Protein purification

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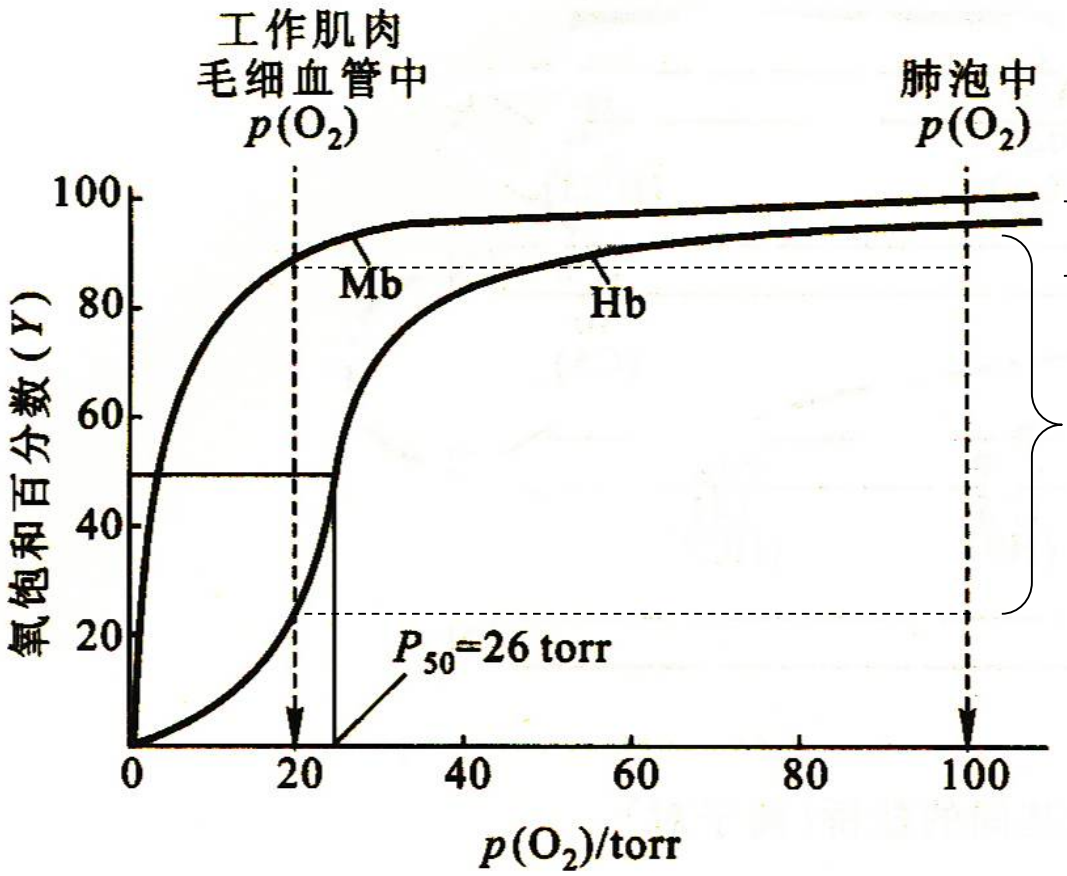
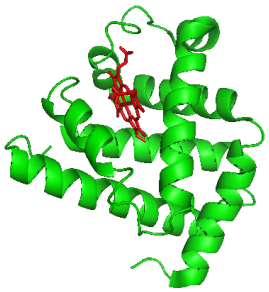
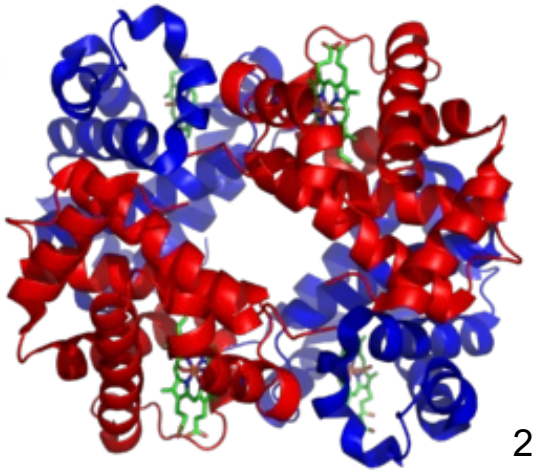
myoglobin *vs.* hemoglobin





myoglobin: O_2 storage

hemoglobin:
O_2 transportation

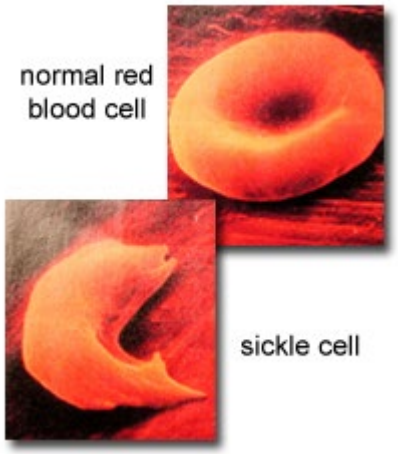


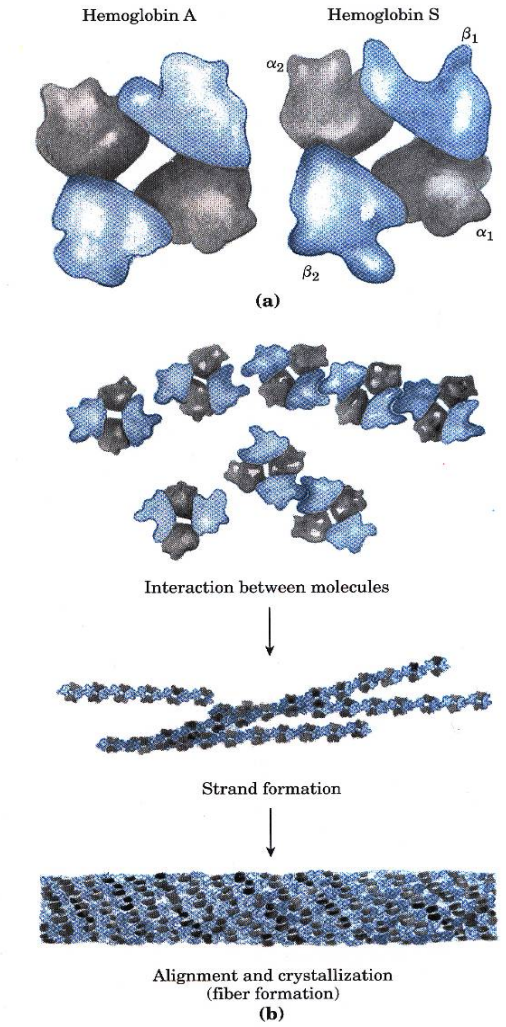
Mb: myoglobin (肌红蛋白);
Hb: hemoglobin (血红蛋白).

Something else to know about hemoglobin

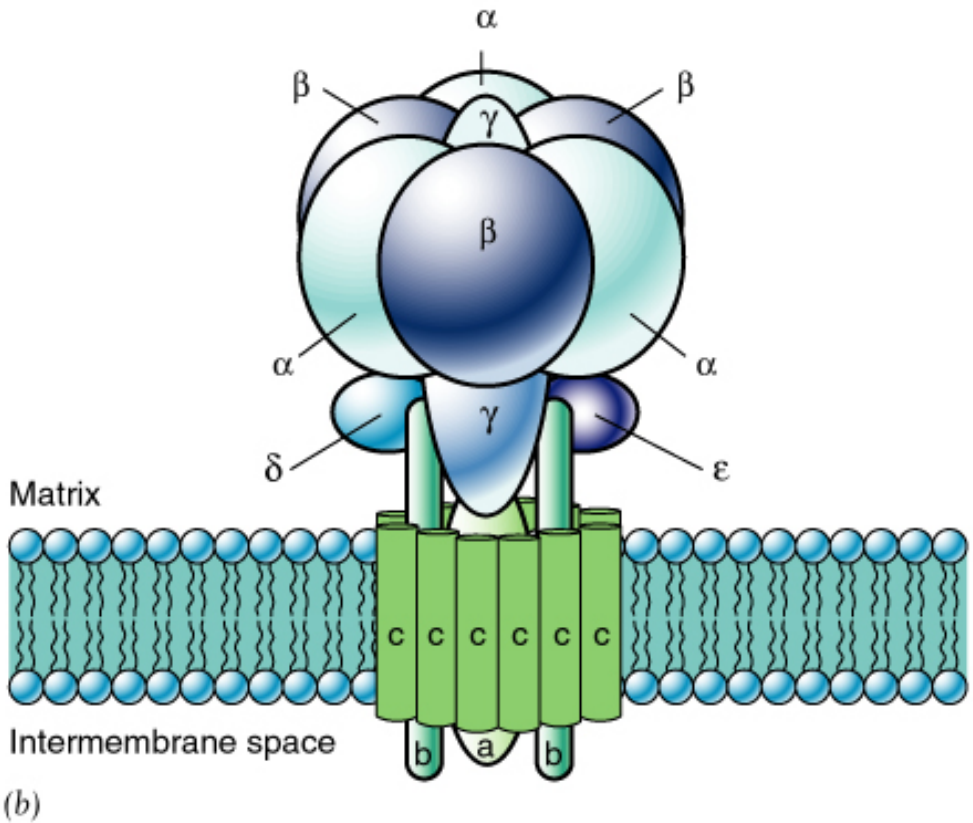
Hemoglobin binds CO with higher affinity（亲和力）;

