Protein Structure Analysis

Laboratory of Bioinformatics I
Module 2

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http://biofold.org/

Biomolecules Folding and Disease

Department of Pharmacy and Biotechnology (FaBiT)
University of Bologna

Molecular biology data

GenBank: 255,669,865

```
>BGAL_SULSO BETA-GALACTOSIDASE Sulfolobus solfataricus.
MYSFPNSFRFGWSQAGFQSEMGTPGSEDPNTDWYKWVHDPENMAAGLVSG
DLPENGPGYWGNYKTFHDNAQKMGLKIARLNVEWSRIFPNPLPRPQNFDE
SKQDVTEVEINENELKRLDEYANKDALNHYREIFKDLKSRGLYFILNMYH
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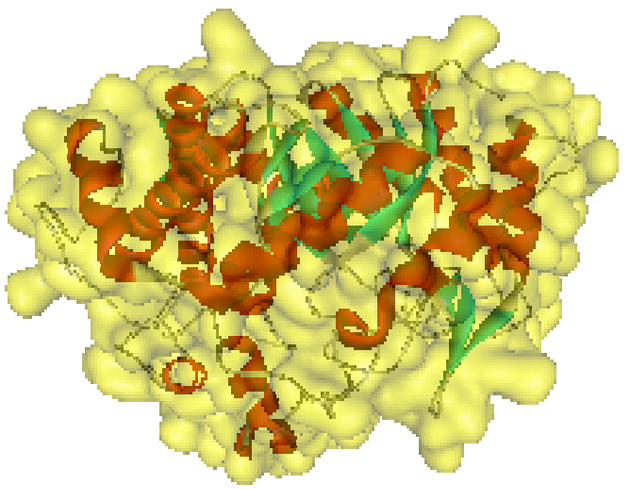
UniRef90: 204,806,910



Swiss-Prot: 572,970

Protein Data Bank: 234,440

Protein: 229,682

