Physics of Life
PHYS-468

Protein purification

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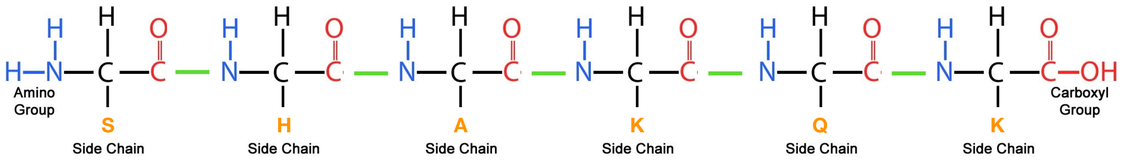
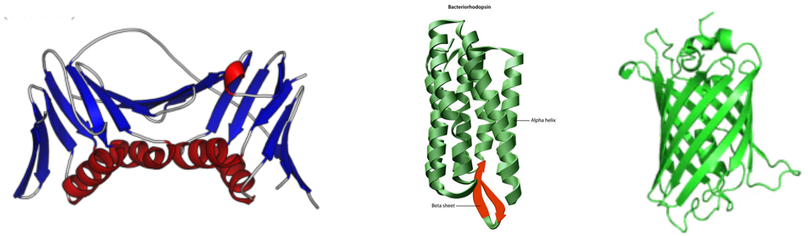
What is a protein?

Primary



Amino acid sequence
(up to 27,000 amino acids)

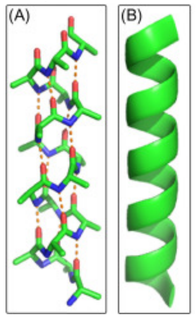
Tertiary



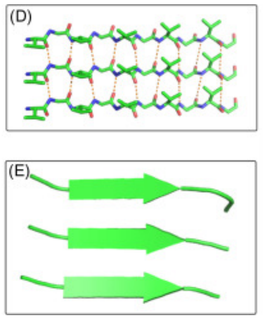
interactions of the side chains
(electrostatic interactions, salt bridges)

Secondary

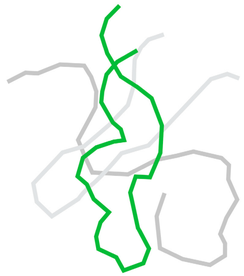
alpha-helix



beta-sheet

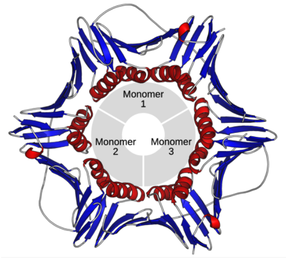


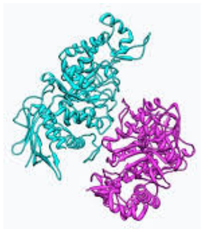
unstructured/disordered

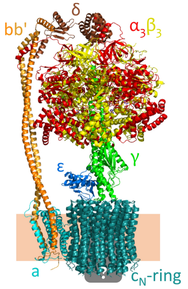


hydrogen bonding of the peptide backbone

Quaternary



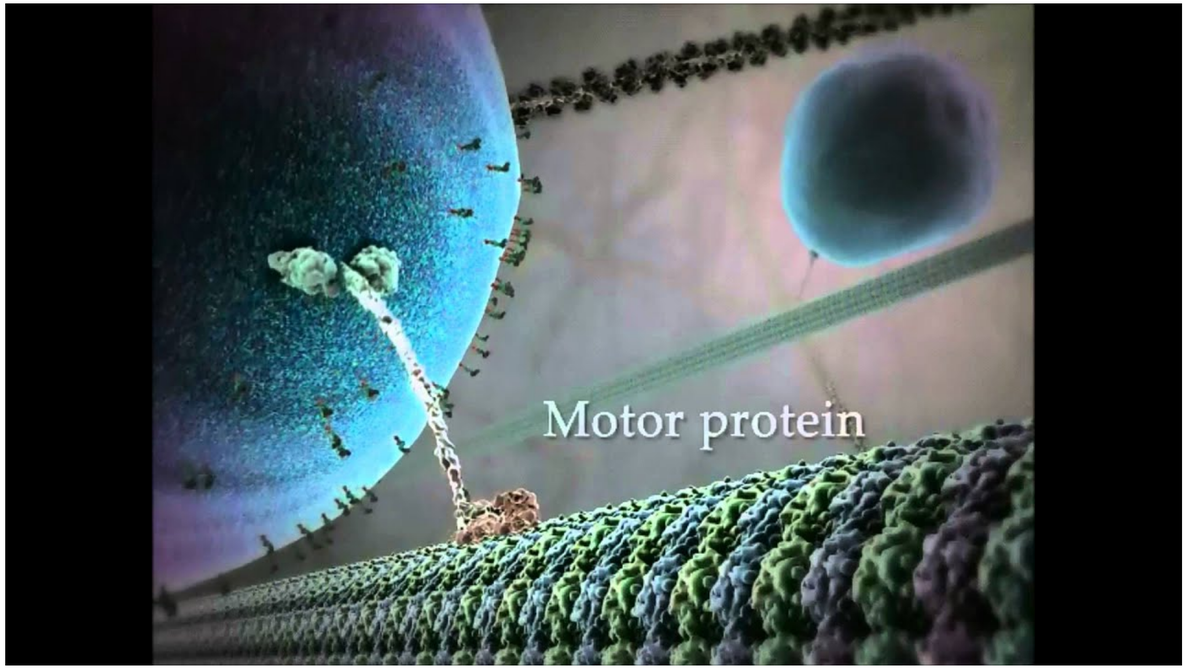




multi-protein complexes

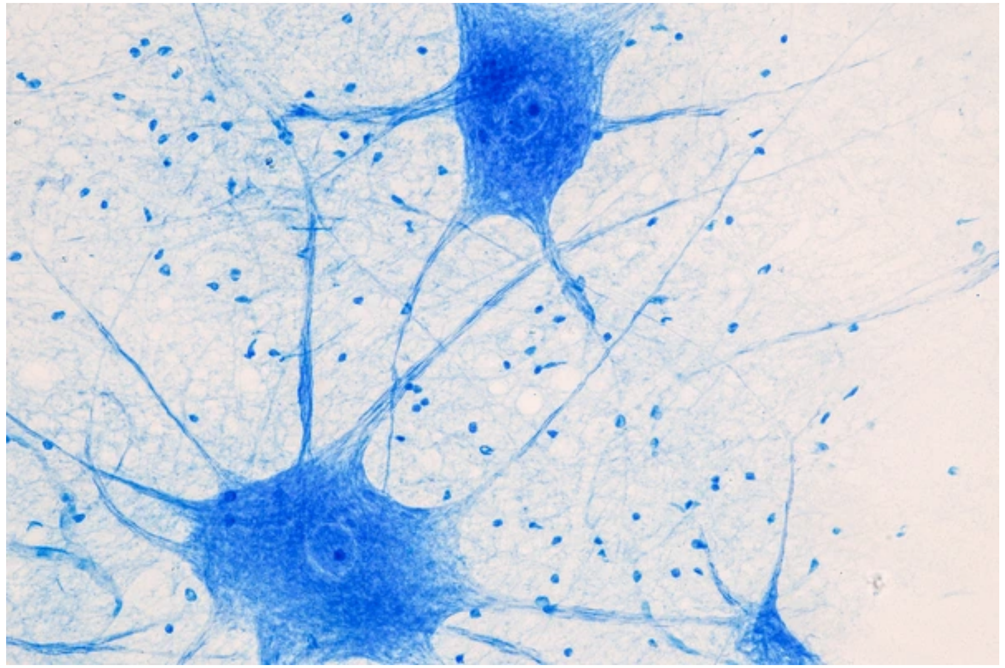
How do we know what proteins do?

Proteins are responsible for everything that happens inside a cell



https://www.youtube.com/watch?v=y-uuk4Pr2i8&t=1s

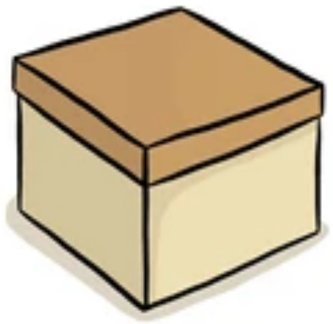
Can’t just look under a microscope



1 cell ~ 20 μm, organelles < 2 μm, protein < 5 nm

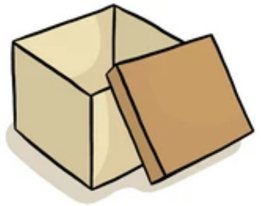
How do we know what proteins do?

There is an object in this box



What does it do?

1. Open the box



2. Remove the object

Purification

3. Look at it

Structural studies

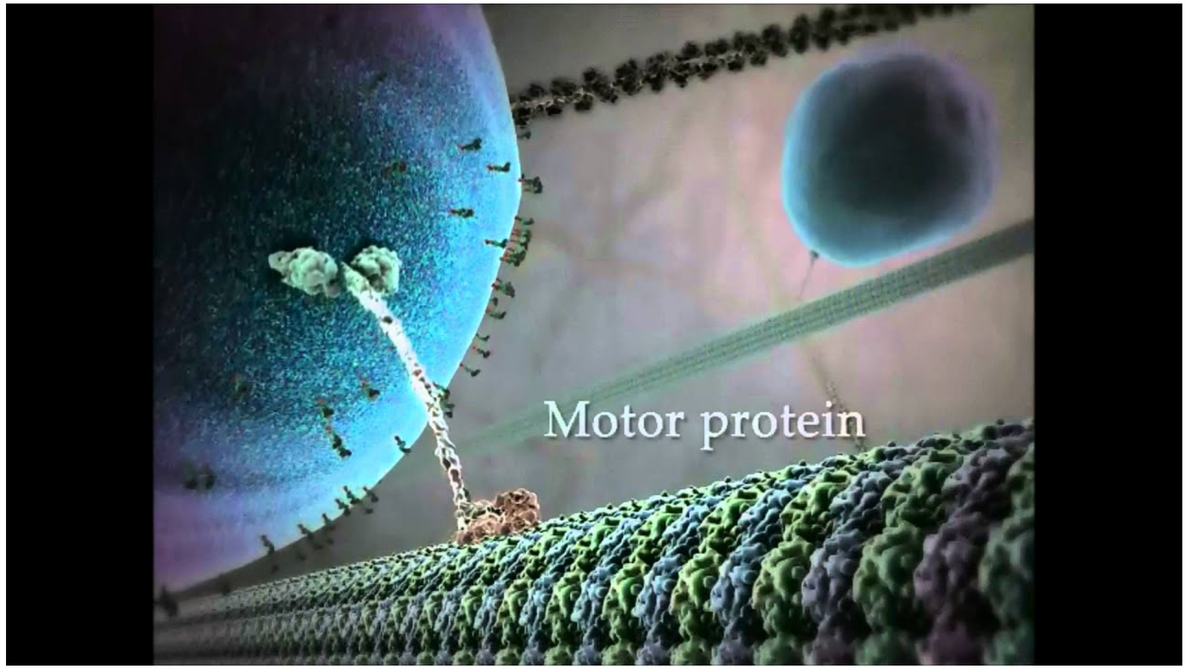
4. manipulate it

Functional studies

How do we know what proteins do?

- Protein biochemistry
- Protein mechanics
- Protein biophysics
- Protein dynamics
- Protein structure

Combining structural and functional knowledge



https://www.youtube.com/watch?v=y-uuk4Pr2i8&t=1s

Requires taking them out of the cell to study in detail

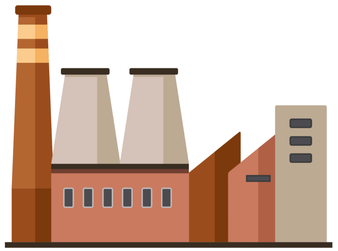
Why purify proteins?

Knowledge

- How do things work?

Biomedicine

- Disease management
- Drug development
- Vaccines
- Diagnostics

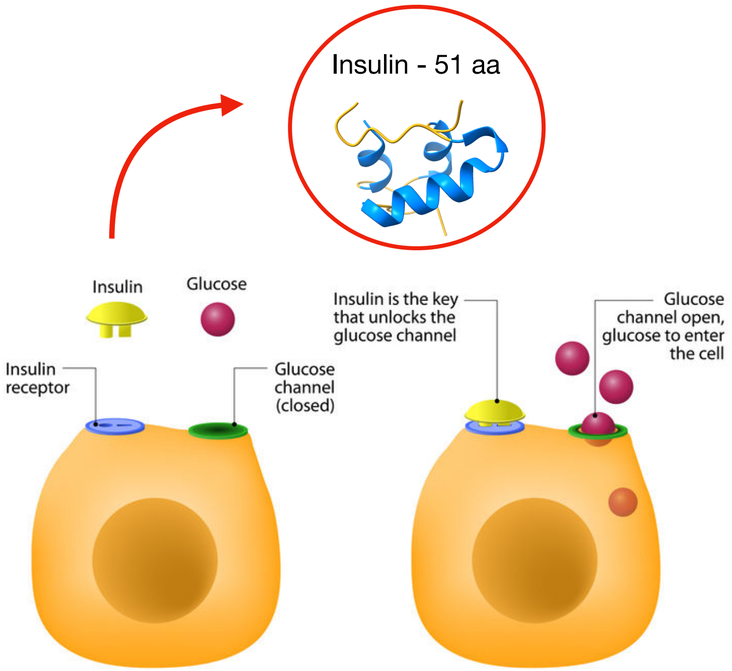


Industrial uses

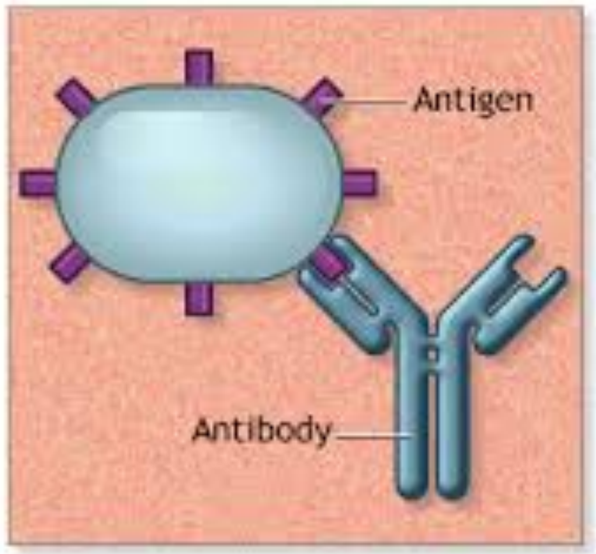
- Enzymes (food processing, textiles biofuels)
- Improving farming practices
- Forensics

Why purify proteins?

- Disease management
- Drug development
- Vaccines
- Diagnostics

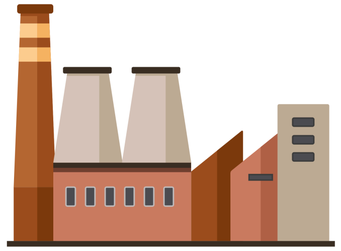


Managing diabetes



Producing vaccines

Serving as reagents in diagnostic tests



Why purify proteins?

- Enzymes (food processing, textiles biofuels)
- Improving farming practices
- Forensics

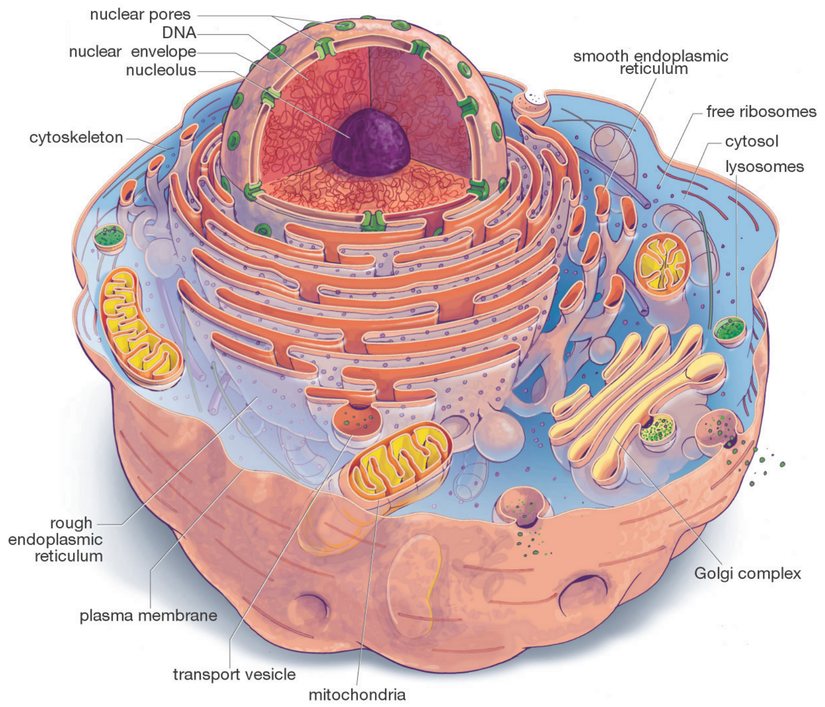
Bio-fuels

Crop protection

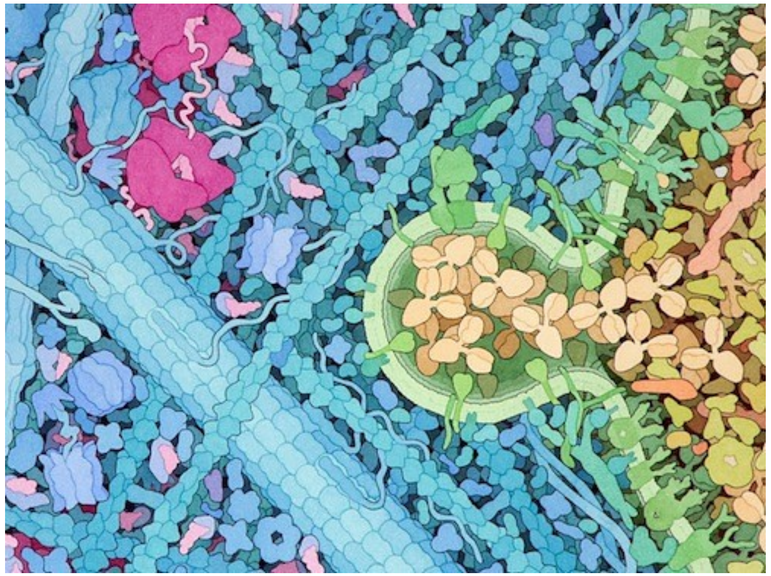
Substance identification

Cells are crowded places

Stylised animal cell



A very crowded environment



In one yeast cell

(Yeast is a eukaryotic cell)

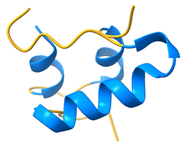
- 42 million protein molecules
- 10,000 different proteins
- Some are plentiful (>500,000 copies)
- Some are very few (10 copies)

How can you pull out just one protein, especially if its rare?

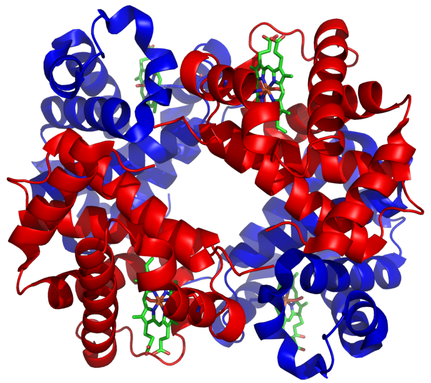
Biophysical properties of proteins

Proteins behave as colloidal particles influenced by hydrophobicity, charge, and size.

Size and shape

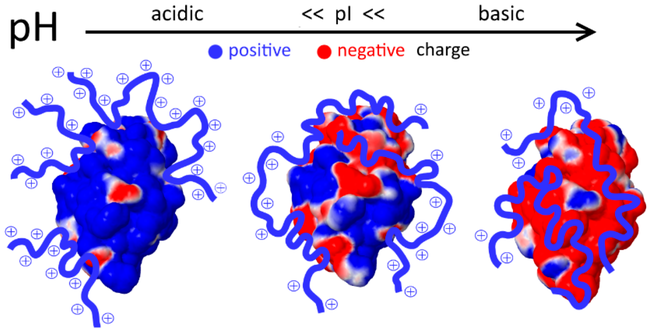


Insulin - 51 aa

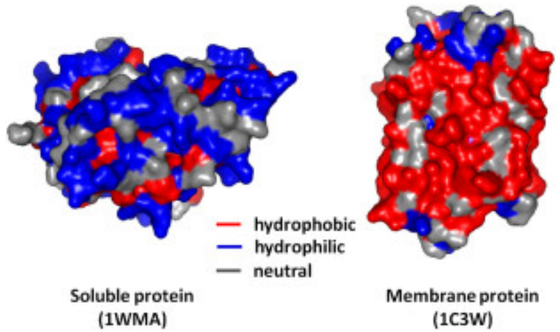


Hemoglobin - 433 aa

Charge



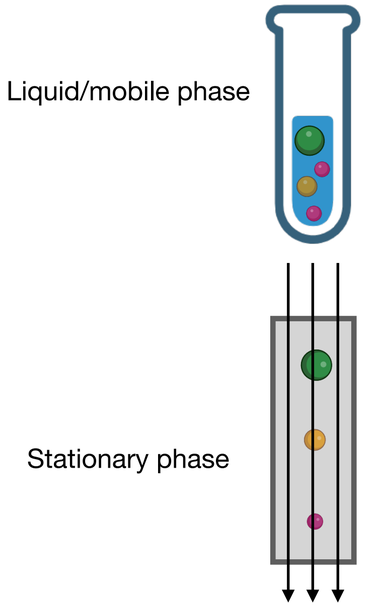
Hydrophobicity



Differences can be exploited for protein purification

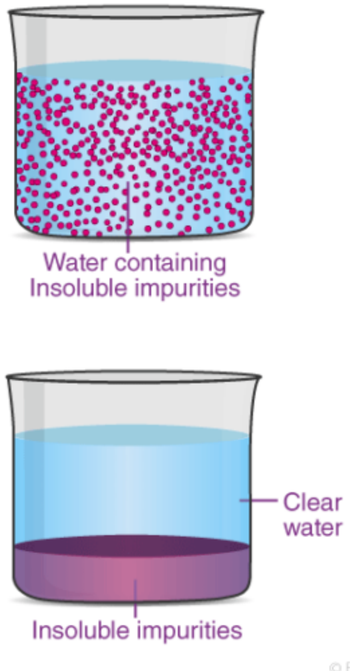
Purification techniques

Chromatography



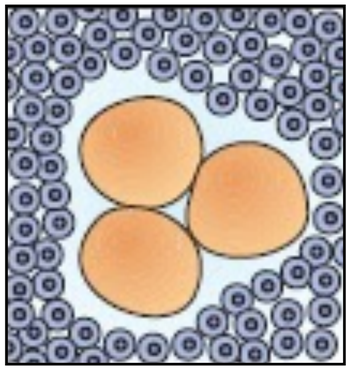
Size/shape/charge

Sedimentation



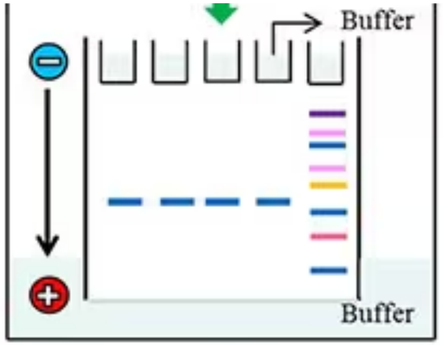
Densities

Precipitation



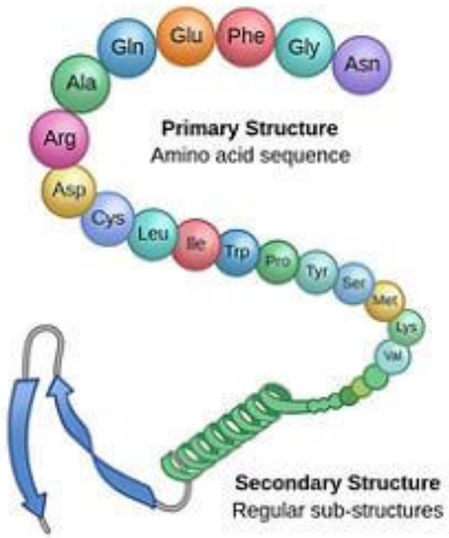
Solubilities

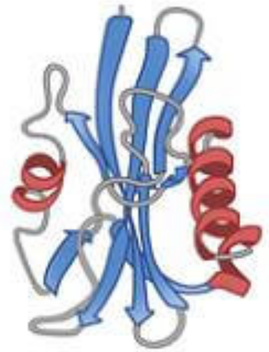
Electrophoresis



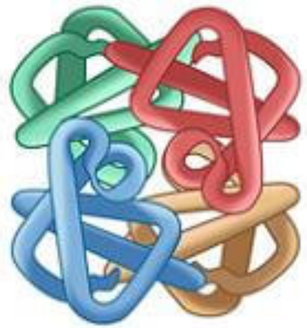
Size/charge - using a current

Proteins are made of amino acids



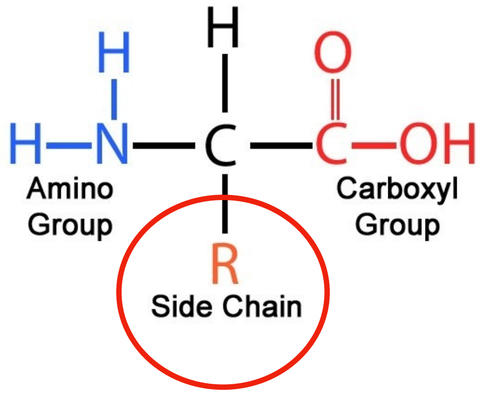


Tertiary Structure
Three-dimensional structure

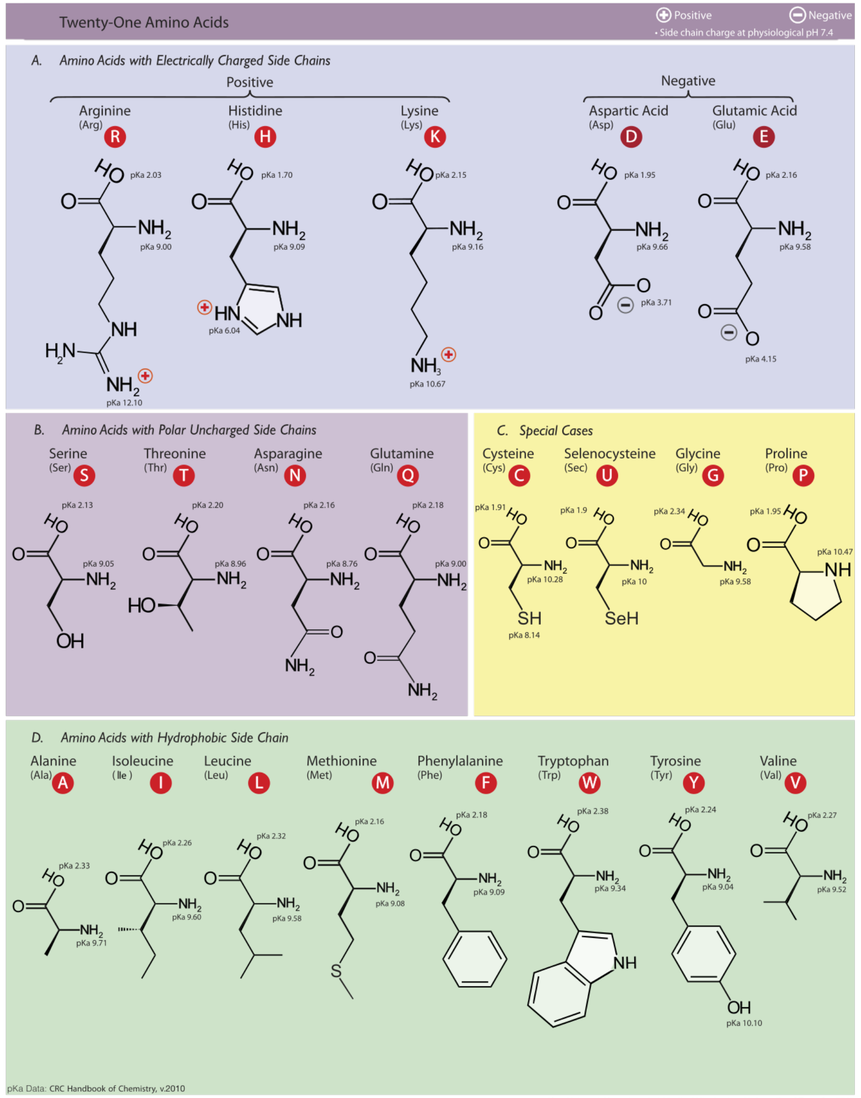


Quaternary Structure
complex of protein molecules

Amino acid



21 different side-chains

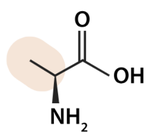


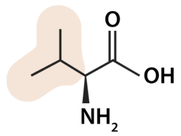
Amino acids have different properties

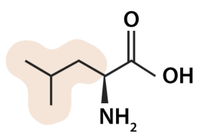
* special cases

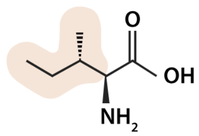
- Its amino N is locked inside a ring
- Imposes constraint (phi angle) in the peptide bond
- Induces bends in a protein structure

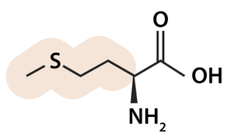
Non-polar side chains, uncharged, hydrophobic

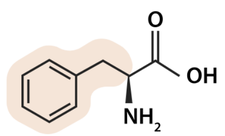

Alanine (Ala, A)

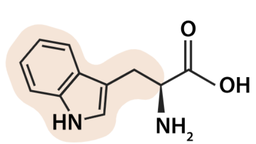

Valine (Val, V)

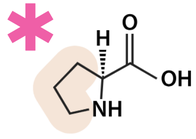

Leucin (Leu, L)


Isoleucine (Ile, I)


Methionine (Met, M)

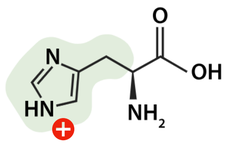

Phenylalanine (Phe, F)

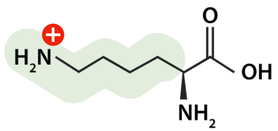

Tryptophan (Trp, W)

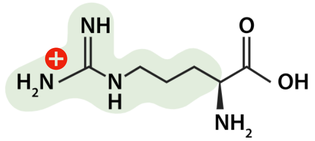

Proline (Pro, P)

Electrically charged side chains

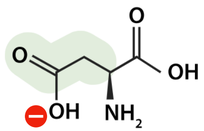
Basic

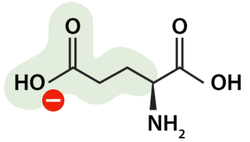

Histidine (His, H)


Lysine (Lys, K)

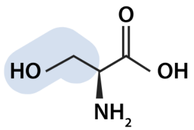

Arginine (Arg, R)

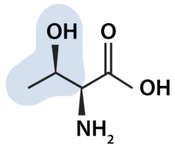
Acidic

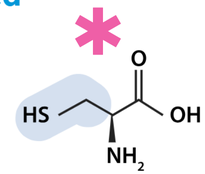

Aspartaic Acid (Asp, D)

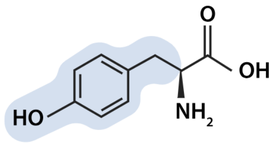

Glutamic Acid (Glu, E)

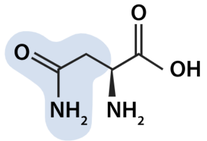
Polar side chains, uncharged

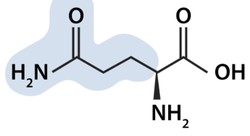

Serine (Ser, S)

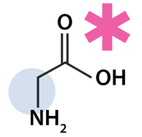

Threonine (Thr, T)


Cysteine (Cys, C)


Tyrosine (Tyr, Y)


Asparagine (Asn, N)


Glutamine (Gln, Q)


Glycine (Gly, G)

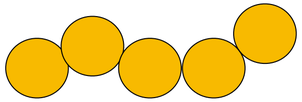
- Forms di-sulfide bond
- stabilises protein structure

- The smallest amino acid
- Enables compaction in a protein

Combination of amino acids will determine the biophysical properties of a protein

Non-polar side chains, uncharged, hydrophobic

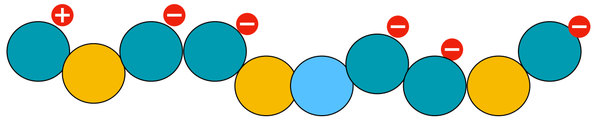
All non-polar amino acids?



