Chromatography

~ a tool to study protein ~

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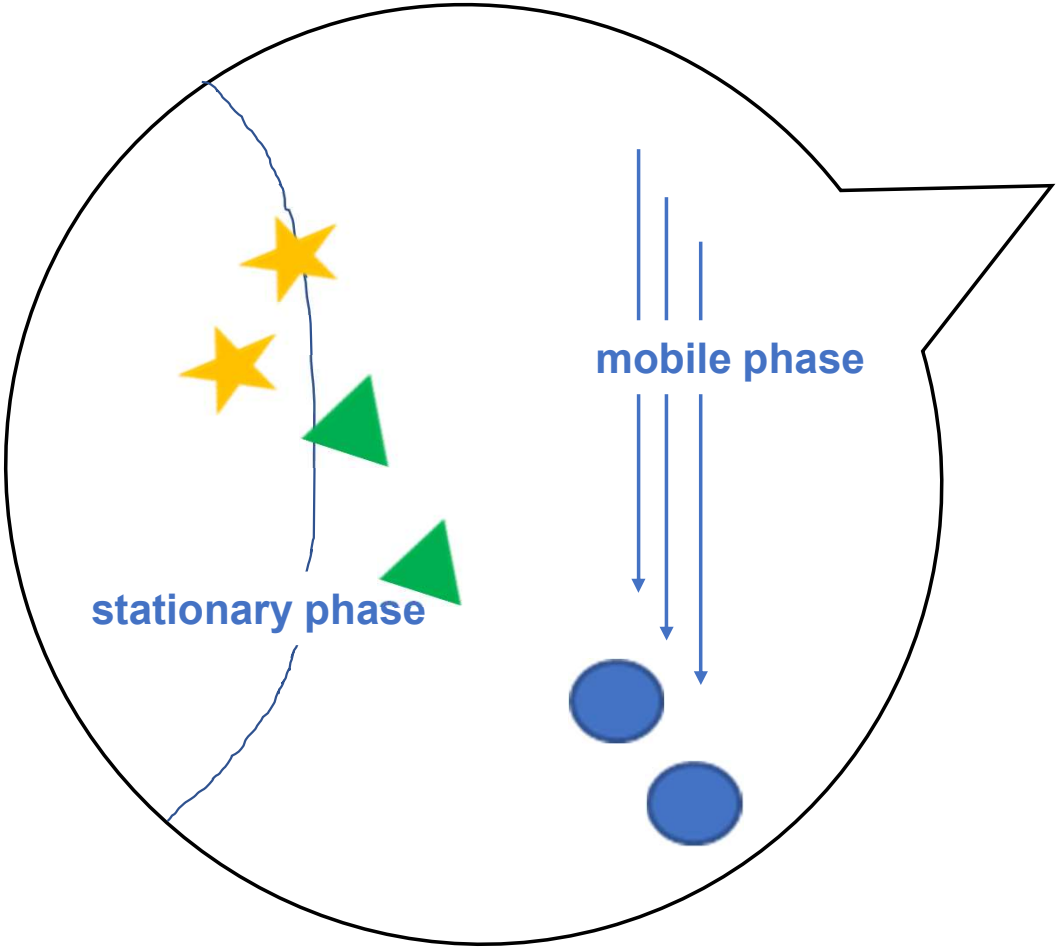
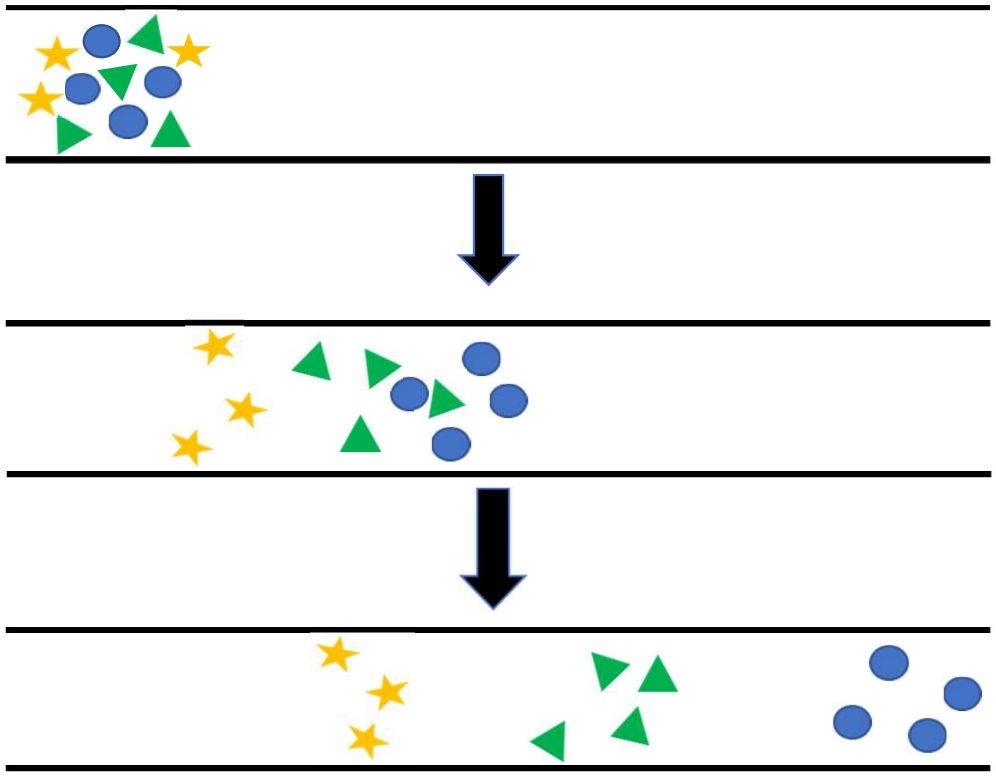
1. Introduction ~ What is chromatography ? ~

