Protein Structure and Variants

CB2-201 – Computational Biology and Bioinformatics

February 23, 2015

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http://biofold.org/emidio

Biomolecules Folding and Disease

Division of Informatics
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Main data types

In molecular biology several type of data are available. Among the most common there are:

- Sequences: string representing the nucleotide and amino acid composition of DNA, RNA and protein.

- Annotations: collection of words with controlled vocabulary that describes property, function, and process in which a biomolecule is involved.

- **Structure: 2D or 3D representation of a molecule describing how it is organized in the space.**

Molecular biology data

GenBank: 179,295,769

```
>BGAL_SULSO BETA-GALACTOSIDASE Sulfolobus solfataricus.
MYSFPNSFRFGWSQAGFQSEMGTPGSEDPNTDWYKWVHDPENMAAGLVSG
DLPENGPGYWGNYKTFHDNAQKMGLKIARLNVEWSRIFPNPLPRPQNFDE
SKQDVTEVEINENELKRLDEYANKDALNHYREIFKDLKSRGLYFILNMYH
WPLPLWLHDPIRVRRGDFTGPSGWLSTRTVYEFARFSAYIAWKFDDLVDE
YSTMNEPNVVGGLGYVGVKSGFPPGYLSFELSRRHMYNIIQAHARAYDGI
KSVSKKPVGIIYANSSFQPLTDKDMEAVEMAENDNRWWFFDAIIRGEITR
GNEKIVRDDLKGRLDWIGVNYYTRTVVKRTEKGYVSLGGYGHGCERNSVS
LAGLPTSDFGWEFFPEGLYDVLTKYWNRYHLYMYVTENGIADDADYQRPY
