Biochemistry

Unit 2 · Lesson 3 · Ramachandran plot and protein folding

The notes for Lesson 3 of Unit 2: chapters 2.5 to 2.6 of the unit book, with their figures, worked examples, exam traps and quizzes, in one file. The full unit book, with every chapter, the exam question bank and the glossary, is linked at the end.

Lesson 3 of 4 | 2 chapters | Chapters 2.5 to 2.6

About these notes

These are the notes for **Lesson 3 · Ramachandran plot and protein folding** of the University free n easy video lessons on Biochemistry Unit 2, Amino Acids and Proteins. They are chapters 2.5 to 2.6 of the unit book, unchanged, so a page here is the same page in the full book.

2.5 Ramachandran plot

Because the peptide bond is flat, each residue of a protein chain can turn only about two bonds, and the angles of those two bonds decide the whole backbone shape. The Ramachandran plot is a map of these two angles that shows which pairs are possible and which would make atoms crash into each other. It was worked out at the University of Madras by G. N. Ramachandran, C. Ramakrishnan and V. Sasisekharan, published in 1963, and it is still the first check made on any new protein structure.

You need: the planar peptide unit (Chapter 2.3) and the α-helix and β-sheet (Chapter 2.4).

The three backbone angles

A torsion (dihedral) angle measures the twist about a bond, from −180° to +180°. Each residue has three:

- **φ (phi)**: rotation about the N–Cα bond.
- **ψ (psi)**: rotation about the Cα–C bond.
- **ω (omega)**: rotation about the peptide C–N bond. It is fixed near 180° (trans) because the bond is partly double, so it is left out of the plot.

So the conformation of a backbone is fully described by one (φ, ψ) pair per residue.

How the plot was made

Ramachandran's group treated atoms as hard spheres and, for every (φ, ψ) pair, checked whether any two atoms of neighbouring peptide units came closer than their normal contact distance. Pairs with no clash are **fully allowed**; pairs that are allowed only if atoms come slightly closer are **partially allowed** (the outer limit); the rest are **disallowed**. Most of the map is disallowed: the backbone oxygen, the amide hydrogen and the side-chain Cβ get in each other's way.