= hydrophobic protein

Electrically charged side chains

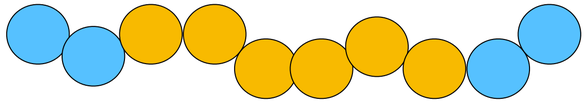
Mostly negatively charged amino acids?



= negatively charged protein

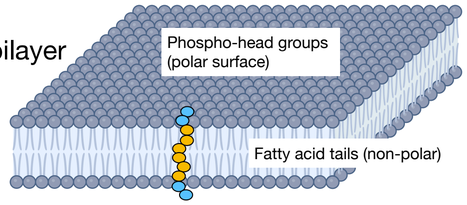
Polar side chains, uncharged

Polar ends, non-polar middle?

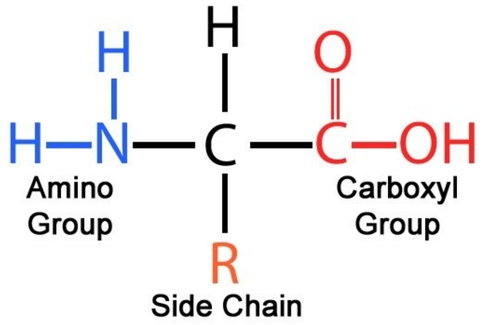


= transmembrane protein

Phospholipid bilayer



Protein size



Periodic Table of the Elements

1 IA 1A	2 IIA 2A	3 IIIB 3B	4 IVB 4B	5 VB 5B	6 VIB 6B	7 VIIB 7B	8	9 VIII 8	10	11 IB 1B	12 IIB 2B	13 IIIA 3A	14 IVA 4A	15 VA 5A	16 VIA 6A	17 VIIA 7A	18 VIIIA 8A
1 H Hydrogen [1.00784;1.00811]																	2 He Helium 4.002602(2)
3 Li Lithium [6.938;6.997]	4 Be Beryllium 9.012183(5)											5 B Boron [10.806;10.821]	6 C Carbon [12.0096;12.0116]	7 N Nitrogen [14.00643;14.00728]	8 O Oxygen [15.99903;15.99977]	9 F Fluorine 18.998403163(6)	10 Ne Neon 20.1797(6)
11 Na Sodium 22.98976928(2)	12 Mg Magnesium [24.304;24.307]											13 Al Aluminum 26.9815386(8)	14 Si Silicon [28.084;28.086]	15 P Phosphorus 30.973761998(5)	16 S Sulfur [32.059;32.076]	17 Cl Chlorine [35.446;35.457]	18 Ar Argon 39.948(1)
19 K Potassium 39.0983(1)	20 Ca Calcium 40.078(4)	21 Sc Scandium 44.955908(5)	22 Ti Titanium 47.867(1)	23 V Vanadium 50.9415(1)	24 Cr Chromium 51.9961(6)	25 Mn Manganese 54.938045(5)	26 Fe Iron 55.845(2)	27 Co Cobalt 58.933194(4)	28 Ni Nickel 58.6934(4)	29 Cu Copper 63.546(3)	30 Zn Zinc 65.38(2)	31 Ga Gallium 69.723(1)	32 Ge Germanium 72.630(8)	33 As Arsenic 74.921595(6)	34 Se Selenium 78.971(8)	35 Br Bromine [79.901,79.907]	36 Kr Krypton 83.798(2)
37 Rb Rubidium 85.4678(3)	38 Sr Strontium 87.62(1)	39 Y Yttrium 88.90584(2)	40 Zr Zirconium 91.224(2)	41 Nb Niobium 92.90637(2)	42 Mo Molybdenum 95.95(1)	43 Tc Technetium <98>	44 Ru Ruthenium 101.07(2)	45 Rh Rhodium 102.90550(2)	46 Pd Palladium 106.42(1)	47 Ag Silver 107.8682(2)	48 Cd Cadmium 112.414(4)	49 In Indium 114.818(1)	50 Sn Tin 118.710(7)	51 Sb Antimony 121.760(1)	52 Te Tellurium 127.60(3)	53 I Iodine 126.90447(3)	54 Xe Xenon 131.293(6)
55 Cs Cesium 132.90545196(6)	56 Ba Barium 137.327(7)	57-71	72 Hf Hafnium 178.49(2)	73 Ta Tantalum 180.94788(2)	74 W Tungsten 183.84(1)	75 Re Rhenium 186.207(1)	76 Os Osmium 190.23(3)	77 Ir Iridium 192.217(3)	78 Pt Platinum 195.084(9)	79 Au Gold 196.966569(5)	80 Hg Mercury 200.592(3)	81 Tl Thallium [204.382;204.385]	82 Pb Lead 207.2(1)	83 Bi Bismuth 208.98040(1)	84 Po Polonium <209>	85 At Astatine <210>	86 Rn Radon <222>
87 Fr Francium <223>	88 Ra Radium <226>	89-103	104 Rf Rutherfordium <267>	105 Db Dubnium <268>	106 Sg Seaborgium <271>	107 Bh Bohrium <272>	108 Hs Hassium <270>	109 Mt Meitnerium <276>	110 Ds Darmstadtium <281>	111 Rg Roentgenium <280>	112 Cn Copernicium <285>	113 Uut Ununtrium unknown	114 Fl Flerovium <289>	115 Uup Ununpentium unknown	116 Lv Livermorium <293>	117 Uus Ununseptium unknown	118 Uuo Ununoctium unknown

Atomic mass values reflect the IUPAC accepted values as of 09/2013.

Masses expressed in [a,b] format show the lower and upper limit of atomic mass depending on the physical and chemical history of the element.

Masses expressed in < > format are the mass numbers of the longest-lived isotope for elements with no stable nucleus.

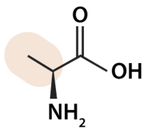
Atomic Number
Symbol
Name
Atomic Mass

Lanthanide Series	57 La Lanthanum 138.90547(7)	58 Ce Cerium 140.116(1)	59 Pr Praseodymium 140.90766(2)	60 Nd Neodymium 144.242(3)	61 Pm Promethium <145>	62 Sm Samarium 150.36(2)	63 Eu Europium 151.964(1)	64 Gd Gadolinium 157.25(3)	65 Tb Terbium 158.92535(2)	66 Dy Dysprosium 162.500(1)	67 Ho Holmium 164.93033(2)	68 Er Erbium 167.259(3)	69 Tm Thulium 168.93422(2)	70 Yb Ytterbium 173.054(5)	71 Lu Lutetium 174.9668(1)
Actinide Series	89 Ac Actinium <227>	90 Th Thorium 232.0377(4)	91 Pa Protactinium 231.03588(2)	92 U Uranium 238.02891(3)	93 Np Neptunium <237>	94 Pu Plutonium <244>	95 Am Americium <243>	96 Cm Curium <247>	97 Bk Berkelium <247>	98 Cf Californium <251>	99 Es Einsteinium <252>	100 Fm Fermium <257>	101 Md Mendelevium <258>	102 No Nobelium <259>	103 Lr Lawrencium <262>

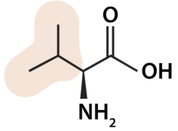
Each element has a mass Atomic mass = molecular weight (MW) = g/mol = dalton

Protein size

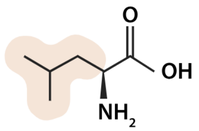
Non-polar side chains, uncharged, hydrophobic



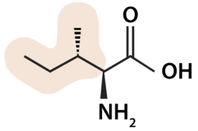
Alanine (Ala, A)
MW: 89,09



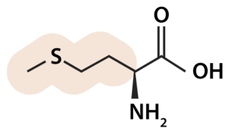
Valine (Val, V)
MW: 117,15



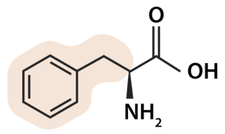
Leucin (Leu, L)
MW: 131,17



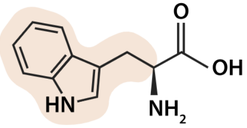
Isoleucine (Ile, I)
MW: 131,17



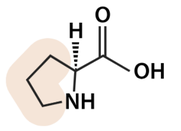
Methionine (Met, M)
MW: 149,21



Phenylalanine (Phe, F)
MW: 165,19



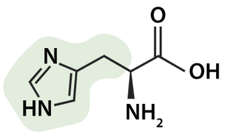
Tryptophan (Trp, W)
MW: 204,23



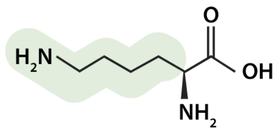
Proline (Pro, P)
MW: 115,13

Electrically charged side chains

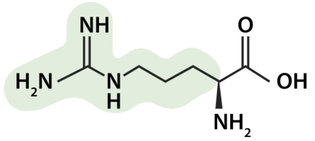
Basic



Histidine (His, H)
MW: 155,16

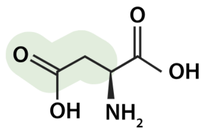


Lysine (Lys, K)
MW: 146,19

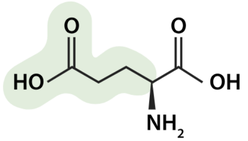


Arginine (Arg, R)
MW: 174,20

Acidic

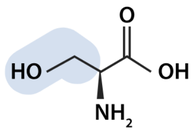


Aspartaic Acid (Asp, D)
MW: 133,1

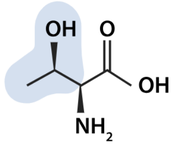


Glutamic Acid (Glu, E)
MW: 147,13

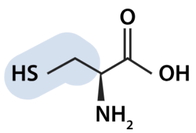
Polar side chains, uncharged



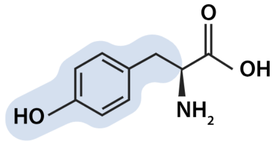
Serine (Ser, S)
MW: 105,09



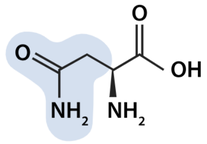
Threonine (Thr, T)
MW: 119,12



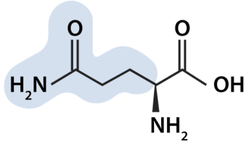
Cysteine (Cys, C)
MW: 121,16



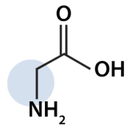
Tyrosine (Tyr, Y)
MW: 181,19



Asparagine (Asn, N)
MW: 132,12



Glutamine (Gln, Q)
MW: 146,15



Glycine (Gly, G)
MW: 75,07

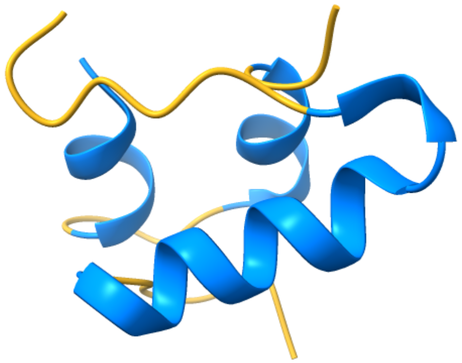
Each amino acid has a different mass

molecular weight (MW) = g/mol = dalton

Protein size

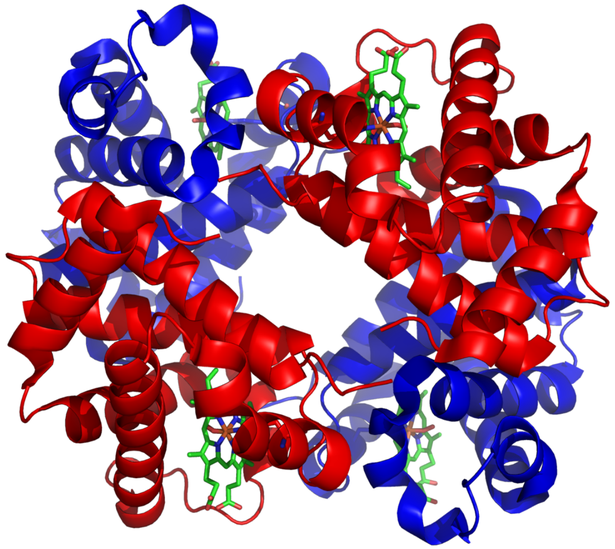
Insulin - 51 aa

5.8 kDa



Hemoglobin - 433 aa → Up to 27,000 aa!

64.5 kDa



Each protein has a different size and mass

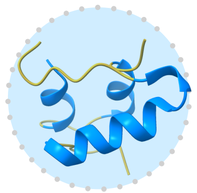
molecular weight (MW) = g/mol = dalton

Protein shape

Variations in protein structure means that proteins have different shapes

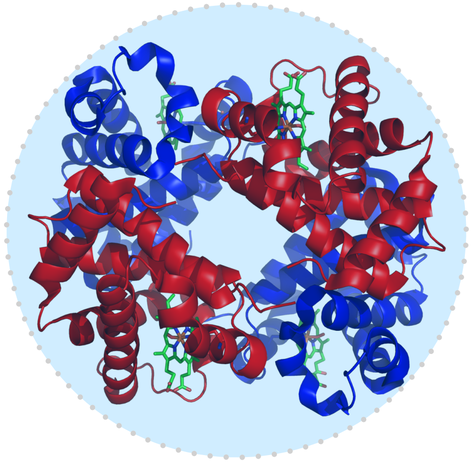
Different shapes will interact with the surrounding liquid differently (hydrodynamics)

Insulin - 51 aa



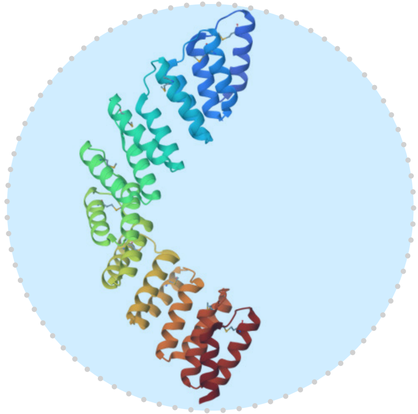
small and compact

Hemoglobin - 433 aa



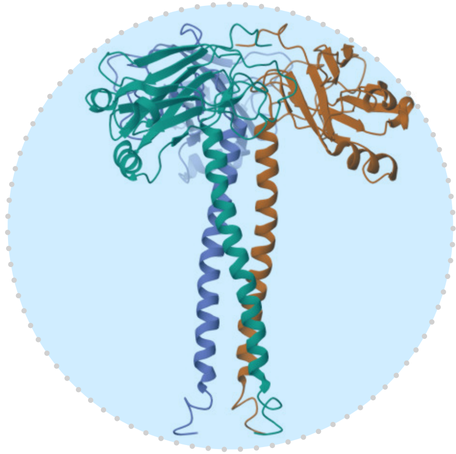
large and compact

PPR protein- 350 aa



elongated

WD40/TRAF protein - 410 aa



Compact region + elongated region

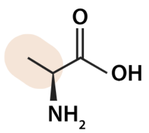
Combination of size and shape

Hydrodynamic radius

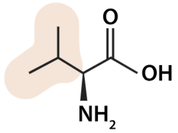
- The radius of a sphere with the same hydrodynamic properties
- Experimentally determined by Stokes Law

Protein charge

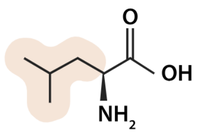
Non-polar side chains, uncharged, hydrophobic



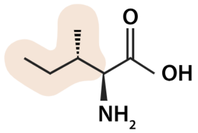
Alanine (Ala, A)



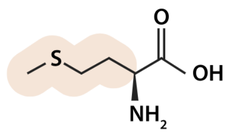
Valine (Val, V)



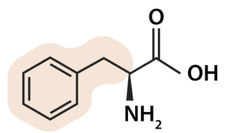
Leucin (Leu, L)



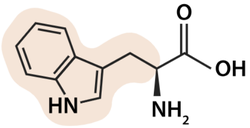
Isoleucine (Ile, I)



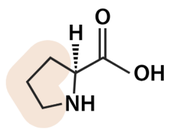
Methionine (Met, M)



Phenylalanine (Phe, F)



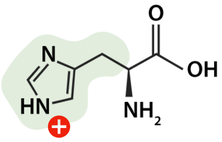
Tryptophan (Trp, W)



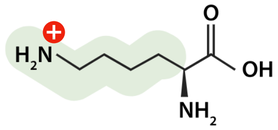
Proline (Pro, P)

Electrically charged side chains

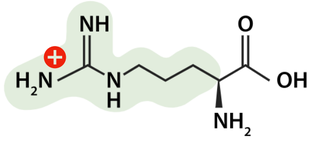
Basic



Histidine (His, H)

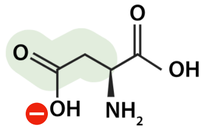


Lysine (Lys, K)

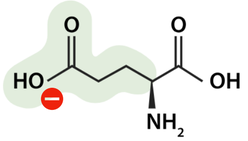


Arginine (Arg, R)

Acidic

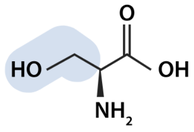


Aspartaic Acid (Asp, D)

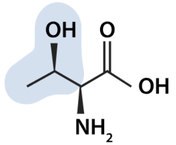


Glutamic Acid (Glu, E)

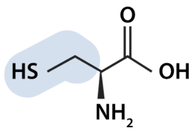
Polar side chains, uncharged



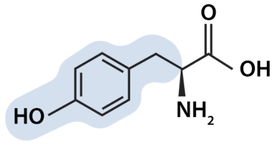
Serine (Ser, S)



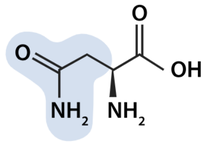
Threonine (Thr, T)



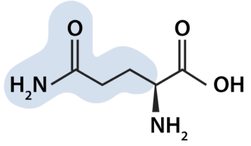
Cysteine (Cys, C)



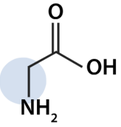
Tyrosine (Tyr, Y)



Asparagine (Asn, N)



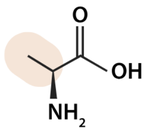
Glutamine (Gln, Q)



Glycine (Gly, G)

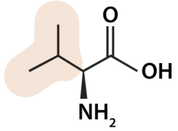
Protein charge

Non-polar side chains, uncharged, hydrophobic



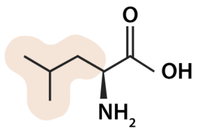
Alanine (Ala, A)

pI: 6,01



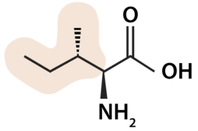
Valine (Val, V)

pI: 6,00



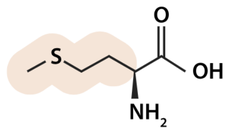
Leucin (Leu, L)

pI: 6,01



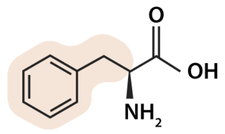
Isoleucine (Ile, I)

pI: 6,05



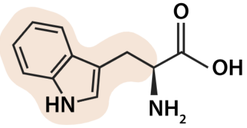
Methionine (Met, M)

pI: 5,74



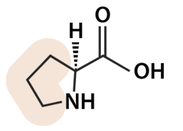
Phenylalanine (Phe, F)

pI: 5,49



Tryptophan (Trp, W)

pI: 5,89

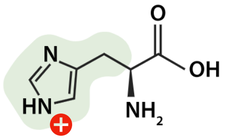


Proline (Pro, P)

pI: 6,30

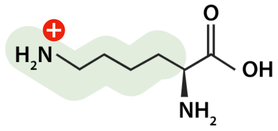
Electrically charged side chains

Basic



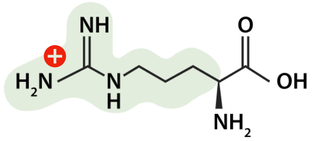
Histidine (His, H)

pI: 7,60



Lysine (Lys, K)

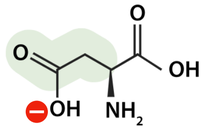
pI: 9,60



Arginine (Arg, R)

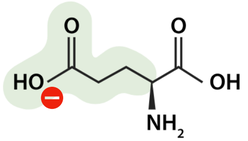
pI: 10,76

Acidic



Aspartaic Acid (Asp, D)

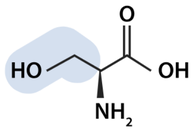
pI: 2,85



Glutamic Acid (Glu, E)

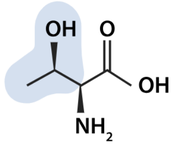
pI: 3,15

Polar side chains, uncharged



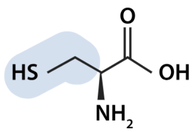
Serine (Ser, S)

pI: 5,68



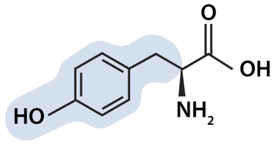
Threonine (Thr, T)

pI: 5,60



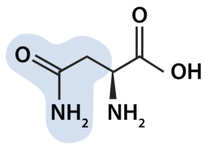
Cysteine (Cys, C)

pI: 5,05



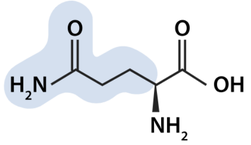
Tyrosine (Tyr, Y)

pI: 5,64



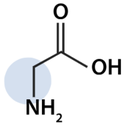
Asparagine (Asn, N)

pI: 5,41



Glutamine (Gln, Q)

pI: 5,65

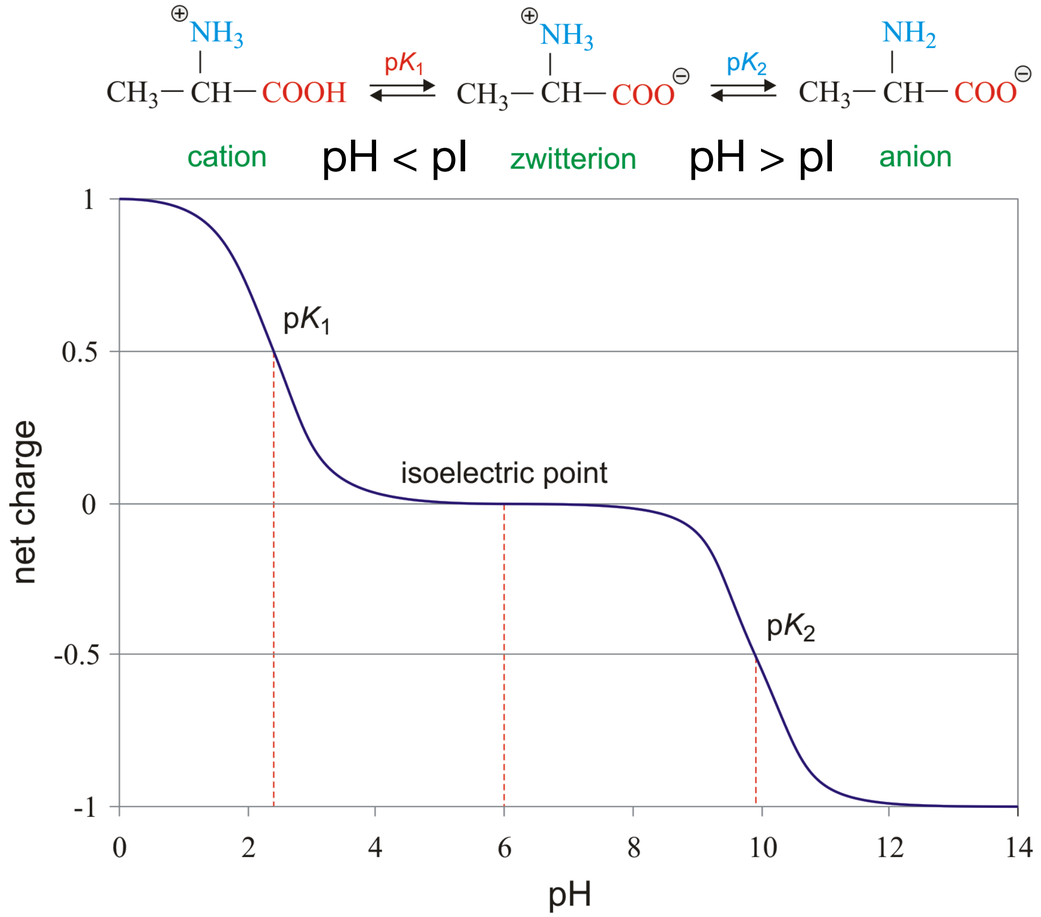


Glycine (Gly, G)

pI: 6,06

Each amino acid has a different level of acidity (charge), expressed as pI

Isoelectric point (pI)

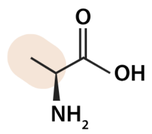


The pH at which the net charge is 0

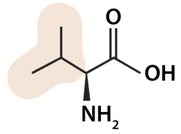
Protein charge

Non-polar side chains, uncharged, hydrophobic

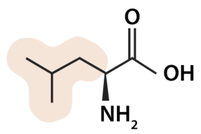
pI's between 5.5-6.3



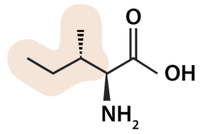
Alanine (Ala, A)
pI: 6,01



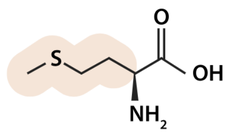
Valine (Val, V)
pI: 6,00



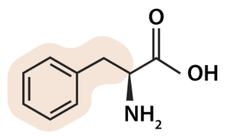
Leucin (Leu, L)
pI: 6,01



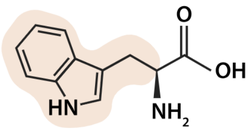
Isoleucine (Ile, I)
pI: 6,05



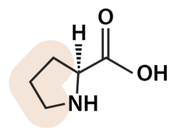
Methionine (Met, M)
pI: 5,74



Phenylalanine (Phe, F)
pI: 5,49



Tryptophan (Trp, W)
pI: 5,89

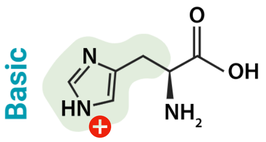


Proline (Pro, P)
pI: 6,30

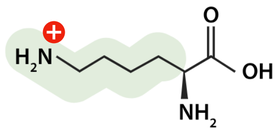
Electrically charged side chains

Extreme pI's

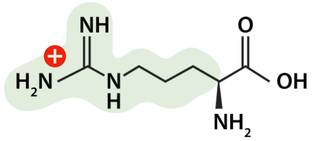
Basic



Histidine (His, H)
pI: 7,60

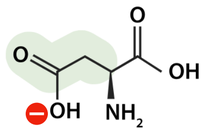


Lysine (Lys, K)
pI: 9,60

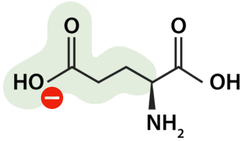


Arginine (Arg, R)
pI: 10,76

Acidic



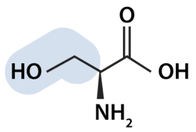
Aspartaic Acid (Asp, D)
pI: 2,85



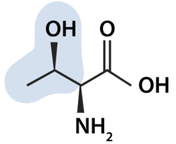
Glutamic Acid (Glu, E)
pI: 3,15

Polar side chains, uncharged

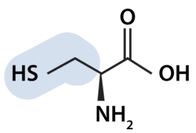
pI between 5 - 6



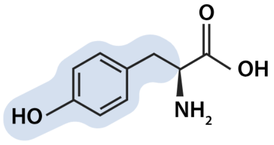
Serine (Ser, S)
pI: 5,68



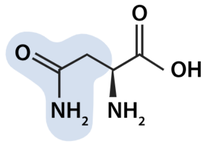
Threonine (Thr, T)
pI: 5,60



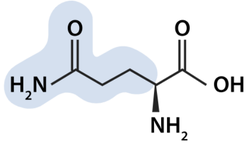
Cysteine (Cys, C)
pI: 5,05



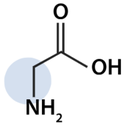
Tyrosine (Tyr, Y)
pI: 5,64



Asparagine (Asn, N)
pI: 5,41



Glutamine (Gln, Q)
pI: 5,65



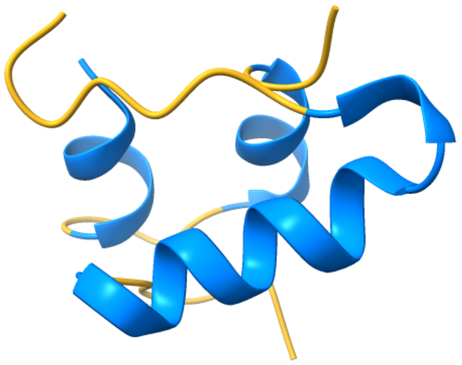
Glycine (Gly, G)
pI: 6,06

At physiological pH (pH 7.4), most amino acids will exist as anion's (negatively charged)

Protein charge

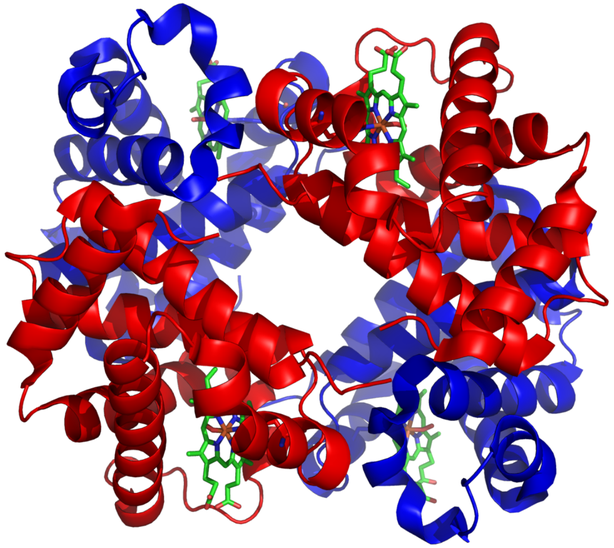
Insulin - 51 aa

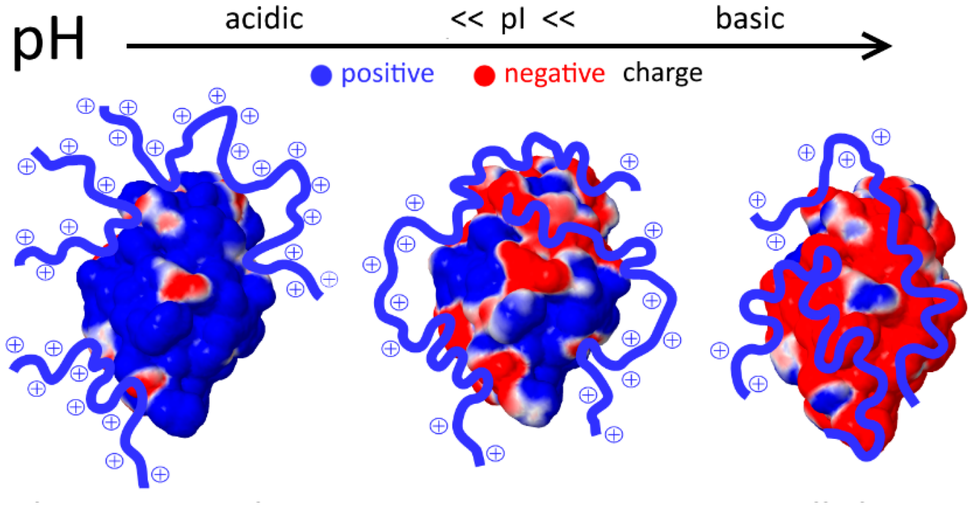
pI - 5.4



Hemoglobin - 433 aa

pI - 7

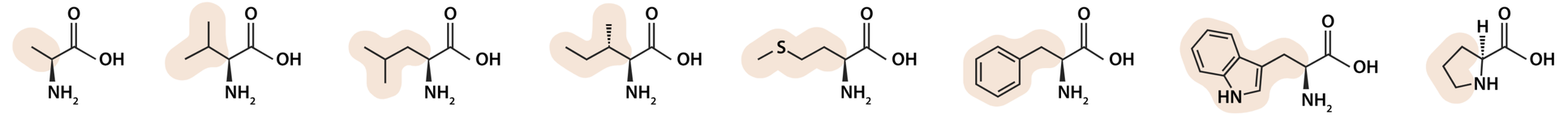




Each protein has a different net charge (pI)

