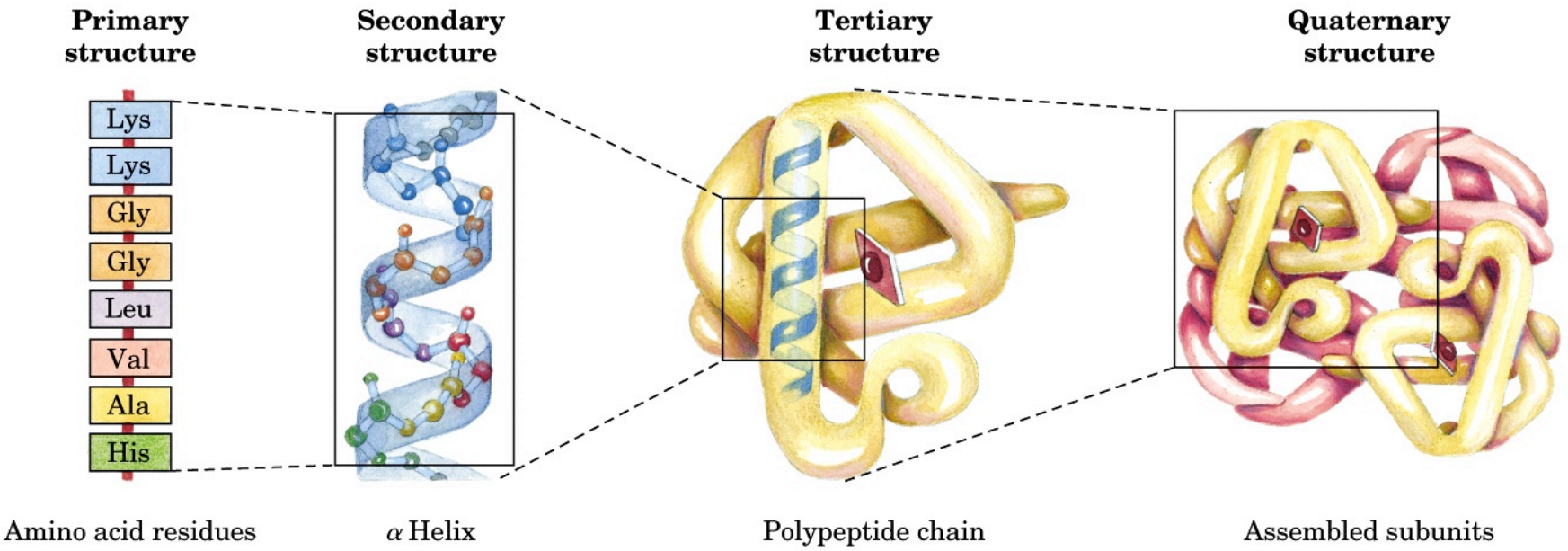
# La sequenza amminoacidica di una proteina ne determina la struttura secondaria, terziaria e quaternaria




**La struttura secondaria:**

**- le catene polipetidiche si possono ripiegare in strutture regolari come l'alfa-elica e foglietto beta**

# PURIFICAZIONE e CARATTERIZZAZIONE DELLE PROTEINE

# Sequenza di purificazione

table 5–5

**A Purification Table for a Hypothetical Enzyme***

| Procedure or step | Fraction volume (ml) | Total protein (mg) | Activity (units) | Specific activity (units/mg) |
|---|---|---|---|---|
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| 5. Affinity chromatog-raphy | 6 | 3 | 45,000 | 15,000 |

*All data represent the status of the sample *after* the designated procedure has been carried out. Activity and specific activity are defined on page 137.

## STRUMENTI NECESSARI PER MONITORARE LA SEQUENZA DI PURIFICAZIONE DI UNA PROTEINA

- Metodo per misurare la quantita' totale di proteine in un estratto grezzo(g/ml)
- Metodo per misurare la attivita' biologica della proteina (U/ml)

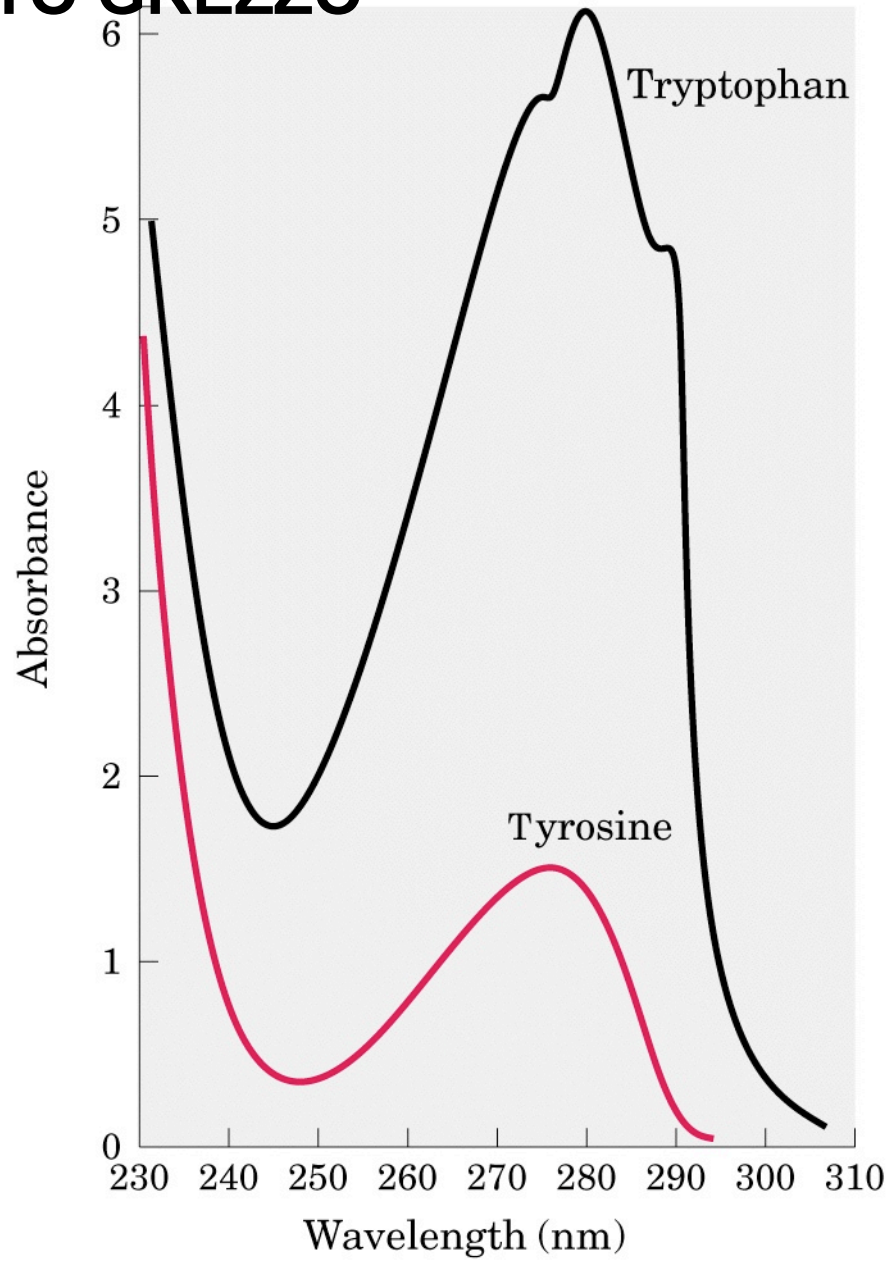
$$\frac{\text{Attivita' biologica della proteina/ml}}{\text{Proteine totali/ml}} = \text{Attivita' specifica}$$

