Protein Markers

What is a protein

- **Proteins** - are large *biological molecules*, or *macromolecules*, consisting of one or more long chains of *amino acid* residues
- Proteins perform a vast array of functions within living organisms, including catalyzing *metabolic reactions*, replicating *DNA*, responding to *stimuli*, and transporting molecules from one location to another
- Proteins differ from one another primarily in their sequence of amino acids

Structure

- **Primary structure**: the *amino acid sequence*. A protein is a *polyamide*.
- **Secondary structure**: regularly repeating local structures stabilized by *hydrogen bonds*. The most common examples are the *alpha helix*, *beta sheet*, and *turns*. Because secondary structures are local, many regions of different secondary structure can be present in the same protein molecule.
- **Tertiary structure**: the overall shape of a single protein molecule; the spatial relationship of the secondary structures to one another. Tertiary structure is generally stabilized by nonlocal interactions, most commonly the formation of a *hydrophobic core*, but also through *salt bridges*, *hydrogen bonds*, and even *posttranslational modifications*. The term "tertiary structure" is often used as synonymous with the term *fold*. The tertiary structure is what controls the basic function of the protein.
- **Quaternary structure**: the structure formed by several protein molecules (polypeptide chains), usually called *protein subunits* in this context, which function as a single *protein complex*.

Function

- Proteins are the chief actors within the cell, said to be carrying out the duties specified by the information encoded in genes
- The best-known role of proteins in the cell is as *enzymes*, which *catalyze* chemical reactions
- Many proteins are involved in the process of *cell signaling* and *signal transduction*
- *Antibodies* are protein components of an *adaptive immune system* whose main function is to bind *antigens*, or foreign substances in the body, and target them for destruction
- Structural proteins confer stiffness and rigidity to otherwise-fluid biological components
Protein measured in the laboratory

- Enzymes – ALT, AST, CK, LIPASE, AMYLASE, ETC
- Transport proteins – CAE, SHBG, TBG, ALB
- Signalling – Hormones
- Structural – collagen, elastin, keratin

And many more

Protein separation

- Properties of protein that aid separation
  - Molecular weight
  - Charge
  - Solubility
  - Affinity
- Methods for protein separation
  - Ultracentrifugation
  - Dialysis
  - Gel filtration
  - SDS PAGE

Protein separation

- Electrophoresis
  - Separation of proteins, nucleic acids, etc. by size, shape, charge
  - Proteins migrate based on their charge-to-mass ratio
  - Proteins visualized (radioactivity or staining)
  - Use gels made of crosslinked polymer (polyacrylamide) or solidified agarose
Protein separation

- Separated by mass to charge ratio
- Based on Electromotive Flow
- Detectors:
  - UV Detector – Beer’s Law
  - Laser Fluorescence – Deriv.
  - MS - electrospray
  - Chemiluminescence
  - Diode Array Detector
  - Indirect
  - Refractive Index
- Compare with HPLC and GC
- Neutral Compounds
- Chiral Compounds

Protein measurements

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<td>Turbidimeter</td>
<td>Measurement of light scattered at right angles to the direction of the incident light as a function of the concentration of the dispersed phase</td>
<td>Scattered light</td>
<td>Spectrophotometer</td>
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<tr>
<td>Nephlometer</td>
<td>Measurement of the intensity of scattered light at right angles to the direction of the incident light as a function of the concentration of the dispersed phase</td>
<td>Transmitted light</td>
<td>Spectrophotometer</td>
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Clinical uses:
- Ag-Ab rxn, immunocomplex rxn, ppts, liver dis, protein in urine or CSF

Specific proteins

- Albumin
  - Albumin is the main protein of human blood plasma. It binds water, cations (such as Ca$^{2+}$, Na$^+$ and K$^+$), fatty acids, hormones, bilirubin, thyroxine (T4) and pharmaceuticals (including barbiturates) - its main function is to regulate the colloidal osmotic pressure of blood.

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Specific Cardiac proteins

**Traditional**
- AST activity
- LDH activity
- LDH isoenzymes
- CK-Total
- CK-MB activity
- CK-isoenzymes

**RECENT**
- CK-MB (mass)
- c.Troponins (I or T)
- Myoglobin

**FUTURE:**
- Ischaemia Modified Albumin
- Glycogen Phosphorylase BB
- Fatty Acid binding Protein
- Highly sensitive CRP

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Immune response

- Immunoglobulins - IgG, IgM, IgA, IgD, IgE
- CRP
  - pentameric protein found in the blood plasma, the levels of which rise in response to inflammation
  - CRP is synthesized by the liver in response to factors released by macrophages and fat cells (adipocytes). It is a member of the pentraxin family of proteins. It is not related to C-peptide (insulin) or protein C (blood coagulation). C-reactive protein was the first pattern recognition receptor (PRR) to be identified.

