CORSO DI LAUREA IN BIOLOGIA PER LA SOSTENIBILITÀ

BIOCHIMICA APPLICATA (6 CFU)

LEZIONE 11

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Analisi conformazionali

- ✓Spettrofotometria: Perturbazione da solvente
- ✓Fluorescenza : Perturbazione da solvente;

  Fluorescenza estrinseca. FRET, FRAP
- ✓Proteine fluorescenti
- ✓Ultracentrifugazione analitica
- ✓Parziale proteolisi
- ✓Dicroismo circolare

CROMOFORI

- I cromofori sono i gruppi chimici all'interno di una molecola che sono responsabili dell'assorbimento della luce

- Spesso sono caratterizzati dalla presenza di doppi legami e sistemi di risonanza

- Lo spettro di assorbimento di un cromoforo è solo parzialmente determinato dalla sua natura chimica

CROMOFORI

- Lo spettro di assorbimento di un cromoforo è solo parzialmente determinato dalla sua natura chimica
- L'ambiente del cromoforo influenza le proprietà dello spettro - pH - polarità del solvente - effetti di orientamento

- I cromofori possono agire da **molecole reporter** che possono dare informazioni circa il loro ambiente .

CROMOFORI

- Effetti di protonazione/deprotonazione dovuti a cambiamenti di pH o effetti di ossidazione/riduzione influenzano la distribuzione elettronica dei cromofori

- polarità del solvente. Gli spettri cambiano in differenti solventi.

- Effetti di orientamento derivano da molecole di cromofori vicini. Ad es. hyperchromicity degli acidi nucleici.

CROMOFORI

I cromofori possono avere degli spostamenti dello spettro verso:

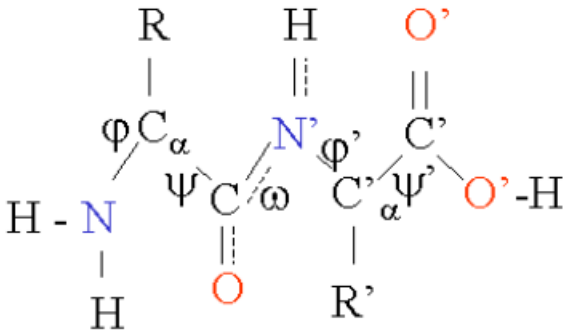
-lunghezze d'onda maggiori, e in questo caso si parla di effetto batocromico o red shift;
-Lunghezze d'onda minori, e in questo caso si parla di effetto ipsocromico o blue shift.

Oppure, si possono osservare:

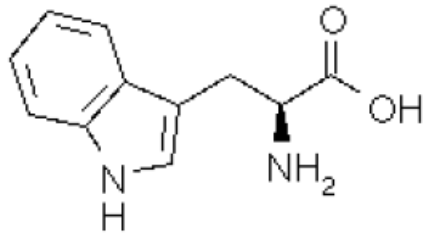
-aumento dell'intensità, e in questo caso si parla di effetto ipercromico;
-diminuzione dell'intensità, e in questo caso si parla di effetto ipocromico.