- *Chroma,* color + *graphein,* to write

- Physical method of separation, components distributed between two phases: **stationary phase** and **mobile phase**

1. Introduction ~ Basic principle ~





1. Introduction

~ Classification of chromatographic methods ~

- **According to mobile and stationary phases**

  Gas-liquid chromatography

  Liquid-liquid chromatography

- **According to the nature of the dominant intereaction**

  Ion exchange chromatography

  Gel filtration chromatography

  Affinity chromatography

  → Main methods to purify proteins

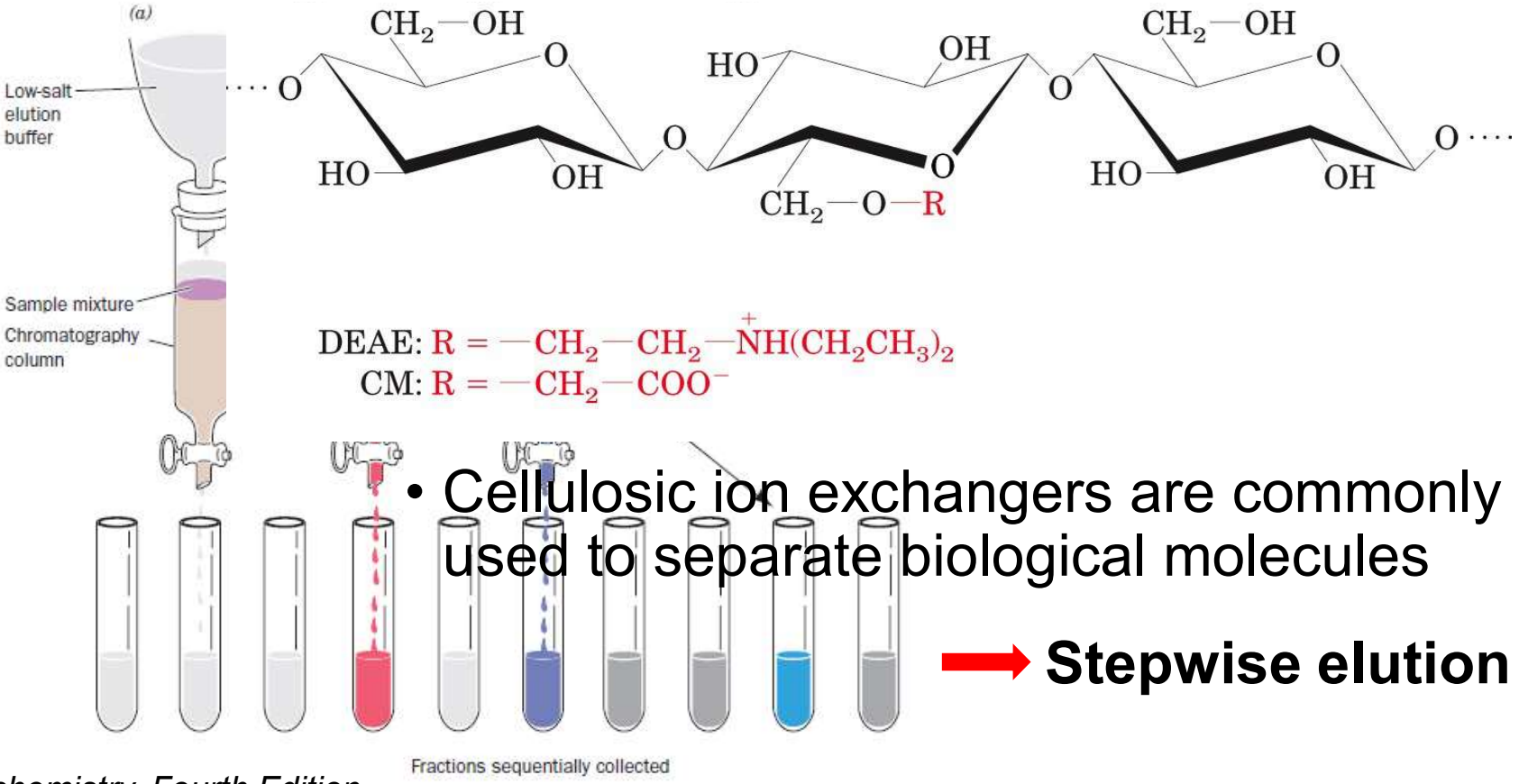
2. Ion exchange chromatography

- Ions that are electrostatically bound to an insoluble and chemically inert matrix are reversibly replaced by ions in solution

$$R^+A^- + B^- \rightarrow R^+B^- + A^-$$

R^+A^- acts as an **ion exchanger**

2. Ion exchange chromatography



- Cellulosic ion exchangers are commonly used to separate biological molecules

➡ **Stepwise elution**

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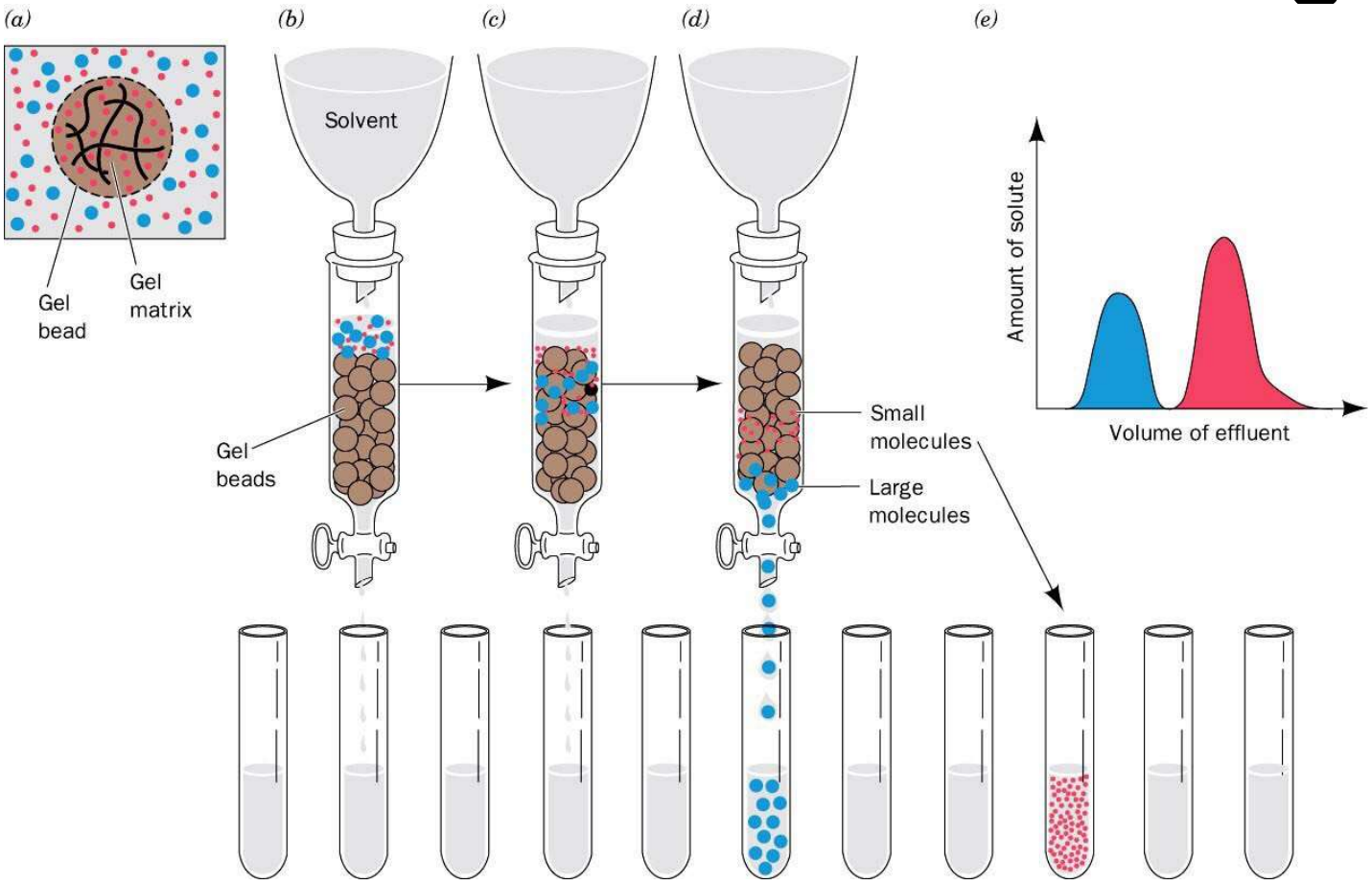
3. Gel filtration chromatography