Sickle-cell anemia（镰刀形贫血症）caused by hemoglobin mutation Eβ(

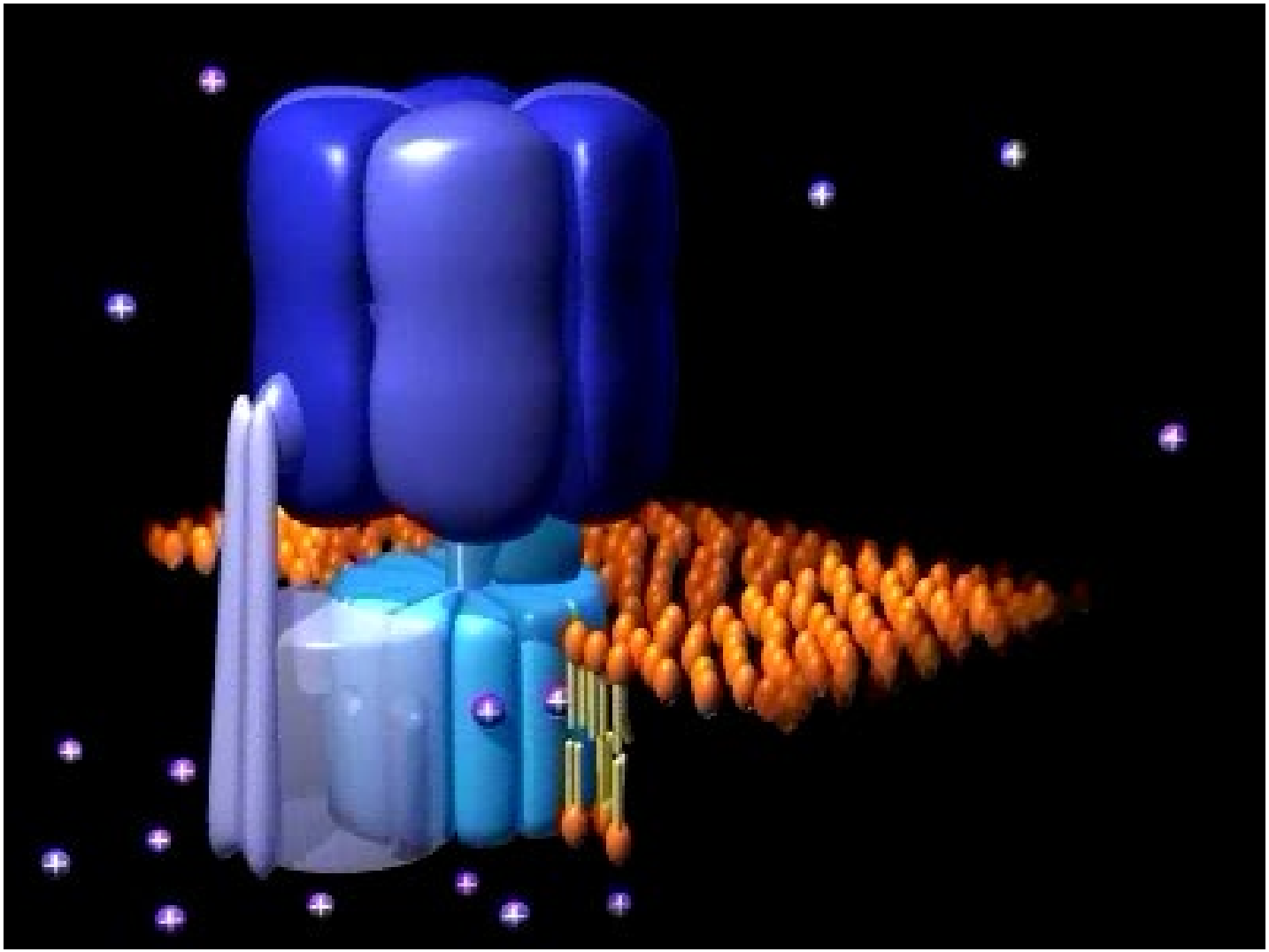


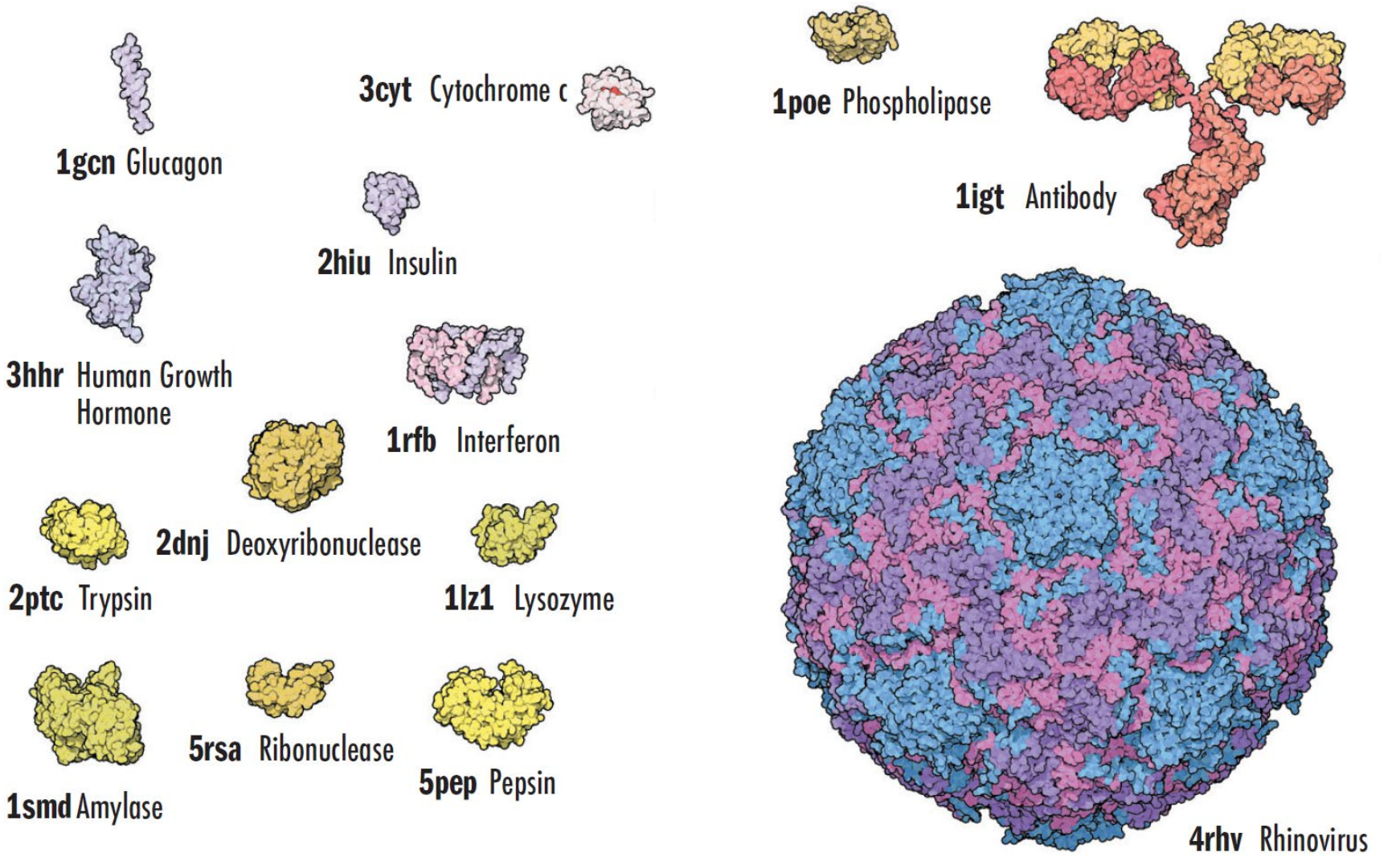


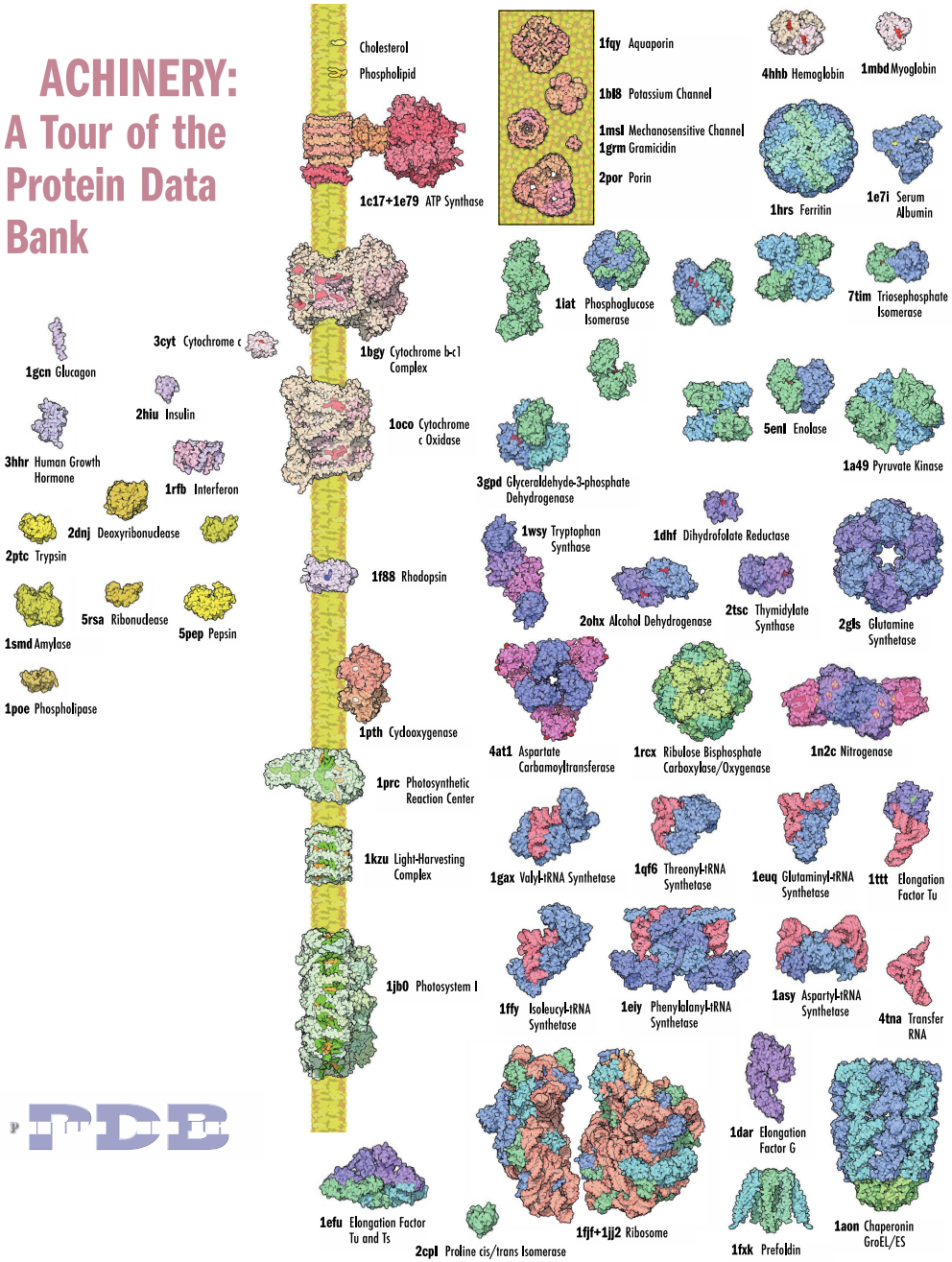
ATP synthase is a highly complex molecular machine.



ATP synthase is a highly complex molecular machine.




1gcn Glucagon
3cyt Cytochrome c
2hiu Insulin
3hhr Human Growth Hormone
1rfb Interferon
2dnj Deoxyribonuclease
2ptc Trypsin
1lz1 Lysozyme
1smd Amylase
5rsa Ribonuclease
5pep Pepsin
1poe Phospholipase
1igt Antibody
4rhv Rhinovirus


ACHINERY:
A Tour of the Protein Data Bank
Cholesterol
Phospholipid
1c17+1e79 ATP Synthase
1fqy Aquaporin
1bl8 Potassium Channel
1msl Mechanosensitive Channel
1grm Gramicidin
2por Porin
4hhb Hemoglobin
1mbd Myoglobin
1hrs Ferritin
1e7i Serum Albumin
1iat Phosphoglucose Isomerase
7tim Triosephosphate Isomerase
3cyt Cytochrome c
1gcn Glucagon
1bgy Cytochrome b-c1 Complex
2hiu Insulin
1oco Cytochrome c Oxidase
3hhr Human Growth Hormone
1rfb Interferon
2dnj Deoxyribonuclease