CROMOFORI delle PROTEINE

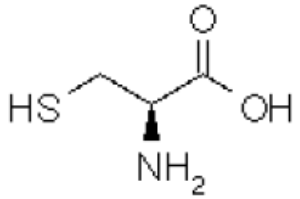
Luce Uv-Vis



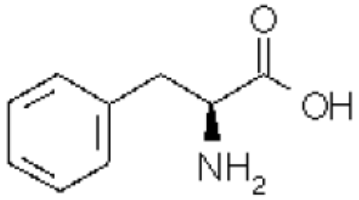
The peptide bond



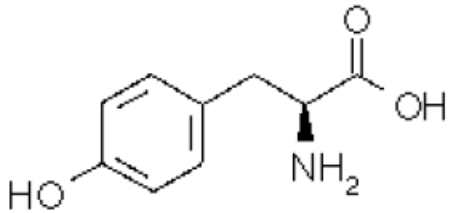
trp w Tryptophan



cys c Cystein



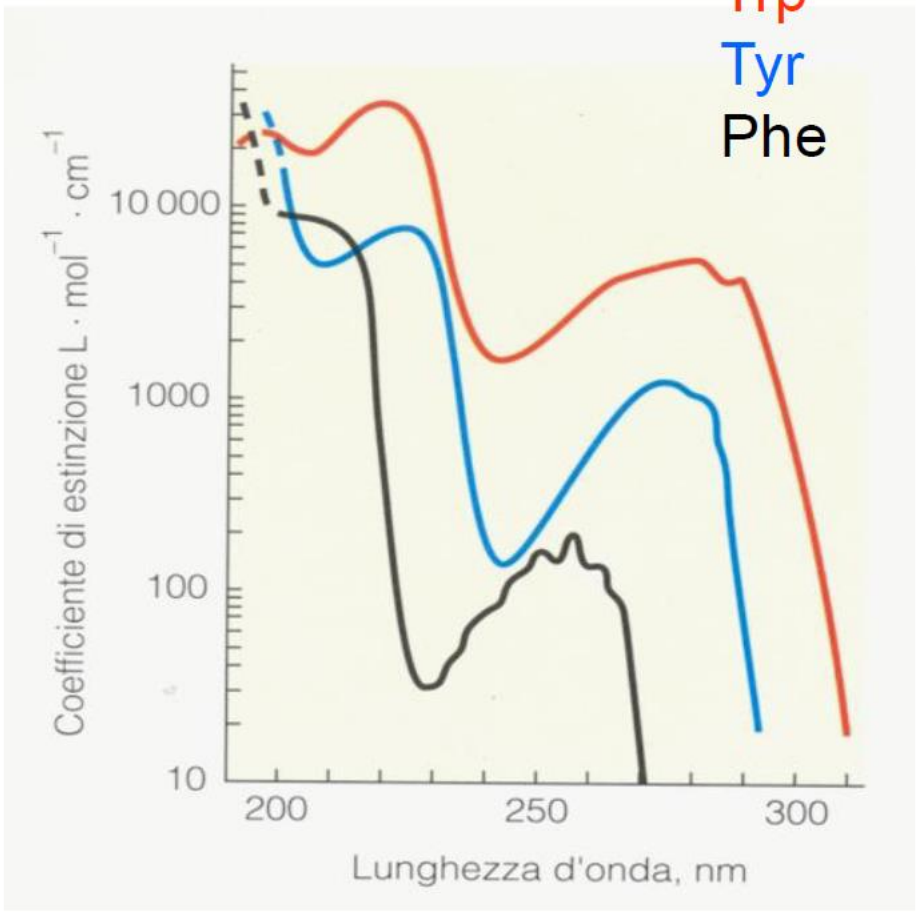
phe f Phenylalanin



tyr y Tyrosin

Spettrofotometria: Perturbazione da solvente

CROMOFORI delle PROTEINE



Luce Uv-Vis

$$A = \varepsilon\, b\, c$$

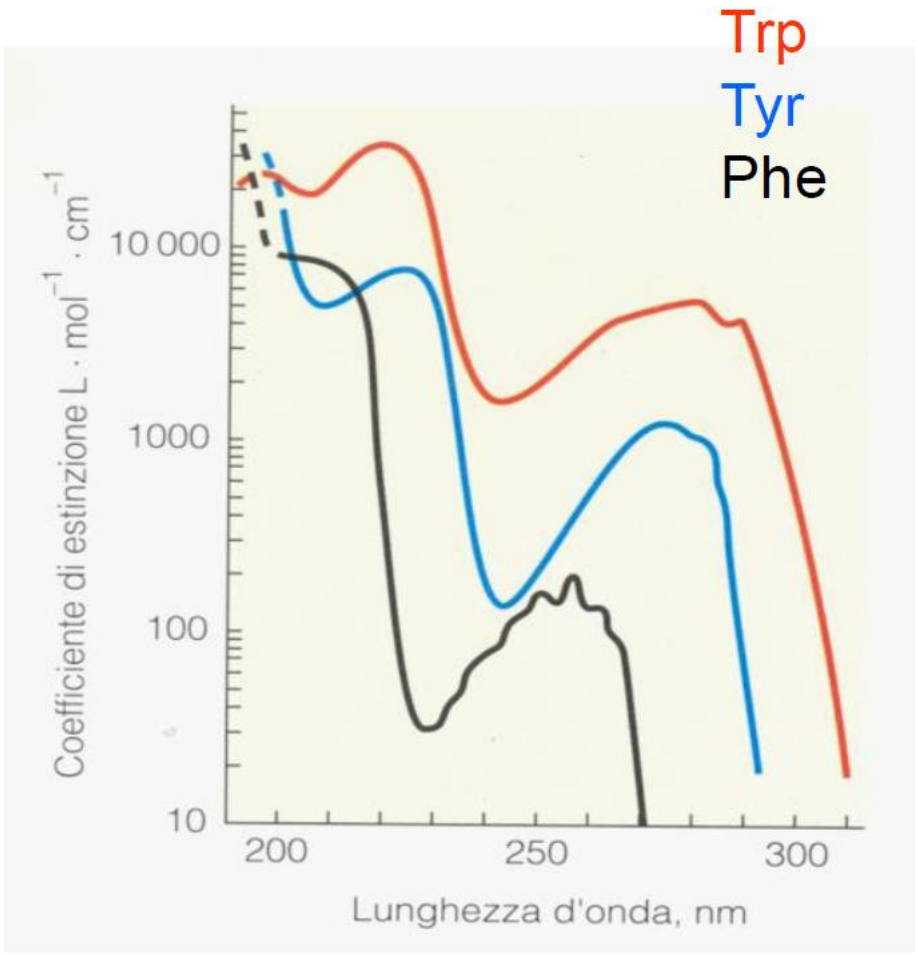
A assorbanza

ε coefficiente di estinzione molare

c concentrazione

CROMOFORI delle PROTEINE

Luce Uv-Vis



$A = \varepsilon bc$

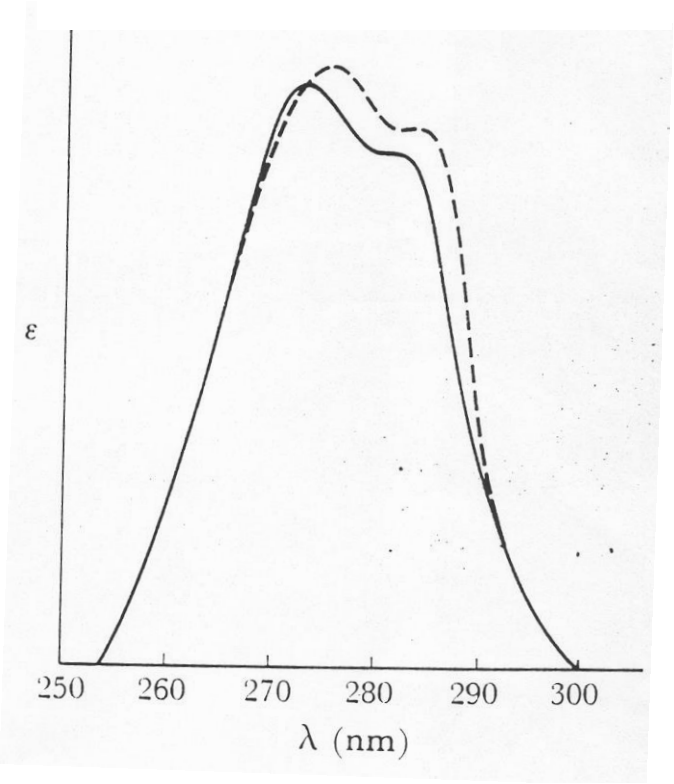
	λ_{max} (nm)	ε ($M^{-1}cm^{-1}$)
tryptophan:	**280**	**5600**
	219	**47000**
tyrosine:	**274**	**1400**
	222	**8000**
	193	**48000**
phenylalanine:	**257**	**200**
	206	**9300**
	188	**60000**

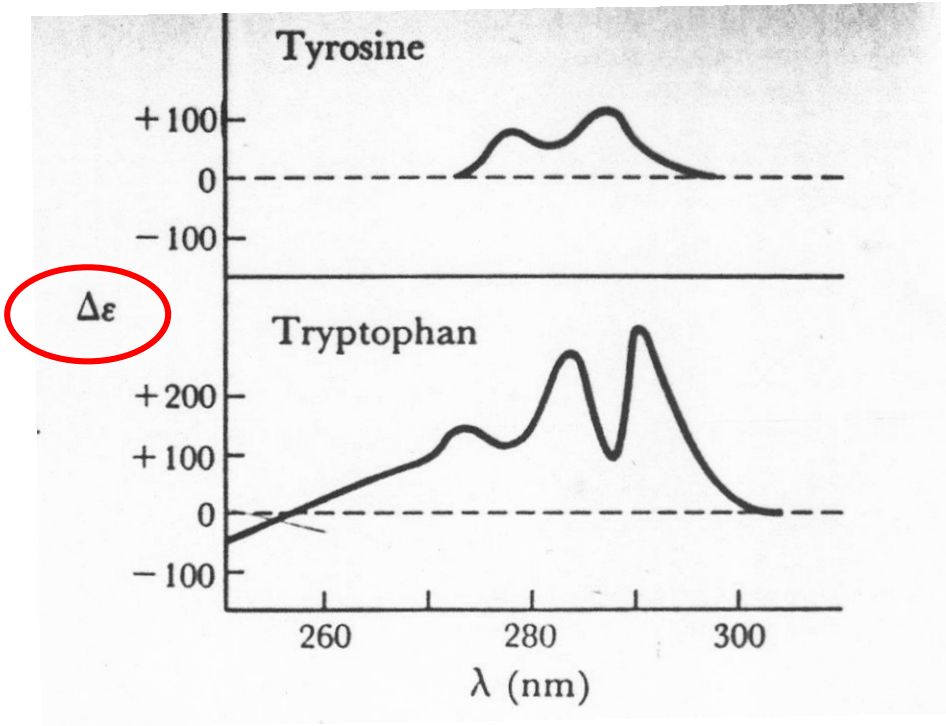
Spettrofotometria: Perturbazione da solvente

Effetto dei solventi sugli spettri di assorbimento

Perturbazione da solvente

Luce Uv-Vis





diminuzione della polarità

aumenta λ (spostamento batocromico)

Difference spectra for tyrosine and tryptophan in H_2O or 20% ethylene glycol. Different spectra can have negative values of Δε.