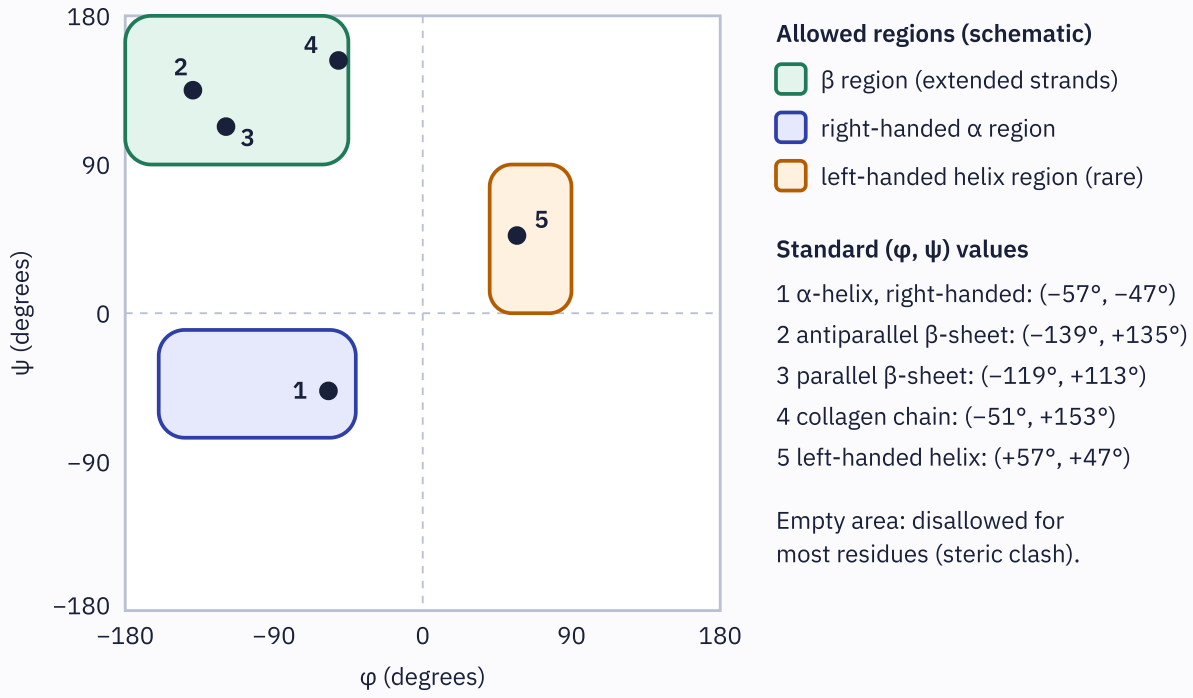


Figure 2.5.1 A schematic Ramachandran plot. The shaded boxes show roughly where the allowed regions lie for a residue with a side chain (real boundaries are curved); the dots are the standard angles of each secondary structure. Notice that both the α-helix and the β-sheet lie at negative φ, on the left half of the map.

Reading the plot

The top-left region holds extended chains: β-strands and the collagen helix. The lower-left region holds the right-handed α-helix. A small region at positive φ holds the left-handed helix, which is rare because the side chain clashes with the backbone there.

Glycine, with only H as its side chain, has a much larger allowed area, including large parts of the right half; this is why glycine is common in tight turns and where a chain must bend sharply. **Proline** is the opposite: its ring locks φ near −60°, so it fits only a narrow vertical band. These two are usually shown on separate plots.

When a new crystal or cryo-EM structure is deposited, programs plot every residue. In a good structure nearly all residues fall in the favoured regions; a residue in a disallowed area points to an error in the model, or to a strained residue that may matter for function.

WORKED EXAMPLE

Question (4 marks). Four residues of a non-glycine, non-proline protein have (φ, ψ) of A (−60°, −45°), B (−130°, +140°), C (+60°, +45°), D (0°, 0°). Assign each to a region and comment.

1. **A**: φ negative, ψ negative and close to (−57°, −47°): right-handed α-helix.
2. **B**: φ negative, ψ large and positive, near (−139°, +135°): β region, probably an antiparallel β-strand.
3. **C**: φ positive, near (+57°, +47°): left-handed helix region. Allowed but rare for a residue with a side chain; check whether it is really glycine, or whether the model is wrong.
4. **D**: (0°, 0°) puts backbone atoms of neighbouring peptide units on top of each other: disallowed. The model must be corrected.

EXAM TRAPS

- ω is not plotted because it is fixed near 180°; do not label the axes ω.
- φ is about N–Cα and ψ about Cα–C; students often swap them.
- The right-handed α-helix has negative φ and ψ. A positive-φ helix is left-handed.
- The examiner's favourite: "Why does glycine have a larger allowed region?" Because its side chain is only H, so there is less to clash.

CHECK YOURSELF

– REMEMBER **Who proposed the Ramachandran plot, and where?** 1 mark

G. N. Ramachandran with C. Ramakrishnan and V. Sasisekharan, University of Madras, 1963.

– REMEMBER **Give the approximate (φ, ψ) of a right-handed α-helix.** 1 mark

About (−57°, −47°).

– UNDERSTAND **Why are large parts of the plot disallowed?** 2 marks

At those angles, atoms of neighbouring peptide units or the Cβ come closer than their van der Waals contact distance, which is not physically possible (steric clash).

– APPLY **A residue has φ = −65°, ψ = +145°. Which region is it in?** 1 mark

The upper-left (β) region, close to the collagen and polyproline value; an extended conformation.