Hydrophobicity

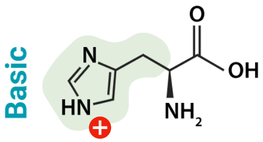
Non-polar side chains, uncharged, hydrophobic



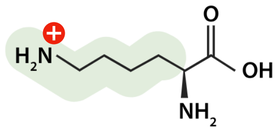
Alanine (Ala, A) **Valine** (Val, V) **Leucin** (Leu, L) **Isoleucine** (Ile, I) **Methionine** (Met, M) **Phenylalanine** (Phe, F) **Tryptophan** (Trp, W) **Proline** (Pro, P)

Electrically charged side chains

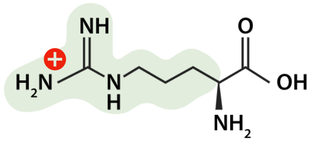
Basic



Histidine (His, H)

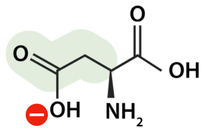


Lysine (Lys, K)

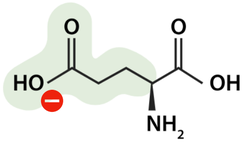


Arginine (Arg, R)

Acidic

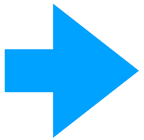


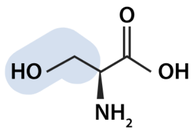
Aspartaic Acid (Asp, D)



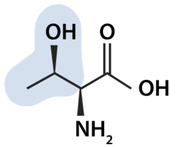
Glutamic Acid (Glu, E)

Polar side chains, uncharged

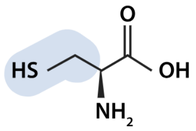




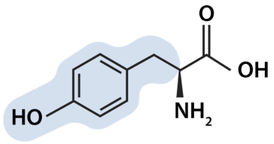
Serine (Ser, S)



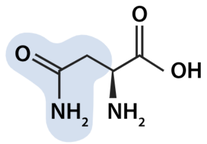
Threonine (Thr, T)



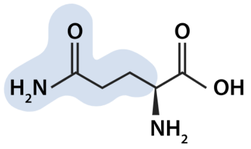
Cysteine (Cys, C)



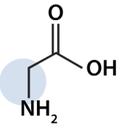
Tyrosine (Tyr, Y)



Asparagine (Asn, N)



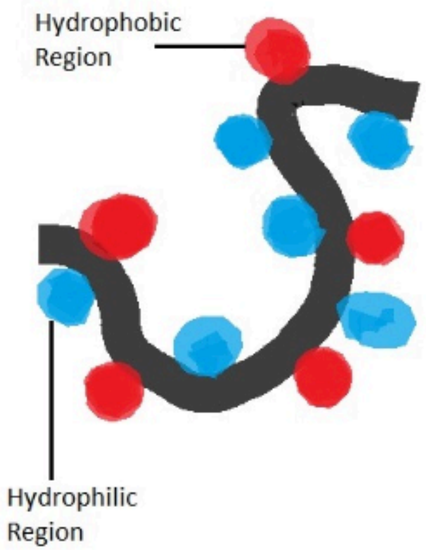
Glutamine (Gln, Q)



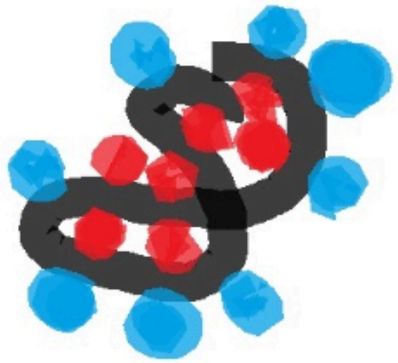
Glycine (Gly, G)

Each amino acid has a different hydrophobicities

Hydrophobicity



Sequence of amino acids



Protein in aqueous solution

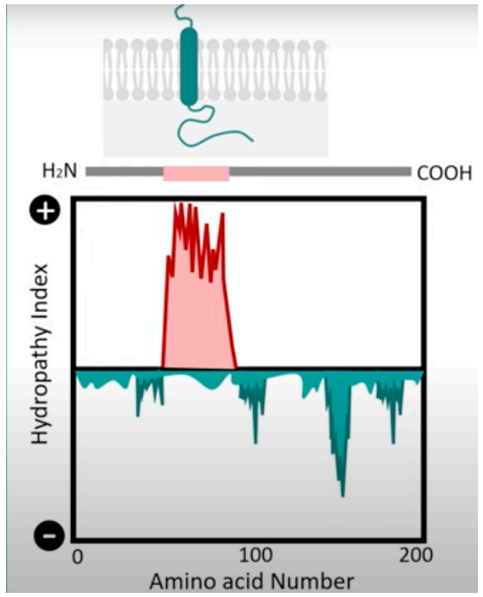
Hydrophobic aa tend to be on the inside, protected by the hydrophilic regions

Hydrophobicity drives protein folding

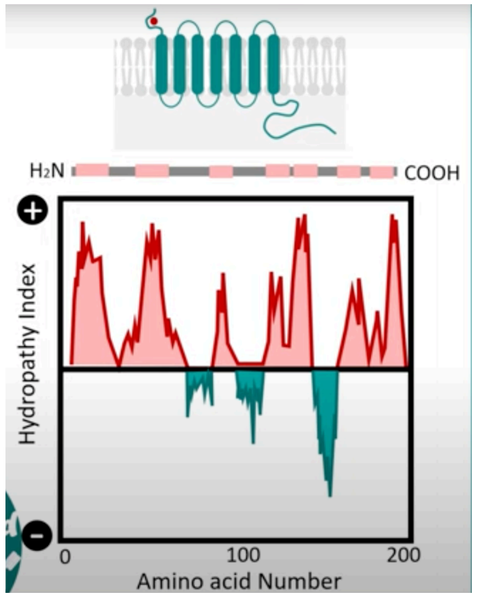
Hydrophobicity

Hyrophobicity of a protein can be predicted from the aa sequence and displayed in a hydropathy plot.

1 transmembrane domain

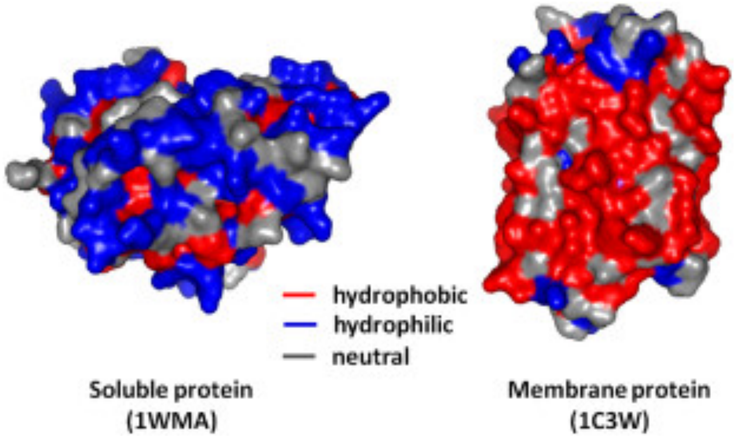


7 transmembrane domains



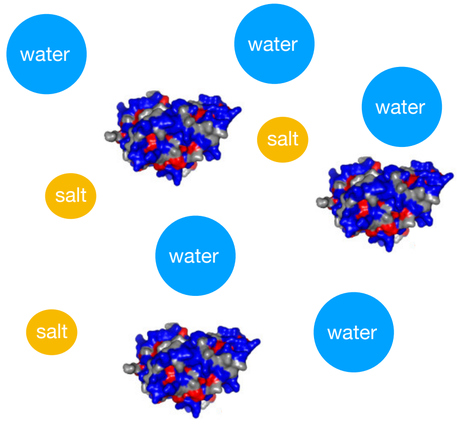
Can be used to predict transmembrane domains in a protein

Hydrophobicity



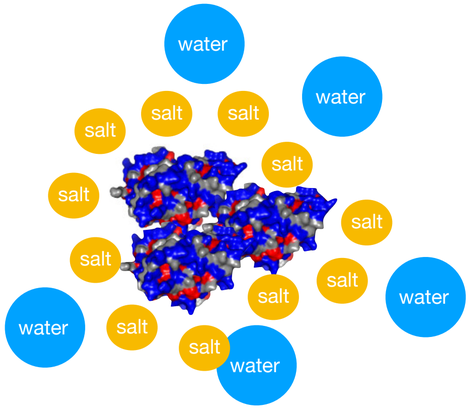
Proteins have different surface hydrophobicity profile

Protein soluble in solution



Change in ionic strength
of solution

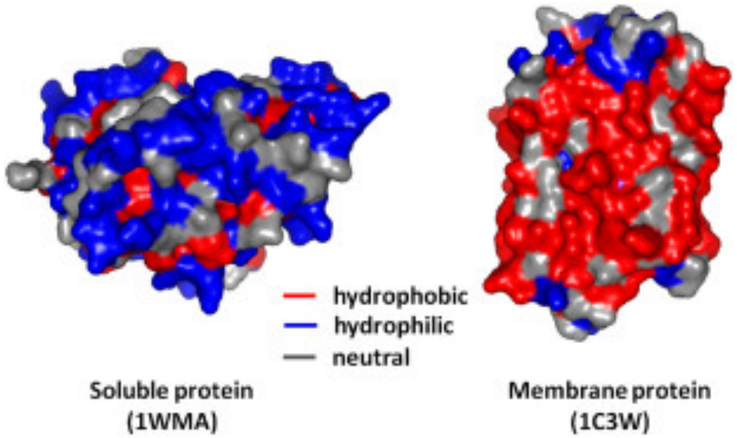
Protein in-soluble in solution



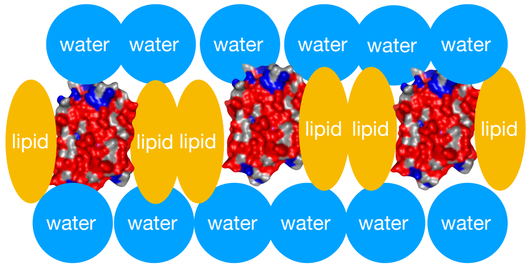
protein aggregation/precipitation

Hydrophobicity profile of a protein can drive its solubility in a given solution

Membrane proteins

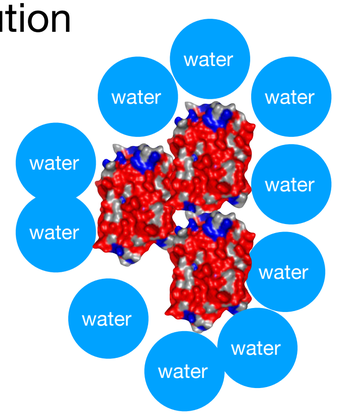


Membrane protein soluble in lipid



Removal of lipids

Membrane protein in-soluble in solution



protein aggregation/precipitation

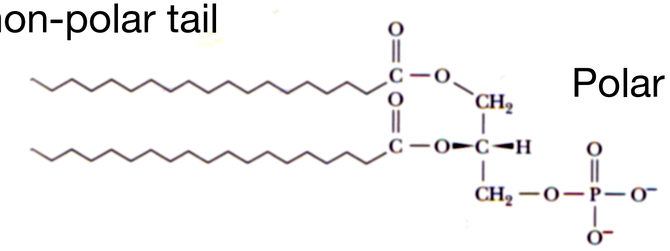
Membrane proteins will aggregate in solution, without the presence of lipids

Detergents

Detergents have a similar structure to lipids and can easily insert into the lipid membrane

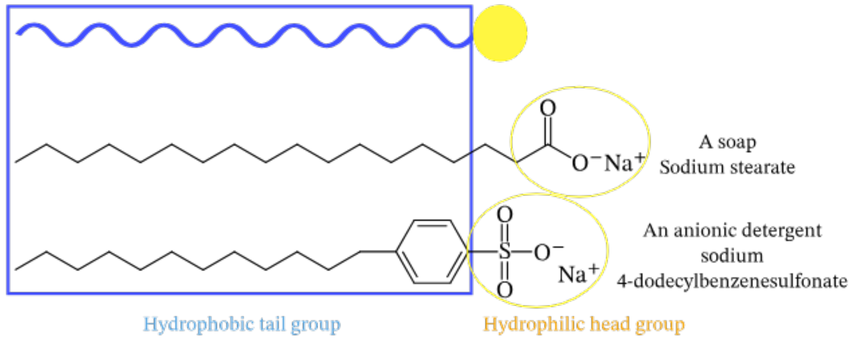
Lipid

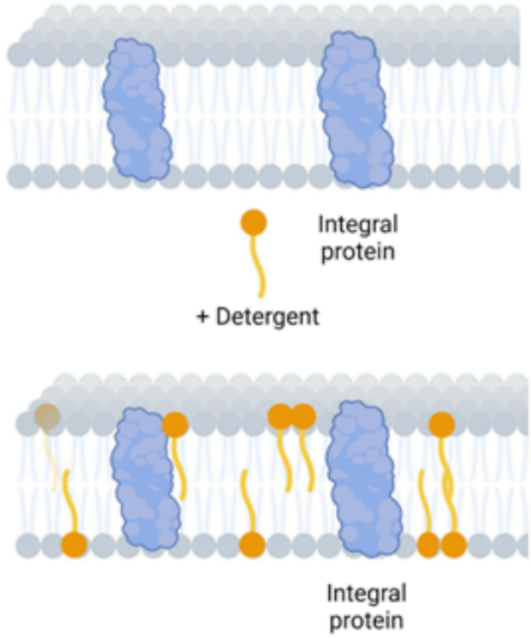
non-polar tail



Polar head

Detergent

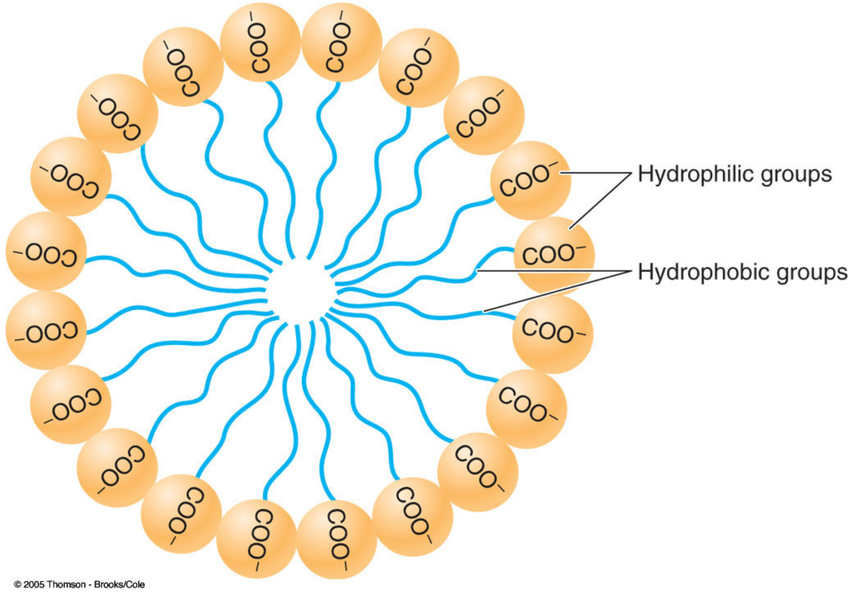




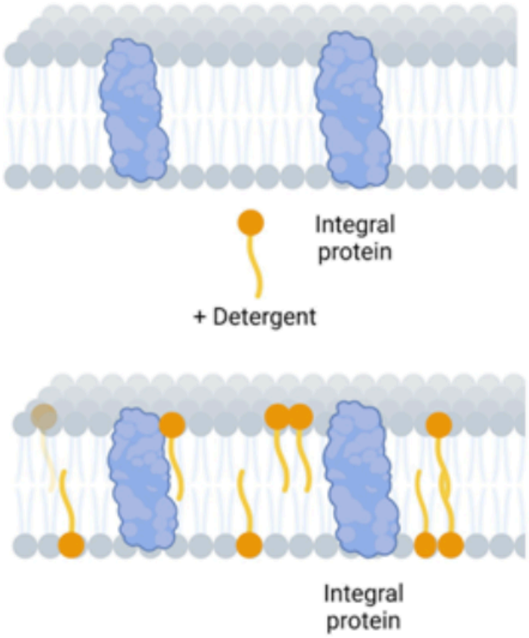
The insertion of the detergents de-stabilises the lipid membrane structure

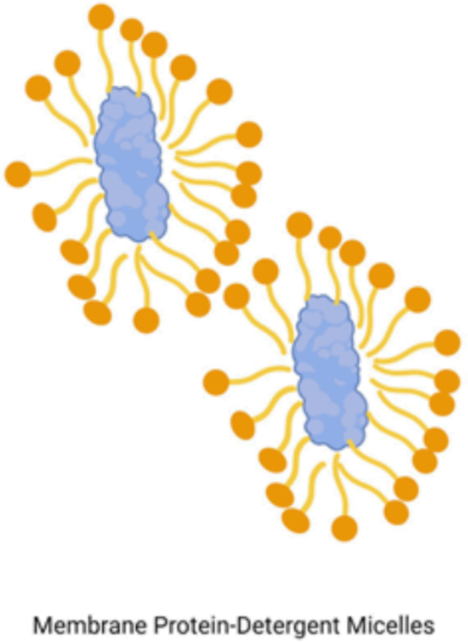
Detergents

Detergents can also form micelles



When the detergent concentration is high enough, it displaces the lipids and forms a micelle around the membrane protein



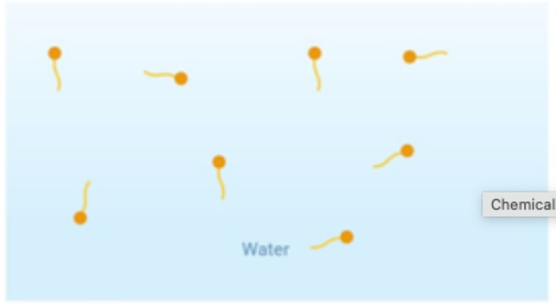


= critical micellar concentration (CMC)

Critical micelle concentration

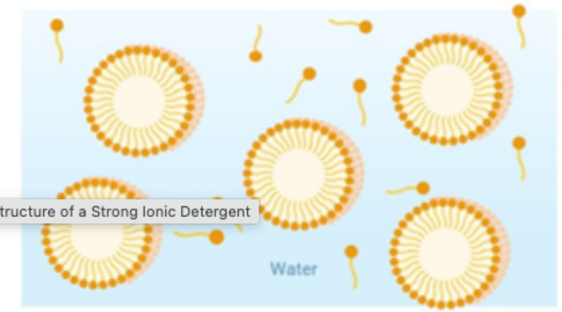
The concentration at which a detergent forms a micelle

below CMC

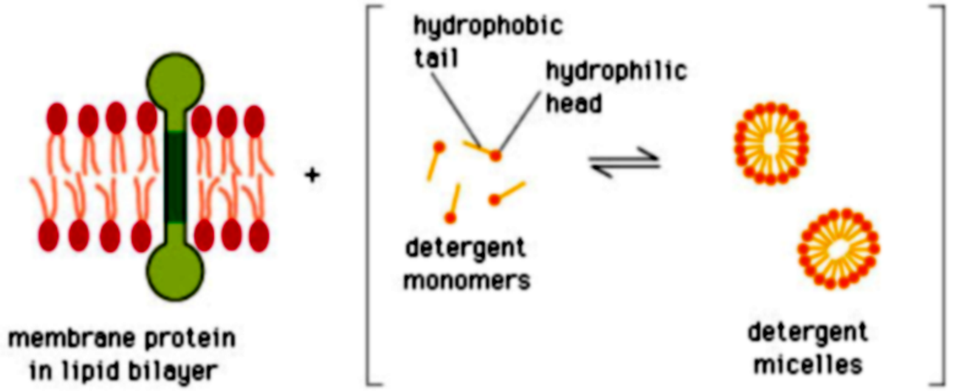


detergent monomers

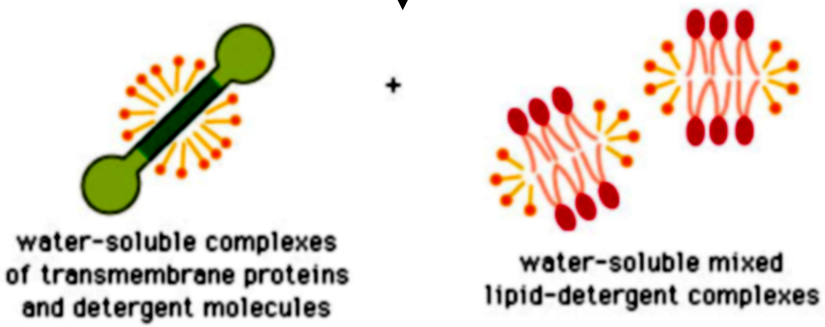
above CMC



detergent monomers
plus detergent micelles

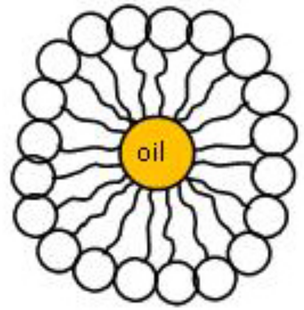


If [detergent] > cmc:



Detergents

Same mechanism for shampoo, dishwashing liquid and soap



Name	Abreviation	M.W. (anhydrous)	CMC (mM)	CMC (%)	Aggregation No.	Average Micellar Weight (Da)
Non-ionic detergents						
APO-10		218.3	4.6	0.100	131	28,000
APO-12		246.4	0.568	0.013	2232	500,000
Big CHAP		878.1	2.9	0.25	10	8,800
Big CHAP, Deoxy		862.1	1.1-1.4	0.12	8-16	10,500
BRIJ® 35	Brij-35	627	0.09	0.0056	40	49,000
$C_{12}E_5$		406.6	0.064	0.002	-	-
$C_{12}E_6$		450.7	0.087	0.0039	-	-
$C_{12}E_8$	$C_{12}E_8$	538.8	0.11	0.0059	123	66,000
$C_{12}E_9$	$C_{12}E_9$	582.8	0.08	0.0046	-	83,000
Cyclohexyl-*n*-ethyl-β-D-maltoside		452.5	120	5.43	-	-
Cyclohexyl-*n*-hexyl-β-D-maltoside		508.6	0.56	0.0284	63	32,000
Cyclohexyl-*n*-methyl-β-D-maltoside		438.5	340	14.909	-	-
7-Cyclohexyl-1-heptyl-β-D-maltoside	Cymal-7	522.5	0.19	0.00992	150	78,300
n-Decanoylsucrose		496.6	2.5	0.124	-	-
n-Decyl-β-D-maltopyranoside	DM	482.6	1.6	0.087	69	-
n-Decyl-β-D-thiomaltoside	DTM	498.6	0.9	0.0448	-	-
Digitonin		1229.3	<0.5		60	74,000
n-Dodecanoylsucrose		524.6	0.3	0.0157	-	-
n-Dodecyl-β-D-glucopyranoside		348.5	0.19	0.0066	200	70,000
n-Dodecyl-β-D-maltoside	DDM	510.6	0.1-0.6	0.009	98	50,000
Dodecyl-trimethyl-ammonium chloride	DTAC	264	17.0	0.488	50	13,200
n-Heptyl-β-D-glucopyranoside		278.3	70	1.9	-	-
n-Heptyl-β-D-thioglucopyranoside	HTG	294.4	79	2.325	-	-
n-Nonyl-β-D-glucopyranoside	NG	306.4	6.5	0.2	133	-
Methyl 6-O-(N-heptylcarbamoyl)-α-D-glucopyranoside	Hecameg	335.4	19.5	0.654		
Nonidet P-40 (octylphenoxypolyethoxyethanol), now IGEPAL CA-630	Nonidet P-40	558.7	0.25	0.014	149	90,000
NP-40 (nonylphenoxypolyethoxyethanol)	NP-40	603.0	0.05-0.3	0.05-0.3	100-155	76,600
n-Octanoyl-β-D-glucosylamine	NOGA	305.4	80	2.443	-	-
n-Octanoylsucrose		468.5	24.4	1.143	-	-
n-Octyl-β-D-glucopyranoside	OG	292.4	10-21	0.3-0.6	84	25,000
n-Octyl-β-D-maltopyranoside		454.5	19.5	0.89	84	38,000
n-Octyl-β-D-thioglycopyranoside	OTG	308.4	9	0.277	-	-
n-Octylpolyoxyethylene	Octyl-POE	174.3	6.6	0.115		
TRITON® X-100	TX-100	625	0.01-0.016	0.015	100-155	80,000
TWEEN® 20	Tween 20	1228	0.059	0.0072	-	-
TWEEN® 80	Tween 80	1310	0.012	0.00157	60	79,000
n-Undecyl-β-D-maltoside	UDM	496.6	0.59	0.0292	-	-
Ionic Detergents						
Amphipol A8-35		9-10	-	20	-	-
Cetyltrimethylammonium Bromide	CTAB	364.5	1.0	0.0364	170	62,000
Cholic Acid, Sodium Salt	Cholate	430.6	9-15		2.0	900
Deoxycholic Acid, Sodium Salt, Na-deoxycholate	DOC	414.6	4-8	0.24	22	1600-4100
Lauroylsarcosine, Sodium Salt		293.4	14.57	0.427	2.0	600
Taurocholic Acid, Sodium Salt		537.7	3-11		4	2100
Zwitterionic Detergents						
CHAPS	CHAPS	614.9	6-10	0.49	10	6000
CHAPSO		630.9	8	0.5	11	7000
Diheptanoyl-*sn*-Glycero-3-Phosphocholine	DHPC	481.5	1.4	0.07	100	50,000
Lauryldimethylamine Oxide, 30% Solution	LDAO	229.4	1-2	0.023	76	17,000
ZWITTERGENT® 3-08 Detergent		279.6	330	10.9	-	-
ZWITTERGENT® 3-10 Detergent		307.6	25-40	1.2	41	12,500
ZWITTERGENT® 3-12 Detergent		335.6	2-4	0.094	55	18,500
ZWITTERGENT® 3-14 Detergent		363.6	0.1-0.4	0.007	83	30,000
ZWITTERGENT® 3-16 Detergent		391.6	0.01-0.06	0.0011	155	60,000

- Many different types of detergents (based on their different structures)
- Each one has a different CMC
- Will interact differently with any given protein depending on the biophysical properties of both (charge/hydrophobicity)

Slide from HS

The biophysical properties of a protein determine how it moves through solution

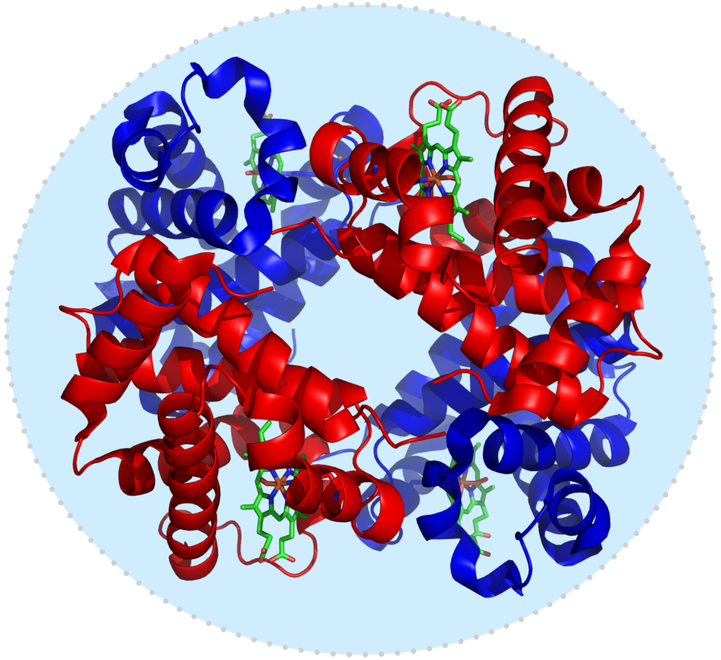
Size (molecular weight)

Charge (pI)

Shape (hydrodynamic radius)

Solubility (hydrophobicity)

Hemoglobin - 433 aa



64.5 kDa

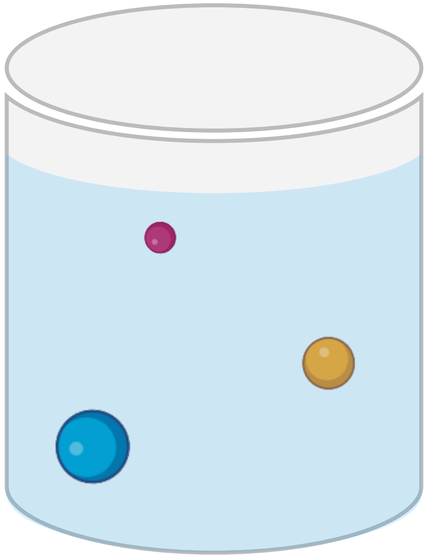
pI - 7

- Differences in behaviour can then be exploited for purification techniques
- Differences in behaviour can be described by diffusion behaviour and Stokes Law

Stokes law

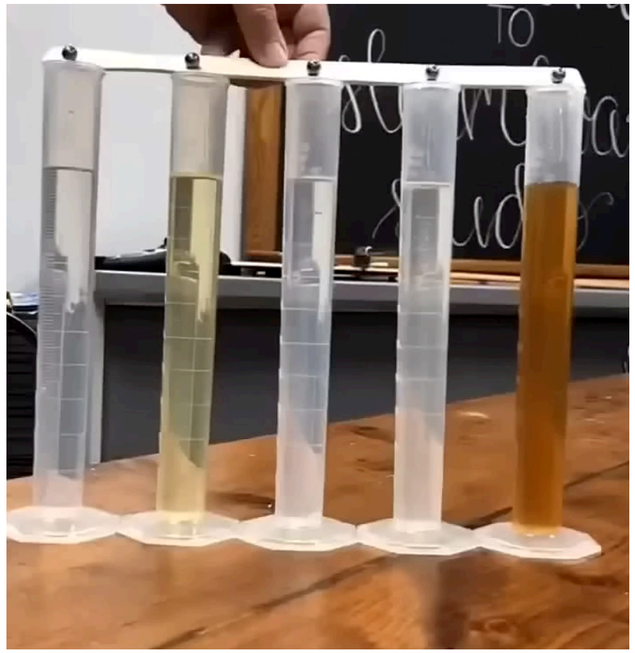
Determines how fast spherical objects fall through a liquid

Size/mass



Bigger/heavier objects push through fluid more easily

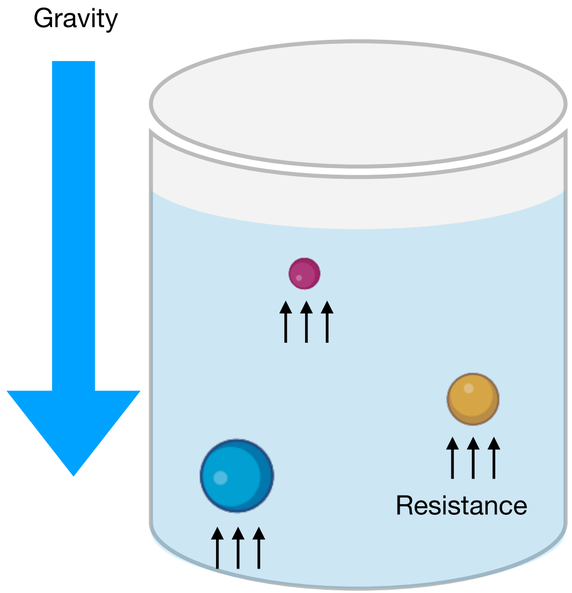
Viscosity of the liquid



https://www.youtube.com/shorts/sDFiWcvNCiI

Thicker fluids slow things down

Drag force



Gravity helps, but fluid resistance pushes back

Stokes law

Determines how fast spherical objects fall through a liquid

The force resisting motion (drag force) can be calculated:

$$F_d = 6\pi\eta r v$$

Where:

η = fluid viscosity (Pa.s)

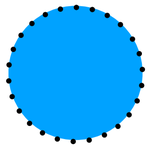
r = radius of the sphere (m)

v = velocity of the sphere (m/s)

Proteins are not always spherical

protein Hydrodynamic radius (r_H)

