Structural bioinformatics KFC/STBI

What is structural bioinformatics?

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Requirements

- Project:
  - Structure analysis, docking, comparison of proteins, prediction of properties from structure, ...
  - 1(max. 2) page-long report with
    - Hypothesis
    - Brief Methodology
    - Conclusions

  ev. ChannelsDB – doplnění 5 struktur do databáze
- Exam:
  - Project-like Questions – problem + discussion about its possible resolution from you side

Content

- Structural bioinformatics, Biomolecules, Structural hierarchy
- Structure determination (X-Ray,NMR,EM), Structure file formats
- Structural databases (PDB, CATH, SCOP, Drugbank)
- Vizualization of structure, structural alignment
- Structure prediction, CASP, AlphaFold ML revolution
- Function prediction, CASA
- Binding prediction – protein-ligand and protein-protein docking
- Challenges of structural bioinformatics - membrane proteins, nucleic acids, protein-protein interactions prediction
- Examples: SARS-CoV-2, Switchable proteins

Bioinformatics

(Molecular) **bio** – informatics: bioinformatics is conceptualising **biology in terms of molecules** (in the sense of physical chemistry) and applying "***informatics techniques***" (derived from disciplines such as applied maths, computer science and statistics) to understand and ***organise*** the ***data and information*** associated with these molecules, on a ***large scale***.

In short, bioinformatics is a management information system for molecular biology and has many ***practical applications***.

Oxford English Dictionary

Structural bioinformatics

Use of structure

- Databases, classification
  - proteins, NA, drugs
- Patterns
  - Active sites, allosteric sites, ...
- Prediction
  - structure, function, active site, channels…
- Docking
  - Fitting of small molecules into the active site
    -> in silico drug design
- Simulations
  - What if…

Problems of structural bioinformatics

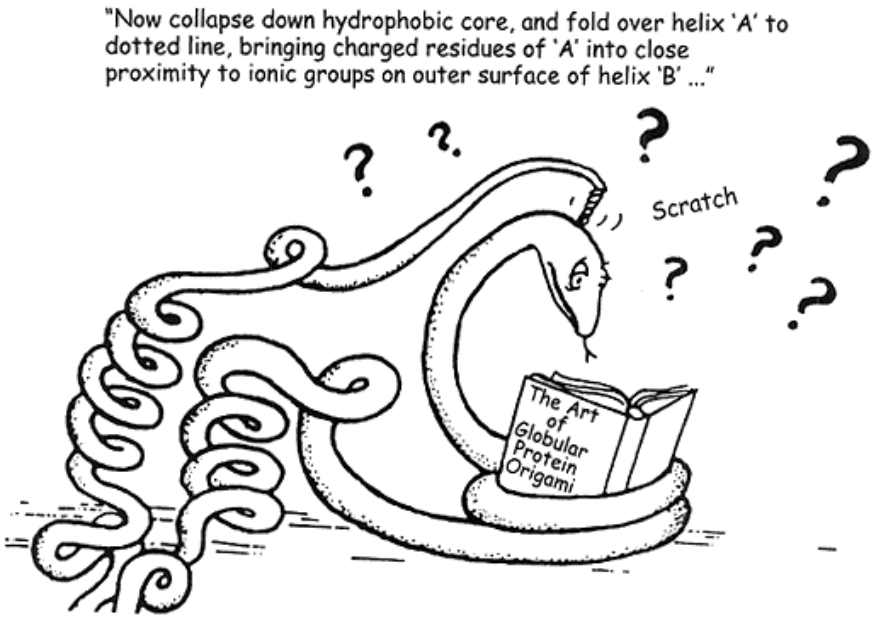
- Structural data are hard to work with:
  - Nonlinear
  - Imprecise from experiment (resolution of structure)
  - 3D representation (3D search)
  - Visualization is not trivial
  - More conserved than sequence data (genomics)
  - Structural genomics prepare structures without annotation
  - Most structures are water soluble globular proteins (most drug targets are membrane proteins)

Challenges

- Target selection
  - Large structures are resource intensive, maybe just one domain might be enough
- Structure methods
  - XRay – crystalisation is not easy
  - NMR – size problem – indistinguishable peaks
  - EM – only recently with atomistic detail
- Validation and Annotation
- Databases
- Correlation of structural data with experimental data

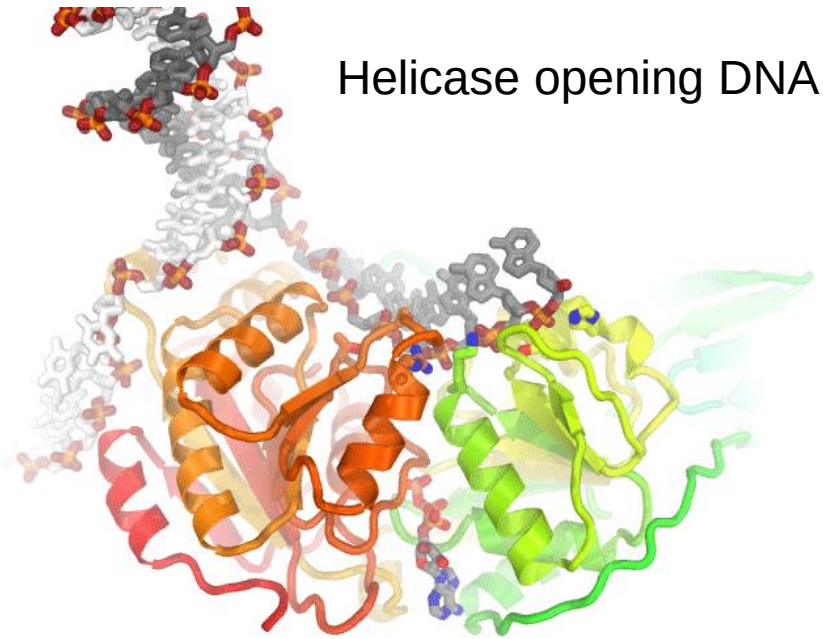
Example 1 : Prediction of protein structure

- Tertiary structure
  - Fold recognition
  - Homolog modelling
    - Structural alignment
  - ab initio modelling
  - ML methods
- Function prediction
  - active sites, channels, pores, allosteric sites, conformations…



Reproduced in U. Tollemar, "*Protein Engineering i USA*", Sveriges Tekniska Attachéer, 1988

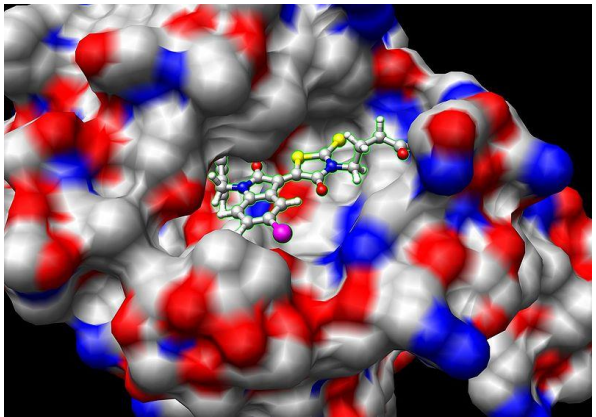
Example 2: Molecular graphics



Helicase opening DNA

- We make nice figs!
- Simulations
  - Structure => Energy
  - Time => Dynamics

- Docking – binding
  - ligands
  - Protein-protein



GOLD docking of compound to acetyltransferase

Structure Description

Coordinate systems

- XYZ (cartesian)
- Inner coordinates (bond lengths, bond angles, torsion angles)
- object representation (secondary structure)

Structure comparison:

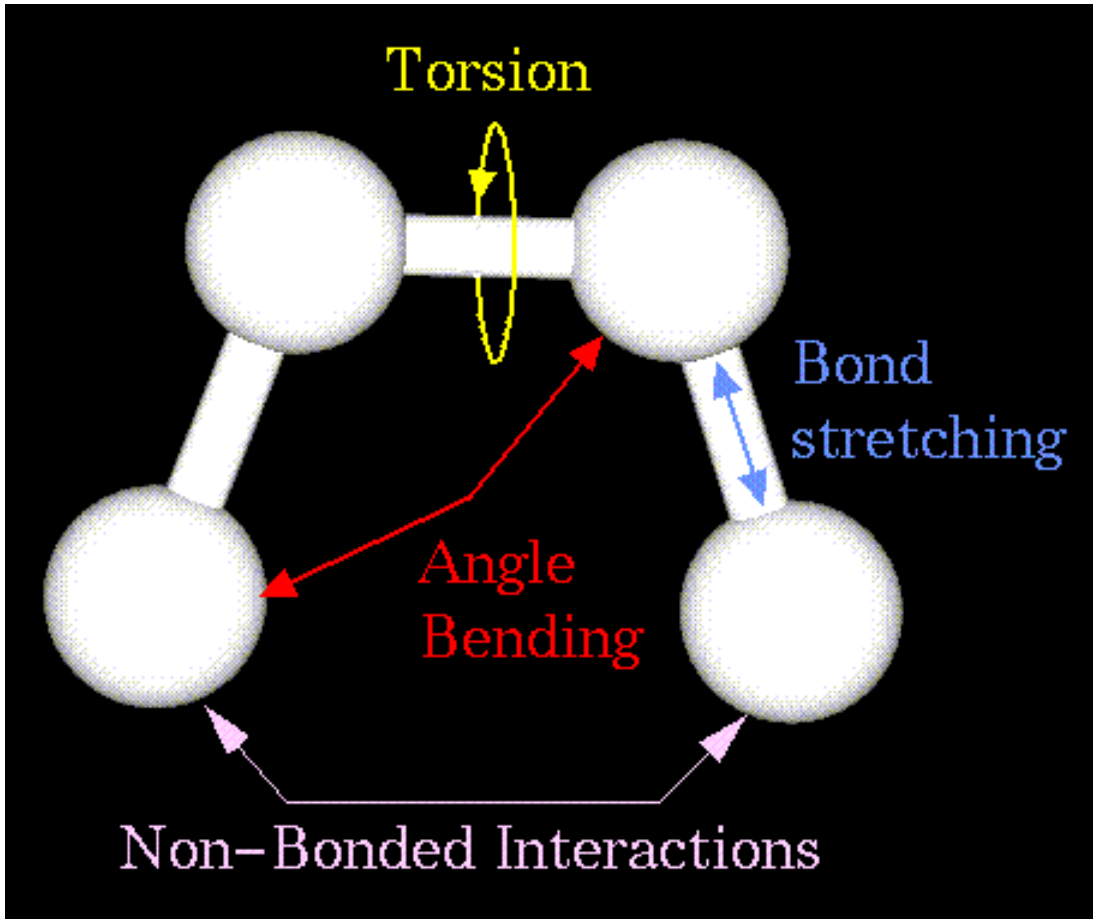
RMSD – root mean square distance

Typical geometrical operations

Bond lengths

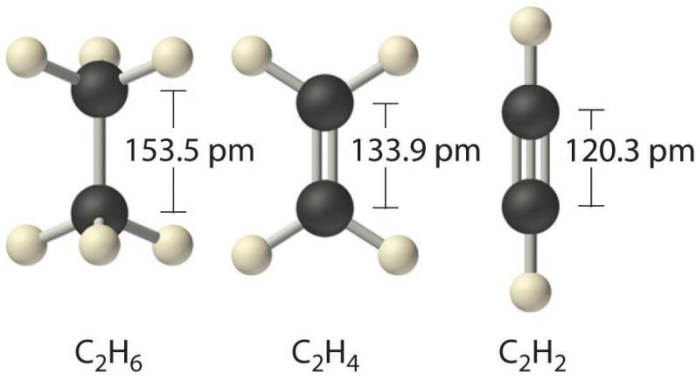
Bond angles

Torsions
(dihedral angles)



Bond Lengths

- function of position of 2 atoms
- Bond length is almost constant
- Type of bond
  - simple C-C
  - double C=C
  - triple C≡C



- Minimal - 1.09 Å (C–H)
- Typical - 1.54 Å (C–C)
- Longer – heteroatoms (sulphur, halogens, metal ions)

Table 9.2 Average Bond Energies (kJ/mol) and Bond Lengths (pm)

Bond	Energy	Length	Bond	Energy	Length	Bond	Energy	Length	Bond	Energy	Length
Single Bonds											
H—H	432	74	N—H	391	101	Si—H	323	148	S—H	347	134
H—F	565	92	N—N	160	146	Si—Si	226	234	S—S	266	204
H—Cl	427	127	N—P	209	177	Si—O	368	161	S—F	327	158
H—Br	363	141	N—O	201	144	Si—S	226	210	S—Cl	271	201
H—I	295	161	N—F	272	139	Si—F	565	156	S—Br	218	225
			N—Cl	200	191	Si—Cl	381	204	S—I	~170	234
C—H	413	109	N—Br	243	214	Si—Br	310	216			
C—C	347	154	N—I	159	222	Si—I	234	240	F—F	159	143
C—Si	301	186							F—Cl	193	166
C—N	305	147	O—H	467	96	P—H	320	142	F—Br	212	178
C—O	358	143	O—P	351	160	P—Si	213	227	F—I	263	187
C—P	264	187	O—O	204	148	P—P	200	221	Cl—Cl	243	199
C—S	259	181	O—S	265	151	P—F	490	156	Cl—Br	215	214
C—F	453	133	O—F	190	142	P—Cl	331	204	Cl—I	208	243
C—Cl	339	177	O—Cl	203	164	P—Br	272	222	Br—Br	193	228
C—Br	276	194	O—Br	234	172	P—I	184	246	Br—I	175	248
C—I	216	213	O—I	234	194				I—I	151	266
Multiple Bonds											
C=C	614	134	N=N	418	122	C≡C	839	121	N≡N	945	110
C=N	615	127	N=O	607	120	C≡N	891	115	N≡O	631	106
C=O	745 (799 in CO_2)	123	O_2	498	121	C≡O	1070	113			

Calculation of atom distance

In Cartesian coordinates:
For two points with coordinates (x_1,y_1,z_1) and (x_2,y_2,z_2)

$$d_{2-1} = \sqrt{[(x_2 - x_1)^2 + (y_2 - y_1)^2 + (z_2 - z_1)^2]}$$

Some distances within protein backbone are **constant** even if not in direct bond:

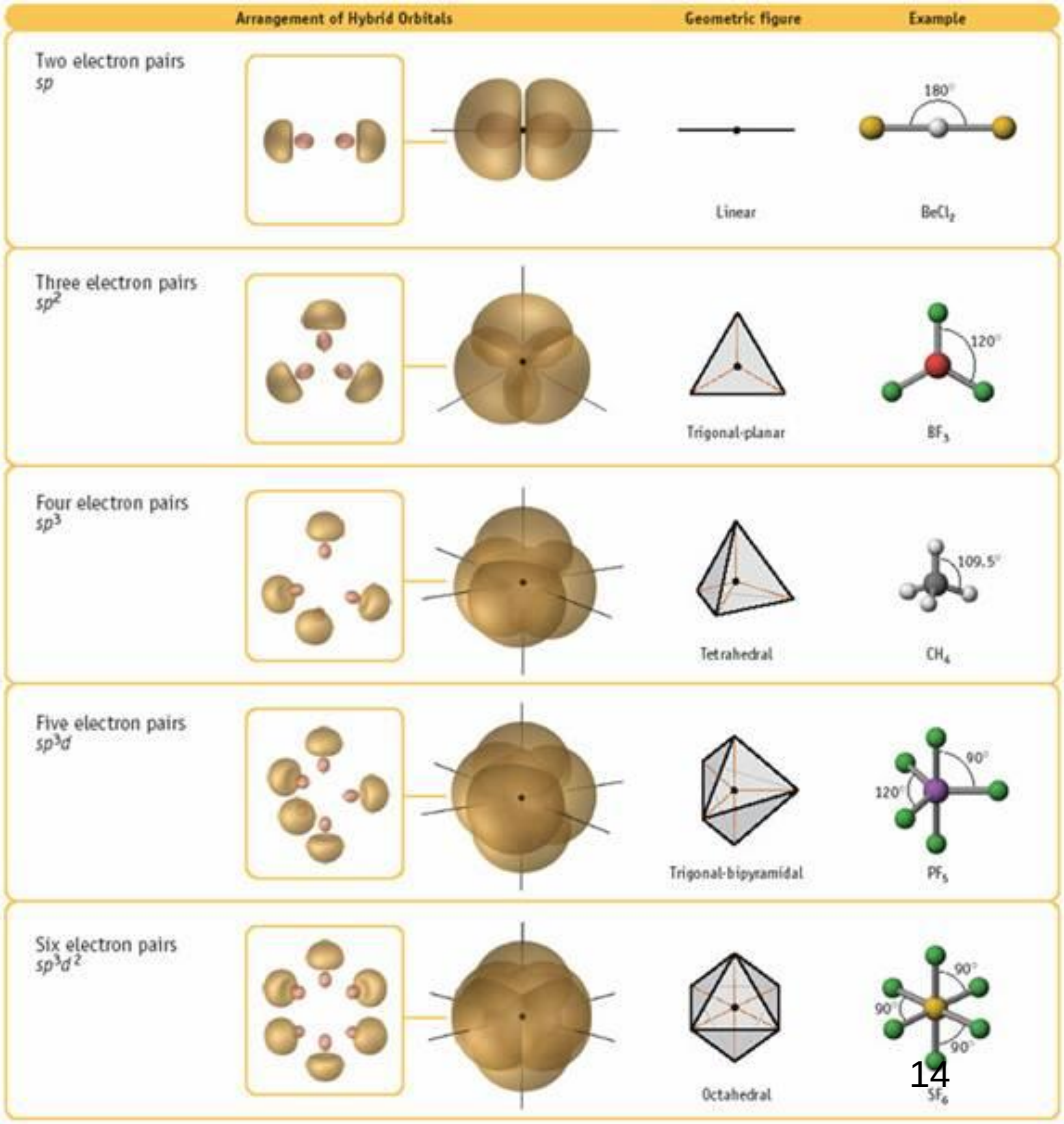
Cα – Cα distance between consecutive amino acids is 3.8 Å

Bond Angles

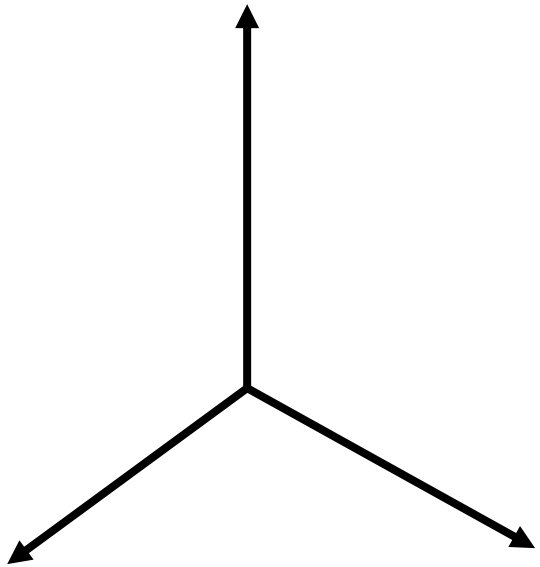
- function of position of 3 atoms
  - Almost constant for given combination of type of atoms

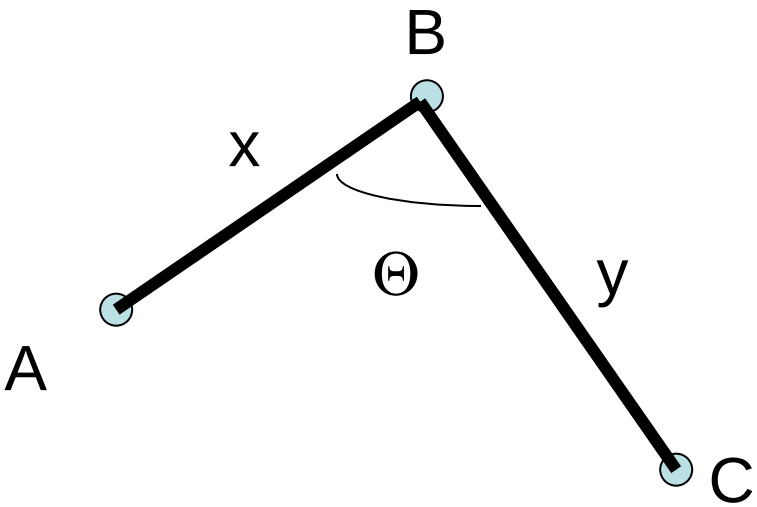
- Depend on atom type and number of electrons in bonding

- Interval from 90 to 180



Calculation of bonding angle





$$X.Y = |X|.|Y|.\cos(\Theta)$$

$$\Theta = \arccos(X.Y/|X|.|Y|)$$

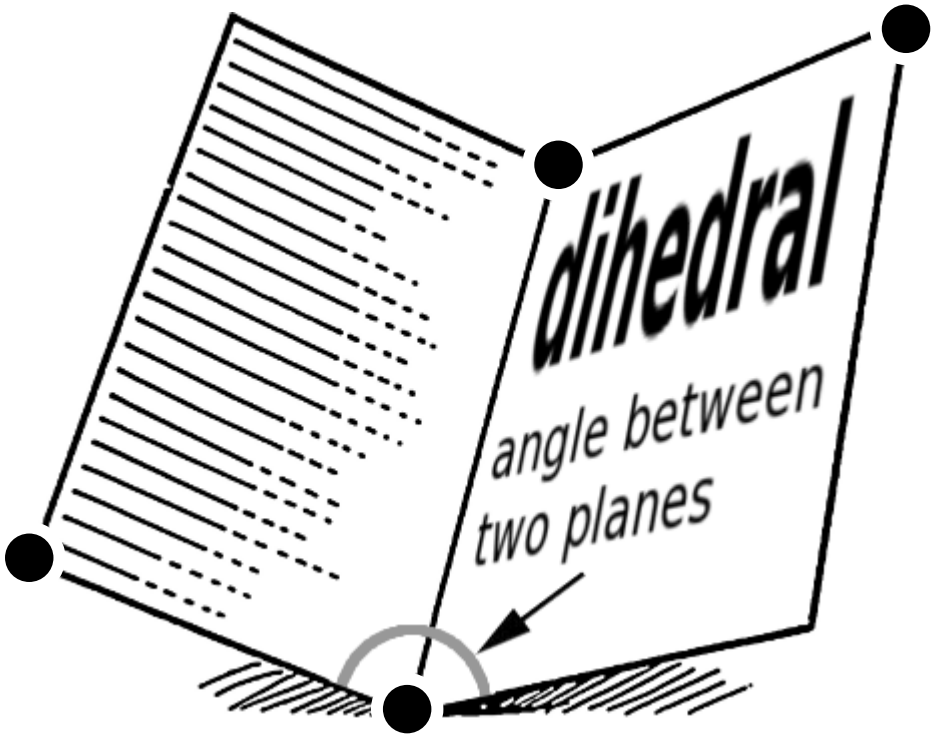
Arccosin of angle between two vectors BA and BC

