

4^a Lezione

Classificazione delle Proteine

Proteine monomeriche

Proteine multimeriche

Proteine semplici

Proteine coniugate

Proteine fibrose

Proteine globulari

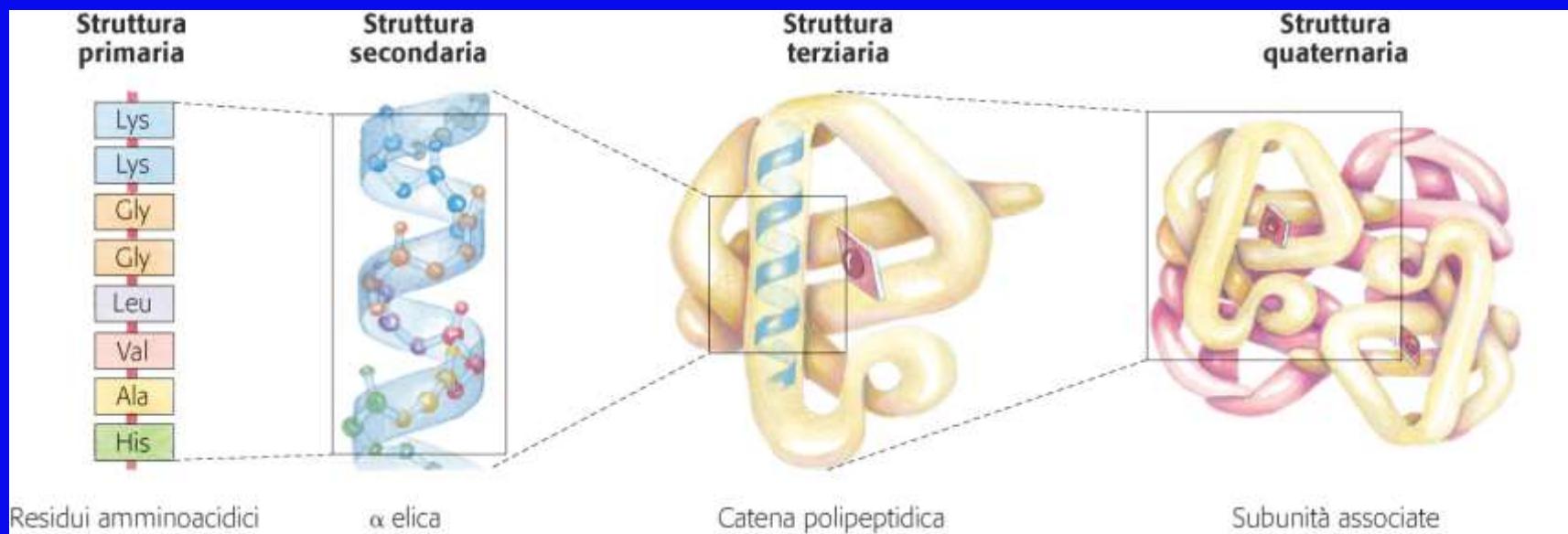
Omomultimeriche

Eteromultimeriche

Lipoproteine

Glicoproteine

Organizzazione strutturale delle proteine



Isolamento e purificazione di una proteina

- Omogenizzazione
- Centrifugazione
 - ad esclusione molecolare
- Cromatografia
 - a scambio ionico
 - ↙ di affinità
- Elettroforesi in SDS (sodiododecilsolfato)
- Analisi quantitativa e qualitativa
- Sequenza amminoacidica

Isolamento e purificazione di una proteina

Omogenizzazione



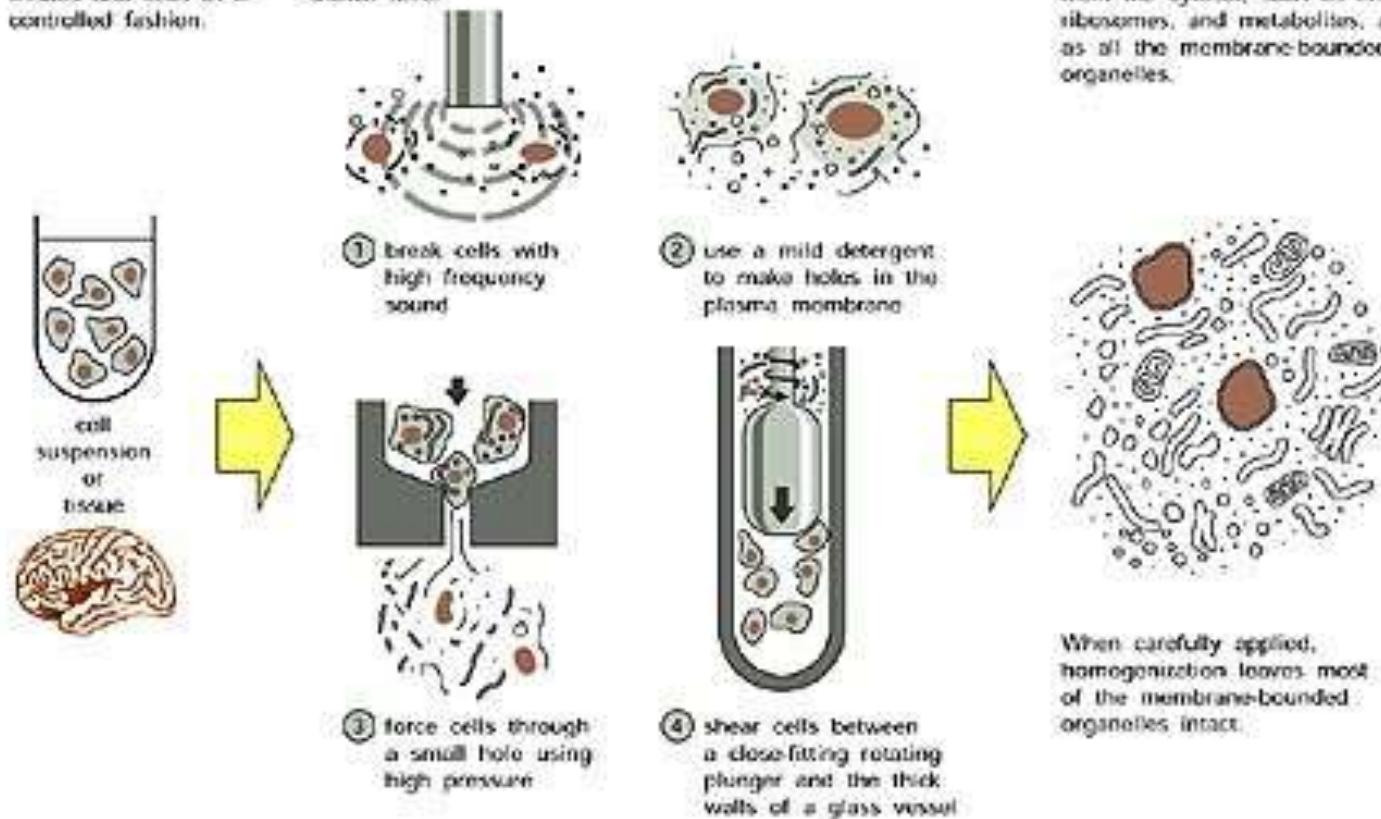
Isolamento e purificazione di una proteina

Omogenizzazione

BREAKING CELLS AND TISSUES

The first step in the purification of most proteins is to disrupt tissues and cells in a controlled fashion.

Using gentle mechanical procedures, called homogenization, the plasma membranes of cells can be ruptured so that the cell contents are released. Four commonly used procedures are shown here.

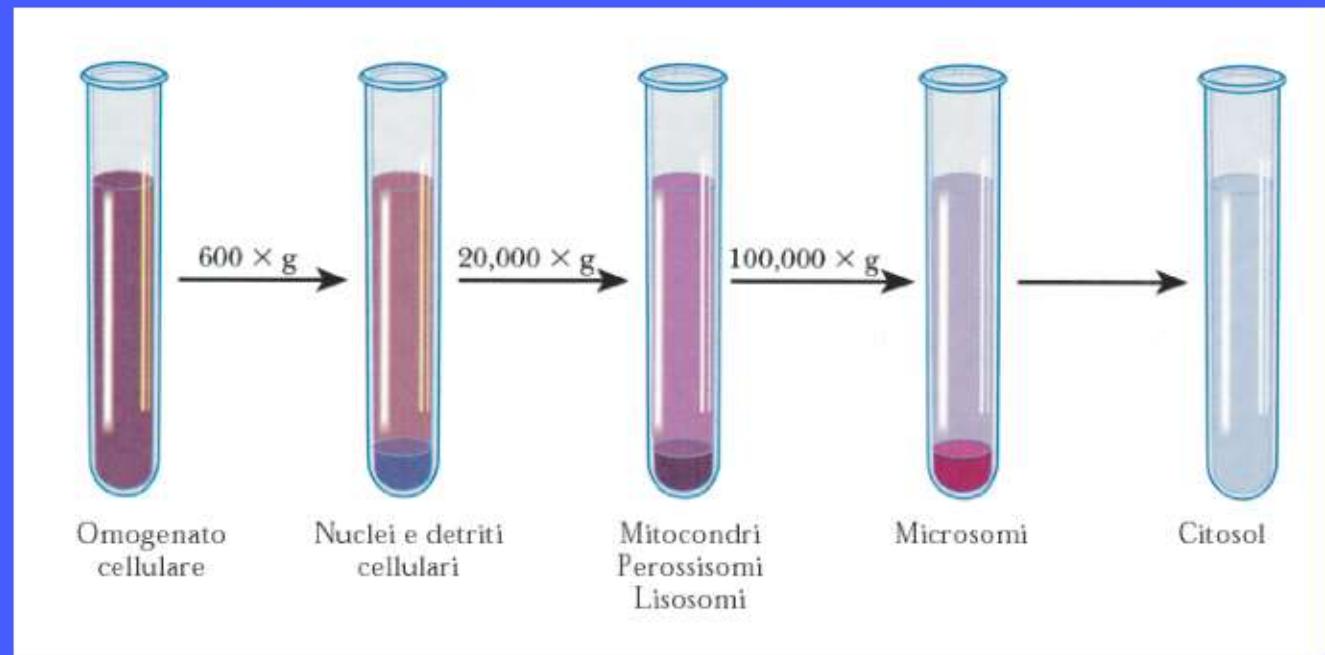


Isolamento e purificazione di una proteina

Omogenizzazione

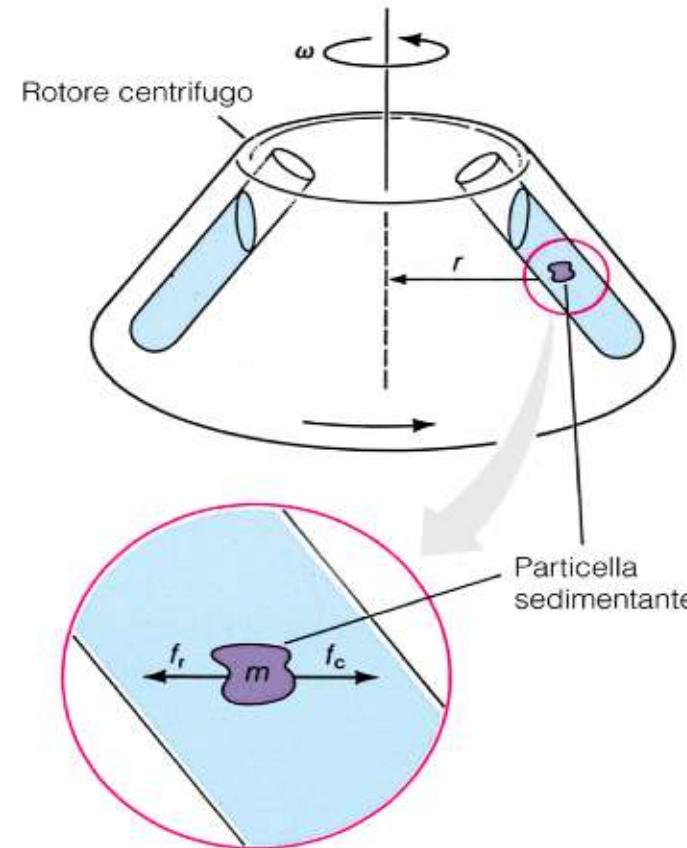
- Ultrasuoni
- Detergenti
- Forza meccanica
- Rotazione forzata di un pestello
nella provetta