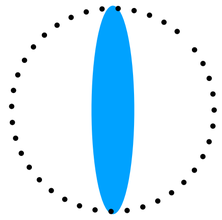
Compact protein



r_H = actual radius

- Protein moves according to Stokes law

Elongated protein

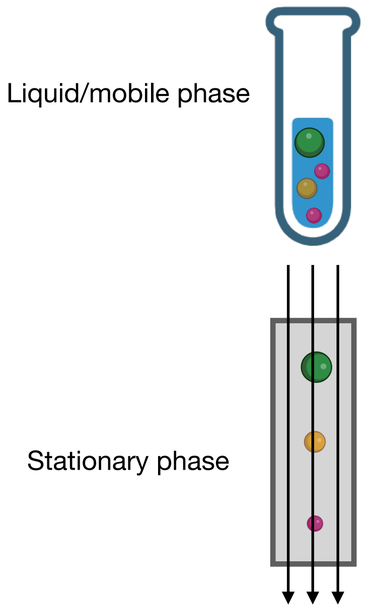


r_H > actual radius

- Protein is subject to an increased drag force
- moves slower than expected for its size

Purification techniques

Chromatography

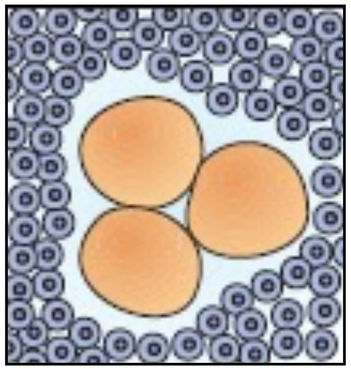


Size/shape/charge

Centrifugation

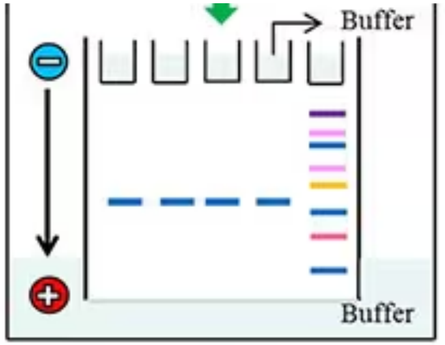
Densities

Precipitation



Solubilities

Electrophoresis



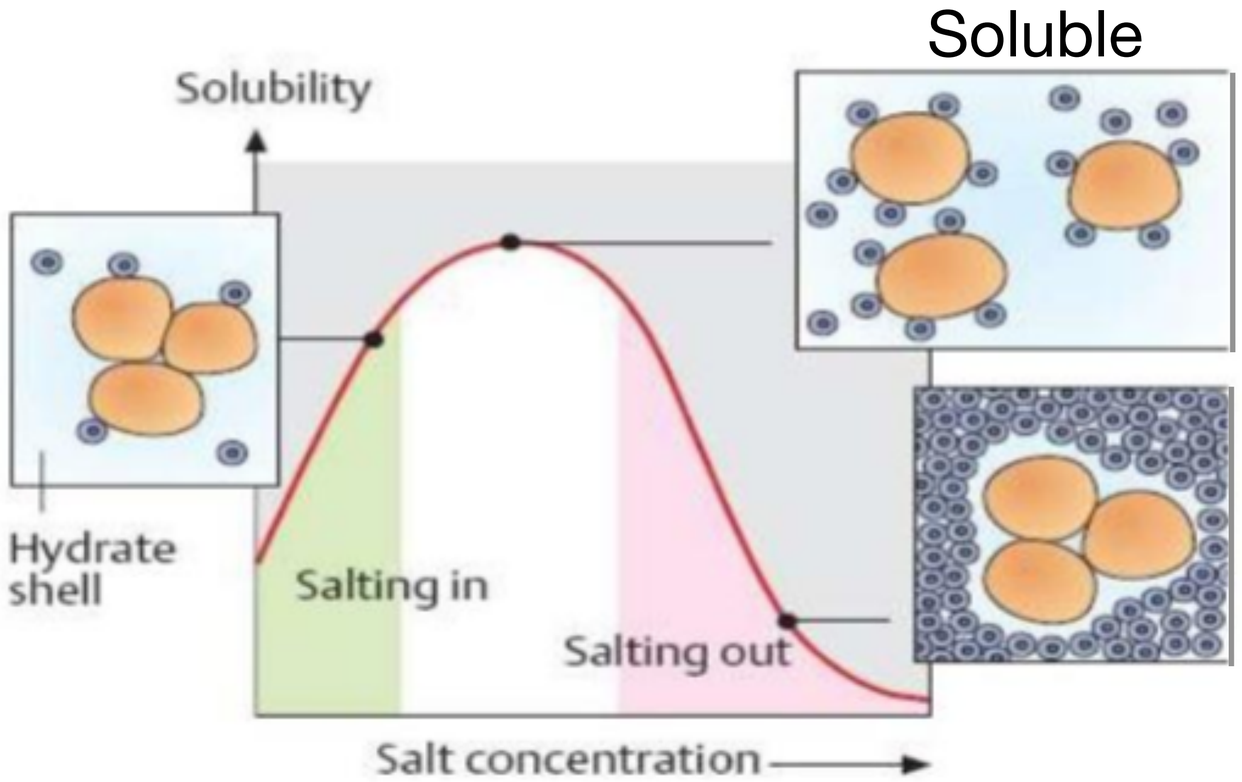
Size/charge - using a current

Precipitation (salting out)

exploits the solubility of a protein using salt

Salting in

- Low concentration of salt increase the hydration surrounding the proteins
- hydrophobic proteins will precipitate



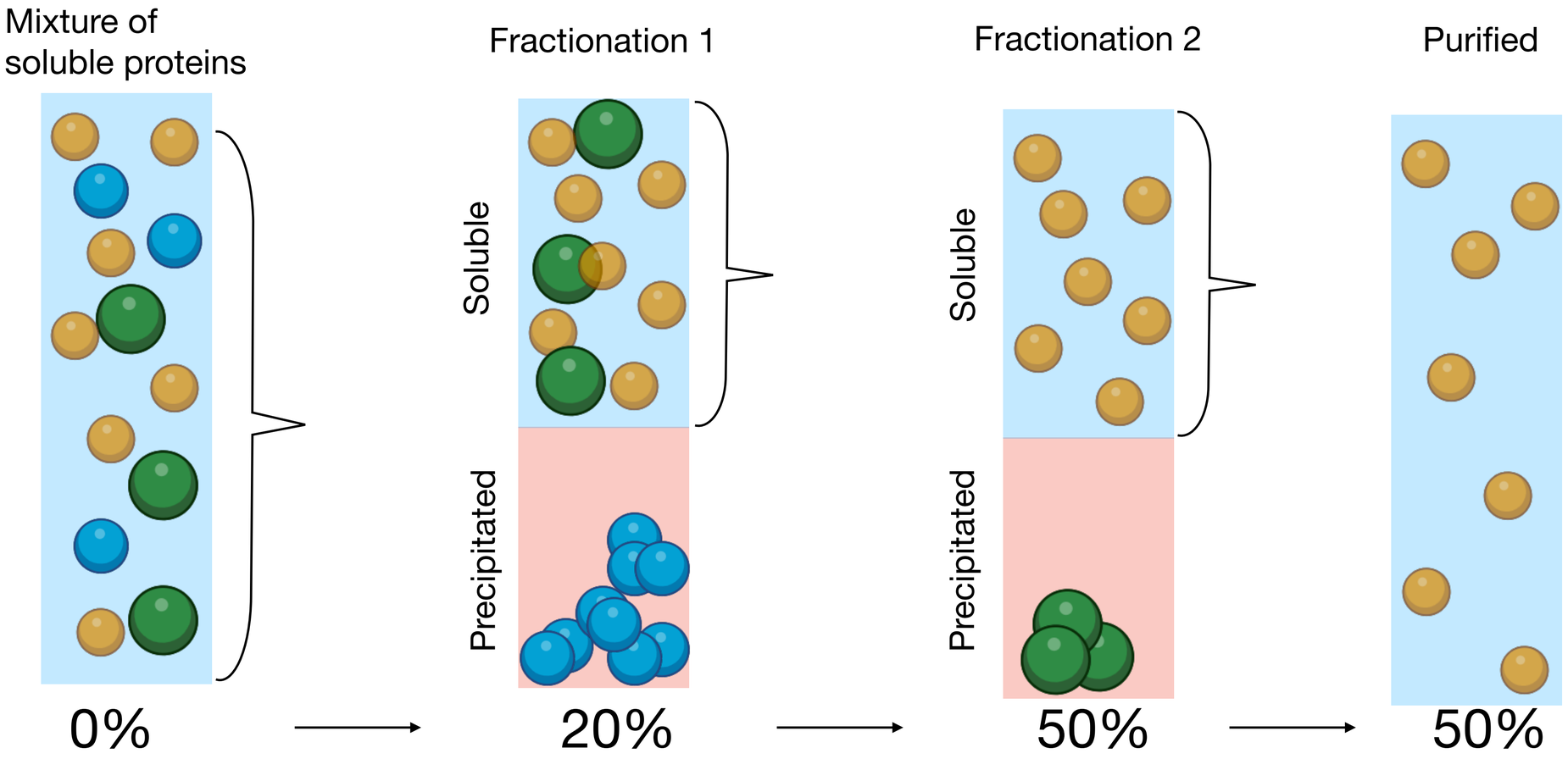
Salt stabilises the protein molecules in solution by deceasing the electrostatic energy between the protein molecules

Salting out

- High concentration of salt competes with the water molecules needed to solvate the proteins.
- the solvation sphere is removed rom the protein
- hydrophilic proteins will precipitate out of solution

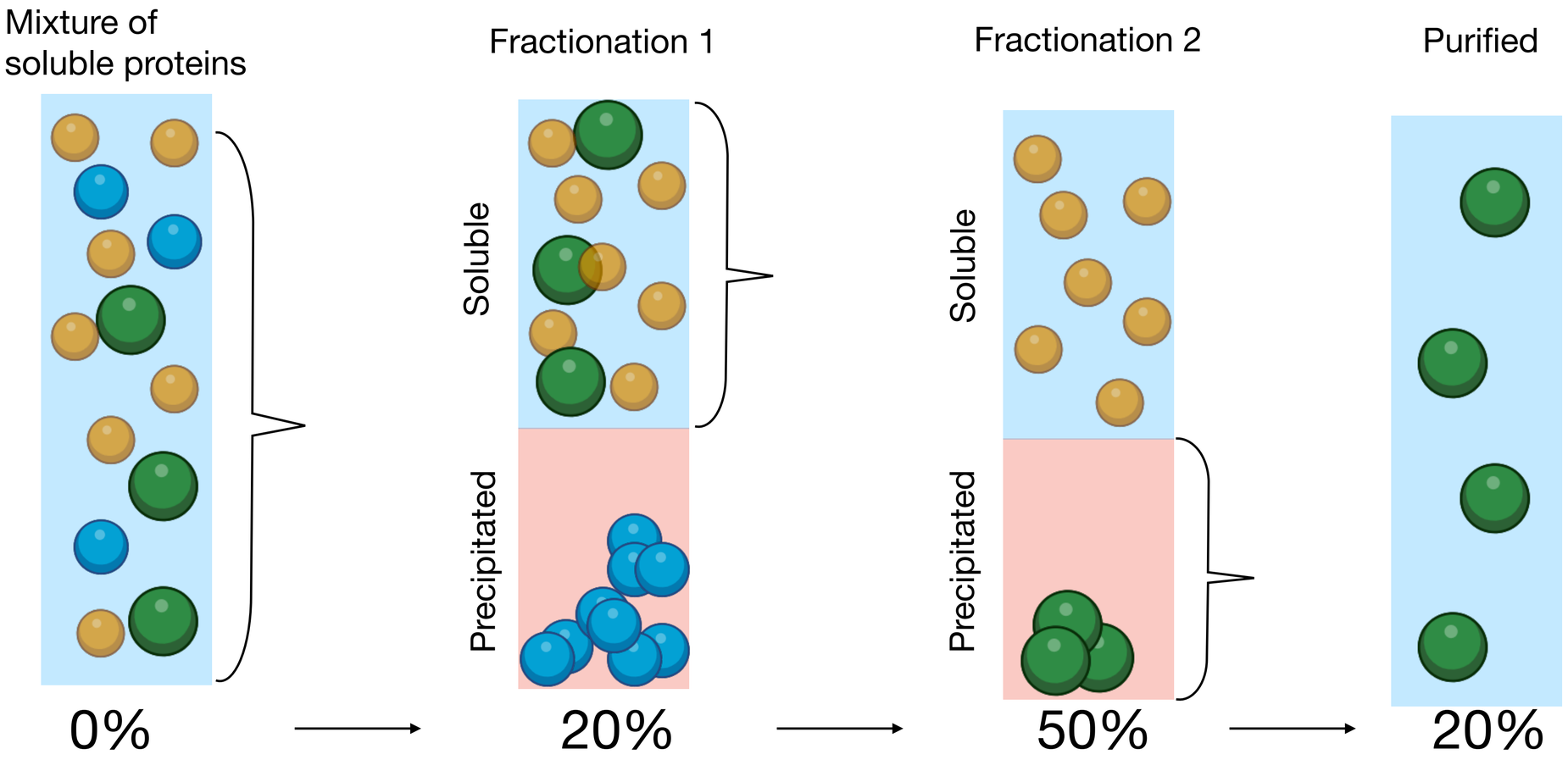
The salt concentration needed to precipitate out a protein depends on the charge (pI) and hydrophobicity of the protein

Precipitation (salting out)



The salt concentration needed to precipitate out a protein depends on the charge (pI) and hydrophobicity of the protein

Precipitation (salting out)

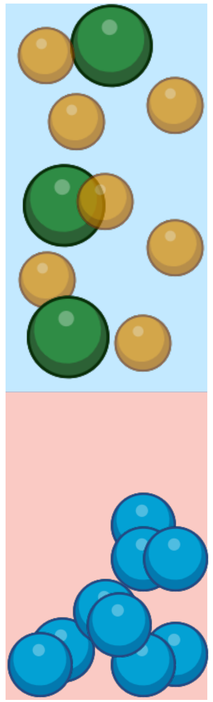


Precipitation can be reversible by reducing salt concentration again

Sedimentation

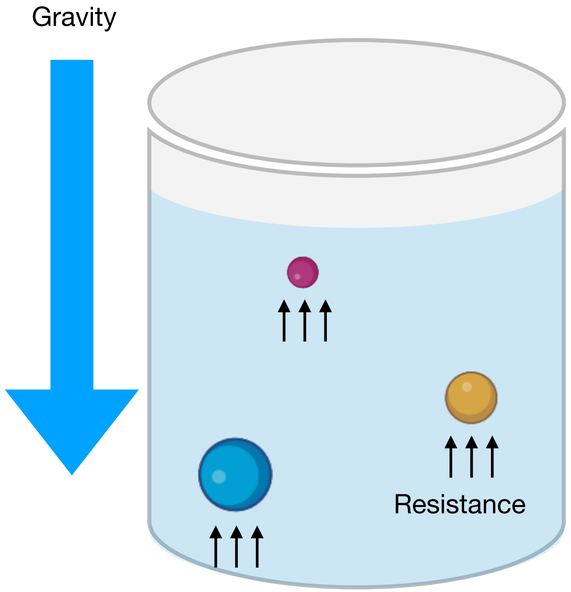
Precipitated proteins will separate naturally due to the process of sedimentation

Fractionation 1

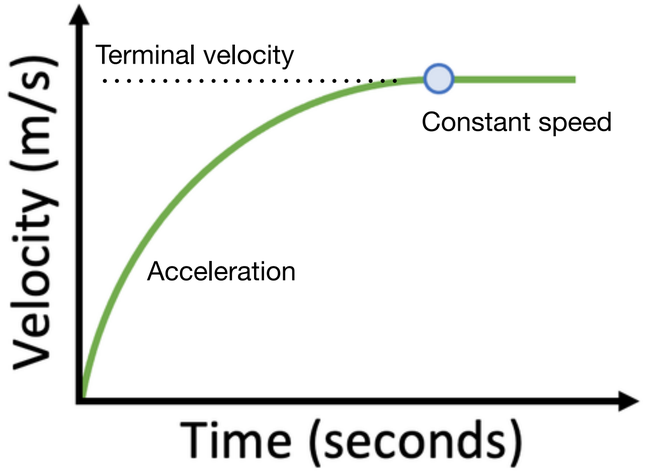


20%

Stokes Law



Terminal velocity



Force of gravity = force of resistance

Sedimentation velocity

Using Stokes' Law, the velocity at which molecules settle (terminal velocity) is:

$$v_s = \frac{2r^2(\rho_p - \rho_m)g}{9\eta}$$

$$v_s = \frac{2(5 \times 10^{-8})^2(1.3 - 1.0)\,(9.81)}{9(1 \times 10^{-3})}$$

Where:

η = fluid viscosity (Pa.s)

r = radius of the sphere (m)

v = velocity of the sphere (m/s)

ρ_p = density of protein (g/cm^3)

ρ_m = density of the medium (g/cm^3)

g = gravitational acceleration (9.81 m/s^2)

cytosol ~1 mPa.s (1×10^{-3} m)

small protein: 50 nm = (5×10^{-8} m)

1.3 g/cm^3

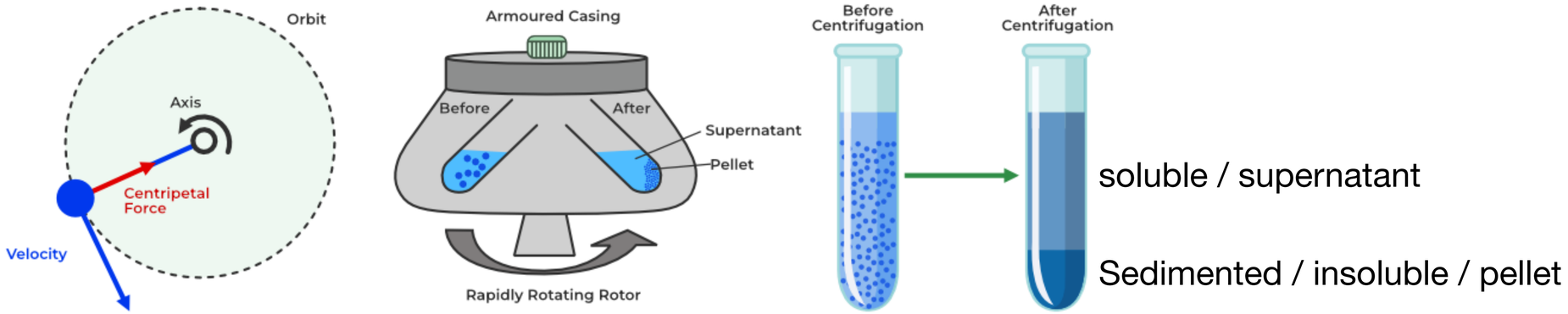
1.0 g/cm^3

$$v_s = 5.4 \times 10^{-9} \text{ m/s}$$
$$= 5.4 \text{ nm/s}$$

This is really slow!

Centrifugation

Increases the force of gravity and speeds up the sedimentation



Relative Centrifugal Force

$$RCF = \frac{\omega^2 r}{g}$$

Where:
r = radius of rotation (distance from centre of rotor to the sample, in cm or m)
ω = angular velocity (radians per second)
g = acceleration due to gravity (9.81 m/s^2)

Centrifugation

In the terminal velocity calculation, gravity is replaced by centrifugal acceleration

$$v_s = \frac{2r^2(\rho_p - \rho_m)\cancel{g}\ a_c}{9\eta}$$

a_c = centrifugal acceleration = $\omega^2 r$

Where:

η = fluid viscosity (Pa.s)

r = radius of the sphere (m)

v = velocity of the sphere (m/s)

ρ_p = density of protein (g/cm^3)

ρ_m = density of the medium (g/cm^3)

~~g = gravitational acceleration (9.81 m/s^2)~~

Where:

r = radius of rotation (distance from centre of rotor to the sample, in cm or m)

ω = angular velocity (radians per second)

and where $\omega = 2\pi$ x (rpm/60)

rpm = revolutions per minute (speed of centrifuge)

$$\text{Relative centrifugal force} = \frac{11.18 \times r \times (\text{RPM})^2}{1000}$$

For a centrifuge running at 100,000 rpm, RCF = 800,000 x g

The extra maths…

Centrifugal acceleration (a_c) is given by:

$$a_c = \omega^2 r$$

where:

- ω = angular velocity in radians per second.
- r = rotational radius (distance from the center of the rotor to the sample, in cm).

The angular velocity is:

$$\omega = 2\pi \times \frac{RPM}{60}$$

So the acceleration can be rewritten as:

$$a_c = \left(2\pi \times \frac{RPM}{60}\right)^2 \times r$$

Simplifying:

$$a_c = \frac{4\pi^2 \times (RPM)^2 \times r}{3600}$$

which in SI units (with $g = 9.81$ m/s² converted to cm/s²) gives:

$$RCF = \frac{4\pi^2}{3600 \times 9.81} \times r \times (RPM)^2$$

Since:

$$\frac{4\pi^2}{3600 \times 9.81} \approx 11.18 \times 10^{-3}$$

we get the **final equation**:

$$RCF = \frac{11.18 \times r \times (RPM)^2}{1000}$$

Sedimentation velocity + centrifugation

Using Stokes' Law, the velocity at which molecules settle (terminal velocity) is:

$$v_s = \frac{2r^2(\rho_p - \rho_m)g}{9\eta}$$

Where:

η = fluid viscosity (Pa.s)

r = radius of the sphere (m)

v = velocity of the sphere (m/s)

ρ_p = density of protein (g/cm^3)

ρ_m = density of the medium (g/cm^3)

g = gravitational acceleration (9.81 m/s^2)

cytosol ~1 mPa.s (1 x 10^{-3} m)

small protein: 50 nm = 5 x 10^{-8} m)

1.3 g/cm^3

1.0 g/cm^3

$$v_s = \frac{2(5 \times 10^{-8})^2(1.3 - 1.0)\,(9.81)}{9(1 \times 10^{-3})}$$

X 800,000

$v_s = 5.4 \times 10^{-9}$ m/s

$= 5.4$ nm/s

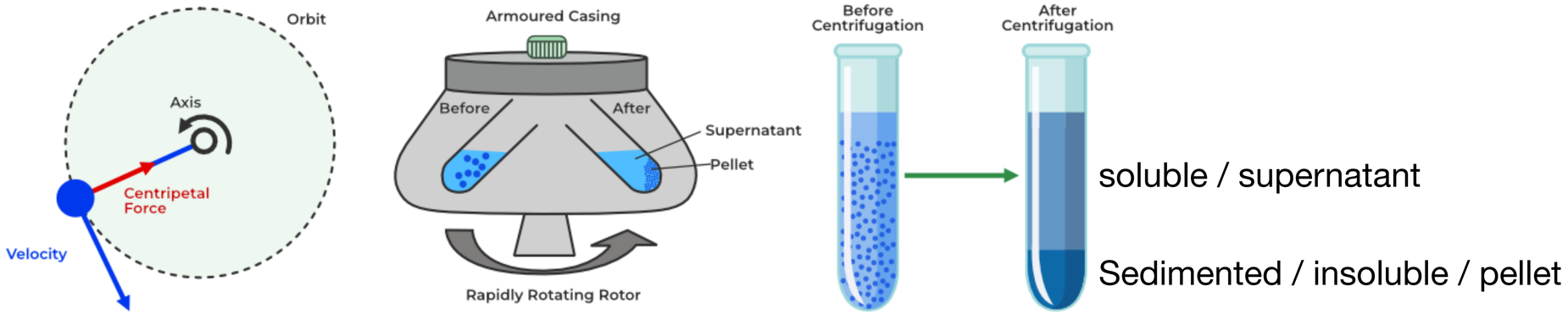
$v_s = 13.08 \times 10^{-7}$ m/s

$= 1308$ nm/s

This is much faster!

Centrifugation

Increases the force of gravity and speeds up the sedimentation



Relative Centrifugal Force

$$RCF = \frac{\omega^2 r}{g}$$

Where:
r = radius of rotation (distance from centre of rotor to the sample, in cm or m)
ω = angular velocity (radians per second)
g = acceleration due to gravity (9.81 m/s^2)

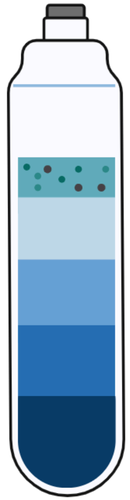
Centrifugation

exploits the different densities of proteins (and other cellular material)

Differential

Removes cellular debris

Density gradient



Separates protein mixtures

Ultracentrifugation

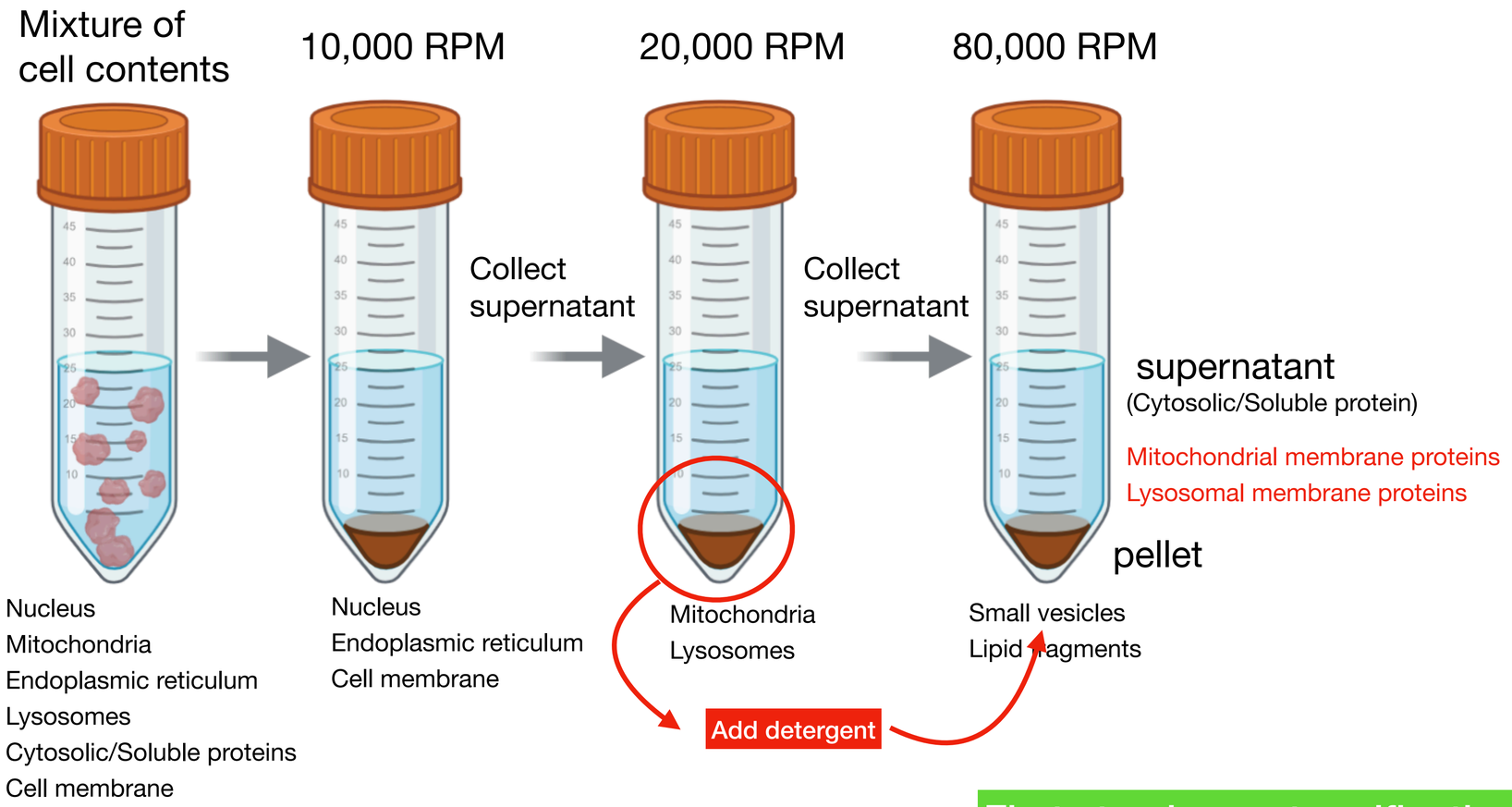
Analytical and preparative

Differential centrifugation

Separation of cellular contents

- **Mechanically**
  - Grinding (mortar and pestle)
  - Sonication (vibrations)
  - Homogenisation (Shearing forces)
  - Freeze-thaw (expansion of water)

- **Chemically**
  - Enzymes
  - Chemicals

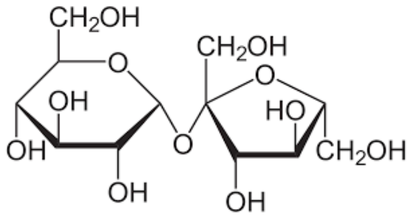


First step in most purification protocols

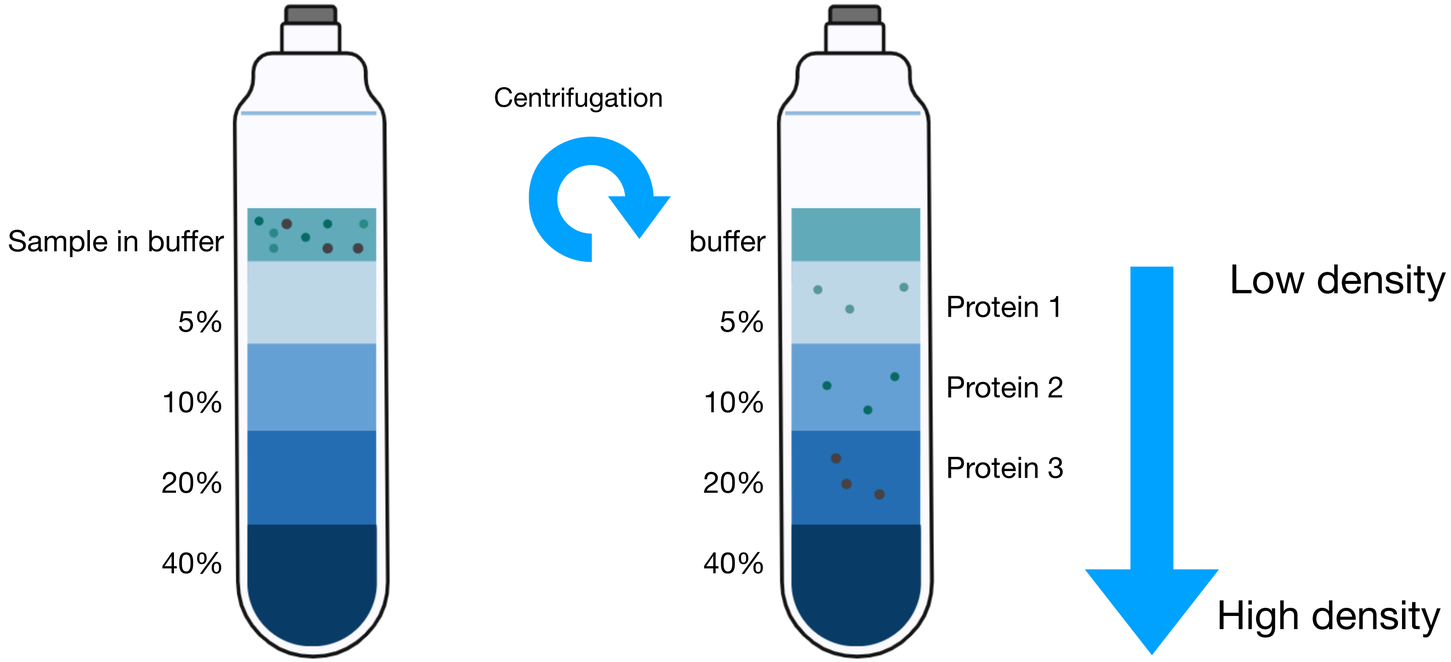
Density Centrifugation

Separates proteins, most commonly using sucrose gradients

Sucrose



- Makes a stable density gradient at different concentrations
- Biologically compatible (non-toxic, non-reactive, gentle for proteins)
- High viscosity ensures a stable gradient without mixing during centrifugation
- Sucrose is easy to remove from proteins



Sucrose provides resistance that slows down particles as they move helping them to separate based on their density

Ultracentrifugation

Spins at speeds up to 100,000 - 1,000,000 x g

Preparative

- **Differential**
- **Density gradient**

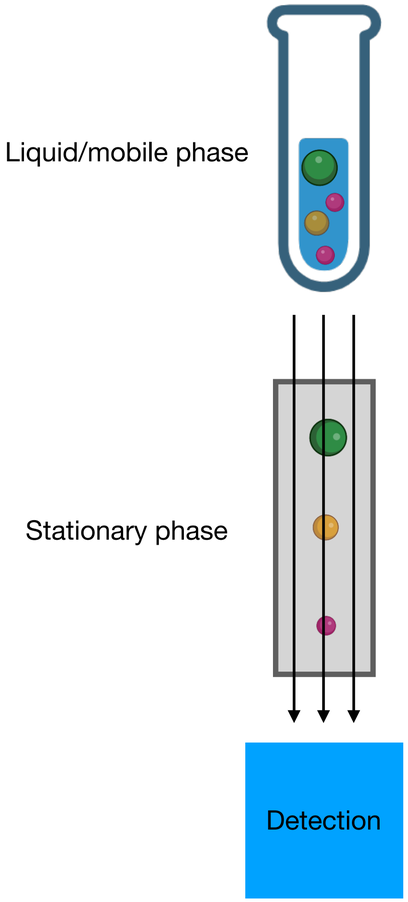
Analytical

Coupled to a detector

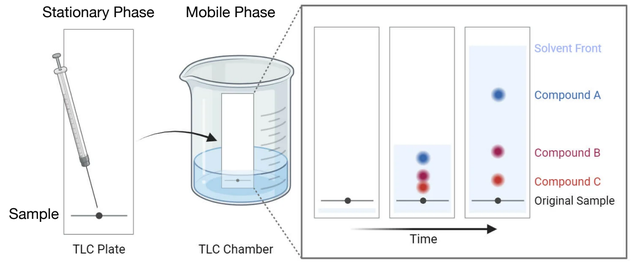
- **Shape:**How spherical is my protein?
- **Diameter:** What's the size of my particle?
- **Mass:** What's the molecular weight of my protein or complex in solution?
- **Stoichiometry:** How many subunits comprise my protein?
- **Purity:** Are there other particles in my sample?
- **Formulation:** How does my protein behave in this buffer?
- **Heterogeneity:** Is my protein bound to other molecules, and what's the configuration of the complex?
- **Aggregation:** Is my protein still in a usable form? Should I expect an immune response with my drug formulation?
- **Association:** Does my protein associate and/or dissociate with other proteins?
- **Conformation:** Does the conformation of my protein change upon binding to a ligand?

