Swiss Institute of Bioinformatics

BIOZENTRUM
Universität Basel

Introduction:
Principles of protein structures

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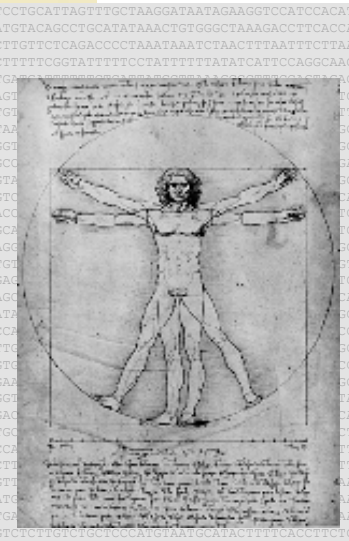
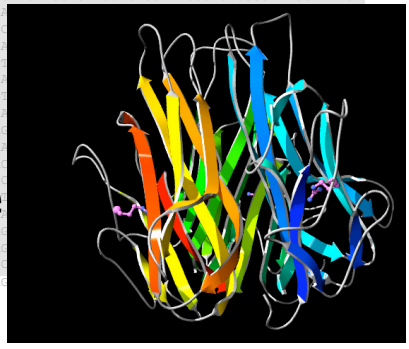
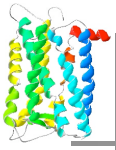


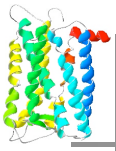


Ceci n'est pas un homme

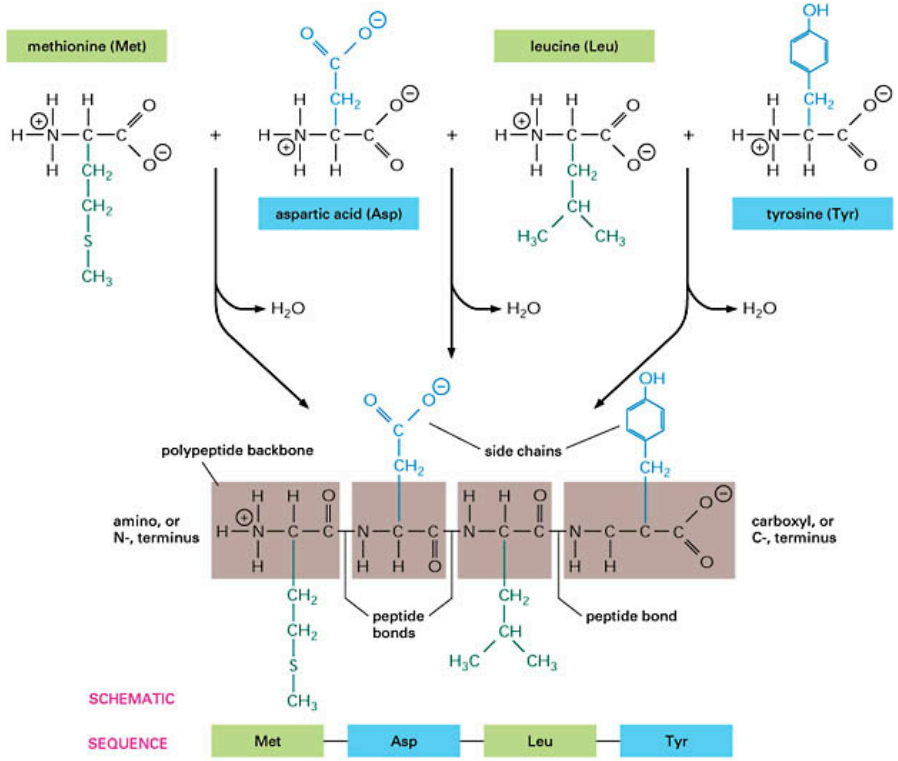


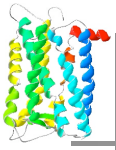
Overview

- Amino Acids and Polypeptides (1)
- Physicochemical Properties of Amino Acid Side Chains (2)
- Protein Folding and Physicochemical Interactions (3)
- Principles of Protein Structure (4)
- Structure Comparison (5)
- Protein Structure Databases (6)



Amino Acids and Polypeptides (1)

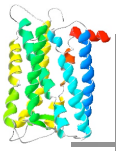




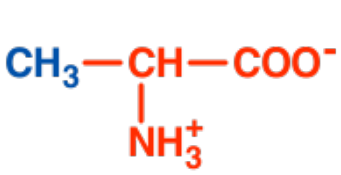
Amino Acids

Three and one letter code:

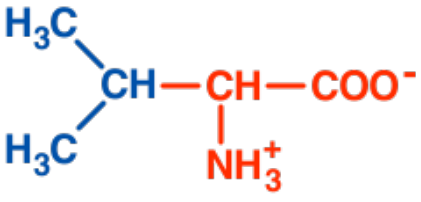
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Asparagine or aspartic acid	Asx	B
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	E
Glutamine or glutamic acid	Glx	Z
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V



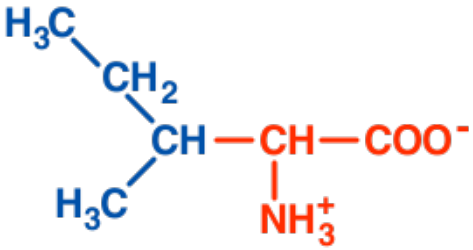
Amino Acids with aliphatic Side-Chains



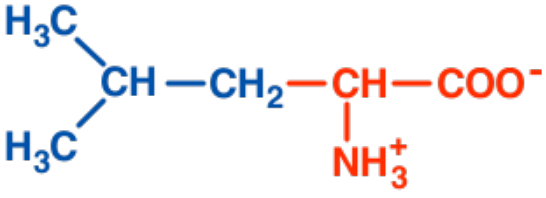
Ala (A)



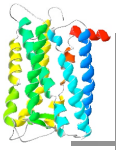
Val (V)



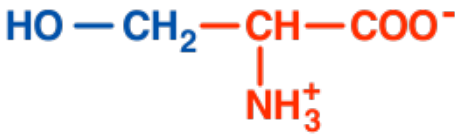
Ile (I)



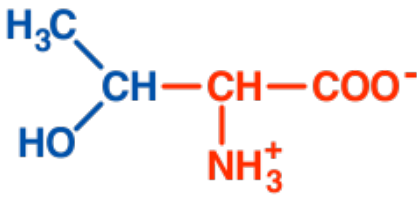
Leu (L)



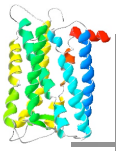
Sidechains with hydroxyl (-OH) groups



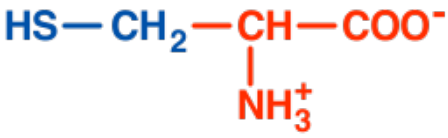
Ser (S)
pK_a=13



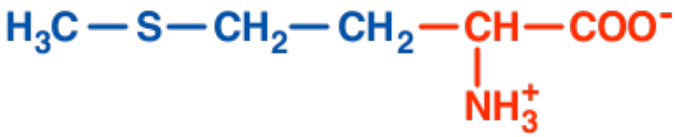
Thr (T)
pK_a=13



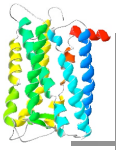
Sidechains containing sulphur



Cys (C)
pK_a=8.3

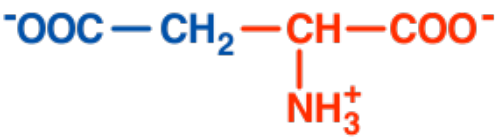


Met (M)

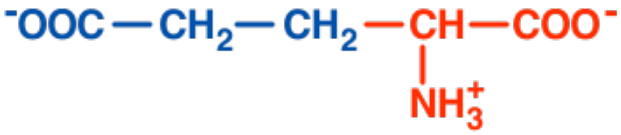


Acidic amino acids

Asp (D)
pK_a=3.9



Glu (E)
pK_a=4.1



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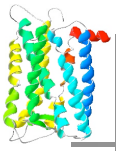
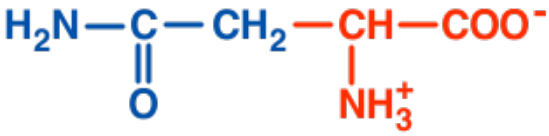
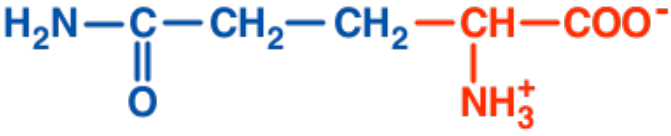


Amides of acidic amino acids

Asn (N)



Gln (Q)



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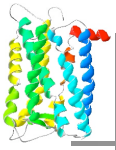
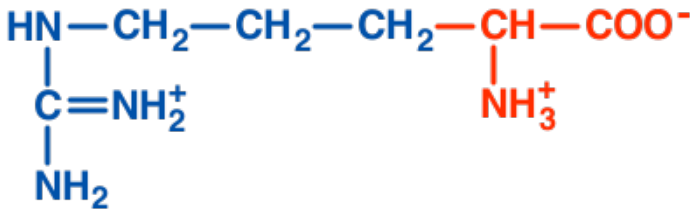
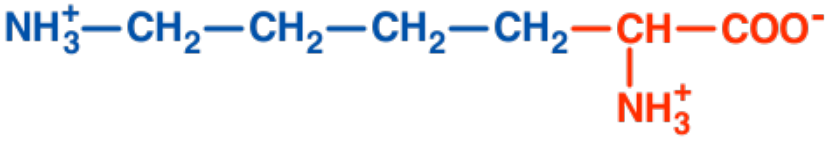


Basic Amino Acids

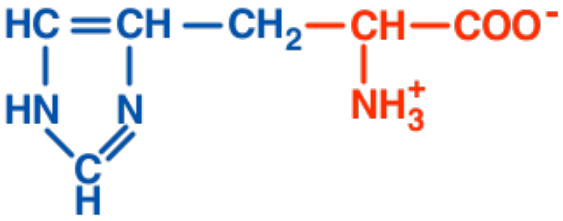
Arg (R)
pK_a=12.5



Lys (K)
pK_a=10.8



His (H)
pK_a=6.0



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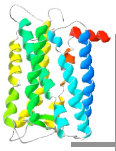
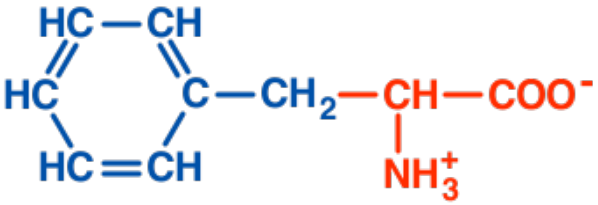
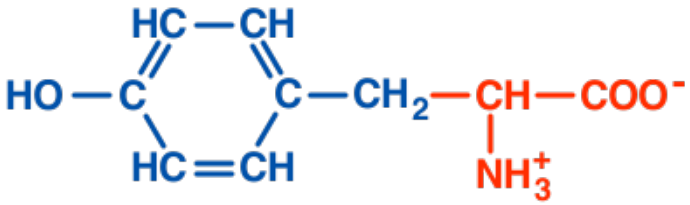