YLVSHVYQVHRAINSGADVRGYLHWSLADNYEWASGFSMRFGLLKVDYNT
KRLYWRPSALVYREIATNGAITDEIEHLNSVPPVKPLRH
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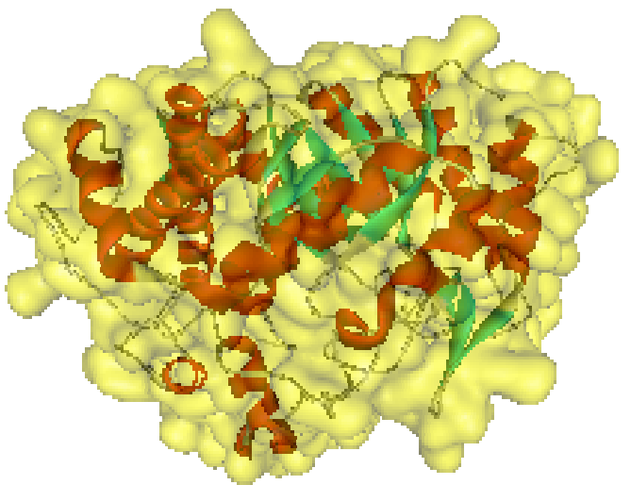
UniRef90: 30,147,837



Swiss-Prot: 547,599

Protein Data Bank: 106,517

Protein: 98,954

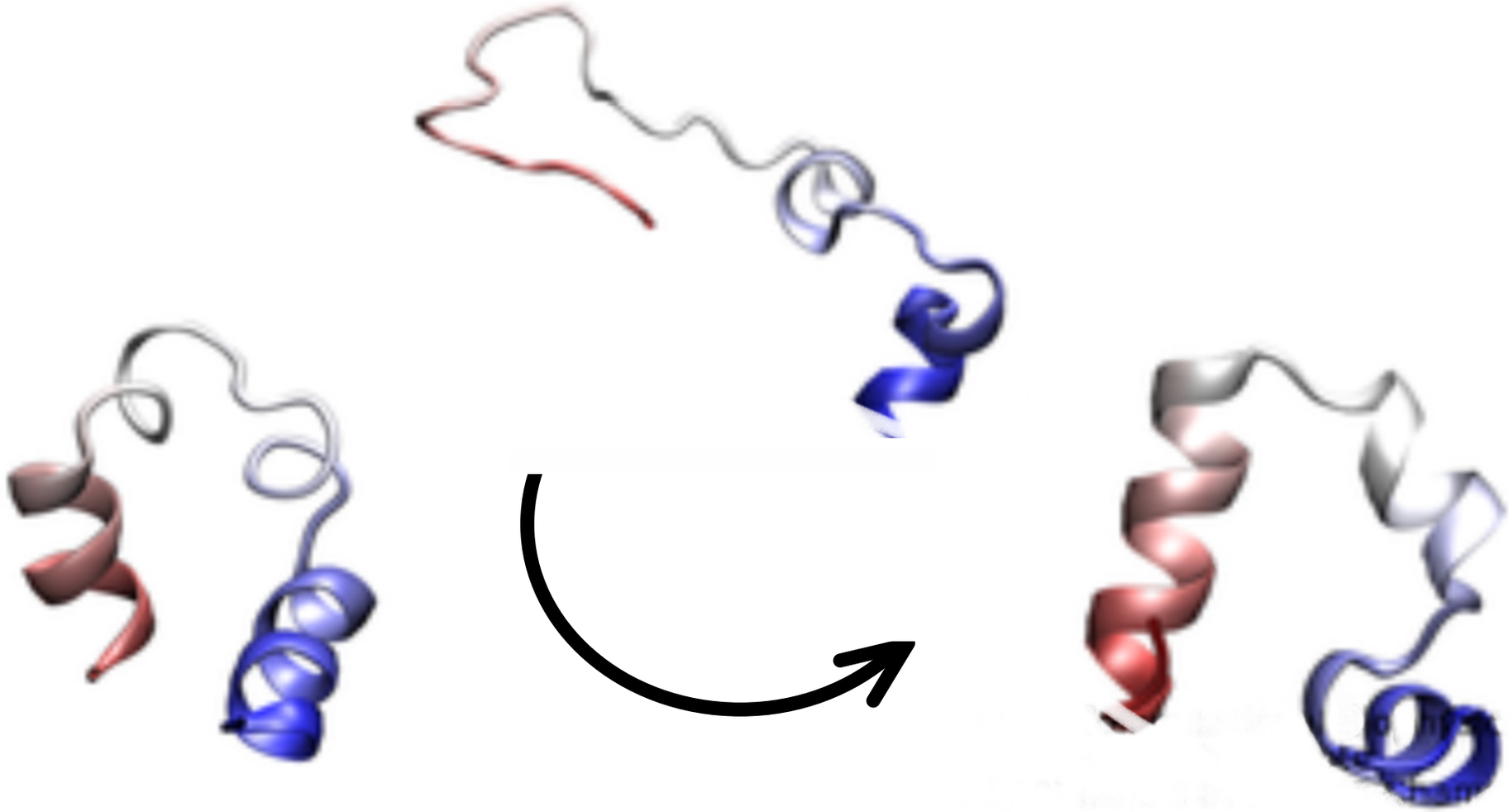
Nucleic Acids: 2,749

Feb 2015

Protein folding

Protein folding is the process by which a protein assumes its native structure from the unfolded structure

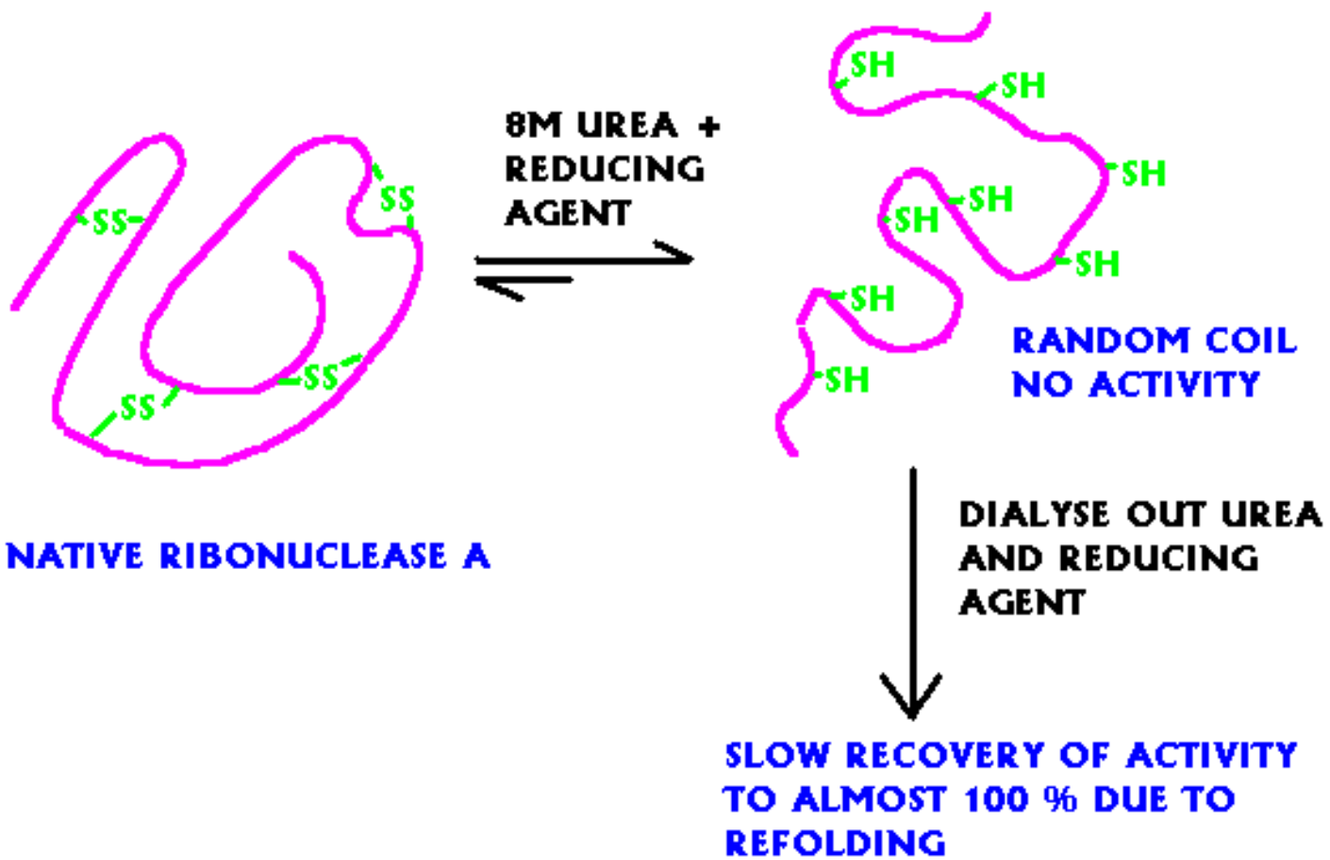
T T C C P S I V A R S N F N V C R L P G T P E A L C A T
Y T G C I I I P G A T C P G D Y A N



The Anfinsen's hypothesis

The sequence contains all the information to specify 3-D structure

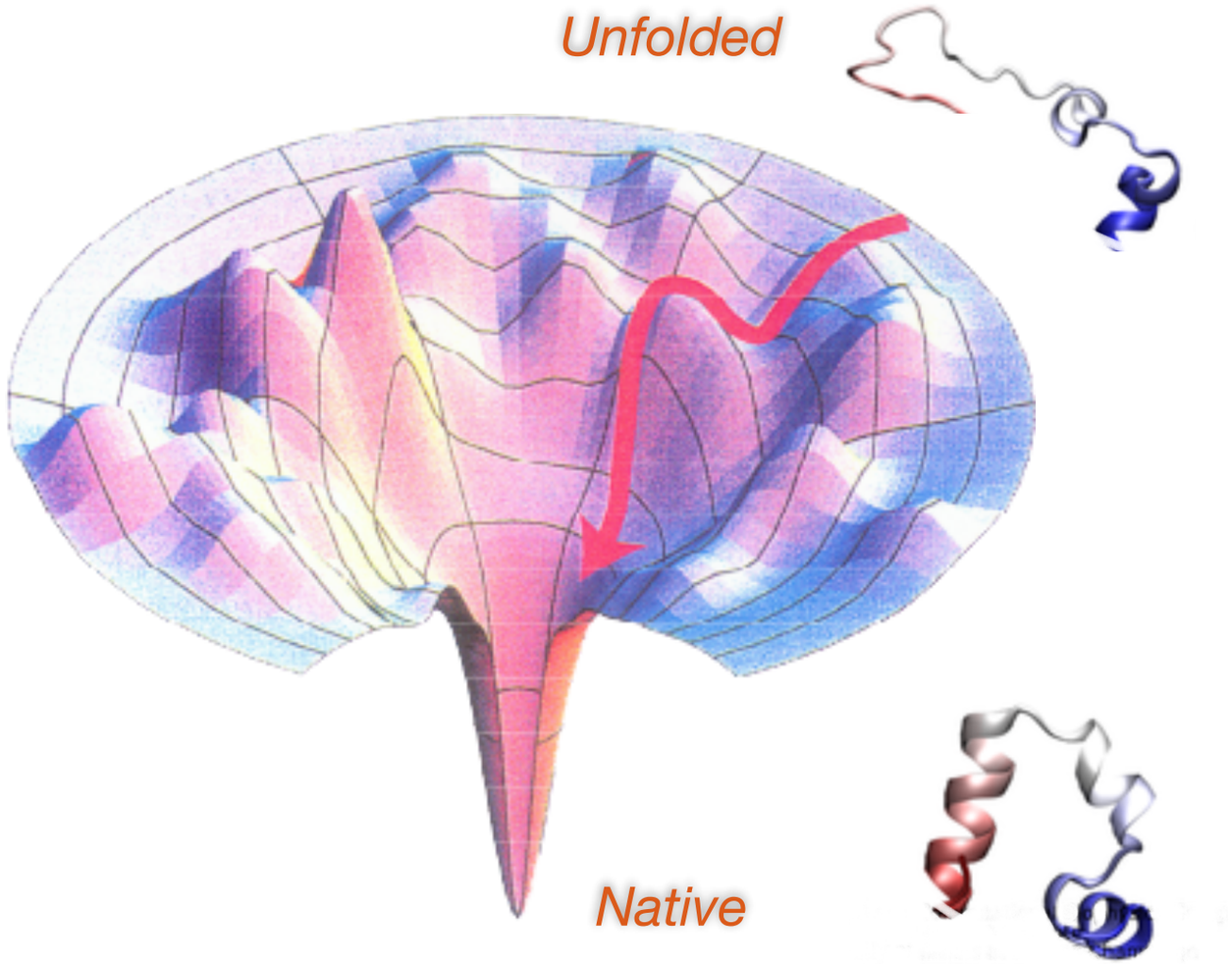
Anfinsen showed that denatured ribonuclease A could be re-activated removing the denaturant.



Anfinsen CB. (1973). Science. 181: 223-230

Levinthal's paradox

A protein chain composed by 100 residues with 2 possible conformations has 2^{100} (10^{30}) possible conformations. Considering a time-step of 10^{-12} s for visiting each conformation, the folding process would take 10^{18} s, that is longer than the age of our Universe (2-3 x 10^{17}s)



Dill et al. (1997). Nature Struct Biology, 4; 10-19

The Anfinsen's Dogma

