Isolasi dan Pemurnian Protein

Tujuan

- Mempelajari fungsi enzim/protein
- Menentukan urutan asam amino
- Mempelajari struktur

- Sel mengandung ribuan protein
- Prosedur pemurnian harus membedakan protein yang diinginkan dari protein yang lain
- Sifat protein merupakan kunci dalam merancang protokol pemurnian

Sifat Protein

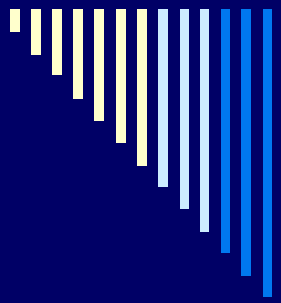
- Muatan disebabkan oleh rantai samping COO^- dan $NH3^+$
- Berat molekul protein bervariasi
- Titik isoelektrik, pI, pH ketika muatan total protein nol

  Jika protein dengan pI tertentu berada dalam larutan dengan pH tertentu, maka

pH < pI < pH
Net+ Net-

Prosedur pemurnian protein dapat dilakukan karena adanya variasi:

Kelarutan, ukuran, muatan, binding specificity (affinity),sifat hidrofobik/hidrofilik



Sel

Lisis

Osmosis
Freeze/Thaw
French press
Grinding glass beads
Sonication (bakteri)

Sentrifugasi

Pelet

Debris sel

Supernatan

Protein terlarut

Isolasi Protein

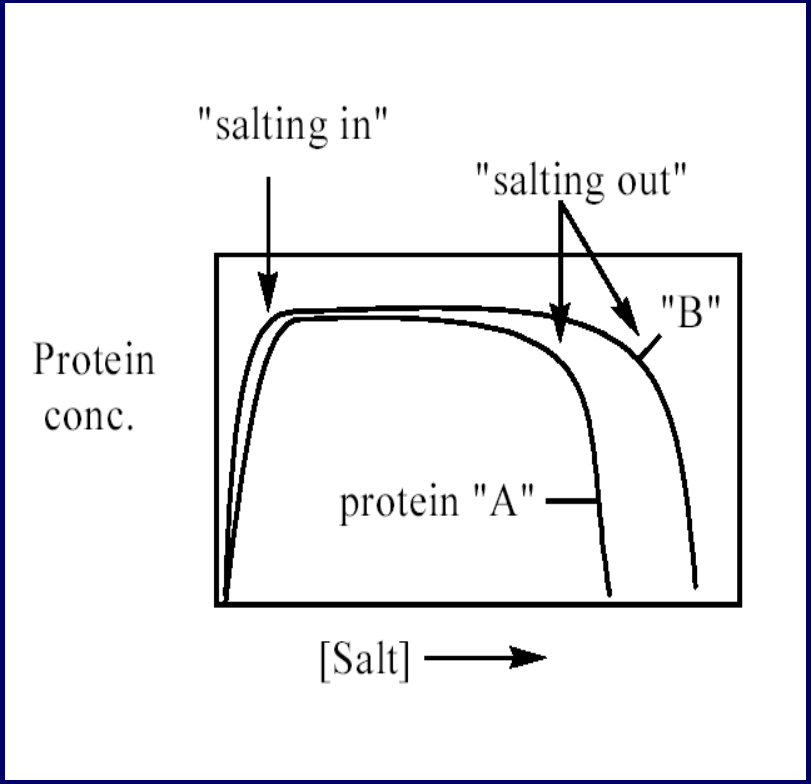
Inhibitor Protease: inaktivasi protease yang dibebaskan selama lisis sel

Nama	Jenis Inhibitor	Target Protease
PMSF (Phenylmethylsulfonyl fluoride)	Ireversibel	Ser (+Cys) protease: terikat pada pusat aktif Ser/Cys
Benzamidine	Reversibel	Ser dan Cys protease: inhibitor kompetitif
EDTA	Reversibel	Metalo enzim: chelate metals
Pestatin (pentapeptida + gugus organik)	Reversibel	Asp'acidic' protease: inhibitor kompetitif (target: pepsin, HIV protease)
Aprotinin (58-mer peptida)	Reversibel	Serin protease, esterase: inhibitor kompetitif (target: tripsin, kimotripsin, plasmin)
Leupeptin (Ac-leu-leu-Arg)	Reversibel	Ser dan Cys protease: inhibitor kompetitif

Stabilisasi: Ketika berada diluar lingkungan aslinya (native), protein menjadi kurang stabil. Meskipun organisme hidup pada temperatur 37^{o}C, pemurnian dilakukan pada temperatur 4^{o}C

