Bioinformatics: Practical Application of Simulation and Data Mining

Protein Folding I

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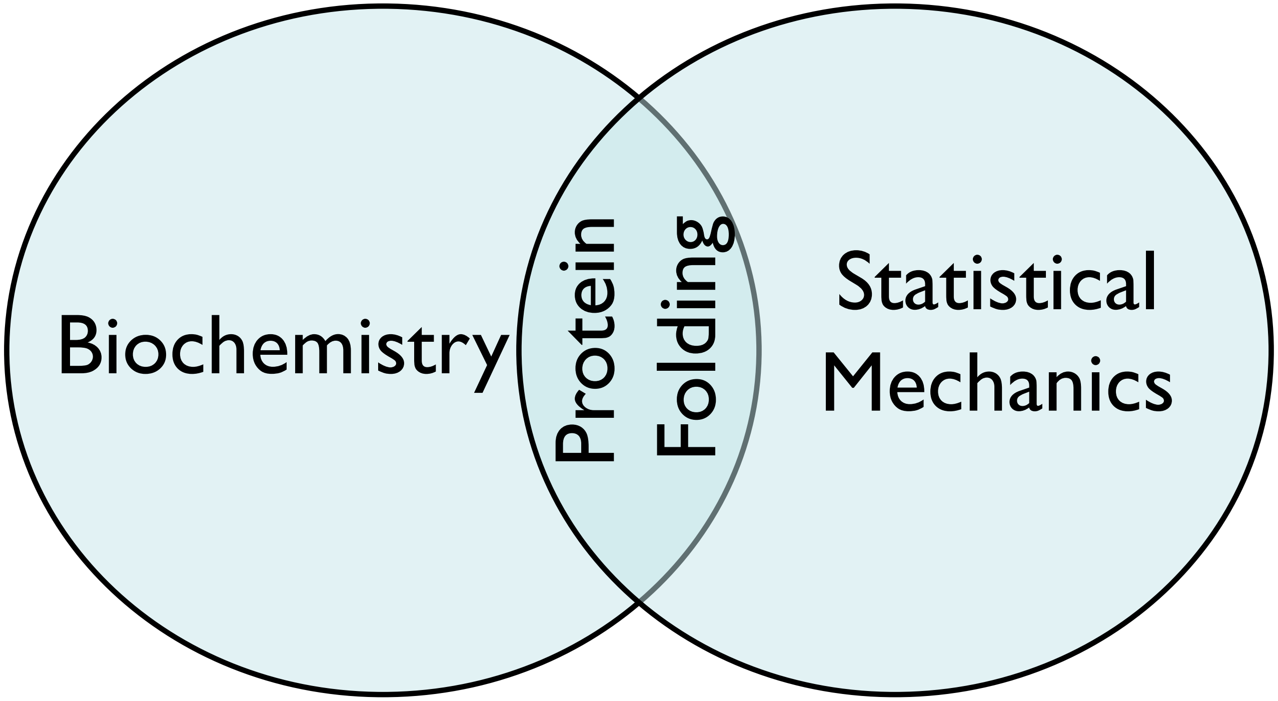
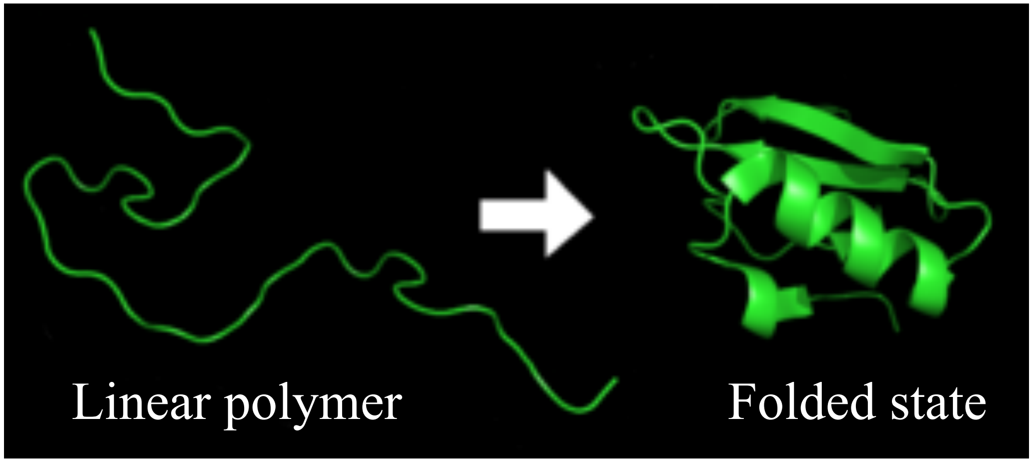
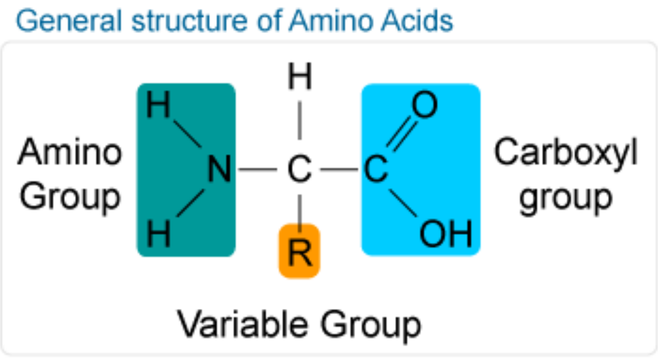

Biochemistry
Protein Folding
Statistical Mechanics

What are proteins?



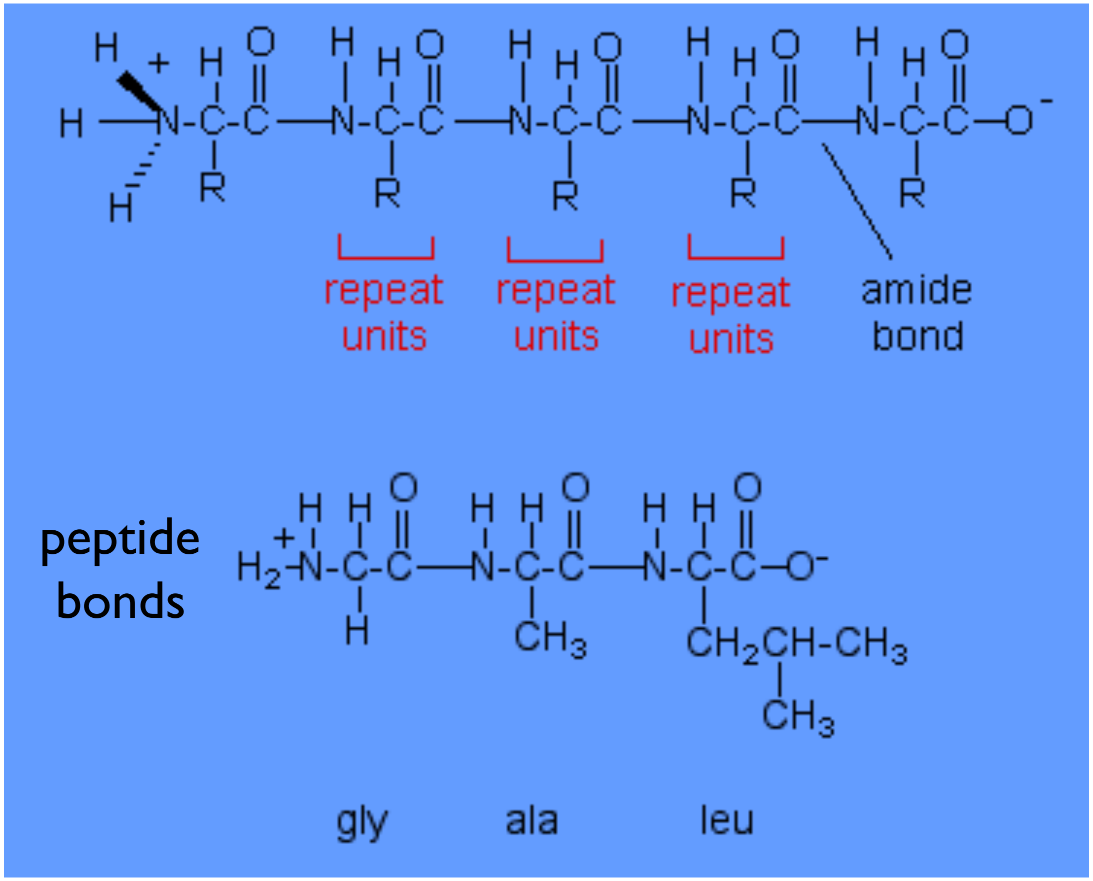
- Proteins are important; e.g. for catalyzing and regulating biochemical reactions, transporting molecules, …
- Linear polymer chain composed of tens (peptides) to thousands (proteins) of monomers
- Monomers are 20 naturally occurring amino acids
- Different proteins have different amino acid sequences
- *Structureless*, extended unfolded state
- Compact, ‘unique’ native folded state (with secondary and tertiary structure) required for biological function
- Sequence determines protein structure (or lack thereof)
- Proteins unfold or denature with increasing temperature or chemical denaturants

Amino Acids I



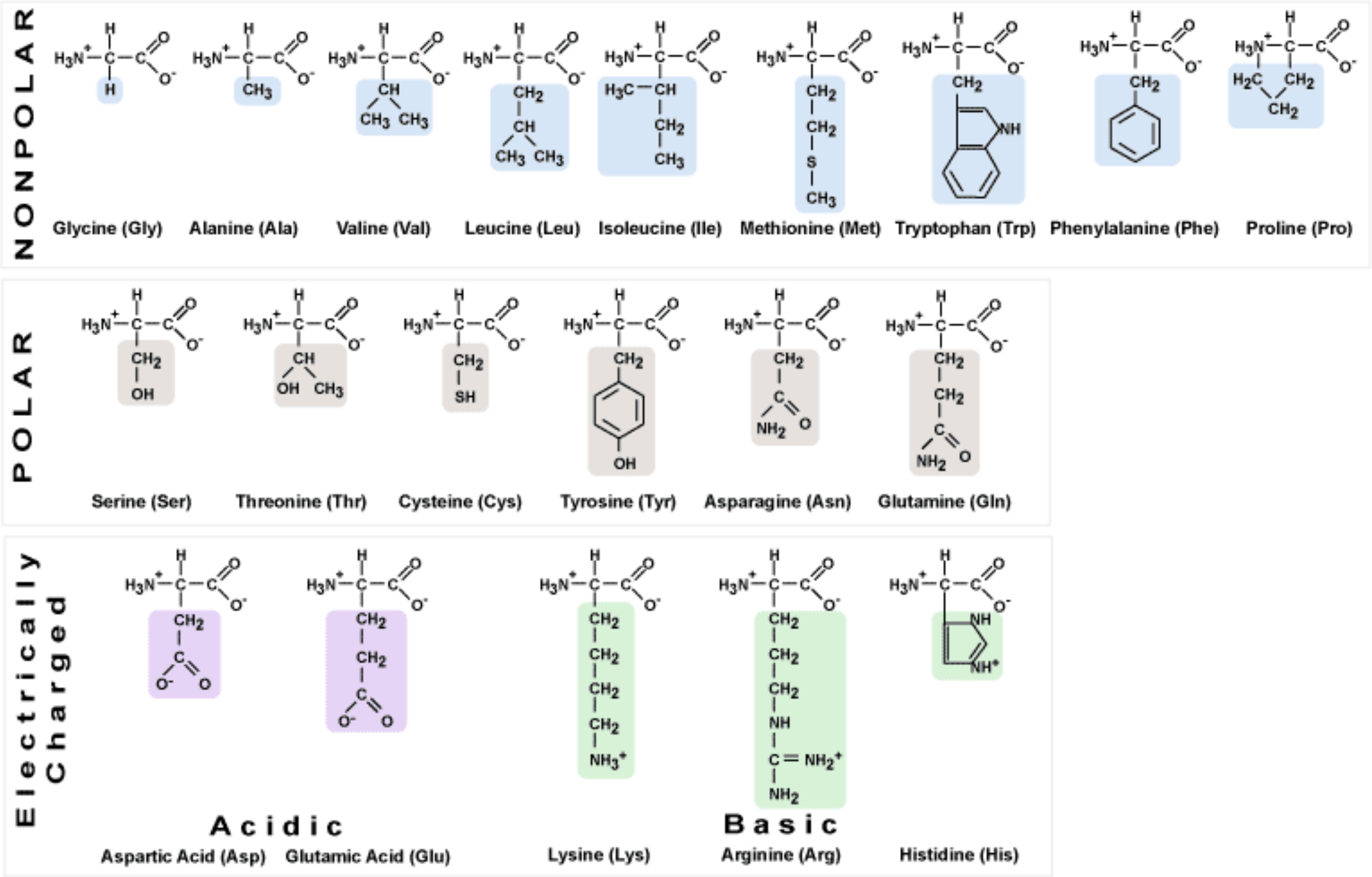
N-terminal C_α C-terminal

R
variable
side chain



- Side chains differentiate amino acid repeat units
- Peptide bonds link residues into polypeptides

