Protein Sequence and Structure

Proteomes Interactomes and Biological Networks

5 and 6 November, 2019

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http://biofold.org/

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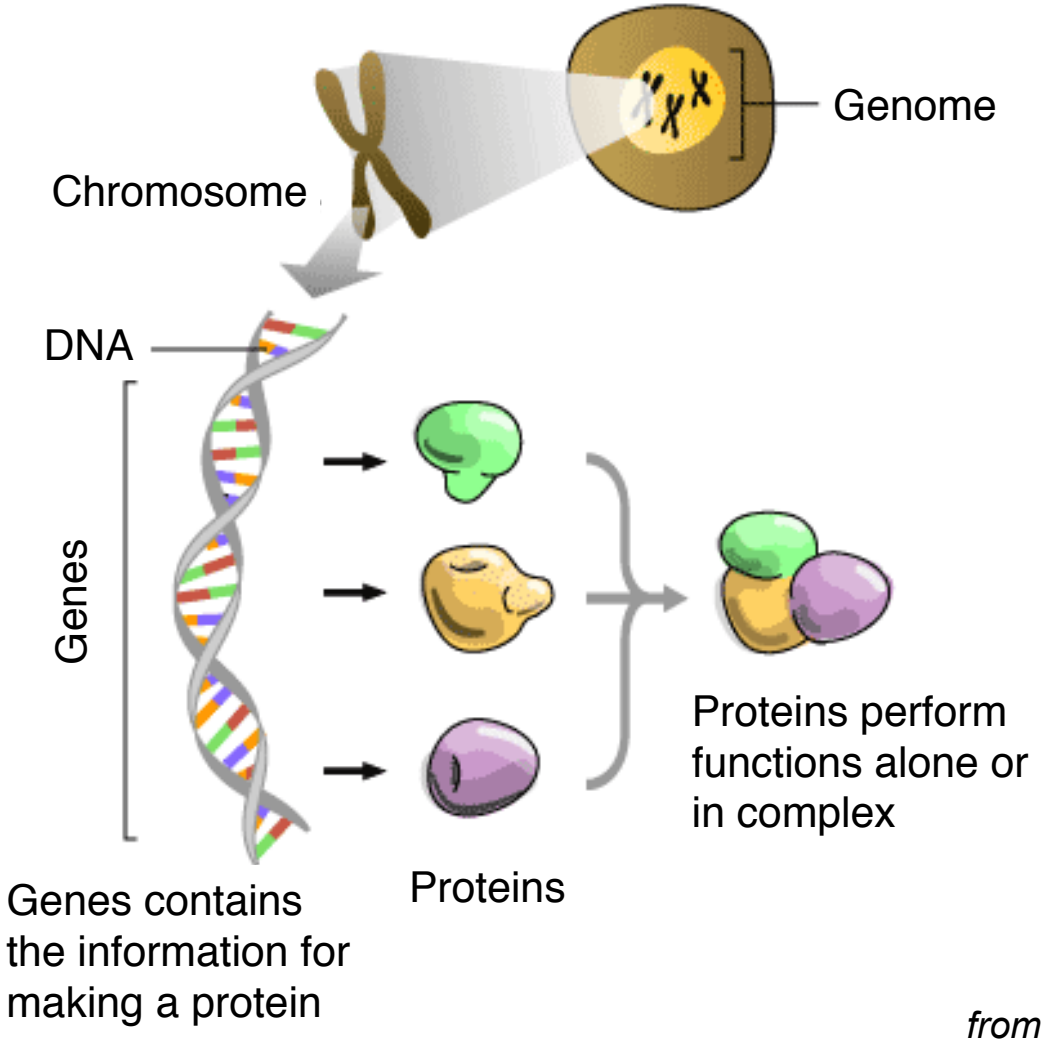
Biotechnology (FaBiT)
University of Bologna

Biomolecules
Folding and
Disease

The Central Dogma

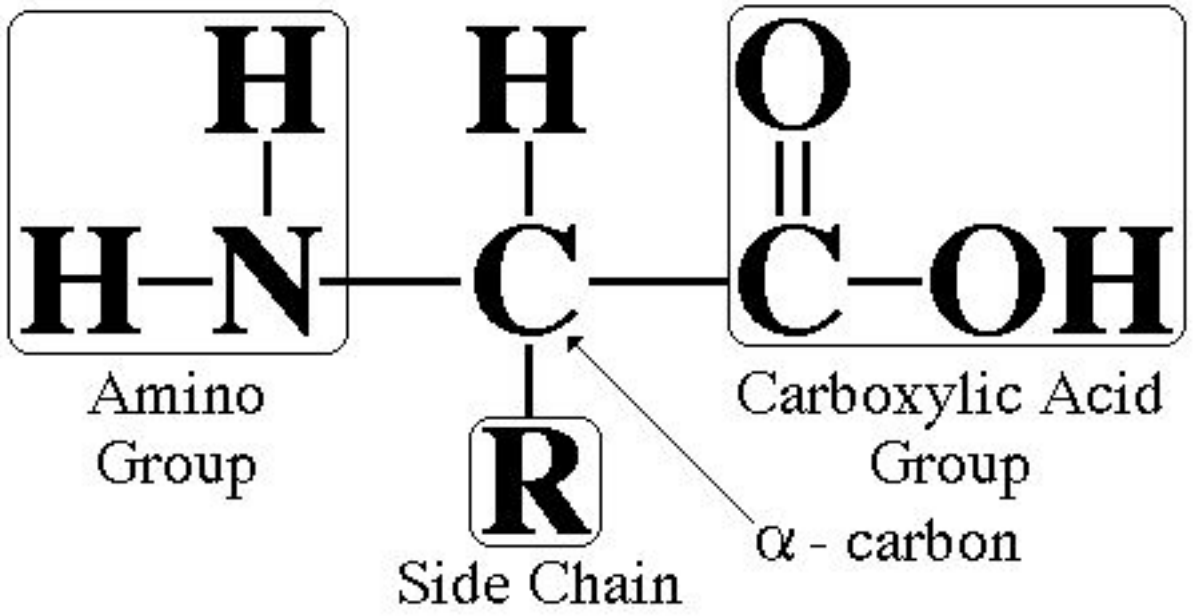


from http://www.scq.ubc.ca

https://www.youtube.com/watch?v=9kOGOY7vthk

Amino Acid

The side chain (R) determines the type of the amino acid

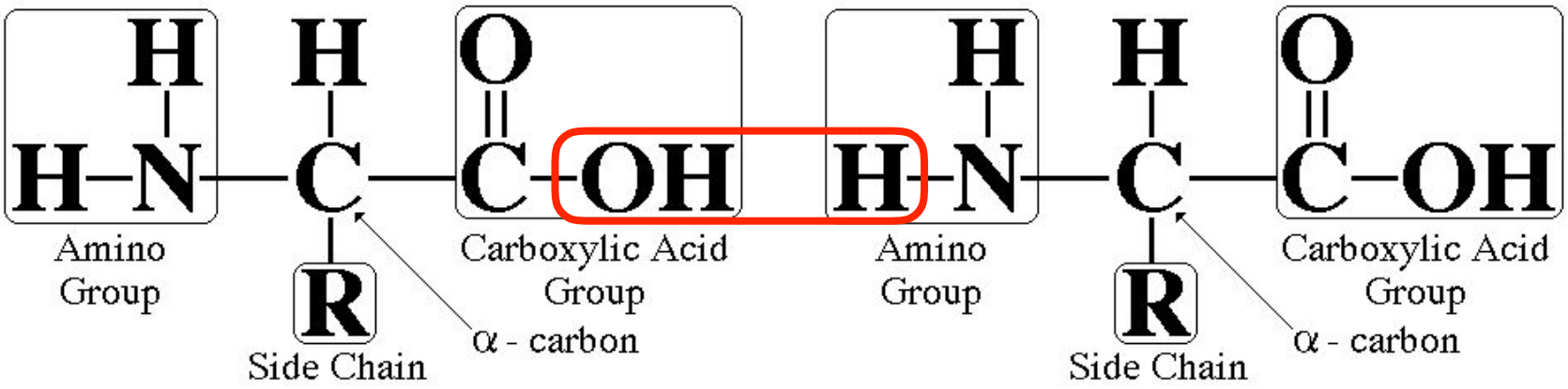


Physico-chemical Properties

The properties of the amino acid depends on the side chain

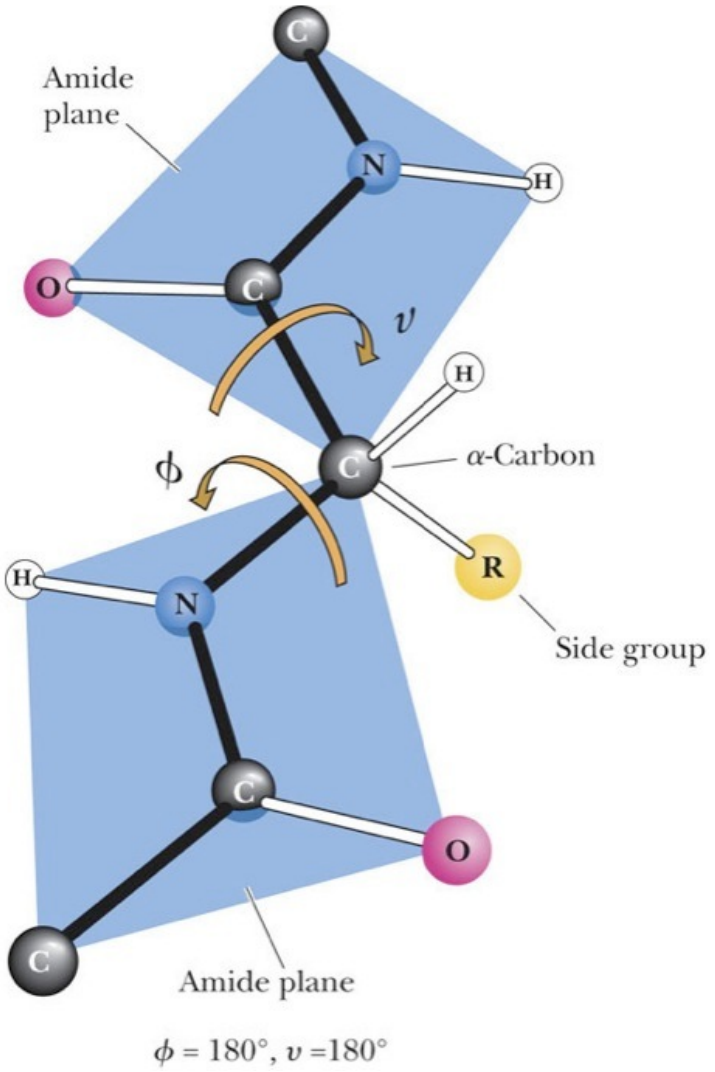
Amino acid	Abbrev.	Side chain	Hydro-phobic	Polar	Charged	Small	Tiny	Aromatic or Aliphatic	van der Waals volume	Codon	Occurrence in proteins (%)
Alanine	Ala, A	$-CH_3$	X	-	-	X	X	-	67	GCU, GCC, GCA, GCG	7.8
Cysteine	Cys, C	$-CH_2SH$	X	-	-	X	-	-	86	UGU, UGC	1.9
Aspartate	Asp, D	$-CH_2COOH$	-	X	negative	X	-	-	91	GAU, GAC	5.3
Glutamate	Glu, E	$-CH_2CH_2COOH$	-	X	negative	-	-	-	109	GAA, GAG	6.3
Phenylalanine	Phe, F	$-CH_2C_6H_5$	X	-	-	-	-	Aromatic	135	UUU, UUC	3.9
Glycine	Gly, G	-H	X	-	-	X	X	-	48	GGU, GGC, GGA, GGG	7.2
Histidine	His, H	$-CH_2-C_3H_3N_2$	-	X	positive	-	-	Aromatic	118	CAU, CAC	2.3
Isoleucine	Ile, I	$-CH(CH_3)CH_2CH_3$	X	-	-	-	-	Aliphatic	124	AUU, AUC, AUA	5.3
Lysine	Lys, K	$-(CH_2)_4NH_2$	-	X	positive	-	-	-	135	AAA, AAG	5.9
Leucine	Leu, L	$-CH_2CH(CH_3)_2$	X	-	-	-	-	Aliphatic	124	UUA, UUG, CUU, CUC, CUA, CUG	9.1
Methionine	Met, M	$-CH_2CH_2SCH_3$	X	-	-	-	-	-	124	AUG	2.3
Asparagine	Asn, N	$-CH_2CONH_2$	-	X	-	X	-	-	96	AAU, AAC	4.3
Proline	Pro, P	$-CH_2CH_2CH_2-$	X	-	-	X	-	-	90	CCU, CCC, CCA, CCG	5.2
Glutamine	Gln, Q	$-CH_2CH_2CONH_2$	-	X	-	-	-	-	114	CAA, CAG	4.2
Arginine	Arg, R	$-(CH_2)_3NH-C(NH)NH_2$	-	X	positive	-	-	-	148	CGU, CGC, CGA, CGG, AGA, AGG	5.1
Serine	Ser, S	$-CH_2OH$	-	X	-	X	X	-	73	UCU, UCC, UCA, UCG, AGU,AGC	6.8
Threonine	Thr, T	$-CH(OH)CH_3$	X	X	-	X	-	-	93	ACU, ACC, ACA, ACG	5.9
Valine	Val, V	$-CH(CH_3)_2$	X	-	-	X	-	Aliphatic	105	GUU, GUC, GUA, GUG	6.6
Tryptophan	Trp, W	$-CH_2C_8H_6N$	X	-	-	-	-	Aromatic	163	UGG	1.4
Tyrosine	Tyr, Y	$-CH_2-C_6H_4OH$	X	X	-	-	-	Aromatic	141	UAU, UAC	3.2