Empirical rules for the interpretation of the absorption spectra of biological macromolecules

If the amino acids tryptophan, tyrosine, phenylalanine, and histidine are shifted to a less polar environment, λ_{max} and ε increase. Hence:

a. If the spectrum of an amino acid in a protein in a polar solvent shows that λ_{max} and ε are higher than they are for the free amino acid in the same solvent, then that amino acid must be in an internal region of the protein ("buried"); and surrounded by nonpolar amino acids.

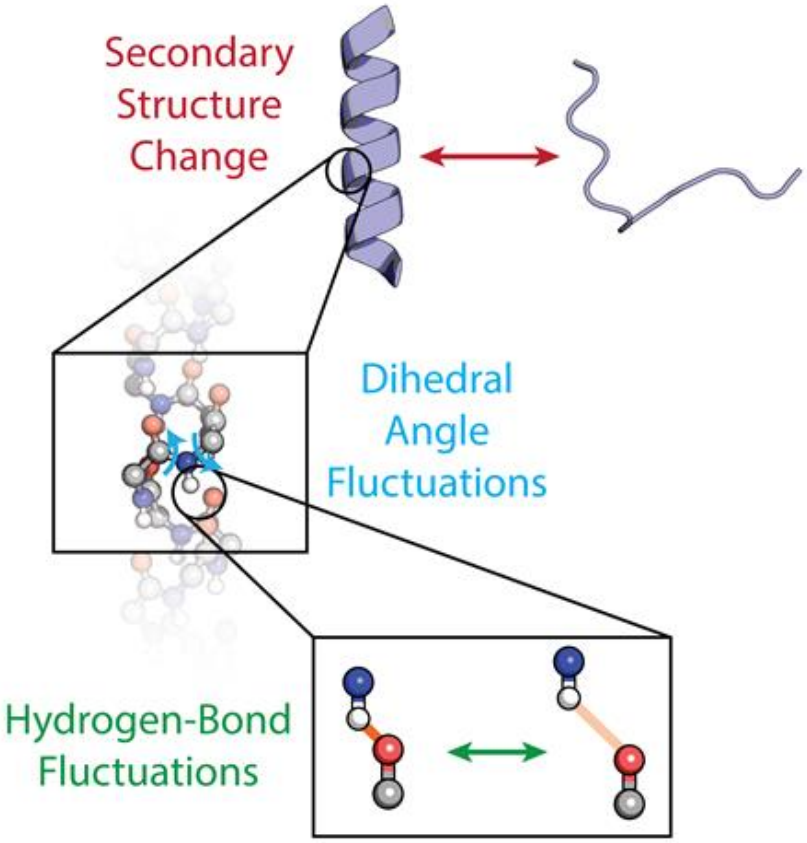
b. If the spectrum of a protein is sensitive to changes in the polarity of the solvent, the amino acid showing the change in λ_{max} and ε must be on the surface of the protein.

It must be ascertained that the change in polarity does not cause a conformational change that could bring an internal amino acid to the surface.