Dihedral Angle

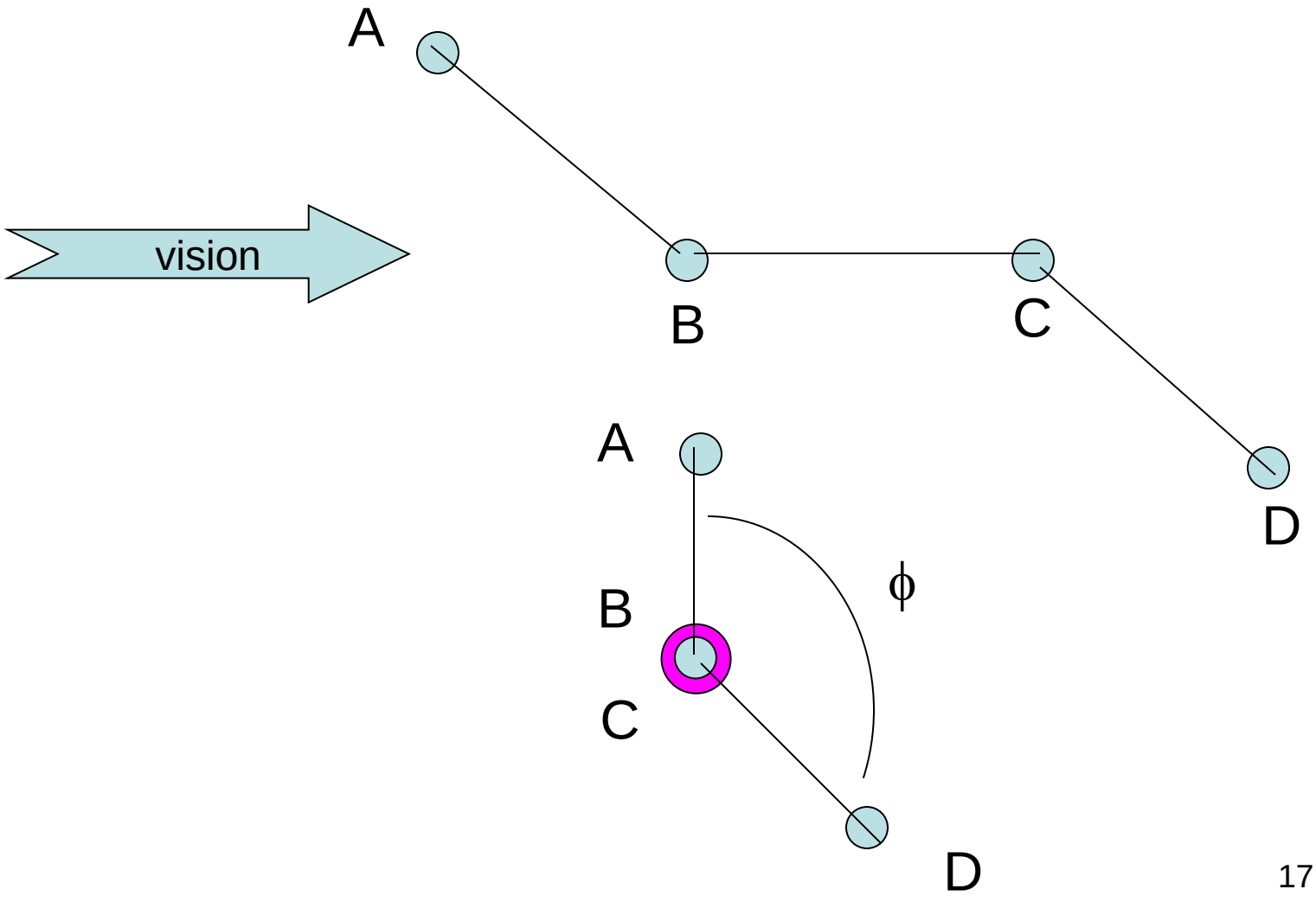
• function of position of 4 atoms

- Quite variable (0 to 360°)

- its change change conformations



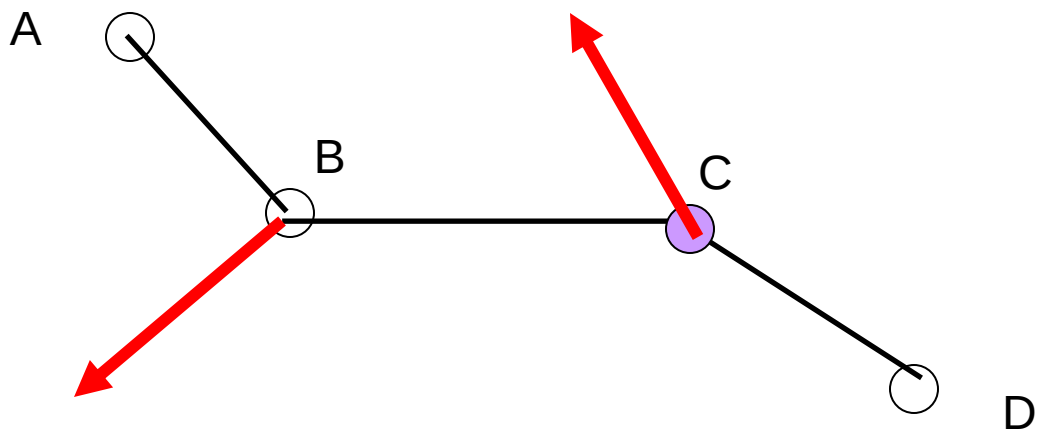
Dihedral Angle



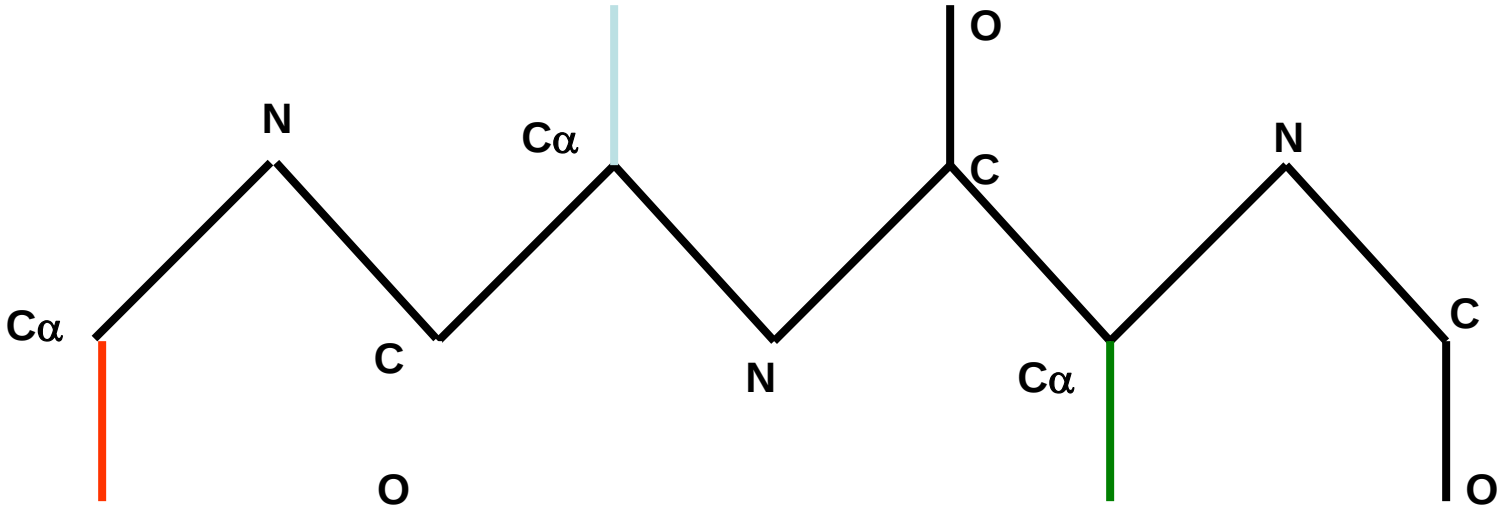
Calculation of dihedral angle

Dihedral angle = Angle between vectors orthogonal to planes defined by vectors:

1) Plane 1 - Vectors BA and CB
2) Plane 2 - Vectors CB and DC



Important dihedral angles in proteins



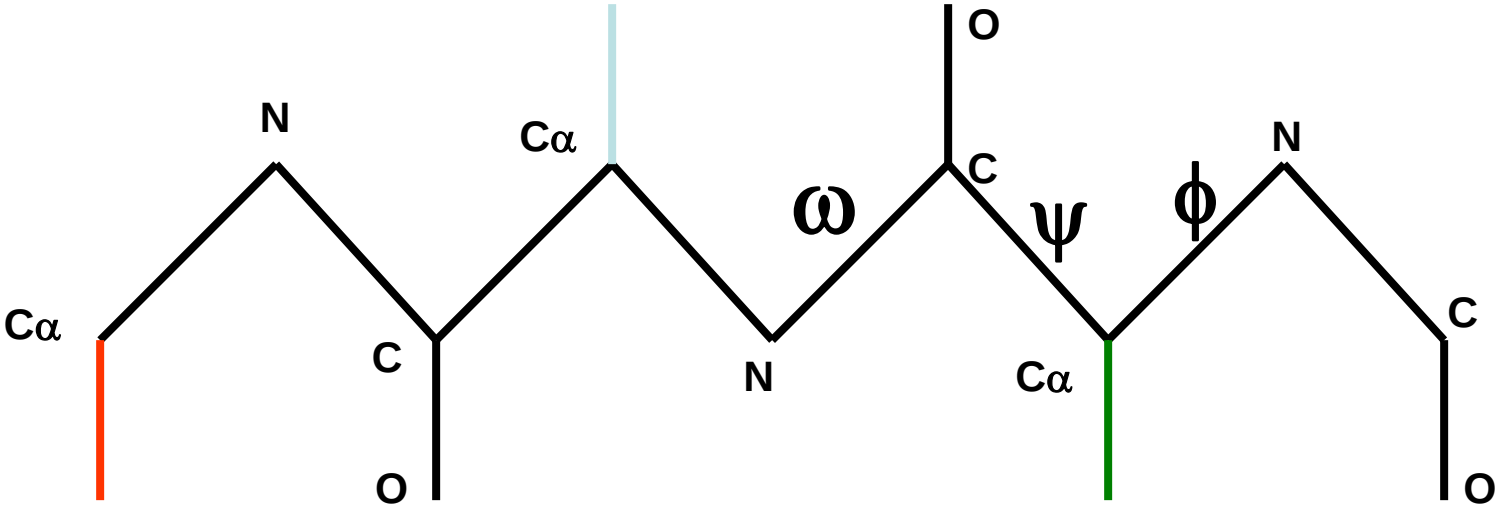
omega ω Cα - Cα

psi ψ N - N

phi ϕ C - C

Important dihedral angles in proteins

- Omega ω is constant = 180 (C-N do not rotate)
- Phi Φ ,Psi Ψ intervals (Cα-N, C-Cα can rotate) restricted to certain areas due to following amino acids



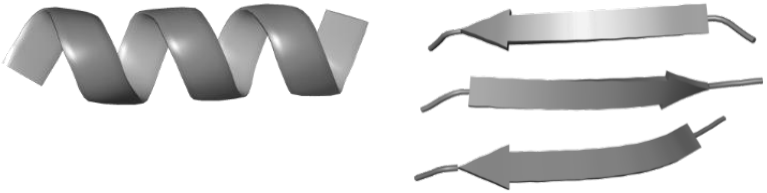
Ramachandran plot

- Typical values of dihedral angles define individual secondary structure elements:

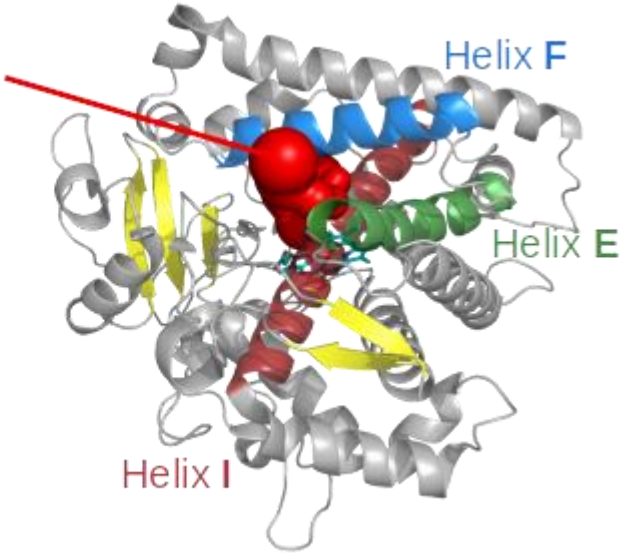
  - α-helix phi = - 57, psi = - 47
  - 3-10 helix phi = - 49, psi = - 26
  - Parallel β-sheet phi = - 119, psi = 113
  - Antiparallel β-sheet phi = - 139, psi = 135

Secondary structure

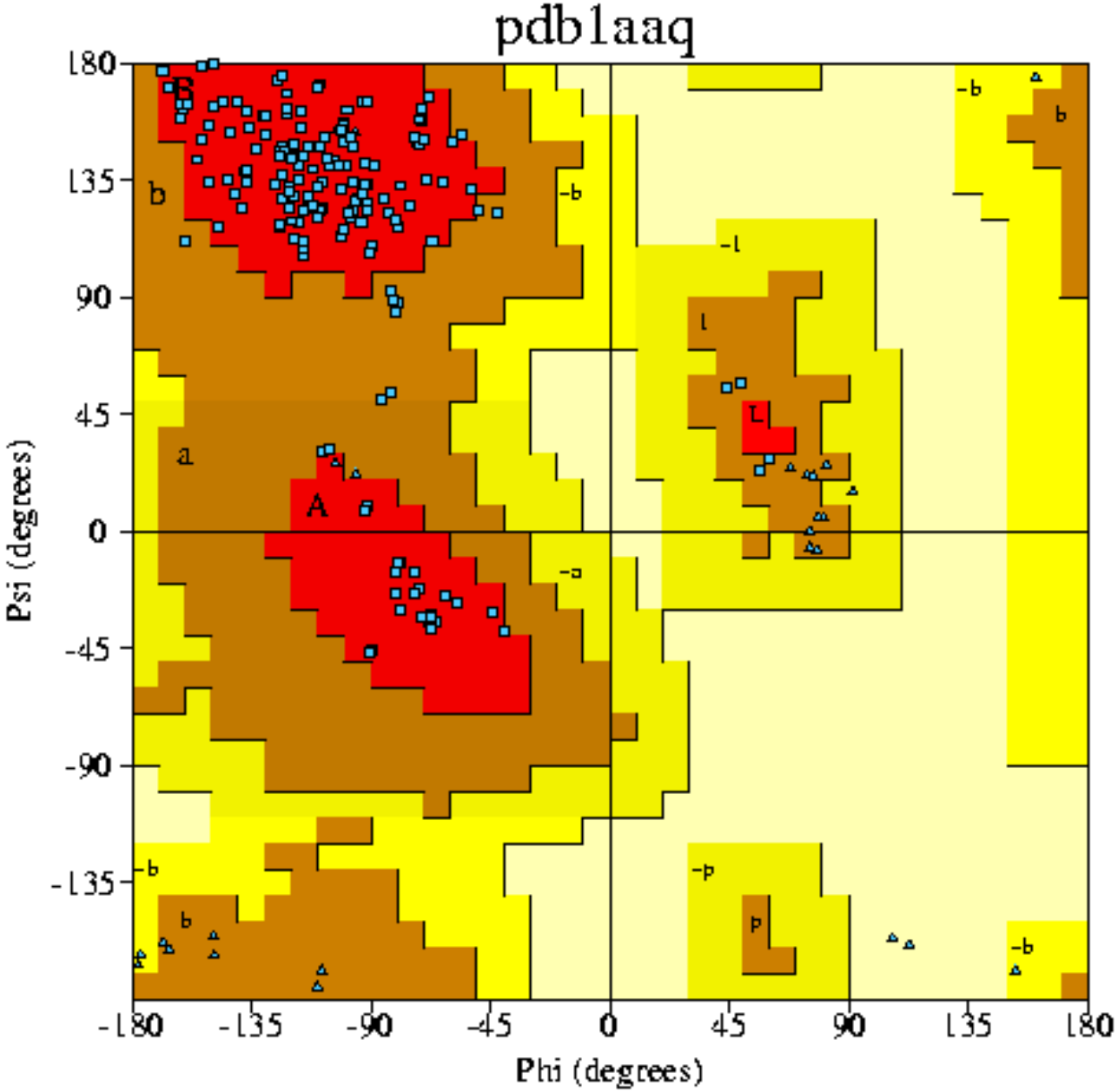
Helices and β-strands = Secondary Structure Elements (SSEs)



- Quite conserved arrangement within a protein family
- Can serve as landmarks, which
  - Help us orient in the structure
  - Help us locate the key regions (active sites, channels…)



Ramachandran plot



Other Coordinate Systems

Cartesian coordinates are orthogonal (x,y,z)

-> used most often

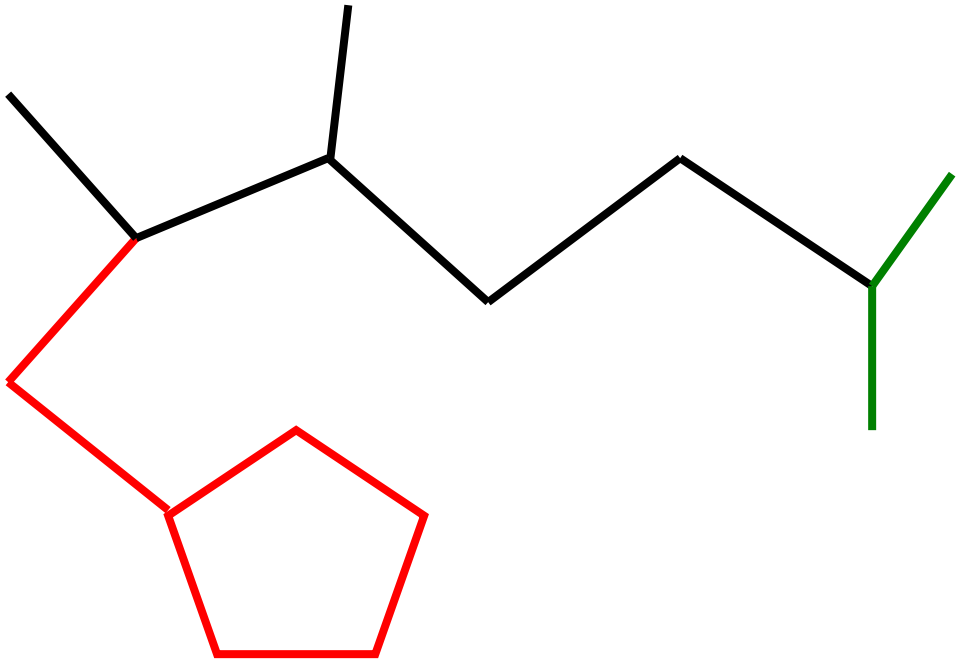
If bond lengths and bond angles are constant -> reduction of coordinates -> only dihedral angles =>

Inner coordinates

If some part of structure can be defined by “rigid” structural element -> solid objects =>

Object-based coordinates

Advantages of Inner Coordinates



3 peptide units = 12 atoms = 36 coordinates OR 6 dihedral angles
3 sidechains = 12 atoms = 36 souřadnic OR 5 dihedral angles

72 cartesians versus 11 inners

Disadvantages of Inner Coordinates

Some calculations are more difficult

Atom-atom distance

Closest atoms toward a point in space

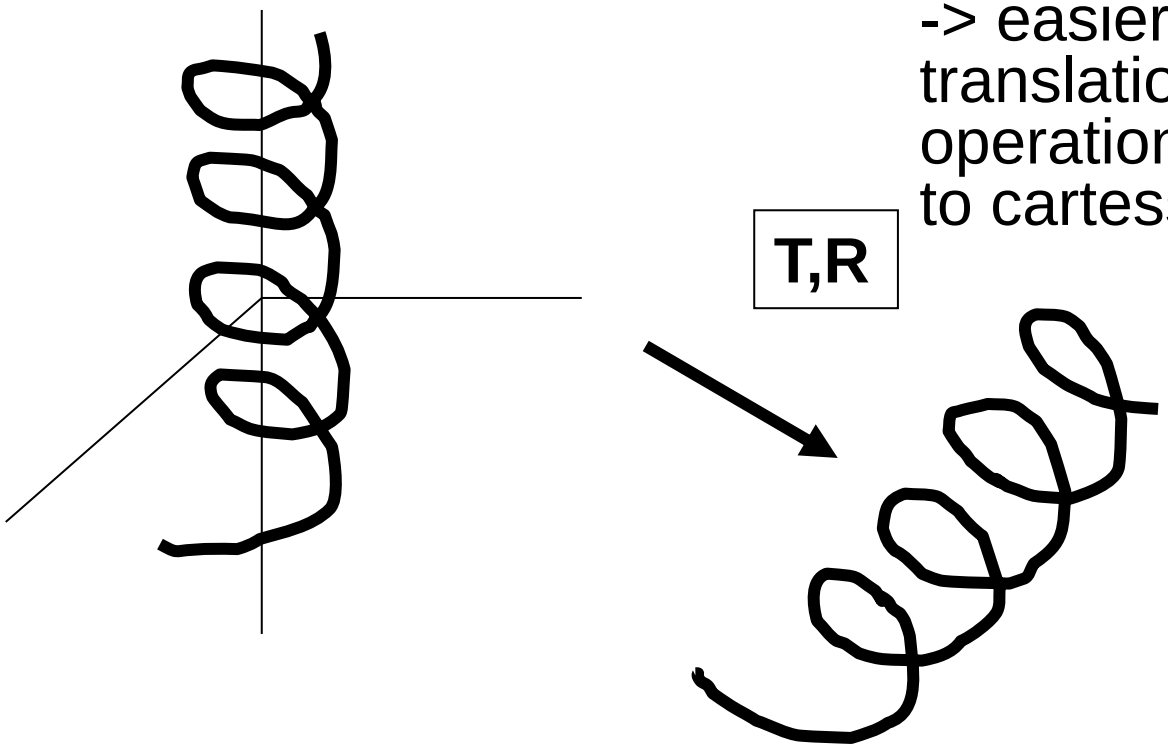
Hard comparison of independent objects (two molecules)

Nonlinear relationships between coordinates => problem for optimizations and simulations

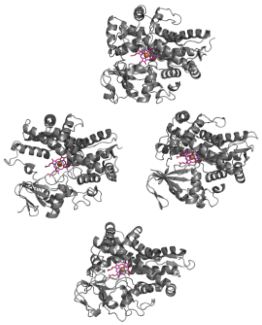
Object-based coordinates

Use of larger objects – secondary structure, subset of atoms…

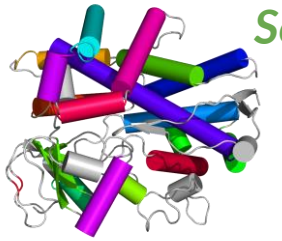
Example -> Helix can be represented as a vector with just 6 coordinates -> easier operations such as translation and rotation (final operation is later transferred to cartessian coordinates)