Side-chains with aromatic rings

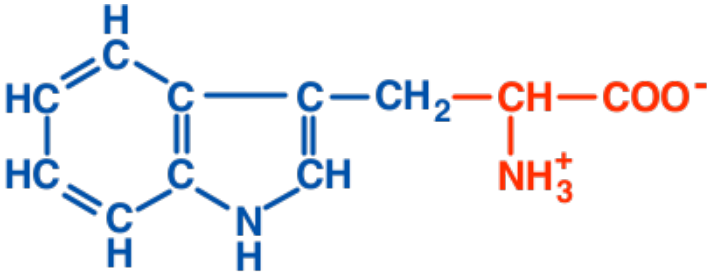
Phe (F)



Tyr (Y)
pK_a=10.1



Trp (W)



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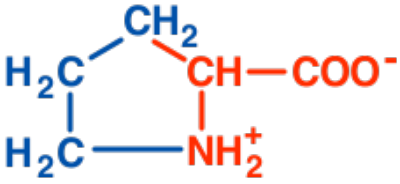
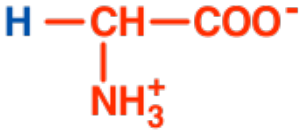
Special cases …

Pro (P)



Imino acid

Gly (G)



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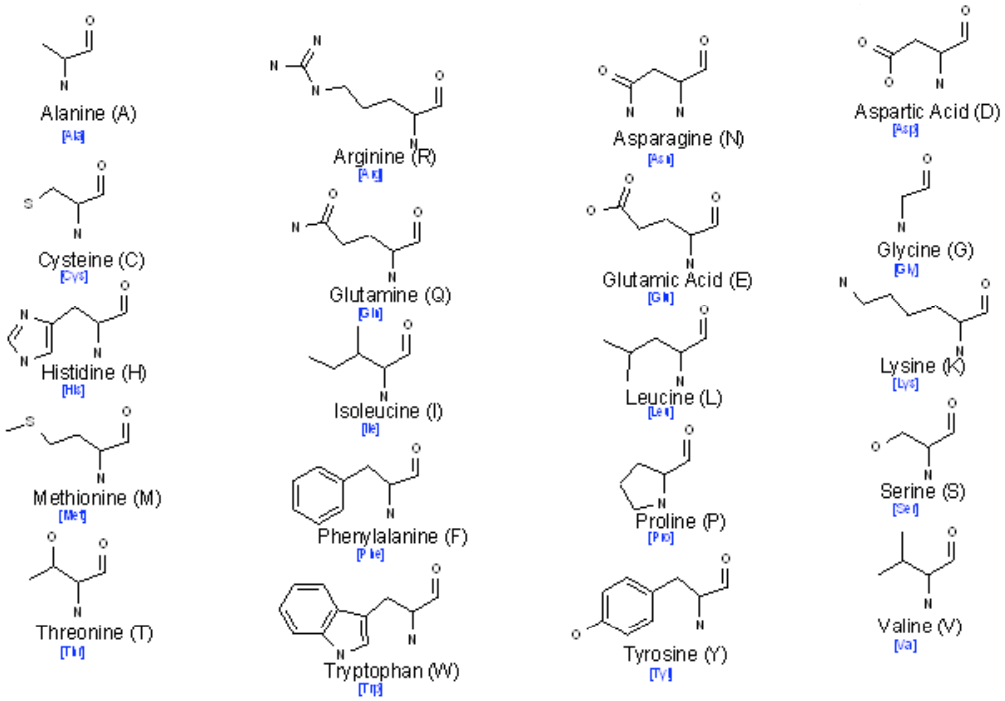
Side Chain Structures



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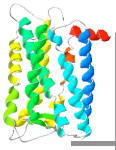


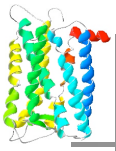
Physicochemical Properties of Amino Acid Side Chains (2)

Neutral Hydrophobic
Alanine
Valine
Leucine
Isoleucine
Proline
Tryptophane
Phenylalanine
Methionine

Neutral Polar
Glycine
Serine
Threonine
Tyrosine
Cysteine
Asparagine
Glutamine

Basic
Lysin
Arginine
(Histidine)

Acidic
Aspartic Acid
Glutamic Acid



pH and pKa

- pH

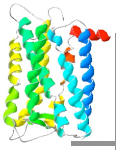
$$pH = -\log [H^+]$$

- Water ion product

$$Kw = [H^+]\,[OH^-] = 10^{-14}$$

$$\log[H^+] + \log[OH^-] = \log(10^{-14})$$

$$pH + pOH = 14$$



pH and pKa

- Dissociation of weak acids

$$HA \leftrightarrow H^+ + A^-$$

$$K_a = \frac{[H^+][A^-]}{[HA]} \qquad [H^+] = K_a \frac{[HA]}{[A^-]}$$

$$\log [H^+] = \log K_a + \log \frac{[HA]}{[A^-]}$$

$$-\log [H^+] = -\log K_a + \log \frac{[A^-]}{[HA]}$$

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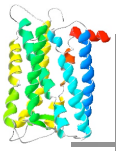

pH and pKa

- Henderson - Hasselbach Equation

$$pH = pKa + \log \frac{[A^-]}{[HA]}$$

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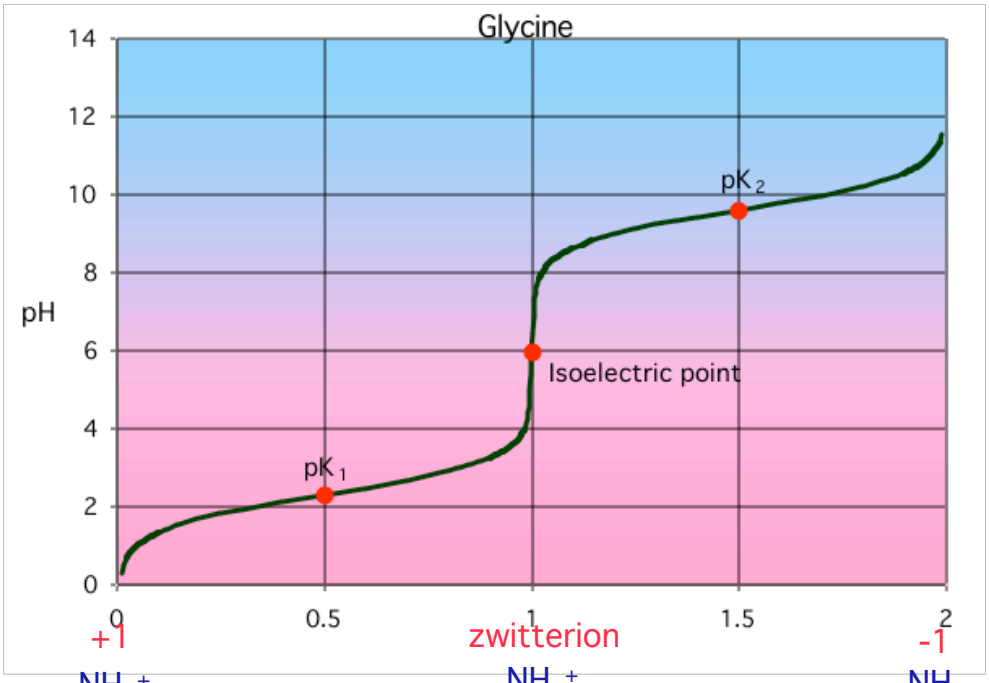
pH and pKa



+1 zwitterion -1

NH_3^+ NH_3^+ NH_2

COOH COO^- COO^-

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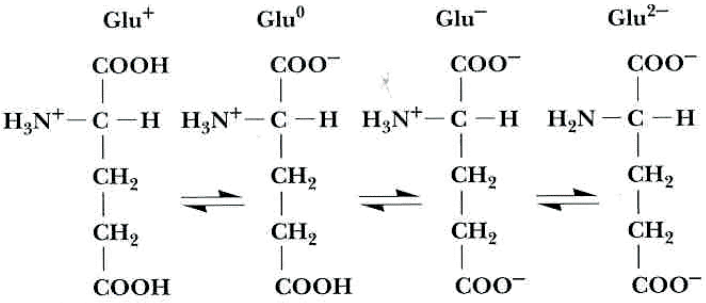
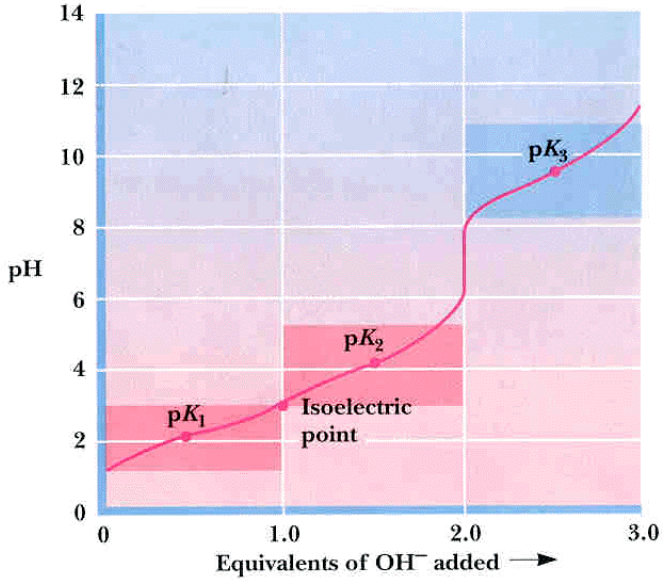
pH and pKa

- Glu





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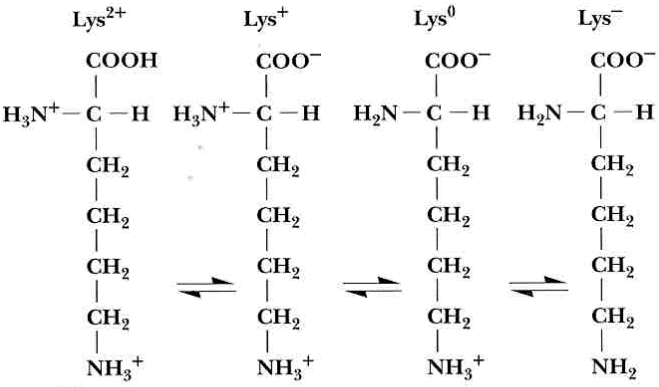
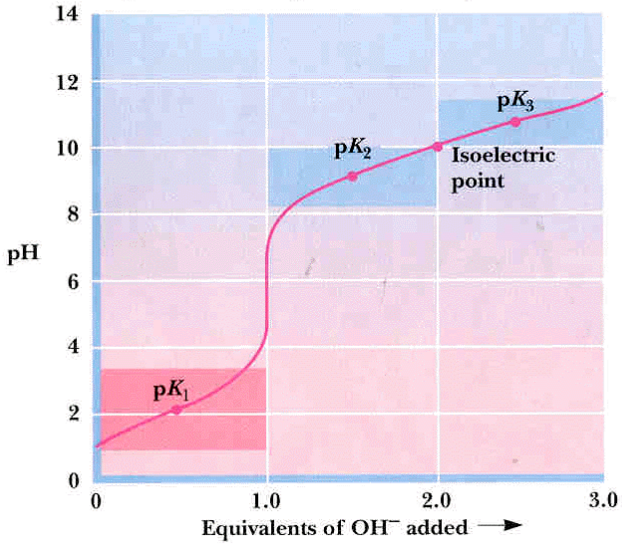
pH and pKa

- Lys





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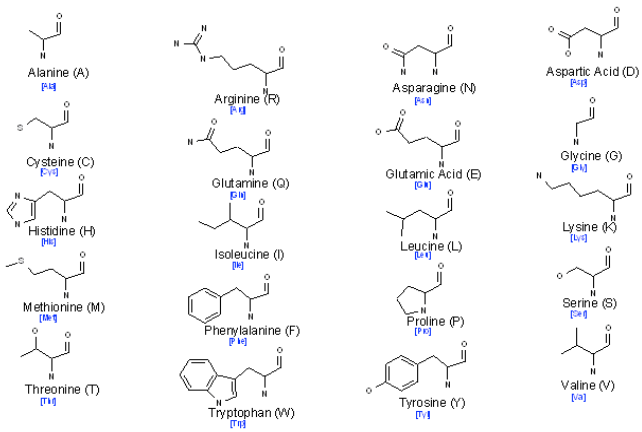
pH and pKa

- Enzymatic reactions often require proton transfer.
  - Q: Which amino-acid(s) are able to change their protonation state under physiological conditions?