**Ad ogni step di purificazione l'attivita' specifica deve aumentare.**

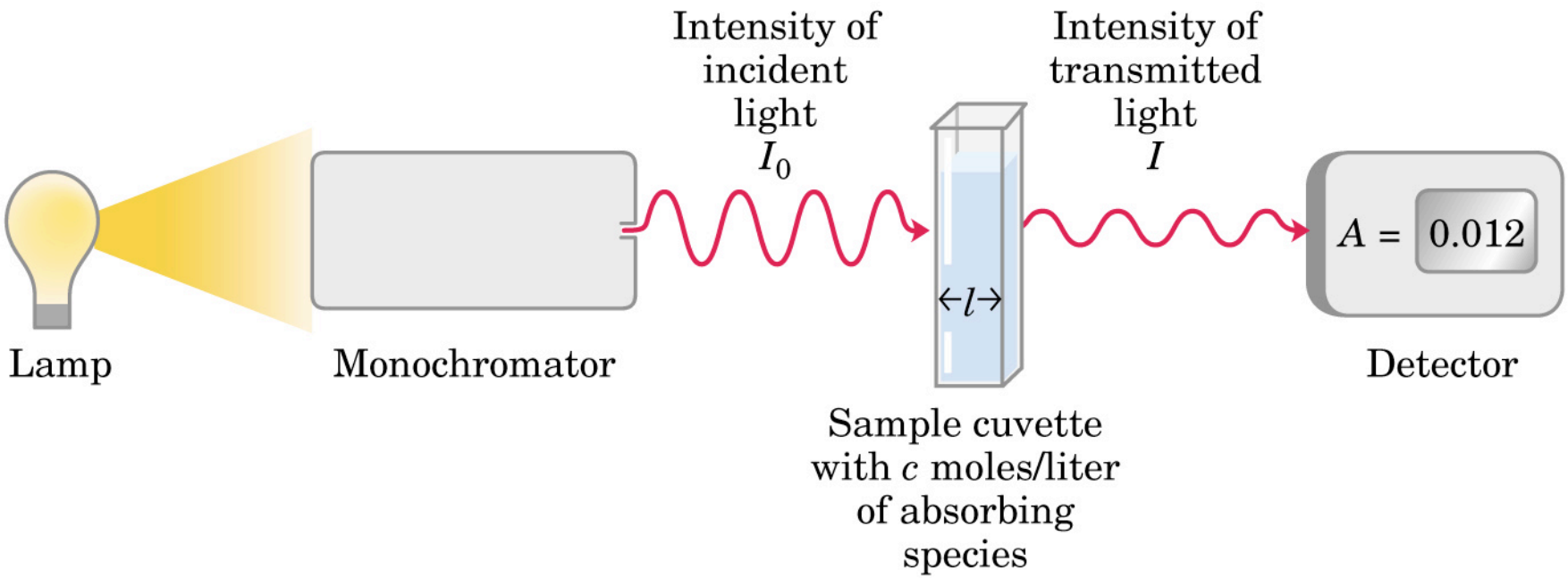
# MISURA DELLA QUANTITA' DI PROTEINA IN UN ESTRATTO GREZZO

## Proprietà spettroscopiche

Le proteine assorbono le radiazioni elettromagnetiche nel campo dell'UV.
Il massimo assorbimento si registra a $\lambda = 280$ nm e dipende Dalla presenza di amminoacidi Aromatici.



# Le molecole assorbono la luce




**LEGGE DI LAMBERT-BEER**

**Log Io/I = ecl**

**Io=luce incidente**
**I =luce trasmessa**
**e =coefficiente di estinzione molare**
**c =concentrazione della sostanza**
**l =cammino ottico**

# Saggio di attivita' biologica
# ……dipende dal tipo di proteina che si vuole purificare

se la proteina e' un enzima:

Attivita' enzimatica = quantita' di substrato trasformato in prodotto nell'unita' di tempo

Enzima: lattico deidrogenasi

*Riduce il piruvato a lattato (reazione red-ox)*

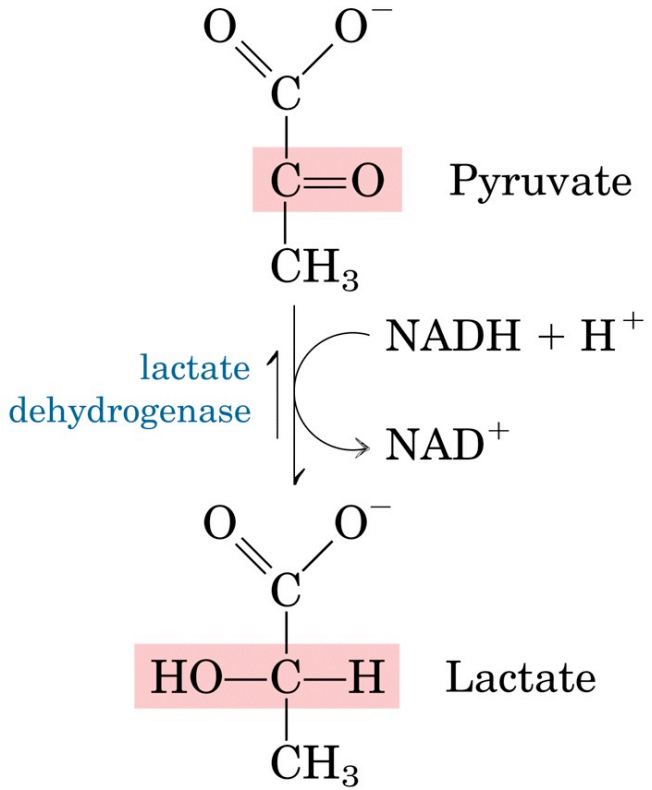
Piruvato+ NADH+$H^+$ $\leftrightarrow$ lattato+ $NAD^+$