2ptc Trypsin
5enl Enolase
1a49 Pyruvate Kinase
3gpd Glyceraldehyde-3-phosphate Dehydrogenase
1wsy Tryptophan Synthase
1dhf Dihydrofolate Reductase
1f88 Rhodopsin
2ohx Alcohol Dehydrogenase
2tsc Thymidylate Synthase
2gls Glutamine Synthetase
5rsa Ribonuclease
5pep Pepsin
1smd Amylase
1poe Phospholipase
1pth Cydooxygenase
4at1 Aspartate Carbamoyltransferase
1rcx Ribulose Bisphosphate Carboxylase/Oxygenase
1n2c Nitrogenase
1prc Photosynthetic Reaction Center
1kzu Light-Harvesting Complex
1gax Valyl-tRNA Synthetase
1qf6 Threonyl-tRNA Synthetase
1euq Glutaminyl-tRNA Synthetase
1ttt Elongation Factor Tu
1jb0 Photosystem I
1ffy Isoleucyl-tRNA Synthetase
1eiy Phenylalanyl-tRNA Synthetase
1asy Aspartyl-tRNA Synthetase
4tna Transfer RNA
1dar Elongation Factor G
1efu Elongation Factor Tu and Ts
1fjf+1jj2 Ribosome
1aon Chaperonin GroEL/ES
2cpl Proline cis/trans Isomerase
1fxk Prefoldin
PDB

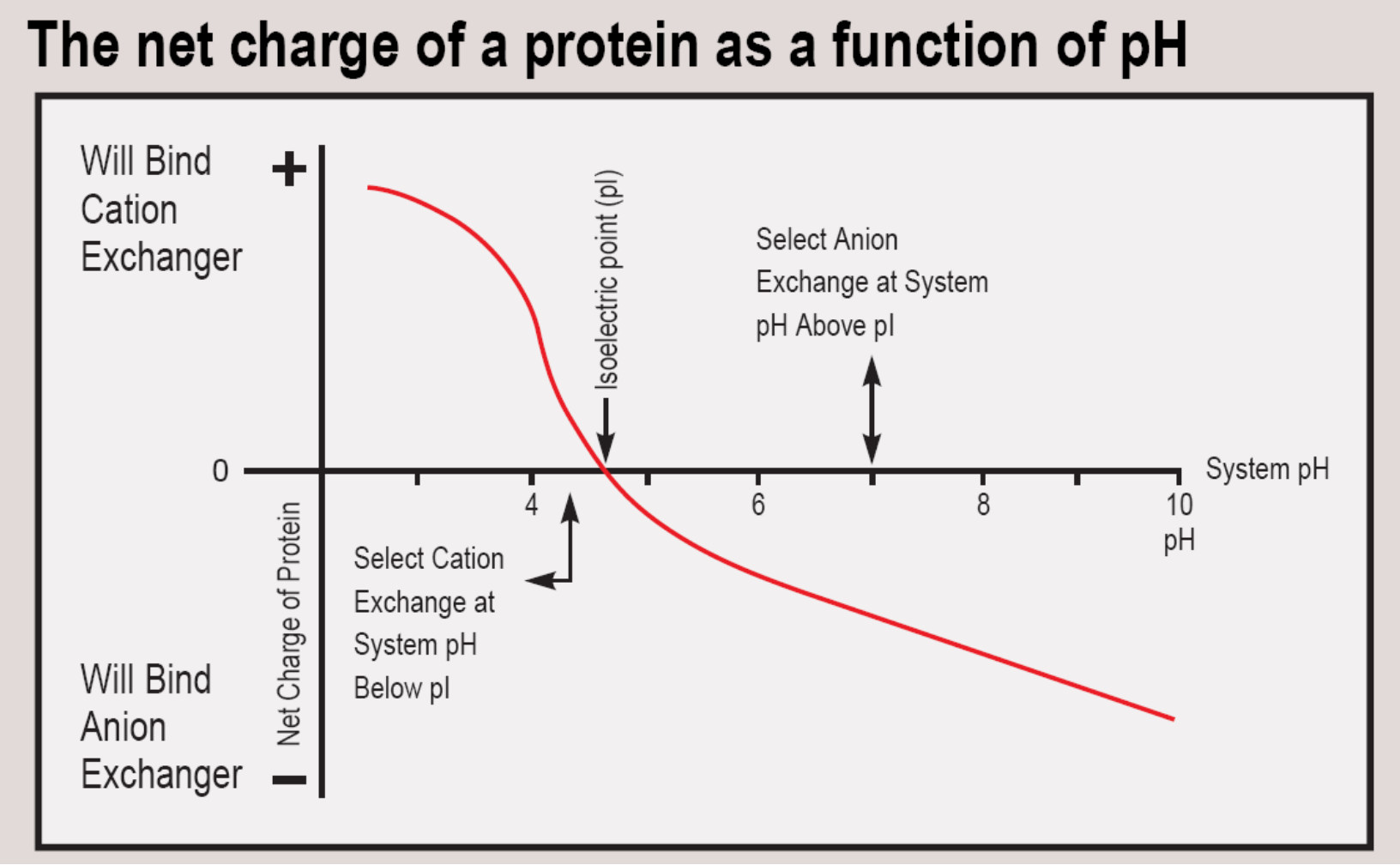
Basic principle of protein purification

The unique property matters!