Solubility (Salting Out)

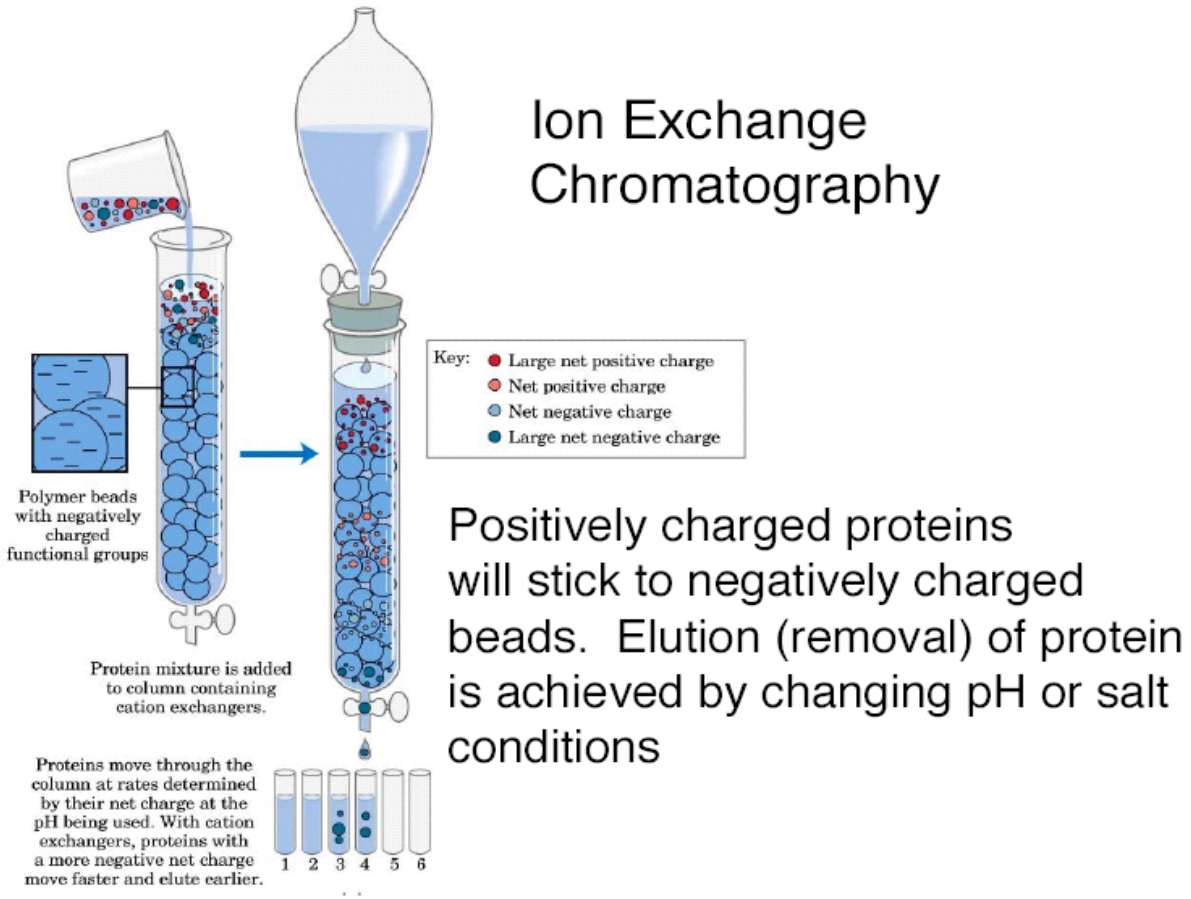
- Garam berkompetisi dengan air
- Protein mengalami agregasi melalui interaksi van der Waals dan hidrofobik dan mengendap dalam bentuk native
- 1-4 M $(NH4)_2SO_4$



