# Proteins

B1.2 · SL and HL

# Why does cooling not uncook an egg?

## Changing a fold and cutting a chain are different processes

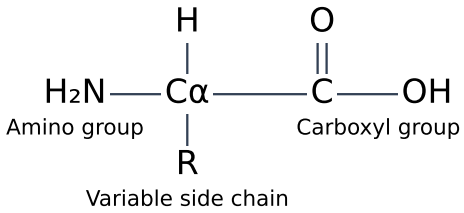
Heating egg white makes an opaque network that ordinary cooling does not reverse. Digestion can still break its proteins into smaller molecules.

Common content: amino acids, peptide formation, dietary requirements, sequence variety and denaturation.

AHL: side-chain chemistry and levels of protein structure.

Short letter sequences and numerical datasets are invented teaching examples.

# Read the four attachments to the alpha carbon




Neutral representation; charge states depend on pH.

> The R-group varies; amino and carboxyl groups belong to the shared framework.

# R is a placeholder, not a new element

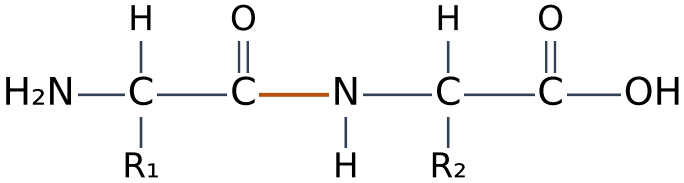
## Distinguish the shared backbone from the variable side chain

The alpha carbon attaches to an amino group, carboxyl group, H and R. Glycine has H as its side chain.

The neutral convention shows $-\mathrm{NH_2}$ and $-\mathrm{COOH}$. A common aqueous form has $-\mathrm{NH_3^+}$ and $-\mathrm{COO^-}$; charge depends on pH.

Draw the carbonyl C=O and OH attached to the same carboxyl carbon. Read bonds rather than copying a decorative shape.

# Locate the peptide C–N link and retain the carbonyl




Orange C–N link: peptide bond.
Two residues; one peptide bond; one water released in the model.

A **peptide bond** joins a carboxyl carbon to an amino nitrogen; each residue retains its R-group.

# Condensation and hydrolysis account for different atoms

## The product is a dipeptide plus water in the simple model

OH from a carboxyl group and H from an amino group account for one released water. The new peptide link is a covalent C–N bond.

Hydrolysis uses water to cut peptide bonds. Partial cleavage can produce shorter peptides rather than only free amino acids.

This is molecular accounting. Ribosomal synthesis uses tRNAs, activated amino acids and energy-transfer reactions; free amino acids do not simply assemble spontaneously.

# Apply residues minus chains before counting water

1. **One chain.** 35 residues give $35 - 1 = 34$ peptide bonds; 120 residues give 119. Complete peptide hydrolysis uses one water per link.
2. **Two chains.** 12 and 18 residues contain $11 + 17 = 28$ peptide bonds. One new end-to-end peptide link gives 29 in a 30-residue chain.
3. **Limit.** Association through other interactions leaves two chains and 28 peptide bonds. Side-chain cross-links are not peptide bonds.

# Essential means dietary supply is needed

## Non-essential does not mean unnecessary

Digestion supplies amino acids for synthesis of the body's own proteins. An intact food protein is not simply transferred into a matching body tissue.

Essential amino acids cannot be synthesized in sufficient amounts. Non-essential amino acids can be synthesized, but both types are needed.

Foods differ in amino-acid proportions. Total protein mass alone does not establish every essential amino acid's adequacy. Vegan diets need attention to supply and balance; plant proteins do contain essential amino acids.

# A food test detects groups, not a complete structure

## Biuret evidence has limits

Add Biuret reagent to the food sample; lilac or purple provides evidence of peptide bonds.

Compare a known protein solution and water as positive and negative controls under the same conditions.

A positive test does not identify the protein, its sequence or its three-dimensional structure.

# A limiting amino acid can restrict complete synthesis

1. **Invented model.** Each peptide needs 2 X residues and 3 Y residues. Supplies: 50 X and 60 Y. Other resources are abundant.
2. **Compare.** X permits $50/2 = 25$ copies; Y permits $60/3 = 20$. The maximum is 20 complete copies.
3. **Account.** X remaining $= 50 - 2(20) = 10$. Real synthesis also depends on energy, other amino acids, enzymes and regulation.

# The order of residues matters as well as the composition

## Primary structure is the sequence in a stated direction

The standard code specifies 20 common amino acids. Chains vary in length and order, producing an enormous range of possible sequences.

Invented A–B–C and A–C–B contain the same types but different orders. They are different primary structures.

Insulin is a hormone, collagen has a structural role, and many enzymes are proteins. Not every protein is an enzyme; some catalysts are RNA.