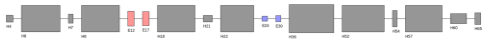


Bigger image

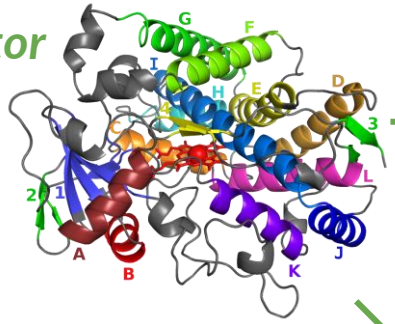


OverProt

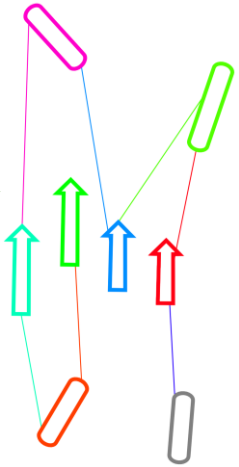




SecStrAnnotator



2DProt



OverProt web server

CATH/PDBe

Precomputed for CYP family and available through SecStrAPI plugin in PyMOL

Annotation of key regions

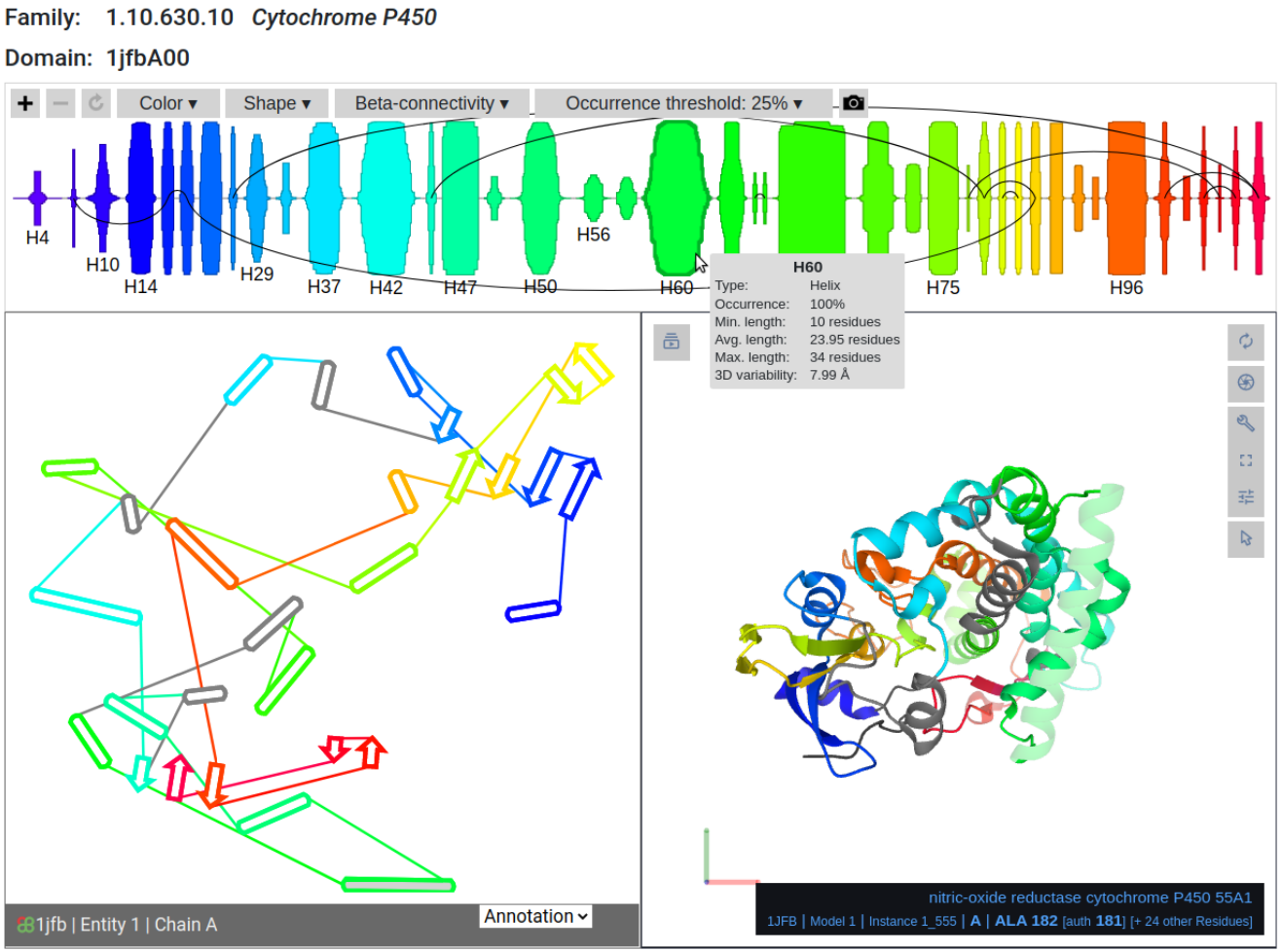
Midlik A, Hutařová Vařeková I, Hutař J, Chareshneu A, Berka K, Svobodová R: **OverProt**: secondary structure consensus for protein families, *Bioinformatics*, 38(14), July 2022, 3648–3650

Midlik A, Navrátilová V, Moturu TR, Koča J, Svobodová R, Berka K: Uncovering of cytochrome P450 anatomy by **SecStrAnnotator**. *Sci Rep* 11, 2021, 12345

Hutařová Vařeková I, Hutař J, Midlik A, Horský V, Hladká E, Svobodová R, Berka K, **2DProts**: database of family-wide protein secondary structure diagrams, *Bioinformatics*, 37(23), 2021, 4599–4601,

OverProt Server – Interactive view

- 1D of the family linked to 2D and 3D of a domain



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Structure Comparison

For comparison of two structures A and B we need:

1. Which atom from A corresponds to which atom from B

=> alignment

2. Atom localization

=> PDB files

3. Comparison criteria

RMSD, energy

RMSD = Root Mean Square Deviation

- Atoms from A and B are taken as equivalent
- Superposition and calculation of differences in distance

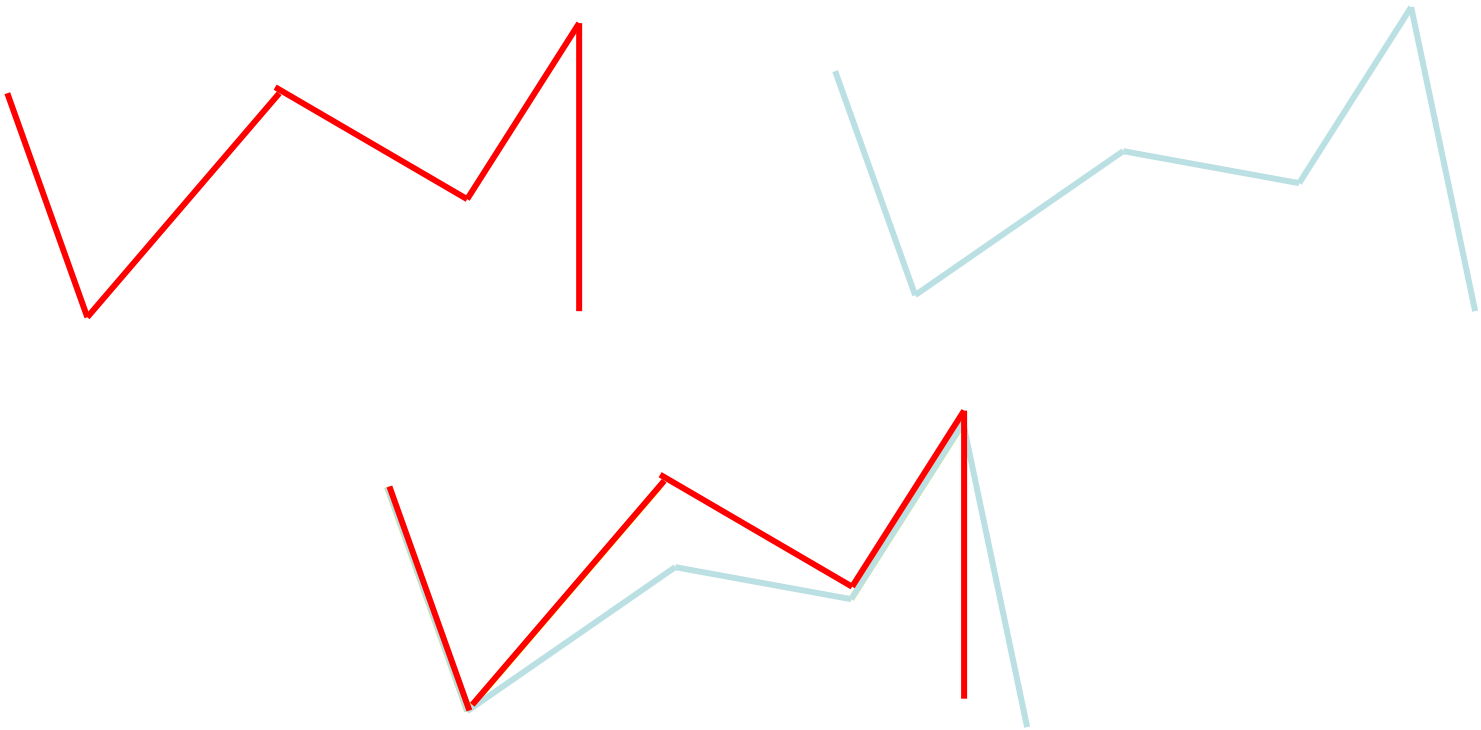
$$\mathbf{RMSD} = \sqrt{\frac{\Sigma\, \mathbf{d}^2_{\mathbf{i}}}{\mathbf{N}}}$$

- If are structures identical -> RMSD = 0
- With more differences between structures -> RMSD increses

N – number of atoms
d_i – distance of two atoms with index *i* from A and B

Structure Comparison

To find minimal RMSD



Calculation of RMSD

- translate and rotate one structure with respect to the other to minimize the RMSD

- Centroid-based solutions

  (Huang,Blostein,Margerum)
- Quaternion-based solutions
  - (rotation-translation) that minimizes the RMSD between two sets of vectors

  (Faugeras a Hebert, Petitjean)
- Matrix Singularity-based methods

  (Arun, Huang, Blostein)

Arun algorithm

- Matrices of pi’ = R.pi + T + Ni
  - pi – 3x1 column matrix of positions
  - R – rotation matrix
  - T – translation vector 3x1 column matrix
  - N – noise vector

- 1) Translation over **centroids**
- 2) Singular value decomposition of matrix to obtain **rotation**

- Arun algorithm is optimal, universal and not iterative

Kabsch algorithm

- 1) Translation over **centroids**
- 2) computation of a **covariance matrix**,
- 3) the computation of the **optimal rotation matrix**.

- Kabsch algorithm is widely used as *fit* function in PyMol, or within VMD
- Algorithm do not recognise similar pairs of residues – these have to be defined iteratively (typically Cα)

Kabsch, W (1976): A solution for the best rotation to relate two sets of vectors. *Acta Cryst.* ***A32*** *(5): 922.*
With a correction in Kabsch, W (1978). "A discussion of the solution for the best rotation to relate two sets of vectors". *Acta Cryst.* ***A34*** *(5): 827–828.*

Advantages and Disadvantages of RMSD

Good behavior, identical structures RMSD = 0

Simple calculation in Cartesian coordinates

Natural units (Ångstroms)

Experience (similar structures have RMSD ~ (1 – 3 Å)

Weight of all atoms is the same

however hydrogens have much smaller effect in practice –> RMSD only for backbone or Cα

Prone to extremities

RMSD of larger protein is larger even if the structure is almost identical

RMSD of 3 Å for 100 residue protein is really bad,
for 1000 residue protein it is sensible.

Other measures

- **global distance test (GDT)**
  - largest set of amino acid residues' Cα atoms in the model structure falling within a defined distance cutoff of their position in the experimental structure.

  $\Rightarrow$Used in structure prediction assessment (CASP)

- **template modeling score (TM-score)**
  - difference between two structures by a score between (0,1]

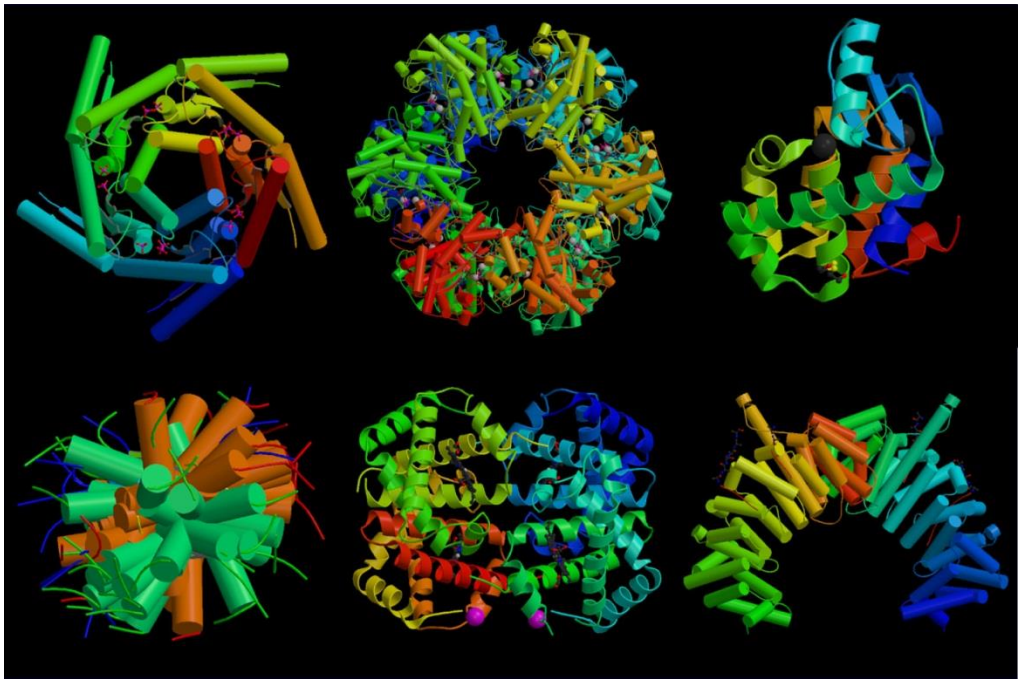
$$\text{TM-score} = \max\left[\frac{1}{L_{\text{target}}}\sum_{i}^{L_{\text{aligned}}}\frac{1}{1+\left(\frac{d_i}{d_0(L_{\text{target}})}\right)^2}\right]$$

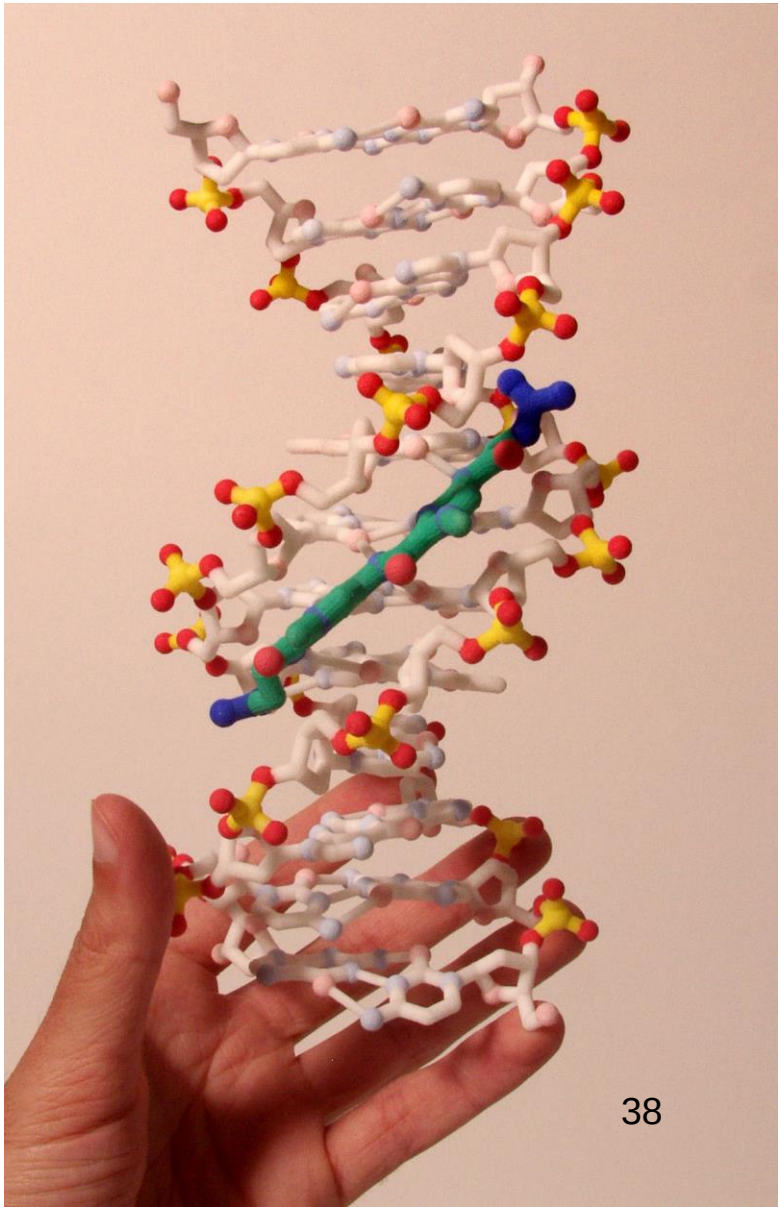
  - TM-score = 1 - perfect match between two structures
  - TM-score > 0.5 assume roughly the same fold
  - TM-score < 0.20 - randomly chosen unrelated proteins

  $\Rightarrow$Used in structure prediction assessment (CASP)

Biomolecules

- proteins
- NA – DNA, RNA
- lipids
- polysaccharides
- Small molecules (hormones, drugs)





Structural Hierarchy

MOLECULAR STRUCTURE

Primary (sequence)

↓

Secondary (local folding)

↓

Tertiary (long-range folding)

↓

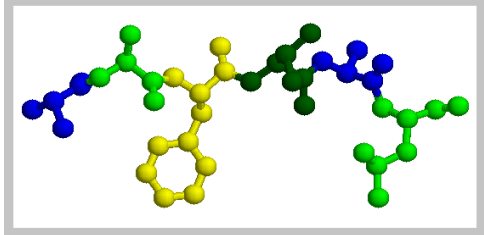
Quaternary (multimeric organization)

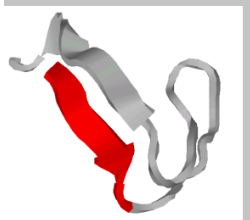
Lodish, Molecular Cell Biology, 5th Ed.

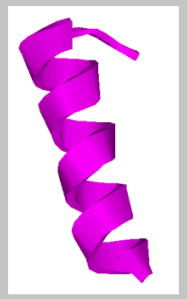
Proteins

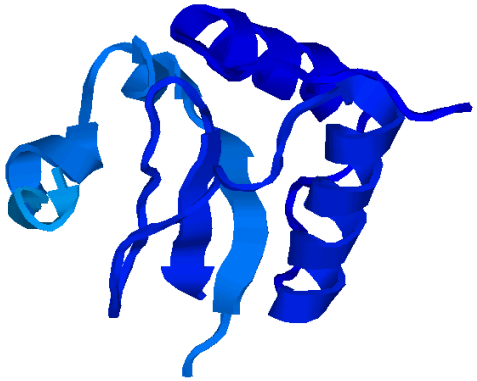
- Amino acids
- Backbone and Sidechains

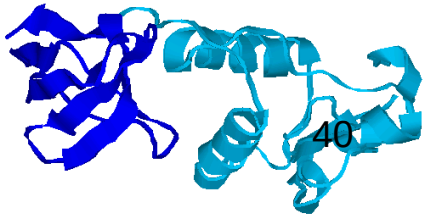
- Primary structure
  - sequence of amino acids
- Secondary structure
  - Local structural patterns
- Tertiary structure
  - Domain Fold
- Quarternary structure
  - Multichain organization







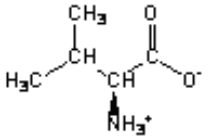




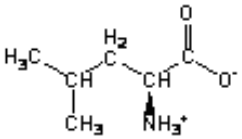
http://cs.wikipedia.org/wiki/Soubor:ProteinStructures.png

Amino acids

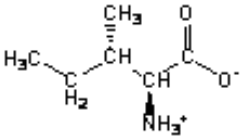
Amino acids with hydrophobic side chains



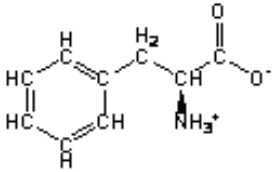
Valine
Val
V



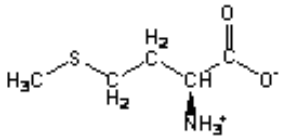
Leucine
Leu
L



Isoleucine
Ile
I

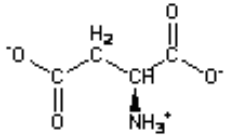


Phenylalanine
Phe
F

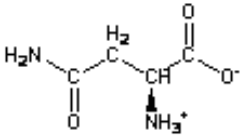


Methionine
Met
M

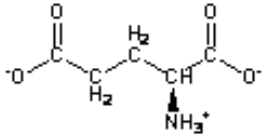
Amino acids with hydrophilic side chains



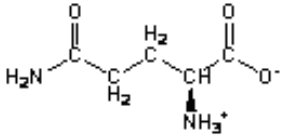
Aspartic acid
Asp
D



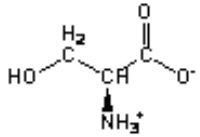
Asparagine
Asn
N



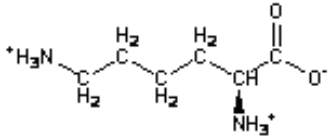
Glutamic acid
Glu
E



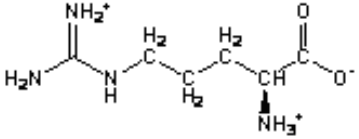
Glutamine
Gln
Q



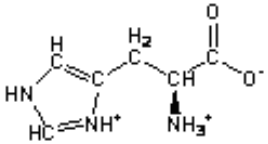
Serine
Ser
S



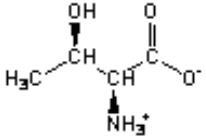
Lysine
Lys
K



Arginine
Arg
R

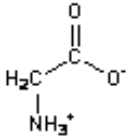


Histidine
His
H

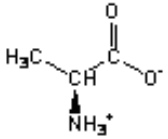


Threonine
Thr
T

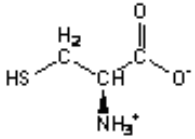
Amino acids with intermediate side chains