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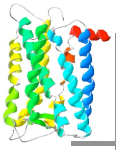
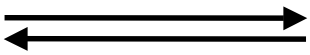
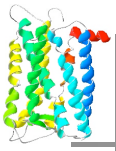


Protein Folding and Physicochemical Interactions (3)

Why do proteins fold?

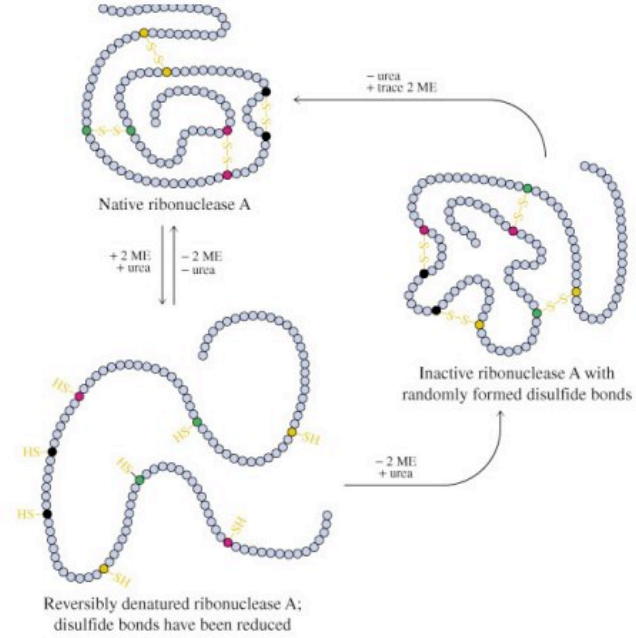
```
MNIFEMLRID EGLRLKIYKD TEGYYTIGIG
HLLTKSPSLN AAKSELDKAI GRNCNGVITK
DEAEKLFNQD VDAAVRGILR NAKLKPVYDS
LDAVRRCALI NMVFQMGETG VAGFTNSLRM
LQQKRWDEAA VNLAKSRWYN QTPNRAKRVI
TTFRTGTWDA YKNL
```





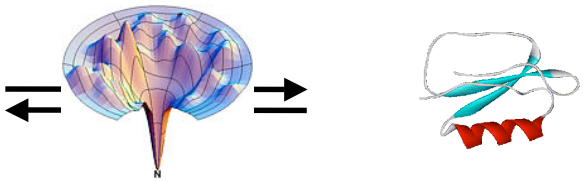
Anfinson's paradigm

- 1957, Nobel Prize 1972



Anfinson's paradigm

```
MNIFEMLRID EGLRLKIYKD TEGYYTIGIG
HLLTKSPSLN AAKSELDKAI GRNCNGVITK
DEAEKLFNQD VDAAVRGILR NAKLKPVYDS
LDAVRRCALI NMVFQMGETG VAGFTNSLRM
LQQKRWDEAA VNLAKSRWYN QTPNRAKRVI
TTFRTGTWDA YKNL
```



All the necessary information for the 3-dimensional structure of an enzyme is contained in the primary structure or sequence of the amino acids.

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Levinthal's Paradox (1968)

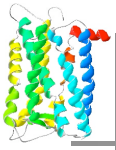
If a chain of a hundred amino acids is considered and it assumed each amino acid can exist in one of three conformations, extended, helical or loop, then there are 3^{100} possible ways to arrange this chain.

This is roughly 10^{48} conformations. Bond rotation can be estimated to occur at a rate of roughly 10^{14} s^{-1}. This means that search for the right conformation through random searching alone would take the order of *10^{34}s or 10^{26} years,* several orders of magnitudes greater than the age of the universe!

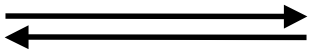
- [*J. Chim. Phys.*, **1968**, *85*, 44]

BIOZENTRUM

SIB



MNIFEMLRID EGLRLKIYKD TEGYYTIGIG
HLLTKSPSLN AAKSELDKAI GRNCNGVITK
DEAEKLFNQD VDAAVRGILR NAKLKPVYDS
LDAVRRCALI NMVFQMGETG VAGFTNSLRM
LQQKRWDEAA VNLAKSRWYN QTPNRAKRVI
TTFRTGTWDA YKNL

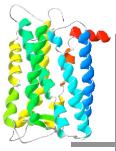


- Many proteins fold spontaneously to their native structure
- Protein folding is relatively fast (nsec – sec)
- Chaperones speed up folding, but do not alter the structure

The protein sequence contains all information needed to create a correctly folded protein.

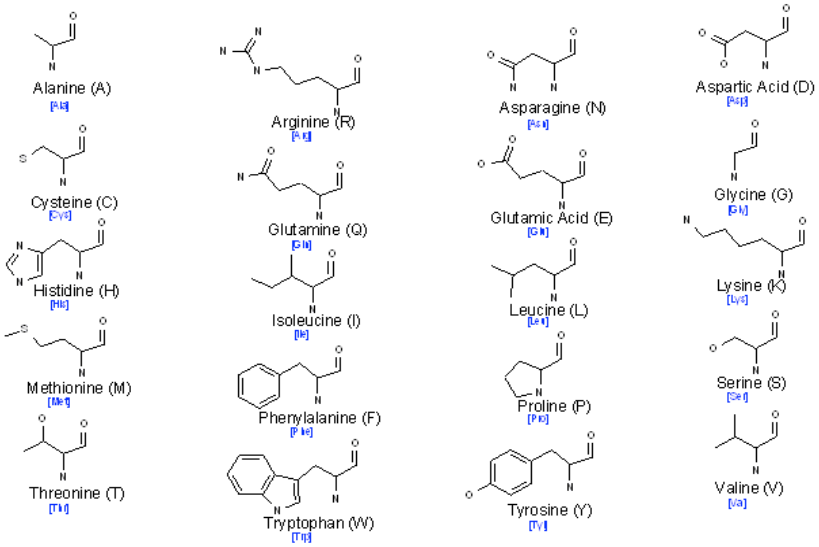
BIOZENTRUM

SIB



Why do proteins fold?

- N - $C_\alpha(HR_1)$ - CO - N - C_α (HR_2) - CO - N - $C_\alpha(HR_1)$ - CO -



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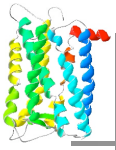


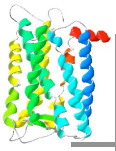
Side Chain Properties

Neutral Hydrophobic
Alanine
Valine
Leucine
Isoleucine
Proline
Tryptophane
Phenylalanine
Methionine

Neutral Polar
Glycine
Serine
Threonine
Tyrosine
Cysteine
Asparagine
Glutamine

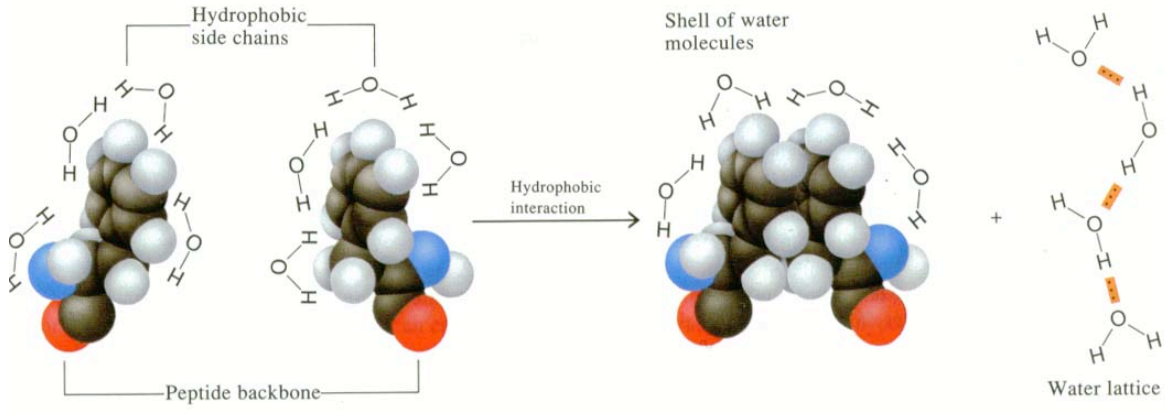
Basic
Lysin
Arginine
(Histidine)

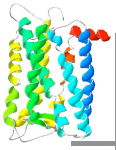
Acidic
Aspartic Acid
Glutamic Acid



Hydrophobic Effects

- main driving force for protein folding

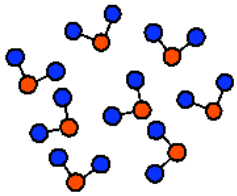


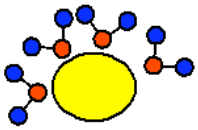


Hydrophobic Effects

- main driving force for protein folding

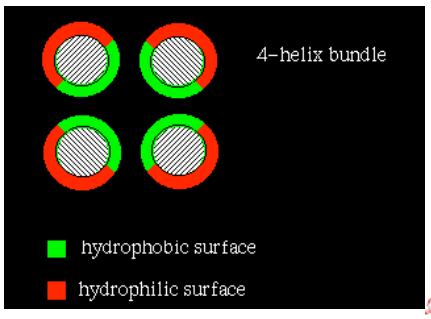
Water molecules in bulk water are mobile and can form H-bonds in all directions.





Hydrophobic surfaces don't form H-bonds. The surrounding water molecules have to orient and become more ordered.

The entropy loss can be minimized by gathering the hydrophobic surfaces together in the core of a protein and separating them from the solvent.