# Count choices at each sequence position

1. **Restricted alphabet.** Three permitted types, repetition allowed, three positions: $3^3 = 27$ possible sequences.
2. **Twenty types.** Four positions give $20^4 = 160000$ possibilities. A fixed length gives a finite count, not literally infinitely many chains.
3. **Interpret.** Having 20 common amino acids does not limit organisms to 20 proteins. Sequence and suitable folding conditions both matter.

# Denaturation can destroy function without cutting the backbone

## A functional conformation depends on conditions

Heating and pH changes can disrupt interactions that stabilize a fold. Denaturation alters the normal functional conformation, often reducing activity.

Ordinary denaturation need not hydrolyse peptide bonds. Digestion cuts those bonds. Severe chemical conditions can cause additional damage, so identify the process tested.

Some proteins can refold under suitable conditions; others aggregate or fail to recover. Heated egg white forms an altered network that ordinary cooling does not reverse.

# Compare activity after every sample returns to the same assay

| **Invented five-minute treatment** | **Activity when assayed at 25°C** |
|---|---|
| Kept at 25°C | 100 units |
| Heated to 45°C | 87 units |
| Heated to 65°C | 20 units |
| Kept cold at 5°C | 98 units |

# Separate treatment temperature from measurement temperature

1. **Calculate.** 65°C-treated sample retains $20/100 \times 100 = 20\%$ activity; loss $= 80\%$.
2. **Infer.** The assay is always at 25°C, so the difference is not merely an immediate temperature effect during measurement. It supports persistent functional damage.
3. **Limit.** Activity alone does not reveal which interaction changed. Use repeats and an unheated control; check evaporation, concentration and timing.

# Test recovery before calling a slow reaction denaturation

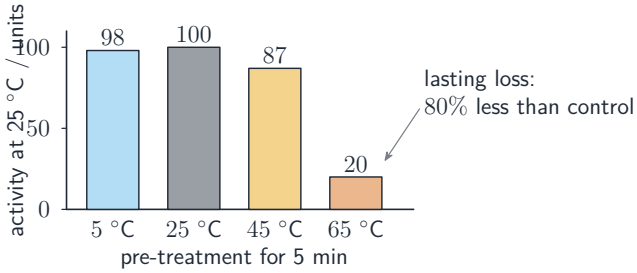
## Restore the same conditions and compare with a control

Low activity while an enzyme is cold can reflect reversible slowing. Test activity after the sample returns to the original conditions.

Control enzyme amount, treatment duration, assay temperature, pH, substrate concentration and measurement interval.

Compare repeated measurements rather than one reading. A functional assay supplies evidence of activity, not a complete molecular image.

# Is the damage permanent?




Cold slows an enzyme; high heat denatures it

# Different side chains support different interactions

| Side-chain property | Consequence for a protein |
|---|---|
| Hydrophobic | Largely non-polar; tends to avoid exposure to water |
| Polar, uncharged | Can interact with water and suitable polar groups |
| Acidic or basic | Charge changes through loss or binding of hydrogen ions |
| Cysteine side chains | Suitable pairs can form covalent disulfide cross-links |

# A substitution's effect depends on chemistry and position

## Sequence constrains a fold without making the molecule rigid

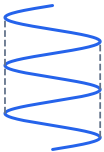
The precise order places side chains at particular positions. Interactions with one another, the backbone and the environment constrain conformation.

Functional proteins can have reproducible structures while moving or changing conformation. Folding may require cellular assistance and suitable conditions.

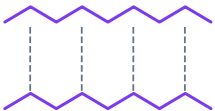
A substitution can alter packing, binding, charge or stability. Its effect may be large, small or undetectable in an assay; one change does not always destroy function.

# Distinguish local coiling from extended strands

Alpha helix



Beta sheet



Solid: backbone paths. Dashed: hydrogen bonds.
Schematic organization; backbone atoms omitted.

> Regular backbone hydrogen bonds stabilize alpha helices and beta sheets.

# Secondary hydrogen bonds are not the peptide links

## Locate the C=O and H–N backbone groups

An alpha helix is a coiled segment with a regular internal hydrogen-bond pattern. A beta sheet has hydrogen bonds between extended strands.

Sheet strands can come from different regions of one chain or from different chains. A sheet does not automatically establish the total chain count.

These regular bonds involve backbone groups, rather than simply R-group-to-R-group contacts. Peptide bonds remain the covalent links connecting residues.