Peptide Bond



Torsion Angles

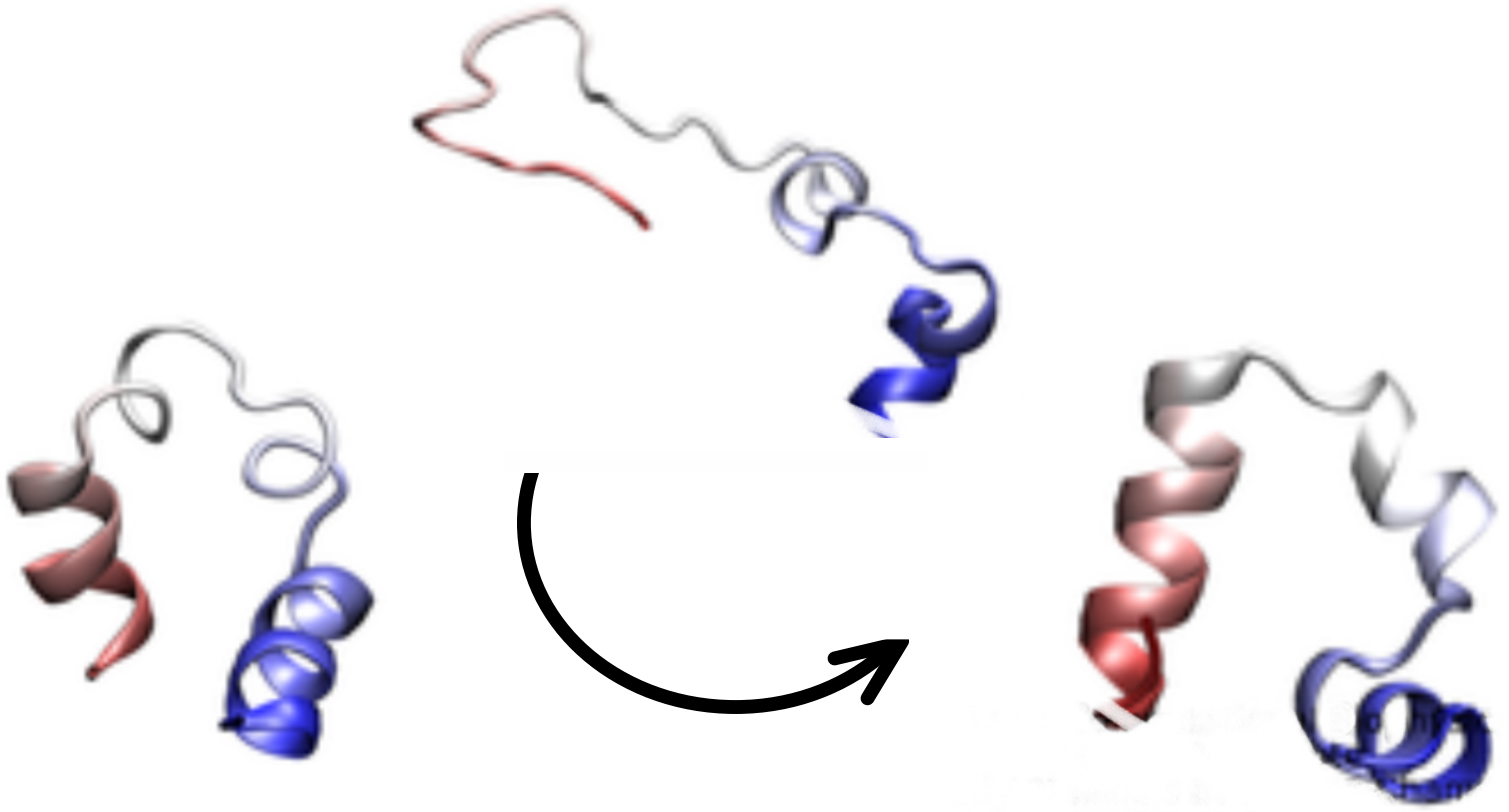
Backbone torsion angles determine the structure of the protein



Protein folding

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

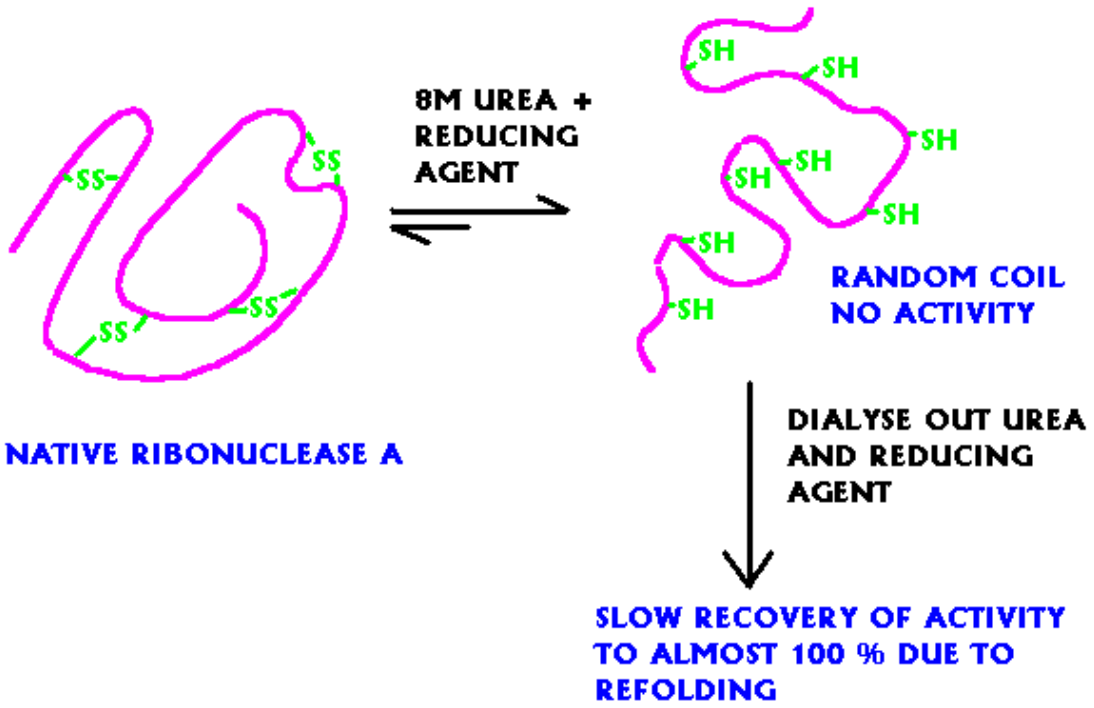
```
T T C C P S I V A R S N F N V C R L P G T P E A L C A T
Y T G C I I I P G A T C P G D Y A N
```



The Anfinsen's hypothesis

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

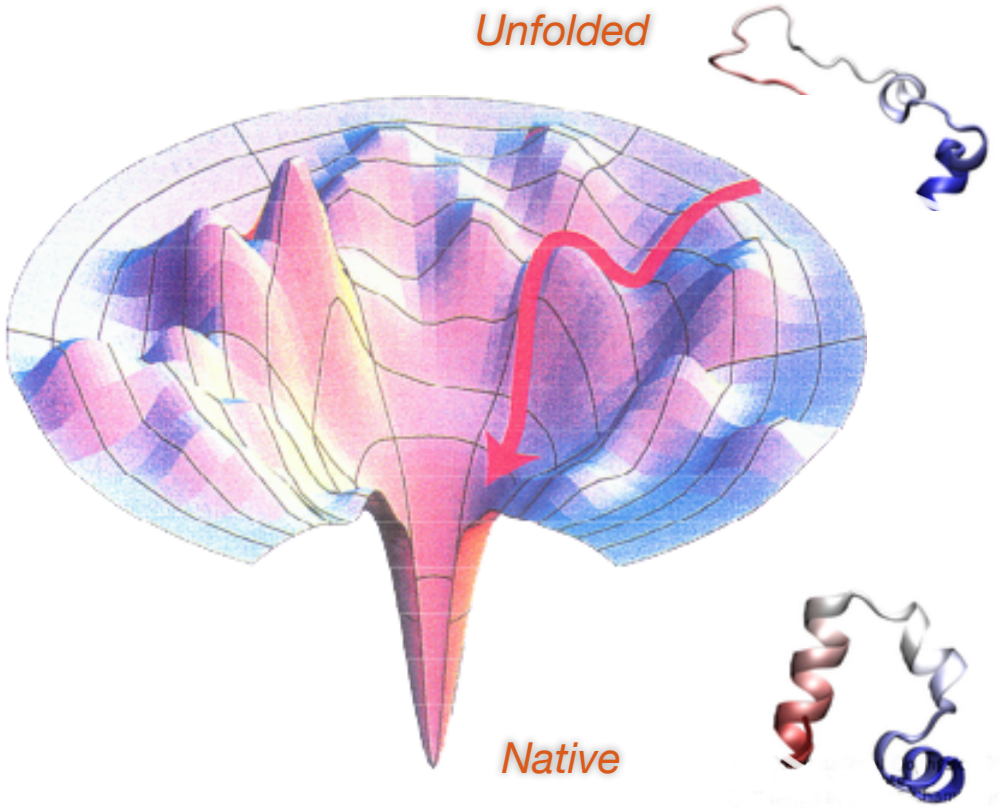
Anfinsen showed that denatured ribonuclease A could be re-activated removing the denaturant.