Uniqueness: requires that the sequence does not have any other configuration with a comparable free energy.

Stability: small changes in the surrounding environment not affect the structure of the stable conformation. This can be pictured as a free energy surface that looks more like a funnel and the free energy surface around the native state must be rather steep and high, in order to provide stability.

Kinetical accessibility: means that the path in the free energy surface from the unfolded to the folded state must be reasonably smooth or, in other words, that the folding of the chain must not involve highly complex changes in the shape.

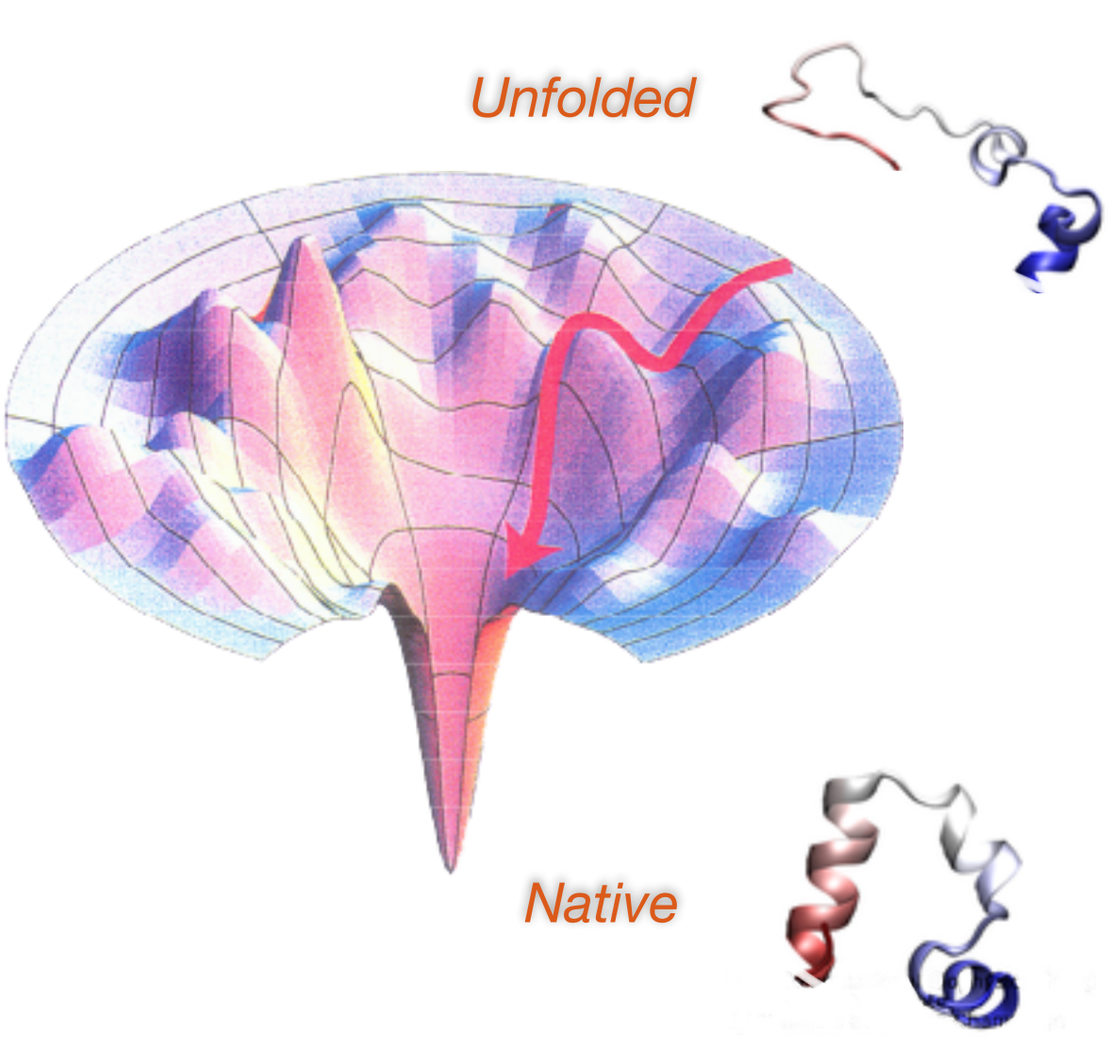
Aspects of the same problem

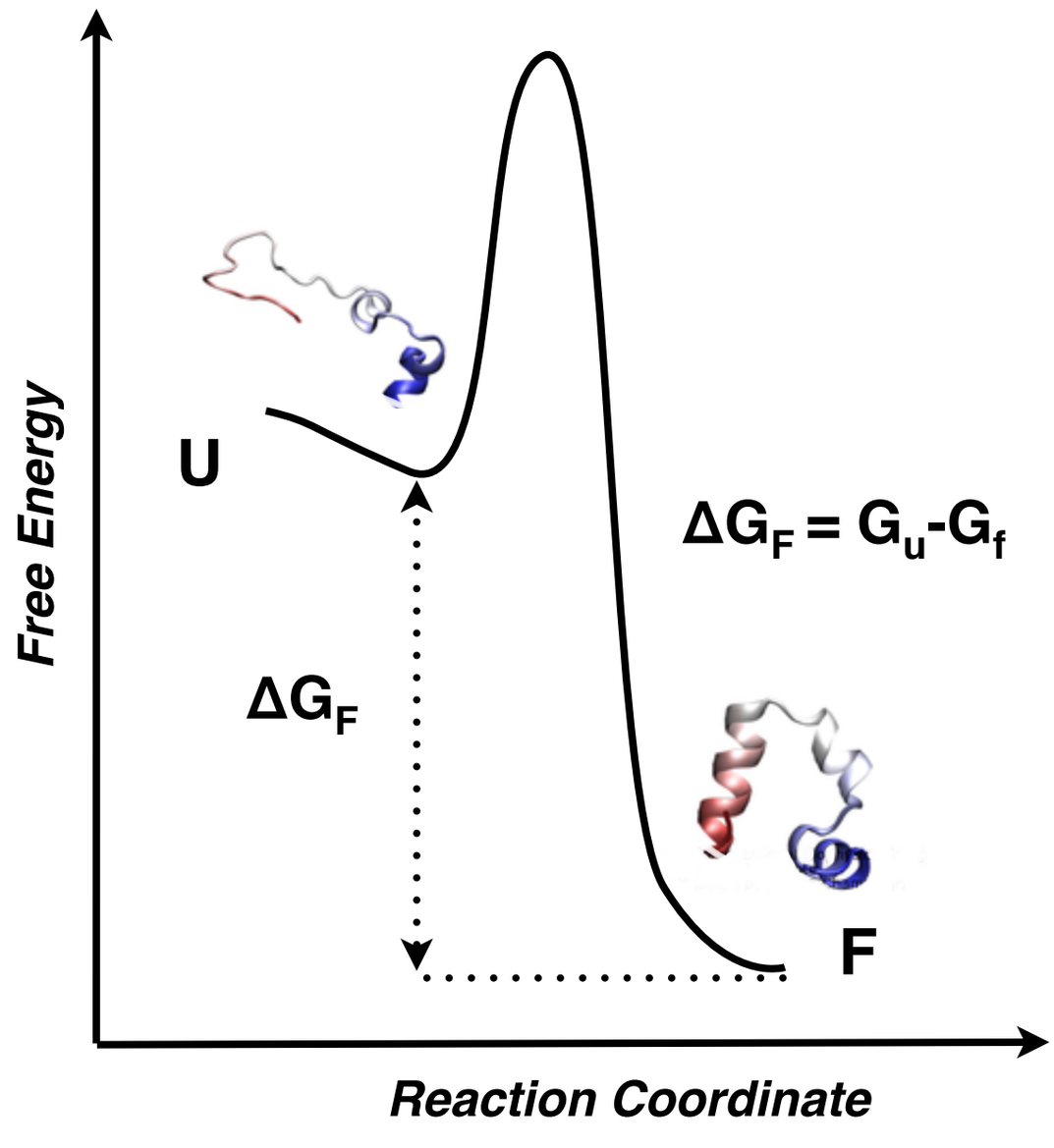
The solution of the protein folding consists in the understanding of three different aspects of the problem:

- Estimate the stability of the native conformation and thermodynamic of the process.
- Define the mechanism and the kinetic of the process.
- Predict the native three-dimensional structure of the protein.

Folding and stability

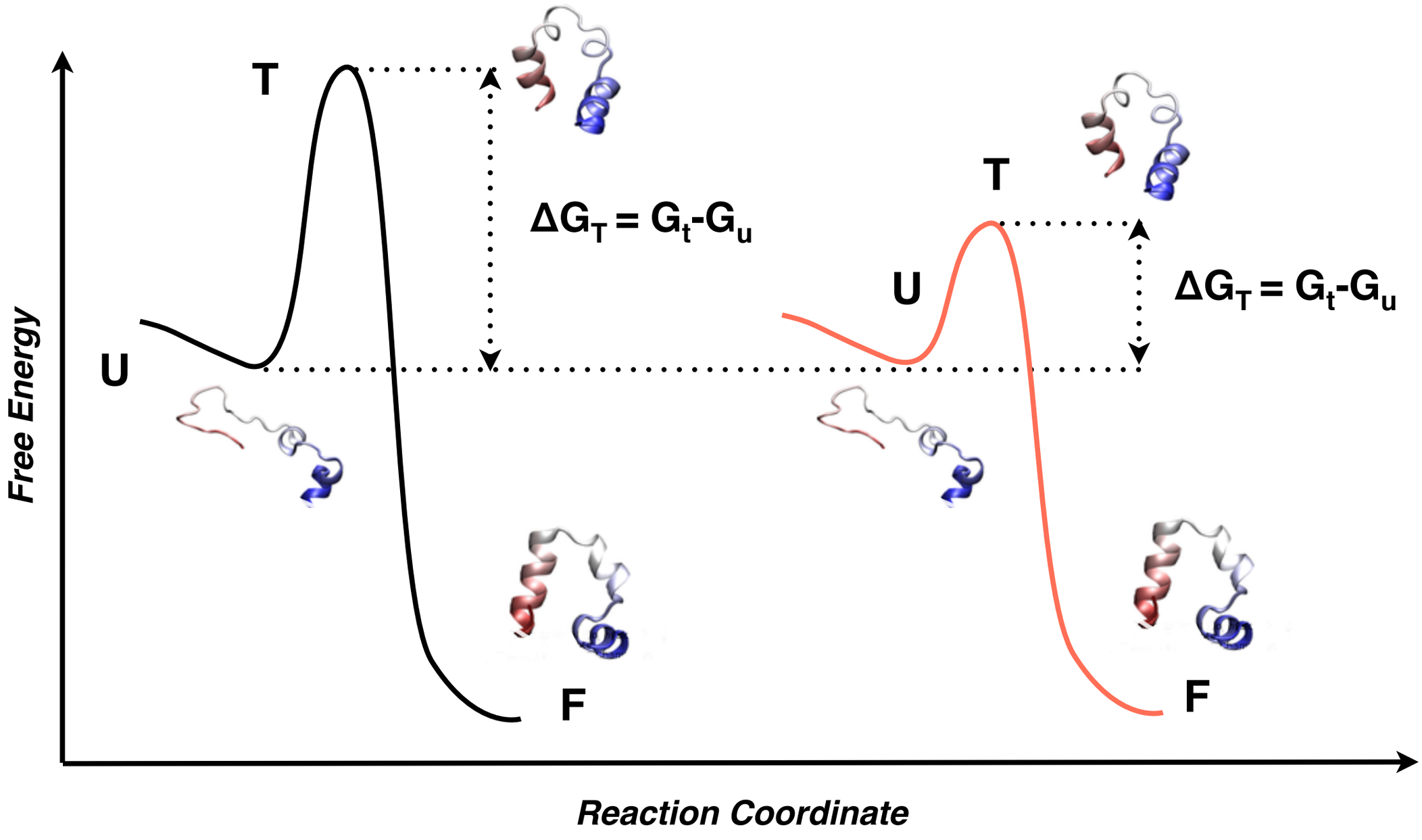
The folding free energy difference, ΔG_F, is typically small, of the order of -5 to -15 kcal/mol for a globular protein (compared to e.g. -30 to -100 kcal/mol for a covalent bond).