Centrifugazione



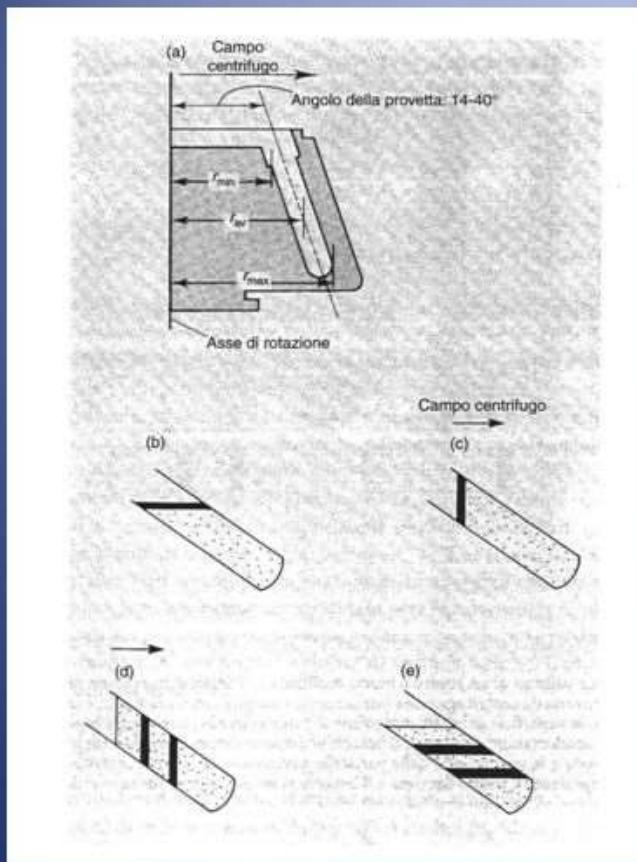


Centrifugazione

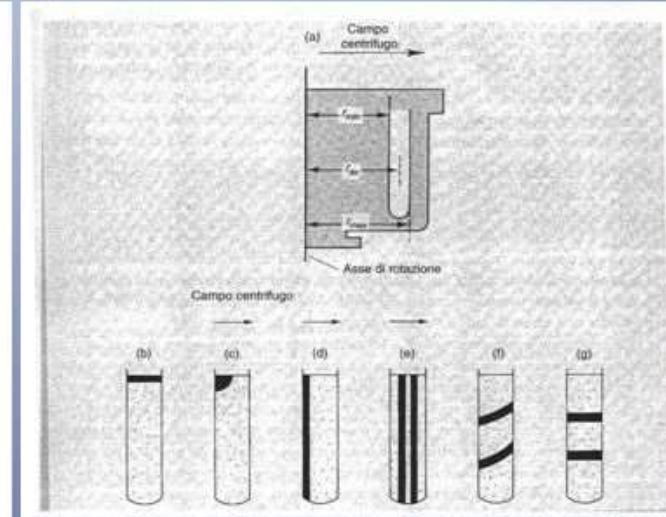


Centrifugazione

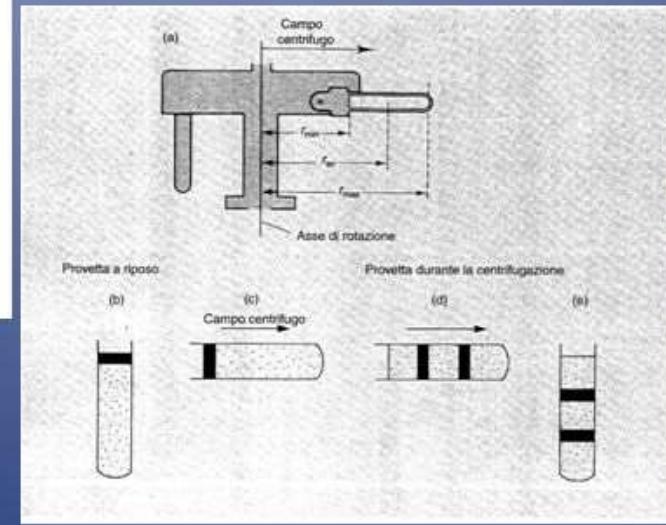
Tipi di rotori



**Rotore ad angolo fisso
separazione differenziale**

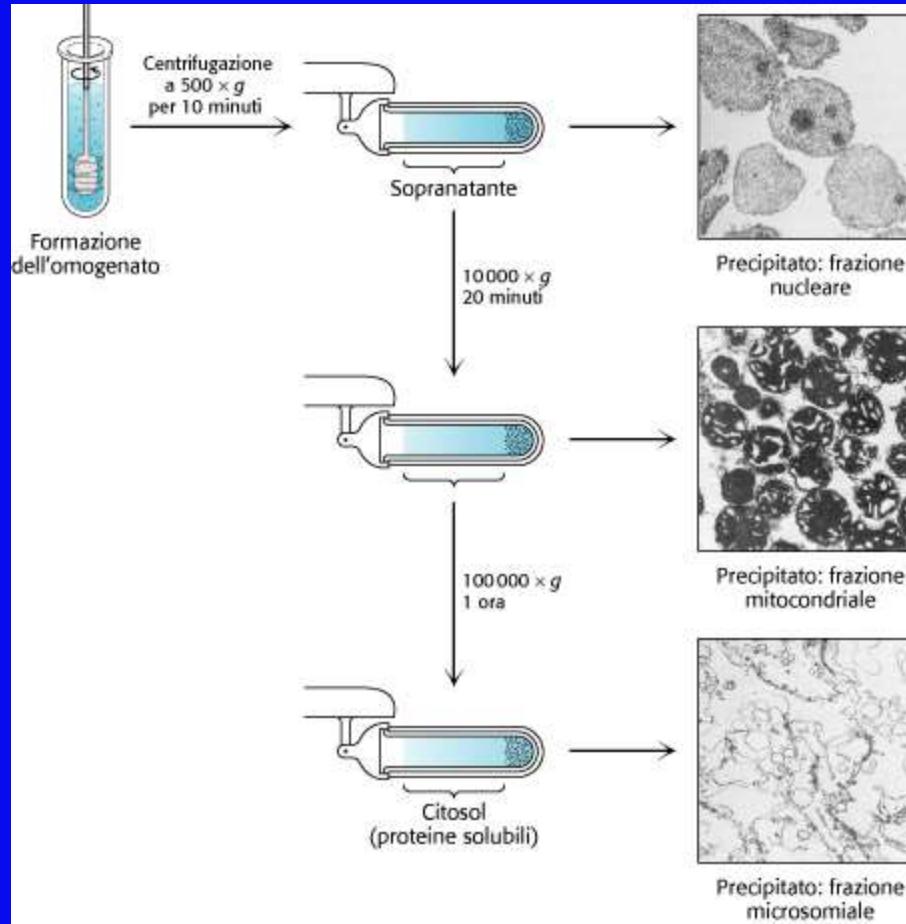


**Rotore
Verticale
Centrifugazione
isopicnica**

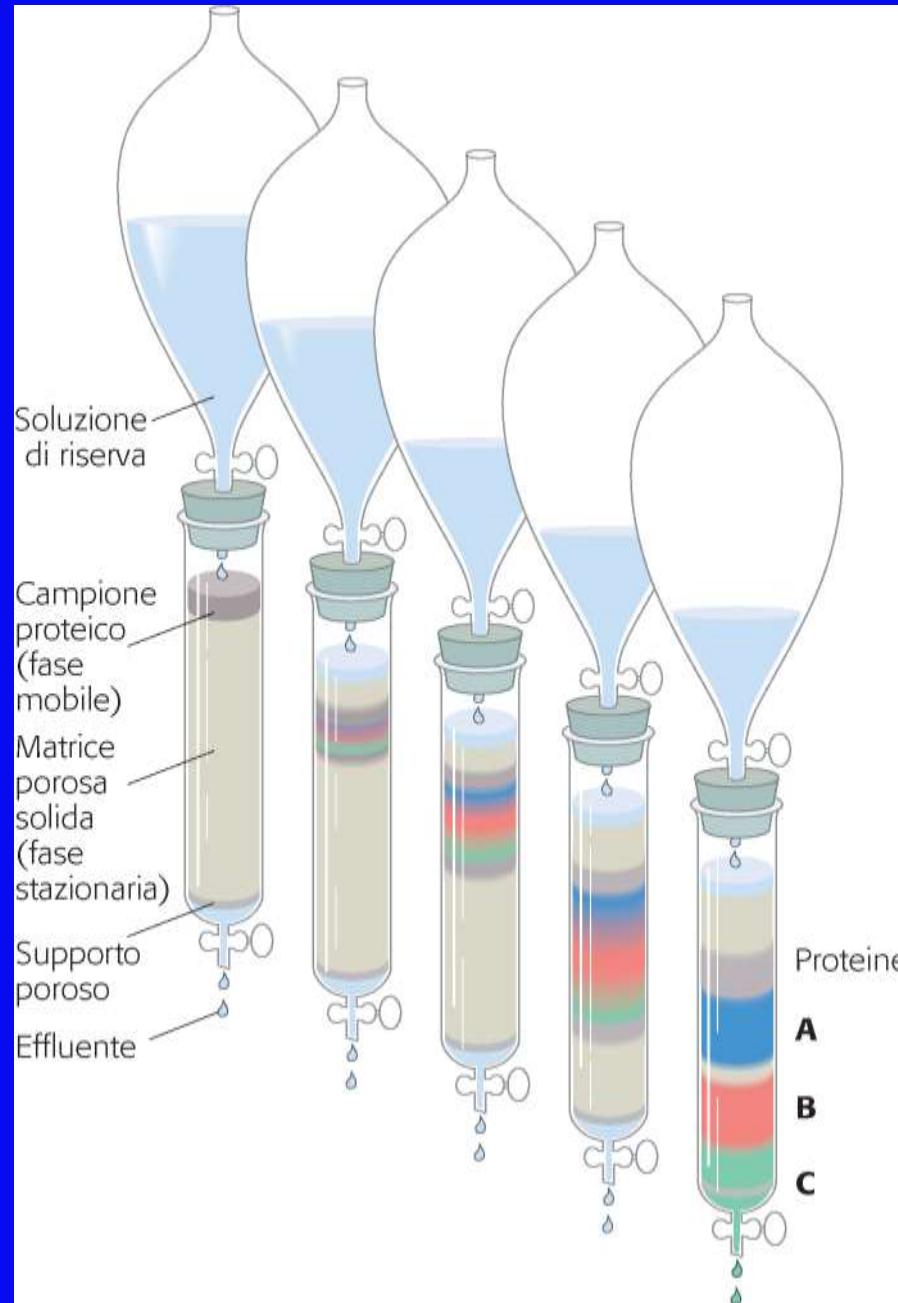


**Rotore a
bracci
Oscillanti
Centrifugazione
isopicnica
massima
risoluzione
delle bande**

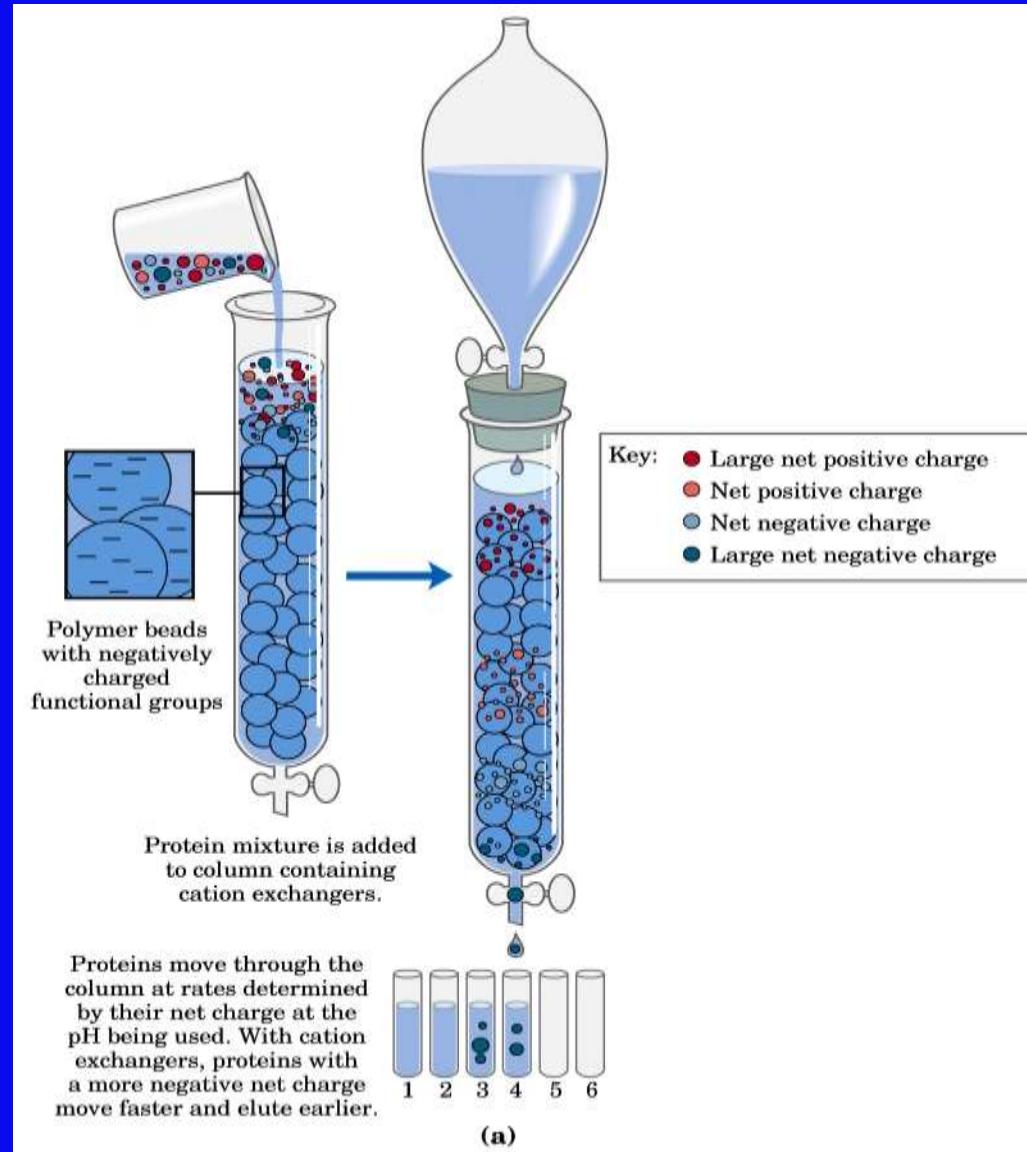
Centrifugazione differenziale



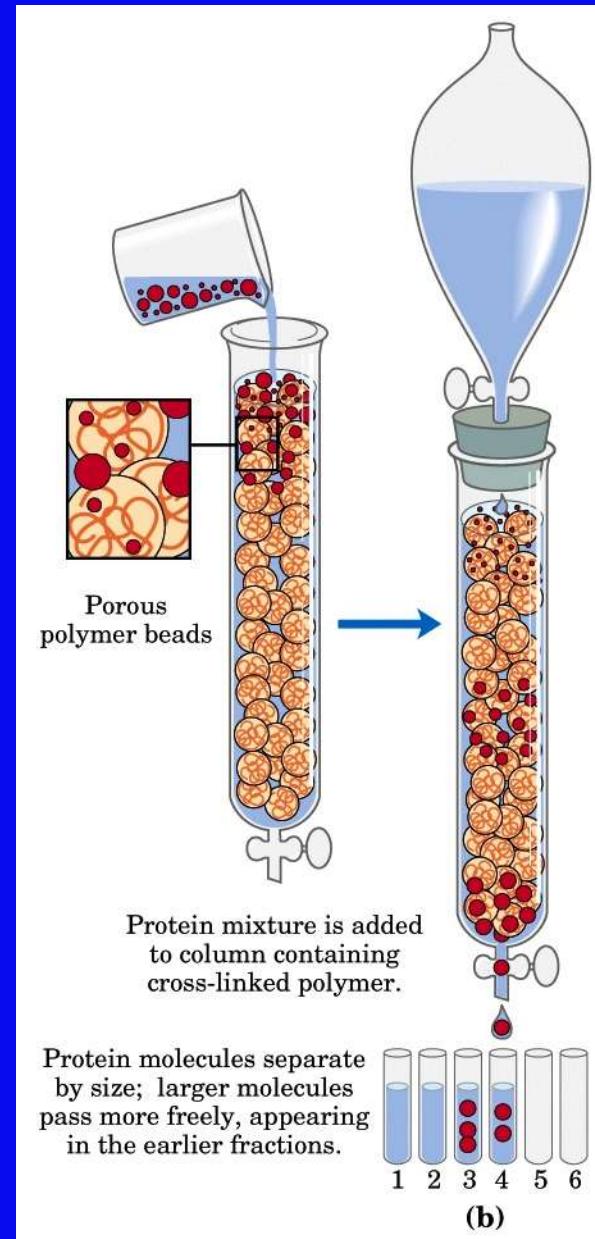
Cromatografia



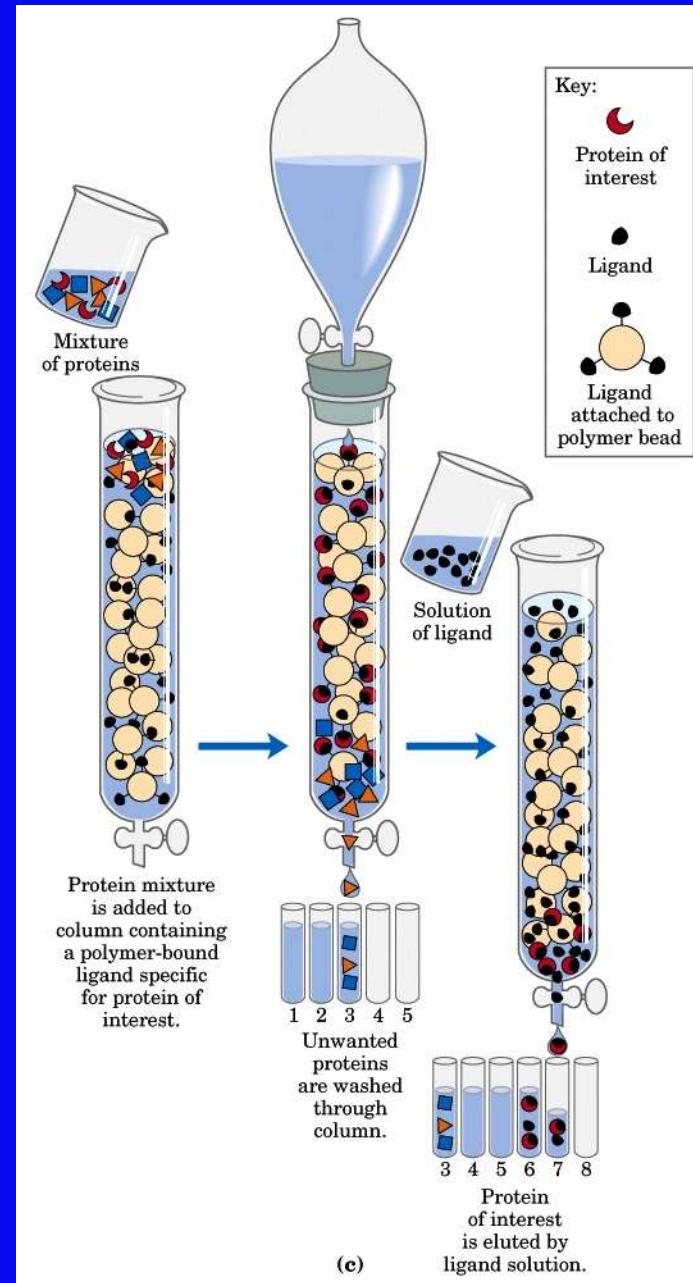
Cromatografia a scambio ionico



Cromatografia per esclusione molecolare



Cromatografia di affinità



Isolamento e purificazione di una proteina

Per poter eseguire una corretta purificazione di una proteina,

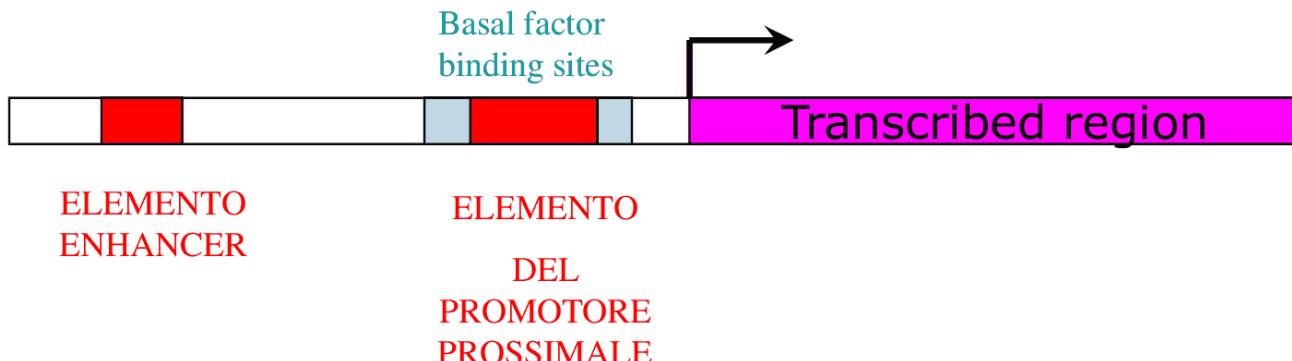
è necessario dotarsi di un saggio di attività.

Il saggio di attività, in genere, dipende dalle proprietà specifiche della stessa proteina.

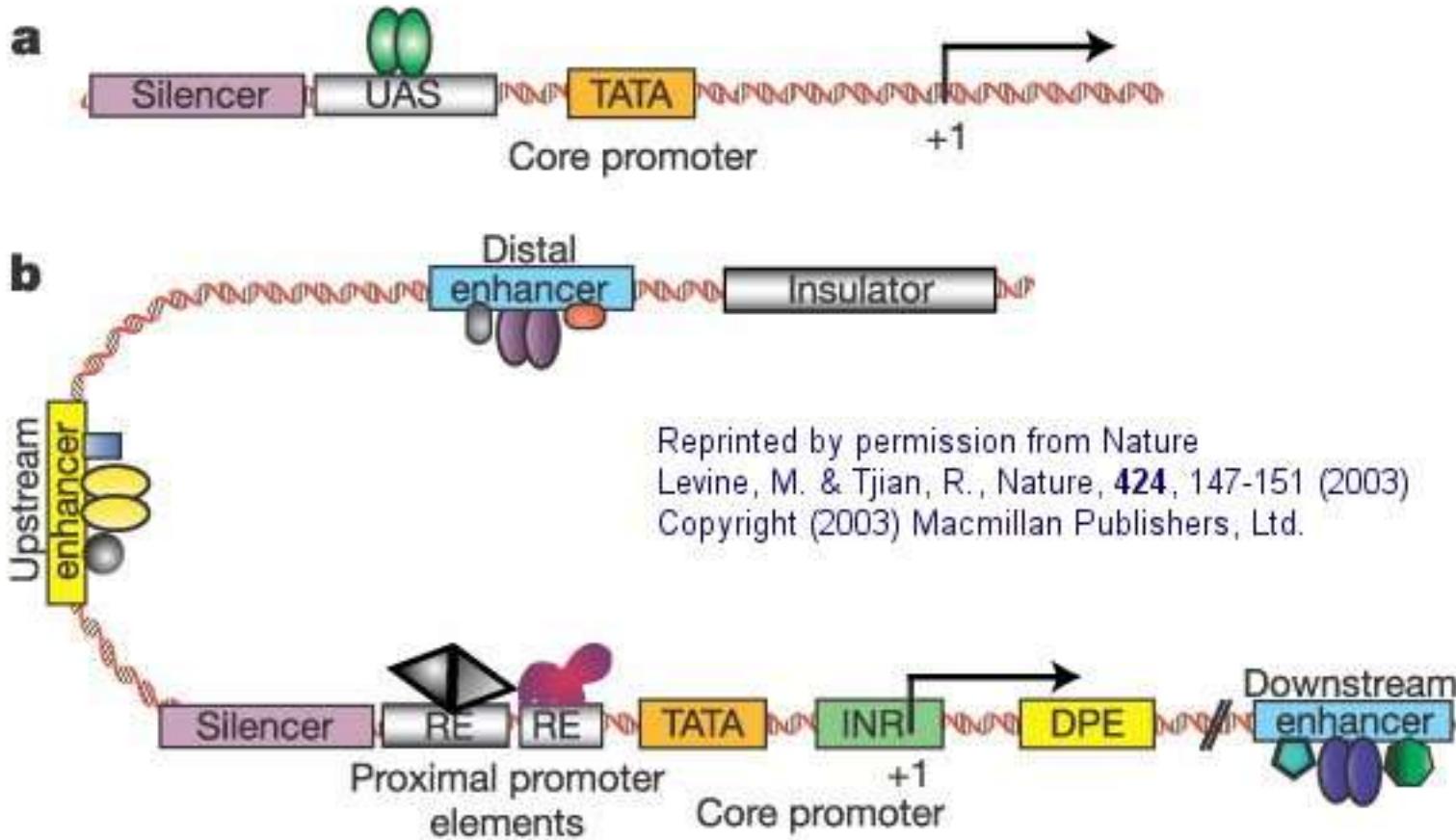
La trascrizione negli Eucarioti

FATTORI DI TRASCRIZIONE

- FATTORI DI TRASCRIZIONE GENERALI (BASALI)
 - RICONOSCONO ELEMENTI "core promoter"
- REGOLATORI SPECIFICI
 - ATTIVATORI
 - REPRESSORI