*Specie ridotta*: piruvato

*specie ossidata*: NAD+

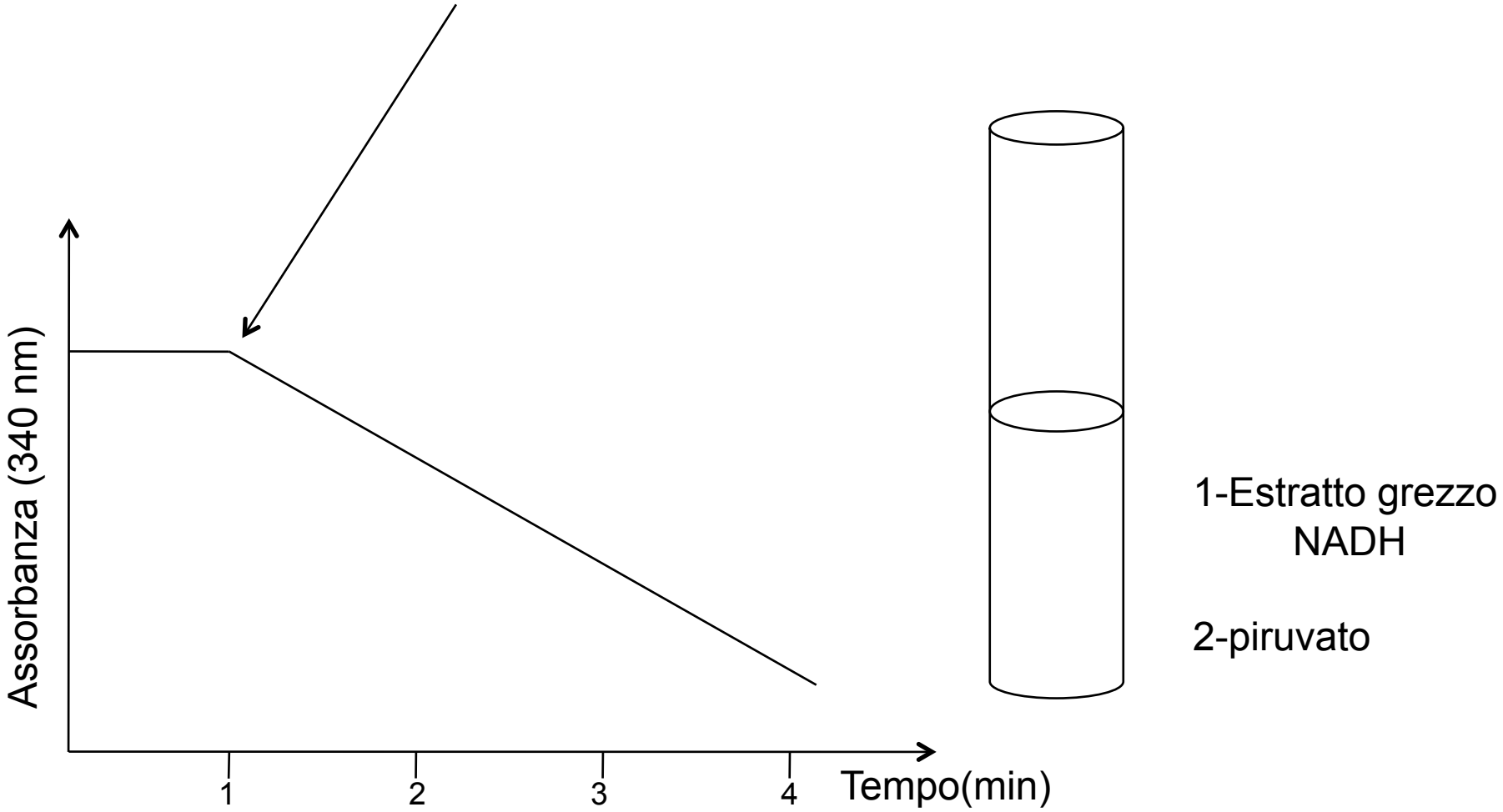
**NADH assorbe a 340 nm**

Attivita' enzimatica = Diminuzione dell'assorbimento a 340 nm nell'unita' di tempo



1- Estratto grezzo ( 1 ml) contenente 10 mg di proteine +NADH in eccesso (100 mM) misuro l'assorbimento a 340 nm