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

Levinthal's paradox

A protein chain composed by 100 residues with 2 possible conformations has 2^{100} ($\sim 10^{30}$) 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.

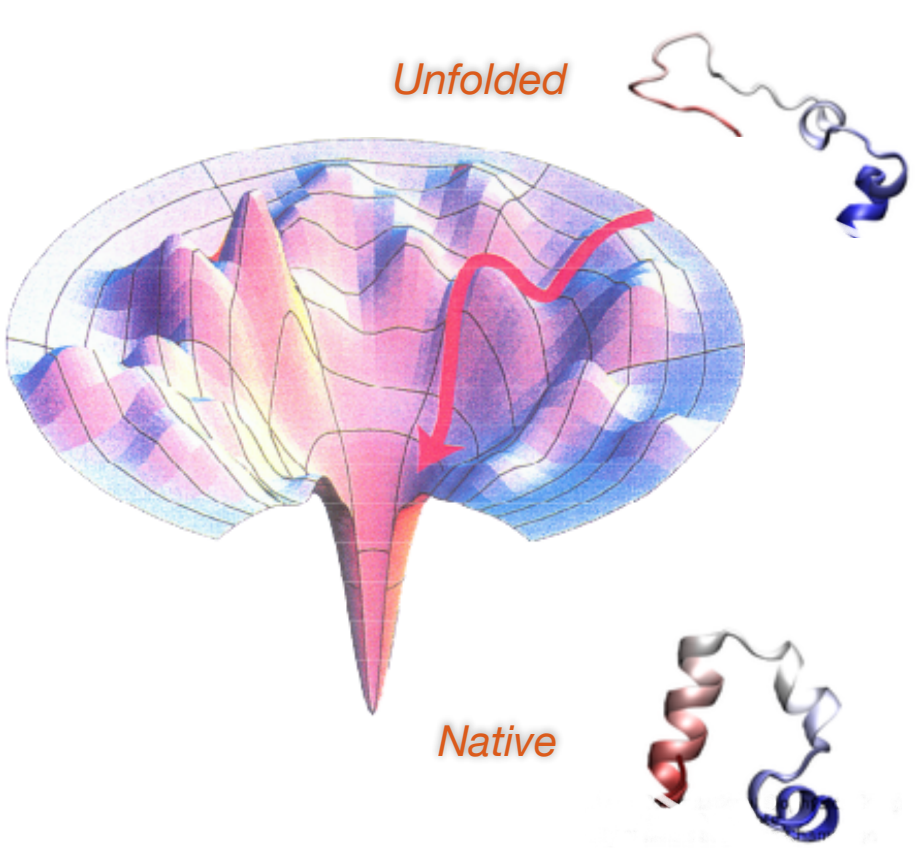
Aspects of the same problem

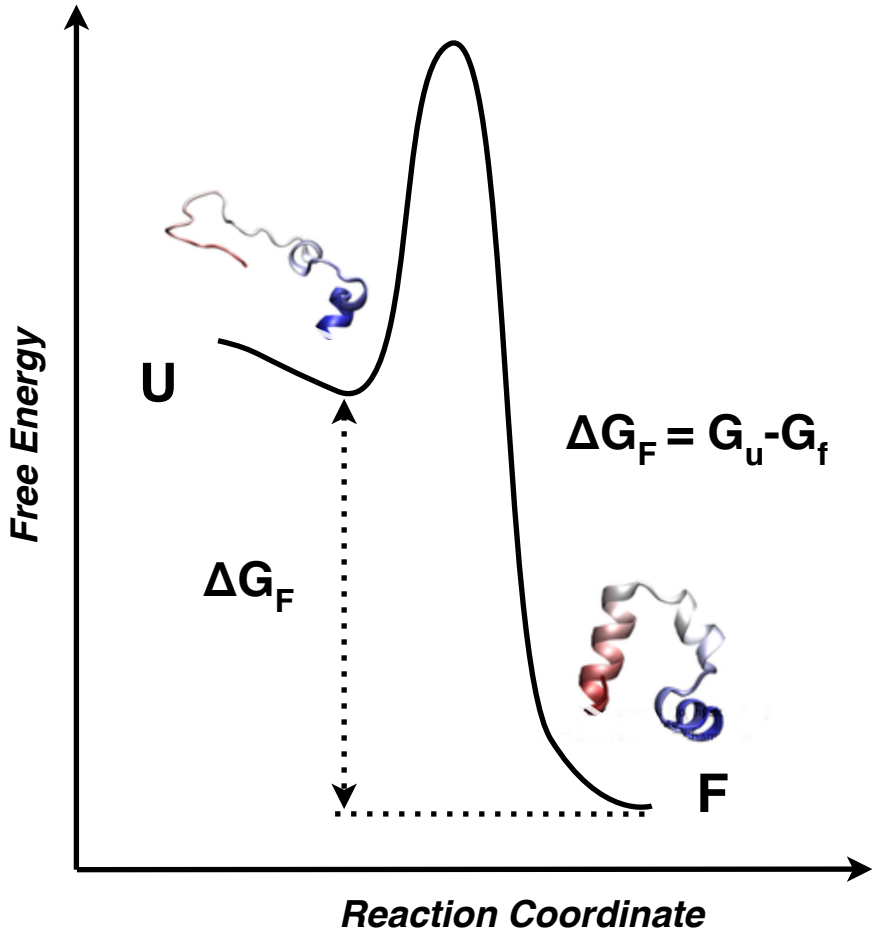
The solution of the protein folding consists in the understanding of three different aspects of the problem:

- 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

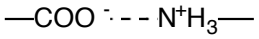
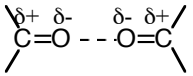
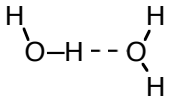
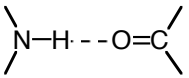
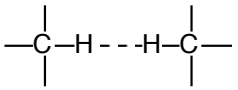
The folding free energy difference, ΔG_F, is typically small, of the order of -5 to -15 kcal/mol for a globular protein (compared to e.g. -30 to -100 kcal/mol for a covalent bond).





