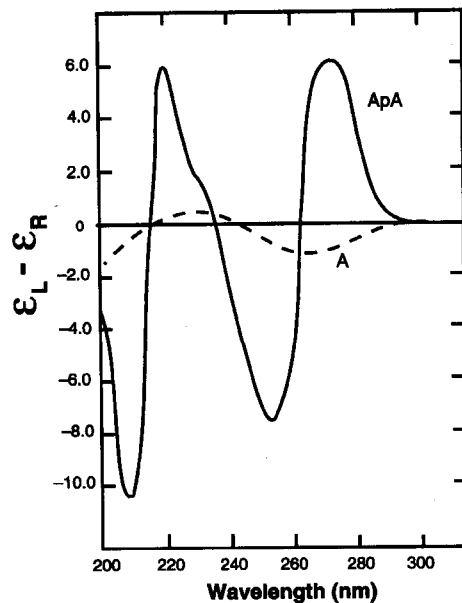


Figure 6-10

Representations of different types of polarized light by an oscillating vector. The tip of the vector indicates the magnitude and direction of the electric field of the light as a function of time and space. On the left is shown linearly polarized light propagating in the z direction. The polarization is along the x axis (a), the y axis (b), 45° from the x axis (c), and 30° from the x axis (d). On the right is shown right circularly polarized light propagating in the z direction. Panels (a) and (b) illustrate that circularly polarized light can be considered as a sum of two perpendicular vectors oscillating 90° out of phase. The resulting tip of the electric vector of the light produces a circle as seen by an observer moving with the light (c); it produces a helix as seen by a stationary observer (d). [Reprinted with permission from Kliger et al., 1990. This figure is from Figures 2-2 and 2-3.]

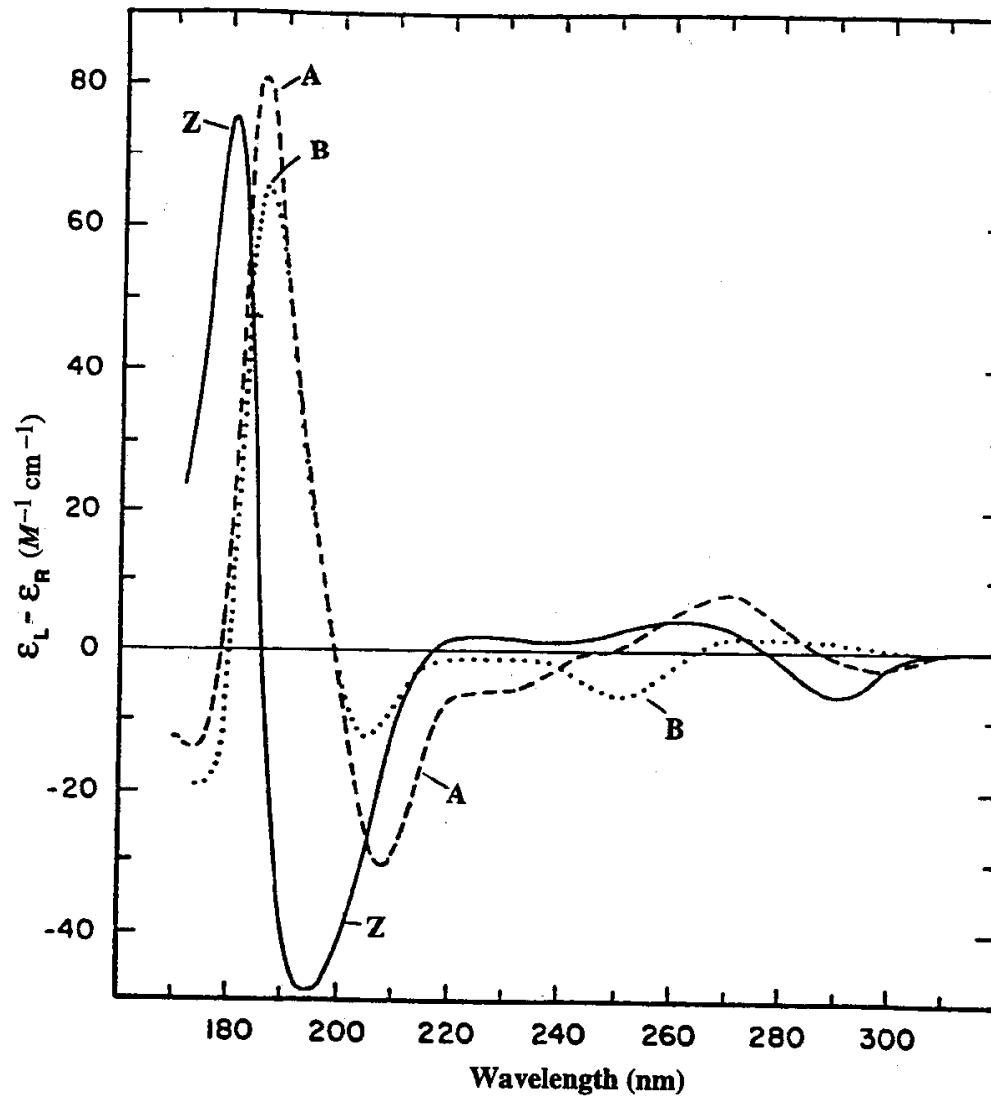
- Ellipticity is also used to measure CD. The ellipse is characterized by an angle, θ , whose tangent is equal to the ratio of the minor to the major axis of the ellipse, and whose sign gives the handedness of the ellipse;
- The molar ellipticity $[\theta] = 3298(\epsilon_L - \epsilon_R)$
Standard unit: $\text{deg M}^{-1} \text{cm}^{-1} \times 100$

