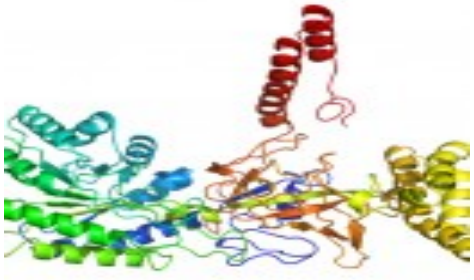
Mizzou
University of Missouri



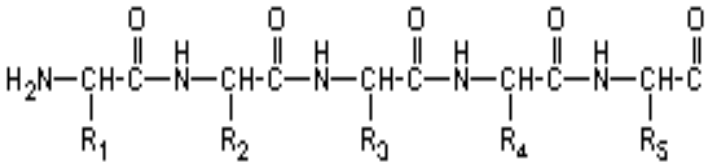
Template Based Protein Structure Modeling

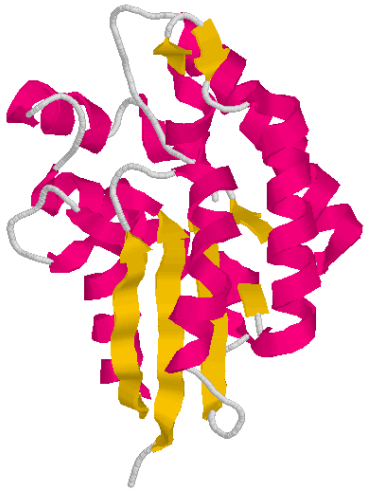
Jianlin Cheng, PhD

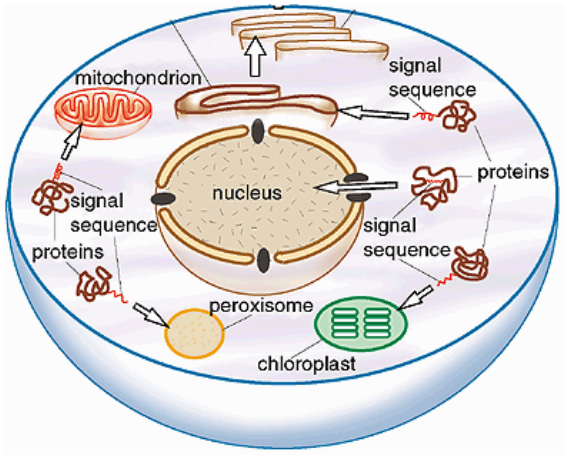
Associate Professor
Computer Science Department
Informatics Institute
University of Missouri, Columbia
2014

Sequence, Structure and Function

AGCWY……





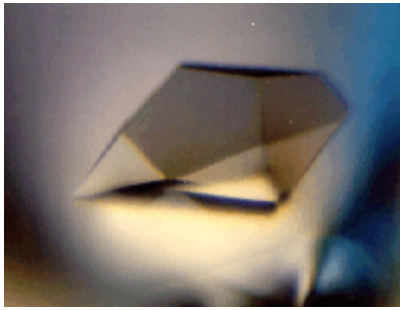


Cell

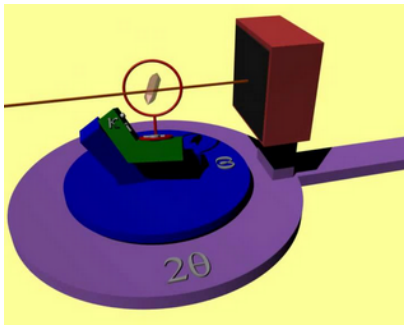
Protein Structure Determination

- X-ray crystallography
- Nuclear Magnetic Resonance (NMR) Spectroscopy
- X-ray: any size, accurate (1-3 Angstrom (10^{-10} m)), sometime hard to grow crystal
- NMR: small to medium size, moderate accuracy, structure in solution

X-Ray Crystallography

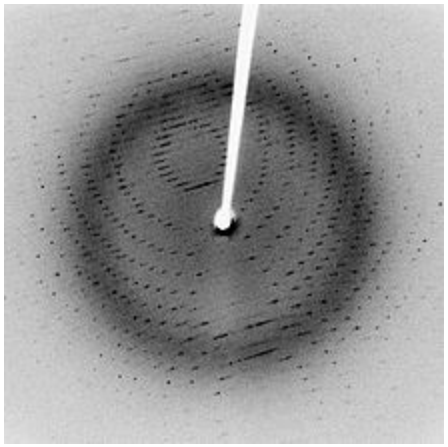


A protein crystal

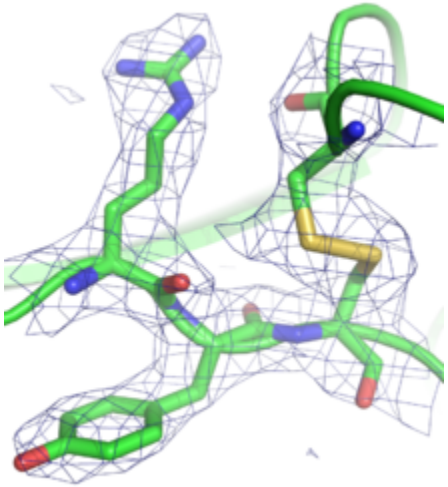


Mount a crystal

Diffractometer



Diffraction



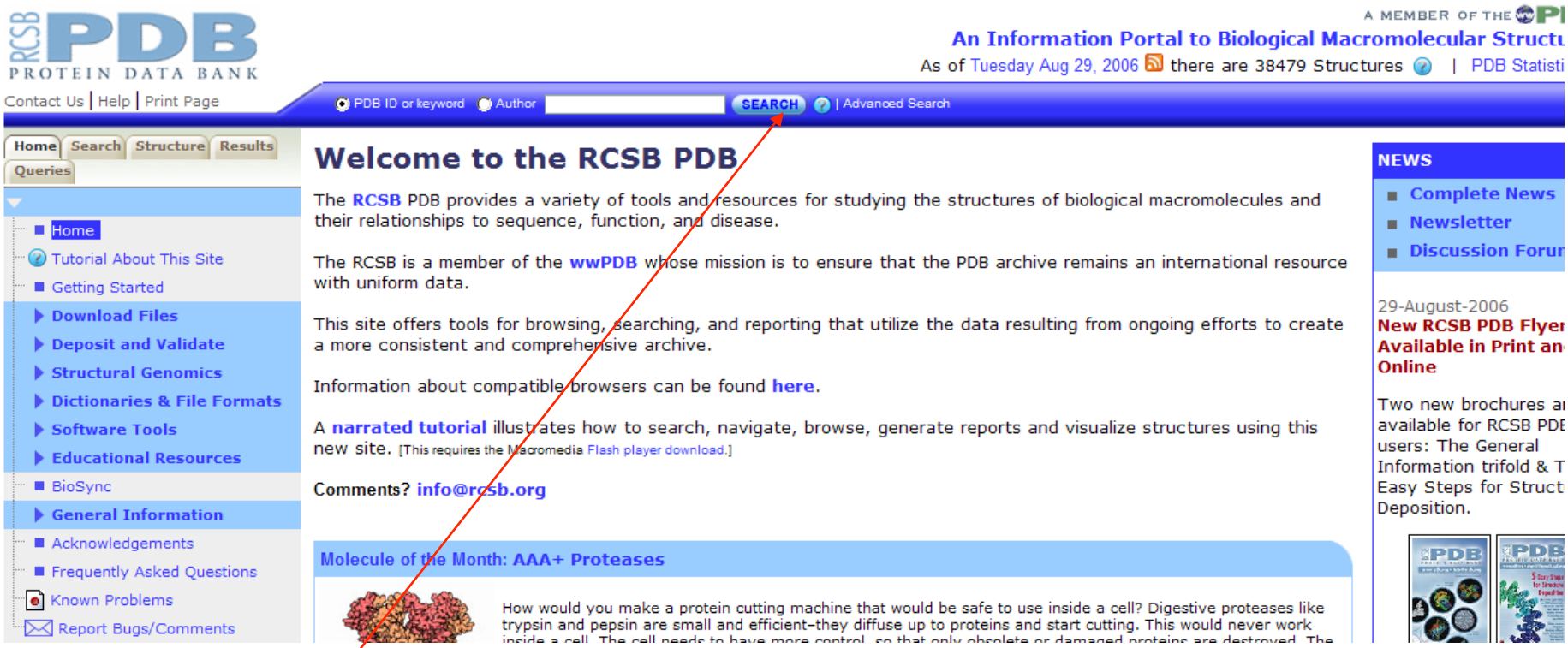
Protein structure

Wikipedia

Pacific Northwest National Laboratory's high magnetic field (800 MHz, 18.8 T) NMR spectrometer being loaded with a sample.

Wikipedia, the free encyclopedia

Storage in Protein Data Bank



Search database

Search protein 1VJG

PDB Format (2C8Q, insulin)