Learning objectives:

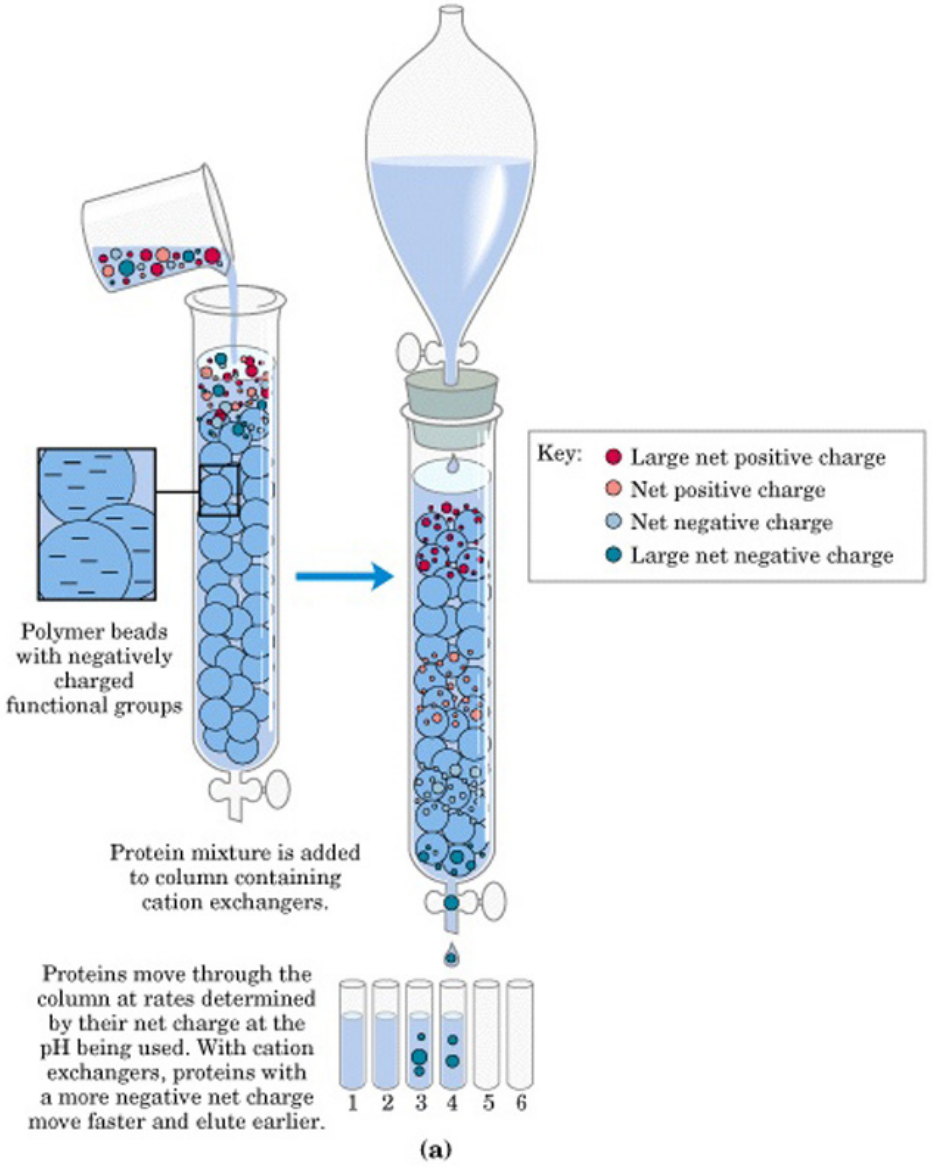
- Explain the ion exchange chromatography （离子交换层析）;
- Explain the gel filtration chromatography（凝胶过滤层析）;
- Choose an appropriate purification technique.

Ion exchange chromatography 离子交换层析

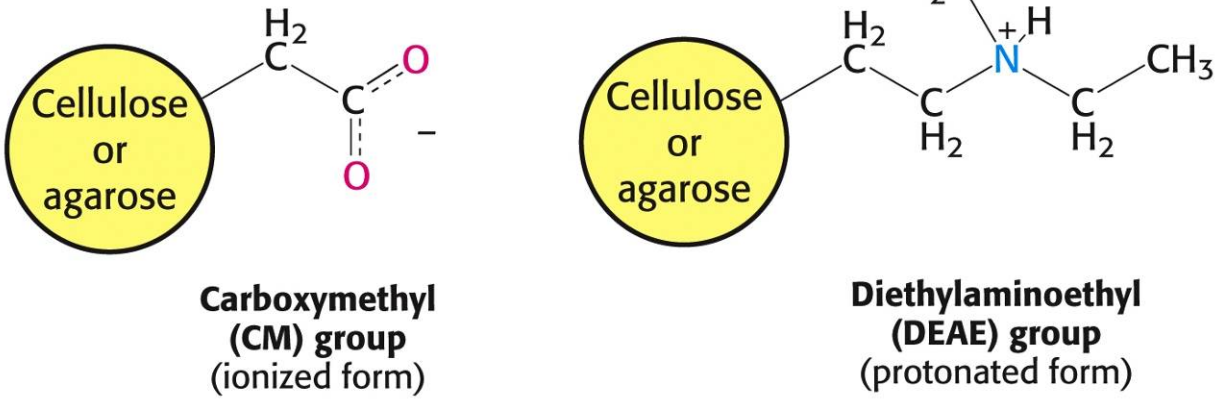


(from GE Healthcare)

Ion exchange chromatography 离子交换层析



Ion exchange chromatography 离子交换层析

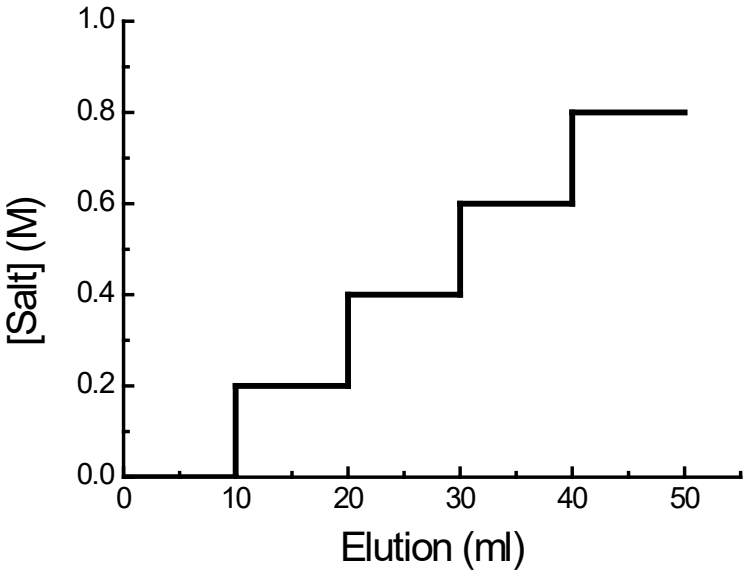


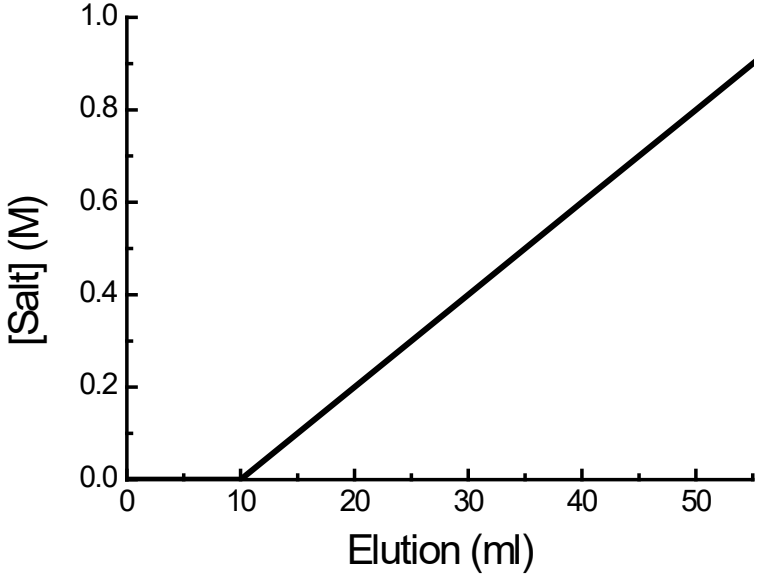
Type of Ion Exchange	Common Abbreviation	Functional group	
Strong Anion	Q	Quarternary Ammonium	— $CH_2N^+(CH_3)_3$
Weak Anion	DEAE	Diethylaminoethyl	— $C_2H_4N^+H(C_2H_5)_2$
Strong Cation	S	Sulfonic Acid	— $CH_2SO_3^-$
Weak Cation	CM	Carboxymethyl	— CH_2COO^-

Elution（洗脱）of ion exchange chromatography

By increasing salt concentration or changing pH condition;

Stepwise elution （阶段洗脱） *vs.* gradient elution（梯度洗脱）;