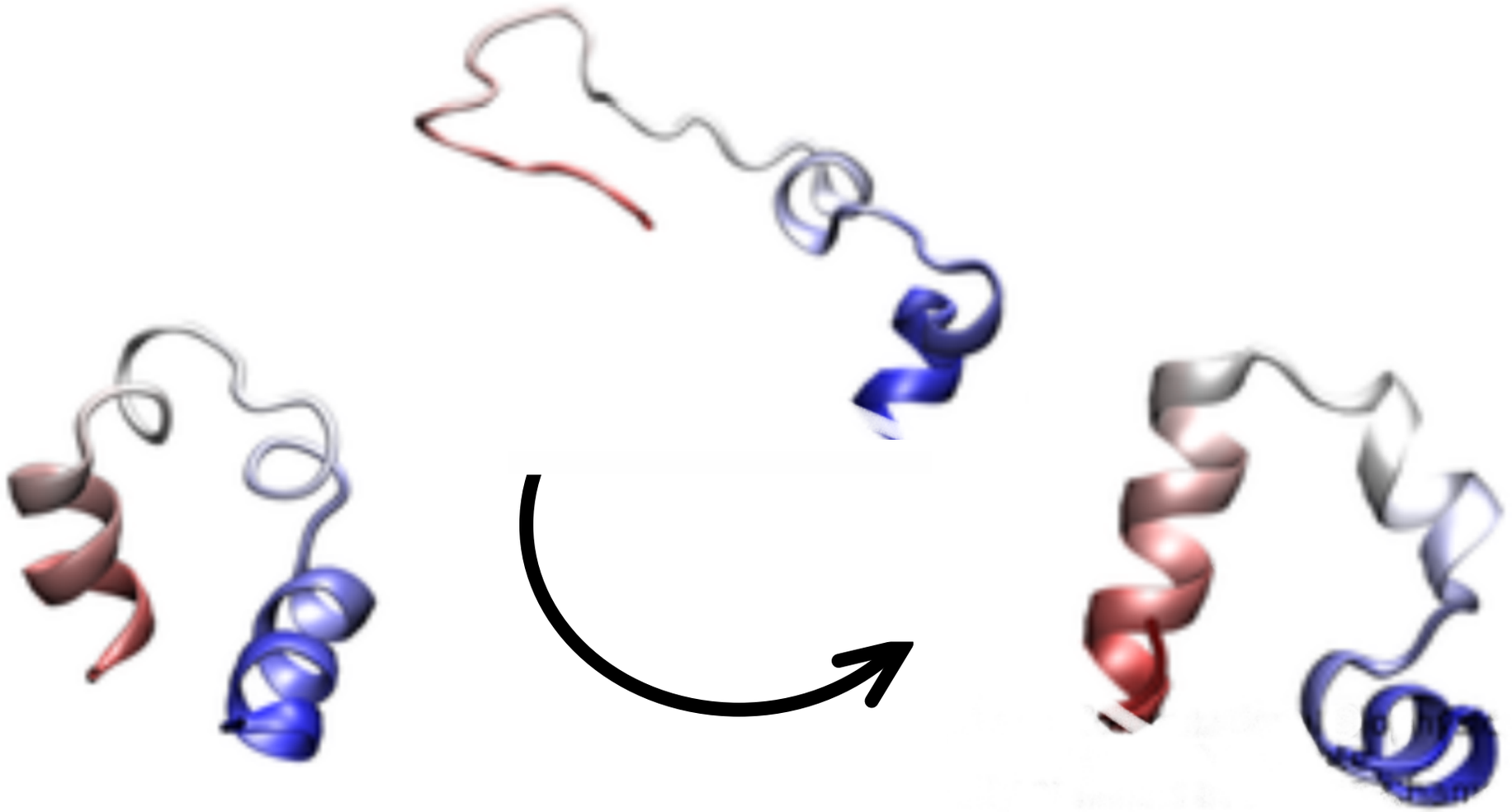
Nucleic Acids: 20,451

April 2025

Protein folding

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

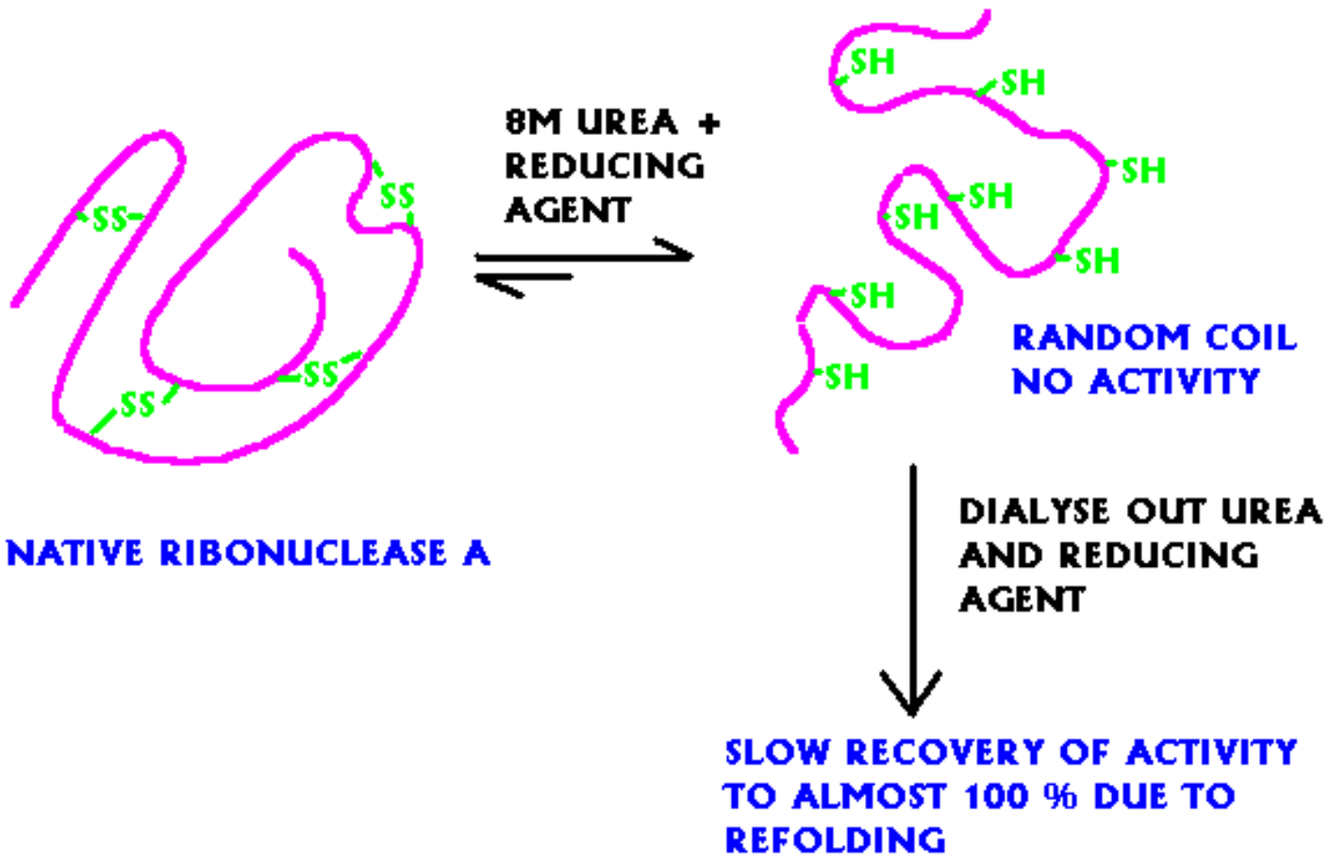
T T C C P S I V A R S N F N V C R L P G T P E A L C A T
Y T G C I I I P G A T C P G D Y A N



The Anfinsen's hypothesis

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

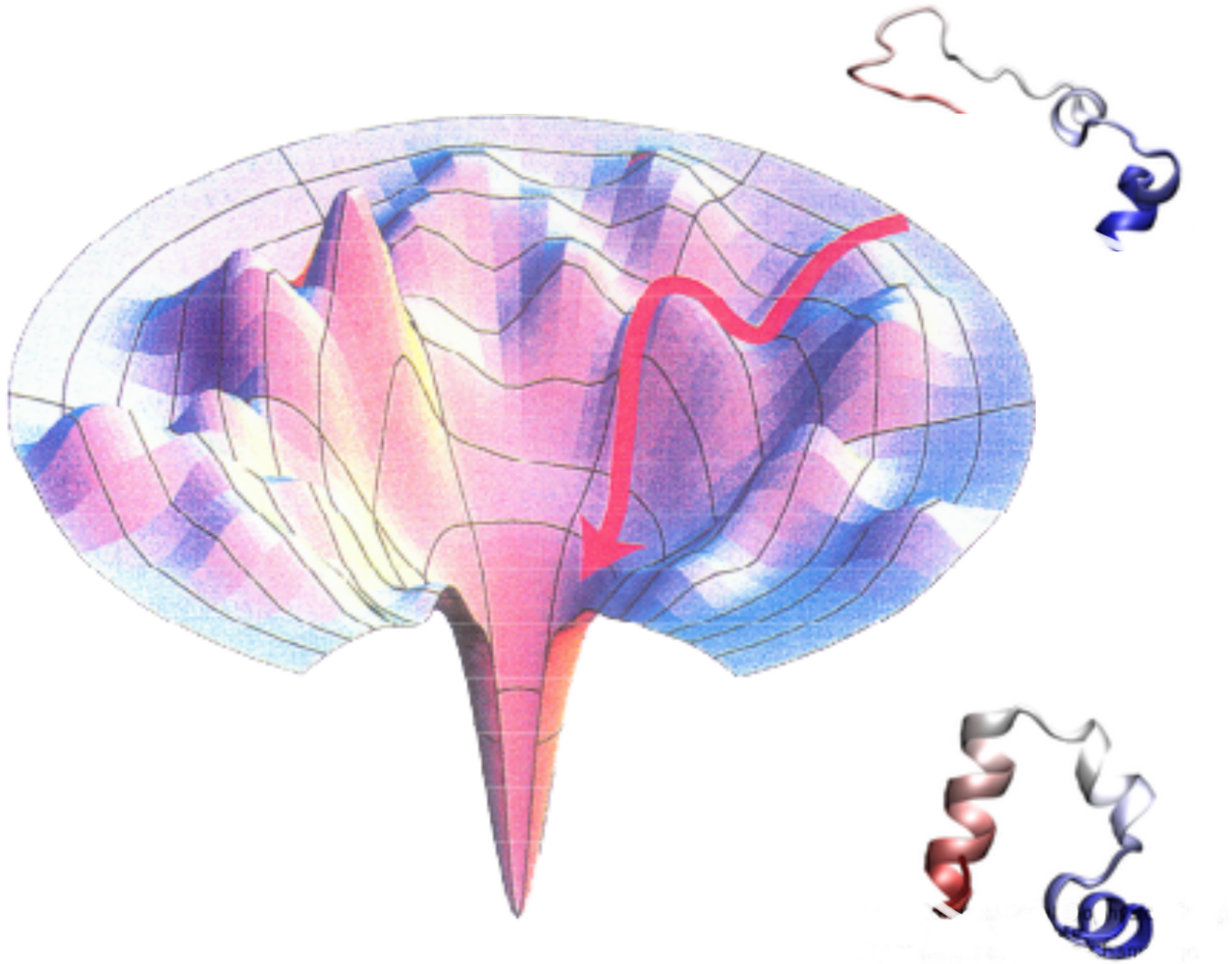
Anfinsen showed that denatured ribonuclease A could be re-activated removing the denaturant.



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.

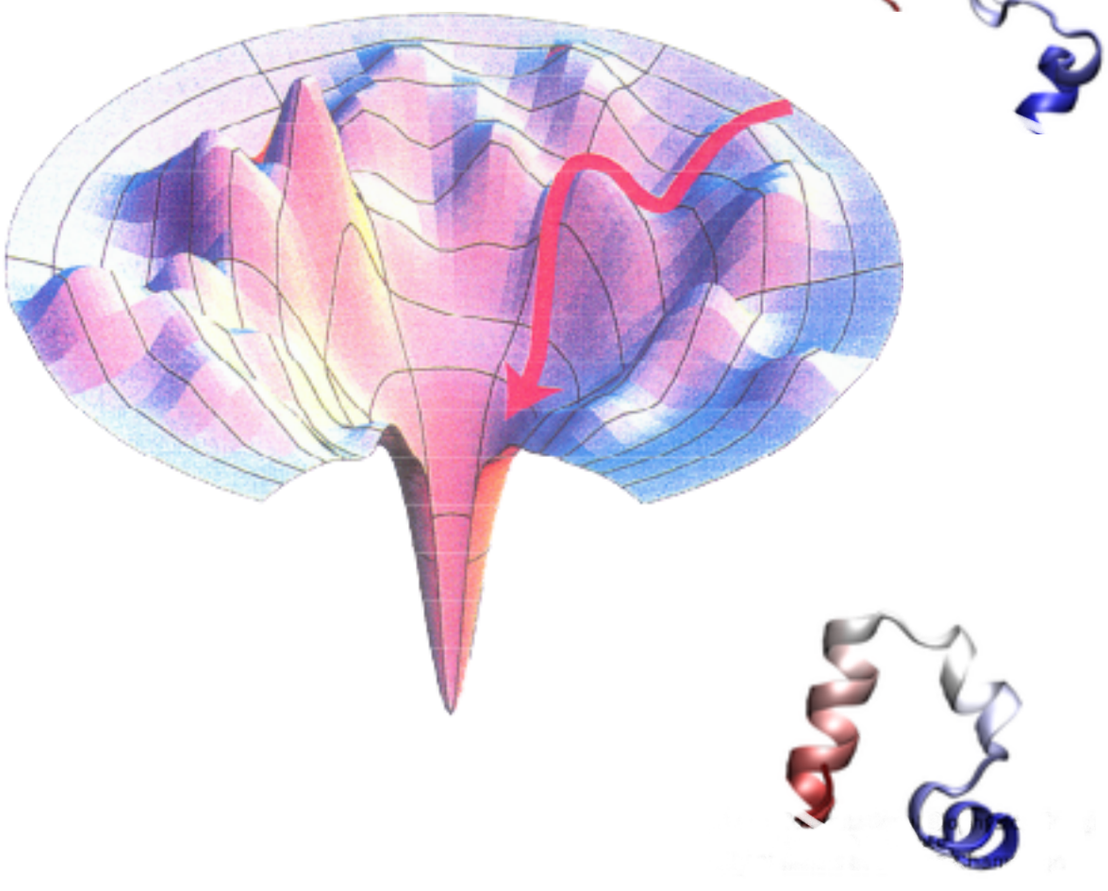
Aspects of the same problem

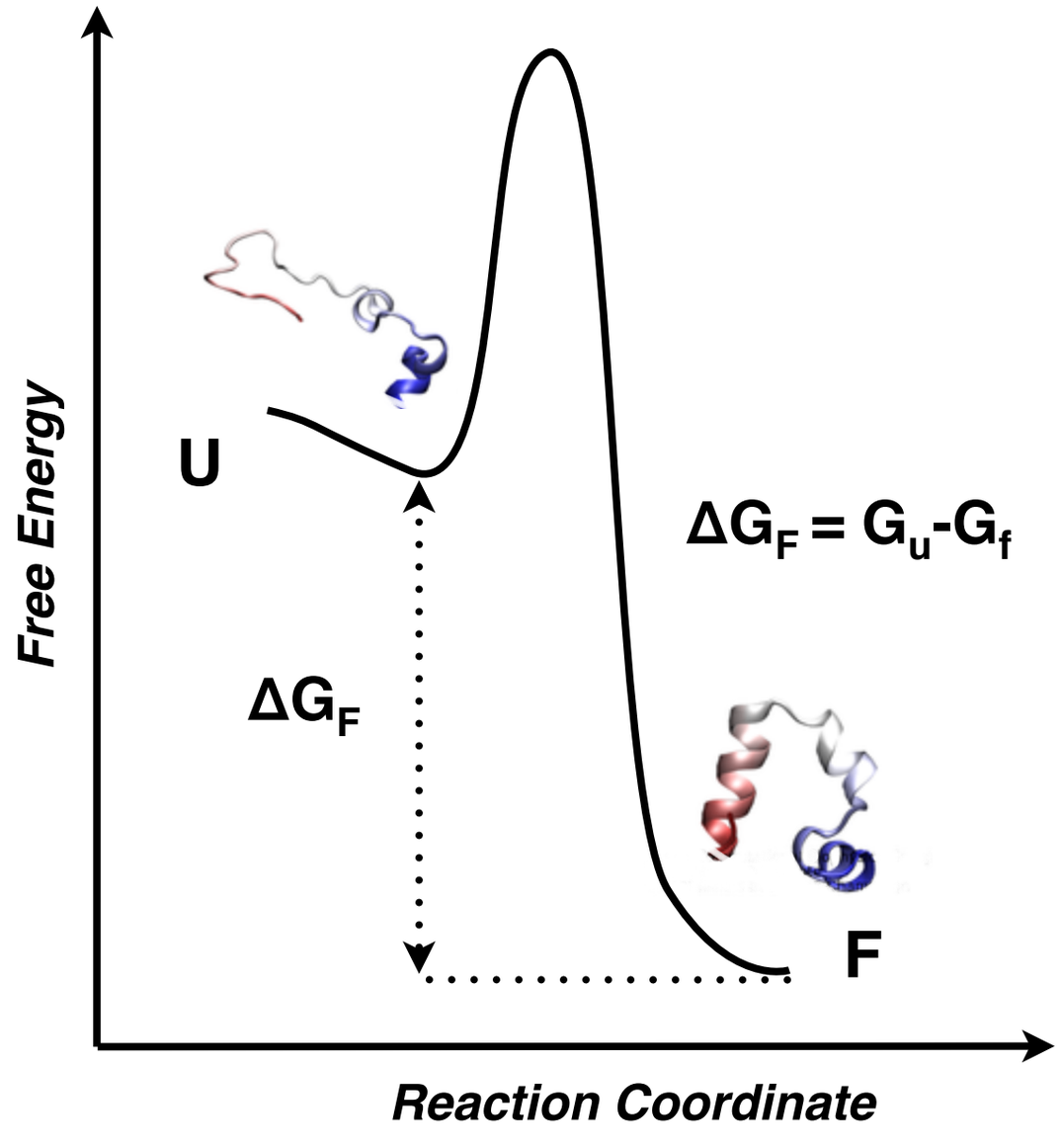
The solution of the protein folding consists in the understanding of three different aspects of the problem:

- 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

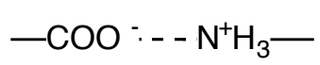
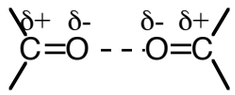
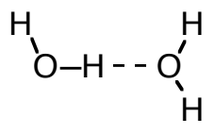
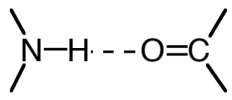
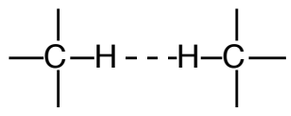
The folding free energy difference, ΔG_F, is typically small, of the order of -5 to -15 kcal/mol for a globular protein (compared to e.g. -30 to -100 kcal/mol for a covalent bond).





Folding interactions

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

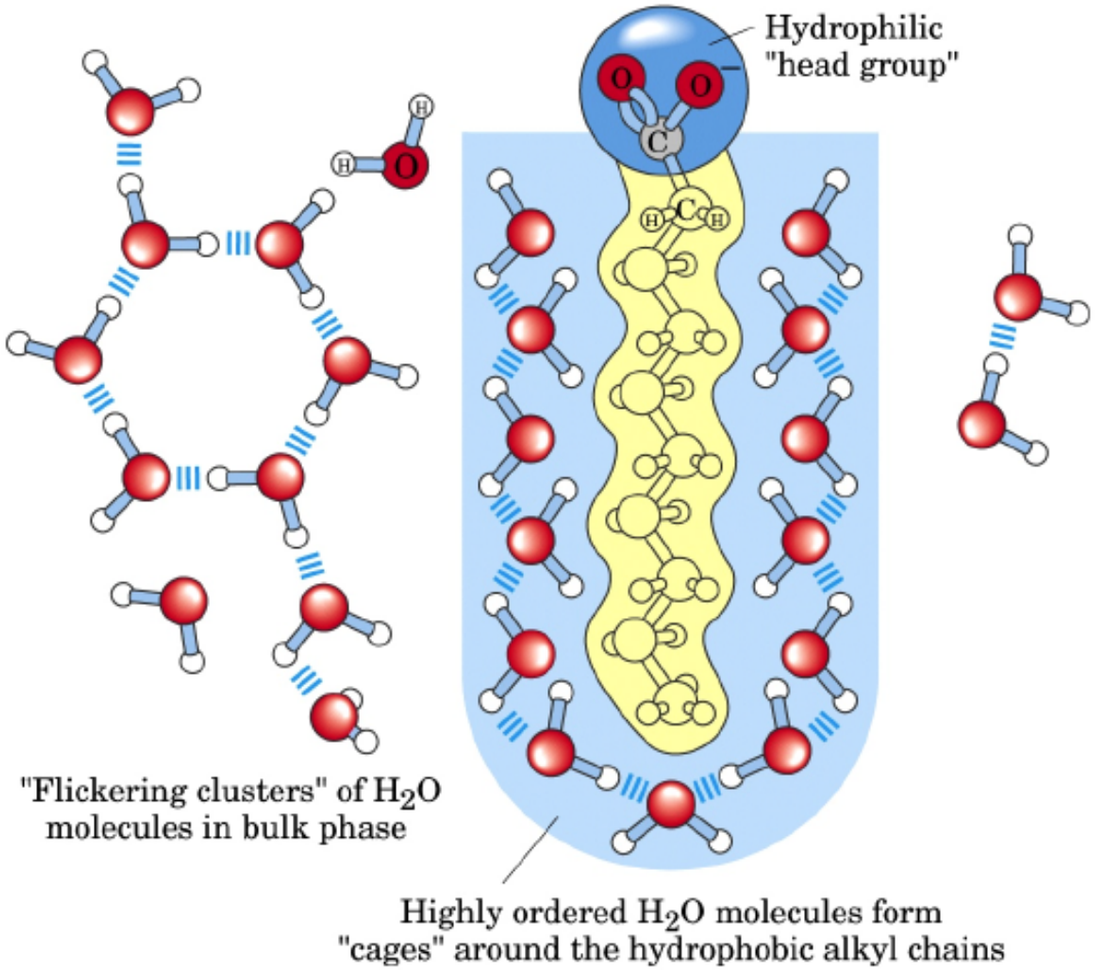
Type	Examples		Binding energy (kcal/mol)	Change of free energy water to ethanol (kcal/mol)