Strategi Pemurnian

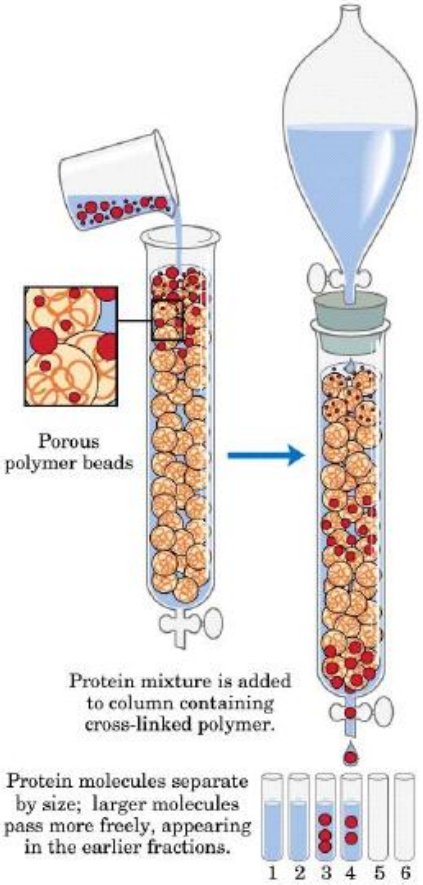
- Muatan: Kromatografi Penukar Ion
- Kepolaran: Kromatografi Interaksi Hidrofobik
- Ukuran: Dialisis, Ultrafiltrasi, Gel elektroforesis, Kromatografi Filtrasi Gel, Ultrasentrifugasi
- Spesifisitas: Kromatografi afinitas

Ion-exchange Chromatography (IEX)

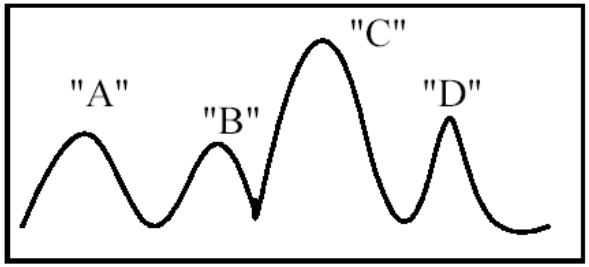


- Cation exchangers contain negatively charged polymer
- Anion exchangers contain positively charged polymer.
- Is effected by pH

Gel Filtration/Size Exclusion Chromatography



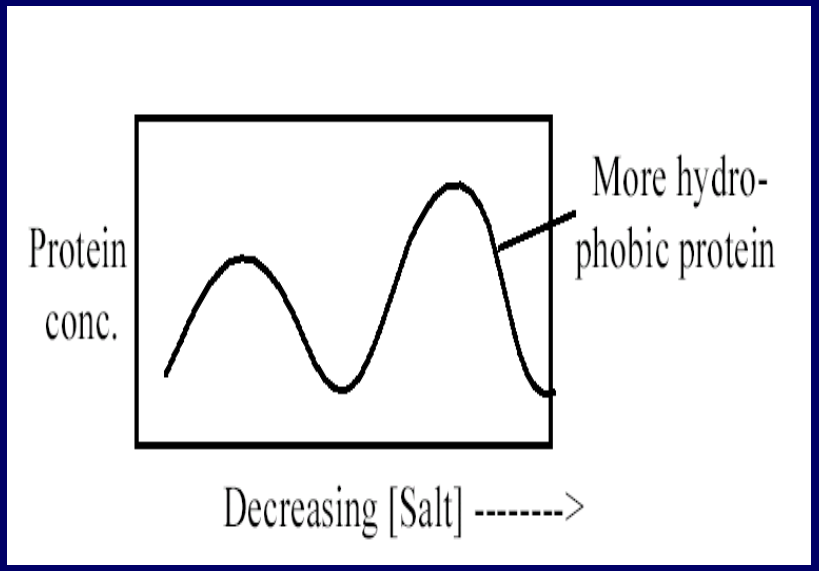
A porous column acts as a "molecular sieve," in which smaller molecules get stuck in pores and take longer to travel through the column.



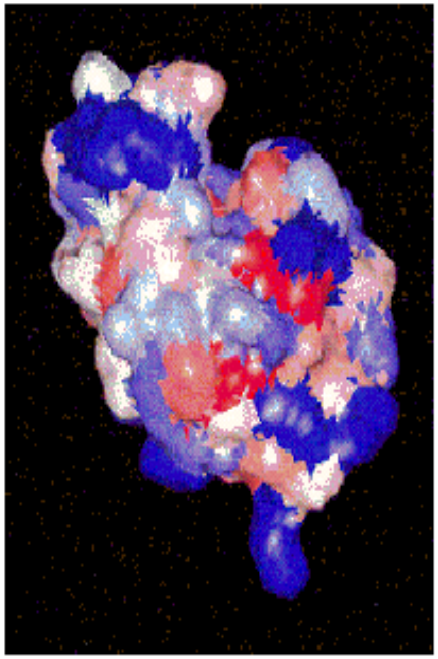
isocratic elution (same buffer)
hi mw ----------> lo mw