2-Aggiungo il substrato (piruvato) in eccesso (100 mM)



# Sequenza di purificazione

table 5–5

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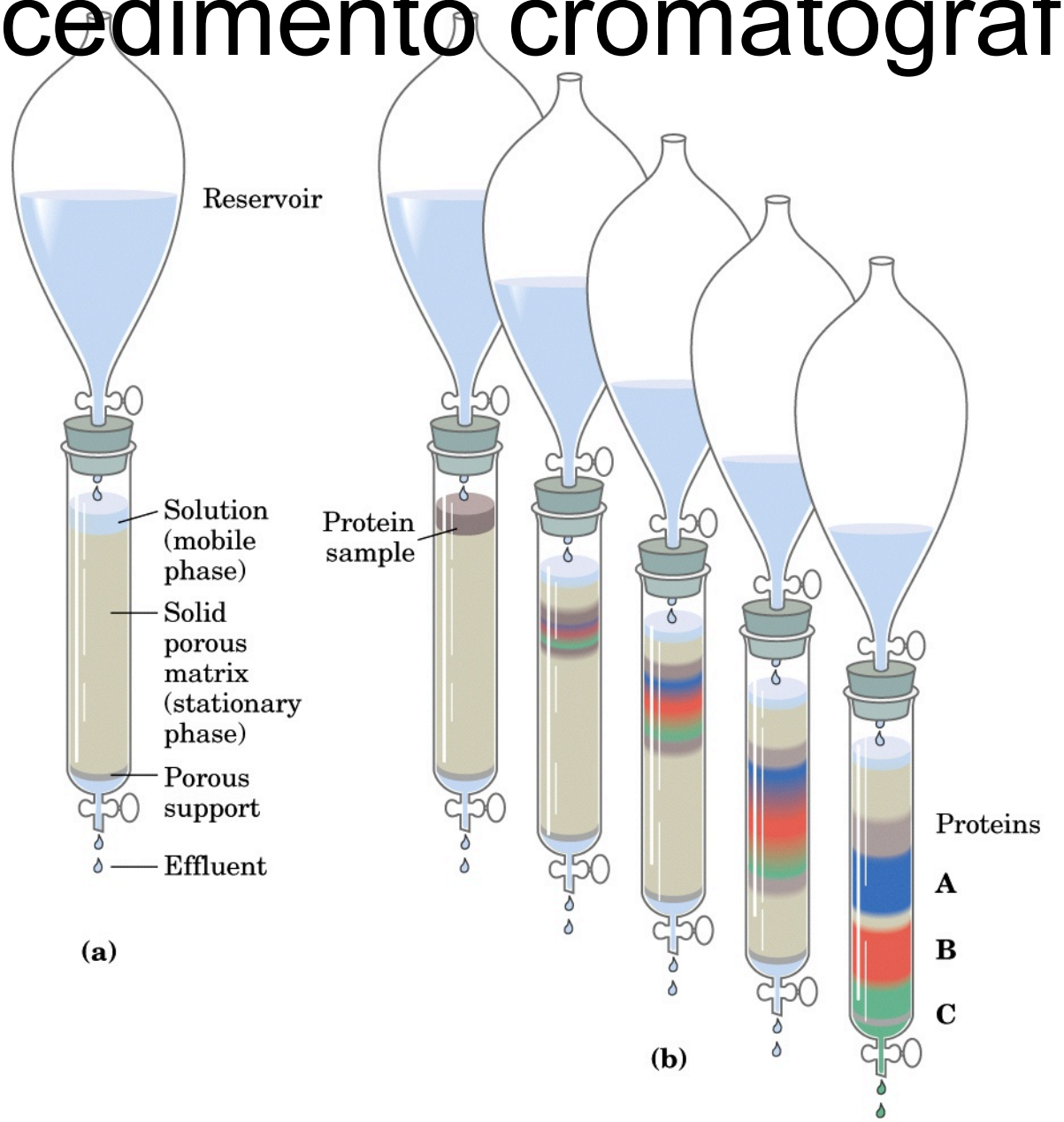
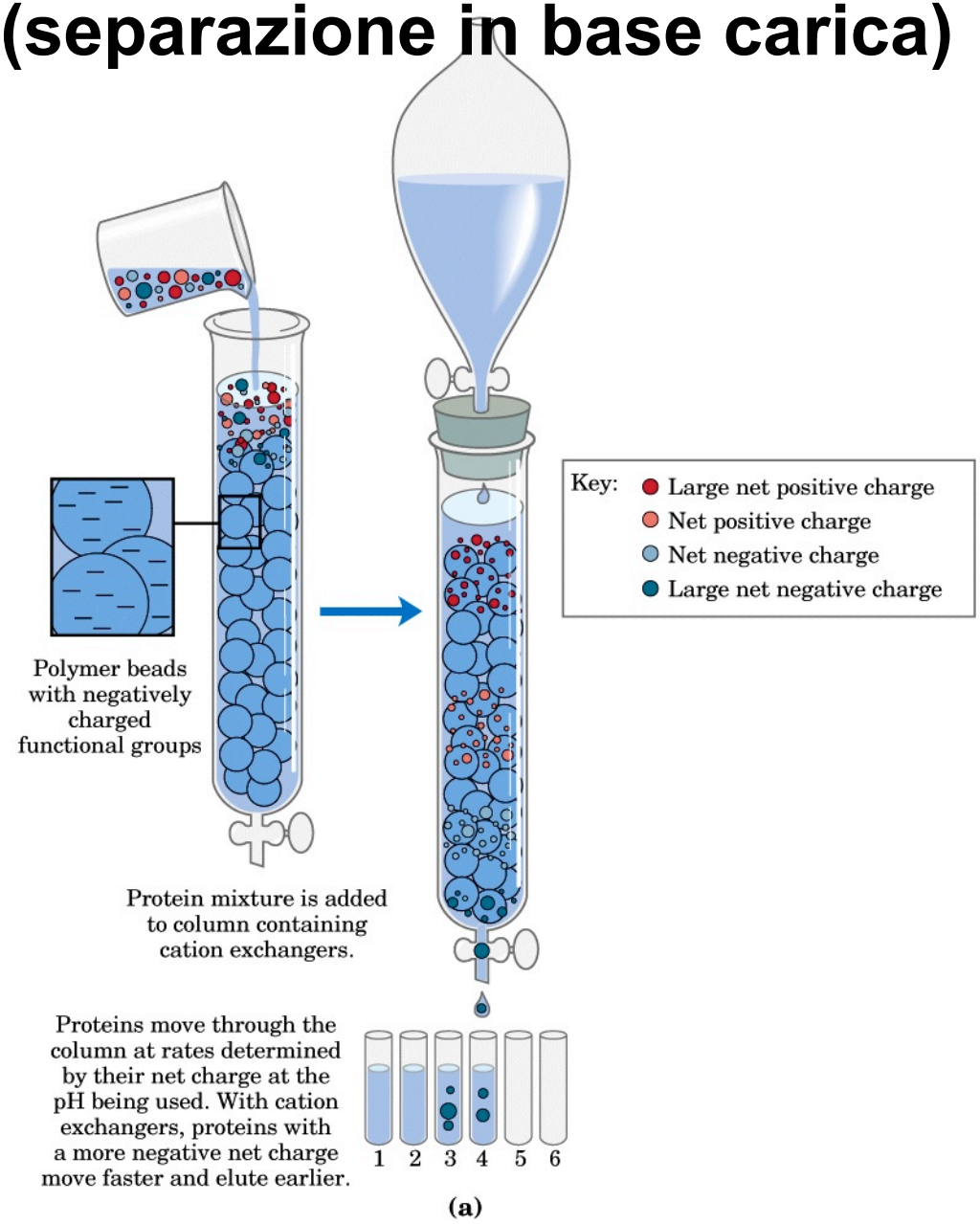
# Procedimento cromatografico



