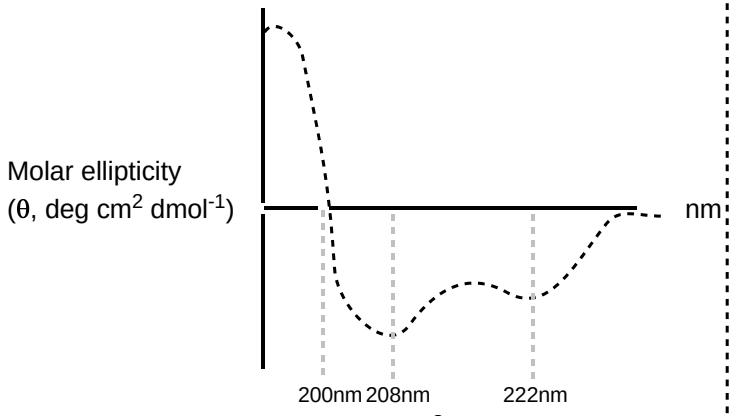
Protein design

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 $f = \frac{-\theta_{obs222}}{\theta_{max222}}$

$\theta_{max} = \frac{n-4}{n}\theta_{\infty}$ $\theta_{\infty} = -40{,}000$ deg cm^2 dmol^{-1}

Coiled form equilibrium

$$C \underset{k_b}{\overset{\sigma k_f}{\rightleftharpoons}} H_1 \underset{k_b}{\overset{k_f}{\rightleftharpoons}} H_2 \underset{k_b}{\overset{k_f}{\rightleftharpoons}} H_n$$

$\sigma \sim 10^{-2}$ -10^{-3} (nucleation parameter)

$s = k_f / k_b = \sim 1\text{-}2$

Designing alpha helices-

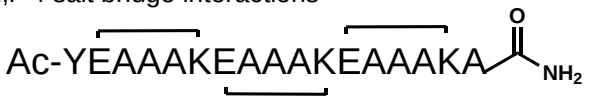
Chou-Fasman Method-

-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 occuring proteins
-results of these studies indicate that alpha helices are preferrentially occupied by Leucine and Alanine

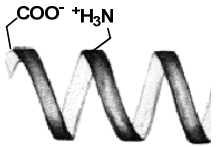
Alpha helix rational design by Baldwin-

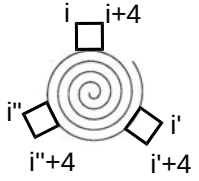
-attempt at *ab initio* alpha helix design based on salt bridge stabilization

i,i+4 salt bridge interactions



-N terminal acetylation to avoid N terminal dipole point charge interaction
-Tyrosine used for UV absorption
-Acetamide to remove carboxylate interaction with helix dipole



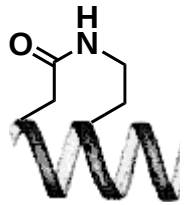


-in principle the i,i+4 salt bridges should increase helix stability
-salt bridge interactions should line all sides of the helix thereby decreasing aggregation potential (right) and increasing overall stability

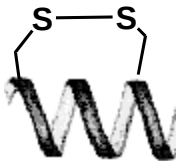
-designed sequence was 75% helical at pH 7
-in principle, lowering pH should destabilize helix by protonating carboxylate groups to discourage salt bridge formation
-however, the helix was highly helical at pH 2
-in general, salt bridges do not have large effects on helix stability but do frequently have important roles in assuring the correct helical orientation
-in this case, stability was largely derived from helical propensity of alanine

Methods to stabilize helical structures by chemical modification of i, i+4 interactions (peptide stapling)