Electrostatic interaction	Salt bridge		-5	-1
	Dipole-dipole		+0.3	
Hydrogen bond	Water		-4	
	Protein backbone		-3	
Dispersion forces	Aliphatic hydrogen		-0.03	
Hydrophobic forces	Side chain of Phe			-2.4

Hydrophobic effect

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

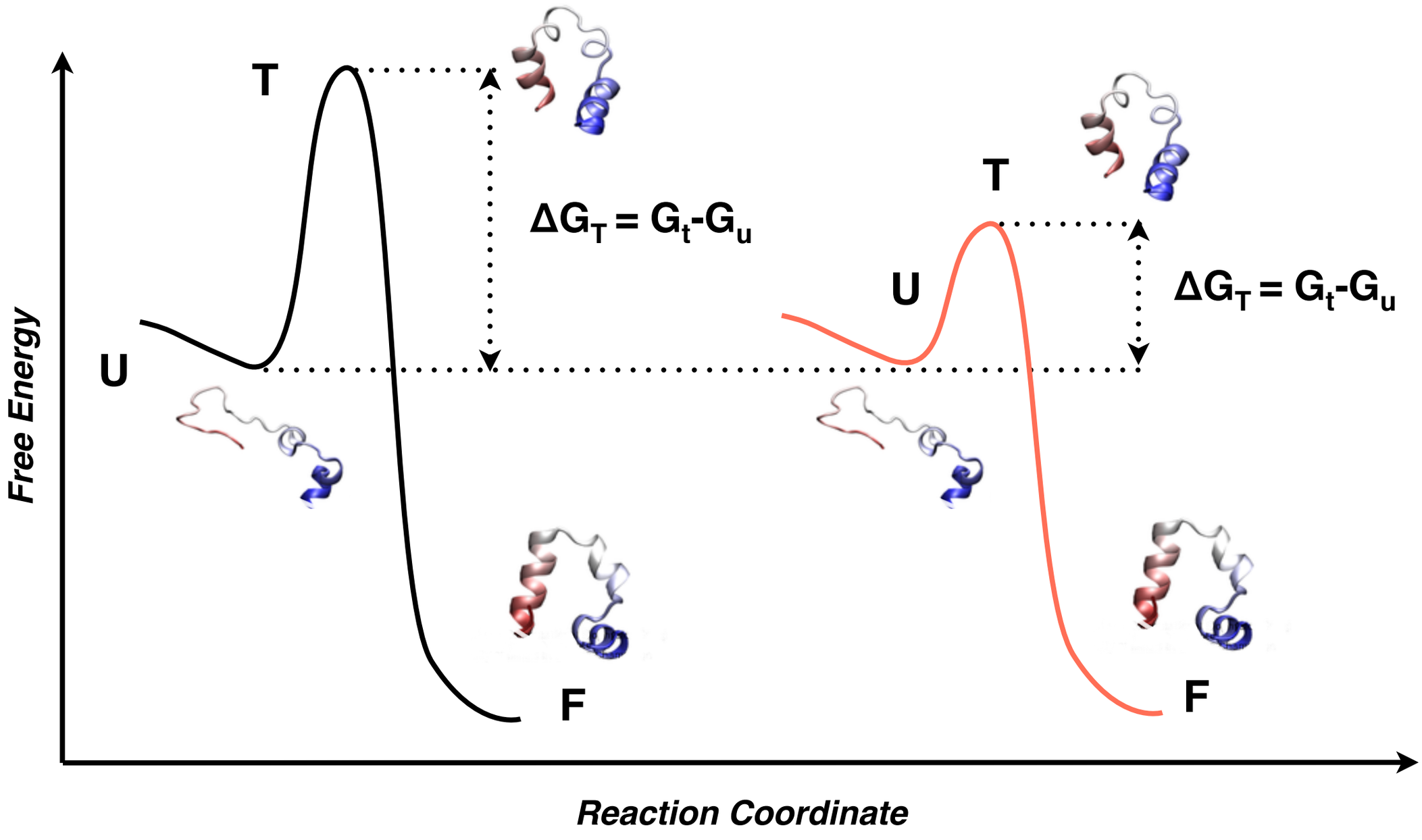
- The positive ΔH is due to the fact that the cage has to be broken to transfer the nonpolar molecule.

- 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.



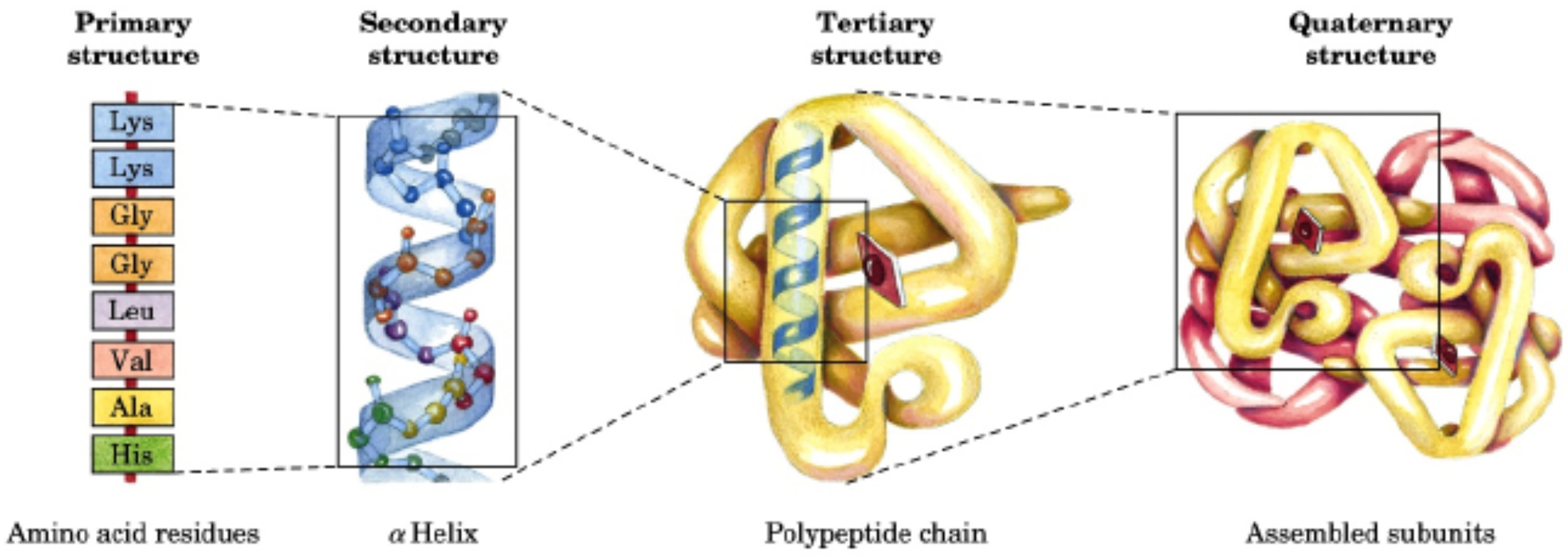
Folding kinetics

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.



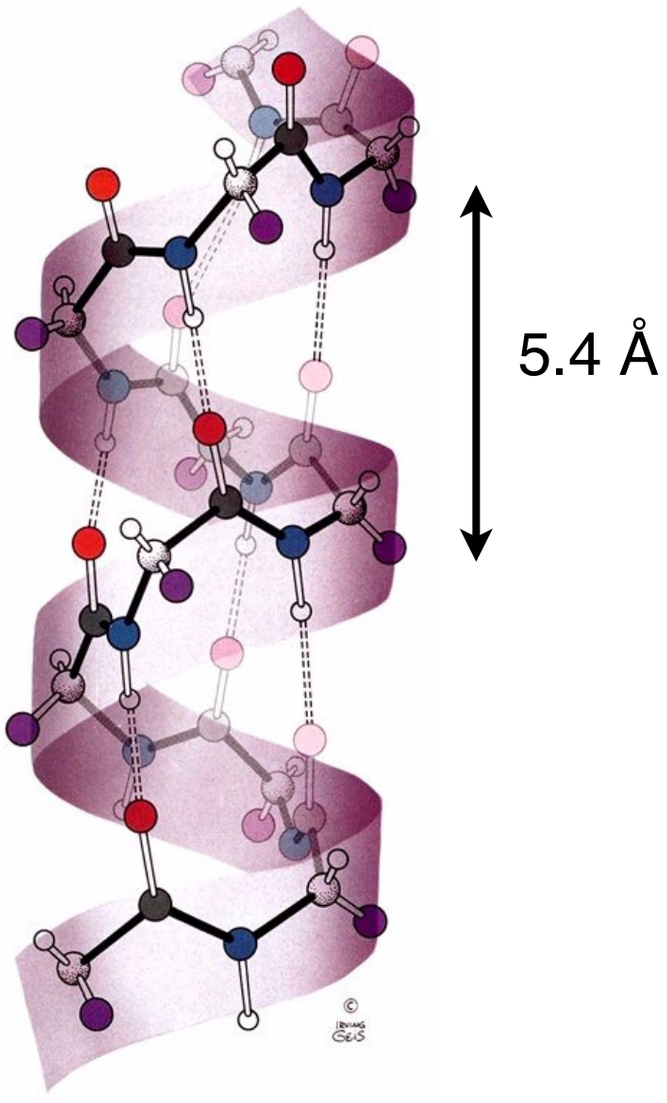
Secondary structure (I)

- 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.