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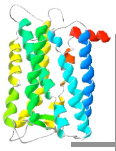

1D-Structure prediction

- Projection onto strings of structural assignments
  - E.g. "Solvent Accessibility"

```
A B C D E F G...
| | | | | | |
e e b b e e e...
```

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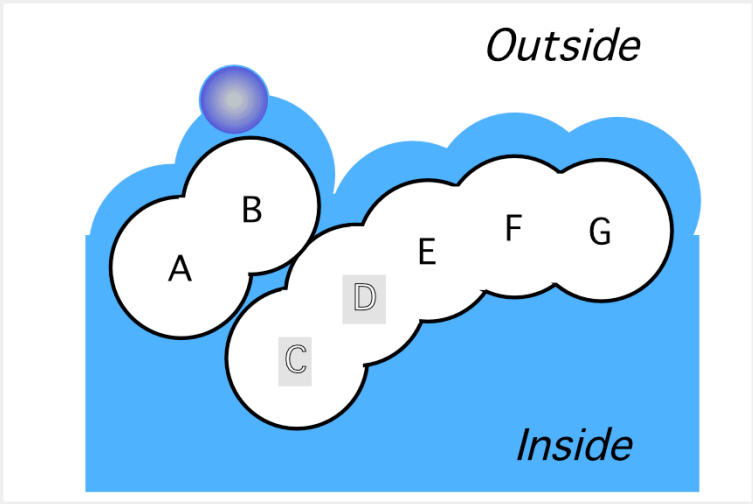
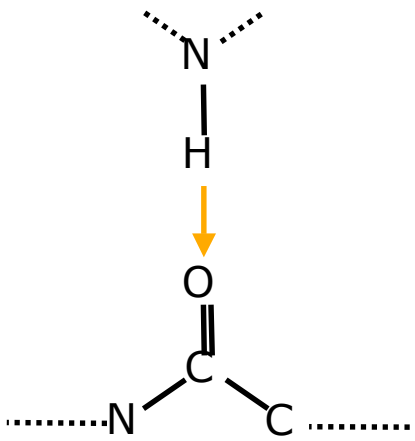
Surface Definitions

- Van der Waals Radius
- Molecular Surface
- Solvent Accessible Surface

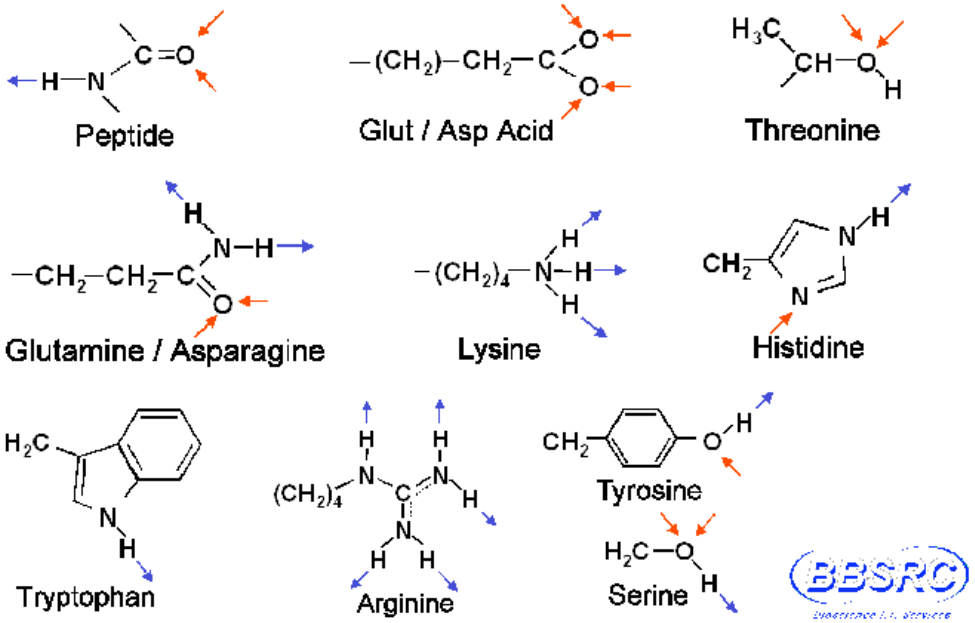


Hydrogen Bonds

- H-atoms bound to electronegative atoms (e.g. N, O) are polarized and can form H-bonds
- H-bonding partners include:
  - main chain atoms
  - side chain atoms
  - water molecules
  - ligands, etc…



Hydrogen bonding amino acids



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Energetics of protein folding

$\Delta G = \Delta H - T\Delta S$

Energetics of protein folding

H-bonds
hydrophobic effects
salt bridges
SS - bonds
loss of solvation
entropy change
dispersion / VdW contacts
conformational energy

- Difference of two very large energetic terms
- Low overall stabilization energy

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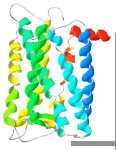
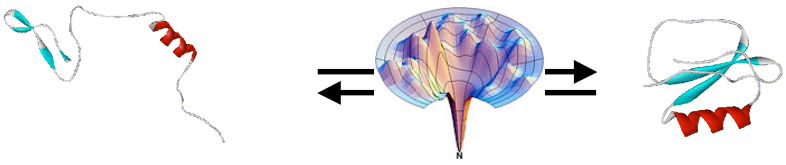
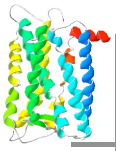


Why do proteins fold?



- Change of energy state from unfolded to folded
- Folded state must have overall lower energy
- Let's assume the folded state is the lowest possible state for this polypeptide



Protein Sequence Space

- How many different proteins are theoretically possible?
- How many of these have been tested during evolution?

Protein sequence space

- Assuming a peptide of length 100 aa

Possible combinations: $n_c = 20^{100} \approx 1.27 * 10^{130}$

Volume of one peptide:

$r_{atom} \approx 2\text{Å}$

$v_{atom} \approx 35\text{Å}^3$

$packing \approx 75\%$

$v_{peptide} \approx 1.3 * 10^5\ \text{Å}^3$

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Protein sequence space

- $1.27*10^{130}$ combinations. For comparison …

Volume of the Earth:

$R \approx 6.4 * 10^3\ \text{km} \approx 6.4 * 10^{16}\ \text{Å}$

$V = \frac{4}{3}\pi R^3 \approx 1.1 * 10^{51}\ \text{Å}^3$

Peptides/Earth:

$$n_p \approx \frac{1.1 * 10^{51}}{1.3 * 10^5} \approx 7.7 * 10^{45}$$

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Protein sequence space

- $1.27*10^{130}$ combinations. For comparison …

Age of the Earth: $3*10^{9}\,\text{years} \approx 2.6*10^{13}\,\text{hours} \approx 9.5*10^{16}\,\text{sec} \approx 9.5*10^{28}\,\text{psec}$

- If the whole planet consisted of peptides, and peptides were renewed every psec...

$$n_T \approx \left(7.7*10^{45}\right)*\left(9.5*10^{28}\right) \approx 7.3*10^{74}$$

Protein sequence space

- Assuming a peptide of length 100 aa

Possible combinations: $n_c = 20^{100} \approx 1.27*10^{130}$

- If the whole planet consisted of peptides, and peptides were renewed every psec...

$$n_T \approx \left(7.7*10^{45}\right)*\left(9.5*10^{28}\right) \approx 7.3*10^{74}$$

$$10^{130} - 10^{75} \approx 10^{130}$$?!?

Principles of Protein Structure (4)

- Primary Structure
- Secondary Structure
- Tertiary Structure
- Quaternary Structure

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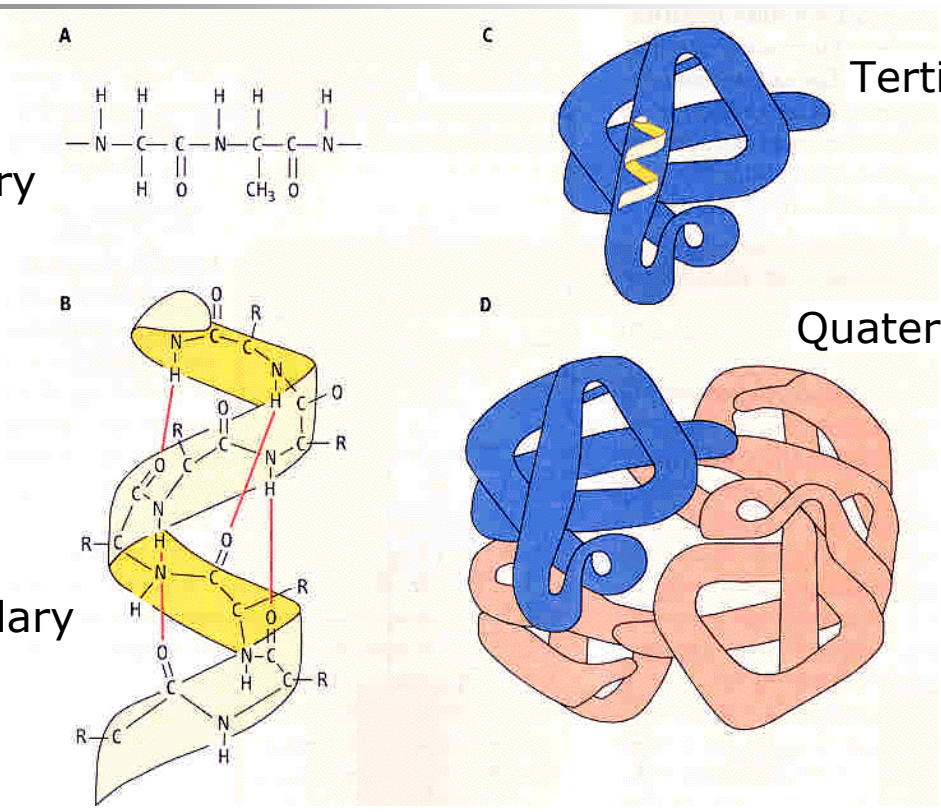
Principles of protein structure



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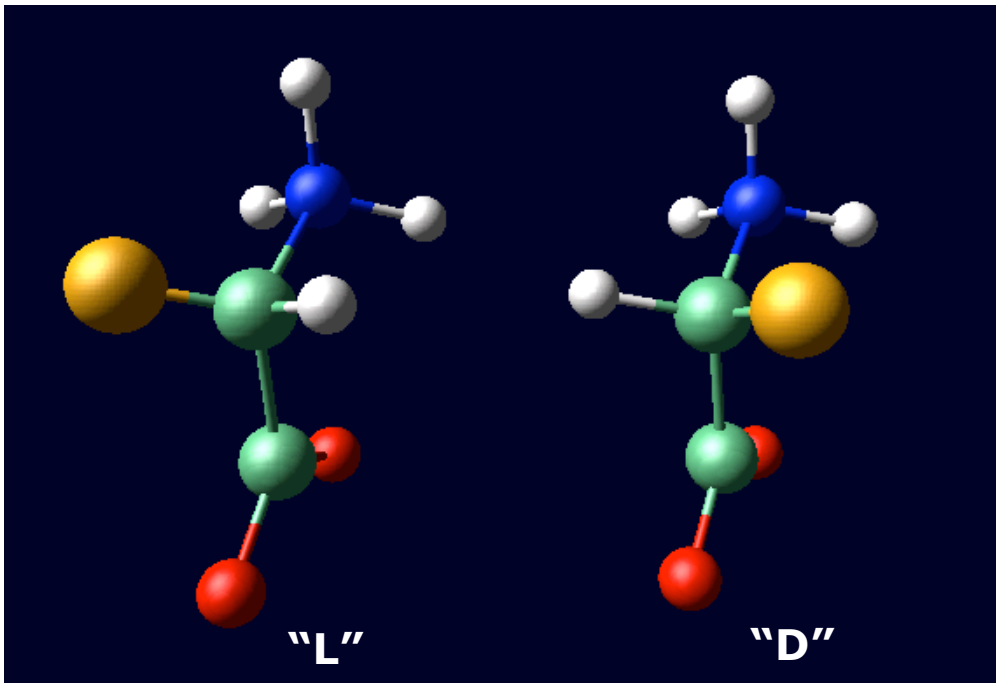
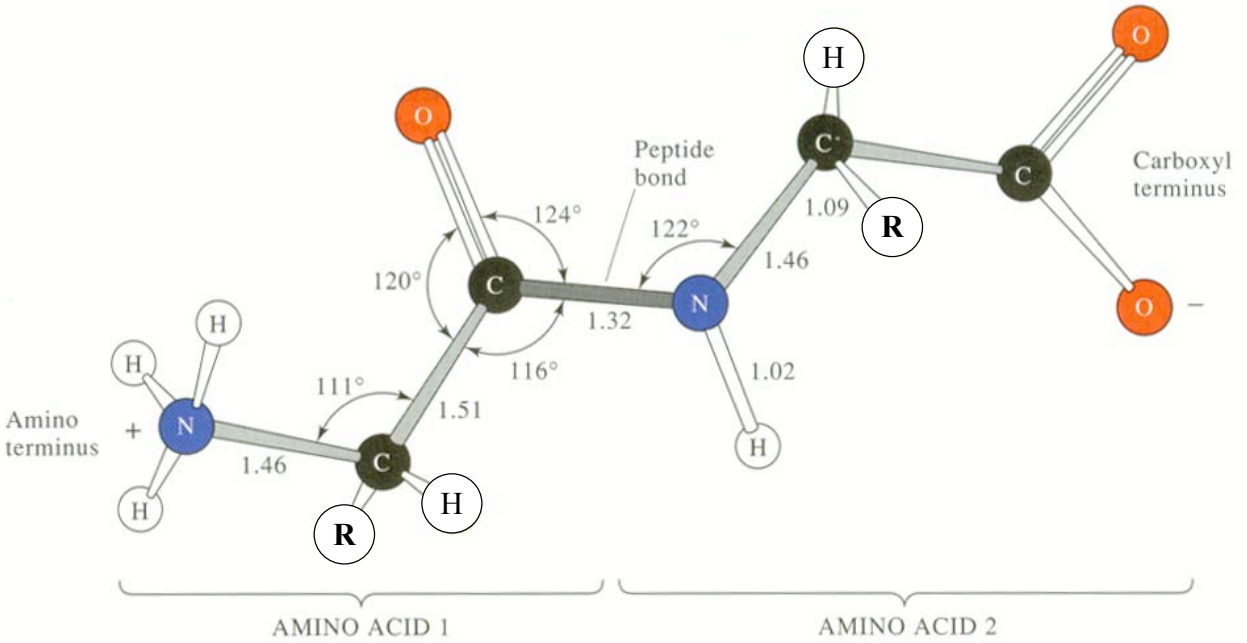
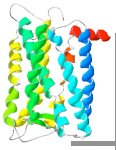
Stereochemistry: L- and D-amino acids