Chromatography

The separation of a mixture into its components

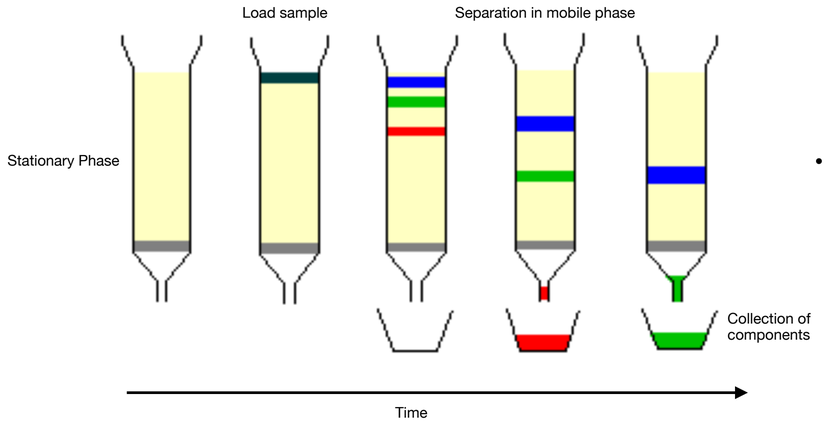


Paper/Thin layer



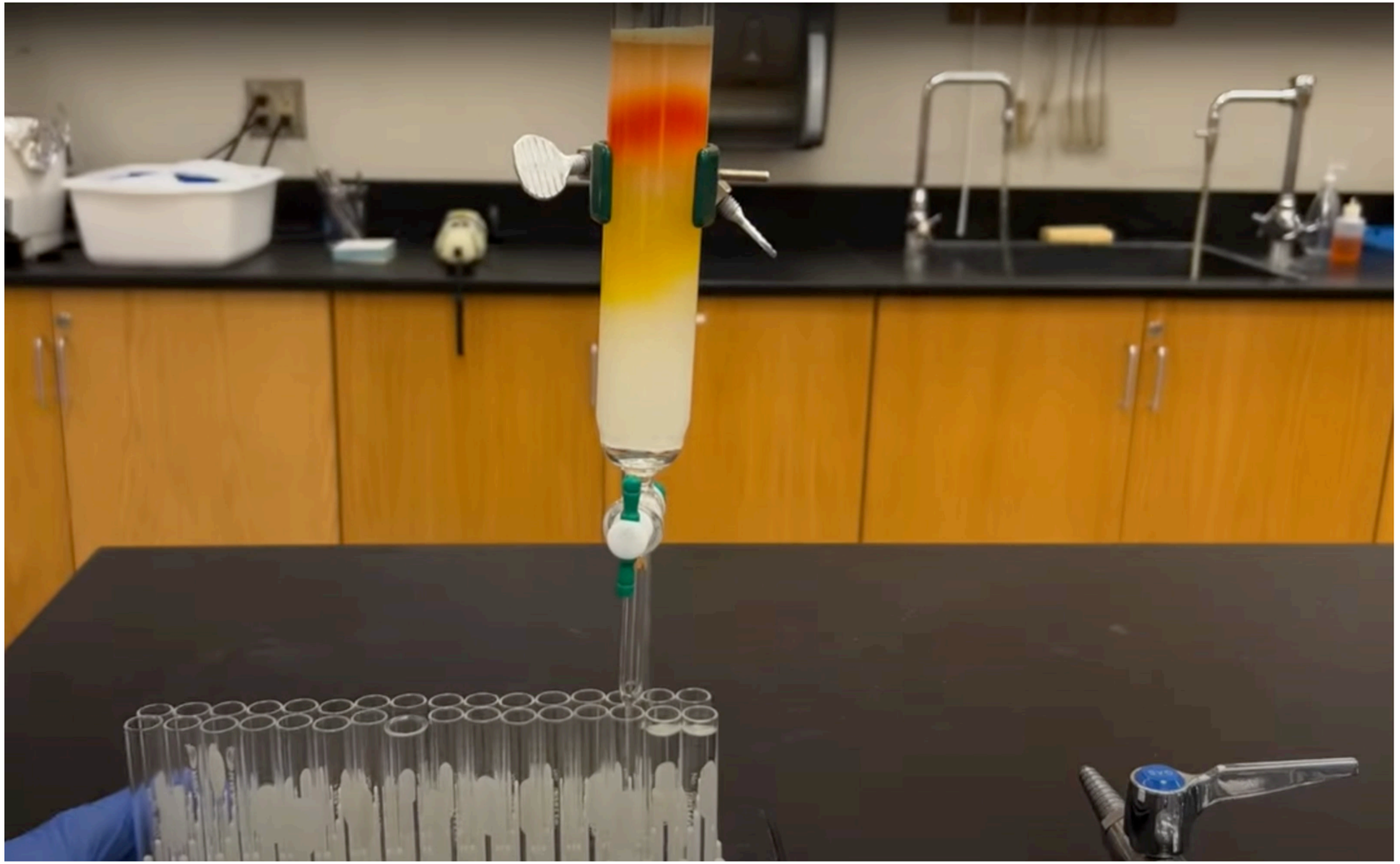
- compound identification
- chemical analysis

Column



- purification

Manual chromatography



https://www.youtube.com/watch?v=KGTZ3XBEfyc

An automated chromatography system

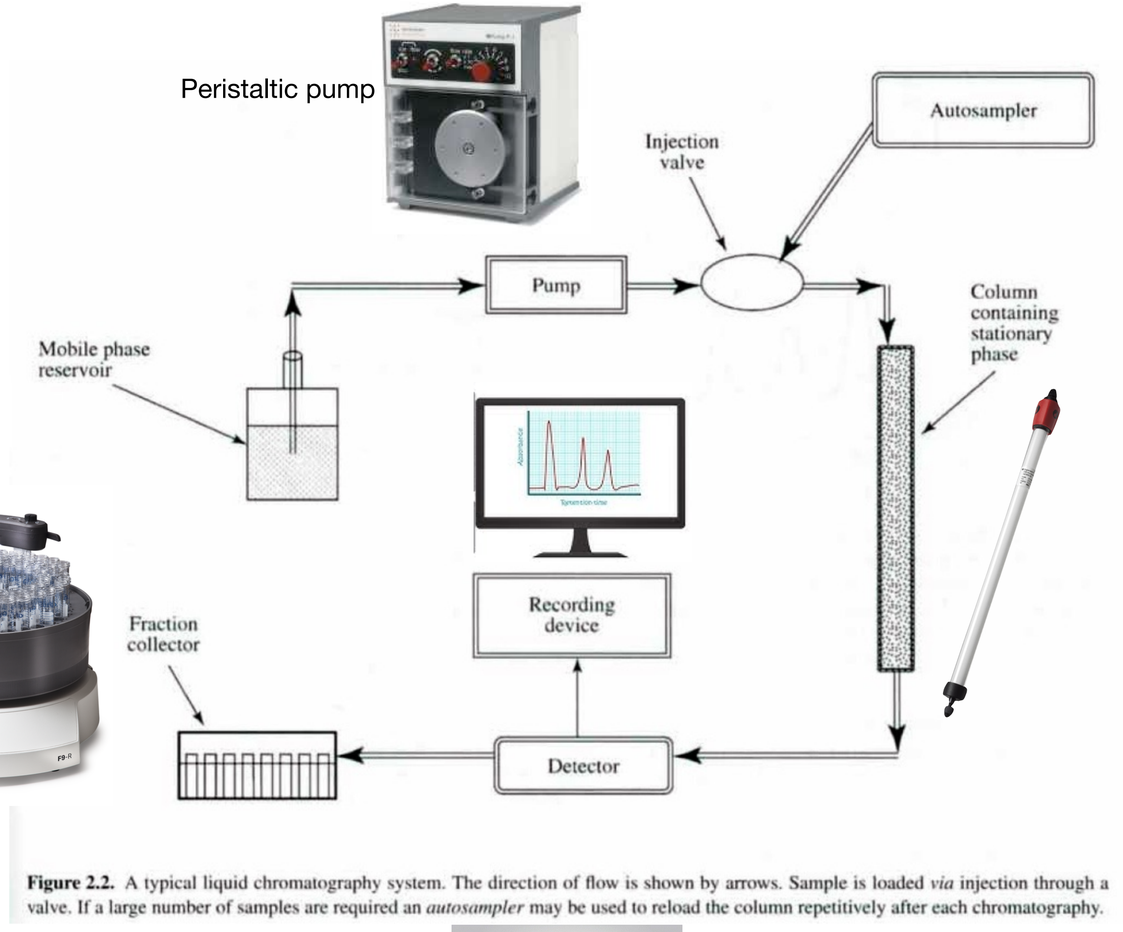
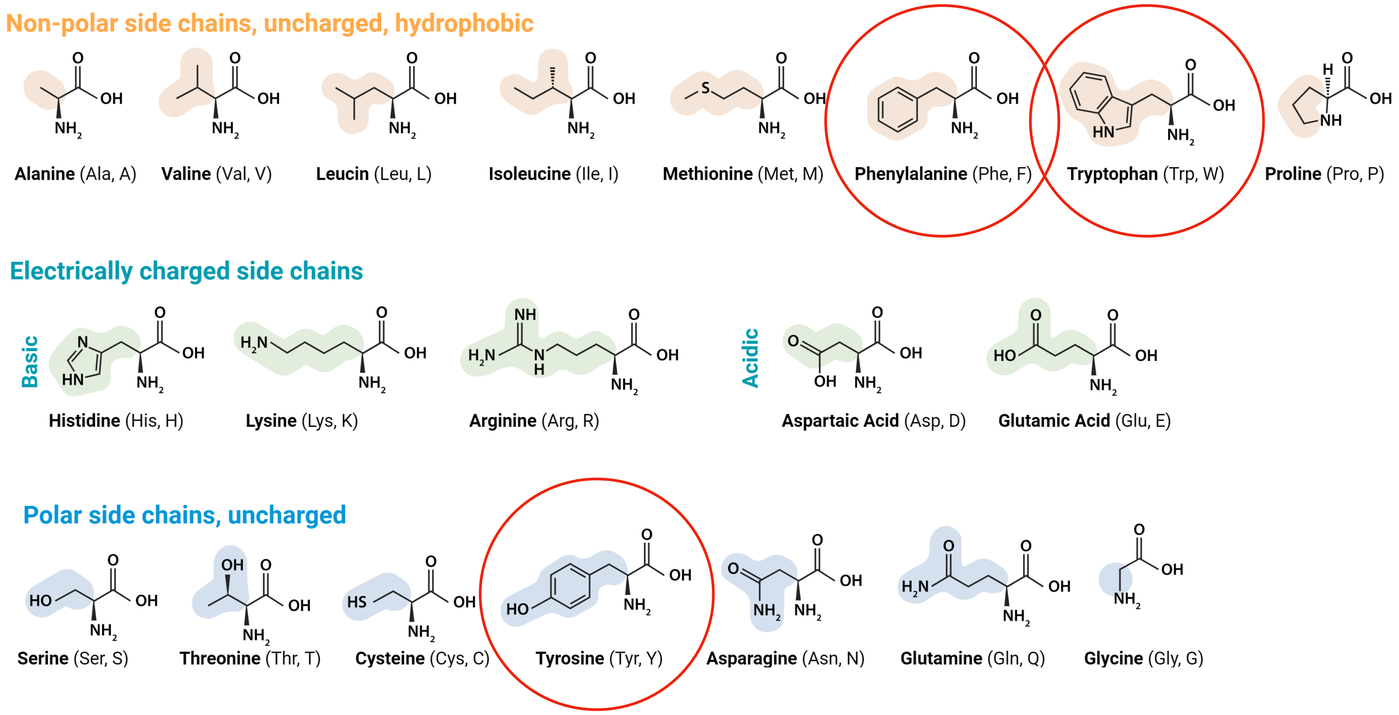


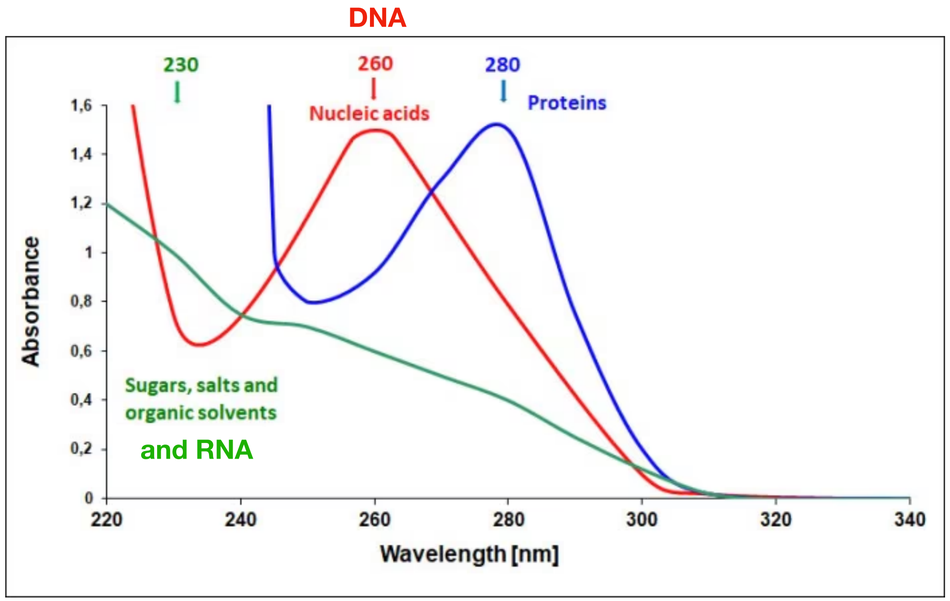
Figure 2.2. A typical liquid chromatography system. The direction of flow is shown by arrows. Sample is loaded *via* injection through a valve. If a large number of samples are required an *autosampler* may be used to reload the column repetitively after each chromatography.

UV/Vis detector

Detection

Proteins absorb at 280 nm due to the presence of aromatic amino acids



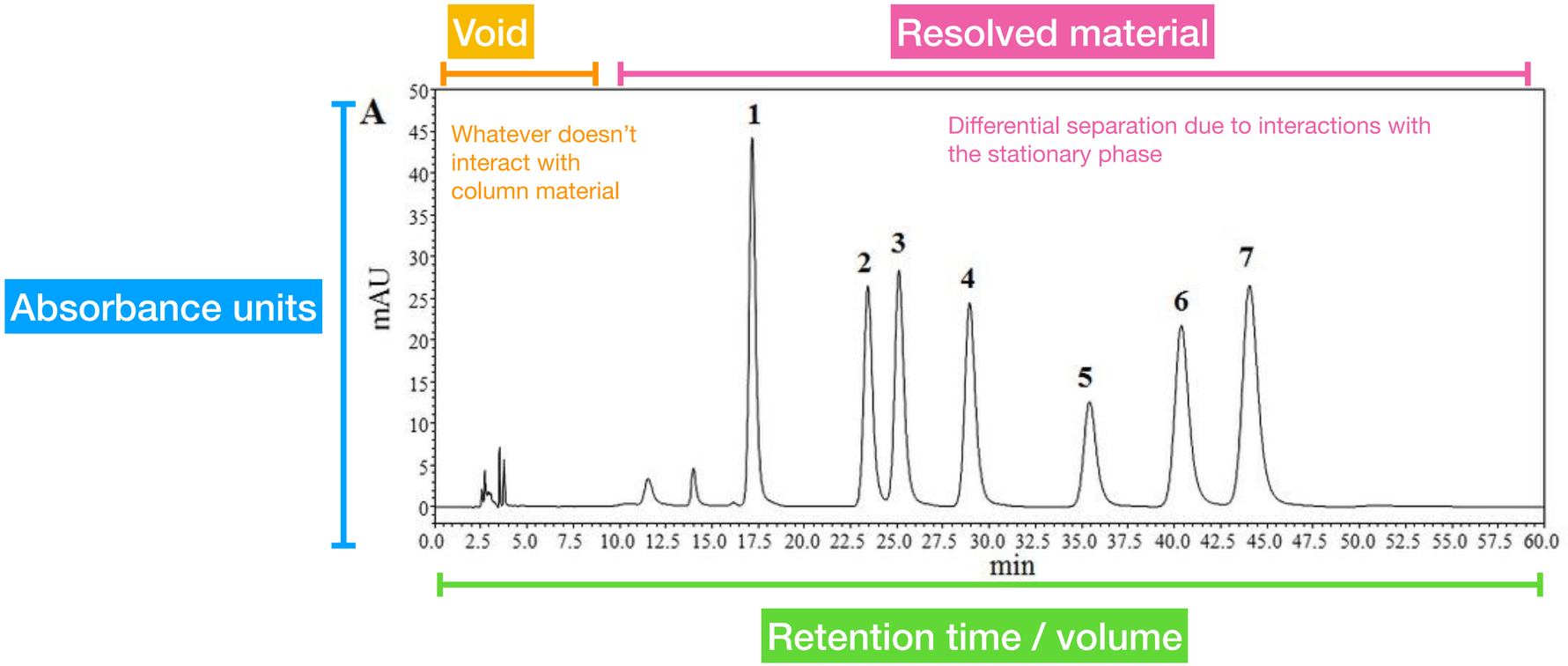


280/260 = ratio of protein:nucleic acid

260/230 = ratio of DNA:RNA

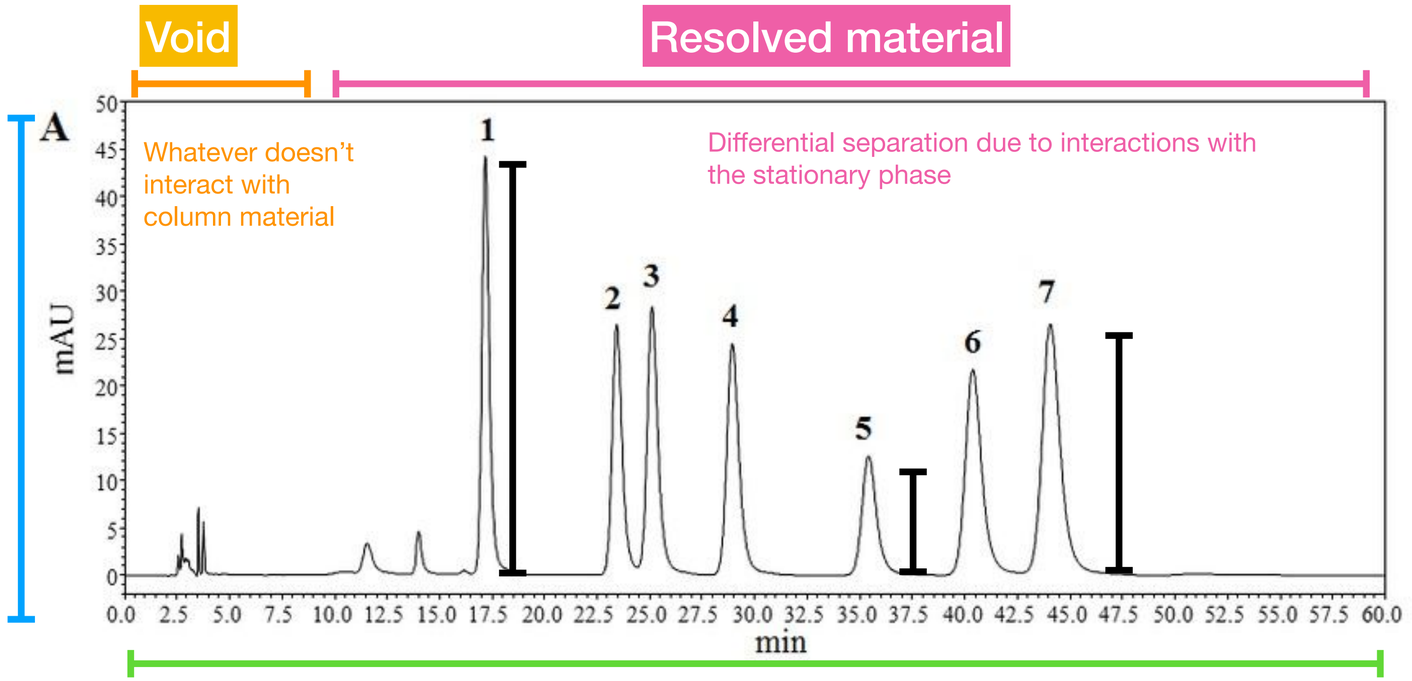
Chromatogram

Protein elution from the column can be simply monitored by A280 nm



Chromatogram

Protein concentration can be also be calculated from its A280 nm



Absorbance units

Retention time / volume

Peak height (A) is proportional to protein concentration

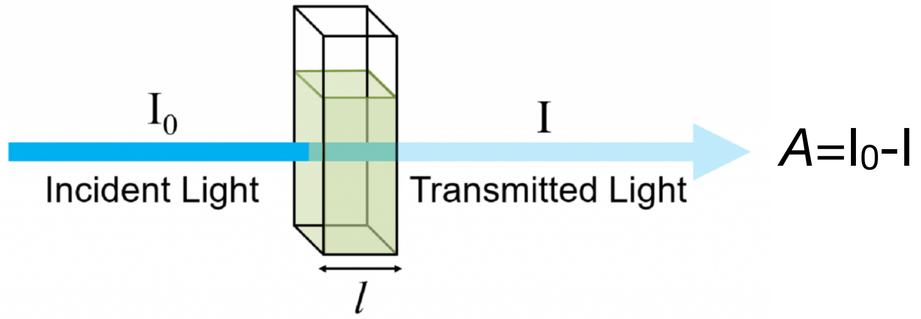
Protein concentration

Beer Lambert Law

The absorbance of a material is directly proportional to its concentration in solution and the path length through which the light travels.

$$A = \varepsilon c l$$

A	Absorbance	
ε	Molar absorption coefficient	$M^{-1}cm^{-1}$
c	Molar concentration	M (g/L)
l	optical path length	cm



A = absorbance at A280 (peak height)

l = path length (determined by detector)

ε = molar absorption coefficient of the protein

ε (protein) = (nW * 5500) + (nY * 1490) + (nC * 125)

W = tryptophan, Y = tyrosine, C = cysteine

$$\text{Protein Concentration (g/L)} = \frac{A}{\varepsilon l}$$

Multi-angle laser light scattering (MALLS)

Viscotec Tetra Detector Array

Detector array consists of

- **Refractometer (refractive index RI)**
  - Refr. index increment *dn*/*dc* [ml/g] (yields concentration)

$$RI.sig \simeq C \cdot \frac{dn}{dc}$$

dn/dc is the refractive index increment. It quantifies how much the refractive index of a solution varies for a given increment in concentration c, expressed as g/mL

- **Viscometer (differential pressure DP)**
  - Intrinsic viscosity *IV* [dl/g] (deciliters per gram; yields shape)
  - Hydrodynamic radius *Rh* [nm] (yields size)

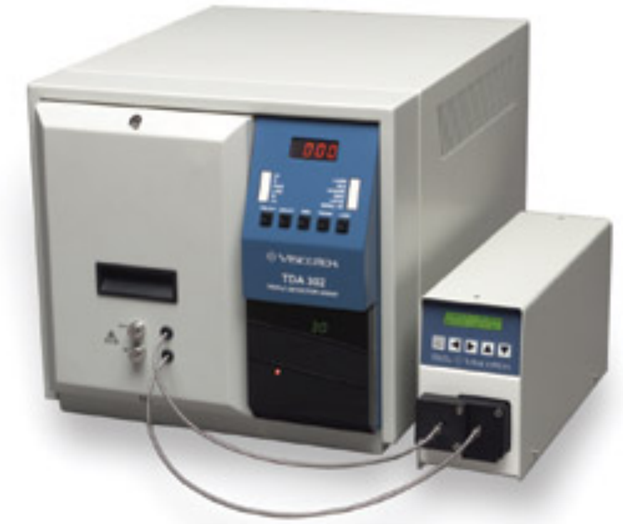
$$Visc.sig \simeq C \cdot IV$$

- **Light Scattering (90° and 7°)**
  - Mass Mw [g/mole] (yields mass, shape)

$$LS.sig \simeq C \cdot \left(\frac{dn}{dc}\right)^2 \cdot M_W$$

- **Absorption (280nm)**
  - Absorption coefficient dA/dc [ml/g] (yields concentration)

$$UV.sig \simeq C \cdot \frac{dA}{dc}$$



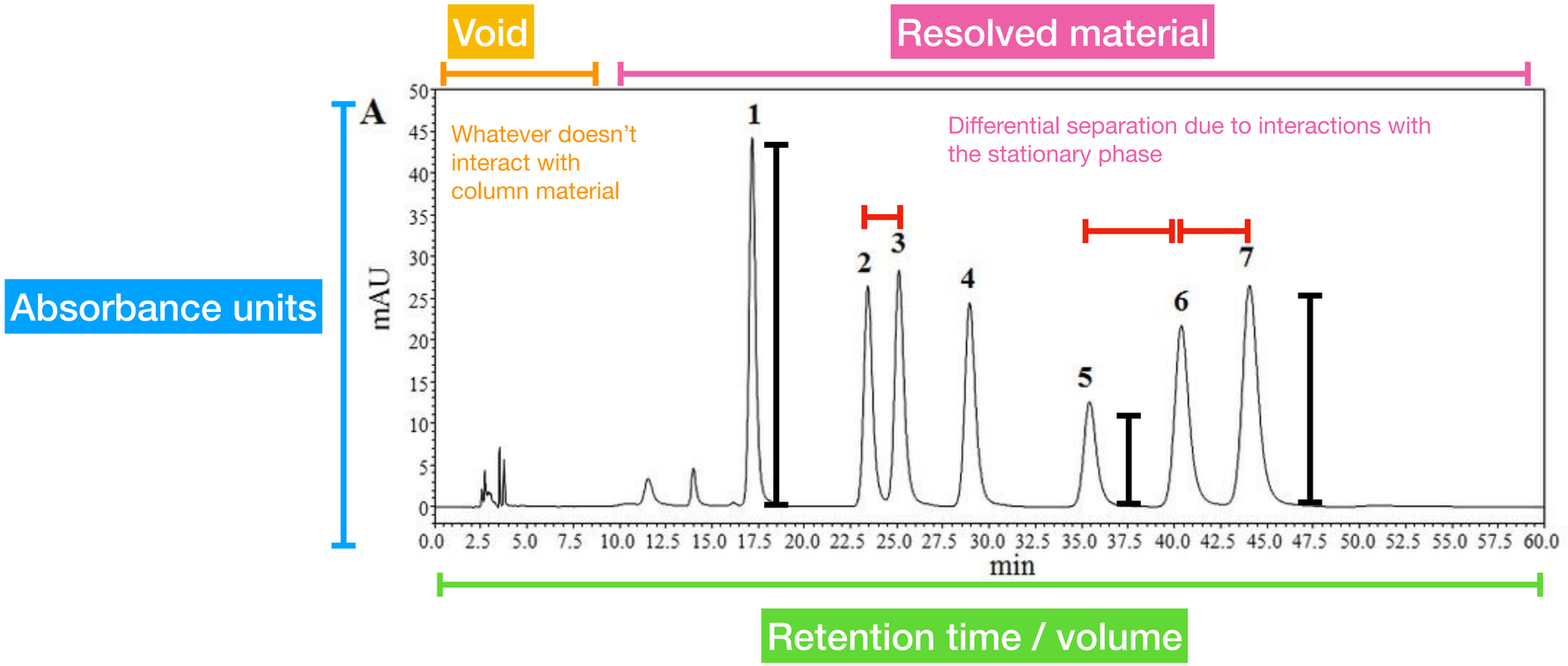
Upon analysis

- **Absolute molecular weight**
- **Radius, Intrinsic viscosity (shape)**
- **% binding partners**
- **concentration**
- **dn/dc**

Slide from HS

Chromatogram

Protein concentration can be also be calculated from its A280 nm



Peak height is proportional to protein concentration

Distance between peaks determines resolution

Resolution of chromatography

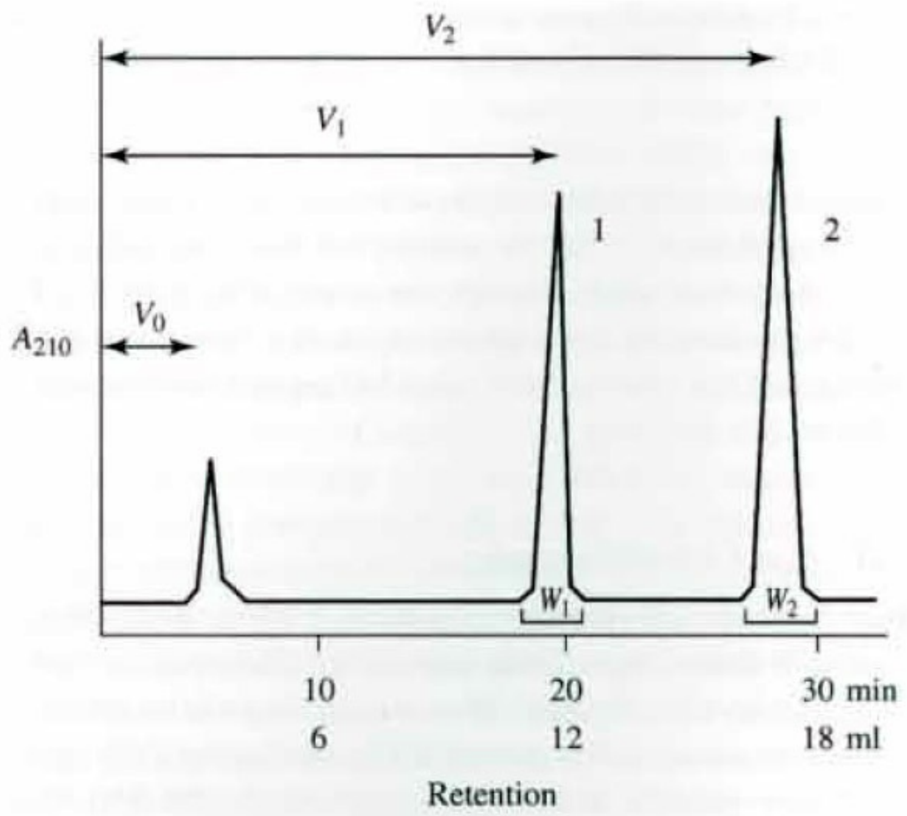


Figure 2.4. Retention in column chromatography. A typical chromatography trace showing the separation of two components; 1 and 2. The retention volumes of the components are shown by V_1 and V_2, respectively, while the base peak widths are denoted by W_1 and W_2. The void volume is denoted by V_0.