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TITLE     INSULINE(1SEC) AND UV LASER EXCITED FLUORESCENCE
COMPND    MOL_ID: 1;
COMPND   2 MOLECULE: INSULIN A CHAIN;
COMPND   3 CHAIN: A;
COMPND   4 MOL_ID: 2;
COMPND   5 MOLECULE: INSULIN B CHAIN;
COMPND   6 CHAIN: B
SOURCE    MOL_ID: 1;
SOURCE   2 ORGANISM_SCIENTIFIC: HOMO SAPIENS;
SOURCE   3 ORGANISM_COMMON: HUMAN;
SOURCE   4 ORGAN: PANCREAS;
SOURCE   5 MOL_ID: 2;
SOURCE   6 ORGANISM_SCIENTIFIC: HOMO SAPIENS;
SOURCE   7 ORGANISM_COMMON: HUMAN;
SOURCE   8 ORGAN: PANCREAS
KEYWDS    LASER, UV, CARBOHYDRATE METABOLISM, HORMONE, DIABETES
KEYWDS   2 MELLITUS, GLUCOSE METABOLISM
EXPDTA    X-RAY DIFFRACTION
AUTHOR    X.VERNEDE,B.LAVAULT,J.OHANA,D.NURIZZO,J.JOLY,L.JACQUAMET,
AUTHOR   2 F.FELISAZ,F.CIPRIANI,D.BOURGEOIS
REVDAT   1   08-MAR-06 2C8Q    0
JRNL        AUTH   X.VERNEDE,B.LAVAULT,J.OHANA,D.NURIZZO,J.JOLY,
JRNL        AUTH 2 L.JACQUAMET,F.FELISAZ,F.CIPRIANI,D.BOURGEOIS
JRNL        TITL   UV LASER-EXCITED FLUORESCENCE AS A TOOL FOR THE
JRNL        TITL 2 VISUALIZATION OF PROTEIN CRYSTALS MOUNTED IN
JRNL        TITL 3 LOOPS.
JRNL        REF    ACTA CRYSTALLOGR.,SECT.D      V.  62   253 2006
JRNL        REFN   ASTM ABCRE6  DK ISSN 0907-4449
REMARK   2
REMARK   2 RESOLUTION. 1.95 ANGSTROMS.
REMARK   3
REMARK   3 REFINEMENT.
REMARK   3   PROGRAM     : REFMAC 5.2.0005
REMARK   3   AUTHORS     : MURSHUDOV,VAGIN,DODSON
REMARK   3
REMARK   3   REFINEMENT TARGET : MAXIMUM LIKELIHOOD
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SEQRES   1 A   21  GLY ILE VAL GLU GLN CYS CYS THR SER ILE CYS SER LEU
SEQRES   2 A   21  TYR GLN LEU GLU ASN TYR CYS ASN
SEQRES   1 B   29  PHE VAL ASN GLN HIS LEU CYS GLY SER HIS LEU VAL GLU
SEQRES   2 B   29  ALA LEU TYR LEU VAL CYS GLY GLU ARG GLY PHE PHE TYR
SEQRES   3 B   29  THR PRO LYS
FORMUL   3  HOH   *31(H2 O1)
HELIX    1   1 GLY A    1  CYS A    7  1                                   7
HELIX    2   2 SER A   12  ASN A   18  1                                   7
HELIX    3   3 GLY B    8  GLY B   20  1                                  13
HELIX    4   4 GLU B   21  GLY B   23  5                                   3
SSBOND   1 CYS A    6    CYS A   11                          1555   1555
SSBOND   2 CYS A    7    CYS B    7                          1555   1555
SSBOND   3 CYS A   20    CYS B   19                          1555   1555
CRYST1   78.608   78.608   78.608  90.00  90.00  90.00 I 21 3        24
ORIGX1      1.000000  0.000000  0.000000        0.00000
ORIGX2      0.000000  1.000000  0.000000        0.00000
ORIGX3      0.000000  0.000000  1.000000        0.00000
SCALE1      0.012721  0.000000  0.000000        0.00000
SCALE2      0.000000  0.012721  0.000000        0.00000
SCALE3      0.000000  0.000000  0.012721        0.00000
ATOM      1  N   GLY A   1      45.324  26.807  11.863  1.00 24.82           N
ATOM      2  CA  GLY A   1      45.123  27.787  12.967  1.00 24.93           C
ATOM      3  C   GLY A   1      43.756  27.627  13.605  1.00 25.16           C
ATOM      4  O   GLY A   1      43.107  26.591  13.438  1.00 25.00           O
ATOM      5  N   ILE A   2      43.313  28.661  14.323  1.00 25.21           N
ATOM      6  CA  ILE A   2      42.050  28.622  15.065  1.00 25.39           C
ATOM      7  C   ILE A   2      40.818  28.303  14.200  1.00 25.69           C