Kromatografi Interaksi Hidrofobik

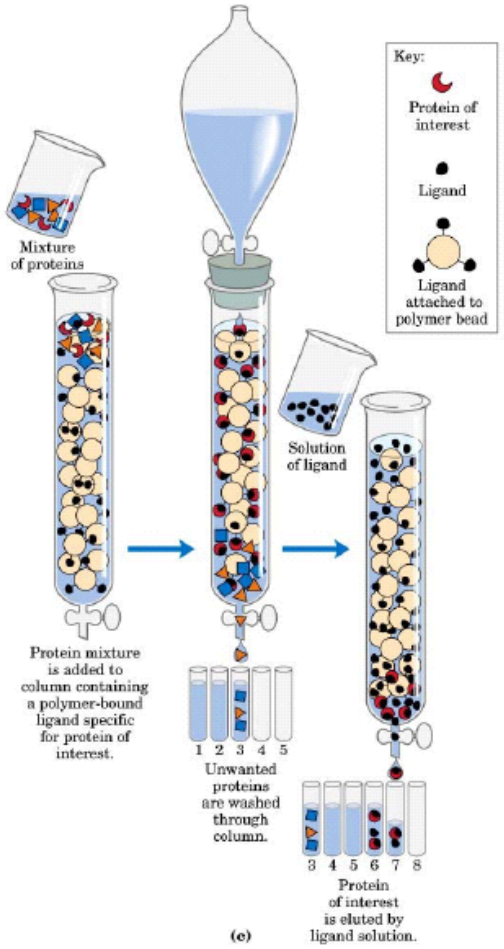
- Protein mempunyai ‘daerah’ hidrofobik dipermukaan yang dapat berinteraksi dengan resin
- Resin yang mengandung gugus fenil atau alifatik mengikat protein: protein yang paling hidrofobik akan terikat paling kuat
- Elusi dilakukan dengan pelarut yang kurang polar atau dengan menurunkan [garam]



Lysozyme

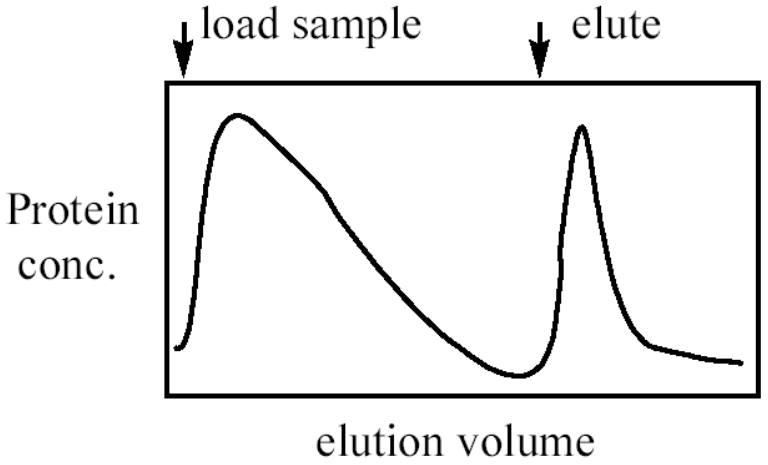


Hydrophobic and hydrophilic parts on the surface of lysozyme:
The most hydrophobic parts are dark red,
the less hydrophobic lighter red.
The most hydrophilic parts are shown in dark blue,
while the less hydrophilic parts are lighter blue