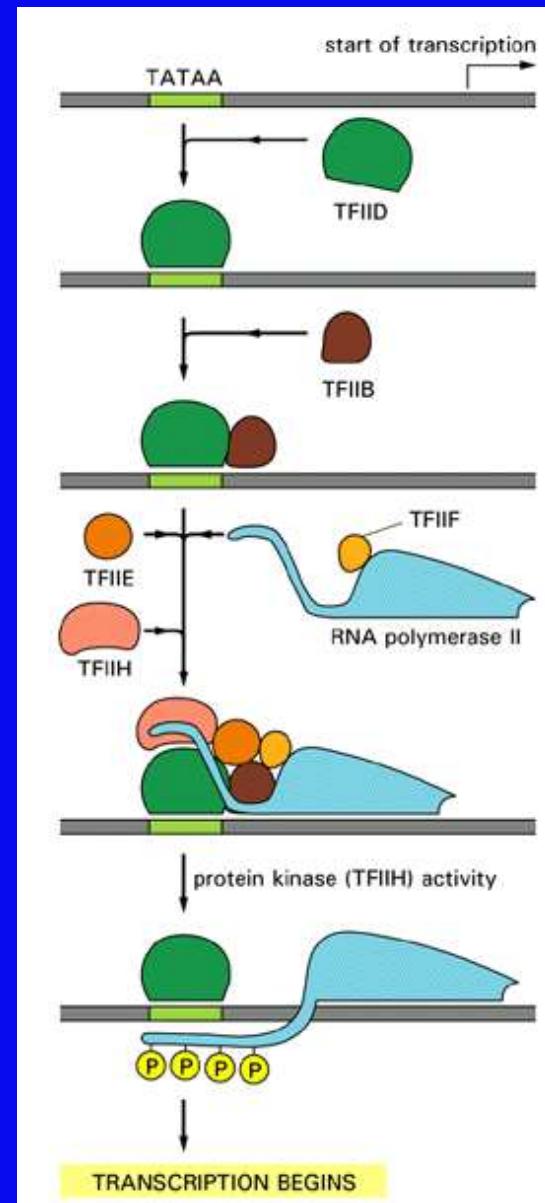


La trascrizione negli Eucarioti

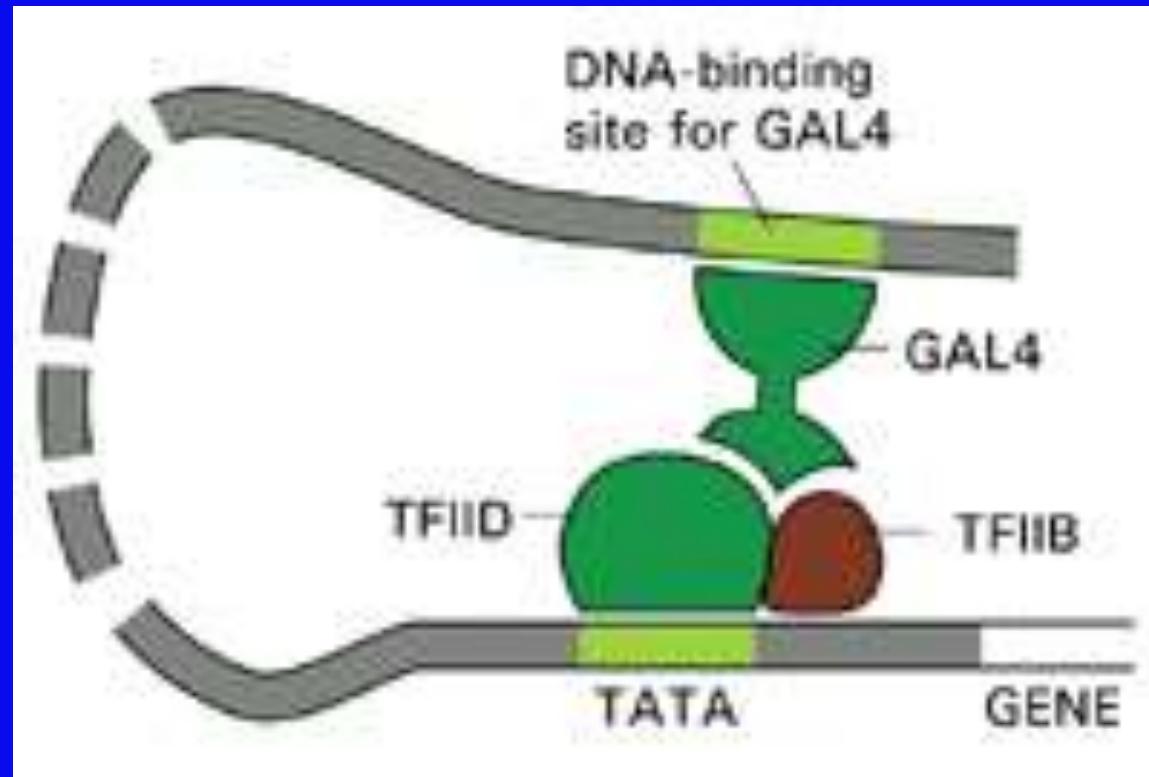


La trascrizione negli Eucarioti

Assemblaggio
del complesso
d' inizio per
la trascrizione
di un gene
eucariote



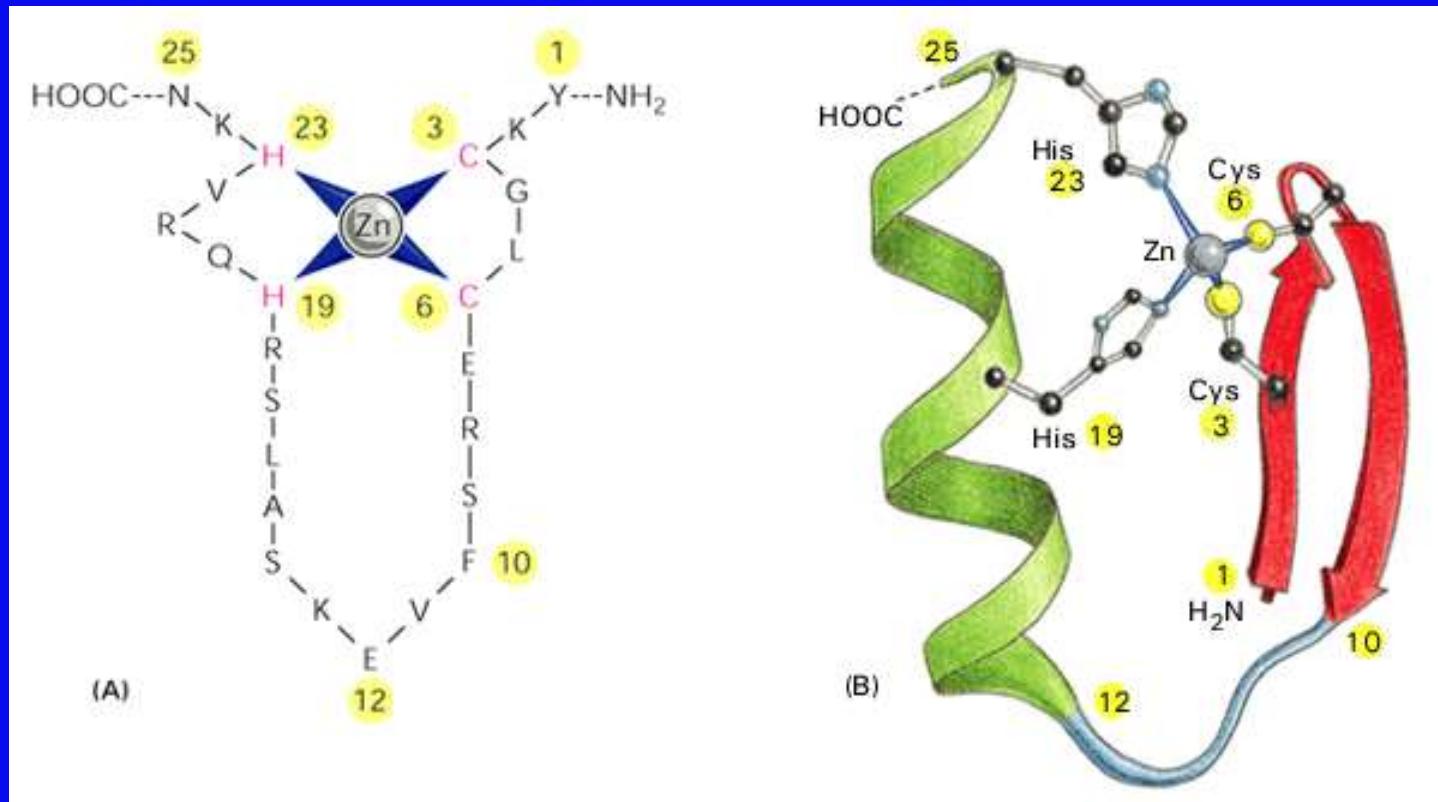
Modello per spiegare il meccanismo d'azione di una proteina attivatrice



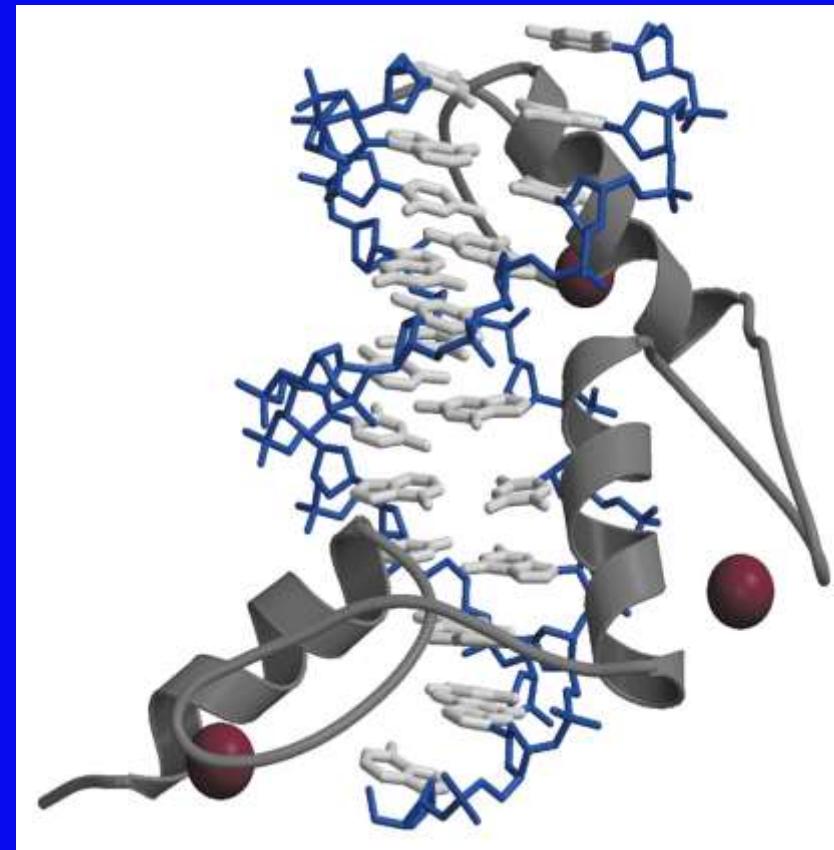
Principali motivi proteici che riconoscono e legano il DNA:

- elica-giro-elica
- omeodominio
- a dito di zinco
- a cerniera di leucine
- elica-ansa-elica

Motivo di legame al DNA a dito di zinco



Nel genoma umano vi sono circa 800 differenti geni che codificano per proteine aventi i motivi ripetuti a dito di zinco del tipo C2-H2 o del tipo C2-C2.



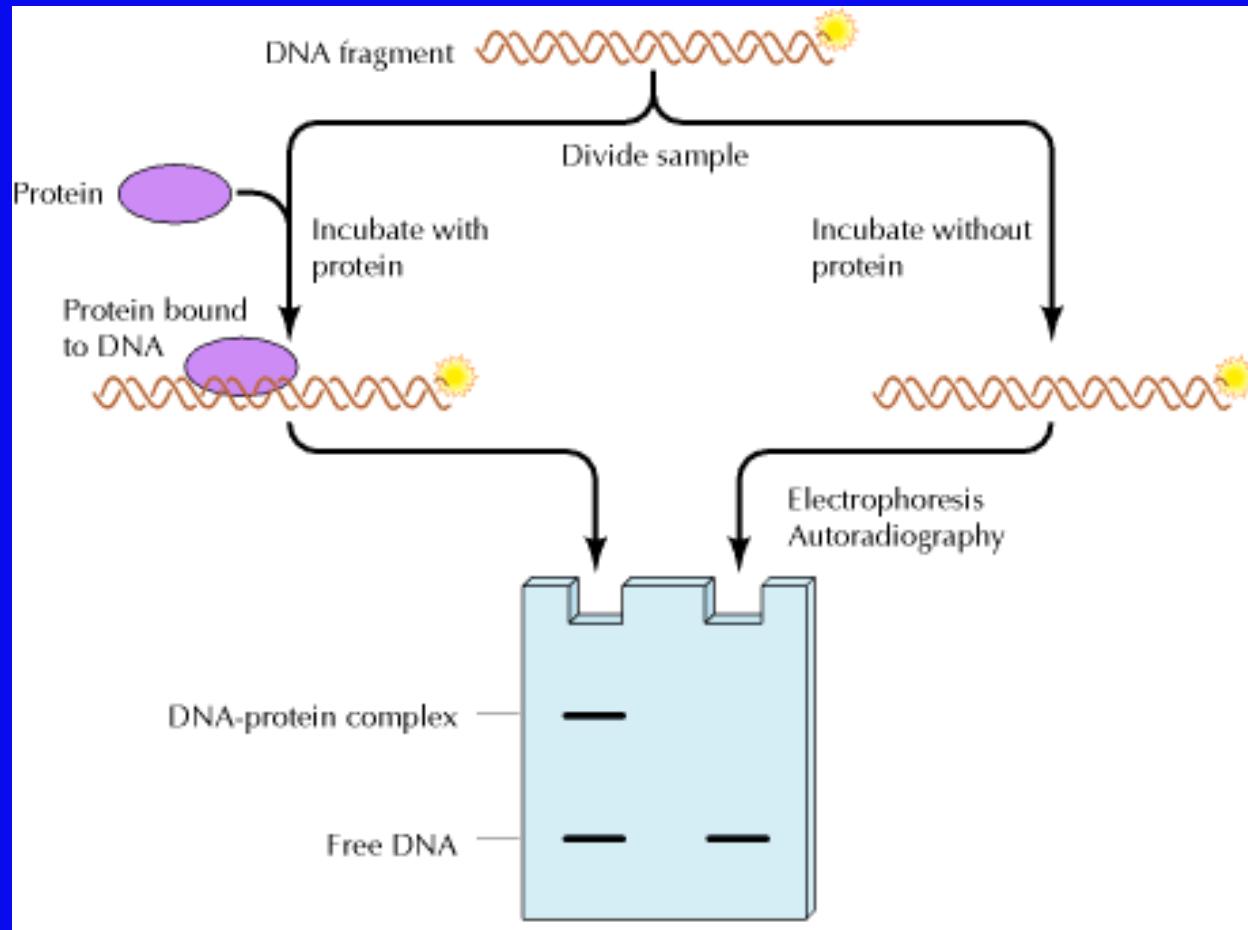
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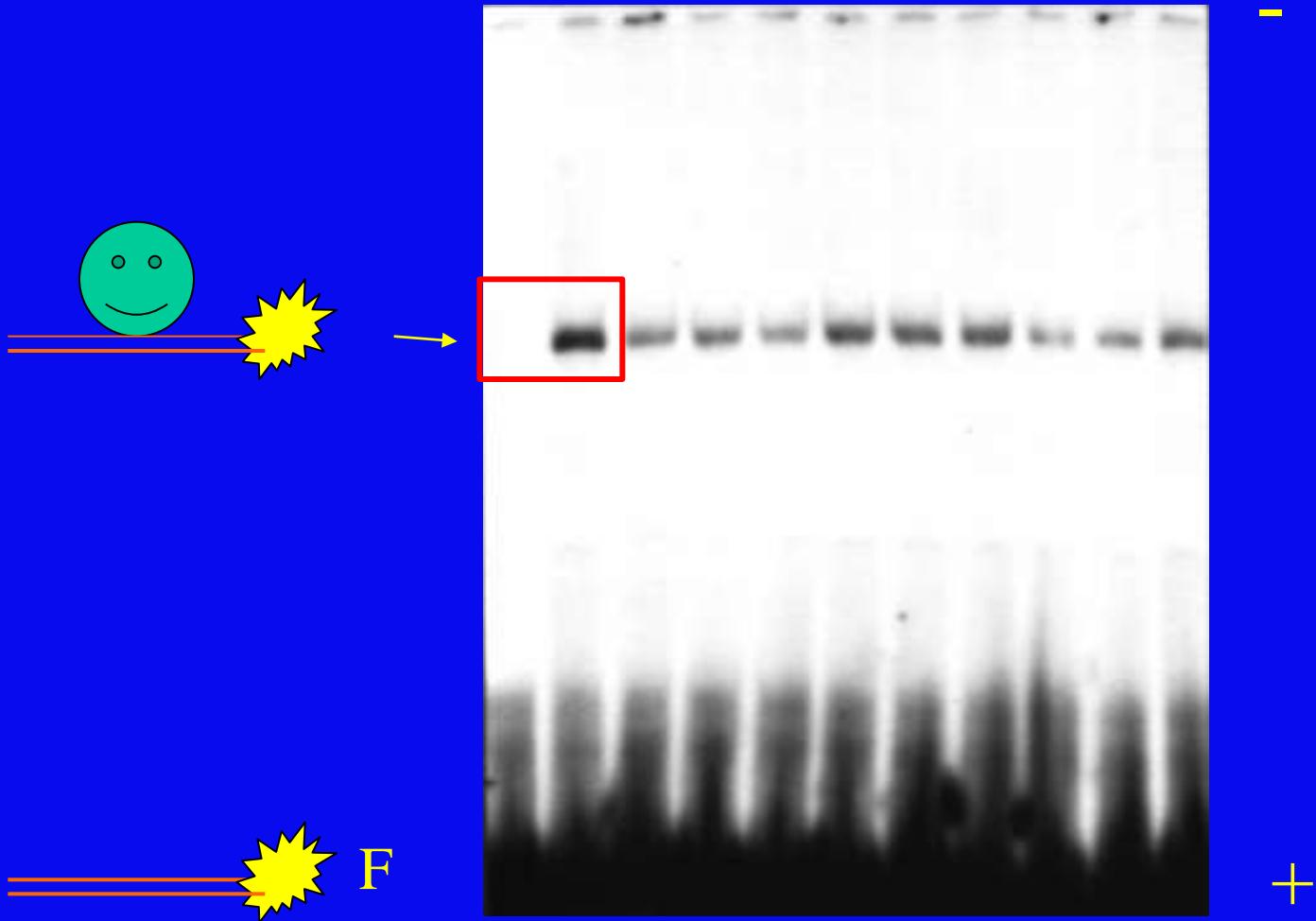
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Gel shift assay

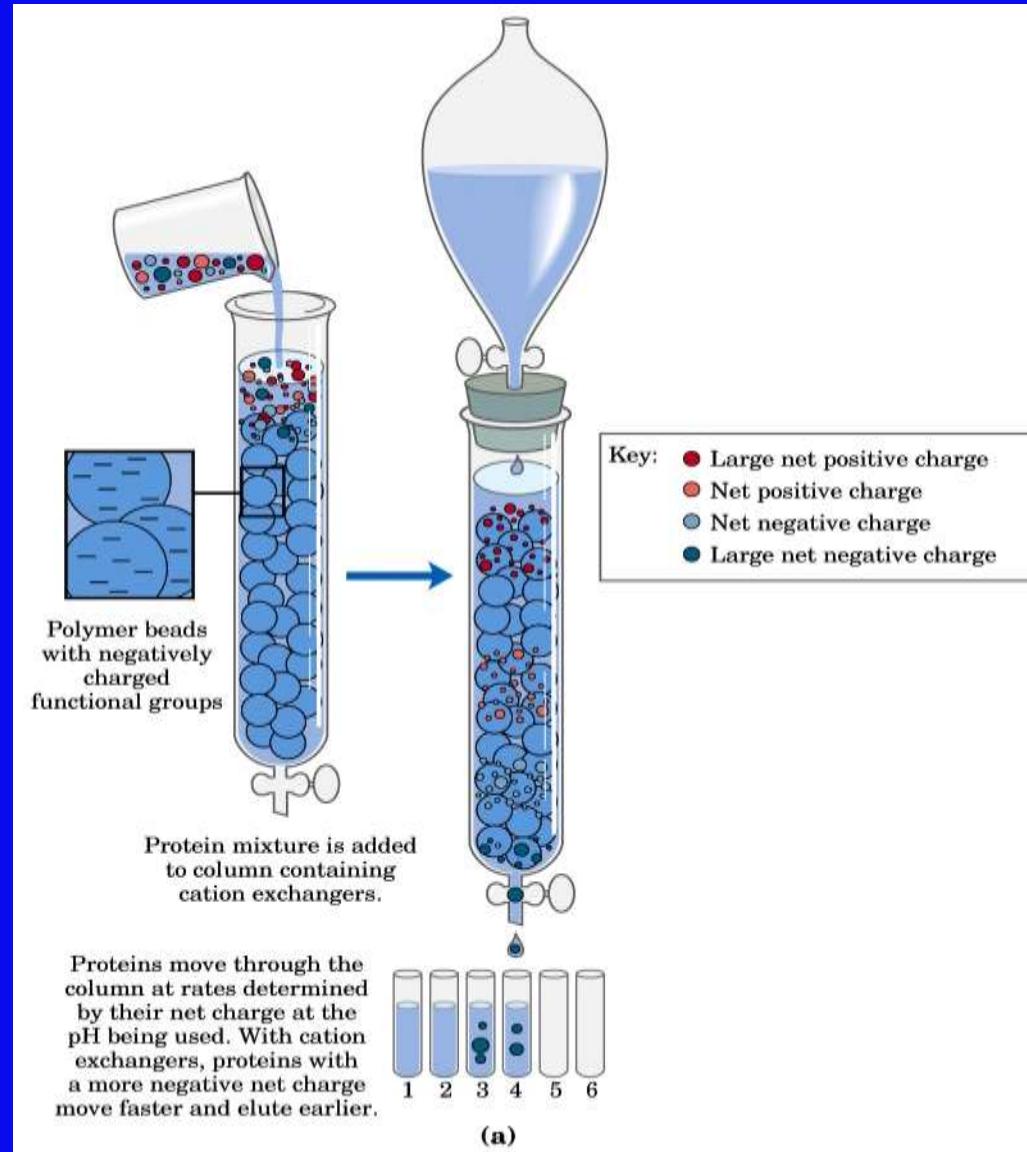


Gel-shift assay on nuclear extracts from proliferating and starved NIH3T3 cells

hrs 2 4 6 8 12 16 20 24
serum % -- 10 0.5 ┌─────────────────┐
 ─



Cromatografia a scambio ionico



DEAE-SEPHAROSE I.E.C.

