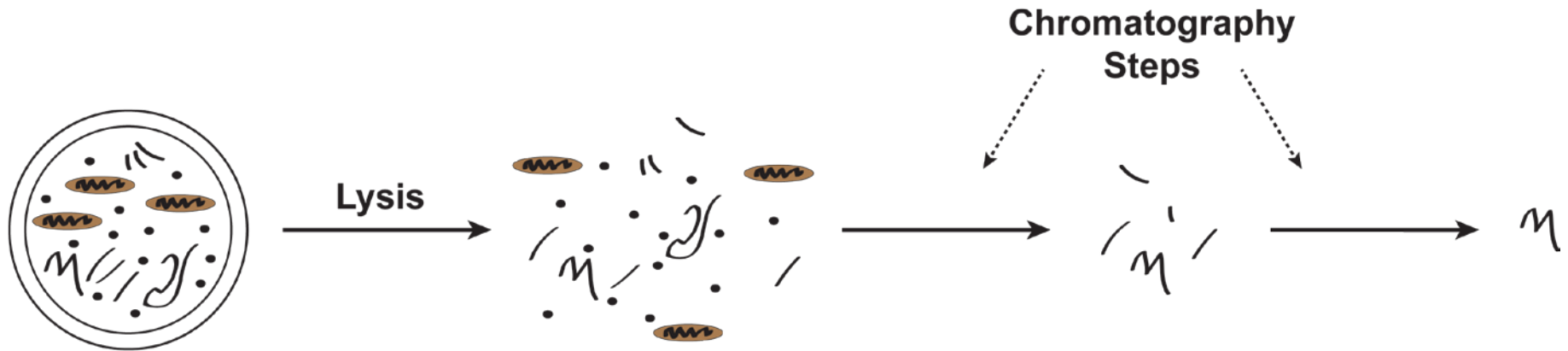
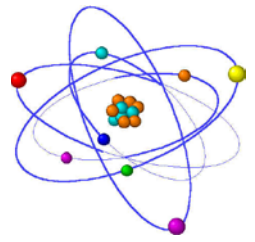


Protein Purification Cell Lysis through Chromatography



Methods of Cell Lysis



Passive Methods:

1. Freeze-thaw
2. Chemical
3. Enzymatic
4. Osmotic Pressure

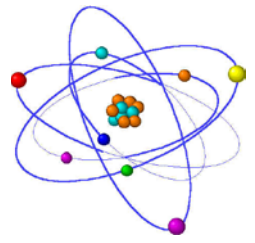
Physical Methods:

1. Sonication
2. Cell Bomb
3. Homogenizer

Additional Considerations:

- | | | |
|--------------|---------------------------------|---------------------|
| 1. Volume | 5. Further Purification Steps | 9. pH |
| 2. Cell Type | 6. Protein Stability | 10. Salt |
| 3. Toxicity | 7. Protease activity/inhibitors | 11. Reducing Agents |
| 4. Cost | 8. Protein location | |

Chemical Lysis



Adding a detergent to solubilize the cell membrane

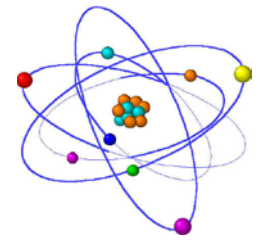
Detergent selection is critical!

Property	SDS	CHAPS	Triton-X	Tween 80
Charge	anionic	amphoteric	non-ionic	non-ionic
Denaturing	yes	no	no	no
Dialyzable	yes	yes	no	no
Ion-exchangable	yes	no	no	no
Strong Abs ₂₈₀	no	no	yes	no
Interference Potential	no	no	yes**	yes*
Expensive	no	yes	no	no

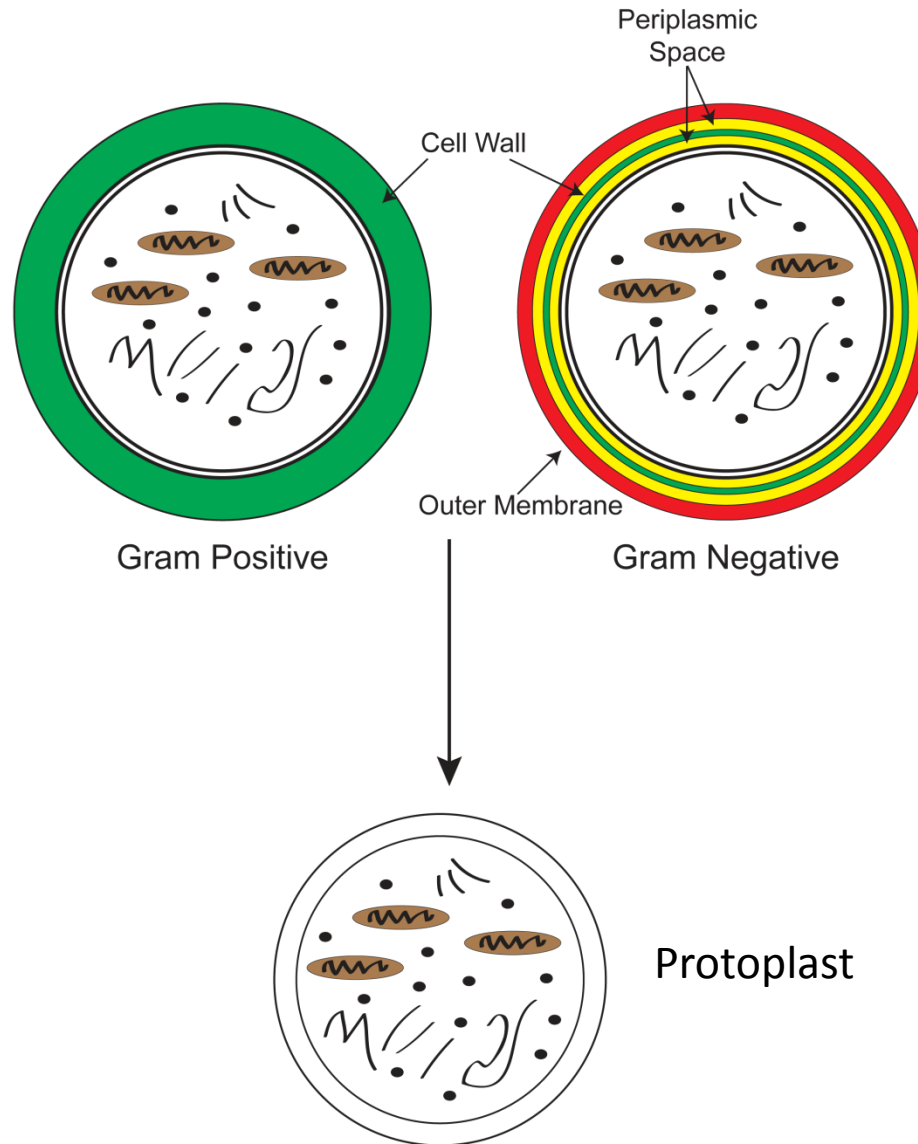
*Gel Filtration, native electrophoresis

**Gel Filtration, native electrophoresis, Lowry Assay

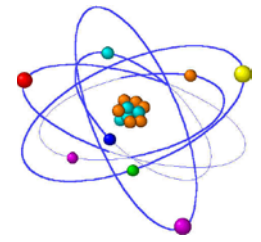
Enzymatic Lysis



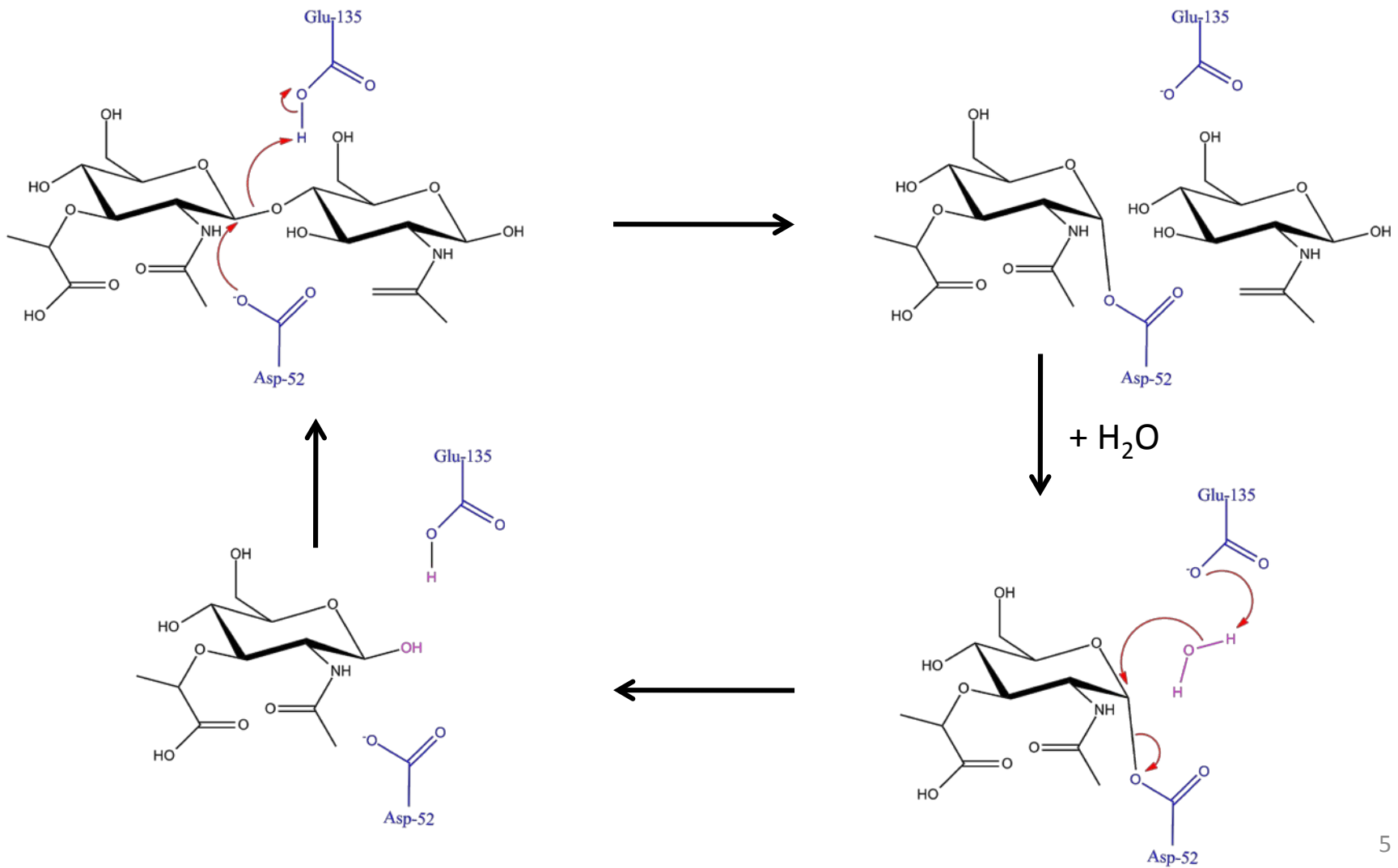
Adding an enzyme to degrade the cell wall



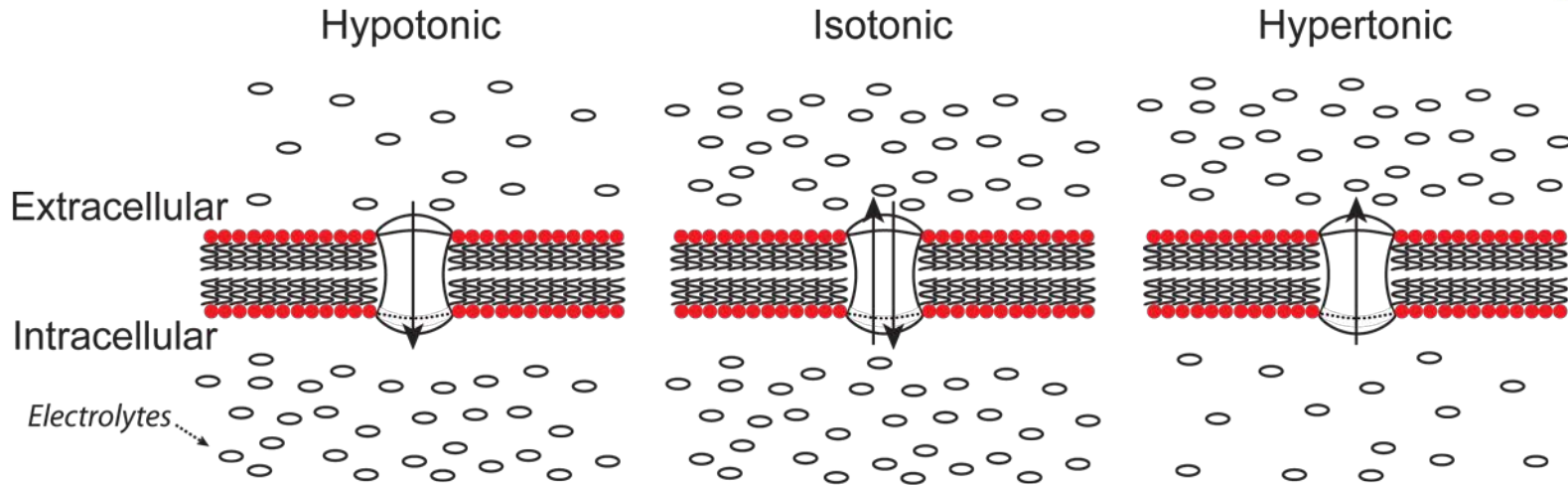
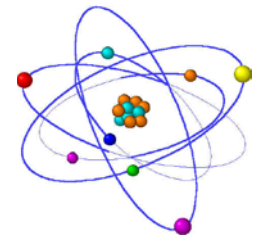
Enzymatic Lysis



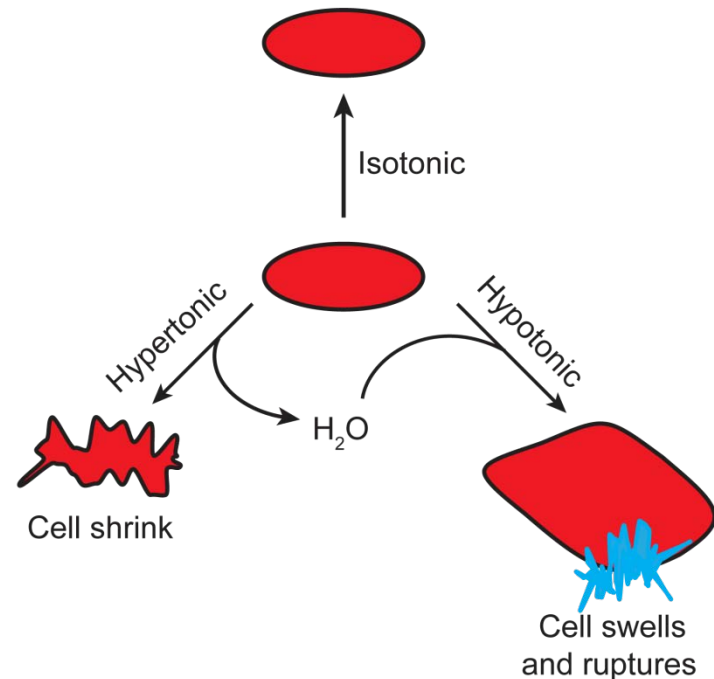
Lysozyme is most commonly used



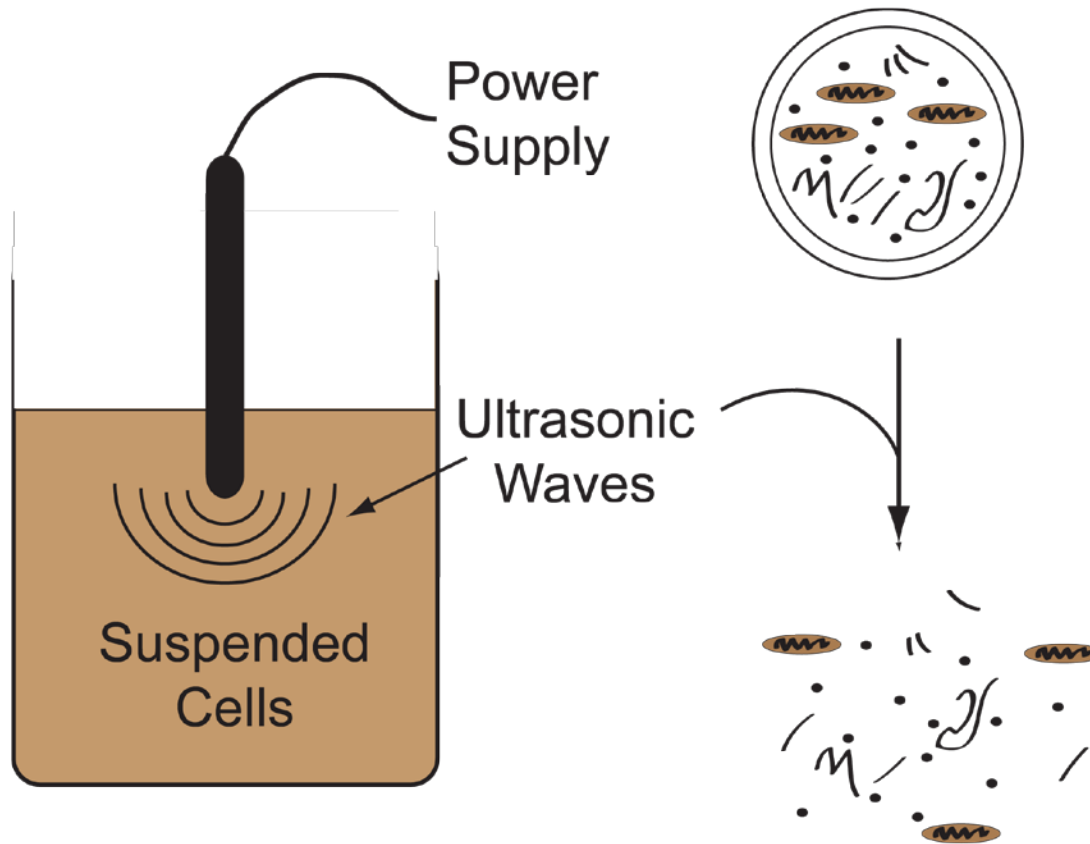
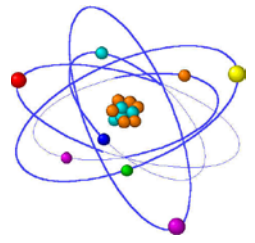
Lysis by Osmotic Pressures



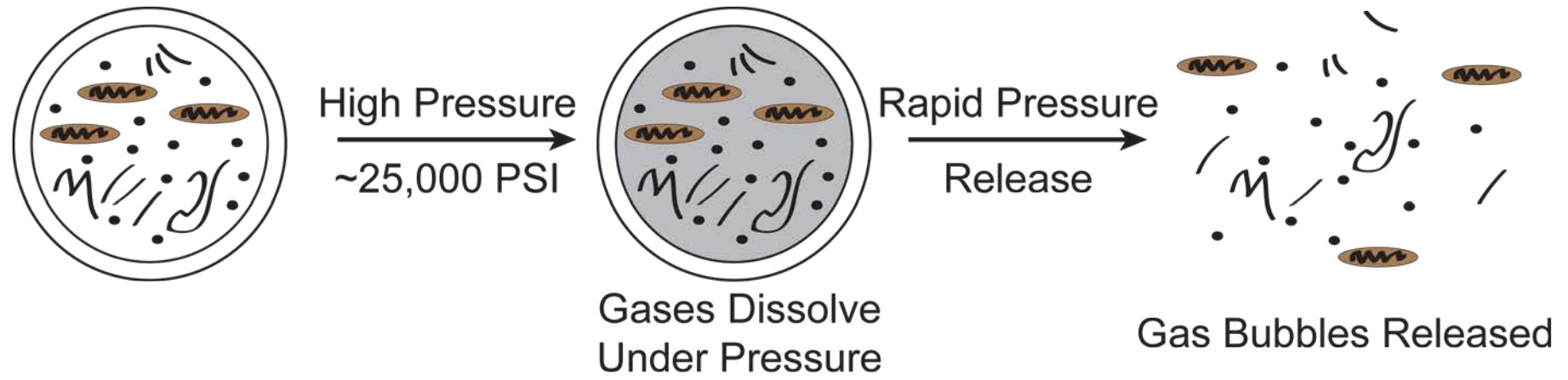
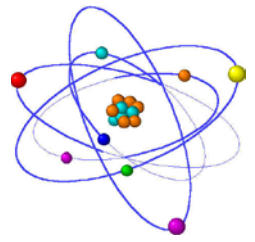
Adjusting the concentration of electrolytes in the lysis buffer can promote lysis through osmotic pressures



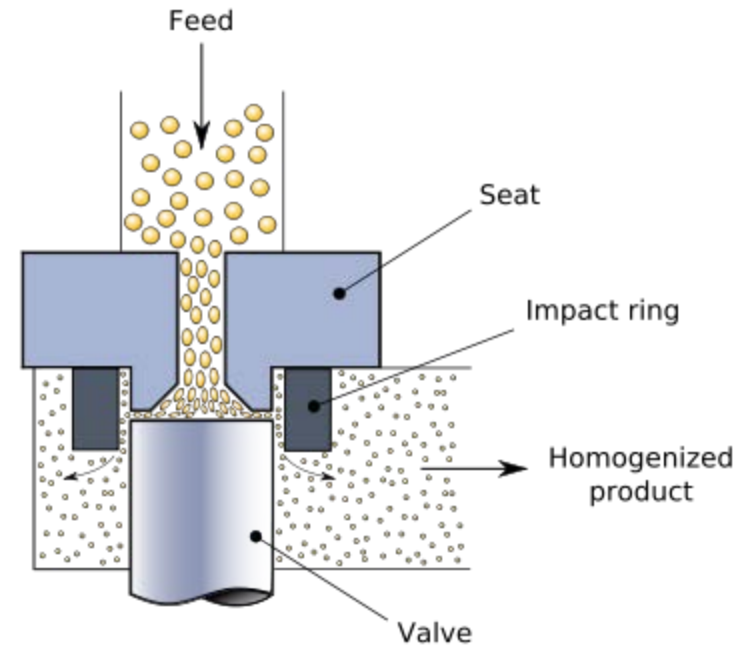
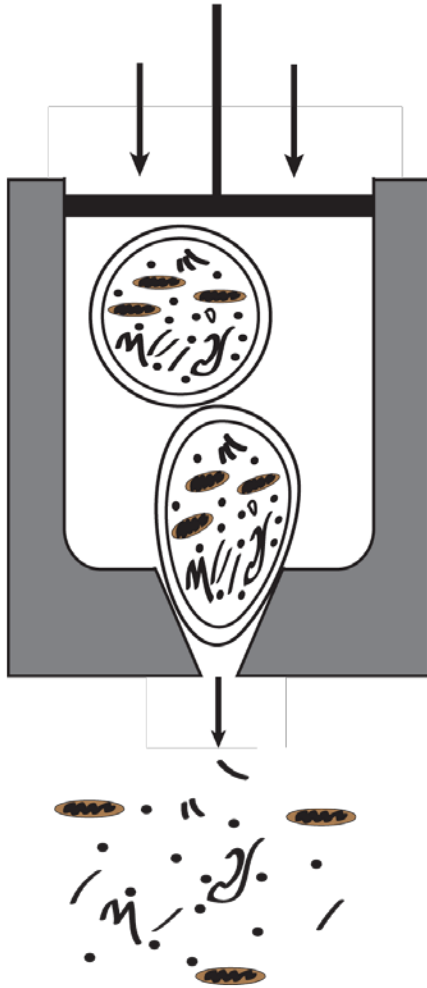
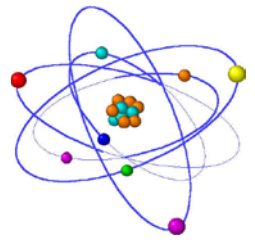
Lysis by Sonication



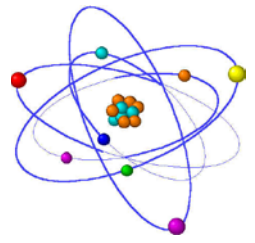
Lysis by Cell Bomb



Lysis by Homogenization



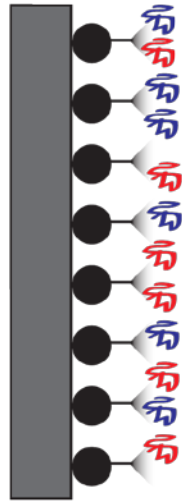
Chromatography



Mixture of proteins



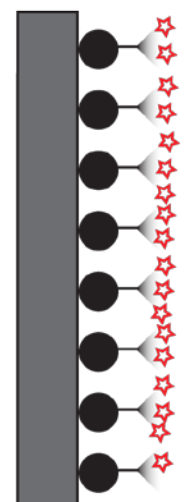
Load



Low competition



High competition



Solid phase support with attached functional group



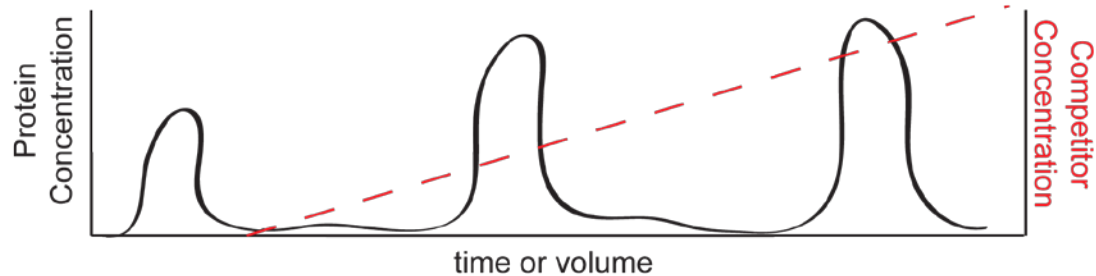
No affinity (Flow Thru)



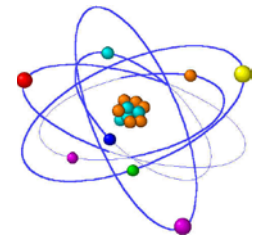
Weak affinity



High affinity

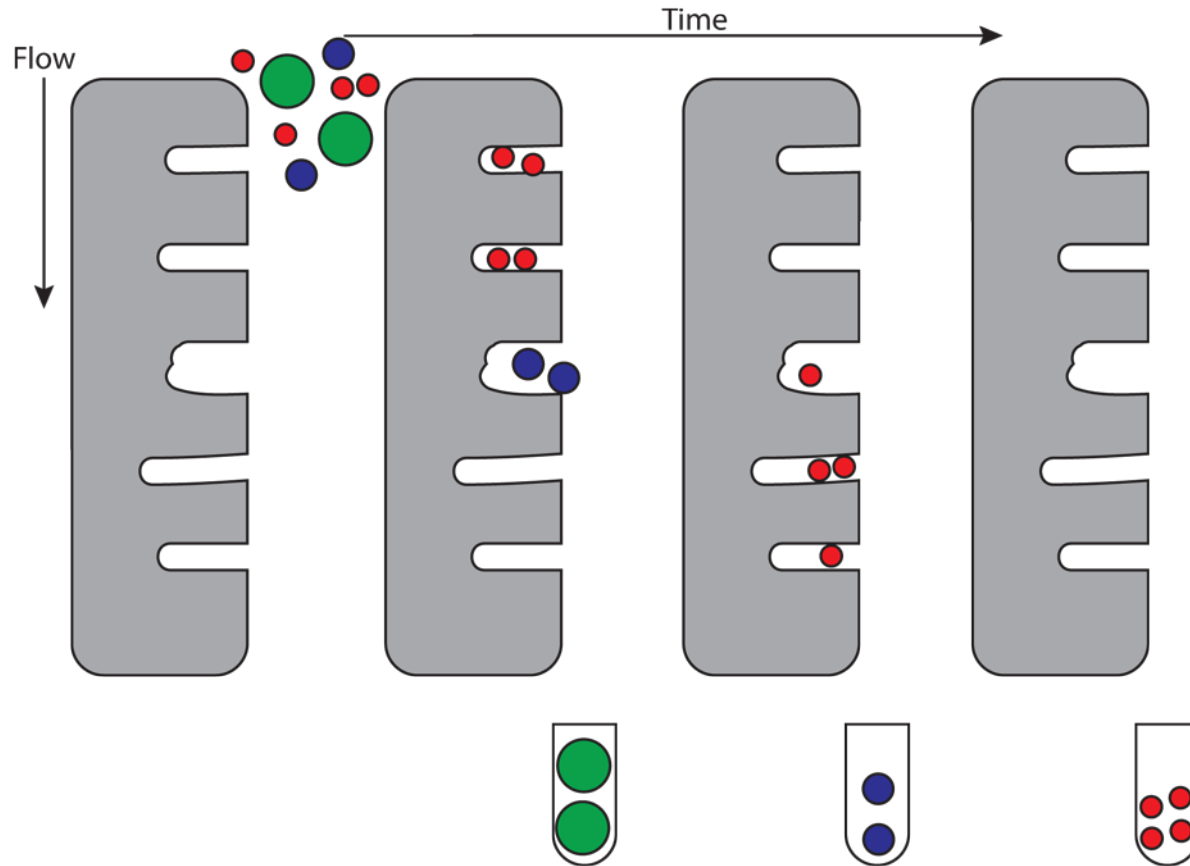
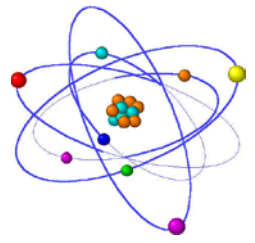


Types of Chromatography

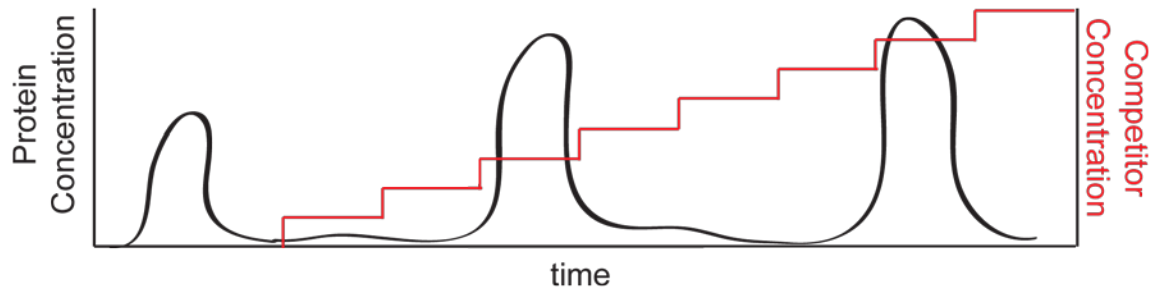
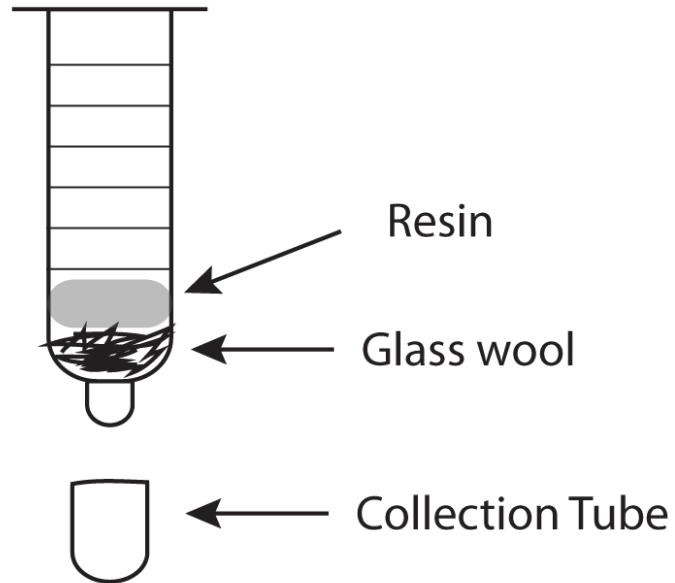
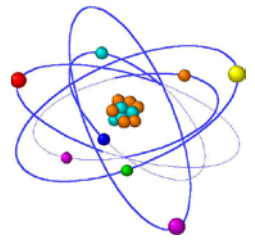


	Representative Functional Group	Competitor
Cation Exchange		NaCl
Anion Exchange		NaCl
Hydrophobic Interaction		$(\text{NH}_4)_2\text{SO}_4^{***}$
Metal Affinity		

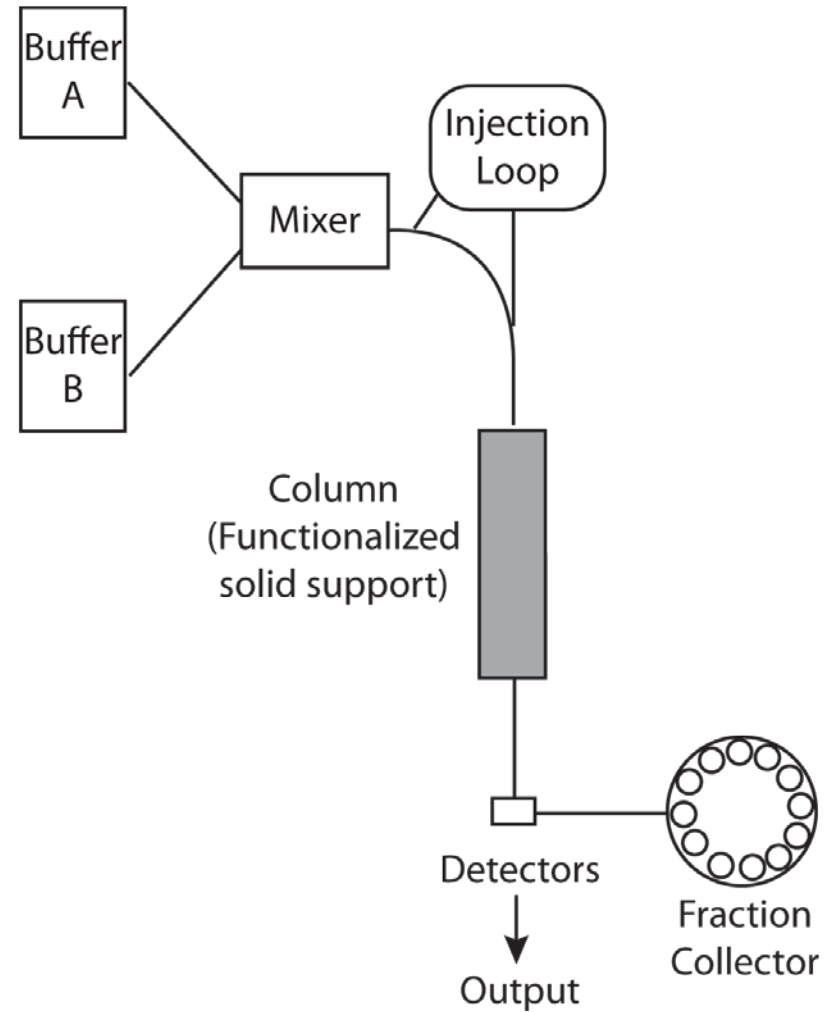
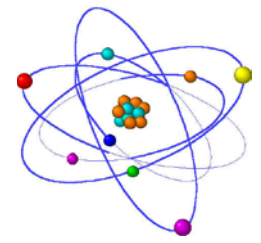
Gel Filtration



Chromatography



FPLC



FPLC - output