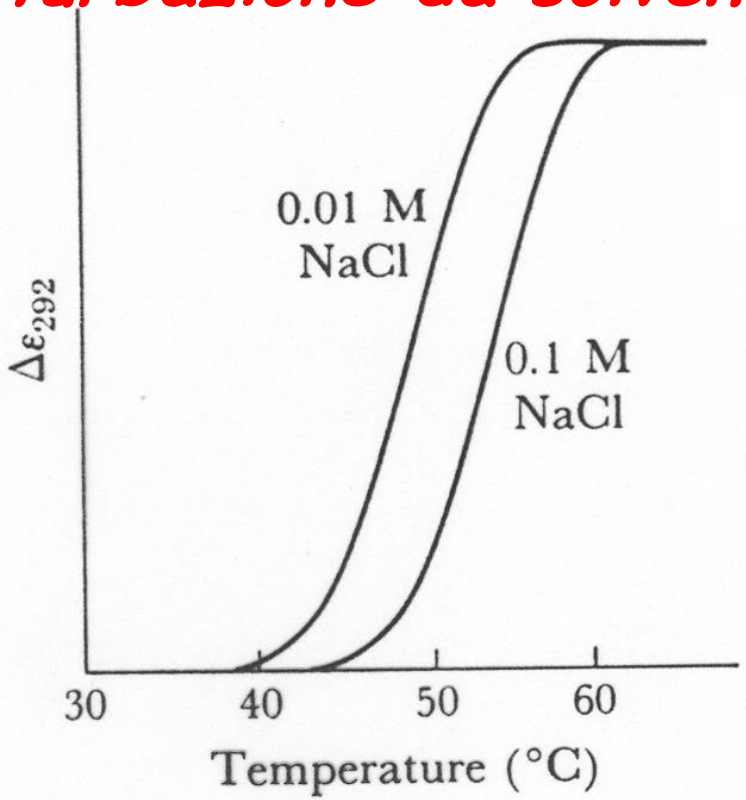
Perturbazione da solvente

Studio della stabilità delle proteine

Helix-coil transition



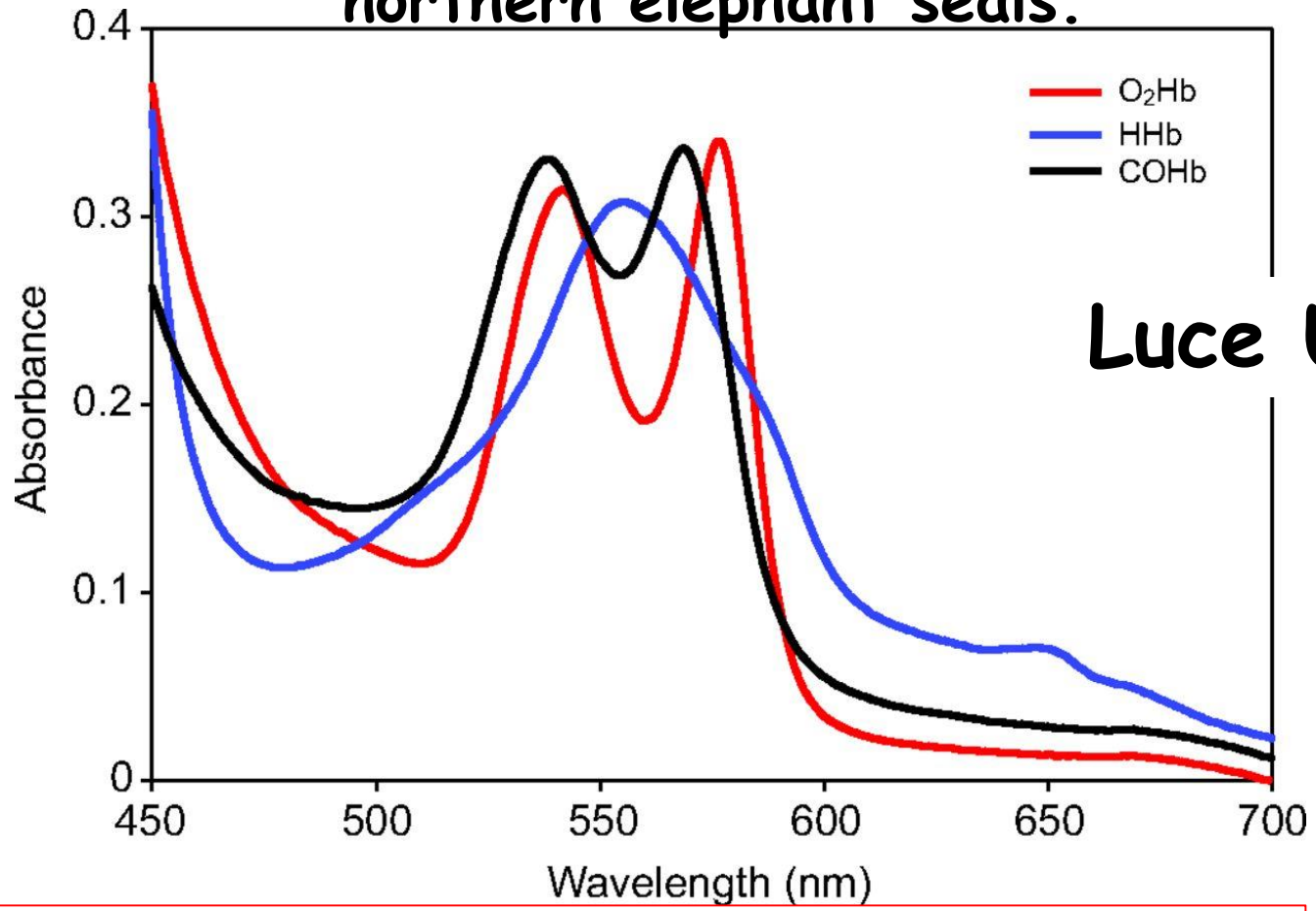
Perturbazione da solvente



Luce Uv-Vis

Helix-coil transition of a hypothetical protein assayed by perturbation difference spectroscopy at a single wavelength using 80% H_2O, 20% ethylene glycol containing two different NaCl concentrations. The reference solution for the difference spectrum is the protein in the ethylene glycol-NaCl solution at 20°C. This protein is more stable at higher NaCl concentration since a higher temperature is needed for unfolding in 0.1 M.

Absorption spectra for oxyhemoglobin (O_2Hb), deoxyhemoglobin (HHb) and carboxyhemoglobin (COHb) in northern elephant seals.



Luce Uv-Vis

Peaks for O_2Hb (542 and 577 nm), HHb (555 nm) and COHb (539 and 569 nm) match those of other mammalian species (human, cow, sheep)

Michael S. Tift et al. J Exp Biol 2014;217:1752-1757

Journal of Experimental Biology

Effetto del pH sullo spettro di assorbimento

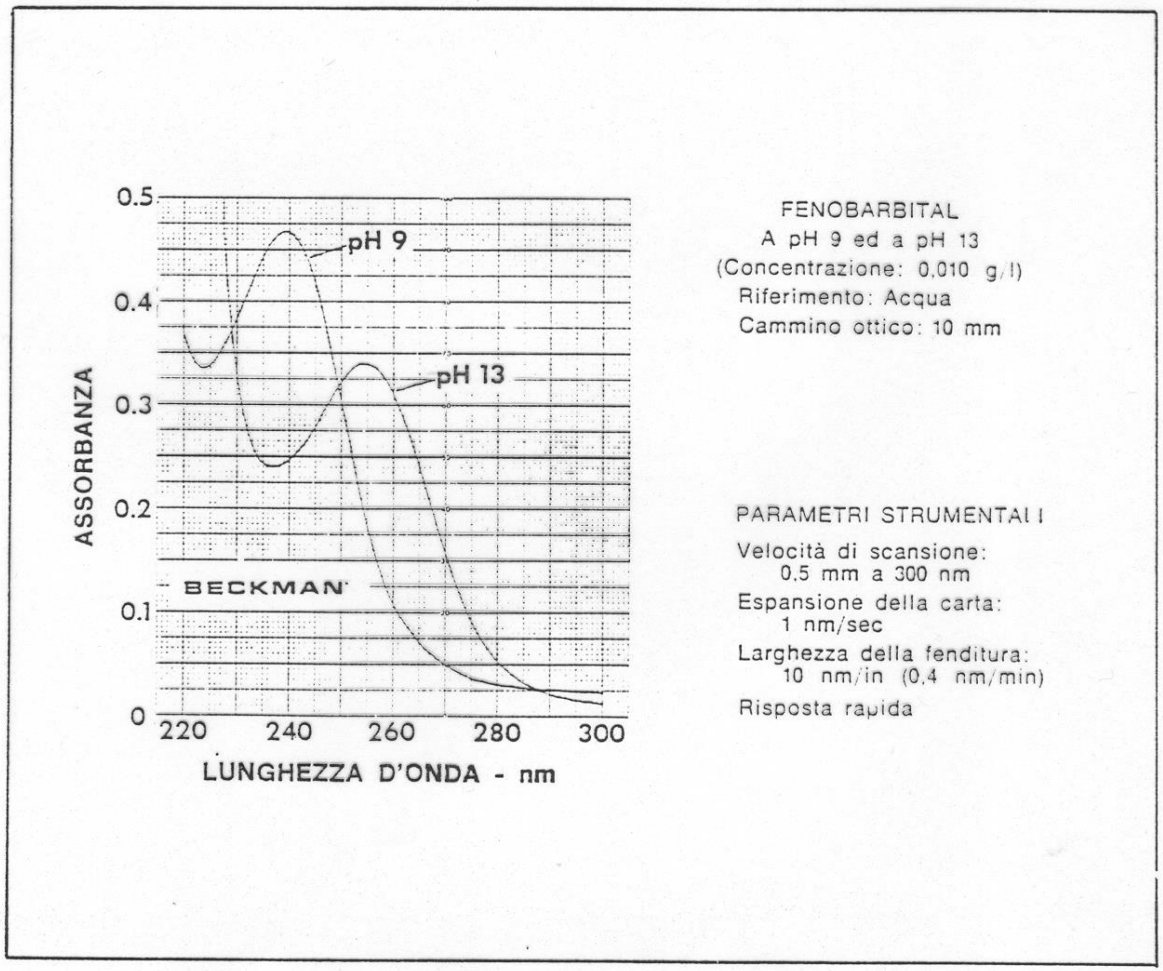
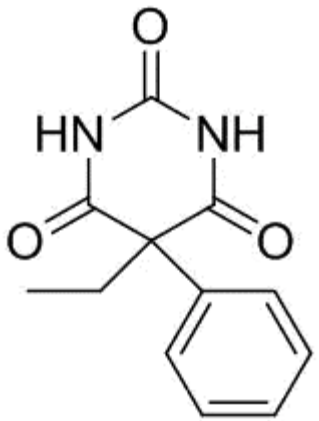