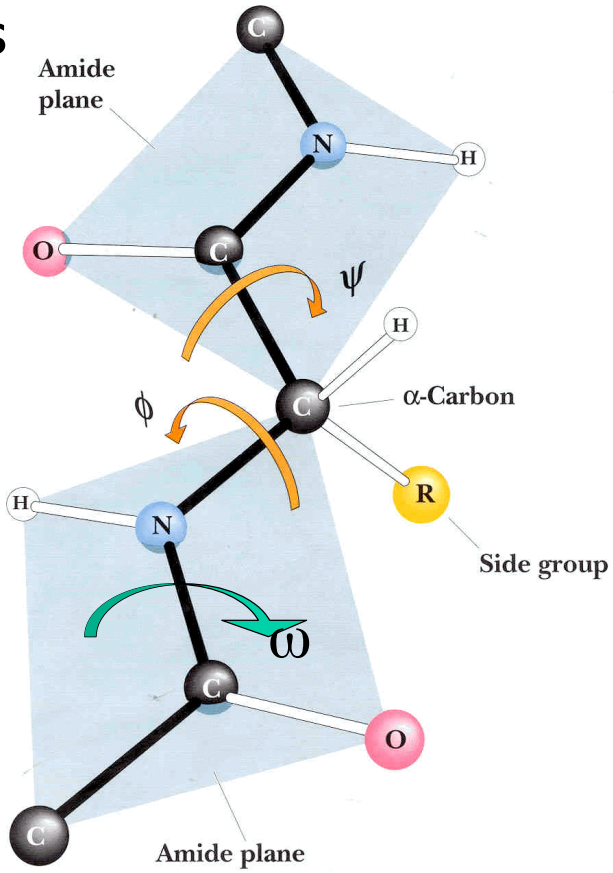
Geometry of a peptide bond





Dihedral angles

Φ, Ψ, and ω



Q: Which values would you you expect for ω?

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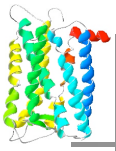
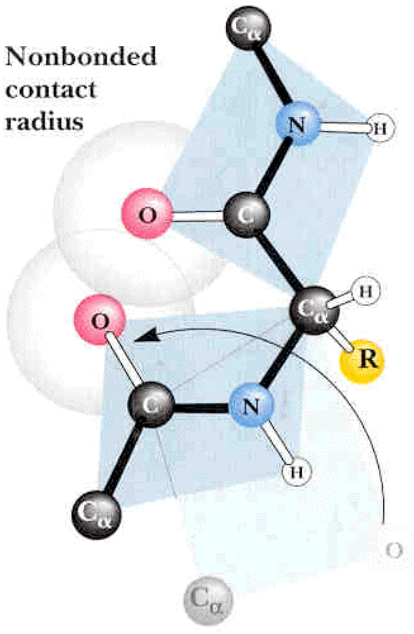
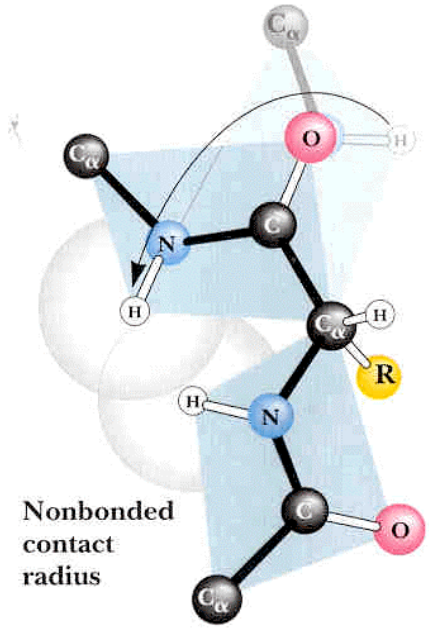


Dihedral angles Φ and Ψ



$\phi = 0°, \psi = 180°$

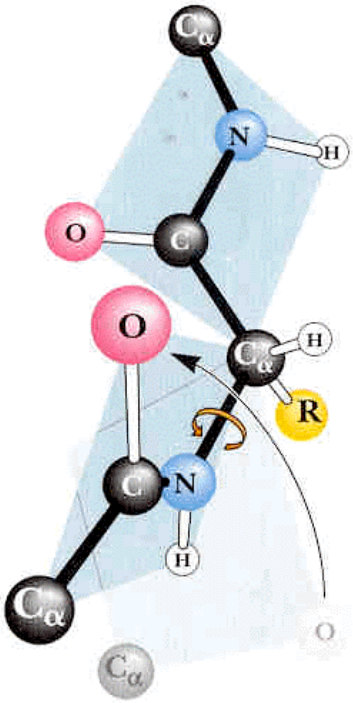


$\phi = 180°, \psi = 0°$

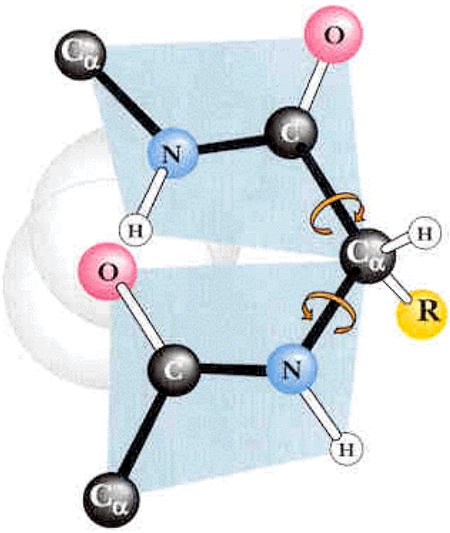
BIOZENTRUM

SIB

Dihedral angles Φ and Ψ

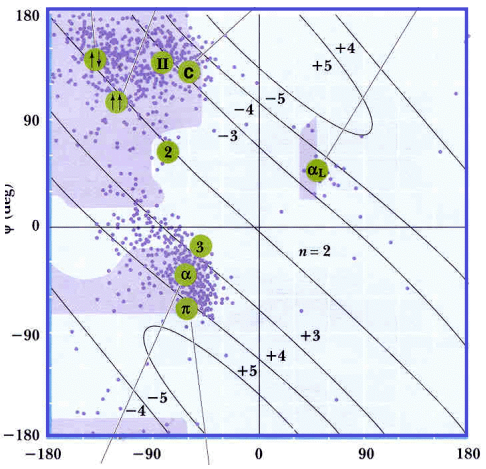


$\phi = -60°, \psi = 180°$



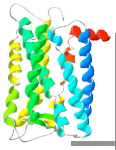
$\Phi = 0°, \Psi = 0°$

Ramachandran Plots

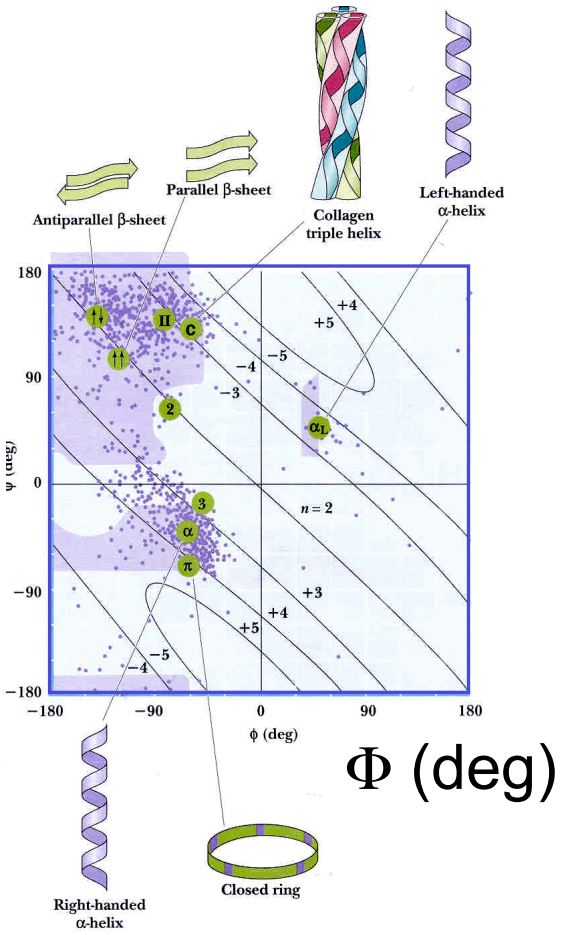


Ψ (deg)

Φ (deg)

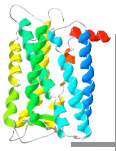


Ramachandran Plots



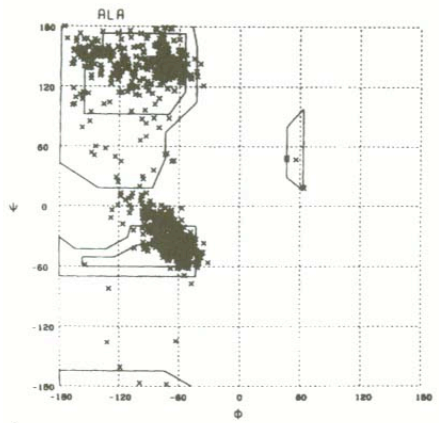
Ψ (deg)

Φ (deg)