– ANALYZE **Explain why glycine and proline have very different Ramachandran plots, and how this links to their roles in proteins.** 4 marks

Glycine has no Cβ, so few clashes and a wide allowed area: it allows sharp turns and flexible loops. Proline's ring fixes φ near −60°, giving a narrow allowed band: it is rigid, breaks helices and is common in turns and in collagen.

BEYOND THE SYLLABUS

Structure-checking tools such as MolProbity use Ramachandran plots built from many high-quality structures, rather than the hard-sphere model, to flag outliers. Protein design and structure-prediction programs use the same angle preferences. Search "Ramachandran outliers" and "MolProbity". This is not for the exam.

2.6 Protein folding and denaturation

A new protein leaves the ribosome as a floppy chain, and within milliseconds to seconds it folds into one exact shape, its native structure. Folding is the process that gets it there; denaturation is the loss of that shape when heat, acid, urea or detergent breaks the weak bonds that hold it. Only the folded protein works, so these two ideas explain why fever is dangerous, why boiled eggs go solid, how paneer is made, and why some diseases come from proteins that fold wrongly.

You need: the bonds that hold proteins and the four levels of structure (Chapter 2.4).

Sequence decides shape: Anfinsen's experiment

Christian Anfinsen worked with ribonuclease A, a small enzyme of one chain with four disulfide bonds. He used it to show that a protein's sequence is enough to fold it.

1. He treated the active enzyme with 8 M urea (which breaks non-covalent bonds) and 2-mercaptoethanol (which reduces the disulfides to –SH). The chain unfolded and lost all

activity.

2. When he removed both reagents slowly by dialysis and let air re-oxidise the –SH groups, the enzyme regained nearly full activity and the correct disulfides re-formed.
3. When instead he let the –SH groups oxidise while urea was still present, the eight cysteines paired at random. Of the 105 possible pairings only one is correct, and this "scrambled" ribonuclease had about 1 percent of normal activity.
4. Adding a trace of 2-mercaptoethanol to the scrambled enzyme without urea let the disulfides swap partners, and activity returned as the chain found its native fold.

Conclusion, the **thermodynamic hypothesis**: the native structure is the most stable shape available to the sequence under normal conditions, so the information for folding is in the primary structure. Anfinsen shared the 1972 Nobel Prize in Chemistry for this work.

How folding happens

Driving forces. The main force is the **hydrophobic effect**: nonpolar side chains are buried, which frees the ordered water molecules that surrounded them and raises the entropy of the water. Hydrogen bonds, salt bridges and van der Waals contacts then fix the details. The folded state wins only narrowly; its free energy is lower than the unfolded state's by just a few tens of kJ/mol, small compared with the energy of one covalent bond. That is why a modest change in temperature or pH can unfold a protein.

Levinthal's paradox. If a chain tried every possible shape at random, folding would take longer than the age of the universe (see the worked example). Real proteins fold fast, so folding cannot be a random search. The current picture is a **folding funnel**: the energy landscape slopes toward the native state, so each small step that forms local structure (a helix, a hydrophobic cluster) lowers the energy and narrows the choices. A partly folded intermediate with most secondary structure but loose packing, the **molten globule**, is often seen on the way.

Helpers in the cell. Inside a crowded cell, hydrophobic patches of half-folded chains can stick to each other. **Molecular chaperones** prevent this. Hsp70 proteins bind exposed hydrophobic segments; **chaperonins** such as GroEL with its lid GroES in *E. coli* give a single chain a closed chamber to fold in, using ATP. Two enzymes speed slow steps: **protein disulfide isomerase** shuffles wrong disulfides, and **peptidyl-prolyl cis–trans isomerase** flips bonds before proline. Chaperones do not carry the shape information; they only stop mistakes.

Misfolding diseases. Some proteins misfold into long, insoluble fibres rich in β-sheet called **amyloid**. Amyloid-β plaques are found in Alzheimer's disease and α-synuclein deposits in Parkinson's disease. In prion diseases such as Creutzfeldt–Jakob disease, a misfolded prion protein converts the normal form into more of itself. In the commonest form of cystic fibrosis,

the CFTR protein lacks one phenylalanine, misfolds and is destroyed before it reaches the cell membrane.