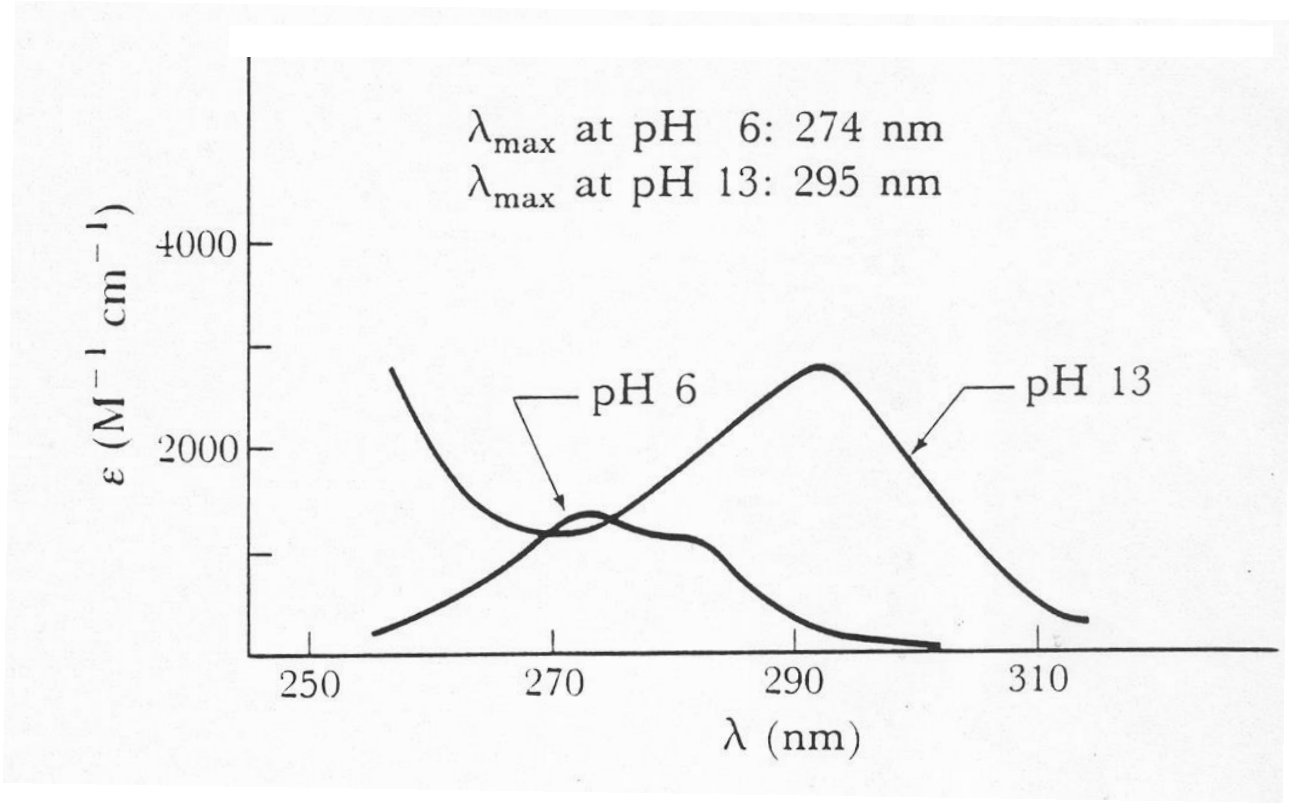
Fig. 3.4 - Effetto del pH sullo spettro di assorbimento

Luce Uv-Vis



Effetto del pH sullo spettro di assorbimento

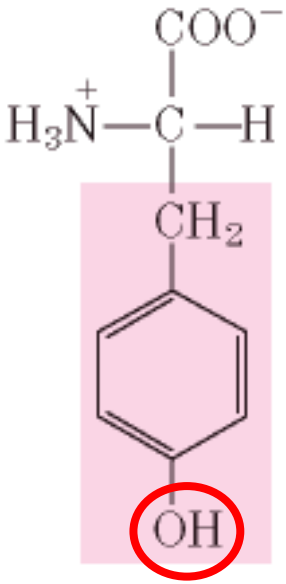
Absorption spectrum or tyrosine at pH 6 and 13



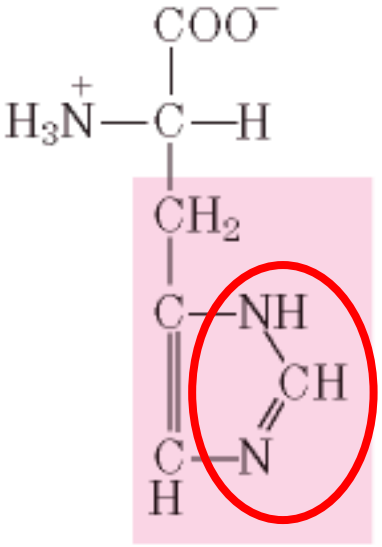
Note that both λ_{max} ε are increased when the phenolic OH is dissociated.

Effetto del pH sullo spettro di assorbimento

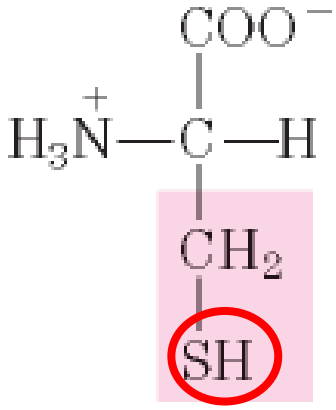
For amino acids, λ_{max} and ε are always increased if a titratable group (e.g., the OH of tyrosine, imidazole of histidine, and SH of cysteine) is charged.



Tirosina



Istidina



Cisteina

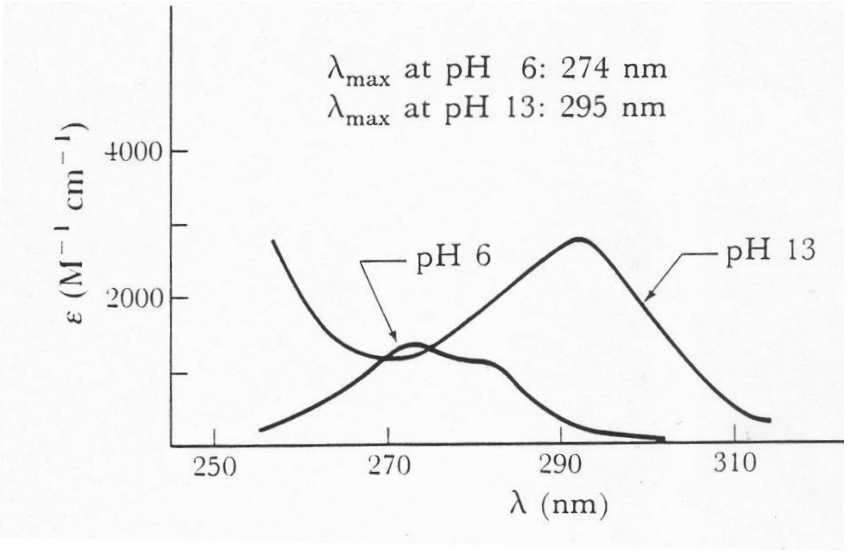
Effetto del pH sullo spettro di assorbimento

For amino acids, λ_{max} and ε are always increased if a titratable group (e.g., the OH of tyrosine, imidazole of histidine, and SH of cysteine) is charged. Hence, when the pH is changed:

a. if no spectral change is observed for one of these chromophores and if the pH change is such that titration of a free amino acid would have occurred, **the chromophore must be buried in a nonpolar region of the protein.**

b. If the spectral change as a function of pH indicates that the ionizable group has the same pK as it would if free in solution, then the amino acid is on the surface of the protein.

c. If the spectral change as a function of pH indicates a very different pK, then the amino acid is likely to be in **a strongly polar environment** (e.g., a tyrosine surrounded by carboxyl groups).



Absorption spectrum or tyrosine at pH 6 and 13.