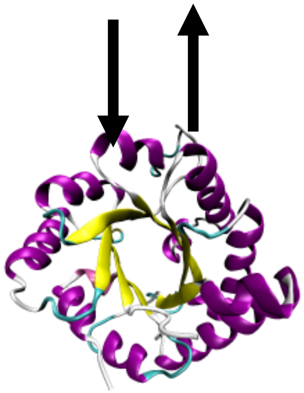
Amino Acids II



The Protein Folding Problem:

What is ‘unique’ folded 3D structure of a protein based on its amino acid sequence? Sequence → Structure

Lys–Asn–Val–Arg–Ser–Lys–Val–Gly–Ser–Thr–Glu–Asn–Ile–Lys– His–Gln–Pro– Gly–Gly–Gly–…



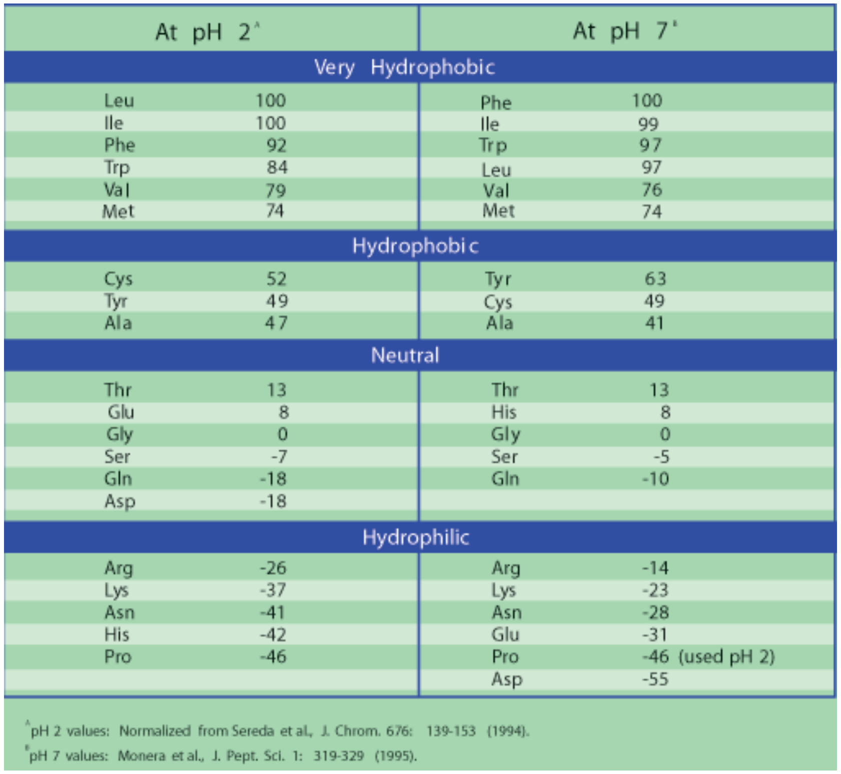
Driving Forces

- Folding: hydrophobicity, hydrogen bonding, van der Waals interactions, …
- Unfolding: increase in conformational entropy, electric charge…

inside H (hydrophobic)

outside P (polar)

Hydrophobicity index

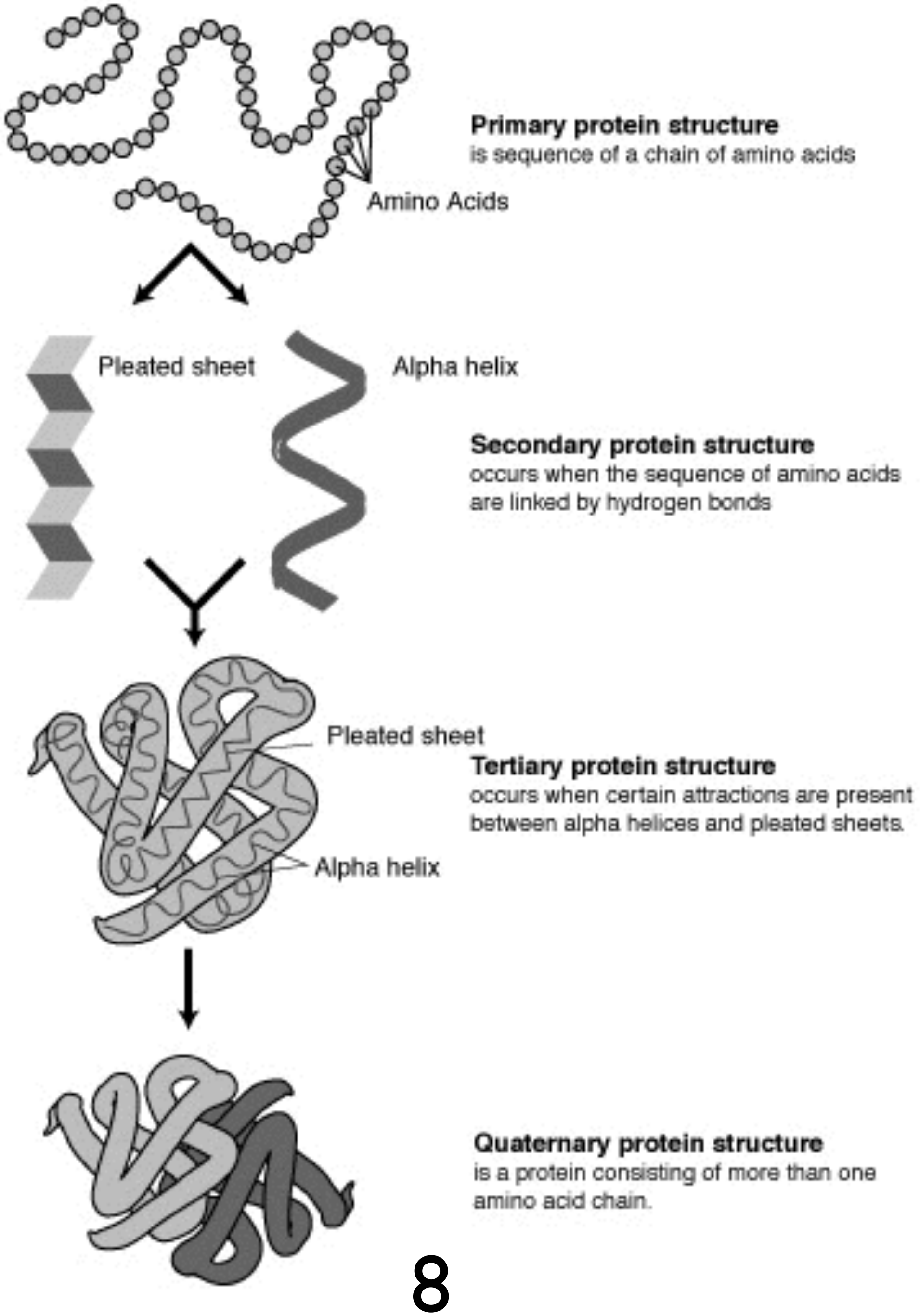


At pH 2[A]		At pH 7[B]	
Very Hydrophobic			
Leu	100	Phe	100
Ile	100	Ile	99
Phe	92	Trp	97
Trp	84	Leu	97
Val	79	Val	76
Met	74	Met	74
Hydrophobic			
Cys	52	Tyr	63
Tyr	49	Cys	49
Ala	47	Ala	41
Neutral			
Thr	13	Thr	13
Glu	8	His	8
Gly	0	Gly	0
Ser	-7	Ser	-5
Gln	-18	Gln	-10
Asp	-18		
Hydrophilic			
Arg	-26	Arg	-14
Lys	-37	Lys	-23
Asn	-41	Asn	-28
His	-42	Glu	-31
Pro	-46	Pro	-46 (used pH 2)
		Asp	-55

[A] pH 2 values: Normalized from Sereda et al., J. Chrom. 676: 139-153 (1994).

[B] pH 7 values: Monera et al., J. Pept. Sci. 1: 319-329 (1995).

Higher-order Structure



Primary protein structure
is sequence of a chain of amino acids

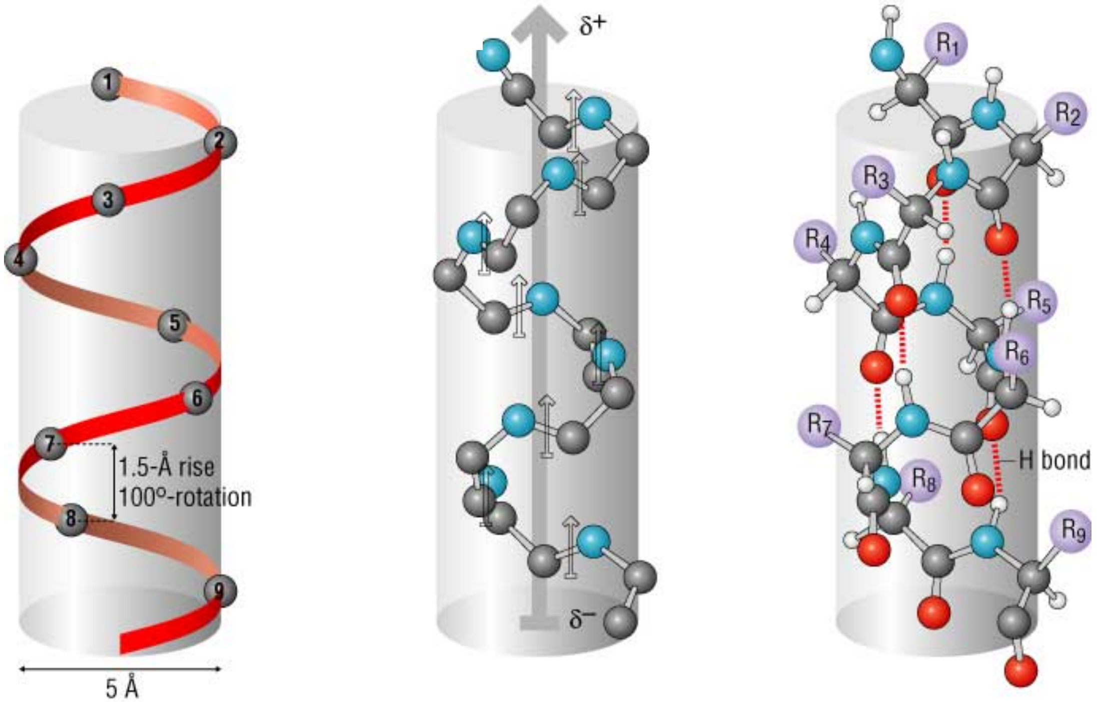
Secondary protein structure
occurs when the sequence of amino acids are linked by hydrogen bonds

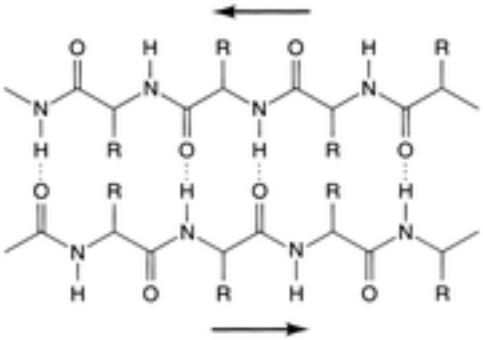
Tertiary protein structure
occurs when certain attractions are present between alpha helices and pleated sheets.