$$V_R = f * t_R$$

V_R = *retention volume*

f = *flow rate*

t_R = *retention time*

$$R.F. = \frac{V_{sample}}{V_{mobile\ phase}}$$

$R.F.$ = *retardation factor*

$V_{mobile\ phase} = V_0$

$$R = \frac{V_2 - V_1}{(W_1 + W_2)/2}$$

R = *resolution*

W = *width at base of peak*

Slide from HS

Peak broadening

Slide from HS

Peak broadening

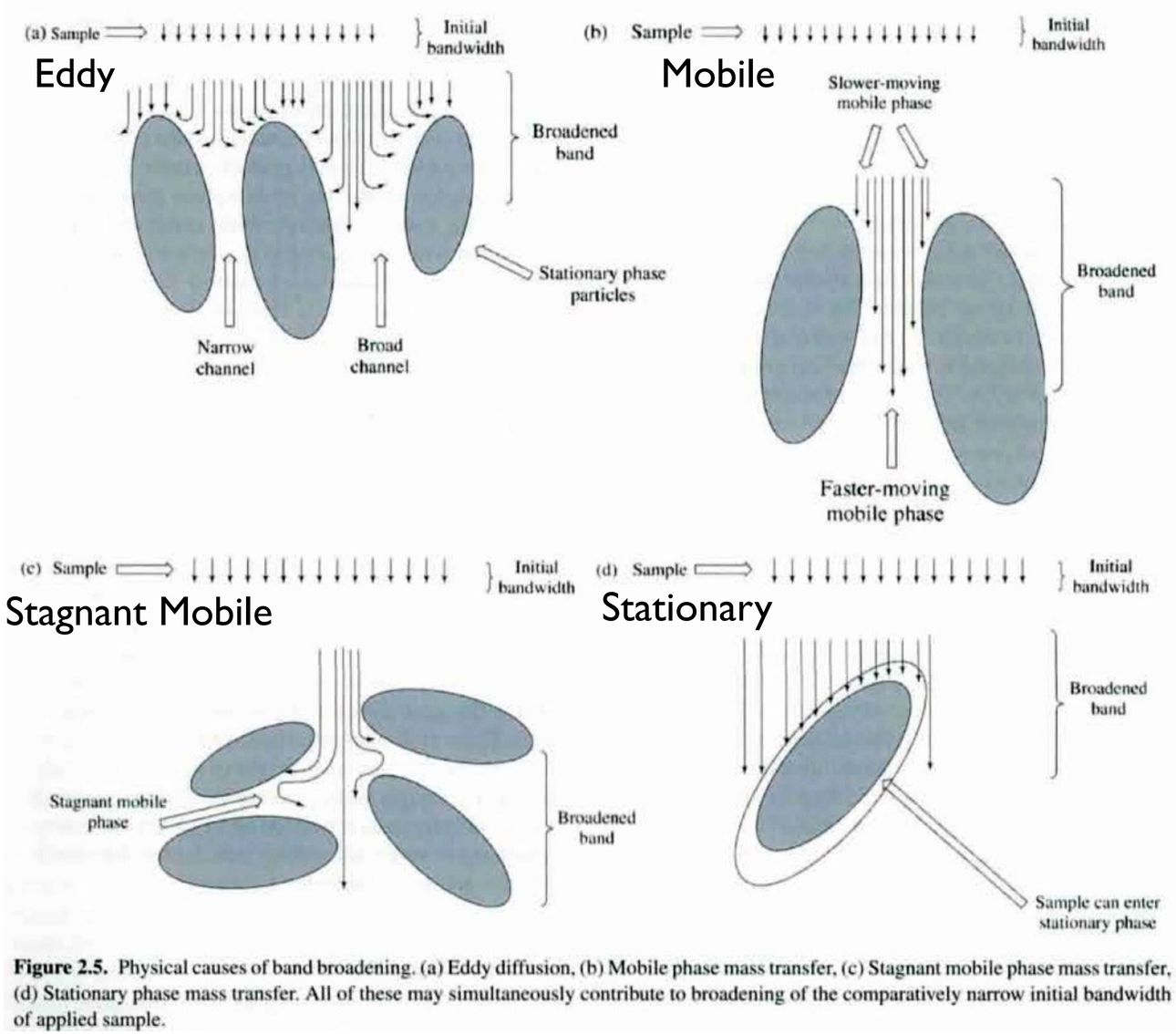


Figure 2.5. Physical causes of band broadening. (a) Eddy diffusion, (b) Mobile phase mass transfer, (c) Stagnant mobile phase mass transfer, (d) Stationary phase mass transfer. All of these may simultaneously contribute to broadening of the comparatively narrow initial bandwidth of applied sample.

a) Eddy diffusion (turbulences)

b) Mobile phase *mass transfer*

c) Stagnant mobile phase *mass transfer*

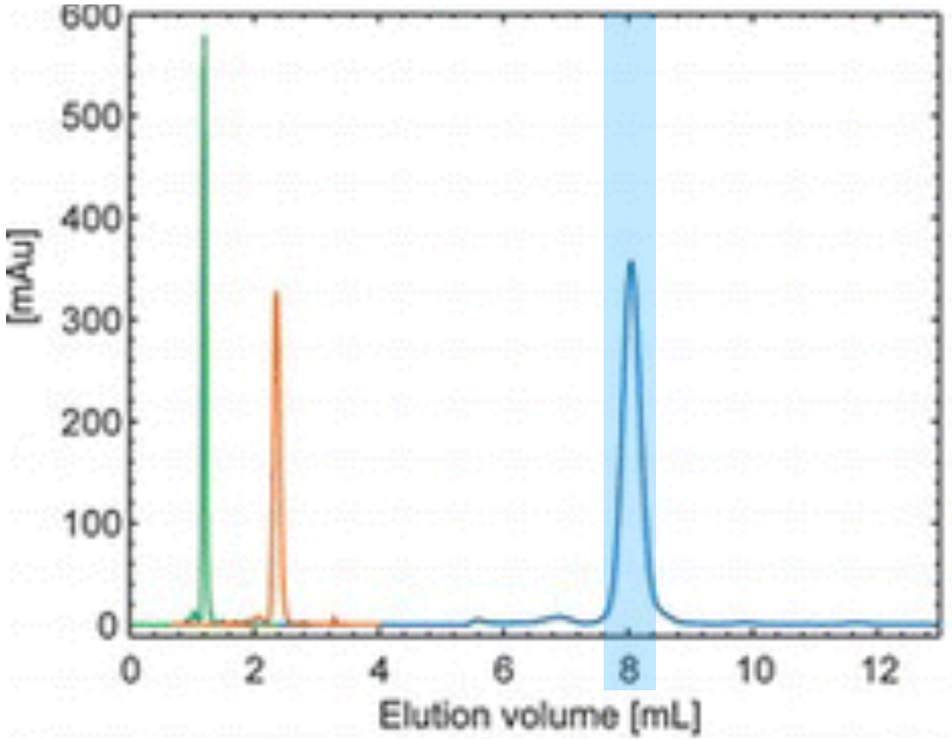
d) Stationary phase *mass transfer*

e) *Longitudinal Diffusion (not shown)*

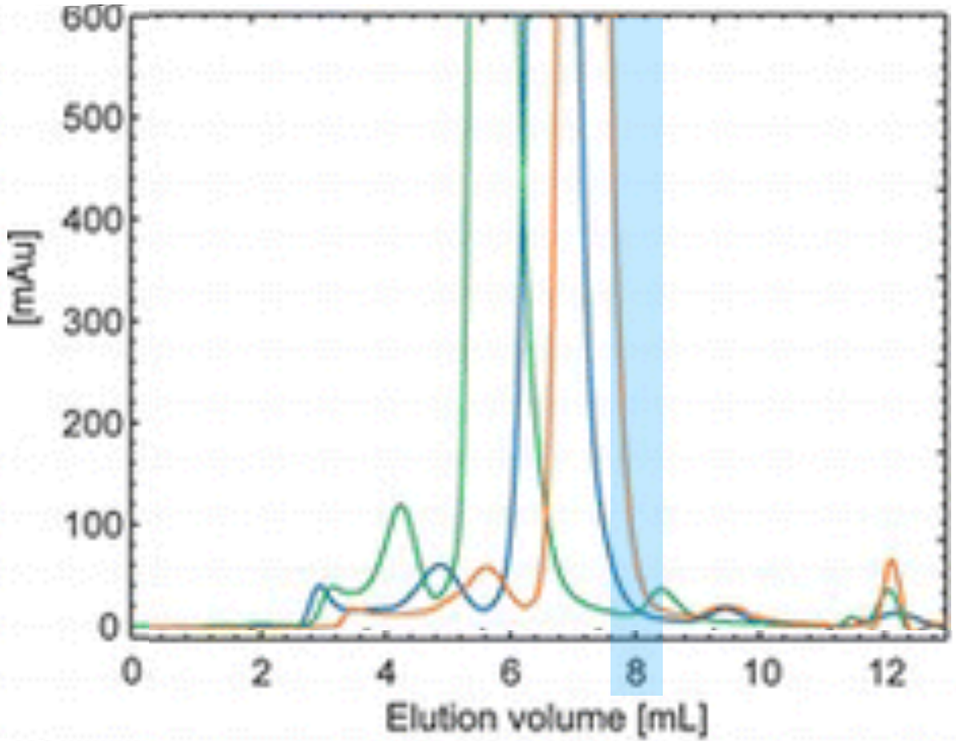
Slide from HS

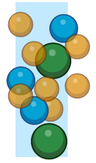
Resolution of chromatography

Well resolved

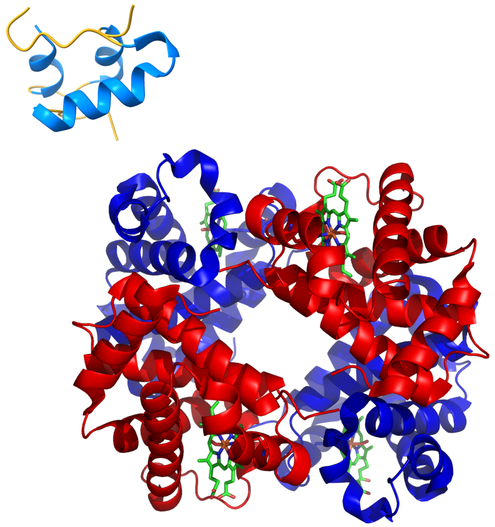
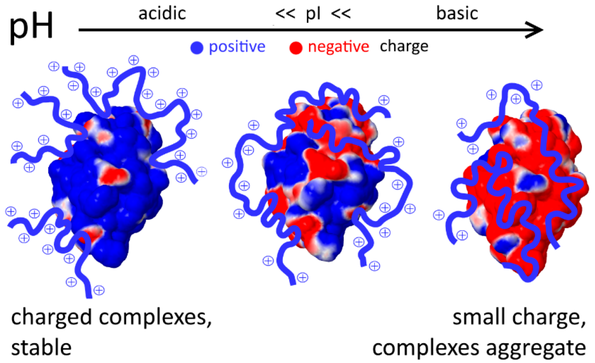
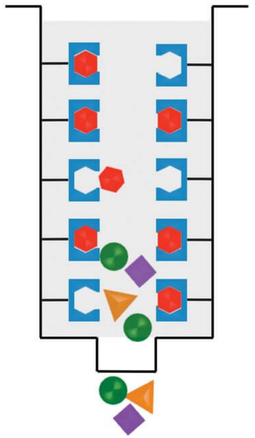
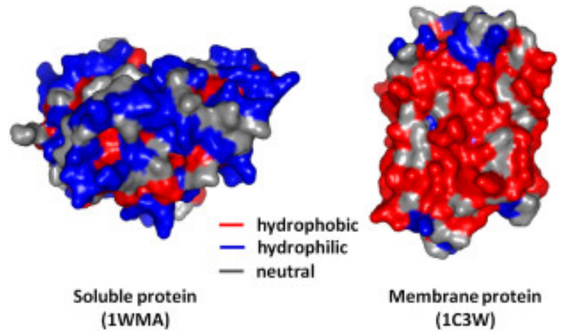


Poorly resolved

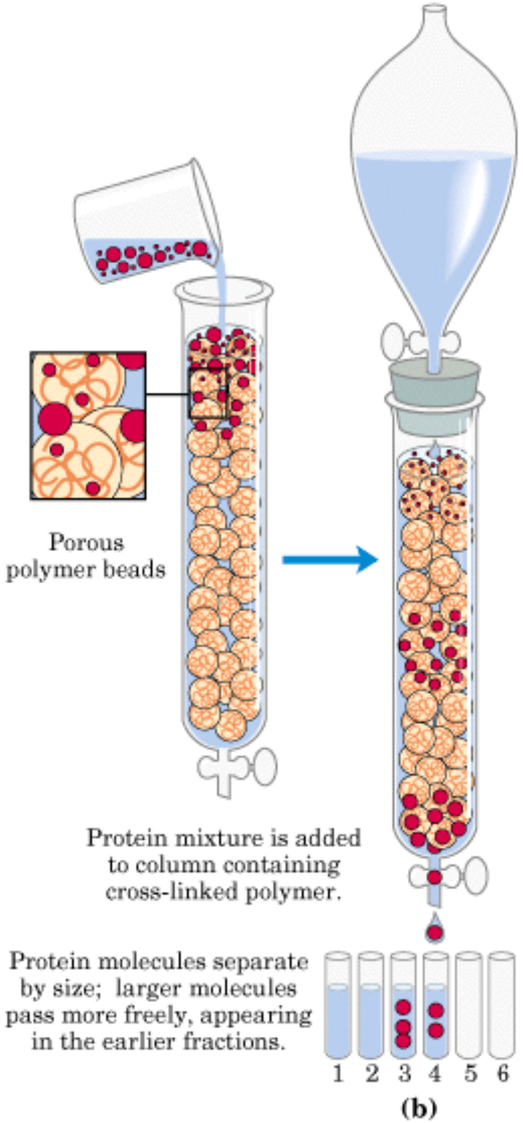


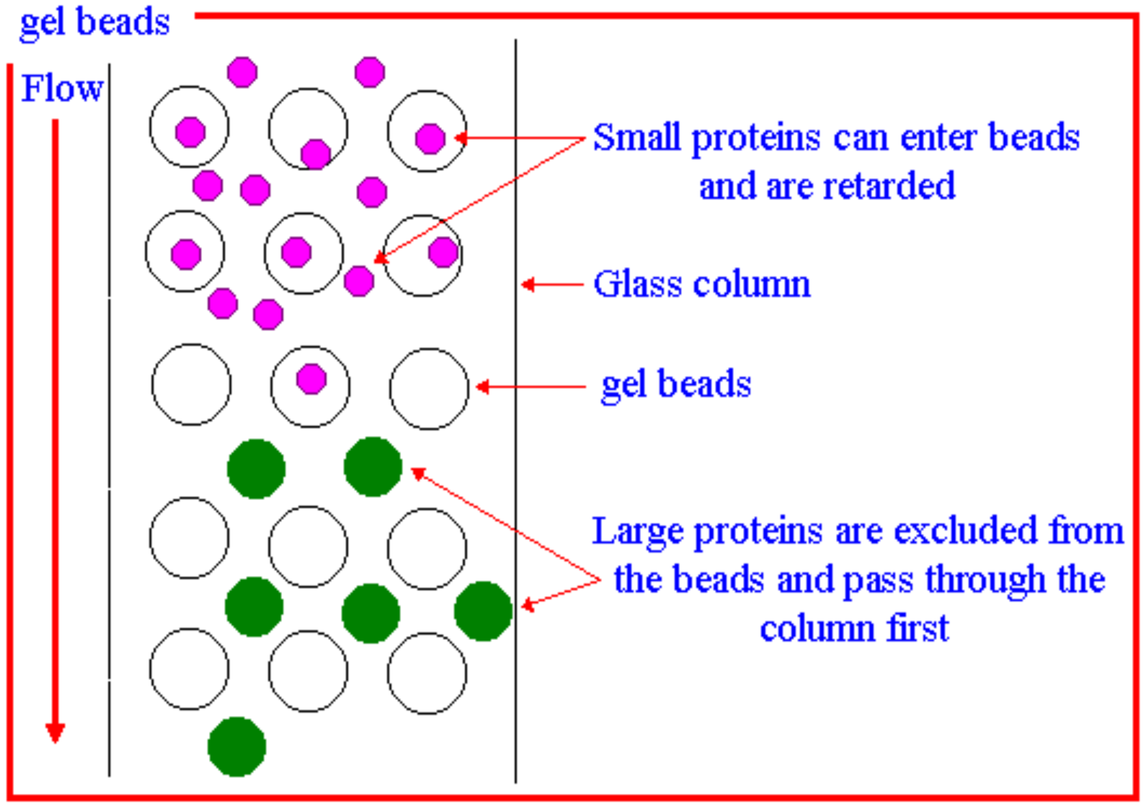


Types of chromatography

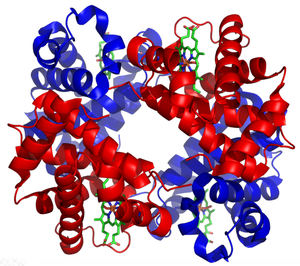
Size-exclusion Chromatography (SEC)	Ion-Exchange Chromatography (IEX)	Affinity chromatography (IMAC)	Hydrophobic interaction chromatography (HIC)
			
Hydrodynamic radius	Charge	binding interactions	Hydrophobic effects

Size exclusion (gel filtration)

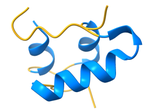


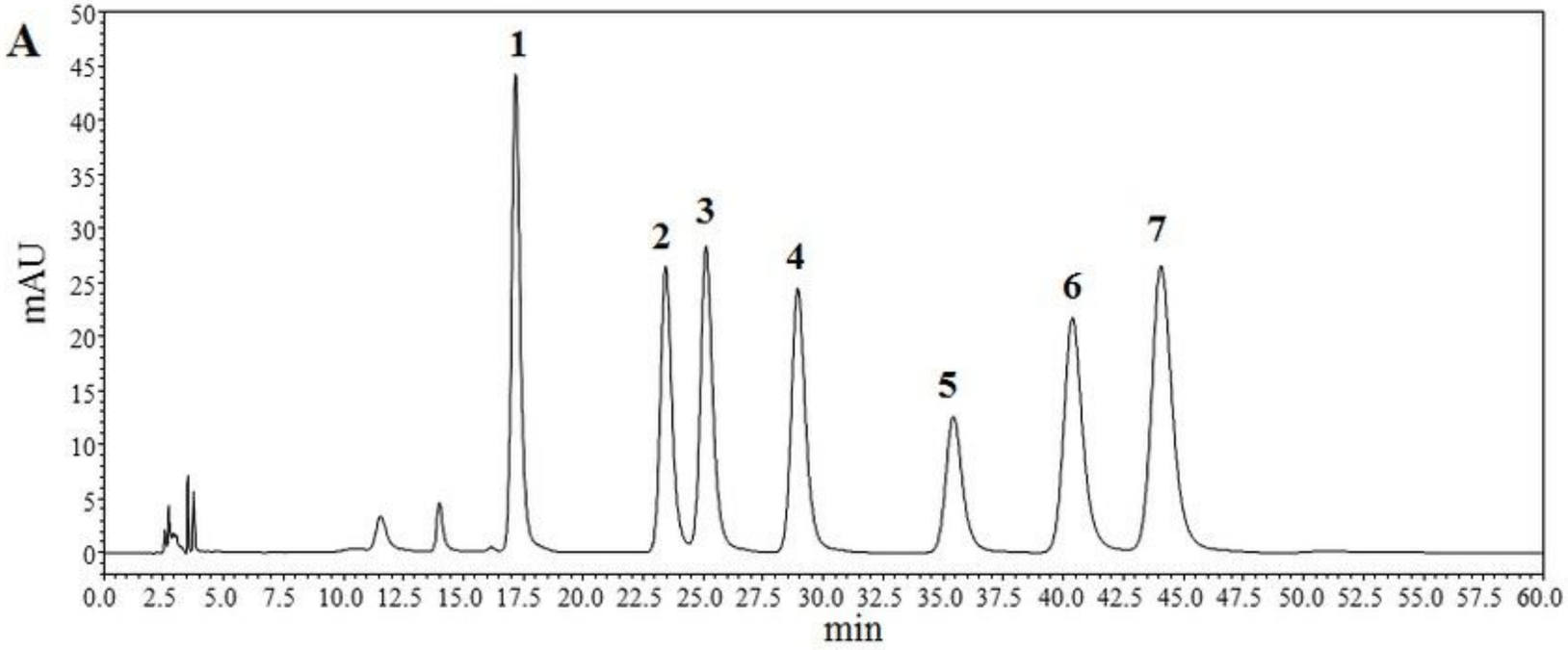


Size exclusion (gel filtration) chromatography



Large → Small

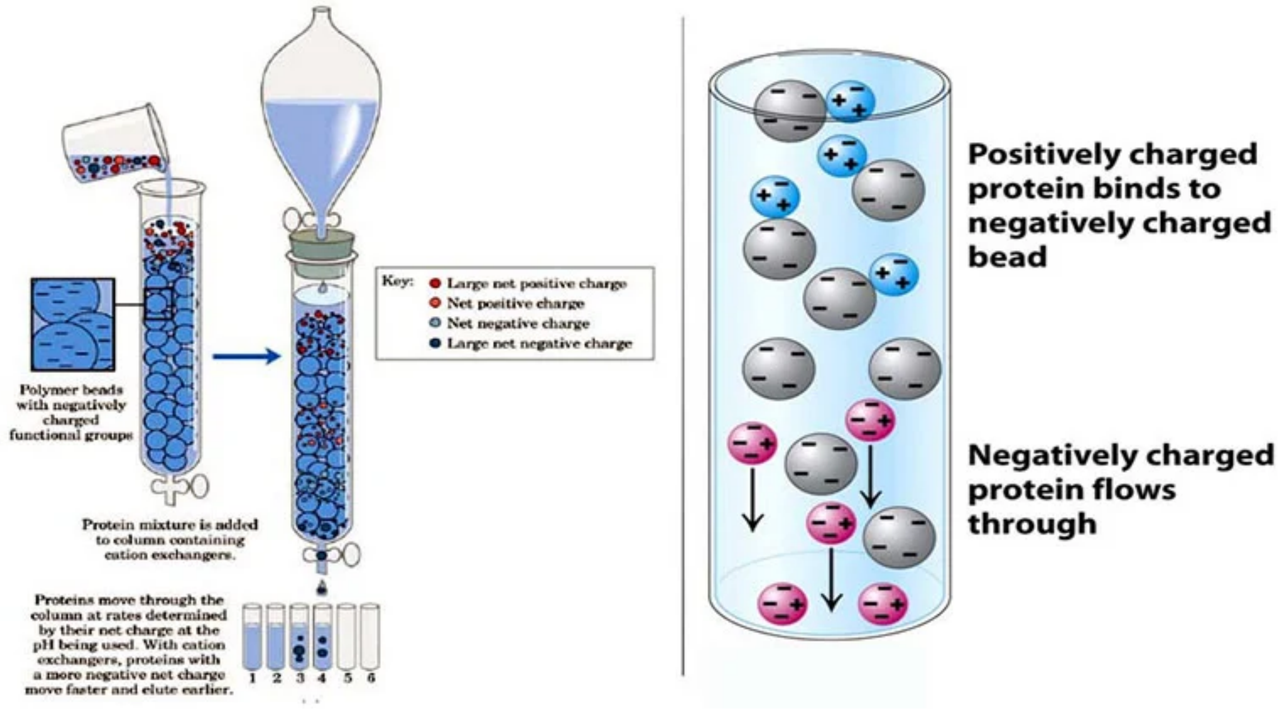




Larger proteins elute faster as they are less easily retained by the porous beads

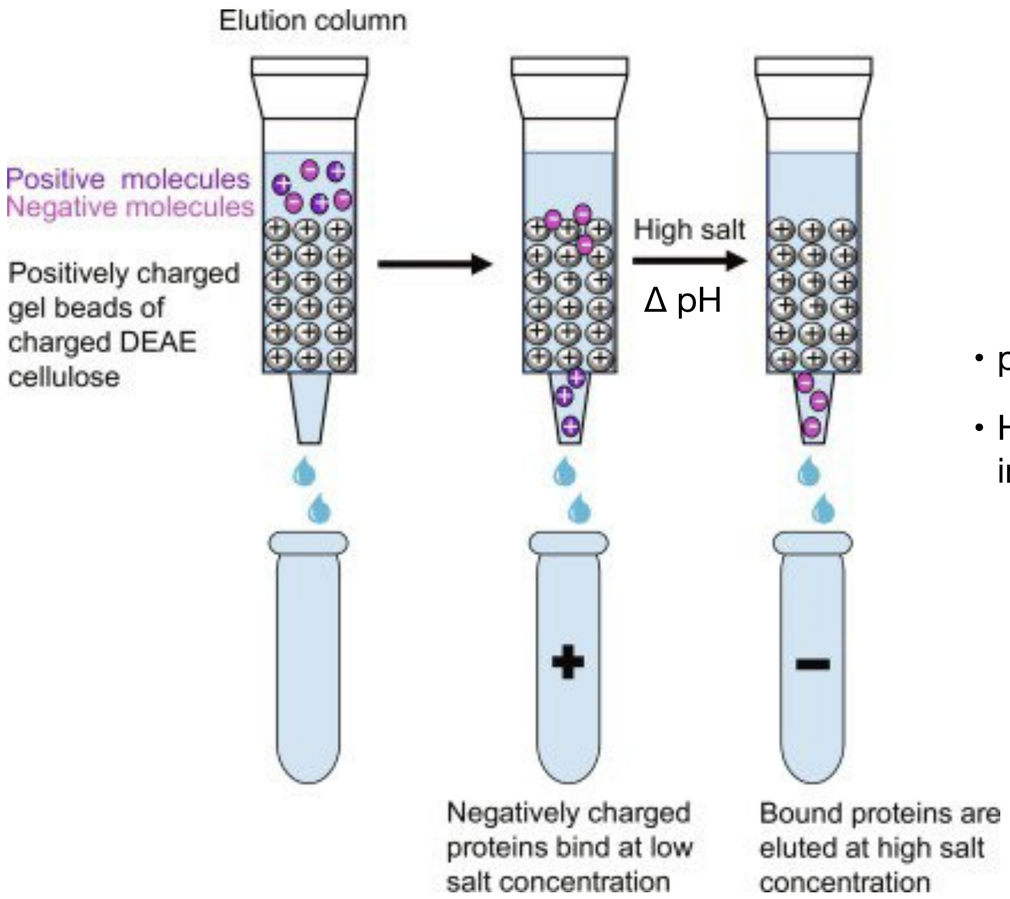
Ion-exchange

pH > pI proteins will have a net -ve charge so bind to an anion exchange column (+ve) beads.



How do you remove the bound proteins?

Ion-exchange



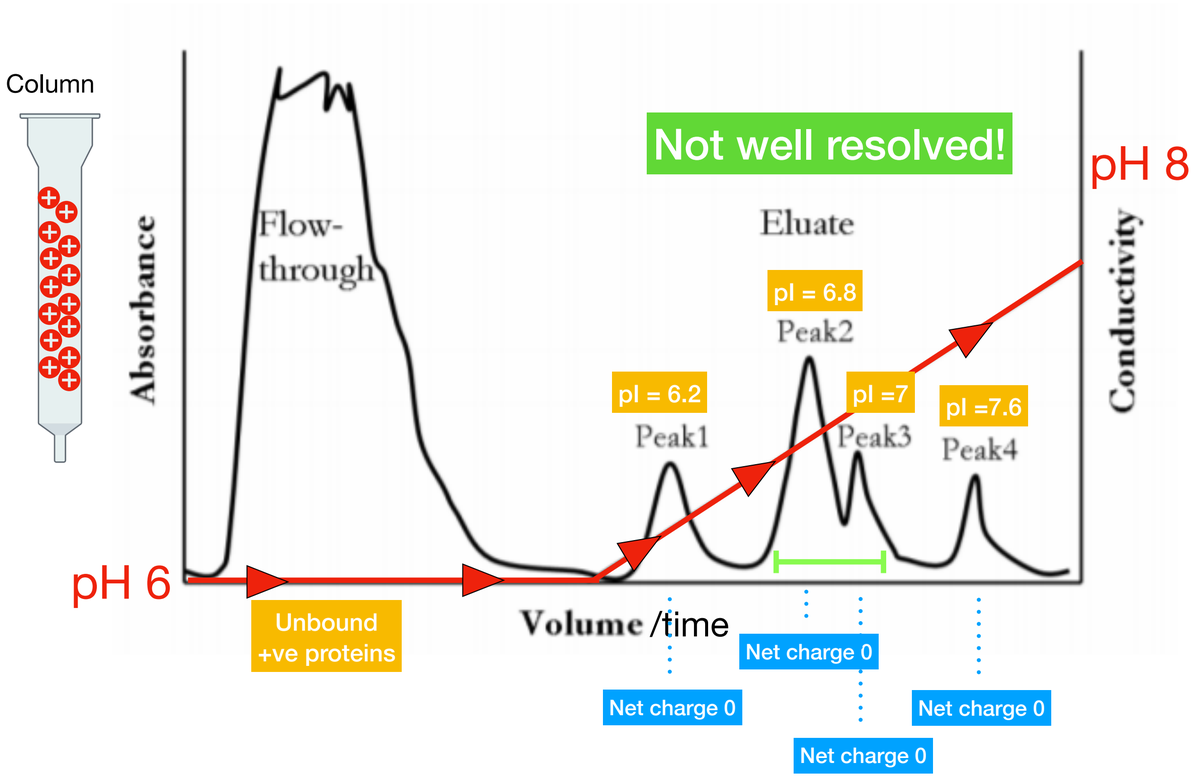
- pH will change the net charge of the protein allowing elution
- High salt carries a stronger charge so will kick off weaker interactions

Changing ionic strength (salt) or pH of the buffer will elute the bound proteins

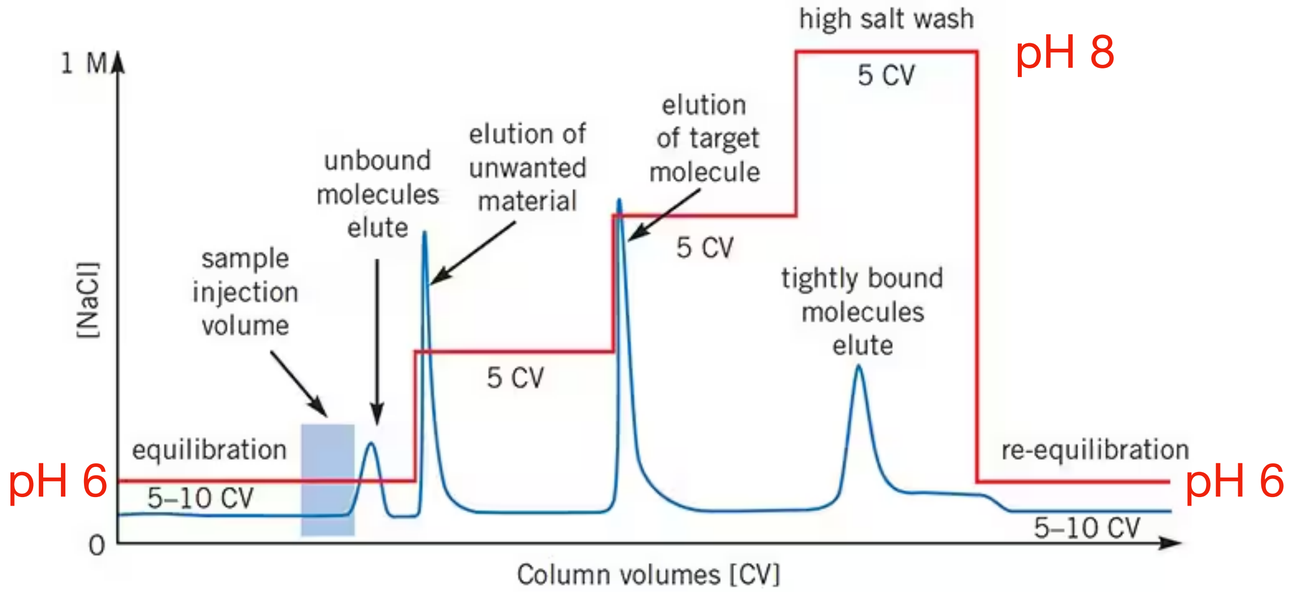
Ion-exchange chromatography (IEX)

Protein of interest has pI of 6.8
At pH 6.8 protein is neutral
At pH < 6.8 protein is +ve
At pH > 6.8 protein is -ve

Gradient elution



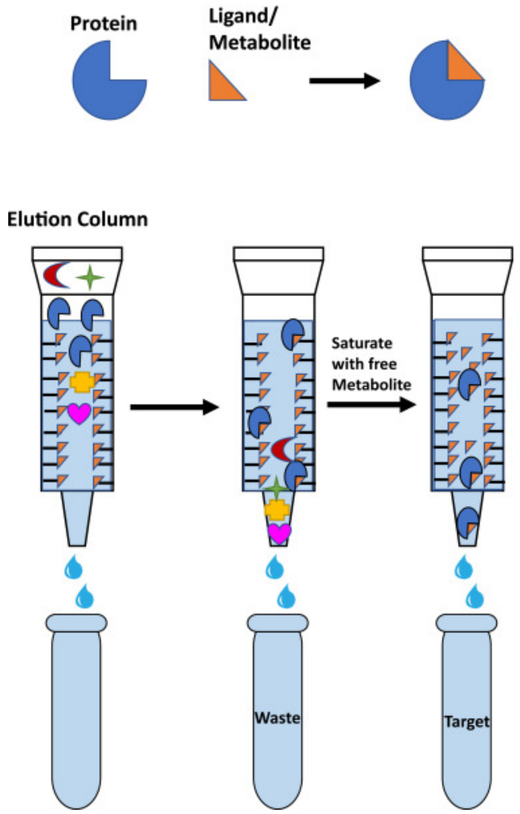
Step-wise elution

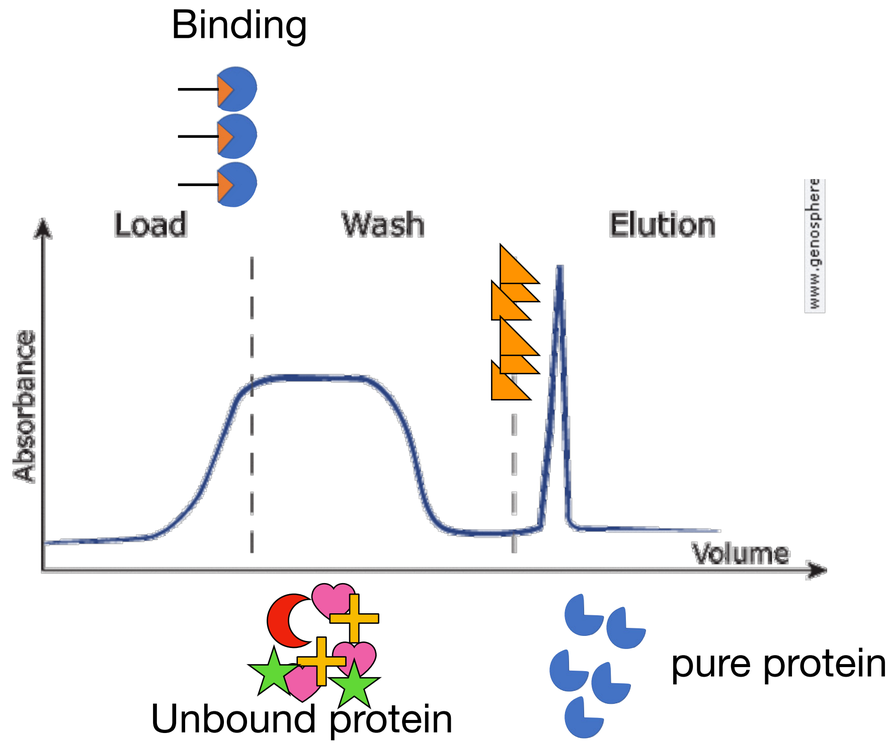


Resolution can be improved

As the pH increases, the proteins will elute when their charge weakens

Affinity chromatography

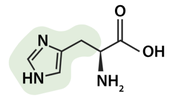




What if a protein has no natural ligand?