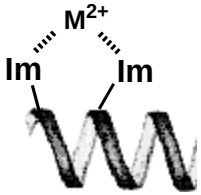
Amide formation-



Disulfide linkage formation with extended Cysteine D,L pairs



Metal coordination with histidines-

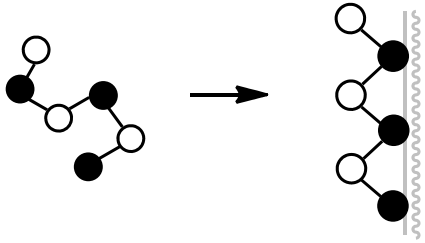


Intermolecular interactions to stabilize helices

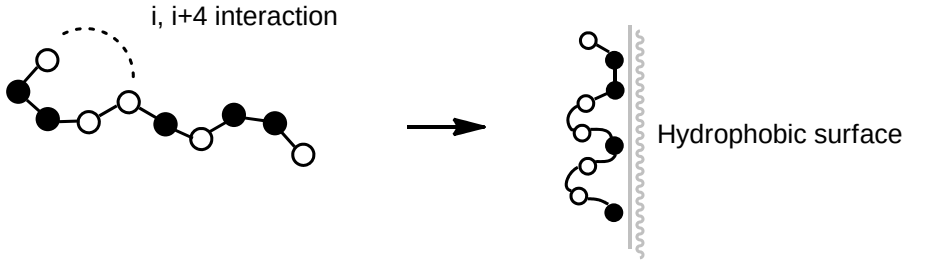
= hydrophobic side chain

=hydrophilic side chain

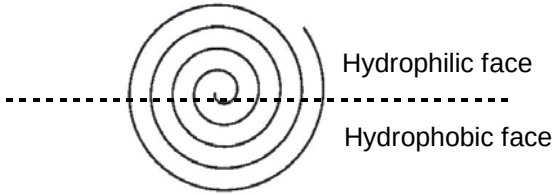
-a peptide with i , i+1 alternating hydrophobic and hydrophilic residues should form a beta sheet like structure when exposed to a hydrophobic surface or hydrophilic surface (e.g. air-water interface).



-a peptide with alternating periodicity of hydrobic 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



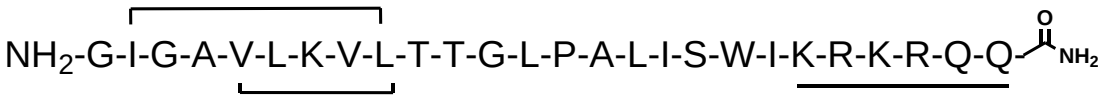
Amphiphilic helix-



Example of helix stabilized by a hydrophobic surface-

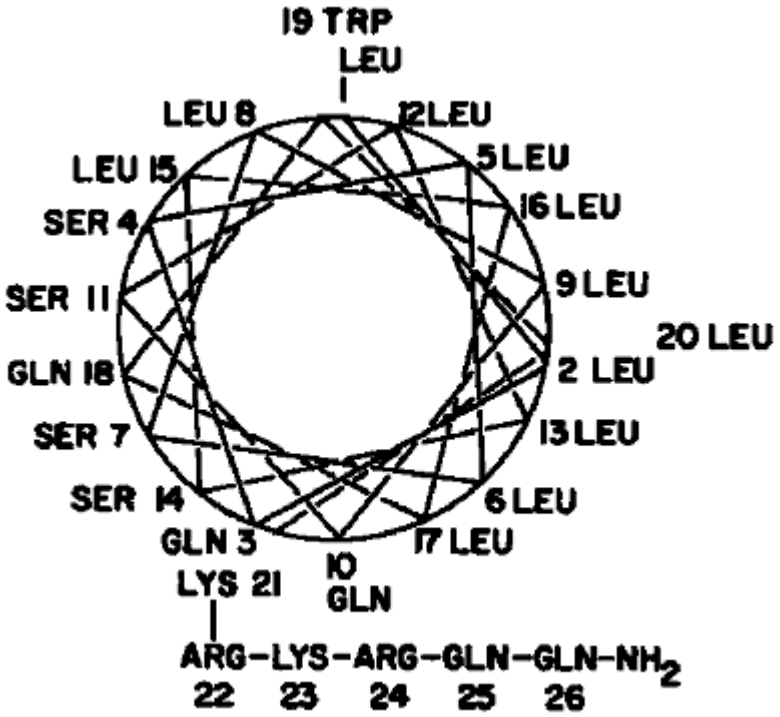
Melittin-
-protein found in bee venom
-helicity of peptide increases upon binding to plasma membranes
-surface-bound molecules induce lysis of erythrocytes

i, i+4 hydrophobic interactions resemble ampiphilic helix



-highly cation rich sequence leads to membrane lysis

Design of synthetic amphiphilic bee venom
-synthetic venom is 2.5x more active at cell lysis

