

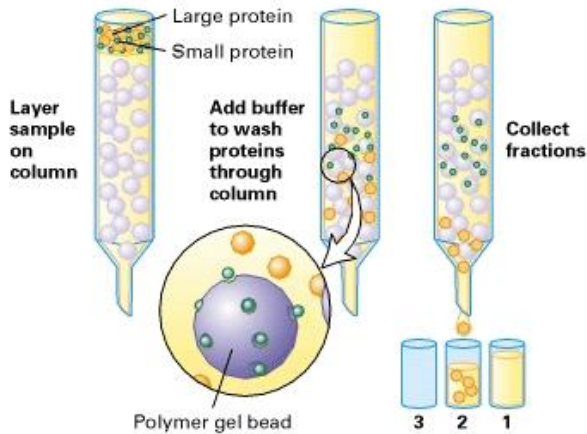
Column Chromatography

Gel Filtration (Size Exclusion)

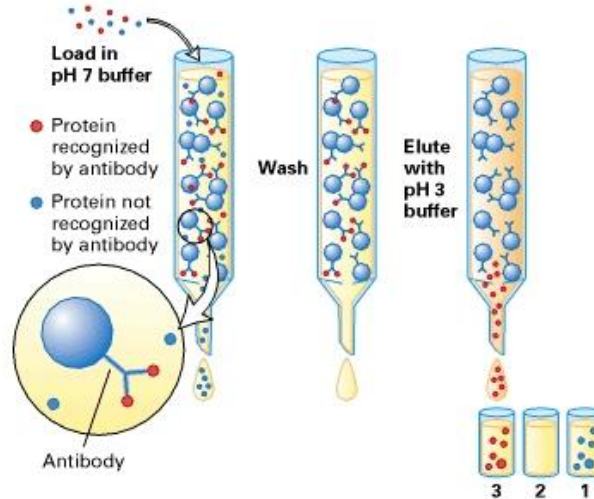
Ion Exchange (Charge)

Affinity (Shape or Functional Group)

