Separation Techniques

Purifying & analyzing macromolecules (e.g., proteins & DNA) from a mixture

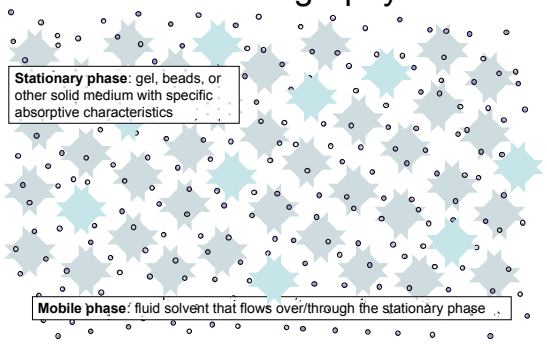
I. **Chromatography**

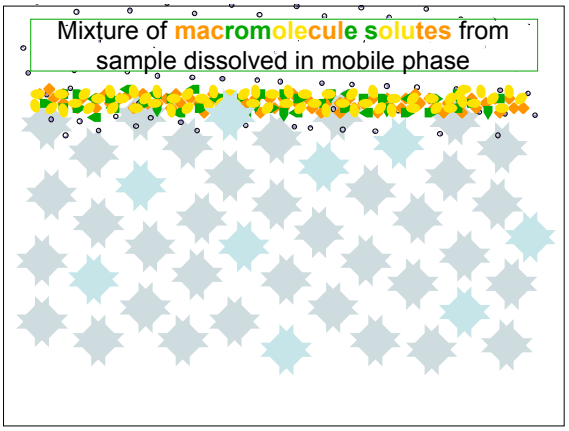
II. **Electrophoresis**

I. Chromatography

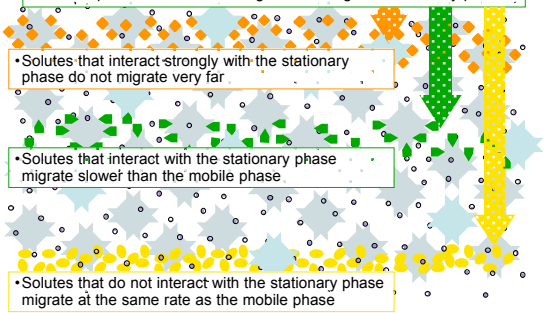
- *Chroma*: "color" + *graph*: "measure"
  - Developed in 19th century to separate pigments from plants and dyes
  - Refined technique now used to separate, analyze, & purify wide range of compounds
    - Still called "chromatography" even though no longer limited to separating colored molecules

Chromatography

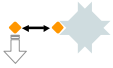




Separation of macromolecule solutes from mixture in original sample —
based upon relative rates of migration through the stationary phase



Solutes interact with stationary phase
↑ interaction ⇒ ⇓ migration rate
⇓ interaction ⇒ ↑ migration rate

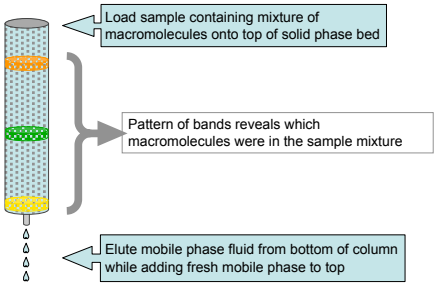


Types of interactions:
- Size
- Charge
- Polarity/hydrophobicity
- Non-specific binding
- Specific binding

Interactions may be modified by manipulating the mobile phase:
- Δ pH
- Δ Ionic strength
- Δ Polarity
- Δ Competitive binding

Column chromatography

- Solid phase as beads or particles packed in a cylinder



Column chromatography

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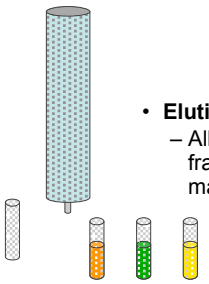


- **Elution**
  - Allows collecting separated fractions containing purified macromolecules

Other types of chromatography

Good for analyzing band patterns, less useful for collecting purified fractions

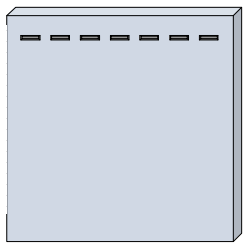
- **Paper chromatography**: mobile phase solvent wicks up paper; cellulose of the paper is the stationary phase. OK for crude separations.
- **Thin layer chromatography (TLC)**: stationary phase of silica particles adhered to glass plates. Allows multiple samples to run side-by-side.
- **Gas chromatography (GC)**: samples are volatile molecules; mobile phase is a carrier gas; stationary phase is a coating on the inside of a capillary tube.