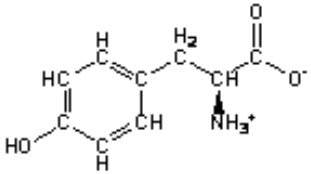
Glycine
Gly
G



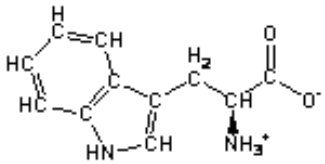
Alanine
Ala
A



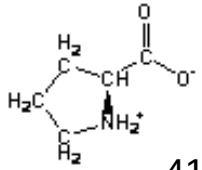
Cysteine
Cys
C



Tyrosine
Tyr
Y

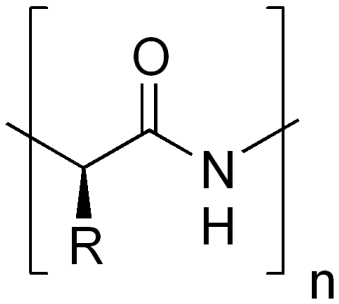


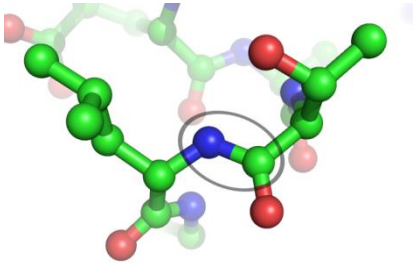
Tryptophan
Trp
W



Proline
Pro
P

Primary Structure of Protein





AMINO ACID			SIDE CHAIN
Aspartic acid	Asp	D	negative
Glutamic acid	Glu	E	negative
Arginine	Arg	R	positive
Lysine	Lys	K	positive
Histidine	His	H	positive
Asparagine	Asn	N	uncharged polar
Glutamine	Gln	Q	uncharged polar
Serine	Ser	S	uncharged polar
Threonine	Thr	T	uncharged polar
Tyrosine	Tyr	Y	uncharged polar

POLAR AMINO ACIDS

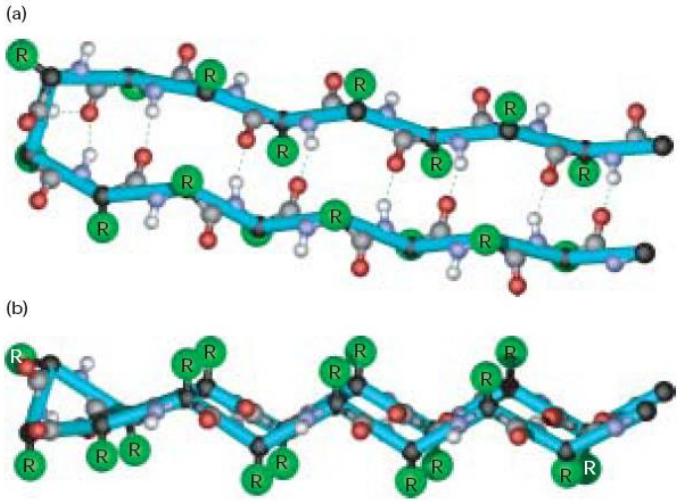
AMINO ACID			SIDE CHAIN
Alanine	Ala	A	nonpolar
Glycine	Gly	G	nonpolar
Valine	Val	V	nonpolar
Leucine	Leu	L	nonpolar
Isoleucine	Ile	I	nonpolar
Proline	Pro	P	nonpolar
Phenylalanine	Phe	F	nonpolar
Methionine	Met	M	nonpolar
Tryptophan	Trp	W	nonpolar
Cysteine	Cys	C	nonpolar

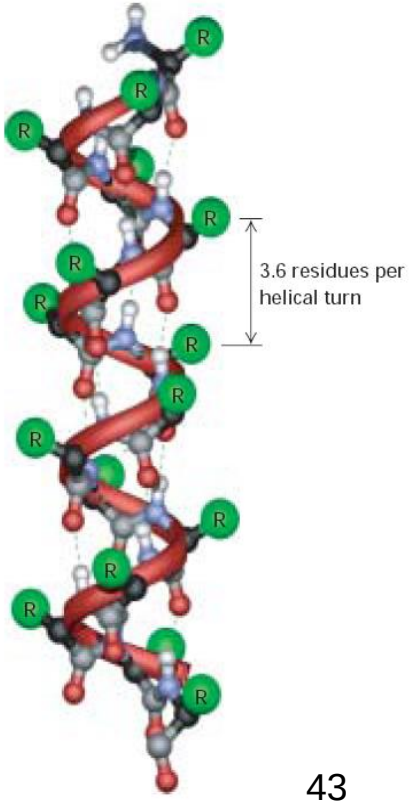
NONPOLAR AMINO ACIDS

Alberts, Molecular Biology of the Cell, 5th Ed.

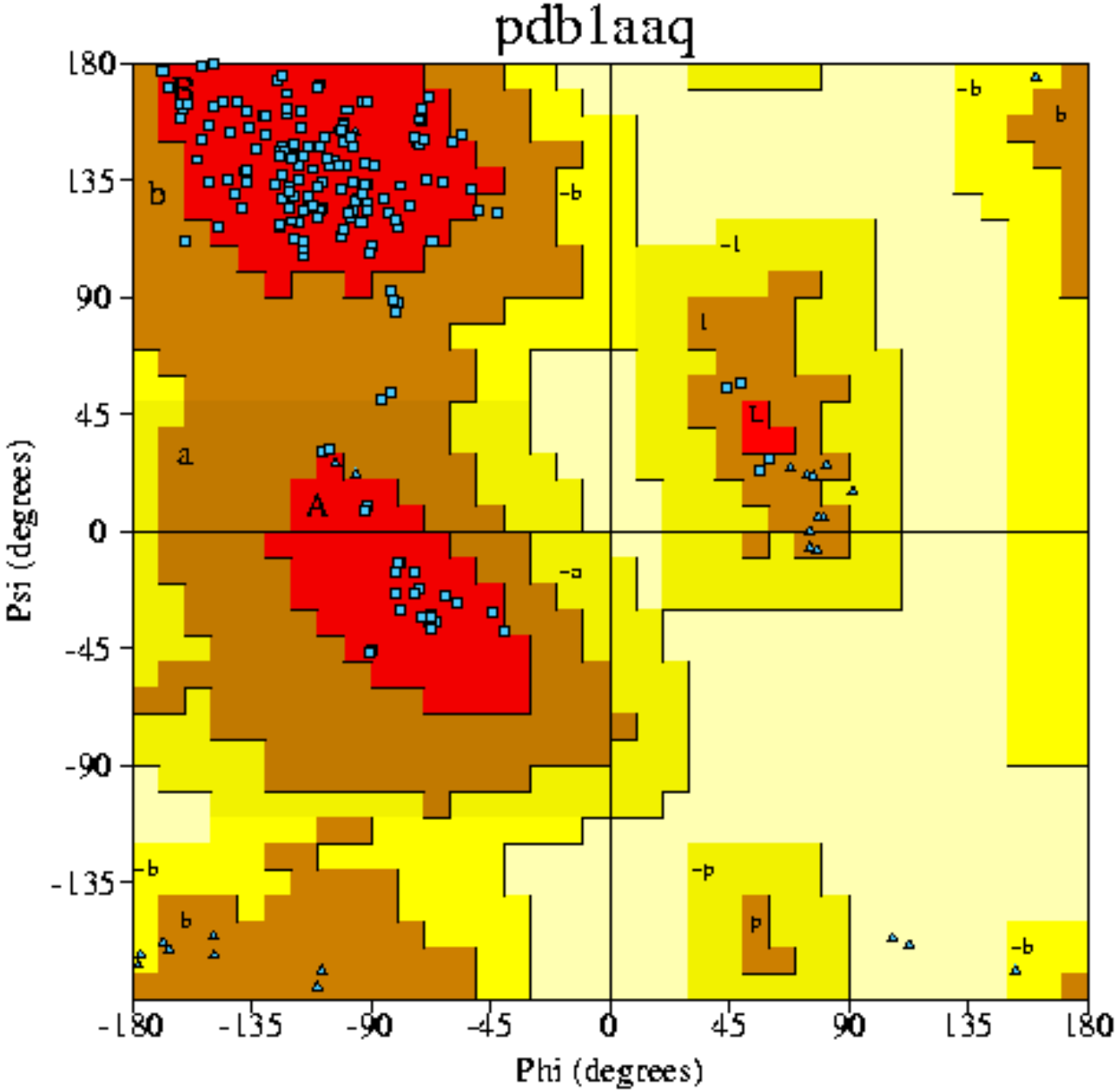
Secondary structure of Proteins

- Local folding
- Secondary structure depends on amino acid sequence
  - **α-helix**
  - **3-10 helix**
  - **β-sheet**
  - **β-turn, loop**





Ramachandran plot



PROCHECK summary for 1aaq

PROCHECK statistics

Ramachandran Plot statistics

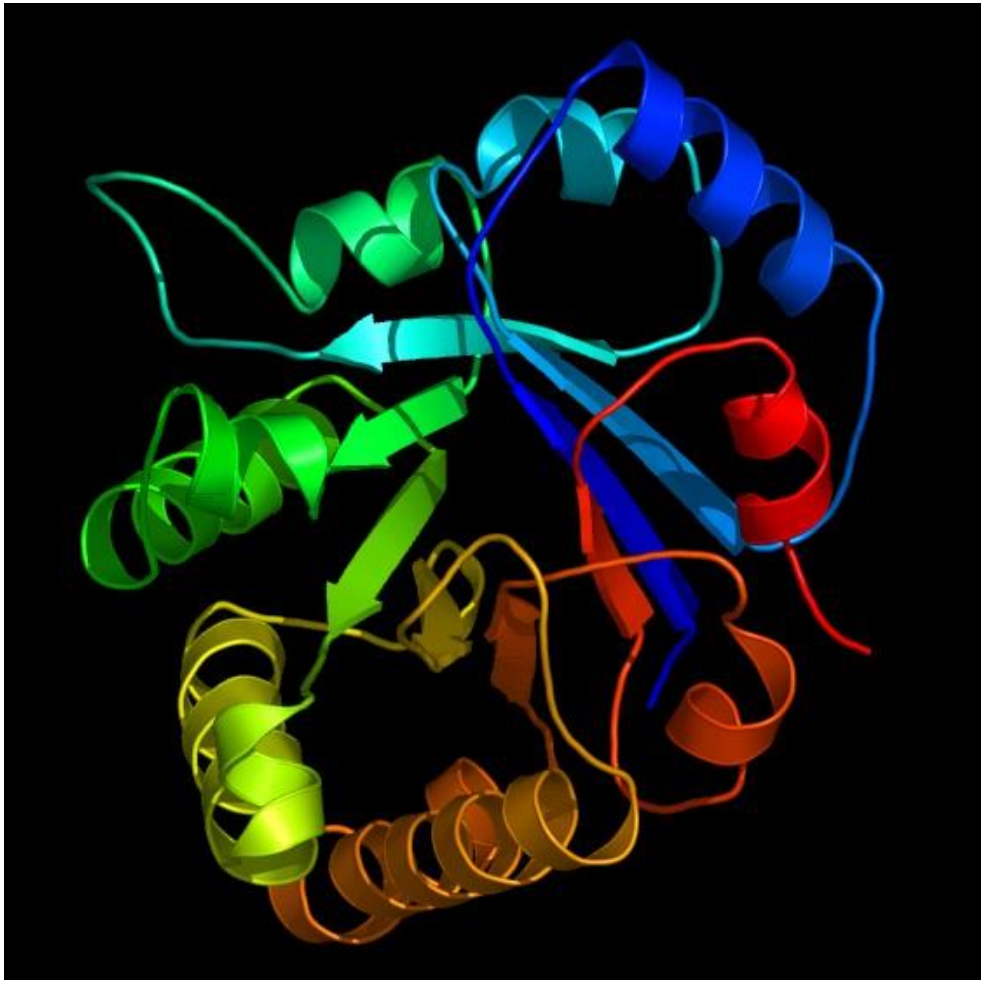
	No. of residues	%-tage
	---	---
Most favoured regions [A,B,L]	146	92.4%
Additional allowed regions [a,b,l,p]	12	7.6%
Generously allowed regions [~a,~b,~l,~p]	0	0.0%
Disallowed regions [XX]	0	0.0%
	---	---
Non-glycine and non-proline residues	158	100.0%

End-residues (excl. Gly and Pro)	2
Glycine residues	26
Proline residues	12

Total number of residues	198

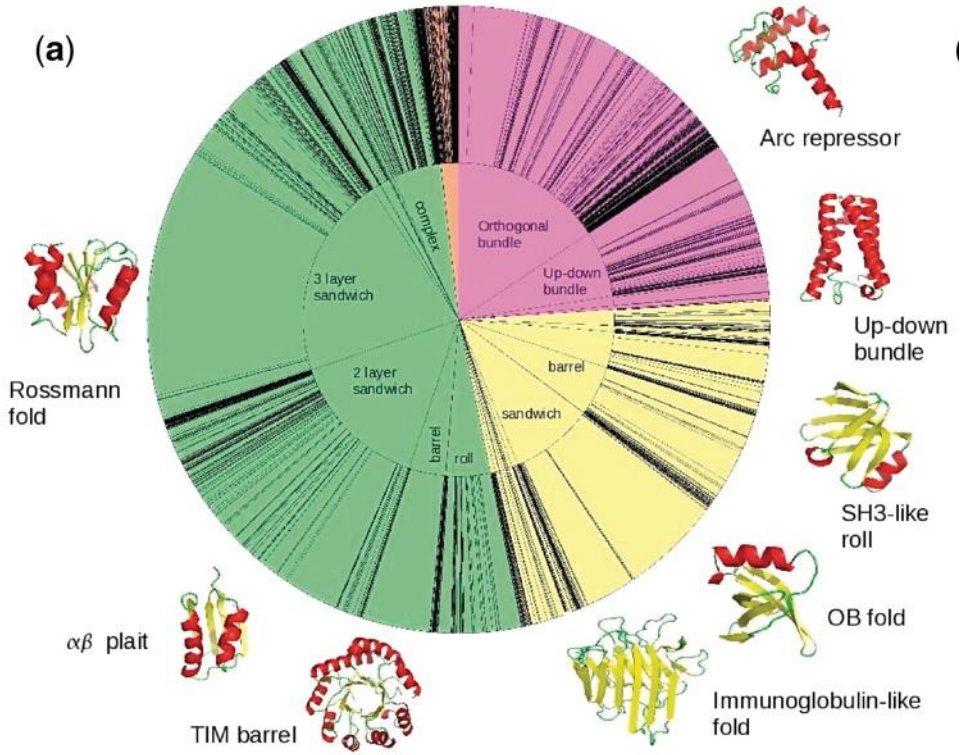
Tertiary Structure

- fold
  - globular
  - membrane
  - Fibrilar
  - IUP



- Necessary for FUNCTION
- domains

‘CATHerine wheels’.



The distribution of all non-homologous structures (2386) within CATH v3.3

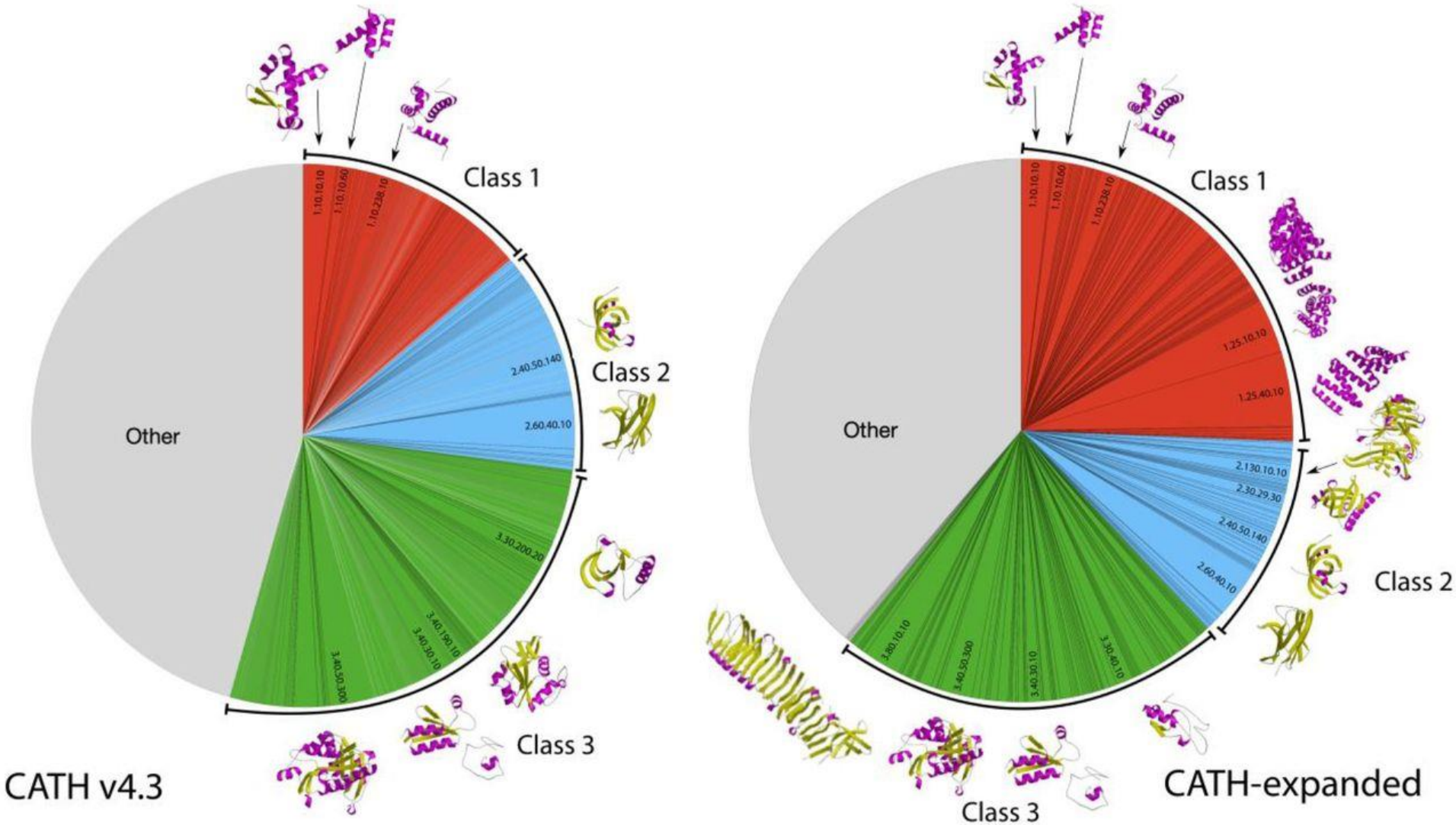
Classes:
pink (mainly α),
yellow (mainly β),
green (αβ)
brown (little secondary structure).

Proportion of structures within any given architecture (inner circle)
Fold group (outer circle).

Cuff A L et al. Nucl. Acids Res. 2011;39:D420-D426

Nucleic Acids Research

CATH update 2022

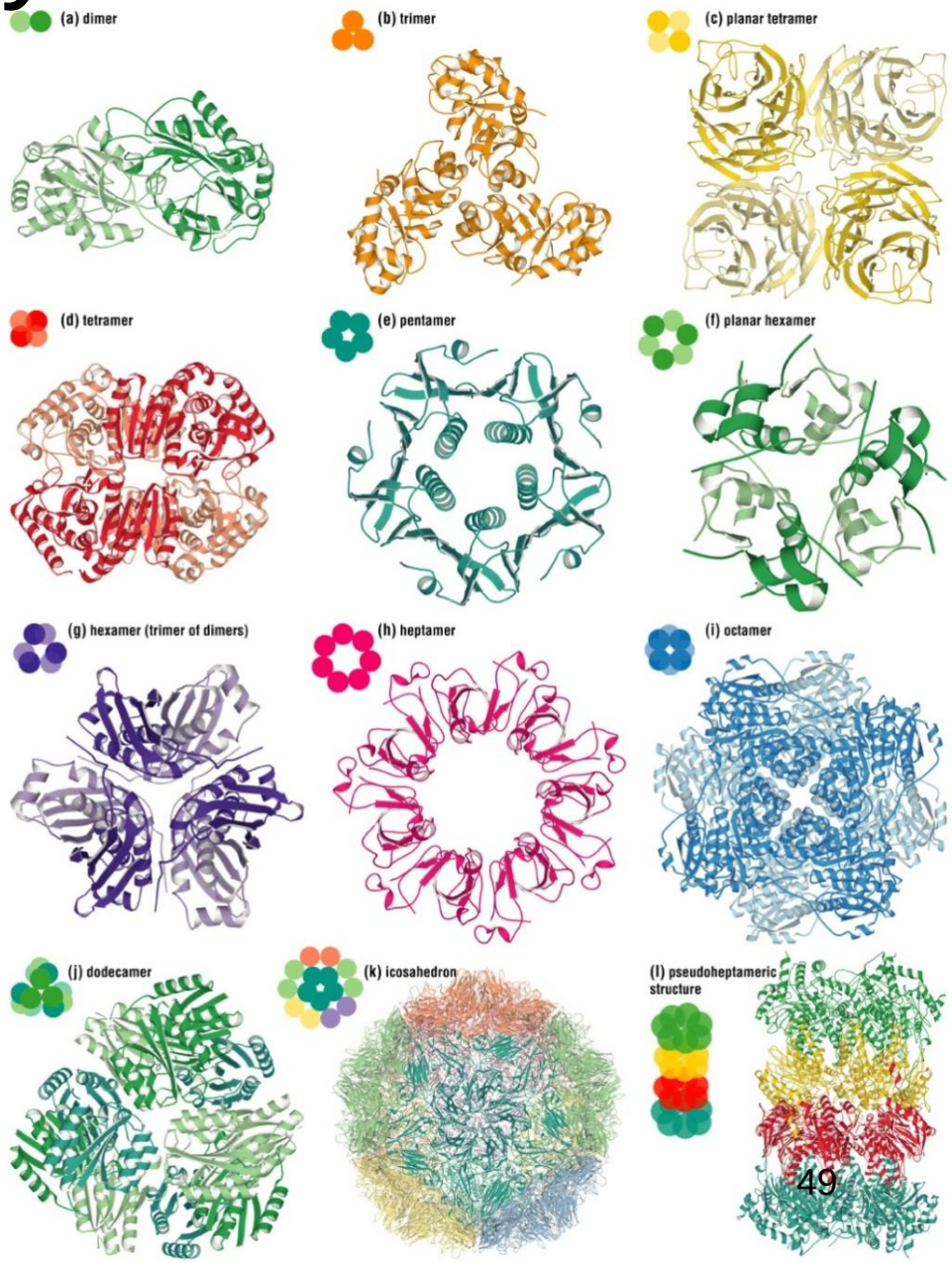


Structural diversity in CATH Superfamilies (left) and expanded by AF2 models (right).