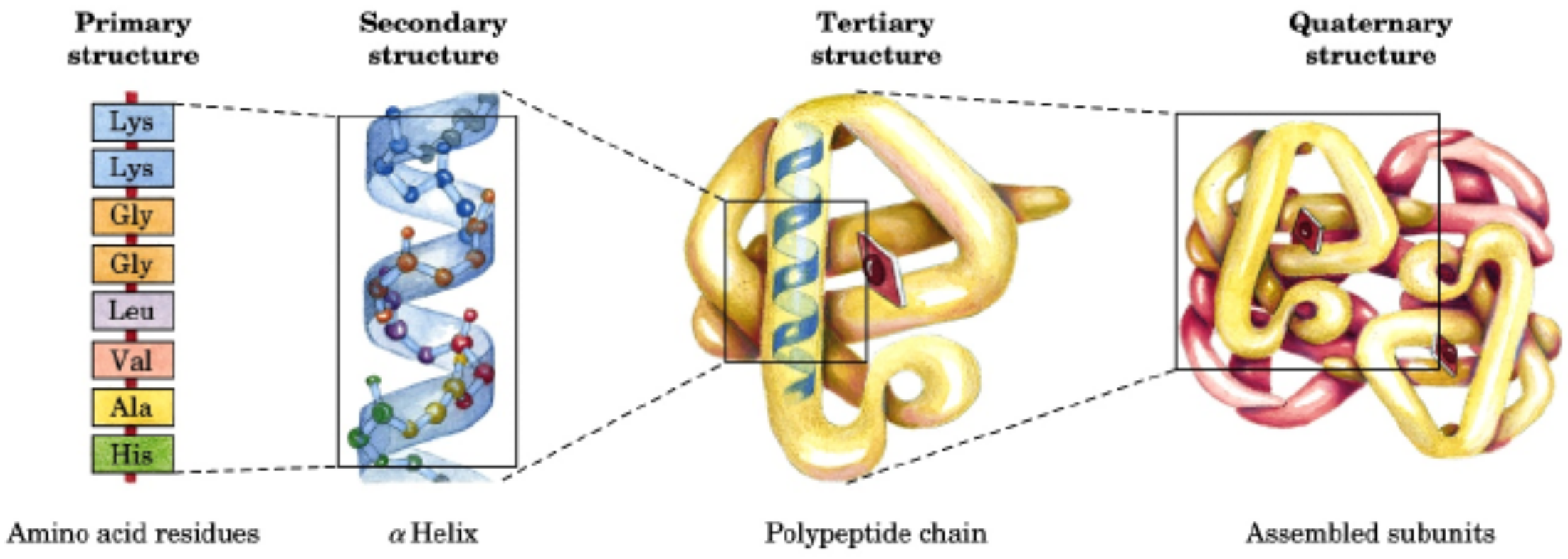
Folding kinetics

The protein folding mechanism depends on the form of the free energy profile.
Higher activation barrier corresponds to longer folding time



Hierarchical organization of protein structure

Protein structure is defined by four levels of hierarchical organization.



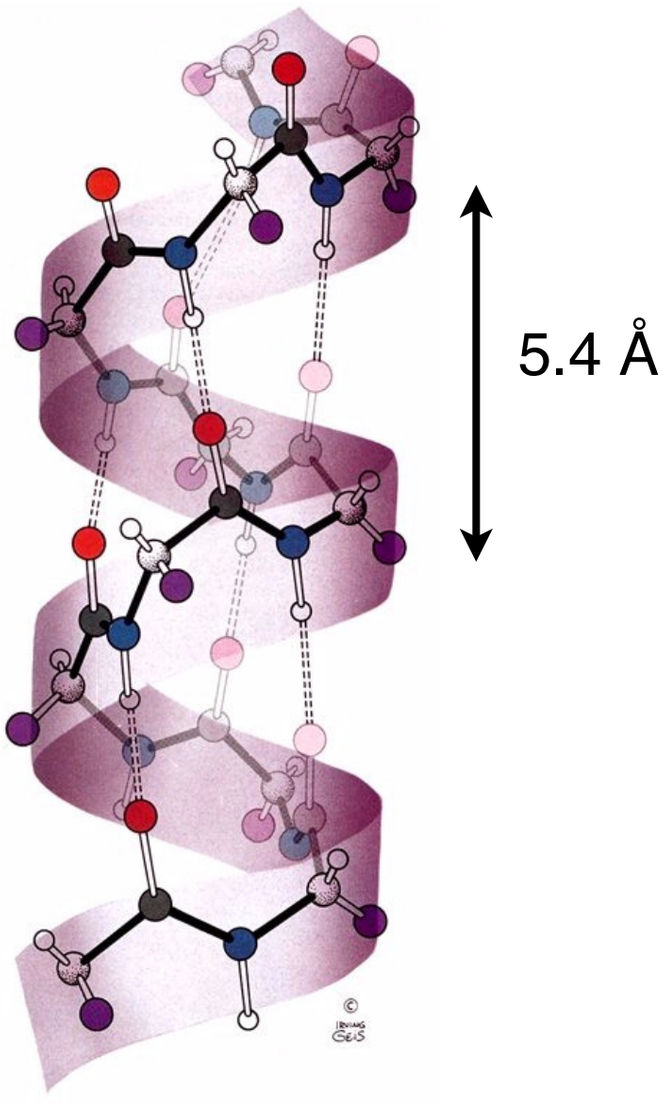
Secondary structure (I)

- Helices observed in proteins are mostly right-handed.

- Typical ϕ, ψ values for residues in α-helix are around -60°; -50°

- Side chains project backward and outward.

- The core of α-helix is tightly packed.



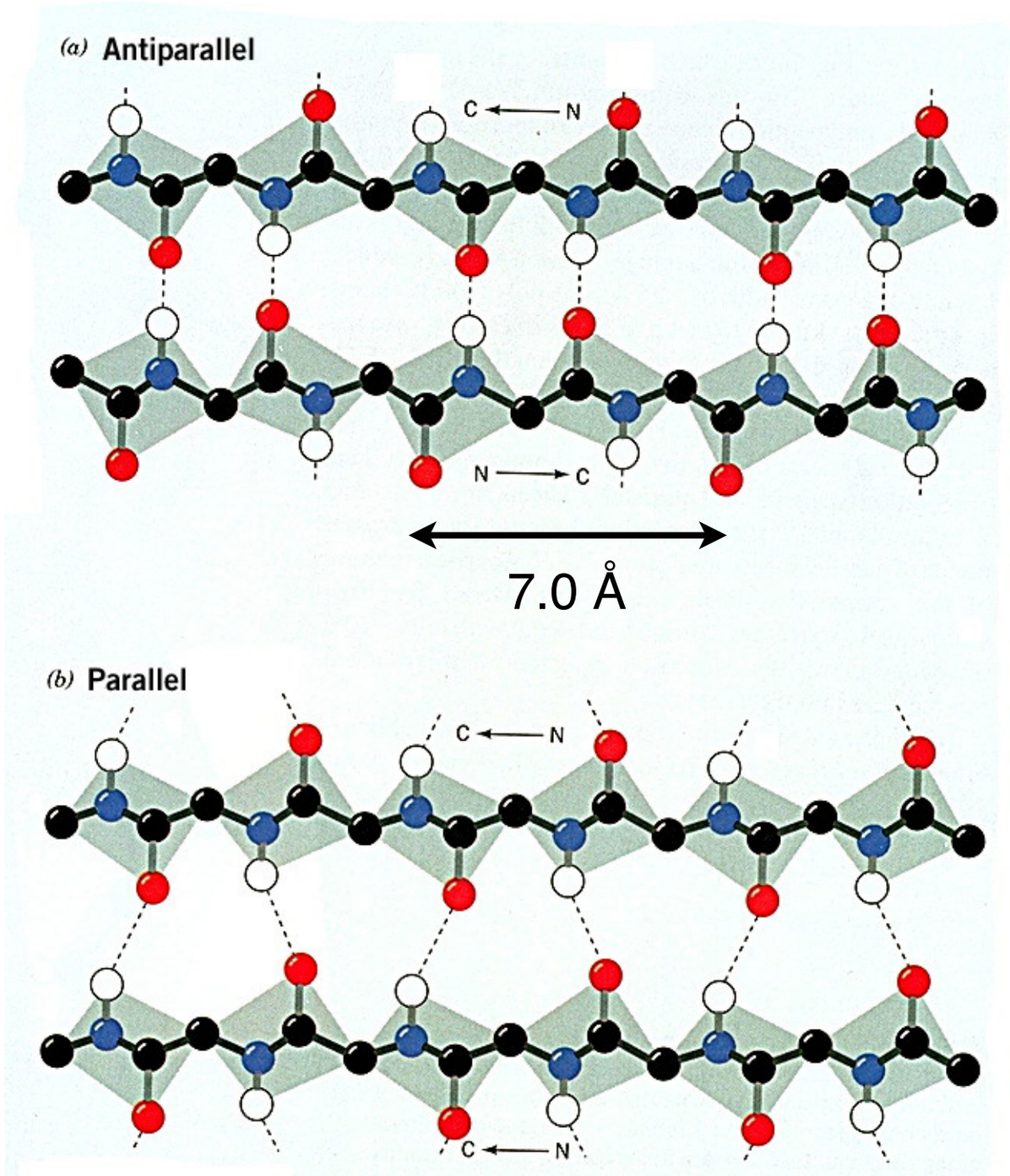
Secondary structure (II)

- Typical ϕ, ψ values for residues in β-sheet are around 140°, -130°

- Side chains of neighboring residues project in opposite directions.

- The polypeptide is in a more extended conformation.