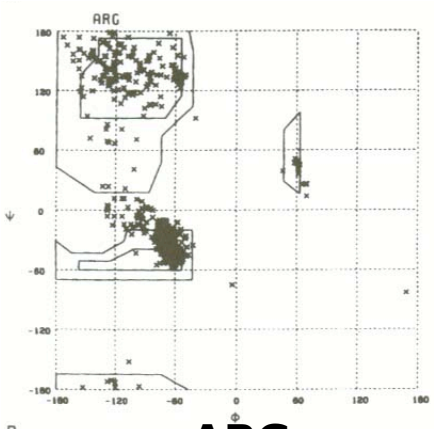


Amino acid preferences

- Alanine and Arginine



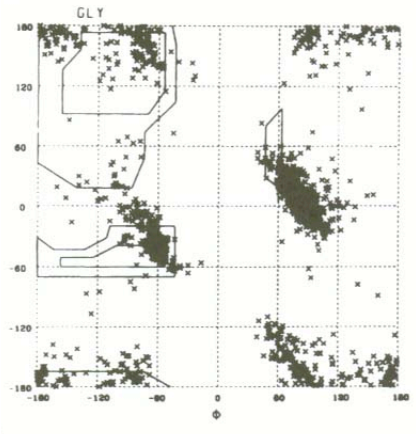
ALA



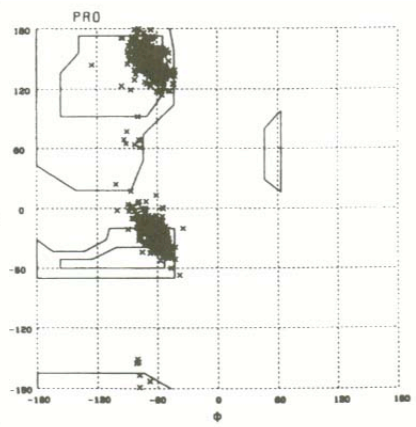
ARG

Amino acid preferences

- Amino acid with special preferences:



GLY



PRO

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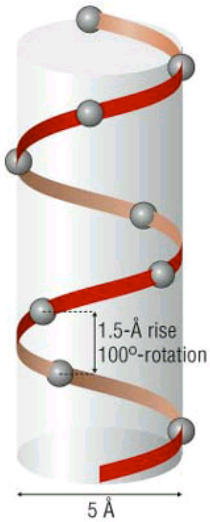
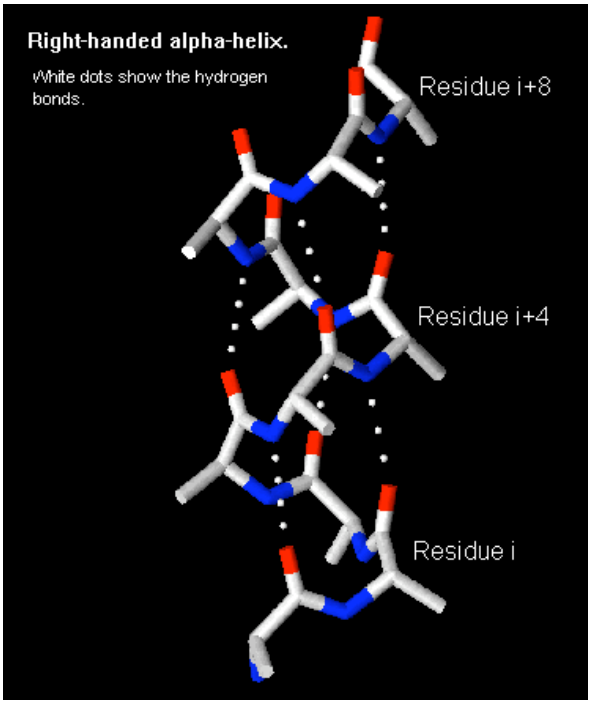
Alpha helices



Right-handed (clockwise) helical conformation



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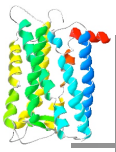
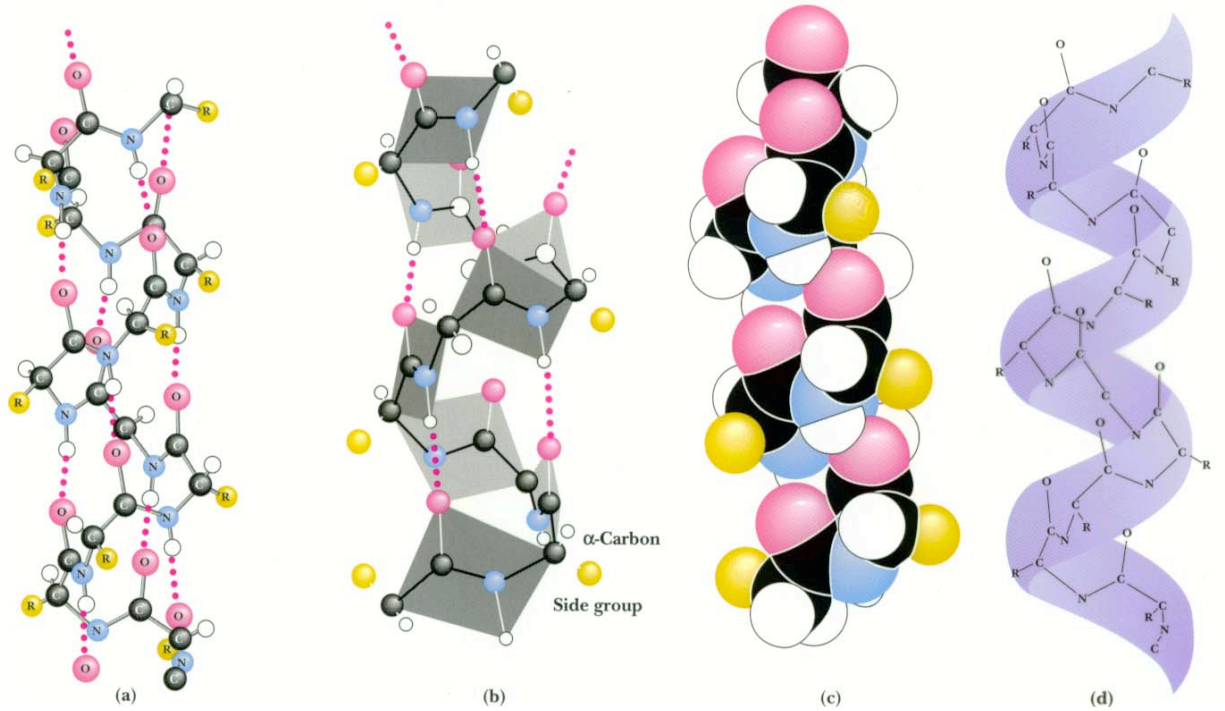


Alpha helices



BIOZENTRUM

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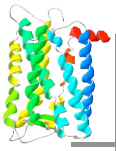
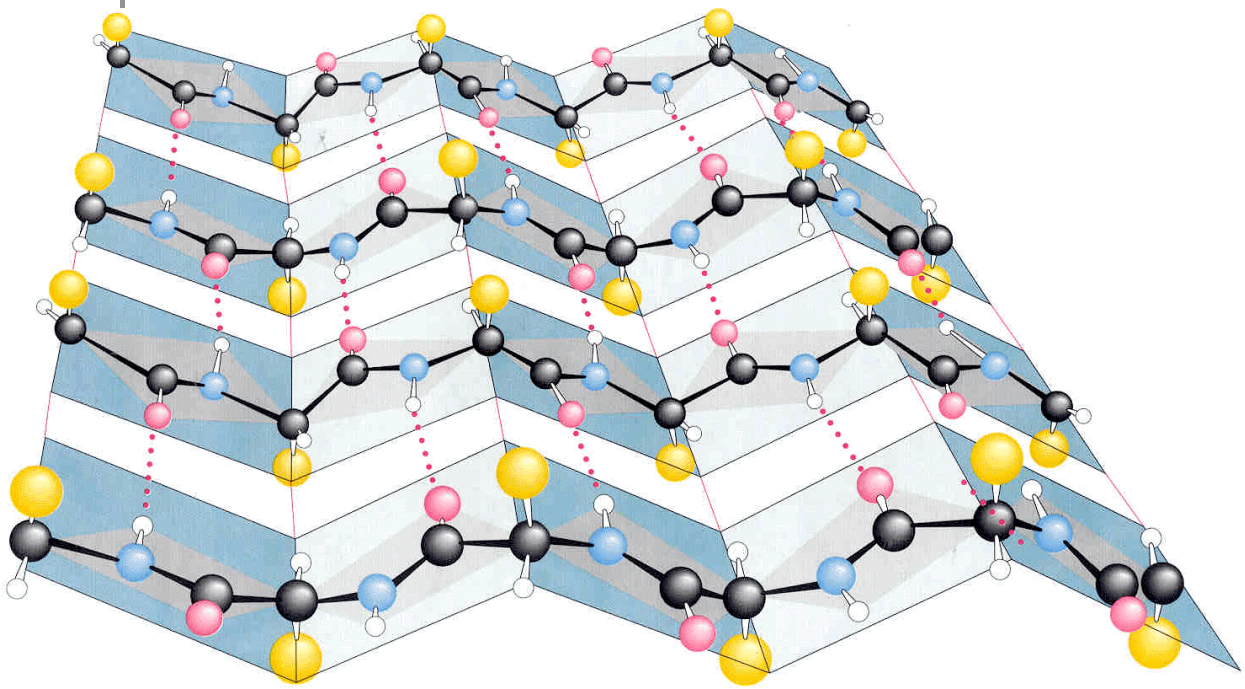


Beta strands / beta sheets



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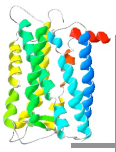
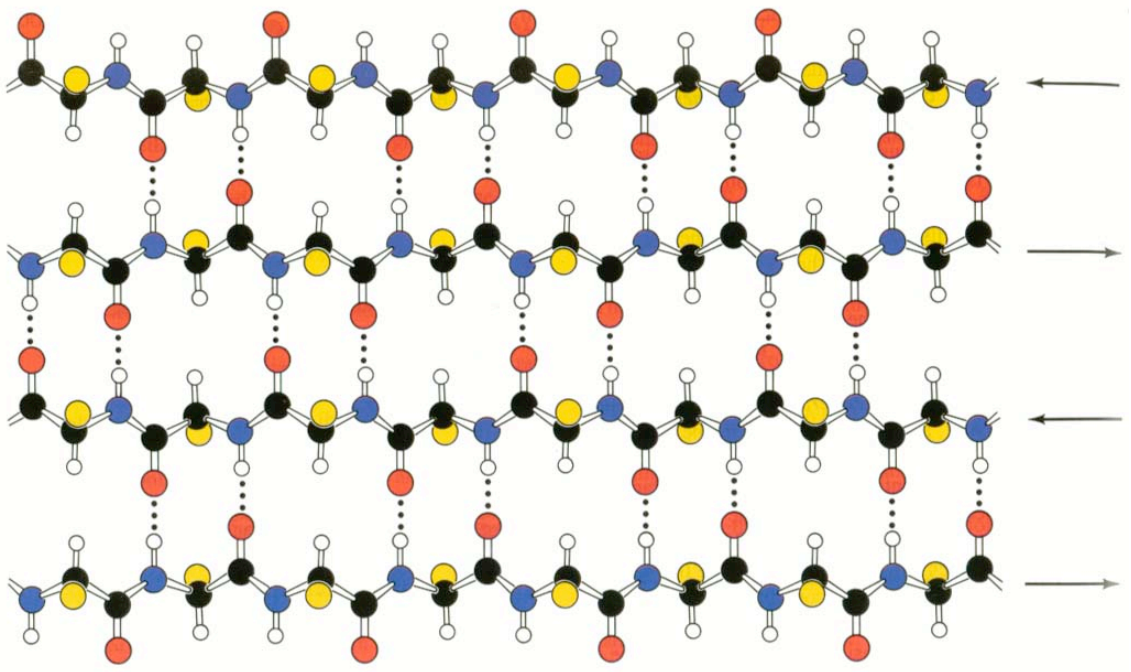