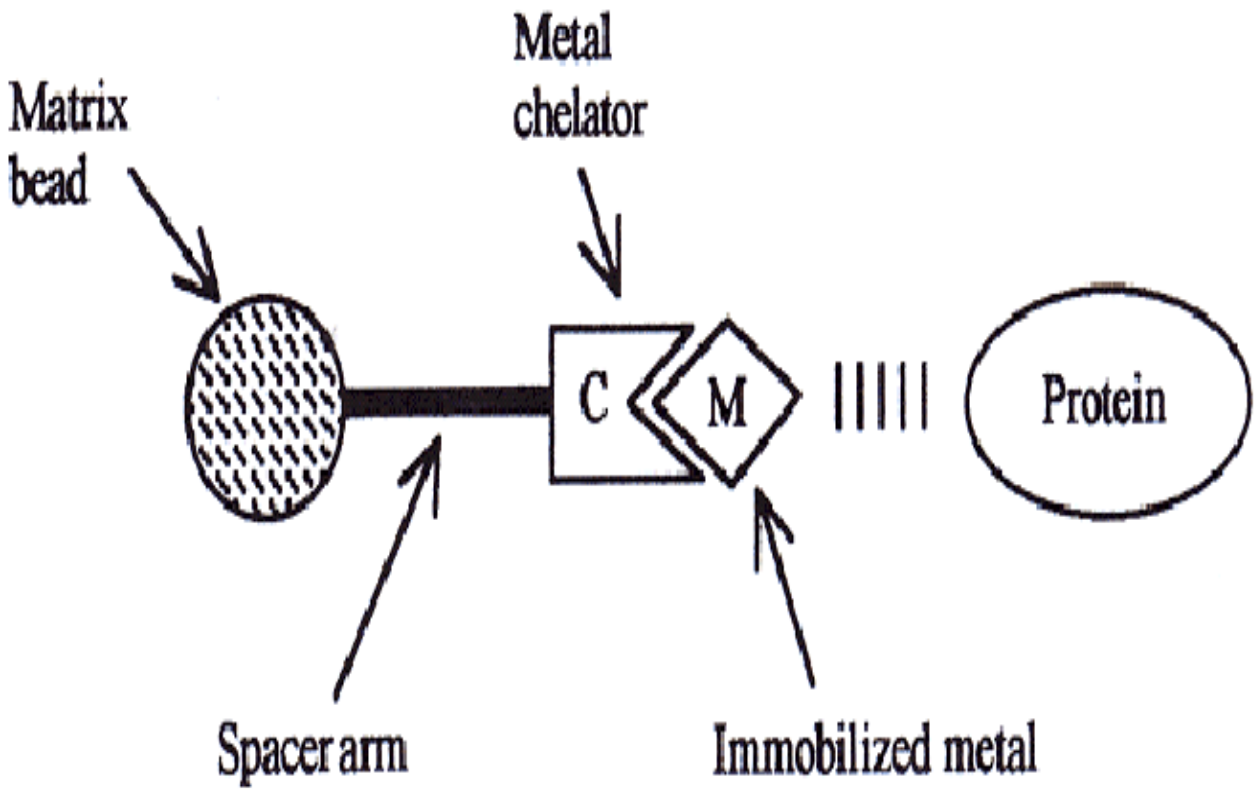


Affinity chromatography

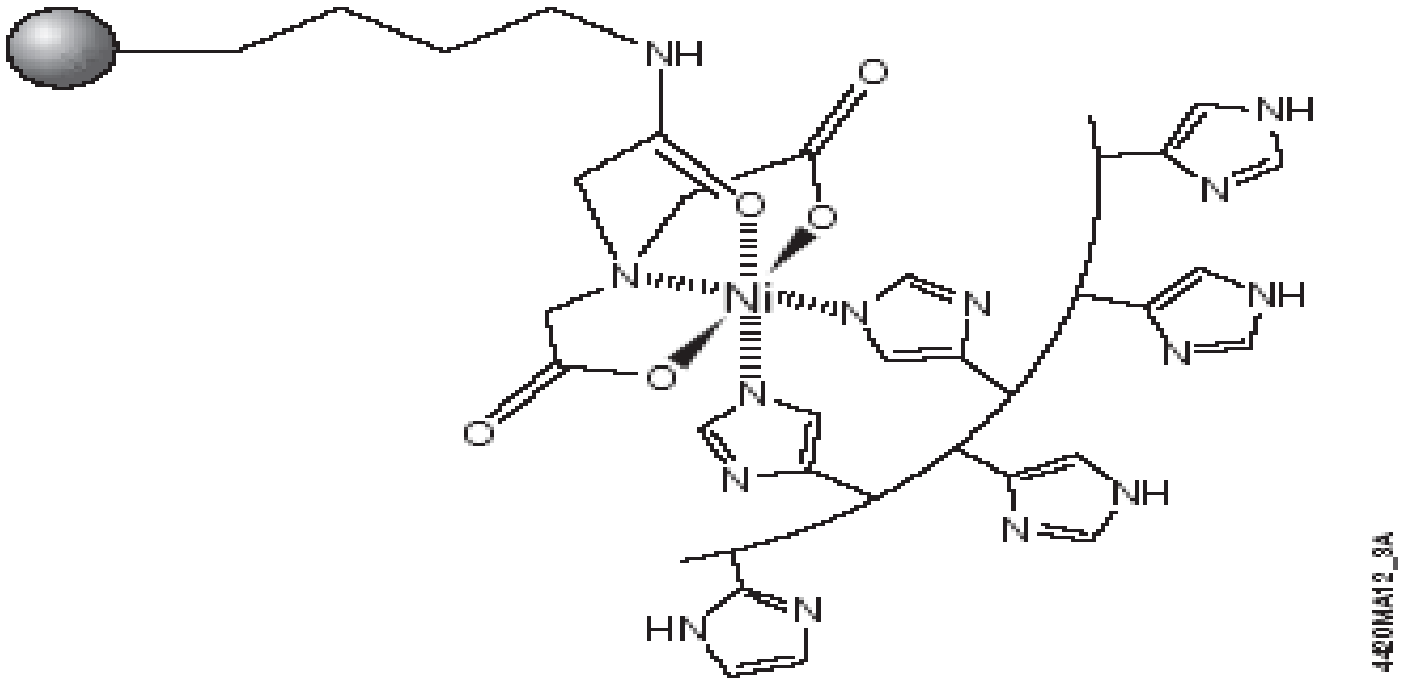
Protein is isolated by a column With a ligand. Example: ATP binding proteins can be Isolated with a column containing bound ATP. Elution (removal) of protein is achieved with a high concentration of free ATP.