ATOM      8  O   ILE A   2      39.935  27.565  14.635  1.00 25.56           O
ATOM      9  CB  ILE A   2      41.816  29.917  15.917  1.00 25.39           C
```

Structure Visualization

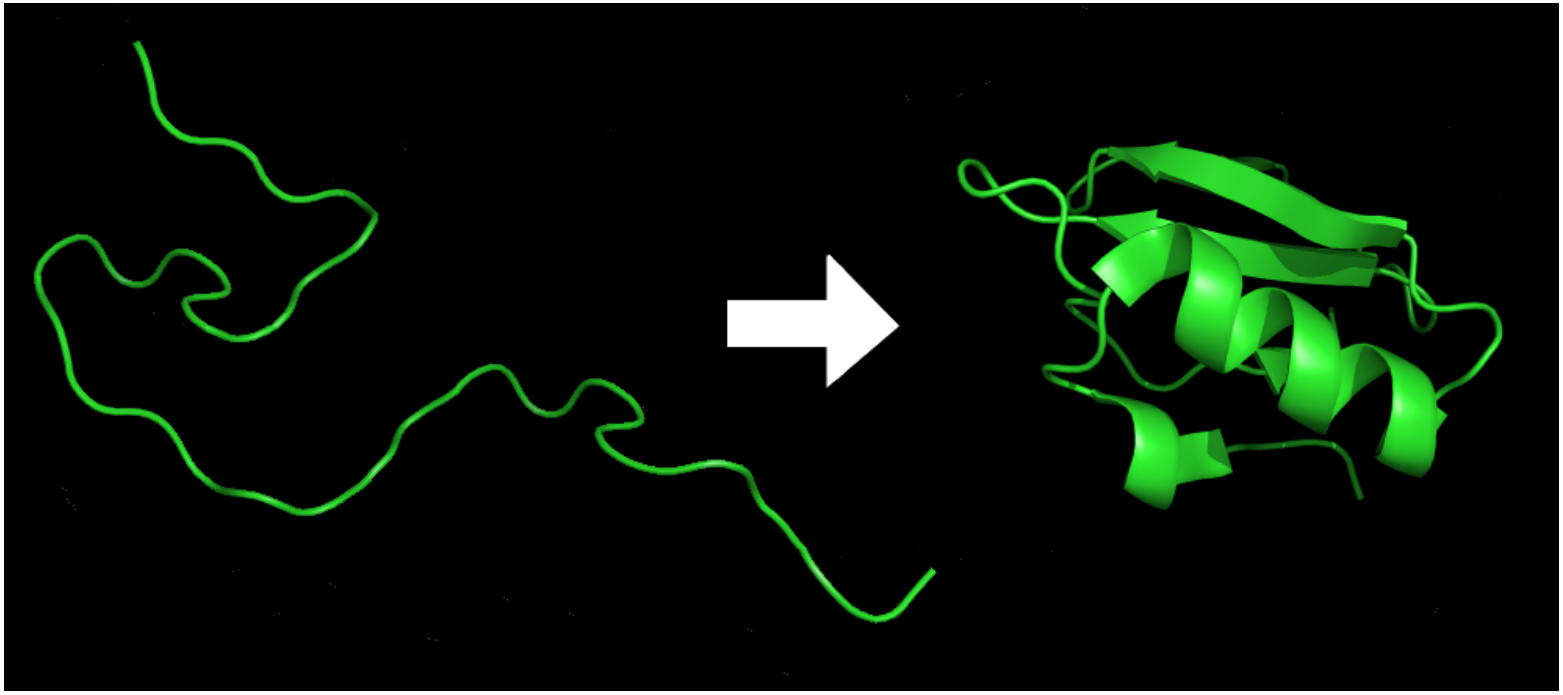
- Rasmol (http://www.umass.edu/microbio/rasmol/getras.htm)
- MDL Chime (plug-in) (http://www.mdl.com/products/framework/chime/)
- Protein Explorer (http://molvis.sdsc.edu/protexpl/frntdoor.htm)
- **Jmol: http://jmol.sourceforge.net/**
- **Pymol: http://pymol.sourceforge.net/**

Jmol Demo (1CRN)

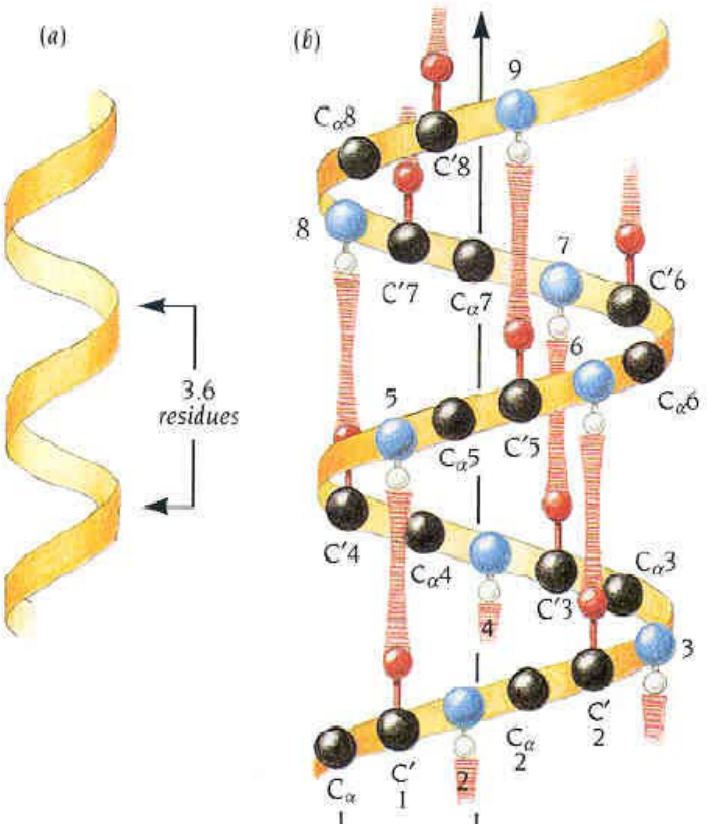
- Identify residues
- Recognize atoms
- Recognize peptide bonds
- Identify backbone
- Identify side chain
- Analyze different visualization style

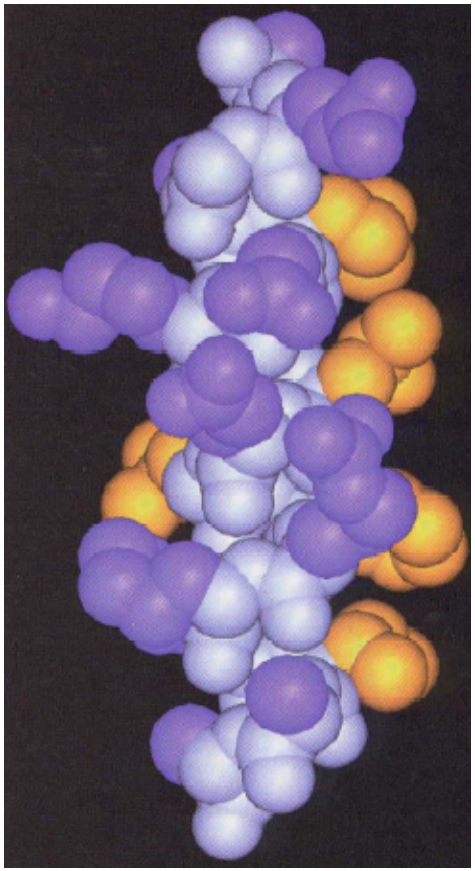
Protein Folding

http://www.youtube.com/watch?v=fvBO3TqJ6FE&feature=fvw



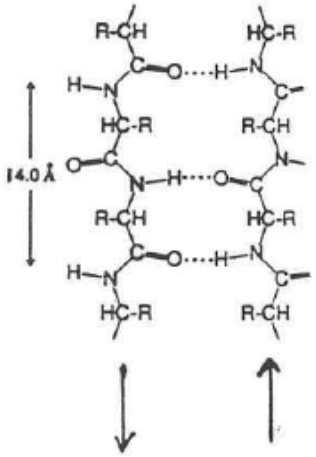
Alpha-Helix

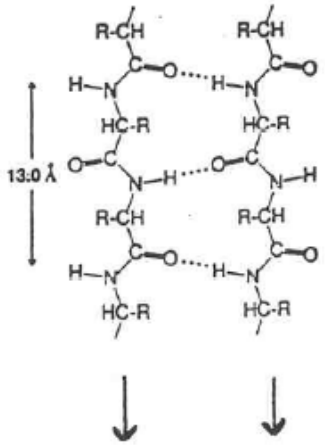


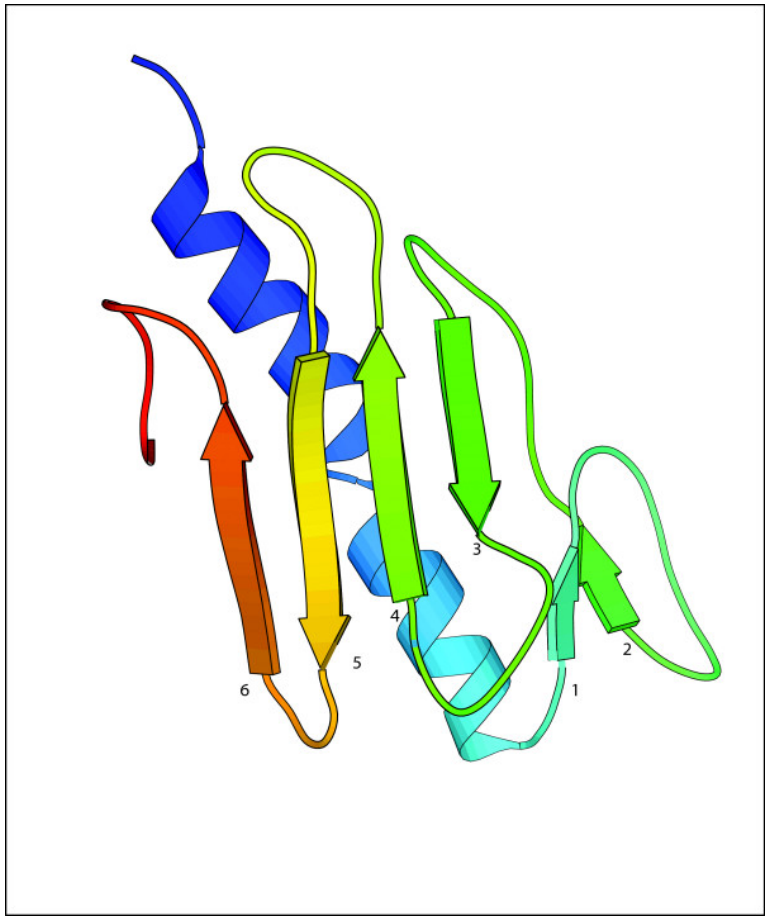


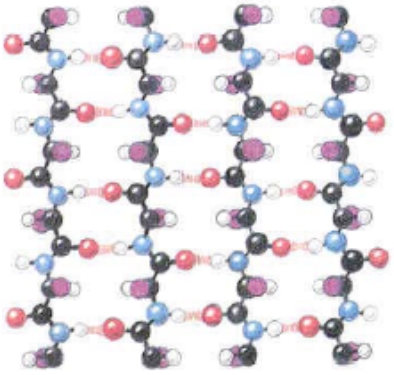
Jurnak, 2003

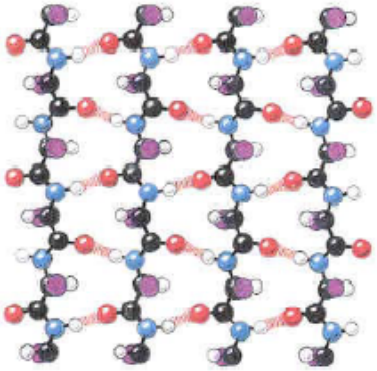
Beta-Sheet







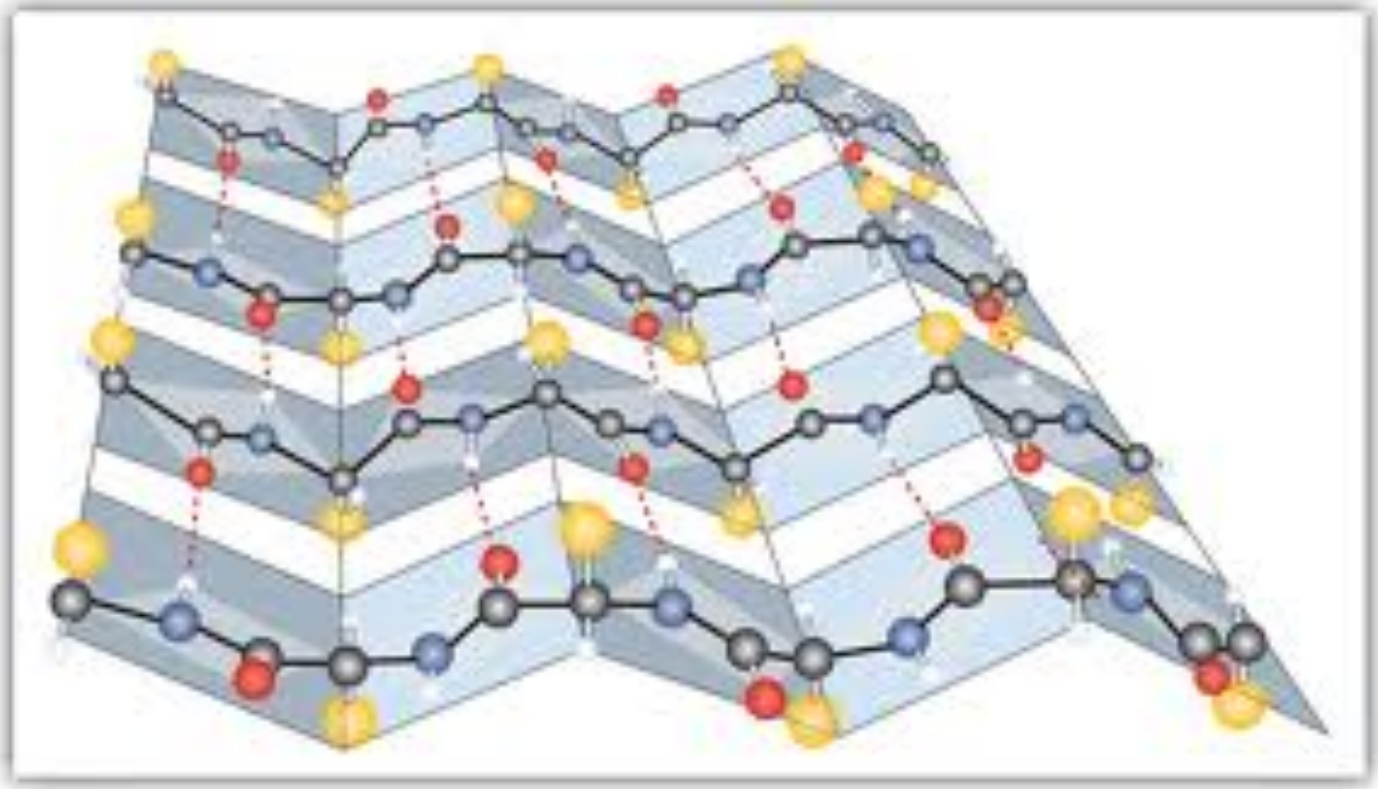




Anti-Parallel

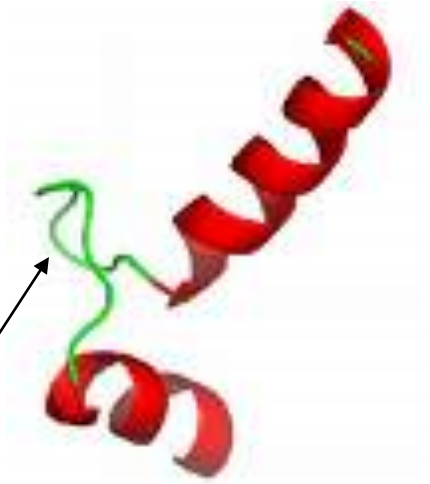
Parallel

Beta-Sheet



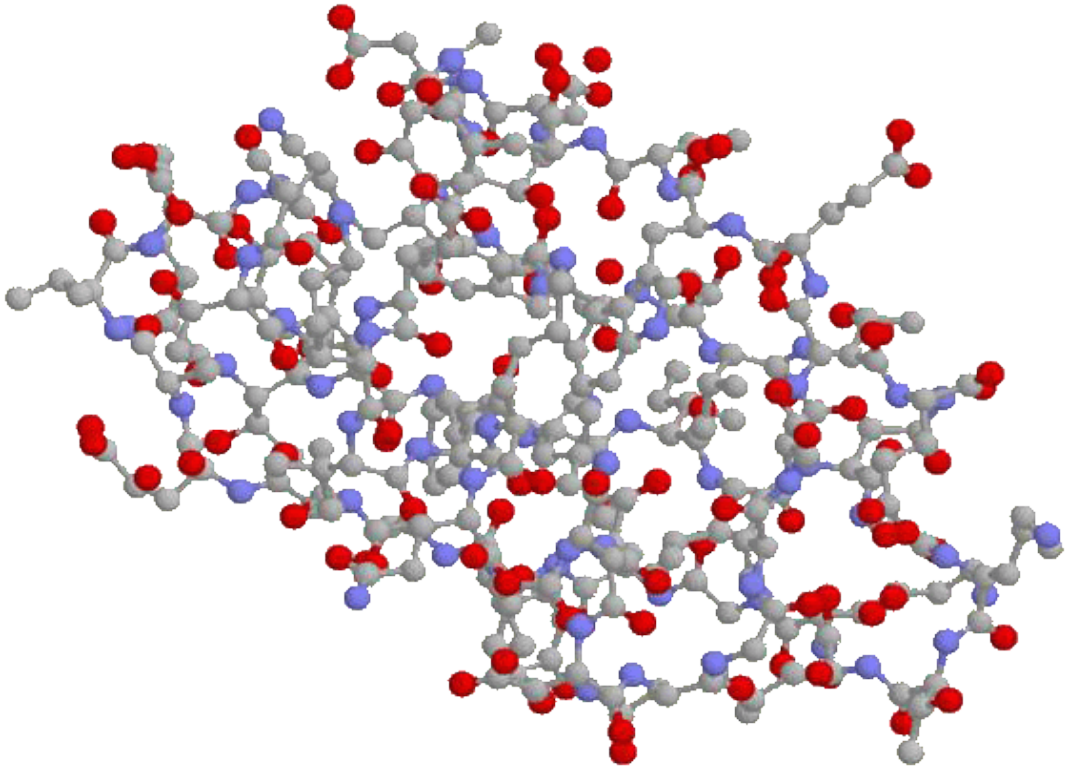
Non-Repetitive Secondary Structure

Beta-Turn



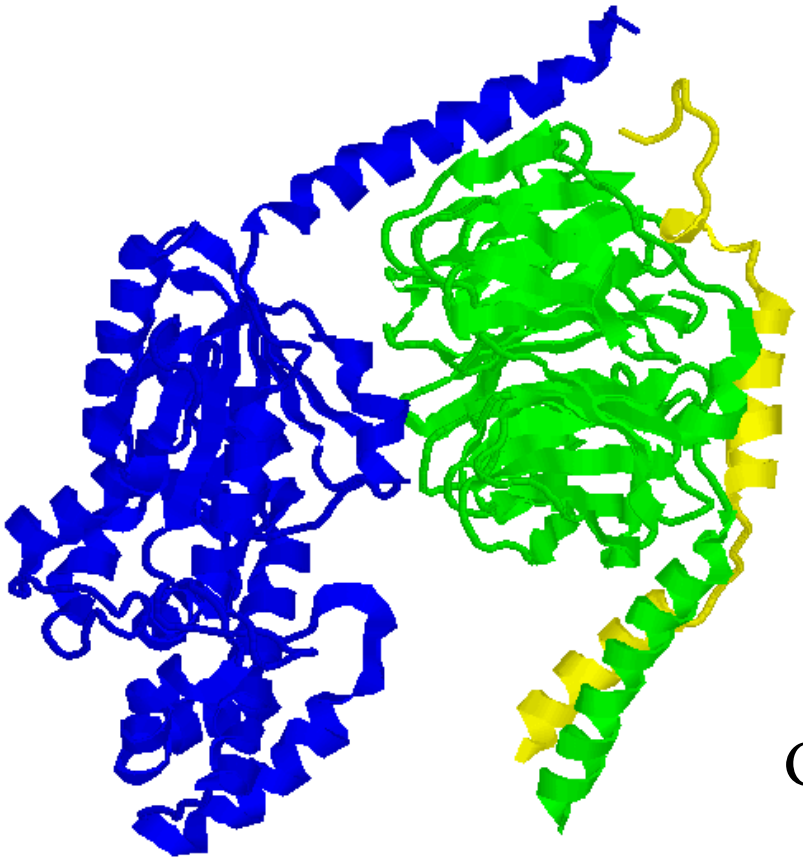
Loop

myoglobin



tertiary structure
(all atom)

Quaternary Structure: Complex



G-Protein Complex

Structure Analysis

- Assign secondary structure for amino acids from 3D structure
- Generate solvent accessible area for amino acids from 3D structure
- Most widely used tool: DSSP (Dictionary of Protein Secondary Structure: Pattern Recognition of Hydrogen-Bonded and Geometrical Features. **Kabsch and Sander, 1983**)

DSSP server: http://bioweb.pasteur.fr/seqanal/interfaces/dssp-simple.html
DSSP download: http://swift.cmbi.ru.nl/gv/dssp/

DSSP Code:
H = alpha helix
G = 3-helix (3/10 helix)
I = 5 helix (pi helix)
B = residue in isolated beta-bridge
E = extended strand, participates in beta ladder
T = hydrogen bonded turn
S = bend
Blank = loop

DSSP Web Service

DSSP : Definition of secondary structure of proteins given a set of 3D coordinates (W.Kabsch, C. Sander)

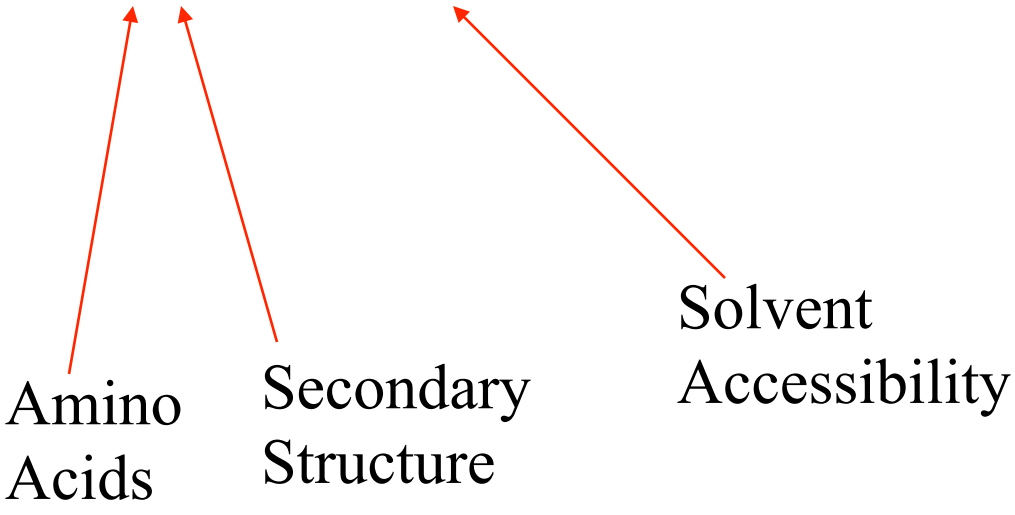
Reset | Run dssp | jianlin.cheng@gmail.com your e-mail

PDB File

1vjg or you can instead enter a PDB id.

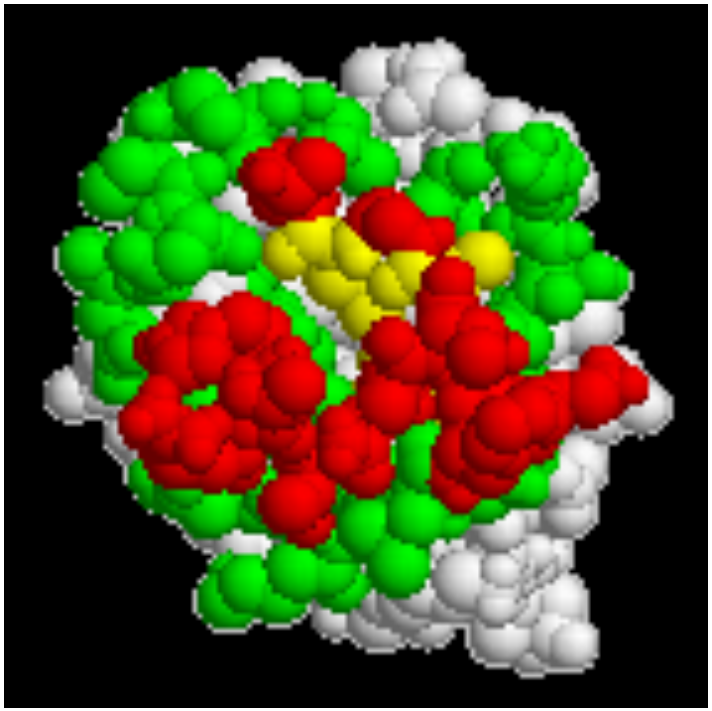
http://bioweb.pasteur.fr/seqanal/interfaces/dssp-simple.html

#	RESIDUE	AA	STRUCTURE	BP1	BP2	ACC	N-H-->O	O-->H-N	N-H-->O	O-->H-N	TCO	KAPPA	ALPHA	PHI	PSI	X-CA	Y-CA	Z-CA
1	5	A	S		0	0	179	0, 0.0	2,-0.0	0, 0.0	0, 0.0	0.000	360.0	360.0	360.0	125.7	-8.6	43.0	43.9
2	6	A	K	-	0	0	123	1,-0.1	2,-0.4	37,-0.1	37,-0.2	-0.235	360.0	-108.7	-87.0	151.4	-7.5	41.4	40.6
3	7	A	T	E -a	39	0A	75	35,-0.6	37,-2.5	1,-0.0	2,-0.3	-0.593	34.7	-132.0	-72.2	128.3	-4.3	39.5	39.6
4	8	A	Q	E +a	40	0A	91	-2,-0.4	69,-0.6	35,-0.2	2,-0.4	-0.639	26.0	179.8	-86.4	132.7	-2.0	41.5	37.4
5	9	A	I	E -ab	41	73A	3	35,-1.9	37,-2.9	-2,-0.3	2,-0.5	-0.991	13.3	-156.5	-129.4	131.5	-0.7	39.9	34.2
6	10	A	R	E -ab	42	74A	48	67,-2.8	69,-1.7	-2,-0.4	2,-0.4	-0.910	14.8	-173.2	-105.2	126.8	1.6	41.6	31.8
7	11	A	I	E -ab	43	75A	0	35,-2.5	37,-2.6	-2,-0.5	2,-0.5	-0.983	11.9	-162.4	-124.9	124.4	1.7	40.3	28.2
8	12	A	C	E -ab	44	76A	0	67,-2.3	69,-2.6	-2,-0.4	2,-0.6	-0.931	6.5	-159.9	-100.8	130.8	3.9	41.2	25.3