Denaturation

Denaturation is the loss of the native secondary, tertiary and quaternary structure, and with it biological activity, without breaking peptide bonds. The primary structure stays intact; breaking it is hydrolysis, not denaturation.

AGENT	WHAT IT BREAKS
Heat	Hydrogen bonds and other weak interactions, by increasing molecular motion
Extreme pH (strong acid or alkali)	Changes side-chain charges; breaks salt bridges and adds charge repulsion
Urea (about 8 M), guanidinium chloride (about 6 M)	Hydrogen bonds; also make it easier for nonpolar groups to be in water
Detergents such as SDS	Bind hydrophobic parts and coat the chain with negative charge
Organic solvents (ethanol, acetone)	Hydrophobic interactions
Heavy metal ions (Hg^{2+}, Pb^{2+}, Ag^{+})	Bind –SH and carboxyl groups
Reducing agents (2-mercaptoethanol, DTT)	Disulfide bridges
Mechanical stress (whipping, shaking)	Unfold proteins at air–water surfaces, as in whipped egg white

Effects: loss of biological activity; lower solubility, often with precipitation or coagulation; higher viscosity; and easier digestion by proteases, which is one reason cooked food is easier to digest. Denaturation can be **reversible** (ribonuclease after urea is removed) or **irreversible**, when unfolded chains tangle and aggregate, as when an egg is boiled. In the kitchen, lemon juice added to hot milk lowers the pH toward the isoelectric point of casein, about 4.6; casein loses its charge and coagulates into paneer.

Heat denaturation of a small protein is usually **cooperative**: it goes from folded to unfolded over a narrow range of temperature, because the bonds support each other. The **melting temperature (Tm)** is the temperature at which half the molecules are unfolded.

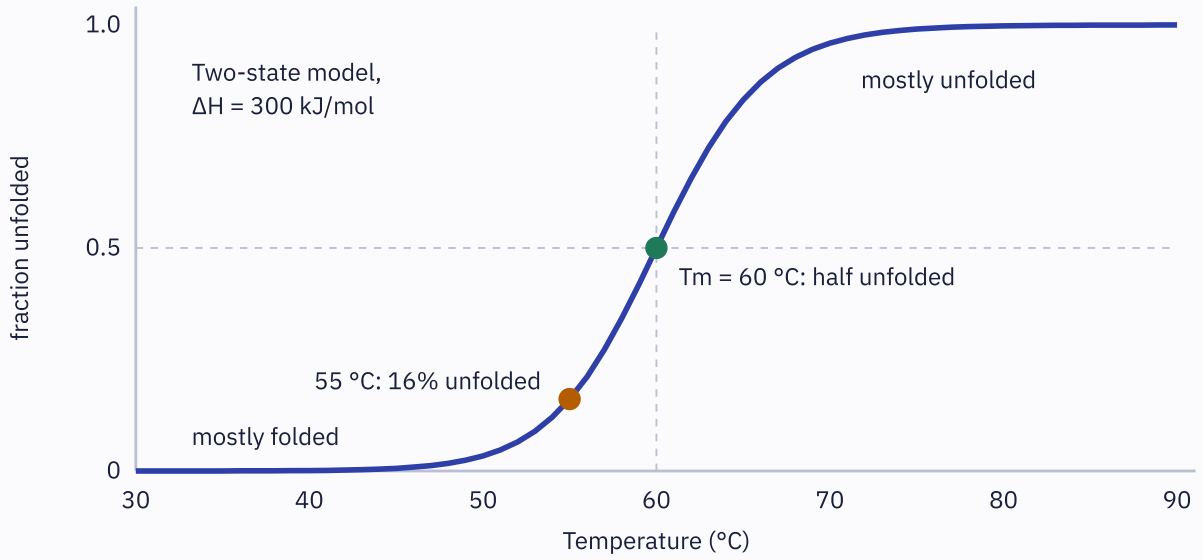


Figure 2.6.1 Computed heat-denaturation curve for a model protein that is either fully folded or fully unfolded (two-state), with Tm = 60 °C and unfolding enthalpy ΔH = 300 kJ/mol. Notice how sharp the change is: most of it happens within about 10 °C of Tm.