Metal-chelate chromatography



Schematic of HisLink and His-tag Interaction



Two site are available for His-tag binding are rapidly coordinated with histidine in the presence of a His-tagged polypeptide

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.

Specific activity

Units: enzyme activity per mg of total protein (U/mg)

Specific activity increases as the target is purified.
It tells the effectiveness of each step.

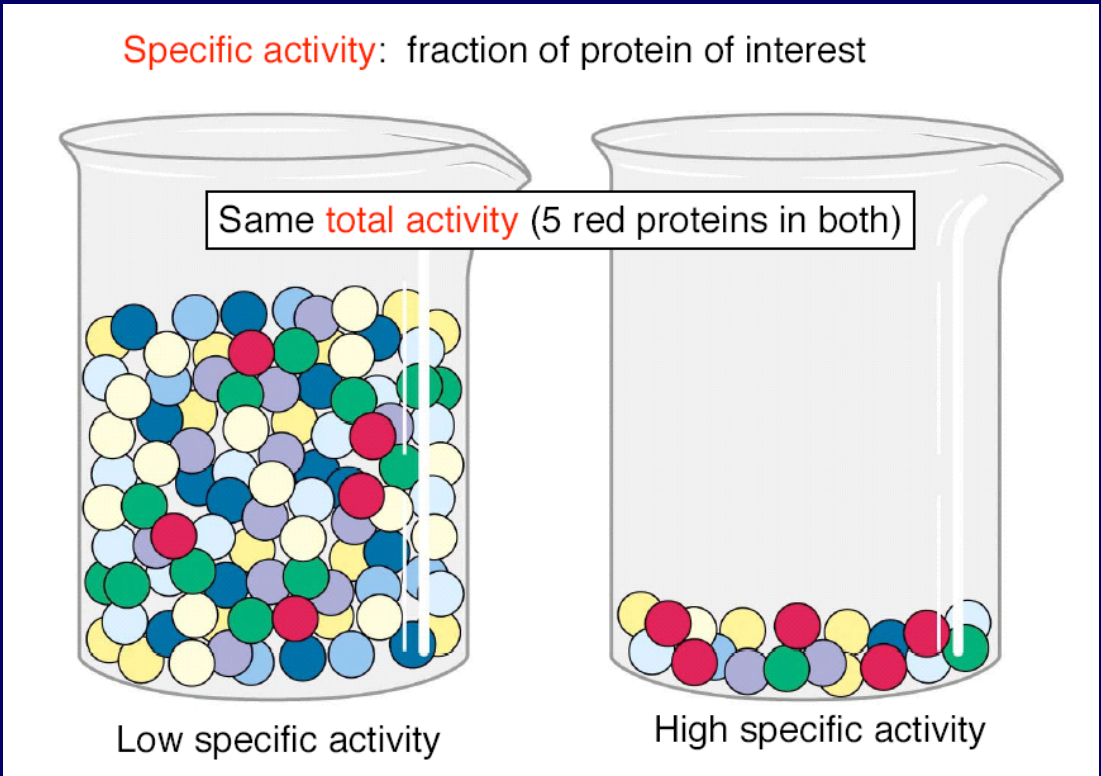
$$\text{Purification (or enrichment)} = \frac{\text{specific activity of purified material}}{\text{specific activity of original material}}$$

Note that it is expressed as fold-purification

$$\%\ \text{Yield (or recovery)} = \frac{\text{enzyme units recovered}}{\text{enzyme units at start}} \times 100$$