9	13	A	F	E -ab	45	77A	0	35,-2.2	37,-3.0	-2,-0.5	2,-0.5	-0.955	13.2	-169.0	-109.5	117.1	2.7	40.2	21.8
10	14	A	V	E +ab	46	78A	0	67,-3.1	69,-2.2	-2,-0.6	2,-0.3	-0.926	34.8	71.1	-116.5	129.9	5.6	40.1	19.4
11	15	A	G	E S-ab	47	79A	0	35,-0.9	37,-1.9	-2,-0.5	69,-0.2	-0.921	70.2	-50.2	169.0	-146.4	5.3	39.9	15.6
12	16	A	D	S >> S-	0	0	4	67,-0.8	4,-2.2	-2,-0.3	3,-0.6	-0.023	78.2	-51.3	-111.5	-151.8	4.2	41.6	12.4
13	17	A	S	H 3>>S+	0	0	7	35,-0.3	5,-1.7	1,-0.2	4,-1.5	0.803	130.2	57.8	-67.3	-28.8	1.2	43.5	11.1
14	18	A	F	H 345S+	0	0	5	2,-0.2	12,-0.5	1,-0.2	-1,-0.2	0.884	108.5	46.5	-68.2	-33.2	-1.2	40.8	12.2
15	19	A	V	H <45S+	0	0	1	-3,-0.6	12,-0.3	64,-0.2	-2,-0.2	0.900	111.1	52.2	-68.9	-41.4	-0.0	41.1	15.7
16	20	A	N	H <5S-	0	0	71	-4,-2.2	-2,-0.2	30,-0.1	-1,-0.2	0.774	110.8	-127.0	-62.6	-26.6	-0.3	45.0	15.4
17	21	A	G	T ><5 -	0	0	5	-4,-1.5	3,-2.2	-5,-0.2	8,-0.4	0.741	36.4	-174.6	83.1	25.3	-3.9	44.5	14.2
18	22	A	T	T 3 < +	0	0	14	-5,-1.7	-1,-0.2	1,-0.3	-2,-0.0	-0.199	68.4	29.2	-54.0	135.4	-3.4	46.6	11.0
19	23	A	G	T 3 S+	0	0	28	1,-0.3	-1,-0.3	159,-0.1	162,-0.2	0.121	86.2	120.8	94.7	-21.4	-6.7	47.0	9.2
20	24	A	D	X -	0	0	9	-3,-2.2	3,-1.2	160,-0.2	-1,-0.3	-0.706	48.9	-160.5	-79.7	117.6	-8.9	46.8	12.4
21	25	A	P	T 3 S+	0	0	91	0, 0.0	-1,-0.2	0, 0.0	159,-0.0	0.677	91.8	60.1	-70.9	-17.3	-10.9	50.1	12.6
22	26	A	E	T 3 S-	0	0	119	-3,-0.0	-2,-0.1	3,-0.0	158,-0.0	0.426	105.0	-132.3	-87.9	-3.3	-11.4	49.4	16.3
23	27	A	C	S < S+	0	0	112	-3,-1.2	-5,-0.1	-6,-0.2	-6,-0.0	0.730	80.2	98.1	62.8	28.1	-7.6	49.4	16.9



Solvent Accessibility

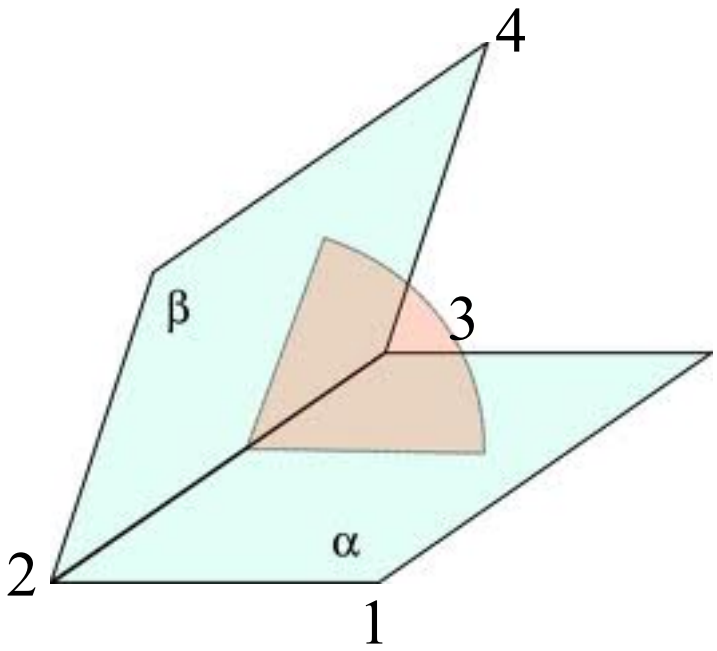
Size of the area of an amino acid that is exposed to solvent (water).

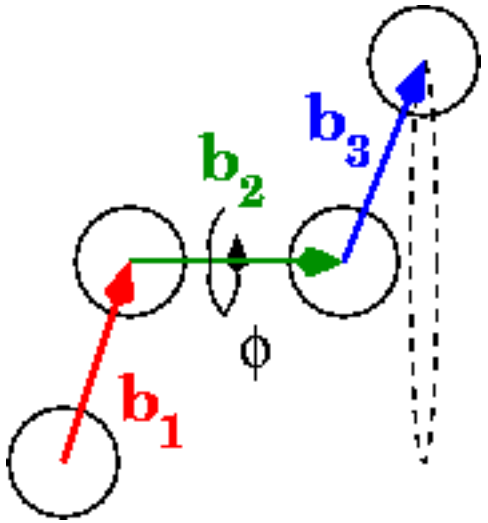


Maximum solvent accessible area for each amino acid is its whole surface area.

Hydrophobic residues like to be Buried inside (interior).
Hydrophilic residues like to be exposed on the surface.

Dihedral Angle

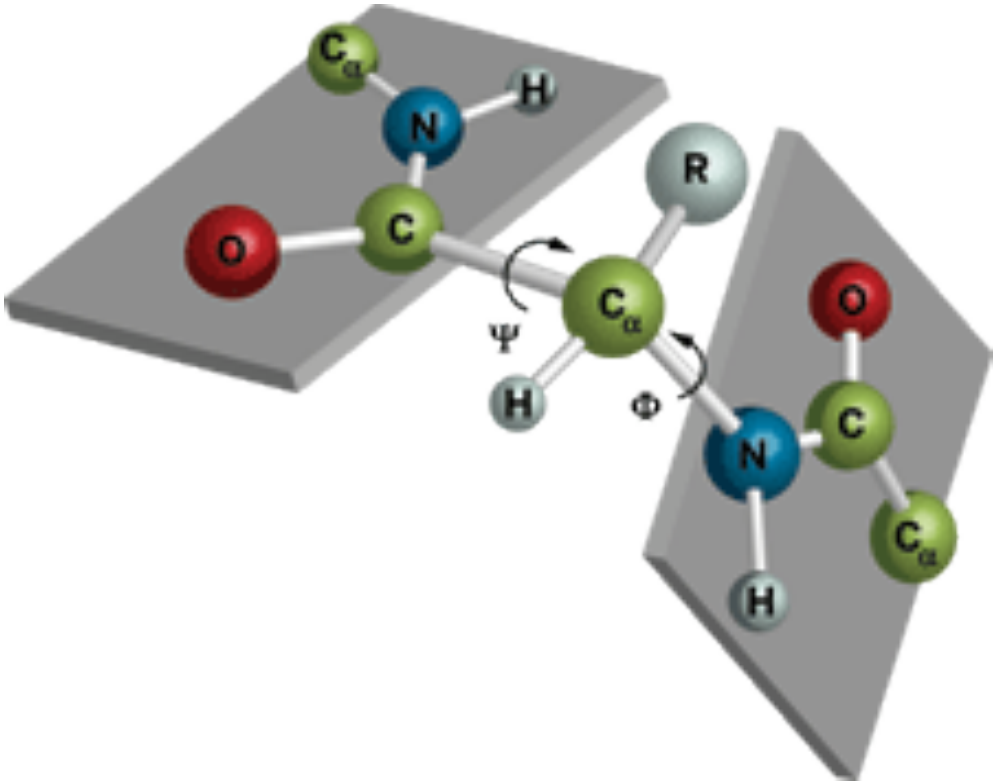




Project Groups

- 15 students
- Form 3 groups

Dihedral / Torsion Angle



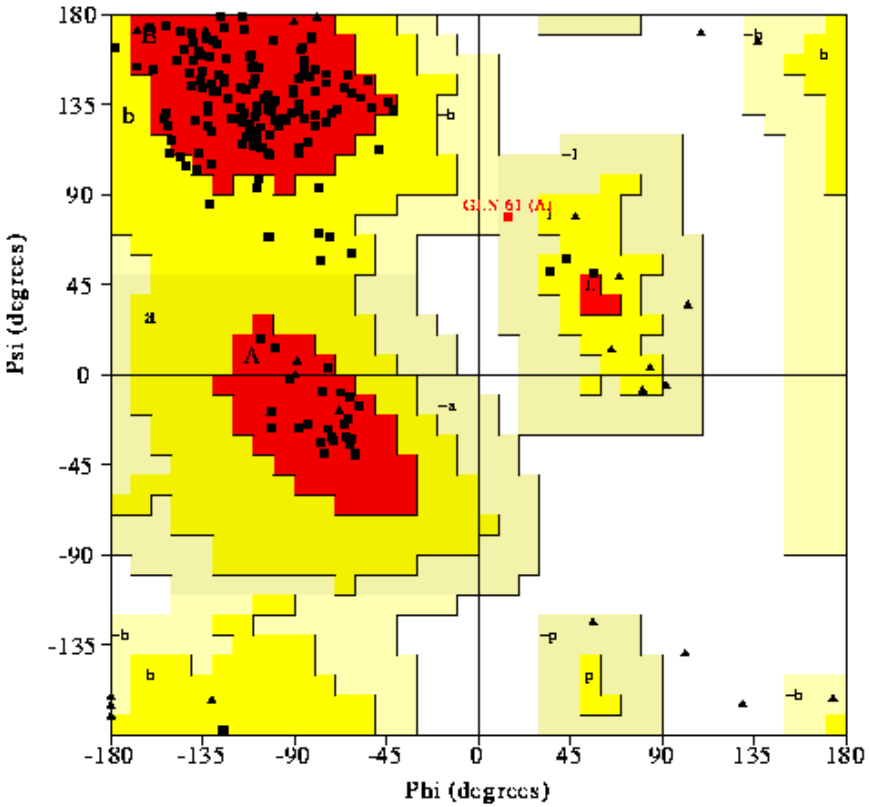
φ (phi, involving the backbone atoms C'-N-Cα-C'), ψ (psi, involving the backbone atoms N-Cα-C'-N)

- http://en.wikipedia.org/wiki/Dihedral_angle

PROCHECK

Ramachandran Plot

1abc

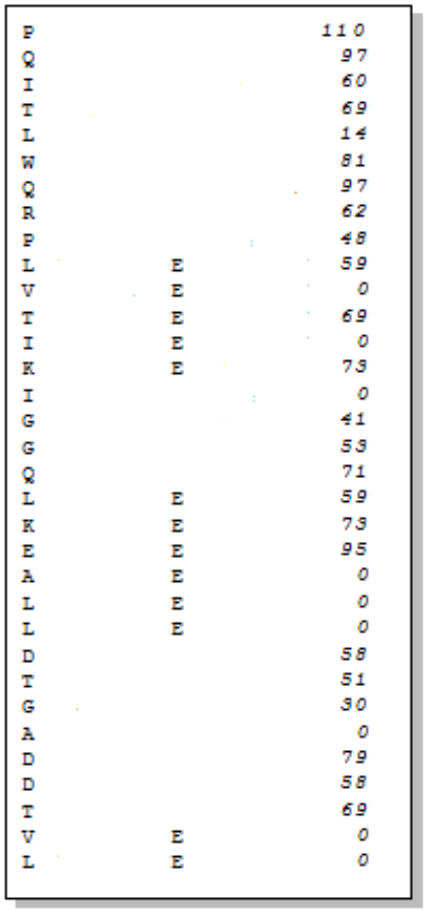


Plot statistics

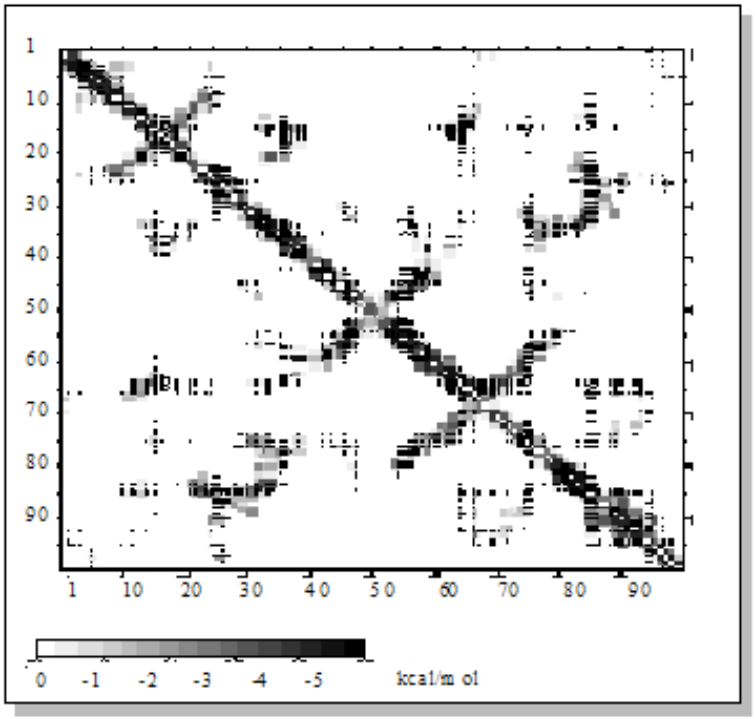
Residues in most favoured regions [A,B,L]	143	89.9%
Residues in additional allowed regions [a,b,l,p]	15	9.4%
Residues in generously allowed regions [~a,~b,~l,~p]	1	0.6%
Residues in disallowed regions	0	0.0%
Number of non-glycine and non-proline residues	159	100.0%
Number of end-residues (excl. Gly and Pro)	5	
Number of glycine residues (shown as triangles)	26	
Number of proline residues	15	
Total number of residues	205	

Based on an analysis of 118 structures of resolution of at least 2.0 Angstroms and R-factor no greater than 20%, a good quality model would be expected to have over 90% in the most favoured regions.

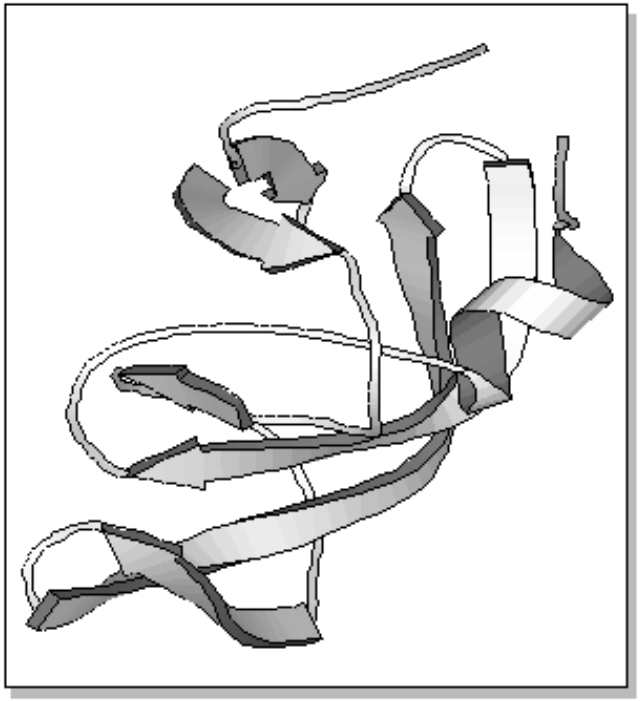
Protein Structure 1D, 2D, 3D



1D



2D



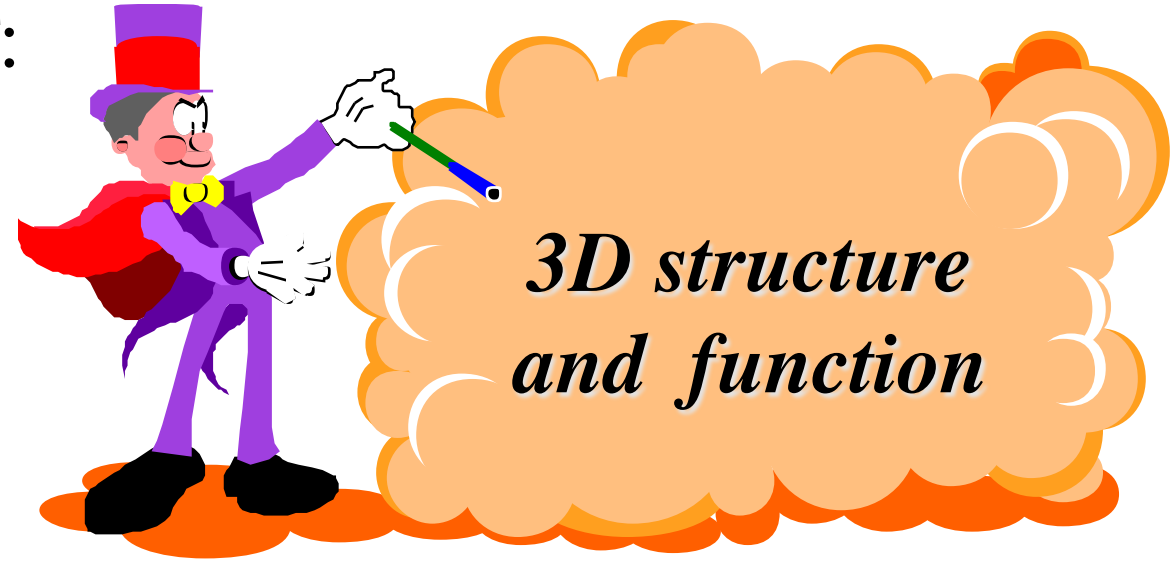
3D

B. Rost, 2005

Goal of Structure Prediction

- Epstein & Anfinsen, 1961:
  sequence uniquely determines structure

- INPUT: sequence
- OUTPUT:



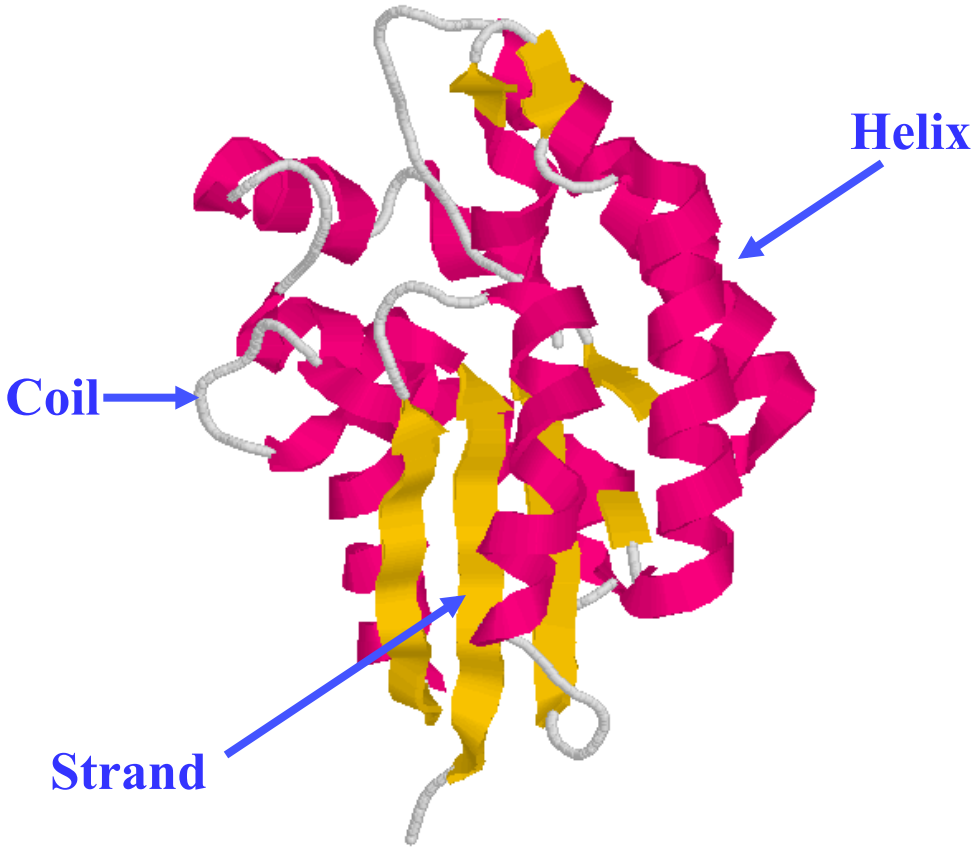
B. Rost, 2005

CASP – Olympics of Protein Structure Prediction