II. **Electrophoresis**

- *Electro*: "electrical field" + *phorus*: "carried by"
  - Developed in mid-20th century
  - Similar principle as chromatography
    - ↑ interaction with stationary phase □ □ migration rate
  - But instead of moving solutes through stationary phase by flowing with mobile phase …
  - Move charged solutes through stationary phase by attraction toward oppositely charged electrical pole

Slab Gel Electrophoresis

- The stationary phase is poured as a liquid into a mold and solidifies into a **gel slab**.



- An advantage of a **slab gel** over a column gel is having multiple sample wells to run several samples simultaneously for comparison.

Slab Gel Electrophoresis

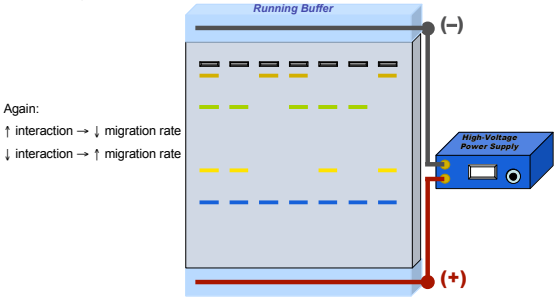
- The stationary phase is poured as a liquid into a mold and solidifies into a **gel slab**.

Types of gels:

- **Starch**. Heterogeneous medium allows resolution of minor differences between allelic or isoenzyme variations of certain proteins. But hard to reproduce between stocks of starch.
- **Agarose**. Highly purified form of agar. Forms gels at low densities to allow separation of large molecules such as DNA fragments or protein complexes.
- **Polyacrylamide**. A plastic polymer that can form thin slabs with precisely determined characteristics. (Thin gels allow smaller sample sizes and efficient cooling.) Used for smaller macromolecules such as nucleotides (DNA sequencing) and most proteins.
  - CAUTION: unpolymerized polyacrylamide is neurotoxic! But once polymerized into the gel it becomes non-toxic.

Slab Gel Electrophoresis

At pH >7, DNA & most proteins have a net **negative** charge
∴ migrate toward **positive** electrode ("run to red")

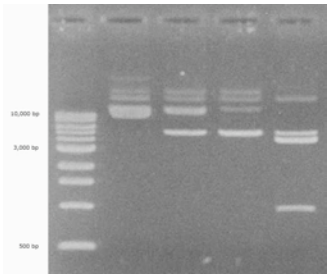


Electrophoresis of DNA

- DNA fragments are very large molecules
  - Use **agarose** gels
- DNA does not chemically interact with agarose
  - Migration rate dependent upon **size** of the DNA fragment
- Since DNA is a linear molecule, size is related to the **length** of the fragment
  - Measured in **# of base pairs** (bp) or **kilobase-pairs** (kb)

Electrophoresis of DNA

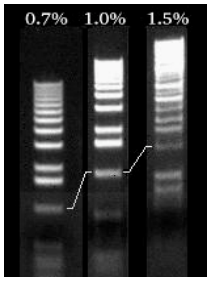
- Gel electrophoresis separates DNA restriction fragments of different lengths



See **Fig. 20.9**

Using different concentration of agarose to resolve different size DNA fragments.

- Notice how the larger fragments are much better resolved in the 0.7% gel, while the small fragments separated best in 1.5% agarose.
- The 1000 bp fragment is indicated in each lane.