Quaternary protein structure
is a protein consisting of more than one amino acid chain.

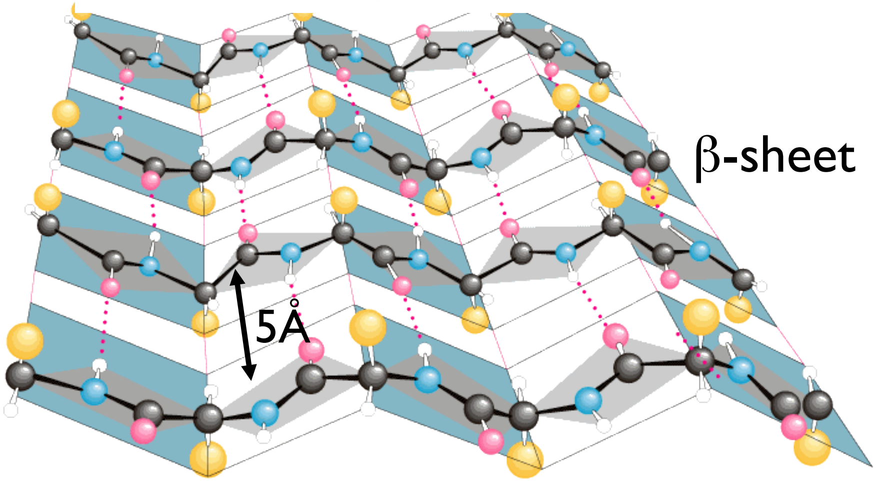
Secondary Structure: Loops, α-helices, β-strands/sheets

α-helix





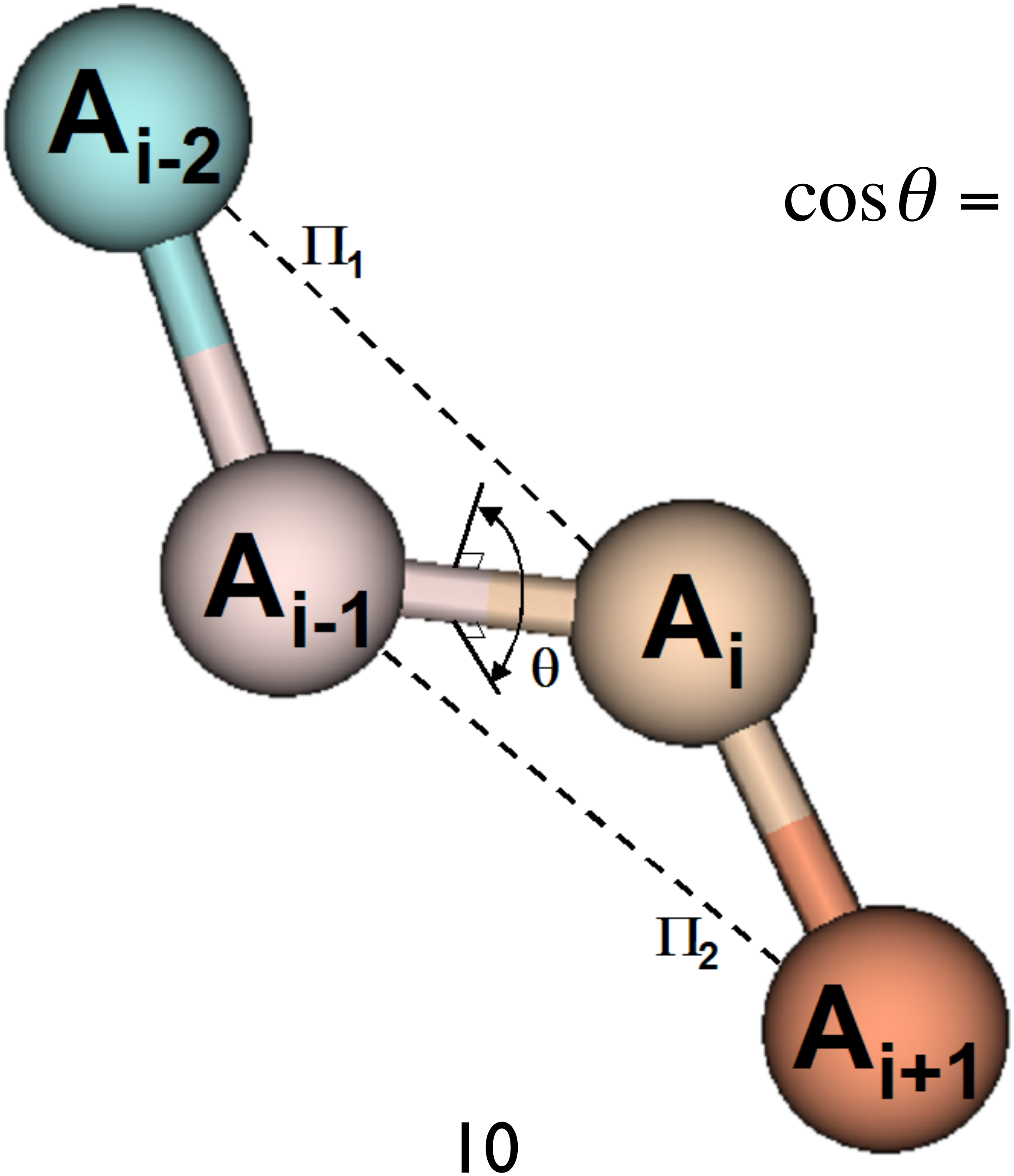
β-strand



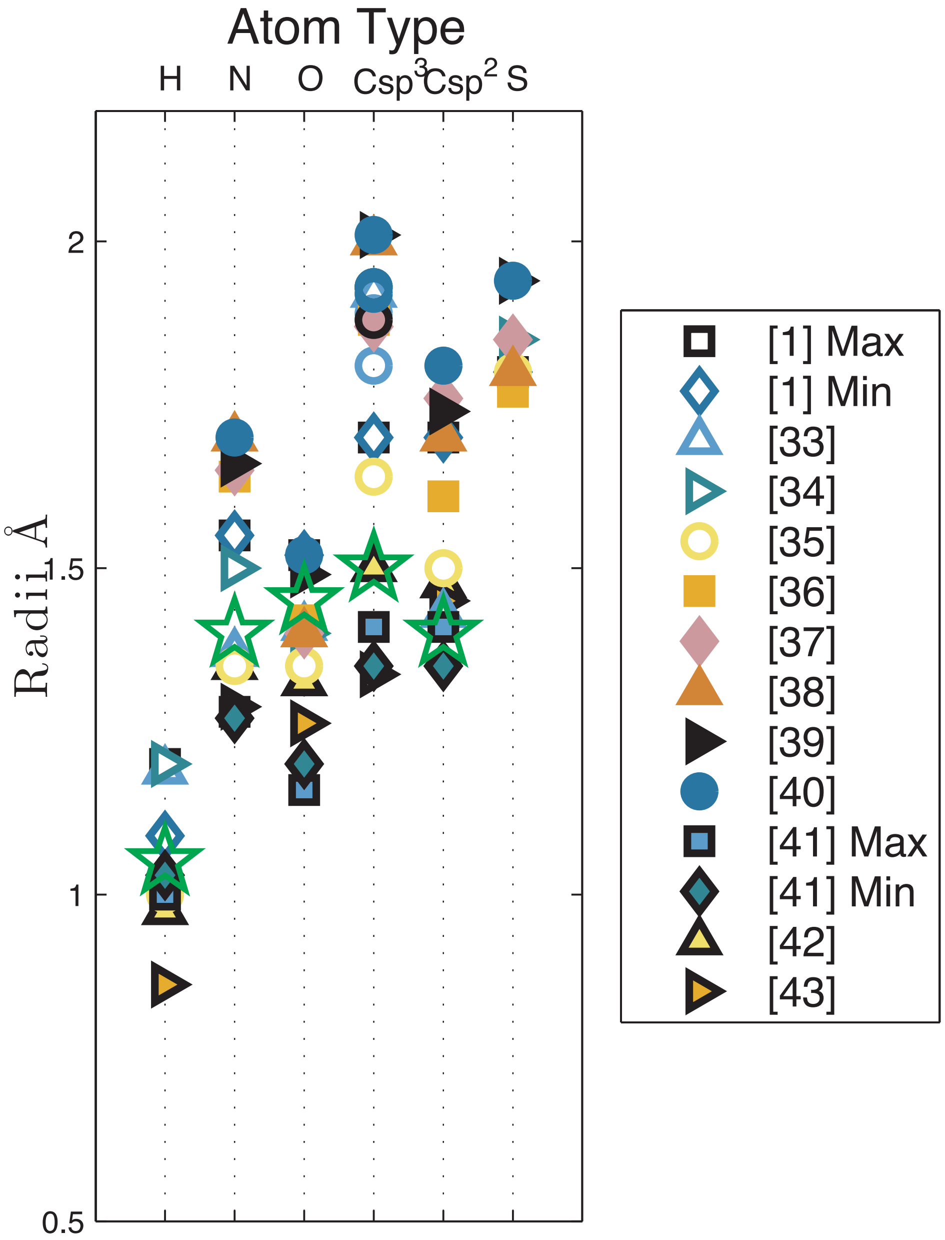
- •Right-handed; three turns
- •Vertical hydrogen bonds between NH_2 (teal/white) backbone group and C=O (grey/red) backbone group four residues earlier in sequence
- •Side chains (R) on outside; point upwards toward NH_2
- •Each amino acid corresponds to 100°, 1.5Å, 3.6 amino acids per turn
- •$(\phi,\psi)=(-60°,-45°)$
- •α-helix propensities: Met, Ala, Leu, Glu