- Critical Assessment of Techniques of Protein Structure Prediction
- 1994,1996,1998,2000,2002,2004,2006, 2008, 2010, 2012
- Blind Test, Independent Evaluation

- CASP ROLL (course project, http://predictioncenter.org/casprol/index.cgi)
- CASP10 (http://predictioncenter.org/casp10/index.cgi)

1D: Secondary Structure Prediction



MWLKKFGINLLIGQSV…

↓

Neural Networks + Alignments

↓

CCCCHHHHHCCCSSSSS…

Cheng, Randall, Sweredoski, Baldi. *Nucleic Acid Research*, 2005

Widely Used Tools (~78-80%)

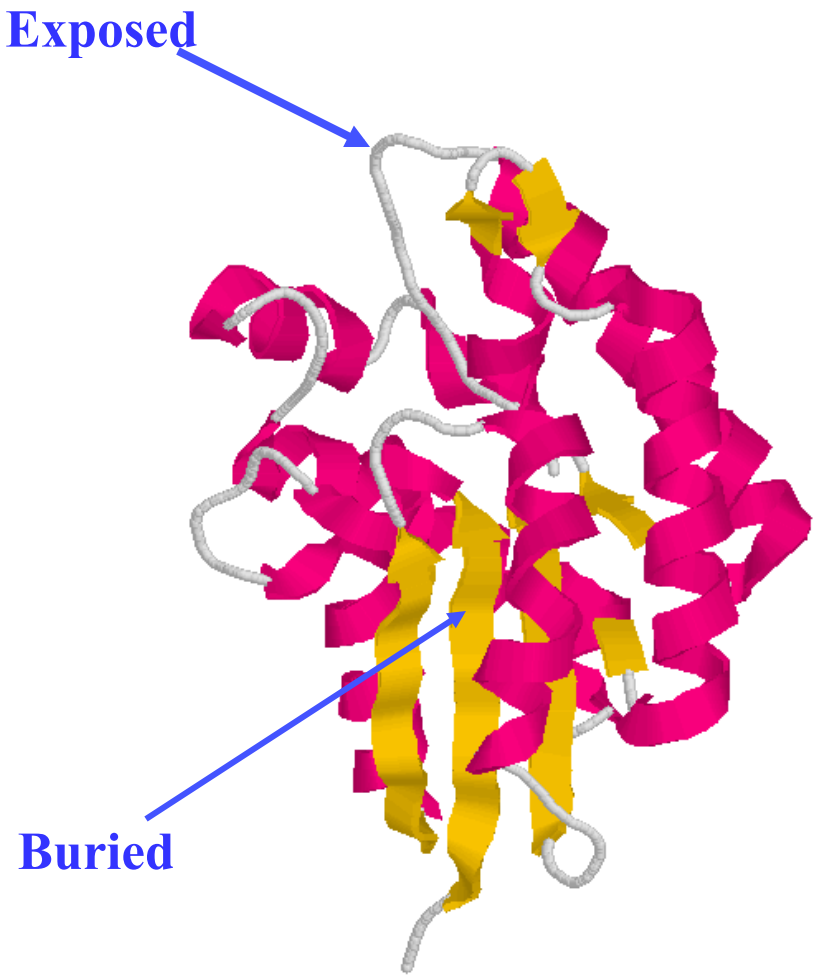
SSpro 4.1: http://sysbio.rnet.missouri.edu/multicom_toolbox/

Distill: http://distill.ucd.ie/porter/

PSI-PRED: http://bioinf.cs.ucl.ac.uk/psipred/psiform.html
software is also available
SAM: http://compbio.soe.ucsc.edu/SAM_T08/T08-query.html

PHD: http://www.predictprotein.org/

1D: Solvent Accessibility Prediction



MWLKKFGINLLIGQSV…

↓

Neural Networks + Alignments

↓

eeeeeeebbbbbbbbeeeebbb…

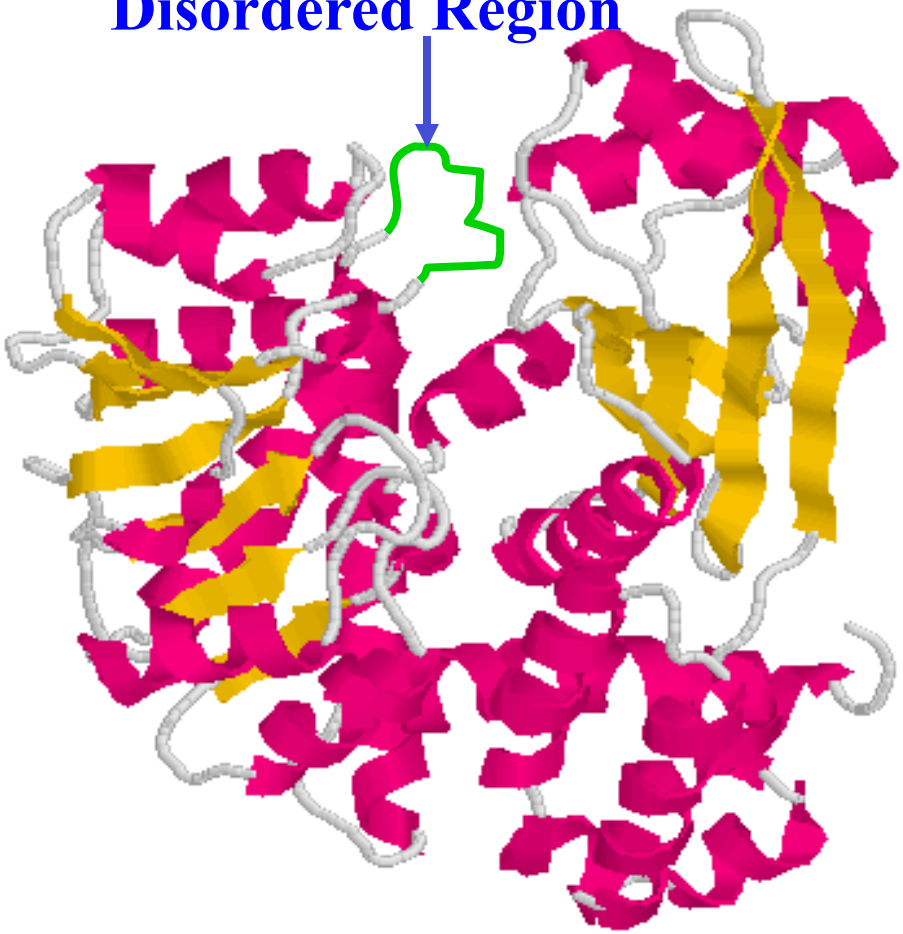
Accuracy: 79% at 25% threshold

Cheng, Randall, Sweredoski, Baldi. *Nucleic Acid Research*, 2005

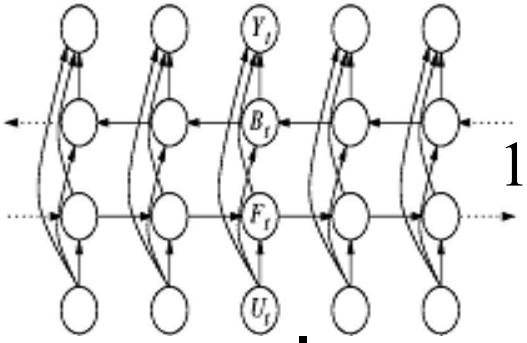
Widely Used Tools (78%)

- ACCpro 4.1: software: http://sysbio.rnet.missouri.edu/multicom_toolbox/
- SCRATCH: http://scratch.proteomics.ics.uci.edu/
- PHD: http://www.predictprotein.org/
- Distill: http://distill.ucd.ie/porter/

1D: Disordered Region Prediction Using Neural Networks



MWLKKFGINLLIGQSV…



1D-RNN

OOOOODDDDOOOOO…

93% TP at 5% FP

Deng, Eickholt, Cheng. BMC Bioinformatics, 2009

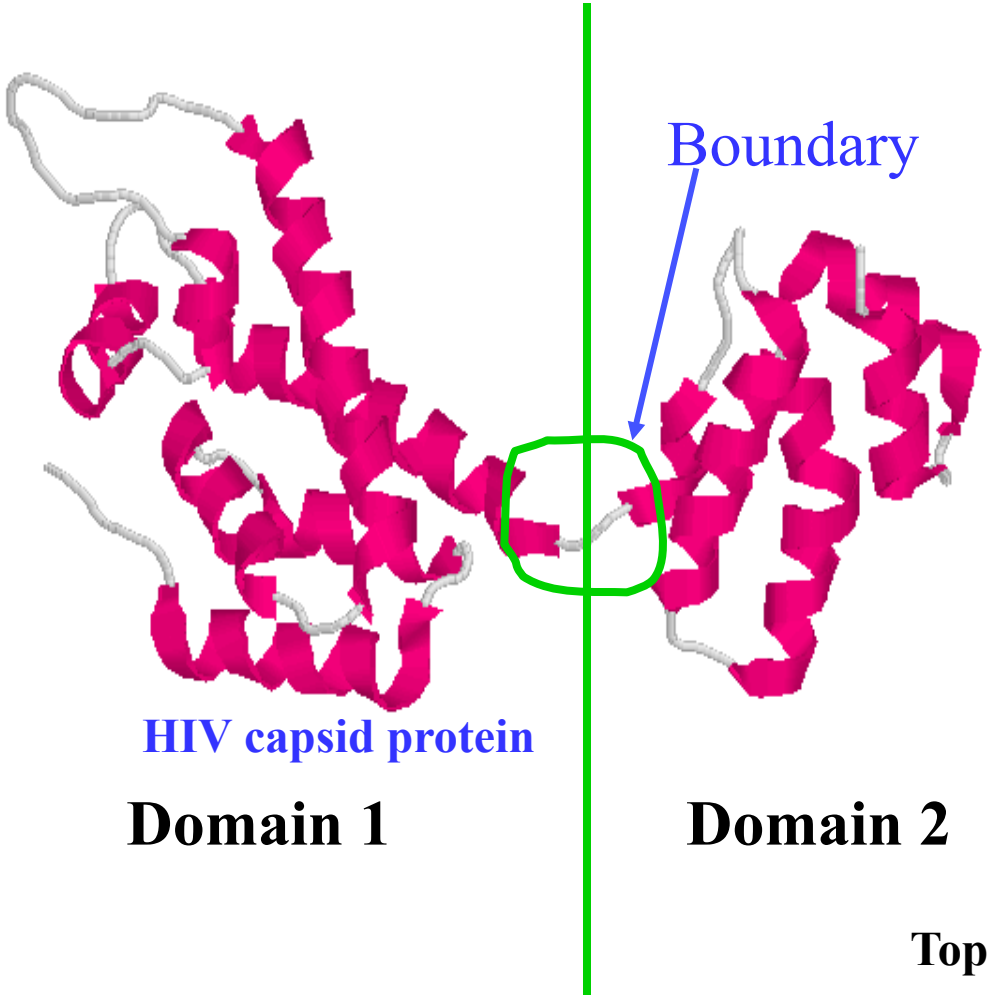
Tools

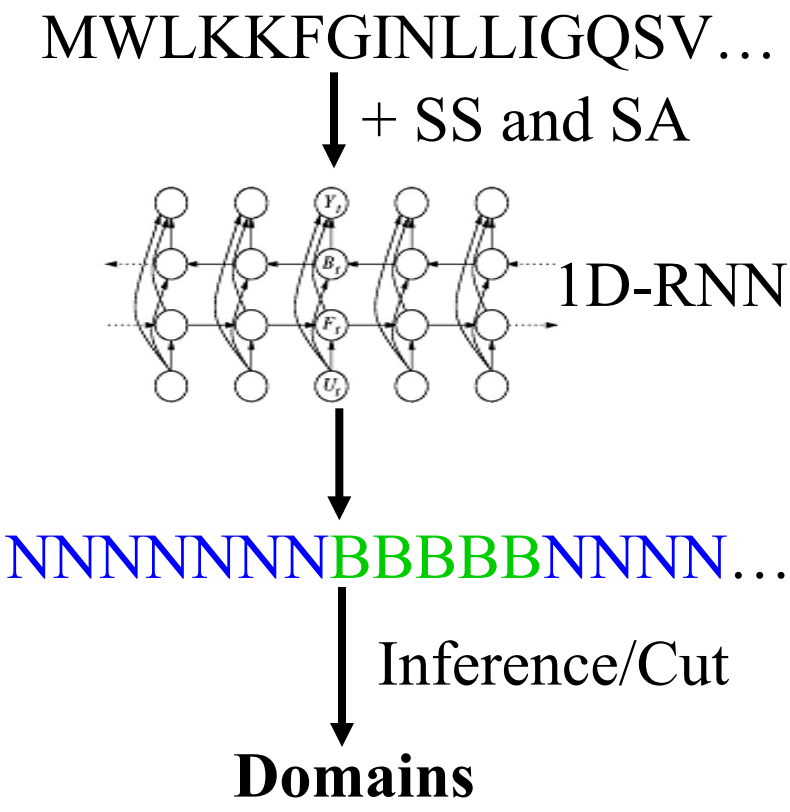
PreDisorder: http://sysbio.rnet.missouri.edu/multicom_toolbox/

A collection of disorder predictors:
http://www.disprot.org/predictors.php

Deng, Eickholt, Cheng. BMC Bioinformatics, 2009 & Mol. Biosystem, 2011

1D: Protein Domain Prediction Using Neural Networks





Top *ab-initio* domain predictor in CAFASP4

Cheng, Sweredoski, Baldi. *Data Mining and Knowledge Discovery*, 2006.

DoBo

Protein domain boundary prediction by integrating evolutionary signals and machine learning

Have a question? Maybe it's answered in the FAQ

Job Details

Job title (optional)

Sequence

Plain sequence. Spaces, newlines and any FASTA header will be ignored.
Mininum sequence length is 90 residues.

Confidence level 60%

Set a minimum threshold for the confidence of domain boundary predictions.

Single/multi-domain classification No

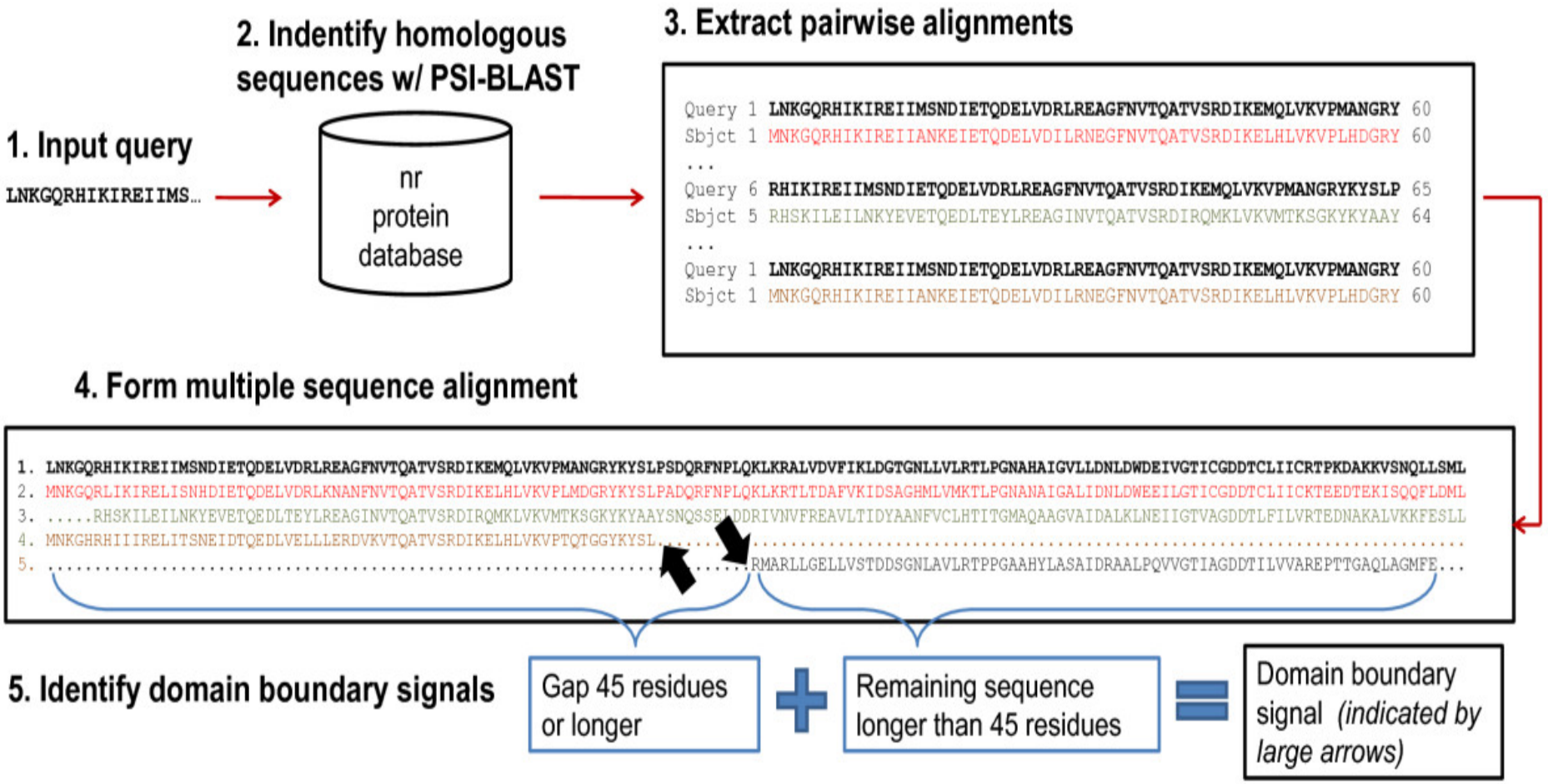
Run an additional check to classify query as a single or multi-domain protein.

Submit Job

Web: http://sysbio.rnet.missouri.edu/multicom_toolbox/index.html

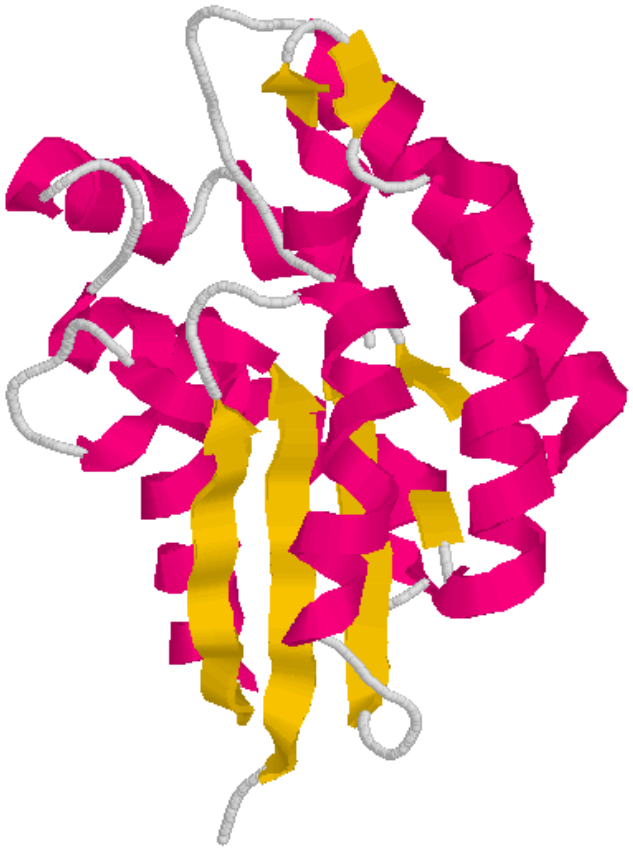
Reference:

J. Eickholt, X. Deng, and J. Cheng. **DoBo: Protein Domain Boundary Prediction by Integrating Evolutionary Signals and Machine Learning.** ***BMC Bioinformatics***. 12:43, 2011.

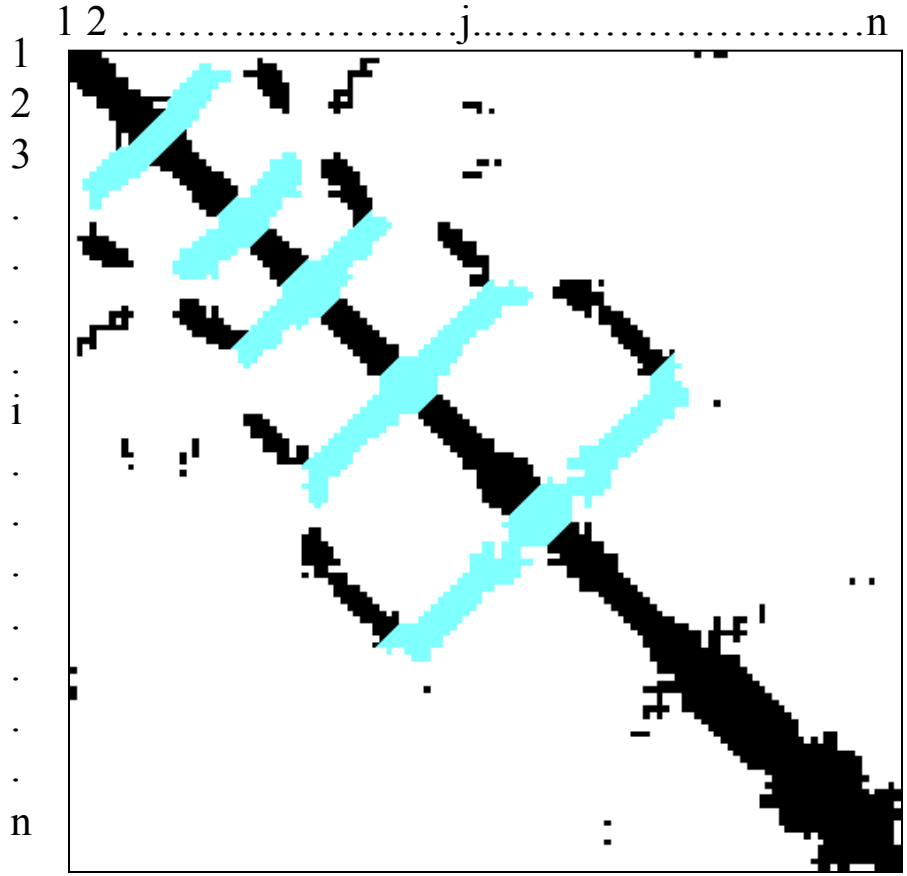

1. Input query
LNKGQRHIKIREIIMS…
2. Indentify homologous sequences w/ PSI-BLAST
nr protein database
3. Extract pairwise alignments
Query 1 LNKGQRHIKIREIIMSNDIETQDELVDRLREAGFNVTQATVSRDIKEMQLVKVPMANGRY 60
Sbjct 1 MNKGQRHIKIREIIANKEIETQDELVDILRNEGFNVTQATVSRDIKELHLVKVPLHDGRY 60
...
Query 6 RHIKIREIIMSNDIETQDELVDRLREAGFNVTQATVSRDIKEMQLVKVPMANGRYKYSLP 65
Sbjct 5 RHSKILEILNKYEVETQEDLTEYLREAGINVTQATVSRDIRQMKLVKVMTKSGKYKYAAY 64
...