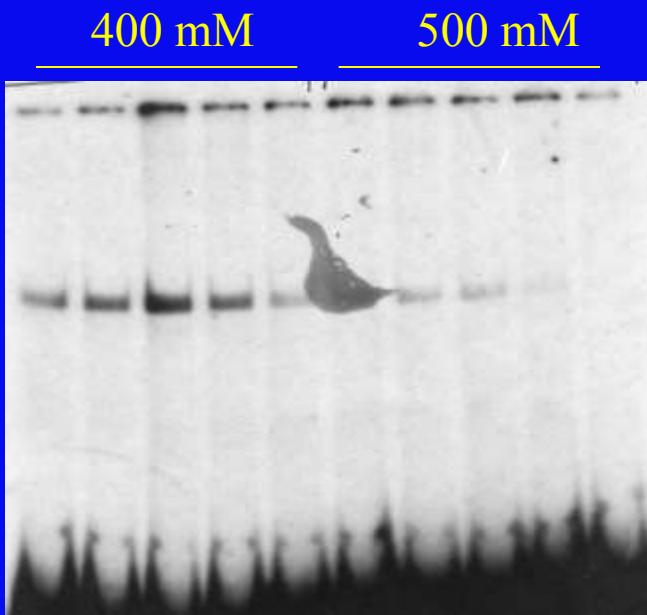
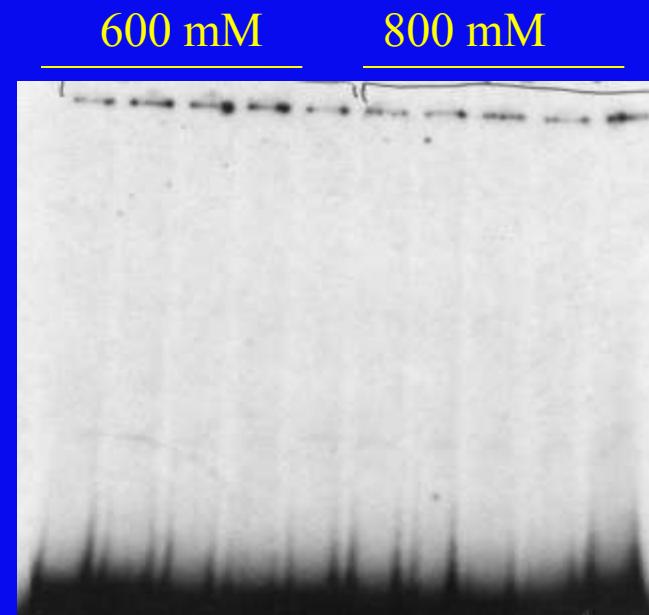
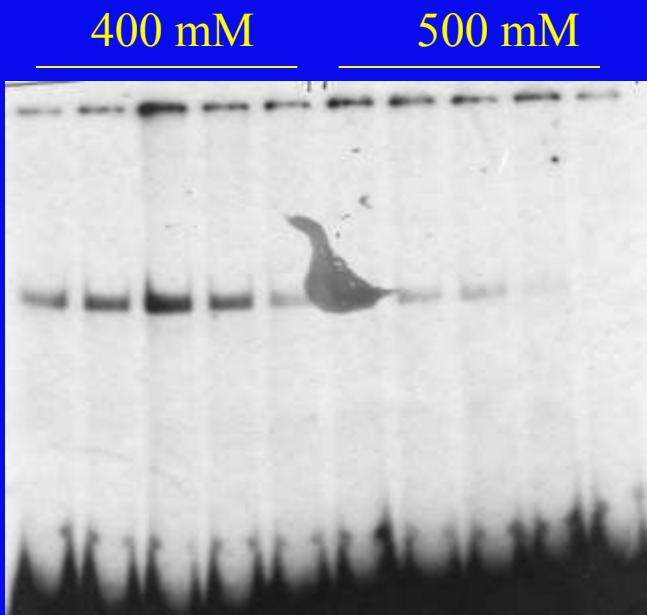
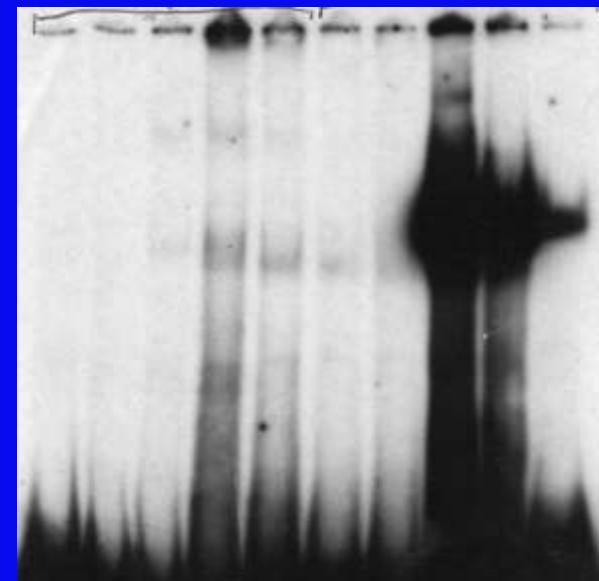
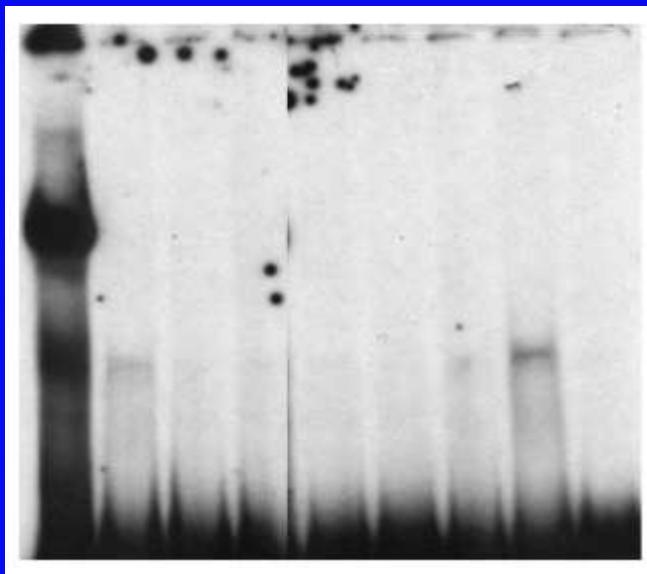
HeLa

FT W

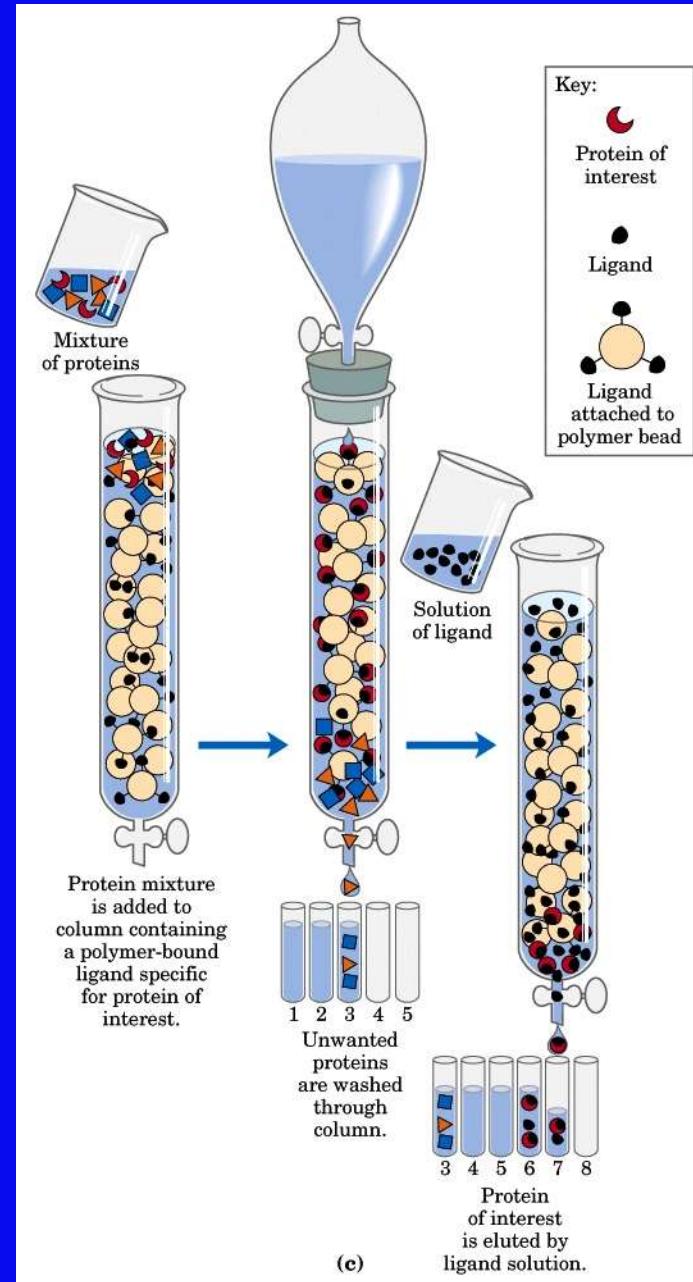
100 mM

200 mM

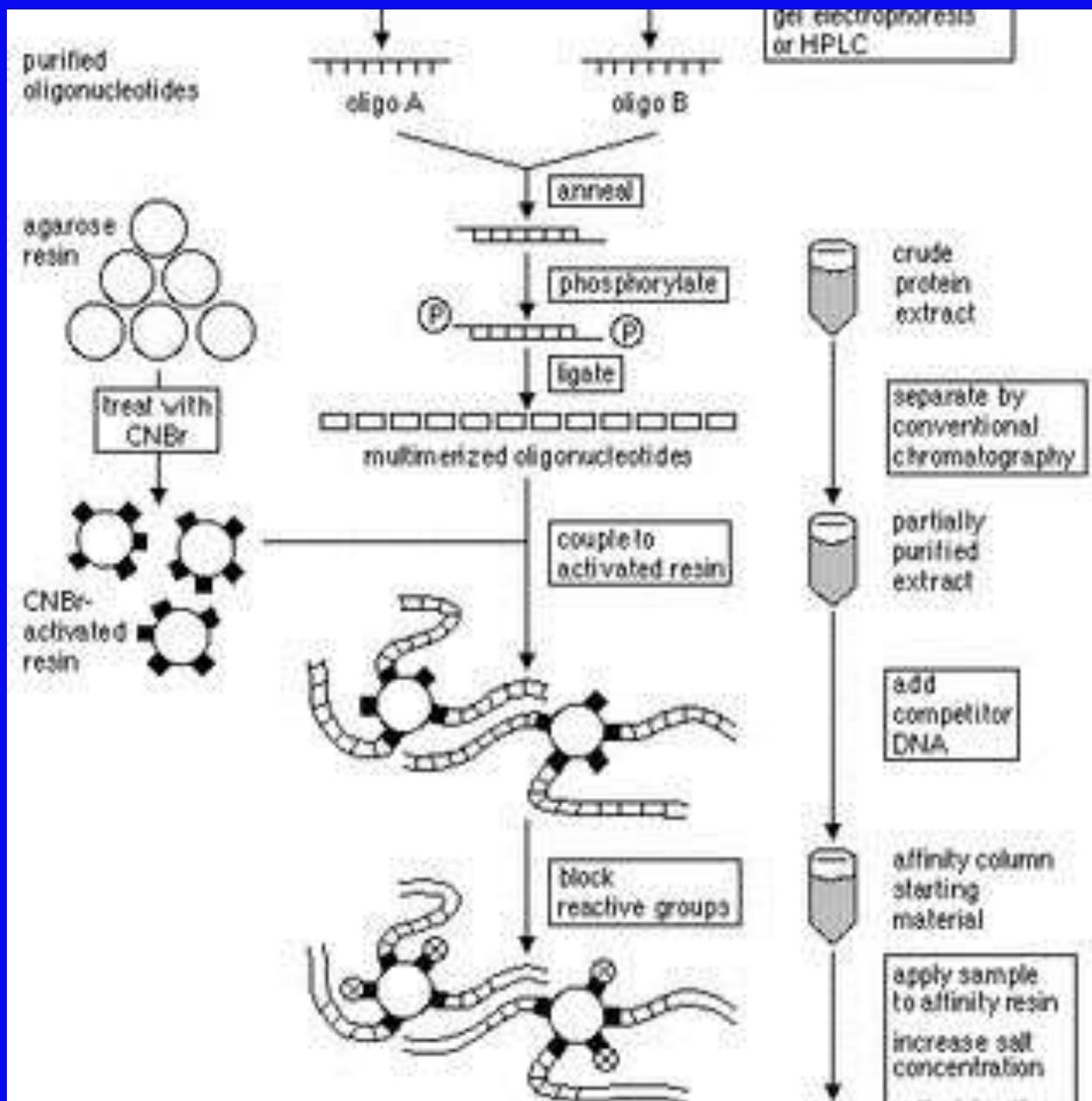
300 mM



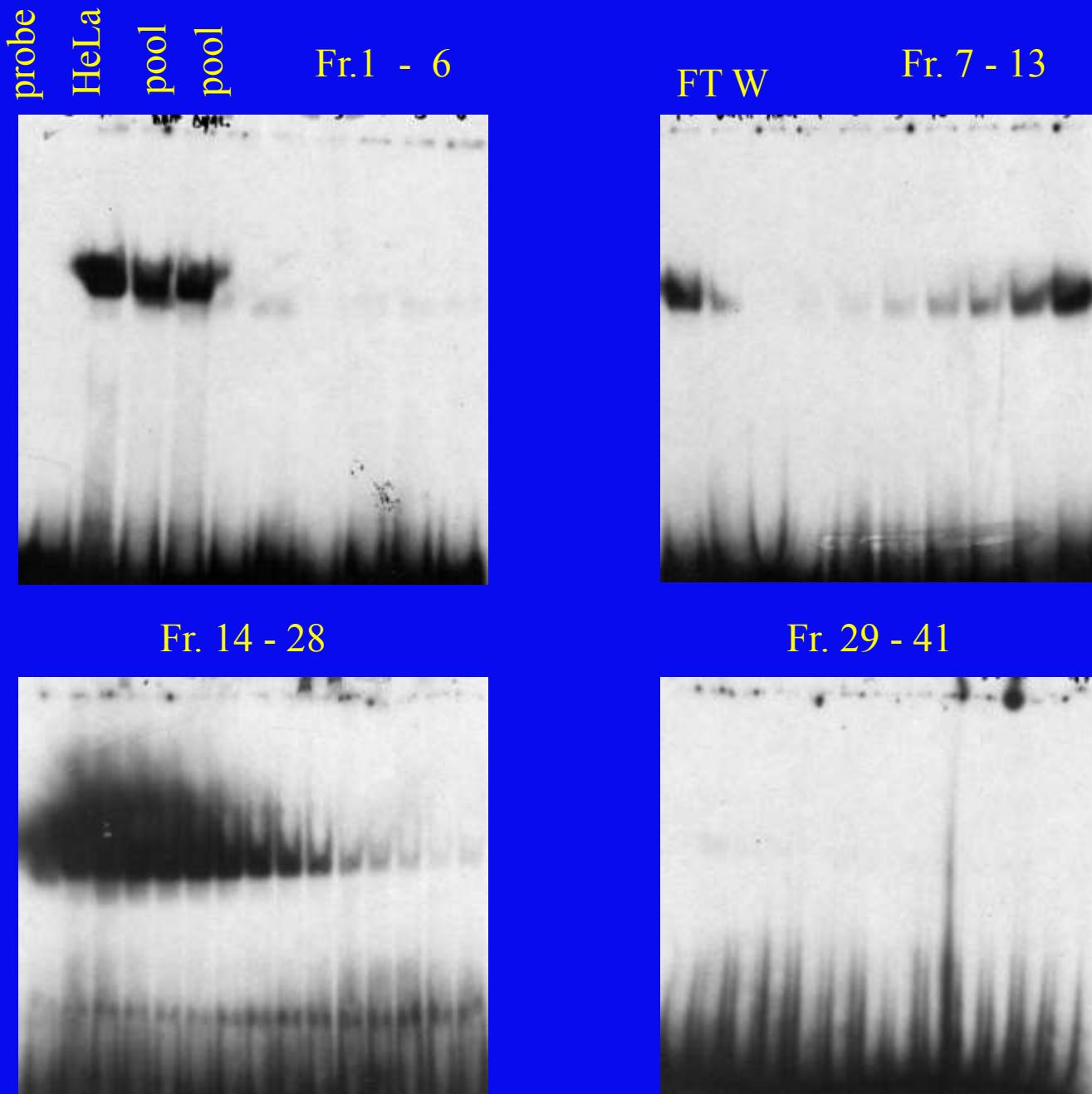
Cromatografia di affinità



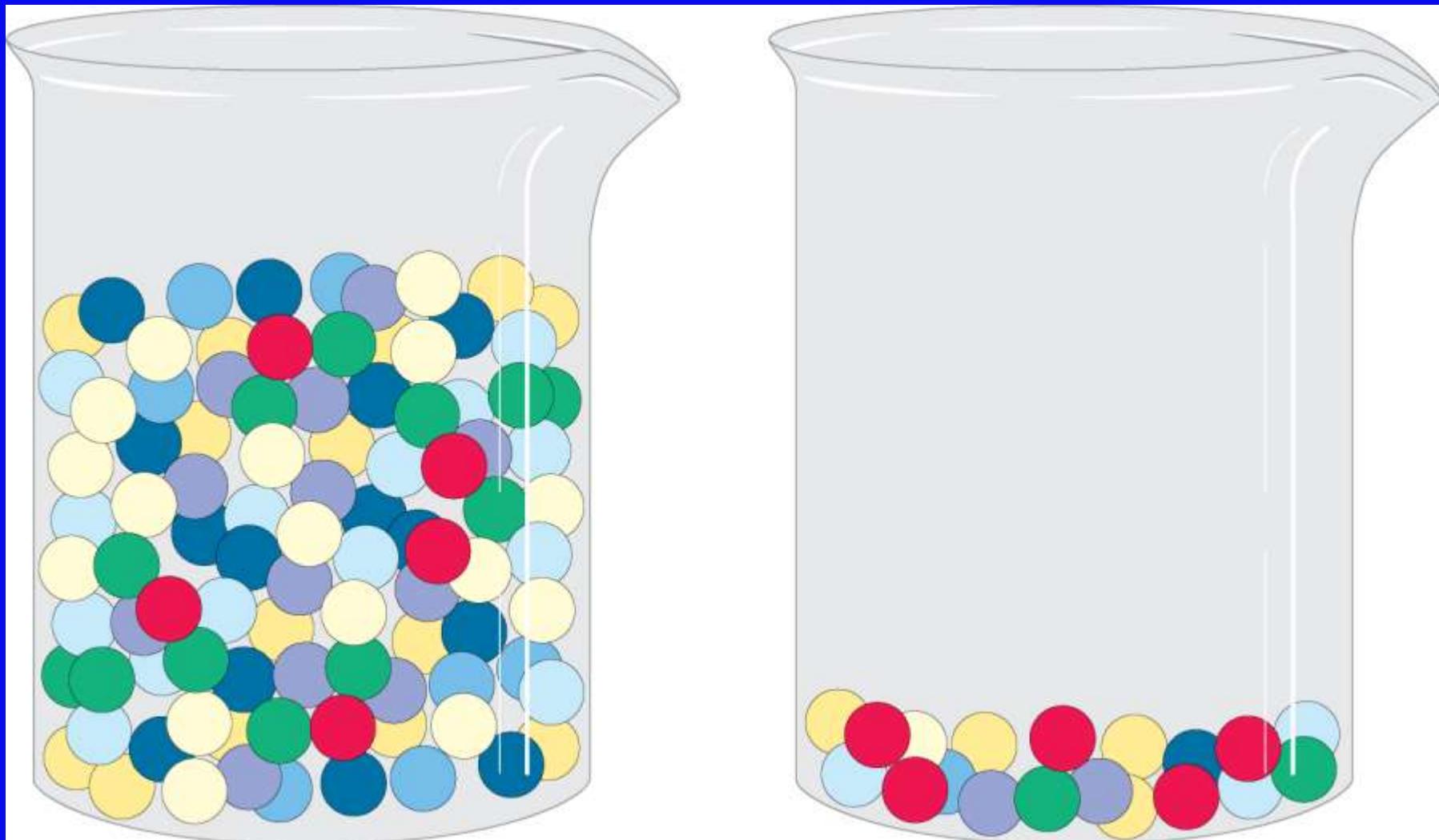
Cromatografia di affinità



DNA-AFFINITY CHROMATOGRAPHY



Isolamento e purificazione di una proteina



Isolamento e purificazione di una proteina

table 5–5**A Purification Table for a Hypothetical Enzyme***

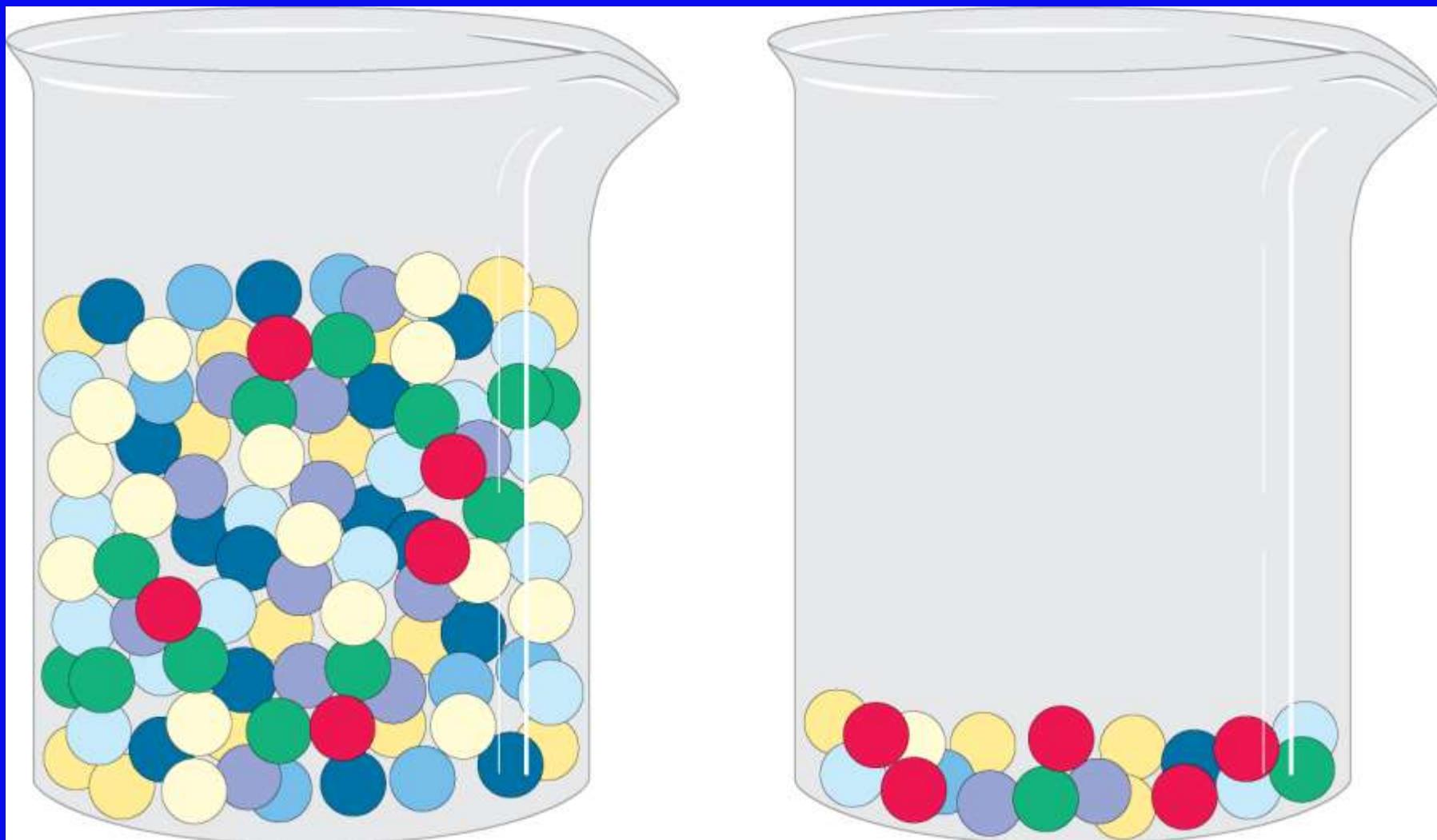
Procedure or step	Fraction volume (ml)	Total protein (mg)	Activity (units)	Specific activity (units/mg)
1. Crude cellular extract	1,400	10,000	100,000	10
2. Precipitation with ammonium sulfate	280	3,000	96,000	32
3. Ion-exchange chromatography	90	400	80,000	200
4. Size-exclusion chromatography	80	100	60,000	600
5. Affinity chromatography	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.

