* Insulin has 2 N-termini and 2 C-termini – bind by disulphide bond
* Oxidise bond to break them
* Protect the disulphide position – cysteine residue – so that they don’t form disulphide bond and stick up again
* Sequence of entire insulin has 3 bonds
* Two chemical methods by which we can detect how many protein chains
  + Dinitrofluorobenzene – DNFB – add DNFB to get polypeptide (shorten it)
  + The aromatic ring can be cleaved out later to get the amino acid
  + Dansyl chloride – cleaves first aa off but the rest of polypeptide also chopped up into polypeptides – don’t get long chains – get free polypeptides
  + Pay attention to these methods
* Carboxyl terminal can be determined by using carboxypeptides – cleave off last residue – leaving new shortened amino acids
* Haemoglobin – both N-terminal of alpha and beta chains are the same – we need to know both chains
* Use beta-mercaptoethanol to break disulphide bond and then iodoacetate to keep them from sticking
* Use performic acid to cleave the bond in one step – form sulphate to prevent bonding
* After having protein chains, need to cut them into small sequences to sequence them
* Two different methods to assemble them back in unambiguous way
* Trypsin and chymotrypsin cleave proteins at different points
* Met-specific – use reagent cyanogen bromide (highly toxic) – only recognise methionine – cut there
* Trypsin cuts after lysine or arginine – only 2 aa that are positively charged in neutral Ph – cut after C=O and create O=C-O-
* Cannot cut residue of proline
* The star will be tested
* R1 is the side chain before peptide (C-N) bond – R2 is after
* R2 forms the new N-terminal
* R1 after cut will be the new C-terminal
* Which enzyme cuts after which site

