Chap 4 Separation by Chromatography

Analytical Biochemistry

3.1 Principle of Separation techniques

3.2 Methods Based on Polarity (3.2.1-3.2.3)

Biochemistry and Molecular Biology

11.5 Partition Chromatography

11.6 Ion Exchange Chromatography

11.7 Gel Filtration Chromatography

11.8 Affinity Chromatography

http://www.waters.com

HPLC (High Performance Liquid Chromatography)

✓Separation and analysis

Biological compounds
Pharmaceuticals
Low- or Non-volatile environmental compound. e.g. PCB,DDT
Genotyping

FPLC (Fast Protein Liquid Chromatography)

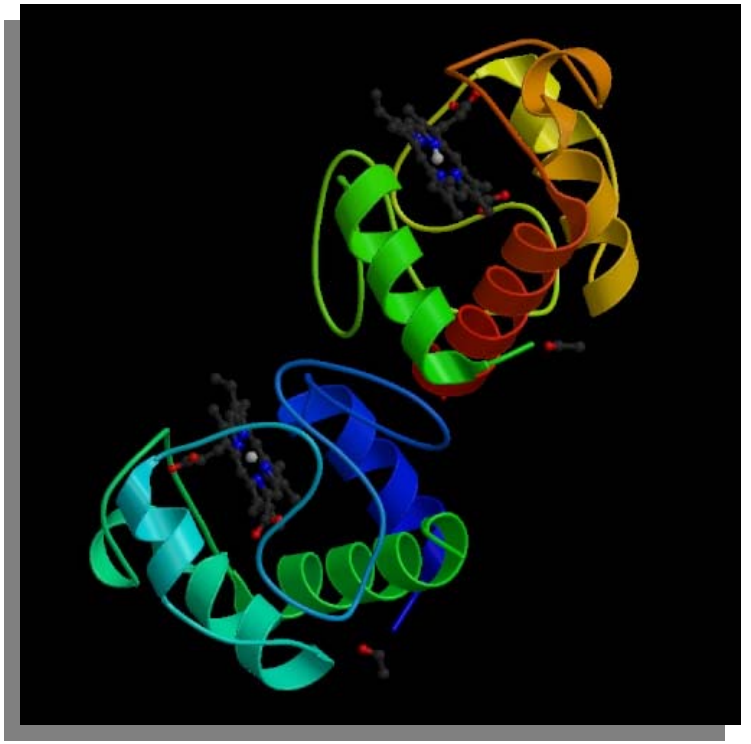
✓Preparative protein purification

(Recovery, Product purity, Capacity)

Purification of Cytochrome c

- Function:
  Redox protein involved in cell apoptosis and respiration

- Structure:
  heme protein
  FW 12,384 (horse)
  Basic protein



Takano, T., Dickerson, R. E.: Redox conformation changes in refined tuna cytochrome c. *Proc. Natl. Acad. Sci. USA* 77 pp. 6371 (1980)

What kind of chromatography method should we use?

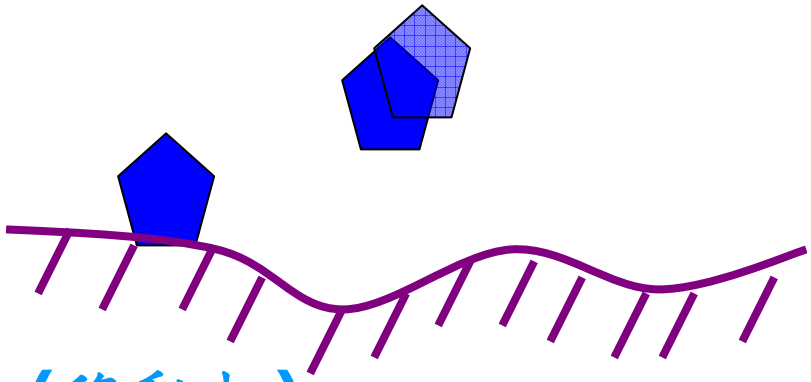
Probing Biological Network by Affinity Purification

Map of protein-protein interactions in yeast. Each point represents a different protein and each line indicates that the two proteins are capable of binding to one another. Only the largest cluster, which contains ~78% of all proteins, is shown.

[H. Jeong, S. P. Mason, A.-L. Barabási & Z. N. Oltvai, "Lethality and centrality in protein networks", Nature, Vol 411, p41, 3 May 2001]

How Does Chromatography Work?

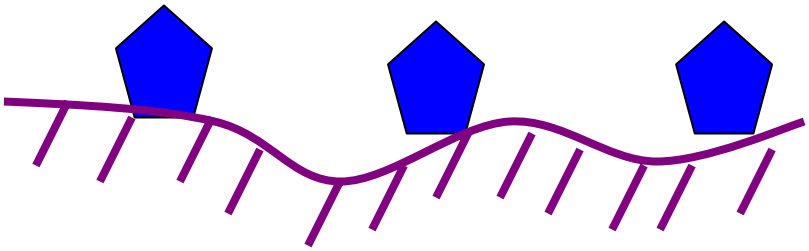
Chromatography is a method for separating the components of a mixture by differential **adsorption** between a stationary phase and a mobile (moving) phase



Mobile Phase (移動相)

Liquid

Liquid



Liquid

Solid

Partition chromatography

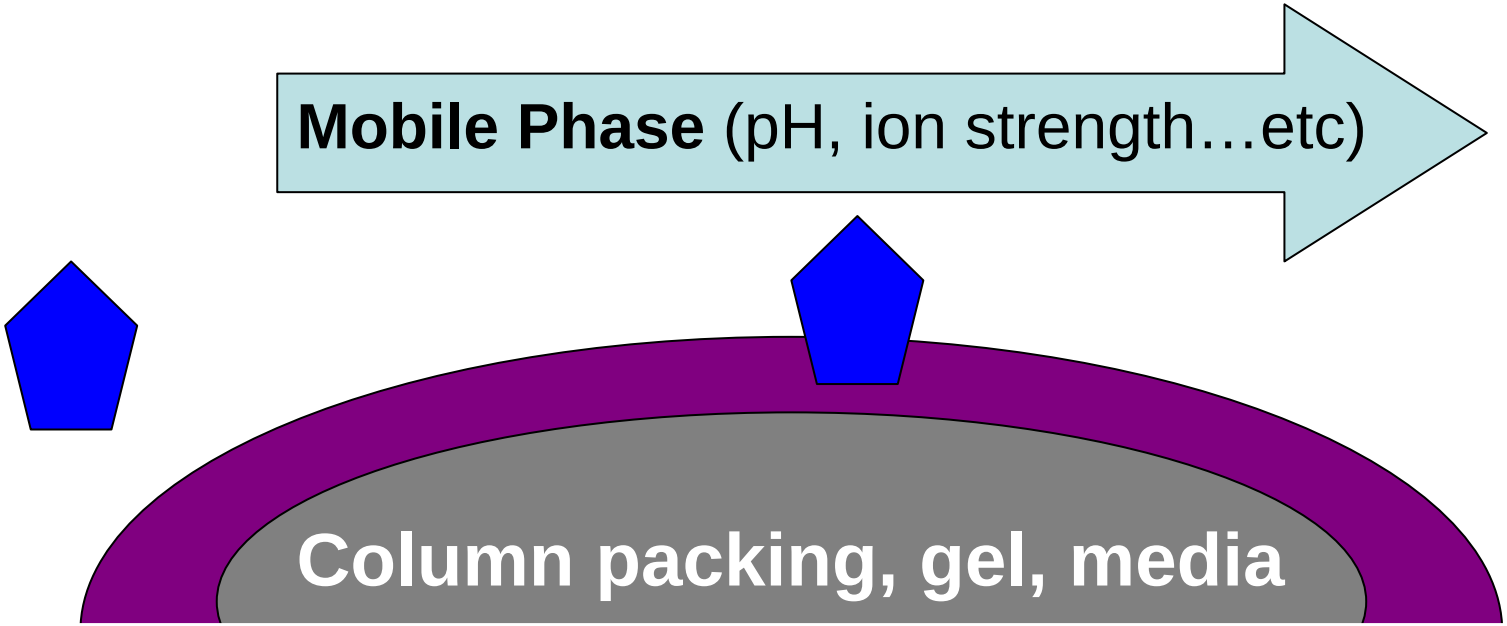
Stationary Phase (固定相)

Adsorption chromatography

1. Analyte enter column in mobile phase

2. Analyte **adsorbs** to functional group in bonded phase in column packing

2. Analyte **desorbs** from bonded phase when mobile phase break their interaction



Stationary Phase

Provide large surface accessible to mobile phase and analyte molecules

Bonded Phase

Provide functional groups to selectively bind analyte molecules

Principles of Separation Techniques

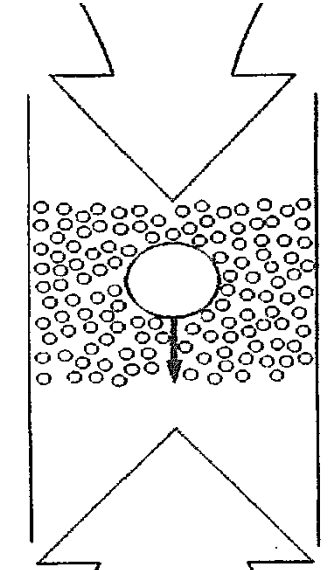
AB 3.1

Molecular Characteristic	Physical property	Separation Technique
Polarity	Volatility	Gas-liquid chromatography
	Solubility	Liquid-liquid chromatography
	Adsorptivity	Liquid-solid chromatography
Ionic	Charge	Ion-exchange chromatography
		Electrophoresis
Size (mass)	Diffusion	Gel permeation chromatography
		Dialysis
Shape	Sedimentation	Ultracentrifugation
	Liquid binding	Affinity chromatography

Factors Involved in Separation

Impelling Force

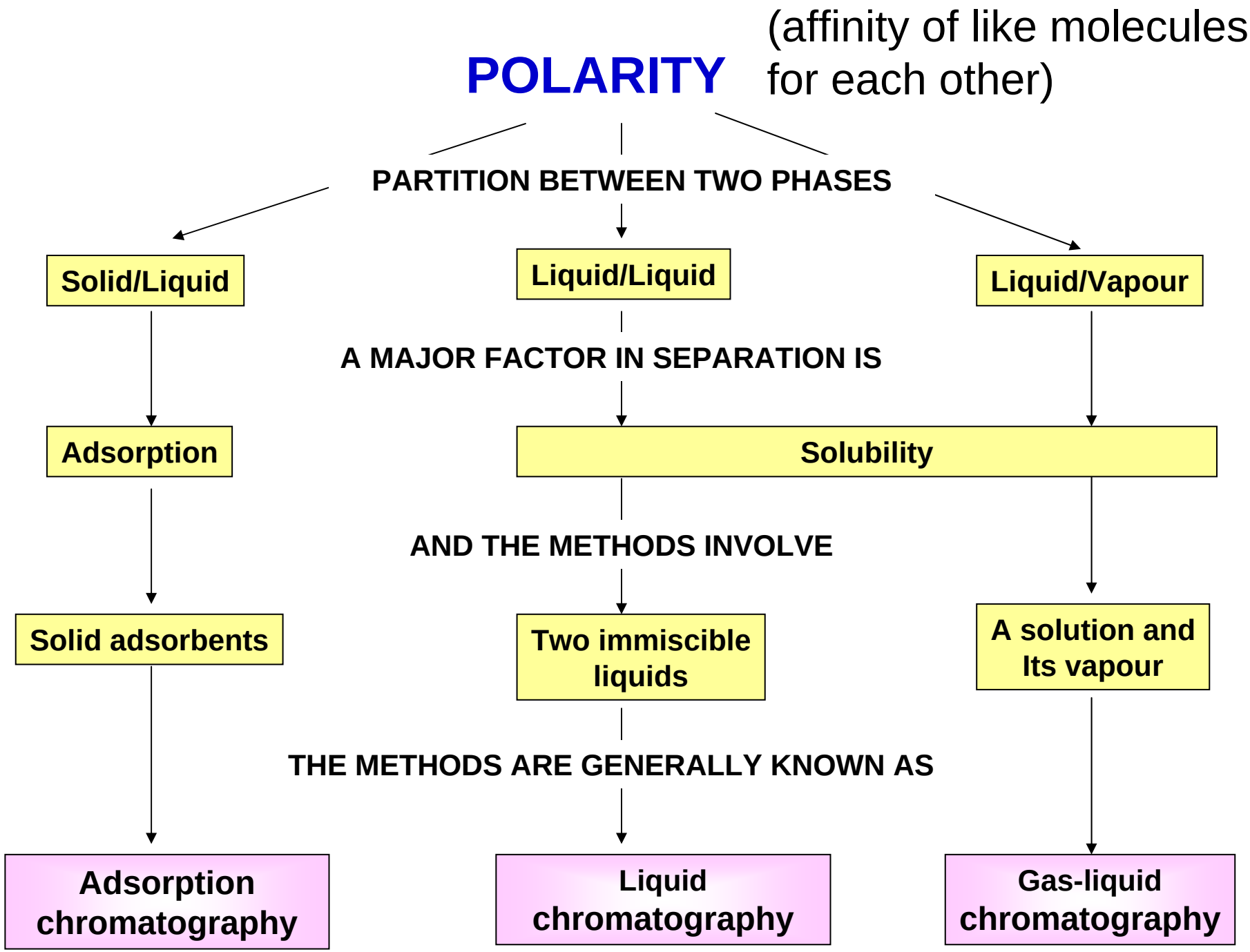
- Gravitational (Ultracentrifugation)
- Electrokinetic (Electrophoresis)
- Hydrodynamic (Chromatography, 沖堤液驅動力)



Retarding Force

Single phase technique
- Molecular friction
- Electrostatic

Dual phase technique
- Adsorption
- Binding
- Ionic interaction


POLARITY
(affinity of like molecules for each other)
PARTITION BETWEEN TWO PHASES
Solid/Liquid
Liquid/Liquid
Liquid/Vapour
A MAJOR FACTOR IN SEPARATION IS
Adsorption
Solubility
AND THE METHODS INVOLVE
Solid adsorbents
Two immiscible liquids
A solution and Its vapour
THE METHODS ARE GENERALLY KNOWN AS
Adsorption chromatography
Liquid chromatography
Gas-liquid chromatography

Different Kinds of Chromatography (characterized by the mobile phase)

- Liquid chromatography (includes column chromatography, thin-layer, and HPLC, mostly liquid-solid)
  - Stationary phase: silica, alumina, etc.
  - Mobile phase (moving phase): organic solvents
  - Important properties: **polarity, hydrophobicity**
- Gas chromatography
  - Stationary phase: a film of a polymer or a wax. The film must have a high boiling point
  - Mobile phase: gas (Helium is the usual carrier gas)
  - Important properties: **boiling point**

Modes of Chromatography

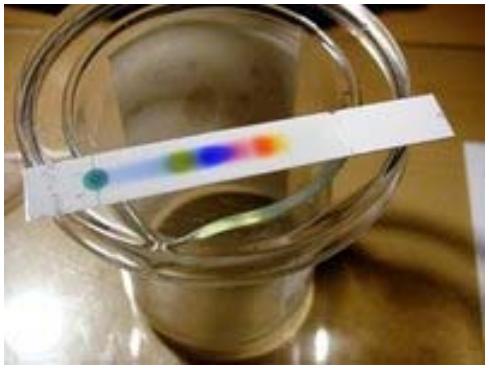
(characterized by shape of Stationary phase

- Column chromatography

- Stationary phase is packed into a column

- Thin-Layer chromatography

- Stationary phase is coated onto glass, metallic or plastic plate.



Liquid-Solid Chromatography (Adsorption)

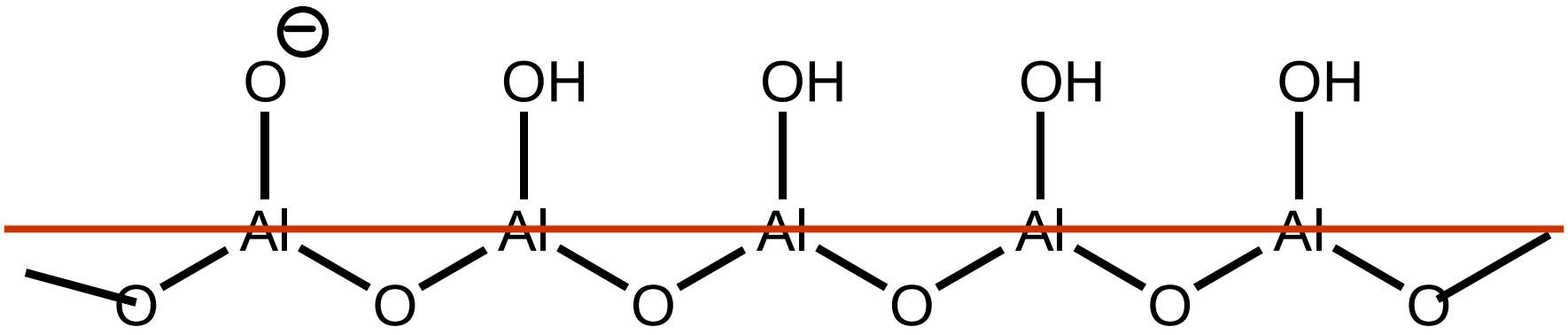
AB 3.2.1

Adsorption (吸附):

Some substances physically bind to the surface of a solid polar substances

- Polar compound
- Large surface for adsorption
- Often by OH (hydroxy group) to form H-bonding

Stationary Phase: Alumina



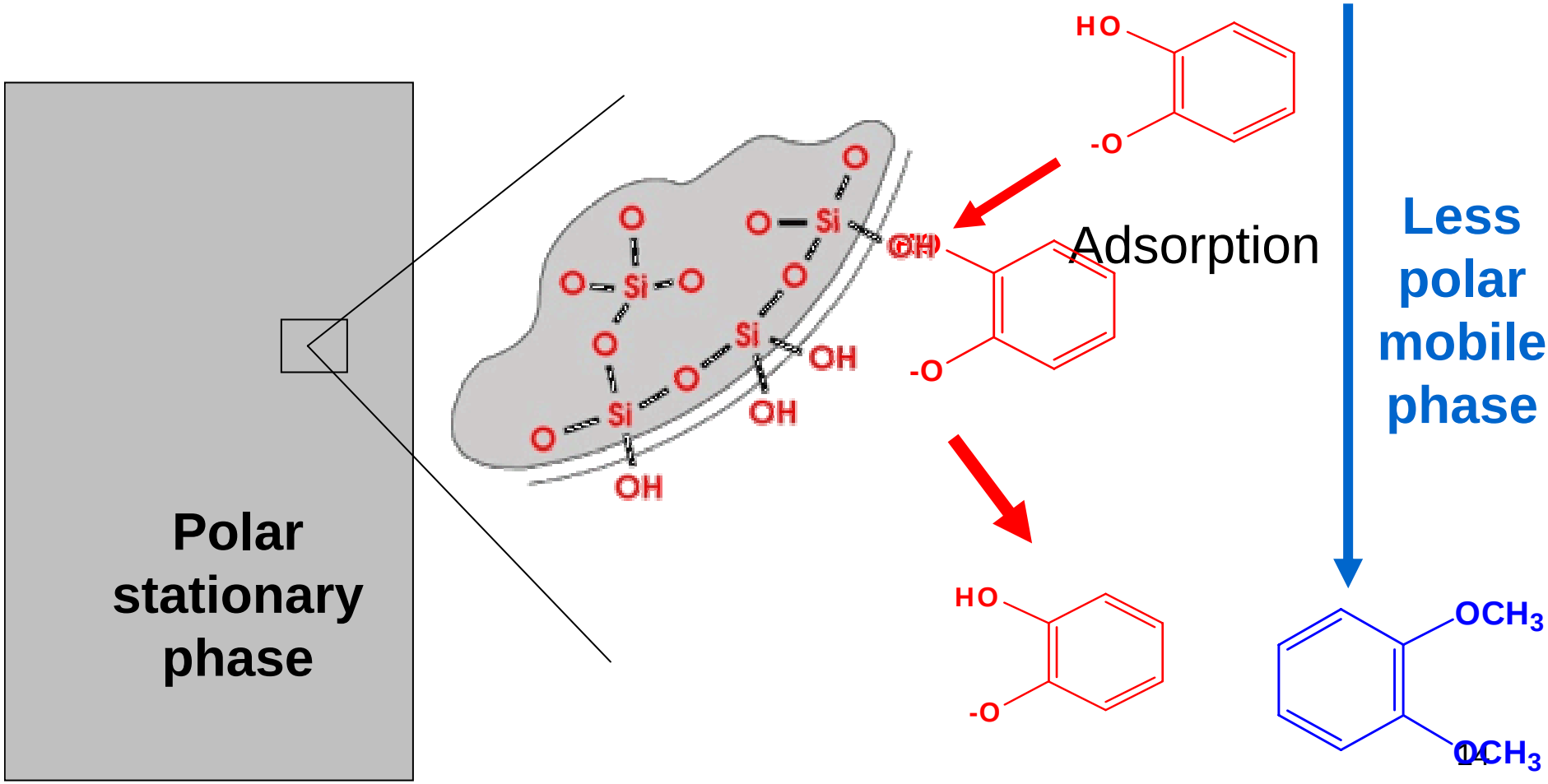
Acidic: -Al-OH

Neutral: -Al-OH + -Al-O$^-$

Basic: -Al-O$^-$

Silica Gel

Silica Gel is a porous, amorphous form of silica (SiO_2). It is composed of a vast network of interconnected microscopic pores. Silica gels have larger pores with a wide range of diameters – typically between 5 Å and 3000 Å.



Examples of Absorbents and Applications

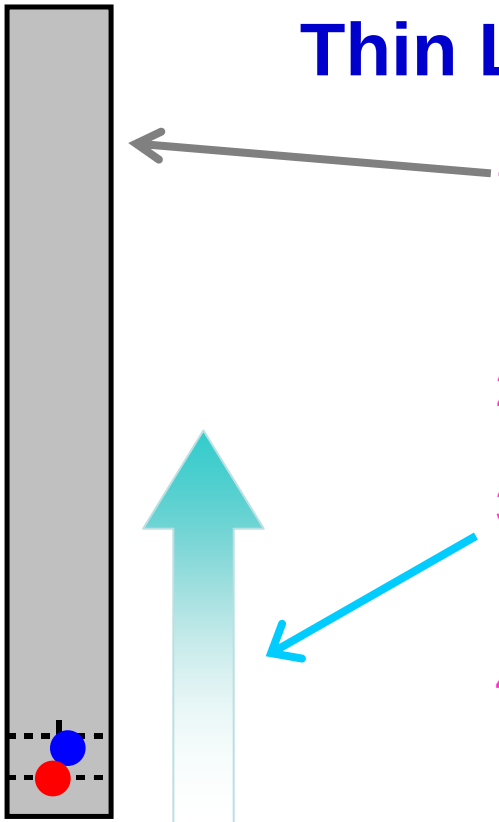
Adsorbent	Strength	Application
Silicic acid(silica gel)	Strong	Steroids,amino acids,lipids
Charcoal	Strong	Peptides,carbohydrates
Aluminium oxide	Strong	Steroids,esters,alkaloids
Magnesium carbonate	Medium	Porphyrins
Calcium phosphate	Medium	Proteins,polynucleotides
Cellulose	Weak	Proteins

- Thin-layer chromatography and column chromatography are different types of **liquid chromatography**.

- The mobile (moving) phase is a liquid. The stationary phase is usually silica or alumina. This phase is *very polar*.

- The principle of operation is the same!

Thin Layer Chromatography (薄層層析法)



1. The surface of the plate consists of a very thin layer of silica on a plastic or aluminum backing. The silica is very polar— the **stationary phase**.

2. Spot the material at the origin (bottom) of the TLC plate.

3. Place the plate into a glass jar with a small amount of a solvent in the glass jar.— the **moving phase**.

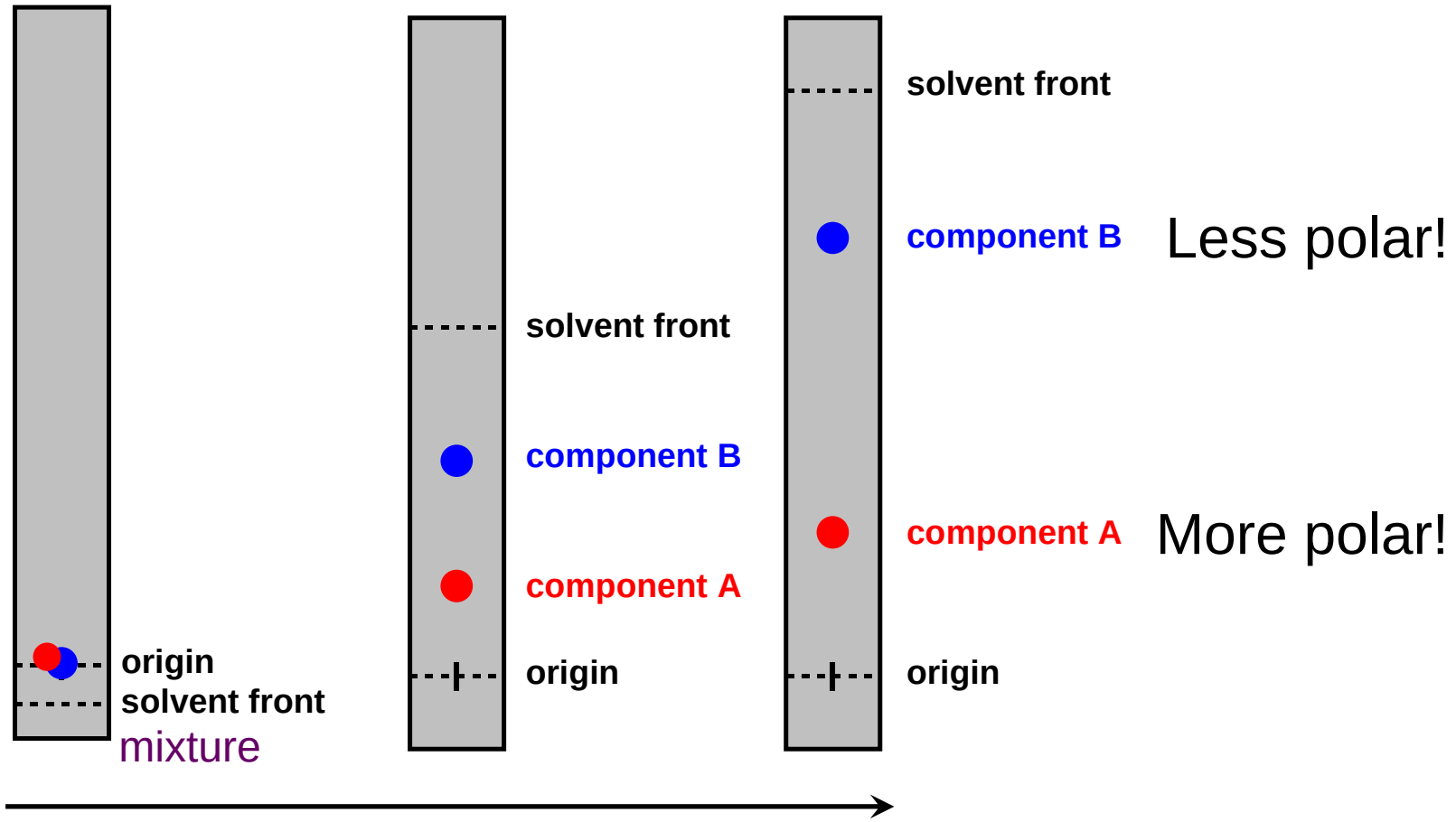
4. Remove the plate from the bottle when the solvent is close to the top of the plate.