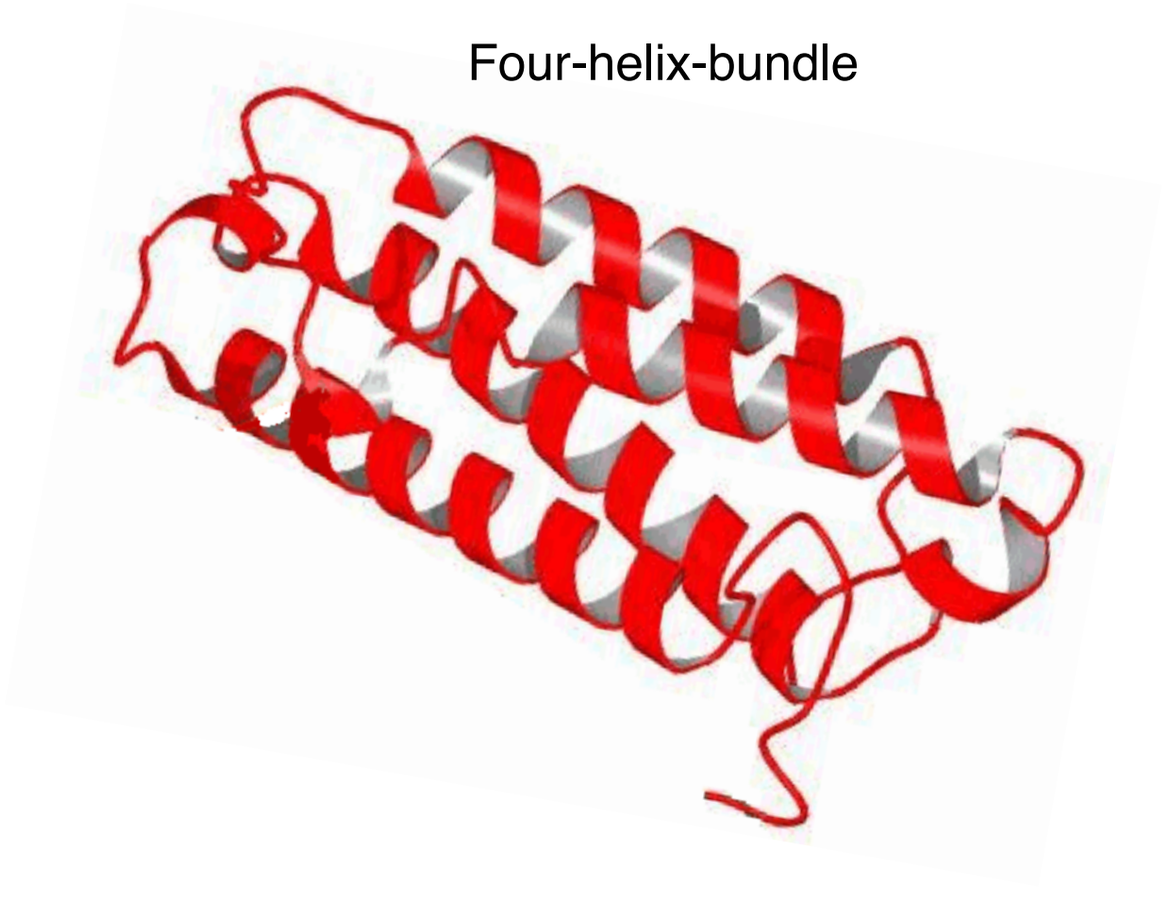
- Parallel β-sheets are less stable than anti-parallel β-sheets.

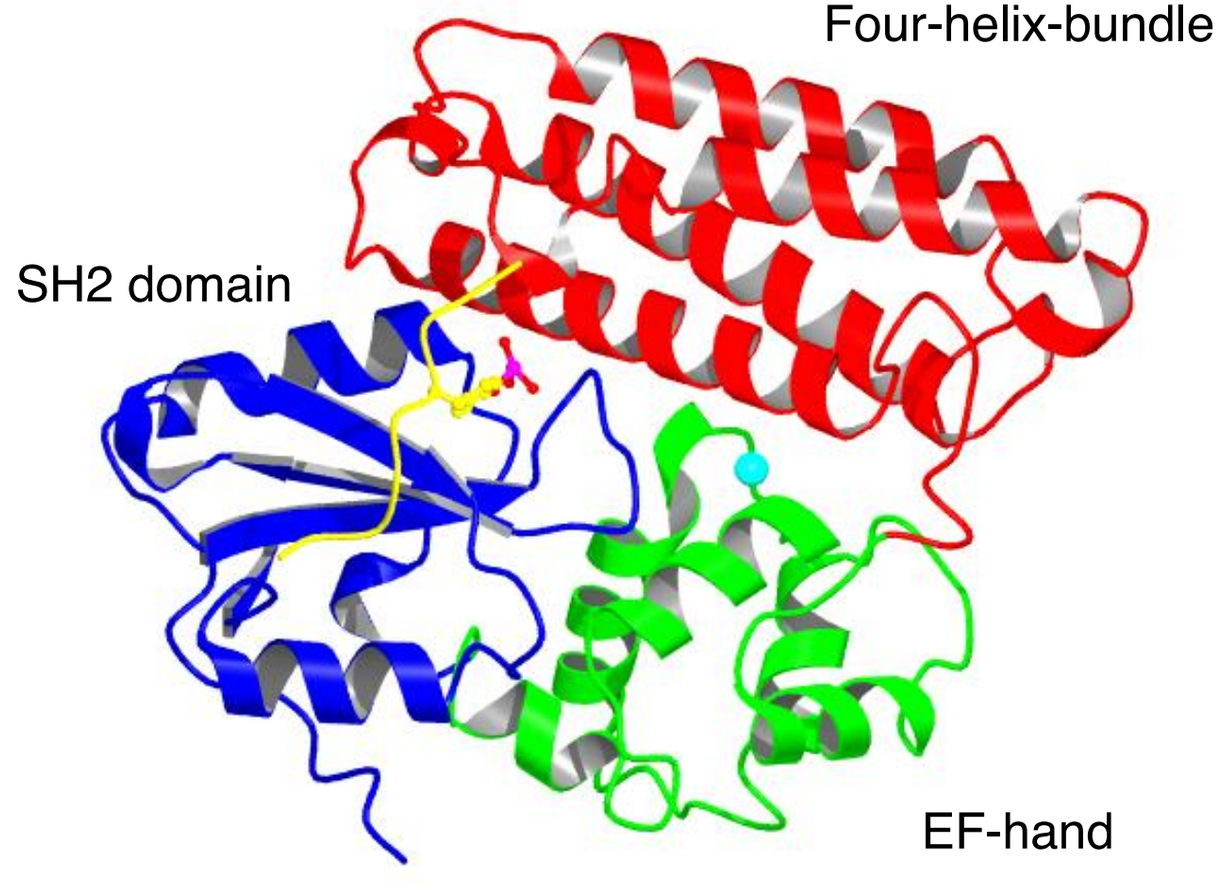


More complex structures

The arrangements of secondary structural elements form the Tertiary Structure of the protein.

The complex of two or more protein domains defines the Quaternary Structure. In the example Four-helix-bundle, EF-hand and SH2 domains together form an integrated phosphoprotein that functions as a negative regulator of many signaling pathways from receptors at the cell surface.

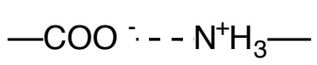
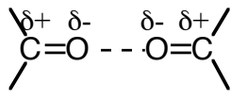
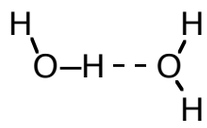
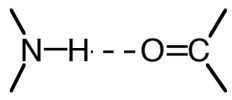
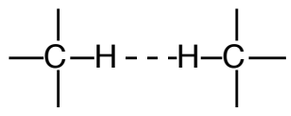




Meng et al. (1999) Nature, 398, 84-90.

Folding interactions

Several electrostatic interactions are contributing to the stability of the native state but they are not the driving forces in the folding process

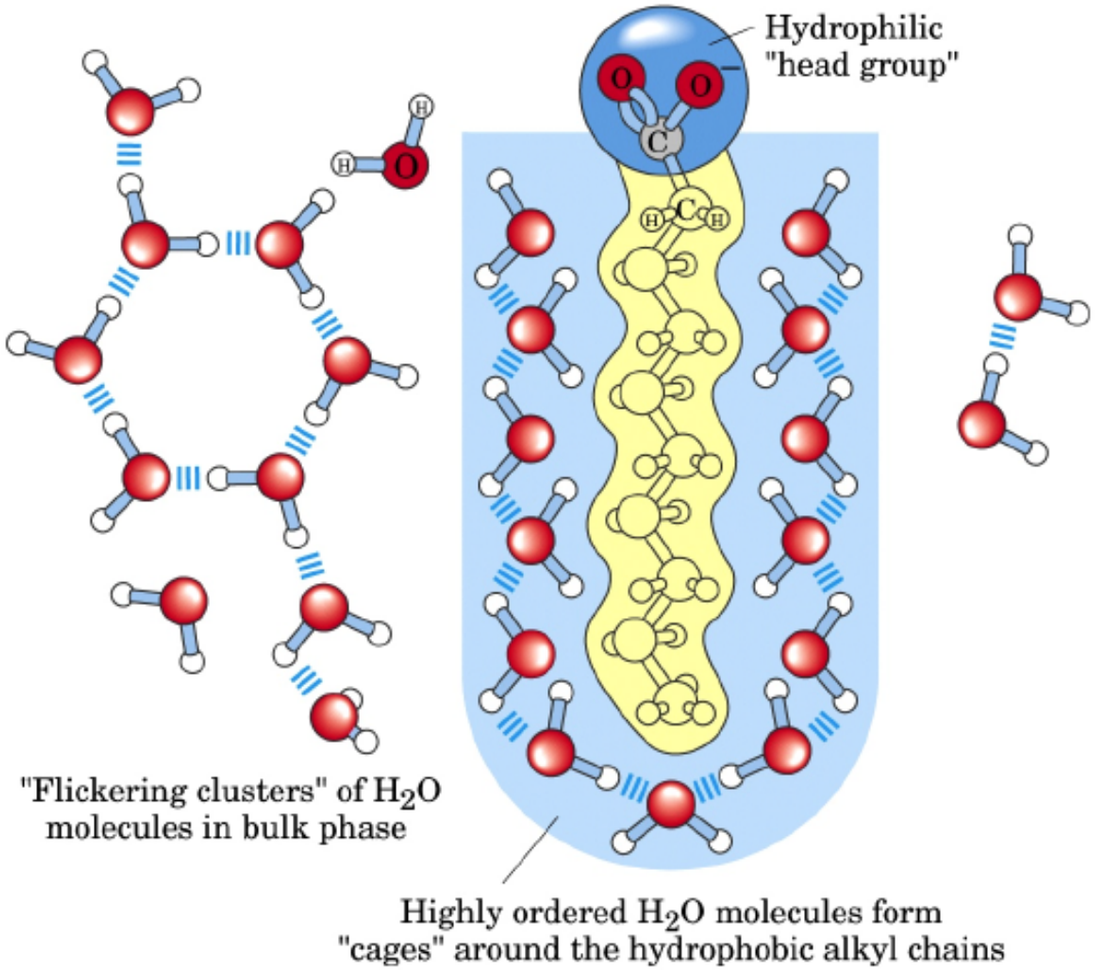
Type	Examples		Binding energy (kcal/mol)	Change of free energy water to ethanol (kcal/mol)
Electrostatic interaction	Salt bridge		-5	-1
	Dipole-dipole		+0.3	
Hydrogen bond	Water		-4	
	Protein backbone		-3	
Dispersion forces	Aliphatic hydrogen		-0.03	
Hydrophobic forces	Side chain of Phe			-2.4

Hydrophobic effect

- Water molecules form a cage-like structure around the nonpolar molecule.