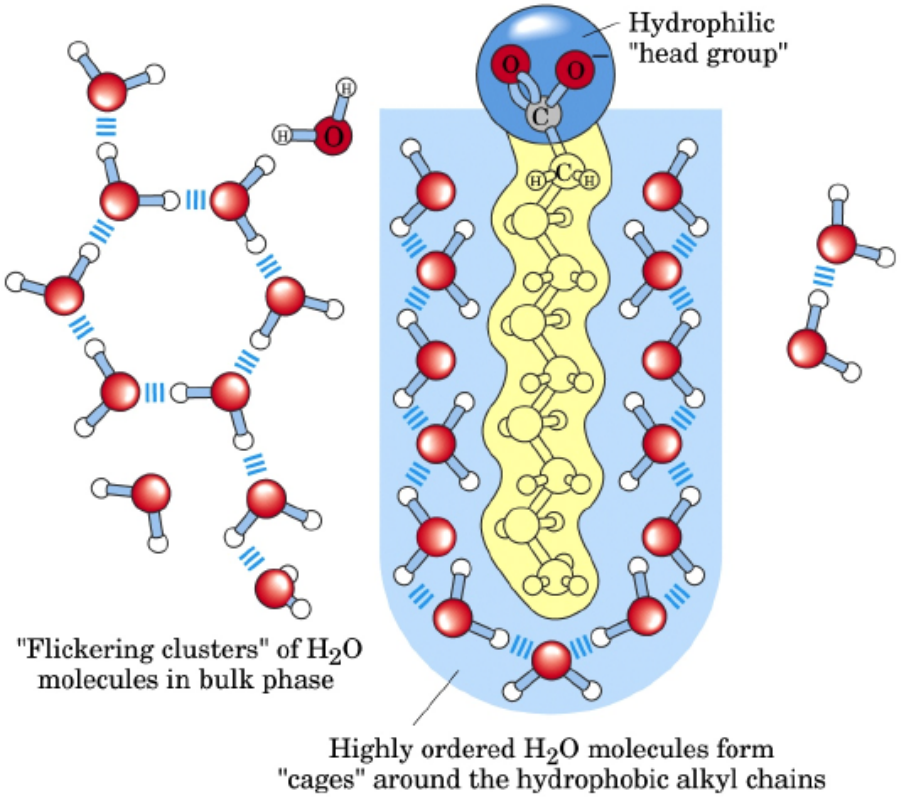
Folding interactions

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

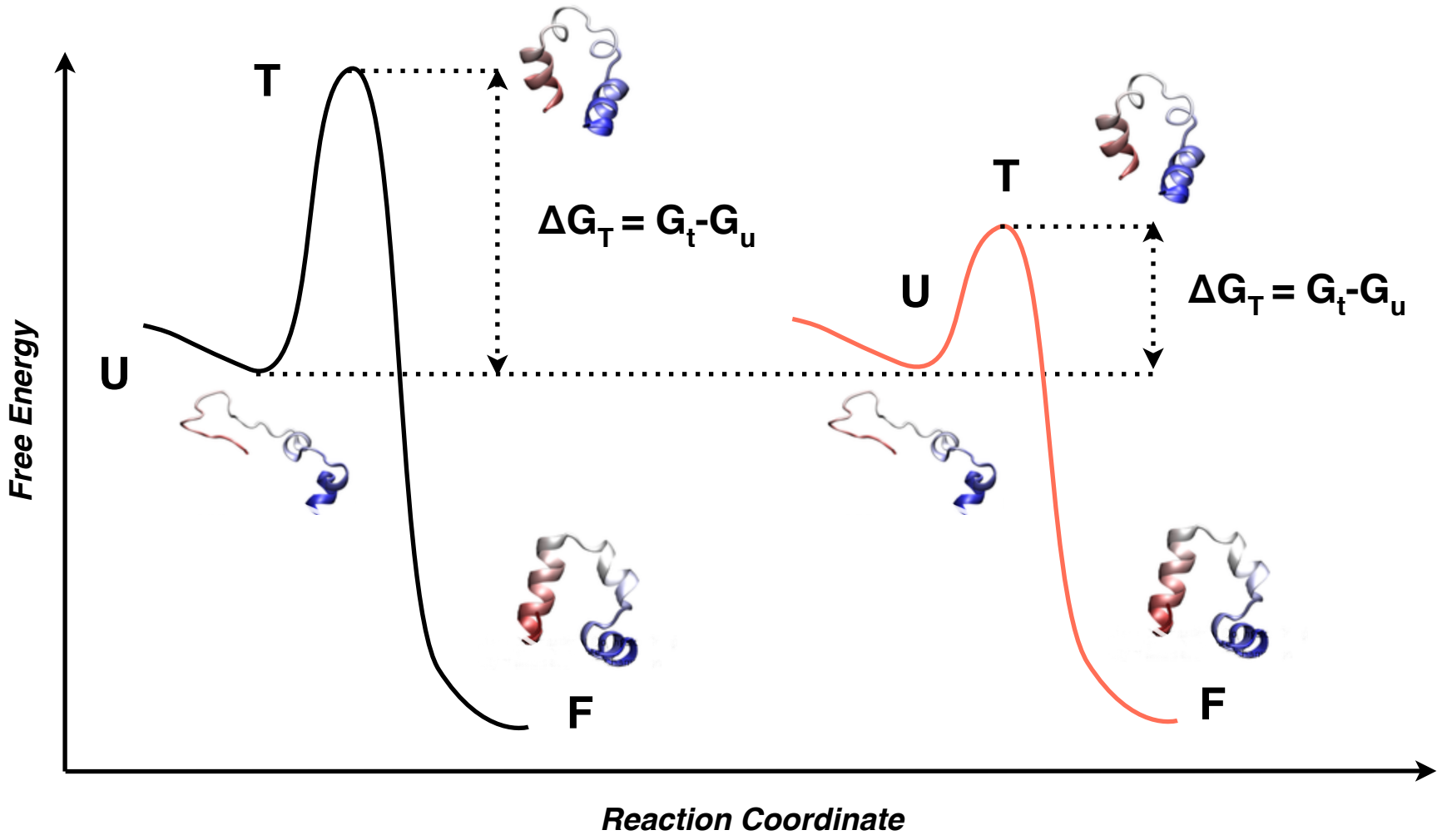
Hydrophobic effect

- 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.



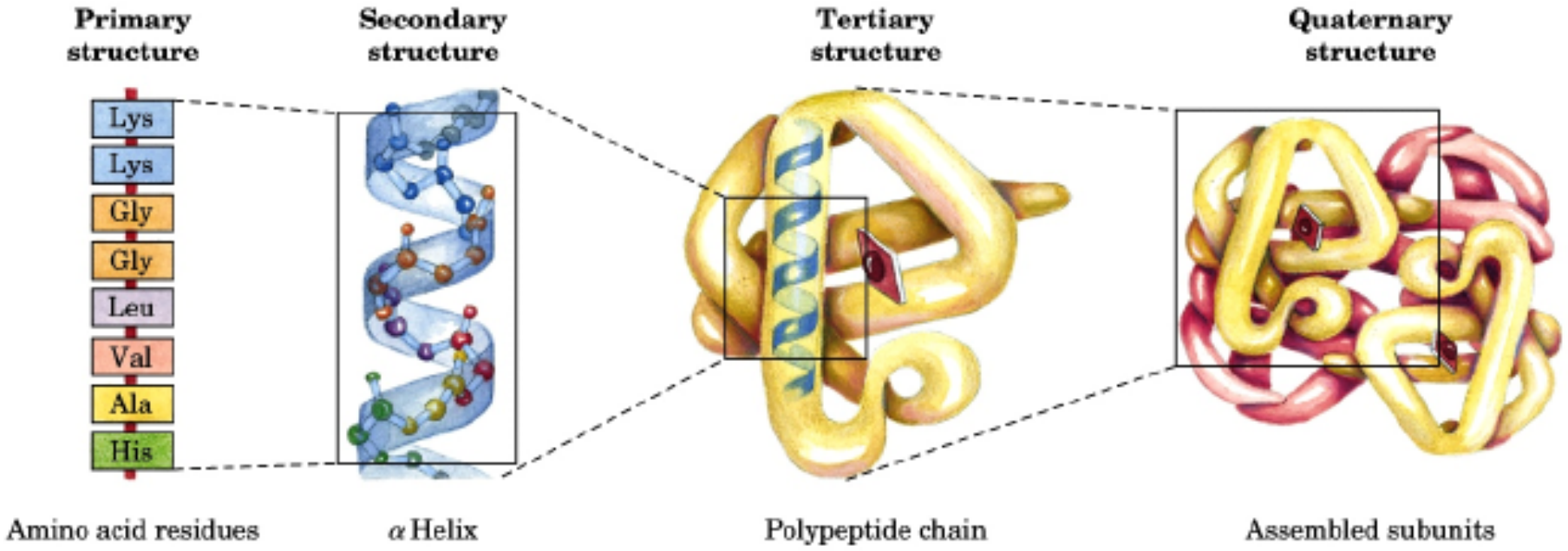
Folding kinetics

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



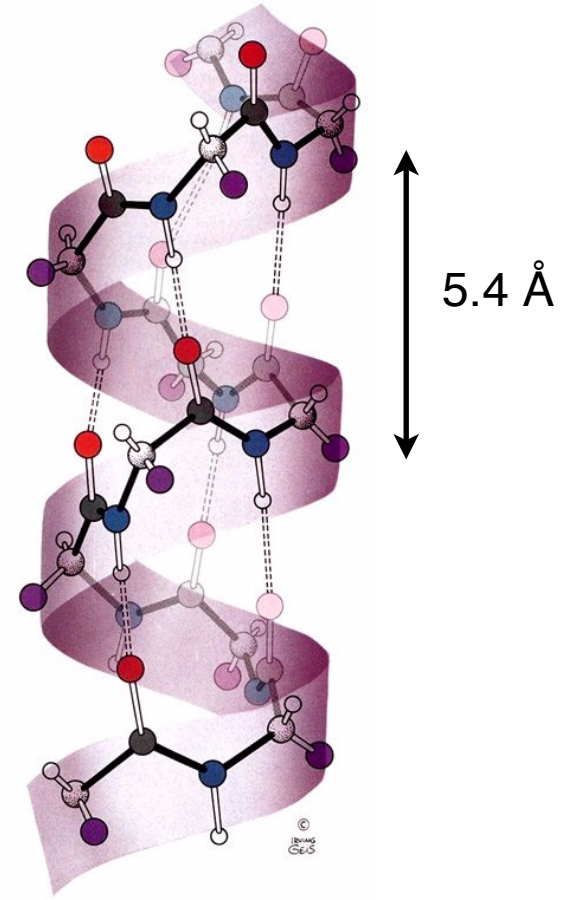
Hierarchical organization of protein structure

Protein structure is defined by four levels of hierarchical organization.



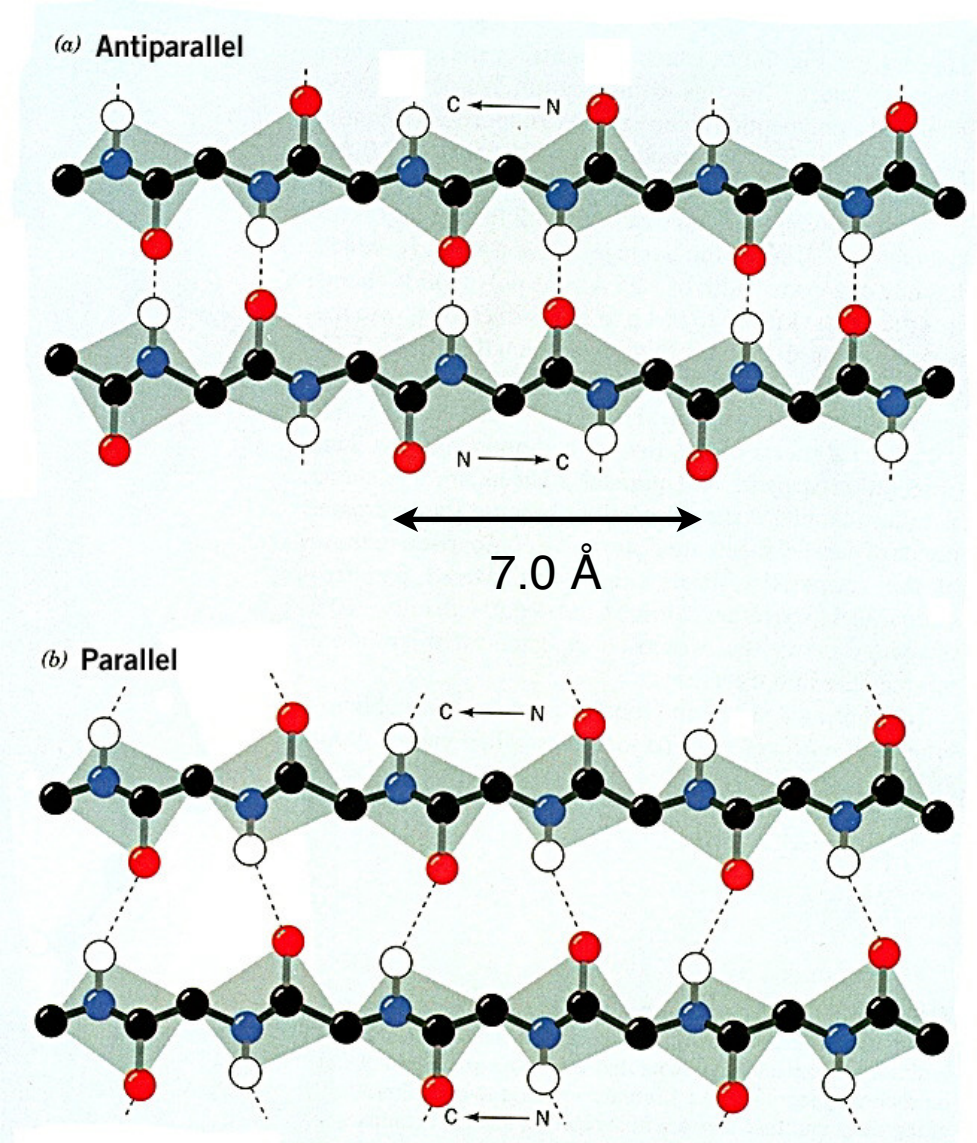
Secondary structure (I)

- 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)