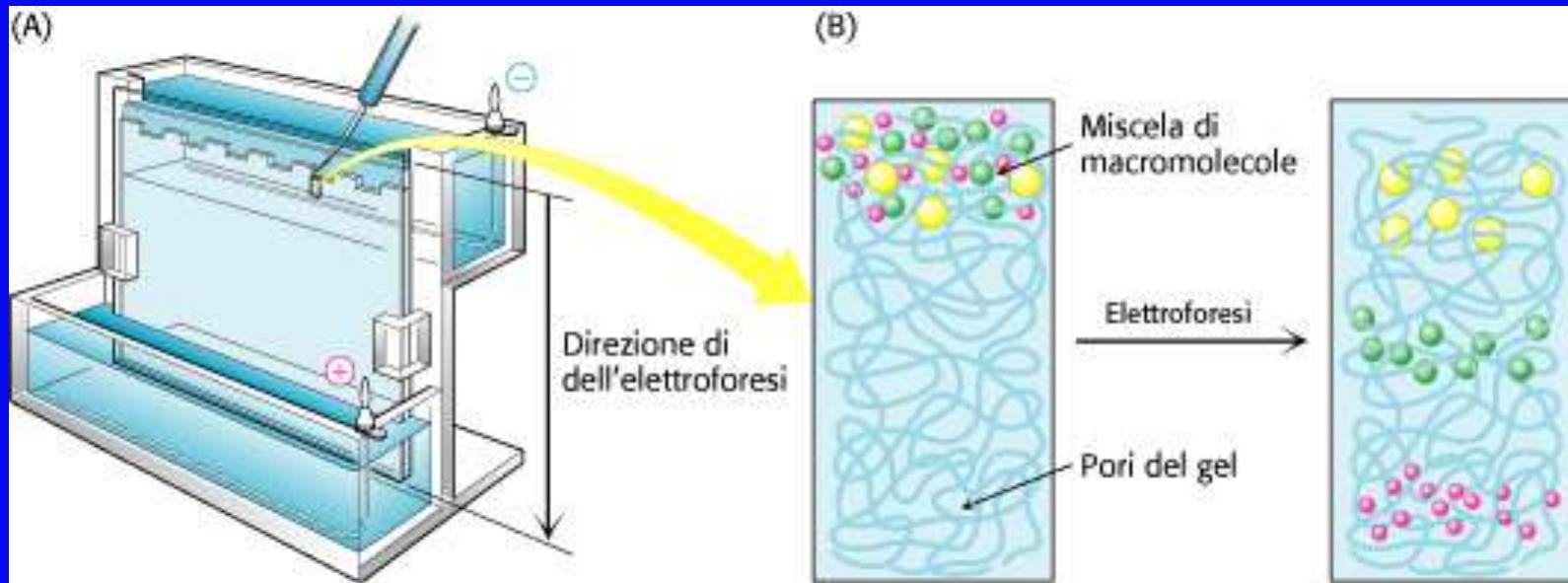
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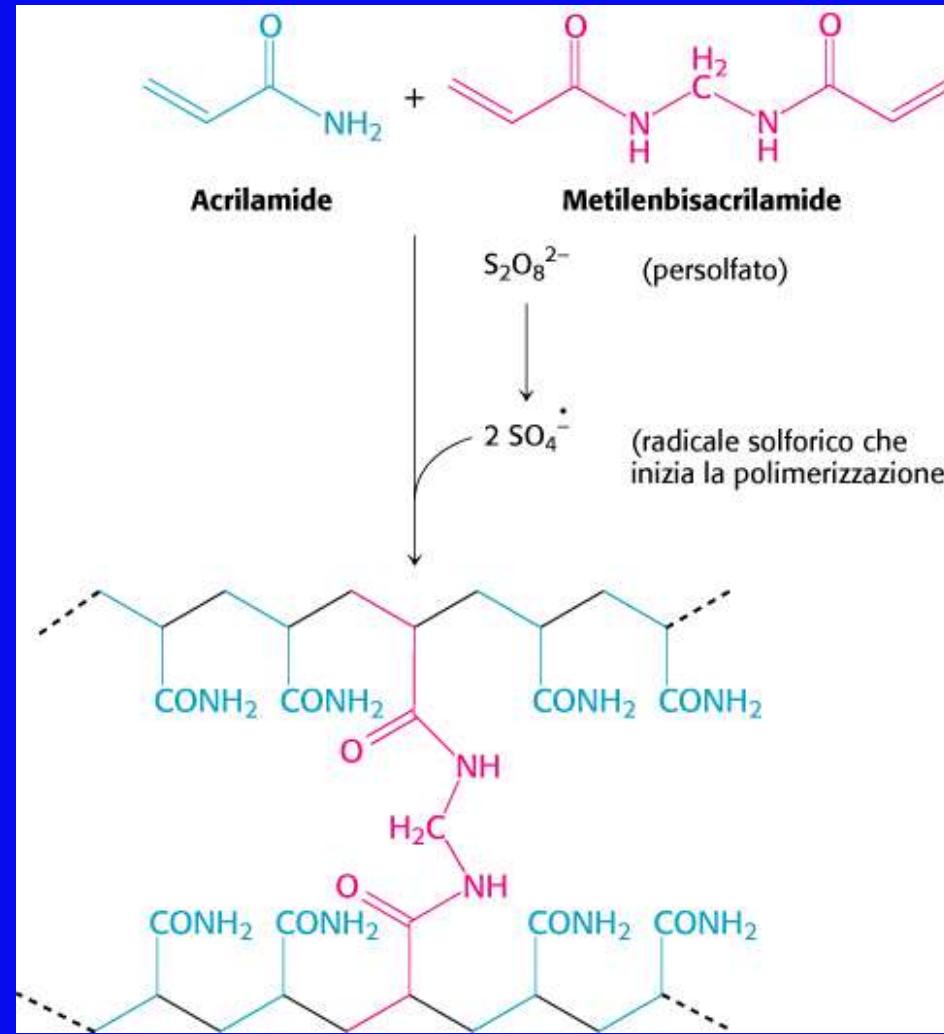
Isolamento e purificazione di una proteina



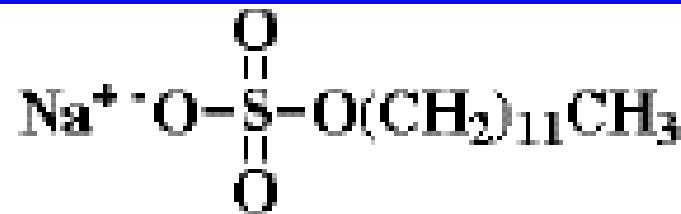
Elettroforesi in poliacrilammide/SDS: Una tecnica analitica



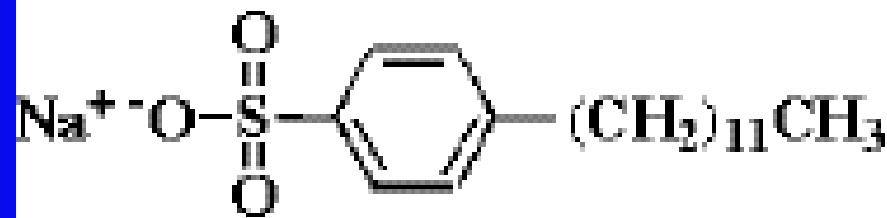
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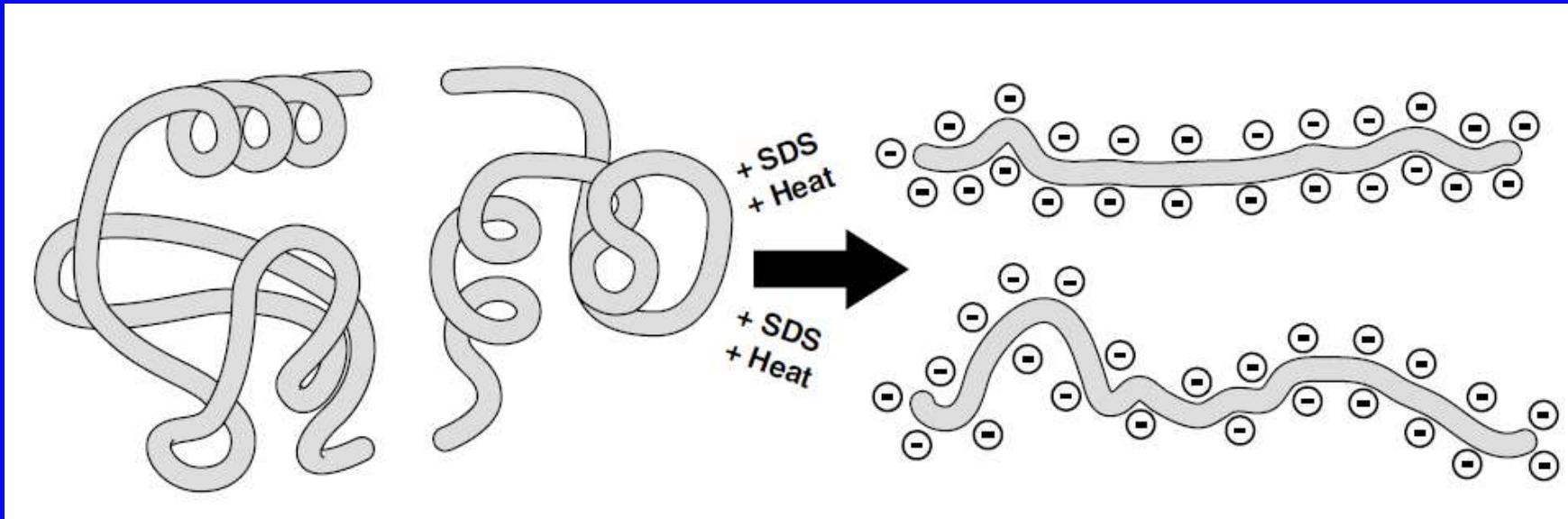


Sodium Dodecyl Sulfate (SDS)



Sodium 4-Dodecylbenzenesulfonate

Elettroforesi in poliacrilammide/SDS: Una tecnica analitica



Elettroforesi in poliacrilammide/SDS: Una tecnica analitica

SDS-PAGE

(Sodium DodecylSulfate Poly-Acrylamide Gel Electrophoresis)

Permette la separazione delle proteine solo in base al peso molecolare

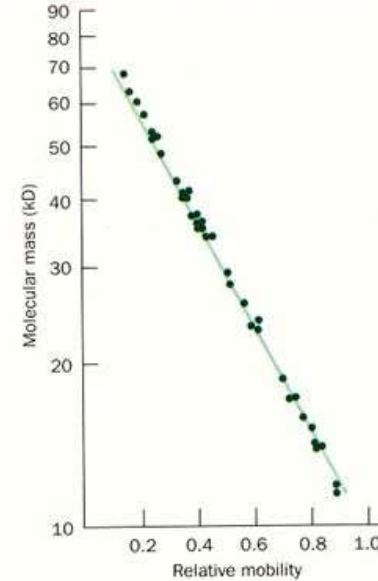
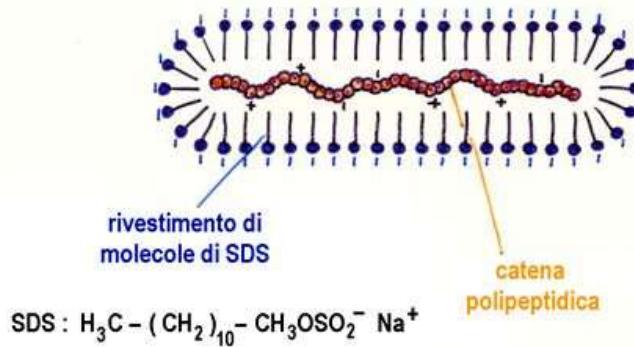
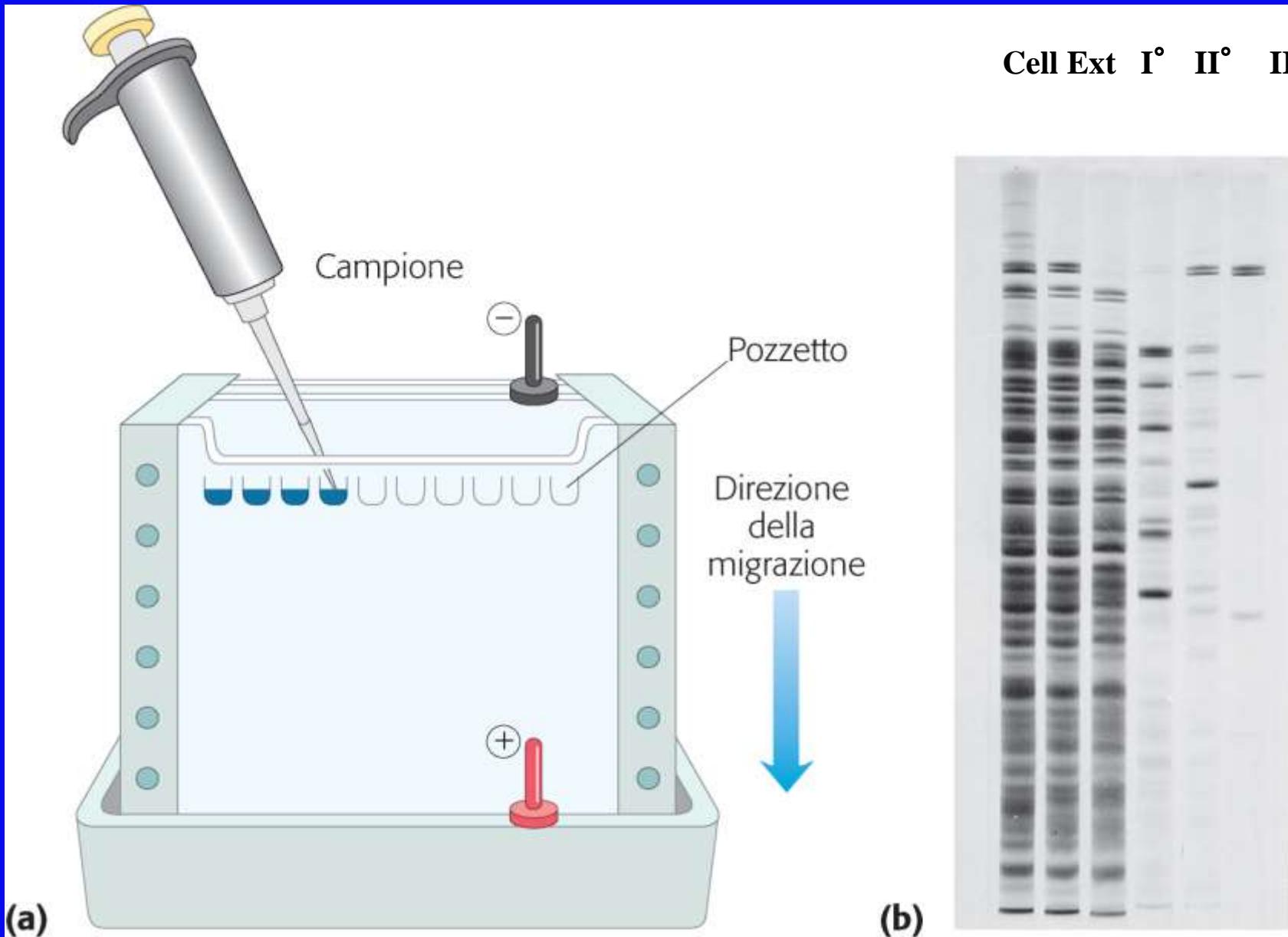


Figure 5-27

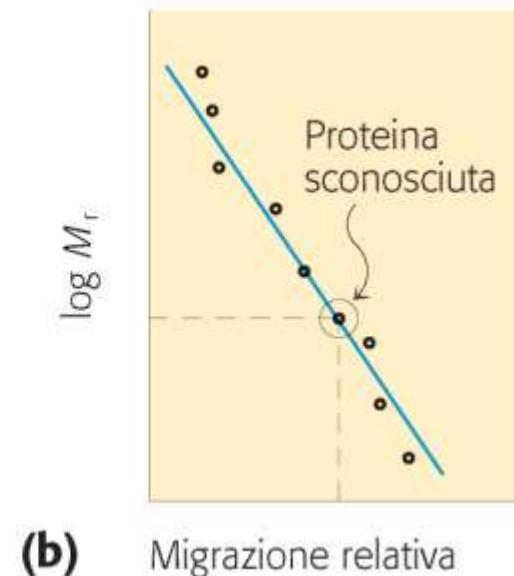
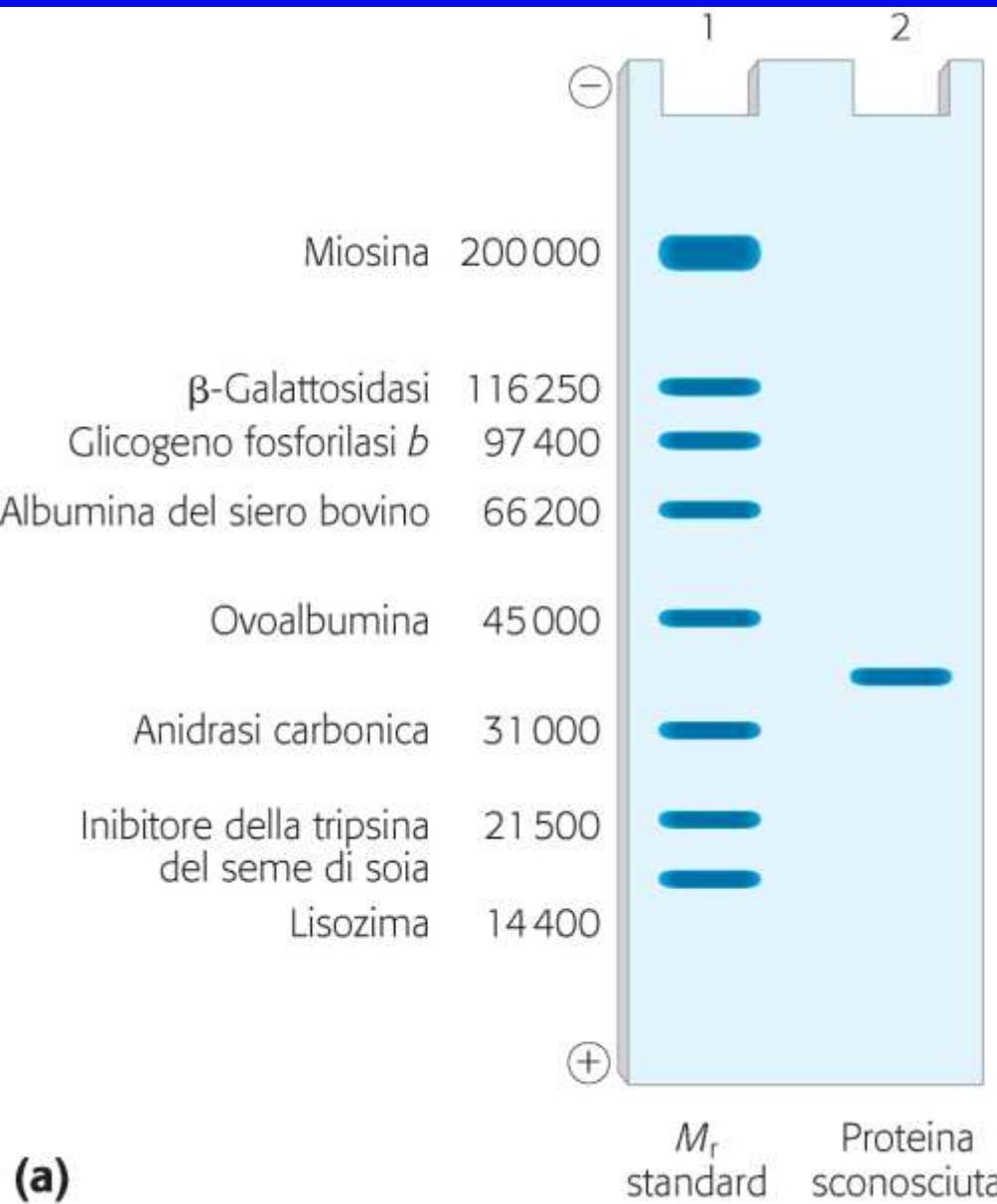
A logarithmic plot of the molecular masses of 37 different polypeptide chains ranging from 11 to 70 kD versus their relative electrophoretic mobilities on an SDS-polyacrylamide gel. [After Weber, K. and Osborn, M., *J. Biol. Chem.* 244, 4406 (1969).]