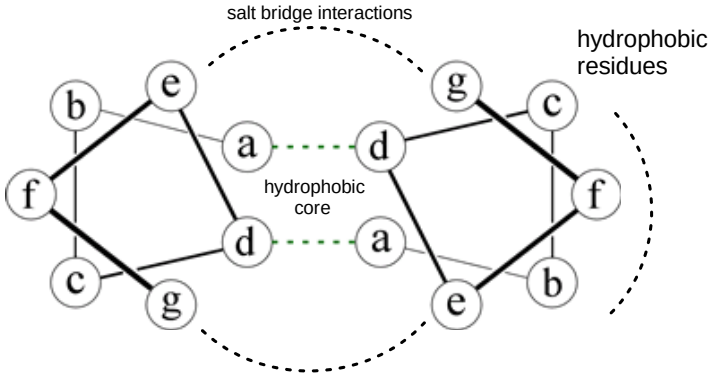


Coiled coils - Stabilizing a helix with another helix

- 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

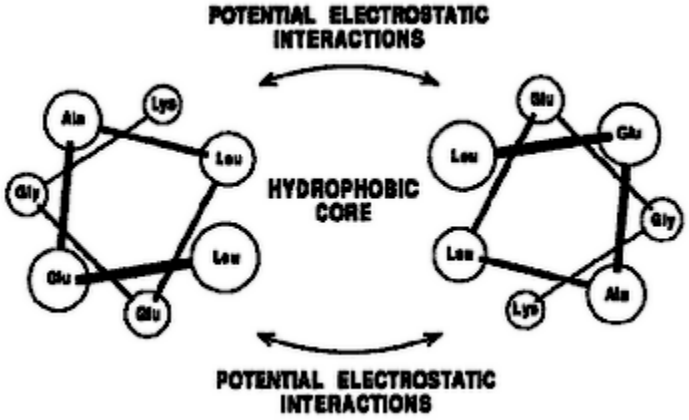


A & D = hydrophobic side chains (Met,Ile, Leu)
B,C & F = hydrophilic side chains (Ser, Gln, etc.)
E & G = salt bridge forming side chains (Lys, Glu, Asp)

-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



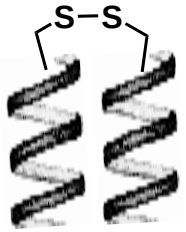
$(HR)_{n=1,2}$ - no helical structure
$(HR)_{n=4,5}$ - dimers formed
$(HR)_{n=5}$ - more stable than tropomyosin

-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

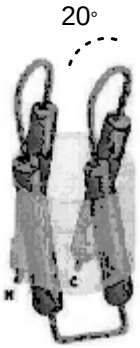
-In general, coiled coils are found in the parallel conformation

4 helix bundles

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

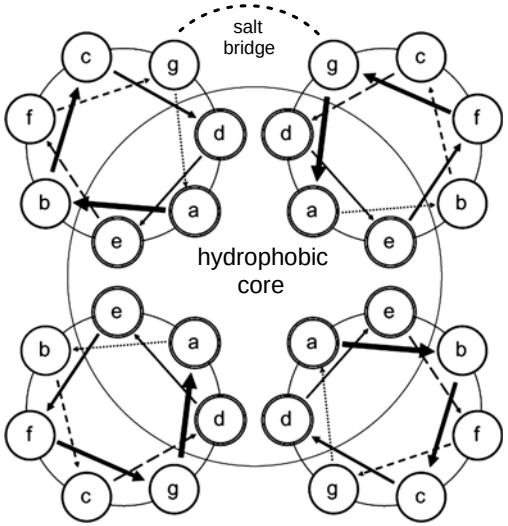
α_1-L-α_2-L-α_3-L-α_4

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



restricted helical segment

DeGrado - Design of 4 helix bundles



A,D = hydrophobic side chains

All else hydrophilic side chains

Helix - GELEELLKKLKGLLKG

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.