Bordin N et al.: AlphaFold2 reveals commonalities and novelties in protein structure space for 21 model organisms. *bioRxiv* 2022, doi:10.1101/2022.06.02.494367v1.full

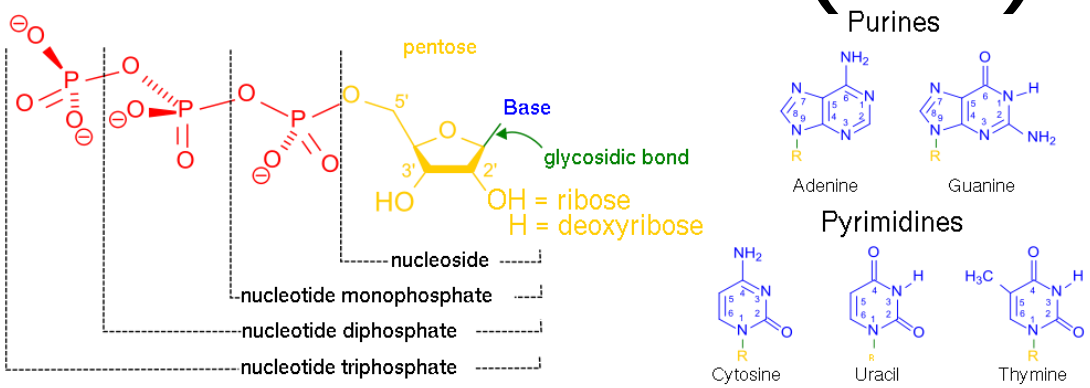
Quarternary Structure

- asociace více řetězců:
  - Kooperativita
    (asociace zesílí vazebné vlastnosti)
    hemoglobin
  - Kolokalizace funkce
    (každá podjednotka dělá něco jiného)
    tryptophansyntáza
  - Kombinace podjednotek
    (přizpůsobování)
    imunoglobuliny
  - Skládání větších struktur
    (podjednotky uspořádávají procesem self-assembly)
    aktin,
    virové kapsidy



Petsko, Ringe – Protein structure and function

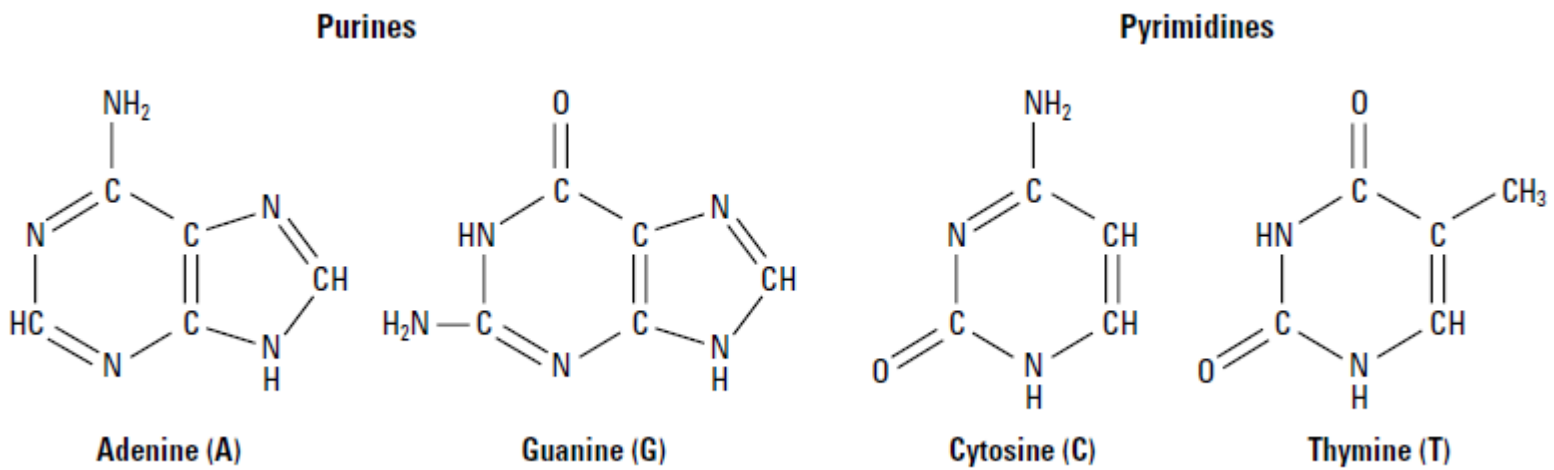
Nucleic Acids (NA)



- Primary structure
  - sequence of NA basis in chains
- Secondary structure
  - set of interactions between nucleic basis
- Tertiary structure
  - 3D localization of atoms
- Quarternary structure
  - Higher organization levels
    - DNA in chromatin
    - Interaction of RNA units in ribosome or spliceosome.

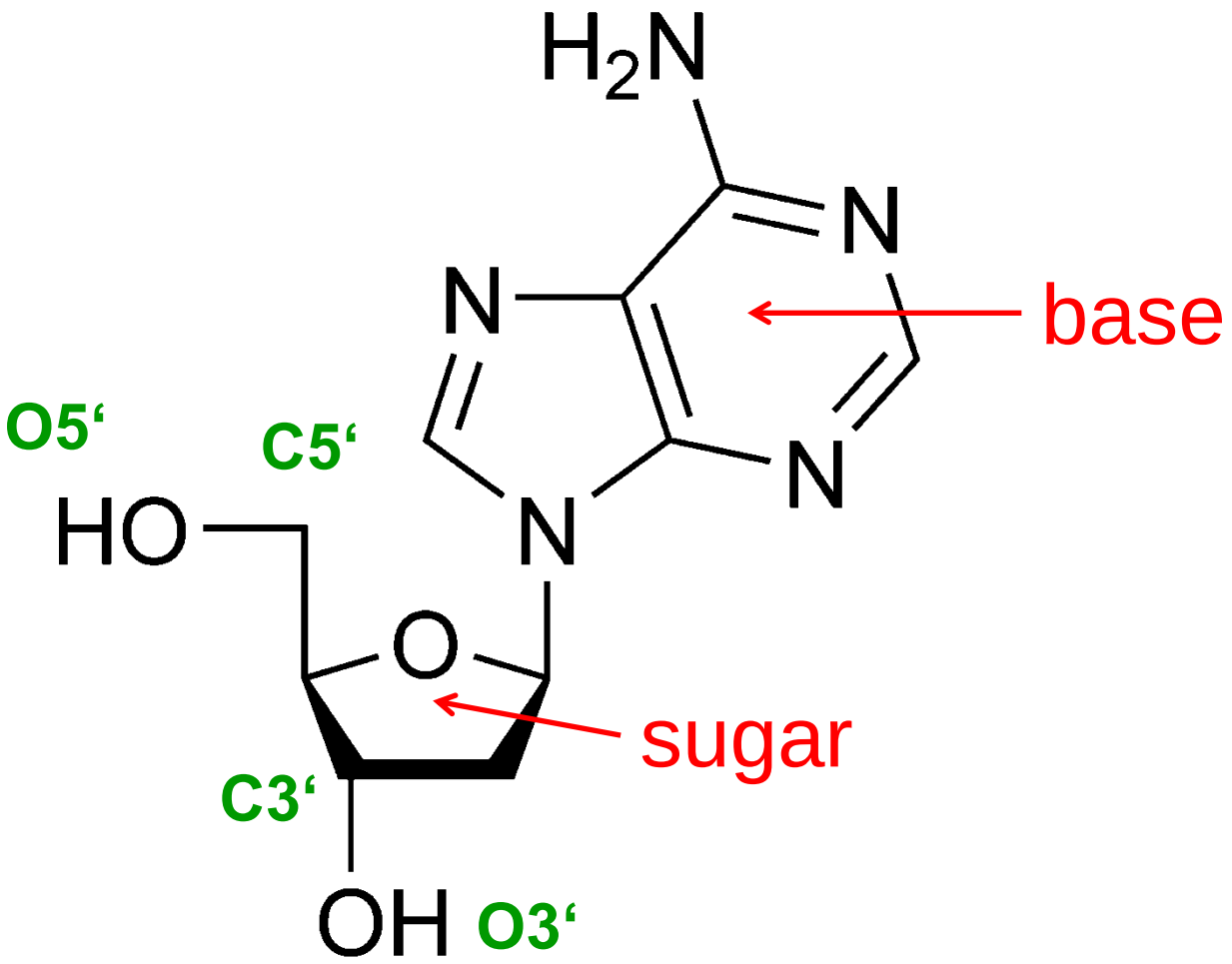
DNA – DeoxyriboNucleic Acid

- bases, deoxyribose sugar, phosphate – nucleotide
- Bases are flat → stacking
- p**Y**rimidines – C, T
- pu**R**ines – A, G



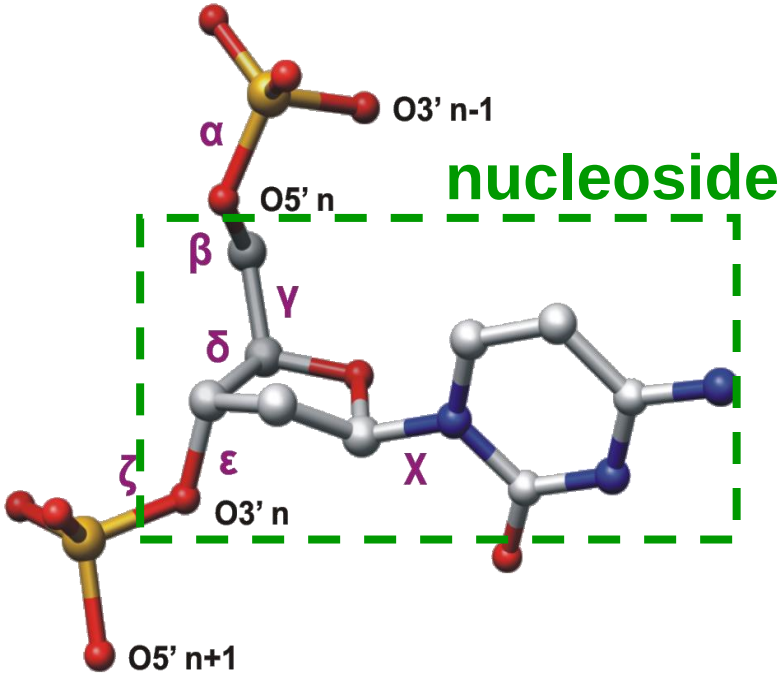
•http://www.umass.edu/molvis/tutorials/dna/, http://ich.vscht.cz/~svozil/teaching.html

Nucleoside



Nucleotide

- nucleosides are interconnected by phospohodiester bond
- nucleotide monophosphate

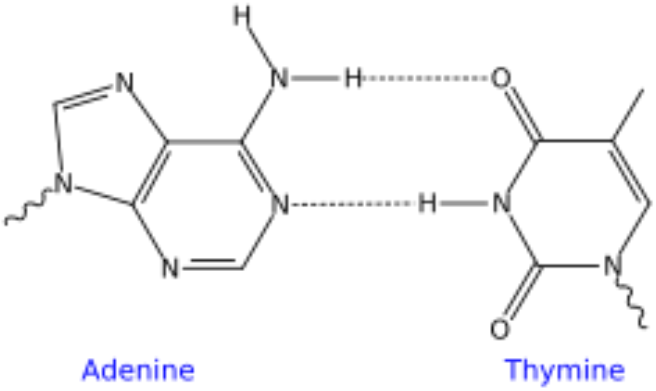


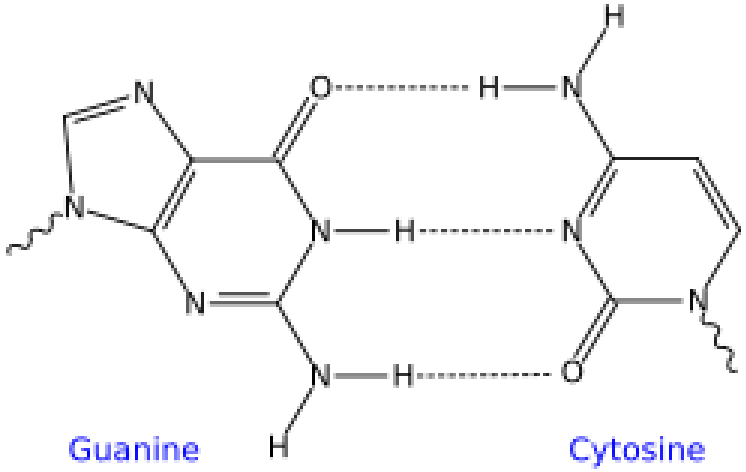
Watson-Crick pairing

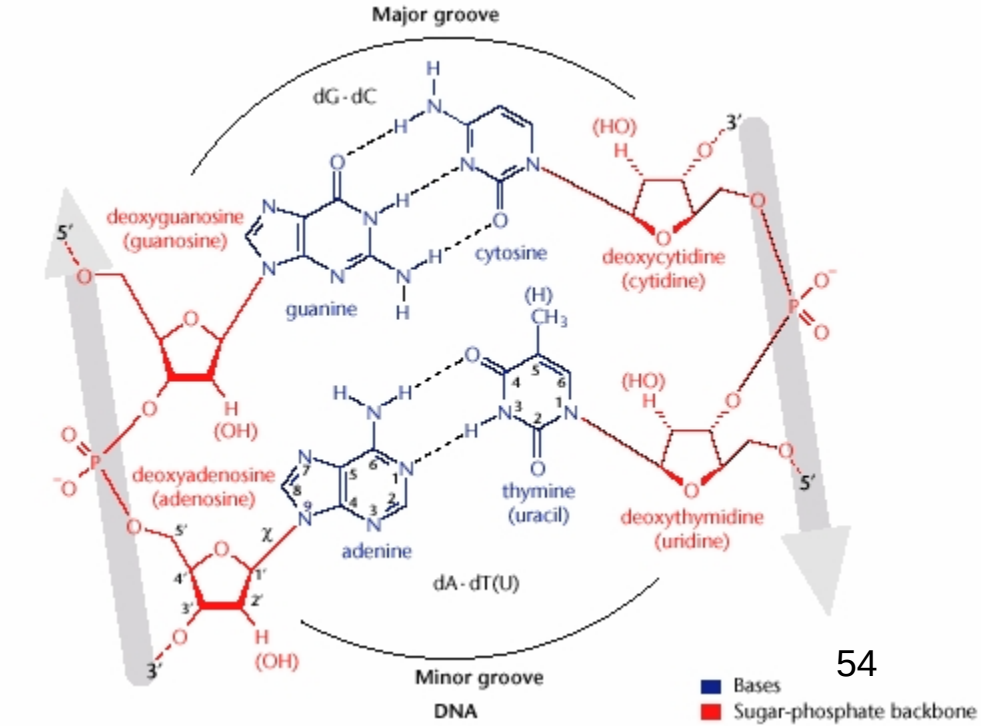
Bases complement each other.