# Overall folding of one chain uses several interactions

| Interaction | Contribution to tertiary structure |
|---|---|
| Hydrogen bonds | Attractions involving suitable polar groups |
| Ionic interactions | Attractions between oppositely charged groups |
| Hydrophobic interactions | Non-polar groups cluster away from water |
| Disulfide bonds | Covalent S–S cross-links between suitable cysteine side chains |

# Hydrophobic does not always mean facing the protein's centre

## Ask whether a surface faces water or lipid tails

A water-soluble globular protein commonly has a hydrophobic core and hydrophilic groups exposed to surrounding water.

A membrane-spanning surface can have hydrophobic side chains facing lipid tails. A water-filled pore can have hydrophilic groups facing its lumen.

A disulfide bond is covalent. It can link regions of one chain or different chains, but is not a branch made of peptide bonds.

# Four levels of protein structure



**primary (1°)**

order of
amino acids



**secondary (2°)**

$\alpha$-helix & $\beta$-sheet
(hydrogen bonds)



**tertiary (3°)**

folded into a
precise 3-D shape



**quaternary (4°)**

two or more
chains together

Quaternary: several chains together

# Count polypeptide chains separately from other components

## Conjugation and chain association answer different questions

Quaternary structure describes the association of two or more polypeptides. One folded chain can have tertiary structure without quaternary structure.

A conjugated protein includes a non-protein component such as a prosthetic group. A non-conjugated protein lacks such a component in this comparison.

Non-conjugated does not mean single-chain, unfolded or without disulfide cross-links. A prosthetic group is not an extra polypeptide.

# Insulin: a compact two-chain signal

## Globular form supports transport and specific receptor binding

Mature human insulin has a 21-residue A chain and a 30-residue B chain. Disulfide bonds stabilize the structure, including links between chains.

The mature hormone is non-conjugated in the syllabus comparison. Zinc-associated storage assemblies are a separate level of organization.

Its specific structure supports binding to appropriate receptors. The RCSB resource linked in the handout distinguishes the mature chains from the precursor.

# Do not apply residues minus one across separate insulin chains

1. **Residues.** A chain: 21. B chain: 30. Total $= 51$ residues, in two separate peptide backbones.
2. **Peptide bonds.** $(21 - 1) + (30 - 1) = 20 + 29 = 49$. Subtracting only one from 51 would incorrectly join the chains by another **peptide bond**.
3. **Distinguish.** Disulfide links between cysteine side chains are covalent cross-links, not extra peptide bonds.

# Collagen: three chains form a fibrous tensile structure

## An elongated triple helix suits a mechanical role

Three polypeptides wind together into a triple helix. Glycine at every third position in the triple-helical region permits close packing.

Associations and cross-links in fibrils support resistance to pulling forces in tendons, skin and other tissues. This is not an alpha helix renamed because three chains are present.

Collagen is the fibrous, non-conjugated comparison. Modification of a residue is not automatically a separate prosthetic group.

# Haemoglobin: four chains plus four haem groups

## Globular form and a non-protein component support oxygen transport

Main adult human haemoglobin has two alpha and two beta globin chains. Each carries an iron-containing haem group that can bind one oxygen molecule reversibly.

Four haem groups give a maximum of four oxygen molecules per haemoglobin. Haem groups do not make eight polypeptide chains.

Haemoglobin is conjugated and has quaternary structure. Subunit interactions help relate oxygen binding at one site to behaviour at others.

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# A ribbon diagram represents an inferred three-dimensional structure

## Technology reveals detail beyond the unaided eye

X-ray crystallography and cryogenic electron microscopy use measurements and reconstruction to study molecular organization.

A ribbon cartoon summarizes a structural model; it is not an ordinary light-microscope photograph.

Compare sequence, chain organization, shape and non-protein components separately. A two-dimensional page does not make the protein flat.

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# Try chain accounting, sequence counting and recovery reasoning

## Pause before the reveal

Two chains contain 12 and 18 residues. Count peptide bonds before and after one new end-to-end peptide link.

With three allowed amino-acid types and four positions, repetition allowed, how many sequences are possible?

A sample has 20 units of activity after treatment and return to the common assay, compared with a 100-unit control. Calculate retention and loss; state the inference limit.

# Reveal: count chains and compare controlled measurements

1. **Bonds.** Before: $11 + 17 = 28$. After one new peptide link: 29. Association without that link leaves 28.
2. **Sequences.** $3^4 = 81$. Order and repetition rules must be stated.
3. **Activity.** Retention 20%; loss 80%. This supports persistent functional damage but does not reveal the entire molecular mechanism.

# Check yourself

## Use bonds, sequence and controlled comparisons

Draw all four alpha-carbon attachments. Identify the carboxyl carbon and amino nitrogen in a peptide link; keep its neighbouring carbonyl oxygen.

State separate chain counts, compare amino-acid supply with demand, and distinguish sequence order from composition.

Explain denaturation as altered functional conformation. Separate pretreatment from assay conditions and test recovery before claiming irreversible loss.