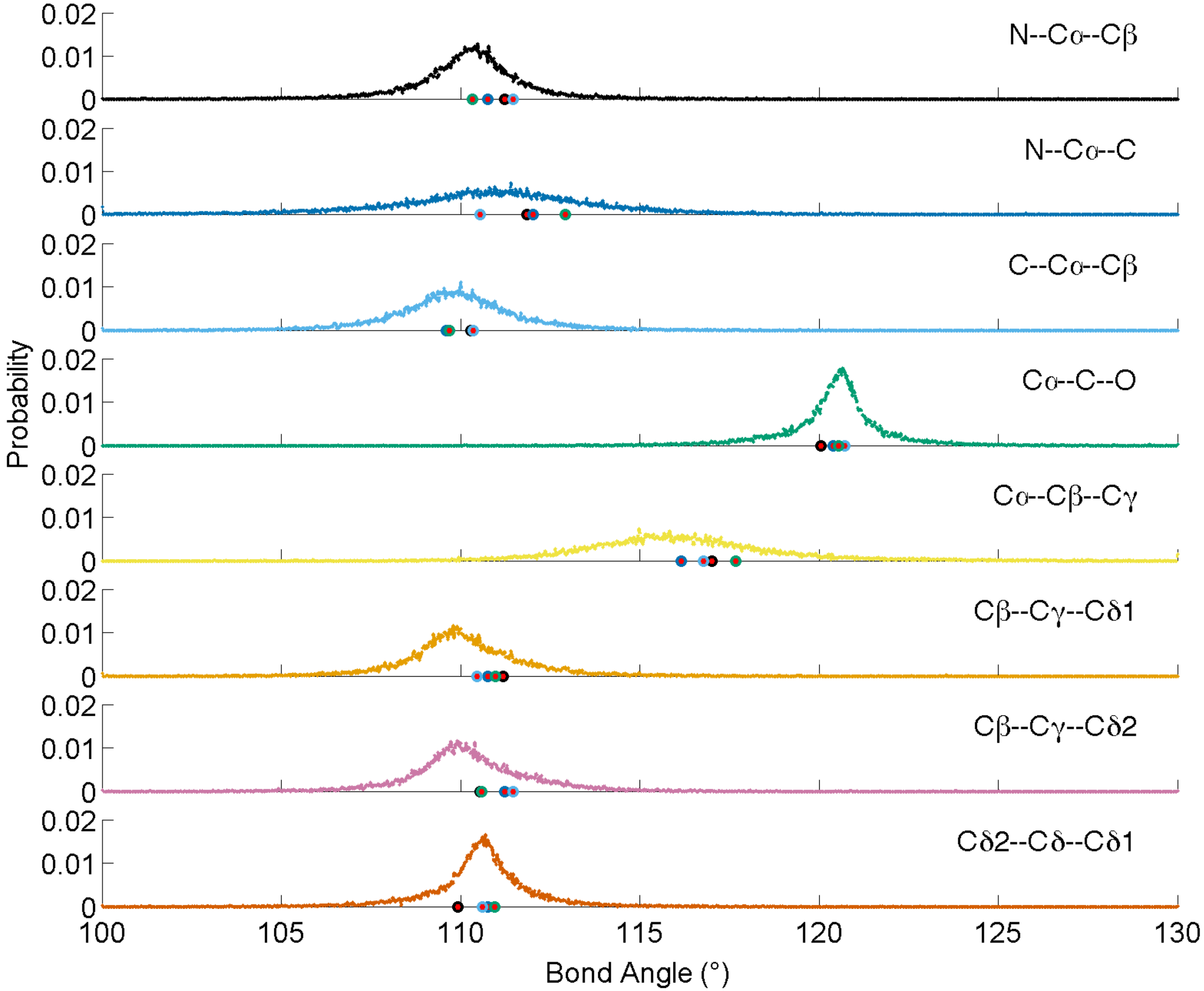
- •5-10 residues; peptide backbones fully extended
- •NH (blue/white) of one strand hydrogen-bonded to C=O (black/red) of another strand
- •C_α ,side chains (yellow) on adjacent strands aligned; side chains along single strand alternate up and down
- •$(\phi,\psi)=(-135°,135°)$
- •β-strand propensities: Val, Thr, Tyr, Trp, Phe, Ile

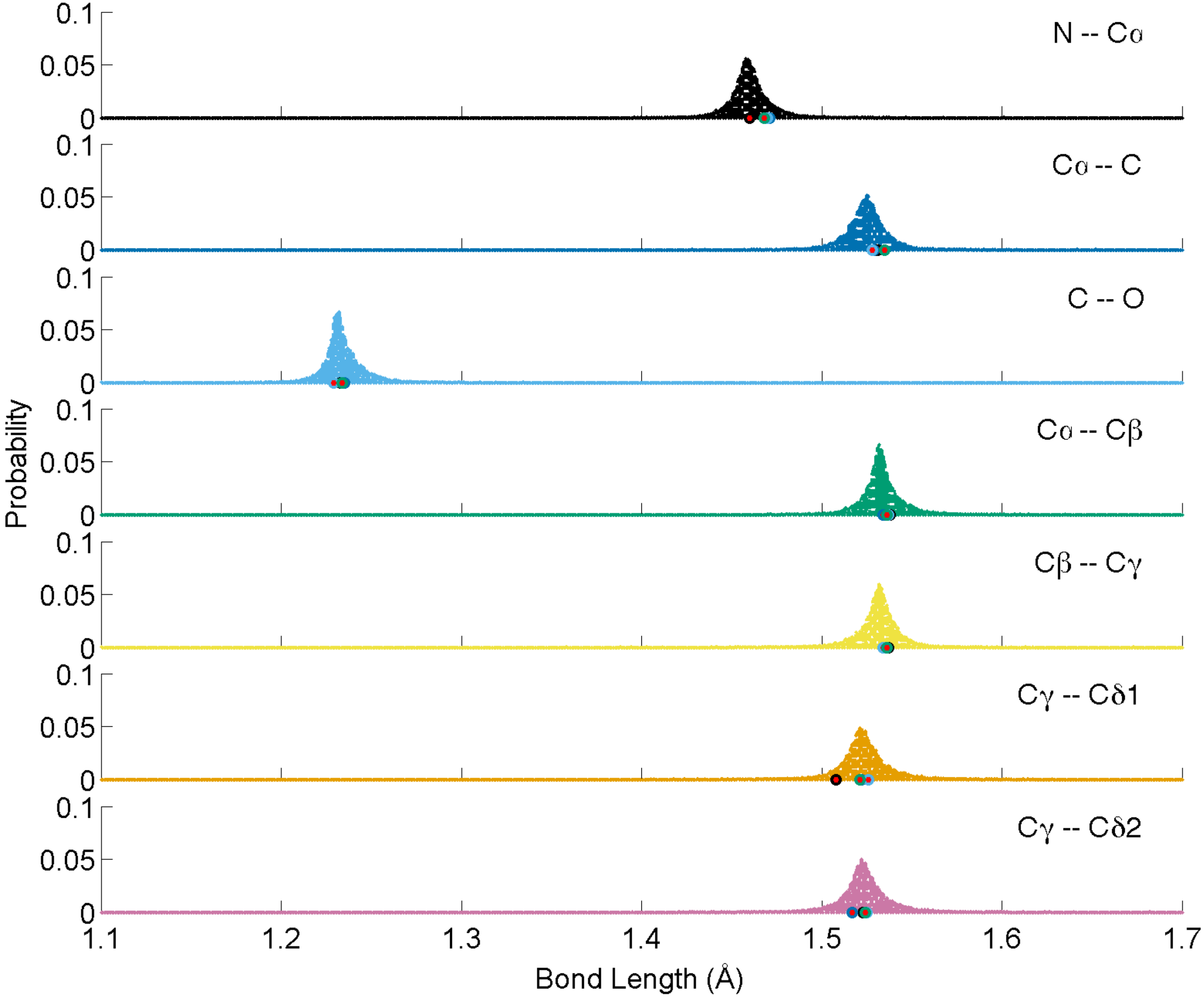
Backbonde Dihedral Angles



$$\cos\theta = \hat{\pi}_1 \bullet \hat{\pi}_2$$

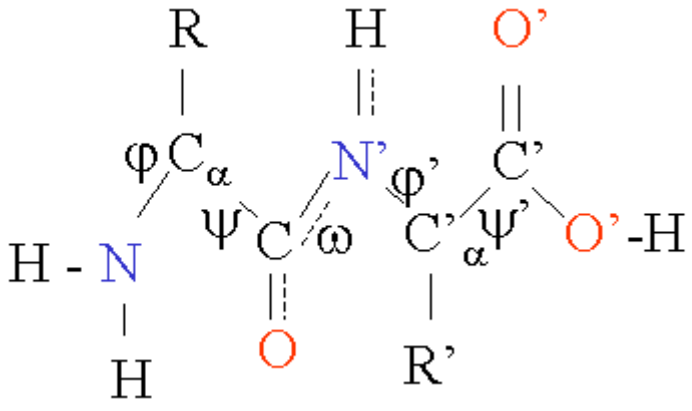

Atom Type
H
N
O
Csp^3
Csp^2
S
Radii Å
2
1.5
1
0.5
[1] Max
[1] Min
[33]
[34]
[35]
[36]
[37]
[38]
[39]
[40]
[41] Max
[41] Min
[42]
[43]


N--Cα--Cβ
N--Cα--C
C--Cα--Cβ
Cα--C--O
Cα--Cβ--Cγ
Cβ--Cγ--Cδ1
Cβ--Cγ--Cδ2
Cδ2--Cδ--Cδ1
Probability
0.02
0.01
0
100
105
110
115
120
125
130
Bond Angle (°)


N -- Cα
Cα -- C
C -- O
Cα -- Cβ
Cβ -- Cγ
Cγ -- Cδ1
Cγ -- Cδ2
Probability
0.1
0.05
0
1.1
1.2
1.3
1.4
1.5
1.6
1.7
Bond Length (Å)

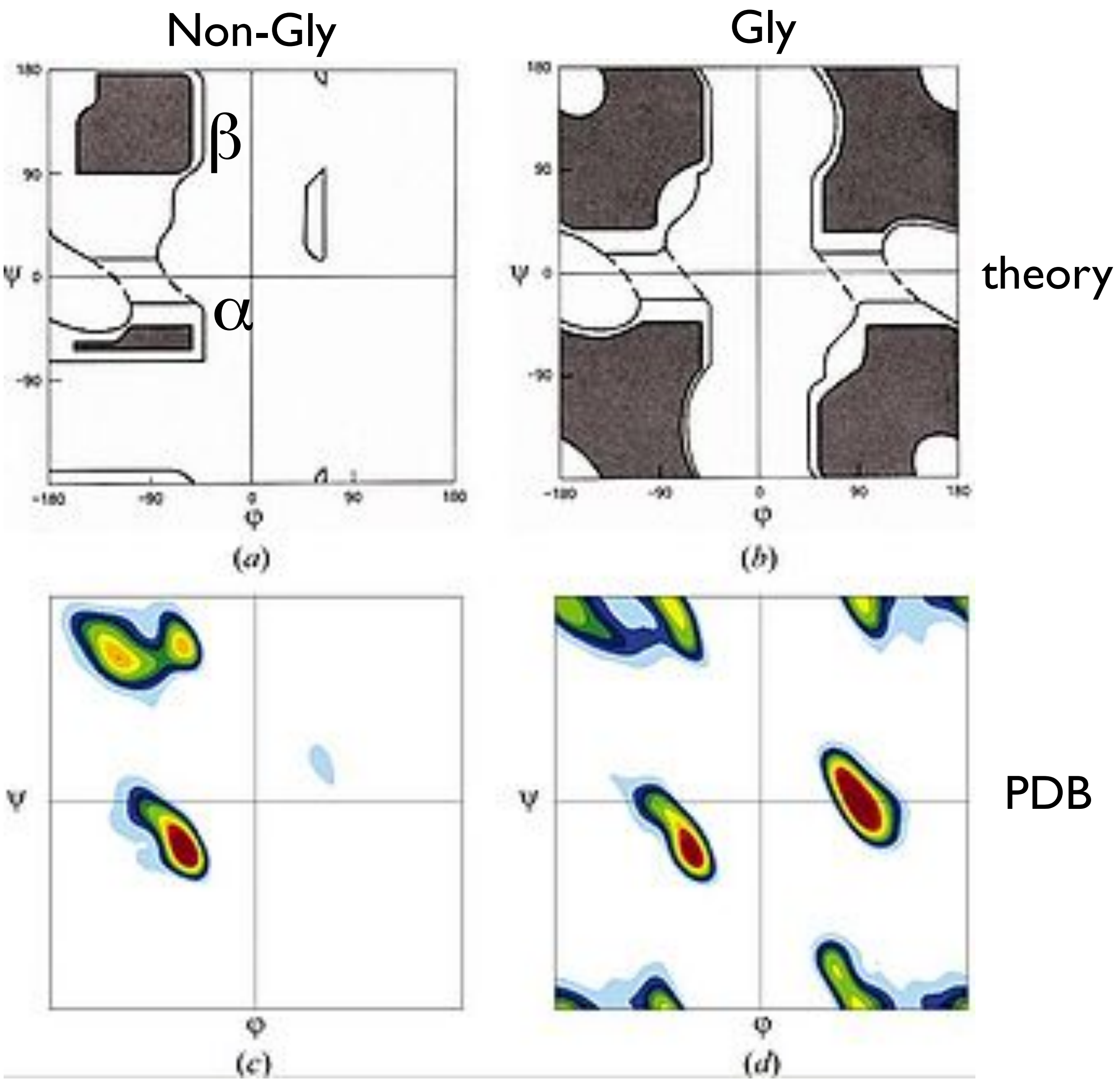
Ramachandran Plot: Determining Steric Clashes

