The Proteome

- Proteome is the protein equivalent of the genome
- The proteome consist of all of the proteins expressed by a cell under specific conditions
- The proteome of a cell depends on the cell type, its developmental stage, environment/stimuli, nutritional and metabolic status etc
- The genome of a cell is fixed, the proteome is dynamic
- The proteome is much larger than the genome. Each gene can translate into mutiple isoforms of proteins

Protein Mass and Concentration

- Protein mass is measured in Daltons (Da) or kDa
- One Dalton is 1/12 the mass of a ^{12}C atom
- On average, the MW of each aa is 110 Da
- Most proteins range from 30 to 80 kDa
- Trp and Tyr have a high ability to absorb light with maximum absorption at 280 nm. Since most proteins contain these aa, protein concentration can be estimated spectrophotometrically.

Protein Purification

- Proteins can be purified from cell or tissue samples
- Samples are homogenized and fractionated by differential centrifugation to isolate the fraction containing protein of interest
- Protein purification is a multi-step procedure
- Need to establish a specific method of identification. Can be enzymatic, binding or activity assay
- With each step, the purification level and specific activity increases and the yield decreases

Protein Biochemistry Methods

- Methods can be analytical or preparative
- Preparative: purification of protein of interest
- Analytical: identification and characterization
- Methods based on solubility, size, charge, binding affinity, activity

Solubility

- **Salting Out**: For most proteins, solubility decreases as salt concentration increases.
- Greater the polarity, greater the solubility.
- Proteins can be fractionated by sequential salt precipitation
- **Isoelectric Precipitation**: Proteins are least soluble when pH = pI. (because at pI, net charge is 0)

Size

- **Dialysis**: Diffusion through a semi-permeable cellulose membrane. Different pore sizes allow removal of molecules smaller than specific MW
- **Gel-Filtration Chromatography**: Column packed with dextran or agarose beads. Smaller molecules get trapped within the porous beads and their flow down the column is retarded. Larger molecules are excluded form the beads and move down between loosely packed bead. Smaller the molecule longer the elution time.

Mass

- **Ultracentrifugation**: Use of centrifugal force to force a particle to settle through a fluid suspension
- The rate of movement of a particle is directly related to its sedimentation coefficient *S* which, in turn, is proportional to its mass, density and shape.
- Gradient ultracentrifugation involves layering a sample over a density gradient of sucrose. After ultracentrifugation, the different components of the sample will separate into different bands based on their sedimentation coefficient

Charge

- **Ion Exchange Chromatography**: Columns are made of charged cellulose particles.
- Carboxymethyl (CM) cellulose: -charge, cation exchange column
- Diethylaminoethyl (DEAE) cellulose: + charge, anion exhange column
- Proteins are eluted using a pH gradient

Binding Affinity

- **Affinity Chromatography**: The column matrix is modified by covalent linkage to a compound with high specific binding affinity to protein of interest
- Eg: Lectins, antibodies, ligands, substrates
- 3 steps: Specific binding of protein, washing unbound proteins, elution of bound protein by specific displacement, high salt or low pH

HPLC

- High Pressure Liquid Chromatography
- Enhanced version of all column chromatography techniques
- Column material are very fine and closely packed for better resolution
- High pressure has to be applied to maintain flow
- Clean, rapid separation of very small samples

Electrophoresis

- Primary analytical technique
- Electrophoresis is the movement of charged proteins in an electric field
- Movement is from the – electrode to + electrode. Migration is related to charge: mass ratio. Generally, smaller proteins migrate further
- Separation on slabs of polyacrylamide cross-linked with methylenebisacrylamide: inert, porous and readily formed
- Visualization by staining (coomassie blue, silver)

Types of Electrophoresis

- **Denaturing gels**: SDS disrupts all non-covalent interactions. Associates with all proteins and imparts high – charge making native charge insignificant. Reducing agent opens disulfide linkages
- **Native gels**: No SDS, native charge contributes to migration, usually protein activity maintained
- **Isoelectric focusing**: Polyampholytes are used to form a pH gradient in the gel. Proteins focus where pH = pI, because at this point they have 0 net charge
- **2D electrophoresis**: IEF + SDS-PAGE. Proteins separated by pI horizontally, then by size vertically for complete resolution of complex samples