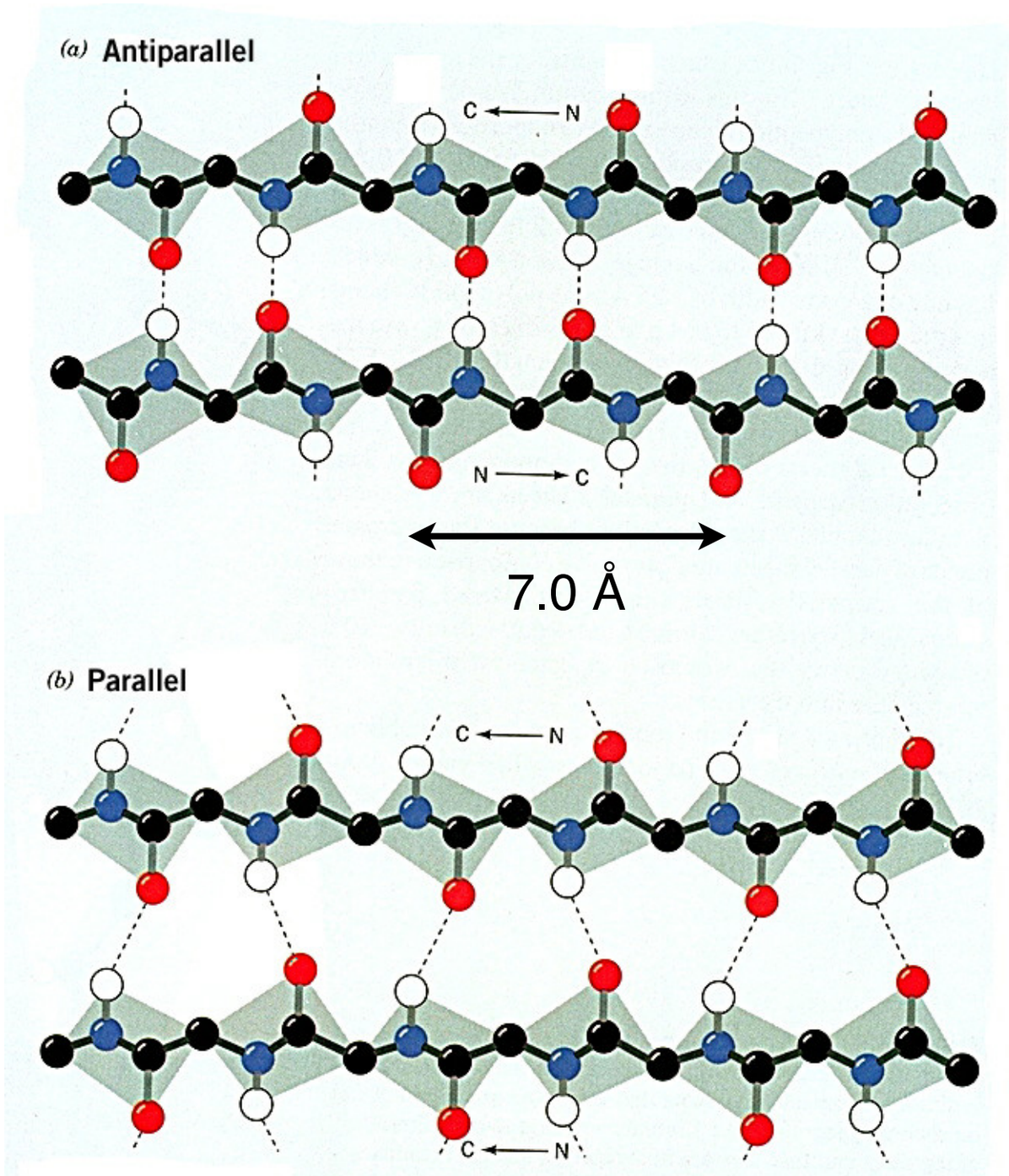
Secondary structure (II)

- 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.

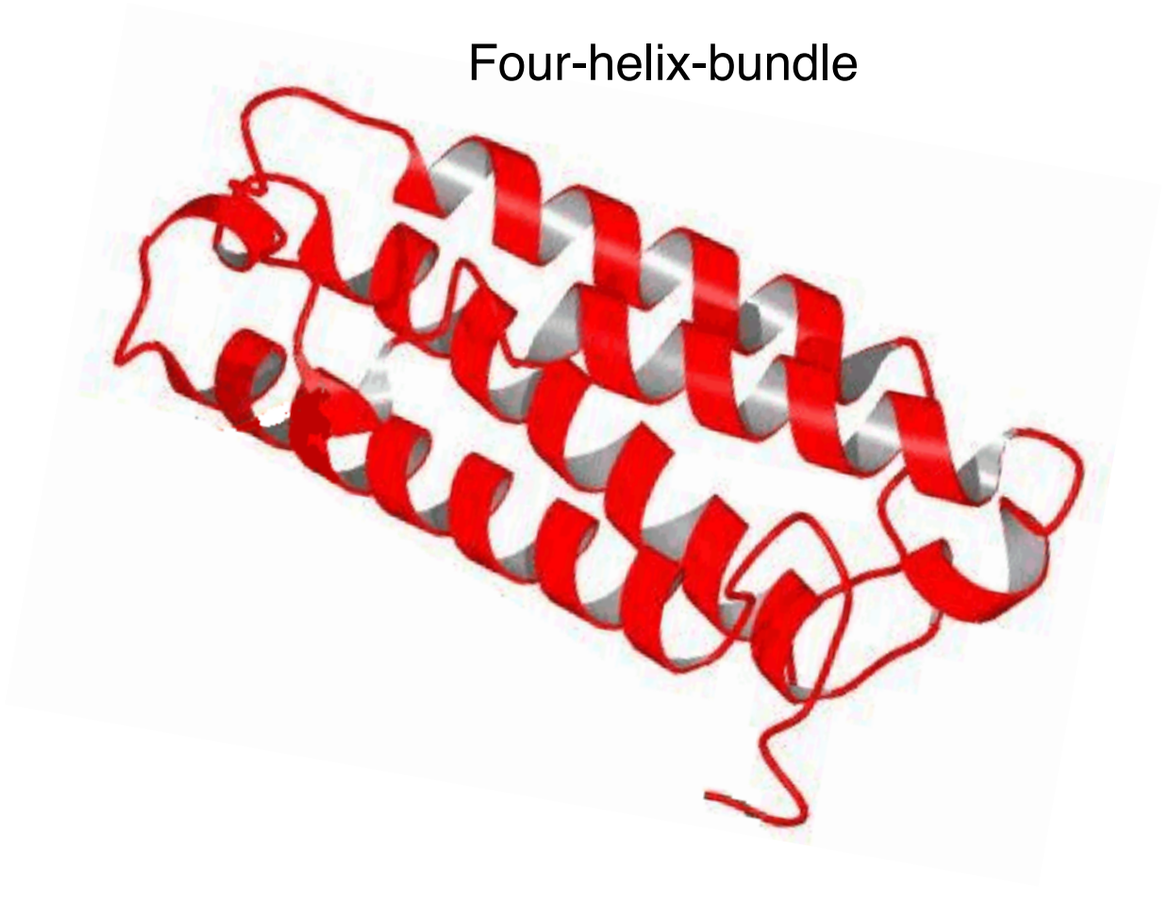
- Parallel β-sheets are less stable than anti-parallel β-sheets.