Query 1 LNKGQRHIKIREIIMSNDIETQDELVDRLREAGFNVTQATVSRDIKEMQLVKVPMANGRY 60
Sbjct 1 MNKGQRHIKIREIIANKEIETQDELVDILRNEGFNVTQATVSRDIKELHLVKVPLHDGRY 60
4. Form multiple sequence alignment
1. LNKGQRHIKIREIIMSNDIETQDELVDRLREAGFNVTQATVSRDIKEMQLVKVPMANGRYKYSLPSDQRFNPLQKLKRALVDVFIKLDGTGNLLVLRTLPGNAHAIGVLLDNLDWDEIVGTICGDDTCLIICRTPKDAKKVSNQLLSML
2. MNKGQRLIKIRELISNHDIETQDELVDRLKNANFNVTQATVSRDIKELHLVKVPLMDGRYKYSLPADQRFNPLQKLKRTLTDAFVKIDSAGHMLVMKTLPGNANAIGALIDNLDWEEILGTICGDDTCLIICKTEEDTEKISQQFLDML
3. .....RHSKILEILNKYEVETQEDLTEYLREAGINVTQATVSRDIRQMKLVKVMTKSGKYKYAAYSNQSSELDDRIVNVFREAVLTIDYAANFVCLHTITGMAQAAGVAIDALKLNEIIGTVAGDDTLFILVRTEDNAKALVKKFESLL
4. MNKGHRHIIIRELITSNEIDTQEDLVELLLERDVKVTQATVSRDIKELHLVKVPTQTGGYKYSL.....
5. .....RMARLLGELLVSTDDSGNLAVLRTPPGAAHYLASAIDRAALPQVVGTIAGDDTILVVAREPTTGAQLAGMFE...
5. Identify domain boundary signals
Gap 45 residues or longer
Remaining sequence longer than 45 residues
Domain boundary signal (indicated by large arrows)

2D: Contact Map Prediction

3D Structure



2D Contact Map

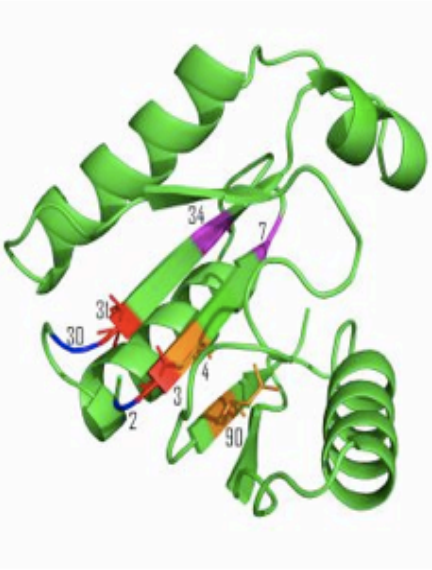


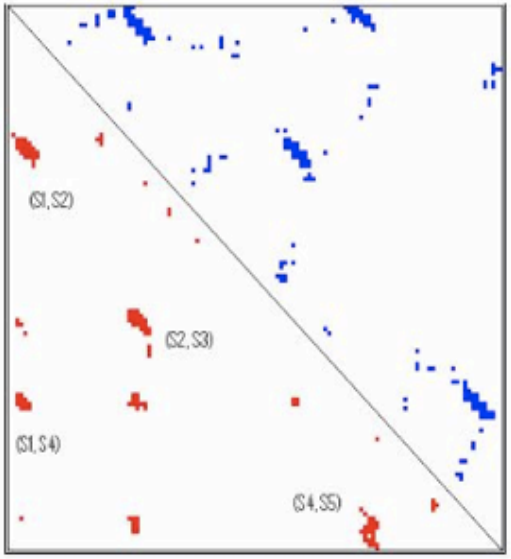
Distance Threshold = 8A°

Cheng, Randall, Sweredoski, Baldi. *Nucleic Acid Research*, 2005

Contact Prediction

- SVMcon: http://casp.rnet.missouri.edu/svmcon.html
- NNcon: http://casp.rnet.missouri.edu/nncon.html
- SCRATCH: http://scratch.proteomics.ics.uci.edu/
- SAM: http://compbio.soe.ucsc.edu/HMM-apps/HMM-applications.html





NNcon: Protein Contact Map Prediction Using Artificial Neural Networks (Help)

Email address(where the prediction will be sent):

Target Name(required):

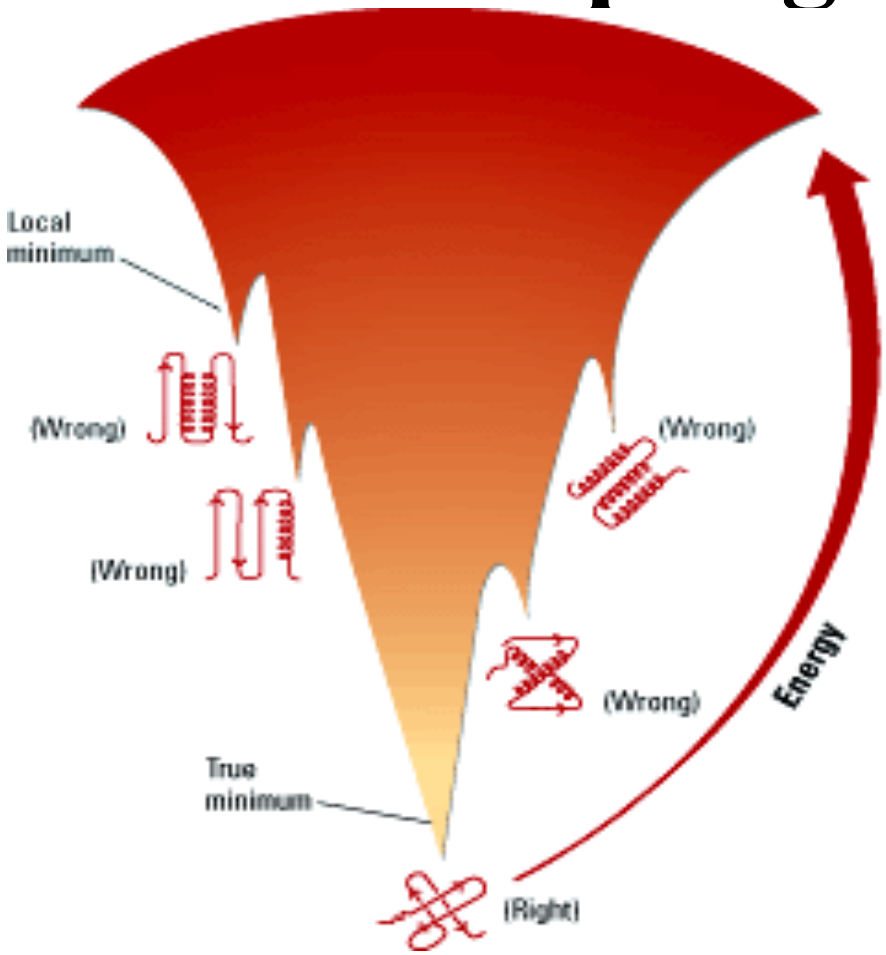
Protein sequence(one plain sequence, no headers, and length < 1000 amino acids; an example sequence is here):

Predict

Tegge, Wang, Eickholt, Cheng, Nucleic Acids Research, 2009

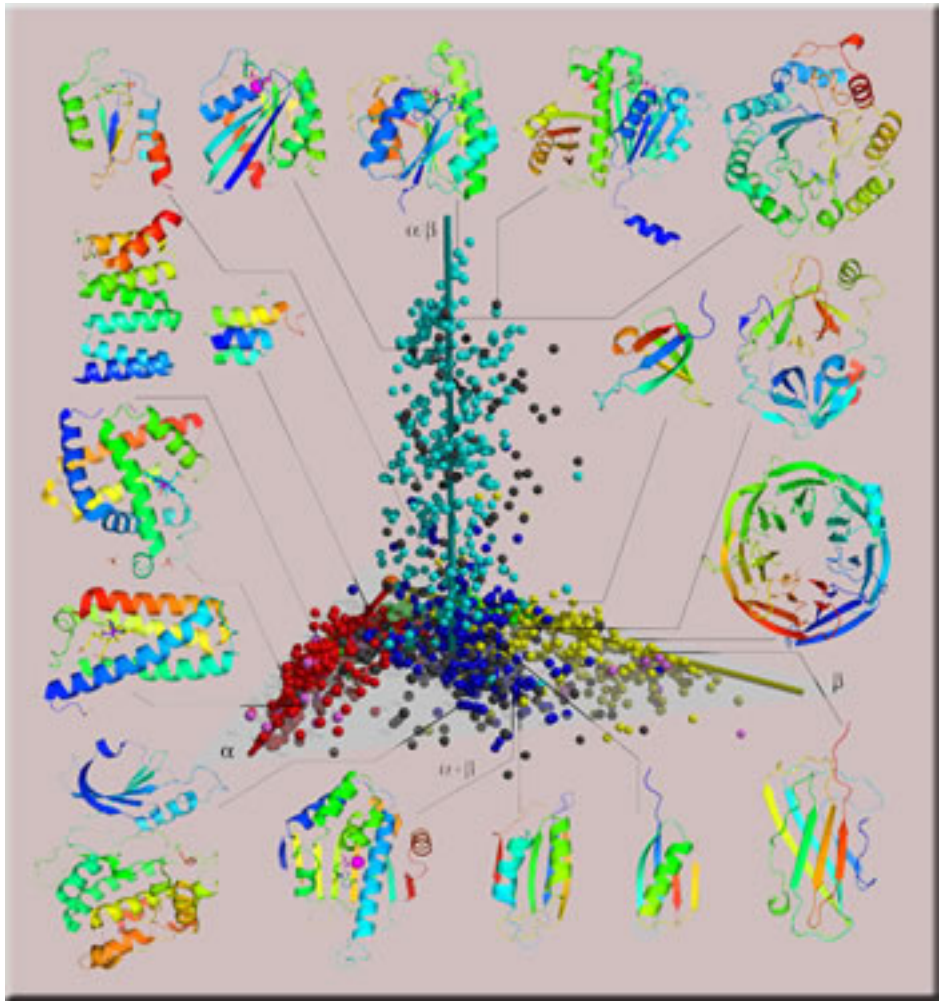
Protein tertiary structure prediction is a space sampling problem.

Protein Energy Landscape & Free Sampling



http://pubs.acs.org/subscribe/archive/mdd/v03/i09/html/willis.html

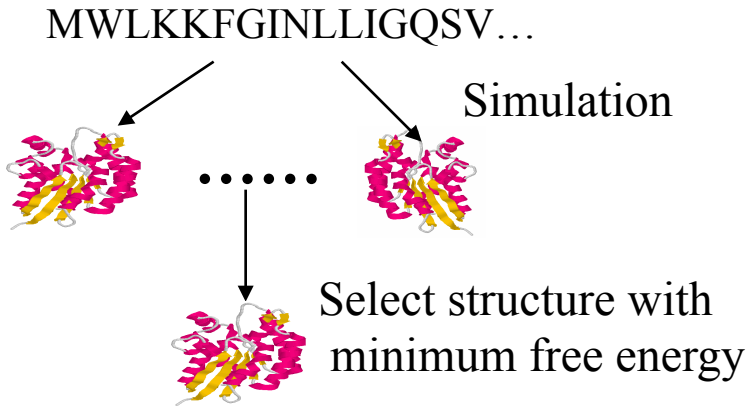
Protein Structure Space & Target Sampling



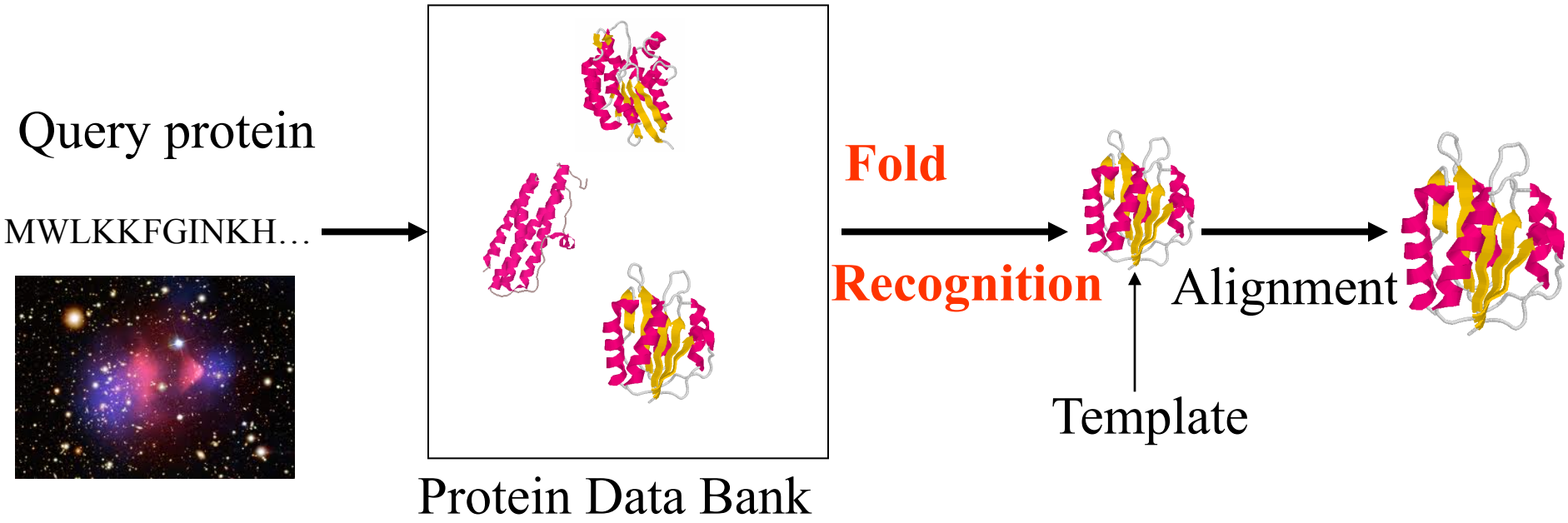
Two Approaches for 3D Structure Prediction

- **Ab Initio Structure Prediction**

  Physical force field – protein folding
  Contact map - reconstruction



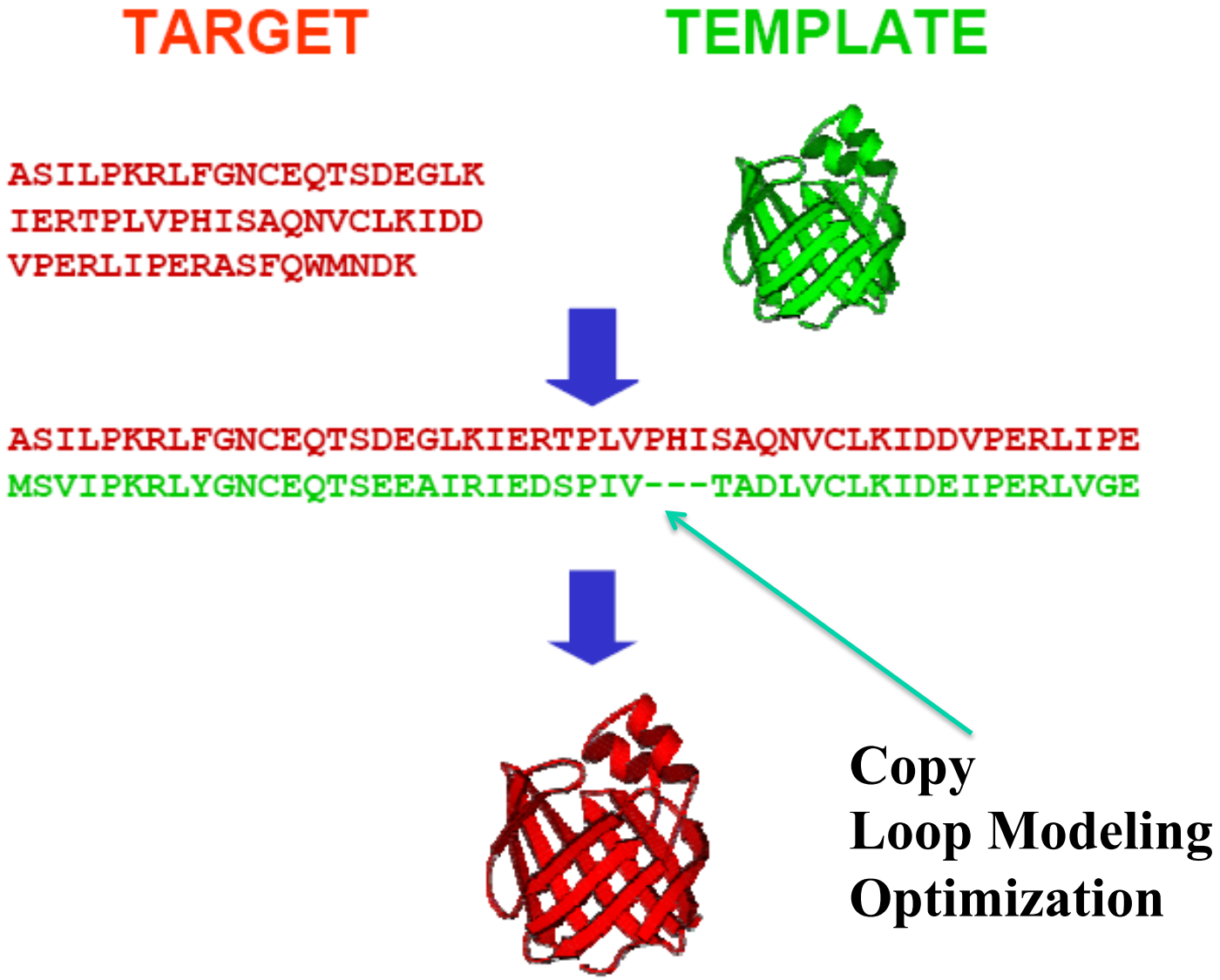
- **Template-Based Structure Prediction**



Template-Based Structure Prediction

1. Template identification
2. Query-template alignment
3. Model generation
4. Model evaluation
5. Model refinement

Notes: if template is easy to identify, it is often called **comparative Modeling or homology** modeling. If template is hard to identify, it is often called **fold recognition**.

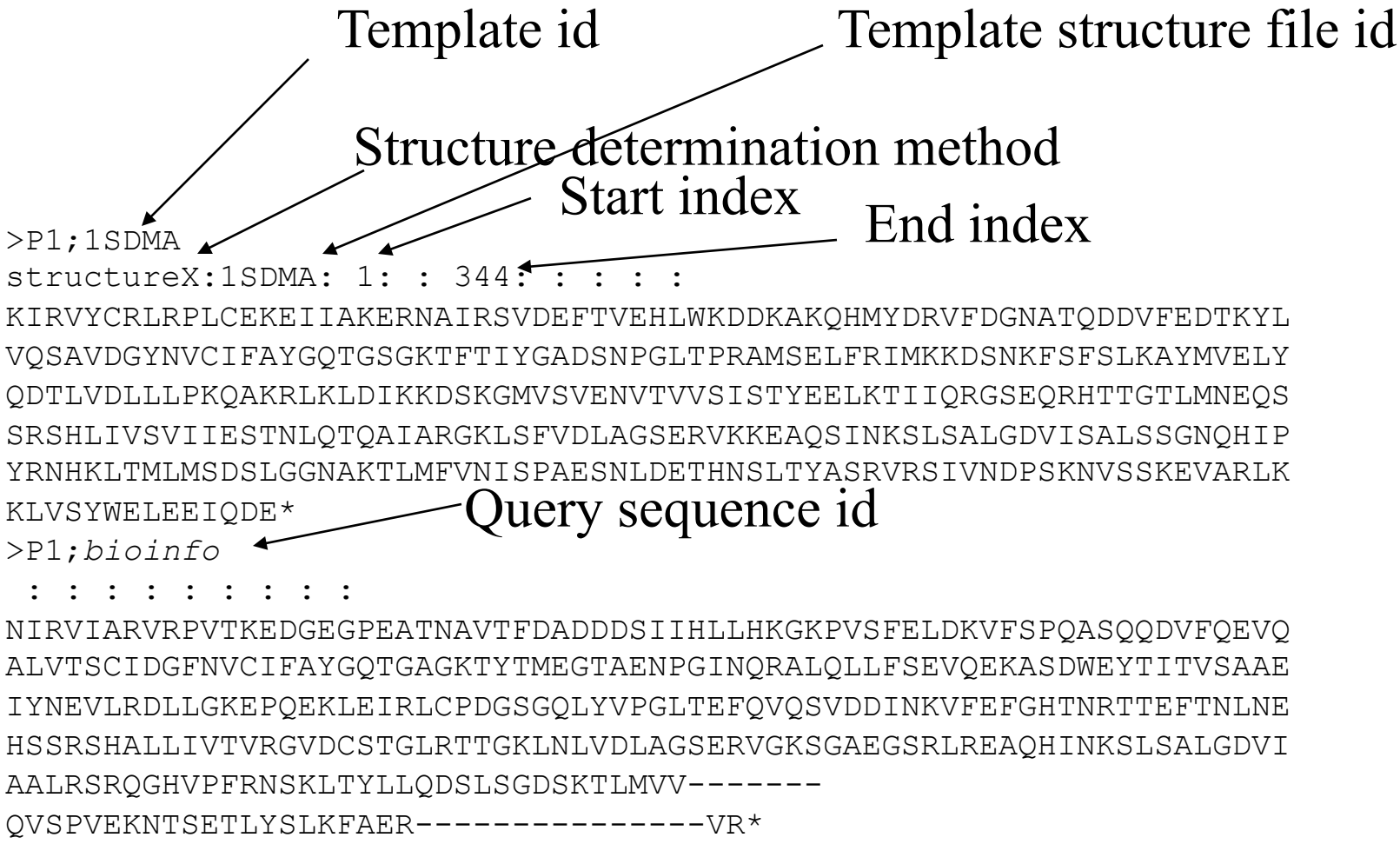


A. Fisher, 2005

Modeller

- Need an alignment file between query and template sequence in the PIR format
- Need the structure (atom coordinates) file of template protein
- You need to write a simple script (Python for version 8.2) to tell how to generate the model and where to find the alignment file and template structure file.
- Run Modeller on the script. Modeller will automatically copy coordinates and make necessary adjustments to generate a model.
- See project step 5-8 for more details.

An PIR Alignment Example



Structure File Example (1SDMA.atm)