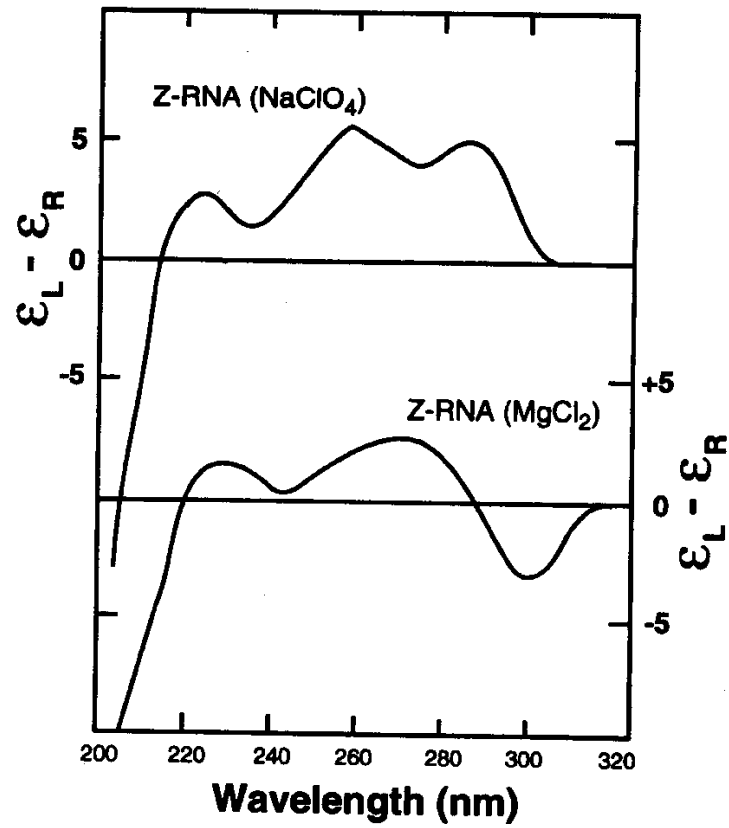
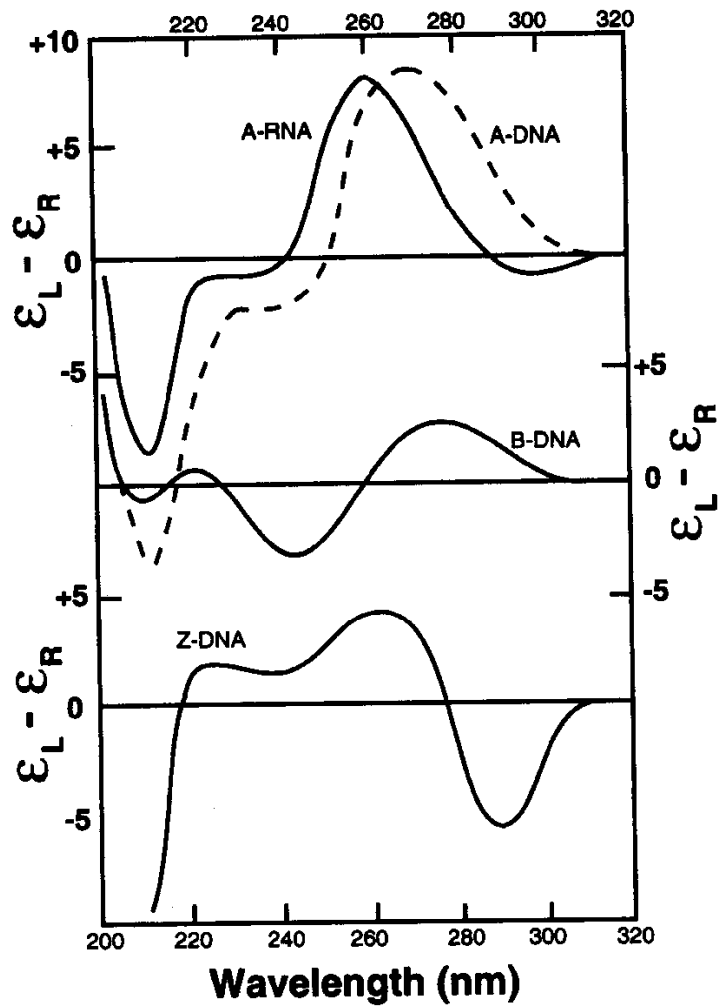
Relation of CD with Nucleic Acids structures