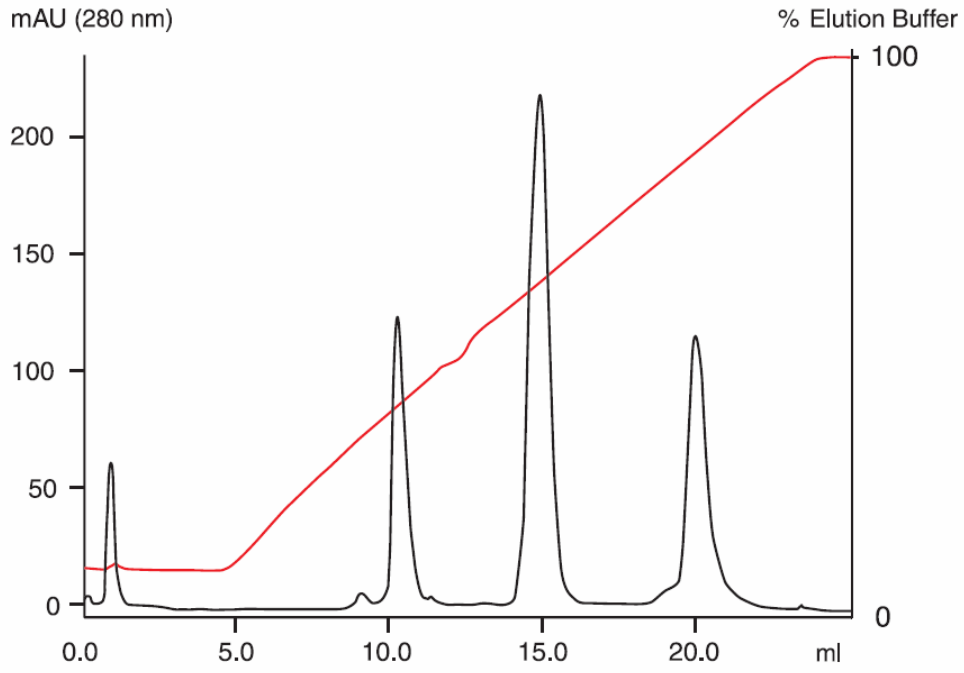




Example: Mono S column from GE Healthcare

Column Mono S 5/50 GL

Sample:	1. Ribonuclease A (1.5 mg/ml)	SIGMA R-5000
	2. Cytochrome C (0.4 mg/ml)	SIGMA C-7752
	3. Lysozyme (0.4 mg/ml)	SIGMA L-6876
Sample volume:	100 µl	
Gradient:	0–100% elution buffer in 20 CV	
Start buffer:	20 mM sodium phosphate, pH 6.8	
Elution buffer:	20 mM sodium phosphate + 0.4 M NaCl, pH 6.8	
Flow rate:	1.0 ml/min (room temperature)	



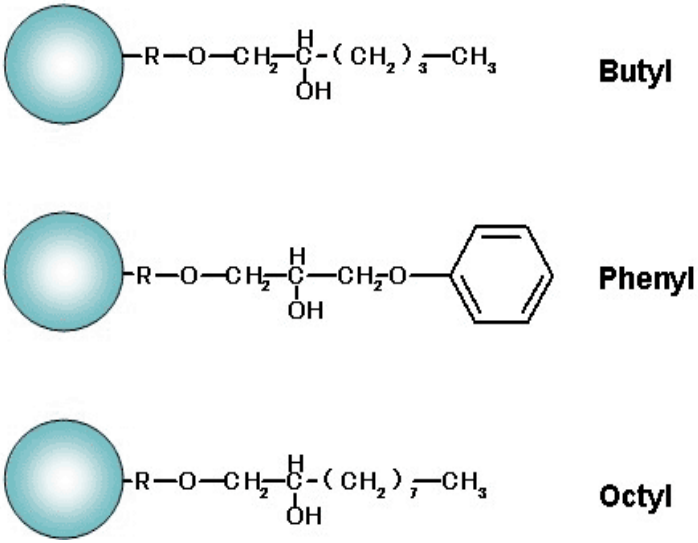
Ribonuclease A
pI=9.45

Cytochrome C
pI=10.0-10.5

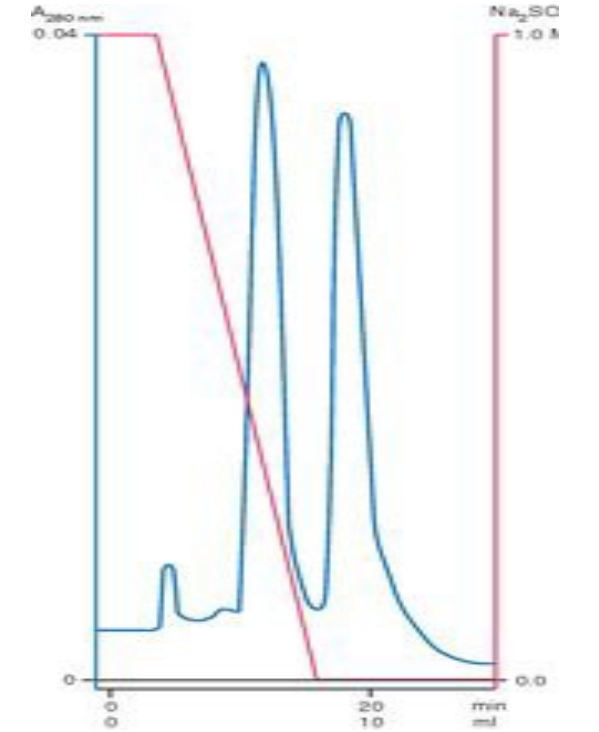
Lysozyme
pI=11.35

Hydrophobic interaction chromatography (HIC)
（疏水相互作用层析）

Hydrophobicity: octyl > phenyl > butyl



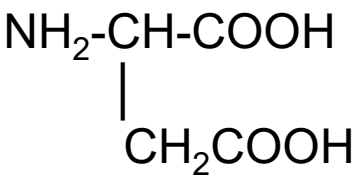
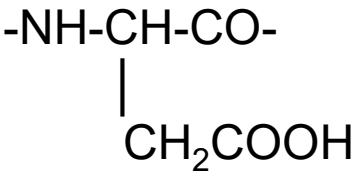
(http://www.chisso.co.jp/fine/en/cellufine/images/hyd_partial.gif)



Lab Pack:	Phenyl Sepharose High Performance
Column:	5 × 50 mm High Performance column
Bed height:	10 mm
Sample:	60 µg active and 60 µg methylamine treated, inactive α_2-macroglobulin
Start buffer (A):	0.02 M sodium phosphate with 1 M Na_2SO_4, pH 7.2
Elution buffer (B):	0.025 M sodium phosphate, pH 7.2
Flow rate:	0.5 ml/min

(from GE Healthcare)

Protein size

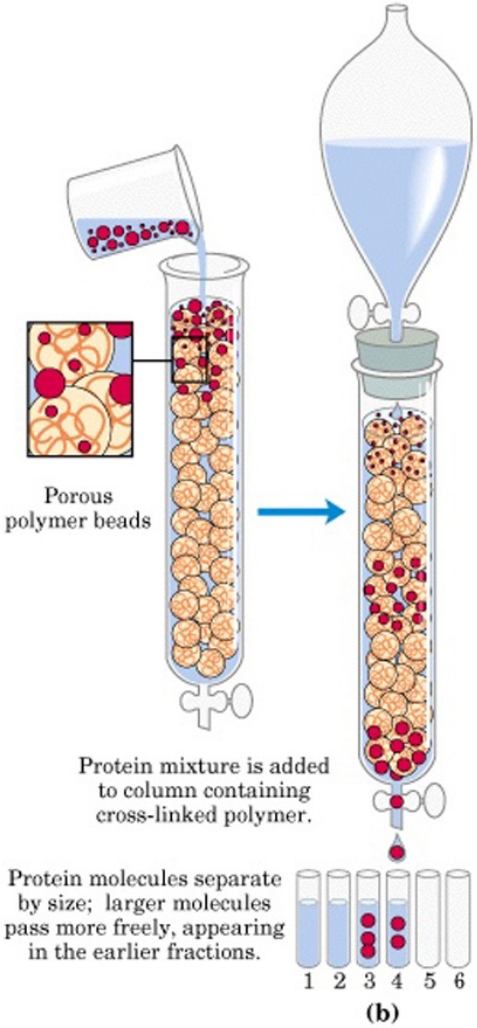
amino acid	residue
	
Mr = 133	Mr = 115

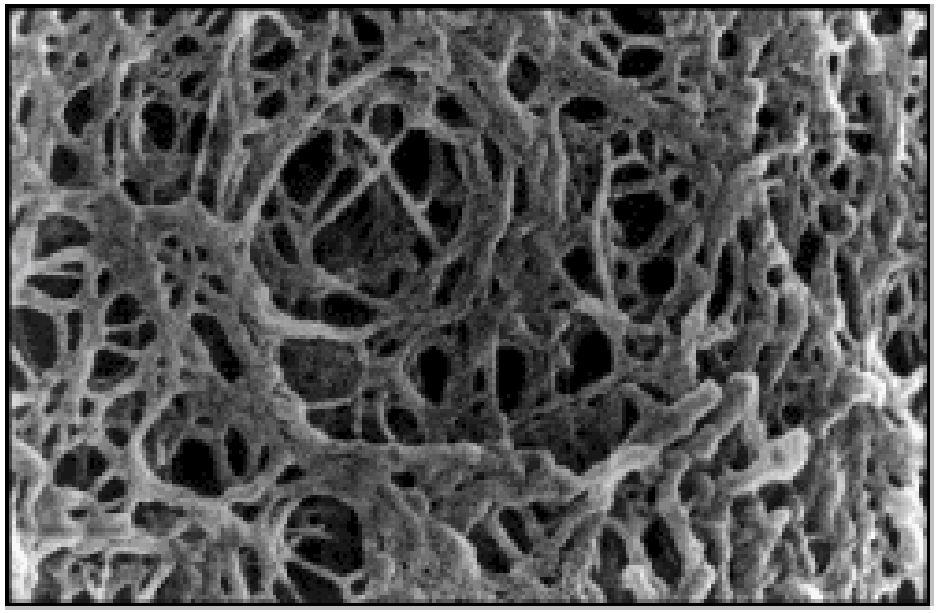
Average molecular weight of residues = 110.