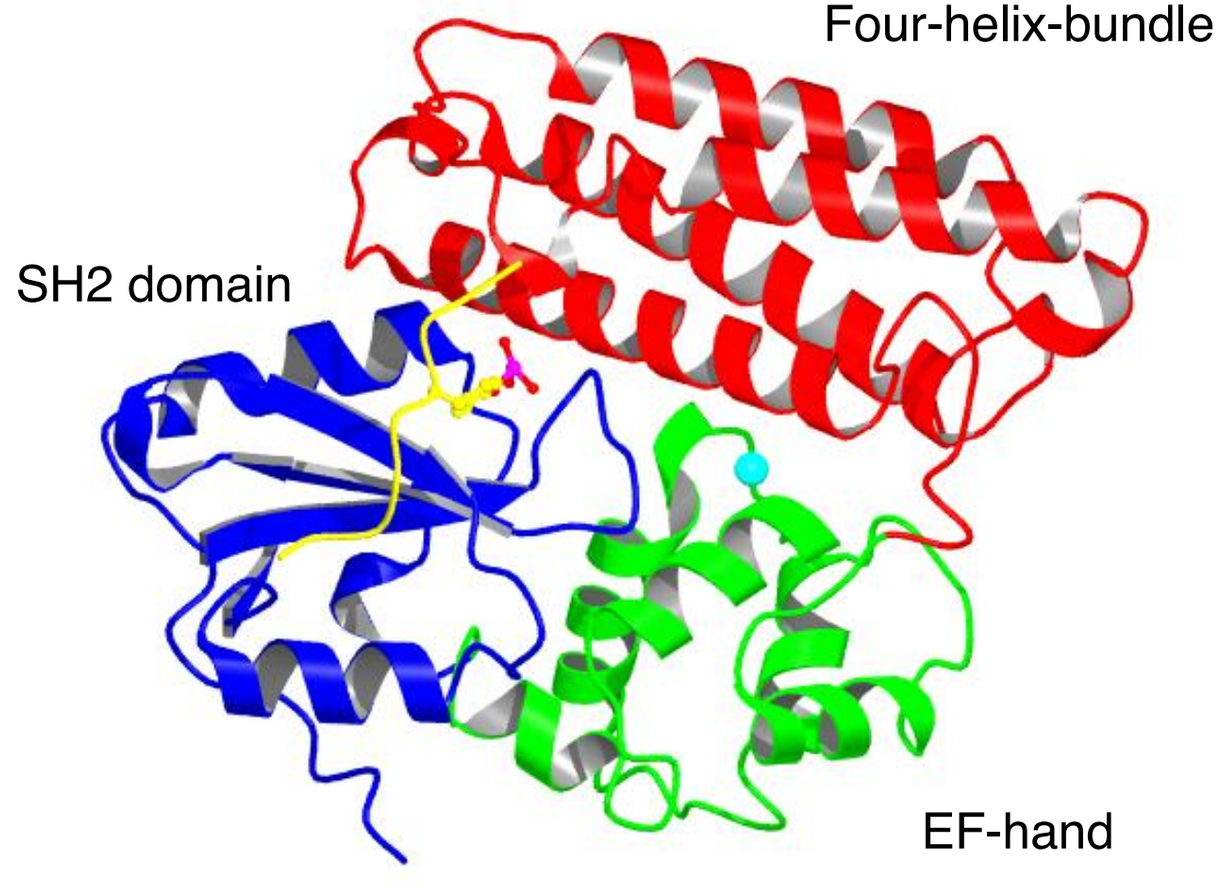


More complex structures

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.

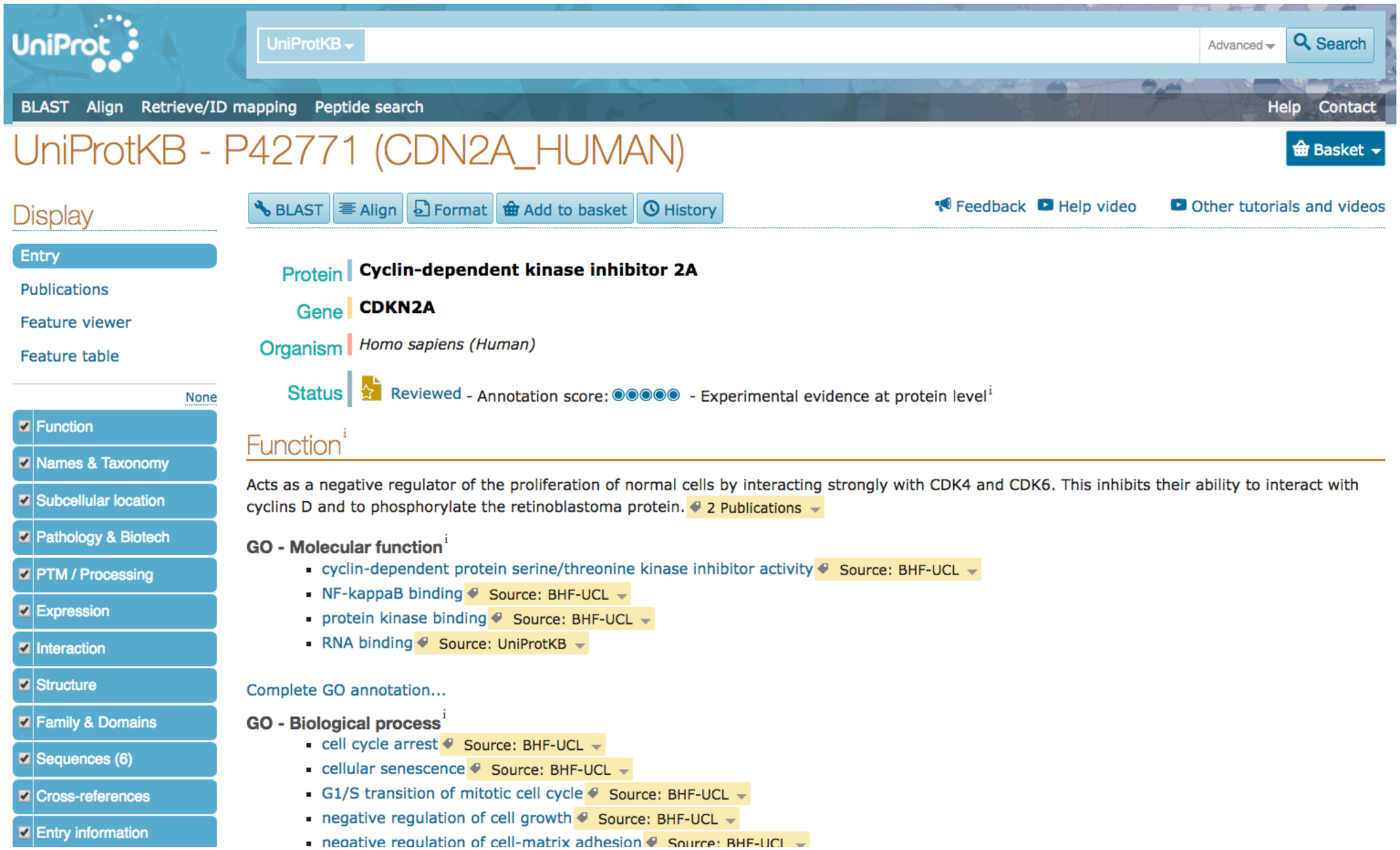




Meng et al. (1999) Nature, 398, 84-90.

CDK inhibitor 2A

The cyclin-dependent kinase inhibitor 2A is a negative regulator of cell proliferation.



CDK inhibitor 2A

Mutation of the CDKN2A have been associated to different forms of melanomas

Display

Entry

Publications

Feature viewer

Feature table

None

Function

Names & Taxonomy

Subcellular location

Pathology & Biotech

PTM / Processing

Expression

Interaction

Structure

Family & Domains

Sequences (6)

Cross-references

Entry information

Miscellaneous

Similar proteins

Top

Pathology & Biotech

Involvement in disease

The association between cutaneous and uveal melanomas in some families suggests that mutations in CDKN2A may account for a proportion of uveal melanomas. However, CDKN2A mutations are rarely found in uveal melanoma patients.

Melanoma, cutaneous malignant 2 (CMM2) 12 Publications

Disease susceptibility is associated with variations affecting the gene represented in this entry.
Disease description: A malignant neoplasm of melanocytes, arising de novo or from a pre-existing benign nevus, which occurs most often in the skin but also may involve other sites.