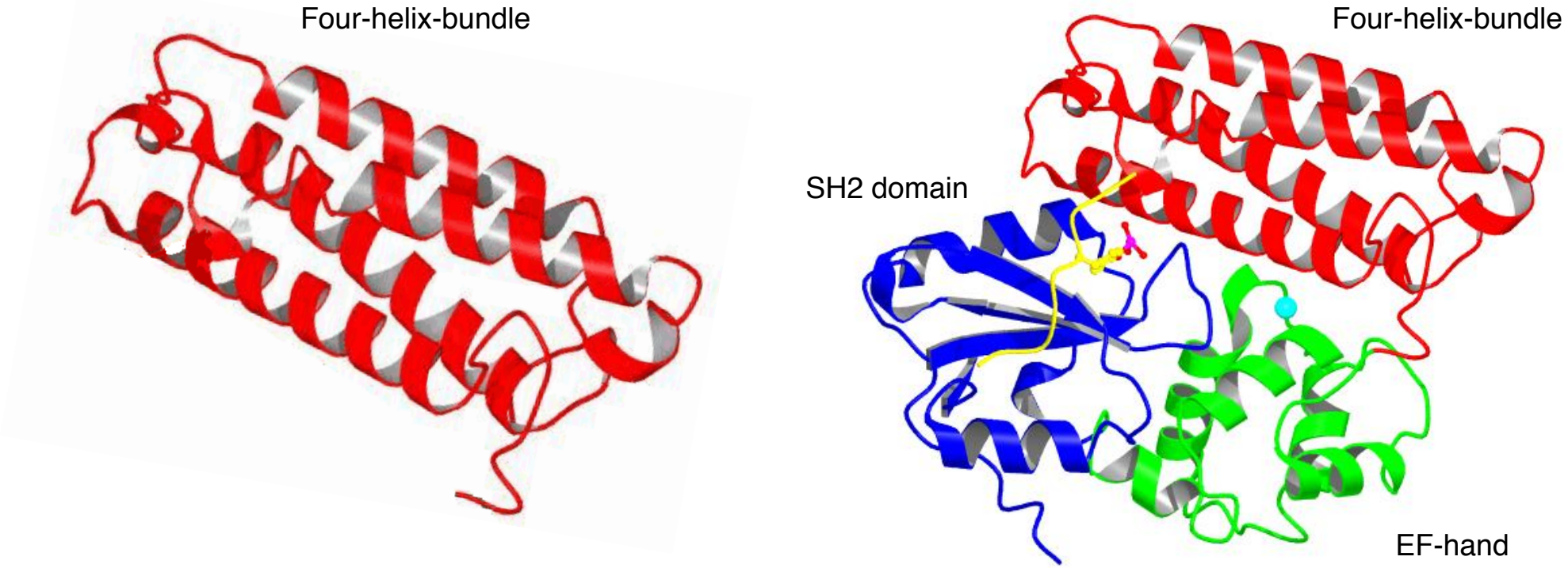
- Typical ϕ, ψ values for residues in β-sheet are around 140°, -130°
- Side chains of neighboring residues project in opposite directions.
- The polypeptide is in a more extended conformation.
- Parallel β-sheets are less stable than anti-parallel β-sheets.



More complex structures

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

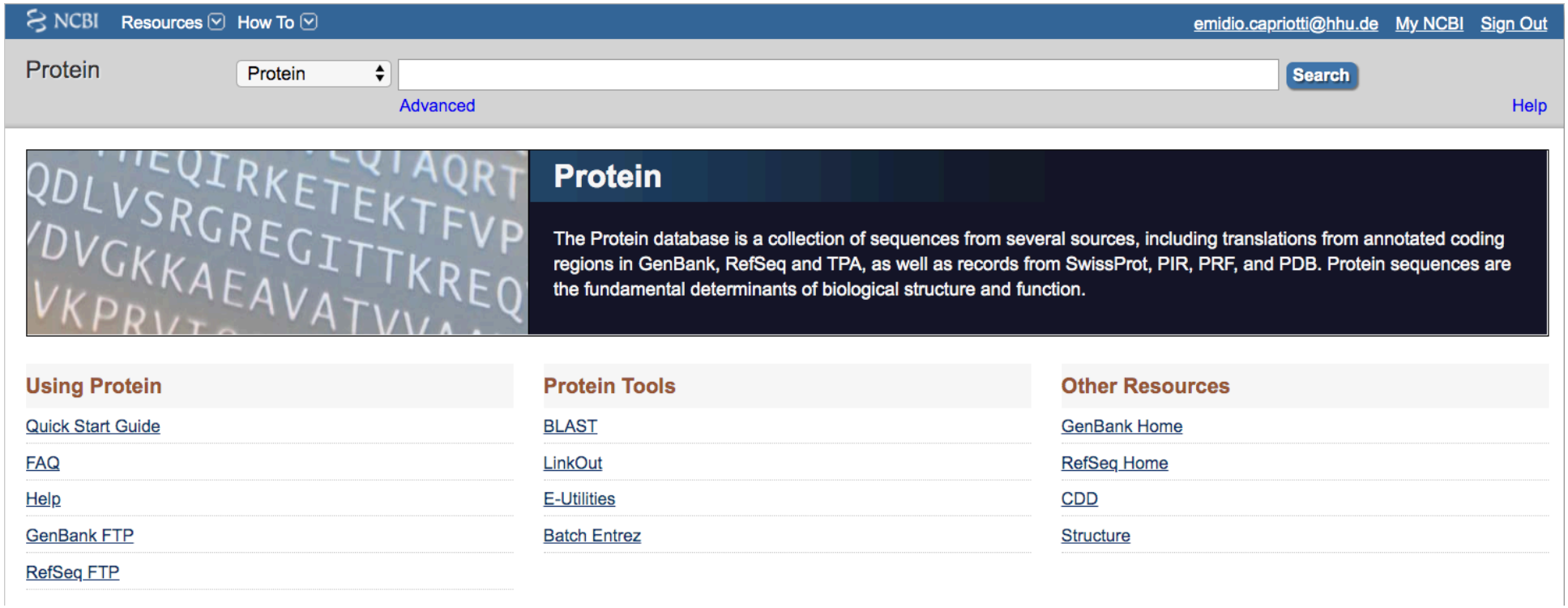
The complex of two or more protein domains defines the Quaternary Structure. In the example Four-helix-bundle, EF-hand and SH2 domains together form an integrated phosphoprotein that functions as a negative regulator of many signaling pathways from receptors at the cell surface.



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

Protein at the NCBI

The Protein database is a collection of sequences from several resources accessible though Entrez



https://www.ncbi.nlm.nih.gov/protein/

Protein search

Using the name of the protein and the organism we can retrive a specific protein

P53 [Homo sapiens]

GenBank: BAC16799.1

Identical Proteins FASTA Graphics

Go to:

```
LOCUS       BAC16799                393 aa            linear   PRI 01-APR-2003
DEFINITION  P53 [Homo sapiens].
ACCESSION   BAC16799
VERSION     BAC16799.1
DBSOURCE    accession AB082923.1
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1
  AUTHORS   Azuma,K., Shichijo,S., Maeda,Y., Nakatsura,T., Nonaka,Y., Fujii,T.,
            Koike,K. and Itoh,K.
  TITLE     Mutated p53 gene encodes a nonmutated epitope recognized by
            HLA-B*4601-restricted and tumor cell-reactive CTLs at tumor site
  JOURNAL   Cancer Res. 63 (4), 854-858 (2003)
   PUBMED   12591737
REFERENCE   2  (residues 1 to 393)
  AUTHORS   Shichijo,S. and Itoh,K.
  TITLE     Direct Submission
  JOURNAL   Submitted (26-MAR-2002) Shigeki Shichijo, Kurume Univ. School of
            Med., Dep. Immunol.; 67-Asahi-machi, Kurume, Fukuoka 830-0011,
            Japan (E-mail:shichijo@med.kurume-u.ac.jp, Tel:81-942-31-7551,
            Fax:81-942-31-7699)
FEATURES             Location/Qualifiers
```

Protein Sequence DB

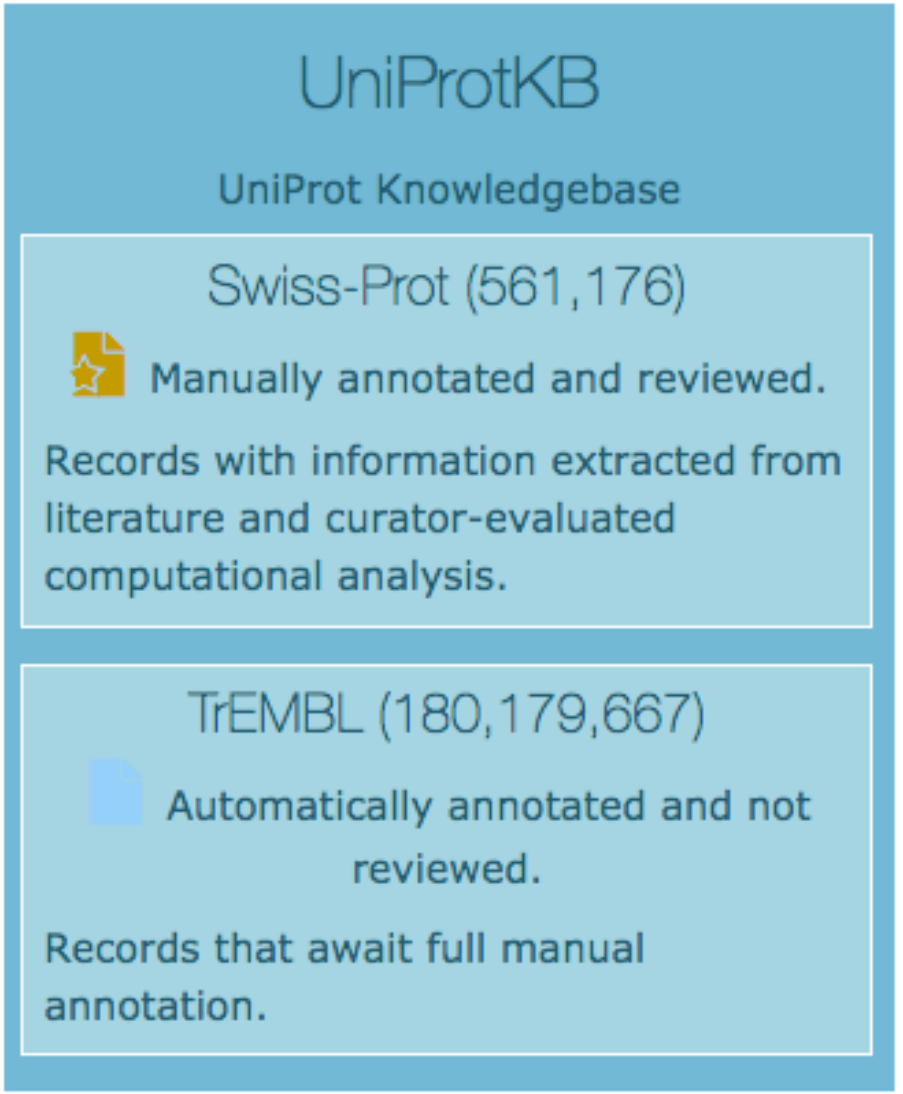
The main database of protein sequences is UniProt which is composed by SwissProt and TrEMBL