5. Visualize the spots (**Ultraviolet light, color reagent…etc**)

Non-polar compounds will be **less strongly** attracted to the plate and will spend more time in the moving phase. This compound will move faster and will appear closer to the top of the plate.

Polar compounds will be **more strongly** attracted to the plate and will spend less time in the moving phase and appear **lower on the plate**.

Thin-Layer Chromatography: A Two-Component Mixture

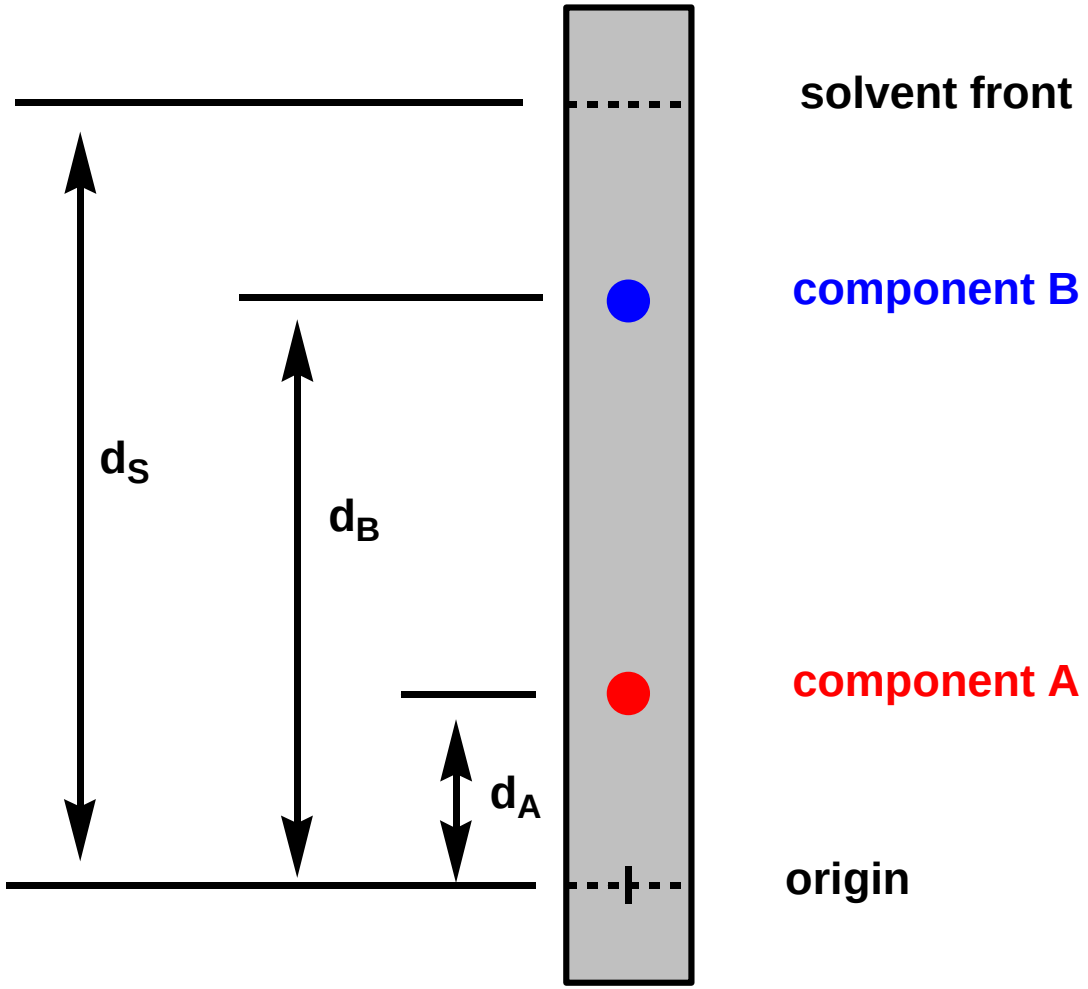


Thin-Layer Chromatography: Determination of R_f Values

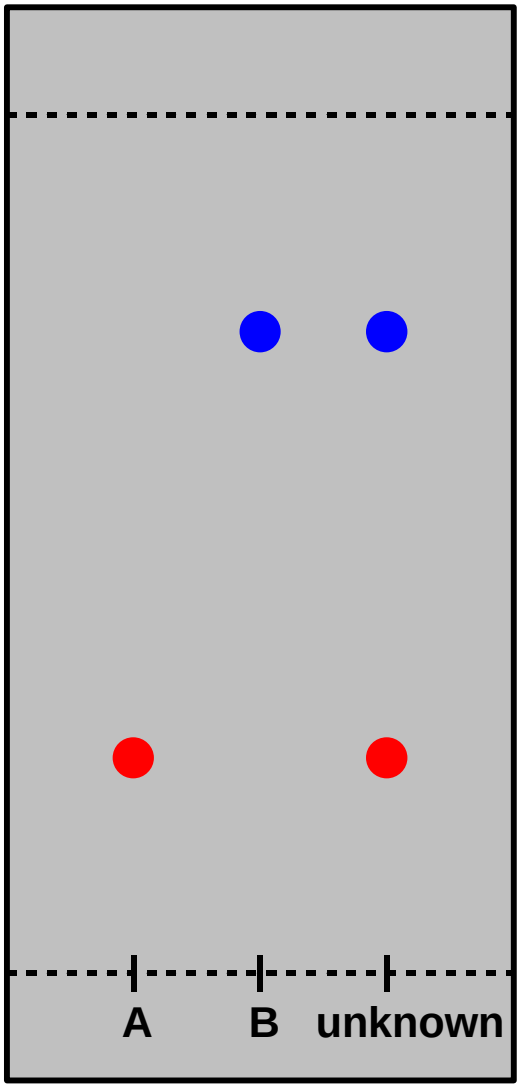
R_f of component A = $\frac{d_A}{d_S}$

R_f of component B = $\frac{d_B}{d_S}$

The R_f value is a decimal fraction, generally only reported to two decimal places



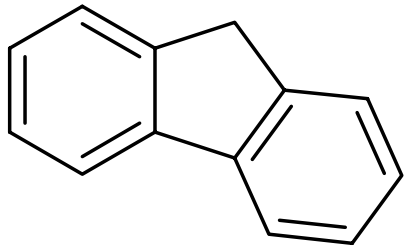
Thin-Layer Chromatography: Qualitative Analysis



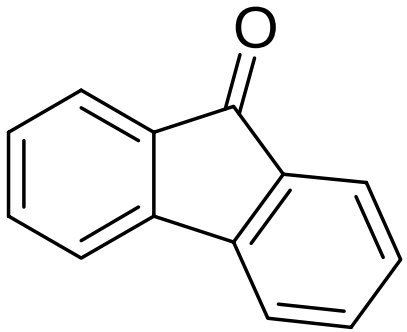
Advantages

- Simple
- Rapid
- Cheap

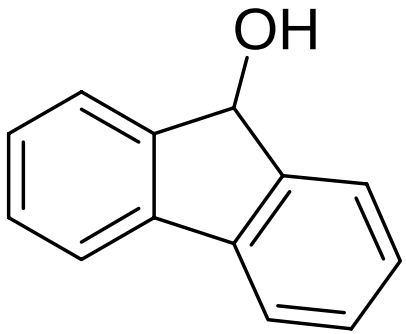
Example: Thin-Layer Chromatography



Fluorene



Fluorenone



Fluorenol

a) Which one of these compounds is the least polar?

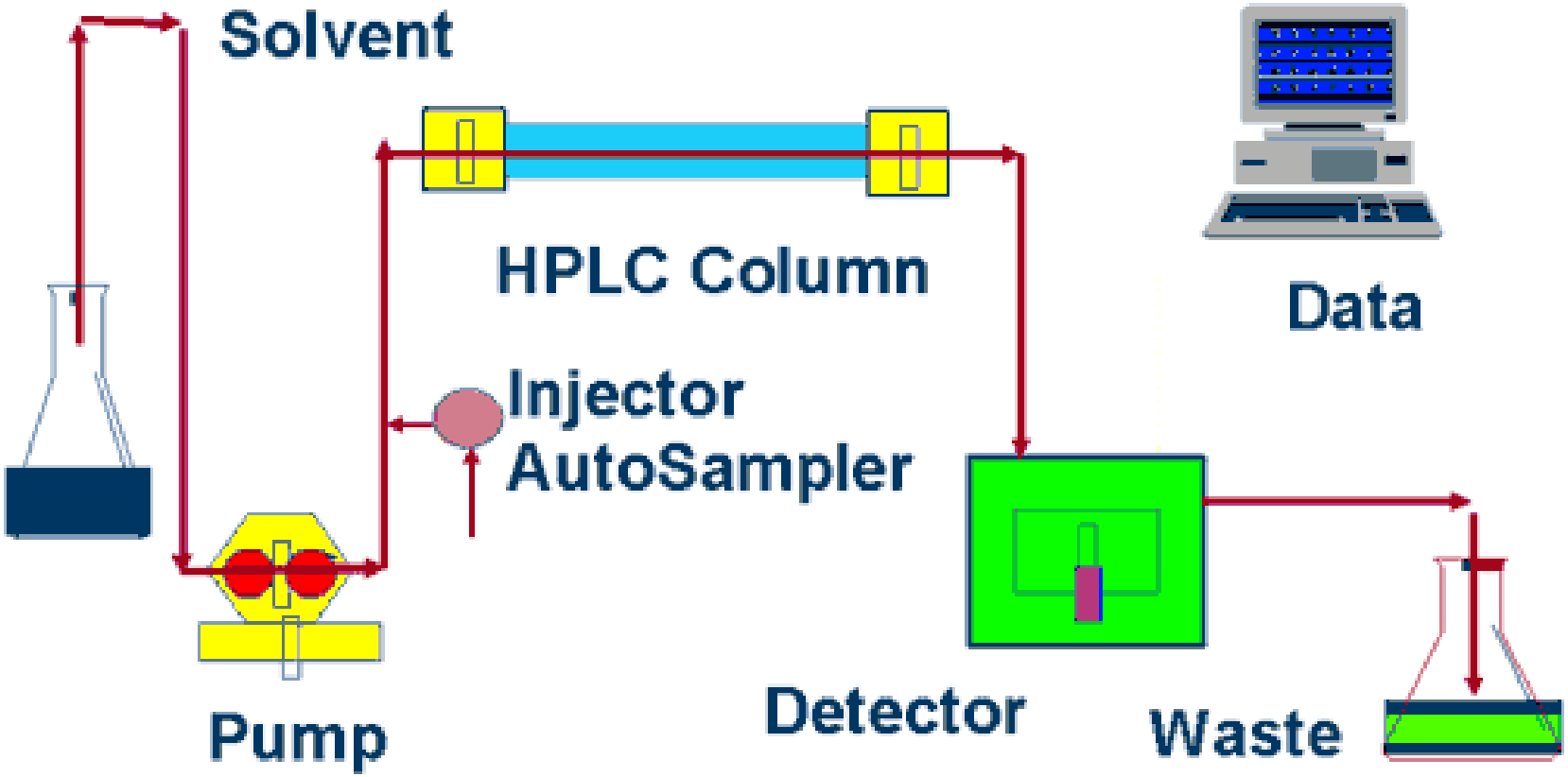
b) Which one of these compounds is the most polar?

c) What would be the relative order of separation on the TLC plate remembering that CH_2Cl_2 is not very polar?

High Performance Liquid Chromatography

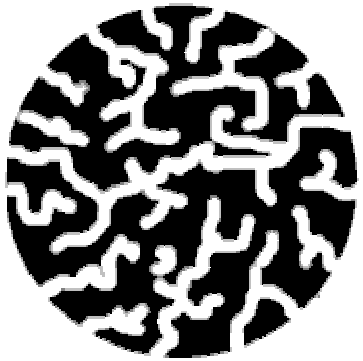
AB 3.2.2

Small and regular support media with stationary phase

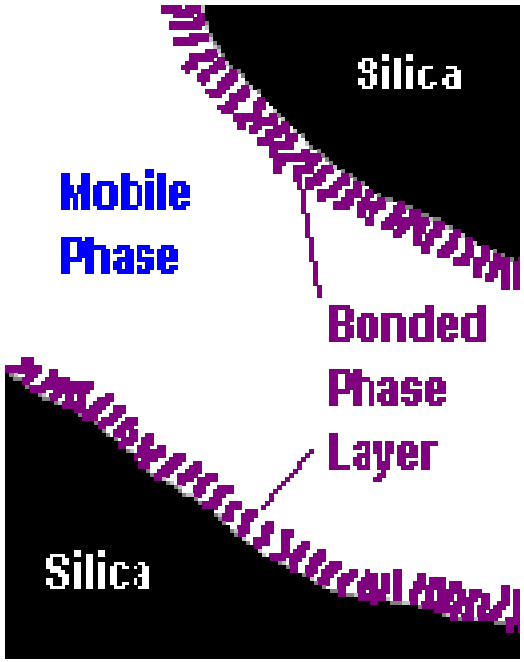


Provide steady solvent flow rate for isocratic or gradient mobile phase

Column

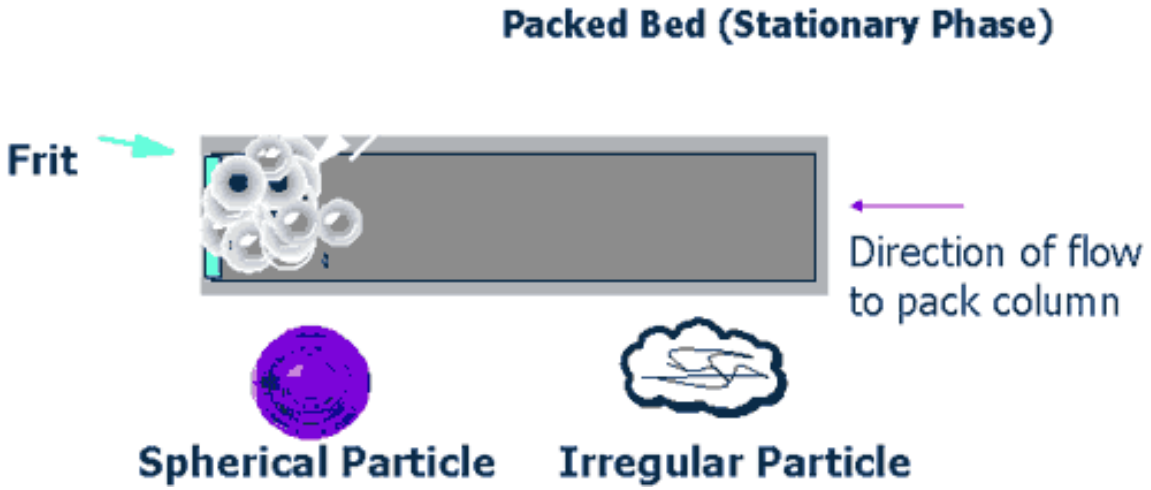


porous.



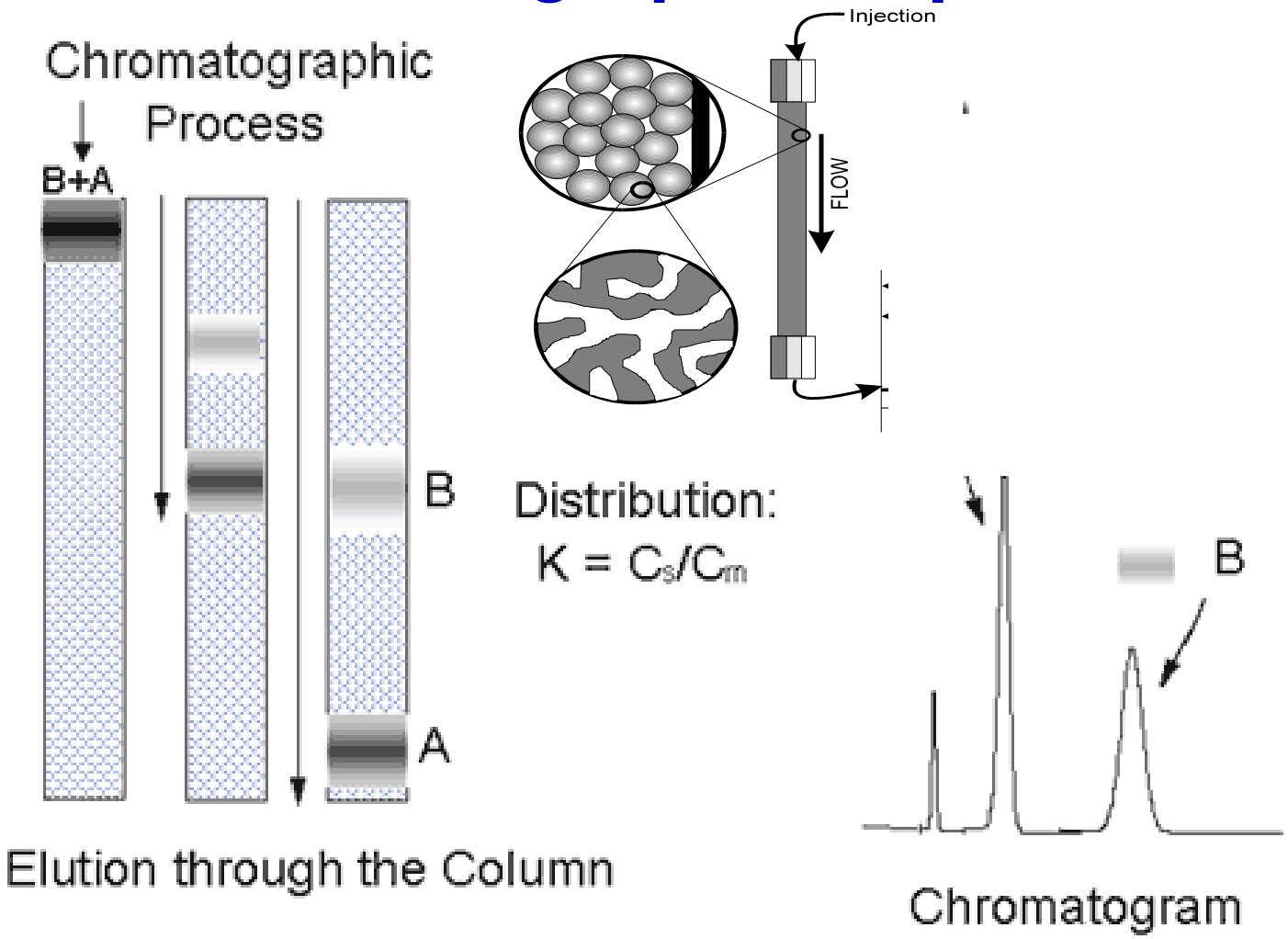
Most HPLC packings are porous. Most of the stationary phase surface area is on the inside of the particles

A layer of alkyl chains bonded to the silica surface



The composition of the mobile phase provides the chemical environment for the interaction of the solutes with the stationary phase.

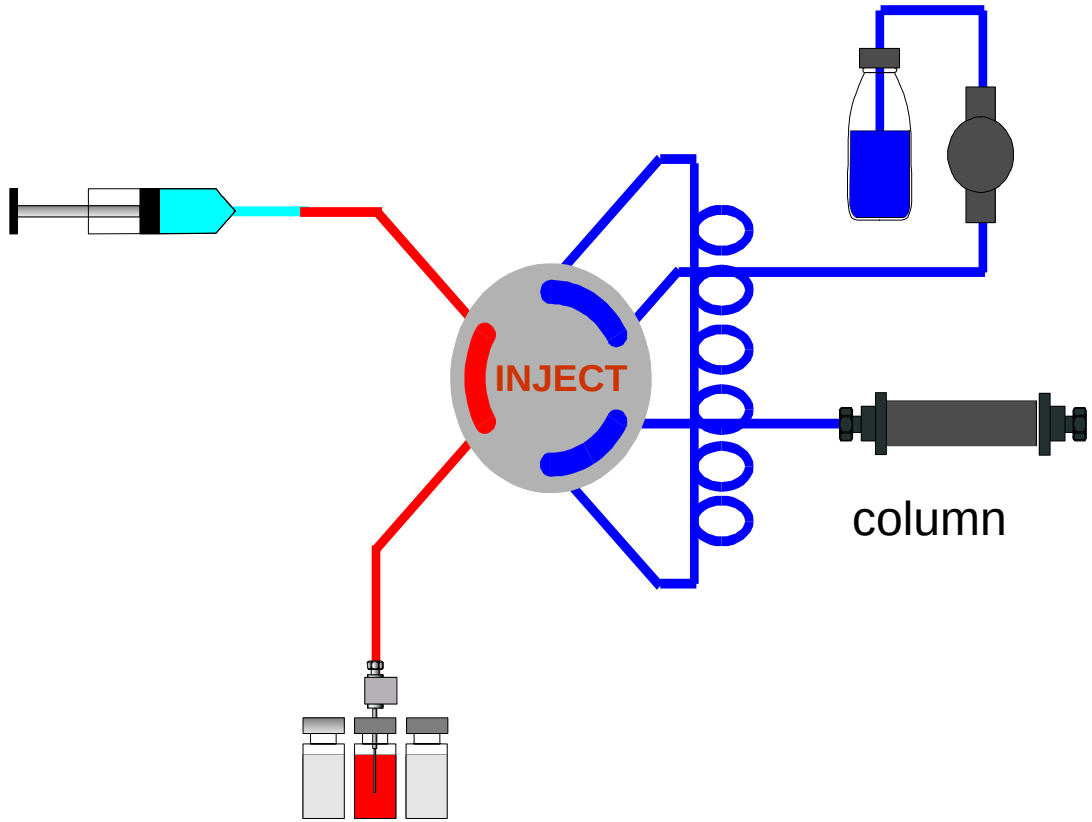
Chromatographic Separation



In a liquid chromatographic process a liquid permeates through a porous solid stationary phase and elutes the solutes into a flow-through detector

FAMOS
Microautosampler

Full Loop Injection



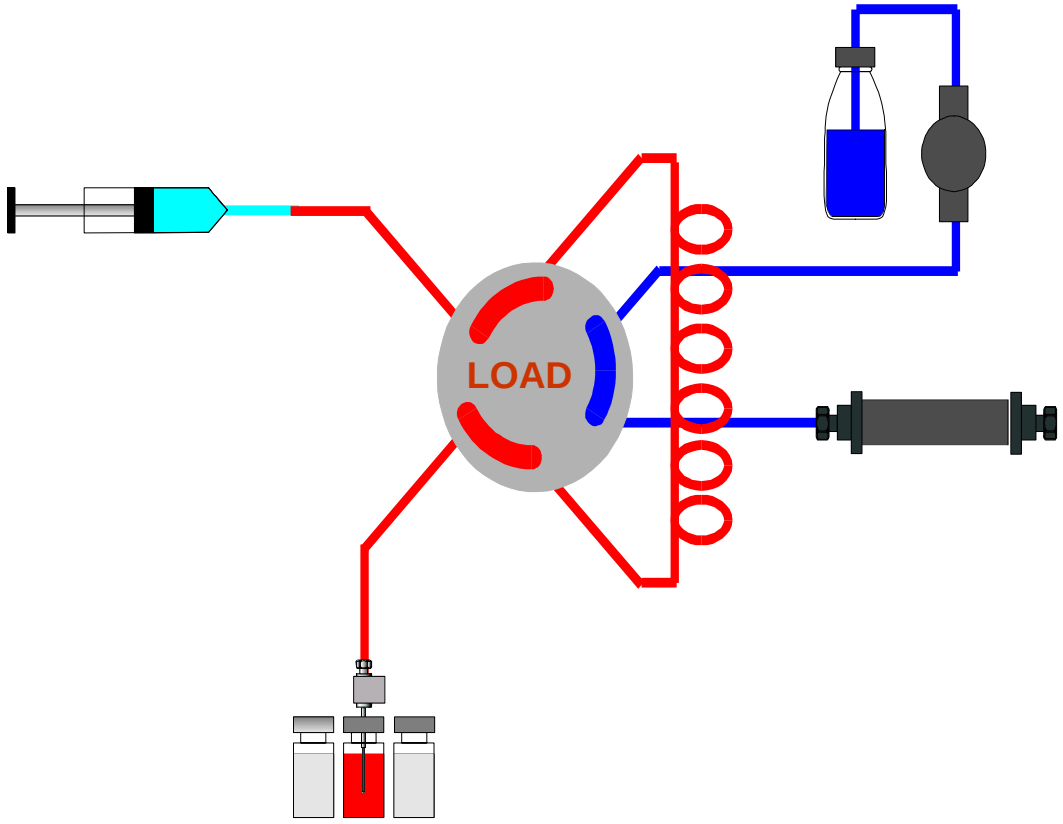
INJECT position

PRE-FLUSH with
2 x needle volume

(= 2 x 2.4 µl)