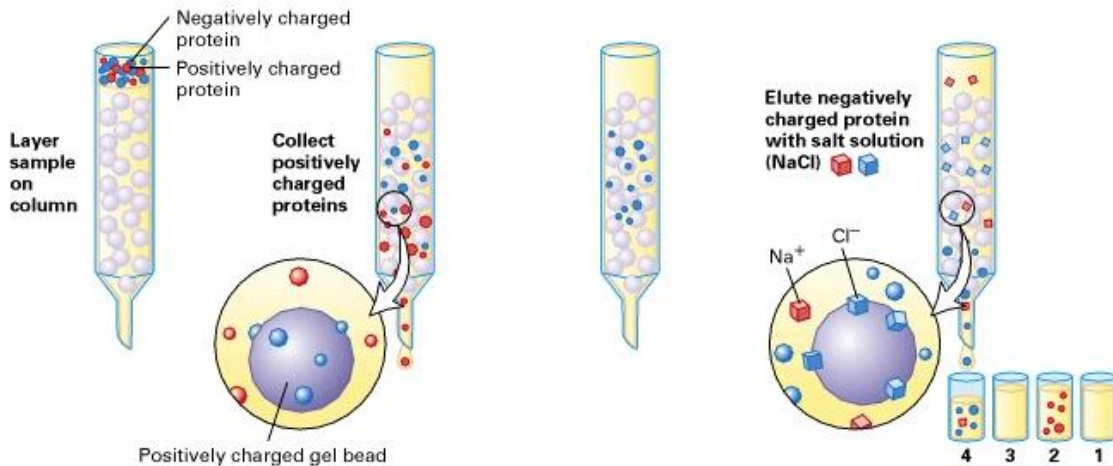
(a) Gel filtration chromatography



(c) Antibody-affinity chromatography

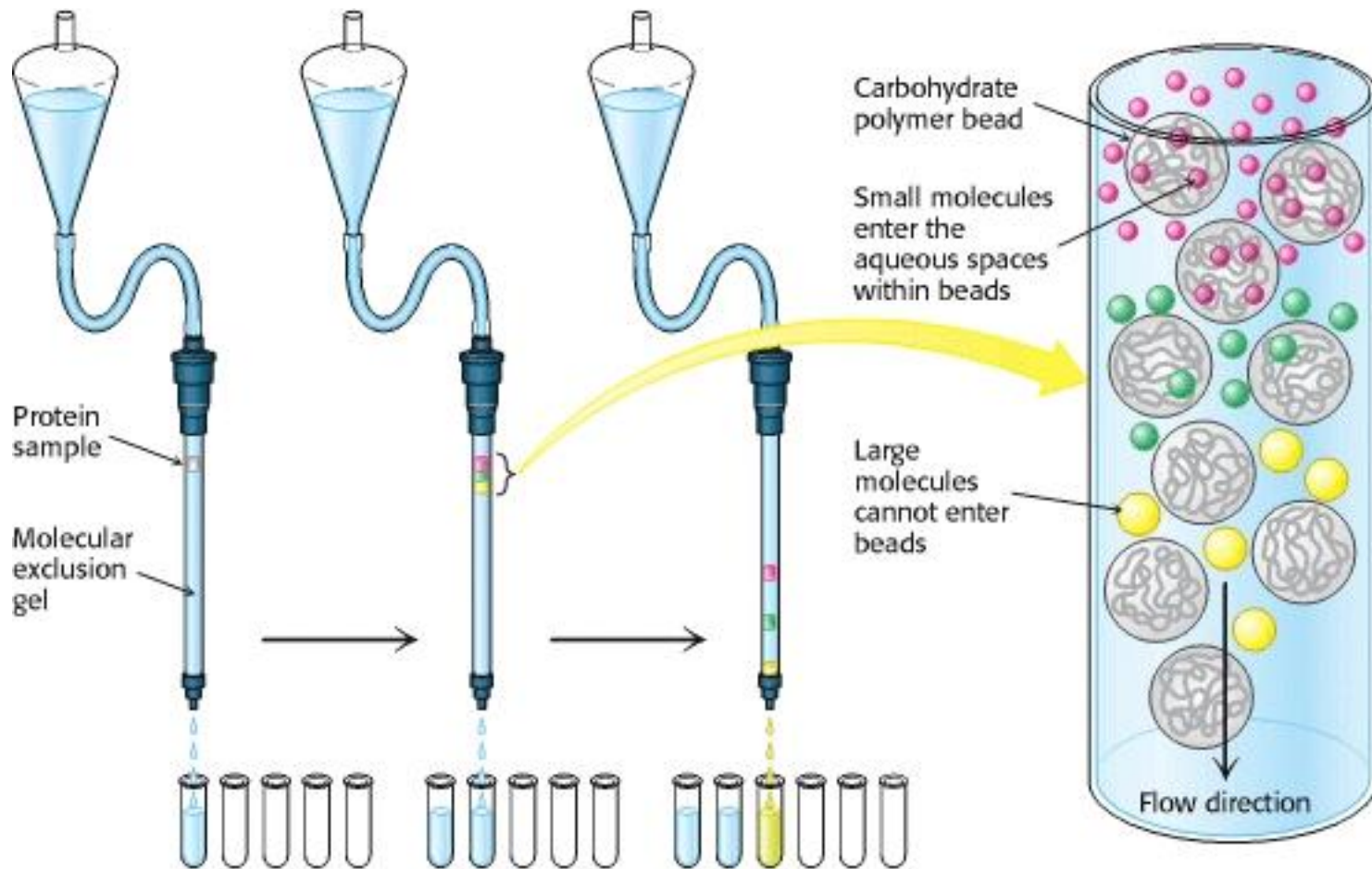


(b) Ion-exchange chromatography



Three commonly used liquid chromatographic techniques

(a) Gel filtration chromatography separates [proteins](#) that differ in size. A mixture of [proteins](#) is carefully layered on the top of a glass cylinder packed with porous beads. Smaller [proteins](#) travel through the column more slowly than larger [proteins](#). Thus different [proteins](#) have different elution volumes and can be collected in separate liquid fractions from the bottom. (b) Ion-exchange chromatography separates [proteins](#) that differ in net charge in columns packed with special beads that carry either a positive charge (shown here) or a negative charge. [Proteins](#) having the same net charge as the beads are repelled and flow through the column, whereas [proteins](#) having the opposite charge bind to the beads. Bound [proteins](#), in this case negatively charged, are eluted by passing a salt gradient (usually of NaCl or KCl) through the column. As the ions bind to the beads, they desorb the [protein](#). (c) In [antibody](#)-affinity chromatography, a specific [antibody](#) is covalently attached to beads packed in a column. Only [protein](#) with high affinity for the [antibody](#) is retained by the column; all the nonbinding [proteins](#) flow through. The bound [protein](#) is eluted with an acidic solution, which disrupts the [antigen-antibody](#) complexes.



Gel Filtration Chromatography

A mixture of proteins in a small volume is applied to a column filled with porous beads. Because large proteins cannot enter the internal volume of the beads, they emerge sooner than do small ones.

Animations of Chromatography

Gel Filtration (or Size Exclusion) Chromatography

- [http://www.gelifesciences.com/aptrix/upp00919.nsf/Content/9D693300F8ECB9EDC1257628001D3796/\\$file/GE_Gel+Filtra+ion.swf](http://www.gelifesciences.com/aptrix/upp00919.nsf/Content/9D693300F8ECB9EDC1257628001D3796/$file/GE_Gel+Filtra+ion.swf)

Ion Exchange Chromatography

- [http://www.gelifesciences.com/aptrix/upp00919.nsf/Content/19A520DC7A58549EC1257628001D37B2/\\$file/GE_Ion+Excha+nge.swf](http://www.gelifesciences.com/aptrix/upp00919.nsf/Content/19A520DC7A58549EC1257628001D37B2/$file/GE_Ion+Excha+nge.swf)

Affinity Chromatography

- [http://www.gelifesciences.com/aptrix/upp00919.nsf/Content/756194071612EF31C1257628001D3779/\\$file/GE_Affinity.swf](http://www.gelifesciences.com/aptrix/upp00919.nsf/Content/756194071612EF31C1257628001D3779/$file/GE_Affinity.swf)

BioRad Teacher Resources

- The site with
- Free posters
- Grant Ideas
- Powerpoints
- http://www.biorad.com/evportal/evolutionPortal.portal?_nfpb=true&_pageLabel=AboutProgramsPage&HtmlFilePath=%2Fwebroot%2Fweb%2Fhtml%2Fse%2Fproducts%2Fprograms%2Fteaching_resources_ed_endy.html&Title=Teaching+Resources&catID=3dec64ef-aa3a-4534-bc63-d33c13fc367c&index=US