```
ATOM      1  N   LYS     1      -3.978  26.298 113.043  1.00 31.75           N
ATOM      2  CA  LYS     1      -4.532  25.067 113.678  1.00 31.58           C
ATOM      3  C   LYS     1      -5.805  25.389 114.448  1.00 30.38           C
ATOM      4  O   LYS     1      -6.887  24.945 114.072  1.00 32.68           O
ATOM      5  CB  LYS     1      -3.507  24.446 114.631  1.00 34.97           C
ATOM      6  CG  LYS     1      -3.743  22.970 114.942  1.00 36.49           C
ATOM      7  CD  LYS     1      -3.886  22.172 113.644  1.00 39.52           C
ATOM      8  CE  LYS     1      -3.318  20.766 113.761  1.00 41.58           C
ATOM      9  NZ  LYS     1      -1.817  20.761 113.756  1.00 43.48           N
ATOM     10  N   ILE     2      -5.687  26.161 115.522  1.00 26.16           N
ATOM     11  CA  ILE     2      -6.867  26.500 116.302  1.00 22.75           C
ATOM     12  C   ILE     2      -7.887  27.226 115.439  1.00 21.35           C
ATOM     13  O   ILE     2      -7.565  28.200 114.770  1.00 20.95           O
ATOM     14  CB  ILE     2      -6.513  27.377 117.523  1.00 21.68           C
ATOM     15  CG1 ILE     2      -5.701  26.563 118.526  1.00 21.13           C
ATOM     16  CG2 ILE     2      -7.782  27.875 118.200  1.00 18.96           C
ATOM     17  CD1 ILE     2      -5.368  27.325 119.787  1.00 21.39           C
ATOM     18  N   ARG     3      -9.120  26.737 115.461  1.00 22.04           N
ATOM     19  CA  ARG     3     -10.214  27.327 114.693  1.00 23.95           C
ATOM     20  C   ARG     3     -10.783  28.563 115.400  1.00 22.82           C
ATOM     21  O   ARG     3     -10.771  28.645 116.629  1.00 22.62           O
ATOM     22  CB  ARG     3     -11.327  26.290 114.510  1.00 26.34           C
ATOM     23  CG  ARG     3     -11.351  25.586 113.161  1.00 30.68           C
ATOM     24  CD  ARG     3     -10.004  25.034 112.771  1.00 35.43           C
ATOM     25  NE  ARG     3     -10.104  24.072 111.672  1.00 43.37           N
ATOM     26  CZ  ARG     3     -10.575  24.350 110.458  1.00 46.04           C
ATOM     27  NH1 ARG     3     -10.997  25.572 110.168  1.00 48.68           N
ATOM     28  NH2 ARG     3     -10.627  23.400 109.532  1.00 48.37           N
ATOM     29  N   VAL     4     -11.278  29.524 114.630  1.00 20.49           N
ATOM     30  CA  VAL     4     -11.853  30.724 115.225  1.00 17.59           C
ATOM     31  C   VAL     4     -13.082  31.211 114.471  1.00 18.31           C
ATOM     32  O   VAL     4     -13.030  31.446 113.264  1.00 16.37           O
ATOM     33  CB  VAL     4     -10.834  31.872 115.272  1.00 19.94           C
ATOM     34  CG1 VAL     4     -11.512  33.168 115.759  1.00 15.64           C
ATOM     35  CG2 VAL     4      -9.668  31.489 116.168  1.00 15.45           C
```

Modeller Python Script
(bioinfo.py)