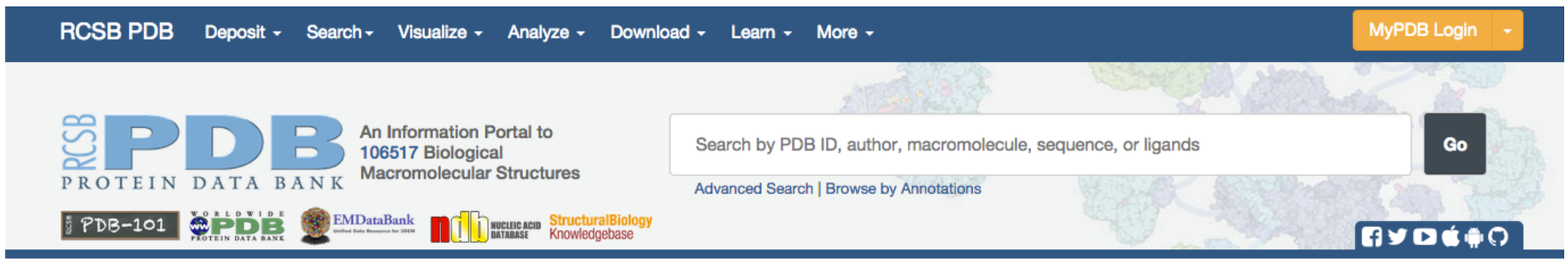
- The positive ΔH is due to the fact that the cage has to be broken to transfer the nonpolar molecule.

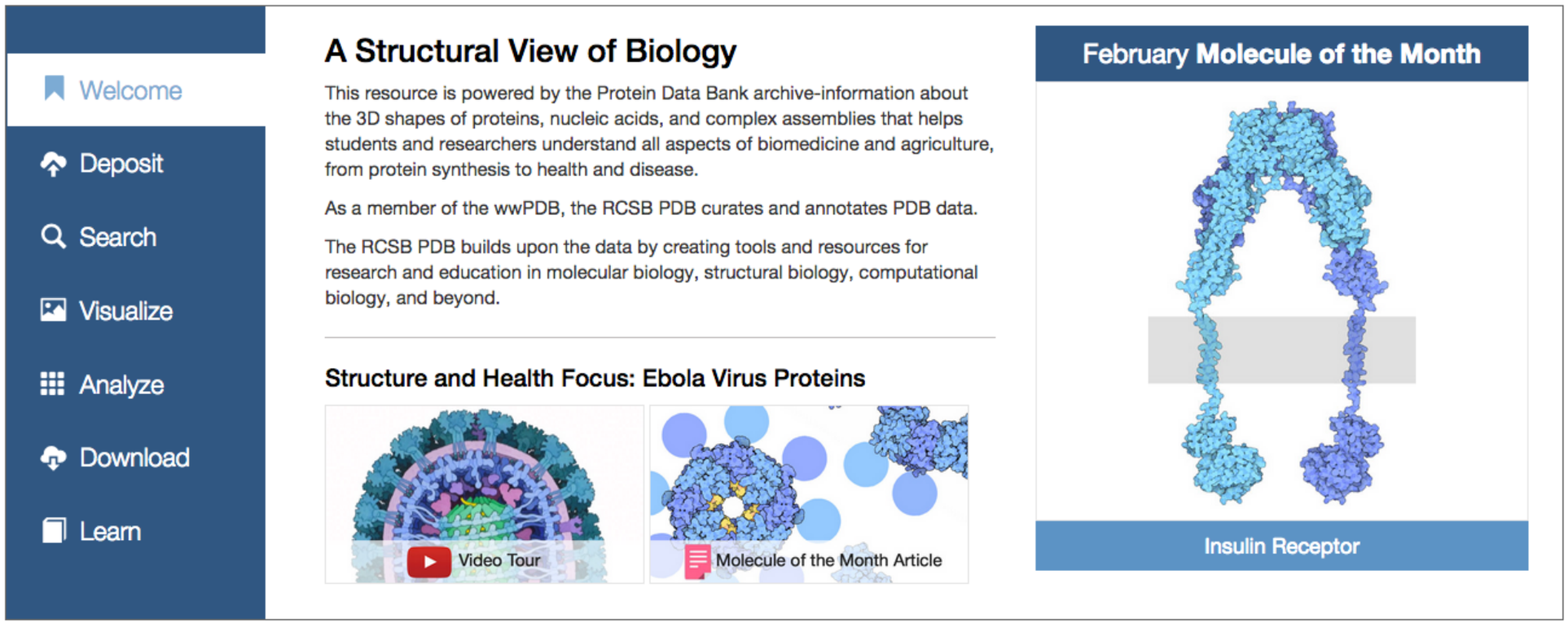
- The positive ΔS is due to the fact that the water molecules are less ordered (an increase in the degree of disorder) when the cage is broken.



The Protein Data Bank

The largest repository of macromolecular structures obtained mainly by X-ray crystallography and NMR