For adenosine, the CD depends on the interaction of adenine with its ribose and phosphates. For ApA, the CD is mainly from the chiral adenine-adenine interactions, which is 10 times larger than that of adenosine.

For double-stranded nucleic acids, the CD below 220 nm is the sense of the helix (A, B, and Z)





Summary of CD

- CD is very useful as a method to compare conformations and to detect changes when the solvent or temperature is changed. It is sensitive to interactions of neighboring bases and to any change in the spectrum of the bases;
- When the solvent or temperature causes a change in CD, a change in conformation is assumed, but the nature of the change is not known. Other methods such as NMR or Raman scattering can be used to define the change.

Calorimetry

- **Differential Scanning Calorimetry (DSC).**

Differential scanning calorimetry measures the heat capacity of the solution present in the calorimeter cell as a continuous function of temperature.

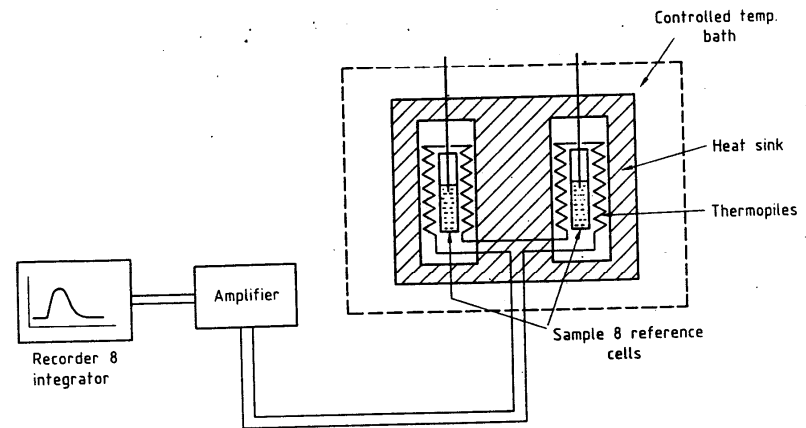


Fig. 7. Schematic of the components of a typical differential scanning calorimeter