# Scambio ionico
## (separazione in base carica)



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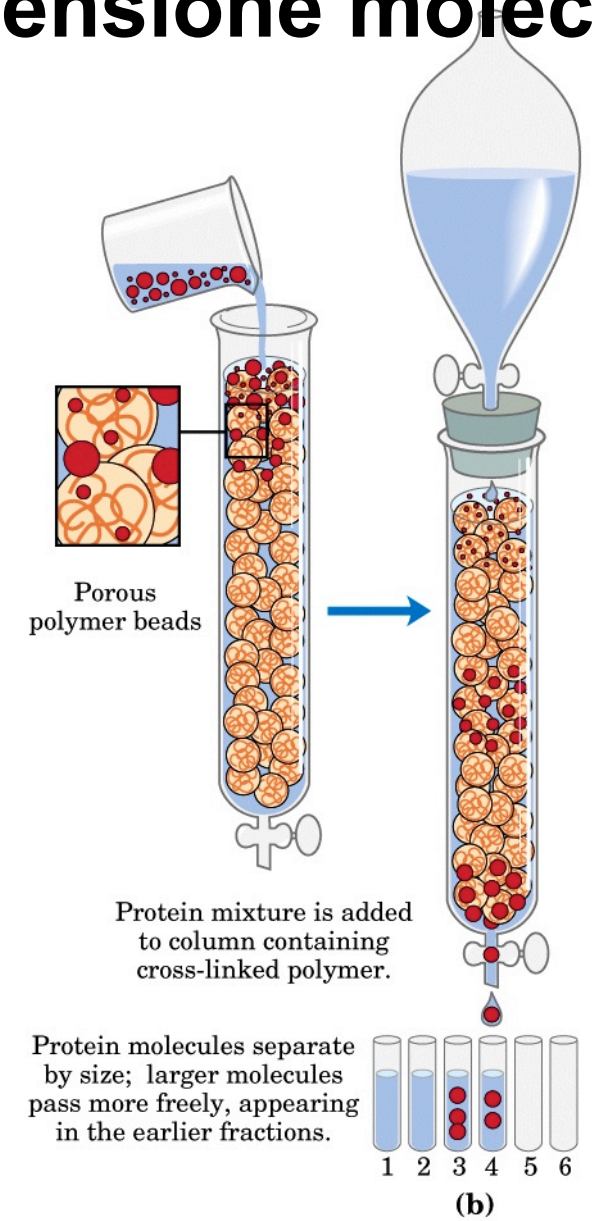
# Gel-filtrazione (separazione in base alla dimensione molecolare)



# Sequenza di purificazione

table 5–5

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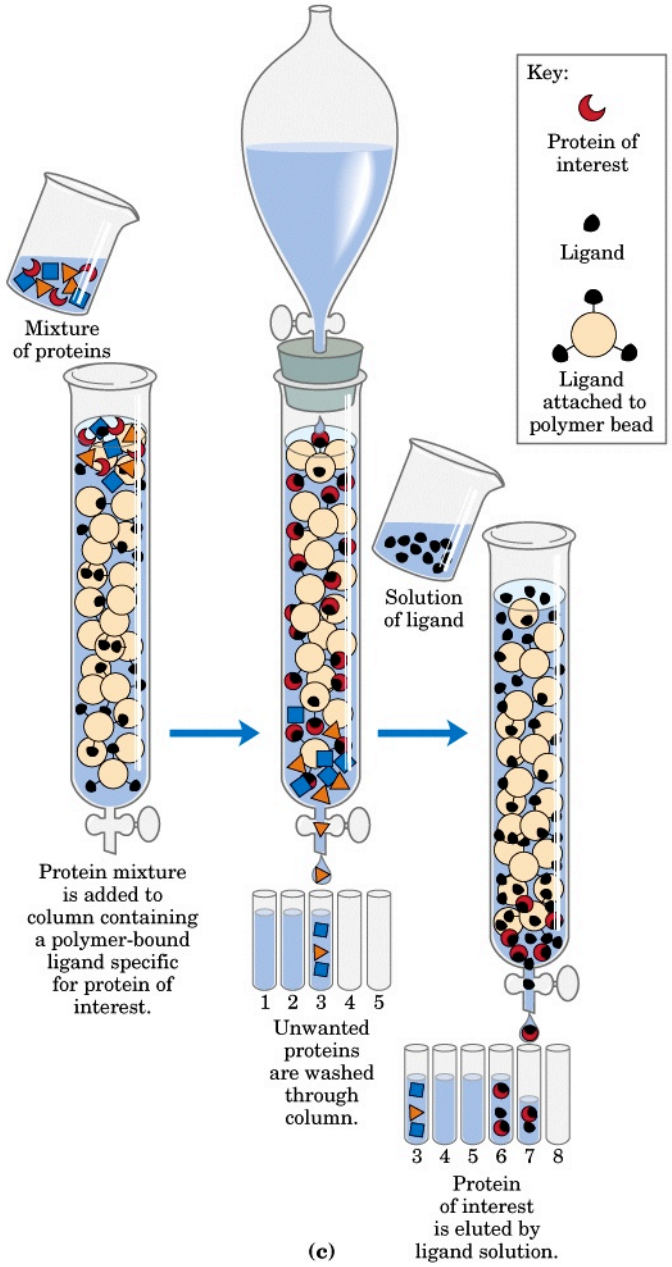
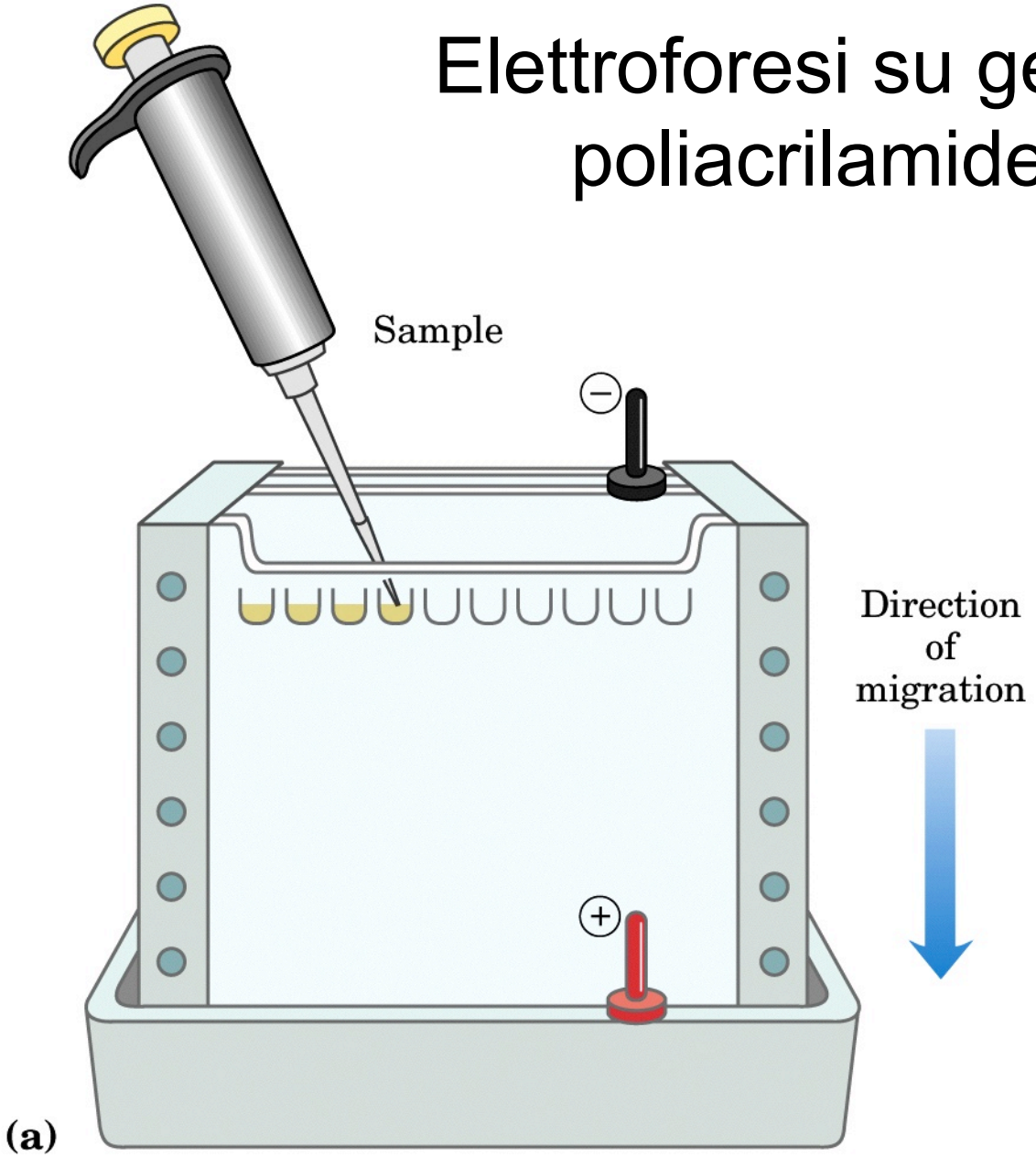
# Cromatografia di affinità

