Lecture 7 & 8: PROTEIN ARCHITECTURE IV: Tertiary and Quaternary Structure
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Tertiary Structure

- Tertiary structure describes how the secondary structure units associate within a single polypeptide chain to give a three-dimensional structure

How to tell a left- vs. right-handed α-helix

Point your thumb up in the direction of the α-helix (N→C).

Look where the “base” of the helix spirals are. If they match the knuckles on your right hand it is a right-handed helix.

----------or----------

If the helix spirals up in a counterclockwise direction, it is a right-handed helix.

Tertiary Structure: Basic Tenets - the “truths”

1). All information for folding is contained in the primary sequence.
2). Secondary structure formation is spontaneous - a consequence of the formation of hydrogen bonds.
3). No protein is stable as a single layer - hence secondary structural elements pack together in sheets.
4). Connections between structural elements are short - minimization of degrees of freedom - keeps structures compact.

Consequences

1). Secondary structures are arranged in a few common patterns - i.e., resulting in protein “families”.
2). Proteins fold to form the most stable structure. Stability arises from:
- formation of large number of intramolecular hydrogen bonds
- reduction in hydrophobic surface area from solvent
Note that some parts of a protein structure are not regular (i.e., helical-like or sheet-like). These are often referred to as disordered or random coil regions. However, a better nomenclature is “natively random.”

Fibrous proteins:
- Filamentous; play a major structural role in cells & tissues
- Compact structures; different folds for different functions

Globular proteins:
- compact structures; different folds for different functions

Membrane Proteins:
- found associated with various membrane systems

Fibrous Proteins
- Share properties that give strength &/or flexibility to the structures in which they occur;
- Fundamental unit is a simple repeating element of secondary structure;
- Insoluble in water; large percentage of hydrophobic amino acids;
- Usually the hydrophobic surfaces are hidden in the elaborate supramolecular complexes;
- mechanically strong; perform important structural functions
- Strength is enhanced by cross-links (disulfide bonds).

Secondary Structures and Properties of Fibrous Proteins

<table>
<thead>
<tr>
<th>Structure</th>
<th>Characteristics</th>
<th>Examples of occurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Helix, Cross-linked by disulfide bonds</td>
<td>Tough, insoluble protective structures of varying hardness and flexibility</td>
<td>α – Keratin of hair, feathers and nails</td>
</tr>
<tr>
<td>β-Conformation</td>
<td>Soft, flexible filaments</td>
<td>Silk fibroin</td>
</tr>
<tr>
<td>Collagen triple helix</td>
<td>High tensile strength, without stretch</td>
<td>Collagen of tendons, bone matrix</td>
</tr>
</tbody>
</table>
**FIBROUS PROTEINS: α-Keratin**

*What:* Part of the “intermediate filament proteins” which have major structural roles in nuclei, cytoplasm and cell surfaces

*Where:* Found in hair, fingernails, claws, horns, animal skin

*Composition:* Long stretches of α-helices (> 300 residues)

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**Coiled-Coils**

- Interactions are stabilized by hydrophobic interactions between the α-helices;
- Heptad repeat (α-b-c-d-e-f-g)<sub>n</sub> where a & d are nonpolar & lie in the center of the coiled coil.
- Evolved for strength; helical nature confers flexibility
- Coiled-coil is a “super twist” left-handed helix
- Distortion of helix to 3.5 residues/turn
- Hydrophobic faces interacting in a close interlocking pattern

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**FIBROUS PROTEINS: β-Keratin**

*What:* Part of the “fibroin proteins”

*Where:* Silk, bird feathers

*Composition:* stacked anti-parallel β-sheets; strength

*Sequence:* Alternating Gly-Ala/Ser

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Contact side chains (red balls) interlock
COLLAGEN

What: Greek for glue; defined as “that constituent of connective tissue which yields gelatin on boiling”

Where: Principal component of mammalian tissue; constitutes ~25% of a mammal’s protein content; more than 30 varieties

Composition: Triple helix
Sequence: Gly-X-Y; X usually Pro, Y usually Pro/HyPro

> 3000Å long; 15Å in diameter

Collagen Primary Structure

• Approx 1000 AA/chain
• Repeats of Gly-X-Y where X is often Pro and Y is often hydroxyproline or proline

Composition

G ~ 35%
A ~ 11%
P/HP ~ 30%

MODIFIED AMINO ACIDS
post-translational modifications add functionality to amino acid

Hyp: stabilizes tropocollagen via intrachain H-bonds
Hyl: stabilizes fibrils via its ability to cross-link; attachment of CHO groups

Consequences of Collagen Primary Structure

Distortion of backbone due to high content of glycines and prolines
Can’t form “normal” secondary structures
Forms triple helix
Every third residue faces inside
Interior is compact; hence interior residue is glycine
**Consequences of Collagen Primary Structure**