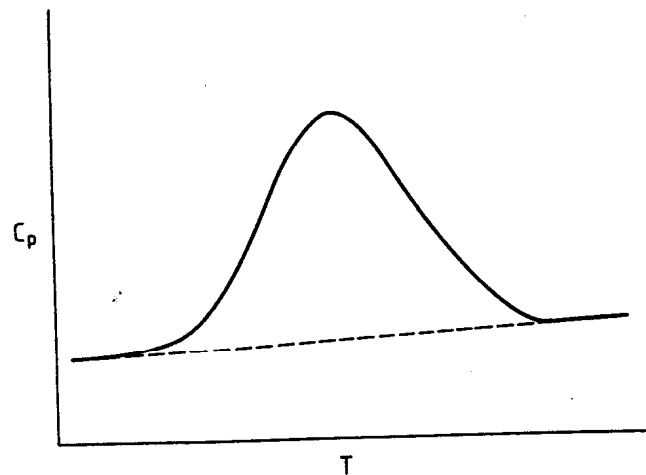
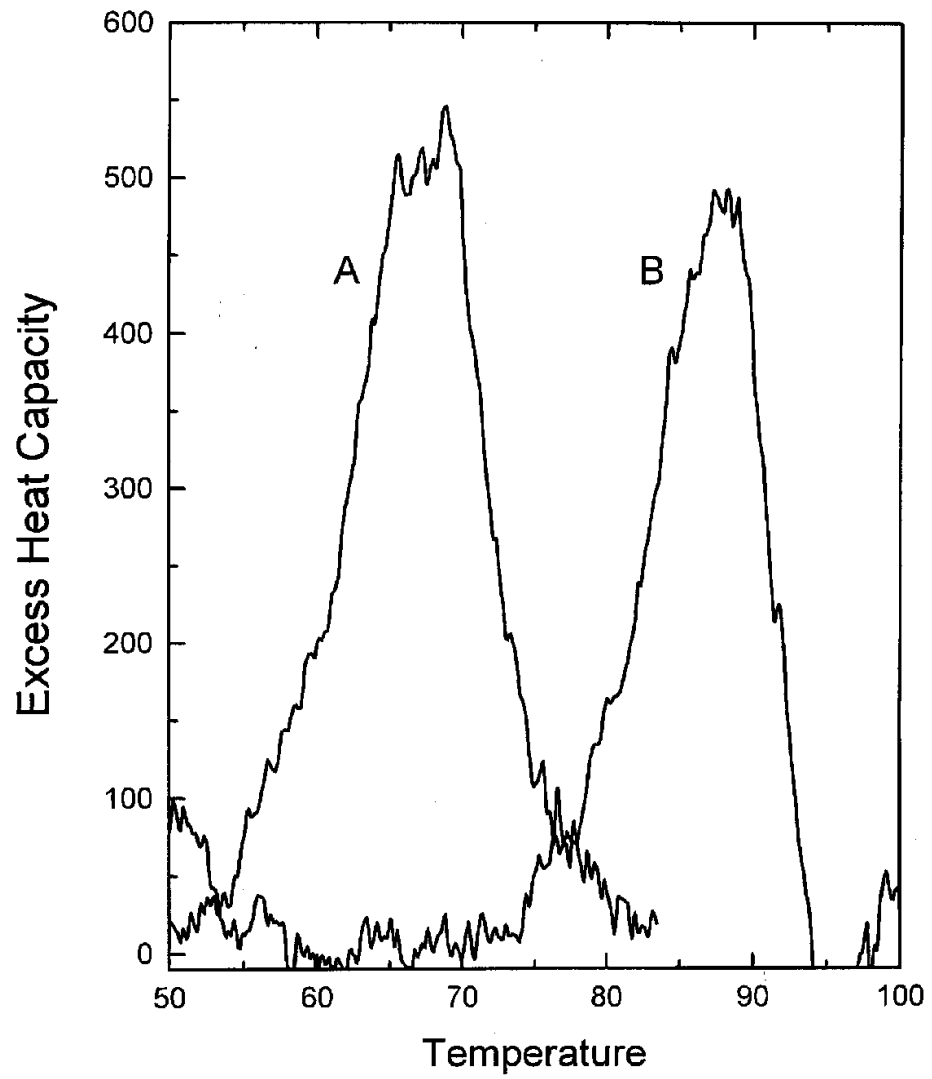