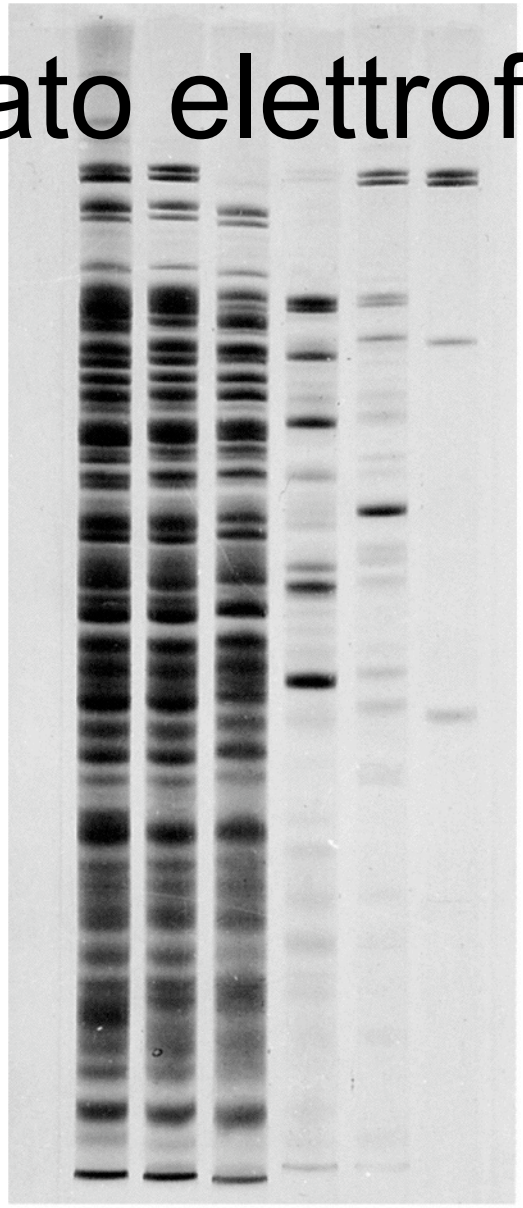


# CRITERI DI PUREZZA

# Elettroforesi su gel di poliacrilamide

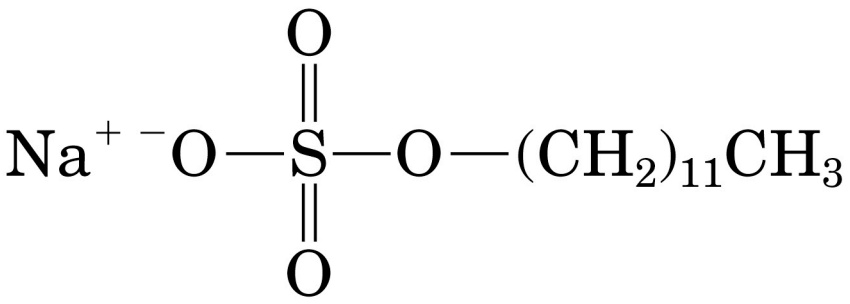


# Tracciato elettroforetico

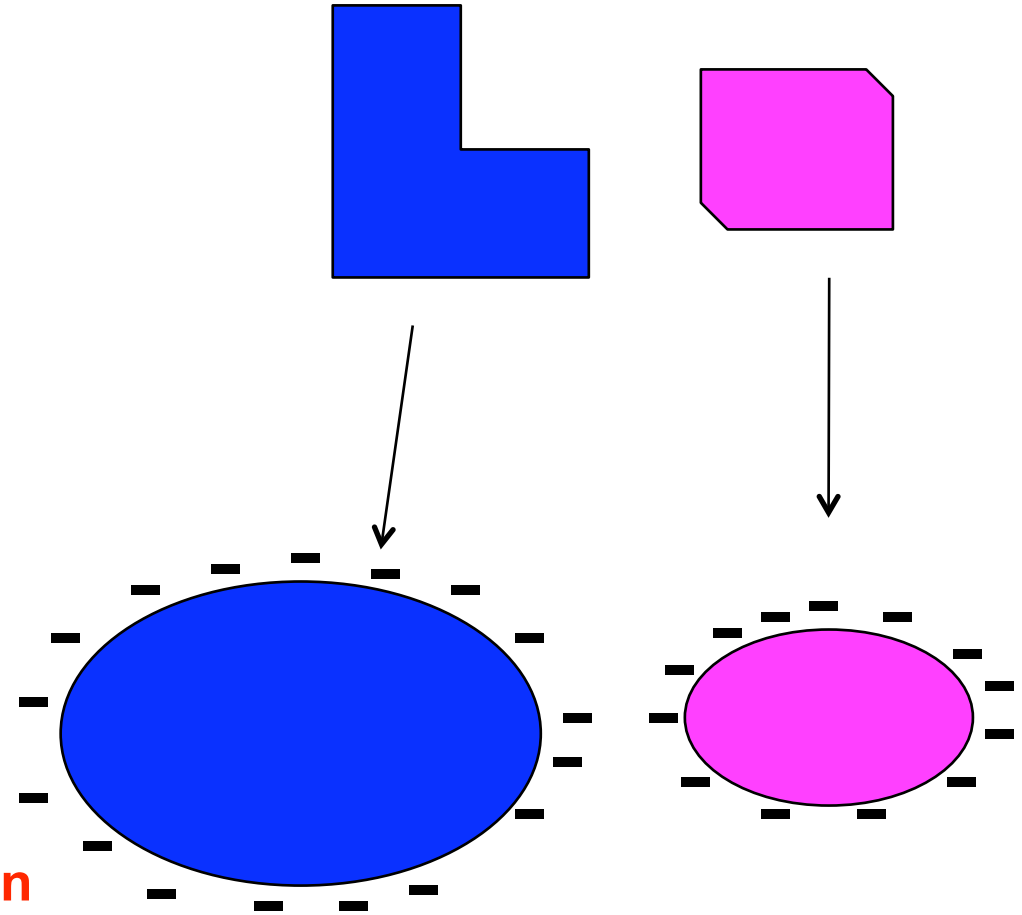


**(b)**

# Determinazione massa molecolare: Elettroforesi su gel di poliacrilammide

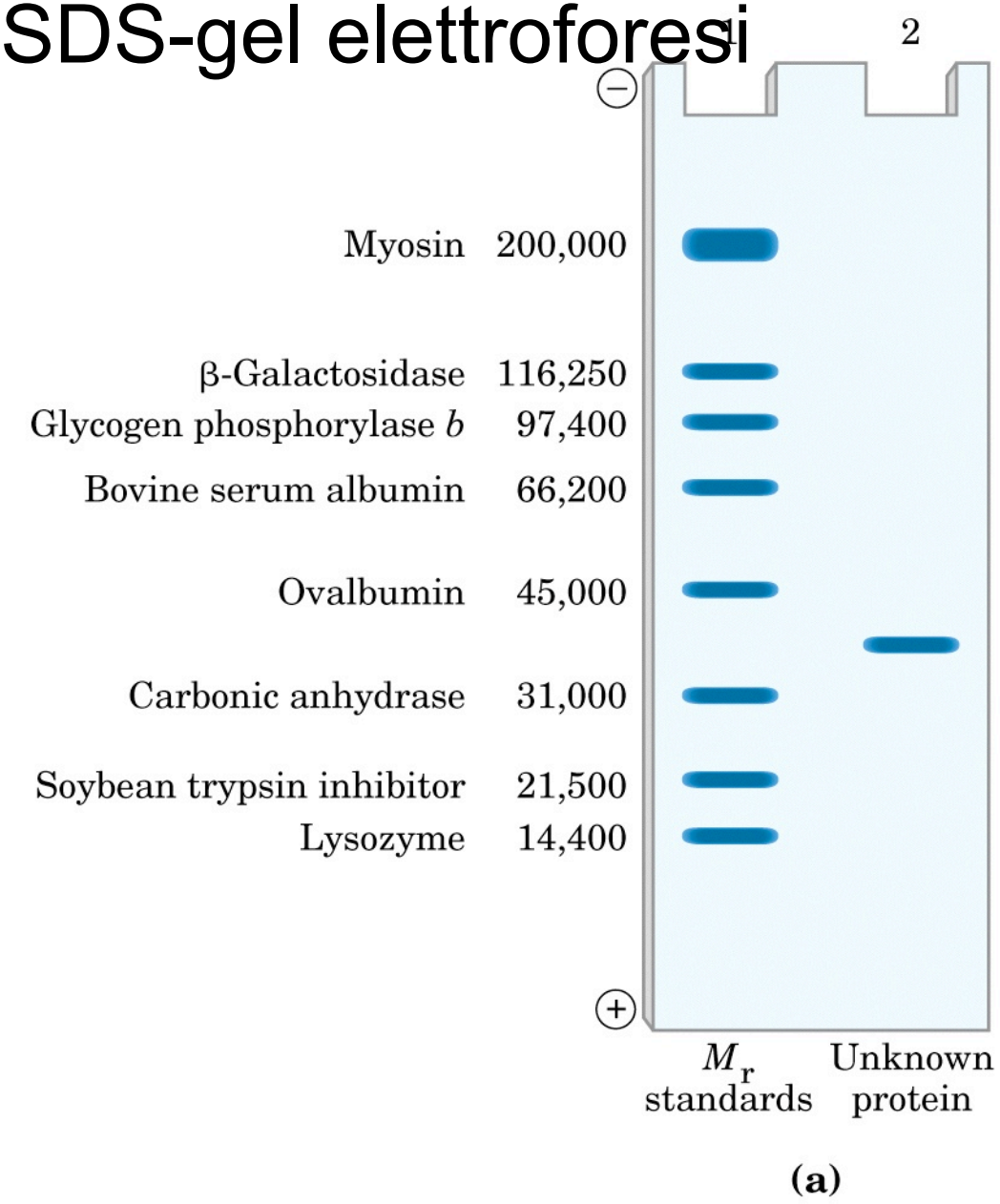



Sodium dodecyl sulfate
(SDS)