Elettroforesi in poliacrilammide/SDS: Una tecnica analitica

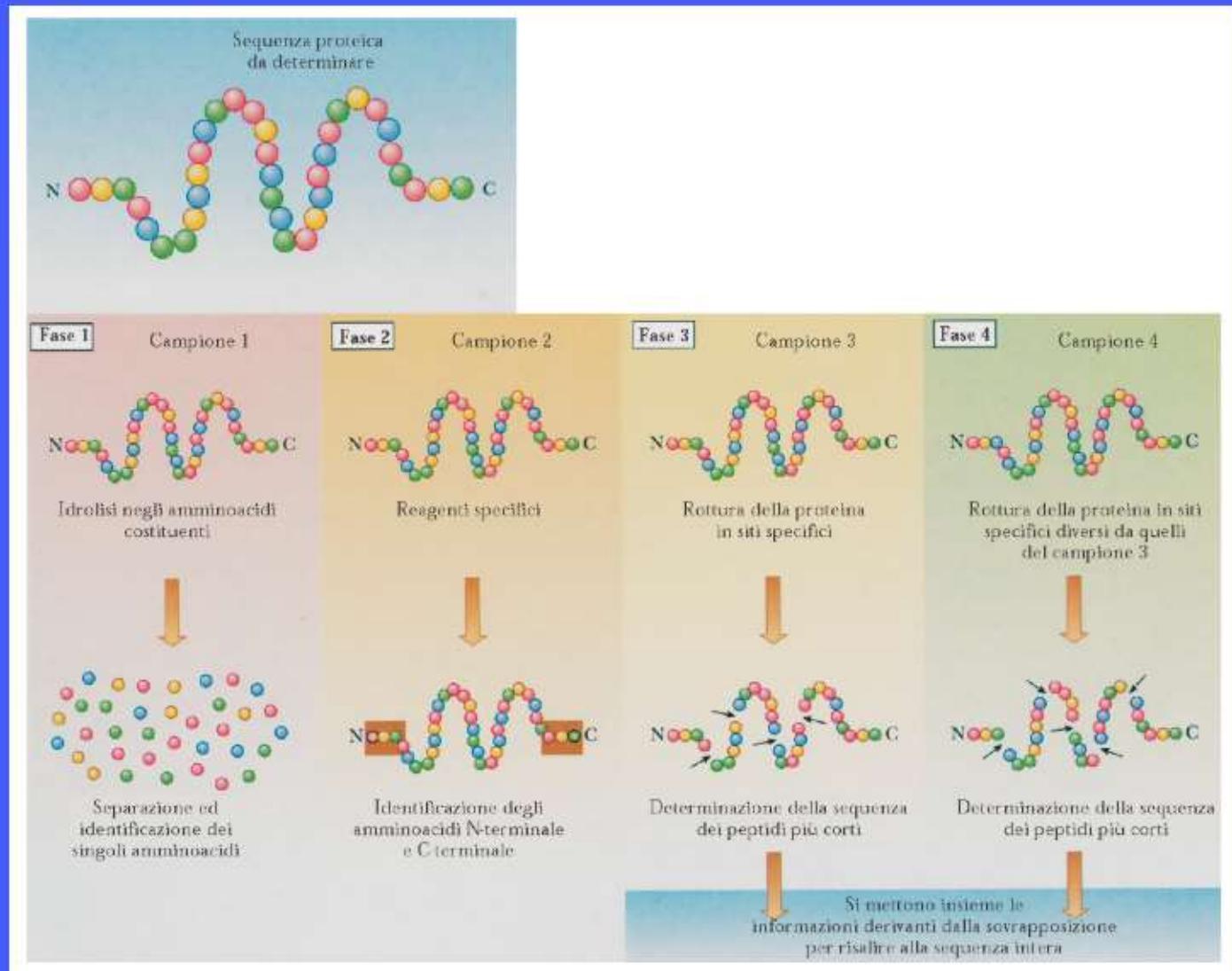


Elettroforesi in poliacrilammide/SDS:

Una tecnica analitica



Sequenziamento di una proteina

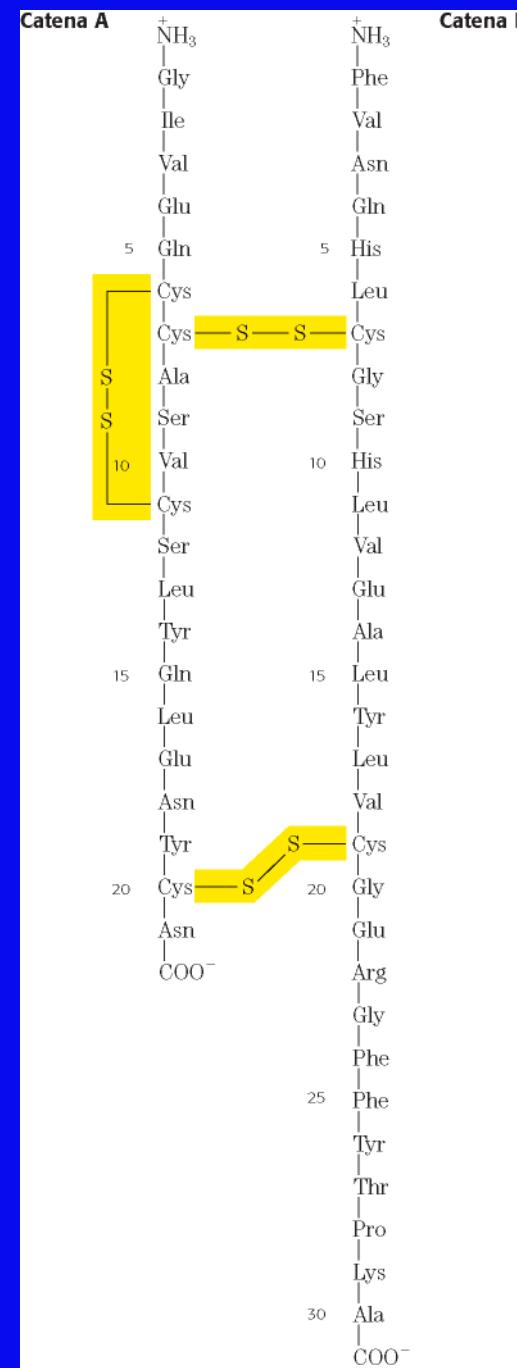


Sequenziamento di una proteina

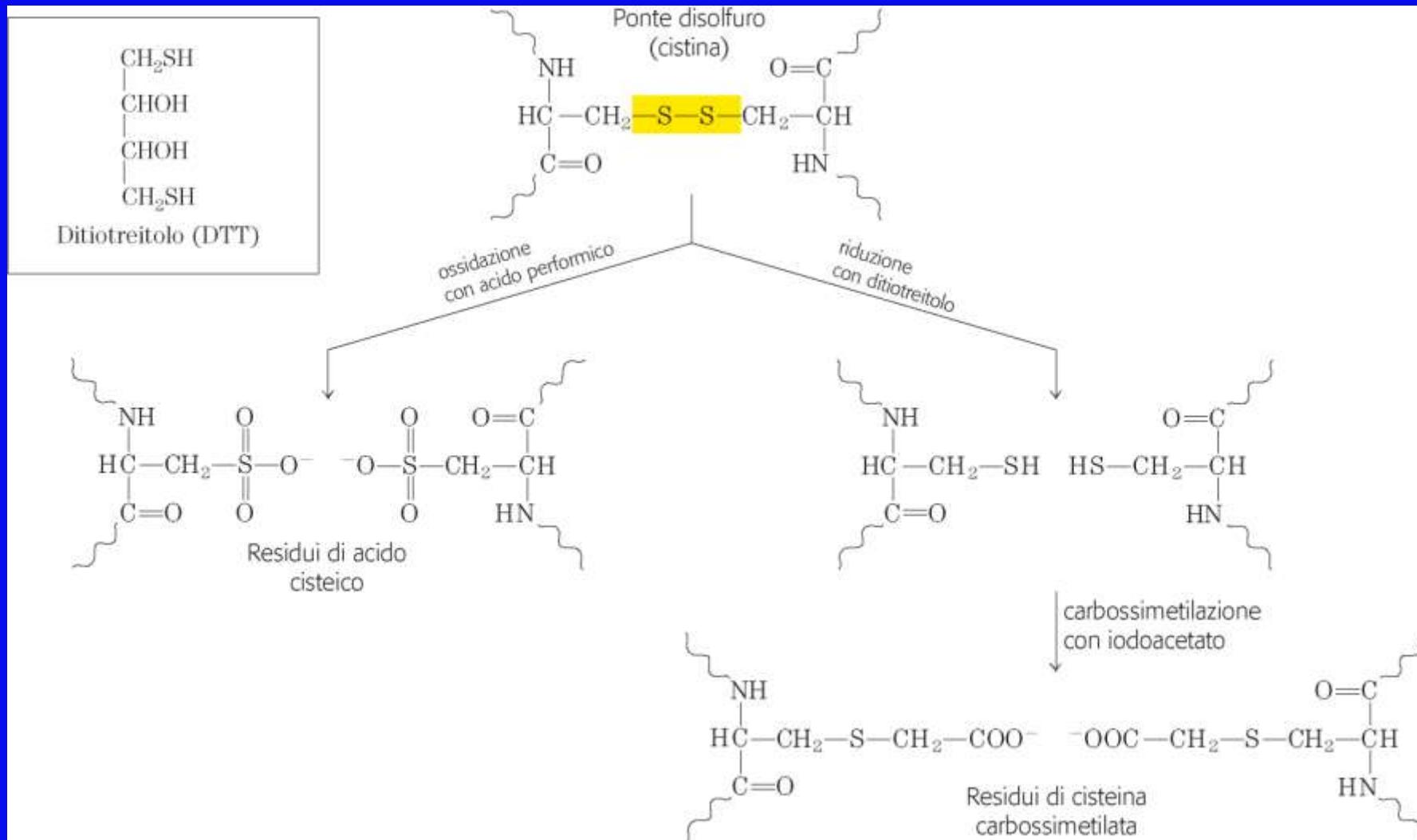
- 1) Separazione delle catene polipeptidiche
(Urea 8M, Guanidina HCl, pH estremi)
- 2) Scissione dei ponti disolfuro
(Acido performico, β -mercaptoetanolo)
- 3) Analisi della composizione amminoacidica
(Idrolisi, cromatografia a scambio ionico,
analizzatori automatici)
- 4) Identificazione dei residui N-terminali e C-terminali
(Reattivo di Sanger, Cloruro di dansile, reattivo di
Edman Idrazinolisi, riduzione mediante LiAlH₄,
carbossipeptidasi)
- 5) Frammentazione della catena polipeptidica
(Metodi enzimatici e chimici).



Scissione dei ponti disolfuro nell'insulina



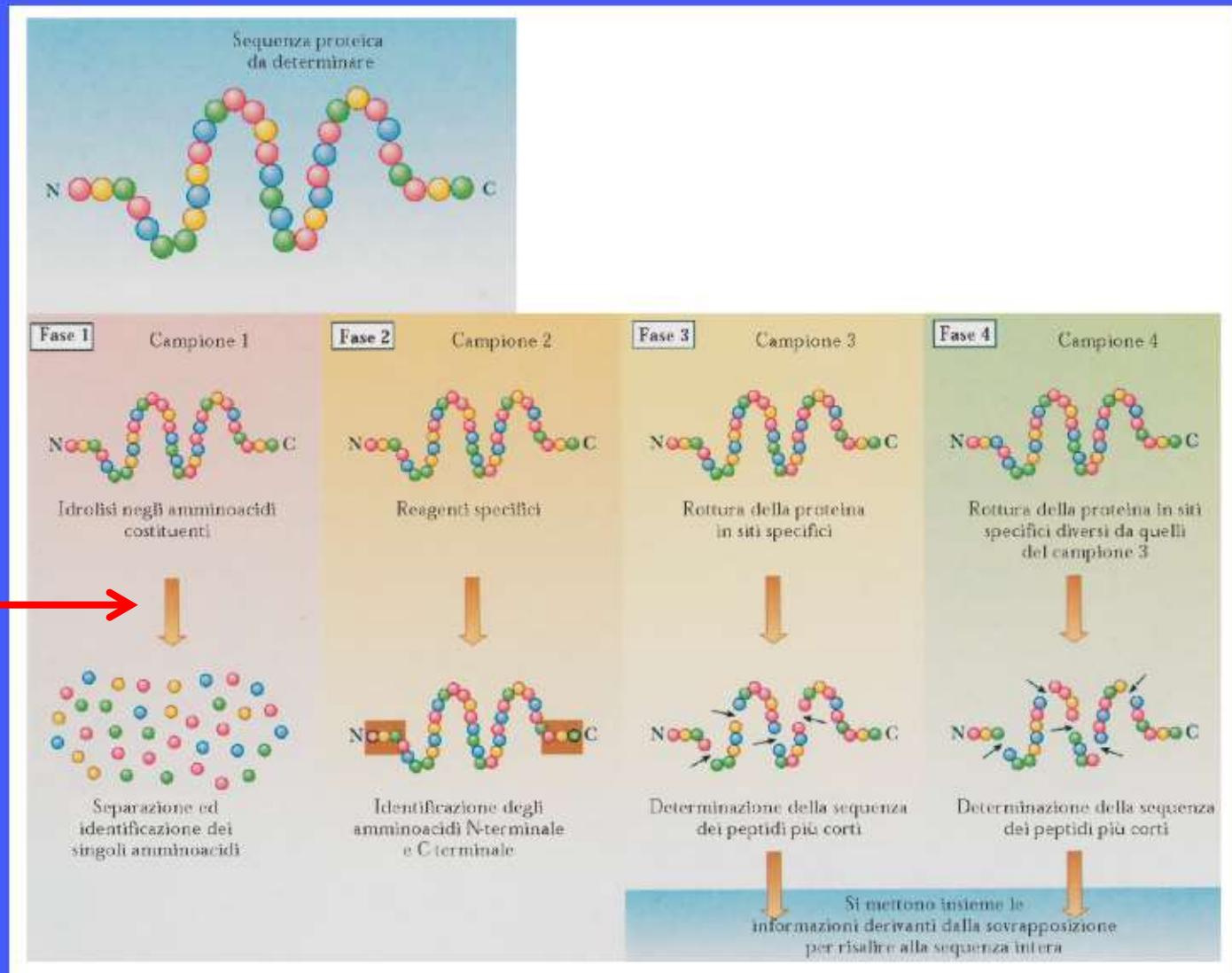
Scissione dei ponti disolfuro



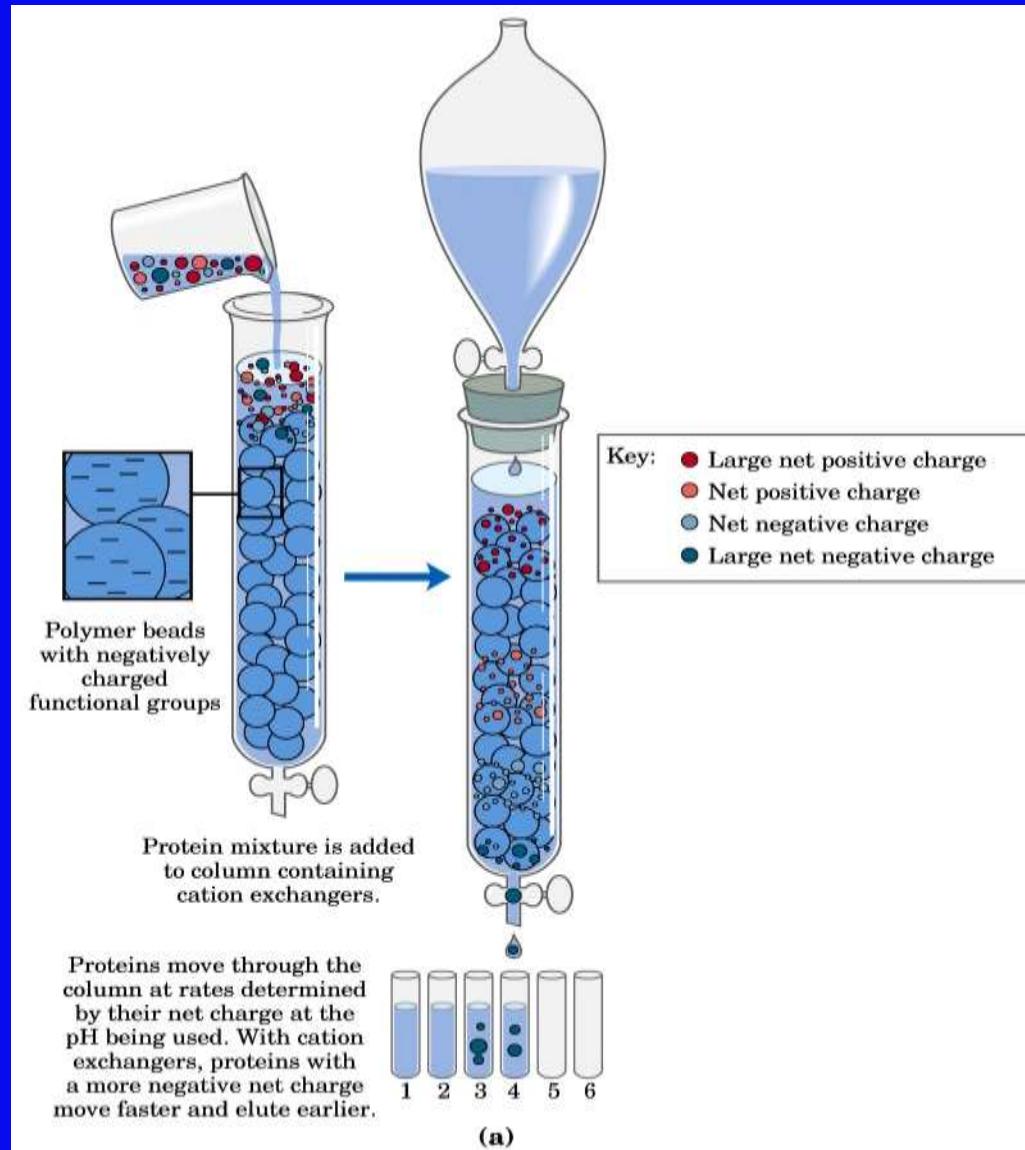
Sequenziamento di una proteina

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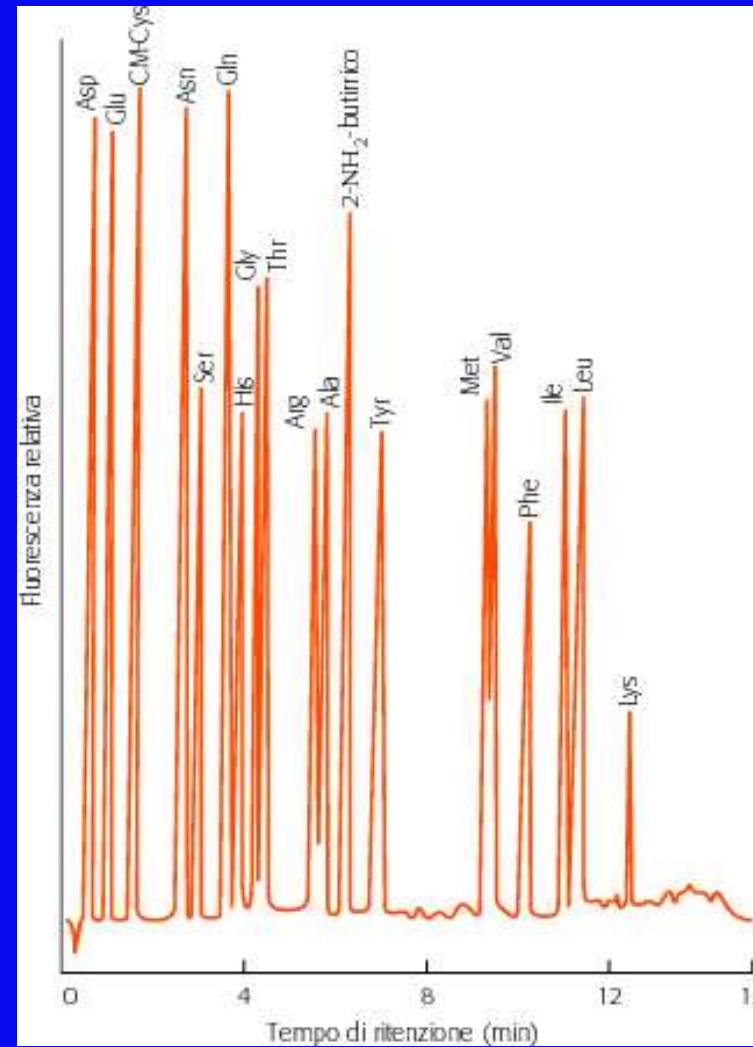
Sequenziamento di una proteina