https://www.uniprot.org

UniProt Composition

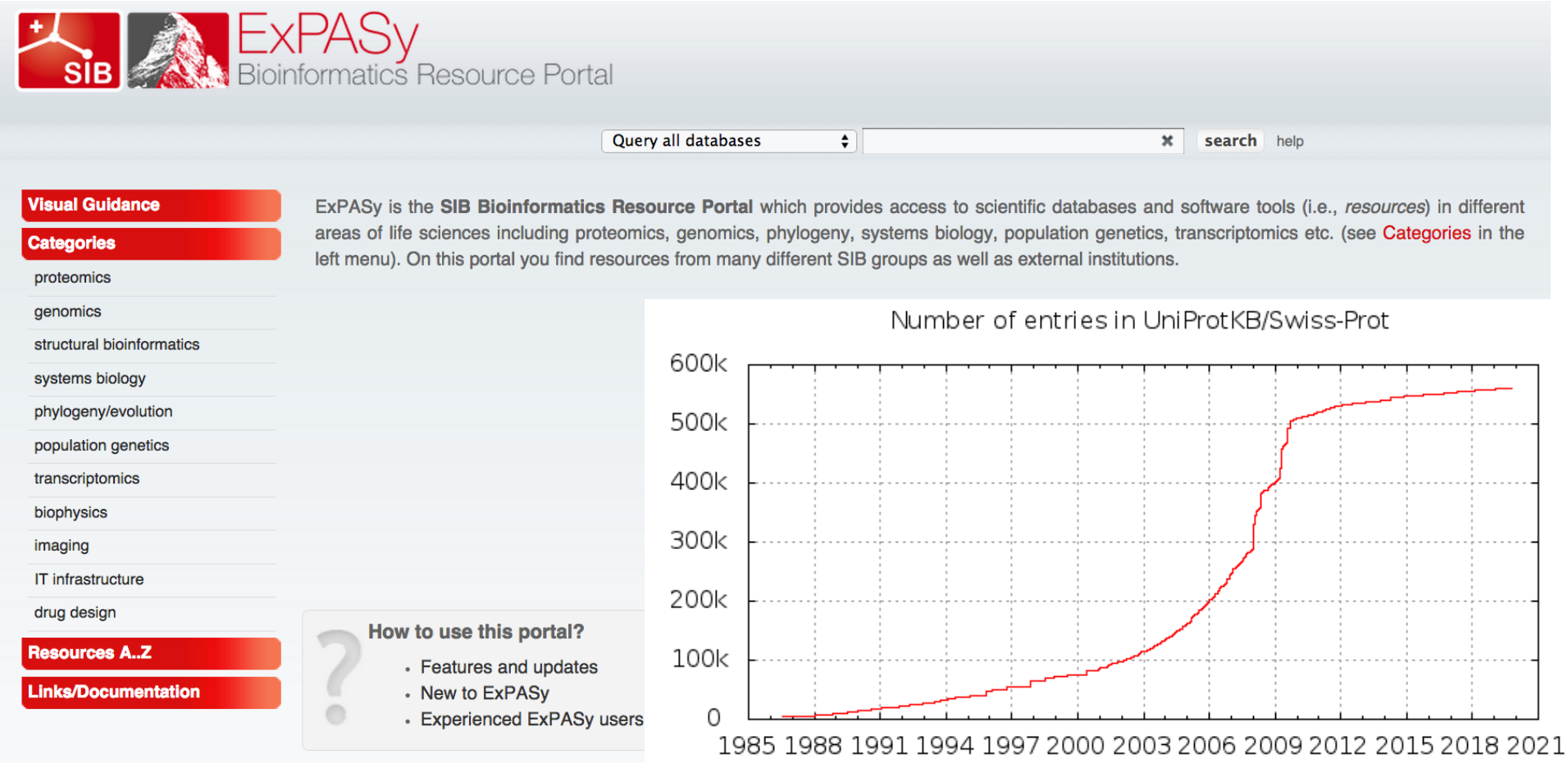
Database of annotated proteins

- Swiss-Prot: Manually annotated ~560K
- TrEMBL: Automatically annotated ~180M



The SwissProt

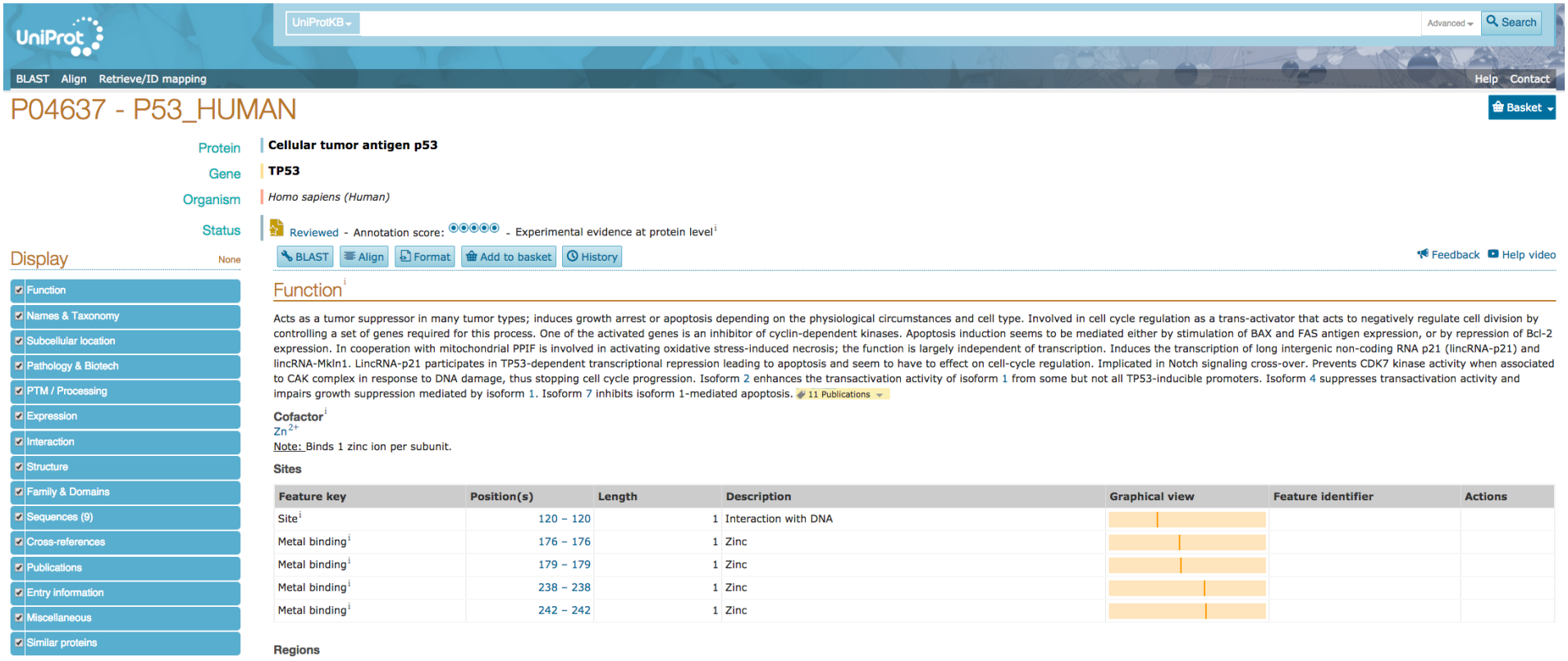
SwissProt contains all the proteins that have been manually annotated using information extracted from literature.



http://www.expasy.org/

The function

Acts as a tumor suppressor in many tumor types; induces growth arrest or apoptosis depending on the physiological circumstances and cell type.
11 publications



Getting the information

The SwissProt fasta file contains all the sequences in the database and the dat file contains all the information including annotation.

The fasta and dat files can be downloaded using the following links

http://www.uniprot.org/uniprot/P53_HUMAN.fasta
http://www.uniprot.org/uniprot/P53_HUMAN.txt

More complex queries:
http://www.uniprot.org/help/programmatic_access

```
ID   P53_HUMAN               Reviewed;         393 AA.
AC   P04637; Q15086; Q15087; Q15088; Q16535; Q16807; Q16808; Q16809;
AC   Q16810; Q16811; Q16848; Q2XN98; Q3LRW1; Q3LRW2; Q3LRW3; Q3LRW4;
AC   Q3LRW5; Q86UG1; Q8J016; Q99659; Q9BTM4; Q9HAQ8; Q9NP68; Q9NPJ2;
AC   Q9NZD0; Q9UBI2; Q9UQ61;
DT   13-AUG-1987, integrated into UniProtKB/Swiss-Prot.
DT   24-NOV-2009, sequence version 4.
DT   04-FEB-2015, entry version 228.
DE   RecName: Full=Cellular tumor antigen p53;
DE   AltName: Full=Antigen NY-CO-13;
DE   AltName: Full=Phosphoprotein p53;
DE   AltName: Full=Tumor suppressor p53;
GN   Name=TP53; Synonyms=P53;
OS   Homo sapiens (Human).
OC   Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC   Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
OC   Catarrhini; Hominidae; Homo.
OX   NCBI_TaxID=9606;
RN   [1]
RP   NUCLEOTIDE SEQUENCE [MRNA] (ISOFORM 1).
RX   PubMed=4006916;
RA   Zakut-Houri R., Bienz-Tadmor B., Givol D., Oren M.;
RT   "Human p53 cellular tumor antigen: cDNA sequence and expression in COS
RT   cells.";
RL   EMBO J. 4:1251-1255(1985).
```

Exercise

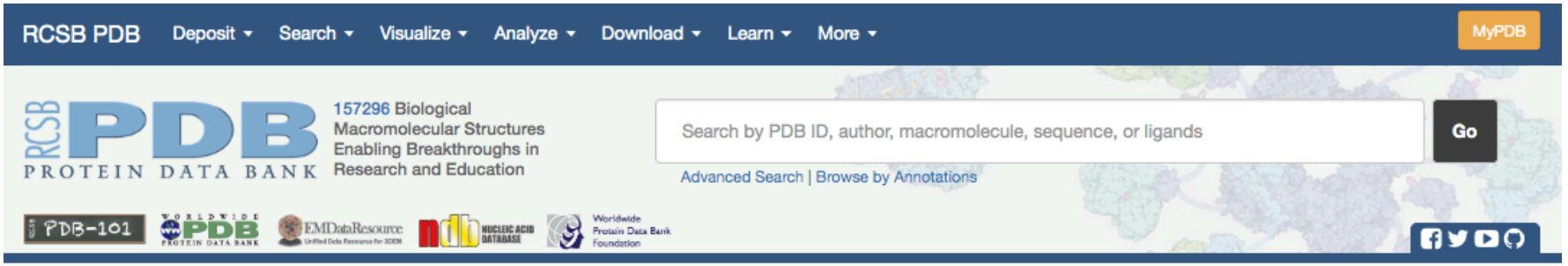
From the UniProt FTP web site (ftp://ftp.expasy.org/databases/uniprot/) download the Human protein UP000005640_9606 in fasta format.