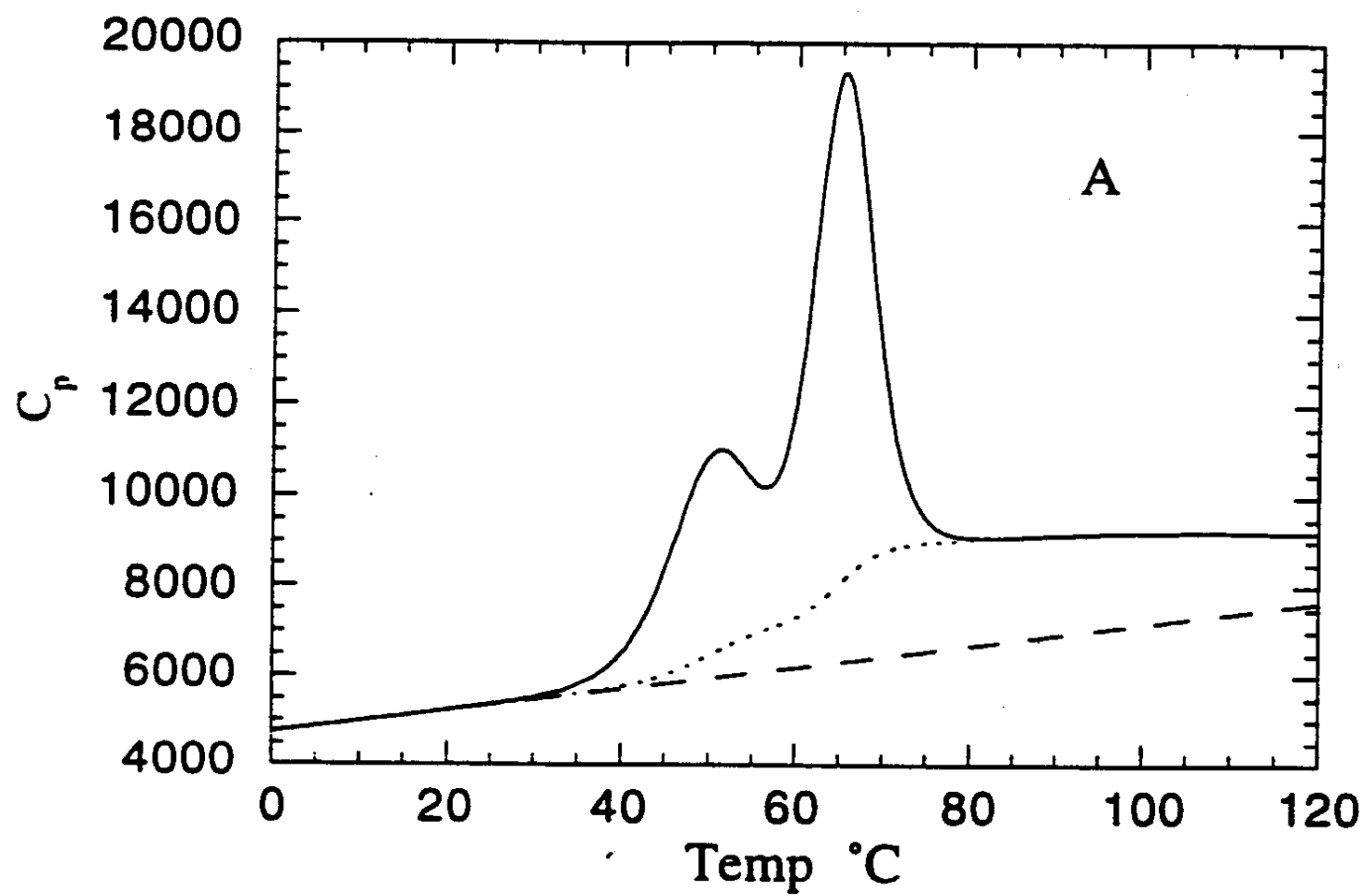