Backbone
dihedral angles



4 atoms define dihedral angle:

$CC_{\alpha}NC$	ϕ
$C_{\alpha}N\ CC_{\alpha}$	$\omega=0,180°$
$N\ CC_{\alpha}N$	ψ

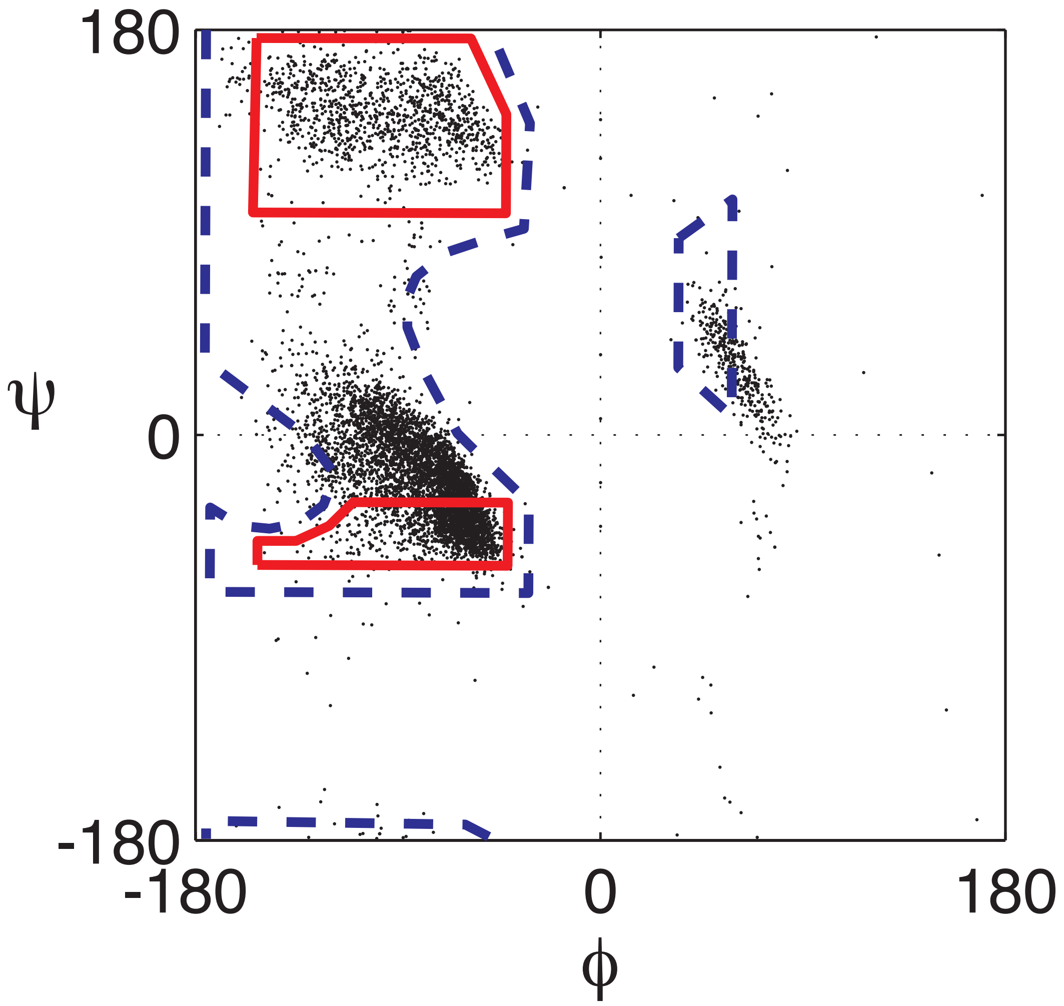


▨ vdW radii

—— < vdW radii

_ _ _ backbone flexibility

Backbone dihedral angles from PDB



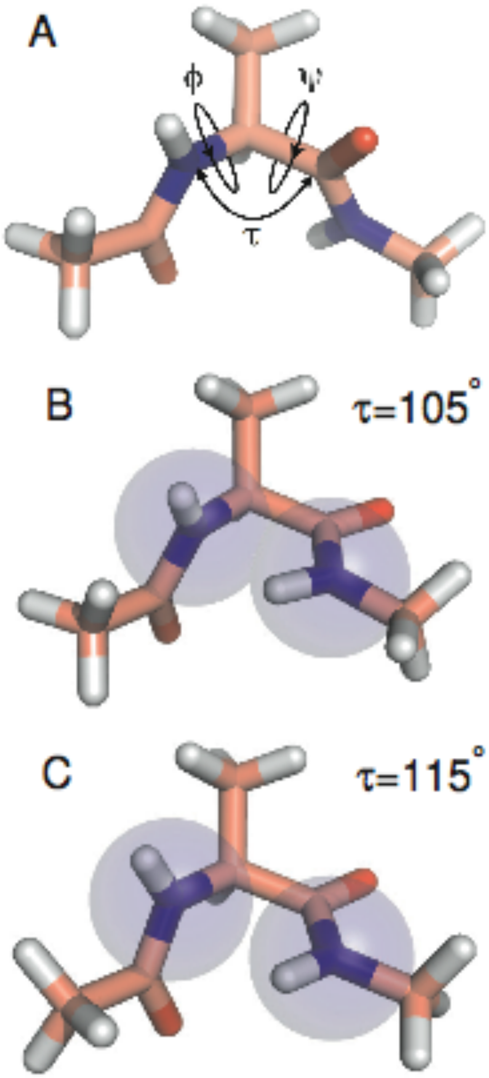


Figure 1: Stick representation of an alanyl dipeptide mimetic. Atom types are color-coded: carbon=pink, nitrogen=blue, oxygen=red, hydrogen=white. **A**: The backbone dihedral angles ϕ and ψ and the bond angle τ are indicated. **B**: $\tau = 105°$, $\phi = -90°$, $\psi = 0°$ (i.e. bridge region values of ϕ and ψ). Blue-shaded spheres indicate steric overlap between main-chain nitrogens for this value of τ. **C**: $\tau = 115°$, $\phi = -90°$, $\psi = 0°$ (i.e. bridge region values of ϕ and ψ). Blue-shaded spheres indicate no steric overlap between main-chain nitrogens for this value of τ.

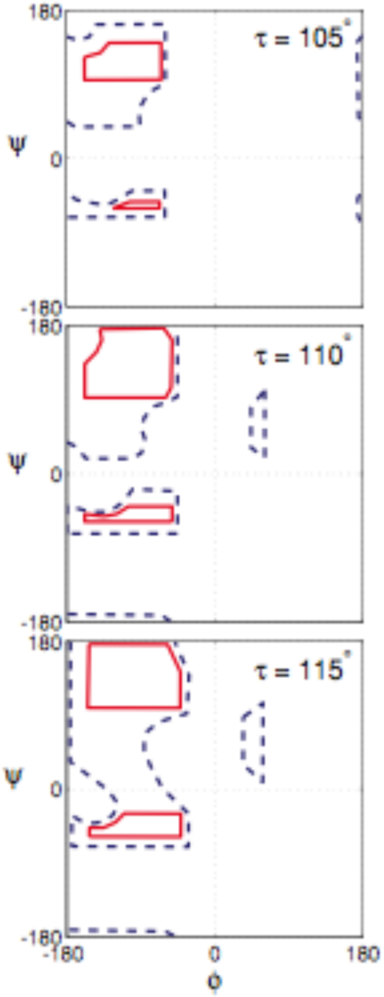
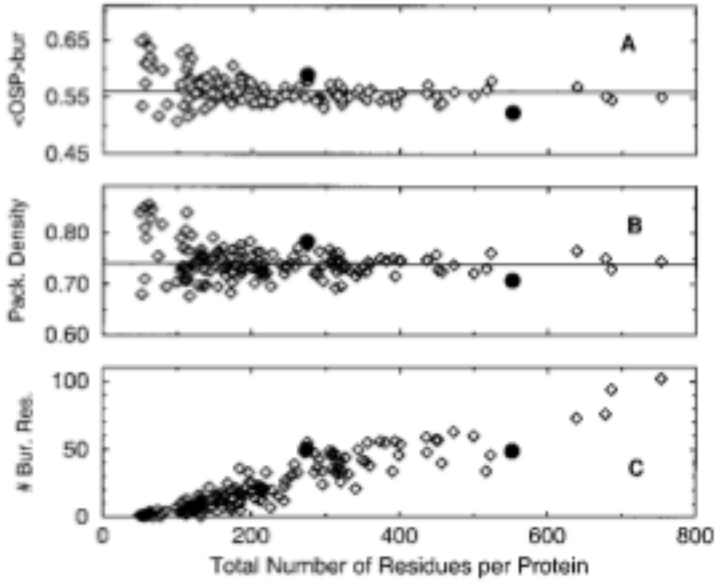
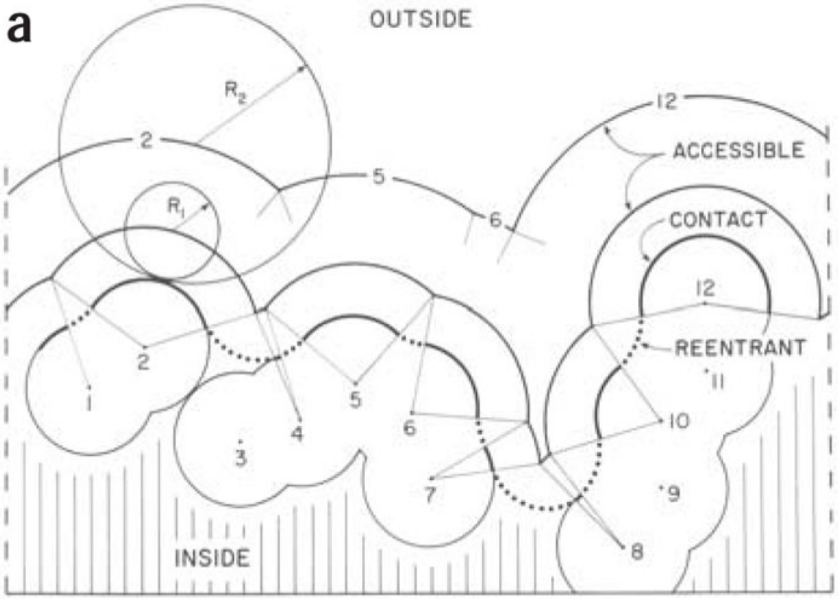
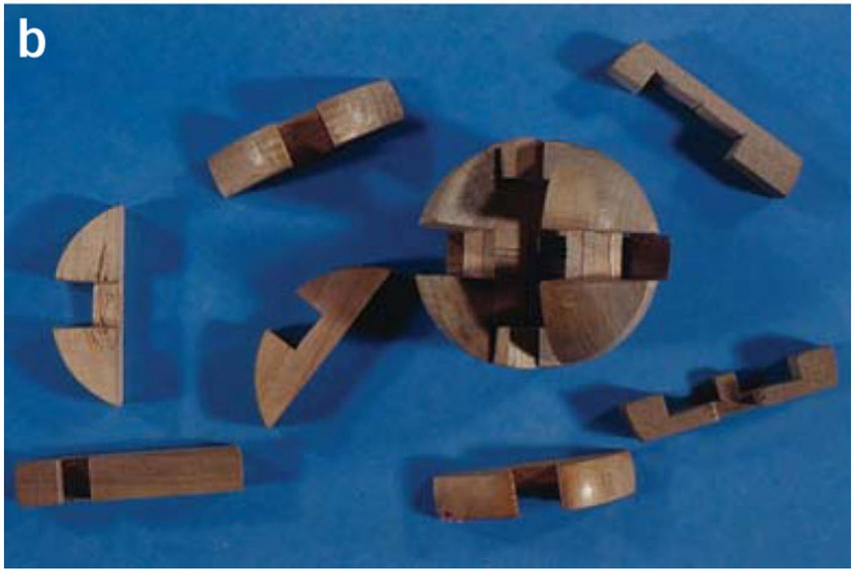


Figure 2: Ramachandran plots of allowed ϕ/ψ combinations for 3 values of τ [2]. The solid red lines enclose the 'normally allowed' ϕ/ψ combinations and the dashed blue line indicates the 'outer limit'.