FAMOS
Microautosampler

Full Loop Injection (cont'd)



LOAD position

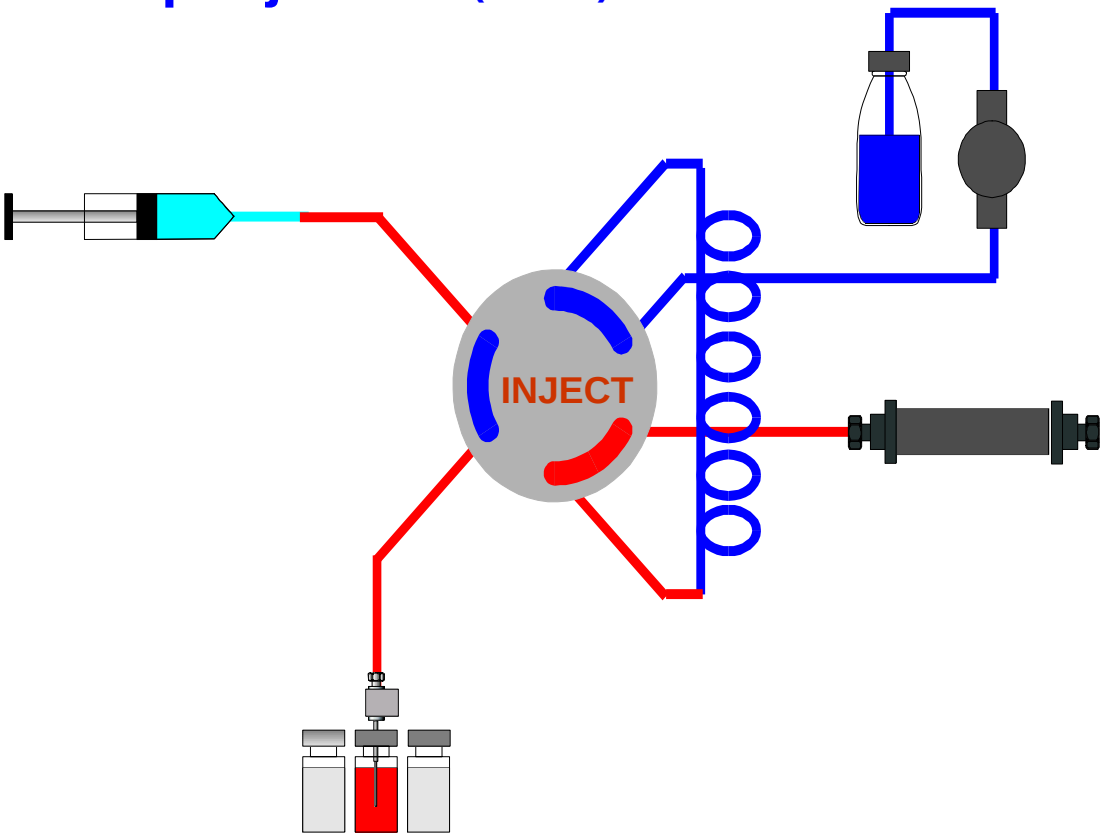
Cut-off the diluted front

Fill 3 x loop volume

(e.g. 3 x 5 µl)

FAMOS
Microautosampler

Full Loop Injection (cont'd)



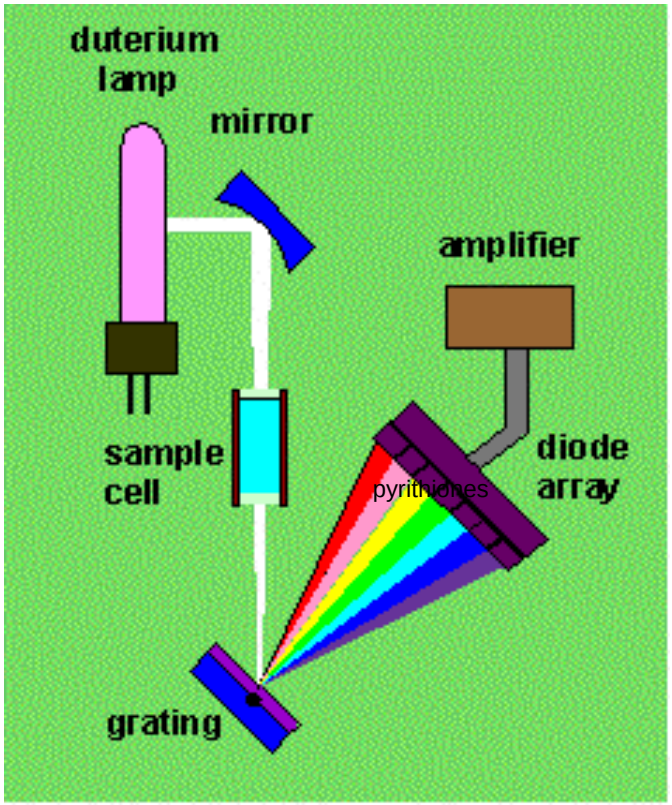
INJECT position

Provides maximum reproducibility

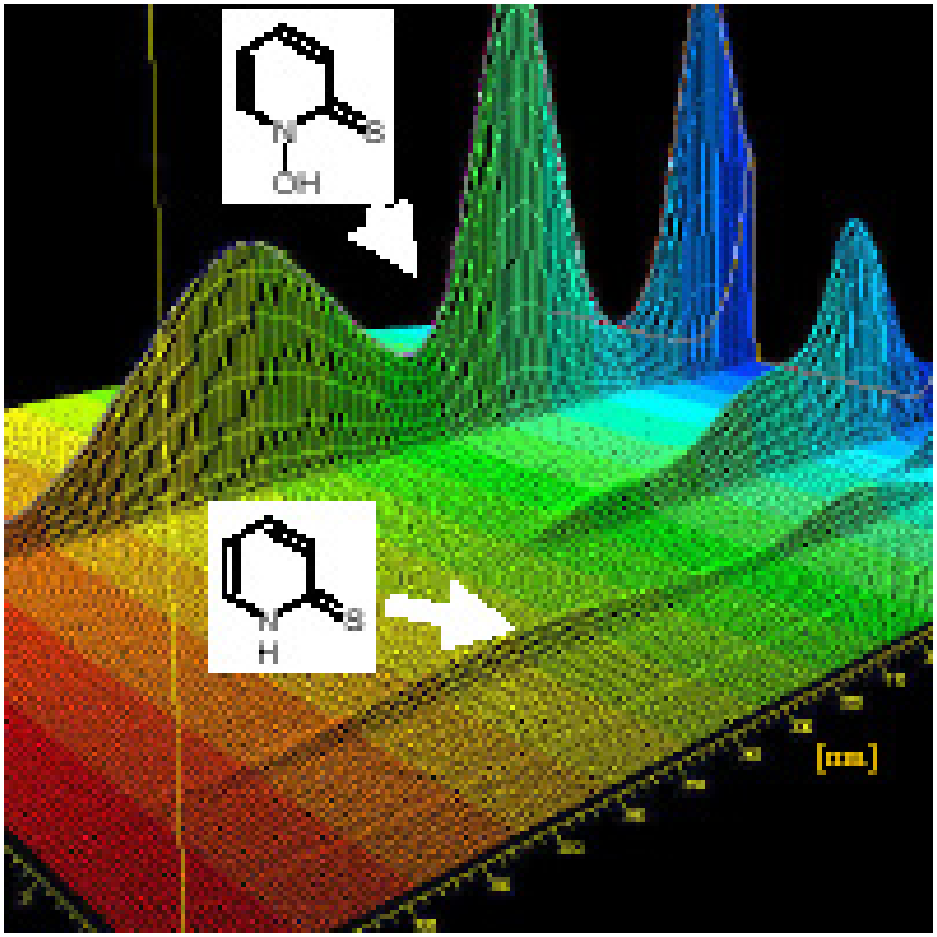
Detection Methods

- UV – Ultraviolet light
  - Lamp
  - Grating/Lens - Wave length
  - FlowCell
  - PhotoDiode - Differential Light Output
- RI – Refractive Index
  - Universal analyte detector
  - Solvent must remain the same throughout separation
  - VERY temperature sensitive
  - Sometimes difficult to stabilize baseline
- FD – Fluorescence
  - Excitation wavelength generates fluorescence emission at a higher wavelength
  - Analytes must have fluorophore group
  - Very sensitive and selective
- MS – Mass Spectrometry
  - Mass to charge ratio (m/z)
  - Allows specific compound ID

Example: HPLC Diode Array Detection



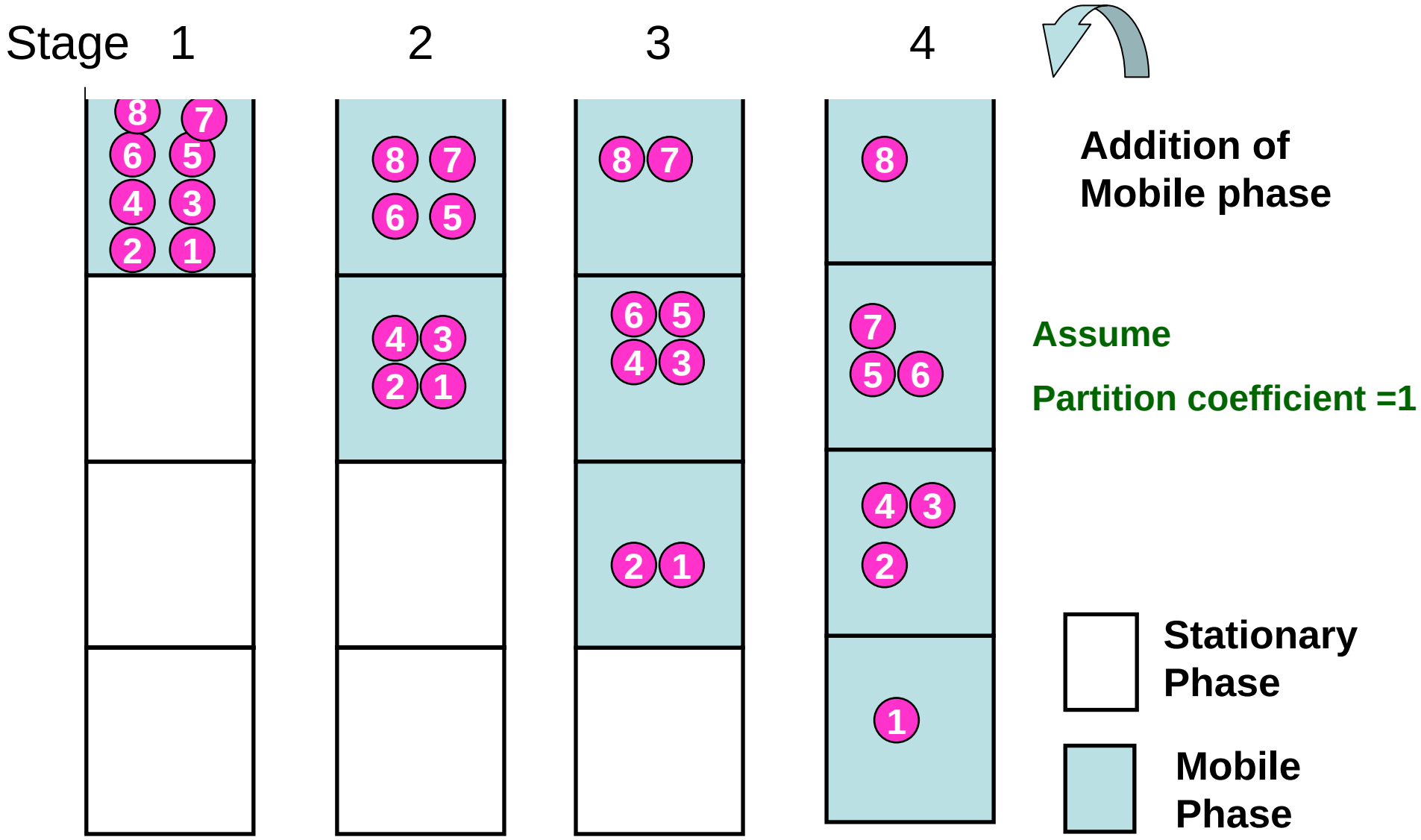
Absorbance is measured at two or more wavelengths



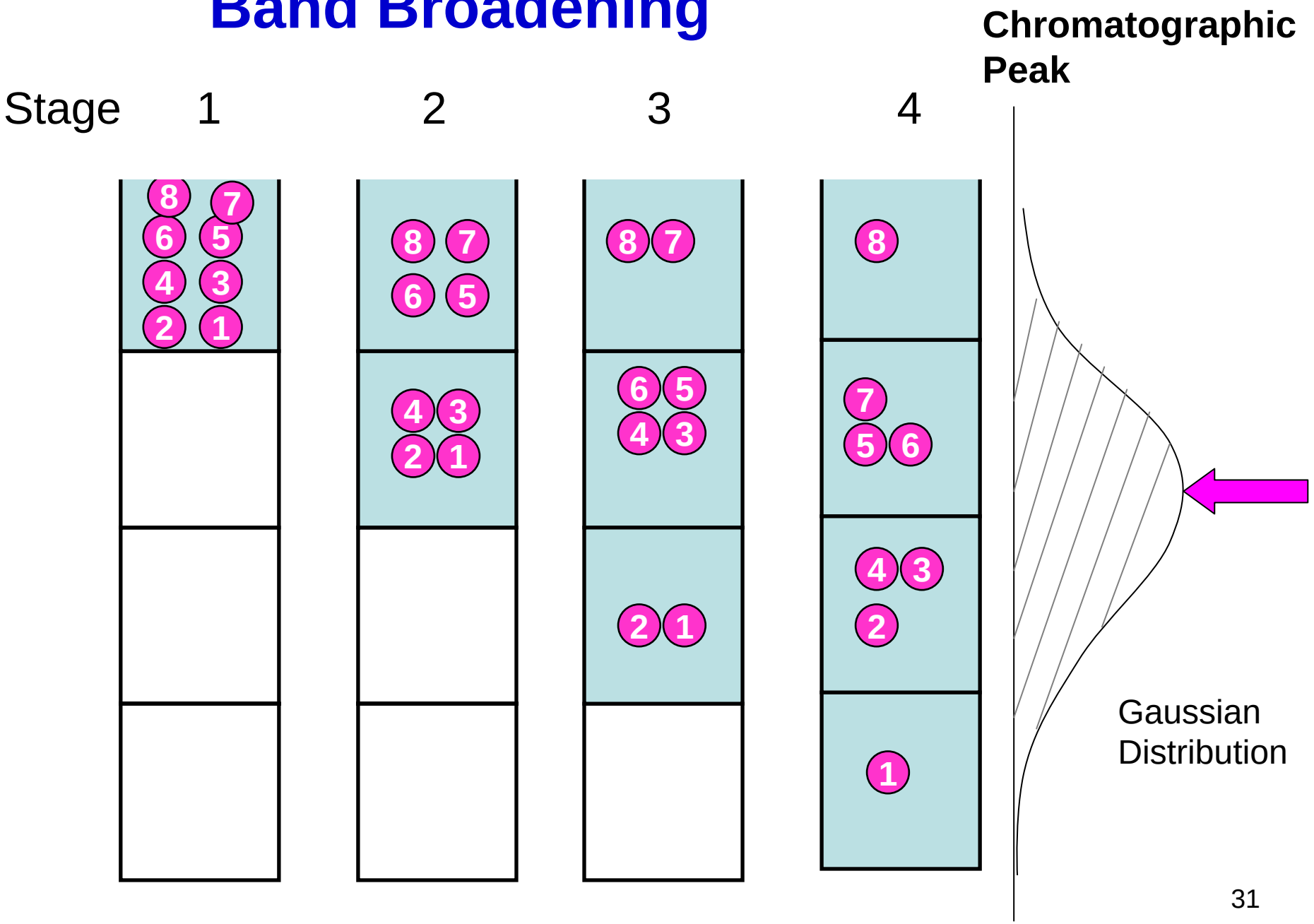
Absorption Wavelength

Pyrithione 是膜運輸過程一種一般抗化劑

Column Chromatographic Separation

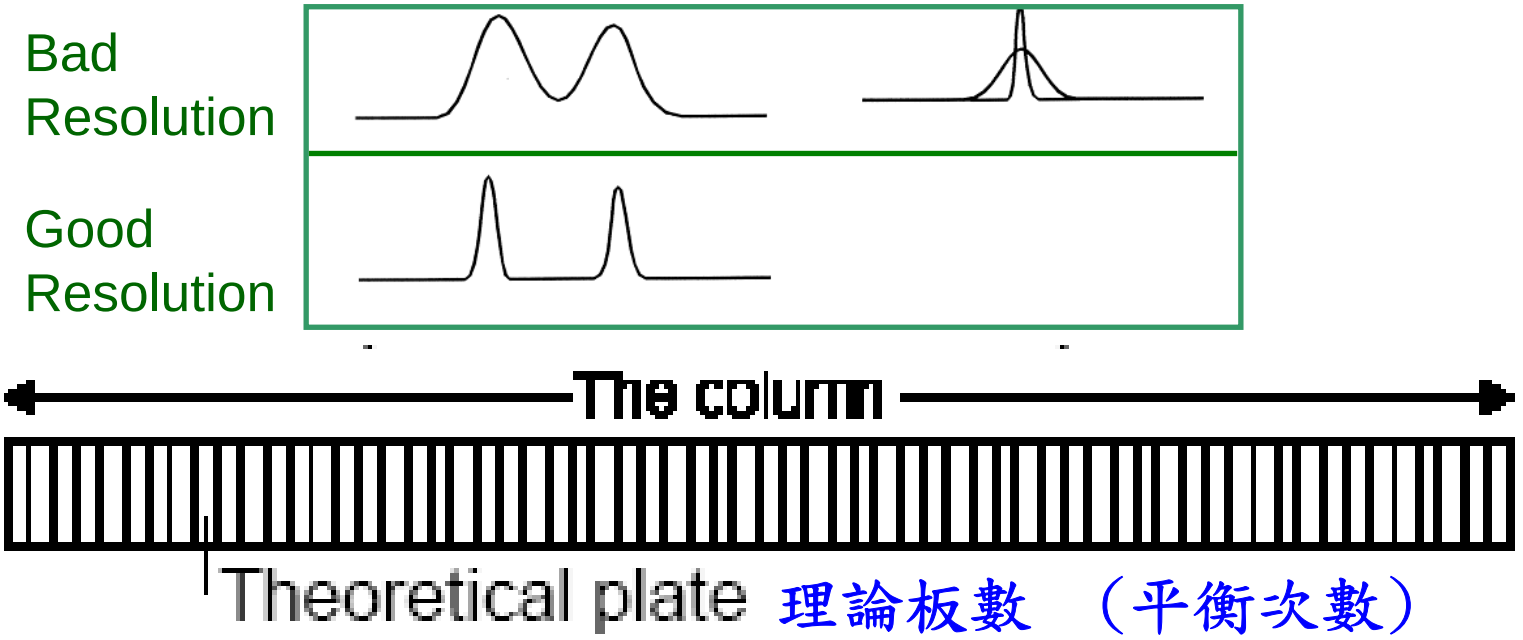


Band Broadening



Separation Efficiency: Plate Theory

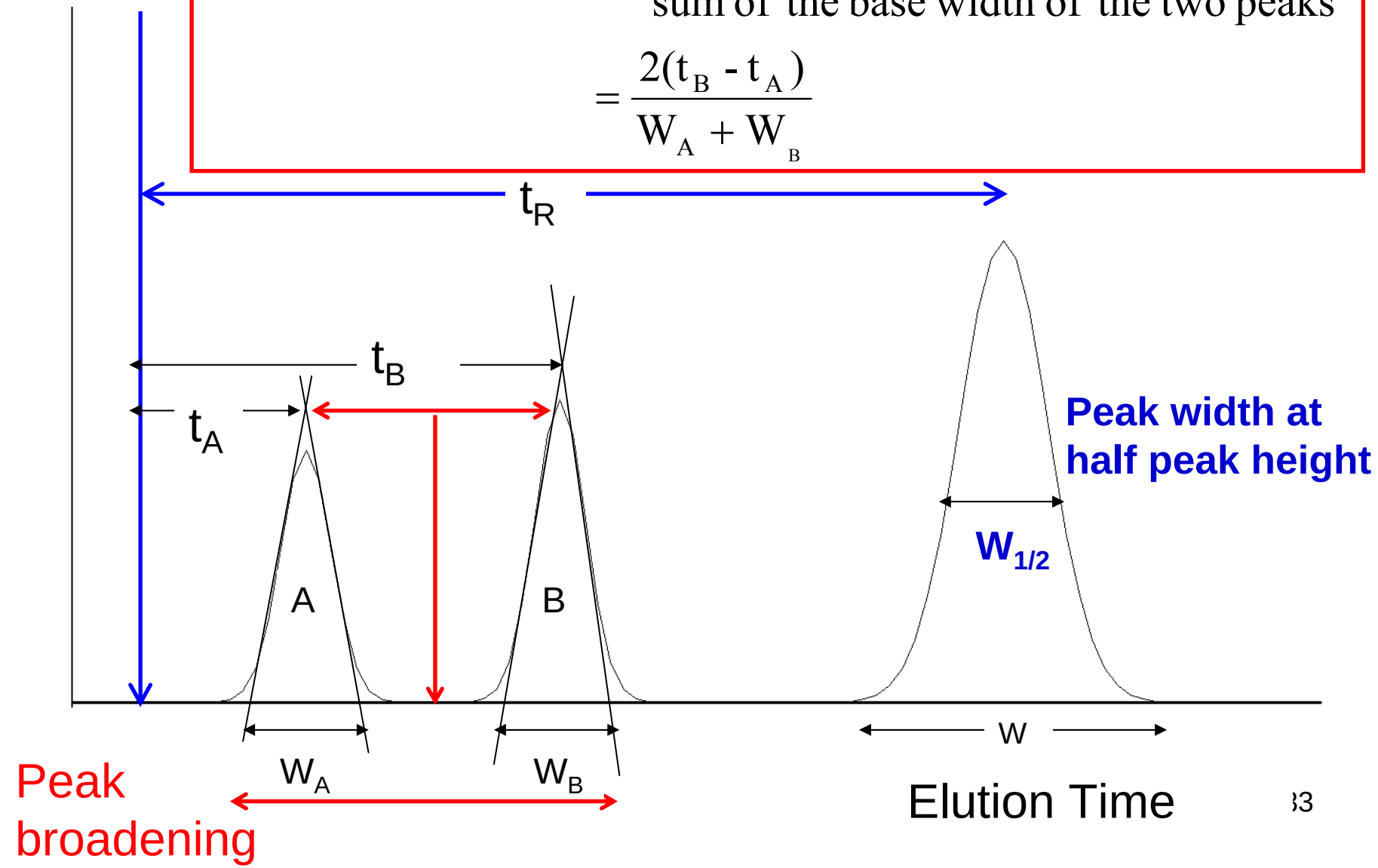
The plate theory suppose that the chromatographic column contains a large number of separate layers, called **theoretical plates**. Separate equilibrations of the sample between the stationary and mobile phase occur in these "**plates**". The analyte moves down the column by transfer of equilibrated mobile phase from one plate to the next.