Anti-parallel beta sheet



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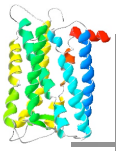
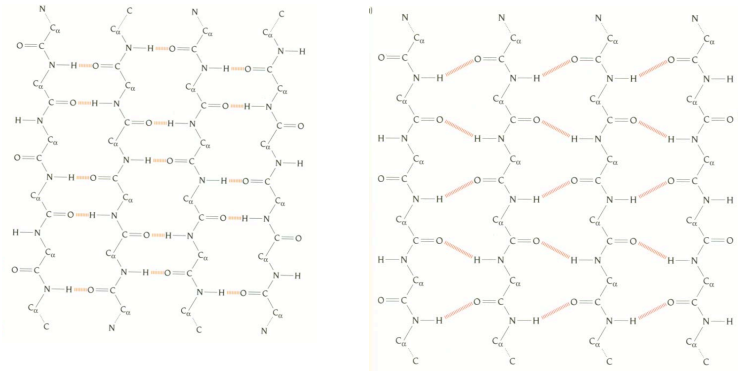
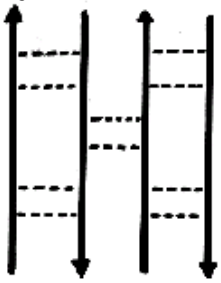
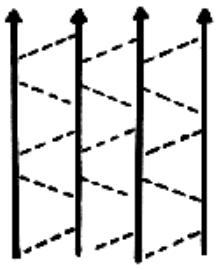


Parallel and anti-parallel beta sheets

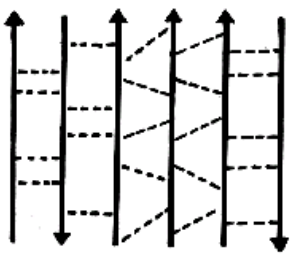


Antiparallel beta-sheet





Parallel beta-sheet



Mixed beta-sheet

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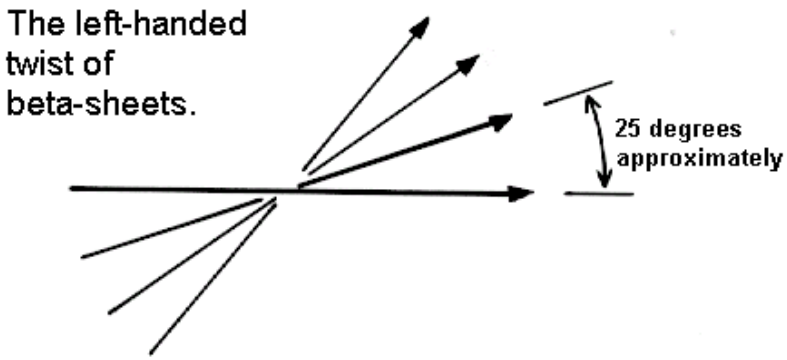
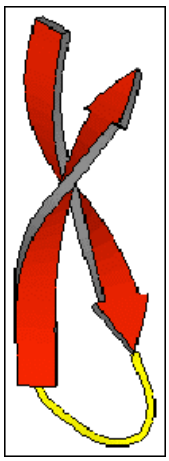
Left-handed twist in beta-sheets





Bovine pancreatic
trypsin inhibitor

0° - 30° per aa

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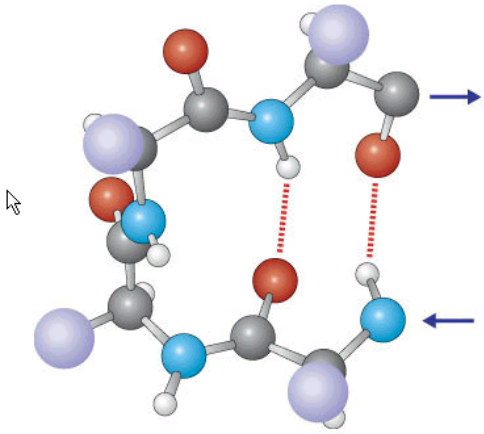
Turns and loops



Schematic diagram showing the interresidue backbone hydrogen bonds that stabilize the reversal of the chain direction. Side chains are depicted as large light purple spheres. Due to the tight geometry of the turn, some residues are found more commonly in turns than others.

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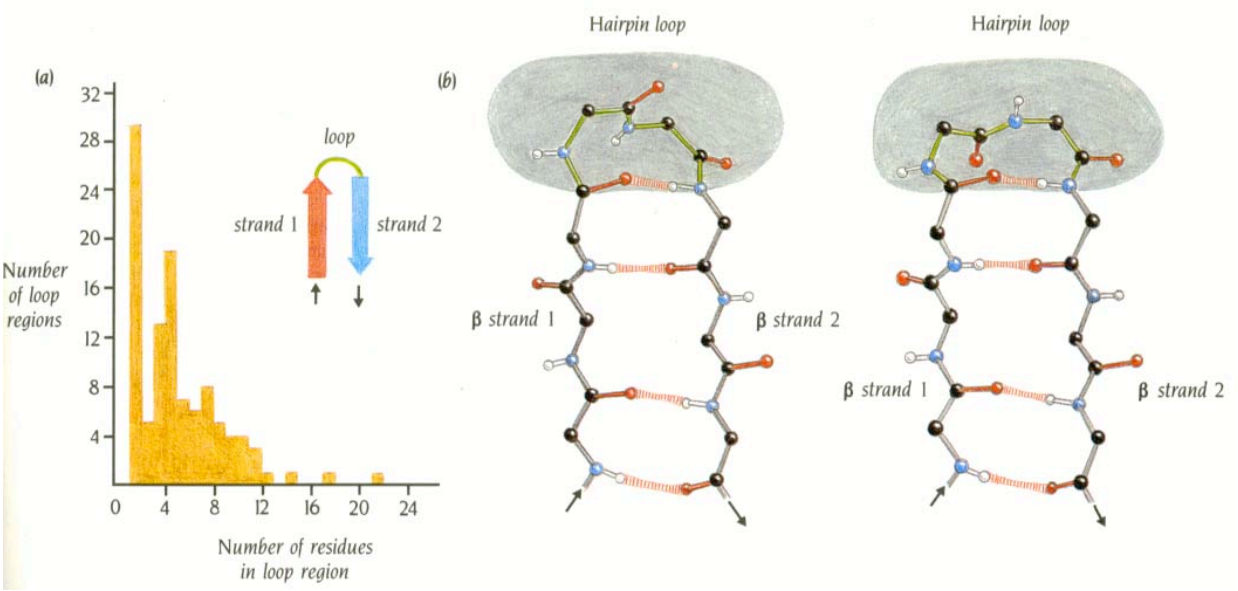
Turns and loops

Hairpin loops



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Conformational Preferences

Conformational Preferences of the Amino Acids

Amino acid	Preference α-helix	β-strand	reverse turn
Glu	**1.59**	0.52	1.01
Ala	**1.41**	0.72	0.82
Leu	**1.34**	1.22	0.57
Met	**1.30**	1.14	0.52
Gln	**1.27**	0.98	0.84
Lys	**1.23**	0.69	1.07
Arg	**1.21**	0.84	0.90
His	**1.05**	0.80	0.81
Val	0.90	**1.87**	0.41
Ile	1.09	**1.67**	0.47
Tyr	0.74	**1.45**	0.76
Cys	0.66	**1.40**	0.54
Trp	1.02	**1.35**	0.65
Phe	1.16	**1.33**	0.59
Thr	0.76	**1.17**	0.90
Gly	0.43	0.58	**1.77**
Asn	0.76	0.48	**1.34**
Pro	0.34	0.31	**1.32**
Ser	0.57	0.96	**1.22**
Asp	0.99	0.39	**1.24**

α

β

RT

Biochimica et Biophysica Acta 916: 200-204 (1987).

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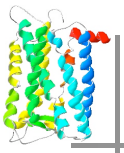
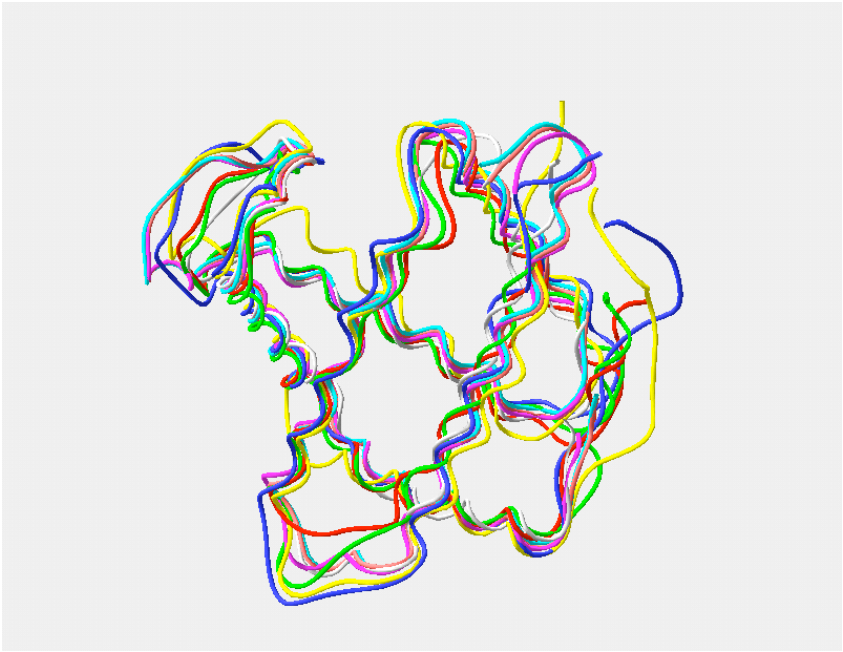
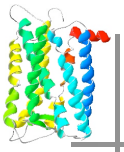


Pair Wise Structure Comparison (5)



BIOZENTRUM

SIB



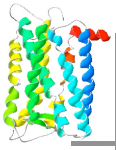
Pair Wise Structure Comparison

- Root mean square deviation
  - Comparing two structures A and B
  - $R_{i,A}$ = Position of atom i in structure A
  - n = Number of equivalent atoms

$$r.m.s.d. = \sqrt{\frac{\sum_{i=0}^{n} (R_{i,A} - R_{i,B})^2}{n}}$$

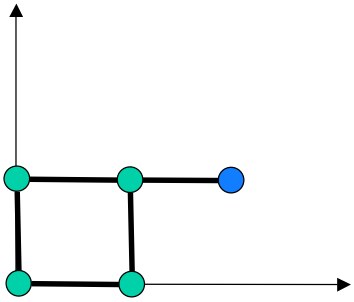
BIOZENTRUM

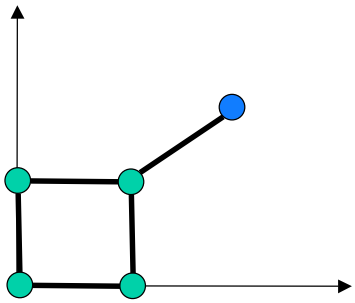
SIB



RMSD

- Comparing two structures

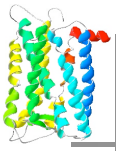




Min: $$r.m.s.d. = \sqrt{\frac{\sum_{i=0}^{n}(R_{i,A} - R_{i,B})^2}{n}}$$

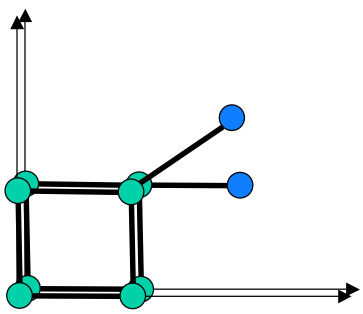
BIOZENTRUM

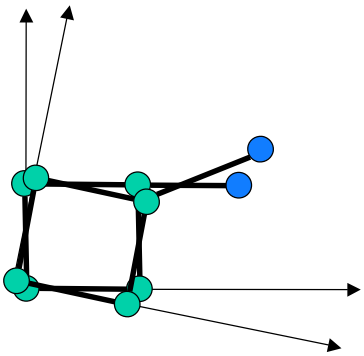
SIB



RMSD

- Comparing two structures





BIOZENTRUM

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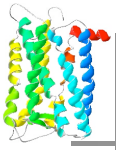