Prof. Fred Richards, Yale



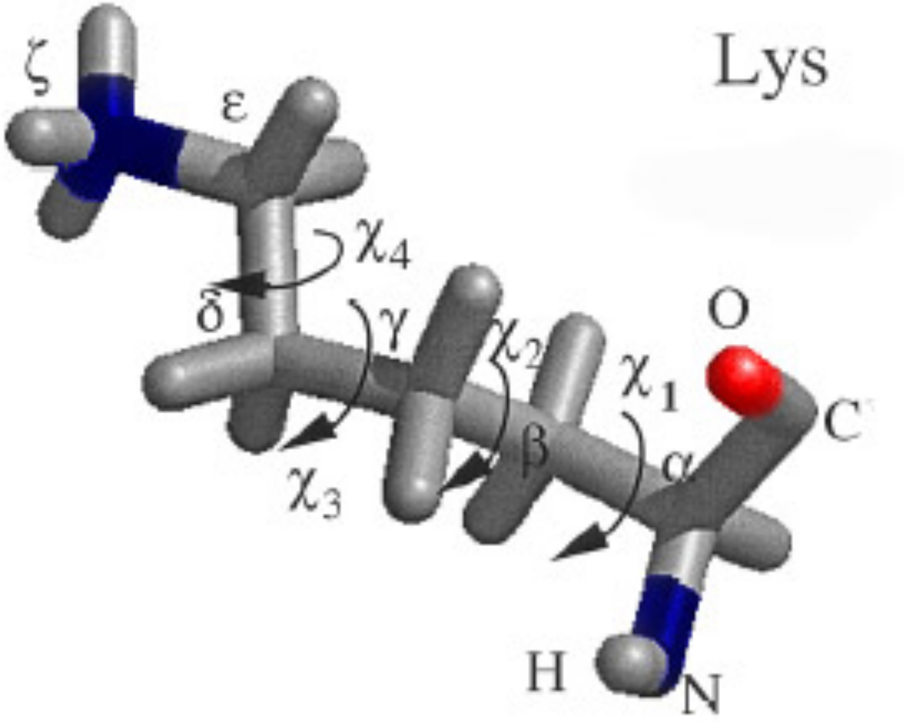




Side-Chain Dihedral Angles

χ_4: Lys, Arg

χ_5: Arg



Side chain: C_α-CH_2-CH_2-CH_2-CH_2-NH_3

Use $NC_\alpha C_\beta C_\gamma C_\delta C_\varepsilon N_\zeta$ to define χ_1, χ_2, χ_3, χ_4

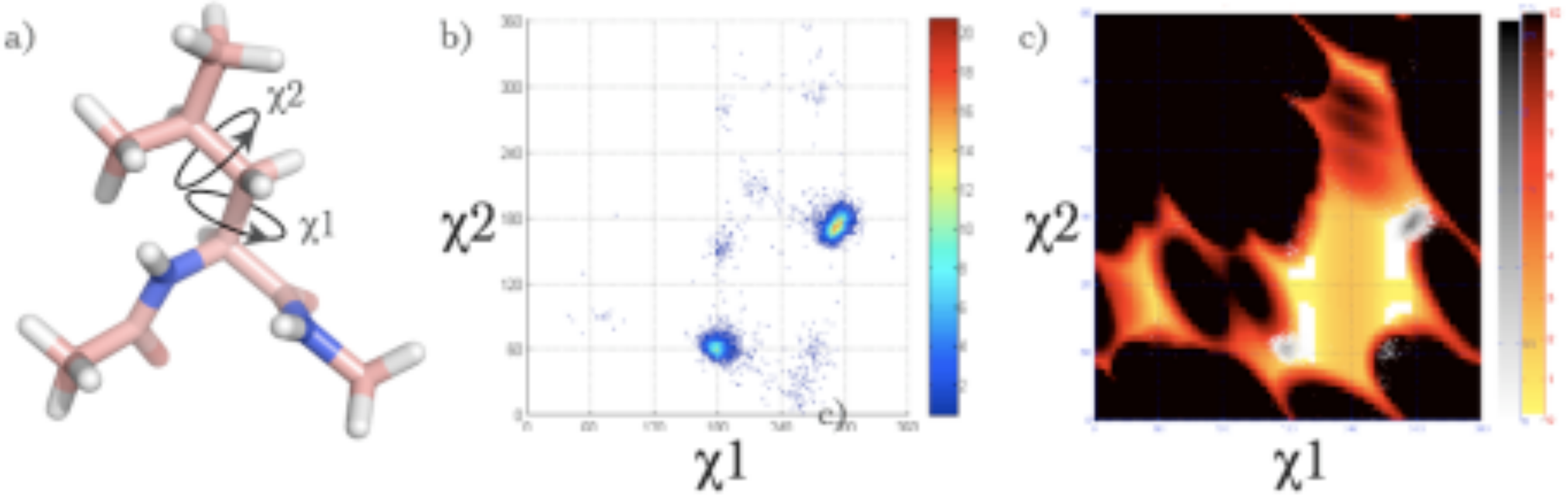
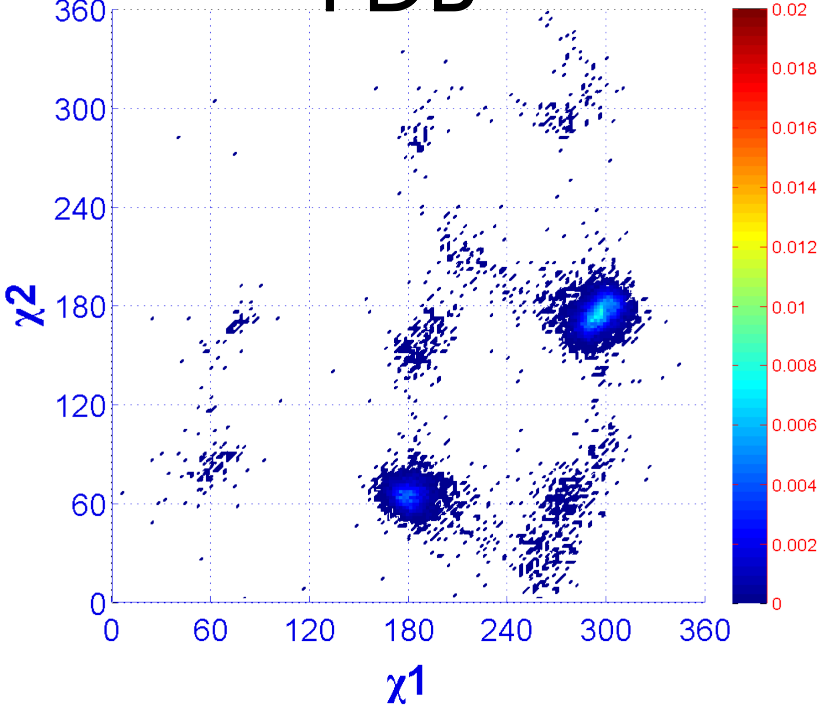


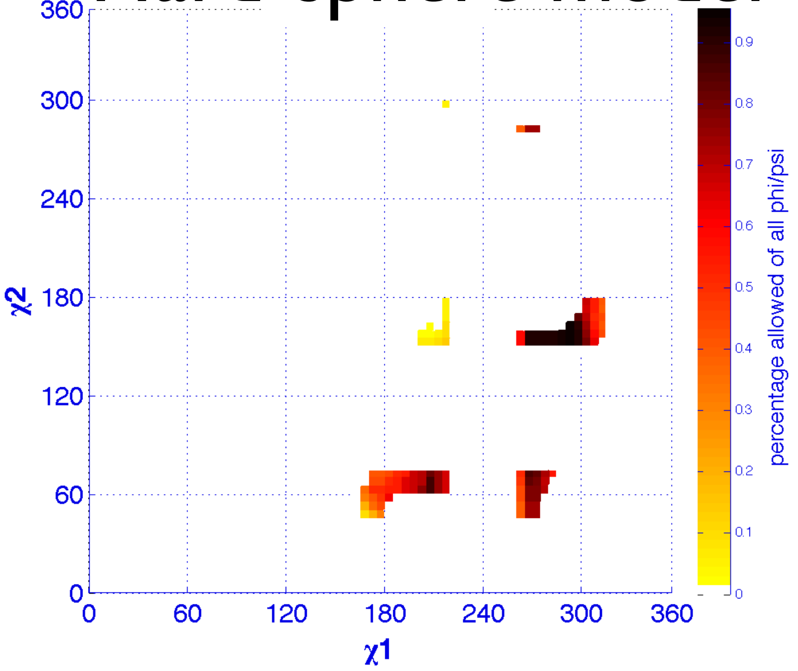
Figure 2: (a) Stick representation of a Leu dipeptide showing the side-chain dihedrals chi1 and chi2 (carbon=pink, nitrogen=blue, oxygen=red, hydrogen=white). (b) Density plot of chi1/chi2 value for every Leu in the Durbrack database [5]. (c) My calculated energy landscape for the Leu dipeptide using the repulsive Lennard-Jones interaction potential overlaid on the Durbrack probability distribution (grey scale). White regions correspond to low-energy minima with energy increasing from yellow to black.

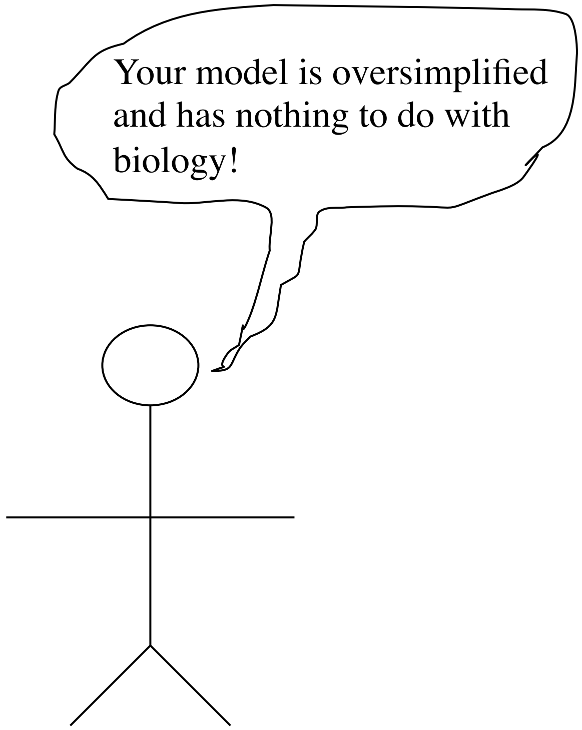
Sidechain Dihedral Angle Distributions for Leu

PDB

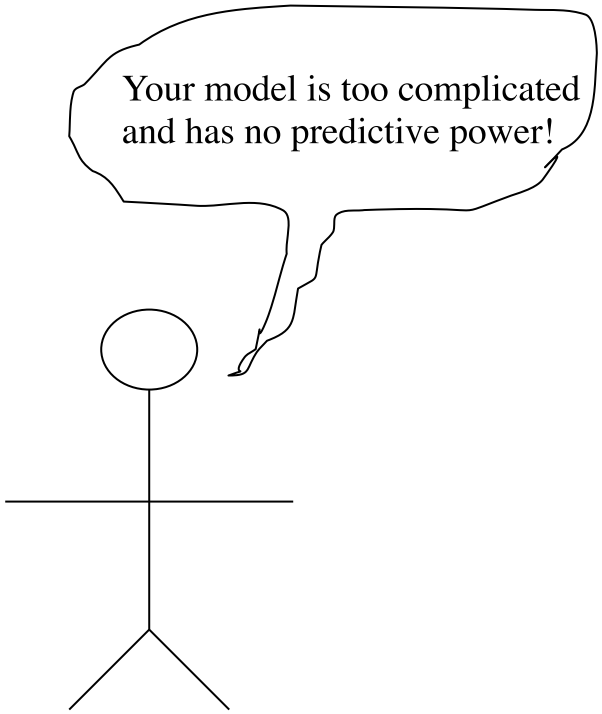


Hard-sphere model




Your model is oversimplified and has nothing to do with biology!