WORKED EXAMPLE

Question (6 marks). (a) A protein of 100 residues; assume each residue can take 3 backbone conformations and the chain tries a new shape every 10^{-13} s. How long would a random search of all shapes take? (b) For the protein in Figure 2.6.1, what fraction is unfolded at 55 °C? Use the two-state relation $K = \exp[-(\Delta H/R)(1/T - 1/Tm)]$ and fraction unfolded $= K/(1 + K)$, with $R = 8.314$ J mol^{-1} K^{-1}.

1. Number of shapes $= 3^{100} \approx 5.15 \times 10^{47}$.
2. Time $= 5.15 \times 10^{47} \times 10^{-13}$ s $= 5.15 \times 10^{34}$ s. One year $\approx 3.16 \times 10^{7}$ s, so time $\approx$ **1.6×10^{27} years**, far longer than the age of the universe (about 1.4×10^{10} years). Real folding takes milliseconds to seconds, so it cannot be a random search.
3. Convert temperatures to kelvin: T = 55 + 273.15 = 328.15 K; Tm = 60 + 273.15 = 333.15 K.
4. $1/T - 1/Tm = 0.0030474 - 0.0030017 = 4.57 \times 10^{-5}$ K^{-1}. $\Delta H/R = 300{,}000 / 8.314 = 36{,}084$ K.
5. Exponent $= -36{,}084 \times 4.57 \times 10^{-5} = -1.65$, so $K = e^{-1.65} = 0.192$.
6. Fraction unfolded $= 0.192 / 1.192 =$ **0.16**, so 16 percent is unfolded and 84 percent still folded, 5 °C below Tm.

EXAM TRAPS

- Denaturation does not break peptide bonds. If you write that the primary structure is destroyed, you lose the mark.
- Urea alone does not break disulfide bonds; a reducing agent is needed. Anfinsen used both.
- Chaperones help folding but do not decide the final shape; the sequence does.
- Use kelvin, not °C, in any thermodynamic formula.
- The examiner's favourite: "Describe Anfinsen's experiment and its conclusion." Give the reagents, the two refolding conditions, the 1 percent activity of scrambled enzyme and the thermodynamic hypothesis.

CHECK YOURSELF

– REMEMBER **Define denaturation.** 2 marks

Loss of a protein's native secondary, tertiary and quaternary structure, and so its activity, without breaking peptide bonds.

– REMEMBER **Name two molecular chaperones.** 1 mark

Hsp70 and the chaperonin GroEL–GroES (also acceptable: Hsp90, protein disulfide isomerase as a folding enzyme).

– UNDERSTAND **Why does a scrambled ribonuclease regain activity when a trace of 2-mercaptoethanol is added after urea is removed?** 3 marks

The trace of thiol lets wrong disulfides break and re-form with new partners. Without urea, the chain can fold, and the native pairing, which belongs to the most stable fold, accumulates.

– UNDERSTAND **What is the hydrophobic effect, and why does it drive folding?** 3 marks

Nonpolar groups in water force nearby water molecules into ordered cages. Burying these groups inside the protein releases that water, raising its entropy and lowering the free energy of the folded state.

– APPLY **Why does adding lemon juice to hot milk give paneer?** 2 marks

The acid lowers the pH to near casein's isoelectric point (about 4.6), where it has no net charge and so stops repelling itself; heat helps the chains unfold and coagulate into curds.