Cromatografia a scambio ionico



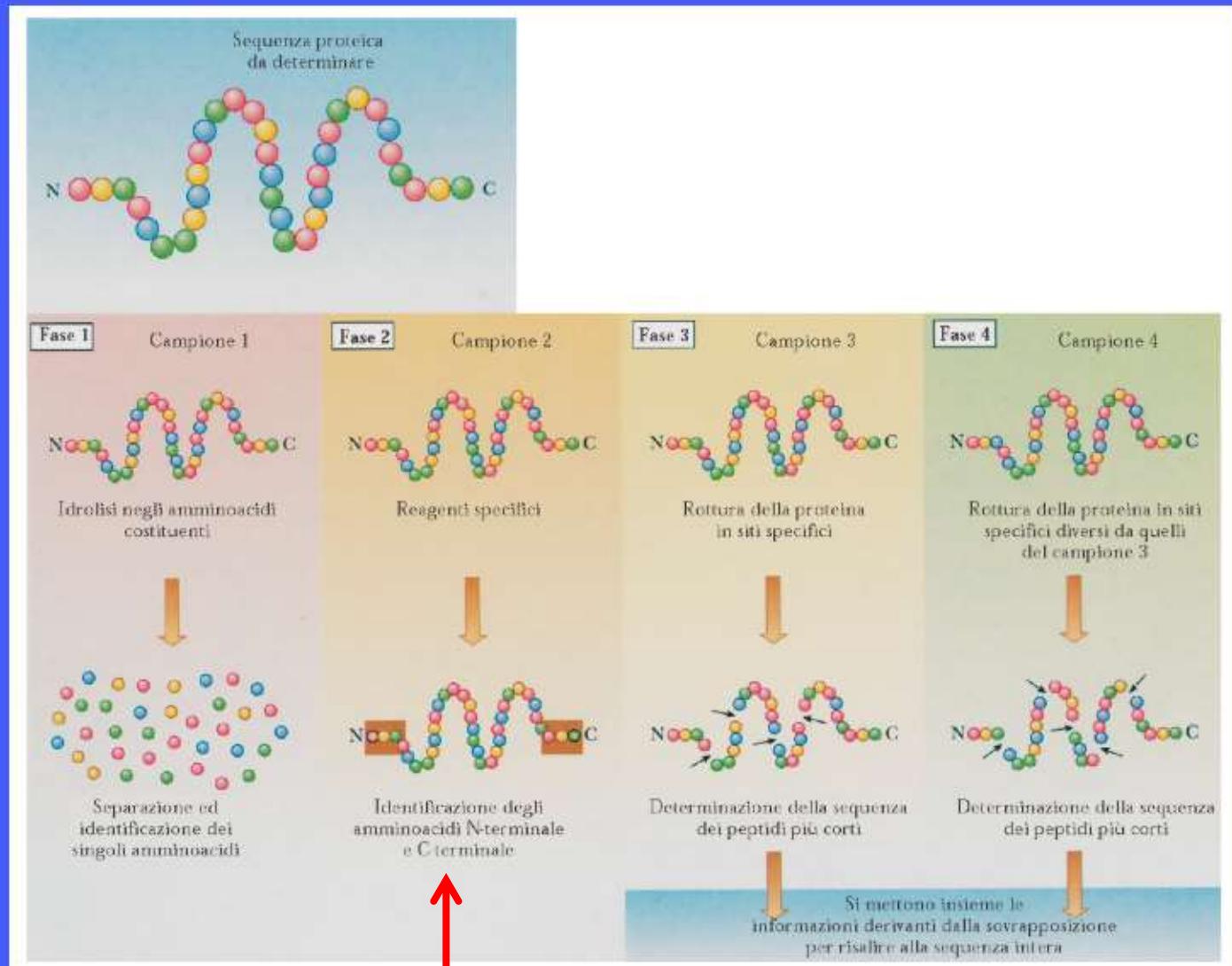
Applicazioni della cromatografia nella identificazione degli aminoacidi in una miscela



Sequenziamento di una proteina

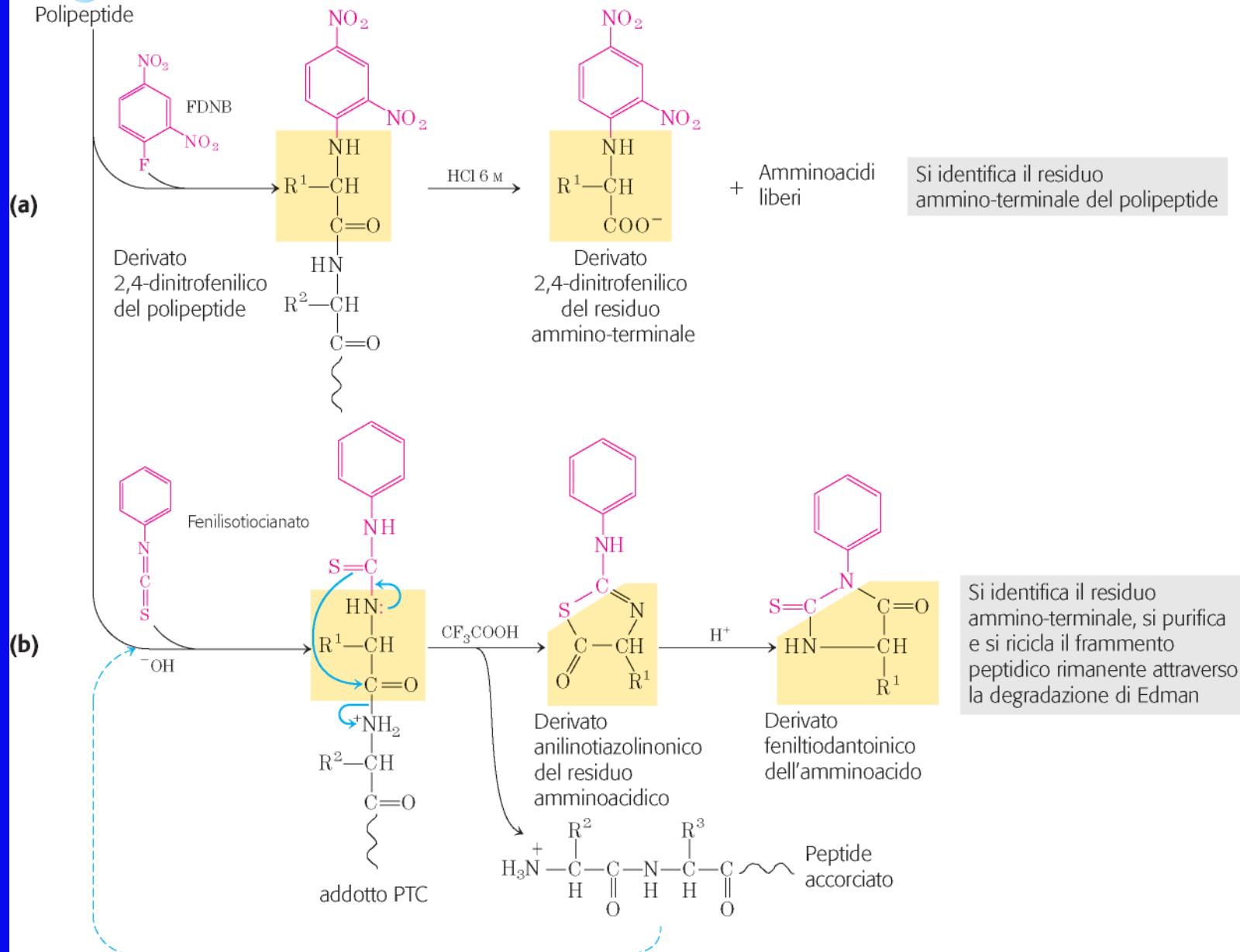
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Sequenziamento di una proteina





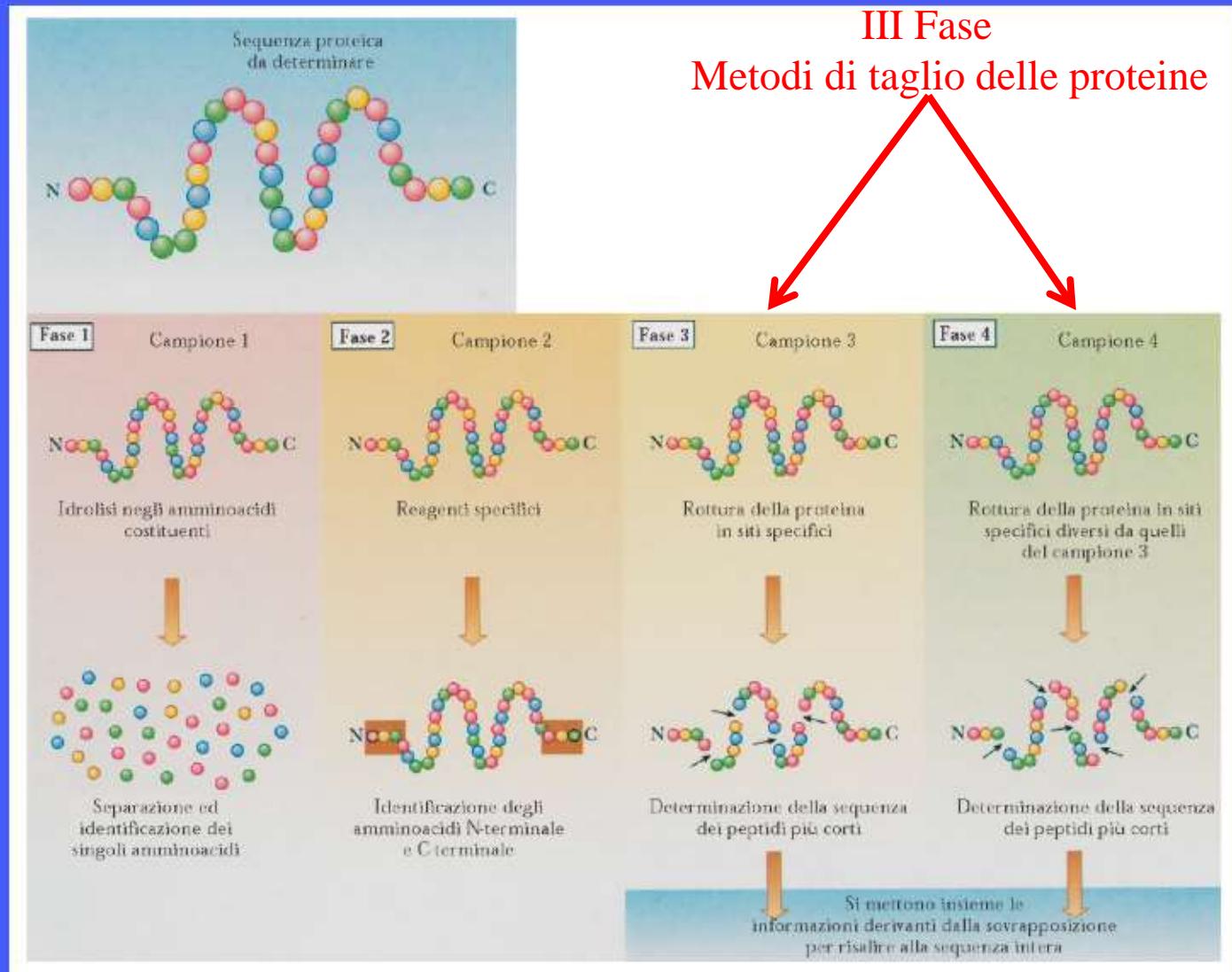
Identificazione dei residui N-terminali

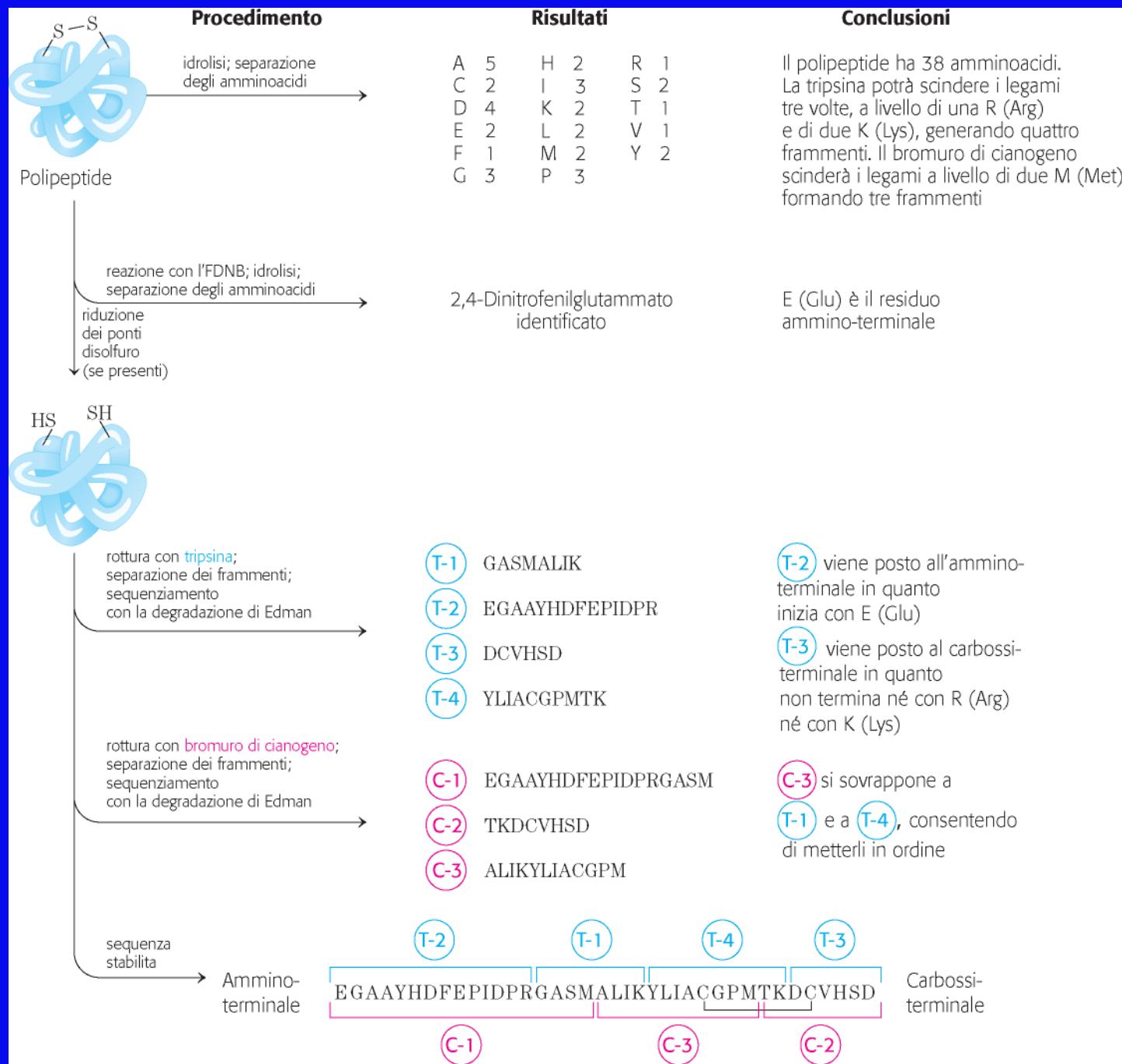


Sequenziamento di una proteina

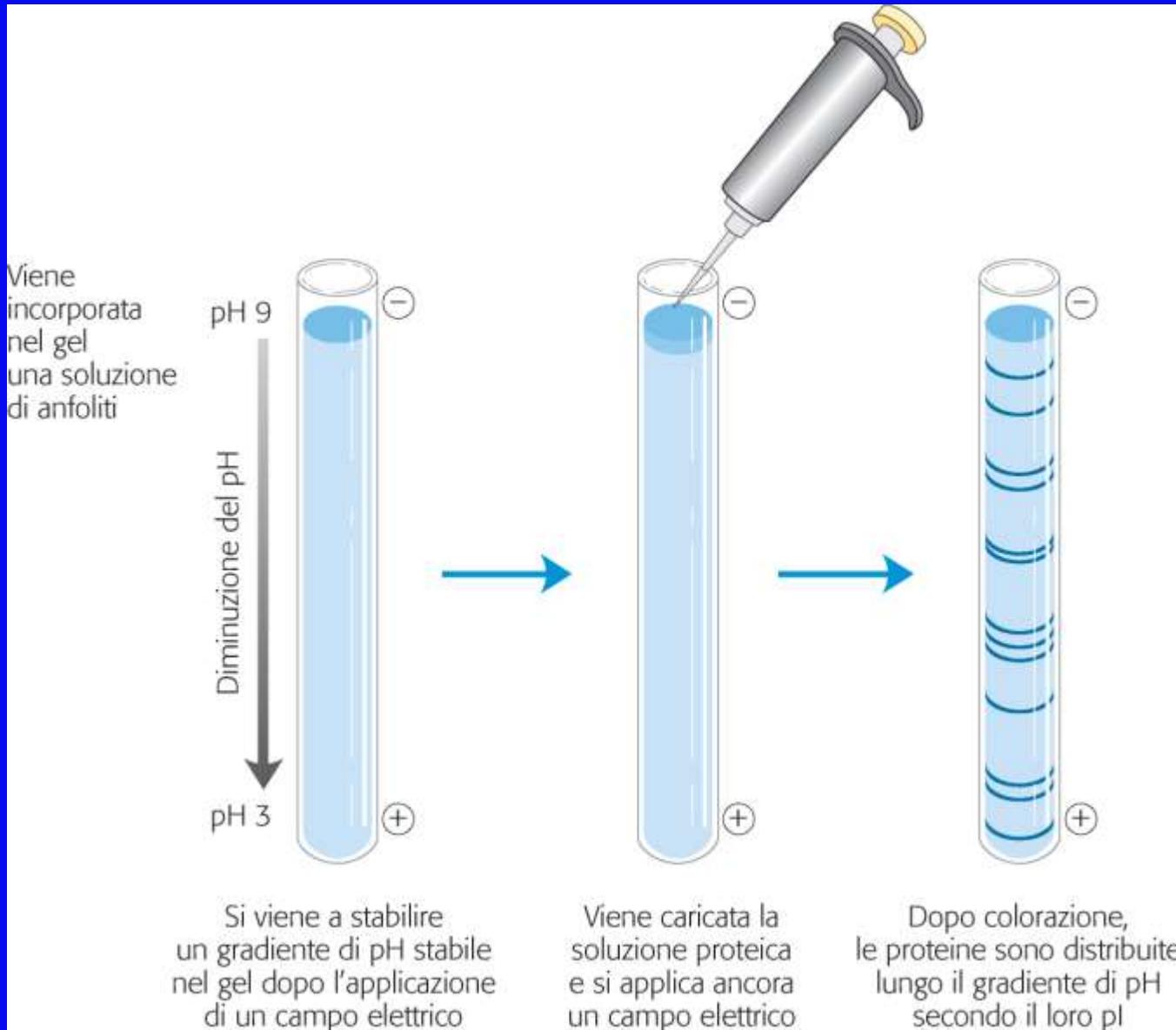
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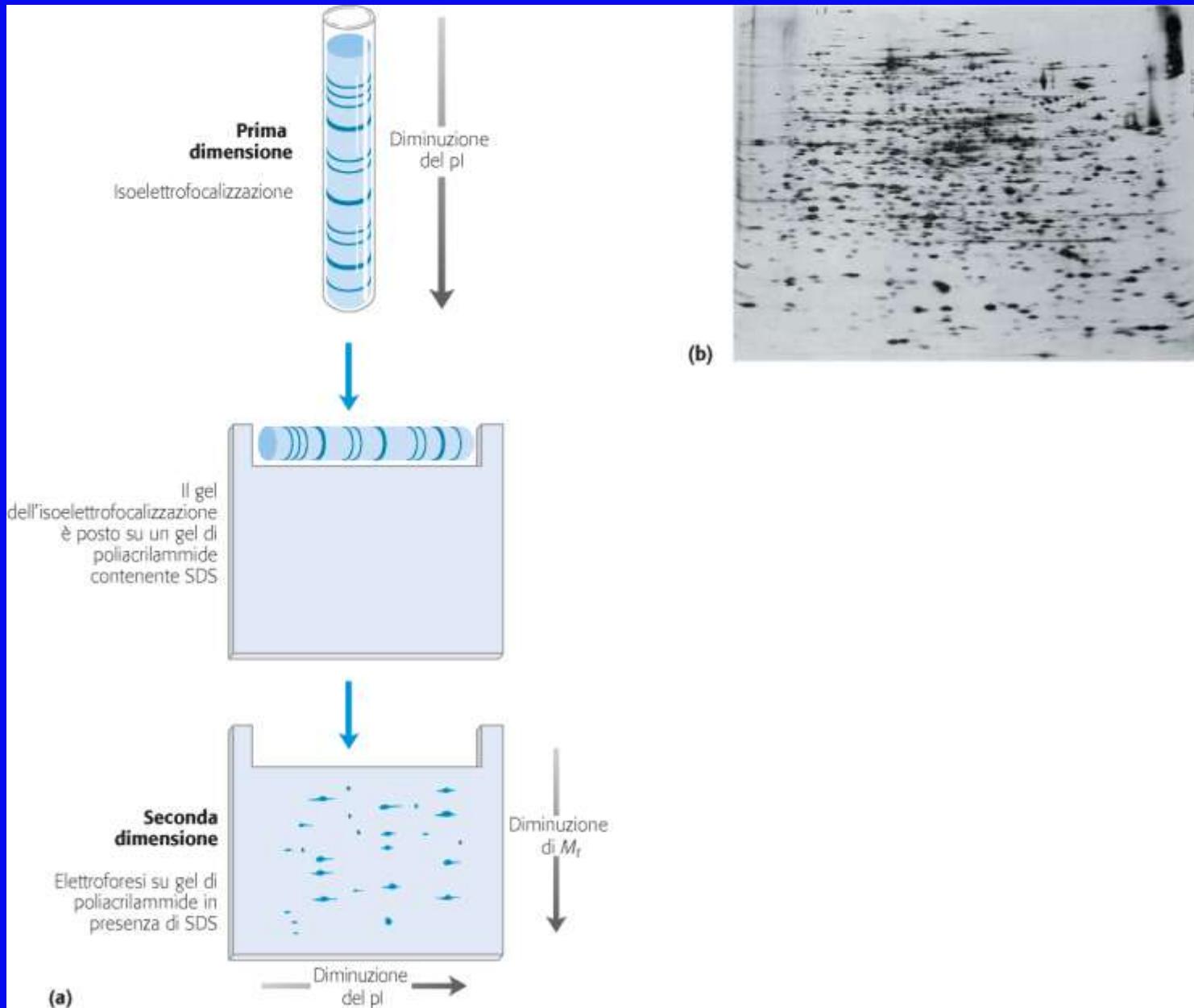