Fig. 8. A typical heat capacity (C_p) vs. temperature transition curve obtained by differential scanning calorimetry





Deconvolution of the partial molar Heat capacity

$$C_p = C_{p,0} + \frac{\partial}{\partial T} \left(\sum_{i=1}^N P_i * \Delta H_i \right)$$

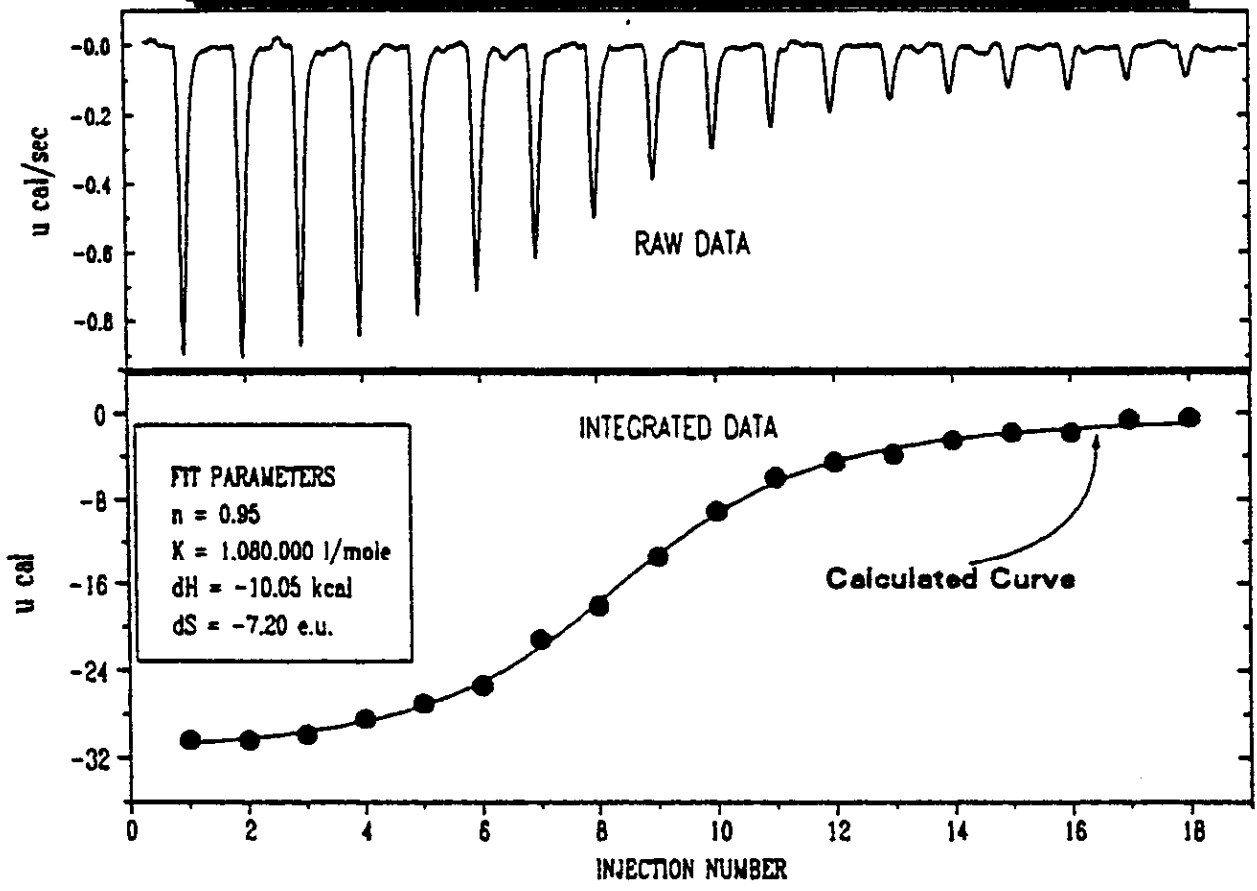
$C_{p,0}$ is the partial molar heat capacity of the reference state; ΔH_i is the relative enthalpy of state i, thus $\Delta H_i = H_i - H_0$; and P_i is the population of state i.