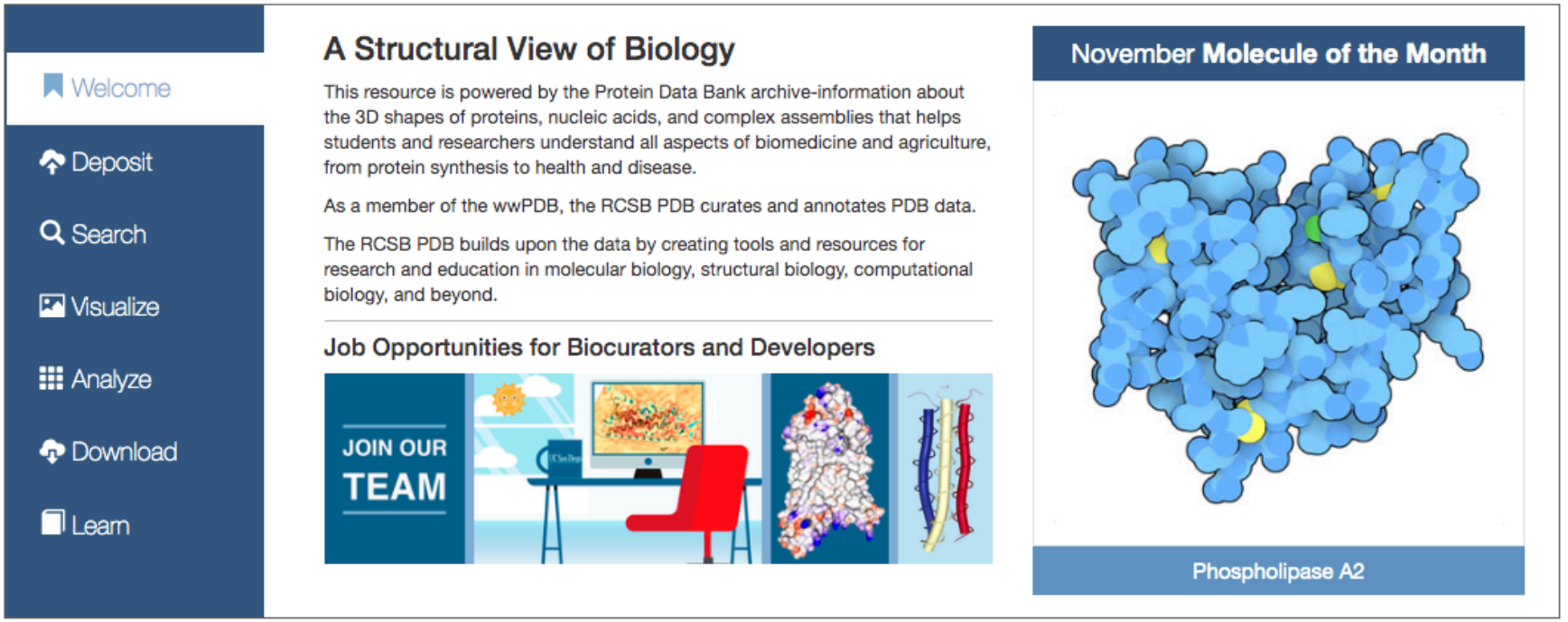
- What is the total number of human proteins in the SwissProt and TrEMBL dataset?

- Given the fasta file containing the protein sequence of P53 what is the total number of residues?

The Protein Data Bank

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



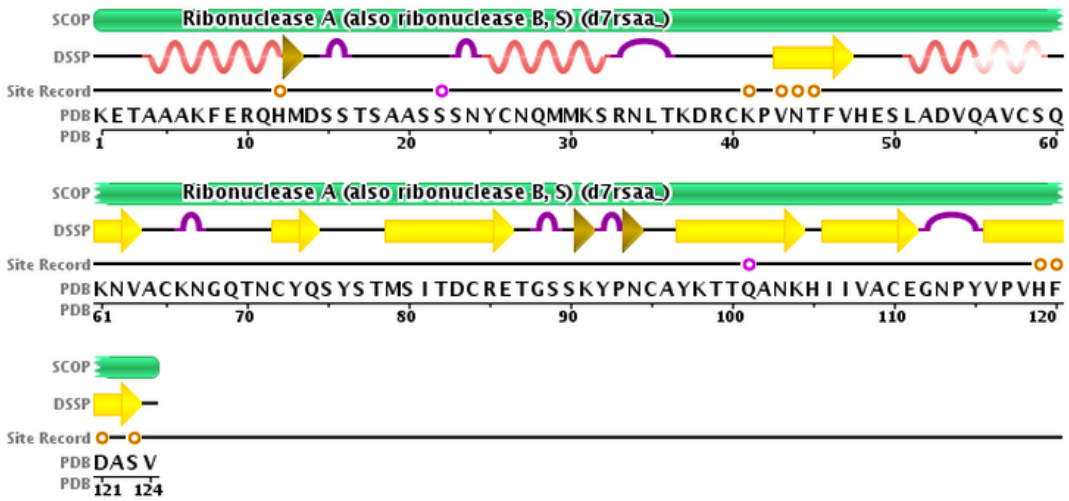


http://rcsb.org

http://ftp.rcsb.org/pub/pdb/

The Bovine Ribonuclease A

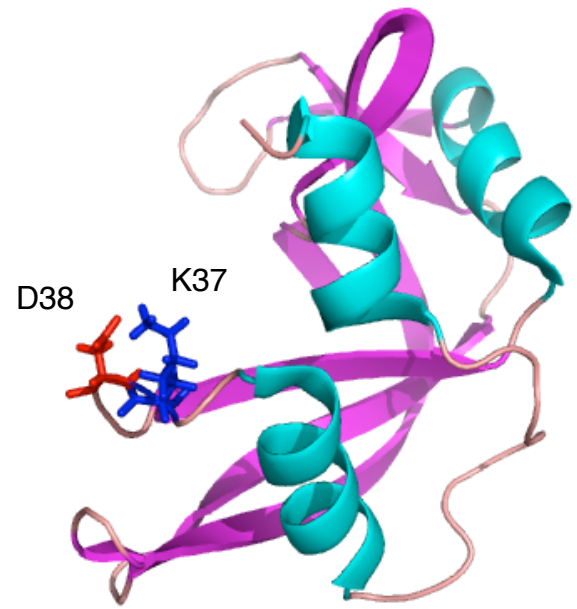
Ribonuclease A (RNase A) is a pancreatic ribonuclease that cleaves single-stranded RNA.

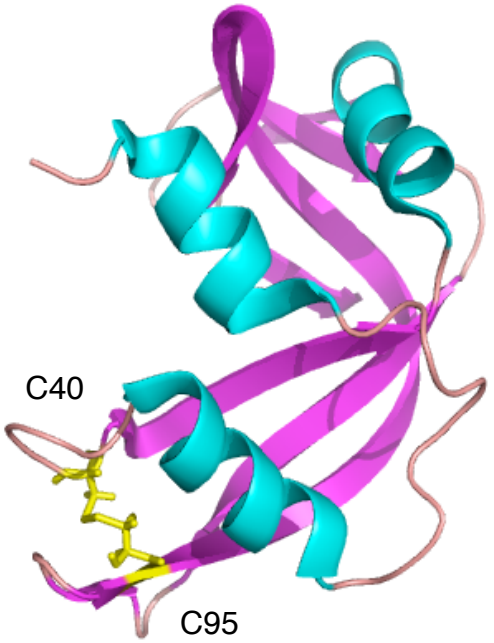


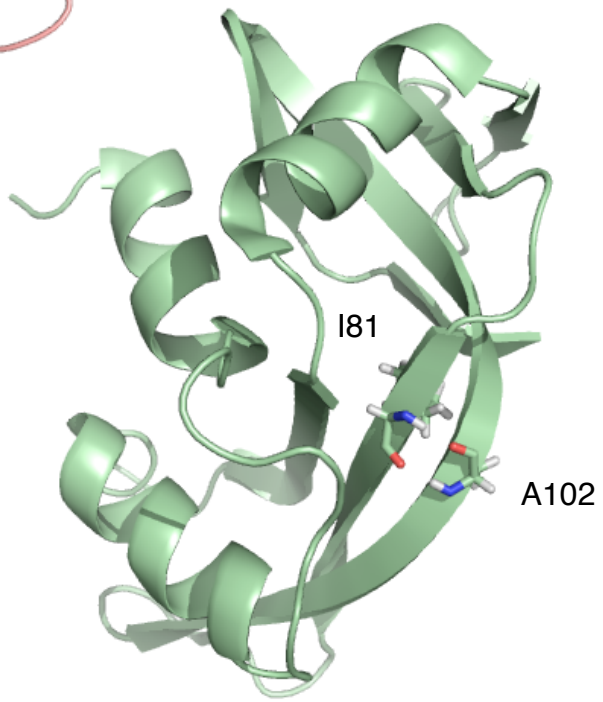
Wlodawer, A. et al. (1988) Biochemistry 27: 2705-2717

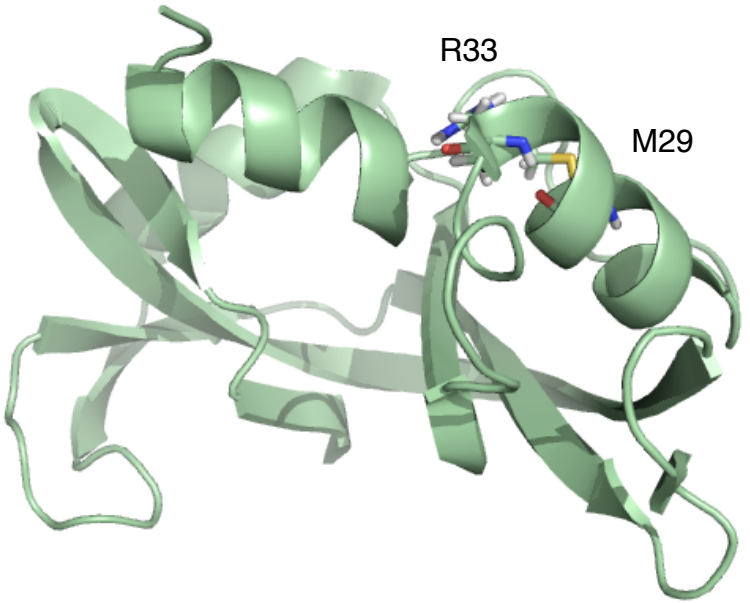
Bonds and interactions

Examples of salt bridge, disulfide bond and hydrogen bonds in ribonuclease A









Exercise

Download the PDB file of the Ribonuclease A (PDB: 7RSA) from the web (http://ftp.rcsb.org/pub/pdb/data/structures/all/pdb/pdb7rsa.ent.gz) and perform the following tasks

- Run a shell command to calculate the number of residues of the protein?
- Write a python script to parse the PDB file.
- Modify the program to calculate the distance between to atoms and residues.
- Calculate the average and standard deviation of the distance between two consecutive α carbons?