Greater theoretical plates → Better separation resolution

Assessment of Column Efficiency

$$\text{Resolution index } (R_s) = \frac{\text{twice the distance between the two peaks}}{\text{sum of the base width of the two peaks}} = \frac{2(t_B - t_A)}{W_A + W_B}$$



Theoretical Plate Number—Resolution

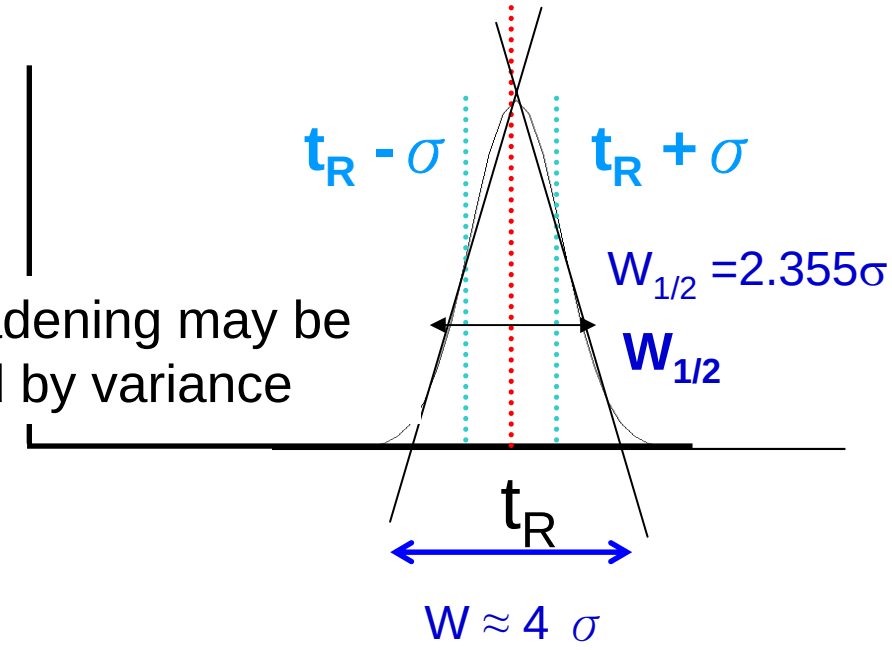
A measure of separation efficiency: How many times the

Analyte $_{mobile}$ → Analyte$_{stationary}$ equilibrium is achieved

$$N = \frac{t_R^{\,2}}{\sigma^2} = \left(\frac{t_R}{\sigma}\right)^2$$

$$N = 16\left(\frac{t_R}{W}\right)^2$$

Peak broadening may be expressed by variance



$$N = \left(\frac{2.355 \times t_R}{\text{peak width at half peak height}}\right)^2$$

$$= \left(\frac{\text{retention distance}}{\text{width at half peak height}}\right)^2 \times 5.54 \qquad \frac{t_R^{\,2}}{}$$

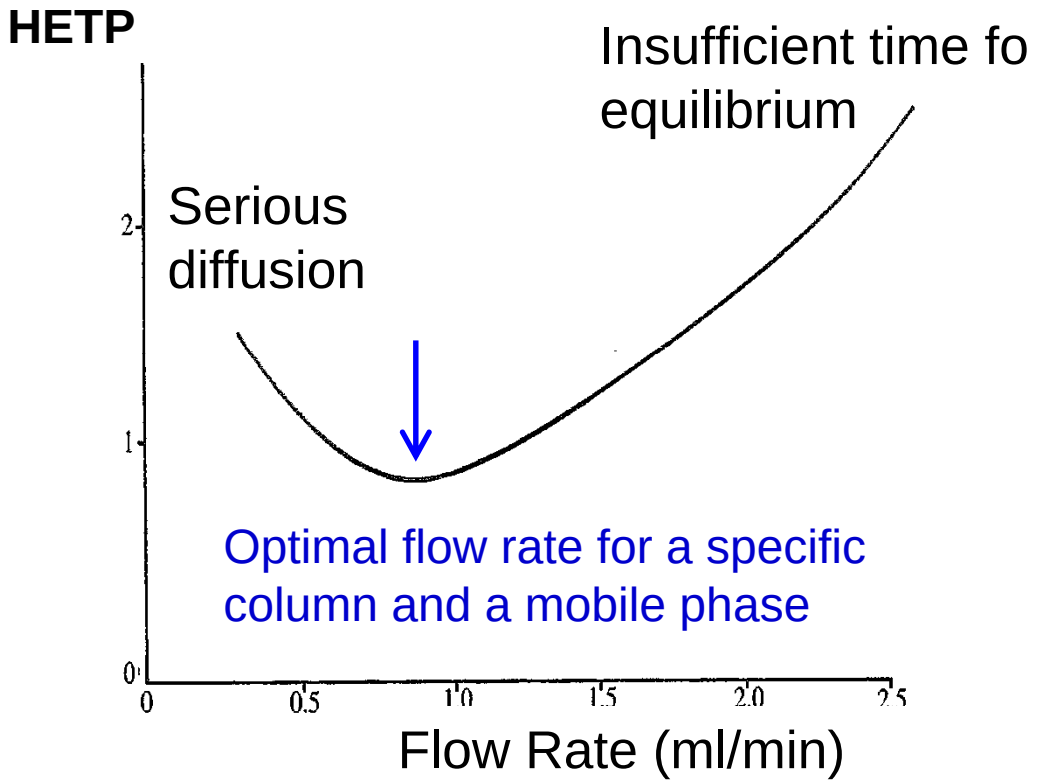
Retention time: measure of effective column volume for analyte

W Base width

Height Equivalent to a Theoretical Plate (HETP)

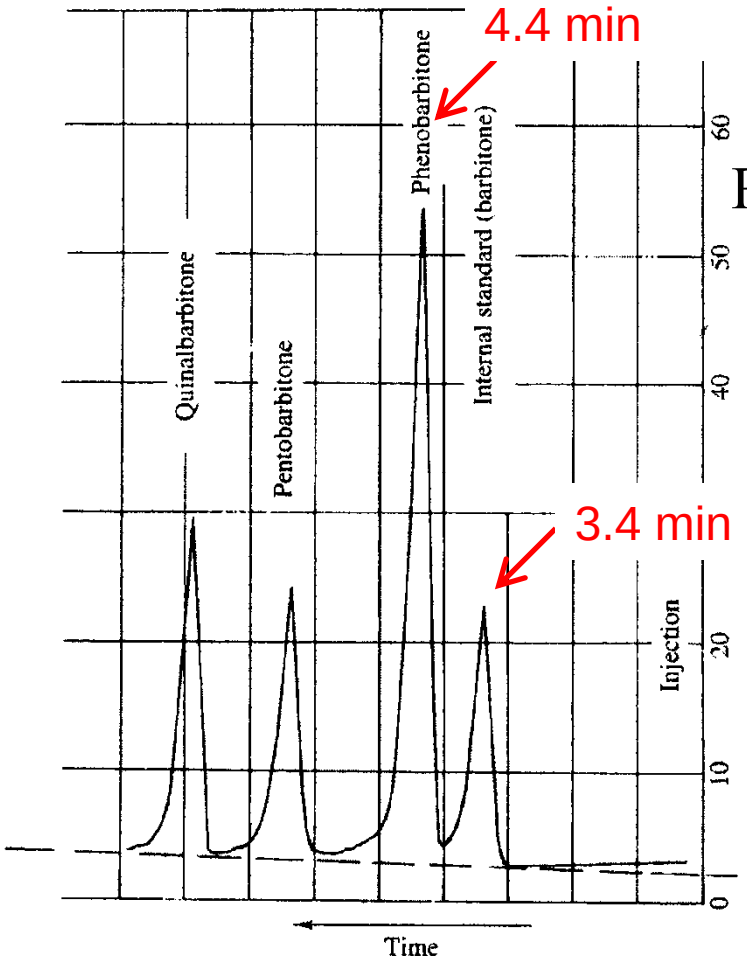
- Length of a column necessary for the attainment of compound distribution equilibrium (measure the efficiency of the column).

$$\mathrm{HETP} = \frac{\text{length of the column}}{\mathrm{N}}$$



Qualitative Analysis

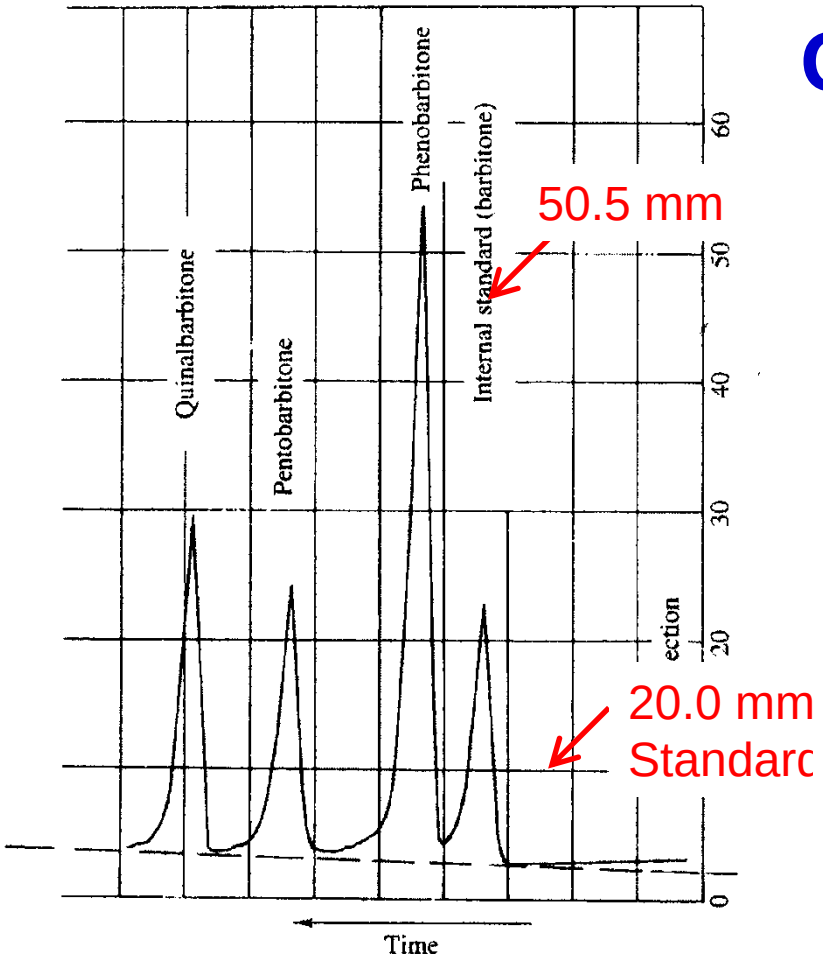
- By comparison with known components, retention time (Distance) is used for identification of a component of a mixture.



$$\text{Relative retention} = \frac{\text{test retention time}}{\text{reference retention time}}$$

$$\text{Relative retention} = \frac{4.4}{3.4} = 1.3$$

Quantitative Analysis



Separation took approximately 15 minutes.

Component	Peak height (mm)
Standard	20.0
Phenobarbitone	50.5
Pentobarbitone	21.0
Quinalbarbitone	26.0

Calculation

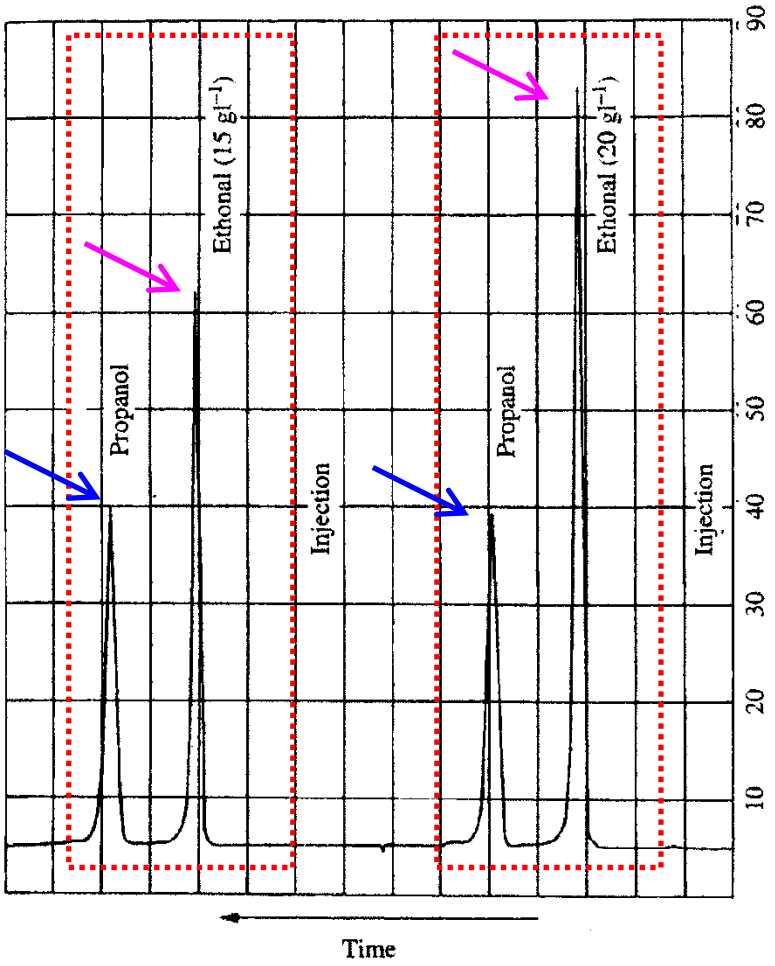
Component	Concentration ($mmol\,l^{-1}$)
Phenobarbitone	$\frac{50.5}{20.0} \times \frac{1.1}{1.0} \times 5.0 \times \frac{0.1}{1.1}$

0.1 ml of the internal standard (barbitone, 5.0 mmol/l) was added to 1.0 ml of sample.

20 uL of the mixture was injected

What is the concentration of phenobarbitone?

Injection 2 Injection 1



What is the concentration of test sample?

Reference: Propanol

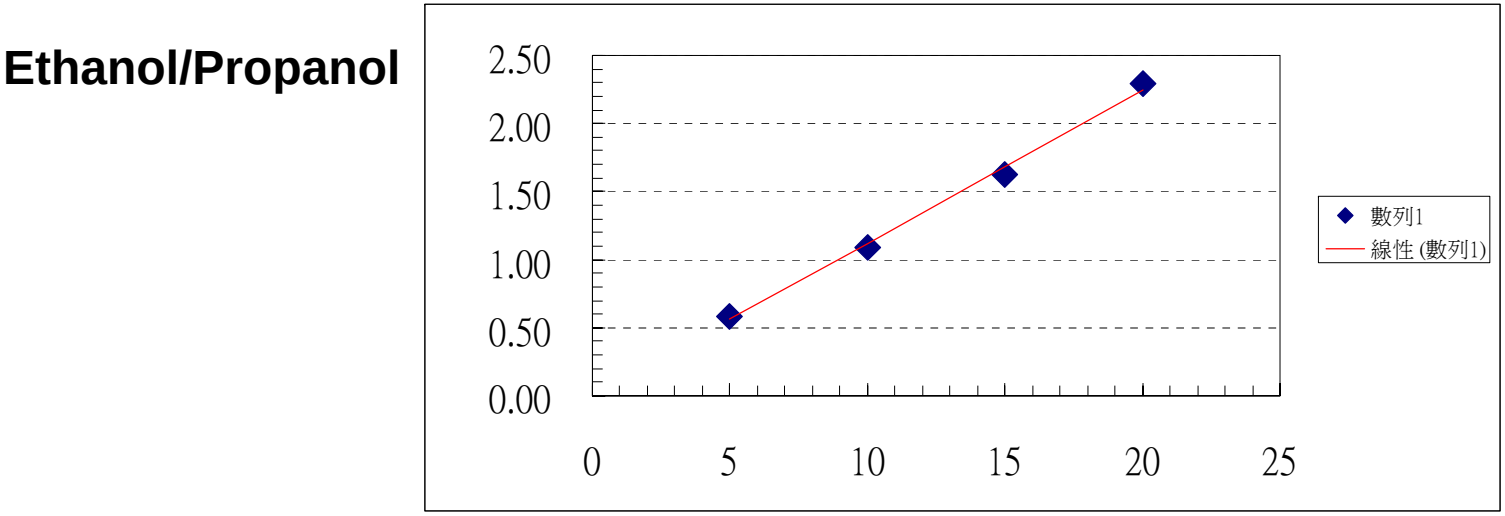
Standard: Ethanol

Normalization with Injected Internal Standard

Injection number	Component 1	Peak height (mm)	Component 2	Peak height (mm)	Peak heights ratio
1	Ethanol (20 gl^{-1})	78	Propanol (1.0 gl^{-1})	34	2.29
2	Ethanol (15 gl^{-1})	57	Propanol (1.0 gl^{-1})	35	1.62
3	Ethanol (10 gl^{-1})	37	Propanol (1.0 gl^{-1})	34	1.08
4	Ethanol (5 gl^{-1})	21	Propanol (1.0 gl^{-1})	36	0.55
5	Test sample	45	Propanol (1.0 gl^{-1})	35	1.28

Reference:

Ethanol Conc.	Peak height	Propanol	Peak height	Ethanol/Propanol
20	78	1	34	2.29
15	57	1	35	1.63
10	37	1	34	1.09
5	21	1	36	0.58
X	45	1	35	1.29



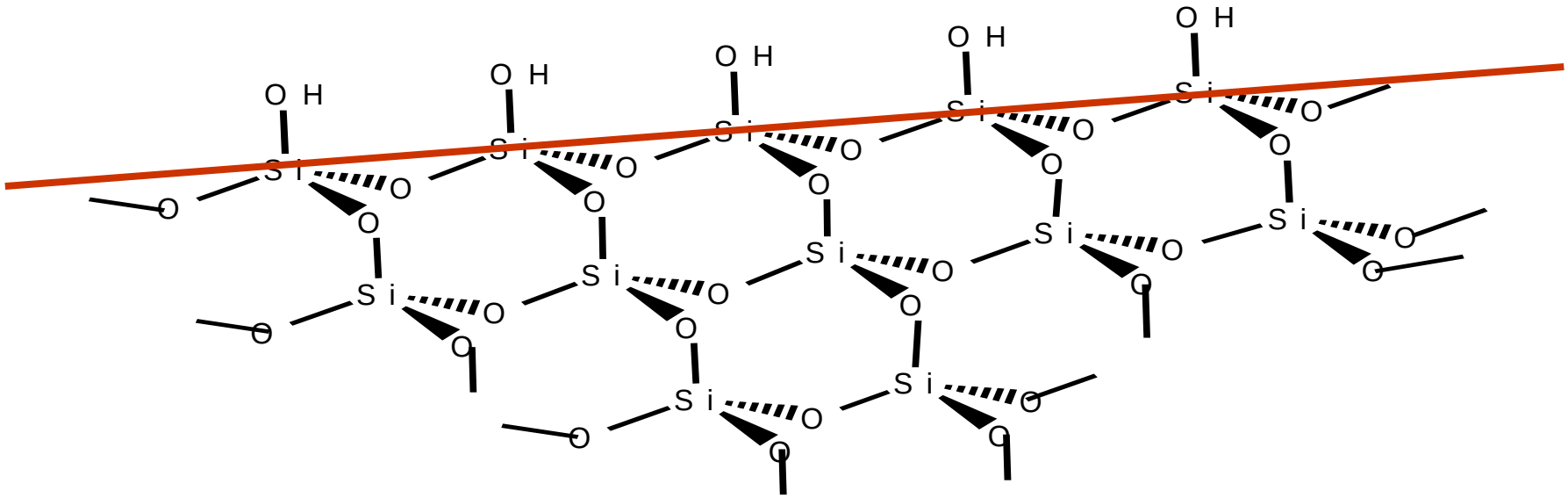
Partition Chromatography BMB 11.5

Partition chromatography is based on differences in capacity factors and distribution coefficients of the analytes using liquid stationary and mobile phases.

- Normal/Reverse Phase Chromatography
- Ion-Exchange Chromatography
- Gel Filtration Chromatography
- Affinity Chromatography

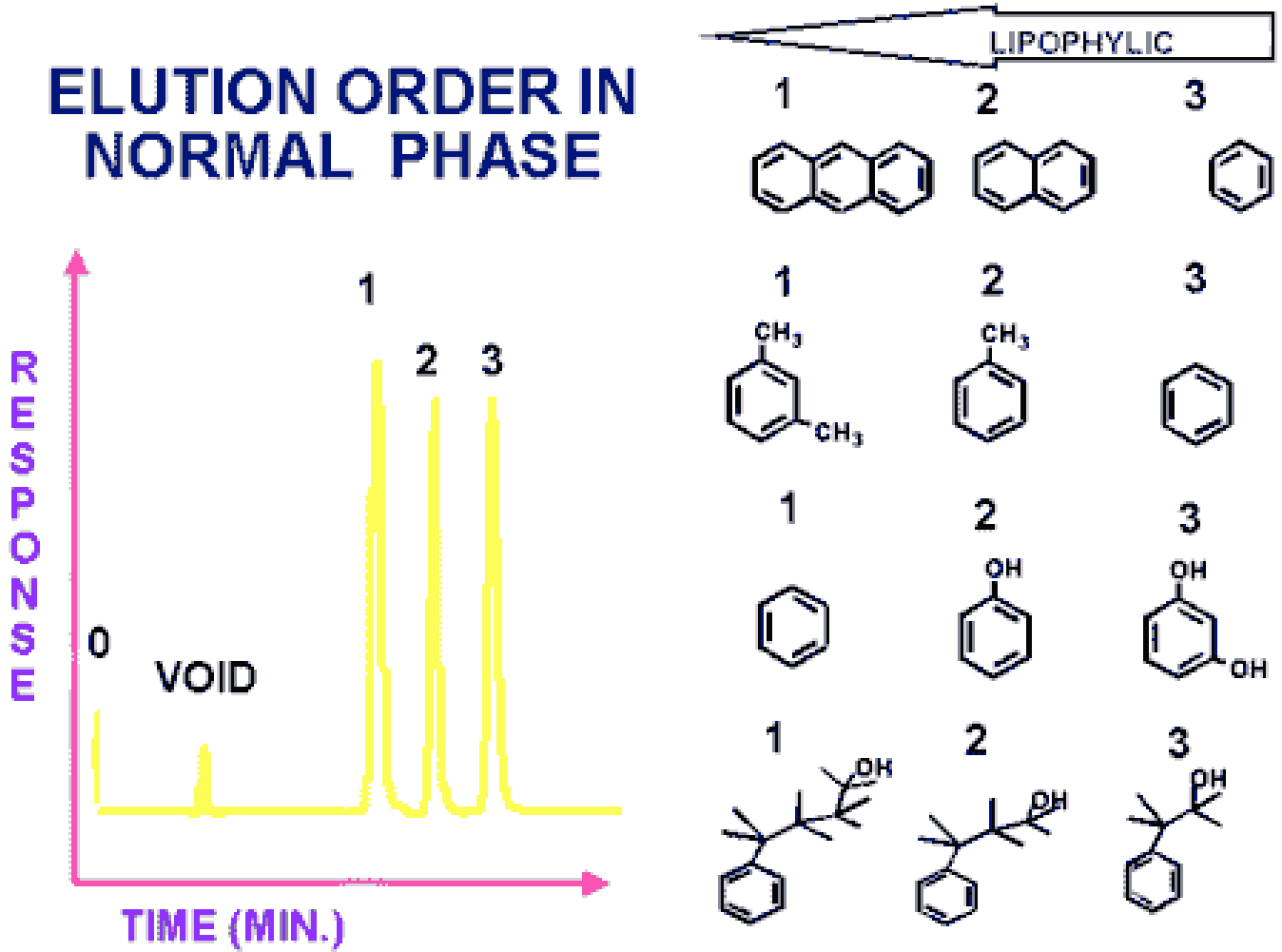
Normal-Phase HPLC

Adsorption of analytes on the polar, weakly acidic surface of silica gel



- Stationary Phase.: Silica (pH 2-8), Alumina (pH 2 - 12), Bonded Diol, and NH_2 **(hydrophilic surface)**
- Mobile Phase: Non-polar solvents (Hexane, $CHCl_3$)
- Applications: hexane soluble; positional isomers.

Normal Phase Liquid Chromatography

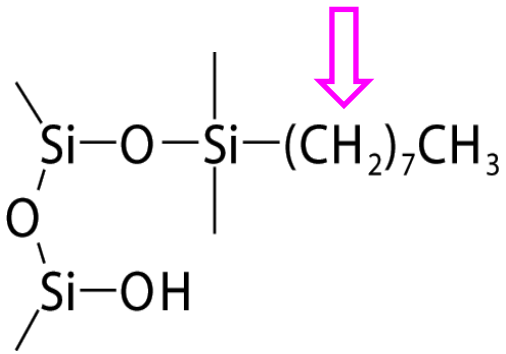


Polar solutes elute later than non-polar lypophilic ones.

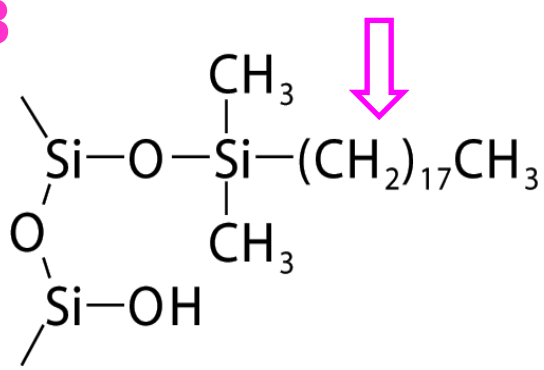
Reversed-Phase HPLC

Partition of analytes between mobile phase and stagnant phase inside the pore space + adsorption on the surface of bonded phase