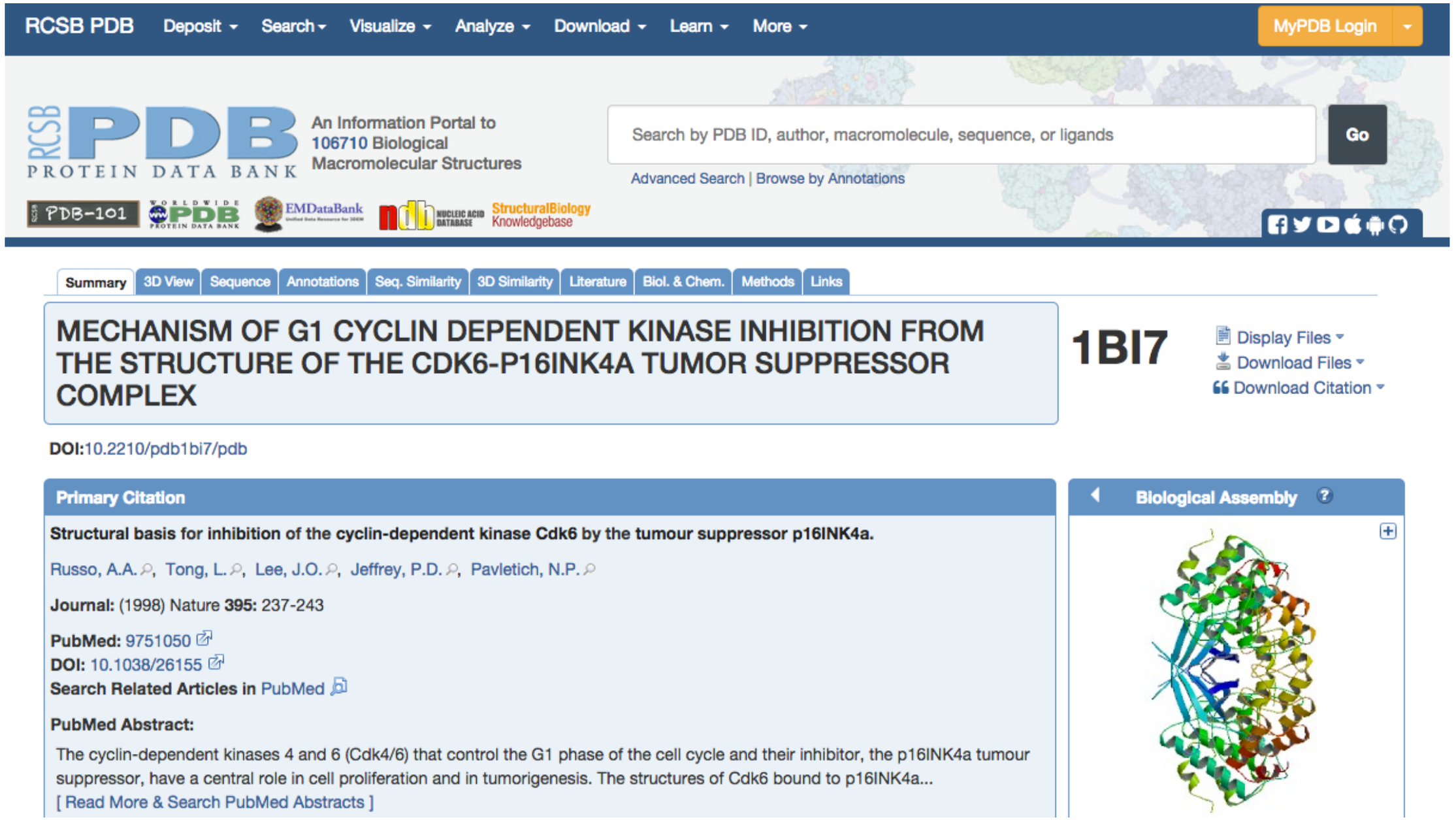




http://www.pdb.org

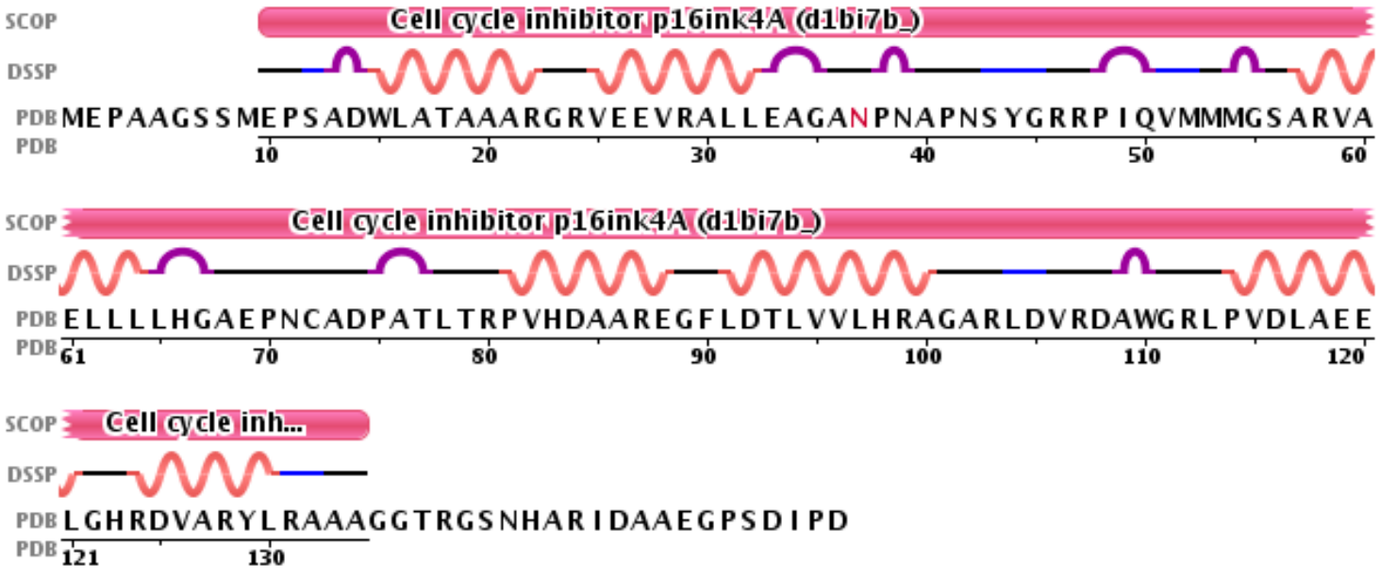
CDK6-P16INK4A

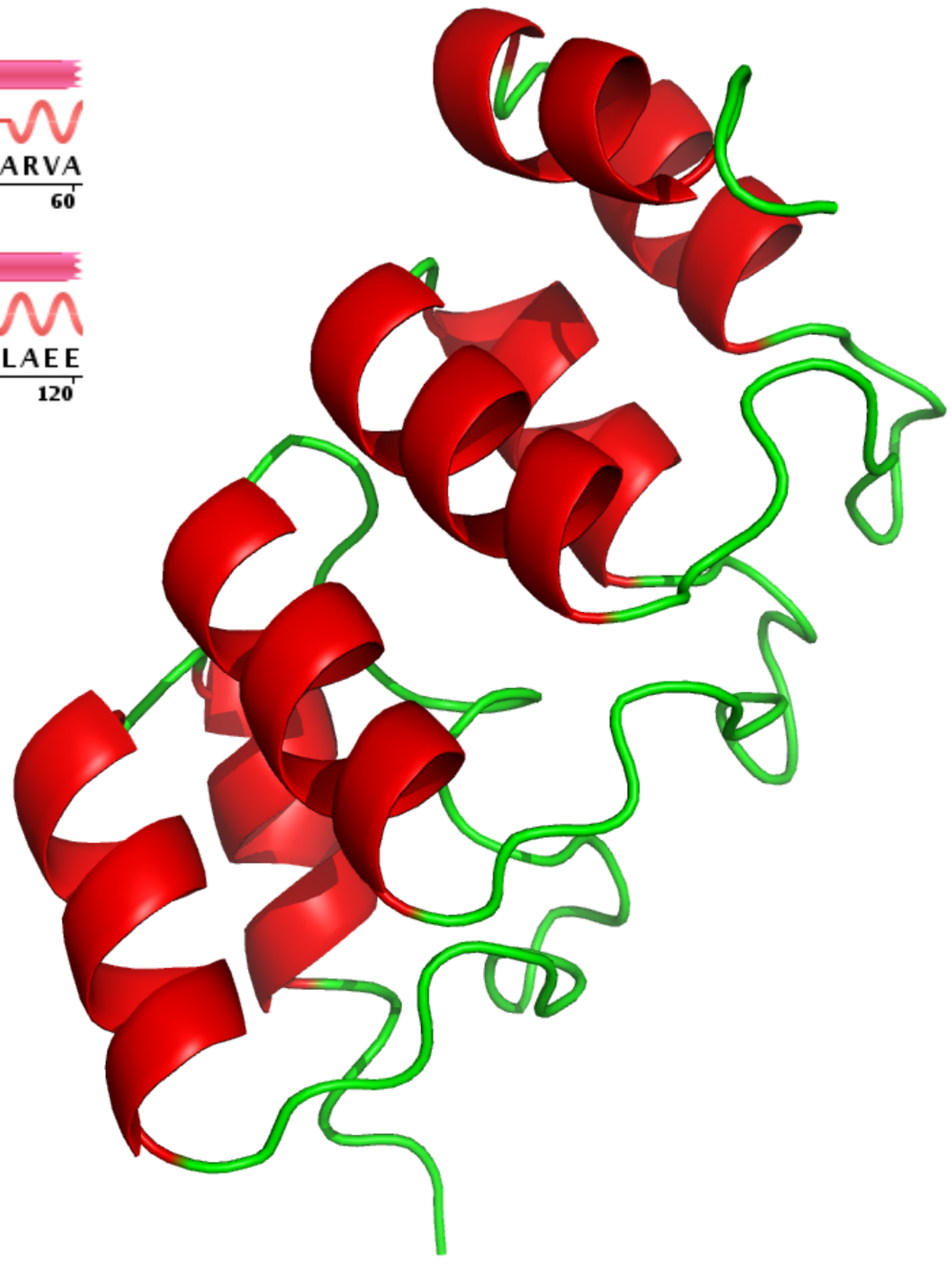
Mechanism of CDK6 inhibition from the complex with tumor suppressor P16INK4A.



P16INK4A

The P16INK4A is a tumor suppressor protein with 7 helixes.





PDB data

The most important information are the atomic coordinates.