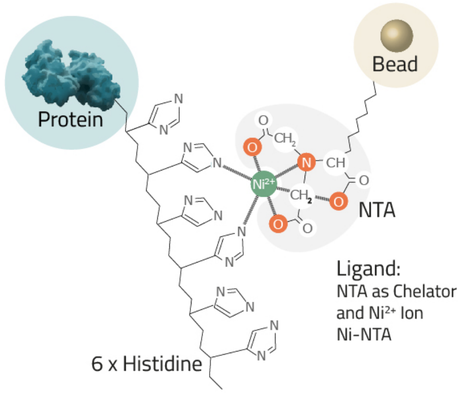
Types of affinity chromatography

Normally utilised with genetically engineered affinity tags

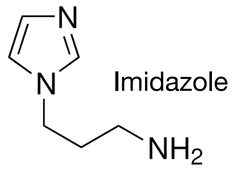


Histidine (His, H)

His-tag



Elution with high concentration of Imidazole

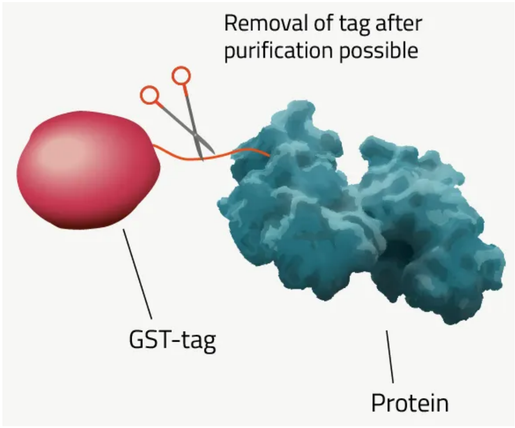


Imidazole

Immobilised metal affinity chromatography (IMAC)

GST-tag

glutathion-S transferase
26 kDa



Column = glutathione
Elution = reduced glutathione

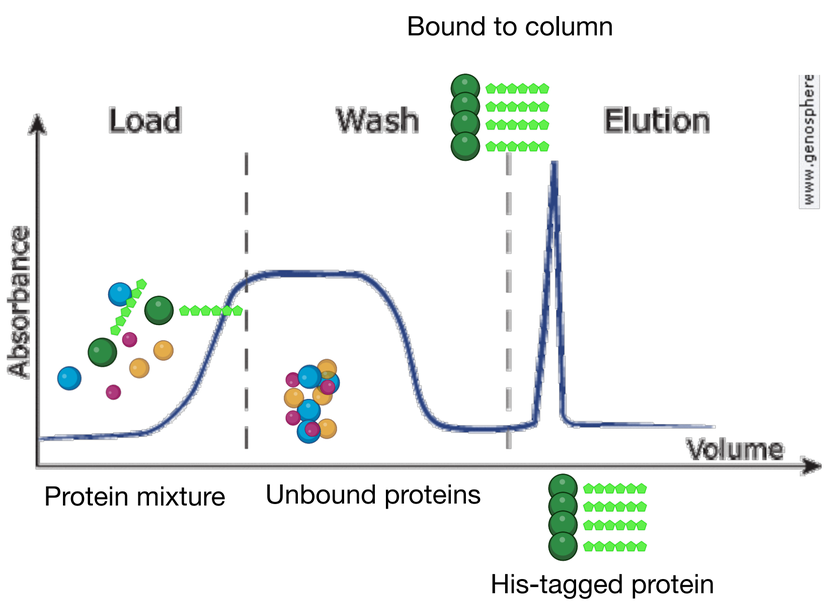
MBP-tag

Maltose binding protein
42 kDa

Column = amylose
Elution = maltose

Tag removal = affinity column, twice

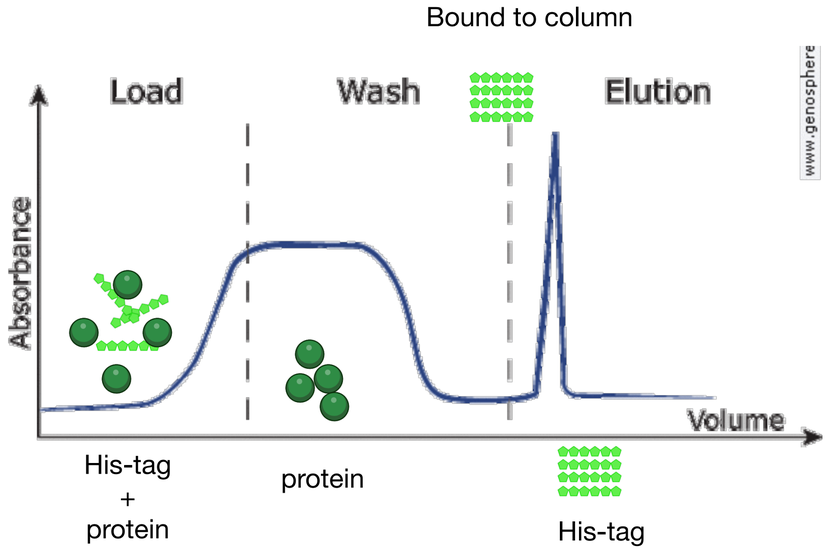
1



2

Add enzyme to protein
to cleave off the tag

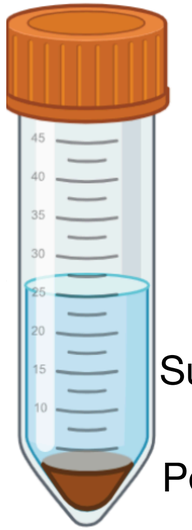
3



Steps of protein purification

1. Cell lysis

2. Removal of cellular debris



Supernatant

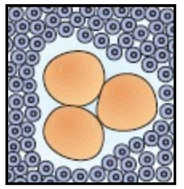
Pellet

Differential centrifugation

3. Purification of proteins

centrifugation

precipitation



chromatography

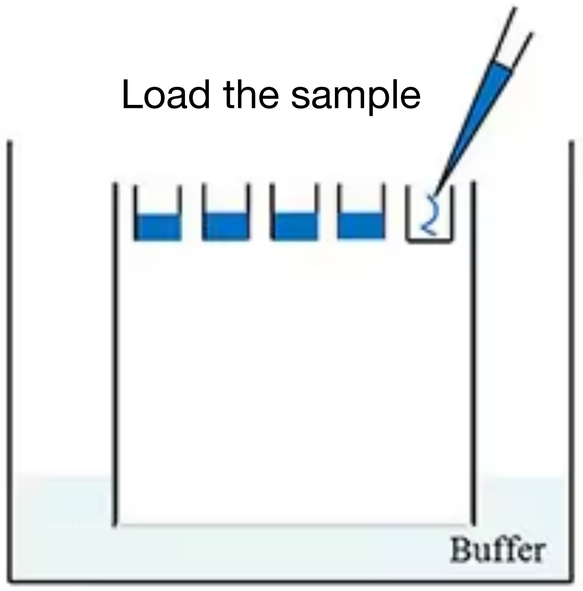
Ni^{2+}

How can we assess the purity of the sample?

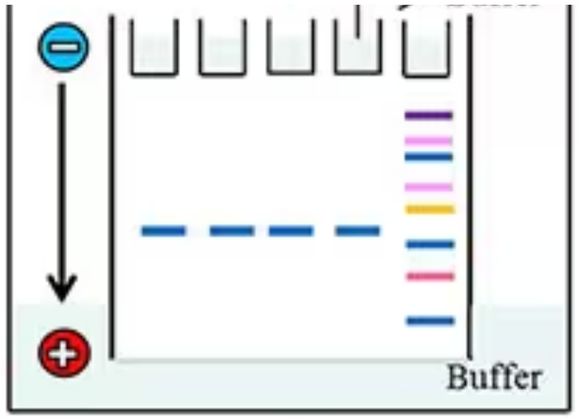
(gel) Electrophoresis

Uses a current to move proteins through a gel or matrix

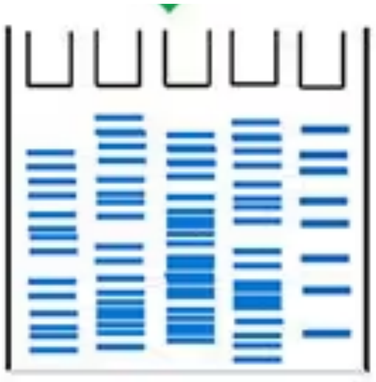
Load the sample



Apply the current

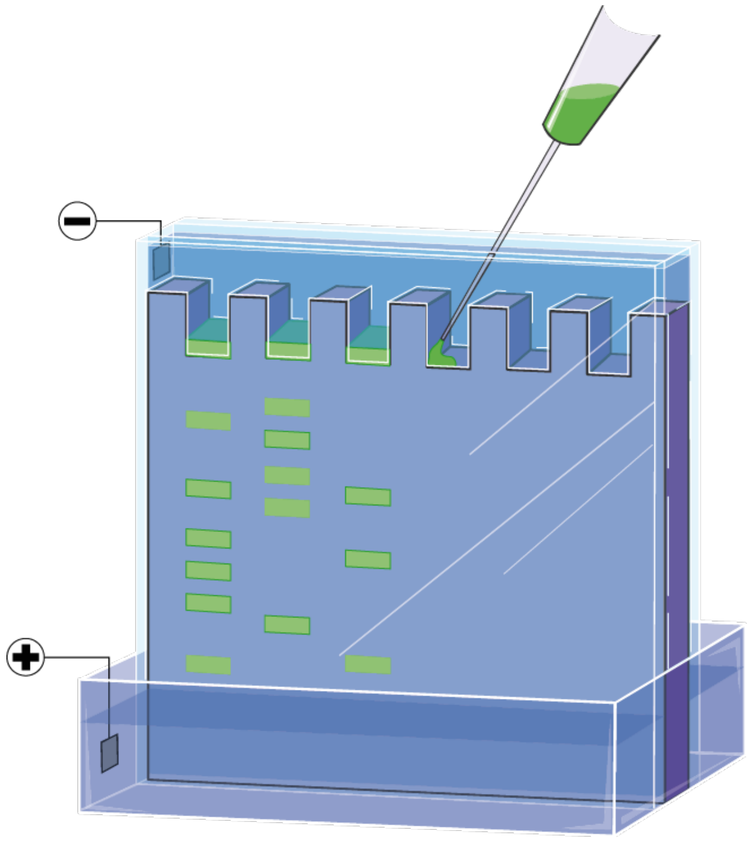


Detect the proteins



(gel) Electrophoresis

Uses a current to move molecules through a gel or matrix



The **electrophoretic mobility** (μ) of a protein in an electric field is calculated using:

$$\mu = \frac{v}{E}$$

where:

- μ = electrophoretic mobility (m²/V·s)
- v = velocity of the protein (m/s)
- E = electric field strength (V/m)

$$v = \frac{d}{t}$$

Where:
d = distance travelled by the protein (m)
t = time taken (s)

$$E = \frac{V}{L}$$

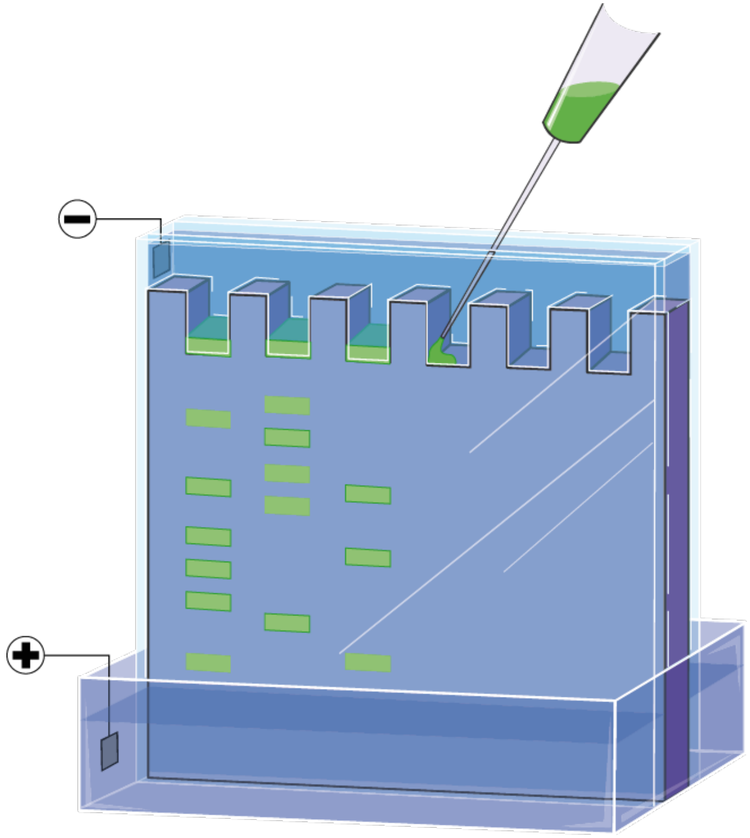
Where:
V = applied voltage (V)
L = distance between the electrodes (m)

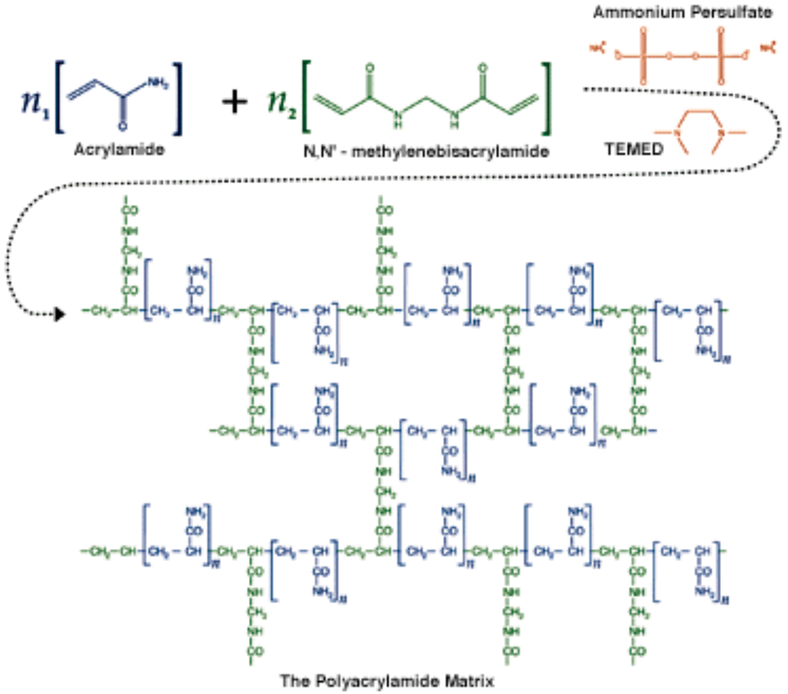
$$\mu = \frac{(d/t)}{(V/L)}$$

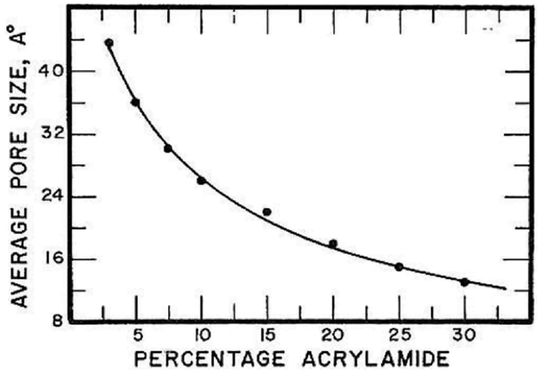
- Pores in the gel allow smaller molecules to move faster than large ones
- separates molecules based on size and charge

(gel) Electrophoresis

Gel is made of polyacrylamide





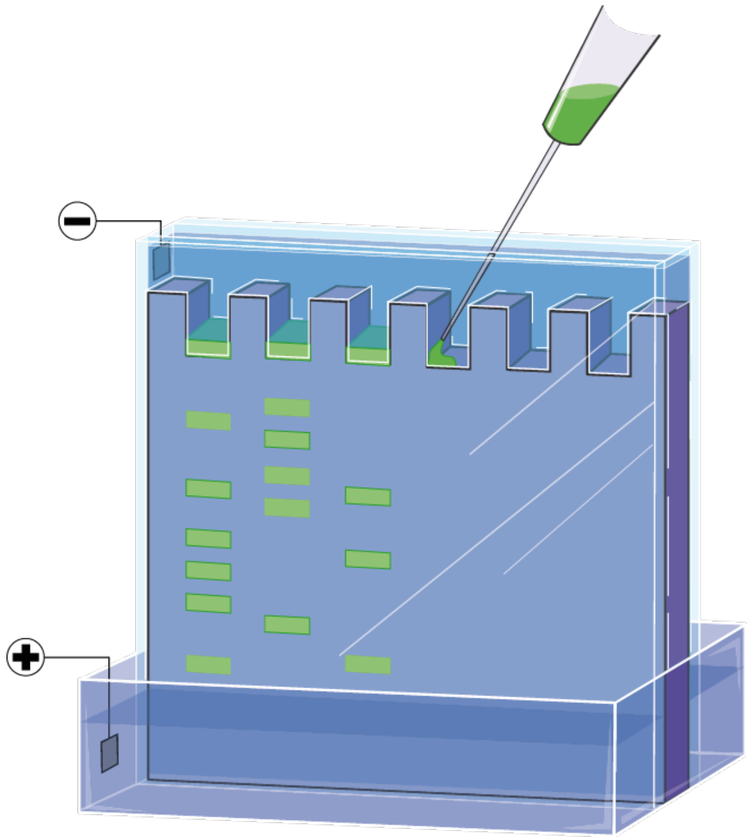


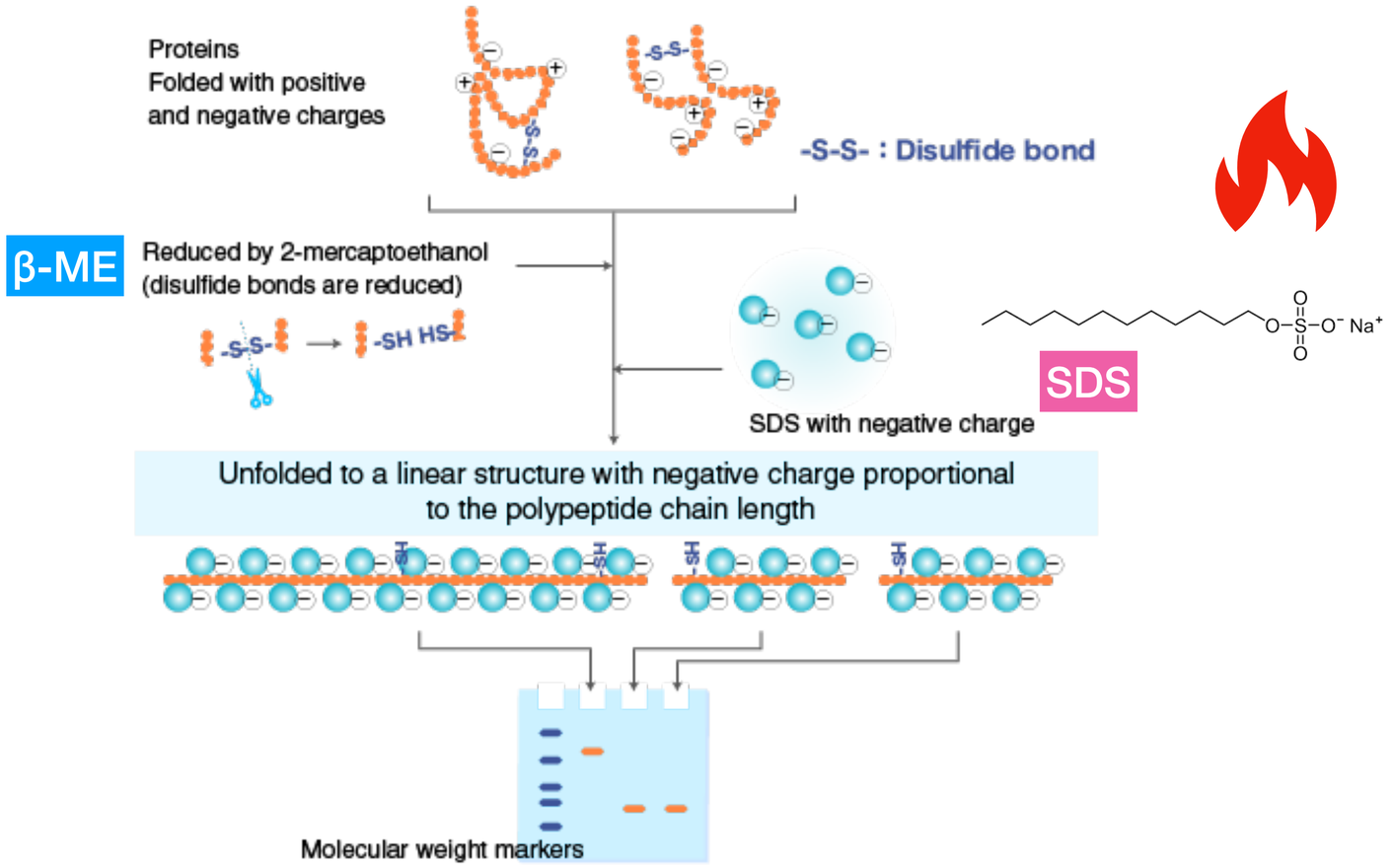
Cross-linking creates a gel with pores

Pore size depends on % acrylamide

(gel) Electrophoresis

Sodium dodecyl-sulfate (SDS) makes all the proteins negatively charged

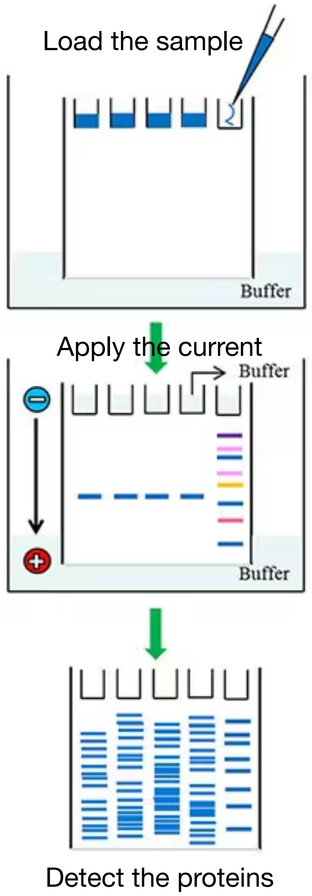




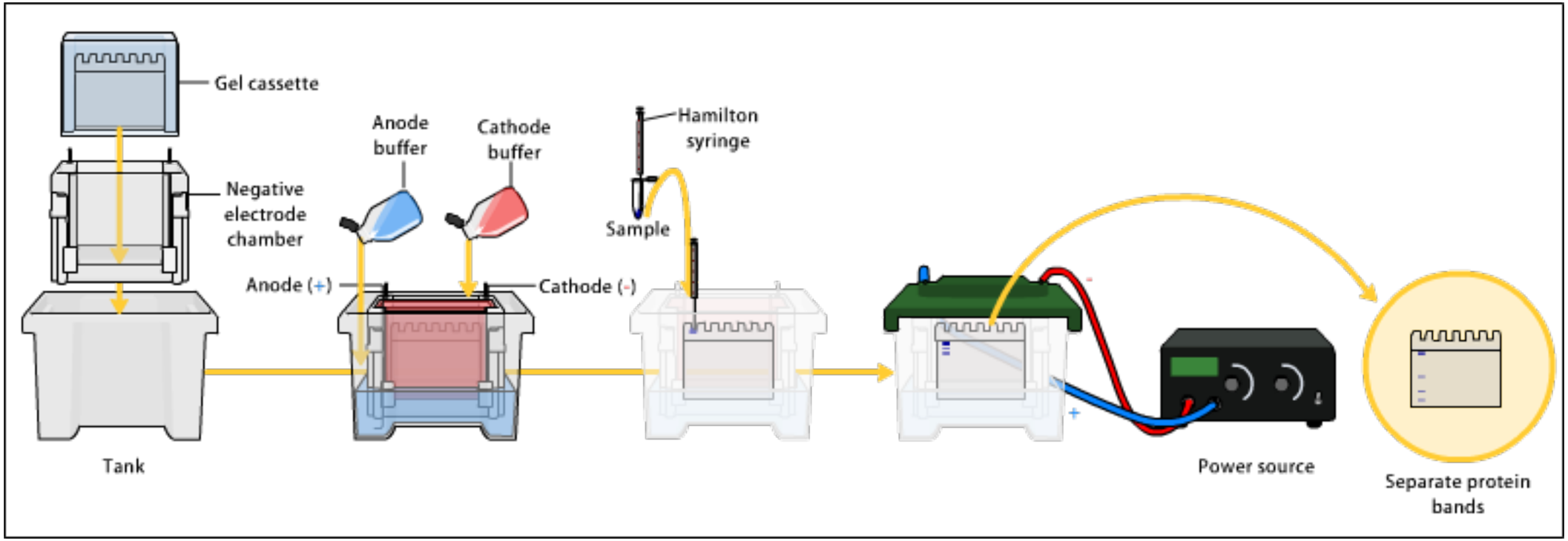
Proteins are unfolded = A denaturing gel

SDS-PAGE

Sodium dodecyl-sulfate - polyacrylamide gel electrophoresis

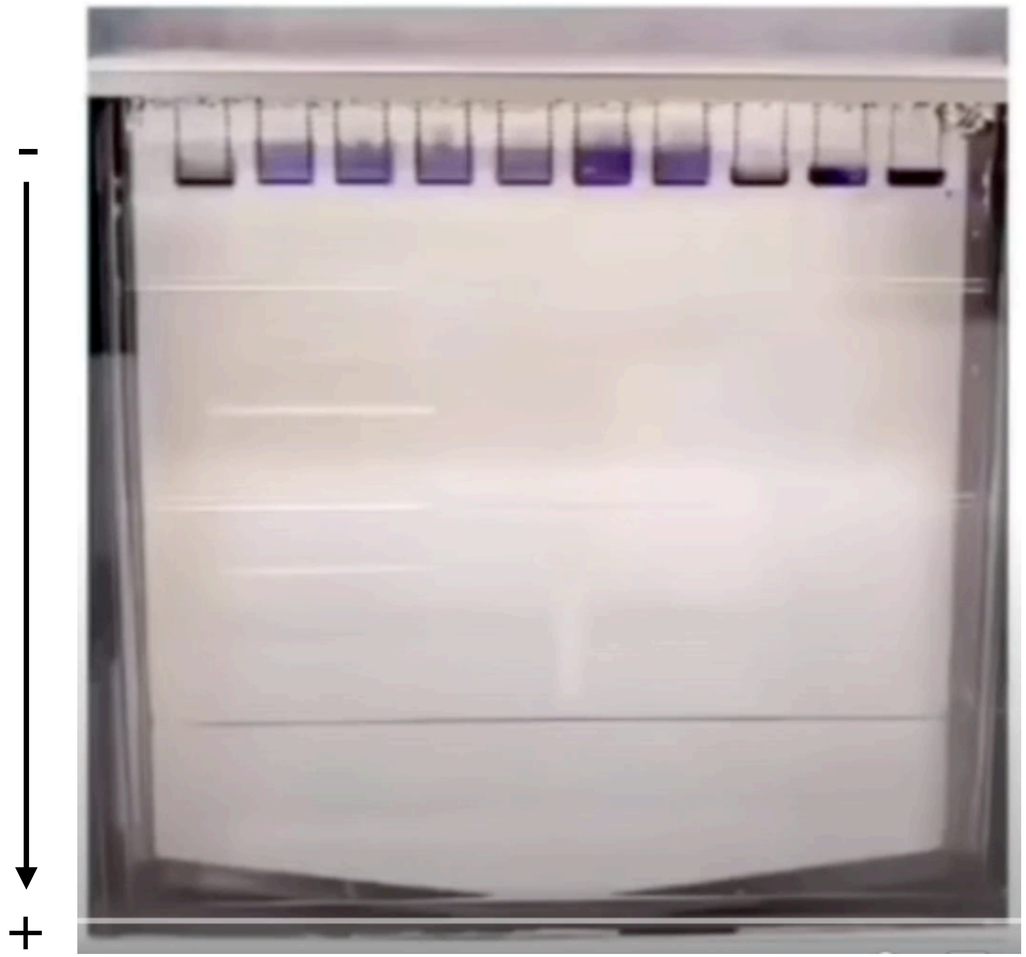


Gel apparatus



SDS-PAGE

Sodium dodecyl-sulfate - polyacrylamide gel electrophoresis



https://www.youtube.com/shorts/BSwEj-U_eSo

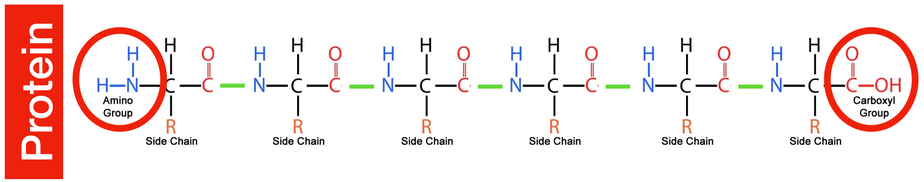
SDS-PAGE -visualization

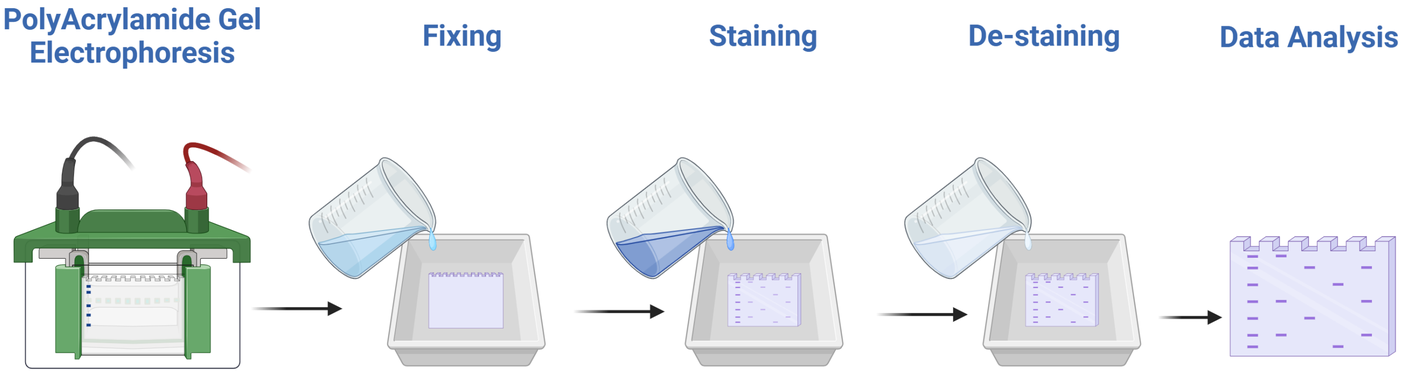
Sodium dodecyl-sulfate - polyacrylamide gel electrophoresis

