* Pearson does not have back button
* Protein from dairy, non-dairy, meat, fish – 1/3 of diet consist of protein
* Protein performs all tasks in cells
* Enzymes are biological catalysts – very specific bio reaction – 10^16 times without enzymes
* Regulatory proteins – 14% - gate keeper – look after many proteins include enzymes – control which genes are turned on and off for transcription and activation
* Proteins generated in ribosome have to find their way outside of ribosome to perform their tasks – transport proteins carry proteins from one location to others
* Membrane transport proteins – facilitate transport ………….
* When supply falls short, storage protein comes into use
* Movement proteins – mobility eg. muscle cell
* Structural proteins – maintain cell wall, constitute skins etc. – very tough, cannot be digested
  + Collagen holds bones etc. together – when collagen hydrolyses, it becomes gelaton
* Signalling proteins – trigger actions for regulation to occur
  + Eg. scaffold or adapter proteins
  + Anchor proteins – hold them down – some proteins should not be moved
* Protective proteins – antibody, immunoglobins
* Defensive/exploitive – toxins and venoms
* Exotics – monellin – 2000 times sweeter than sugar
* pH and temperature affect protein stability – pH can cause proteins to aggregate or unfold (stop their functions)
* Assay – purify or quantify proteins
* Chemistry of side chains to maintain proteins and stabilise them
* Need to understand the protein we’re dealing with
* Presence of other enzymes, etc.
* A few degrees are enough to kill proteins
* Most times work in 0 degrees C
* Degradative enzymes – break cell wall and everything will spill out
* It’s because we are extracting proteins from cells – 0 degrees not 37 degrees
* Proteins are usually purified based on specific functions – solubility, ionic charge, mass, size, binding specificity
  + Will try to maintain optimum pH
  + Ionic charge – ion exchange chromatography is best
  + Affinity chromatography – not used in this unit
* Proteins are not fully soluble
  + Charge 0 is isoelectric point - pI
  + Solubility increases for small amount of addition of salts – salts make protective layers around proteins – salts charge so repel with proteins
  + Too much salt – super saturated – everything will settle down – salt prefers water so will remove water around proteins – proteins will find each other and stabilise
* Salting-out: Chuck in a lot of salt to get proteins out – ammonium sulphate is what we’ll use
* Electrophoresis depends on size and charge
  + Protein will be positively charged in acidic condition
  + Protons neutralise in alkaline/basic conditions – proteins will be negatively charged
  + Proteins are colourless
* PAGE uses gel filtration as well as movement – voltage will make charges moved – proteins at pI will not move – gel electrophoresis
* Ion exchange chromatography – the same as purifying water – hold back proteins that are oppositely charged
* Gel electrophoresis – about 3 hours
* Isoelectric focussing – separate charges – proteins move on gels bases on their pI value
* Isopycnic solution – vary from low to high density – spin the proteins – proteins will be settled based on their density – high density lower down
* Ultracentrifugation – tubes that can spin and then punctured – cannot reuse the tubes after punctured
* Chromatography – originally used to dye clothes – separate things based on colours – for amino acid mixture etc. – 4 to 5 hours to work – use porous substances – presence of proteins monitored by absportion spectrophotometry
* Column chromatography – add mixture to the top of the buffer – as mixture passes through – molecules fall down – molecules that don’t stick come down first
* Size exclusion chromatography – small gel beads that are porous – large molecules come out first – small ones get stuck in pores – can monitor appearance through chromatography
* Affinity chromatography – property of binding to another molecule – choose the molecule specifically – affinity matrix specifically chosen – His tag immobilises the proteins to the column – can later recover
* Purification – break the cells open – everything will spill out (proteins, cell membranes, etc) – separate soluble and insoluble – use affinity chromatography to remove the ones with His tag – apply ion chromatography – concentrate and purify proteins
* Column 5 of figure 5A – standards – compare mass of the proteins – is it right proteins or broken out into fragments – add the sequences to get the mass (eg. C, N, O)
* Dalton/Kilo Dalton is the mass
* Mass does not tell the function
* Whole genome sequencing
* Interested to find functions of proteins – use bioinformatics
* Compare human myoglobin to human alpha chain of haemoglobin – not 100% but quite similar – good match – probability of this match is very small – not by chance
* At least 25% similar – homologous
* If the side chains are similar – 37% like – can substitute each other
* Lots of gaps are not good – conserve functional parts but getting changed over time
* Best matches have very low scores
* Homology search can be done against multiple organisms to understand evolution
* Cytochrome c in animals and plants – very similar
* Help us understand mitochondrial respiration – organisms have highly similar sequences – highly conserved protein – highly conserved sequences
* If it’s absolutely conserved in the 27 sequences, there will be no change – these positions are very important – cannot mutate because will lose function
* Help us understand diseases such as cystic fibrosis (first to be found as genetic disease) – caused by one single change in amino acid – the whole codon is missing – because of that, protein does not function