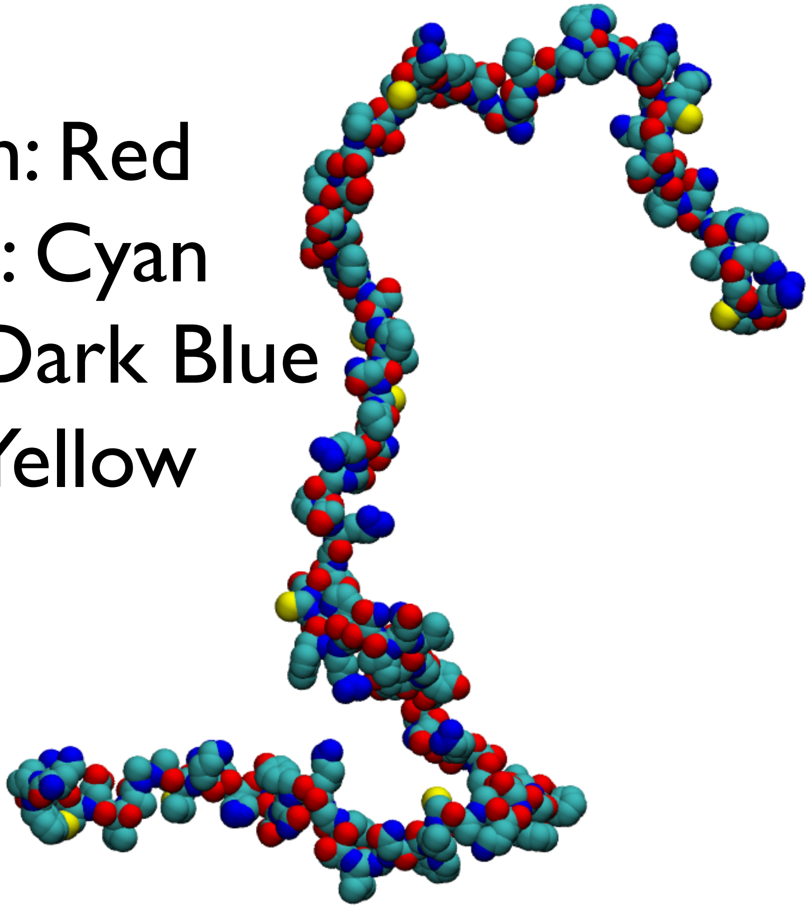
Molecular biologist



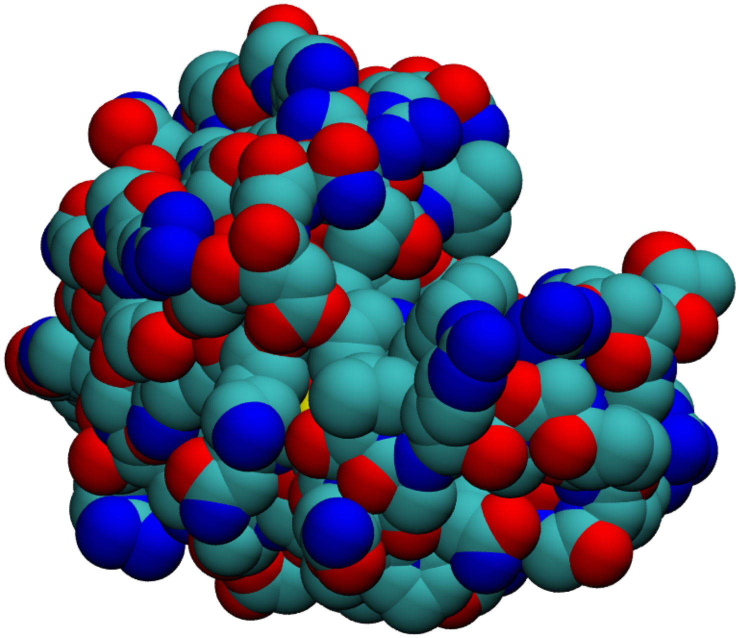
Biological Physicist

Folding Transition

Oxygen: Red
Carbon: Cyan
Nitrogen: Dark Blue
Sulfur: Yellow



$T>T_m$



$T<T_m$

Possible Strategies for Understanding Protein Folding

- For all possible conformations, compute free energy from atomic interactions within protein and protein-solvent interactions; find conformation with lowest free energy…e.g using all-atom molecular dynamics simulations

Not possible?, limited time resolution

- Use coarse-grained models with effective interactions between residues and residues and solvent

General, but qualitative

Why do proteins fold (correctly & rapidly)??

Levinthal's paradox:

For a protein with N amino acids, number of backbone conformations/minima

$$N_c \sim \mu^{2N}$$

μ = # allowed dihedral angles

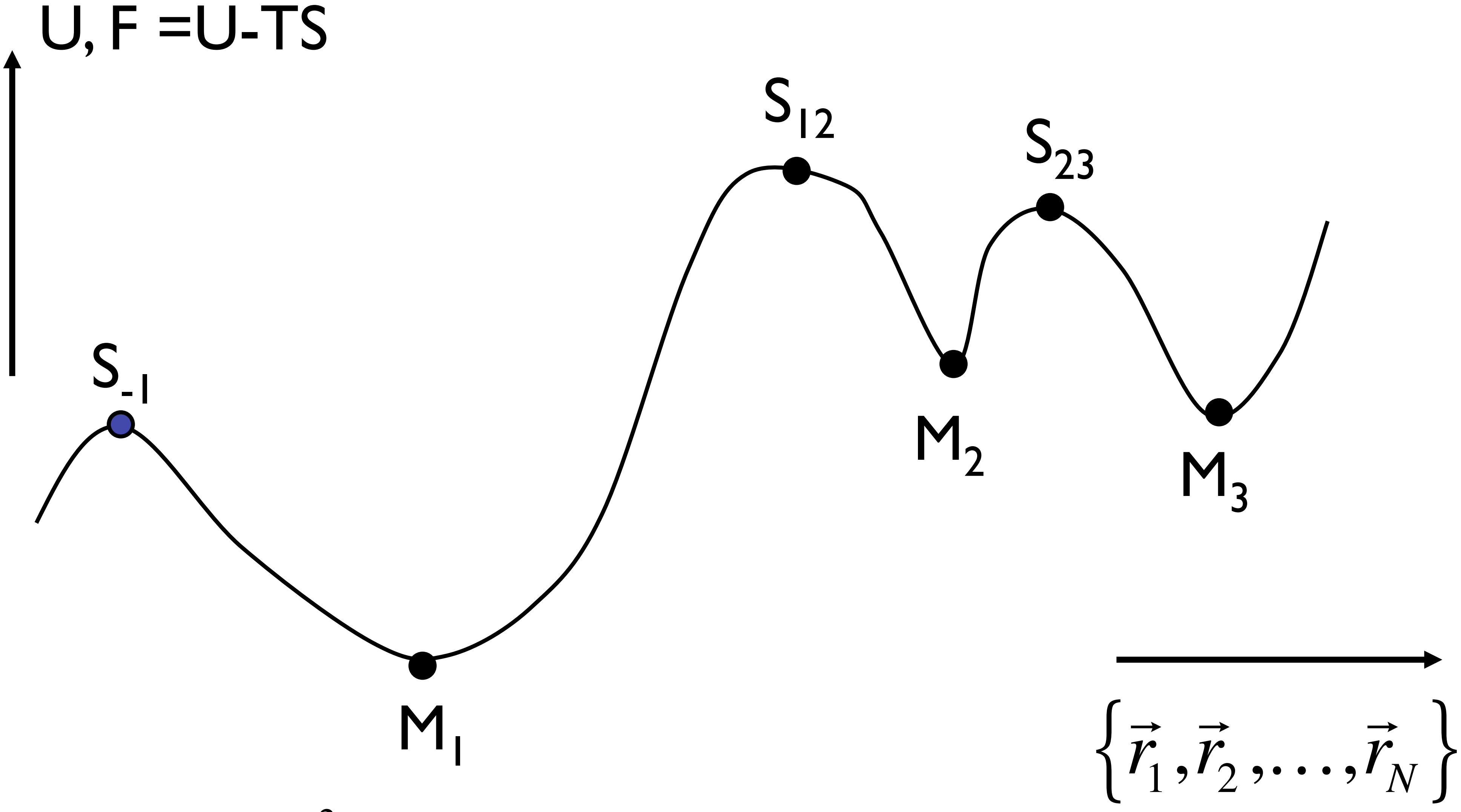
How does a protein find the global optimum w/o global search? Proteins fold much faster.

$N_c \sim 3^{200} \sim 10^{95}$

$\tau_{fold} \sim N_c\, \tau_{sample} \sim 10^{83}$ s vs $\tau_{fold} \sim 10^{-6}\text{-}10^{-3}$ s