See also OMIM:155601

Feature key	Position(s)	Description	Actions	Graphical view	Length
Natural variant (VAR_058549)	19	A → ATA in CMM2; loss of CDK4 binding.			1
Natural variant (VAR_001413)	24	R → C in CMM2.			1
Natural variant (VAR_001414)	24	R → P in CMM2. 1 Publication Corresponds to variant dbSNP:rs104894097	Ensembl, ClinVar.		1
Natural variant (VAR_001416)	32	L → P in CMM2. 1 Publication			1
Natural variant (VAR_001418)	35	G → A in CMM2; also found in a biliary tract tumor and a patient with uveal melanoma; partial loss of CDK4 binding. 1 Publication Corresponds to variant dbSNP:rs746834149	Ensembl, ClinVar.		1
Natural variant (VAR_001419)	35	G → E in CMM2. 1 Publication Corresponds to variant dbSNP:rs746834149	Ensembl, ClinVar.		1
Natural variant (VAR_058551)	35	G → V in CMM2; loss of CDK4 binding. 1 Publication			1
Natural variant (VAR_001420)	48	P → L in CMM2; also found in head and neck tumor; somatic mutation. 1 Publication			1
Natural variant (VAR_001423)	50	Q → R in CMM2. 1 Publication			1
Natural variant (VAR_001424)	53	M → I in CMM2. 3 Publications Corresponds to variant dbSNP:rs104894095	Ensembl, ClinVar.		1
Natural variant (VAR_001427)	59	V → G in CMM2. 1 Publication Corresponds to variant dbSNP:rs104894099	Ensembl, ClinVar.		1
Natural variant (VAR_001430)	62	L → P in CMM2.			1

CDK6-P16INK4A

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

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MECHANISM OF G1 CYCLIN DEPENDENT KINASE INHIBITION FROM THE STRUCTURE OF THE CDK6-P16INK4A TUMOR SUPPRESSOR COMPLEX

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DOI:10.2210/pdb1bi7/pdb

Primary Citation

Structural basis for inhibition of the cyclin-dependent kinase Cdk6 by the tumour suppressor p16INK4a.

Russo, A.A., Tong, L., Lee, J.O., Jeffrey, P.D., Pavletich, N.P.

Journal: (1998) Nature **395:** 237-243

PubMed: 9751050
DOI: 10.1038/26155
Search Related Articles in PubMed

PubMed Abstract:

The cyclin-dependent kinases 4 and 6 (Cdk4/6) that control the G1 phase of the cell cycle and their inhibitor, the p16INK4a tumour suppressor, have a central role in cell proliferation and in tumorigenesis. The structures of Cdk6 bound to p16INK4a...
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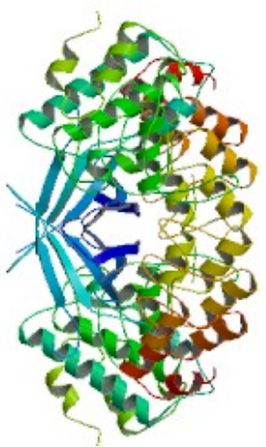
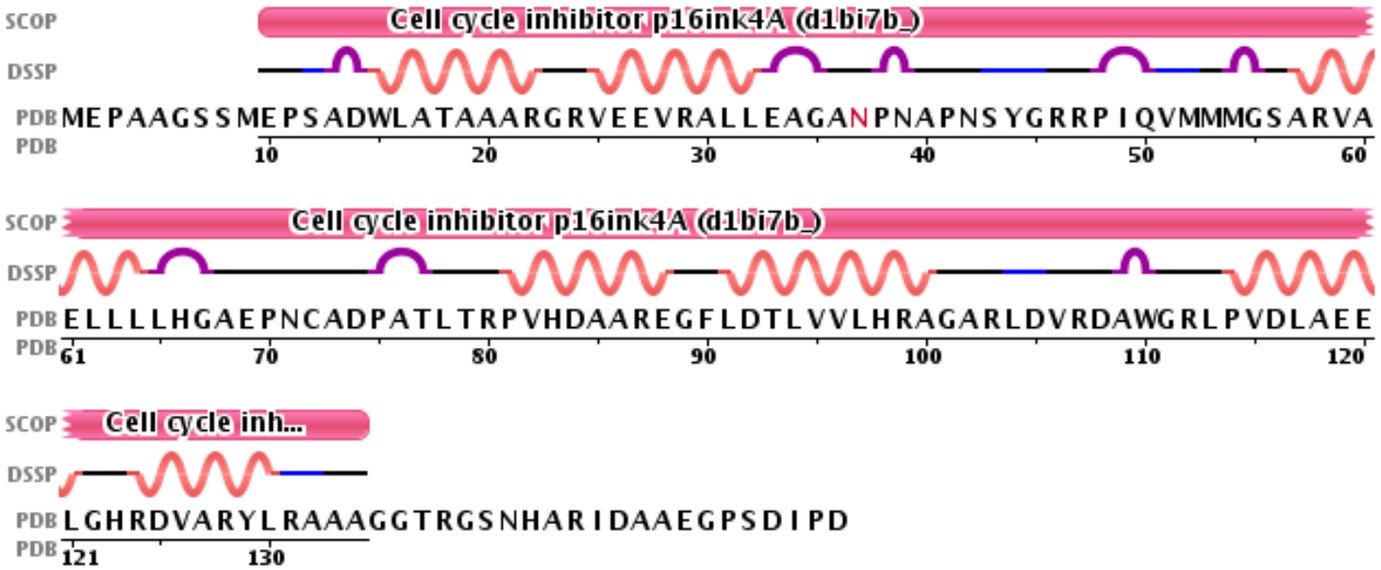
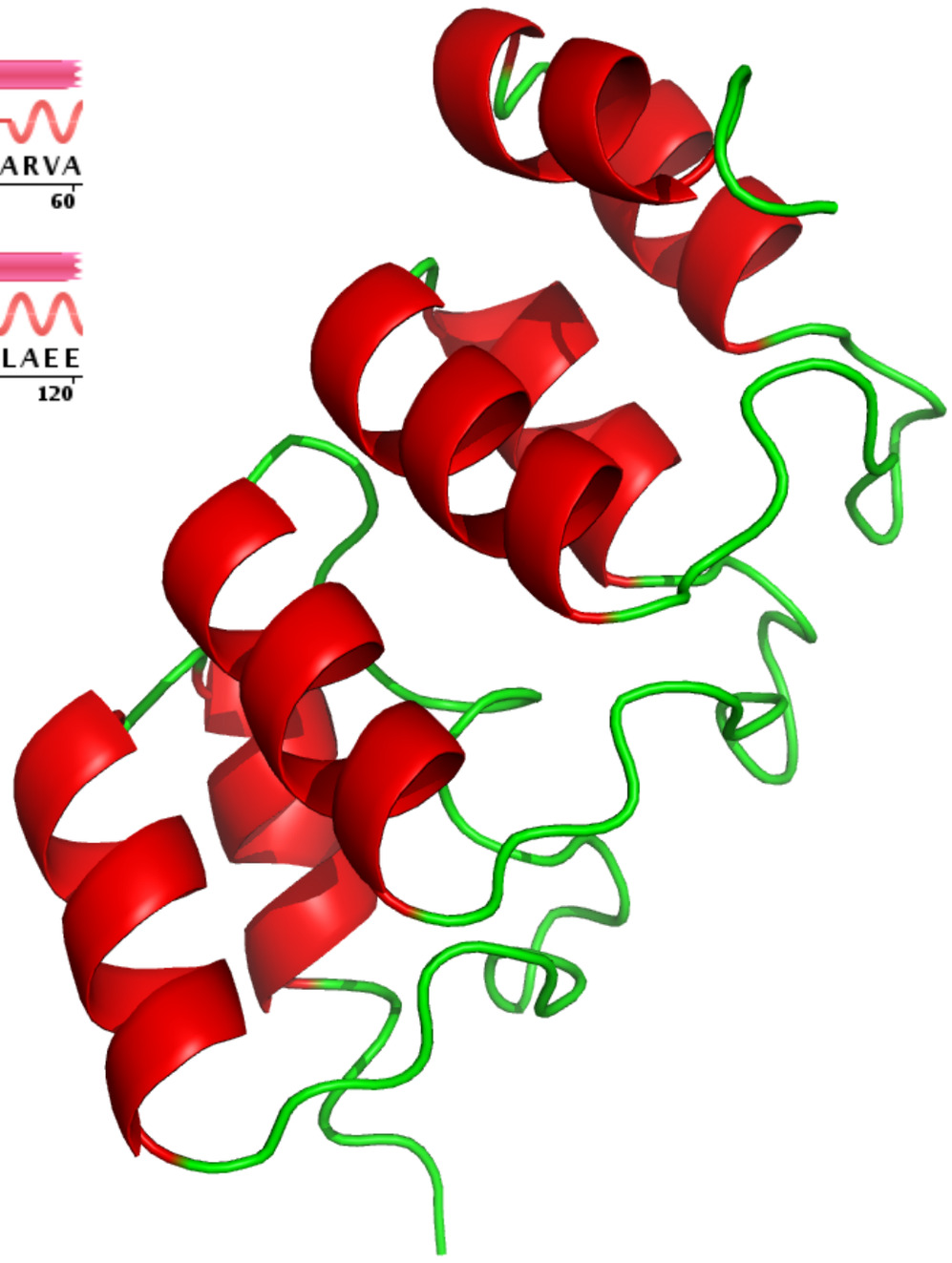
Biological Assembly



P16INK4A

The P16INK4A is a tumor suppressor protein with 7 helixes.