C8

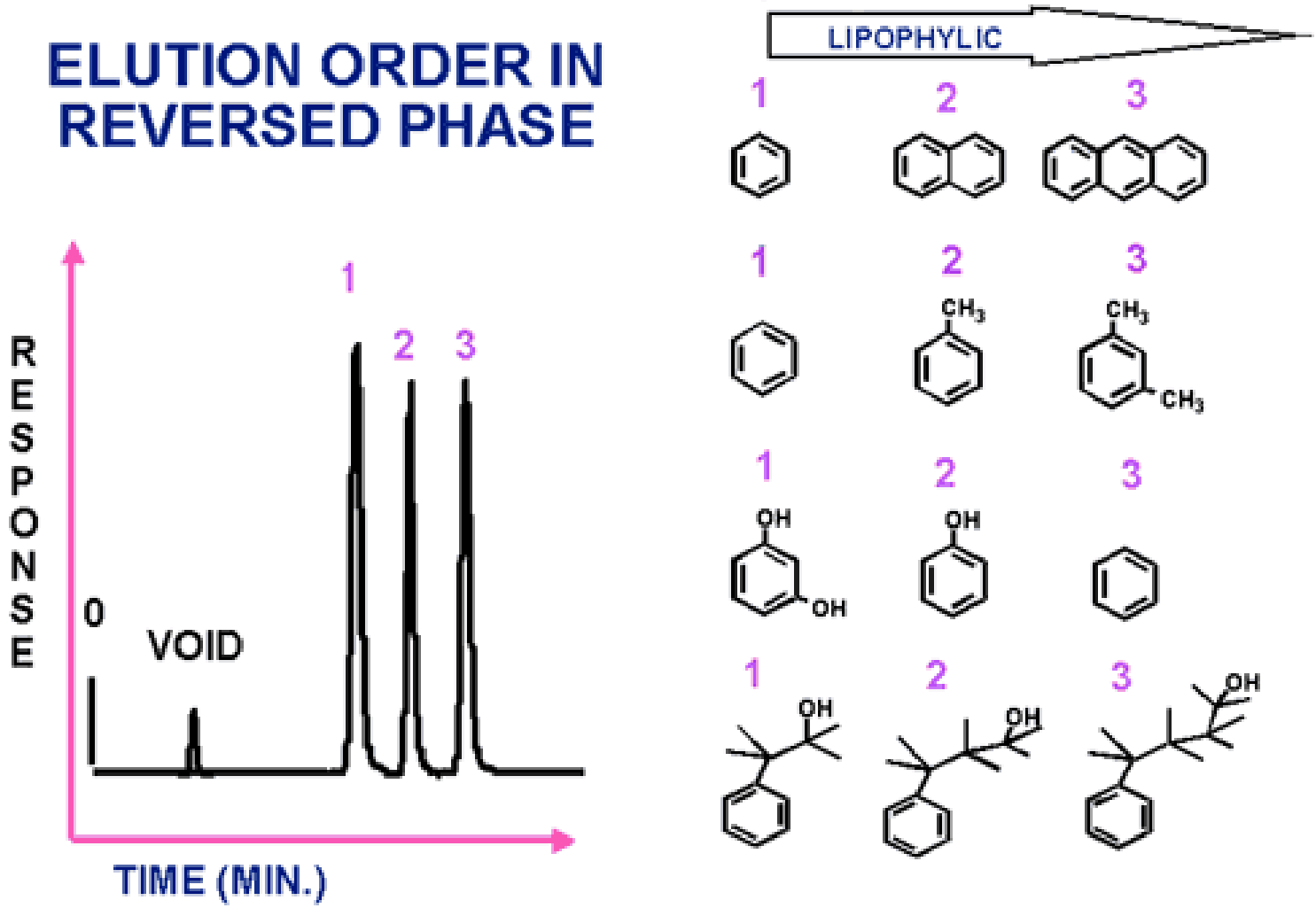


C18



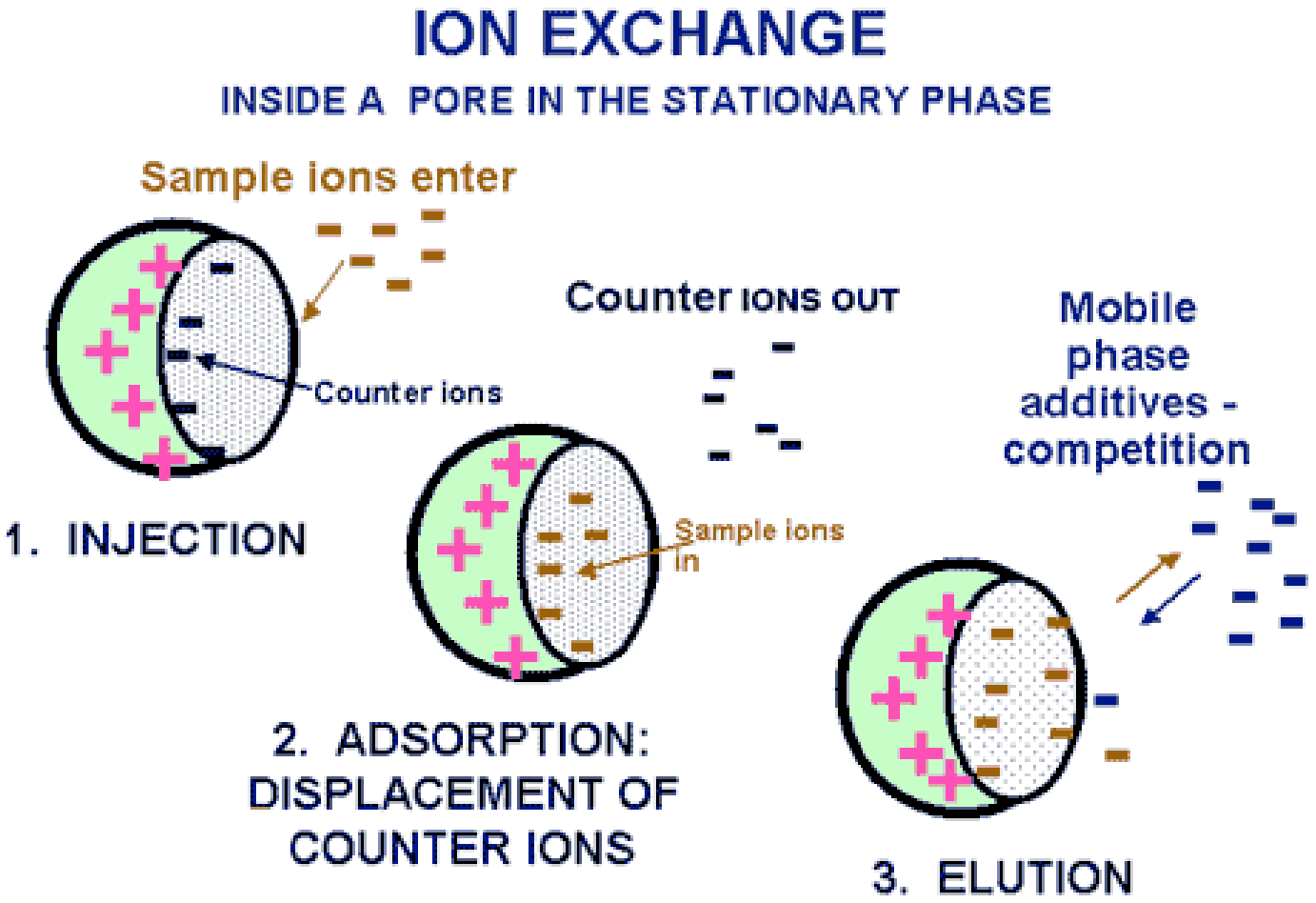
- Stationary Phase: **Hydrophobic surfaces** of moieties bonded on silica (C18, C8, C5, Phenyl, CN)
- Mobile phase: Methanol or Acetonitrile and Water.
- Applications: ~80% of all separations done on RP HPLC.

“Reverse” Phase Liquid Chromatography



In Reversed Phase separations organic molecules are separated based on their degree of hydrophobicity. There is a correlation between the degree of lipophylicity and retention in the column.

Ion Exchange Liquid Chromatography

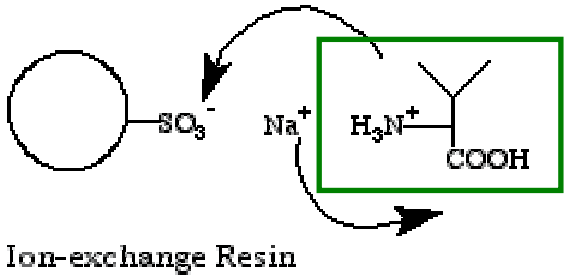


Elution order in ion exchange chromatography is determined by the charge density (charge/radius) of the hydrated ion. In organic acids and bases the elution order is determined by their pKa or pKb (strength of acid or base).

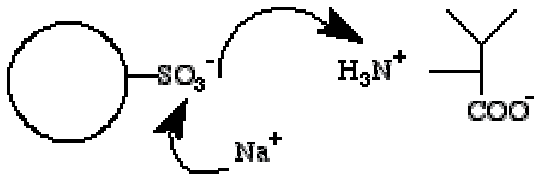
Different Types of Ion Exchange Resins

Cation exchanger

Charge of Analyte



Ion-exchange Resin



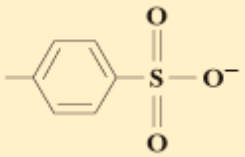
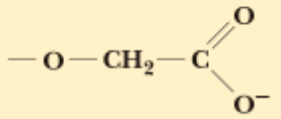
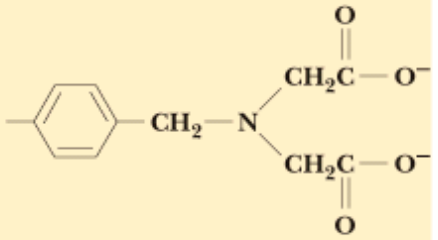
Anion exchanger.

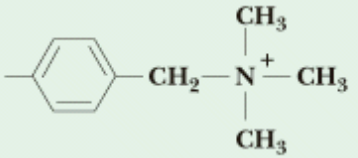
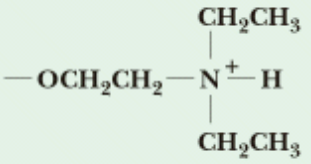
+ charge

pH2

pH4.5

- charge

(a) Cation Exchange Media	Structure
Strongly acidic, polystyrene resin (Dowex–50)	
Weakly acidic, carboxymethyl (CM) cellulose	
Weakly acidic, chelating, polystyrene resin (Chelex–100)	

(b) Anion Exchange Media	Structure
Strongly basic, polystyrene resin (Dowex–1)	
Weakly basic, diethylaminoethyl (DEAE) cellulose	

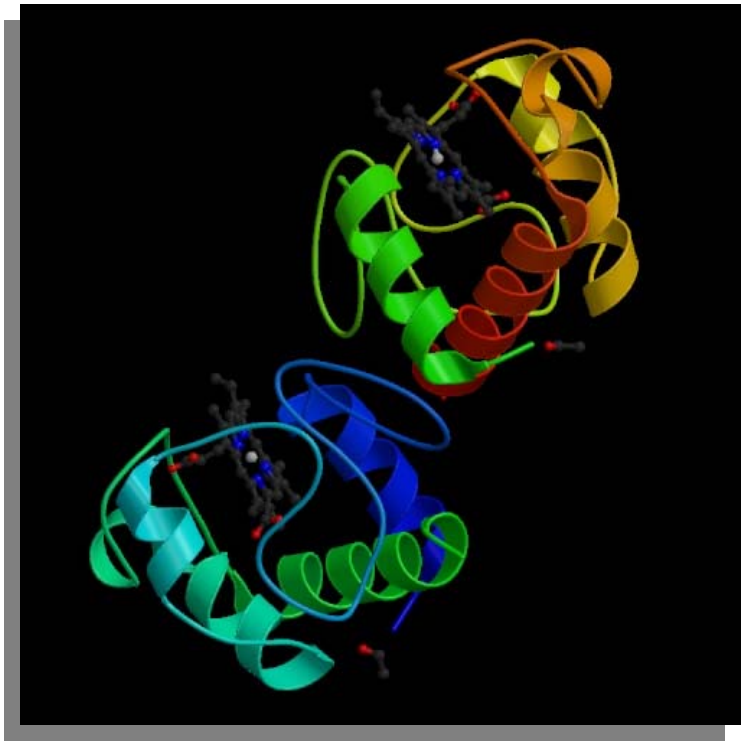
Purification of Cytochrome c

- Function:
  Redox protein involved in cell apoptosis and respiration

- Structure:
  heme protein

  FW 12,384 (horse)

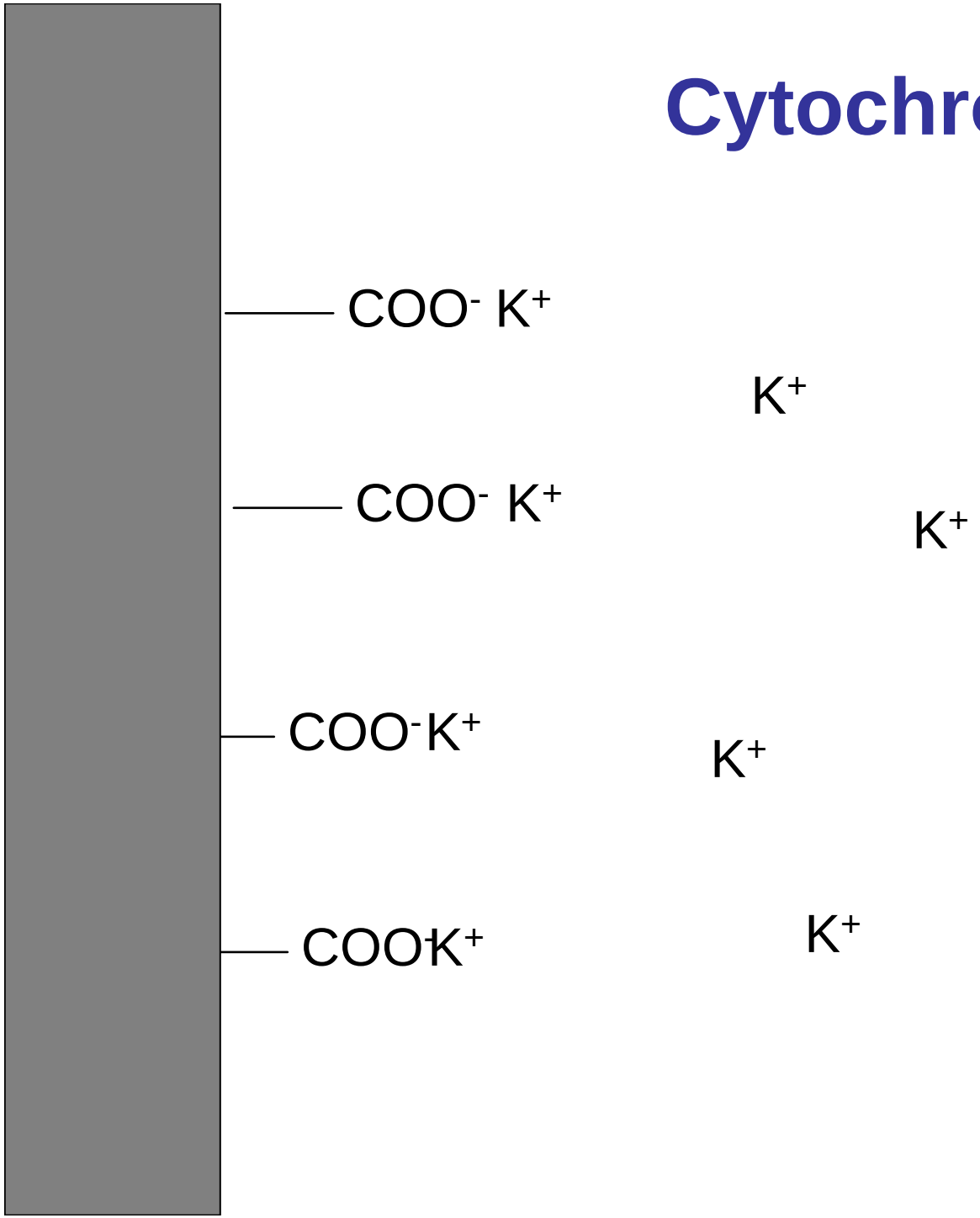
  Basic protein



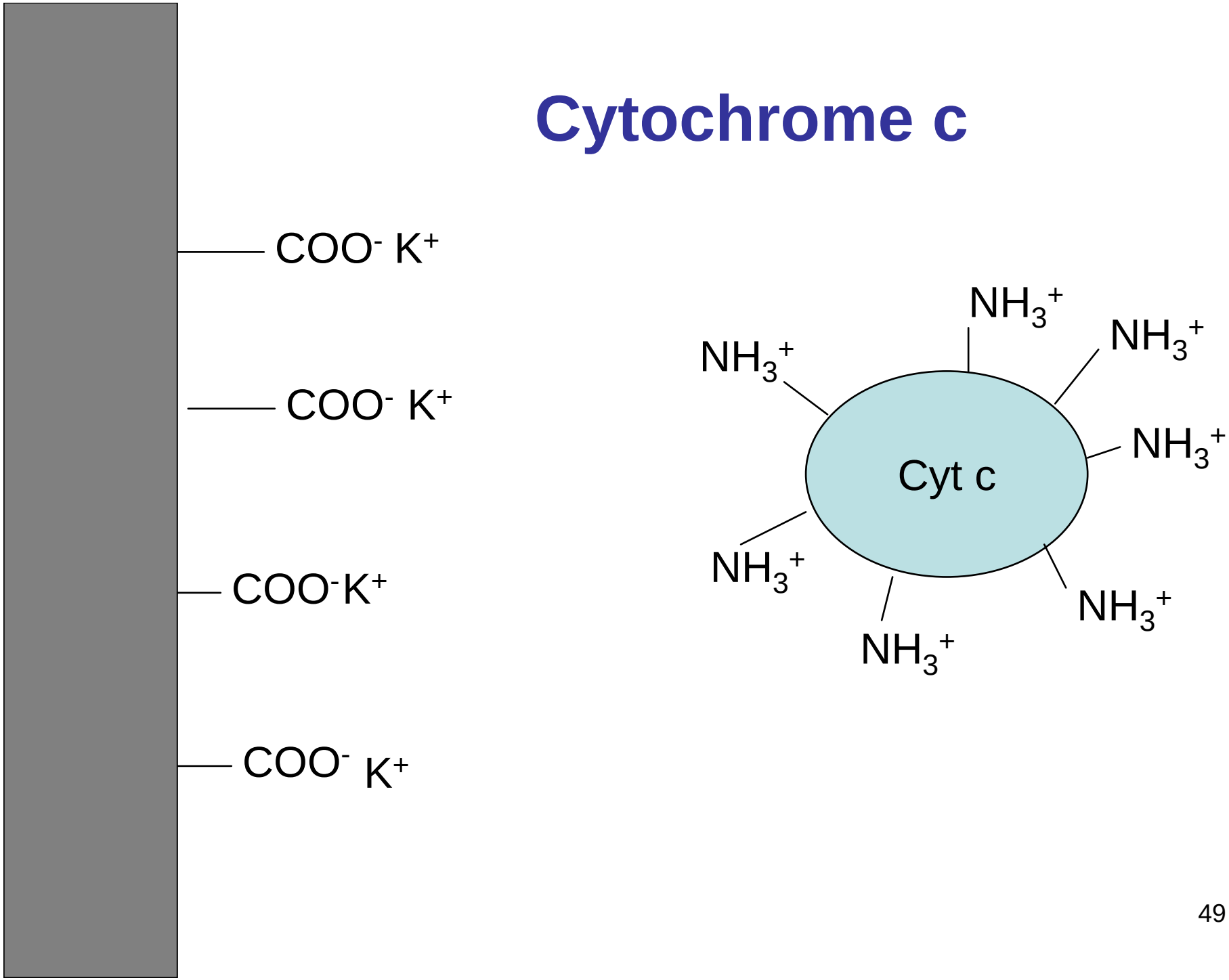
Takano, T., Dickerson, R. E.: Redox conformation changes in refined tuna cytochrome c. *Proc. Natl. Acad. Sci. USA* 77 pp. 6371 (1980)

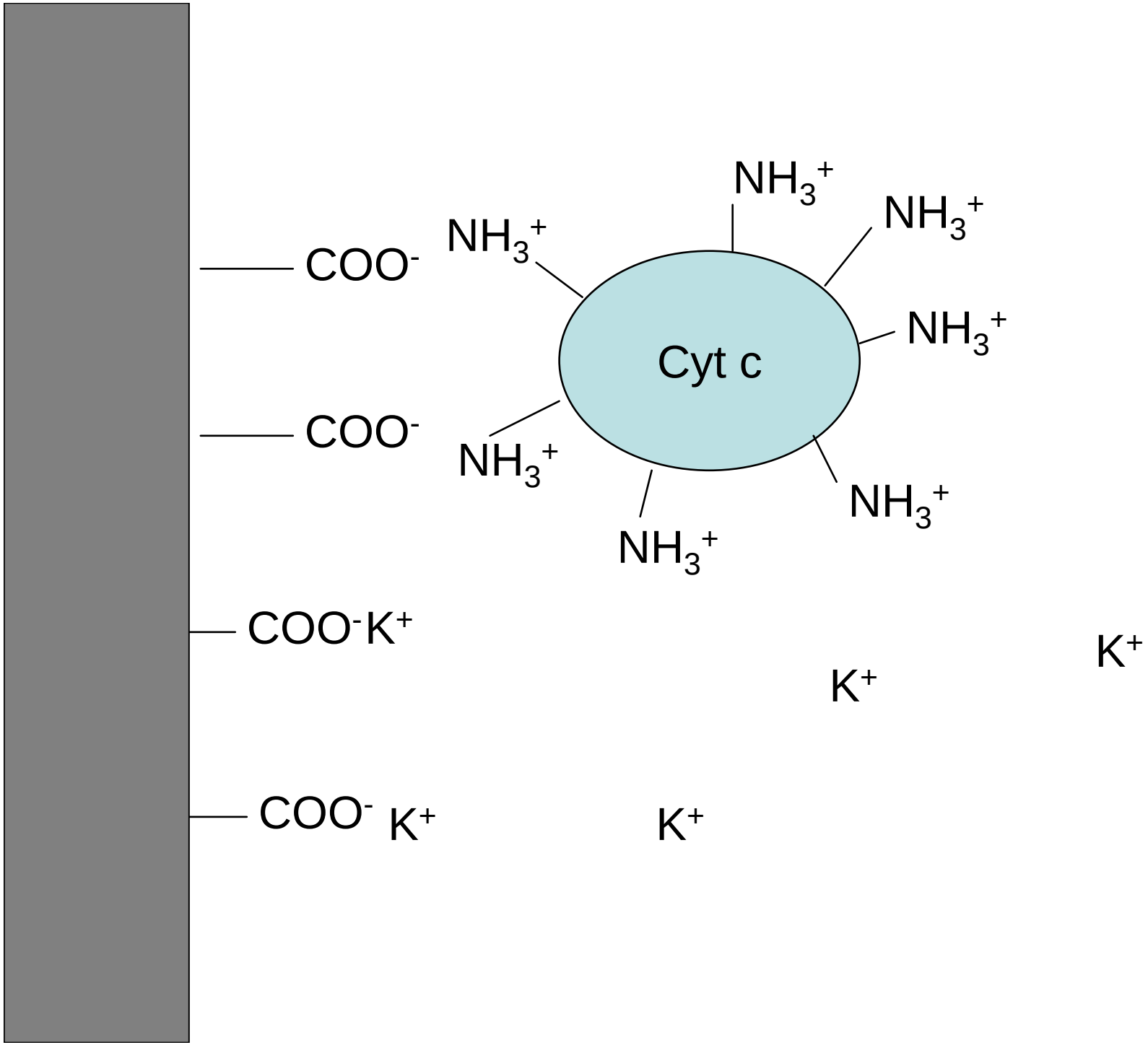
What kind of chromatography method should we use?

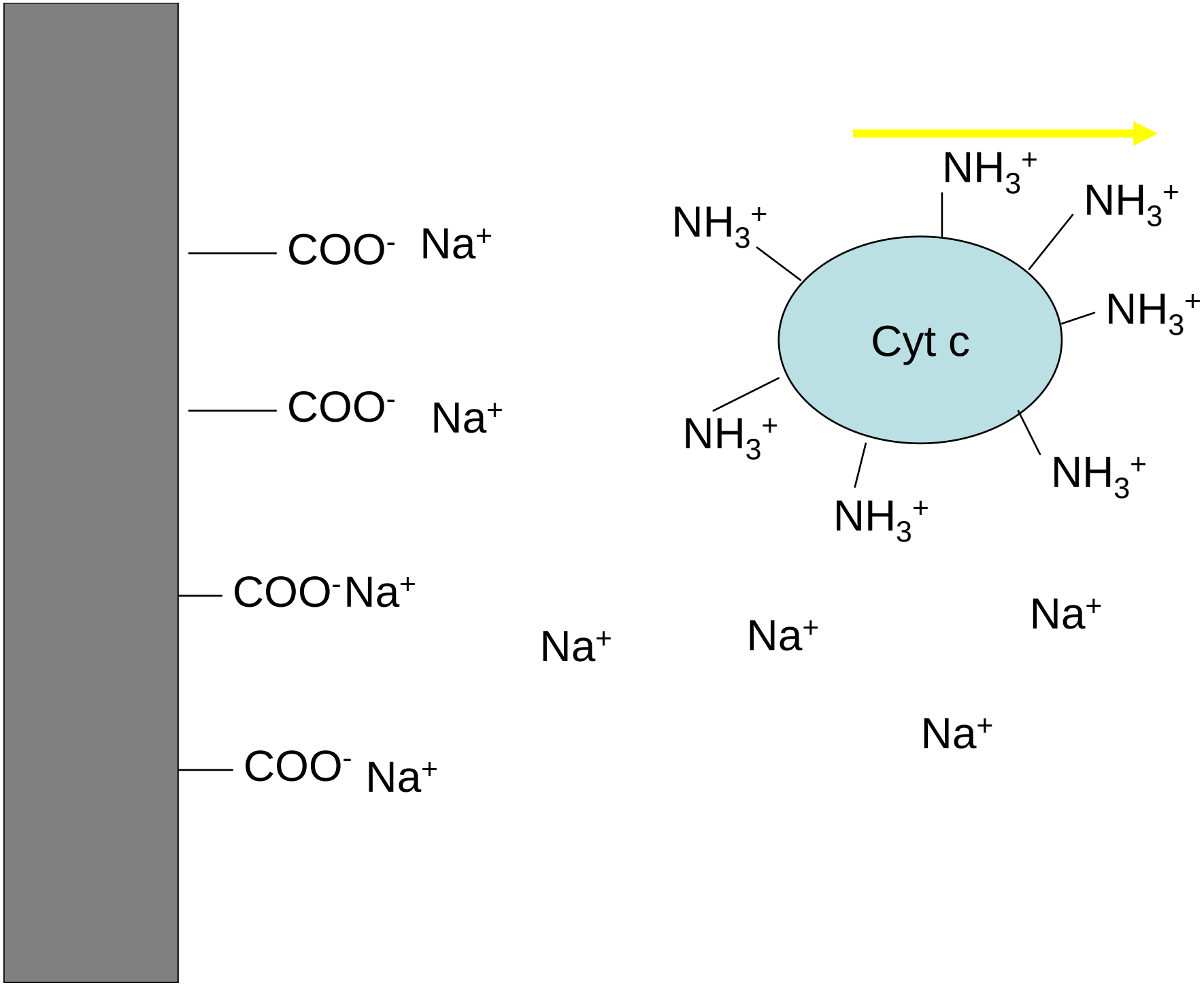
Cytochrome c



Cytochrome c

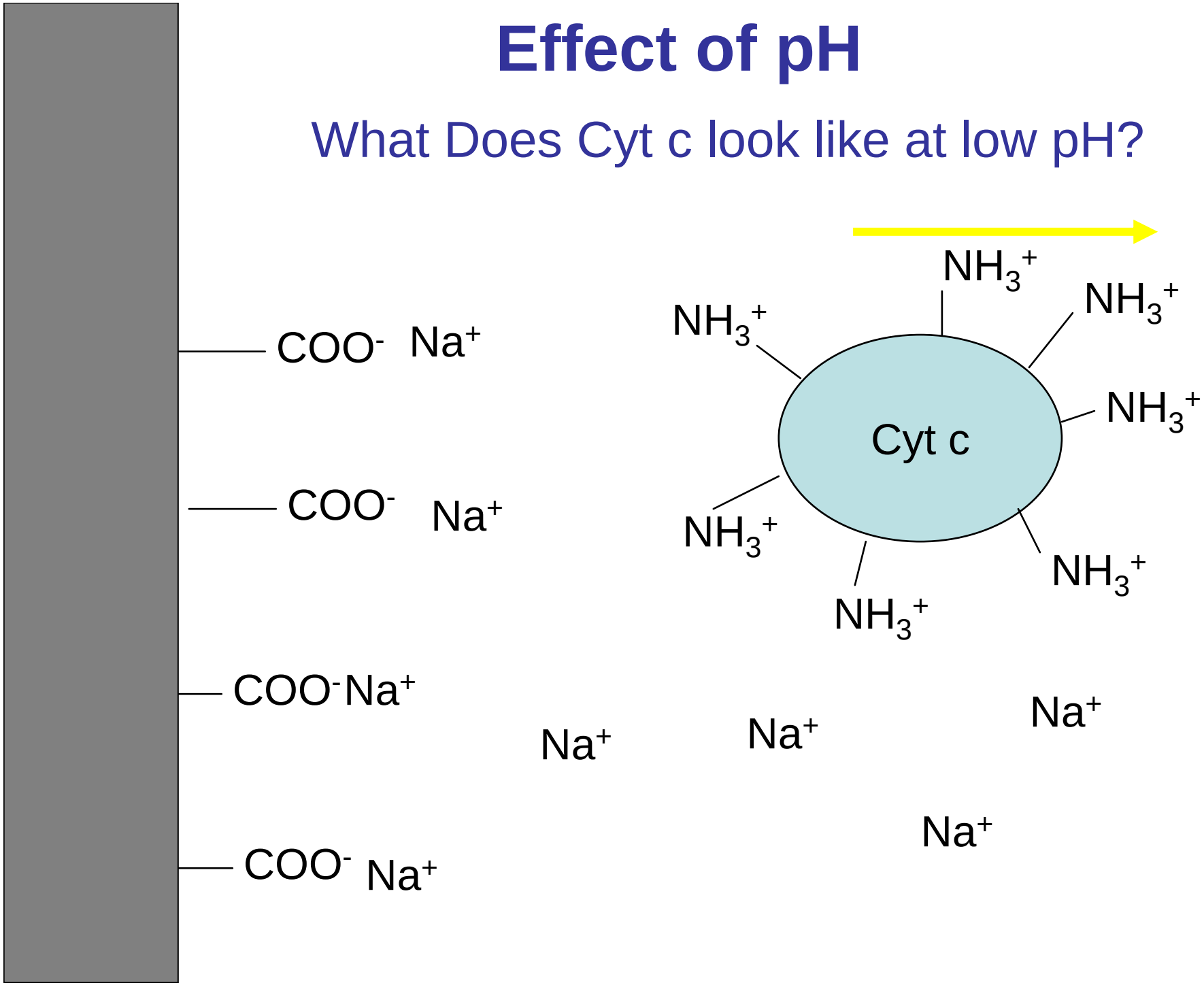



COO-
COO-
COO-K+
COO- K+
NH3+
NH3+
NH3+
NH3+
NH3+
NH3+
Cyt c
K+
K+
K+


COO- Na+
COO- Na+
COO- Na+
COO- Na+
Na+
Na+
Na+
Na+
NH3+
NH3+
NH3+
NH3+
NH3+
NH3+
Cyt c