$\tau_{universe} \sim 10^{17}$ s

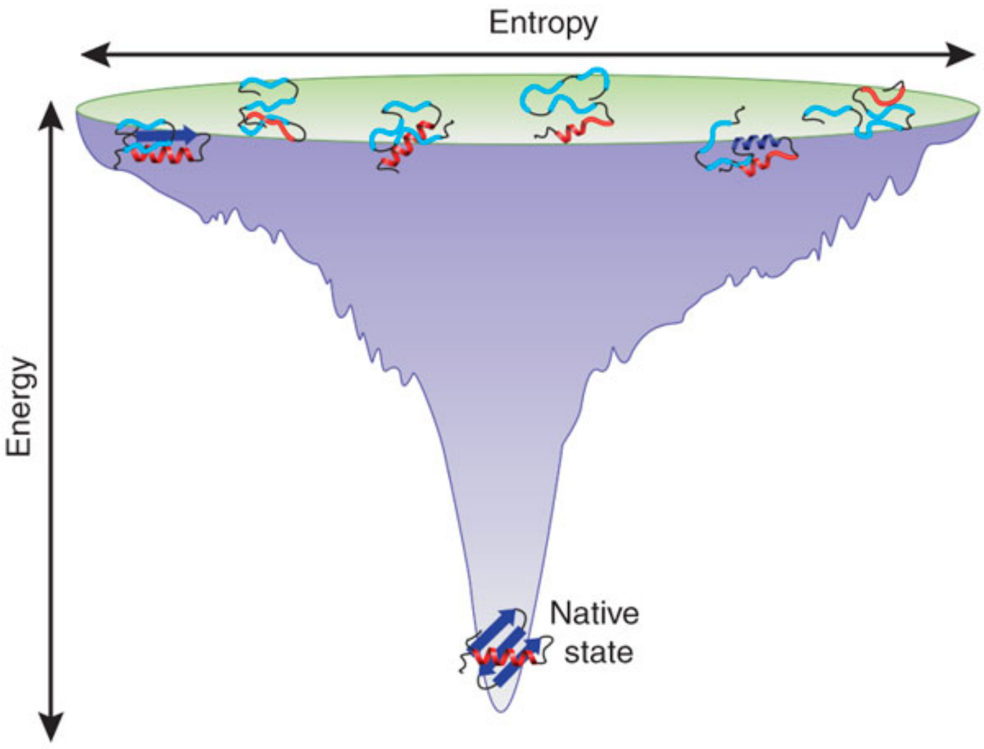
Energy Landscape



all atomic coordinates; dihedral angles

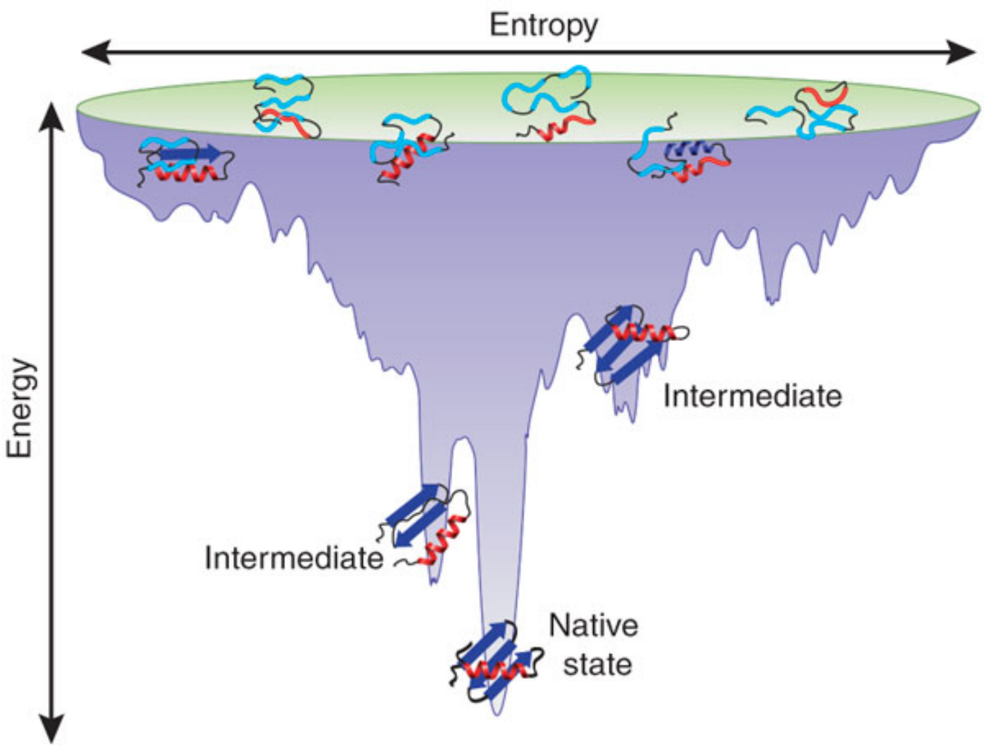
$$\vec{\nabla} U = 0 \quad \begin{cases} \nabla^2 U > 0 & \text{minimum} \\ \nabla^2 U = 0 & \text{saddle point} \\ \nabla^2 U < 0 & \text{maximum} \end{cases}$$

Roughness of Energy Landscape



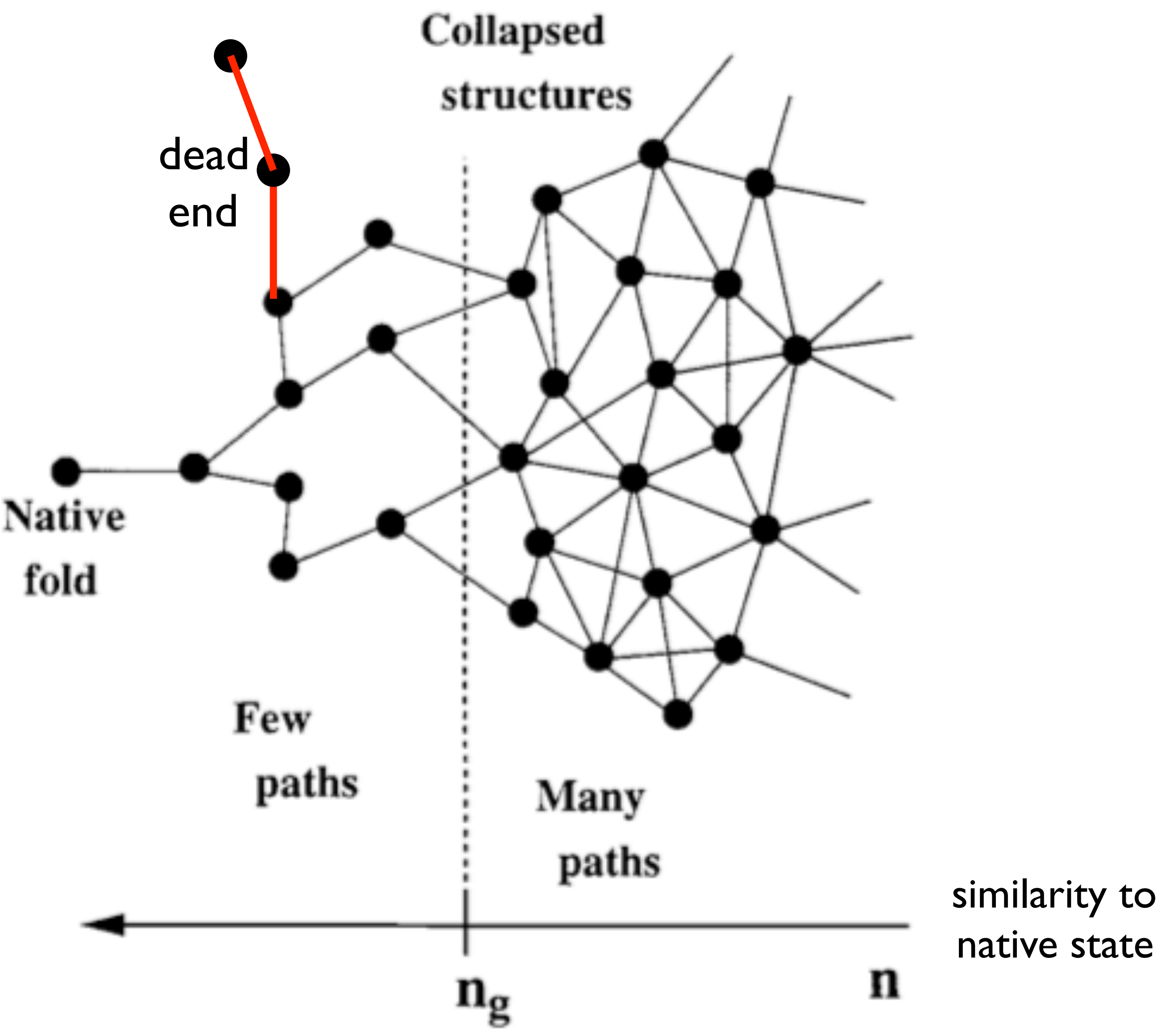
smooth, funneled

(Wolynes et. al. 1997)

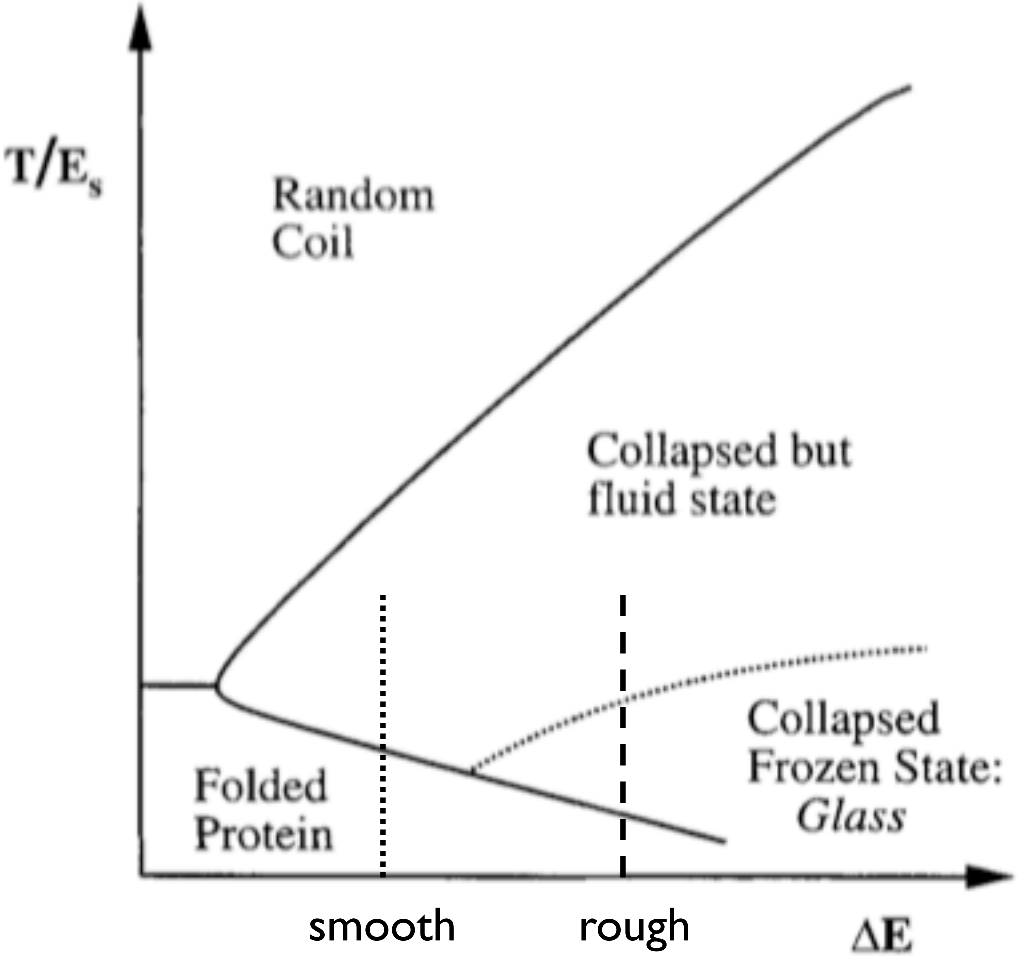


rough

Folding Pathways



Folding Phase Diagram



Open Questions

- What differentiates the native state from other low-lying energy minima?

- How many low-lying energy minima are there? Can we calculate landscape roughness from sequence?

- What determines whether protein will fold to the native state or become trapped in another minimum?

- What are the pathways in the energy landscape that a given protein follows to its native state?

NP Hard Problem!

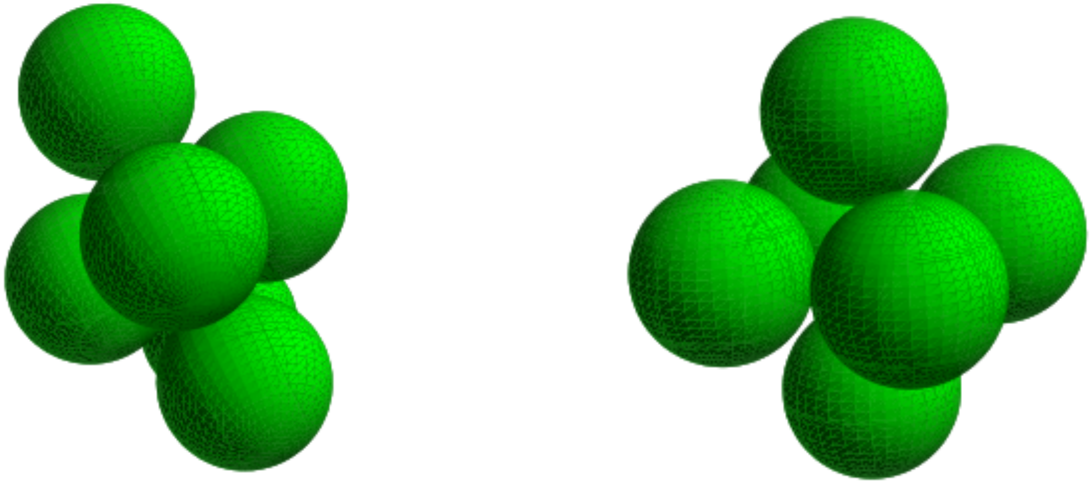
Digression---Number of Energy Minima for Sticky Spheres

N_m	N_s	N_p
4	1	1
5	1	6
6	2	50
7	5	486
8	13	5500
9	52	49029
10	-	-

N_s~exp(aN_m);

N_p~exp(bN_m) with b>a

sphere packings



polymer packings