Note that both λ_{max} ε are increased when the phenolic OH is dissociated.

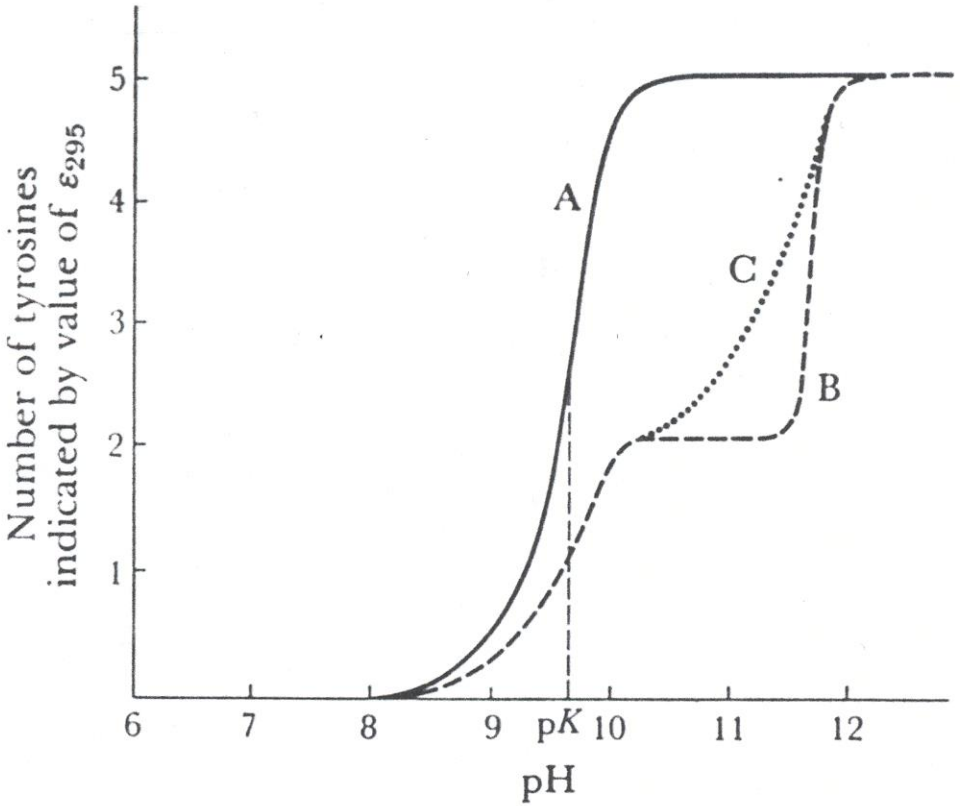


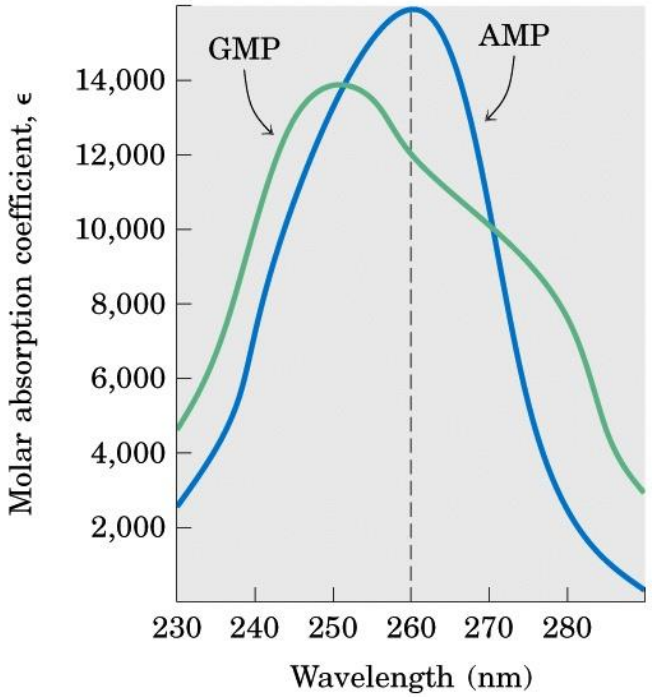
Figure 14-18

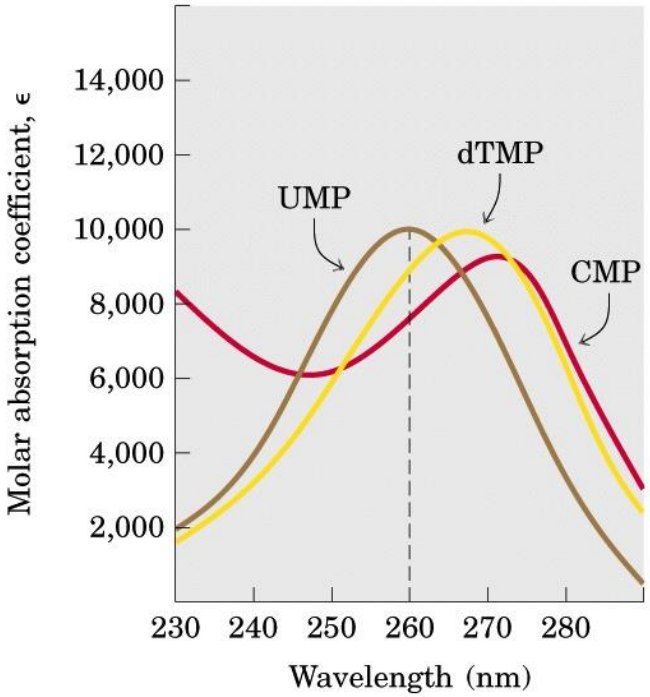
pH titration curves for tyrosine, using ε_{295} as an assay. The hypothetical protein contains five tyrosines. In curve A, all five are on the surface. In curve B, two are on the surface and the remaining three are internal—in a non-polar environment and therefore not titratable. In curve C, the three internal ones are in a polar environment and accessible to the solvent.

Spettrofotometria: Effetto del pH

CROMOFORI degli acidi nucleici

Basi azotate, nucleosidi, nucleotidi





Molar absorption coefficient at 260 nm, ϵ_{260} ($M^{-1}cm^{-1}$)	
AMP	15,400
GMP	11,700
CMP	7,500
UMP	9,900
dTMP	9,200

Spettrofotometria:

For purines and pyrimidines, ε decreases as their ring systems become parallel and nearer to one another (more stacked).

The value of ε decreases in the following series: free base> base in an unstacked single-stranded polynucleotide > base in a stacked single-stranded polynucleotide > base in a double-stranded polynucleotide.