Example: a protein of 22 KD → about 200 residues;

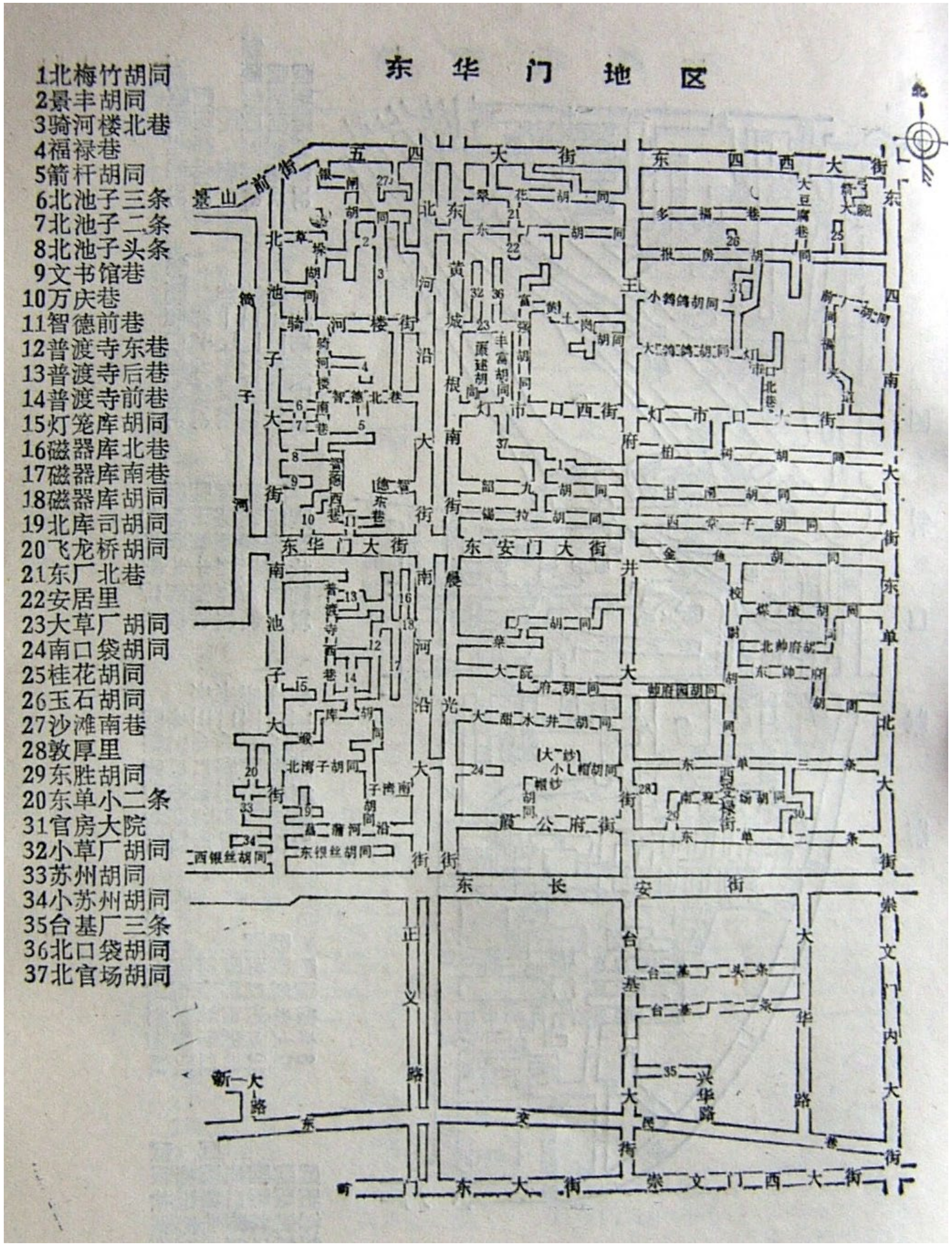
→ cDNA of about 600 bp.

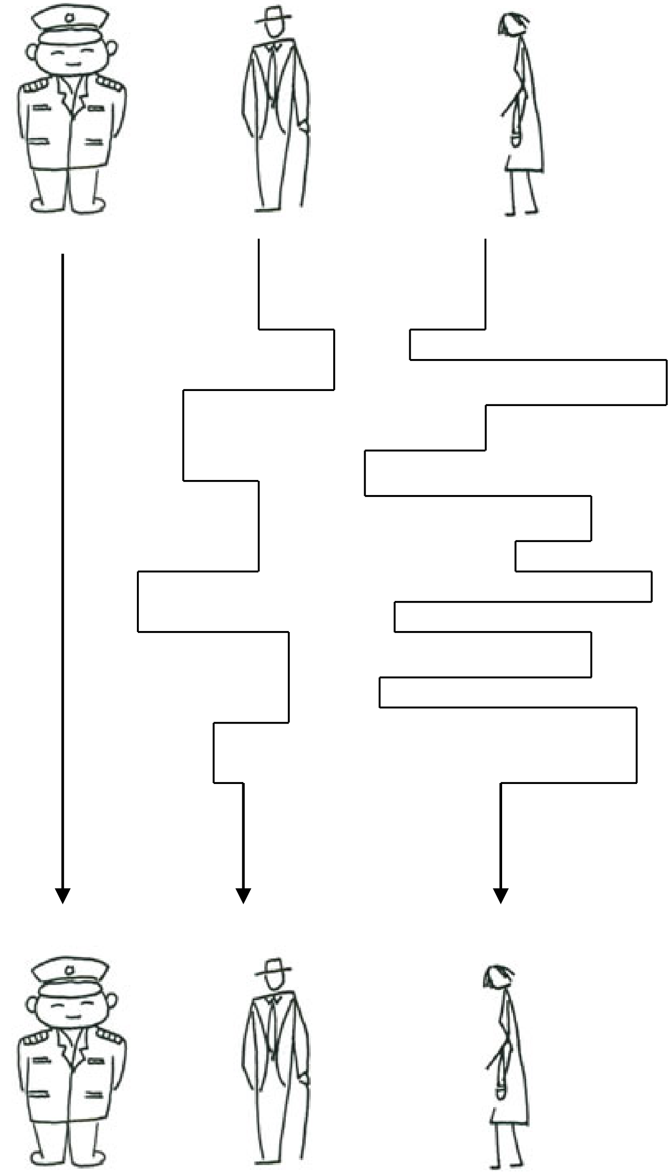
Gel filtration chromatography 凝胶过滤层析



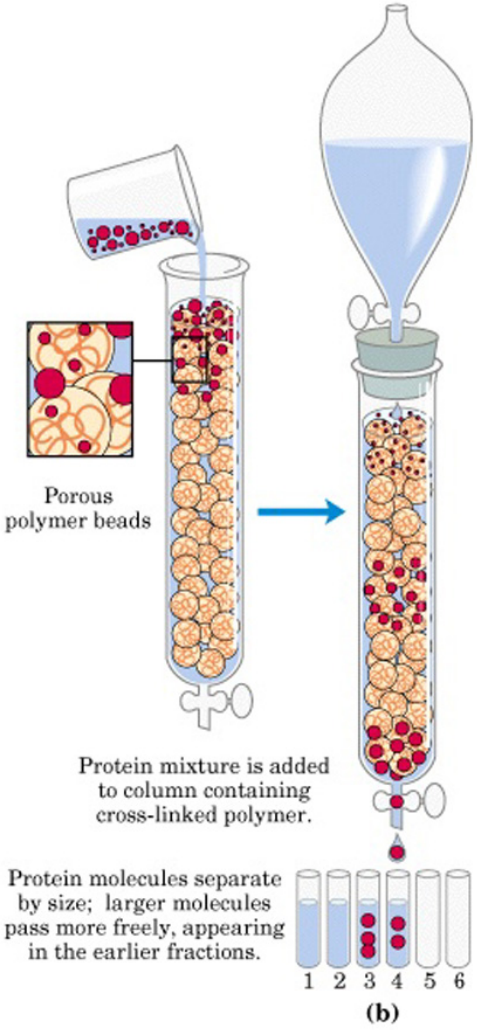


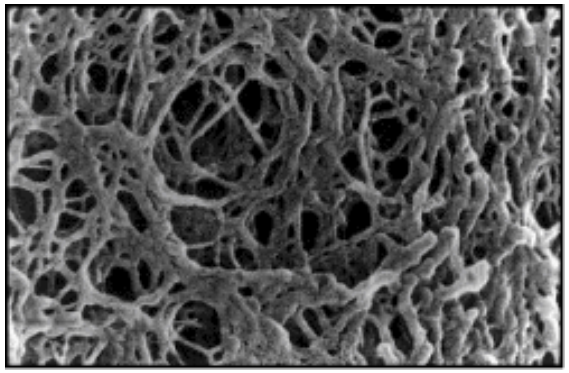
Scanning electron micrograph of an agarose gel. Magnification x 50,000. (R*ef. Anders S. Medin,PhD Thesis, Uppsala University 1995*)