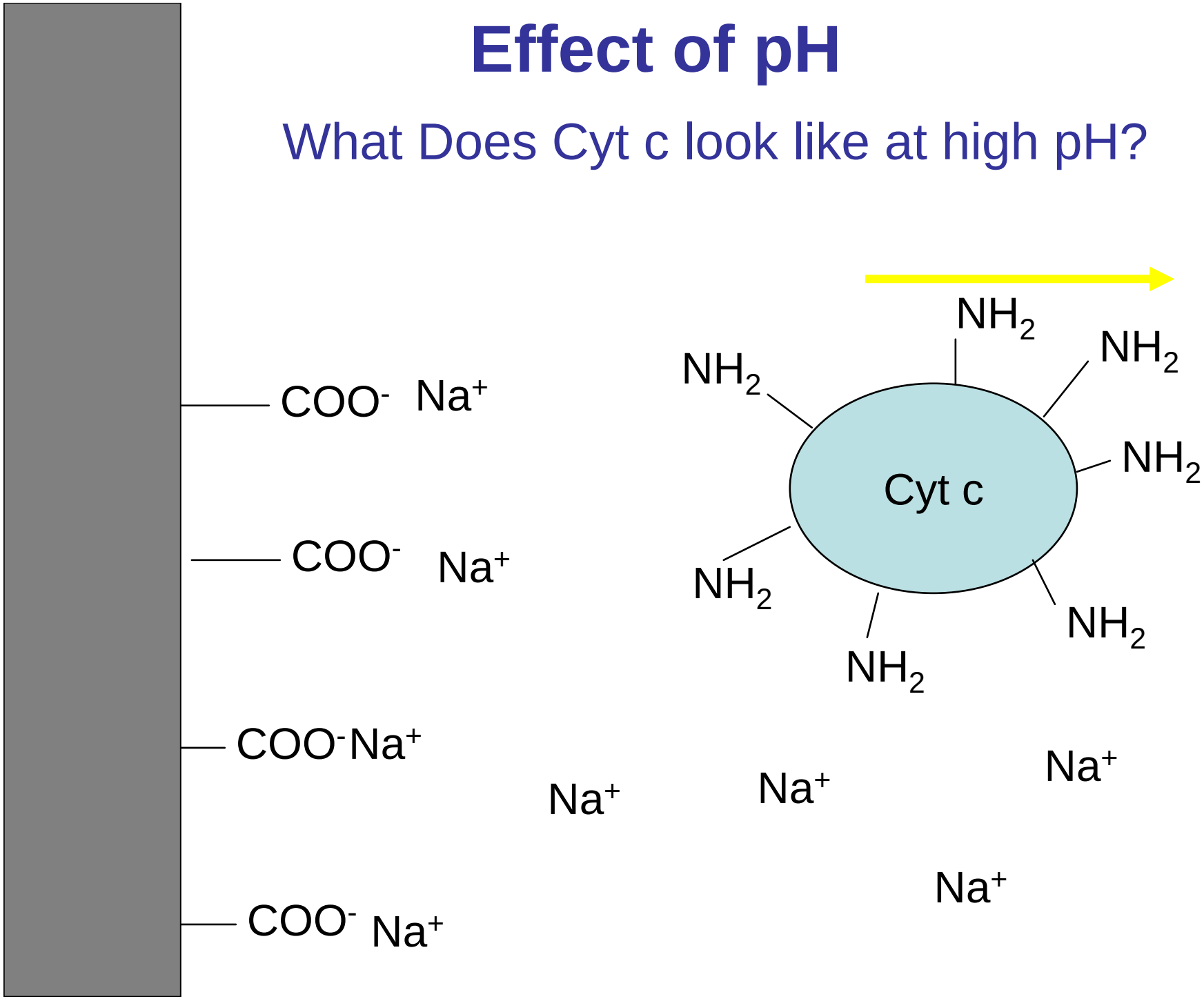
Effect of pH

What Does Cyt c look like at low pH?



Effect of pH

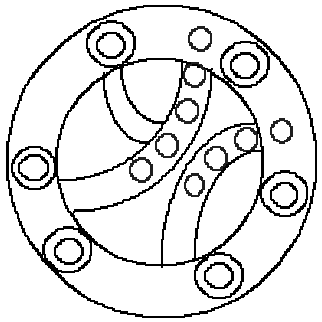
What Does Cyt c look like at high pH?



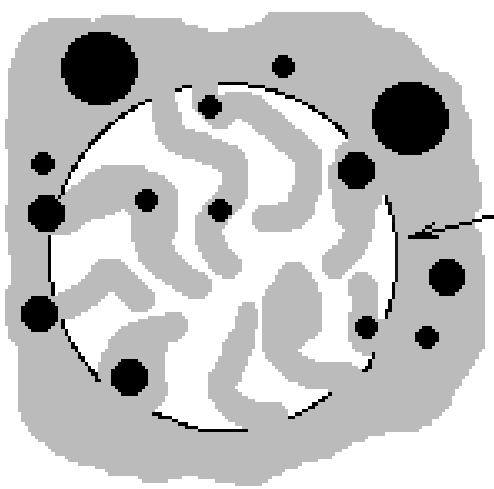
Effect of pH

So low pH more effective for cation exchange than high pH

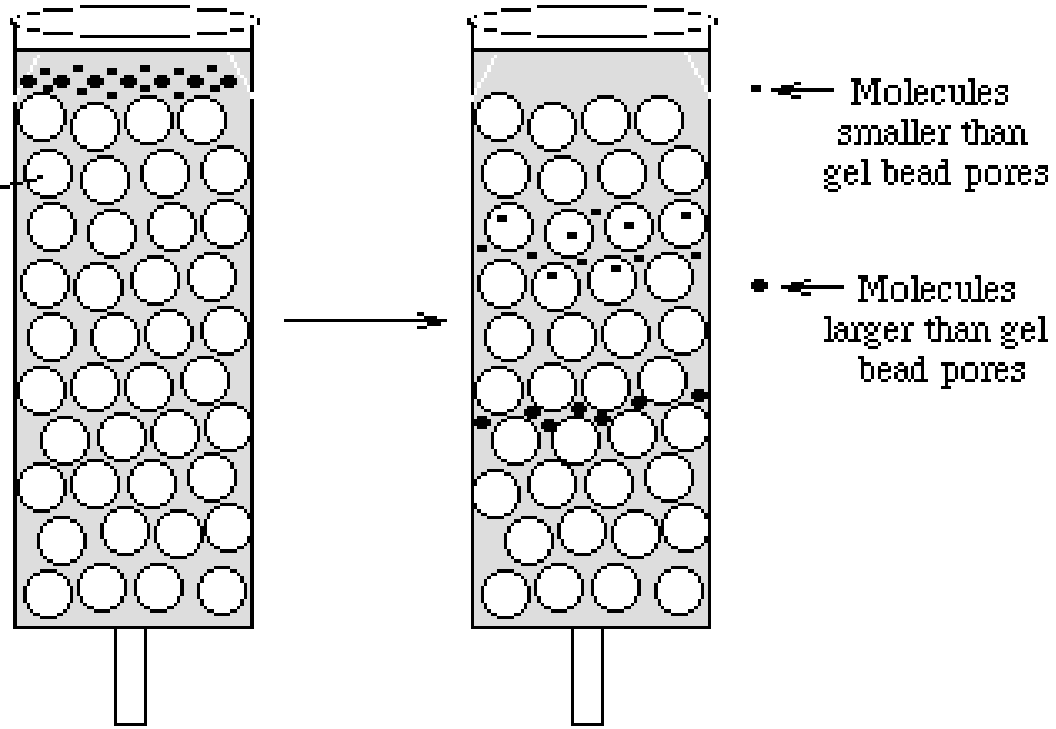
Gel Permeation Chromatography -- Molecular Sieve Chromatography



The separation is based on the molecule size and shape by the molecular sieve properties of a variety of porous material



Gel beads have pores in them of a defined size range which allows smaller molecules to enter but excludes molecules larger than the pore diameters.

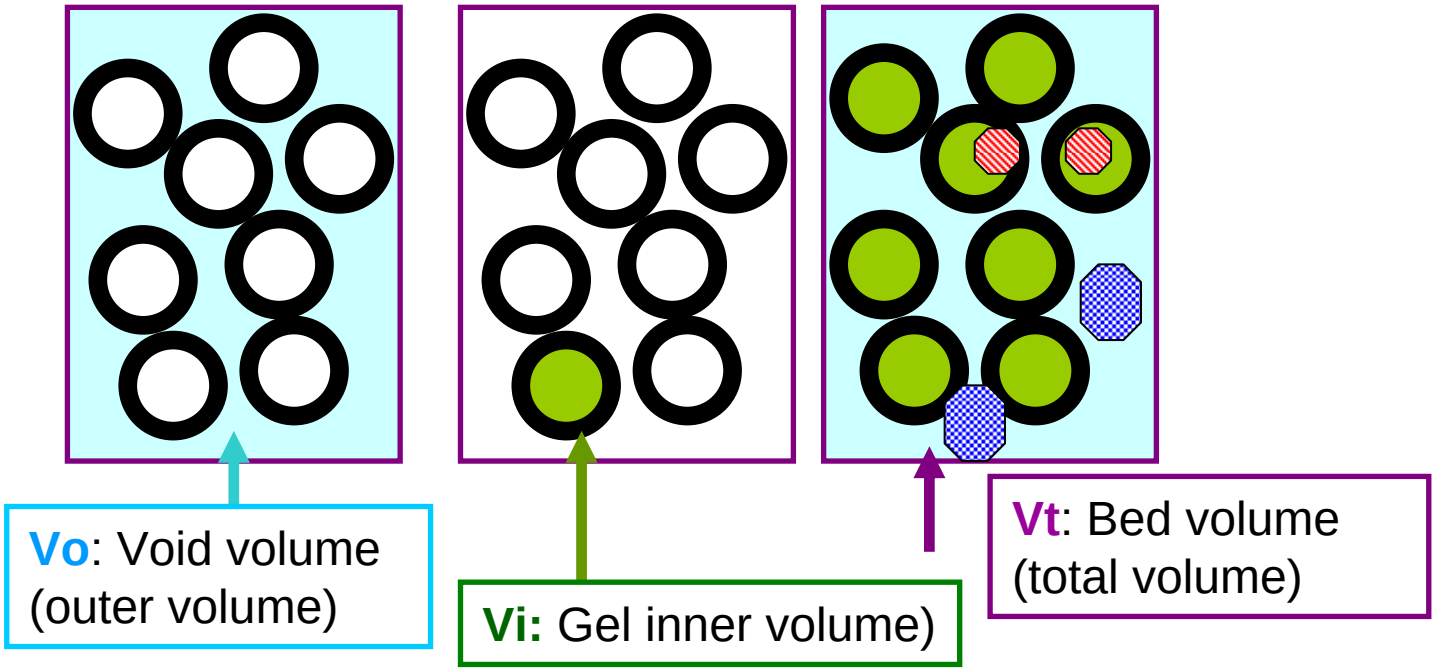


Gel Permeation Chromatography (GPC)

- Also known as ‘size exclusion chromatography’ and ‘gel filtration chromatography’
- Separates molecules on the basis of **molecular size**
- Separation is based on the use of a **porous matrix**. Small molecules penetrate into the matrix more, and their path length of elution is longer.
- Large molecules appear first, smaller molecules later

Mass measurement by Gel Permeation Chromatography

AB 3.4



Ve: Effluent volume (Elution volume of the desired protein)

$Ve = Vo + Kd \times Vi$

$Vi \approx Vt - Vo$

$$Kd = \frac{Ve - Vo}{Vt - Vo}$$

Kd: partition constant of solute between gel matrix and solvent

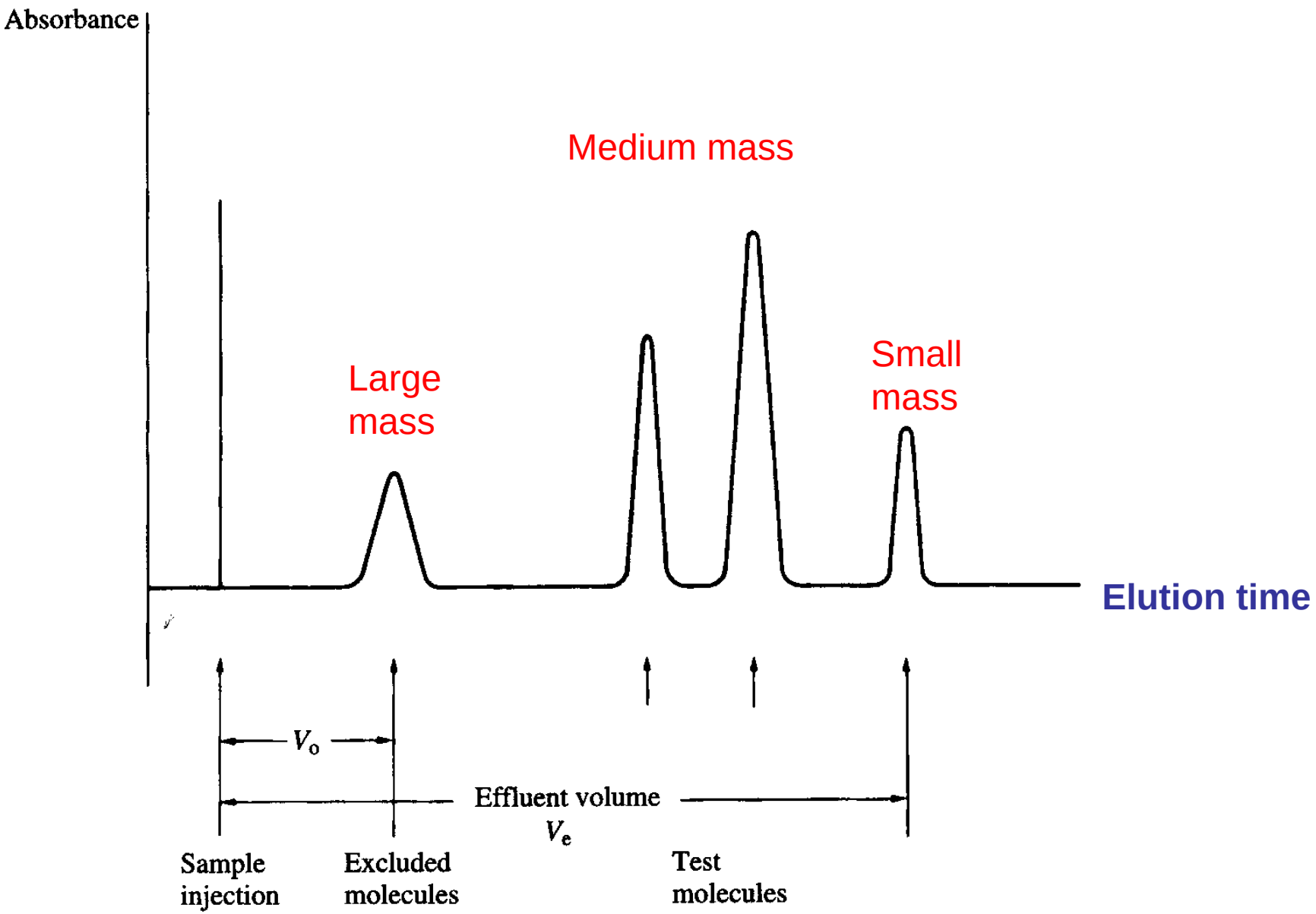
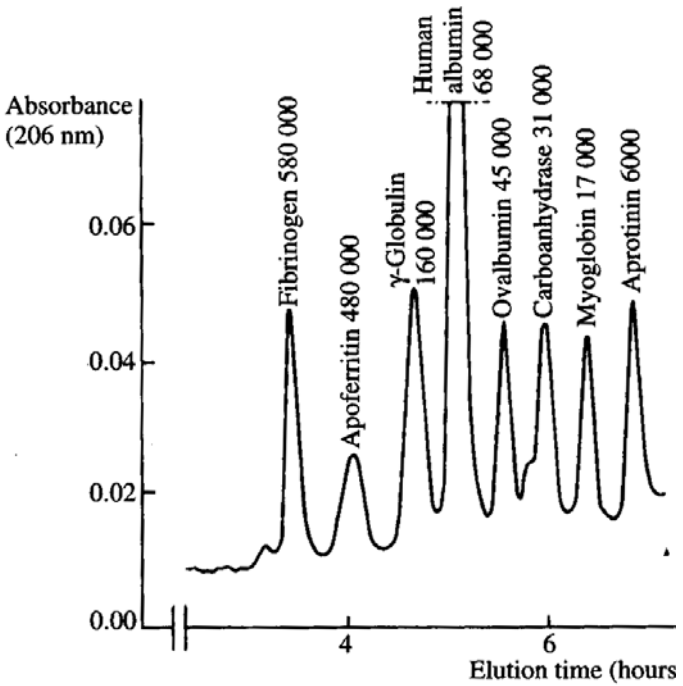
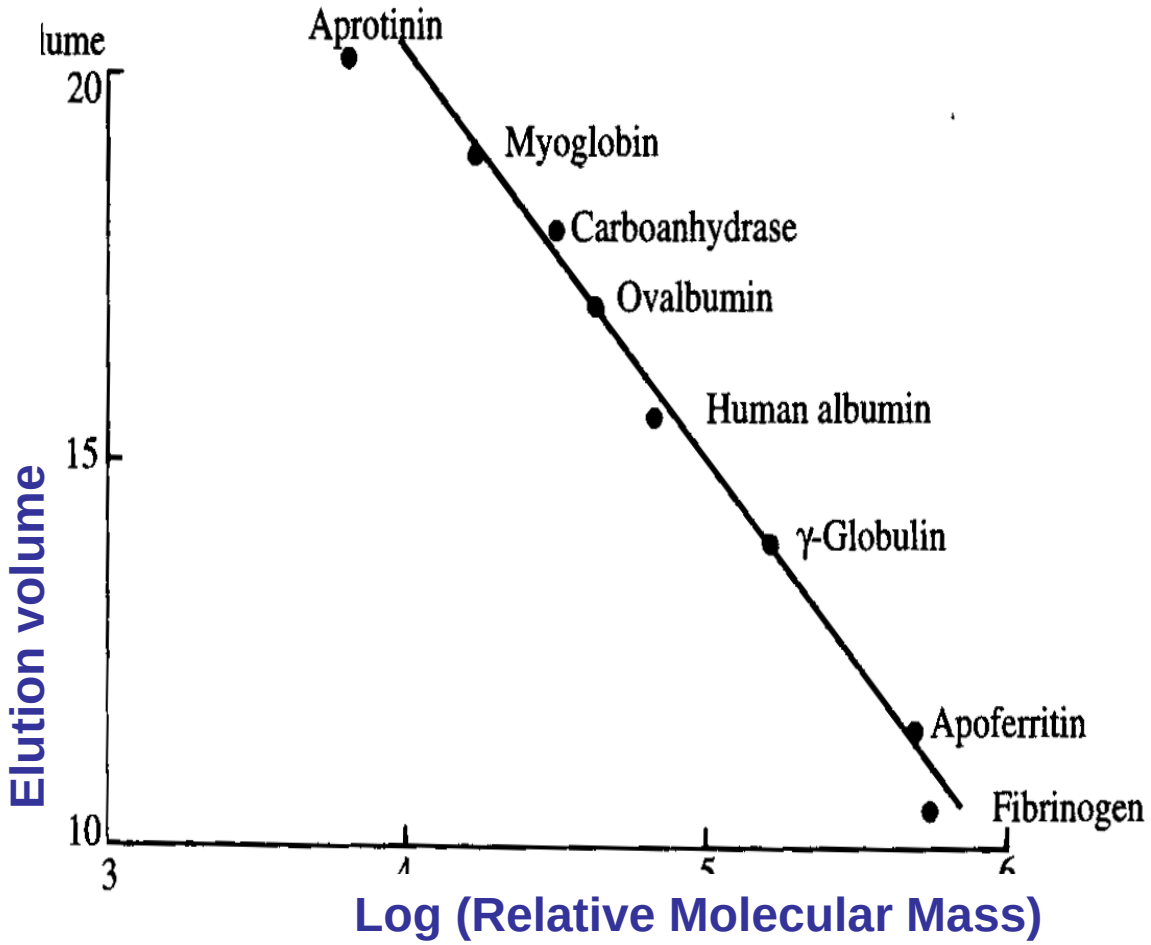


Figure 3.36 **Gel permeation chromatogram.** All molecules larger than the exclusion limit of the gel appear at V_o (the void volume). Molecules which can gain access to the gel structure to varying degrees are eluted in order of decreasing size.