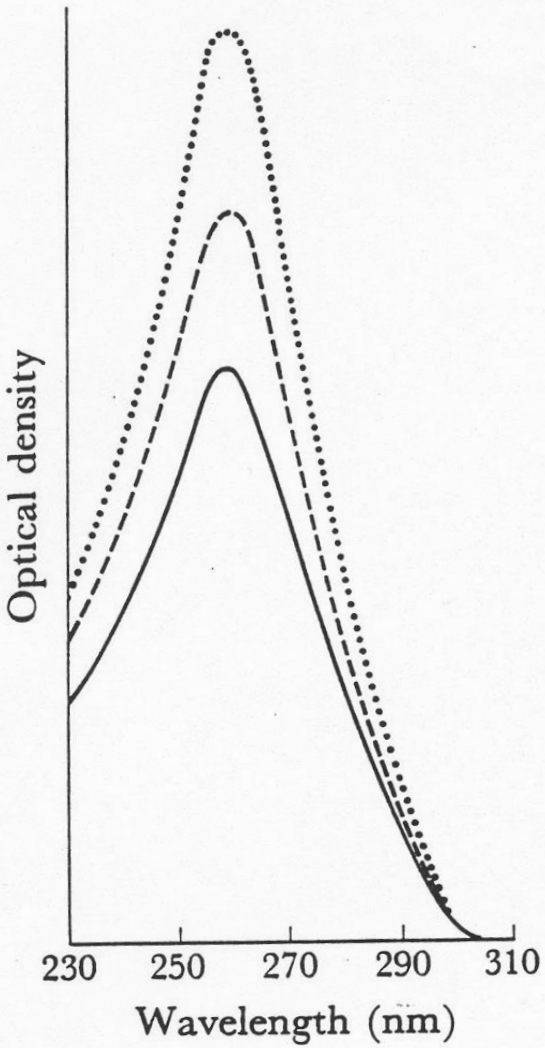
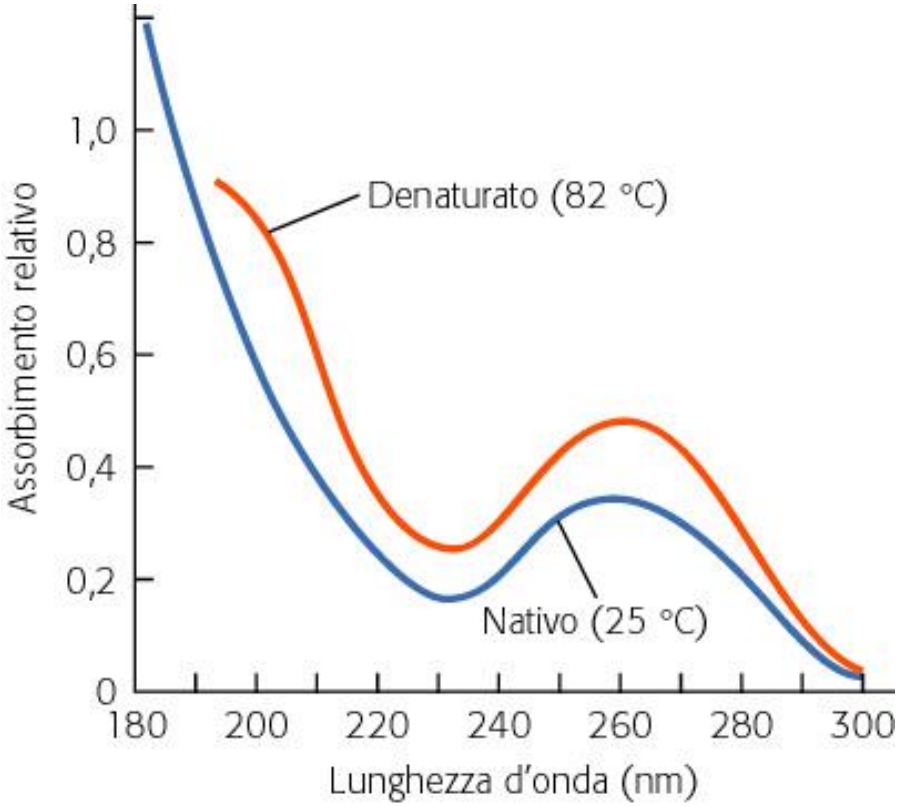


Figure 14-9
Spectra of T7 DNA as a double-stranded DNA (solid line), as a single-stranded DNA (dashed line), or after hydrolysis to free nucleotides (dotted line), showing the decrease in optical density (hypochromicity) that accompanies the formation of a more ordered structure. All spectra were obtained at the same concentration.

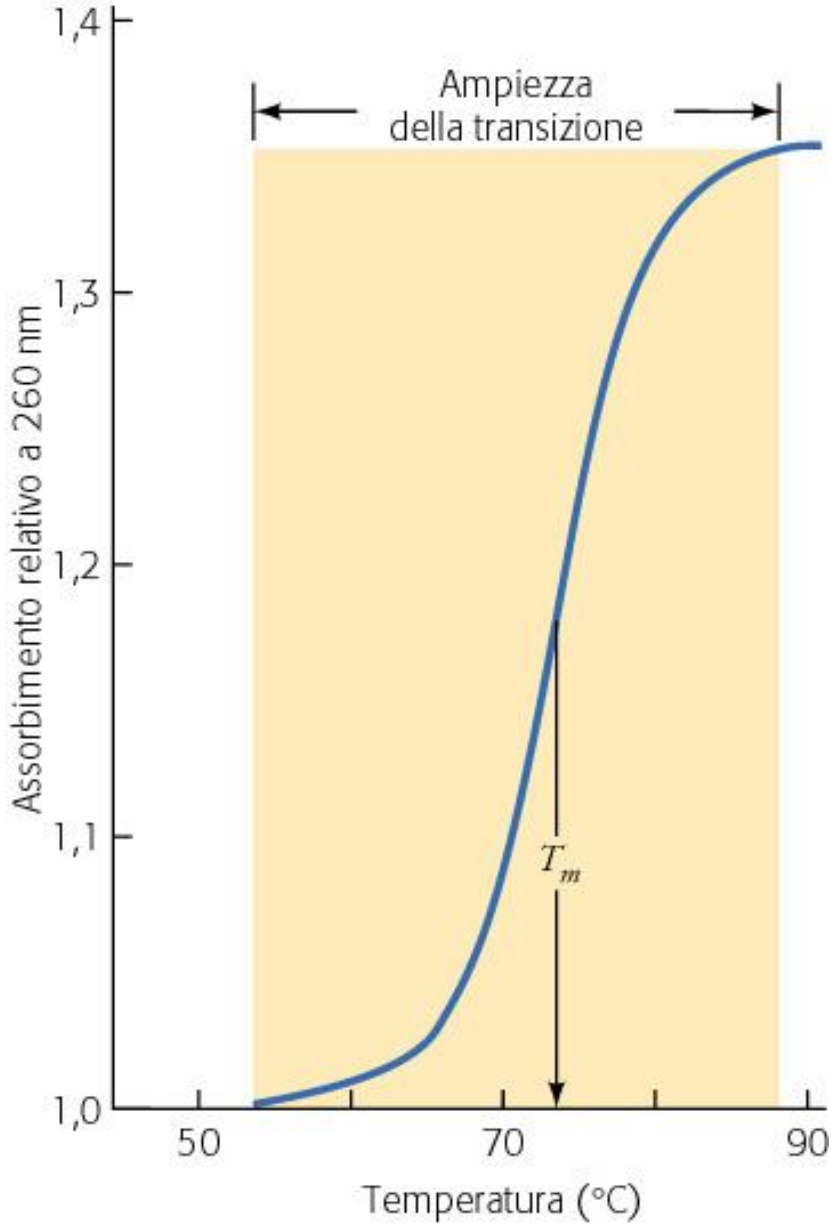
Spettrofotometria:



Spettro di assorbimento agli UV di DNA di *E.coli* nativo e denaturato. La denaturazione non cambia la forma complessiva della curva di assorbimento ma solo la sua intensità

Spettrofotometria:

Curva di fusione del DNA



L'assorbimento relativo è il rapporto dell' assorbimento alle temperature indicate con quello a 25°C.
La temperatura di fusione, T_m, è la temperatura a cui si raggiunge la metà dell'incremento massimo dell'assorbimento.