东 华 门 地 区
1北梅竹胡同
2景丰胡同
3骑河楼北巷
4福禄巷
5箭杆胡同
6北池子三条
7北池子二条
8北池子头条
9文书馆巷
10万庆巷
11智德前巷
12普渡寺东巷
13普渡寺后巷
14普渡寺前巷
15灯笼库胡同
16磁器库北巷
17磁器库南巷
18磁器库胡同
19北库司胡同
20飞龙桥胡同
21东厂北巷
22安居里
23大草厂胡同
24南口袋胡同
25桂花胡同
26玉石胡同
27沙滩南巷
28敦厚里
29东胜胡同
20东单小二条
31官房大院
32小草厂胡同
33苏州胡同
34小苏州胡同
35台基厂三条
36北口袋胡同
37北官场胡同
东华门大街
东安门大街
东长安街
王府井大街
东单北大街
正义路
台基厂大街
崇文门内大街
东交民巷
崇文门西大街



Gel filtration chromatography 凝胶过滤层析





Scanning electron micrograph of an agarose gel. Magnification x 50,000. (R*ef. Anders S. Medin,PhD Thesis, Uppsala University 1995*)

Also known as size exclusion chromatography;

Exclusion limit（排阻限度）;

Effective separation range (globular proteins);（有效分离范围）

Column volume and void volume（外水体积）;

Limited sample volume.

Example: Superdex 75 from GE Healthcare

Bed dimensions	10 × 300–310 mm	
Bed volume	Approximately 24 ml	
	Superdex 75	**Superdex 200**
Exclusion limit, M_r, globular proteins	Approx. 1×10^5	Approx. 1.3×10^6
Optimum separation range globular proteins, M_r	3 000–70 000	10 000–600 000
dextrans	500–30 000	1000–100 000
Flow rate (water at room temperature) recommended	0.5–1.0 ml/min	0.25–0.75 ml/min

Column Superdex 75 10/300 GL

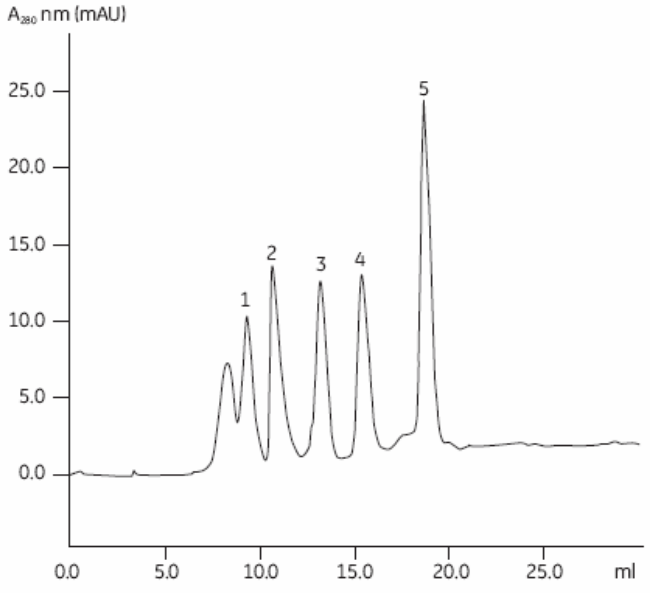
Sample:
1. BSA (M_r 67 000) 8 mg/ml
2. Ovalbumin (M_r 43 000) 2.5 mg/ml
3. Ribonuclease A (M_r 13 700) 5 mg/ml
4. Aprotinin (M_r 6 512) 2 mg/ml
5. Vitamin B12 (M_r 1355) 0.1 mg/ml

Sample volume: 500 µl

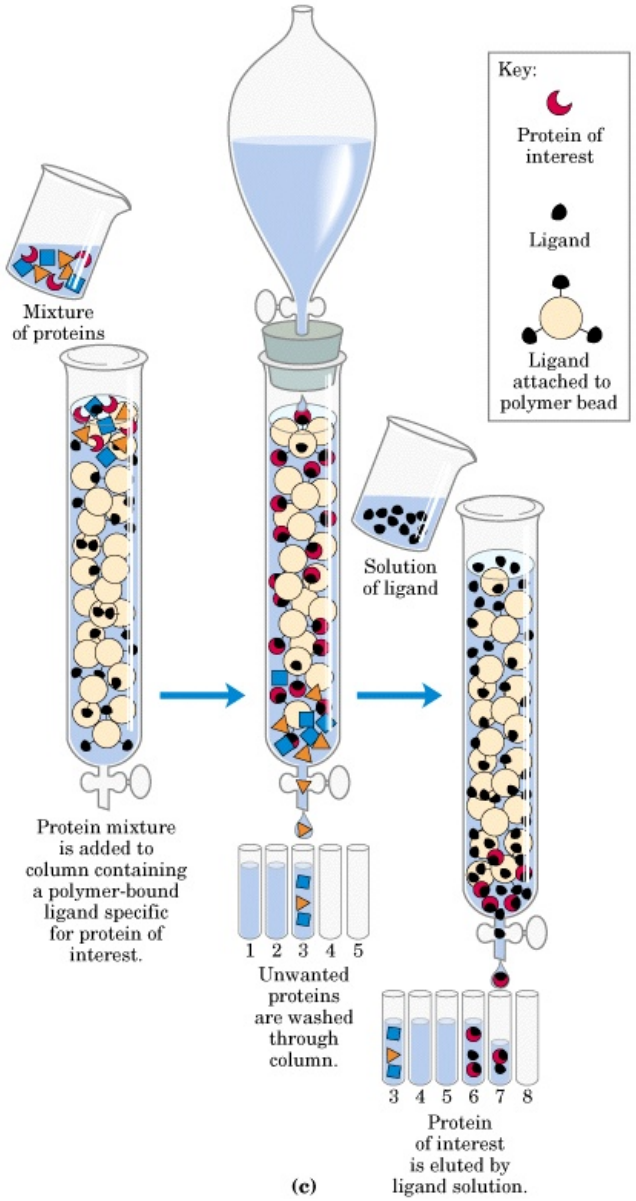
Eluent: 0.05 M phosphate buffer, 0.15 M NaCl, pH 7.0