Chargaffs' rules

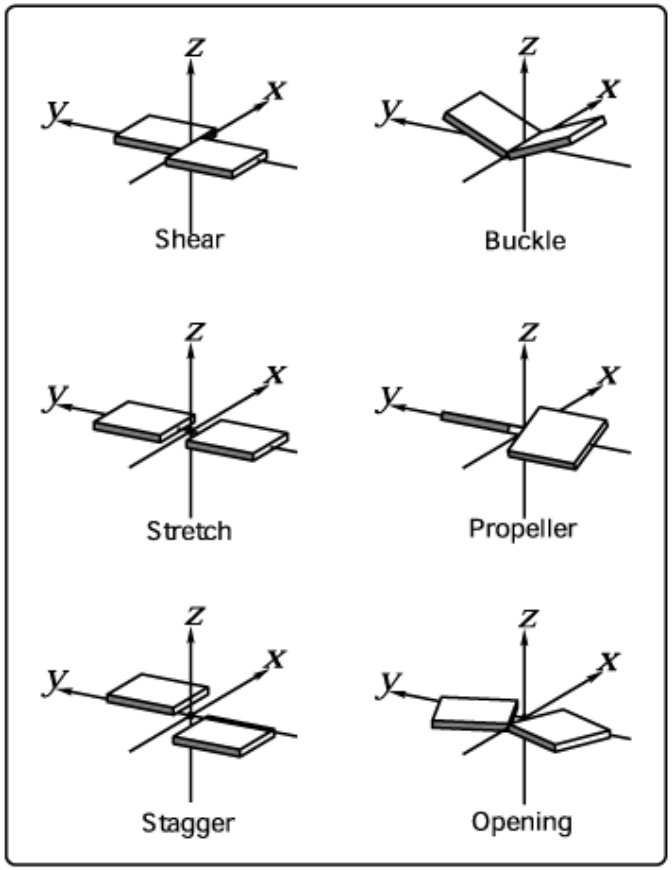
- amount of G = C
- amount of A = T

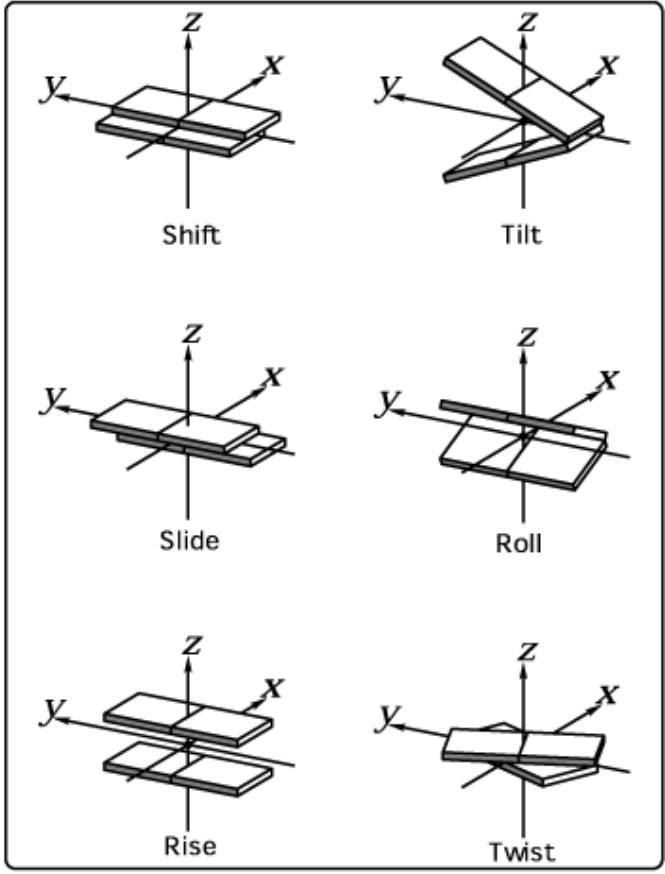


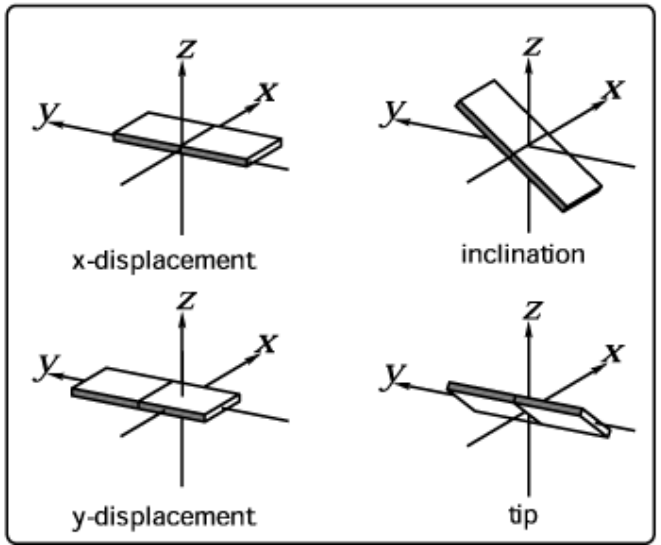


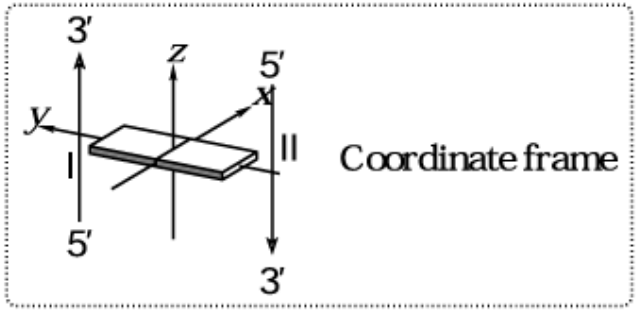


Párování



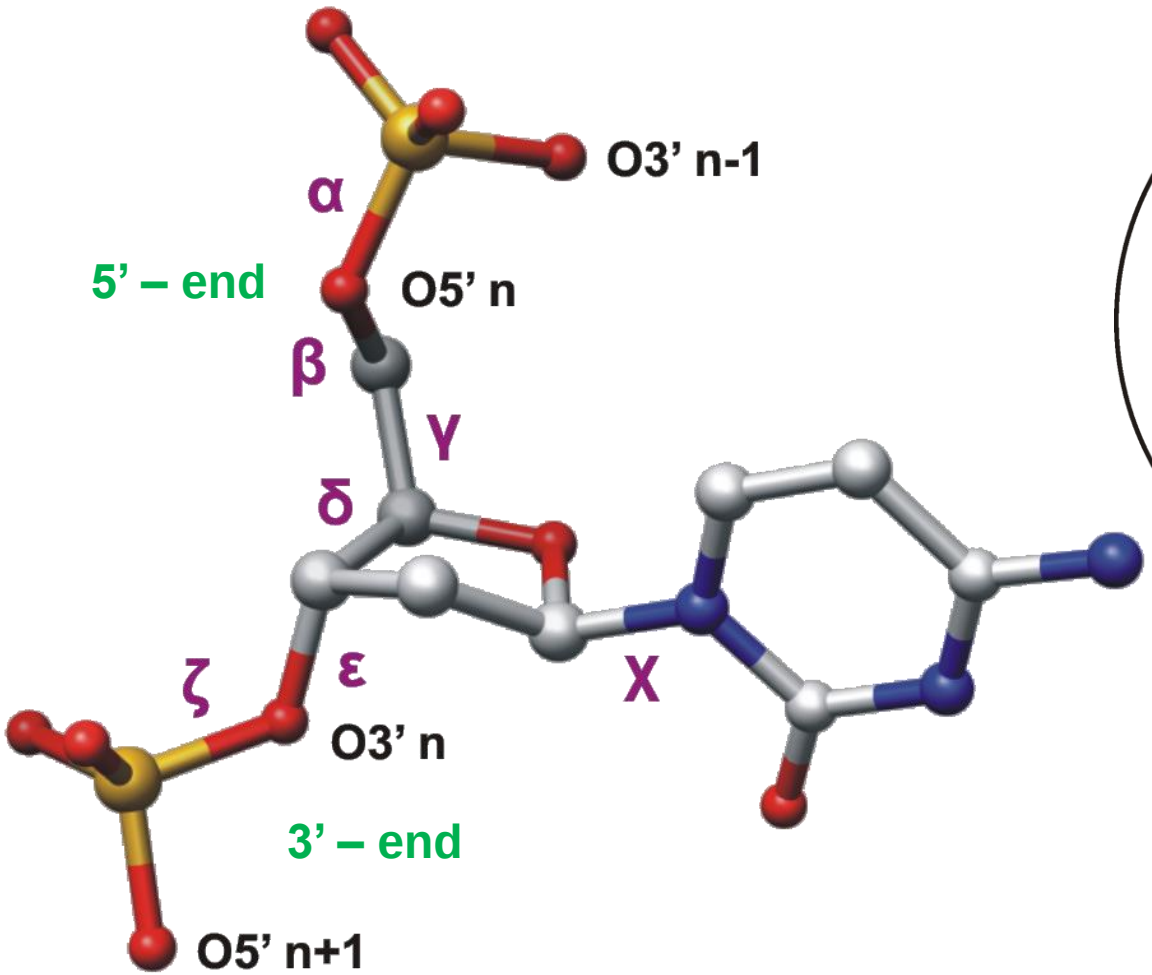


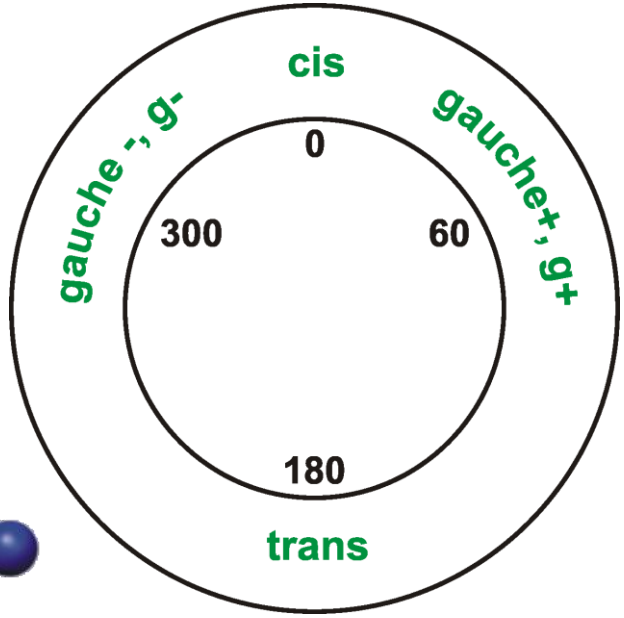




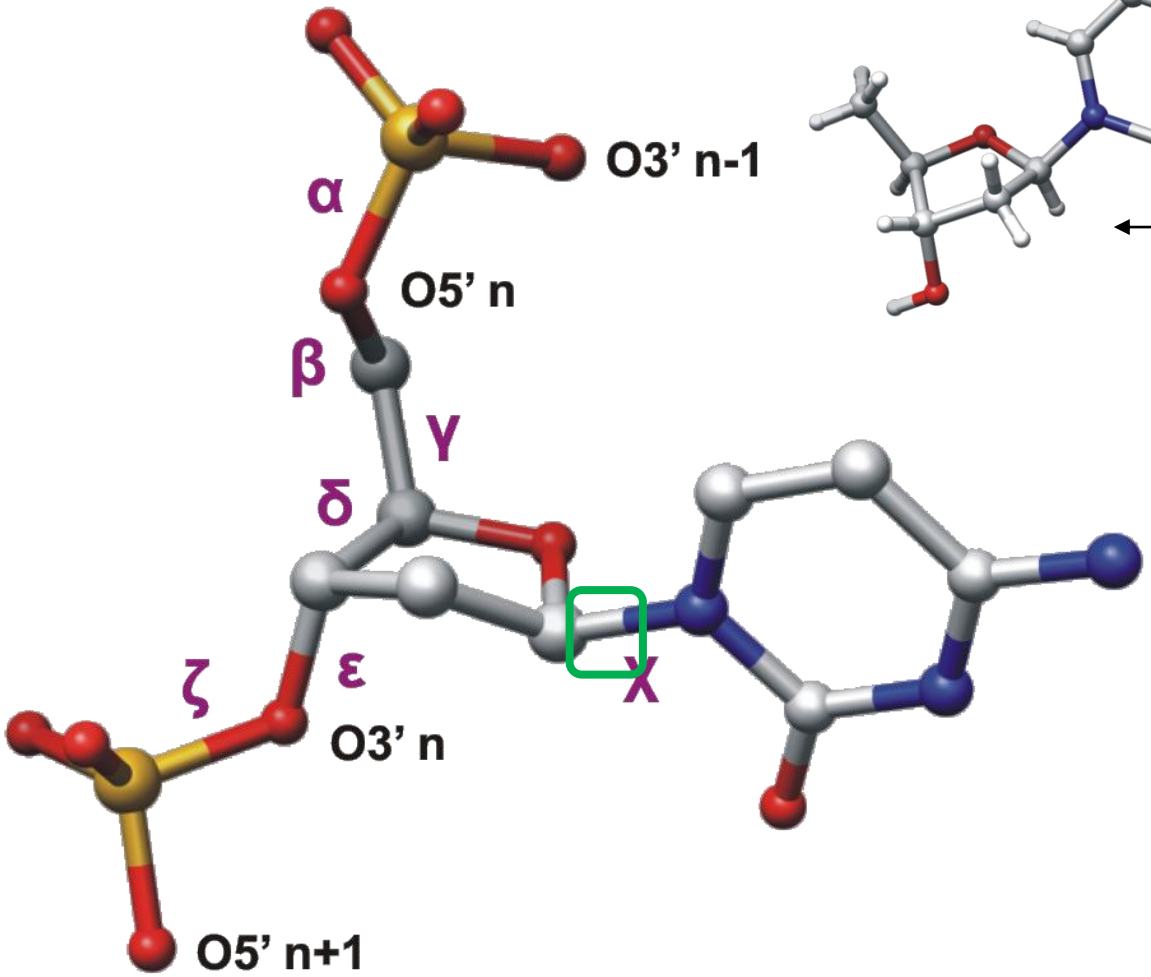
Images created with 3DNA illustrating positive values of designated parameters

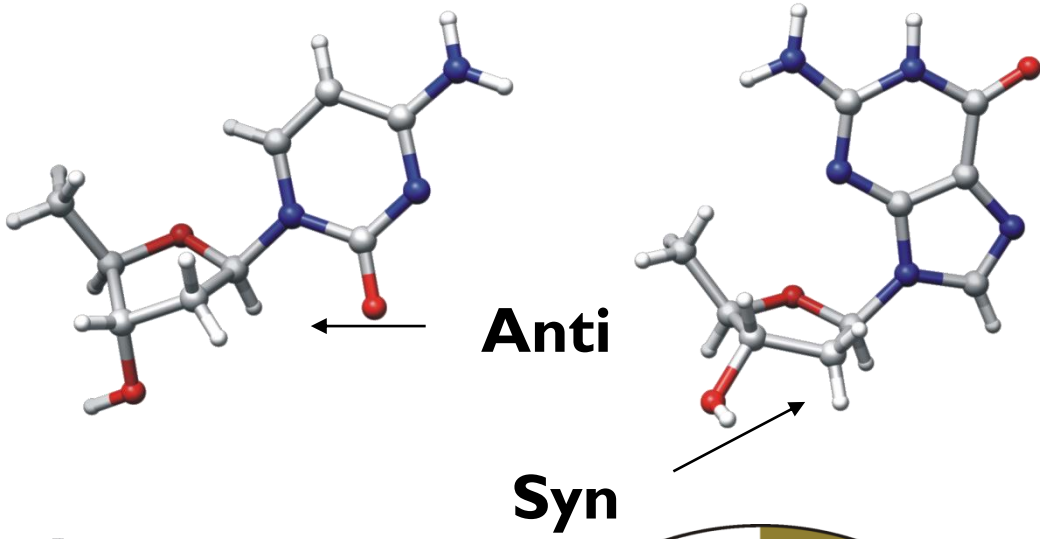
DNA backbone

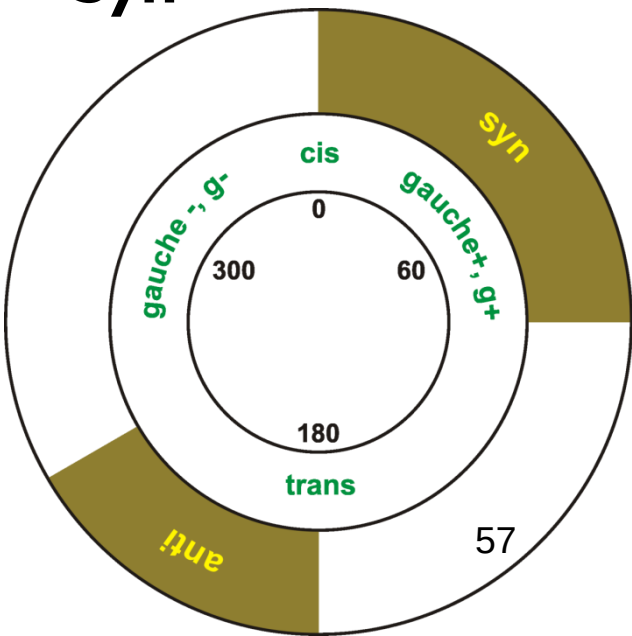




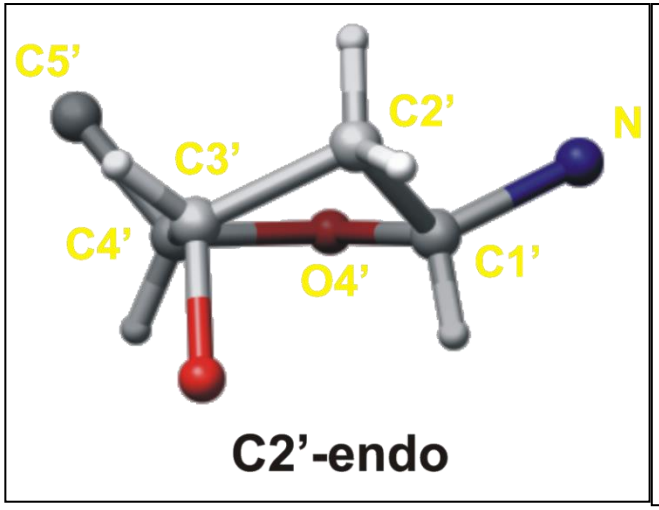
Base at sugar dihedrals

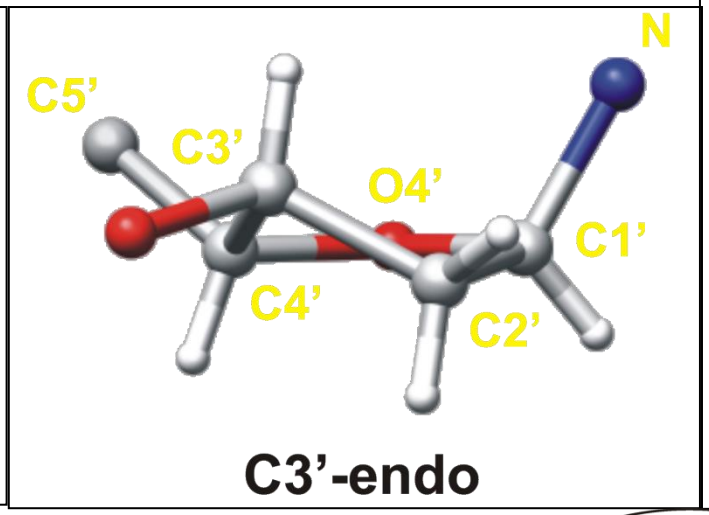


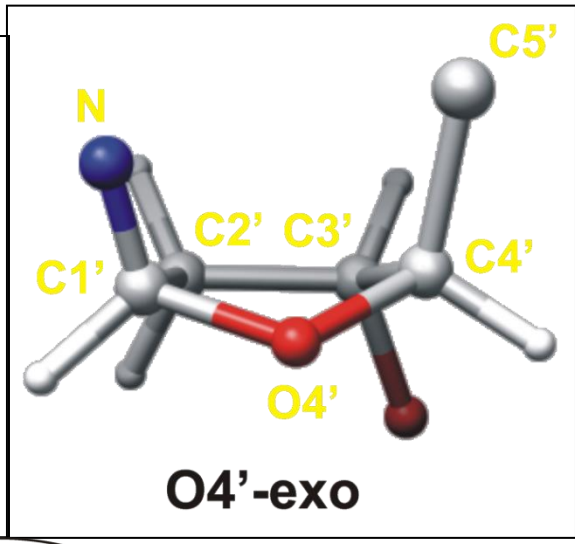




Sugar conformation

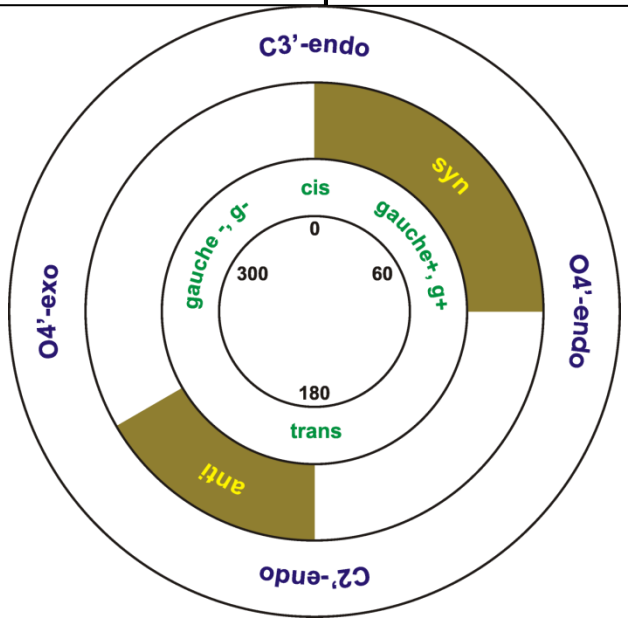




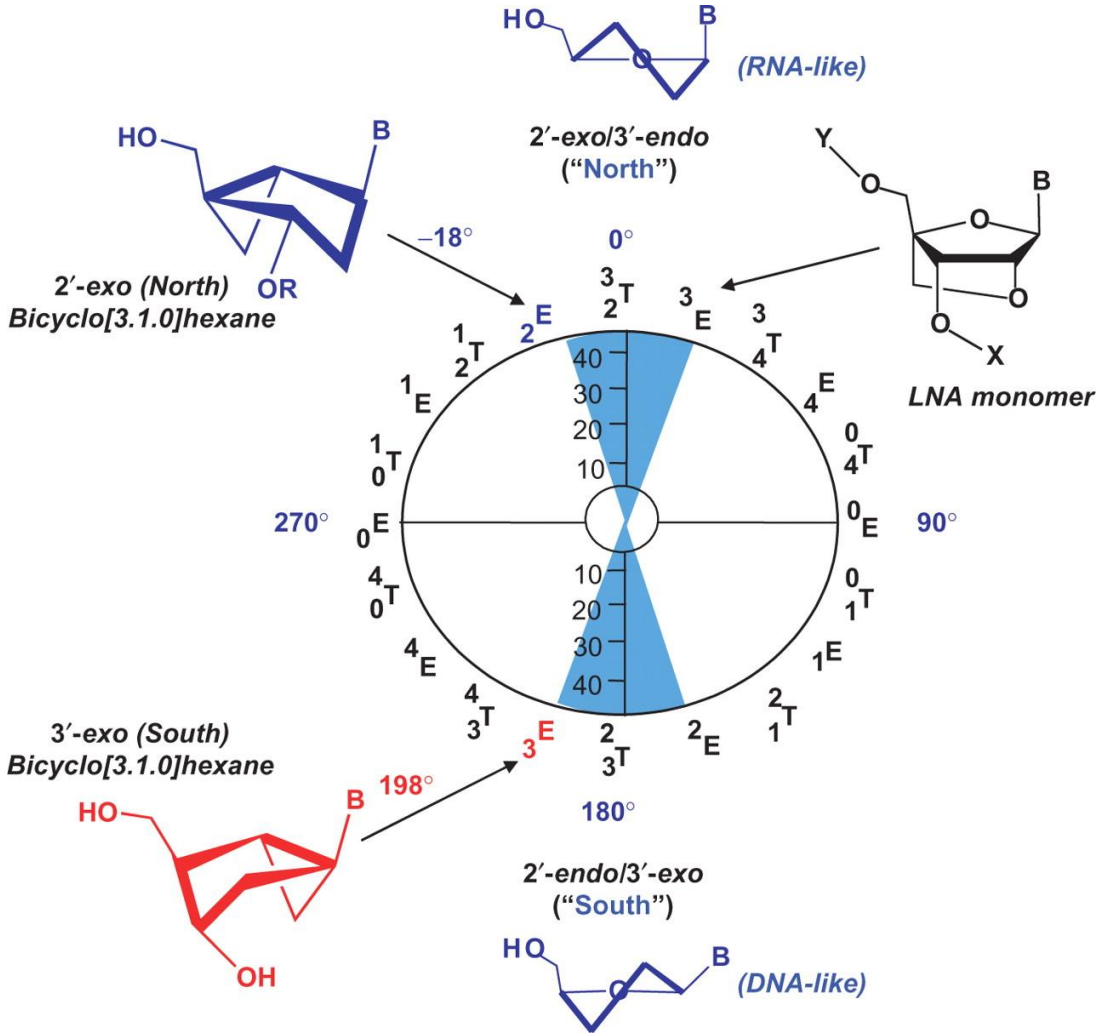


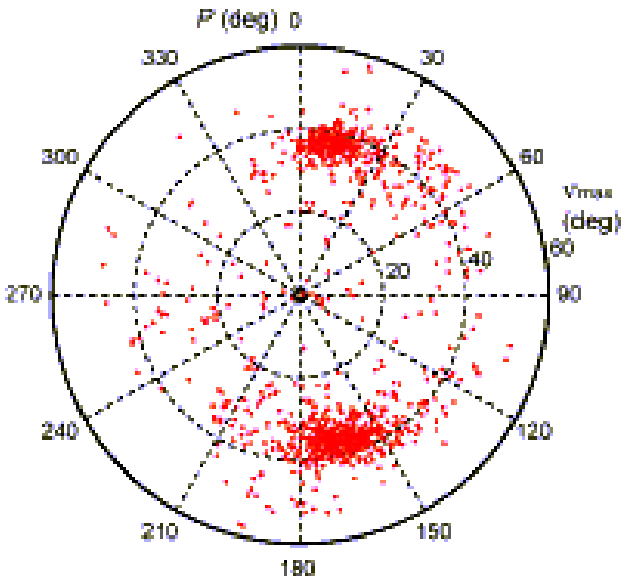
orientation with respect to C5'

- same side – endo
- opposite side – exo



Pseudorotational cycle for furanose ring puckers.





Pucker conformation of sugars in CSD database from PROSIT server

Maderia M et al. Nucl. Acids Res. 2007;35:1978-1991

Nucleic Acids Research

DNA Double helix

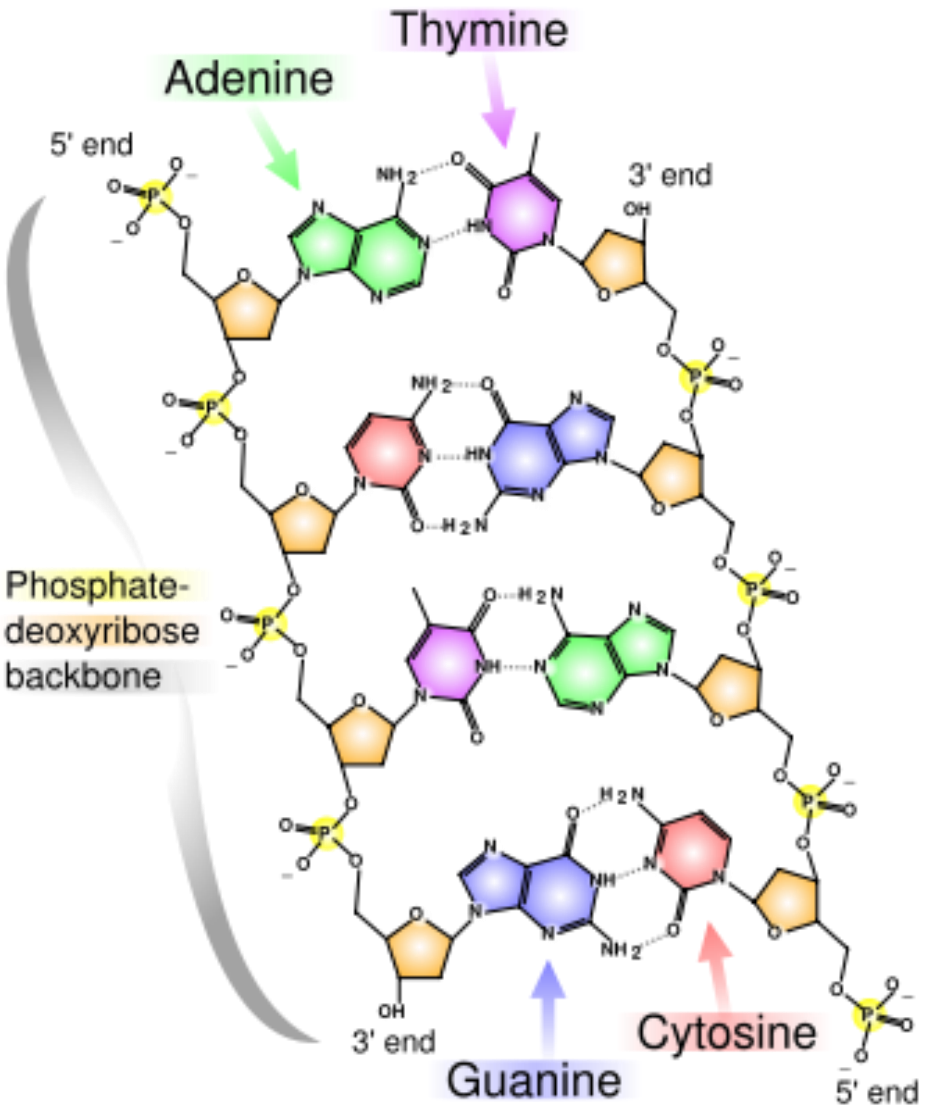
5’ ⟶ 3’

AATCGCTA

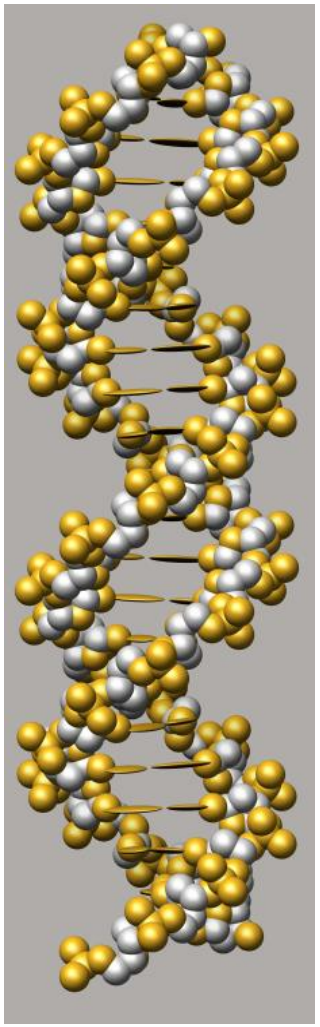
TTAGCGAT

3’ ⟵ 5’

