Protein design

Methods to characterize the secondary structure of proteins:
- NMR
- X-ray crystallography
- Circular Dichroism (CD)

Circular Dichroism
- Proteins absorb circularly polarized light unequally as a result of AA chirality
- Alpha helices display a double minima on a molar ellipticity plot at 208nm and 222nm

Fraction alpha helical
\[
f = \frac{-\theta_{obs,222}}{\theta_{max,222}}
\]

Chou-Fasman Method-
- Empirical technique for prediction of secondary structure in proteins
- The method relies on examining the relative abundancies of specific amino acids within secondary structures of naturally occurring proteins
- Results of these studies indicate that alpha helices are preferrentially occupied by Leucine and Alanine

Alpha helix rational design by Baldwin-
- Attempt at ab initio alpha helix design based on salt bridge stabilization

\[
\text{Coiled form equilibrium}
\]

\[
C \rightleftharpoons H_1 \rightleftharpoons H_2 \rightleftharpoons H_n
\]

\[
\sigma \sim 10^{-2} - 10^{-3} \quad \text{(nucleation parameter)}
\]

\[
s = \frac{k_f}{k_b} \sim 1 - 2
\]
Methods to stabilize helical structures by chemical modification of i, i+4 interactions (peptide stapling)

**Amide formation**

**Disulfide linkage formation with extended Cysteine D,L pairs**

**Metal coordination with histidines**

**Intermolecular interactions to stabilize helices**

- A peptide with i, i+1 alternating hydrophobic and hydrophilic residues should form a beta sheet-like structure when exposed to a hydrophobic surface or hydrophilic surface (e.g., air-water interface).

- A peptide with alternating periodicity of hydrophobic and hydrophilic residues in the i, i+4 configuration (as below) should form an amphipathic helix which has its hydrophobic side bound to a hydrophobic surface.

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Example of helix stabilized by a hydrophobic surface-

Melittin-
-protein found in bee venom
-helicity of peptide increases upon binding to plasma membranes
-surface-bound molecules induce lysis of erythrocytes


\[ \underline{i, i+4 \text{ hydrophobic interactions resemble amphiphilic helix}} \]

Design of synthetic amphiphilic bee venom
-synthetic venom is 2.5x more active at cell lysis
Coiled coils - Stabilizing a helix with another helix

- Coiled coils are frequently found in natural proteins (e.g. myosin, tropomyosin, GCN4, GP41, etc.)
- Left-handed super helix composed of repeating right-handed alpha helices composed of heptad repeats

Interhelical angle 10°

General configuration of alpha helix heptad repeats

A & D = hydrophobic side chains (Met, Ile, Leu)
B, C & F = hydrophilic side chains (Ser, Gln, etc.)
E & G = salt bridge forming side chains (Lys, Glu, Asp)

-salt bridges are only slightly stabilizing but frequently lead to selective dimer orientation
-therefore the +, - configuration is highly preferred over the +, + configuration

Talbot and hodges - Design of synthetic coiled coils

Heptad repeat (HR) : Leu, Glu, Ala, Leu, Glu, Gly, Lys

(HR)_{n=1,2} - no helical structure
(HR)_{n=4,5} - dimers formed
(HR)_{n=5} - more stable than tropomyosin

-more stable at pH 2.5 than at pH 7
-increasing ionic strength increases stability

How to determine if helix is parallel or anti-parallel

-insert a cysteine in first heptad repeat to form an interhelical disulfide

HR = KLEALEG
HR' = KCAELEG
peptide seq = HR'(HR)_{5}

-crosslinking indicates helices are parallel

-In general, coiled coils are found in the parallel conformation
**4 helix bundles**

- 4 helices connected by loops
- composed of repeating helix-loop-helix motif

\[ \alpha_1-L-\alpha_2-L-\alpha_3-L-\alpha_4 \]

- frequently found in nature (e.g. hGH, GCSF)
- helices diverge due to larger interhelical angle

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**DeGrado - Design of 4 helix bundles**

- A,D = hydrophobic side chains
- All else hydrophilic side chains

**Helix**

- GELEELLKLLG

Glycine used to break helix

**Loop**

- Pro, Arg, Arg

Loops composed of Pro and Arg to discourage interpeptide loop-loop interactions. Proline introduces kinks after each helix, and Arginine's charge discourages loop-loop interactions.

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**Bundle assembly from \( \alpha_1 \beta \)**

\[ \Delta G = -22 \text{ kcal/mol} \]

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**Bundle assembly from \( \alpha_2 \)**

\[ \Delta G = -13 \text{ kcal/mol} \]

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**Bundle assembly from \( \alpha_4 \)**

\[ \Delta G = -15.4 \text{ kcal/mol} \]
DeGrado - Design of 4 helix bundles continued

-Applying NMR, HD exchange, and ANS binding to the above designed 4 helix bundle, it was found that the core of the protein was a molten globule (not well packed, resembling oil)

Attempts to stabilize a folded state would require that the core be better packed.

One such attempt uses Histidine residues coordinating a zinc cation to lock the conformation of the protein.

Hydrogen-Deuterium exchange-

- Measurable rate of exchange of hydrogen for deuterium in protein backbone due to relative acidity of amide NH
- Amides exposed to solvent exchange faster than those buried in protein core

\[
\text{NH} \quad \rightleftharpoons \quad \text{ND}
\]

ANS Binding

- Anilino-naphthalene-8-sulfonic acid, ANS
- Fluorescent hydrophobic compound binds to hydrophobic surfaces of proteins

NMR-

- Rapid exchange leads to low dispersity in NMR (peak averaging)

- Additional methods to assess secondary structure

A, D = Leu
E = Lys
B, F = Lys, Glu
C, G = His

\[
\text{no Zn}^{2+} \quad \Delta G = -2.5 \text{ kcal/mol}
+ \text{Zn}^{2+} \quad \Delta G = -10.3 \text{ kcal/mol}
\]

- NMR is highly dispersed
- Slow HD exchange
- Still binds ANS