$$\begin{aligned} C_p &= C_{p,0} + \left\{ \left[\sum_{i=0}^N \Delta H_i^2 * \exp(-\Delta G_i / RT) / Q \right] - \left[\sum_{i=0}^N \Delta H_i * \exp(-\Delta G_i / RT) / Q \right]^2 \right\} / RT^2 + \sum_{i=0}^N P_i * \Delta C_{p,i} \\ &= C_{p,0} + \{ \langle \Delta H^2 \rangle - \langle \Delta H \rangle^2 \} / RT^2 + \sum_{i=0}^N \Delta C_{p,i} \\ &= C_{p,0} + \langle C_{p, transition} \rangle + \Delta C_p \end{aligned}$$

Isothermal Titration Calorimetry (ITC)

Isothermal Titration Calorimetry (ITC) is a technique which allows the investigator to study the heat of interaction between two molecules. In ITC a syringe containing a "ligand" is titrated into a cell containing a solution of the "macromolecule". As the two elements interact, heat is released or absorbed in direct proportion to the amount of binding that occurs. When the macromolecule in the cell becomes saturated with added ligand, the heat signal diminishes until only the background heat of dilution is observed.

OVOTRANSFERRIN: Interaction of Isolated N & C Domains



Fluorescence Resonance Energy Transfer (FRET)

- Singlet excitation energy of one chromophore can transfer to another chromophore, which occurs by emission from one molecule and absorption by another. This transfer requires the resonance interaction by these two molecules (Forster-transfer). FRET is a dipole-induced dipole interaction, thus is a short distance interaction (proportional to R^{-6} of donor and acceptor, where R is their distance);

- The efficiency of energy transfer, E , is defined as the probability that deexcitation of the donor occurs by resonance transfer to the acceptor:

$$E = 1/(1+(R/R_0)^6)$$

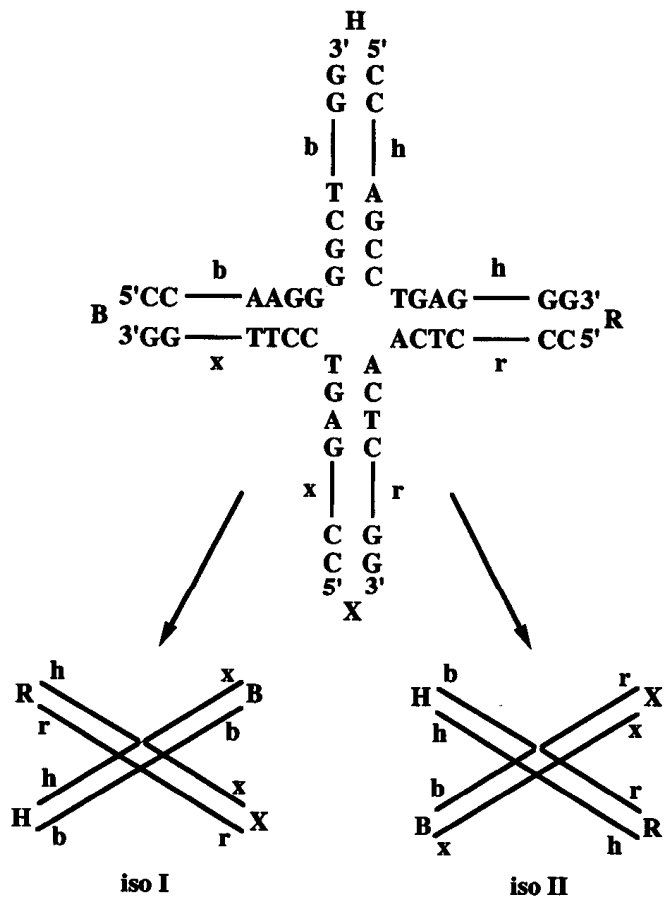
R_0 is the distance at which transfer is half-efficient. Usually, R_0 is from 15 to 45 Å;

- The efficiency of energy, E , is measured either by the decrease in donor fluorescence in the presence of the acceptor, or the increase in acceptor fluorescence.

$$E = 1 - \phi_{D+A}/\phi_D$$

$$E = 1 - \tau_{D+A}/\tau_D$$

- FRET can be used to measure the distance of two molecules between 10 to 80 Å (spectroscopic ruler).



(a) Acceptor

(b) Acceptor

(c) Donor

(d) Donor