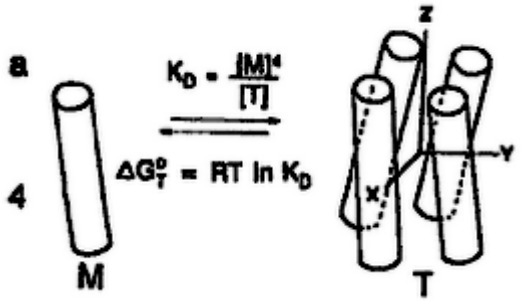
$\alpha_1\beta$ - Ac-helix-$C(=O)NH_2$

α_2 - Ac-helix-loop-helix-$C(=O)NH_2$

α_4 - Ac-helix-loop-helix-loop-helix-loop-helix-$C(=O)NH_2$

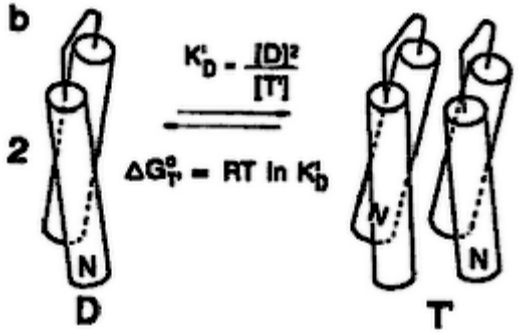
bundle assembly from $\alpha_1\beta$

ΔG = -22 kcal/mol



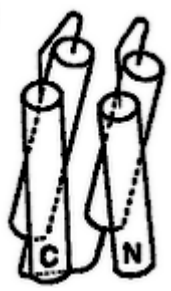
bundle assembly from α_2

ΔG= -13 kcal/mol



bundle assembly from α_4

ΔG= -15.4 kcal/mol

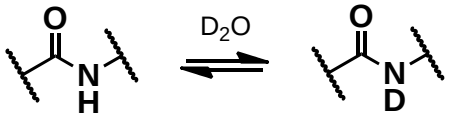


Additional methods to assess secondary structure-

Hydrogen-Deuterium exchange-

-measurable rate of exchange of hydrogen for deuterium in protein backbone due to relative acidity of amide NH

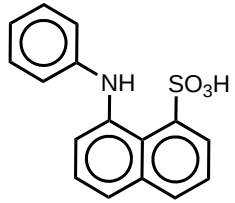
-amides exposed to solvent exchange faster than those buried in protein core



ANS Binding

-1- Anilino-naphthalene-8-sulfonic acid, ANS

-Fluorescent hydrophobic compound binds to hydrophobic surfaces of proteins



NMR-

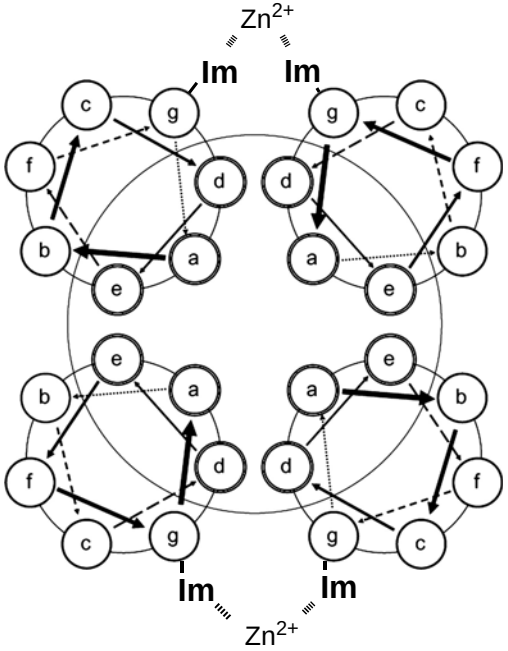
-Rapid exchange leads to low dispersity in NMR (peak averaging)

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



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

no Zn^{2+} ΔG= -2.5 kcal/mol

\+ Zn^{2+} ΔG= -10.3 kcal/mol

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