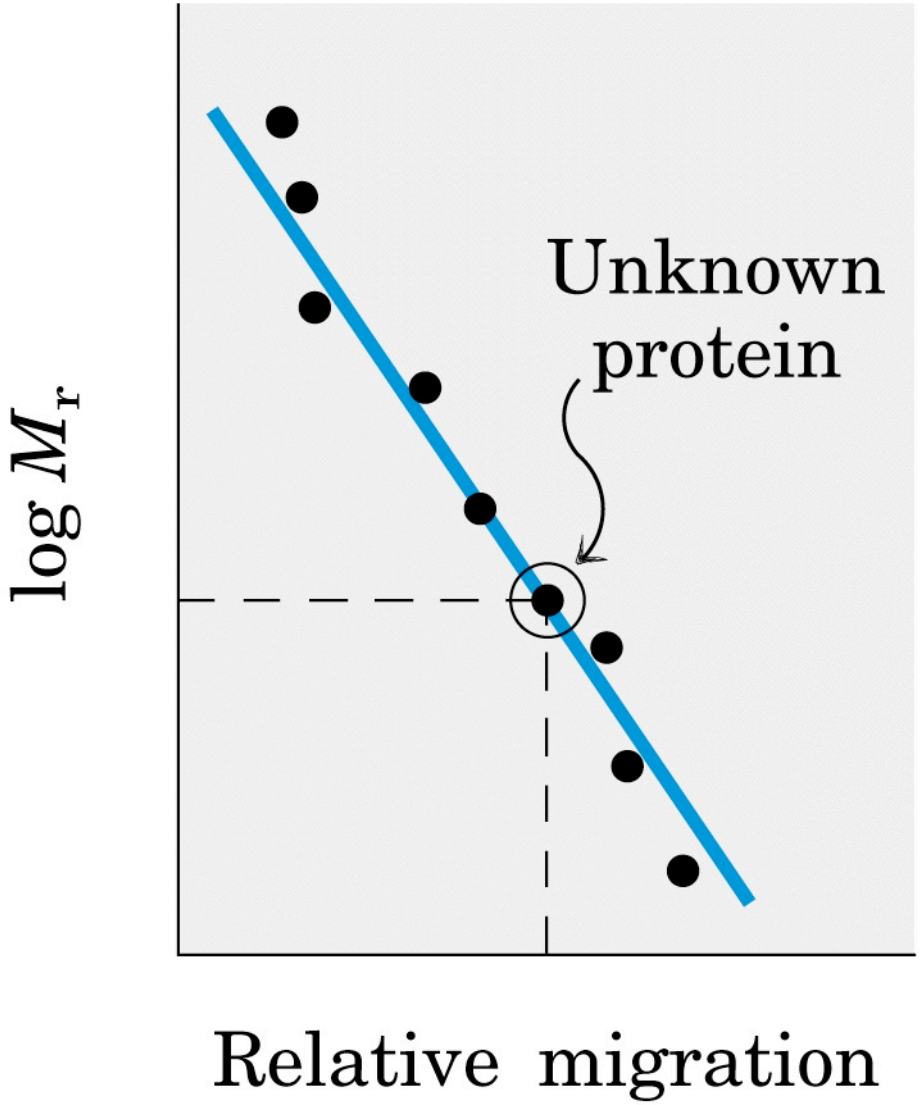


**Forma interazioni idrofobiche con tutte le proteine**

# SDS-gel elettroforesi



# Determinazione Mr (peso molecolare)



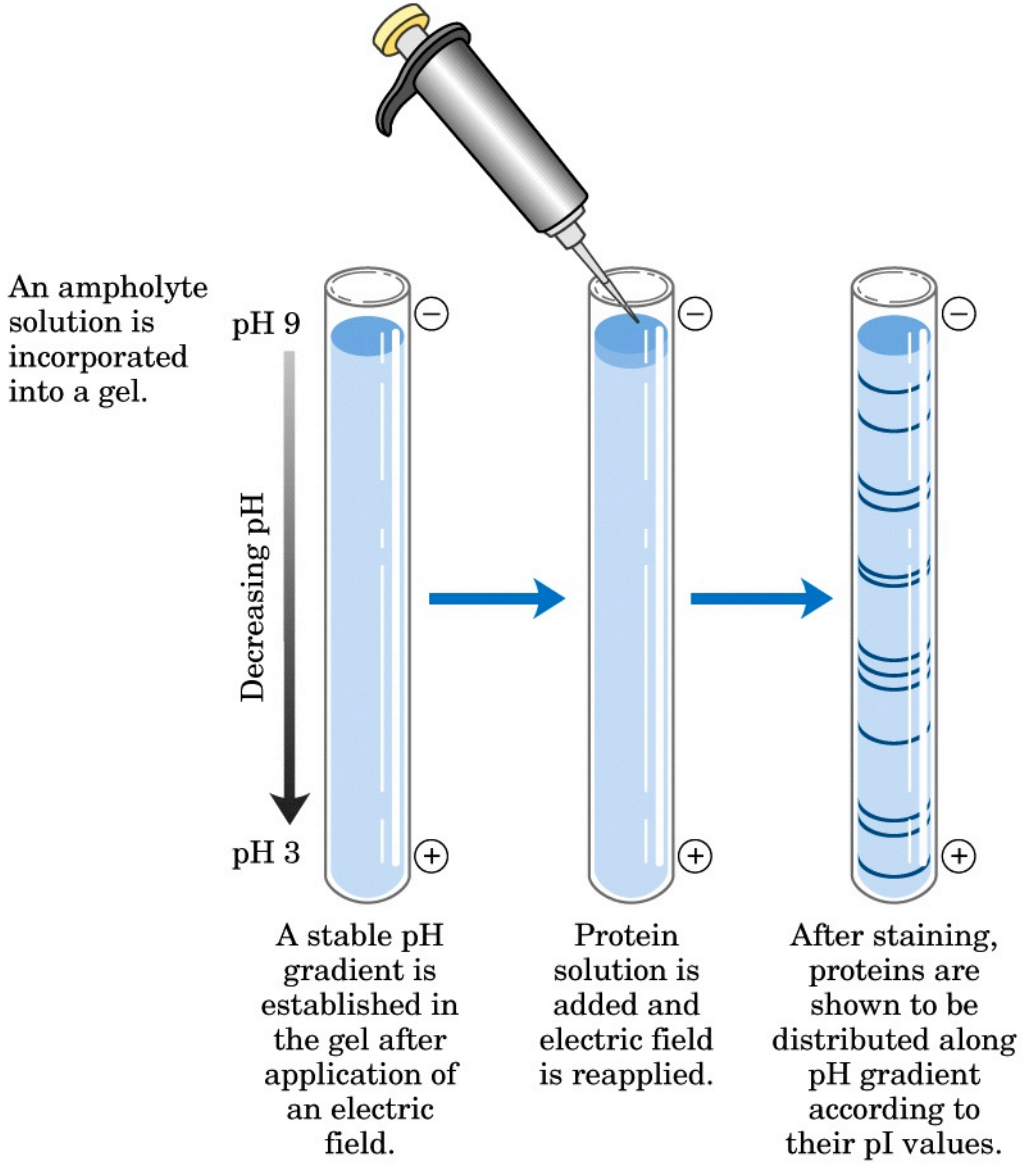
# Punto isoelettrico

table 5–6

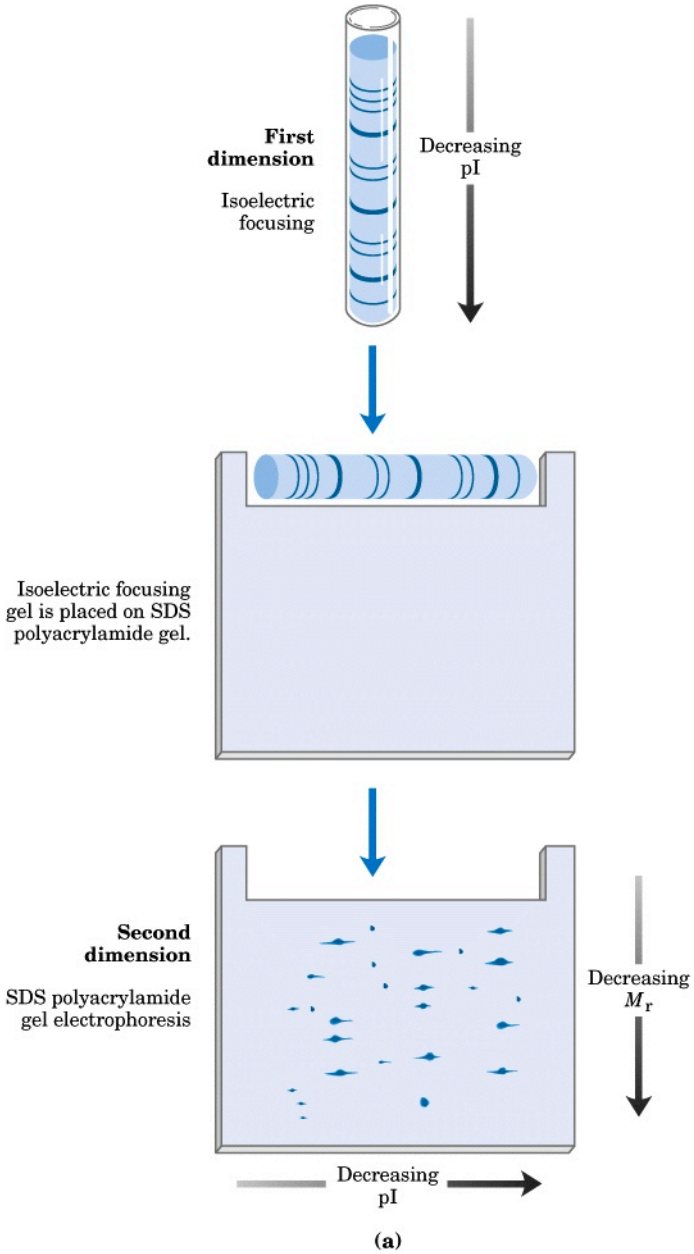
**The Isoelectric Points of Some Proteins**