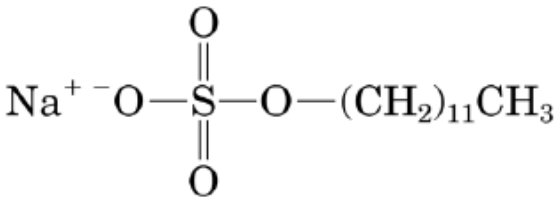
Note that it is expressed as %

Aktivitas Vs. Aktivitas Spesifik

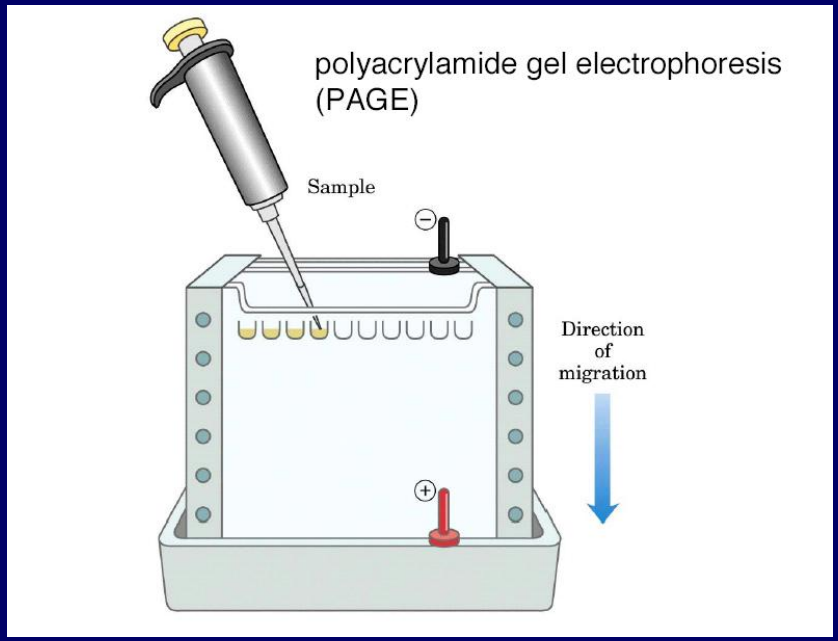


Elektroforesis SDS-PAGE

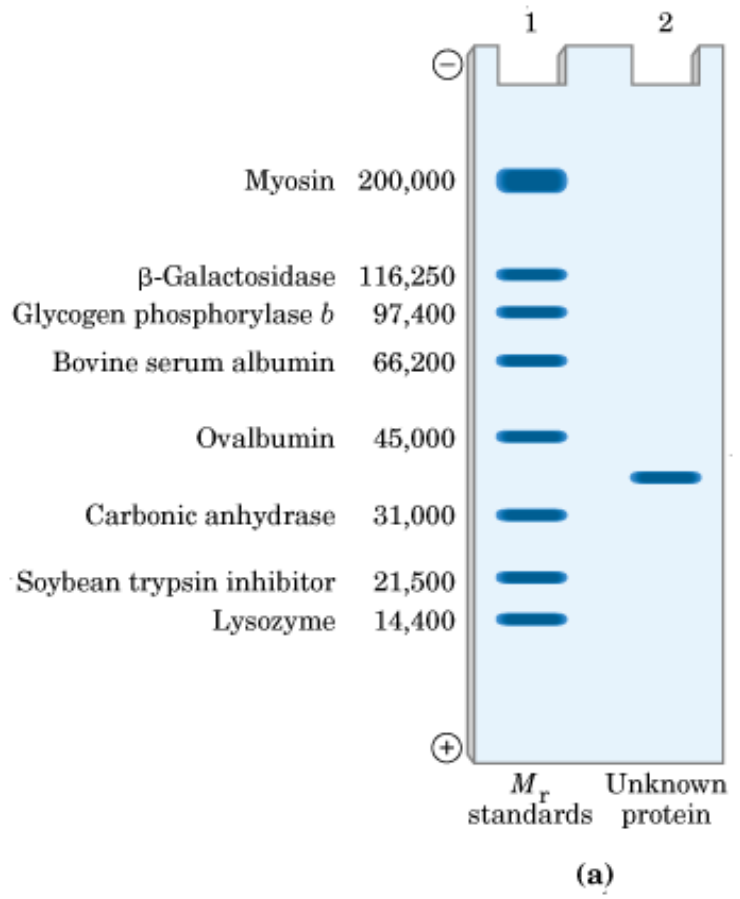
- Pemisahan protein (molekul bermuatan berdasarkan ukuran dalam medan listrik
- Protein didenaturasi dengan detergen anion