```
# Homology modelling by the automodel class

from modeller.automodel import *    # Load the automodel class

log.verbose()    # request verbose output
env = environ()  # create a new MODELLER environment to build this model in

# directories for input atom files
env.io.atom_files_directory = './:../atom_files'

a = automodel(env,
              alnfile  = 'bioinfo.pir',  # alignment filename
              knowns   = '1SDMA',        # codes of the templates
              sequence = 'bioinfo')      # code of the target
a.starting_model= 1                 # index of the first model
a.ending_model  = 1                 # index of the last model
                                    # (determines how many models to calculate)
a.make()                            # do the actual homology modelling
```

Where to find structure file

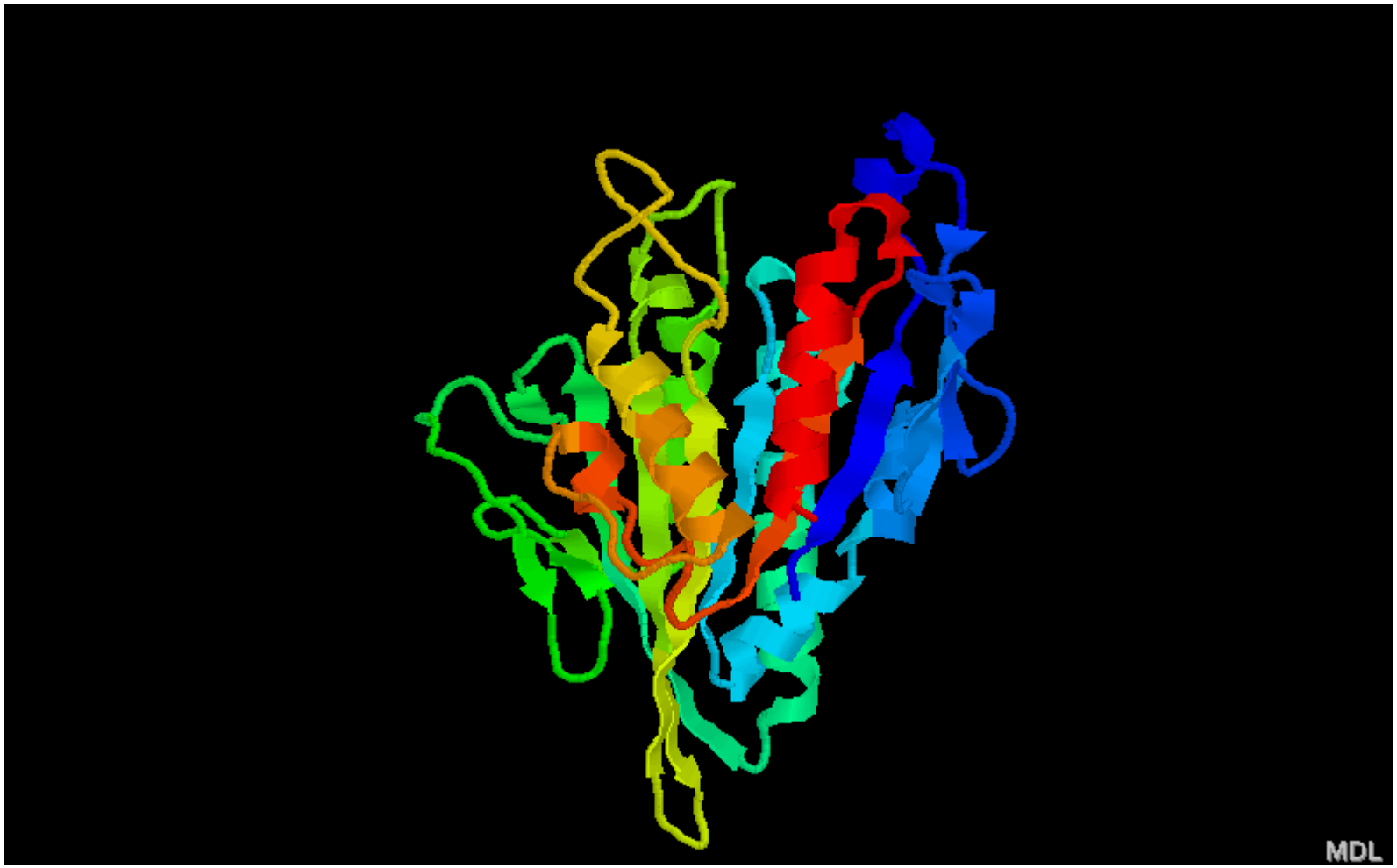
PIR alignment file name

Template structure file id

Query sequence id

Output Example

Command: mod8v2 bioinfo.py



Template Based Modeling Methods

- Comparative Protein Modeling by Satisfaction of Spatial Restraints by Andrej Sali and Tom L. Blundell
- 3D Model is obtained by satisfying spatial restraints derived from alignment with a known structure and expressed as probability density functions (pdfs) for the features restrained.

Probability Density Functions of Features

- Ca – Ca distances
- Main-chain N-O distance
- Main-chain dihedral angles
- Side-chain dihedral angles
- A protein pdf is a combination of individual pdfs of features of the whole protein

Optimization Procedure

- Objective: the pdf of a protein
- Initial input: initial (x, y, z) of each residue satisfying bond length / angle restraints
- Optimization: adjust x, y, z to maximize the pdf (i.e. probability), i.e. reduce the violations of feature restraint as much as possible

Topic 1 – Template Based Modeling

- CASP10 TBM targets
- Known templates at CASP10 web sites
- Develop a homology-based algorithm / tool to build models from templates
- Assess the quality of models
- Implement from scratch
- **Form your group (you did!)**

Feature Restraints

- A database of 17 family alignments including 80 proteins was constructed to obtain feature statistics.
- Feature constraint is represented as conditional distribution. E.g. P(ca-ca distance in target | ca-ca distance in template,…), P(psi angle of a residue in target | psi angle of an equivalent residue in template, …)

Side Chain & Main Chain

- P(X1 | residue type, phi, psi)
- Main-chain and side-chain modeling can be separated or carried out simultaneously

Function Fitting from Known Data

- $P(x|a,b,c) \sim Wx,a,b,c \sim f(x,a,b,c,q)$
- W is a multi-dimensional table calculated from relative frequencies in the data
- *f* is a function that fits W by minimizing root mean square difference
- Fitting algorithm: *Levenberg-Marquardt* algorithm for non-constrained least-squares fitting of a non-linear multidimensional model implemented in the program LSQ.

Features in Multi-Dimensional Table (MDT) Program

- Residue type
- Main-chain dihedral angle of a residue
- Secondary structure of a residue

1	r	Amino acid residue type
2	Φ	Main-chain dihedral angle Φ
3	Ψ	Main-chain dihedral angle Ψ
4	t	Secondary structure class of a residue
5	M	Main-chain conformation class of a residue
6	α	Fractional content of residues in the main-chain conformation class A
7	χ_i	Side-chain dihedral angle χ_i, $i = 1, 2, 3, 4$
8	c_i	Side-chain dihedral angle χ_i class, $i = 1, 2, 3, 4$
9	a	Residue solvent accessibility
10	$\bar{a}$	Average accessibility of two residues in one protein
11	s	Residue neighbourhood difference between two proteins
12	$\bar{s}$	Average residue neighbourhood difference between two proteins
13	i	Fractional sequence identity between two proteins
14	d	C^α–C^α distance
15	Δd	Difference between two C^α–C^α distances in two proteins
16	h	Main-chain N–O distance
17	Δh	Difference between two main-chain N–O distances in two proteins
18	b	Average residue B_{iso}
19	R	Resolution of X-ray analysis
20	g	Distance of a residue from a gap in alignment
21	$\bar{g}$	Average distance of a residue from a gap

Main Chain Conformation Class

Parameters of the main-chain conformation classes

	Mean (°)		Standard deviation (°)	
	Φ_i	$\bar{\Psi}_i$	$\sigma_i(\Phi)$	$\sigma_i(\Psi)$
A	−65	−41	15	15
B	−130	135	15	20
P	−65	140	15	15
G	60	40	10	10
L	90	−10	15	10
E	130	180	25	25

Usefulness of Features

- The most useful pdf is the one that predicts the unknown feature most accurately
- Measured by the entropy of a pdf

Stereochemical Restraints (Generic)

- Obtained from sequence of a protein
- Bond distance, bond angle, planarity of peptide groups, side-chain rings, chiralities of Ca atoms and side-chains, van der Waals contact distance (radii values)
- Mean value and standard deviations for bond lengths, bond angles, and dihedral angles are obtained from GROMOS86

Bond Length and Angles (harmoic model)

The classical harmonic model for the bond length between two atoms gives the vibrational potential energy of the bond as:

$$E(b) = \tfrac{1}{2}c(b-b_o)^2. \tag{19}$$

$$p^b(b) = \frac{1}{\sigma_b\sqrt{2\pi}} \exp\left[-\frac{1}{2}\left(\frac{b-\bar{b}}{\sigma_b}\right)^2\right] = N(\bar{b}, \sigma_b).$$

Van der Waals Repulsion (only non-harmonic feature)

(ii) *van der Waals repulsion*

van der Waals repulsion is the only stereochemical feature which is not described by the harmonic model. Instead, the following pdf is used for two atoms:

$$p^v(d) = c \cdot \begin{cases} N(d_o, \sigma_w); & d \leq d_o \\ \dfrac{1}{\sigma_w \sqrt{2\pi}}; & d_o < d < d_{\max}, \end{cases} \qquad (22)$$

where d is the distance between the two atoms, d_o is the sum of their van der Waals radii and σ_w is the standard deviation of the Gaussian part of the whole pdf (usually 0·05 Å). $d_{\max}$ is the maximal possible linear dimension of a protein and constant c is chosen so that $p^v(d)$ integrates to 1. This pdf does not differentiate between contact distances larger than d_o, but it does select against distances smaller than d_o. This is achieved by imposing a repulsive harmonic potential on atoms that are less than d_o apart.

Ca-Ca Distance Features

$$p^{d}(d/\bar{g}, i, \bar{a}', d') = \frac{1}{\sigma(\bar{g}, i, \bar{a}', d')\sqrt{2\pi}} \times \exp\left[-\frac{1}{2}\left(\frac{d-d'}{\sigma(\bar{g}, i, \bar{a}', d')}\right)^{2}\right]$$

Standard deviation depends on solvent accessibility, gaps of alignment, and sequence identity.

Combine pdfs of a Feature (Ca-Ca distance) from Multiple Templates

- Weighted sum of the same type of pdfs from multiple known structures

The last step in the derivation of the feature pdf is to include the van der Waals restraint. Since all stereochemical restraints have to be satisfied in all structures, these restraints are multiplied into the feature pdf and we obtain the final feature pdf:

$$p^D(d) = [\omega_1 p_1^d(d) + \omega_2 p_2^d(d)]p^v(d).$$

(ii) *Derivation of a molecular pdf from feature pdfs*

The last stage in the derivation of a molecular pdf is to combine all feature pdfs into a molecular pdf. The 3D structure of a protein is uniquely determined if a sufficiently large number of its spatial features, f_i, are specified. The goal is to find the 3D structure that is consistent with the most probable values of individual features f_i. The molecular pdf should give a probability for occurrence of any combination of these features simultaneously. Then the model for the 3D structure of the unknown would correspond to the maximum of the molecular pdf. Assuming that feature pdfs are independent of each other, the molecular pdf is simply a product of feature pdfs defined in equations (29) to (33):

$$P = \prod_i p^F(f_i). \tag{34}$$

Optimization

The function that is actually optimized is a transformation of the molecular pdf P:

$$F = -\ln(P), \tag{35}$$

where all the features are expressed in terms of atomic Cartesian co-ordinates. Function F is referred to as the objective function. The same Cartesian co-ordinates that maximize P also minimize F. However, F is computationally better suited for optimization than P, since multiplication of terms in the product of equation (34) is substituted by their addition in equation (35) and since the problem of floating point overflow is smaller for F than for P.

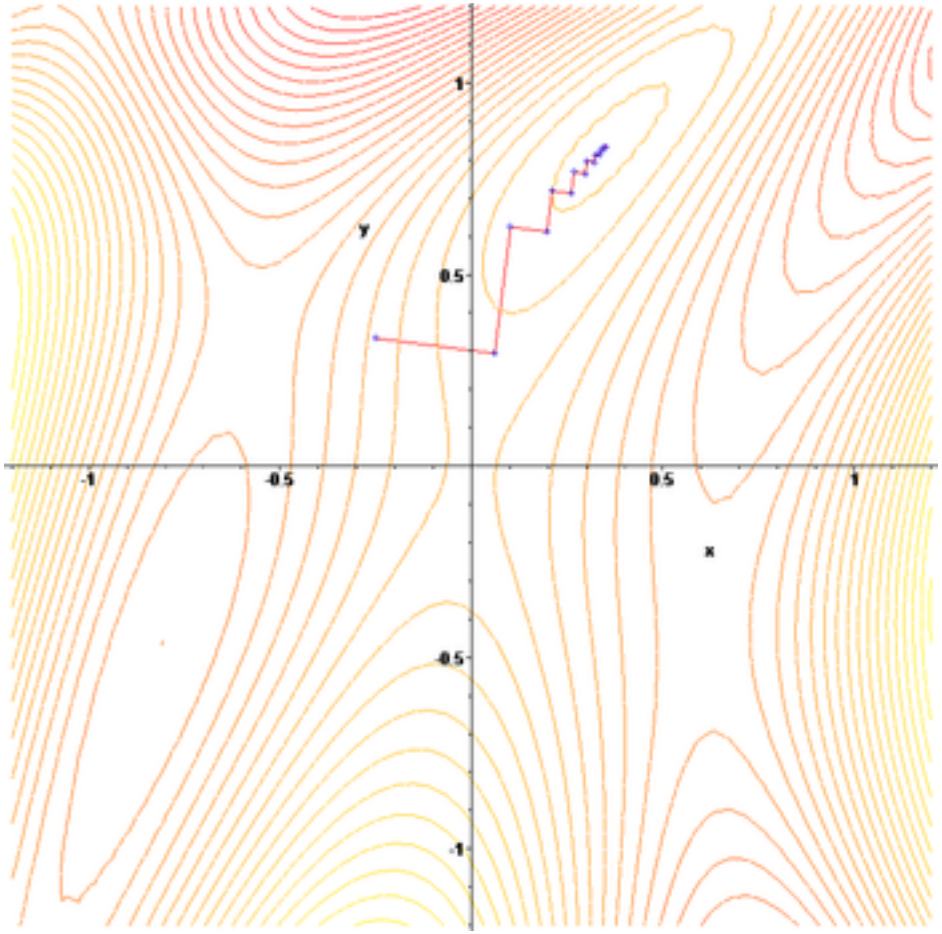
dihedral angles. Following the variable target function method, the optimum of the molecular pdf is found by successive optimizations of increasingly more complex "target" functions, culminating in the true molecular pdf at the end. This series is obtained by starting with sequentially local restraints and then introducing more and more long-range restraints, finally arriving at the true molecular pdf incorporating all the restraints. More precisely, the target function $P(\Delta r)$ is defined as a function of an integer variable $\Delta r = 1, \ldots, N$, where N is the number of residues in the sequence being modelled. The target function $P(\Delta r)$ is obtained in the same way as the molecular pdf, except that only those restraints whose atoms originate from residues not more than Δr residues apart in the sequence are included. The whole calculation consists of a

included. The whole calculation consists of a number of conjugate gradient optimizations (Press *et al.*, 1986) of target functions $P(\Delta r)$ with increasing Δr values. The starting conformation for $P(1)$ optimization is either an extended structure or a conformation derived from an extended chain by rotation around the main-chain and side-chain dihedral angles. In the subsequent steps of the variable target function method, the starting conformation is the final model from the previous step. *An ensemble of different final models is obtained by* using different initial conformations.

Spatial restraints used to model trypsin

Type	Basis pdfs[a]	Feature pdfs[b]	Violations[c]	r.m.s.[d]	r.m.s.[e]
Bond lengths	1659	1659	0 (0·1 Å)	0·005 Å	0·005 Å
Bond angles	2250	2250	5 (10°)	2·00°	2·00°
Dihedral angles[f]	919	919	1 (20°)	3·40°	3·40°
van der Waals contacts[g]	531	531	0 (0·2 Å)	0·02 Å	0·02 Å
C^{α}–C^{α} distances	23,538	11,914	26 (1·5 Å)	0·22 Å	0·47 Å
Main-chain N–O distances	7480	3832	19 (1·5 Å)	0·31 Å	0·51 Å
Main-chain Φ dihedral angles	1110	222	2 (20°)	10·8°	21·2°
Main-chain Ψ dihedral angles	1332	222	9 (20°)	10·6°	20·3°
Side-chain χ_1 dihedral angles	528	176	5 (25°)	8·4°	16·8°
Side-chain χ_2 dihedral angles	264	103	3 (25°)	10·2°	13·0°
Side-chain χ_3 dihedral angles	92	32	2 (25°)	11·9°	48·1°
Side-chain χ_4 dihedral angles	48	16	0 (25°)	4·5°	21·9°
Disulphide bridge bonds	6	6	0 (0·1°)	0·007 Å	0·007 Å
Disulphide bridge angles	12	12	0 (10°)	3·7°	3·7°
Disulphide bridge dihedral angles	6	12	0 (20°)	10·0°	12·9°
cis-Peptides[h]	0	0			

Gradient Descent



Wikipedia

Conjugate Gradient Descent

Consider the following n variables unconstrained optimization problem:

$$\min_{x \in \mathbb{R}^n} f(x), \tag{1.1}$$

where $f : \mathbb{R}^n \to \mathbb{R}$ is smooth and its gradient $g(x)$ is avaible. The nonlinear conjugate gradient (CG) method for (1.1) is designed by the iterative form

$$x_{k+1} = x_k + \alpha_k d_k, \quad k = 0, 1, 2, \ldots, \tag{1.2}$$

where x_k is the kth iterative point, $\alpha_k > 0$ is a steplength, and d_k is the search direction defined by

$$d_k = \begin{cases} -g_k + \beta_k d_{k-1}, & \text{if } k \geq 1, \\ -g_k, & \text{if } k = 0, \end{cases} \tag{1.3}$$

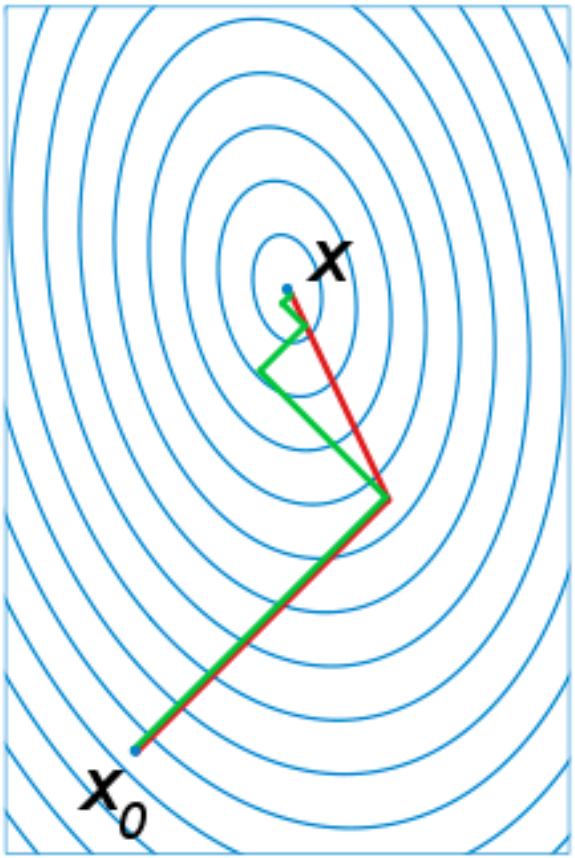
where $\beta_k \in \mathbb{R}$ is a scalar which determines the different conjugate gradient methods [1, 2], and g_k is the gradient of $f(x)$ at the point x_k. There are many well-known formulas for β_k, such as the Fletcher-Reeves (FR) [3], Polak-Ribière-Polyak (PRP) [4], Hestenses-Stiefel (HS) [5], Conjugate-Descent (CD) [6], Liu-Storrey (LS) [7], and Dai-Yuan (DY) [8]. The CG method is a powerful line search method for solving optimization problems, and it remains very popular for engineers and mathematicians who are interested in solving large-scale problems [9–11]. This method can avoid, like steepest descent method, the computation and storage of some matrices associated with the Hessian of objective functions. Then there are many new formulas that have been studied by many authors (see [12–20] etc.).

The following formula for β_k is the famous FR method:

$$\beta_k^{\mathrm{FR}} = \frac{\|g_{k+1}\|^2}{\|g_k\|^2}, \tag{1.4}$$

Wikipedia

where g_k and g_{k+1} are the gradients $\nabla f(x_k)$ and $\nabla f(x_{k+1})$ of $f(x)$ at the point x_k and x_{k+1}, respectively, $\|\cdot\|$ denotes the Euclidian norm of vectors. Throughout this paper, we also denote $f(x_k)$ by f_k. Under



A comparison of the convergence of gradient descent with optimal step size (in green) and conjugate vector (in red) for minimizing a quadratic function associated with a given linear system. Conjugate gradient, assuming exact arithmetic, converges in at most *n* steps where *n* is the size of the matrix of the system (here *n*=2).

Group Formation

- **Group 1**:
- **Group 2**:
- **Group 3**:

Group 1

- **Caiwei Wang**
- **Haipei Fan**
- **Puneet Gaddam**
- **Sean Lander**
- **Xiaokai Qian**
- **Stephen Koonce**

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Group 2

- **Kishore Banala**
- **Kommidi Sai Ram**
- **Maruthi Donthi**
- **Samantha Warren**
- **Shravya Ramisetty**
- **Zainab Al-Taie**

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Group 3

- **Lingfei Xu**
- **Pei Hu**
- **Rui Wang**
- **Shiyuan Chen**
- **Abhimanyu Vemulapalli**
- **Zhiluo Gao**

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Project 1

- Design and develop a template-based protein structure modeling tool
- Assess its performance on a few TBM targets used in CASP benchmark

Project Directory

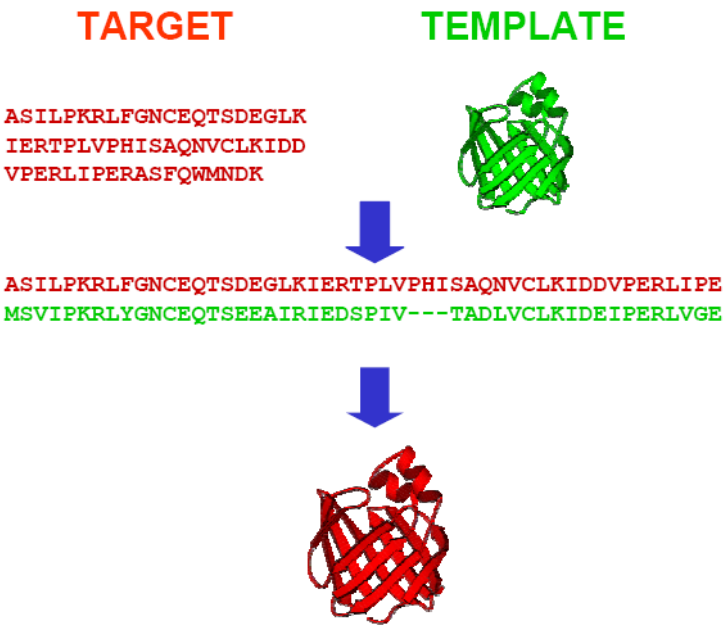
- Project1
- ---- src: source code
- ---- bin: binary
- ---- lib: library
- ---- data: data
- ---- training: training
- ---- test: test cases
- ---- doc: document / references / presentation / report
- ---- other: third-party programs

Discussion of Your Project Plan

- Data preparation
- Algorithm development (initialization, restraints extraction & representation, sampling, optimization): creative, alternative, plural
- Implementation: interface, design, platform, languages, code base / from scratch, task assignment, timeline, progress track
- Evaluation plan (metrics, tools, data, objective, comprehensive, expectation)
- Challenges, Technical Hurdles, Feasibility, Strength, weakness, Risks
- Software Package (installation, test cases)

Useful Tools

- Loop modeling: http://www.math.unm.edu/~vageli/codes/codes.html



- Tools convert between (x,y,z) Coordinates and (phi, psi) angles

Key Milestones of the Project

- Plan presentation on Feb. 12 (only two days)
- 10 days, initial results discussion on Feb. 19 (a results and assessment report, doc file)