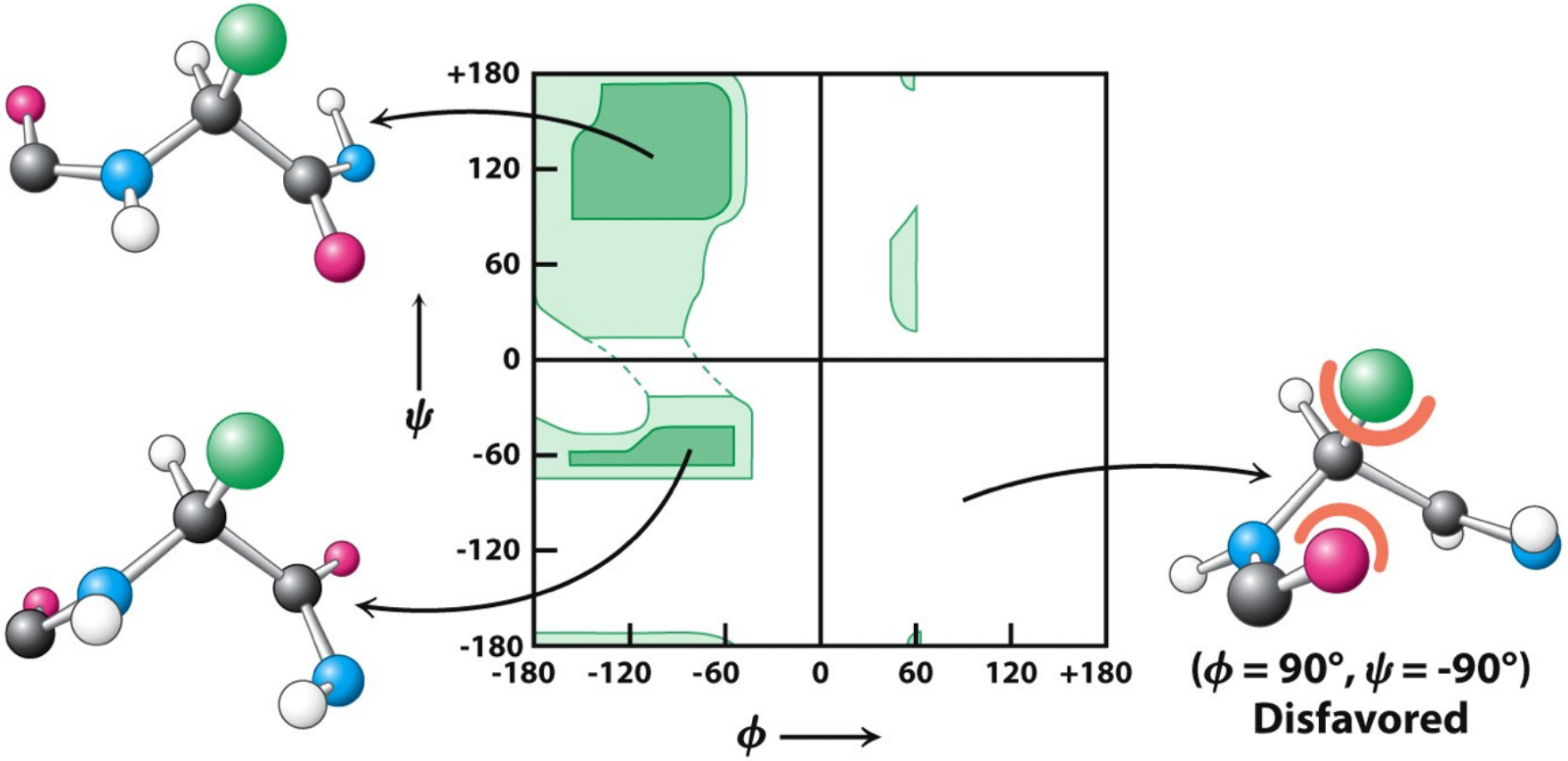
Defining protein structure

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.

Important columns in DSSP file of a protein are:

Secondary structure 17 (H=helix, E=Extended)

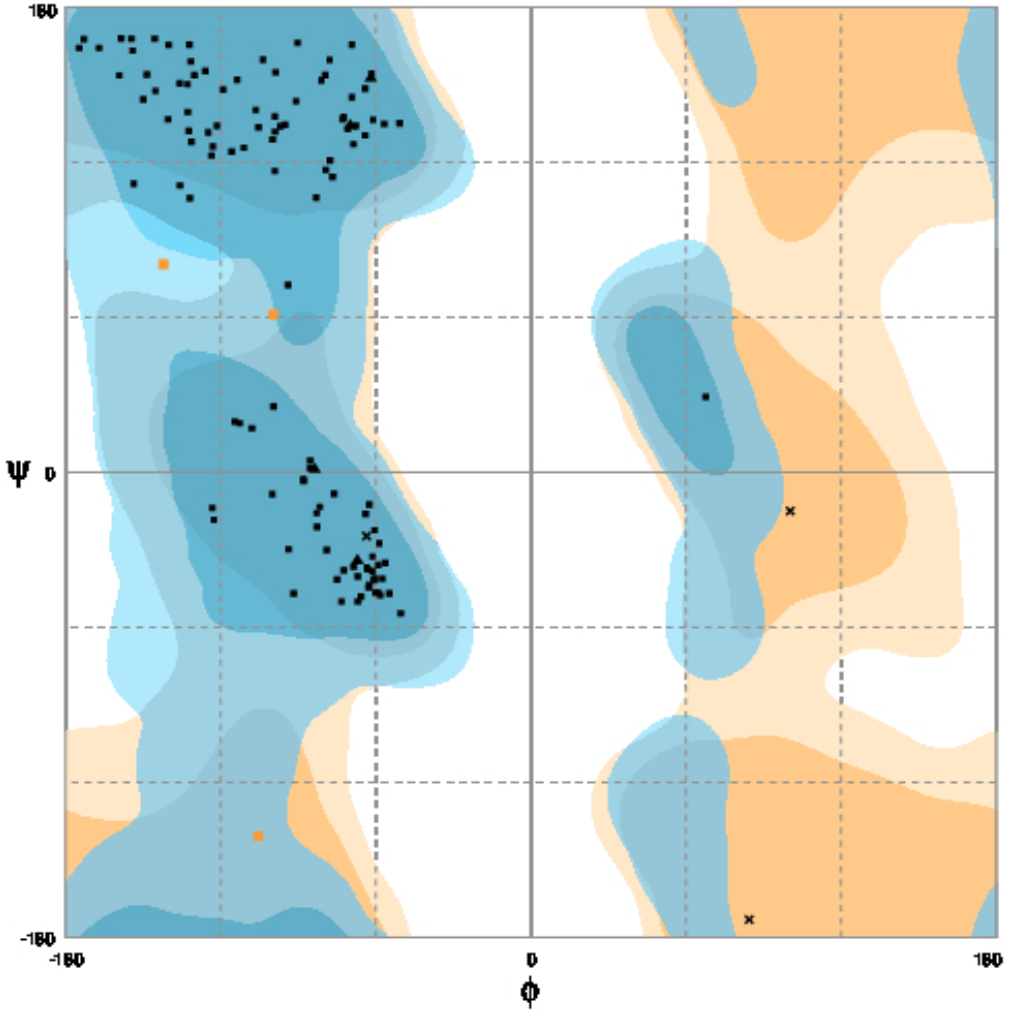
Accessibility 36-38

Phi angle 104-109 and Psi angle 110-115

more details at https://swift.cmbi.umcn.nl/gv/dssp/

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

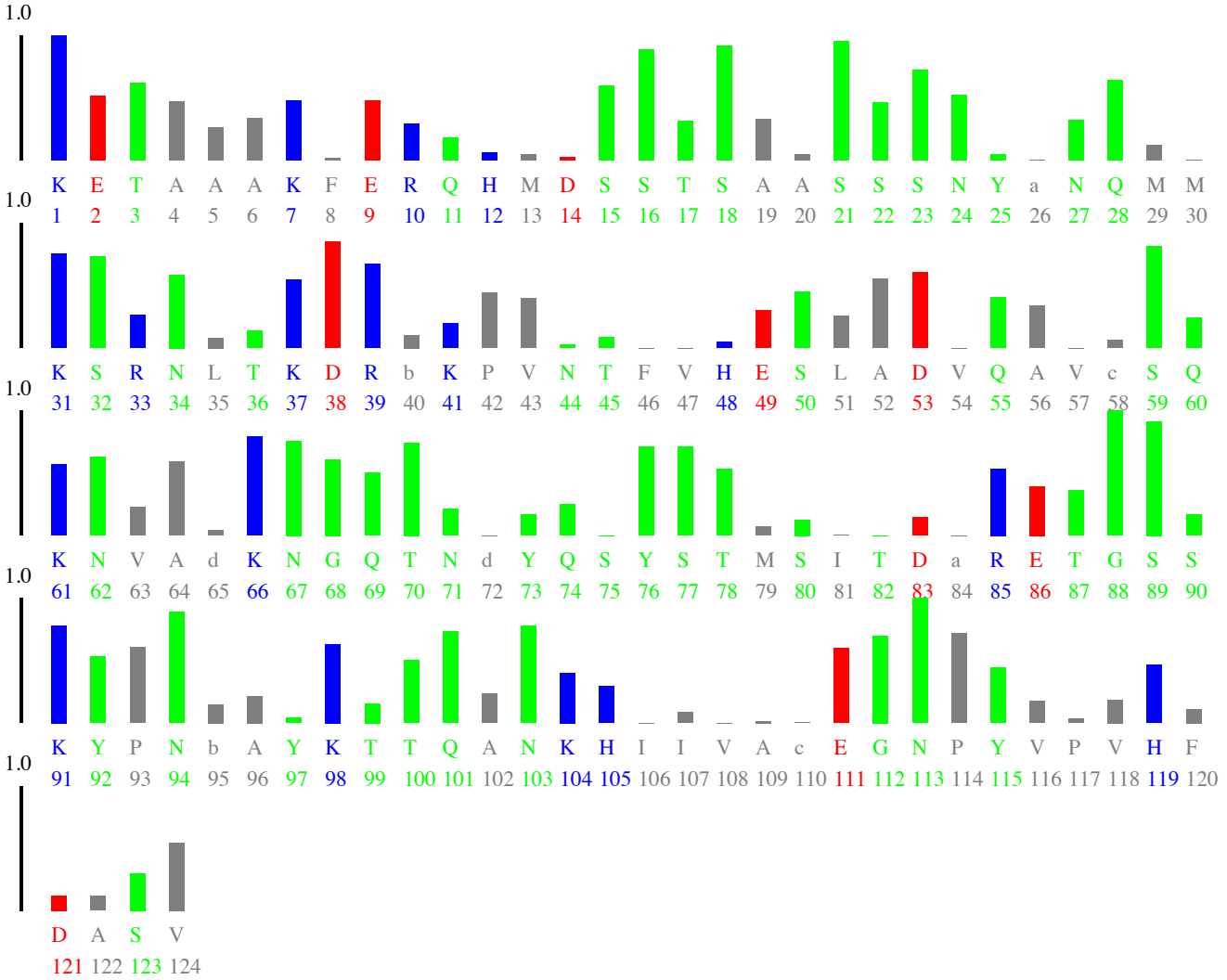
Ramachandran plot



http://mordred.bioc.cam.ac.uk/~rapper/rampage2.php

Relative solvent accessibility

The relative solvent accessible area is obtained dividing the accessible area of the residue by an estimation of the its maximum accessible surface.



http://gibk26.bio.kyutech.ac.jp/jouhou/shandar/netasa/asaview/

Exercise

Download the DSSP file of the Ribonuclease A (PDB: 7RSA) from the web (ftp://ftp.cmbi.umcn.nl//pub/molbio/data/dssp/7rsa.dssp) and answer the following questions

- What is the total number of residues in helical and extended conformations?
- What is the average value of the ϕ and ψ angles for the residues in helical and extended conformations?
- Are the average values falling the the correct region of the Ramachandran plot?
- Considering the solvent accessibility values reported in the DSSP file, calculate the relative solvent accessible area for Lysine (205), Valine (142) and Glutamine (198).
- Are this value compatible with the physico-chemical properties of the residues?