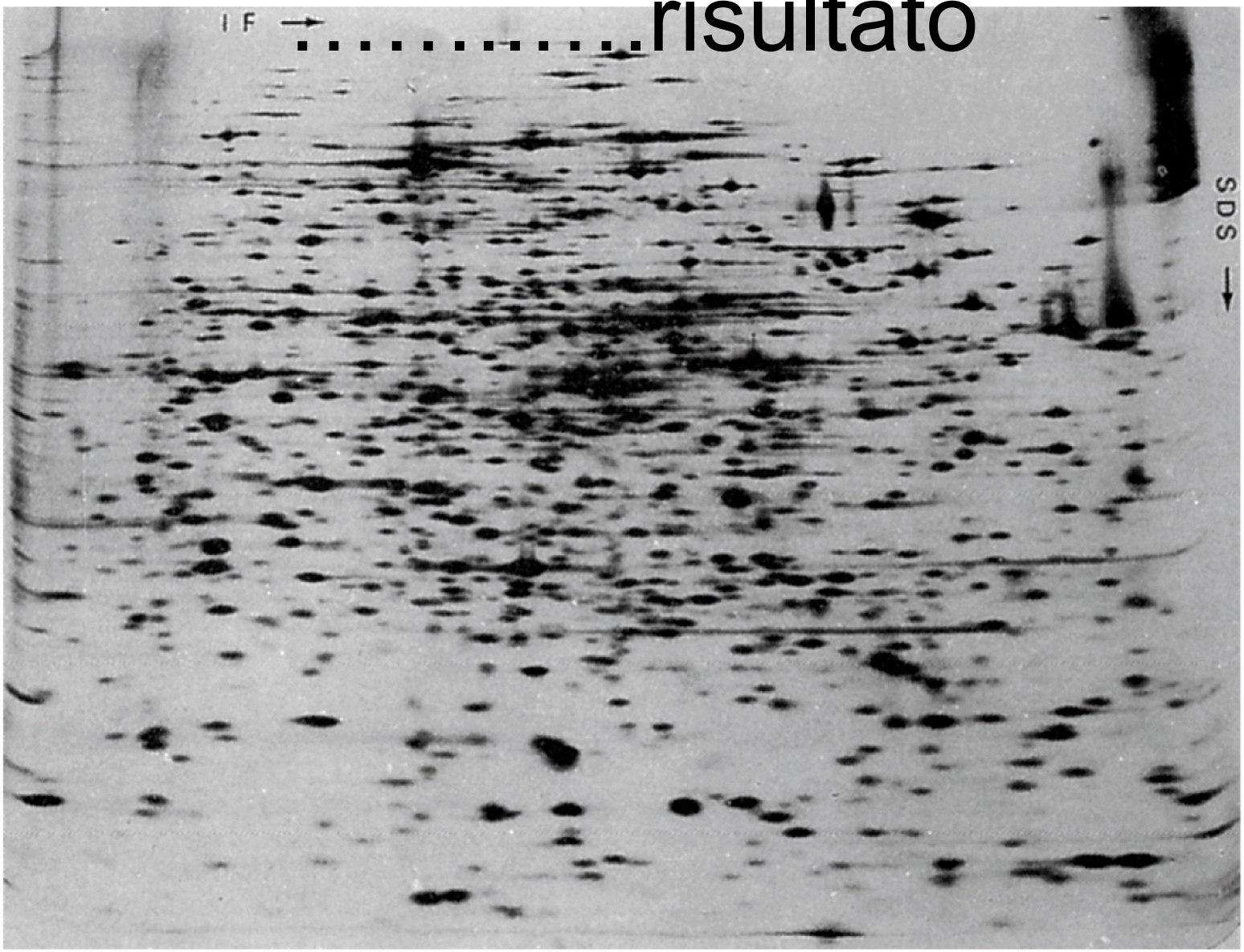
| Protein | pI |
|---|---|
| Pepsin | ~1.0 |
| Egg albumin | 4.6 |
| Serum albumin | 4.9 |
| Urease | 5.0 |
| β-Lactoglobulin | 5.2 |
| Hemoglobin | 6.8 |
| Myoglobin | 7.0 |
| Chymotrypsinogen | 9.5 |
| Cytochrome *c* | 10.7 |
| Lysozyme | 11.0 |

# Isoelettrofocalizzazione



# Elettroforesi bidimensionale



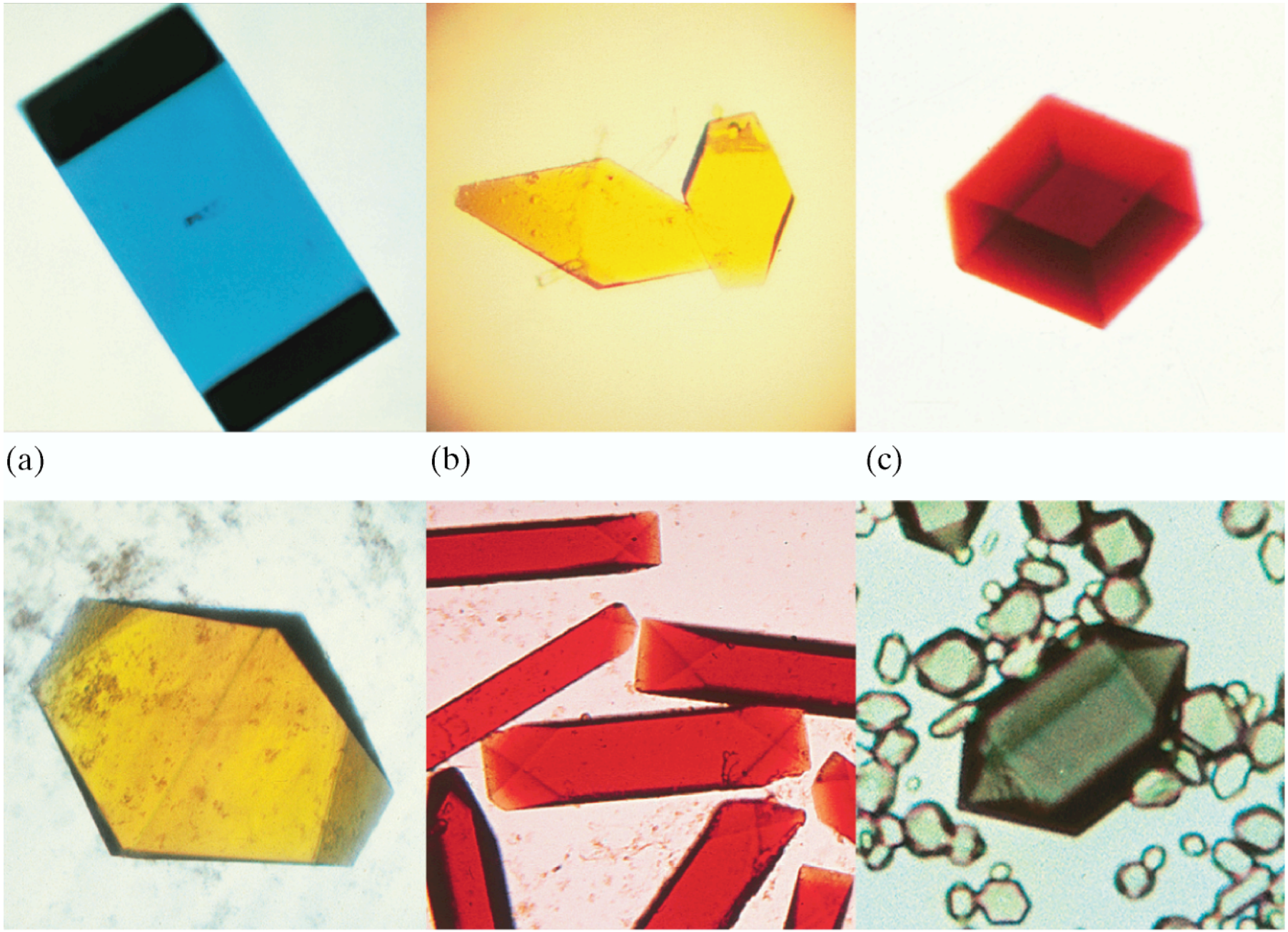



.....risultato

**(b)**

I componenti di una miscela proteica separati
mediante elettroforesi bidimensionale
possono essere identificati mediante

**analisi proteomica**


(a)
(b)
(c)
(d)
(e)
(f)