- Also called “size exclusion” or “molecular sieve chromatography”
- Separate molecules according to their size and shape

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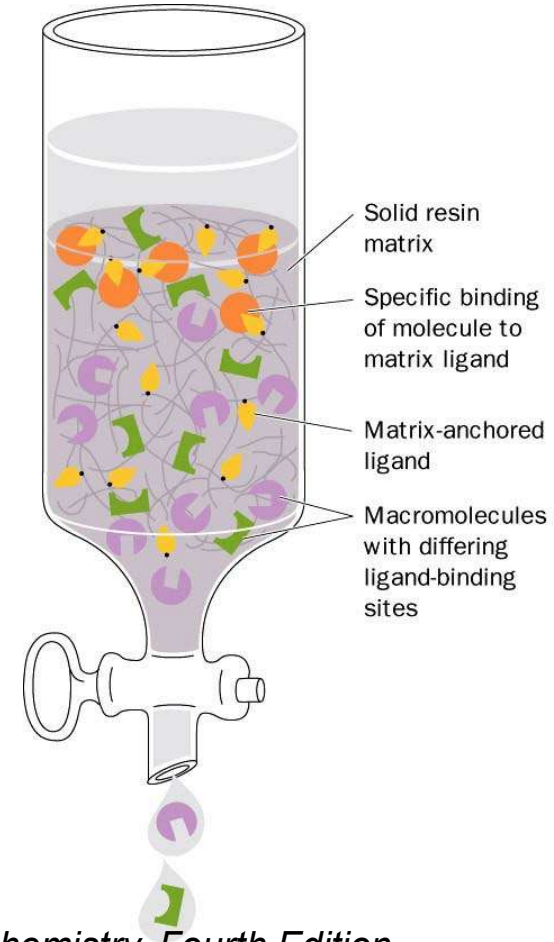
4. Affinity chromatography