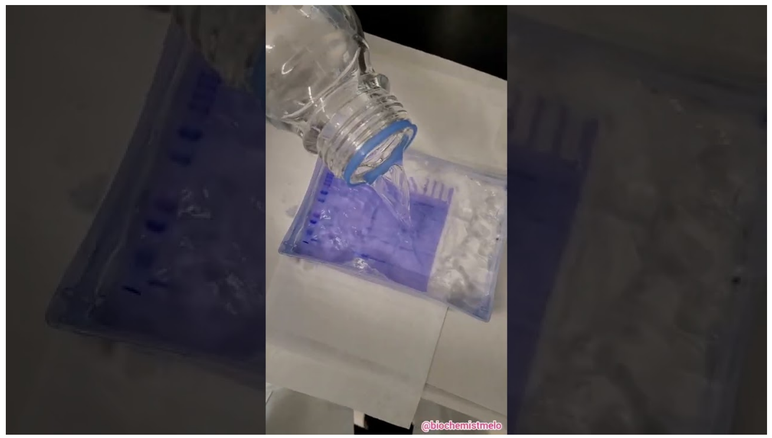
Coomassie brilliant blue G-250

Solid Coomassie brilliant blue G

Brilliant blue G in isopropanol solution

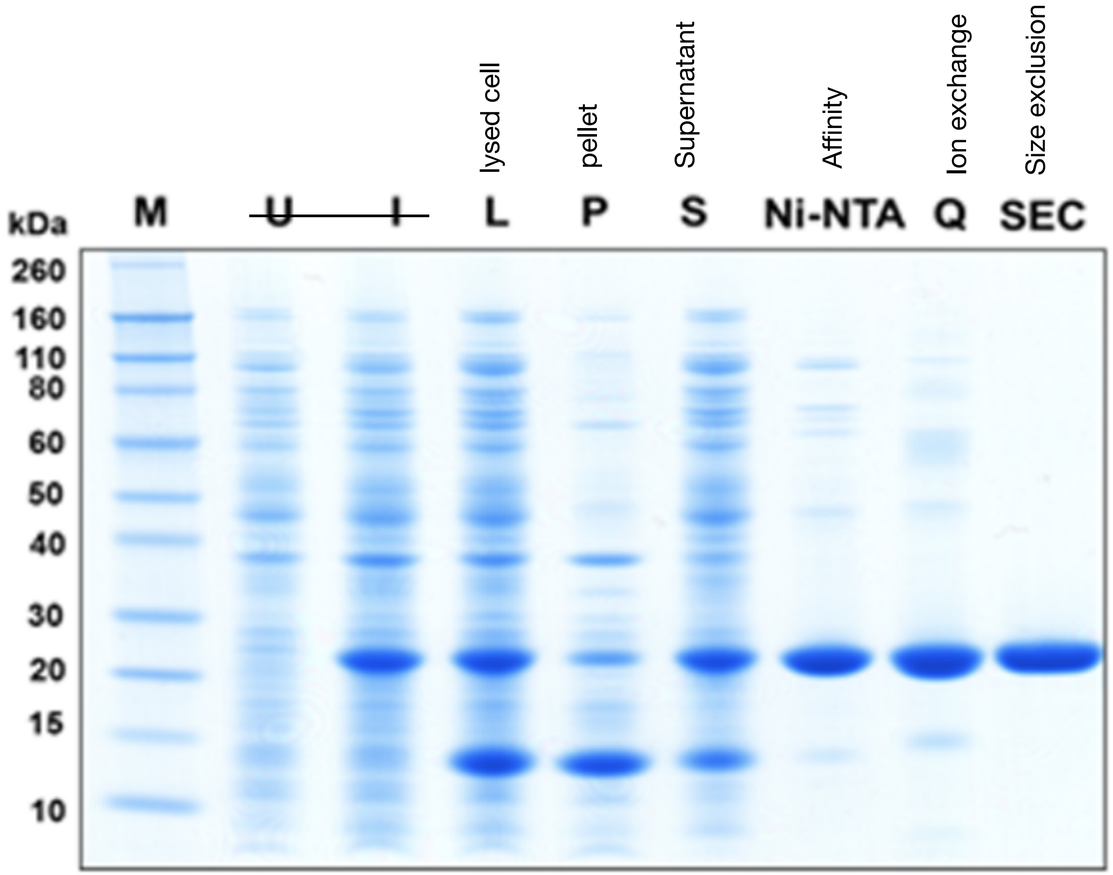






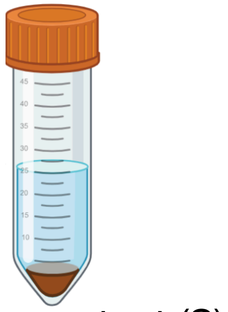
https://www.youtube.com/shorts/5zFDhlUgp7E

Visualising protein purification



Protein of interest

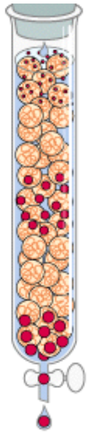
lysed cell
L



Supernatant (S)
Pellet (P)

Affinity
Ni-NTA

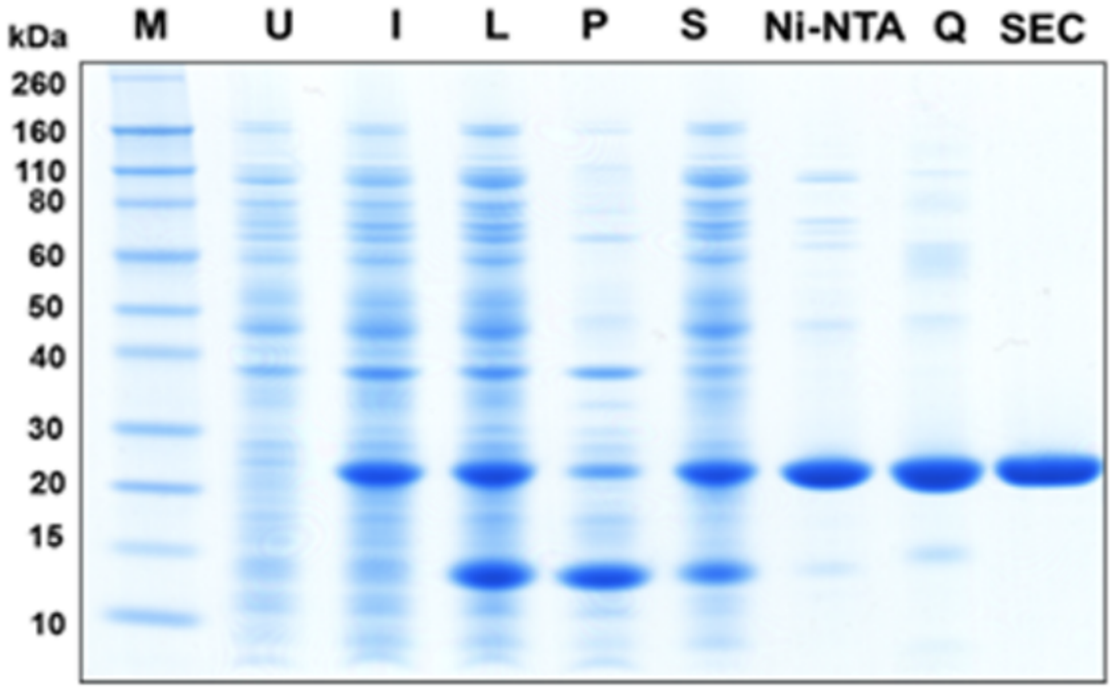
Ion exchange
Q



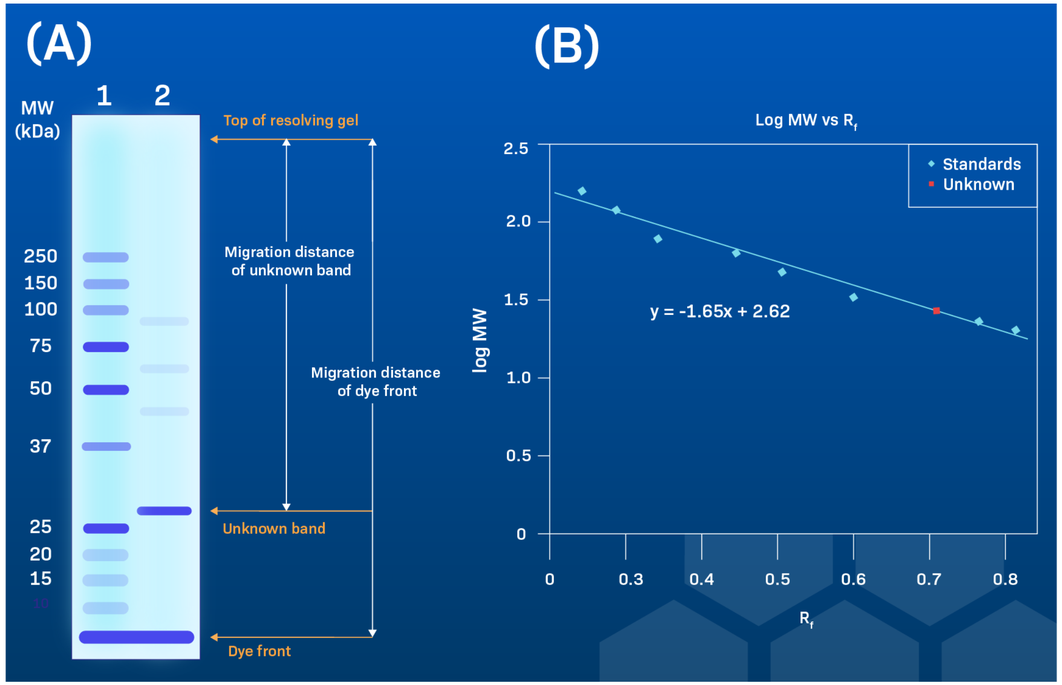
Size exclusion
SEC

Different proteins are removed at each step until the protein is pure

Molecular weight determination



molecular weight (MW) marker

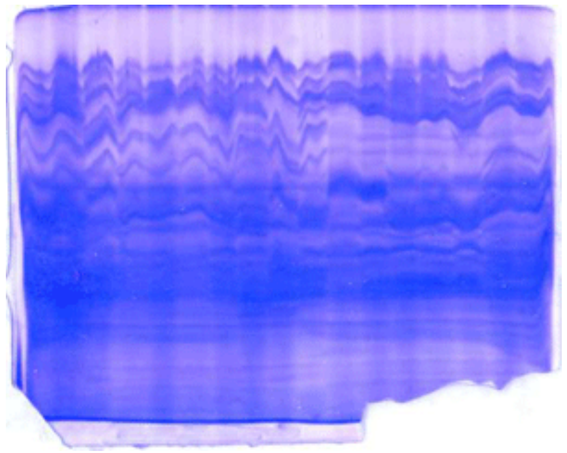


Protein ladder - mixture of purified proteins of known MW

Migration distance of each band in the protein ladder used to calculate MW of sample

SDS-PAGE - hall of shame

Overheating

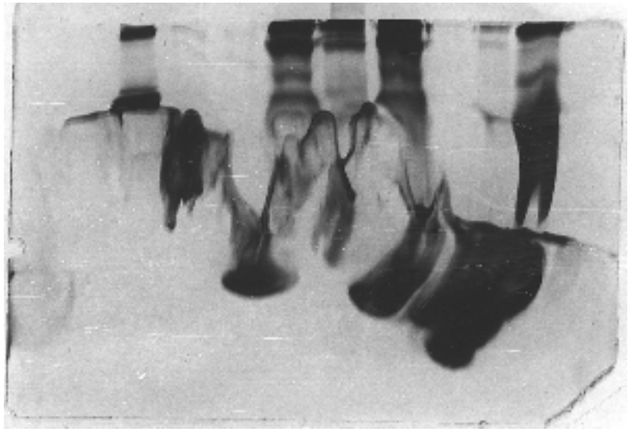


Voltage too high

Incorrect mixing

Acrylamide % too high

Incorrect mixing

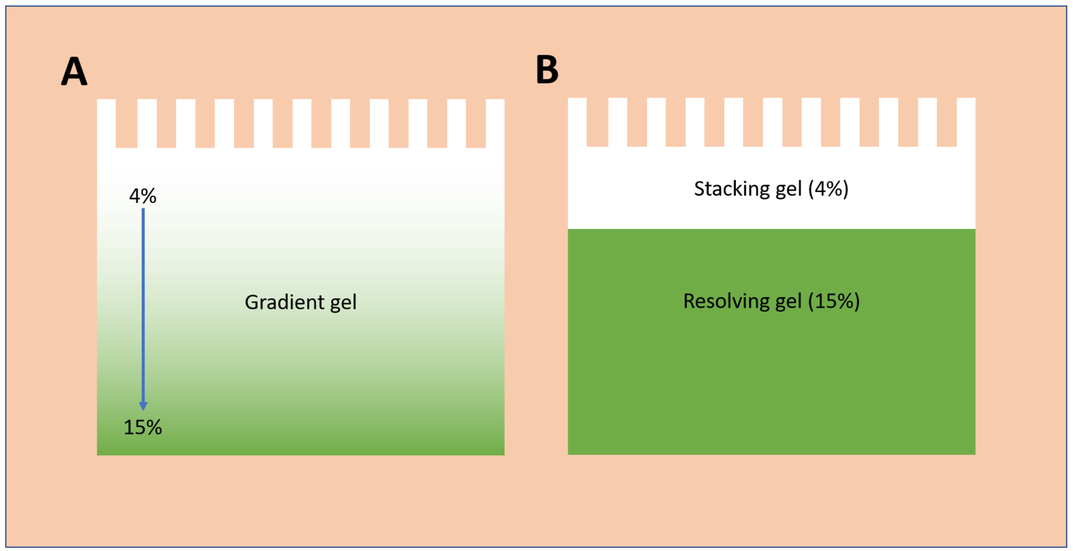


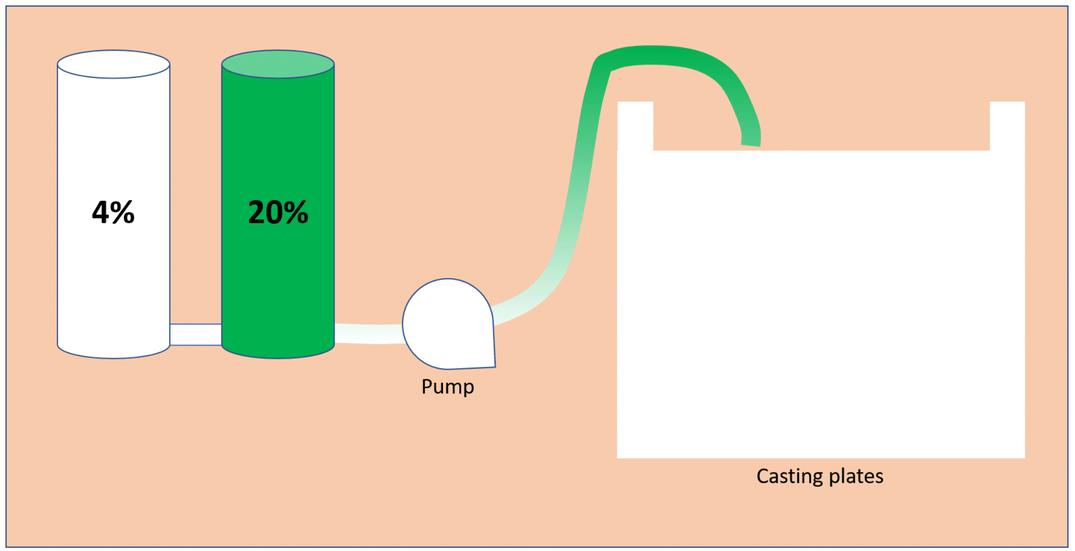
Protein overloading

https://www.ruf.rice.edu/~bioslabs/studies/sds-page/sdsgoofs.html

SDS-PAGE

Single % vs gradient gels





Advantages of a gradient gel

- You have a nicer looking gel with sharper bands
- You can resolve a broader range of protein sizes on one gel (so a big protein and a small one)
- You can better separate similar-sized proteins.

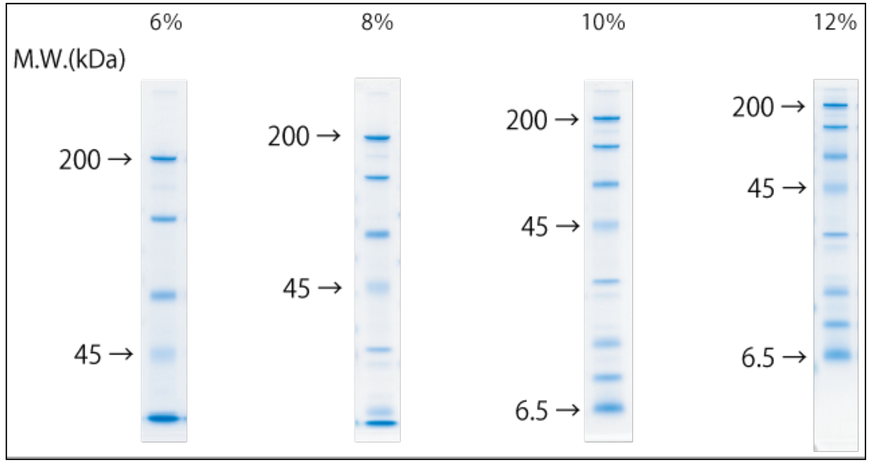
SDS-PAGE

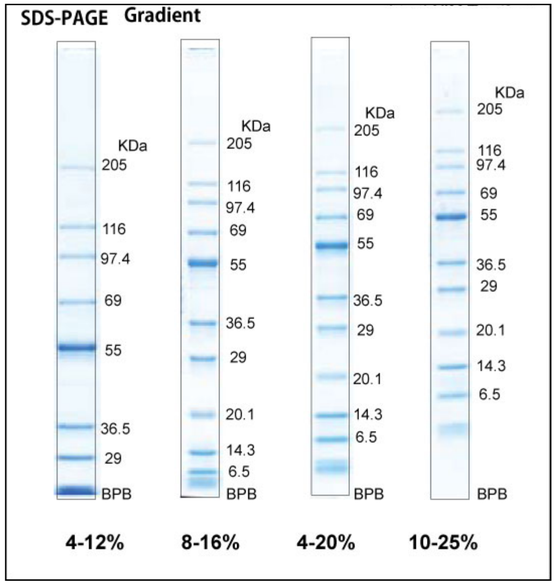
Single % vs gradient gels

Table 1: Protein sizes resolved by different gel percentages

PROTEIN SIZE	GEL PERCENTAGE
4-40 kDa	Up to 20%
12-45 kDa	15%
10-70 kDa	12.5%
15-100 kDa	10%
50-200 kDa	8%
>200 kDa	4-6%

Range of protein sizes	Low / High acrylamide percentages	Application
4 – 250 kDa	4% / 20%	Discovery work; you are looking for everything under the sun
10 – 100 kDa	8% / 15%	A more targeted approach, but you want to avoid multiple gels
50 – 75 kDa	10% / 12.5%	You are trying to resolve similarly sized proteins





native-PAGE

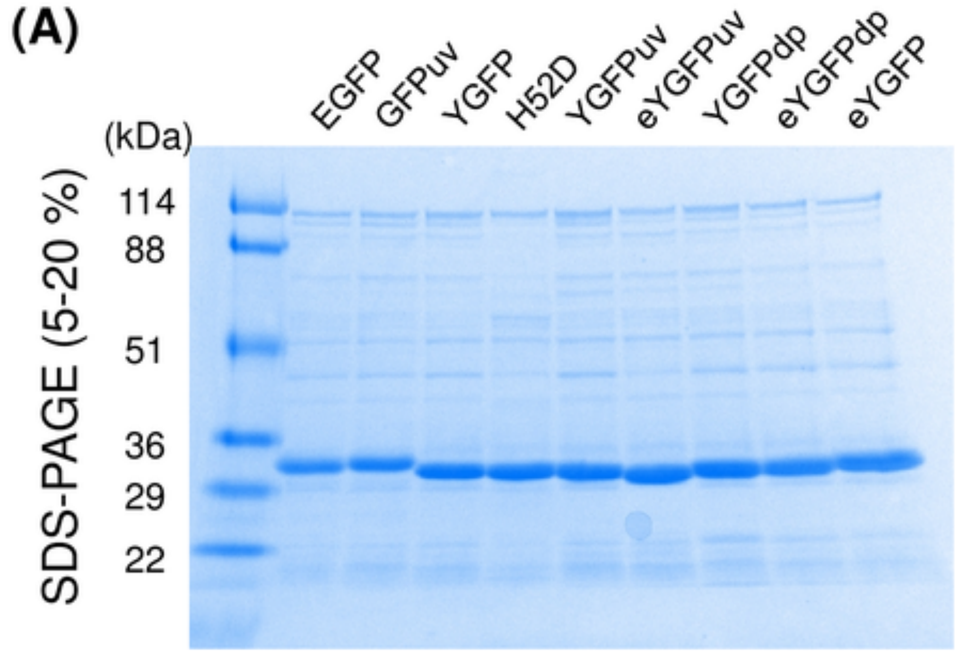
polyacrylamide gel electrophoresis

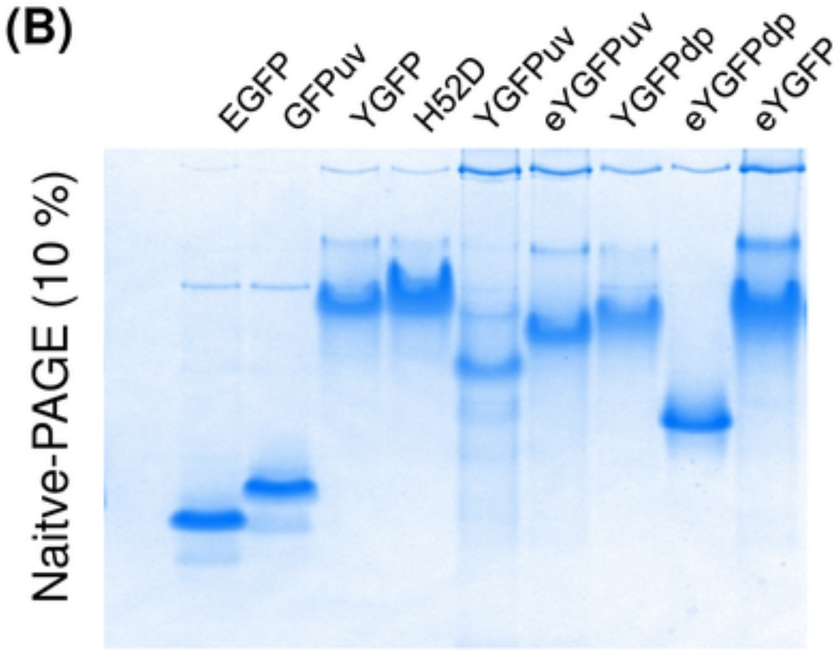
Denaturing

non-Denaturing

SDS

β-ME





NO SDS

NO β-ME

NO

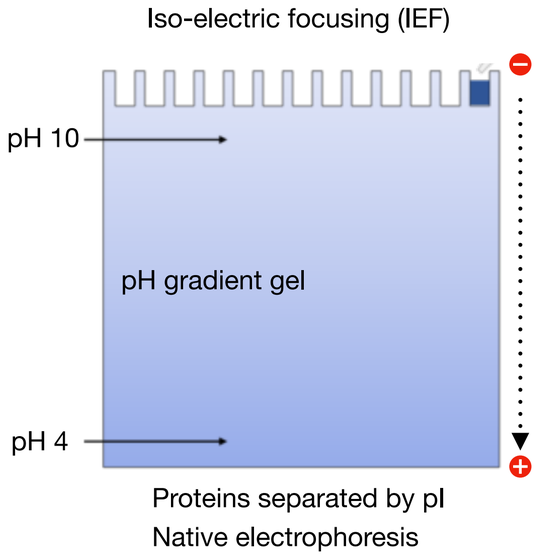
Protein complexes will be separated

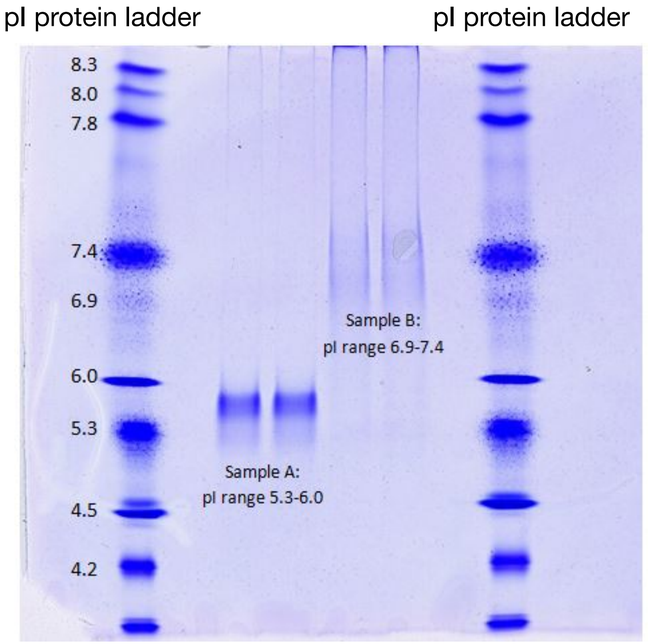
- Separation based on size (MW)
- Protein identification

Protein complexes will stay together.

- Separation based on charge, shape and size (H_R)
- Protein characterization

Iso-electric focusing (IEF)

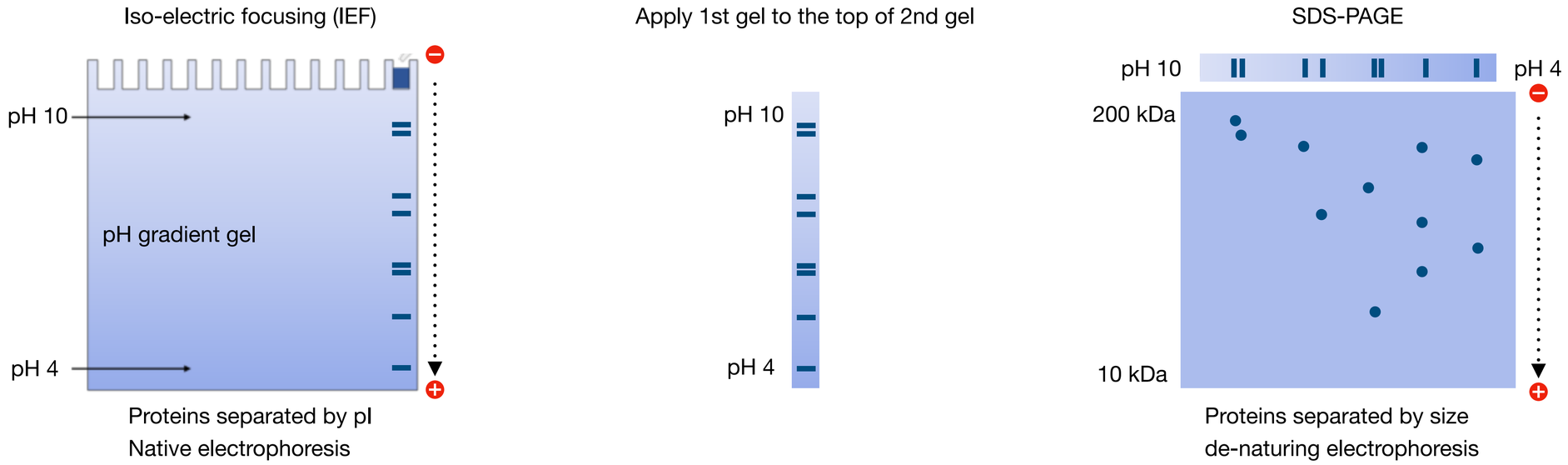




Protein ladder - mixture of purified proteins of known pI

Migration distance of each band in the protein ladder used to calculate pI of sample

2D-electrophoresis



- Characterization/analysis of complex protein mixtures

Challenges of protein purification

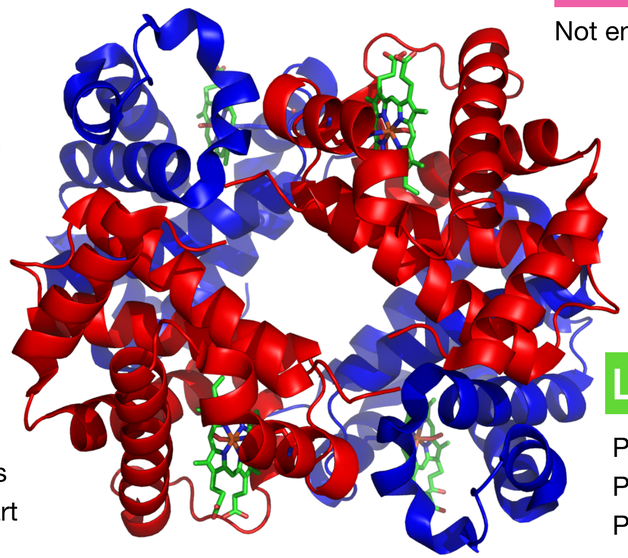
Proteins should be purified in their native state so they are functional

Stability

Protein unfolding
Uncontrolled aggregation/precipitation

Contaminants

Not enough purification steps



Degradation

Molecular scissors chopping up proteins
Protein multimers/complexes falling apart

Loss of function

Protein has unfolded (loss of structure)
Protein complexes have fallen apart
Protein is not in right environment for functionality

number and type of purification steps, a well as purification buffers (pH and salt) need to be highly optimised for each protein