Pair wise protein structure comparison methods

•(Iterative) Least squares superposition (e.g. LSQ-Kabsch)

- uses RMSD (root mean square deviation) as similarity measure
- useful if sequence alignment is unambiguous
- only useful for similar structures (rmsd < 5 Å)

•DALI (Distance Matrix Alignment)

- http://www.ebi.ac.uk/dali/
- Represents structures as 2-dimensional array of distances between Cα positions
- Compare two structures by overlapping these arrays
- Similarity expressed as z-score vs. database background distribution

•CE (Combinatorial Extension of the optimal path)

- http://cl.sdsc.edu/ce.html
- start by finding octameric fragments in both structures that can be overlapped
- extend aligned fragment pairs (AFPs) to find optimal alignment
- Similarity expressed as z-score vs. database background distribution

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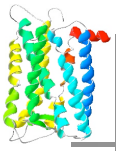
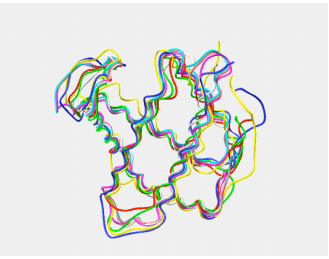
SIB



Structural Alignments



- **Protein Structure is better conserved than sequence**
- Structural alignments establish equivalences between amino acid residues based on the 3D structures of two or more proteins
- Structure alignments therefore provide information not available from sequence alignment methods
- Structural alignments can be used to guide sequence alignments (see: T_COFFEE / SAP)

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Protein Structure Databases (6)

- PDB
- EBI-MSD
- SCOP
- CATH

RCSB PDB PROTEIN DATA BANK

PDB Current Holdings

PDB Home Contact us

This page contains:

PDB Holdings List: Breakdown of the contents of the PDB, following the latest update
PDB Content Growth: Data on the growth in the number of structures in the PDB

PDB Holdings List: 13-Apr-2004

		Molecule Type				
		Proteins, Peptides, and Viruses	Protein/Nucleic Acid Complexes	Nucleic Acids	Carbohydrates	total
Exp. Tech.	X-ray Diffraction and other	19696	969	727	14	21406
	NMR	3031	97	577	4	3709
	Total	22727	1066	1304	18	25115

BIOZENTRUM SIB

Fold Classification Databases

- Number of folds is limited
  - according to C. Chothia ca. 1000 – 5000 folds

Fold databases systematically classify protein structure folds

- **FSSP**
  - *Fold classification based on Structure-Structure alignment of Proteins*
  - http://www2.ebi.ac.uk/dali/fssp/
  - Contains structurally aligned proteins based in DALI
- **SCOP**
- **CATH**

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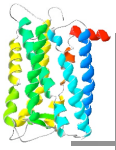
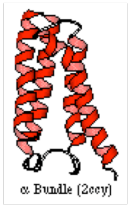
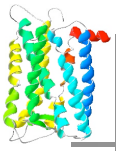
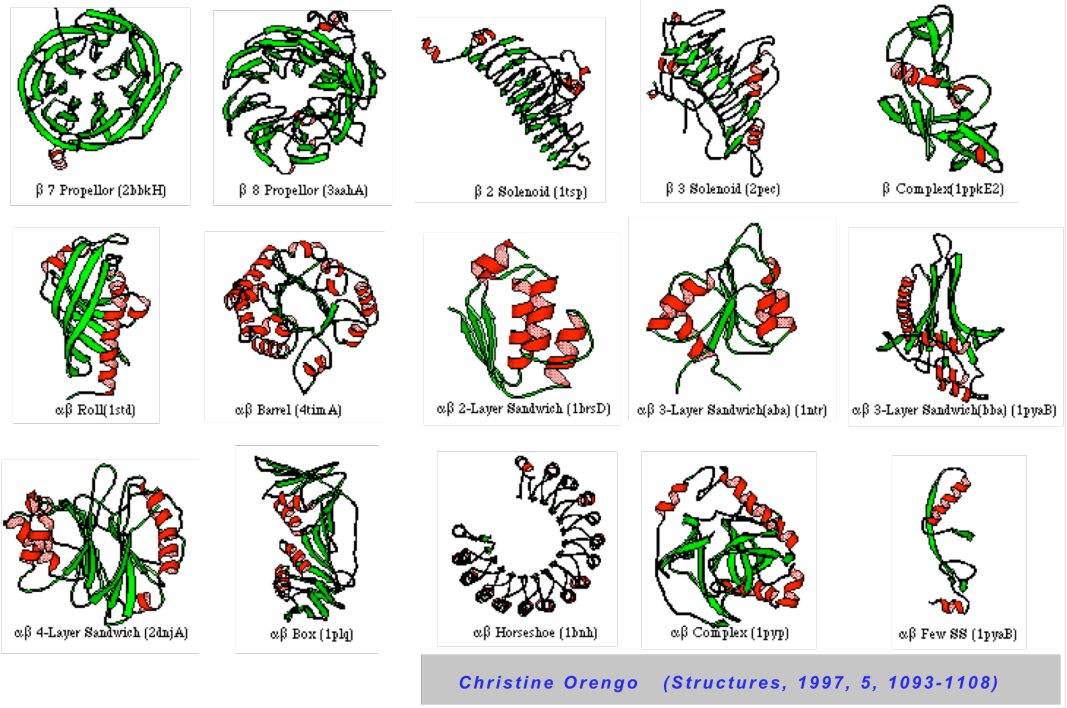




Christine Orengo (Structures, 1997, 5, 1093-1108)





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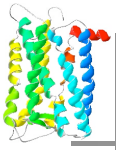
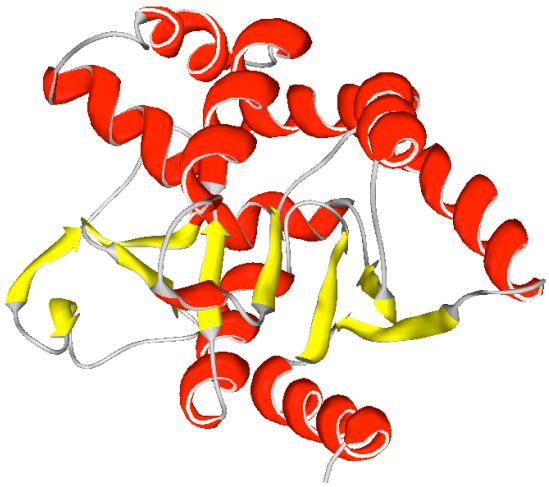
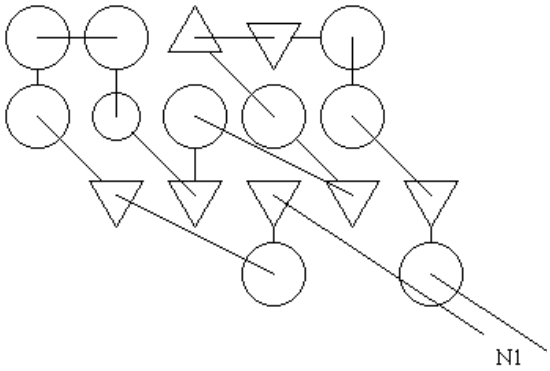


Topology Cartoons

- TOPS: Topology of Protein Strcuture
  - http://www.tops.leeds.ac.uk/





BIOZENTRUM

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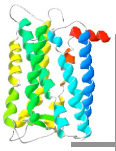
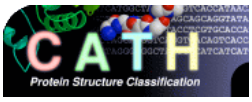


CATH - Protein Structure Classification

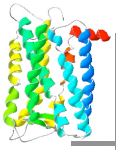


- Hierarchical classification of protein domain structures
- UCL, Janet Thornton & Christine Orengo
- clusters proteins at four major levels:
  - Class(C)
  - Architecture(A)
  - Topology(T)
  - Homologous superfamily (H)

[http://www.biochem.ucl.ac.uk/bsm/cath_new/]

BIOZENTRUM

SIB



CATH - Protein Structure Classification

- **Class(C)**

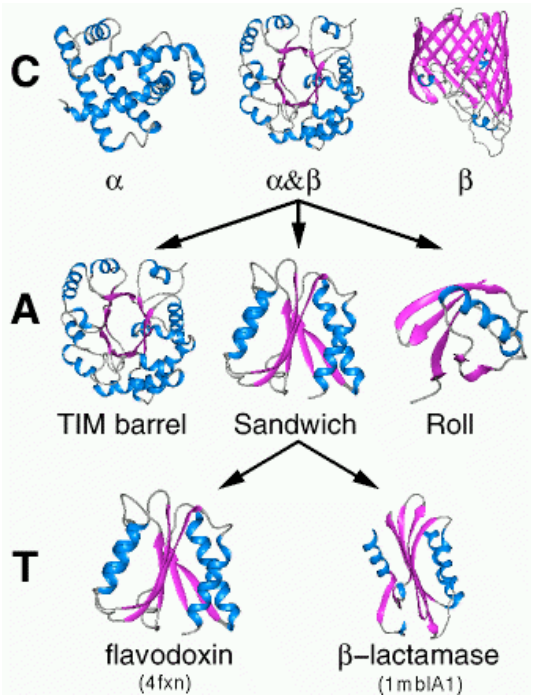
  derived from secondary structure content is assigned automatically

- **Architecture(A)**

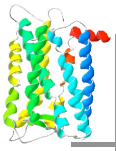
  describes the gross orientation of secondary structures, independent of connectivity.

- **Topology(T)**

  clusters structures according to their topological connections and numbers of secondary structures



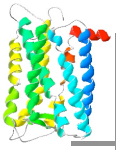
[http://www.biochem.ucl.ac.uk/bsm/cath_new/]



SCOP - Structural Classification of Proteins

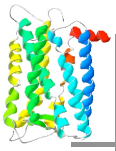
- MRC Cambridge (UK), Alexey Murzin, Brenner S. E., Hubbard T., Chothia C.
- hierarchical classification of protein domain structures
- created by manual inspection
- comprehensive description of the structural and evolutionary relationships
- organized as a tree structure
  - Class
  - Fold
  - Superfamily
  - Family
  - Species

[http://scop.mrc-lmb.cam.ac.uk/scop/]



Protein Structure / Fold Databases

- PDB: http://www.pdb.org
- EBI-MSD http://www.ebi.ac.uk/msd/
- SCOP http://scop.mrc-lmb.cam.ac.uk/scop/
- CATH http://www.biochem.ucl.ac.uk/bsm/cath_new/



References

- I. Branden, J. Tooze. Introduction to Protein Structure, Garland Publishing.
- P.E.Bourne, H. Weissig. Structural Bioinformatics, Wiley-Liss and Sons.
- G.A. Petsko, D. Ringe. Protein Structure and Function, New Science Press.