Amino Acid Composition

- Complete hydrolysis for 24 hr at 110 ^{o}C in 6 M HCl
- Separation of amino acids by ion exchange chromatography on sulfonated polystyrene resin
- Elution using a pH gradient
- Detection of amino acids by reaction with ninhydrin or fluorescamine (spectrophotometry)
- Identification of amino acids by position of peak on chromatogram
- Determination of amino acid ratios by size (height) of each peak

Identification of N-terminal amino acid

- The N-terminal aa can be identified by Sanger's method. This method involves modification of the N-terminal residue by flurodinitrobenzene followed by complete hydrolysis of the peptide.
- More recently, fluorescent compounds such as dansyl chloride or dabsyl chloride are used because of their higher sensitivity.
- The N-terminal aa is the only modified aa and it is identified by chromatography.
- The peptide is completely hydrolyzed and cannot be reused

Amino acid sequencing

- Peptides are sequenced by 'Edman Degradation' method
- Amino acids are removed and identified sequentially one residue at a time from the N-terminus
- The N-terminal amino acid is modified by phenyl isothiocyanate
- Mild hydrolysis releases the tagged amino acid as a cyclic derivative phenylthiohydantion-aa (PTH-aa) which is identified by HPLC ion-exchange chromatography
- The rest of the peptide remains intact, just one aa short
- The next cycle releases residue 2. It is possible to identify ~50 aa from each sample by this method

Specific Cleavage of Polypeptides

- Proteins larger than 50 aa are first hydrolyzed into shorter peptides
- Chemical or enzymatic methods hydrolyze proteins at specific sites
- Peptides are separated by chromatography
- Peptides generated by 2 or more cleavage methods are each sequenced separately.
- Sequences of individual peptides are overlapped together to deduce the entire protein sequence

TABLE 4.3 Specific cleavage of polypeptides

Reagent	Cleavage site
Chemical cleavage	
Cyanogen bromide	Carboxyl side of methionine residues
O-Iodosobenzoate	Carboxyl side of tryptophan residues
Hydroxylamine	Asparagine–glycine bonds
2-Nitro-5-thiocyanobenzoate	Amino side of cysteine residues
Enzymatic cleavage	
Trypsin	Carboxyl side of lysine and arginine residues
Clostripain	Carboxyl side of arginine residues
Staphylococcal protease	Carboxyl side of aspartate and glutamate residues (glutamate only under certain conditions)
Thrombin	Carboxyl side of arginine
Chymotrypsin	Carboxyl side of tyrosine, tryptophan, phenylalanine, leucine, and methionine
Carboxypeptidase A	Amino side of C-terminal amino acid (not arginine, lysine, or proline)

Protein Sequencing Example

Method 1 (Trypsin):

ser-glu-phe-his-lys

ala-ile-cys-asp-tyr-thr-ala

gly-leu-pro-arg

Method 2 (staphylococcal protease):

gly-leu-pro-arg-ser-glu

phe-his-lys-ala-ile-cys-asp

tyr-thr-ala

Overall protein sequence:

Gly-leu-pro-arg-ser-glu-phe-his-lys-ala-ile-cys-asp-tyr-thr-ala

Proteins with disulfide linkages

- Disulfides are reduced using DTT
- -SH groups are blocked by treatment with iodoacetate to form carboxymethylated derivatives
- Position of disulfide linkage is determined by diagonal elecrophoresis
- Initially, peptide mixture with intact disulfides are resolved in one direction by paper electrophoresis.
- The paper support is treated with formic acid which oxidizes the disulfides to charged sulfites
- Electrophoresis in perpendicular direction shows displacement of sulfite-containing peptides from a single diagonal line

Peptide Synthesis

- The C-terminal aa's –NH_2 group is blocked using a tertiary butyloxycarbonyl (t-boc) group
- The C-terminal aa is covalently attached to a solid support via its carboxlate group
- The t-boc group is removed (aa is deprotected)
- The n-1 aa is protected with t-boc.
- The –COOH group of n-1 aa is activated by dicyclohexylcarbodiimide (DCC)
- The two are reacted to form a peptide bond
- The 2nd cycle starts with deprotection of n-1 NH_2
- After multiple cycles, the synthetic peptide is released from the resin by HF hydrolysis