Fit occurs because Gly\textsubscript{strand1} lies adjacent to X\textsubscript{strand2} and Y\textsubscript{strand3}

Stabilization from hydrogen bonds

\[ \text{Gly}_{\text{strand1}}\text{N-H to } X_{\text{strand2}}\text{C}=O \text{ hydrogen bond} \]

Hydroxyproline forms hydrogen bonds

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**Globular Protein**

- Folding of 2° structural elements;
- Side chain location varies with polarity:
  - Non-polar are inside
    - (A, V, L, I, M & F);
  - Charged on the surface
    - (D, E, K, R, H);
  - Uncharged polar mostly surface, but interior as well
    - (S, T, N, Q, Y & W);
  - Nearly all H-bond donors have an H-bond acceptor;
  - Interior of a structure is TIGHTLY packed;
- 3° structures are frequently built of domains.

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**Core Proteins: α-Helices and β-Sheets**

**I: antiparallel α-helix**

- Packs in bundles; Left-handed twist
- Usually regular, uniform; Usually 4-helix bundles
- Globin proteins

**II: Parallel or mixed β-sheet proteins**

- Parallel β-sheets have hydrophobes on both sides of sheet ---> these must be core structures

**III. Antiparallel β-sheet structures**

- Antiparallel β-sheets have hydrophobes on one side of sheet and polar residues on the other side. These β-sheet structures can be surface exposed.

**IV: Metal & Disulfide rich proteins**

- Small, < 100 a.a. Structure dependent upon either the metal or disulfide
GLOBULAR PROTEINS AND α-HELICES: return of the helical wheel!

SURFACE HELIX: AMPHIPATHIC

INTERIOR HELIX: HYDROPHOBIC

GLOBULAR PROTEINS AND α-HELICES: return of the helical wheel!

β-sheets: an example of IFABP

SOLVENT-EXPOSED HELIX: POLAR/CHARGED
POLYPEPTIDE CHAINS HAVE A RIGHT-HANDED TWIST

SINGLE “LAYER” STRUCTURES ARE NOT STABLE

GLOBULAR PROTEIN STRUCTURE: other considerations

1). Proteins are tightly packed

2). Proteins have natively random structure

3). Proteins have flexible segments

Proteins Are Dynamic: X-ray vs NMR Structures

A chain of insulin

Static structure

Solution structure “family” of structures
MOTIONS COVER A RANGE OF TIME: seconds to femtoseconds

MULTIMERIC PROTEINS

- Quaternary structure describes how two or more polypeptide chains associate to form a native protein structure (but some proteins consist of a single chain).

Closed Quaternary Structures
The association stoichiometry is finite

Hb tetramer: 2 "alpha chains" and 2 "beta chains"

Isologous vs. Heterologous Associations
How the packing interfaces of the monomers come together
OPEN QUATERNARY STRUCTURE

**tubulin**

**HIV-1 particle**

300 nm x 18 nm
2134 copies of coat protein!

STABILIZATION OF QUATERNARY STRUCTURE

Quaternary Structure are Stable!

\[ K_d = 10^{-8} \text{ to } 10^{-16} \text{ M} \]

or

50 to 100 kJ/mol in stability!

alcohol dehydrogenase

Chemical interactions offset loss of entropy

OPEN QUATERNARY STRUCTURE

**Coat protein**

40 kD

**RNA genome in red** surrounded by helical array of subunits

Tobacco mosaic virus

WHY HAVE QUATERNARY STRUCTURE:
ADVANTAGES OF HAVING ASSOCIATING SUBUNITS

- Subunit association can shield hydrophobic groups from water
- Cooperativity: Binding of a substrate to one subunit can influence the binding of a second substrate to another subunit.
- Genetic Economy and Efficiency: less DNA is used to code for a protein
- Bringing catalytic sites together: important for proteins with multiple catalytic functions - more efficient in terms of "handing off" substrates from one site to another for subsequent chemistry.
1). Tertiary structure describes the three-dimensional structure of a polypeptide chain.
2). The 3 major classes of 3rd structure are fibrous proteins, globular proteins, and membrane proteins.
3). Fibrous proteins are hydrophobic proteins that give strength and flexibility.
4). Coiled-coils are stabilized by hydrophobic interactions.
5). Globular proteins constitute the majority of proteins, consist of α-helices and β-sheet and have a hydrophobic core.
6). Polypeptide chains have a right-handed twist.
7). Globular proteins have layers.
8). Globular proteins are densely packed.
9). Globular proteins can have flexible regions.
10). Proteins display motions (returning to the idea that life is dynamic!)
11). Quaternary structures describe the association between polypeptide chains.
12). Quaternary associations can be "open" or "closed"
13). Quaternary structures are stable (an interplay between entropy and chemical interactions) and confer certain advantages to an organism.