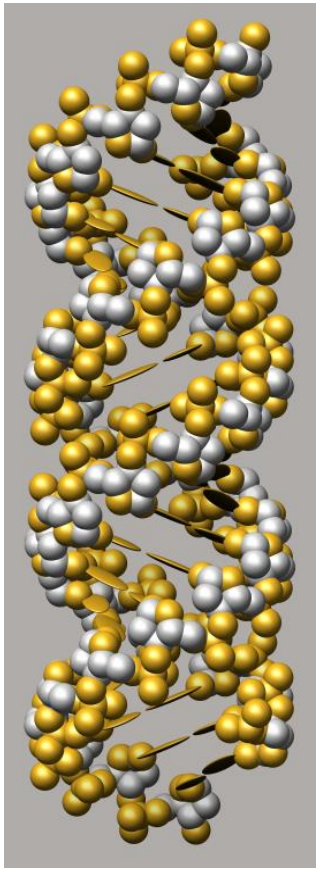
antiparallel



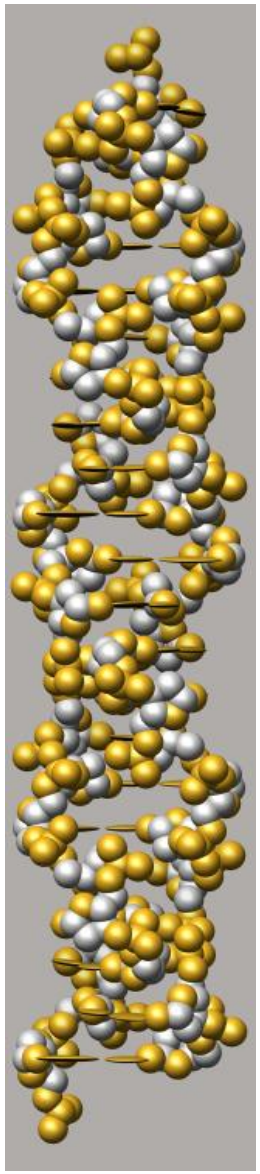
Types of DNA



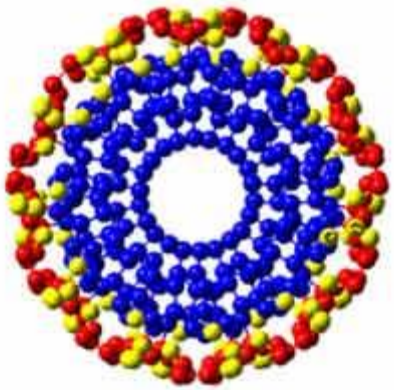
B-DNA



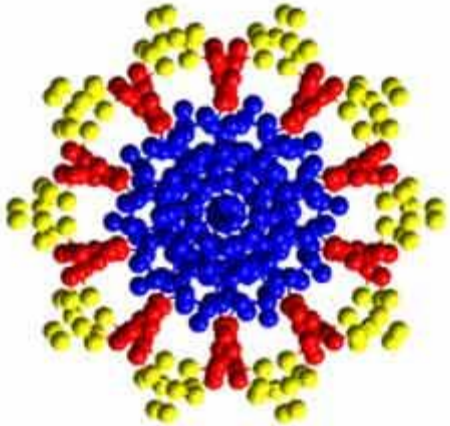
A-DNA



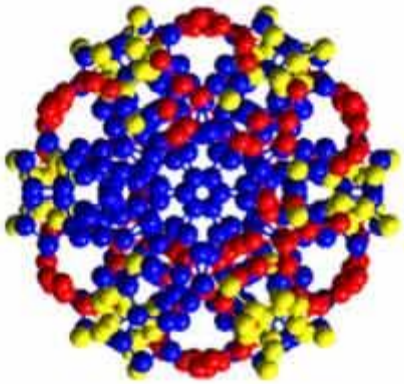
Z-DNA



A



B



Z

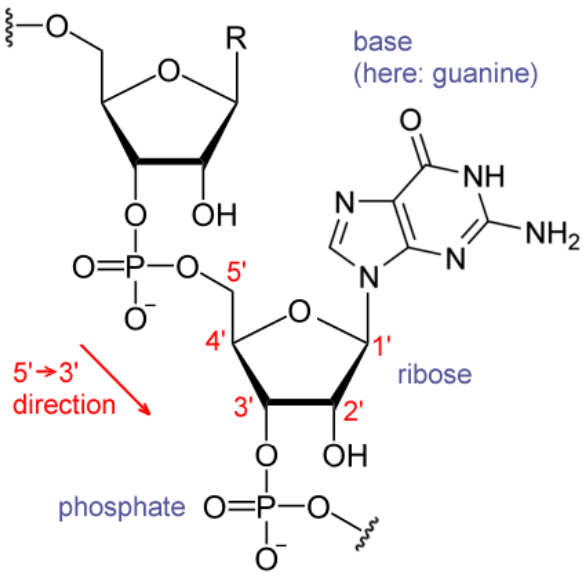
Biological role of different DNAs

- B-DNA
  - canonical DNA
  - predominant
- A-DNA
  - Conditions of lower humidity, common in crystallographic experiments. However, they’re artificial.
  - In vivo – local conformations induced e.g. by interaction with proteins.
- Z-DNA
  - No definite biological significance found up to now.
  - It is commonly believed to provide torsional strain relief (supercoiling) while DNA transcription occurs.
  - The potential to form a Z-DNA structure also correlates with regions of active transcription.

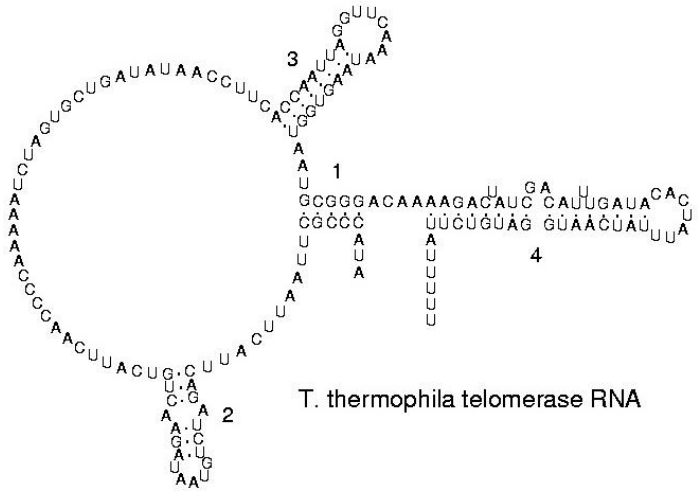
Different sets of DNA

- nuclear DNA
  - cell's nucleus
  - majority of functions cell carries out
  - sequencing the genome – scientists mean nuclear DNA
- mitochondrial DNA
  - *mt*DNA
  - circular, in human very short (17 kbp) with 37 genes (controling cellular metabolism)
  - all *mt*DNA comes from mom
- chloroplast DNA
  - *cp*DNA
  - circular and fairly large (120 – 160 kbp), with only 120 genes
  - inheritance is either maternal, or paternal

RNA - ribonucleic acid

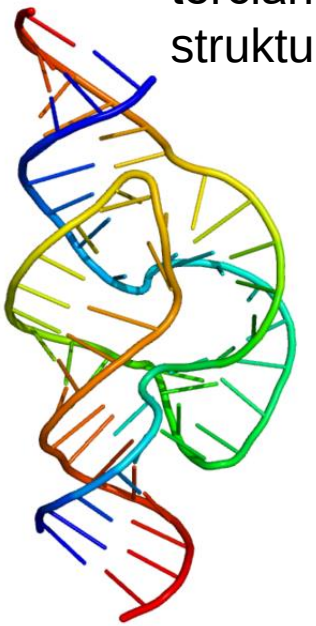


primární struktura



sekundární struktura

terciární struktura



hammerhead ribozyme 2GOZ

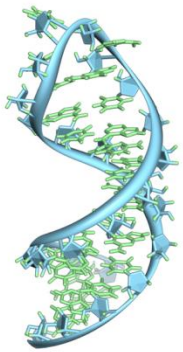
RNA

RNAs involved in protein synthesis

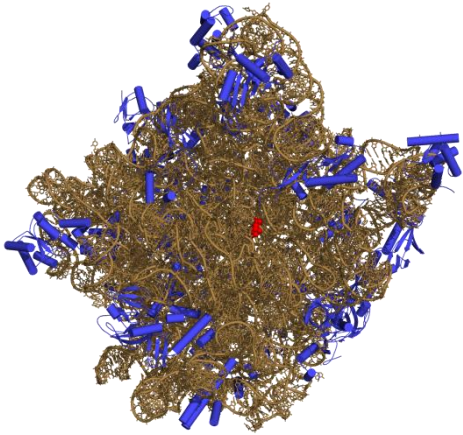
Type	Abbr.	Function	Distribution	Ref.
Messenger RNA	mRNA	Codes for protein	All organisms	
Ribosomal RNA	rRNA	Translation	All organisms	
Signal recognition particle RNA	7SL RNA or SRP RNA	Membrane integration	All organisms	[1]
Transfer RNA	tRNA	Translation	All organisms	
Transfer-messenger RNA	tmRNA	Rescuing stalled ribosomes	Bacteria	[2]

RNAs involved in post-transcriptional modification or DNA replication

Type	Abbr.	Function	Distribution	Ref.
Small nuclear RNA	snRNA	Splicing and other functions	Eukaryotes and archaea	[3]
Small nucleolar RNA	snoRNA	Nucleotide modification of RNAs	Eukaryotes and archaea	[4]
SmY RNA	SmY	mRNA trans-splicing	Nematodes	[5]
Small Cajal body-specific RNA	scaRNA	Type of snoRNA; Nucleotide modification of RNAs		
Guide RNA	gRNA	mRNA nucleotide modification	Kinetoplastid mitochondria	[6]
Ribonuclease P	RNase P	tRNA maturation	All organisms	[7]
Ribonuclease MRP	RNase MRP	rRNA maturation, DNA replication	Eukaryotes	[8]
Y RNA		RNA processing, DNA replication	Animals	[9]
Telomerase RNA		Telomere synthesis	Most eukaryotes	[10]



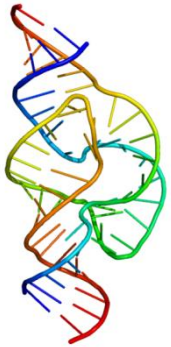
pre-mRNA hairpin



50S-ribozome

Regulatory RNAs

Type	Abbr.	Function	Distribution	Ref.
Antisense RNA	aRNA	Transcriptional attenuation / mRNA degradation / mRNA stabilisation / Translation block	All organisms	[11][12]
Cis-natural antisense transcript		Gene regulation		
CRISPR RNA	crRNA	Resistance to parasites, probably by targeting their DNA	Bacteria and archaea	[13]
Long noncoding RNA	Long ncRNA	Various	Eukaryotes	
MicroRNA	miRNA	Gene regulation	Most eukaryotes	[14]
Piwi-interacting RNA	piRNA	Transposon defense, maybe other functions	Most animals	[15][16]
Small interfering RNA	siRNA	Gene regulation	Most eukaryotes	[17]
Trans-acting siRNA	tasiRNA	Gene regulation	Land plants	[18]
Repeat associated siRNA	rasiRNA	Type of piRNA; transposon defense	Drosophila	[19]
7SK RNA	7SK	negatively regulating CDK9/cyclin T complex		



hammerhead ribozyme

2GOZ

Parasitic RNAs

Type	Function	Distribution	Ref.
Retrotransposon	Self-propagating	Eukaryotes and some bacteria	[20]
Viral genome	Information carrier	Double-stranded RNA viruses, positive-sense RNA viruses, negative-sense RNA viruses, many satellite viruses and reverse transcribing viruses	
Viroid	Self-propagating	Infected plants	[21]
Satellite RNA	Self-propagating	Infected cells	

Other RNAs

Type	Abbr.	Function	Distribution	Ref.
Vault RNA	vRNA	Expulsion of xenobiotics, maybe		[22]

http://en.wikipedia.org/wiki/List_of_RNAs

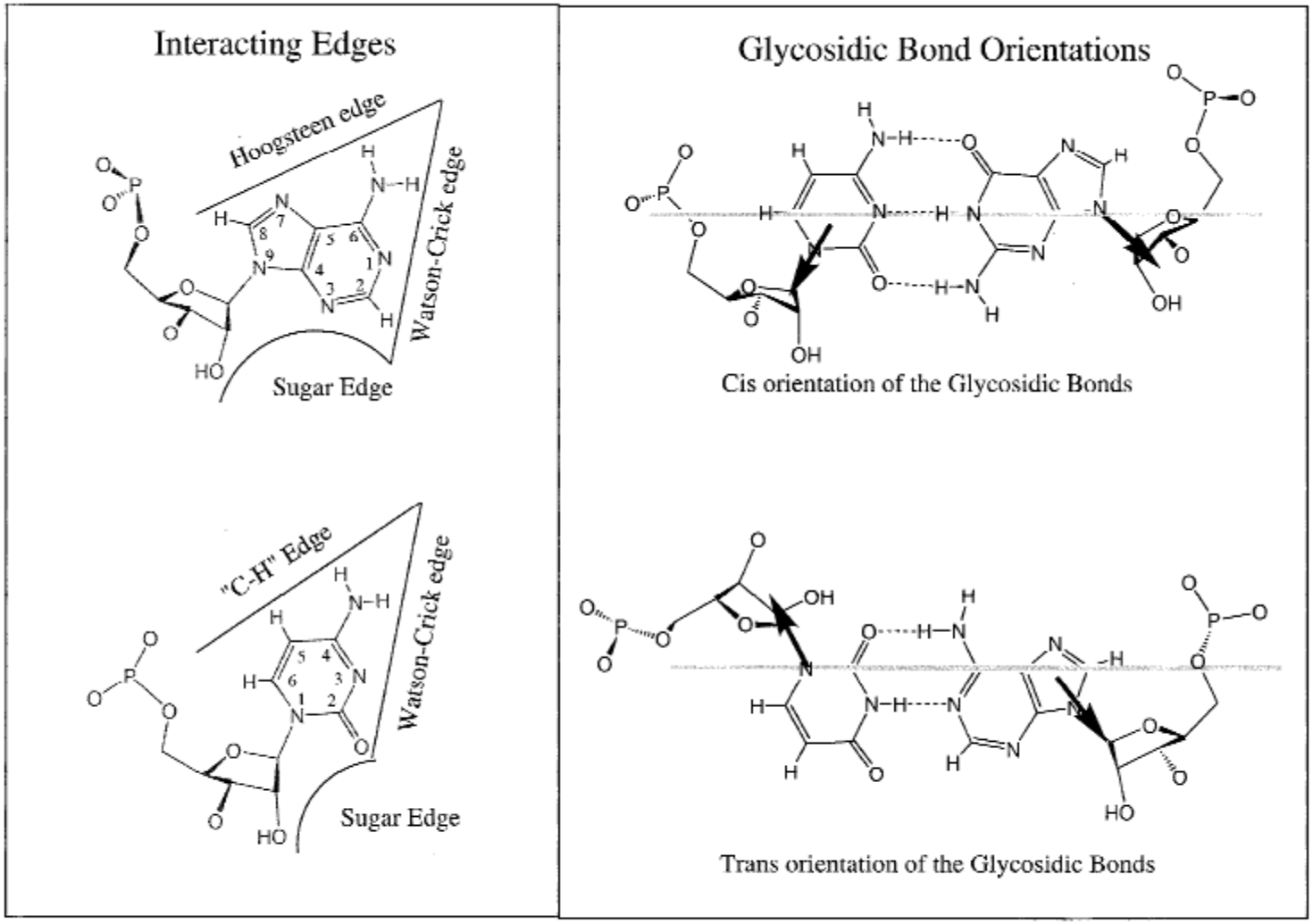


FIGURE 1. Left panel: Purine (A or G, indicated by "R") and pyrimidine (C or U, indicated by "Y") bases provide three edges for interaction, as shown for adenosine and cytosine. The Watson–Crick edge comprises A(N6)/G(O6), R(N1), A(C2)/G(N2), U(O4)/C(N4), Y(N3), and Y(O2). The Hoogsteen edge comprises A(N6)/G(O6), R(N7), U(O4)/C(N4), and Y(C5). The Sugar-edge comprises A(C2)/G(N2), R(N3), Y(O2), and the ribose hydroxyl group, O2′. Right panel: The *cis* and *trans* orientations are defined relative to a line drawn parallel to and between the *base-to-base* hydrogen bonds in the case of two hydrogen bonds or, in the case of three hydrogen bonds, along the middle hydrogen bond.

N. B. Leontis, E. Westhof, RNA (2001), 7:499-512

Annotation of 2D RNA Structures

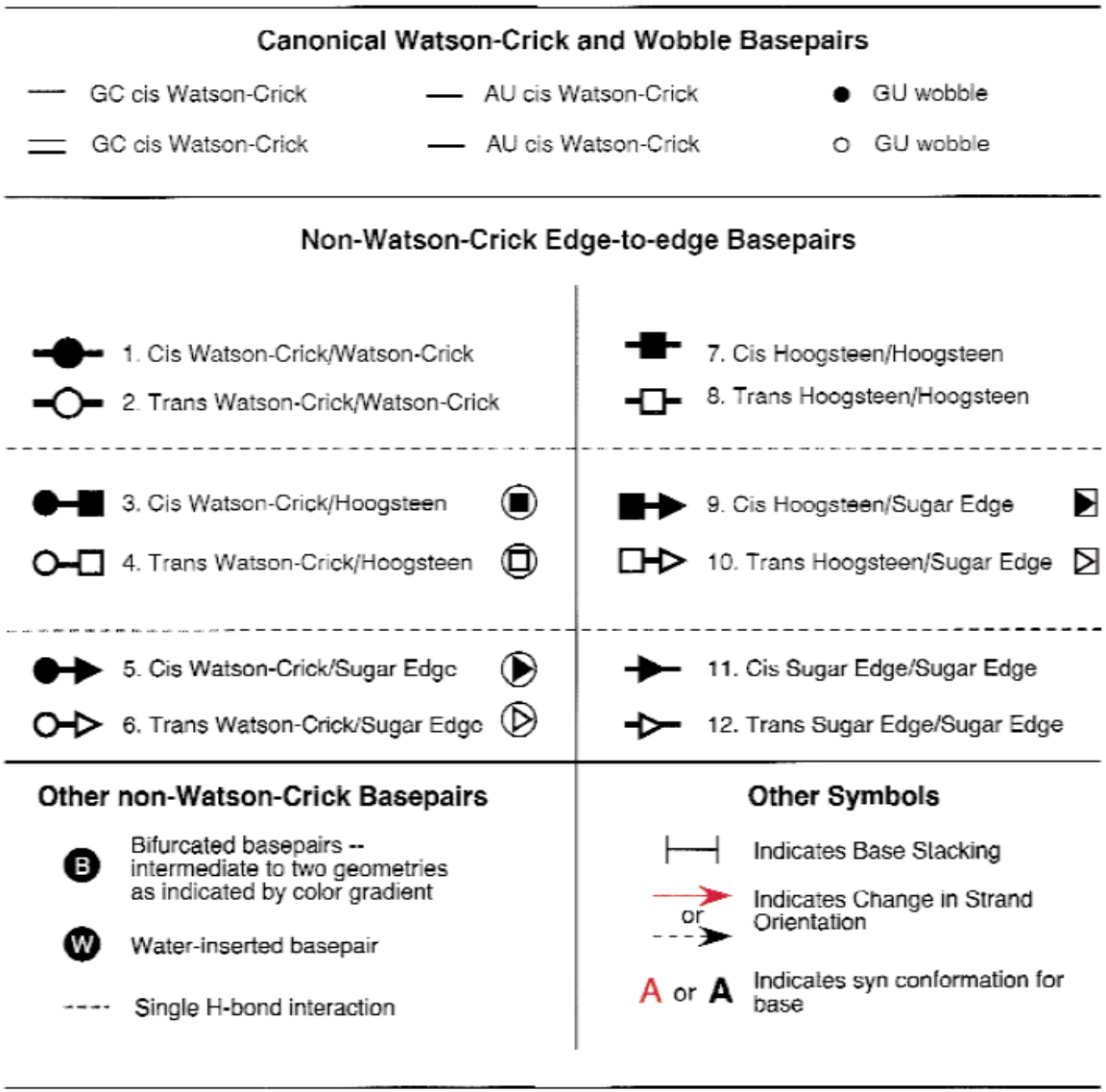
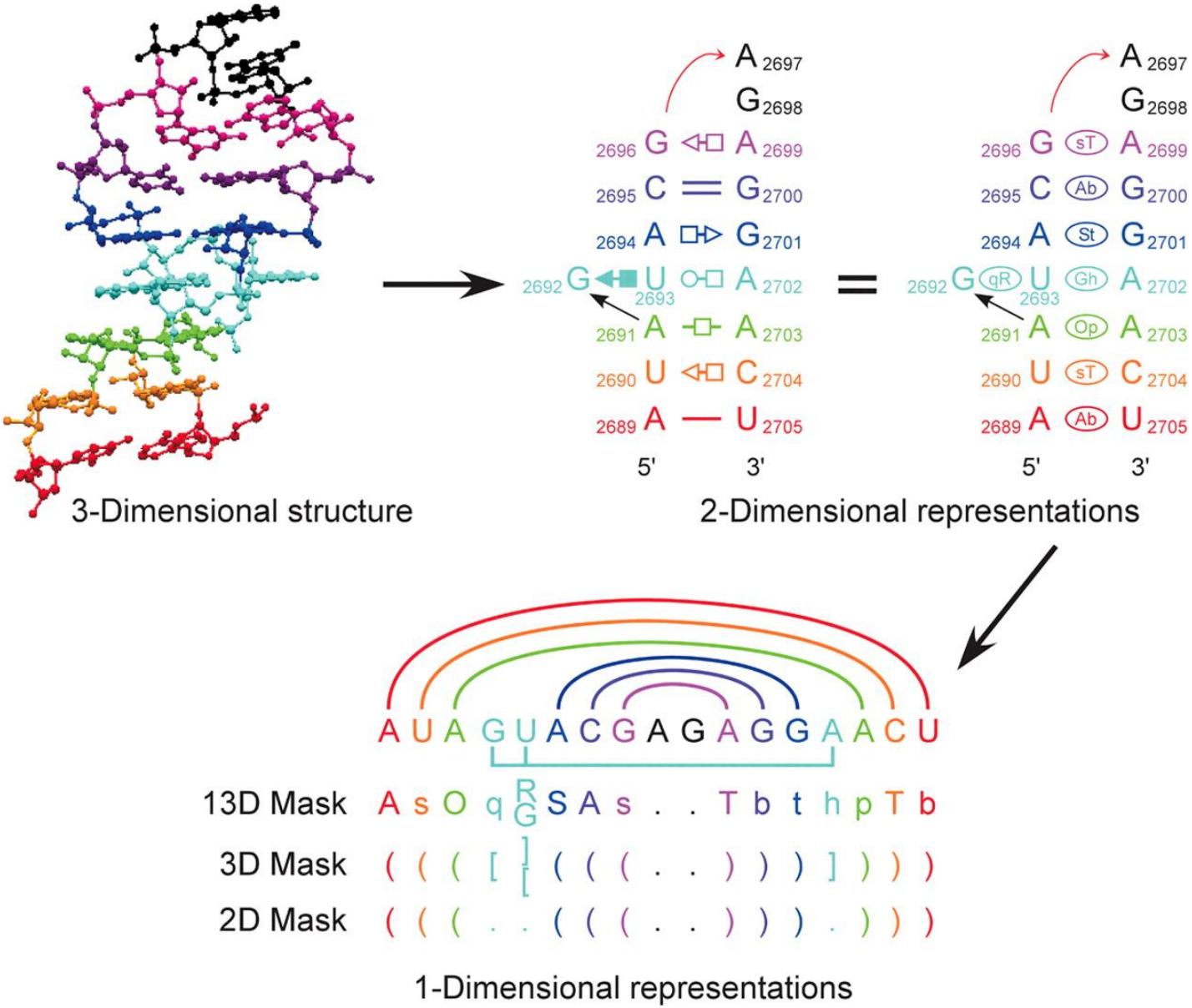


FIGURE 6. Suggested symbols for indicating tertiary interactions and other three-dimensional structural features in two-dimensional representations of RNA structures.