Spettrofotometria:

Curve di fusione del DNA

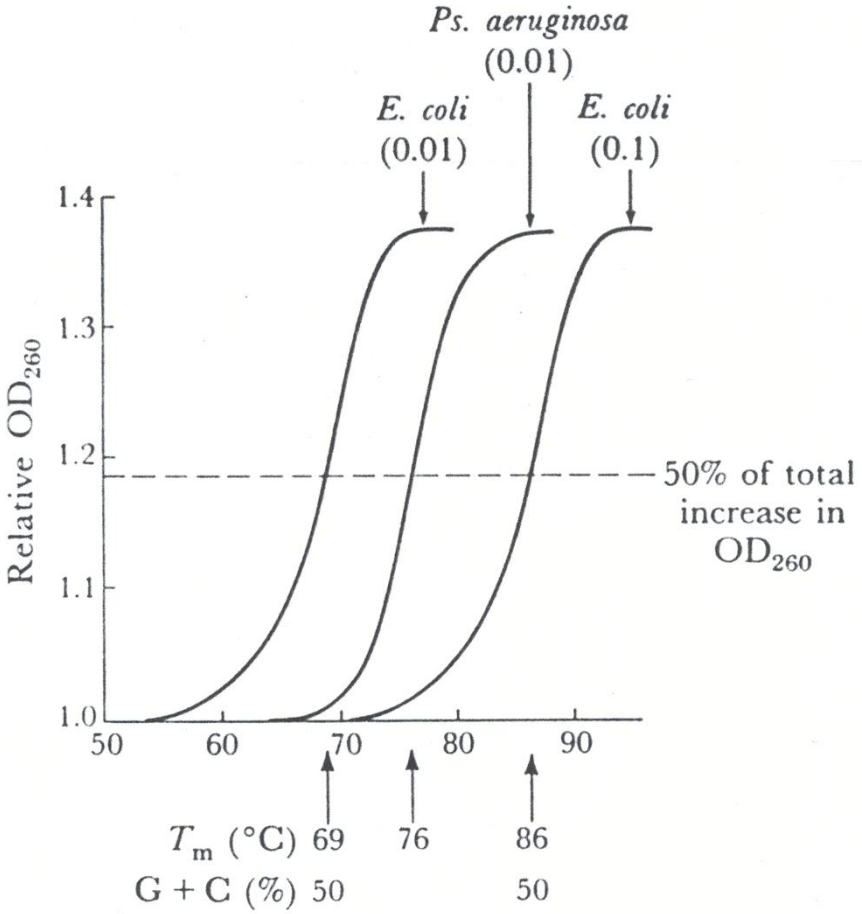
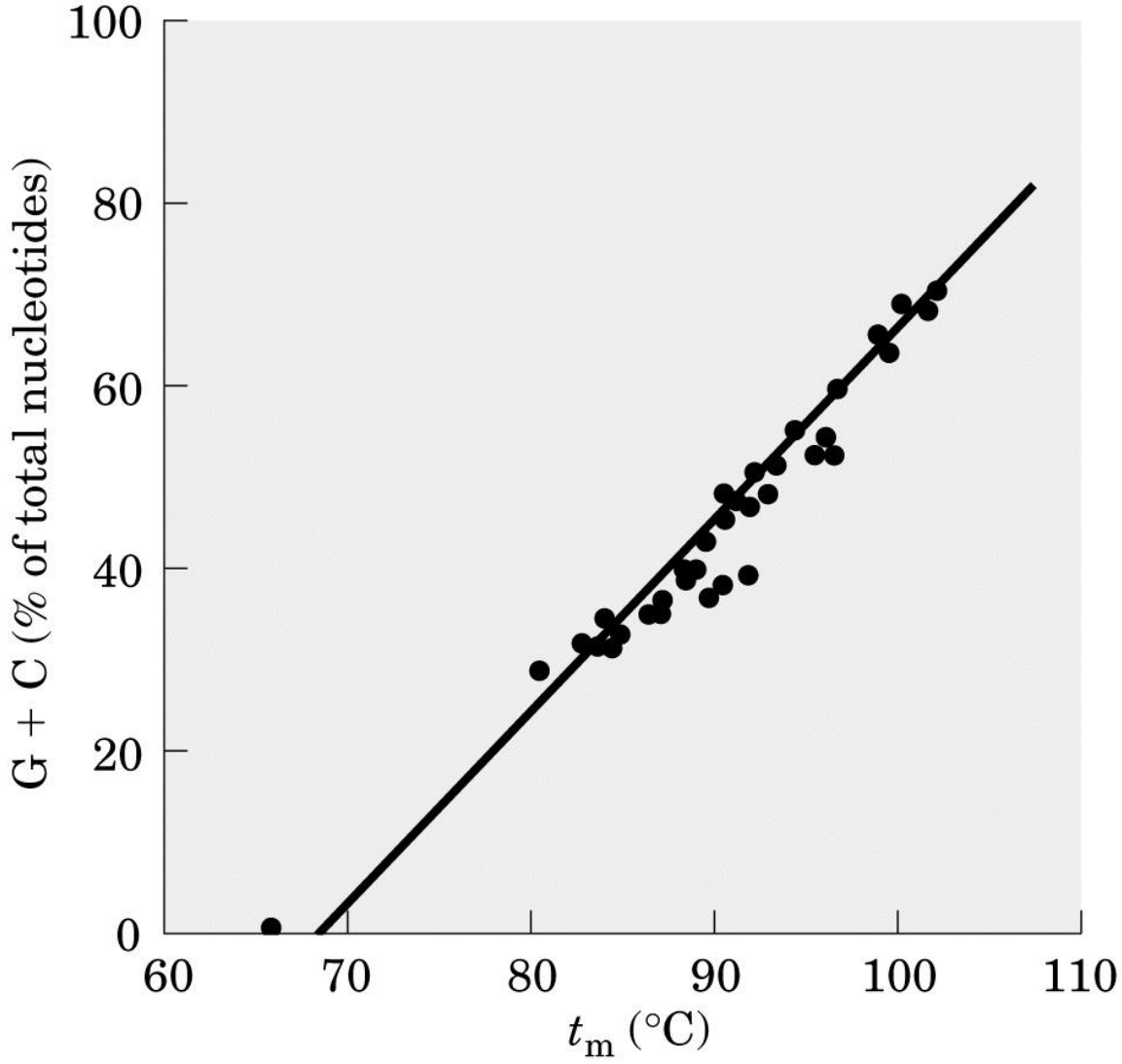


Figure 14-13
Optical density of three DNA solutions as a function of temperature: *E. coli* DNA (40% G + C) in 0.01 M PO_4, pH 7.8; *Pseudomonas aeruginosa* DNA (68% G + C) in 0.01 M PO_4, pH 7.8. The temperature at which the absorbance change is 50% complete is T_m, the melting temperature. Note that T_m increases with ionic strength and with G + C content.

Spettrofotometria:



Variazione delle temperature di fusione, T_m, di vari DNA in funzione del loro contenuto in G+C

Spettrofotometria: