Protein Purification

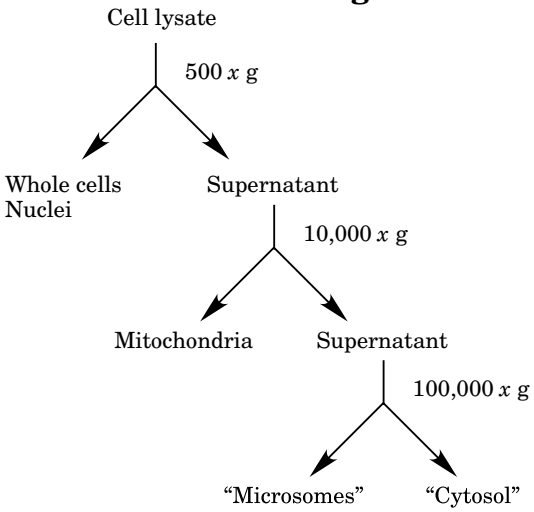
Advantages

Disadvantages

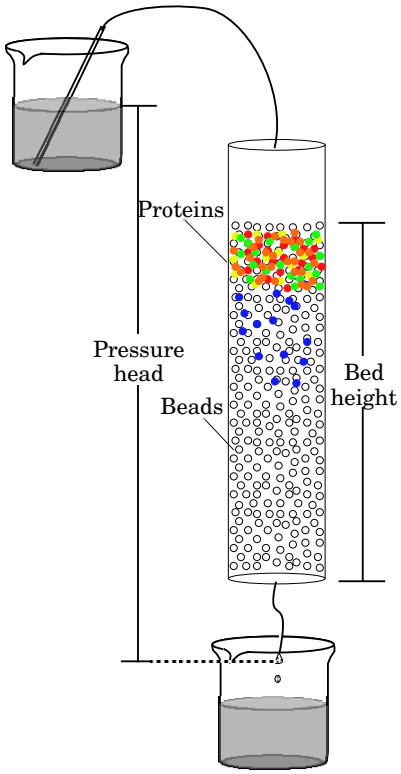
Sources

Strategy

Differential Centrifugation



Chromatography



Resolution

$$R_s = \frac{\sqrt{N}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k_B}{1 + k_B}\right)$$

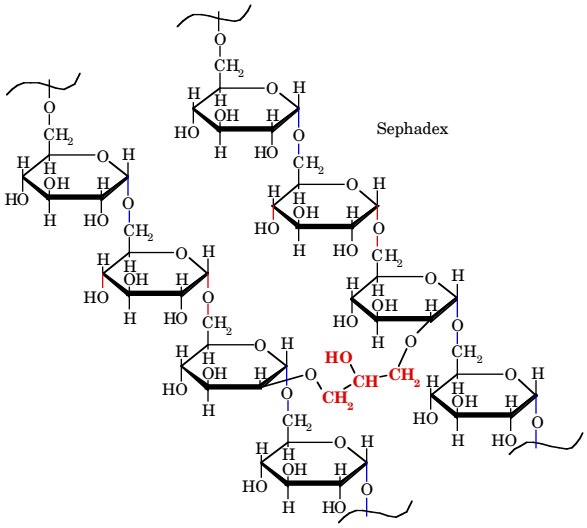
$$R_s = \frac{\sqrt{N}}{4}\left(\frac{t_{r_b} - t_{r_a}}{t_{r_b}}\right)$$

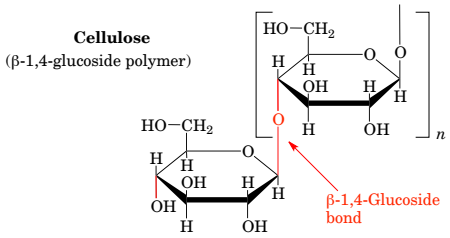
$$\alpha = \frac{k_b}{k_a} = \frac{t_{r_b} - t_m}{t_{r_a} - t_m} = \frac{K_b}{K_a}$$

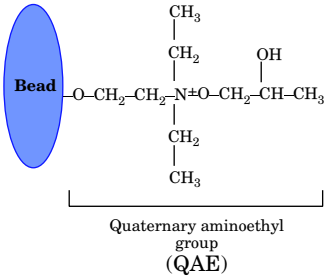
$\alpha \geq 1$ (although if α = 1 then the analytes are not separating)

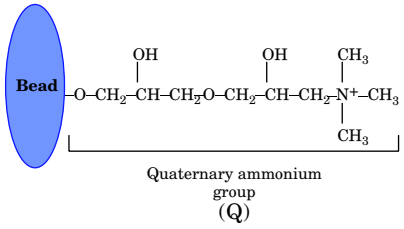
Protein Chromatography Resins

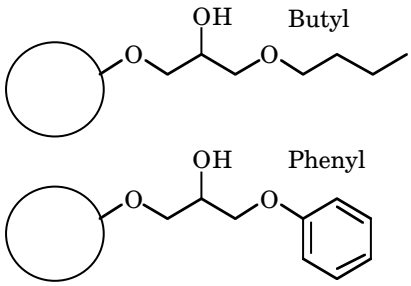
Resin	Carbohydrate	Cross-linking agent
Cellulose	Cellulose	none
Biogel	Polyacrylamide	Bisacrylamide
Sephacel	Cellulose	Epichlorhydrin
Sephacryl	Dextran	Bisacrylamide
Sephadex	Dextran	Epichlorhydrin
Sepharose	Agarose	none
Superdex	Dextran, Agarose	Highly cross-linked
Superose	Agarose	Highly cross-linked

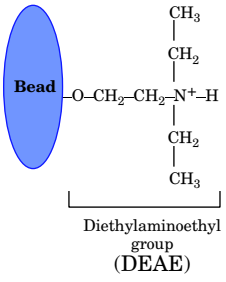


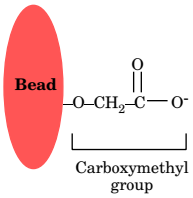












Chromatography types

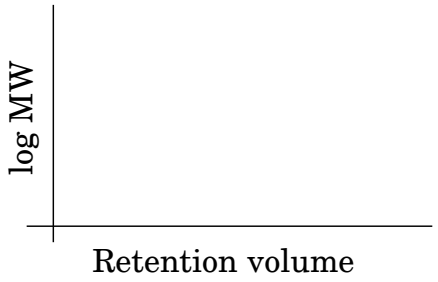
Type	Capacity	Resolution	Expense
Ion exchange			
Hydrophobic interaction			
Gel filtration			
Affinity			

Gel Filtration Chromatography

Preparative

Analytical

Stokes radius



Hofmeister series (effect on hydrophobic interactions)

Anions: $PO_4^{3-} > SO_4^{2-} > CH_3COO^- > Cl^- > Br^- > NO_3^- > ClO_4^- > I^- > SCN^-$

Cations: $NH_4^+ > Rb^+ > K^+ > Na^+ > Cs^+ > Li^+ > Mg^{2+} > Ca^{2+} > Ba^{2+}$

Effect on water surface tension

$Na_2SO_4 > K_2SO_4 > (NH_4)_2SO_4 > Na_2HPO_4 > NaCl > LiCl >$ others $> KSCN$

Affinity Chromatography

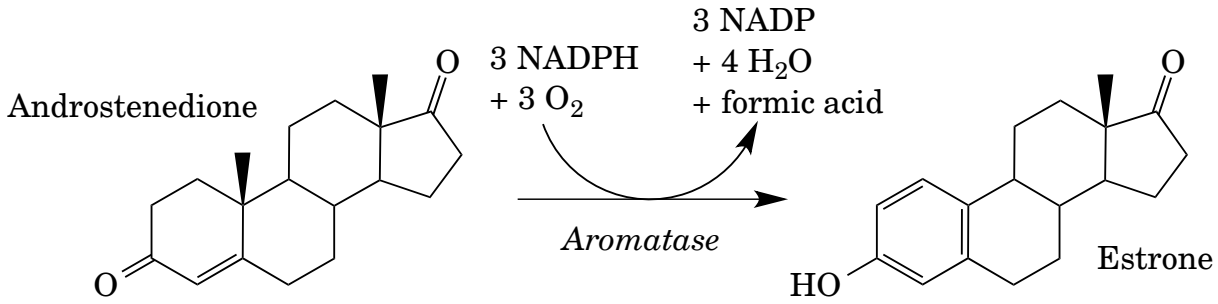
His-tag

Maltose-binding protein

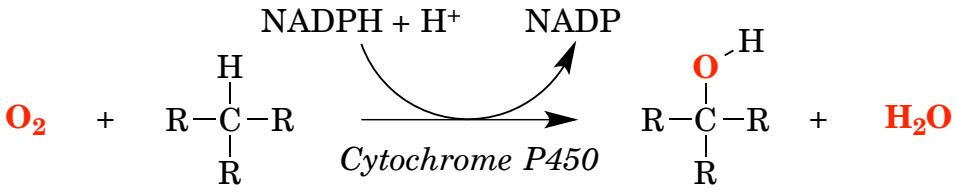
Ligands

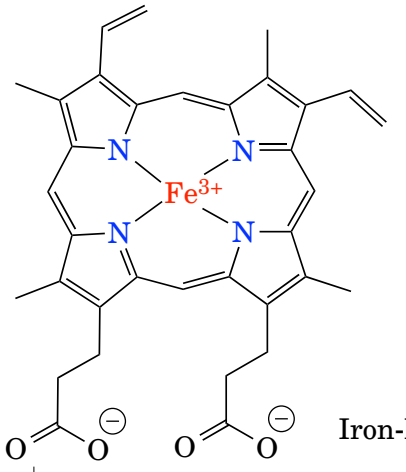
Antibodies

Aromatase

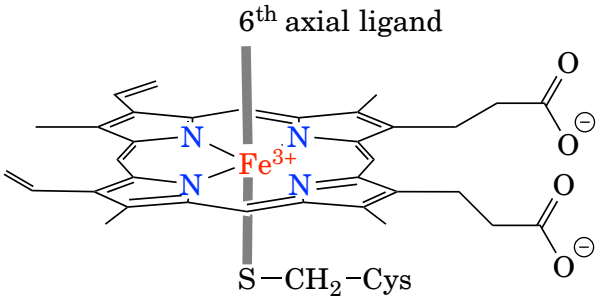


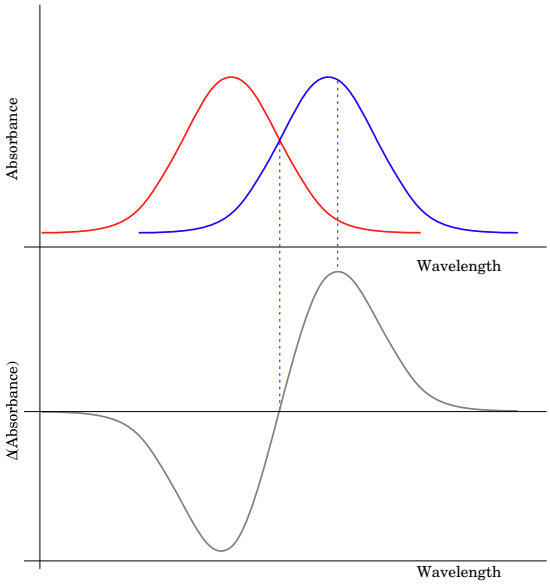
Cytochrome P450 Enzymes

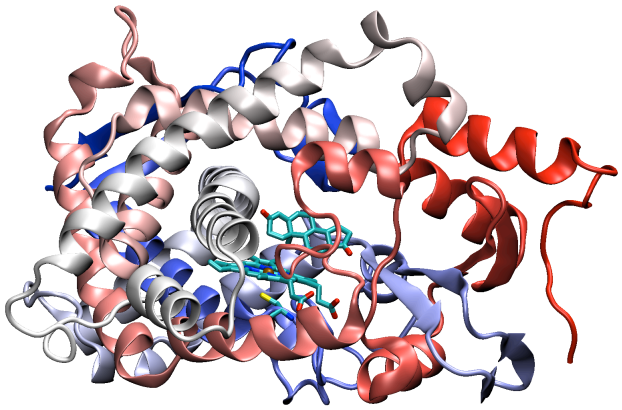




Iron-Protoporphyrin IX







$P450_{arom}$ Aromatase bound to androstenedione (PDB ID 3EQM)