PDB data

The most important information are the atomic coordinates.

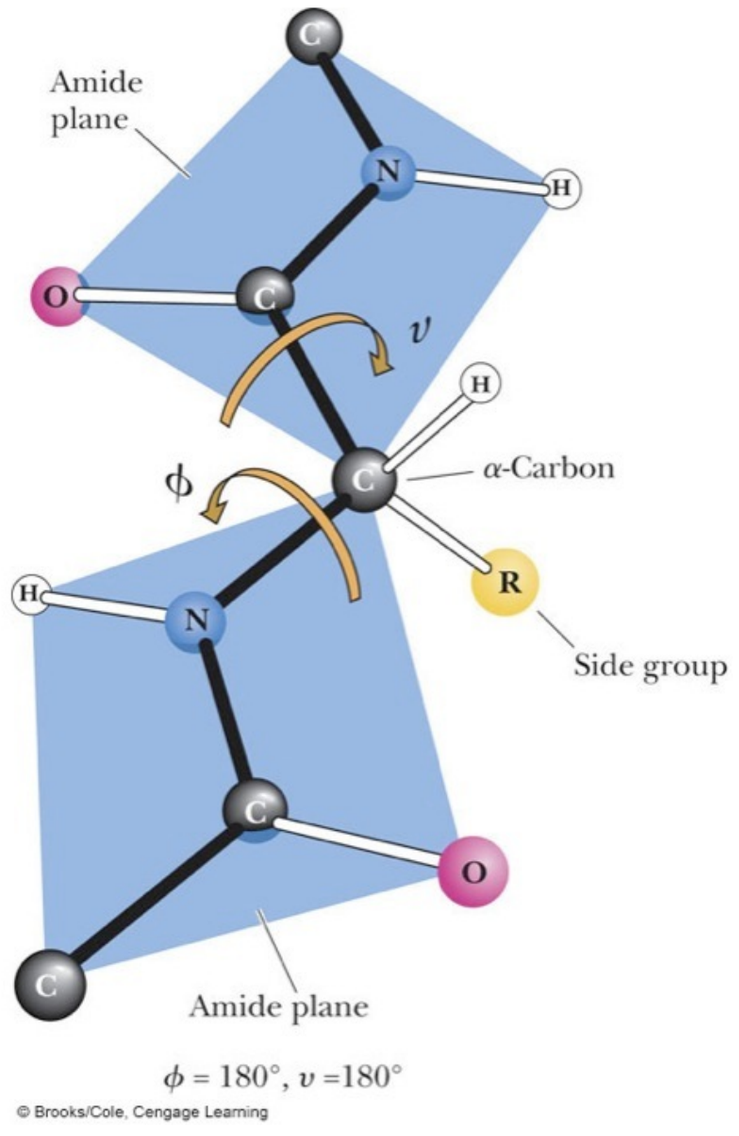
		AT	RES	CH	POS	X	Y	Z			
ATOM	2145	N	GLU	B	10	150.341	72.309	103.145	1.00	99.90	N
ATOM	2146	CA	GLU	B	10	150.096	71.519	101.907	1.00	99.90	C
ATOM	2147	C	GLU	B	10	150.425	70.046	102.190	1.00	99.90	C
ATOM	2148	O	GLU	B	10	151.326	69.770	102.983	1.00	99.90	O
ATOM	2149	CB	GLU	B	10	150.963	72.057	100.790	1.00	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

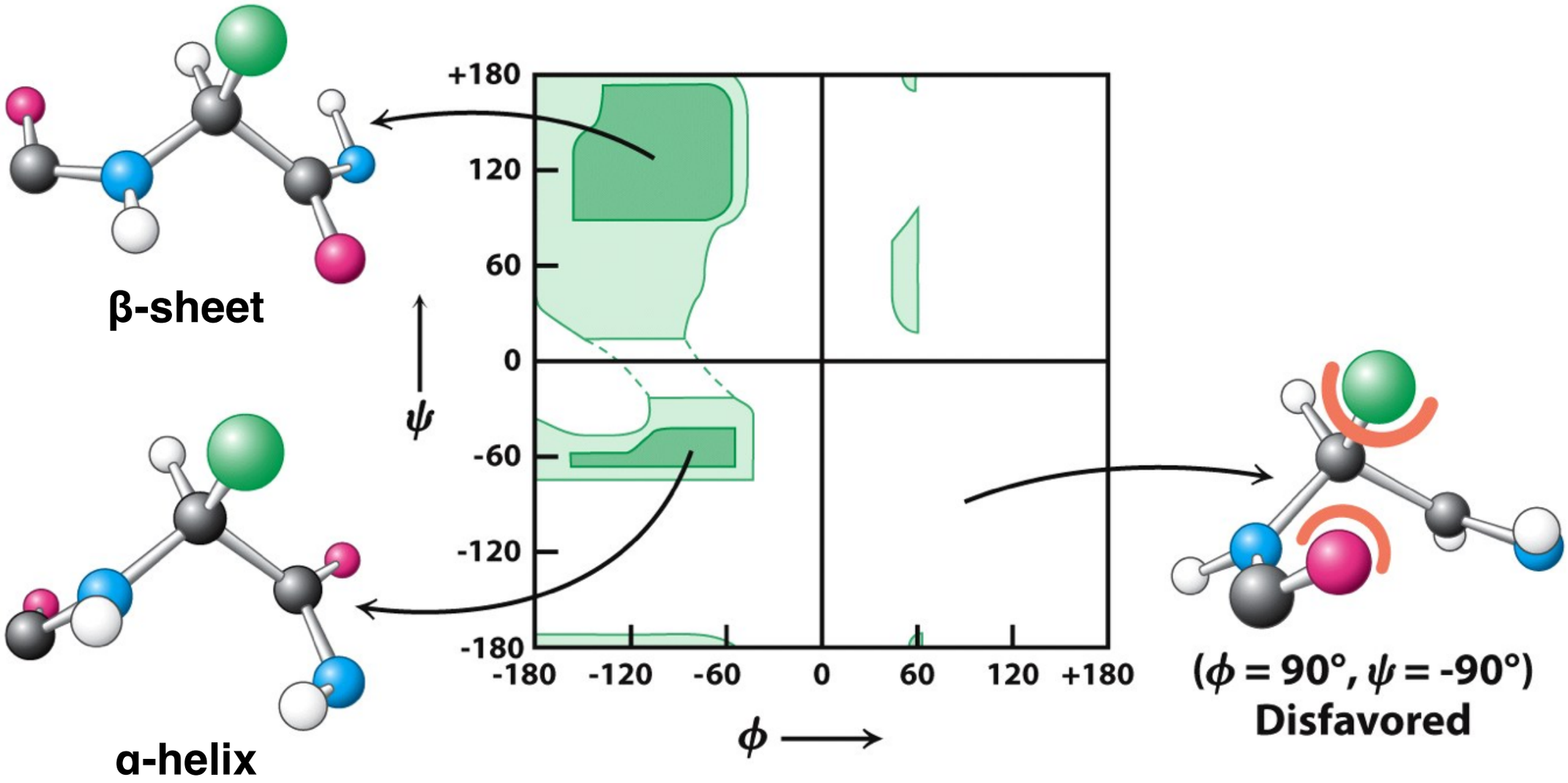
Basic information for the characterization of the protein three-dimensional structures are:

- 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: more details at wikipedia

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 mutated sites 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.