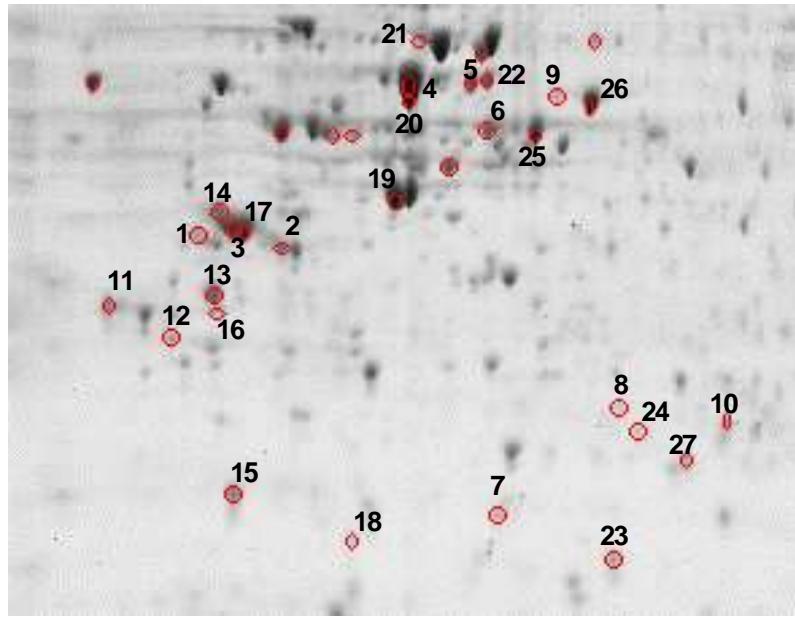


Elettroforesi Bidimensionale: prima dimensione, l' isoeletrofocalizzazione

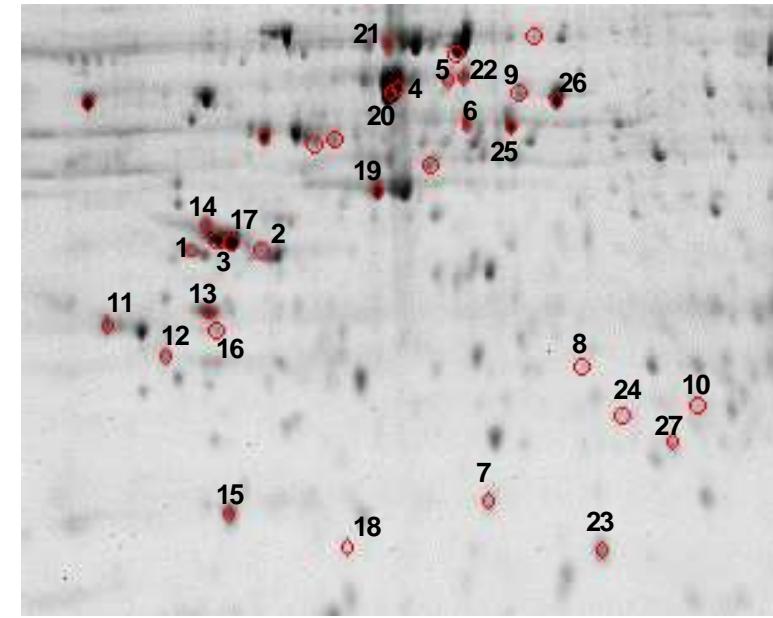


Elettroforesi Bidimensionale: seconda dimensione





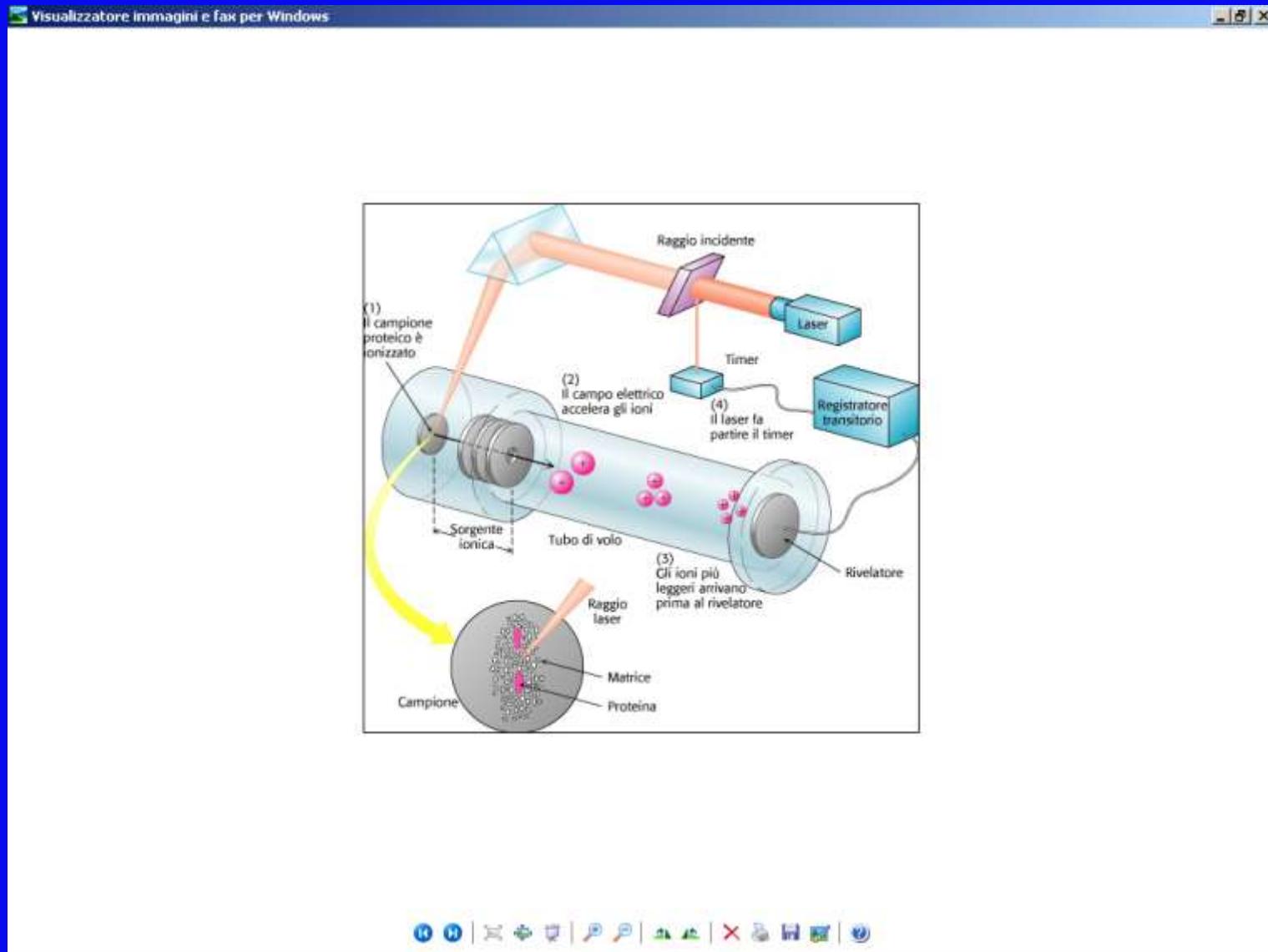
Untreated



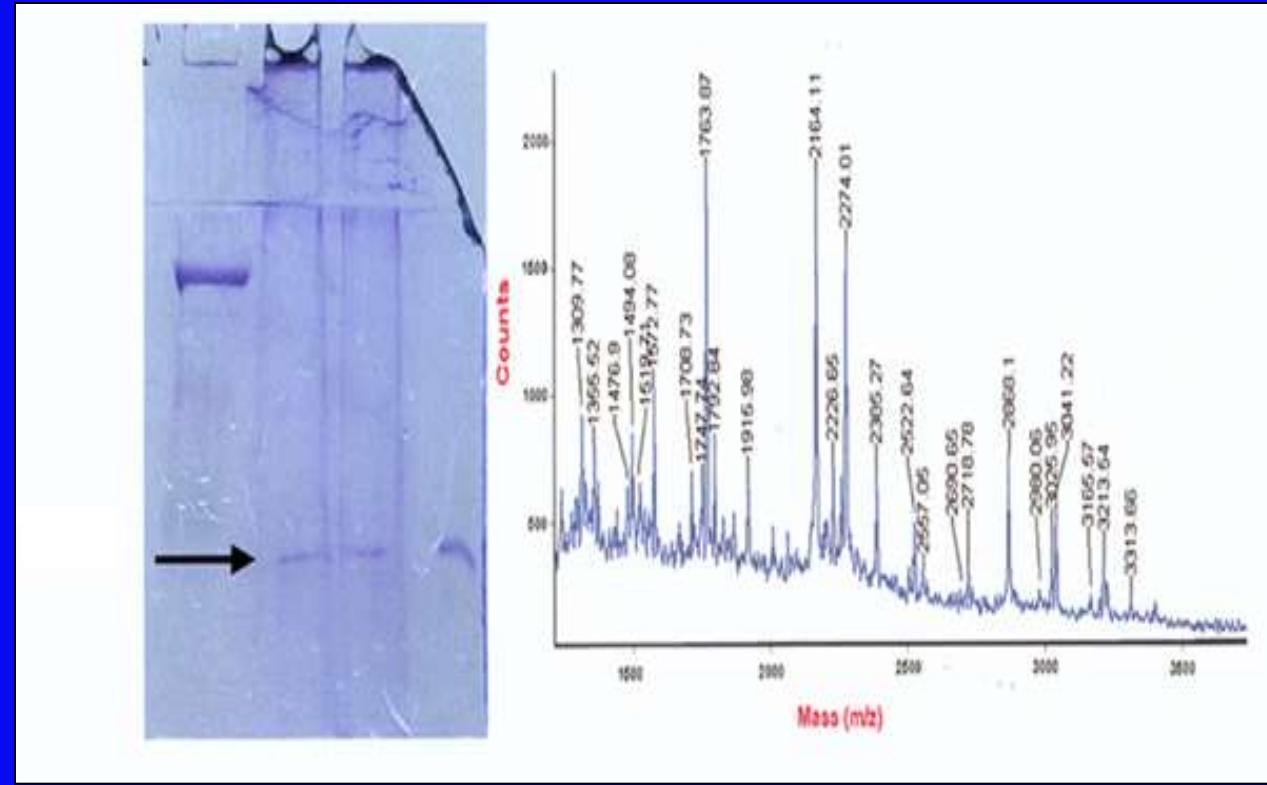
Cladosporol A 20 mM

Suppl.data 2

MALDI-TOF technology



MALDI analysis of purified transcription factor



Sequencing analysis of purified transcription factor

Sequenza ammino-acidica (proteina)

Gln –Tyr –Pro –Thr –Ile –Trp

Sequenza
del DNA (gene)

CAGTATCCTACGATTGG

ZNF224 cDNA sequence

1 GAGTCCAAACATTGGAGTCGGACACTTCCGCTCGGGACTGAGGTTGCTGCAGTTTCCGCGATAGTTG
 101 CCTTCTGAATTCTGGACCTACGCATTGGATCCTCAAAGAACTGCTGAATACCACTAGAAACATACTGTAACCAG
 201 CAGCAAGGAAGCCCACGTACCAAGGGGCTGCTTGGCACAAATTCTGCTTCCAGGAACCTGCATCACTCAGGACTCTG
 301 AAATGACCACGTTCAAGGAGGCAATGACCACTCAAGGACGTTGCTGTTCAACTGAGGAAGAGCTGGGGCTGCTG
 M T T F K E A M T F K D V A V V F T E E E E L G L L
 401 TCGAGATGTGATGCTGGAGAACCTCAGGAACCTGCTCTCAGTGGACATCAAGCATTCCACAGGGATACCTTCCACT
 R D V M L E N F R N L L S V G H Q A F H R D T F H I
 501 ATGATGAAGACAGCAATCCAAGGGAAGGGATTCAAGGAGACAAGATCCAACACTGAGATGGAGACTGTTCAGAACGC
 M M K T A I Q R E G N S G D K I Q T E M E T V S E A
 601 TCCAGCAAATCTGGGAAAAAAATTGCAAGTGATTIAACCAGGTCTCAAGAAGACTGGTGATAAAATAGCTCTCAGTTCTCC
 Q Q I W E K I A S D L T R S Q D L V I N S S S Q F S
 701 GACTGAGGCAGGACTATCTGTAATTCACACAAGACAGAAATCTCCAGGGCAATGGATATAAACCATCCTTCAGTG
 T E A G L S V I H T R Q K S S Q G N G Y K P S F S I
 801 CAACAATTACACTCAGGAGAGAAATCTCATACGTGTGATGAGTGTGGAAAGAACCTTTGTTACATCTCAGCCCTTCG
 Q Q L H S G E K S H T C D E C G K N F C Y I S A L R
 901 GAGAGAAAATGCTATAAGTGTGACGTGTGGTAAGGAATTCAAGTCAGAGTTCACATCTGCAAACATCATCAGAGAGTC
 E K C Y K C D V C G K E F S Q S S H I L Q T H Q R V
 1001 ATGTGTGGAATGTGGGAAAGGCTTCAGTCGTAGATCAGCACTTAATGTTCATCACAAATTACACACAGGAGAGAAC
 C V E C G K G F S R R S A L N V H H K L H T G E K I
 1101 AAGGCCTTCATTCAAGATTCCAGCTTCAGAACATCAGAGAACATCCATACGGGGGAGAACGCCATTCAAATGTGATAT
 K A F I H D S O L O E H O R I H T G E K P F K C D I
 1201 GATCAAGACTTAATAGGCATTCCATGGTCACACGGCAGAGAACCATTCGGATGTGATACGTGTGATAAGAGCTTT
 S R L N R H S M V H T A E K P F R C D T C D K S F
 1301 TCATCGCATGATCCACACAGGAGAGAACCATACAAATGTGAGGAGTGTGGAAAAGGCTTATTGTAGGCAGAGATC
 H E M I H T G E K P Y K C E E C G K G F I C R R D I
 1401 ACGGGAGAAAAGCCATATAATTGTAAGAGTGTGGGAAGAGCTTCAGATGGCCTCGTGTCTTGAAACATCAGCG
 T G E K P Y N C K E C G K S F R W A S C L L K H Q R
 1501 TCAAATGTGAAGAACATGTGGGAAGGGATTACACAAATTCAACATGCTATTCCACCAAGAGATCCCATAAGTGGAGAA
 K C E E C G K G F Y T N S Q C Y S H Q R S H S G E
 1601 TGGGAAGGGCTACAAAAGGAGGTTGGATCTTGACCTTCACAGCGCGTCCATACAGGAGAGAACACTGTATAATTGTA
 G K G Y K R R L D L D F H Q R V H T G E K L Y N C I
 1701 CGGGCCCCATGTCTTTGAAACATGAGAGACTCCACAGTGGAGAAAACCATTCAAATGTGAAGAGTGTGGGAAGAG
 R A P C L L K H E B L H S G E K P F Q C E E C G K R
 1801 ATTCCCATCAGAGAGTTCACACTGGAGAAAAGCCATACAAATGTGAGAAGTGTGGAAAAGGGCTACAATAGTAAGTT
 S H Q R V H T G E K P Y K C E K C G K G Y N S K F
 1901 CCACACAGGAGAGAGACCATACAATTGTAAGGAATGTGGGAAGAGTTTGCGTGGCCTCGTGTCTTTGAAACATC
 H T G E R P Y N C K E C G K S F G W A S C L L K H C
 2001 CCTTTCAAATGTGAAGAGTGTGGAAAAGATTACTCAGAATTCAACAGCTTCATTCTCATCAAAGAGTGCACACTGG
 P F K C E E C G K R F T Q N S Q L H S H Q R V H T G
 2101 AGTGTGGGAAGGGCTTCAGCTGGCCCTCAACTCGTCTGACCCATCAGAGACGCCACAGCAGAGAACACCTCTCAA
 C G K G F S W S S T R L T H Q R R H S R E T P L K
 2201 TGTAAGAATTCTATTCTAAAGTGCAGGAAAGGGATTCAAGTGTAGAAAAGCCATACAAATGTGAGGAGACTGTGGGA
 V O N S F S K V O E K V H S V E K P Y K C E D C G I
 2301 CTTGATATGCATCAGAGGGTCCACATGGGAGAGAAAACATGGAAGTGTAGGGAGTGTGATATGTGCTTTAGTCAGGC
 L D M H Q R V H M G E K T W K C R E C D M C F S Q A
 2401 ATGTTCATGTTGGAGAAAACCTTAGTGTGATGGTGCATAAAAGTCTTCACTCAGTCTTCATG 2466
 V H V G E K P *

Bioinformatic analysis of a sequence protein by a comparison between species

E. coli TGNRTIAVYDLGGGTFDISIIEIDEVDGEKTFEVLATNGDTHLGGEDFDSRLIHYL
B. subtilis DEDQTILLYDLGGGTFDVSILELGDG TFEVRSTAGDNRLGGDDFDQVIIIDHL

Intervallo

Bioinformatic analysis of a sequence protein by a comparison between species

Archebatteri

Eucarioti

Batteri Gram-positivi

Batteri Gram-negativi



Halobacterium halobium
Sulfolobus solfataricus
Saccharomyces cerevisiae
Homo sapiens
Bacillus subtilis
Escherichia coli

Sequenza di identificazione	
I G H V D H G K S T M V G R L L Y E T G S V P E H V I E Q H	
I G H V D H G K S T L V G R L L M D R G F I D E K T V K E A	
I G H V D S G K S T T T G H L I Y K C G G I D K R T I E K F	
I G H V D S G K S T T T G H L I Y K C G G I D K R T I E K F	
I G H V D H G K S T M V G R	I T T V
I G H V D H G K T T L T A A	I T T V



Bioinformatic analysis of a sequence protein by a comparison between species

Bioinformatic analysis of a sequence protein by a comparison between species