Determination of Mass



The elution volume is approximately a linear function of the logarithm of the relative molecular mass



Affinity Chromatography

- Affinity chromatography is based on a (not necessarily biologically relevant) interaction between a protein of interest, and a ligand immobilized on a stationary phase substrate or product analogue
  - Antigen v.s. Antibody:
  - Enzyme v.s. Inhibitor /Substrate / Cofactor/coenzyme
- Specific protein is eluted by adding reagent which competes with binding

Affinity Matrices

Gel exclusion stationary phase matrices work well in affinity chromatography because

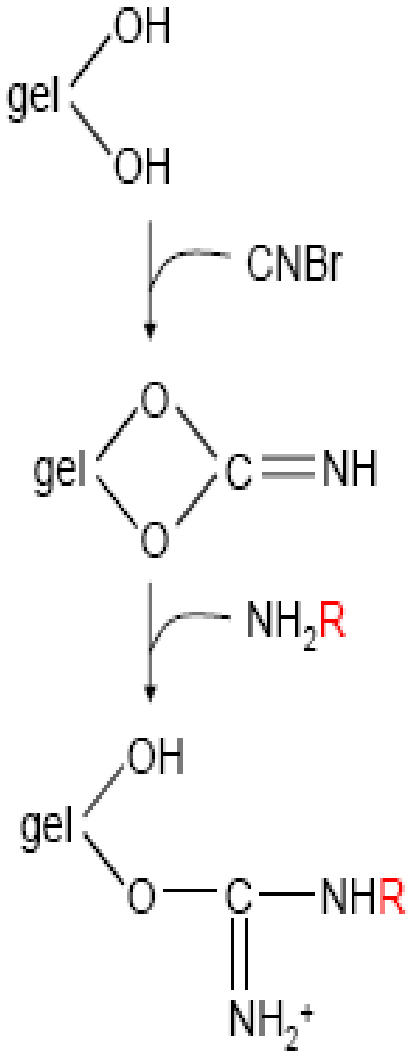
1. Physically and chemically stable under most experimental conditions
2. Relatively free of non-specific adsorption effects
3. Very large pore sizes
4. Reactive functional groups for ligand attachment

Some matrices used include

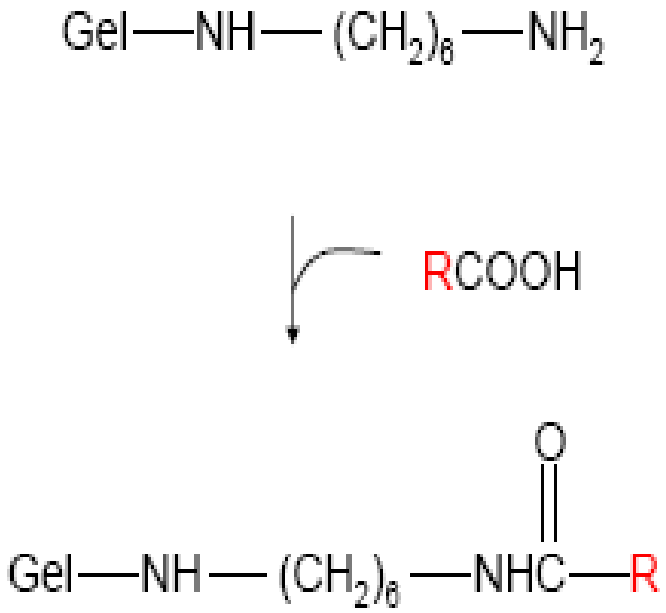
- Agarose (eg. Sepharose 4B)
- Polyvinyl
- Polyacrylamide (Bio-Gel A 150)
- Controlled porosity glass

Examples:

Attachment to CNBr-activated agarose

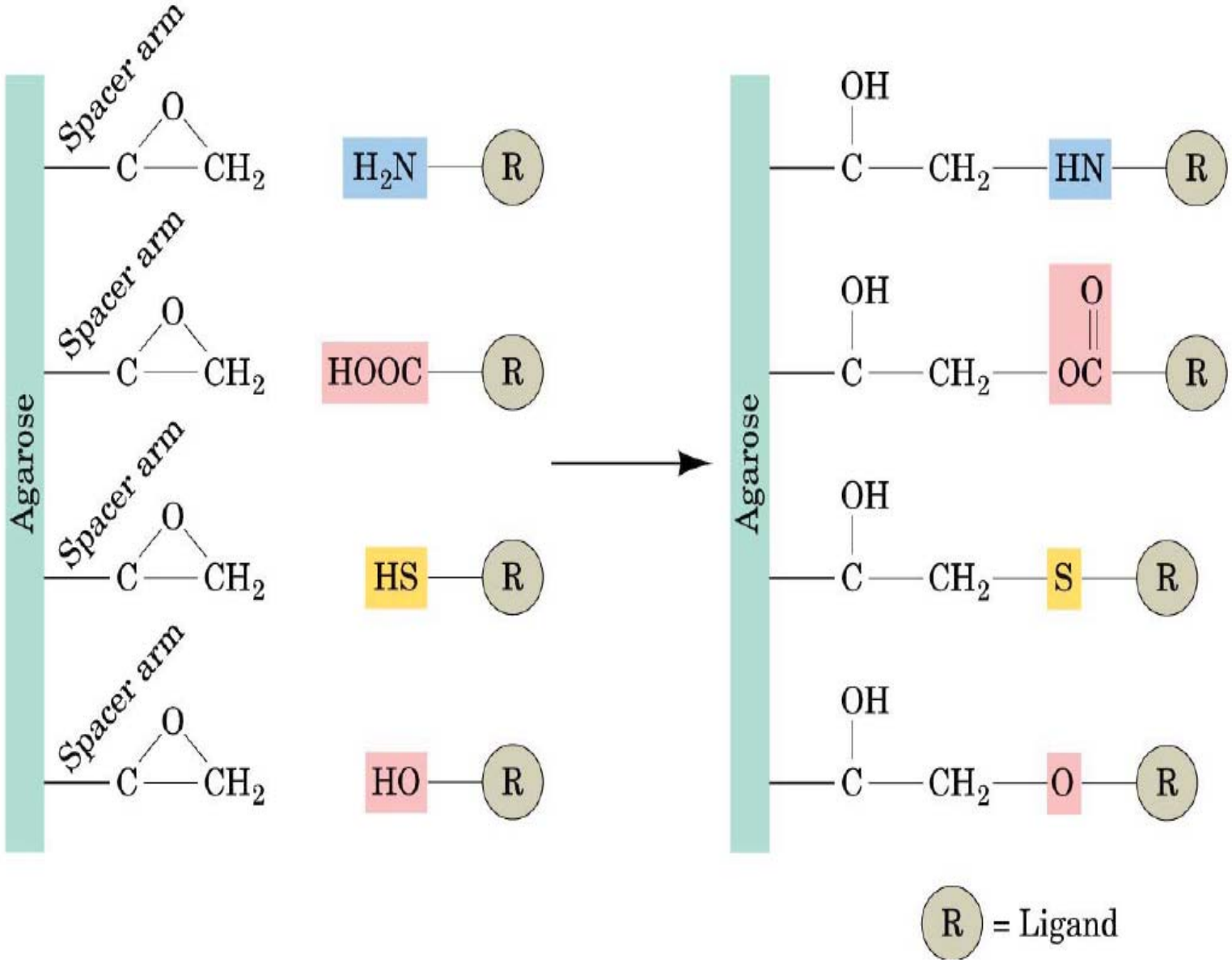


Attachment to 6-aminohexanoic (AH) activated agarose



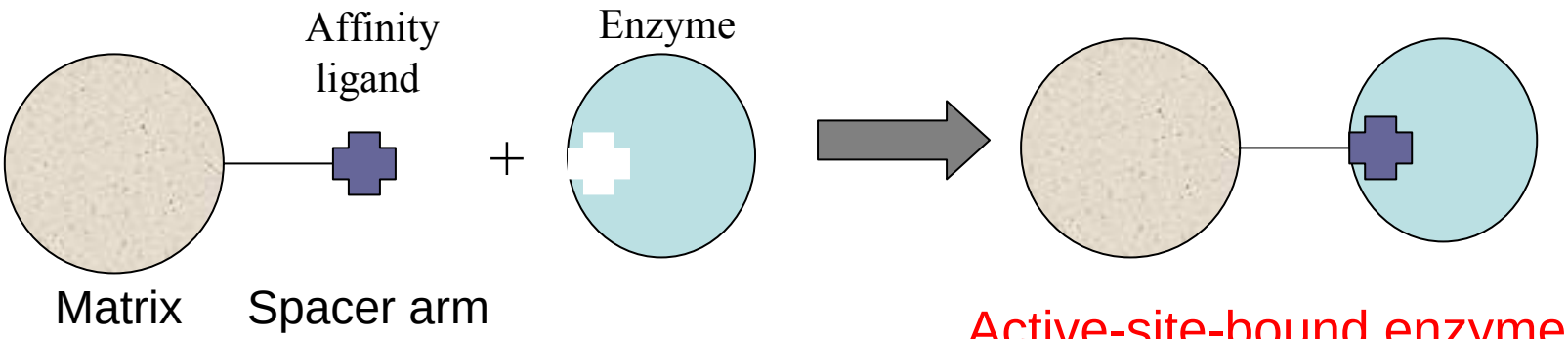
Covalent Attachment of Ligand to the Matrix

Derivation of Epoxy-Activated Agarose

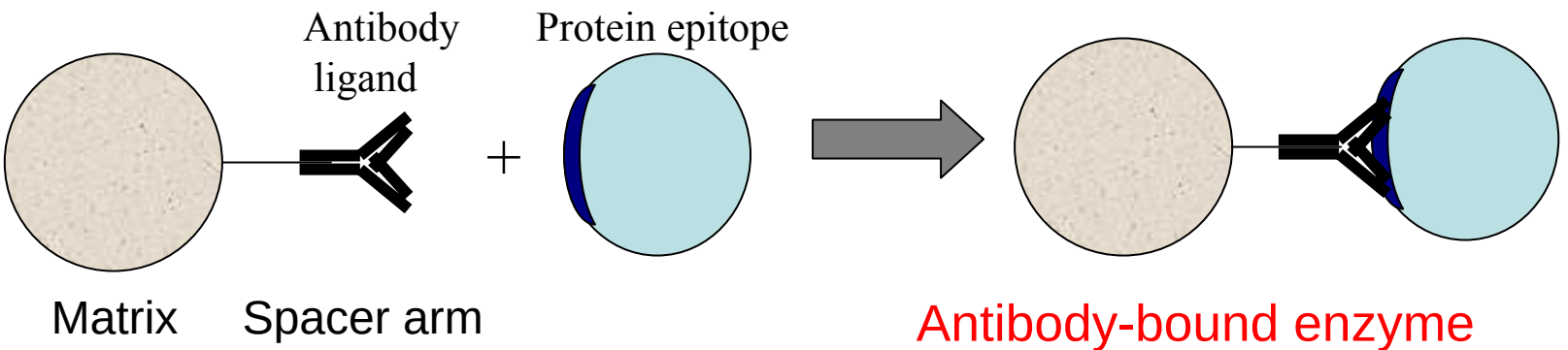


Affinity chromatography

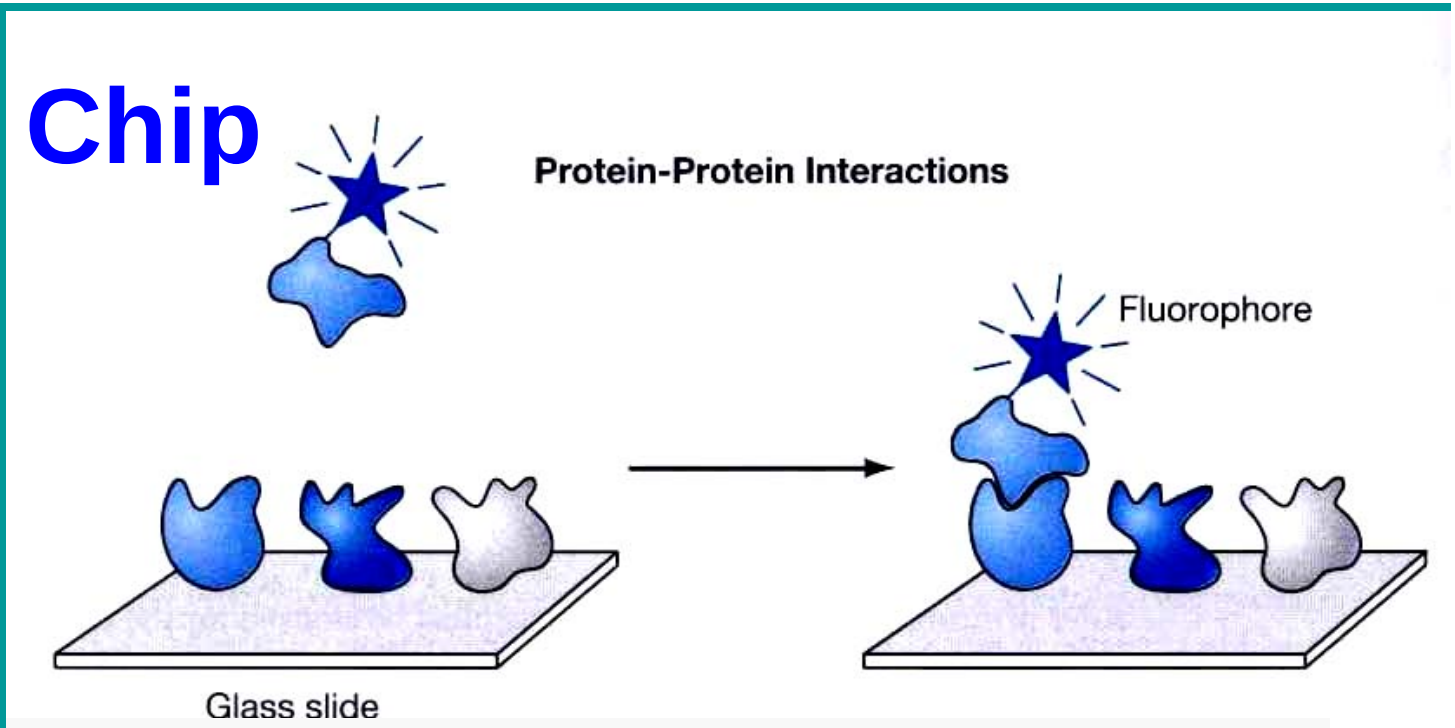
- Substrate analogue affinity chromatography

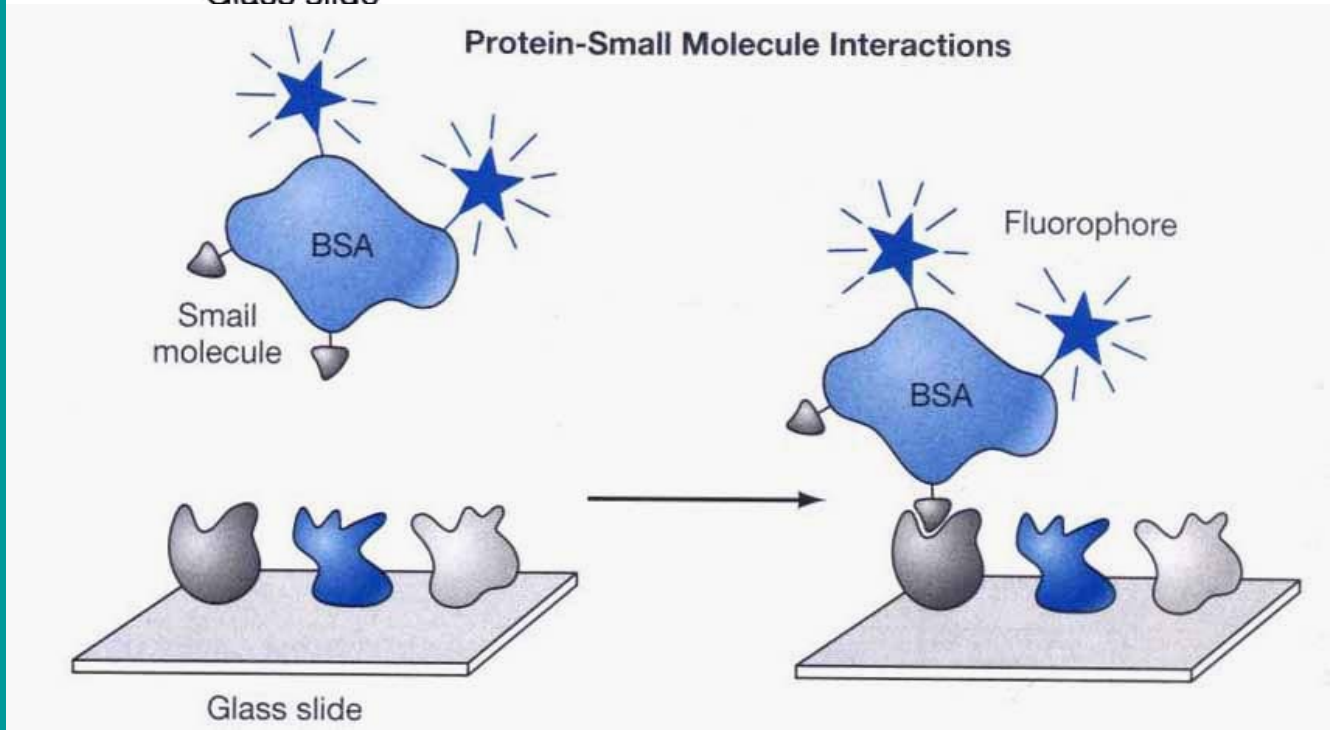


- Immunoaffinity chromatography

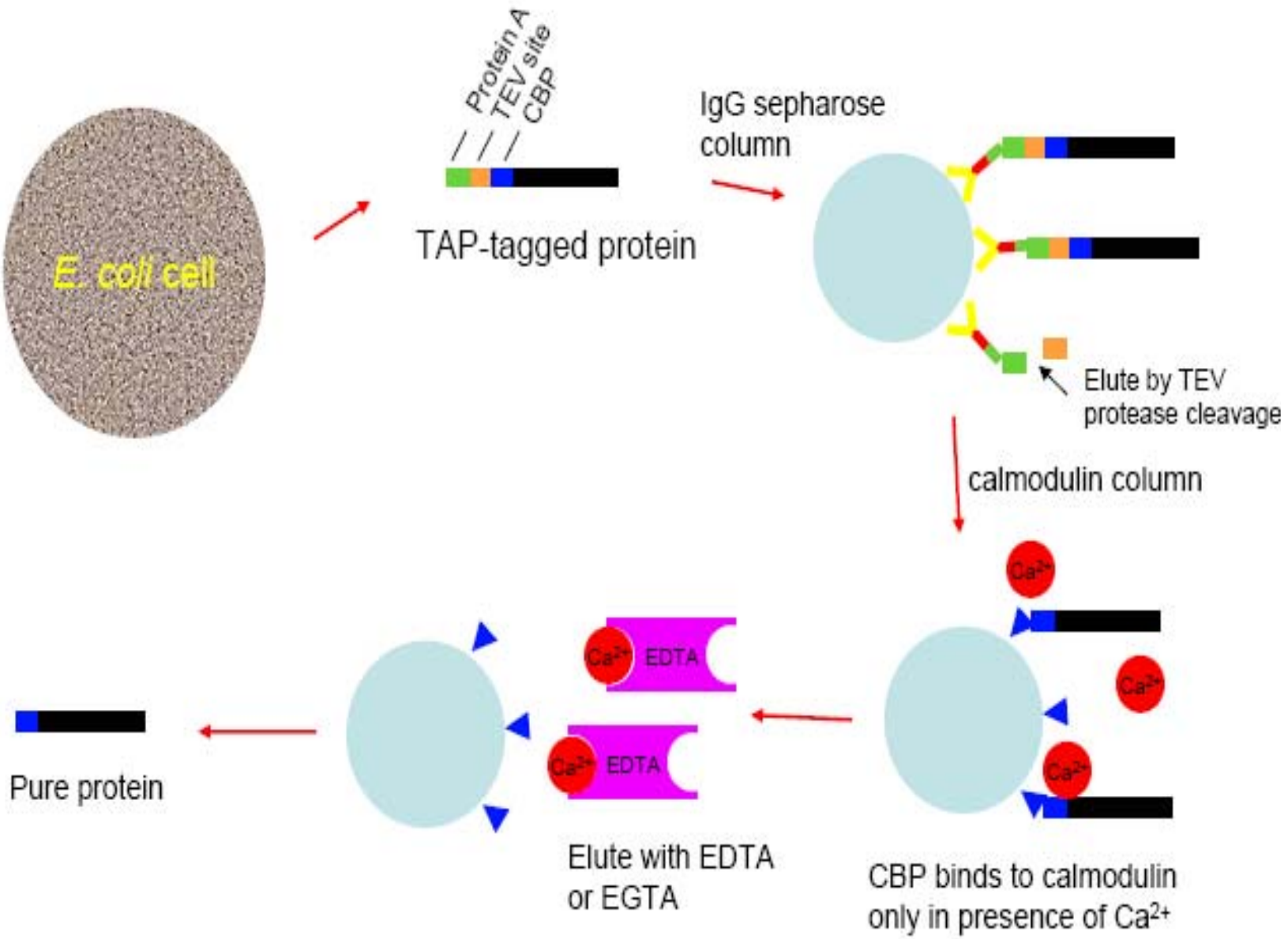


Protein Chip





Tandem Affinity Purification (TAP)



Functional organization of the yeast proteome by systematic analysis of protein complexes

Anne-Claude Gavin*, Markus Bösche*, Roland Krause*, Paola Grandi*, Martina Marzioch*, Andreas Bauer*, Jörg Schultz*, Jens M. Rick*, Anne-Marie Michon*, Cristina-Maria Cruciat*, Marita Remor*, Christian Höfert*, Malgorzata Schelder*, Miro Brajenovic*, Heinz Ruffner*, Alejandro Merino*, Karin Klein*, Manuela Hudak*, David Dickson*, Tatjana Rudi*, Volker Gnau*, Angela Bauch*, Sonja Bastuck*, Bettina Huhse*, Christina Leutwein*, Marie-Anne Heurtier*, Richard R. Copley†, Angela Edelmann*, Erich Querfurth*, Vladimir Rybin*, Gerard Drewes*, Manfred Raida*, Tewis Bouwmeester*, Peer Bork†, Bertrand Seraphin†‡, Bernhard Kuster*, Gitte Neubauer* & Giulio Superti-Furga*†

* *Cellzome AG, Meyerhofstrasse 1, 69117 Heidelberg, Germany*
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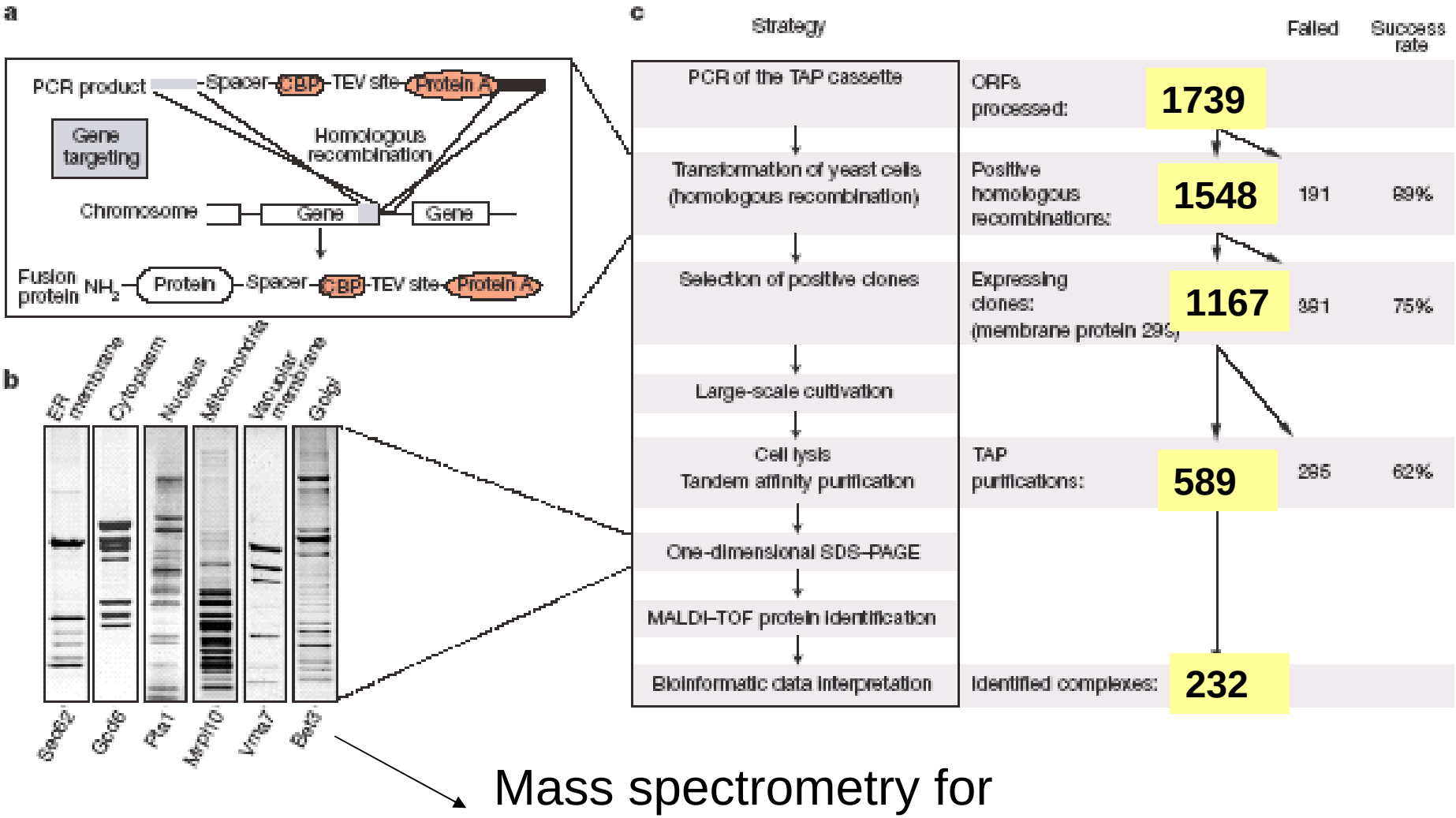
‡ *CGM-CNRS, 91198 Gif sur Yvette Cedex, France*

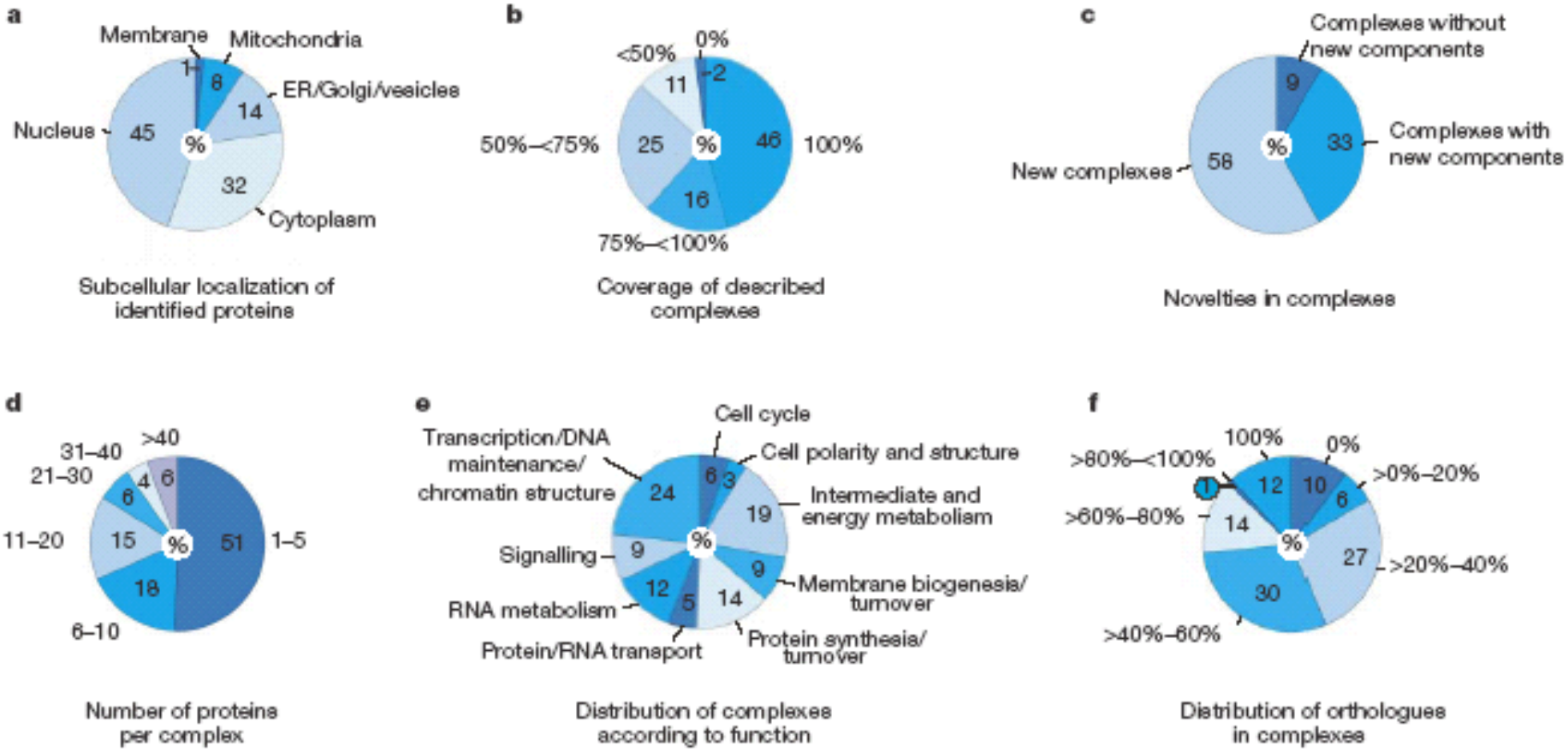
Most cellular processes are carried out by multiprotein complexes. The identification and analysis of their components provides insight into how the ensemble of expressed proteins (proteome) is organized into functional units. We used tandem-affinity purification (TAP) and mass spectrometry in a large-scale approach to characterize multiprotein complexes in *Saccharomyces cerevisiae*. We processed 1,739 genes, including 1,143 human orthologues of relevance to human biology, and purified 589 protein assemblies. Bioinformatic analysis of these assemblies defined 232 distinct multiprotein complexes and proposed new cellular roles for 344 proteins, including 231 proteins with no previous functional annotation. Comparison of yeast and human complexes showed that conservation across species extends from single proteins to their molecular environment. Our analysis provides an outline of the eukaryotic proteome as a network of protein complexes at a level of organization beyond binary interactions. This higher-order map contains fundamental biological information and offers the context for a more reasoned and informed approach to drug discovery.