- Proteins can bind specific molecules tightly but noncovalently

→ Proteins can be purified by affinity chromatography

- A ligand is covalently attached to inert and porous matrix

→ The desired protein binds to the immobilized ligand



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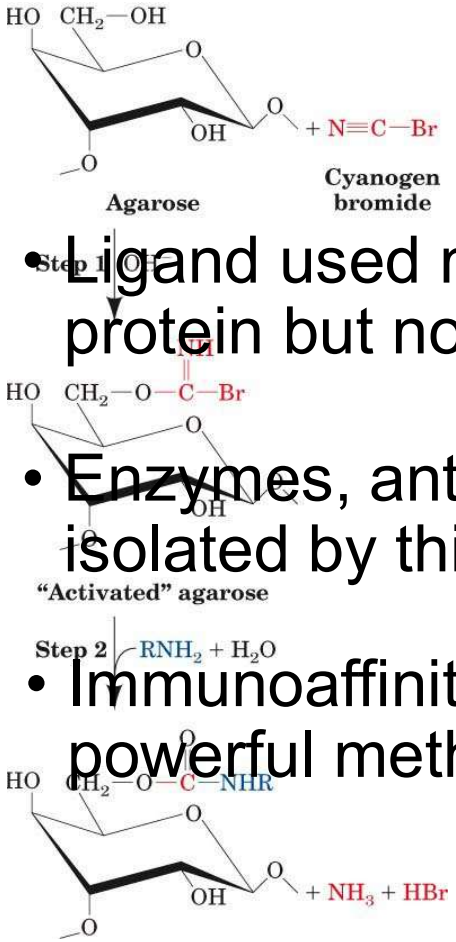
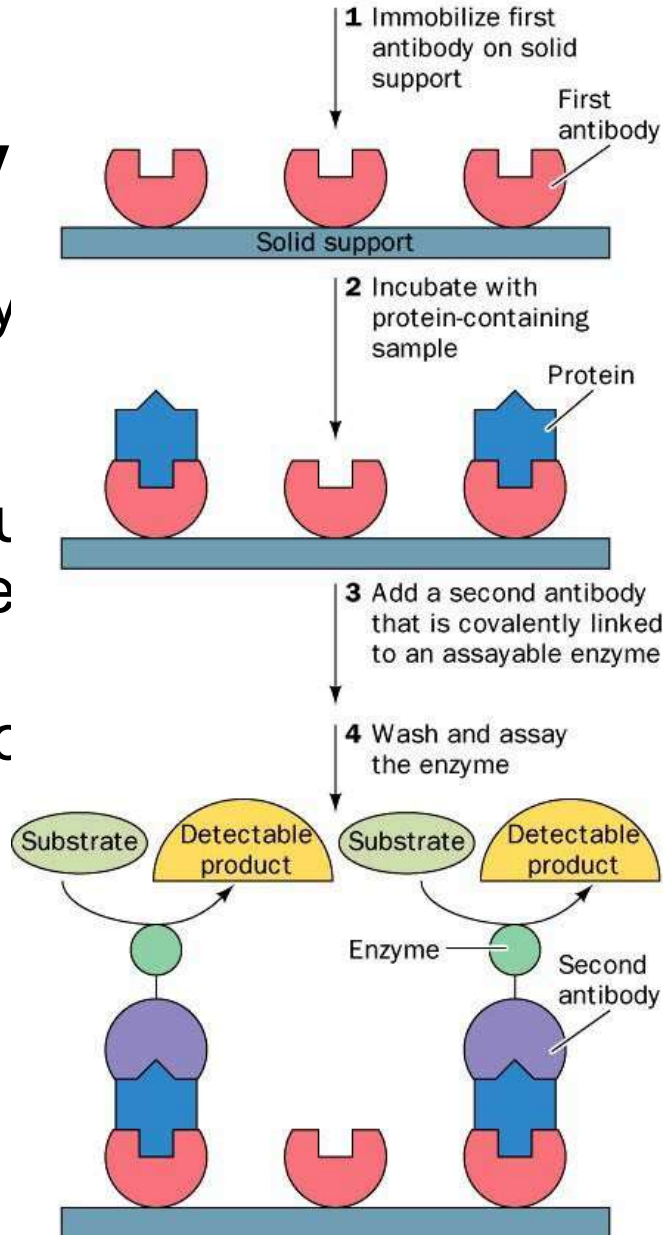
4. Affinity chromatography



- Agarose is used most widely as chromatographic matrix
- Ligand used must have an affinity high enou protein but not too high since we need to se
- Enzymes, antibodies, transport proteins, etc isolated by this method
- Immunoaffinity chromatography is a powerful method



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