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Acute phase proteins

- Complement regulatory proteins: e.g. C1 inhibitor, C4 binding protein, Factor H, Factor I, complement receptor CR1, decay accelerating factor.
- Acute phase proteins e.g. protease inhibitors, ceruloplasmin.
- PGE$_2$, TGF$eta$, Prostaglandins
- IL-10
- sIL-1R
Acute phase response

- A1AT
- CAE
- Transferrin
- C1 esterase inhibitor
- Antithrombin 3
- Haptoglobin
- orosomucoid

Tumour Markers

- AFP
- Calcitonin
- CA125
- CA15-3
- CA19-9
- CEA
- HCG
- Paraproteins
- PSA
- Thyroglobulin

Tumour markers

- Benign conditions giving rise to tumour markers
  - Acute cholangitis (CA19-9)
  - Acute hepatitis (CA125, CA15-3)
  - Cholangiocarcinoma (CA125, CA15-3, CA19-9)
  - Carcinoid syndrome (CEA)
  - Cholelithiasis (CA125, CA15-3)
  - Colorectal cancer (CA125, CA19-9)
  - Congestive heart failure (CA125)
  - Congenital heart failure (CA125)
  - Congestive heart failure (CA125)
  - Dermatological conditions (CA19-9)
  - Diarrhoea (CA125, CA15-3)

Tumour markers

- Benign conditions giving rise to tumour markers
  - Endometriosis (CA125)
  - Acne fulminans (CA125)
  - Irritable bowel syndrome (CA125, CA15-3, CA19-9, CA)
  - Inflammatory bowel disease (CA125)
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Urine proteins

- Causes of proteinuria

- Prerenal causes: Heavy exercise, Fever, hypertension, multiple myeloma, eclampsia
- Renal – acute & chronic glomerulonephritis, Renal tubular dysfunction, Polycystic kidney, nephrotic syndrome
- Post renal – acute & chronic cystitis, tuberculosis cystitis

Microalbuminuria (MALB)

- The level of albumin protein produced by microalbuminuria cannot be detected by urine dipstick methods. In a properly functioning body, albumin is not normally present in urine because it is retained in the bloodstream by the kidneys. Microalbuminuria is diagnosed either from a 24-hour urine collection

Microalbuminuria (MALB)

- An indicator of subclinical cardiovascular disease
- An important prognostic marker for kidney disease
- In diabetes mellitus
- In hypertension
- Increasing microalbuminuria during the first 48 hours after admission to an intensive care unit predicts elevated risk for acute respiratory failure, multiple organ failure, and overall mortality

Bence Jones proteins

- A Bence Jones protein is a monoclonal globulin protein or immunoglobulin light chain found in the urine, with a molecular weight of 22-24 kDa. Detection of Bence Jones protein may be suggestive of multiple myeloma or Waldenström’s macroglobulinemia.
- Bence Jones proteins are particularly diagnostic of multiple myeloma in the context of end-organ manifestations such as renal failure, lytic (or “punched out”) bone lesions, anemia, or large numbers of plasma cells in the bone marrow of patients
Myeloma detection

- Guidelines for detection of M-protein
  - Serum and urine should both be analysed
  - Done by agarose gel or capillary electrophoresis (CZE) that provide separation of beta-1 and beta-2 globulins
  - Immunofixation should be performed with anti-sera against immunoglobulin G, A, M and kappa and lambda light chains
  - IGE ad IGD when a light chain is present with no associated heavy chain
  - Examination of urine for BIP performed by Agarose gel
  - Urine immunofixation when a band is detected

- Guideline for M-protein quantification
  - Quantification should be made by densiometric measurement or CZE
  - Measurement of heavy chains are subject to variation only used when M-protein co-migrates with beta band
  - Repeat quantification with time is must be reproducible
  - The measurement of free light chains in urine and serum is available as a referral test.

Myeloma detection

- Guidelines state
  - Screening normal population for clinical purposes is not recommended
  - Serum and urine should always be requested where there is clinical suspicion of an underlying plasma cell dyscrasia/ B cell malignancy
  - Electrophoresis of serum and urine should requested in all patients with persistent elevated ESR above 30mm/h, anaemia, renal failure or hypercalcaemia with no other obvious explanation
  - The laboratory should perform electrophoresis when there are abnormally high or low immunoglobulins

- Stop electrophoresis of all immunoglobulin requests
- Perform electrophoresis on all abnormal immunoglobulin results
- Electrophoresis if clinical suspicion
- Stop electrophoresis on under 30 years age unless clinical suspicion
- Quantify M-protein even when band is on beta region
**CSF proteins**

- **Increased protein**: CSF protein may rise to 0.50 g/l in bacterial meningitis.

- A **more moderate increase** (0.15-0.20 g/l) occurs in inflammatory diseases of meninges (meningitis, encephalitis), intracranial tumors, subarachnoid hemorrhage, and cerebral infarction.

- A **more severe increase** occurs in the Guillain-Barré syndrome and acoustic and spinal schwannoma.

**Myeloma detection guidelines**

- **Increased IgG**: CSF protein is normal or mildly increased.
- Increased IgG in CSF, but not in serum (IgG/albumin index normally 10:1).
- 90% of MS patients have oligoclonal IgG bands in the CSF.
- Oligoclonal bands occur in the CSF only not in the serum.
- The CSF in MS often contains myelin fragments and myelin basic protein (MBP).
- MBP can be detected by radioimmunoasay. MBP is not specific for MS. It can appear in any condition causing brain necrosis, including infarcts.