Tandem-affinity purification (TAP)

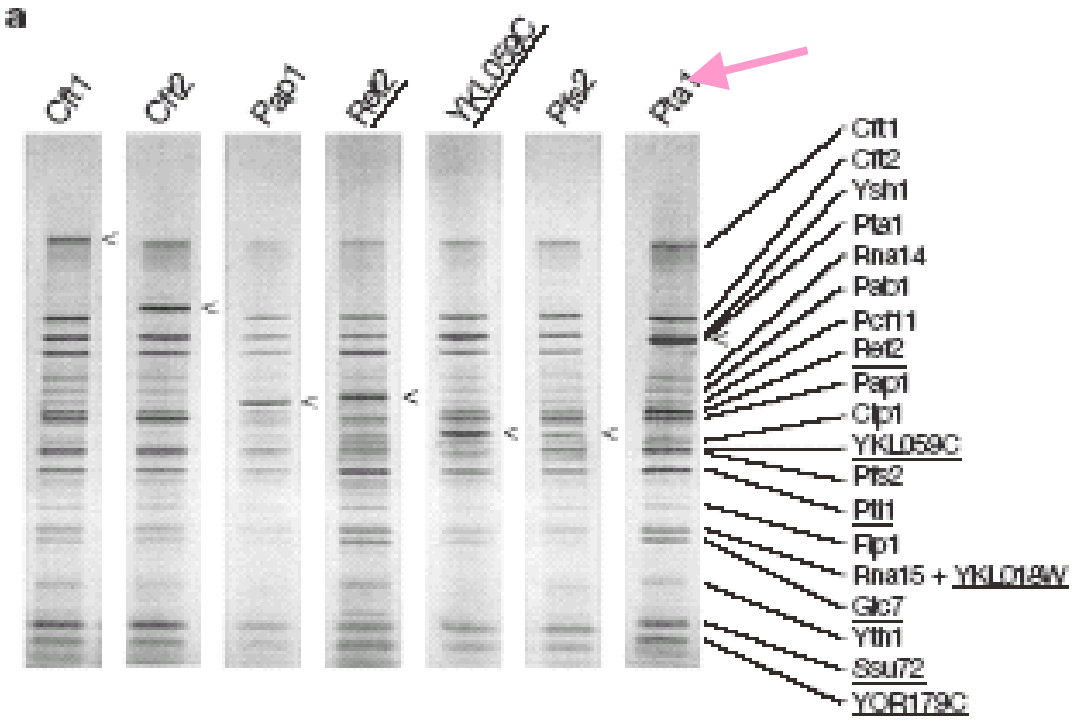


Mass spectrometry for component identification

Statistics of identified proteins and complexes

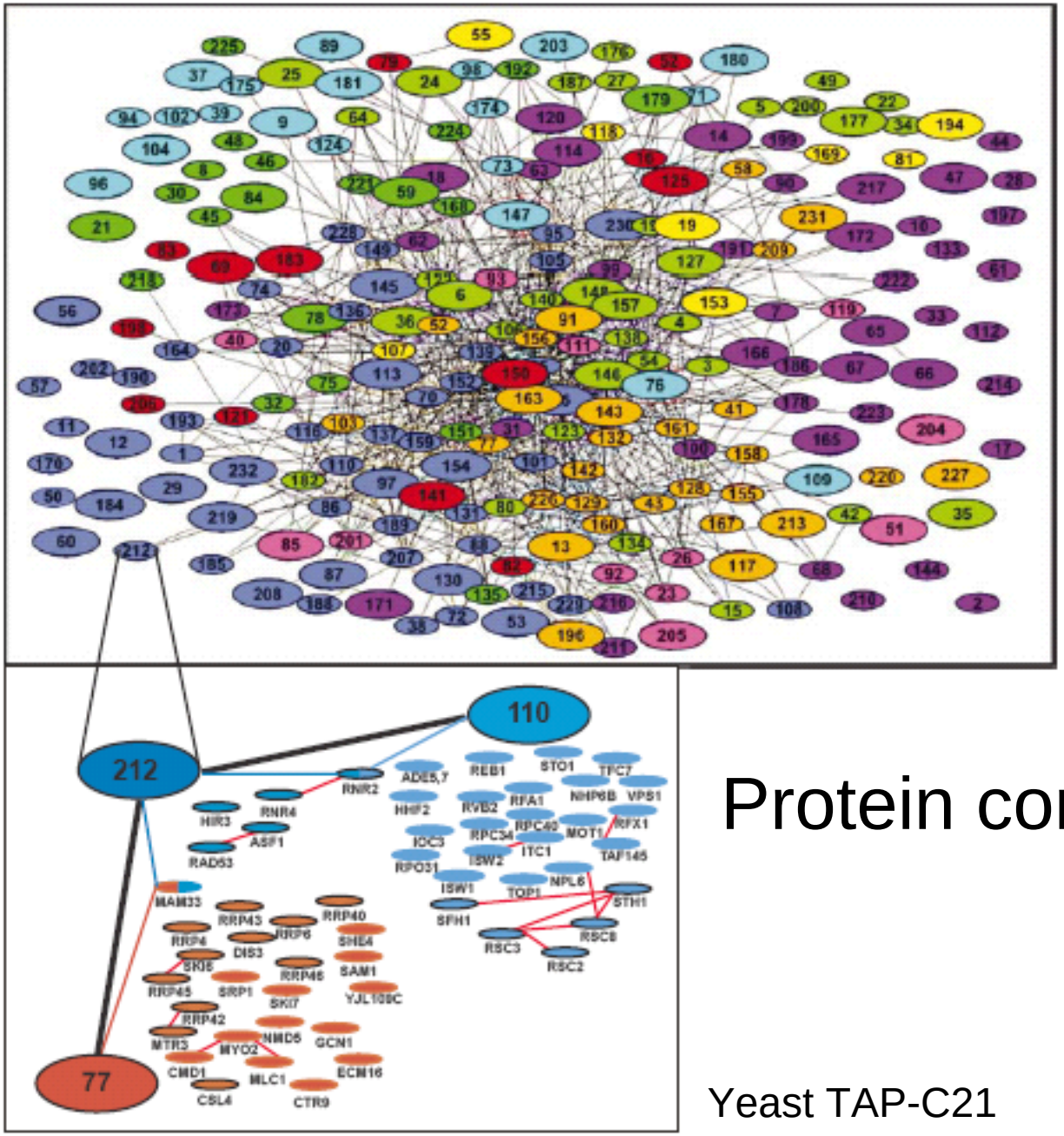


Primary validation of complex composition by `reverse' purifcation



The bands of the tagged proteins are indicated by arrowheads.

New components



Protein complex network

Yeast TAP-C21

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Journal of Chromatography B, 817 (2005) 35–47

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Review

Multidimensional separation of peptides for effective proteomic analysis

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It is generally accepted that no single chromatographic or electrophoretic procedure is capable of resolving the complex mixture of peptides. Therefore, combining two or more **orthogonal** (multimodal) separation procedures dramatically improves the overall resolution and results in a larger number of peptides being identified from complex proteome digests.

Chromatographic Modes of Protein Purification

Chromatographic Mode	Acronym	Separation Principle
Non-interactive modes of liquid chromatography		
Size-exclusion chromatography	**SEC**	Differences in molecular size
Slalom chromatography (for DNA)	-	Diff. in length and flexibility
Interactive modes of liquid chromatography		
Ion-exchange chromatography	**IEC**	Electrostatic interactions
Normal-phase chromatography	**NPC**	Polar interactions
Reversed-phase chromtography	**RPC**	Dispersive interactions
Hydrophobic interaction chromatography	**HIC**	Dispersive interactions
Affinity chromatography	**AC**	Biospecific interaction
Metal interaction chromatography	**MIC**	Complex w/ an immobilized metal

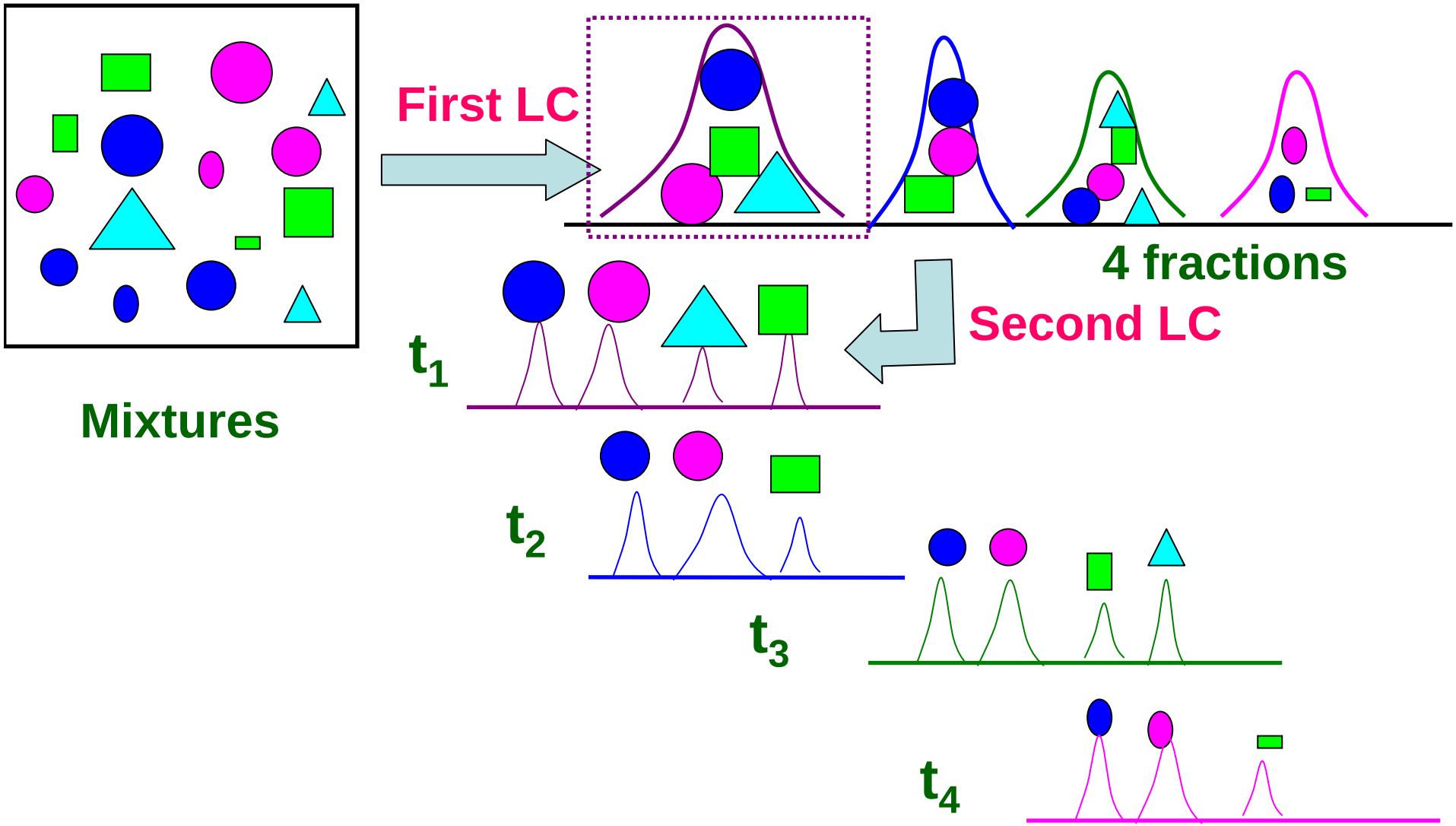
(Christian G. Huber, Biopolymer Chromatography, Encylcopedia in analytical chemistry, 2000)

Multidimensional-Chromatography

- Transferring a fraction or fractions from one chromatographic medium (usually a column) to a secondary (or additional) chromatographic medium (column or columns) for further separation. The technique can be used for further resolution of complex mixtures that cannot be separated entirely on a single medium.

  - IEF-SCX
  - SCX-RP
  - SCF-Affinity

Two-dimensional Chromatography (2D-LC)



血液中含有許多和疾病相關的標記蛋白質

- 疾病的預防及早期診斷
- 藥物治療的成效
- 研究疾病的機制
- 癌症篩檢

臨床癌症篩檢項目

癌症標記蛋白質	(1). 甲種胎兒蛋白 α-FP	肝細胞癌。
	(2). 癌胚抗原（CEA）	大腸癌、胃癌、食道腺癌、胰臟癌、膽道癌、肺癌、乳癌、肝細胞癌、卵巢癌、甲狀腺隨樣癌。
	(3). CA19-9 抗原	胰臟癌、大腸癌、胃癌、肺癌、膽道癌、卵巢癌、肝癌、食道癌、乳癌、淋巴癌。
	(4). 前列腺特異抗原 PSA	前列腺癌。
	(5). CA125	卵巢癌、子宮頸癌、胰臟癌、肺癌、大腸癌、膽道癌、肝癌、子宮內膜癌、胃癌、良性卵巢腫瘤。
	(6). CA153	乳癌。
C反應蛋白檢測	CRP	當體內有急性炎症或細菌感染

血漿中蛋白質的組成複雜，濃度差異很大

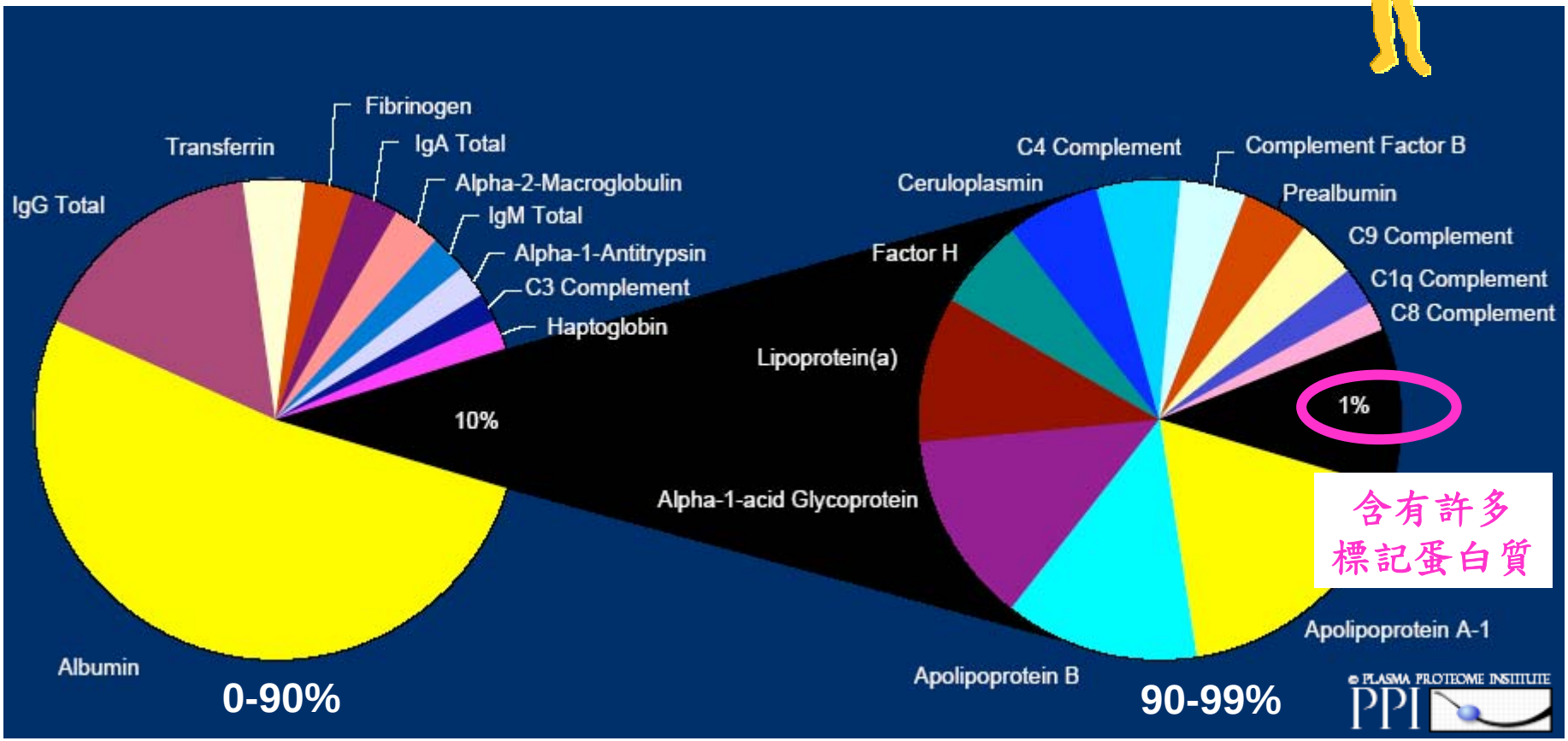
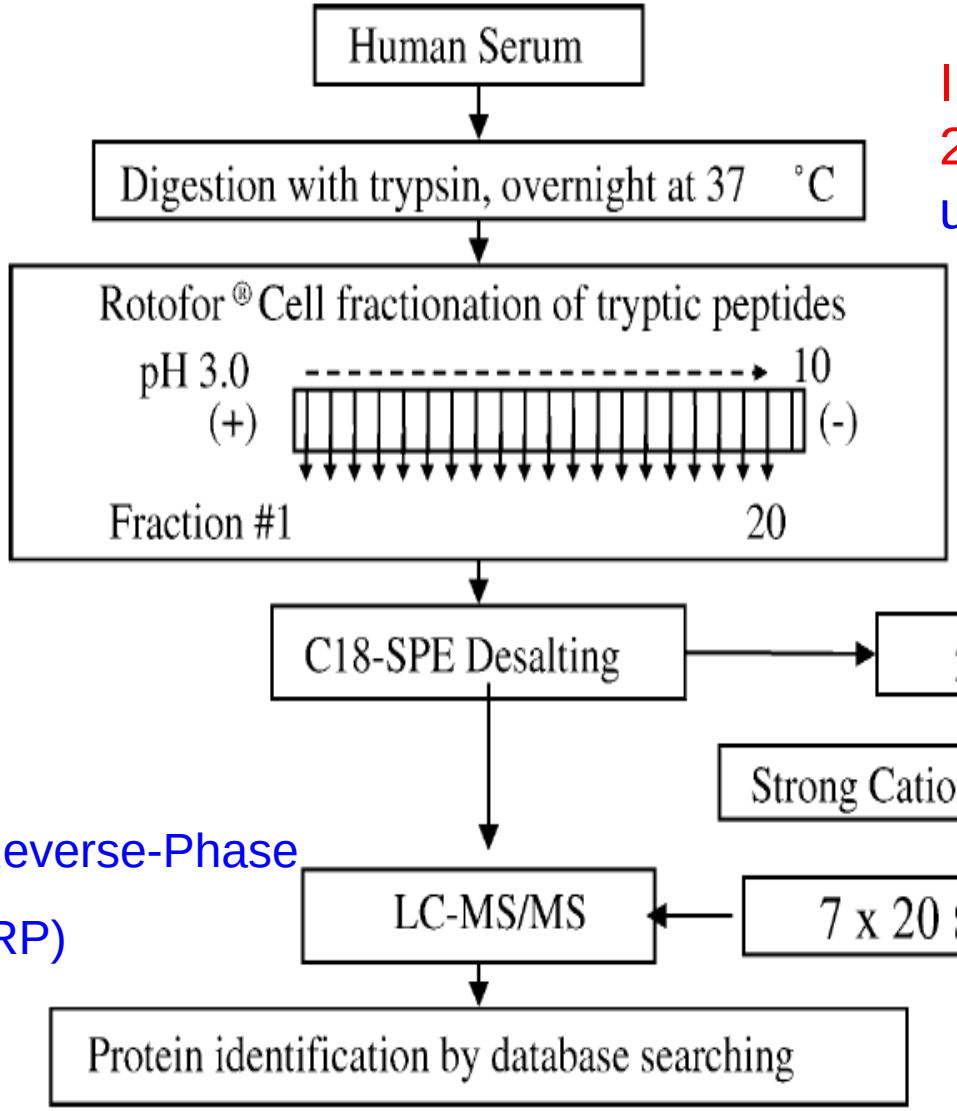


Fig. **Pie chart** representing the relative contribution of proteins within plasma.
Twenty-two proteins constitute 99% of the protein content of plasma

Ref: www.plasmaproteome.org
Molecular & Cellular Proteomics 2:1096–1103, 2003

3D LC for Global Analysis of Serum Proteome



IEF-SCX-RP

2071 peptides (identification of 1143 unique proteins).

Iso-electric Focusing

(IEF)

Strong-Cation Exchange

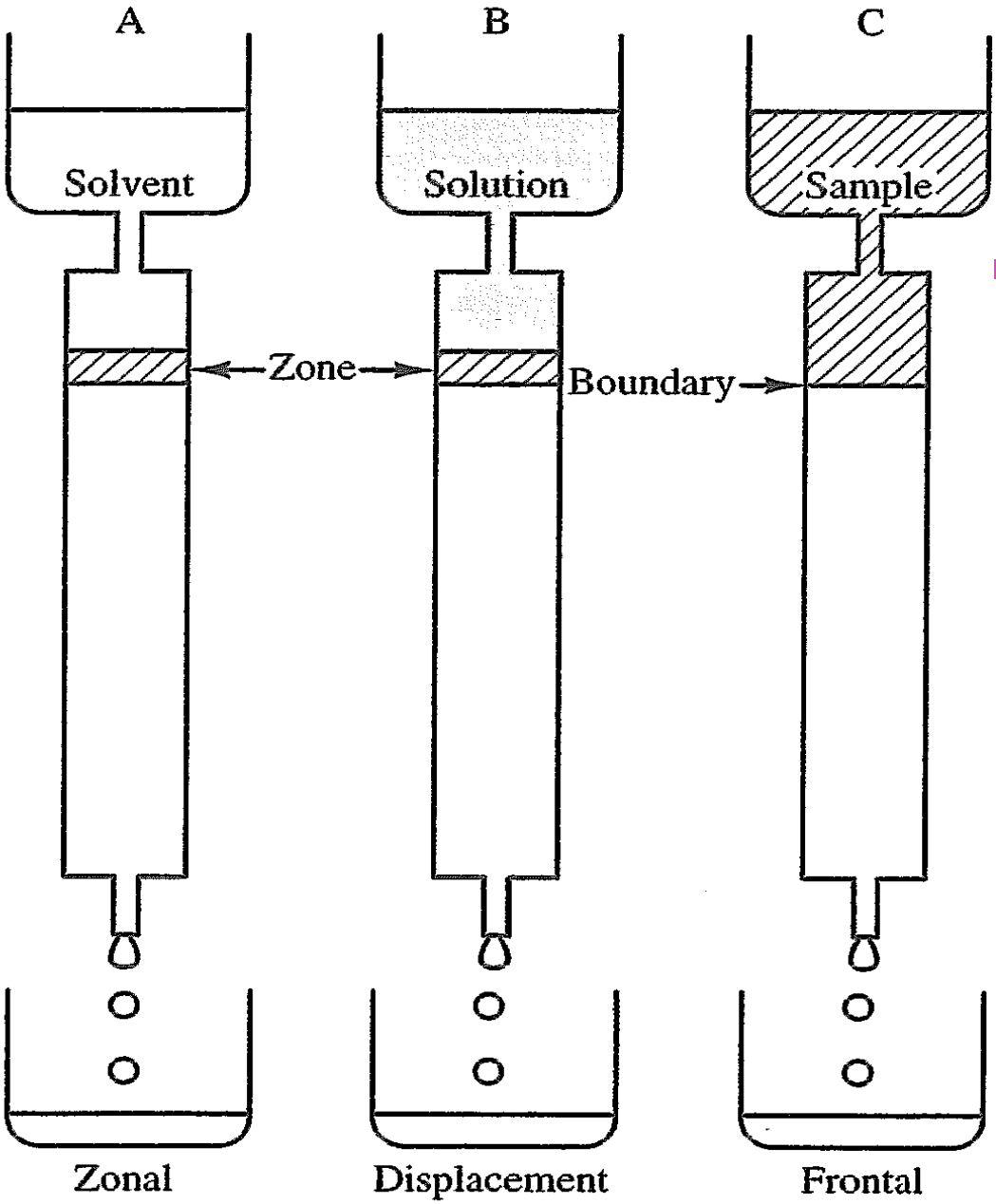
(SCX)

Reverse-Phase

(RP)

Clin. Proteomics, 1 (2004) 101.

Three Major Methods in Chromatography



- **Zonal (區帶)**
  A band (zone) in a solvent system
  -- Change in pH, size…etc

- **Displacement**
  A band (zone) in two-phase solvent system
  ---Different affinity for the solid support (stationary/mobile phase)

- **Frontal (前端)**
  Large sample containing in mobile phase