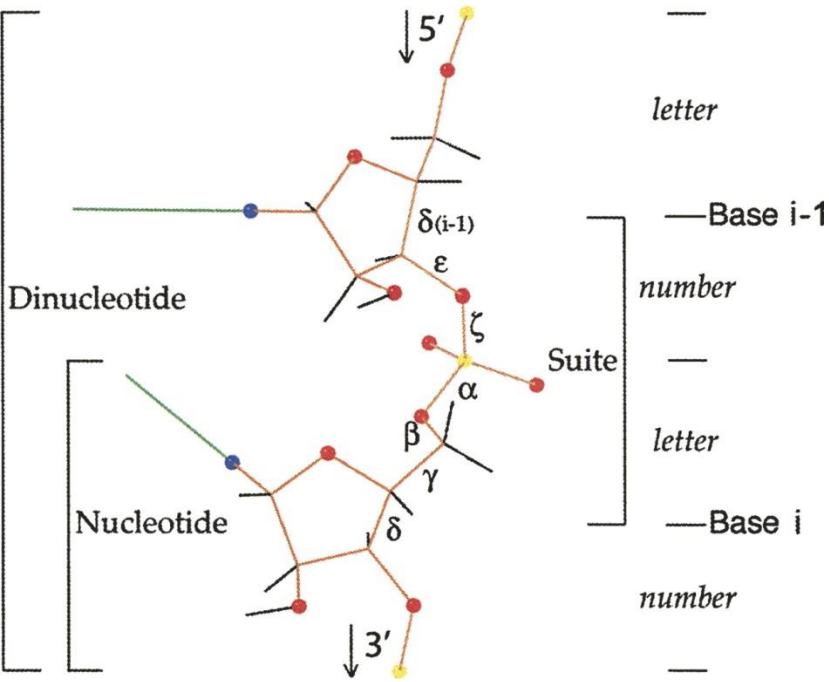
N. B. Leontis, E. Westhof, RNA (2001), 7:499-512

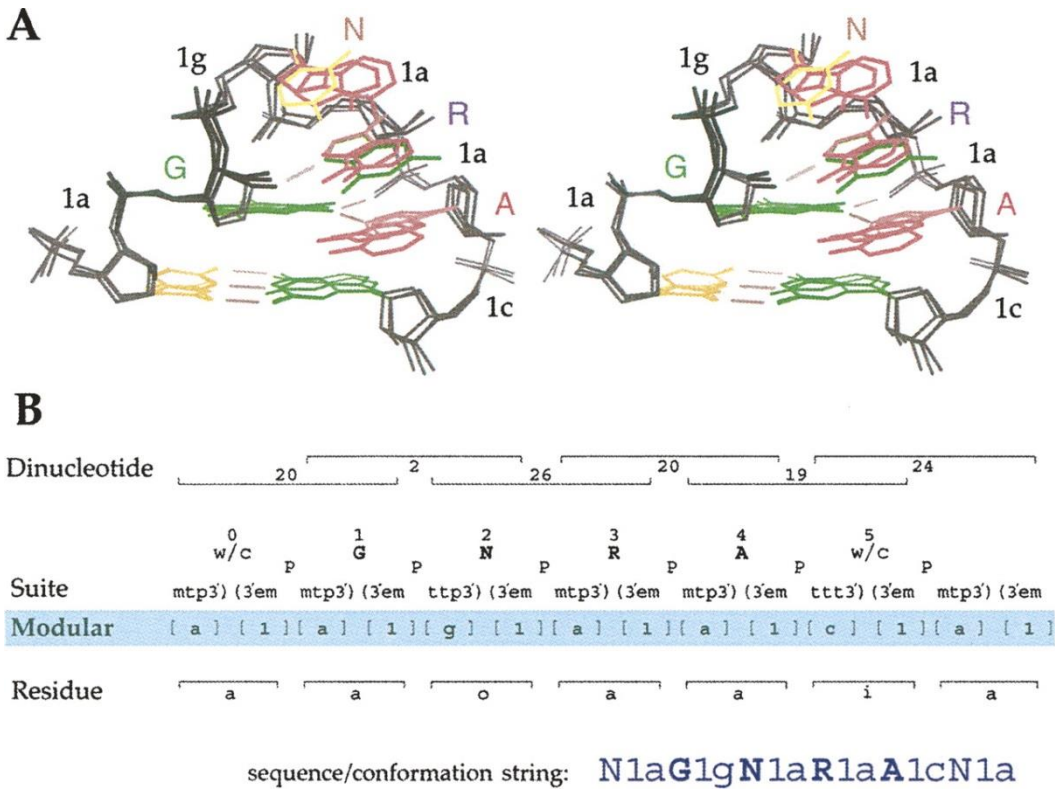
RNA Representations



Mokdad A , Leontis N B Bioinformatics 2006;22:2168-2170

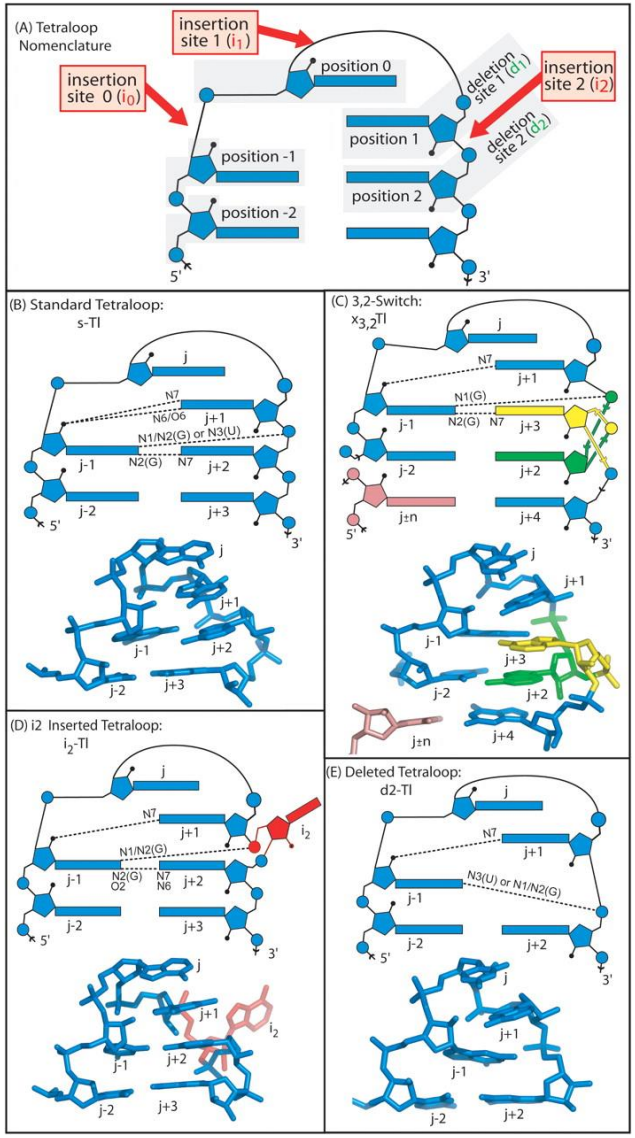
RNA Backbone

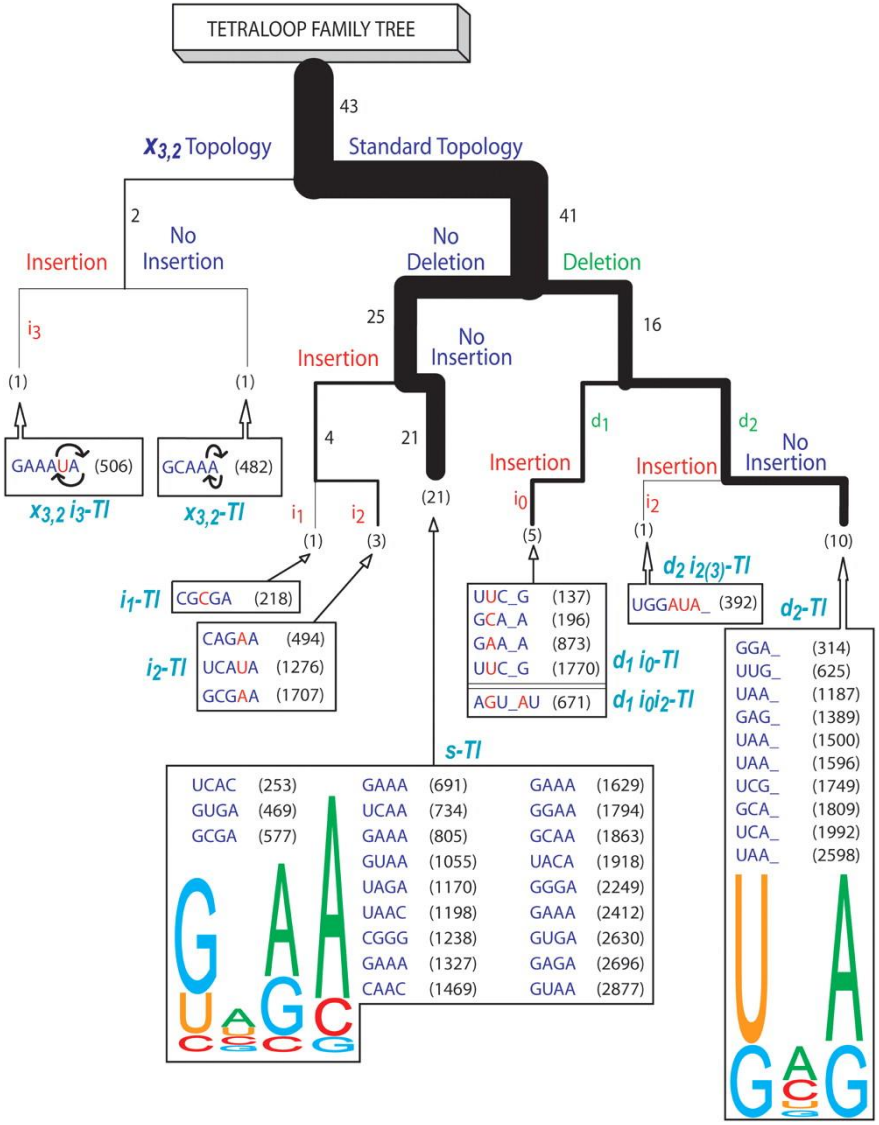




Richardson J S et al. RNA 2008;14:465-481

RNA Tetraloop Family Tree.

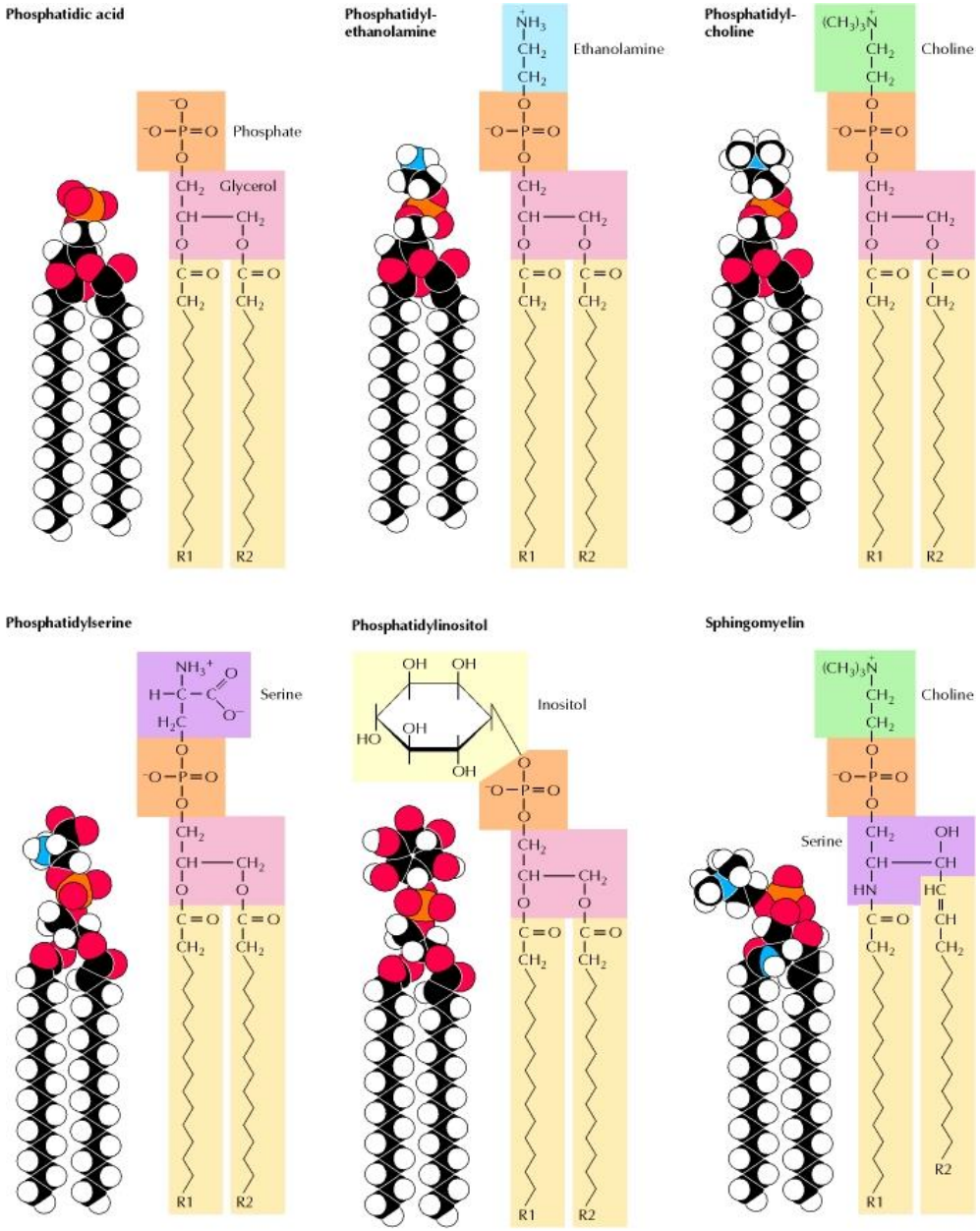


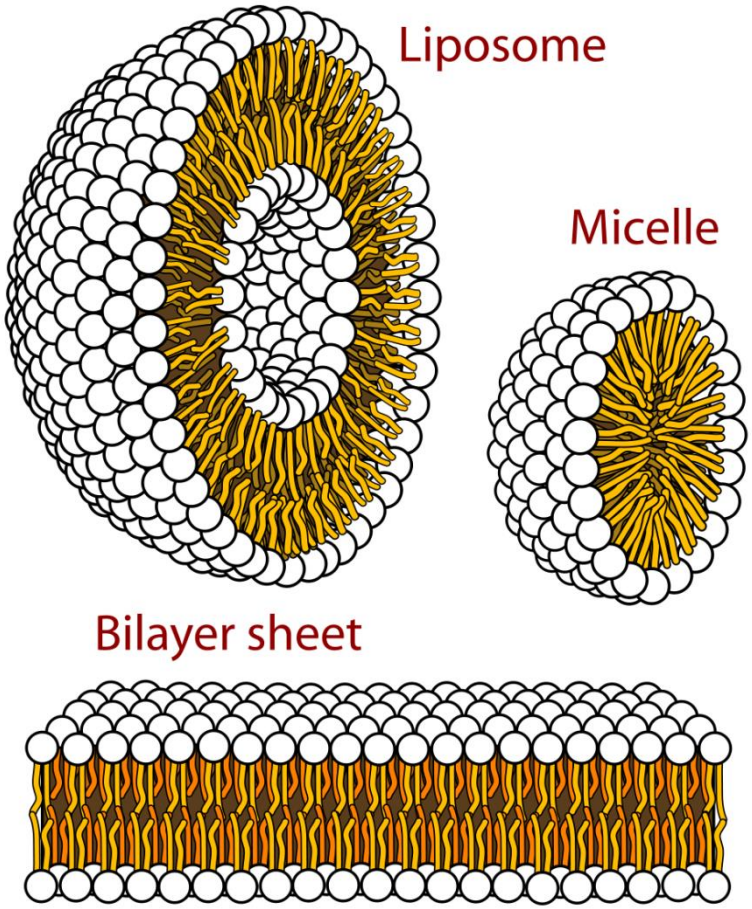


Nucleic Acids Research

Hsiao C et al. Nucl. Acids Res. 2006;34:1481-1491

Lipids





main phospholipids

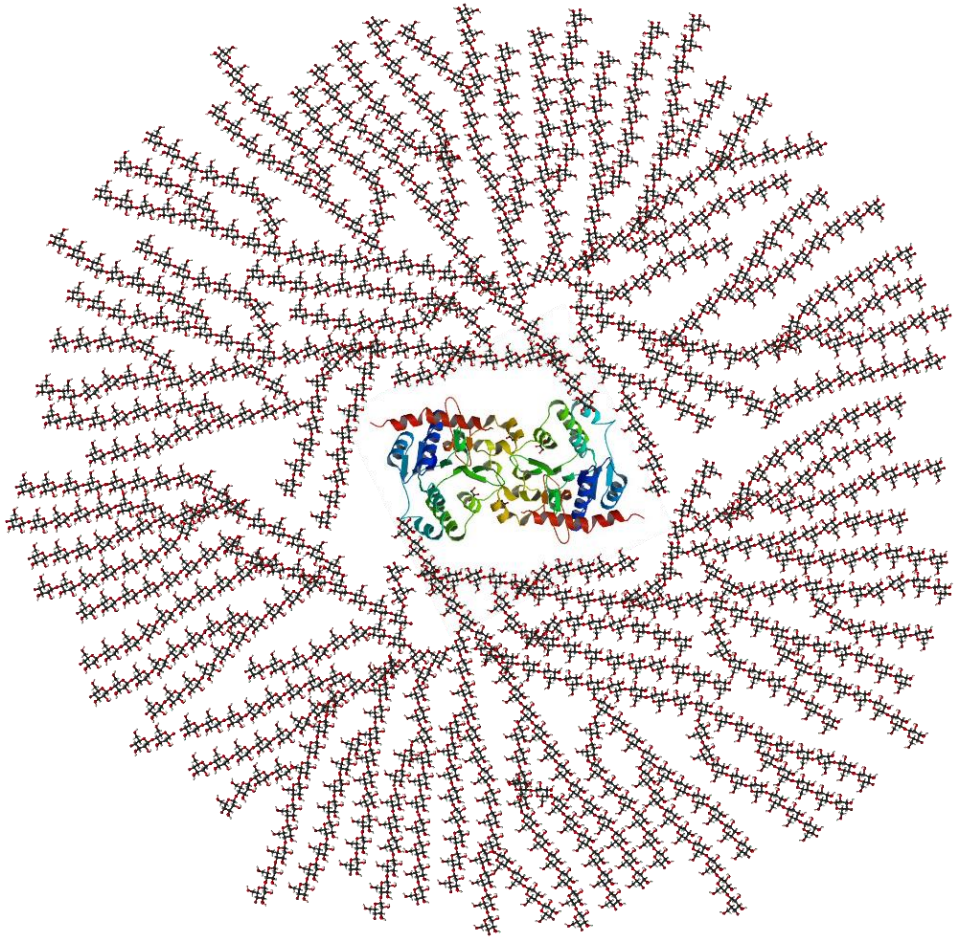
M. Paloncyová, Lipid membranes report, 2010

Polysaccharides

- role:
  - Energy storage
  - Molecular recognition

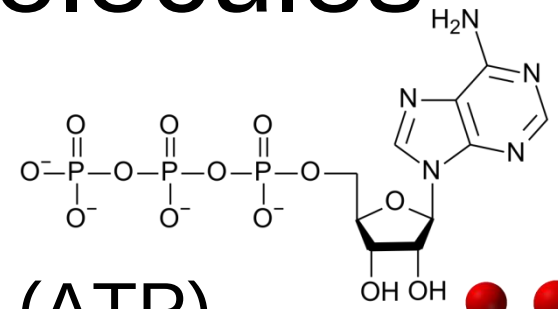
- Harder to read in sequences than NA or proteins

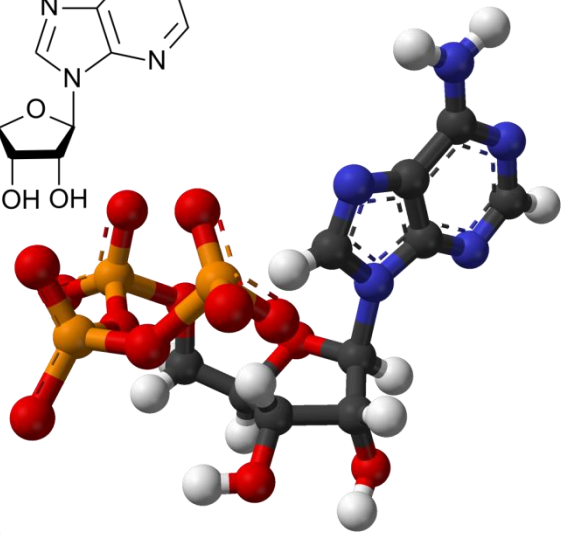
- Quite often on extracellular proteins



glycogen

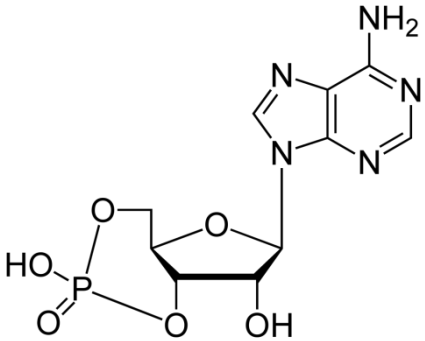
Small molecules

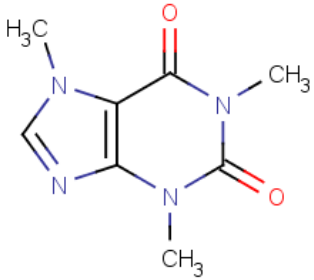




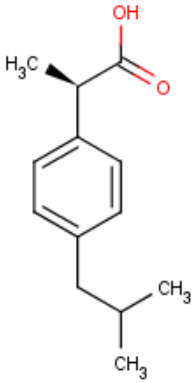
- NTP
  - Cell energy transporter (ATP)
  - Basic stones for NA

- Messengers, Agonists, antagonists
  - (cAMP, xenobiotics)





caffeine



ibuprofen