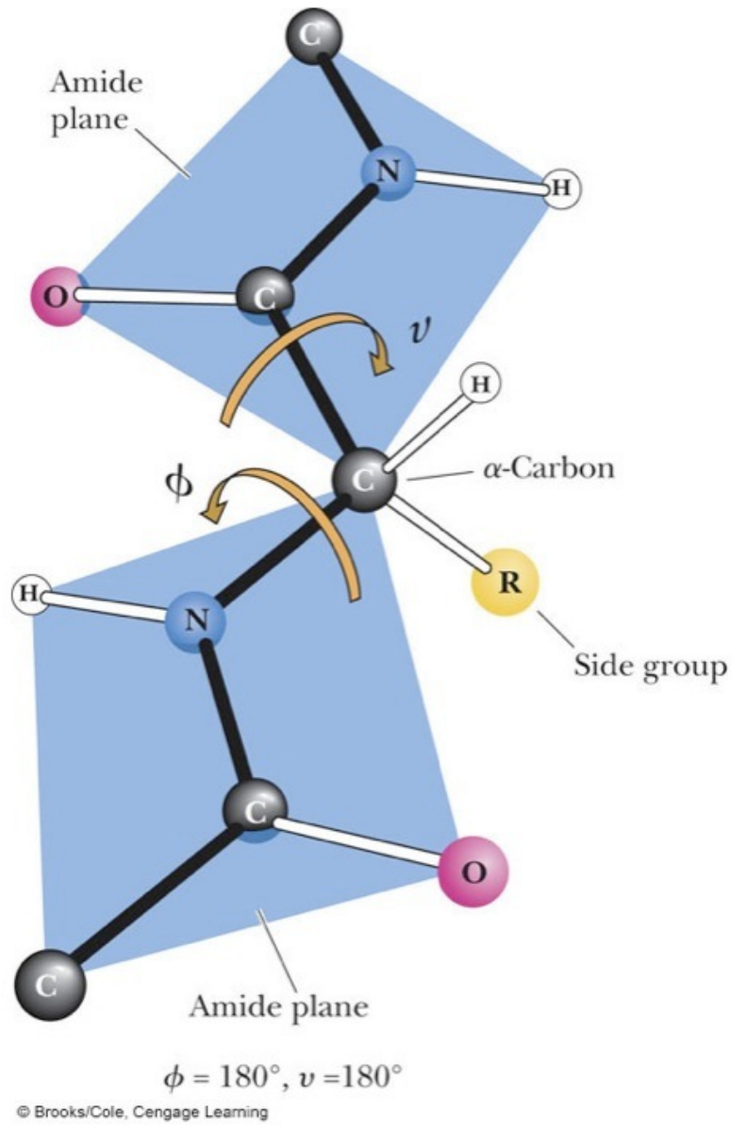
		AT	RES	CH	POS	X	Y	Z			
ATOM	2145	N	GLU	B	10	150.341	72.309	103.145	1.00	99.90	N
ATOM	2146	CA	GLU	B	10	150.096	71.519	101.907	1.00	99.90	C
ATOM	2147	C	GLU	B	10	150.425	70.046	102.190	1.00	99.90	C
ATOM	2148	O	GLU	B	10	151.326	69.770	102.983	1.00	99.90	O
ATOM	2149	CB	GLU	B	10	150.963	72.057	100.790	1.00	99.90	C
ATOM	2150	N	PRO	B	11	149.661	69.092	101.595	1.00	99.90	N
ATOM	2151	CA	PRO	B	11	149.856	67.644	101.778	1.00	99.90	C
ATOM	2152	C	PRO	B	11	150.783	66.845	100.844	1.00	99.90	C
ATOM	2153	O	PRO	B	11	151.938	66.593	101.185	1.00	99.90	O
ATOM	2154	CB	PRO	B	11	148.425	67.108	101.722	1.00	99.90	C
ATOM	2155	CG	PRO	B	11	147.816	67.948	100.672	1.00	99.90	C
ATOM	2156	CD	PRO	B	11	148.333	69.350	101.000	1.00	99.90	C
ATOM	2157	N	SER	B	12	150.258	66.422	99.691	1.00	99.90	N
ATOM	2158	CA	SER	B	12	150.965	65.585	98.710	1.00	99.90	C
ATOM	2159	C	SER	B	12	150.922	64.167	99.292	1.00	99.90	C
ATOM	2160	O	SER	B	12	150.493	63.222	98.632	1.00	99.90	O
ATOM	2161	CB	SER	B	12	152.410	66.042	98.440	1.00	99.90	C
ATOM	2162	OG	SER	B	12	152.907	65.499	97.219	1.00	99.90	O

http://deposit.rcsb.org/adit/docs/pdb_atom_format.html

Defining protein structure

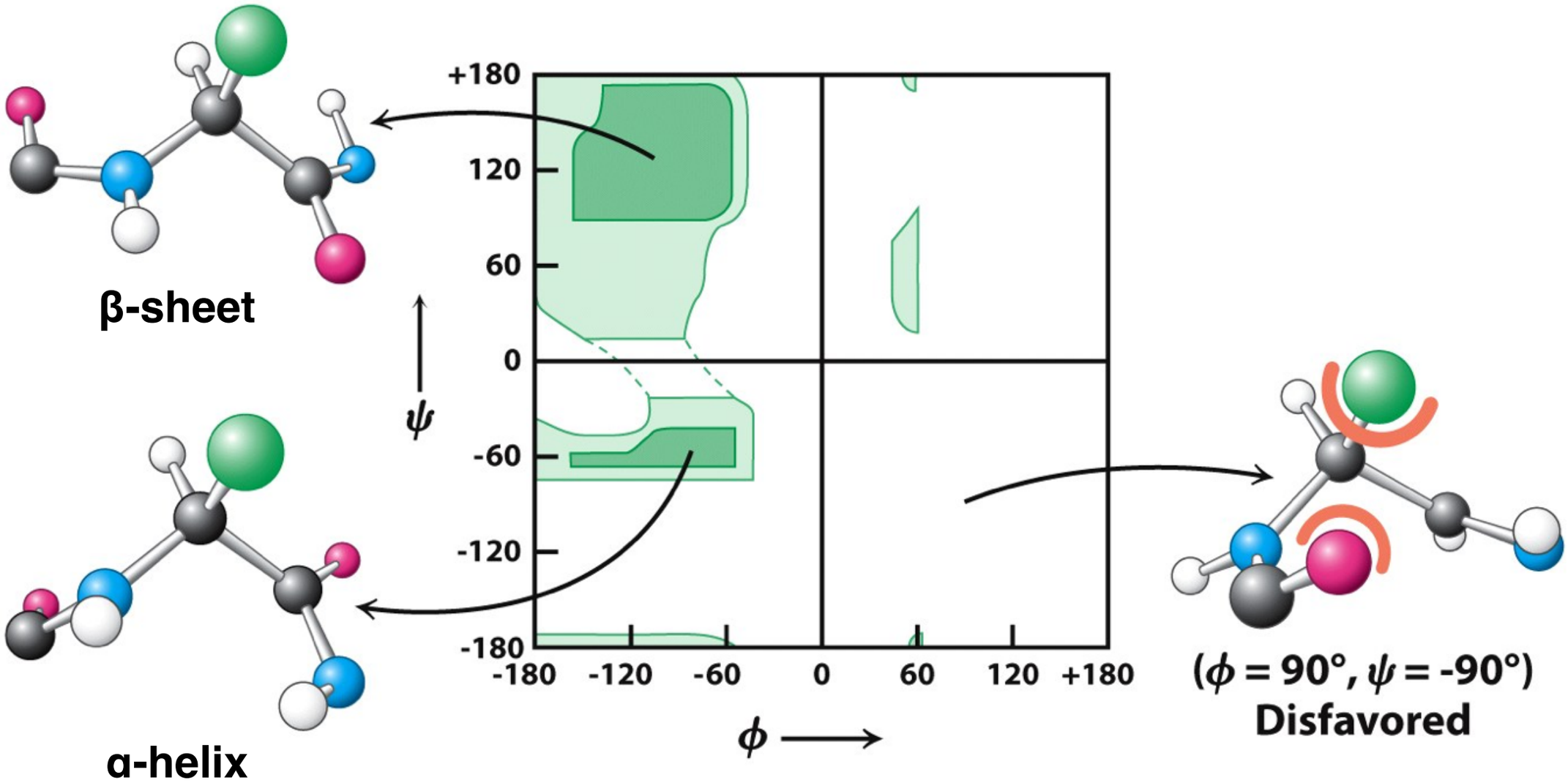
Basic information for the characterization of the protein three-dimensional structures are:

- ϕ, ψ values for each residue in the protein chain
- secondary structure
- solvent accessible area



Ramachandran Plot

The backbone of the protein structure can be defined providing the list of ϕ, ψ angles for each residue in the chain.



Berg JM et al. (2012). Biochemistry VII Ed.

DSSP program

Program that implements the algorithm “Define Secondary Structure of Proteins”.

The method calculates different features of the protein structure such as the ϕ, ψ angles for each residue, its secondary structure and the solvent accessible area.

#	RESIDUE	AA	STRUCTURE	BP1	BP2	ACC	...	PHI	PSI	X-CA	Y-CA	Z-CA
1	10	B	E	0	0	153	...	360.0	144.2	150.1	71.5	101.9
2	11	B	P +	0	0	83	...	-90.2	-84.0	149.9	67.6	101.8
3	12	B	S S >> S+	0	0	60	...	77.6	-51.1	151.0	65.6	98.7
4	13	B	A T 34 S+	0	0	6	...	-82.3	73.7	151.3	62.7	101.2
5	14	B	D T 3> S+	0	0	39	...	-154.6	-41.3	147.5	62.2	100.9
6	15	B	W H <> S+	0	0	170	...	-60.8	-41.6	148.0	61.1	97.3
7	16	B	L H X S+	0	0	0	...	-62.9	-38.5	150.2	58.6	98.9
8	17	B	A H > S+	0	0	3	...	-62.0	-58.1	147.4	57.5	101.3
9	18	B	T H X S+	0	0	72	...	-56.4	-34.0	144.9	56.8	98.6

SS **SAA** **PHI** **PSI**

DSSP: ftp://ftp.cmbi.ru.nl/pub/software/dssp
more details at http://www.cmbi.ru.nl/dssp.html

Kabsch W, and Sander C, (1983). Biopolymers. 22 2577-2637.

Problem 1a

Write a program that parse the DSSP file and for each residue extract:

- the secondary structure (col: 17)
- the solvent accessible area (cols: 36-38)
- phi and psi angles (cols: 104-109 and 110-115)

The program groups the different types of secondary structure in the there main ones (Helix, Beta and Coil) and calculate the relative solvent accessible area.

```
Norm_Acc={"A" :106.0,  "B" :160.0,
          "C" :135.0,  "D" :163.0,  "E" :194.0,
          "F" :197.0,  "G" : 84.0,  "H" :184.0,
          "I" :169.0,  "K" :205.0,  "L" :164.0,
          "M" :188.0,  "N" :157.0,  "P" :136.0,
          "Q" :198.0,  "R" :248.0,  "S" :130.0,
          "T" :142.0,  "V" :142.0,  "W" :227.0,
          "X" :180.0,  "Y" :222.0,  "Z" :196.0}
```

Problem 1b

Write a script that takes in input a list of mutations and a DSSP file and chain, and returns for each mutation the secondary structure and the relative solvent accessible area.

How many mutated sites occurs in buried regions (relative solvent accessible area<20%)?

Run the script on the DSSPs obtained from the whole PDB and only from chain B to find possible mutation at the interface.