Flow rate: 0.4 ml/min, room temperature

Detection: 280 nm

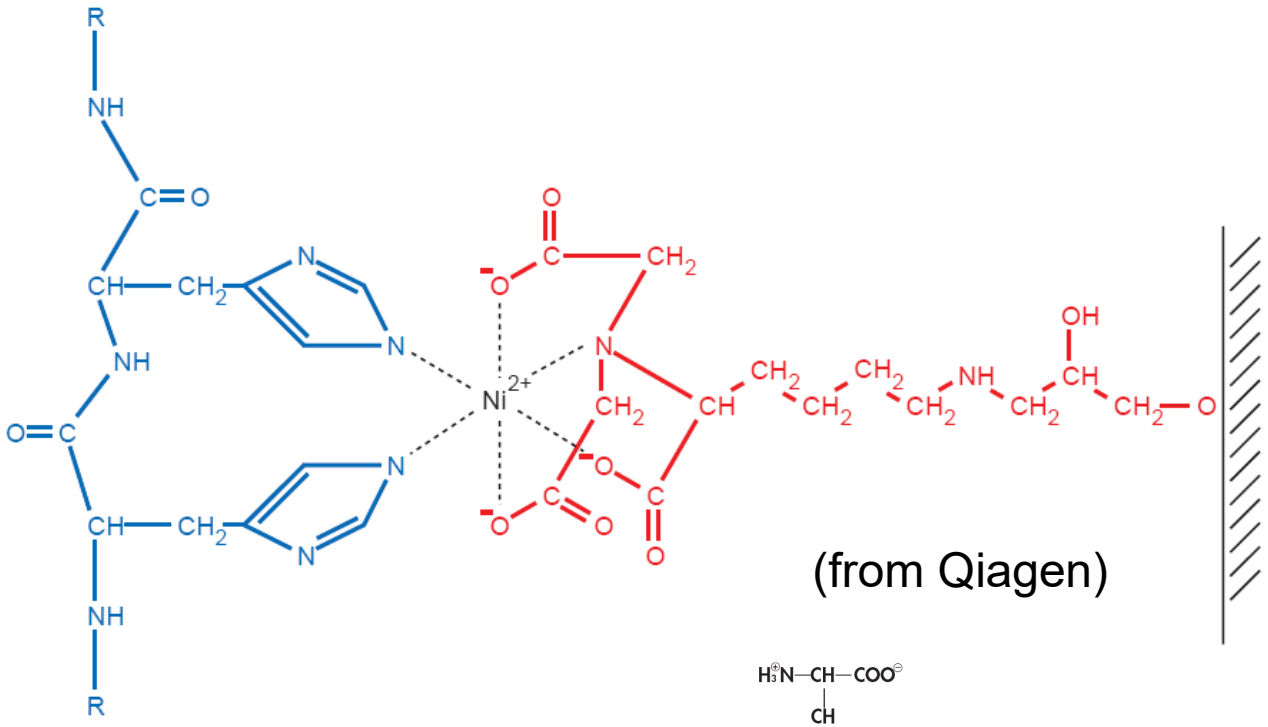


Affinity chromatography 亲和层析



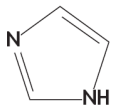
Flash

Immobilized metal chelating chromatography (IMAC)
（金属螯合层析）

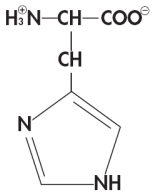


(from Qiagen)

Imidazole to elute;



Imidazole



Histidine

More options of some other metal ions (Co^{2+}, Cu^{2+});

Popularly used for $(His)_6$-tagged proteins.

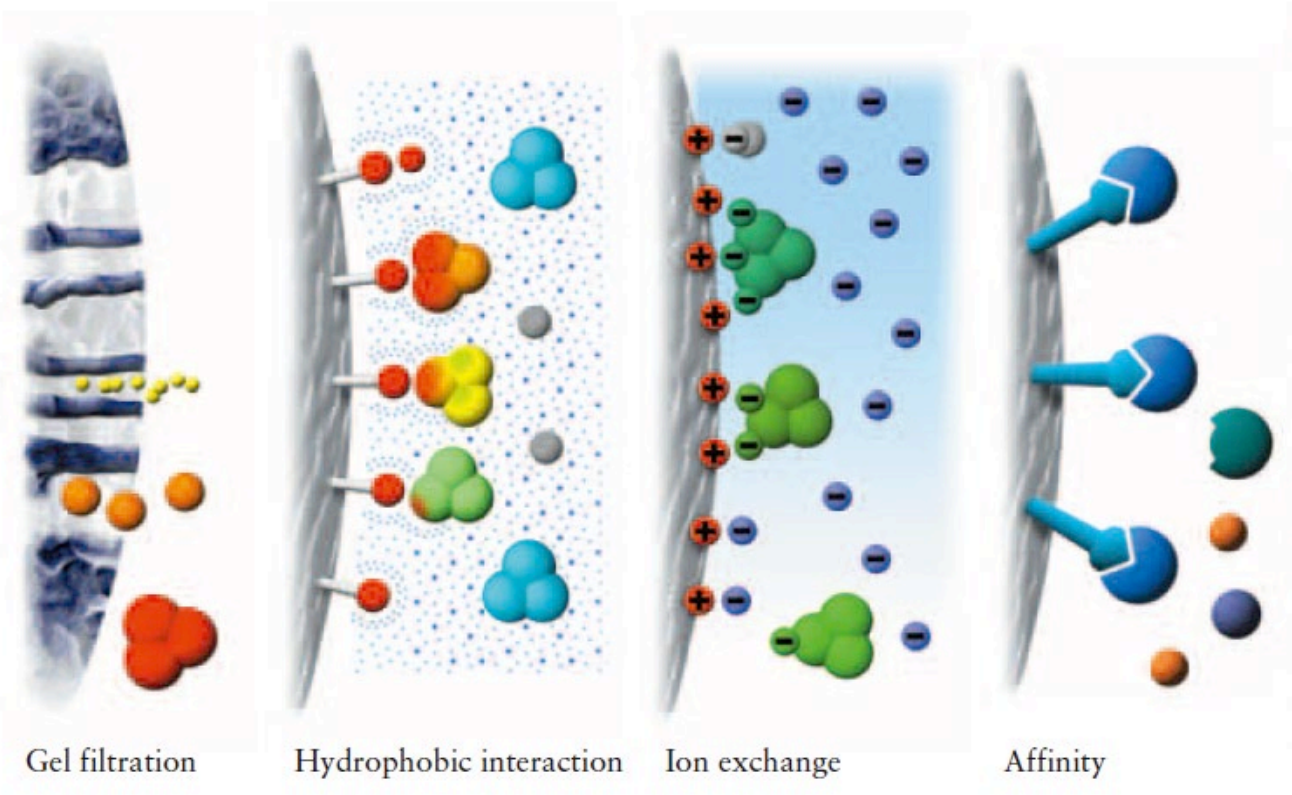
protein purification techniques

- Based on physical and chemical characteristics:

size	gel filtration chromatography
charge	ion exchange chromatography
hydrophobicity	hydrophobic interaction chromatography

- Based on biological characteristics:

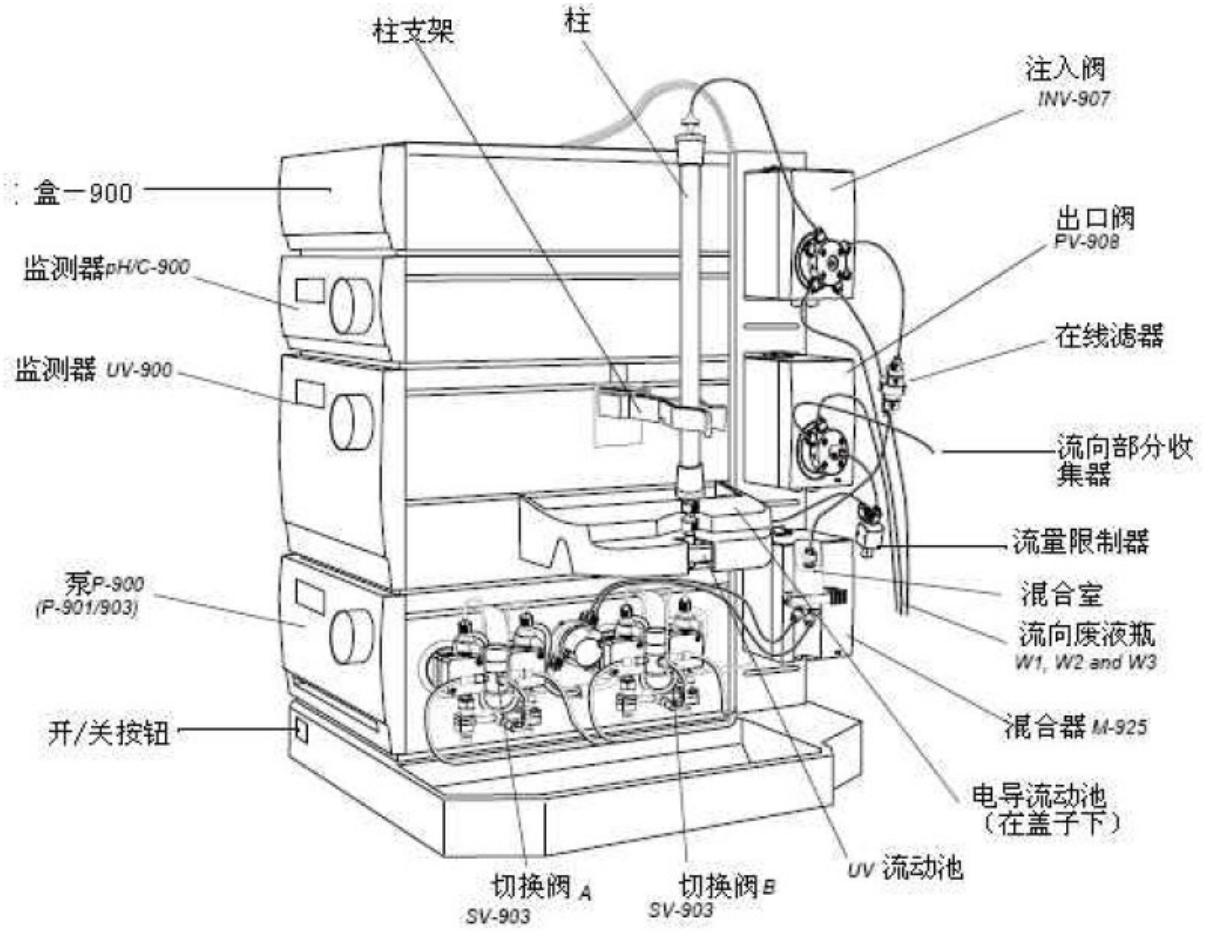
affinity chromatography



	Gel filtration	Hydrophobic interaction	Ion exchange	Affinity
Change condition to elute	No	Yes	Yes	Yes
Sample volume limited	Yes	No	No	No

AKTA purifier 10 from GE Healthcare

AKTA purifier 10 from GE Healthcare



Next time:

Protein characterization.