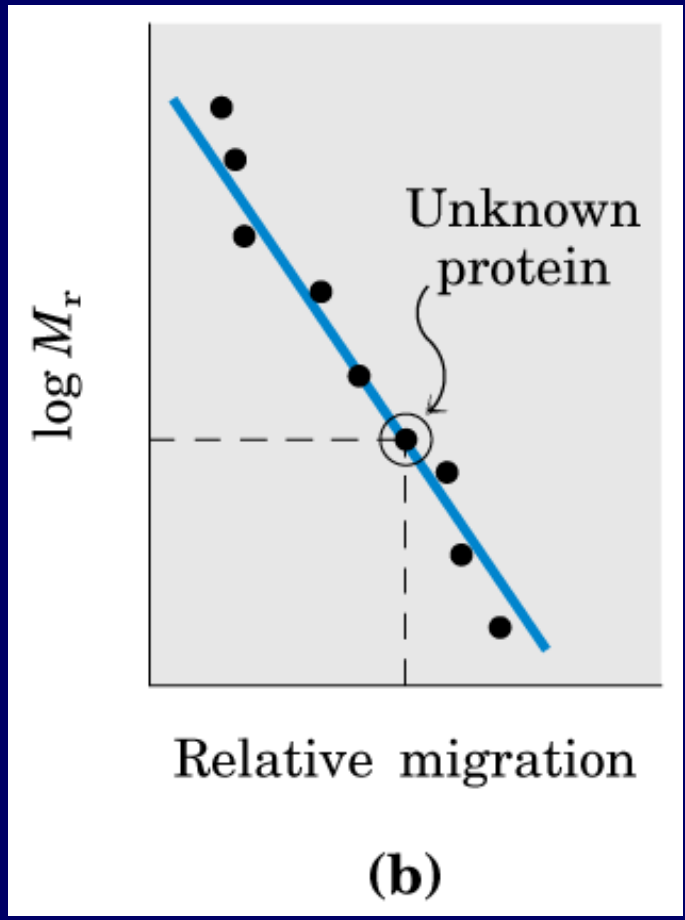


Sodium dodecyl sulfate
(SDS)

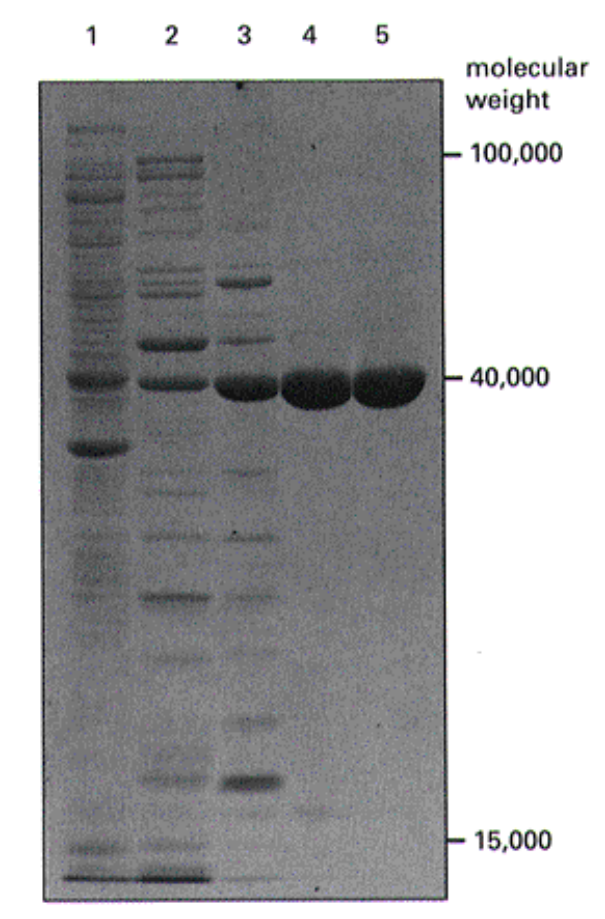


Penentuan Massa Molekul Protein





Analisa Tingkat Kemurnian dengan SDS-PAGE



1. Ekstrak kasar
2. Pengendapan dengan garam
3. Kromatografi penukar ion
4. Kromatografi filtrasi gel
5. Kromatografi afinitas

10µg protein/lajur