– APPLY **Using the model of Figure 2.6.1, what fraction is unfolded at 65 °C?** 3 marks

1/338.15 − 1/333.15 = −4.44 × 10^{-5} K^{-1}; exponent = +1.60; K ≈ 4.96; fraction unfolded = 4.96/5.96 ≈ 0.83.

– ANALYZE **Compare reversible and irreversible denaturation with an example of each, and explain what decides which occurs.** 4 marks

Reversible: ribonuclease in urea refolds when urea is removed. Irreversible: egg albumin on boiling. If unfolded chains stay separate, they can refold; if exposed hydrophobic groups make chains stick together (aggregation), or covalent changes occur, they cannot return to the native state.

BEYOND THE SYLLABUS

Biopharma companies, including Indian biosimilar makers, spend great effort keeping therapeutic proteins such as insulin and monoclonal antibodies folded during production, storage and transport, since aggregates lower activity and can cause immune reactions. Recombinant proteins made in *E. coli* often form insoluble inclusion bodies that must be solubilised in urea and refolded, the Anfinsen experiment at industrial scale. Search "inclusion body refolding" and "differential scanning fluorimetry". This is not for the exam.

Key terms of this lesson

TERM	MEANING OR FORMULA	CHAPTER
Ramachandran angles	φ about N–Cα, ψ about Cα–C; α-helix about (−57°, −47°), β about (−139°, +135°)	2.5
Two-state unfolding	K = exp[−(ΔH/R)(1/T − 1/Tm)]; fraction unfolded = K/(1 + K)	2.6

Glossary (terms used in this lesson)

Amyloid Insoluble, β-sheet-rich fibres formed by misfolded proteins.

Chaperone A protein that prevents wrong folding and aggregation of other proteins.

Denaturation	Loss of native shape and activity without breaking peptide bonds.
Dialysis	Removal of small molecules through a semipermeable membrane that keeps proteins.
Disulfide bridge	A covalent –S–S– bond between two cysteine side chains.
Hydrophobic effect	The tendency of nonpolar groups to cluster away from water, which drives folding.
Levinthal's paradox	A random search of all shapes would take far too long, so folding must follow guided paths.
Molten globule	A partly folded state with secondary structure but loose packing.
Peptide bond	The amide bond between the α-carboxyl of one amino acid and the α-amino group of the next.
φ and ψ	Torsion angles about the N–Cα and Cα–C bonds of each residue.
Residue	An amino acid unit within a peptide chain.

The full unit book

Every chapter of Unit 2, the unit summary, the exam question bank with model answers and the full glossary: Biochemistry · Unit 2 · Amino Acids and Proteins (one file, works offline).

Sources and further reading

Textbooks

- David L. Nelson and Michael M. Cox, *Lehninger Principles of Biochemistry*, W. H. Freeman; chapters on amino acids, peptides and proteins, and on protein structure (from memory; check). Source of the pKa values used here.
- Jeremy M. Berg, John L. Tymoczko, Gregory J. Gatto Jr. and Lubert Stryer, *Biochemistry*, W. H. Freeman; chapters on protein composition and structure and on exploring proteins (from memory; check).
- Donald Voet and Judith G. Voet, *Biochemistry*, Wiley (from memory; check).
- U. Satyanarayana and U. Chakrapani, *Biochemistry*, Elsevier India; widely prescribed in Indian universities for the classification of proteins and colour reactions (from memory; check).

Open resources opened for this book

- G. N. Ramachandran, C. Ramakrishnan and V. Sasisekharan, "Stereochemistry of polypeptide chain configurations", *Journal of Molecular Biology* 7 (1963) 95–99, record at the Indian Academy of Sciences repository: repository.ias.ac.in/49564.
- C. B. Anfinsen, "Principles that govern the folding of protein chains", *Science* 181 (1973) 223–229 (the Nobel lecture): PDF copy. Source of the ribonuclease details in Chapter 2.6.
- Nobel Prize page for Anfinsen's lecture, 1972: nobelprize.org.

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