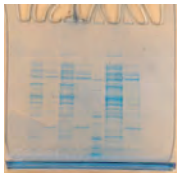


Electrophoresis of Proteins

- Proteins are structurally very complex
  - Unpredictable interactions with most media
  - Use **polyacrylamide** gels
    - **PAGE** (polyacrylamide gel electrophoresis)
- To separate based upon **size only** of the proteins, need to neutralize most of the chemical interactions
  - Denaturation — unfold protein structure
  - Detergent binding — uniform charge

SDS-PAGE

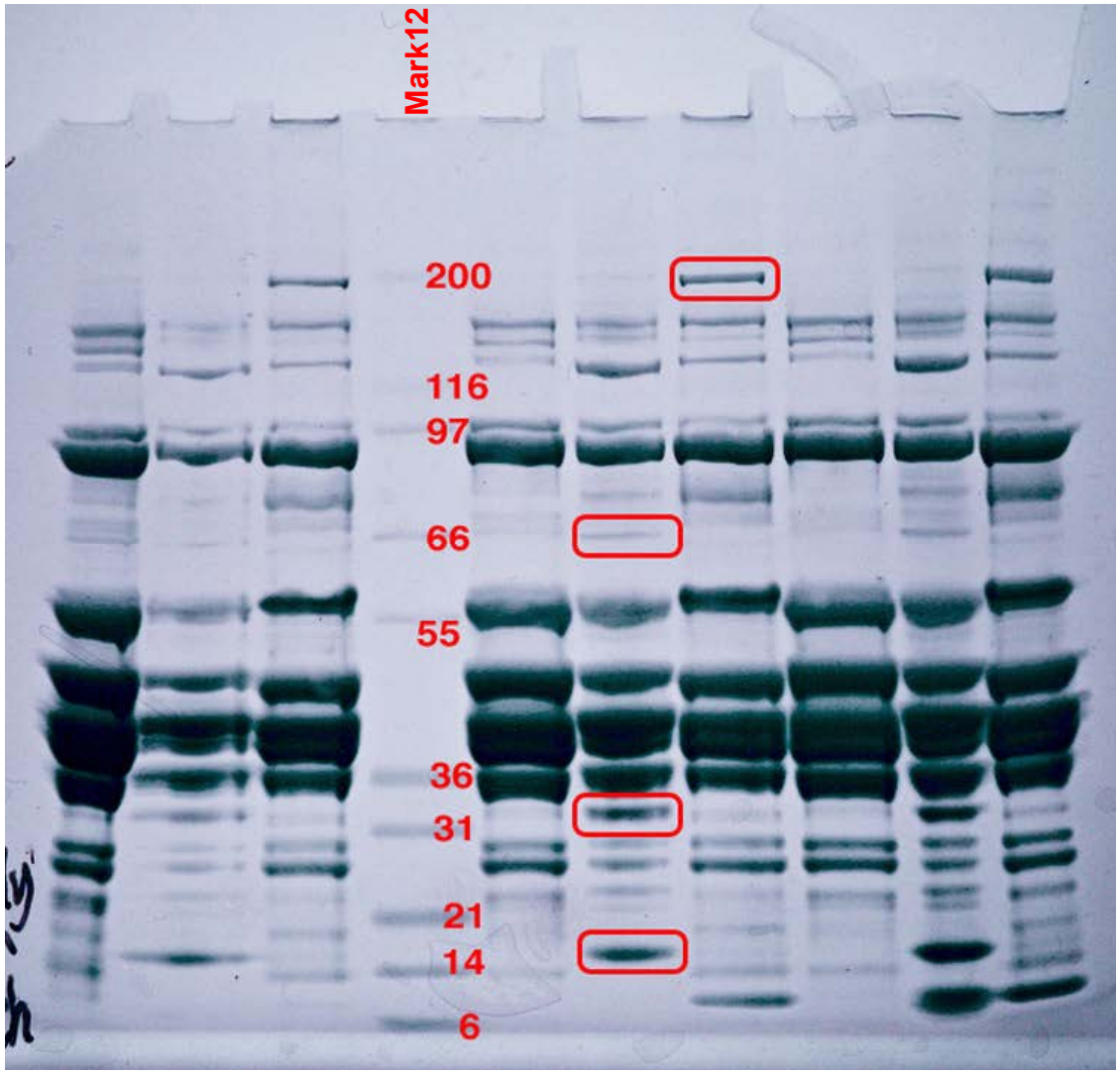
- Detergent: **S**odium **d**odecyl **s**ulfate (SDS)
  - (aka, laurel sulfate)
  - We also use lithium dodecyl sulfate, but traditionally the method is still called SDS-PAGE
- Size of proteins is expressed in terms of molecular weight in **kilodaltons** (kD).



C.f., Molecular Cell Biology tutorial

SDS-PAGE of fish muscle proteins

- Different fish species have some proteins different



Mark12 Protein MW Standard