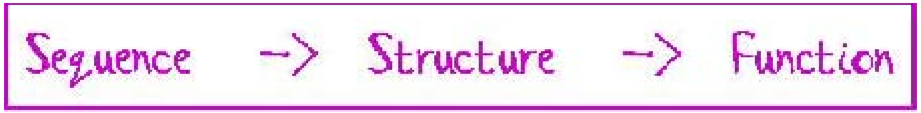
Protein structures

Protein Structure

- Why protein structure?
- The basics of protein
- Basic measurements for protein structure
- Levels of protein structure
- Prediction of protein structure from sequence
- Finding similarities between protein structures
- Classification of protein structures

Why protein structure?

- In the factory of living cells, proteins are the workers, performing a variety of biological tasks.
- Each protein has a particular 3-D structure that determines its function.



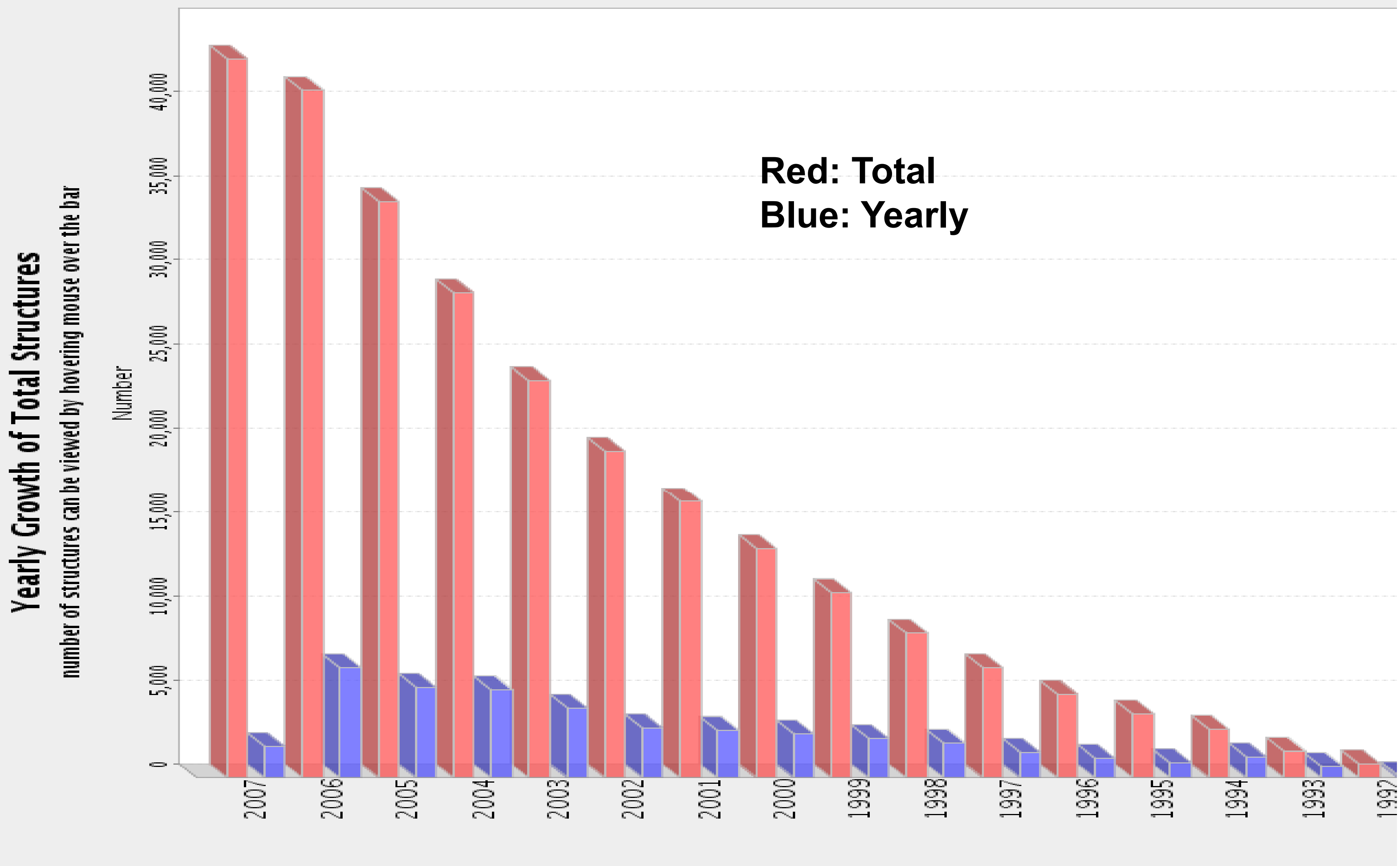
Sequence -> Structure -> Function

- Protein structure is more conserved than protein sequence, and more closely related to function.

Structural information

- Protein Data Bank: maintained by the Research Collaboratory of Structural Bioinformatics(RCSB)
  - http://www.rcsb.org/pdb/
  - > 42752 protein structures as of April 10
  - including structures of Protein/Nucleic Acid Complexes, Nucleic Acids, Carbohydrates
- Most structures are determined by X-ray crystallography. Other methods are NMR and electron microscopy(EM). Theoretically predicted structures were removed from PDB a few years ago.

PDB Growth

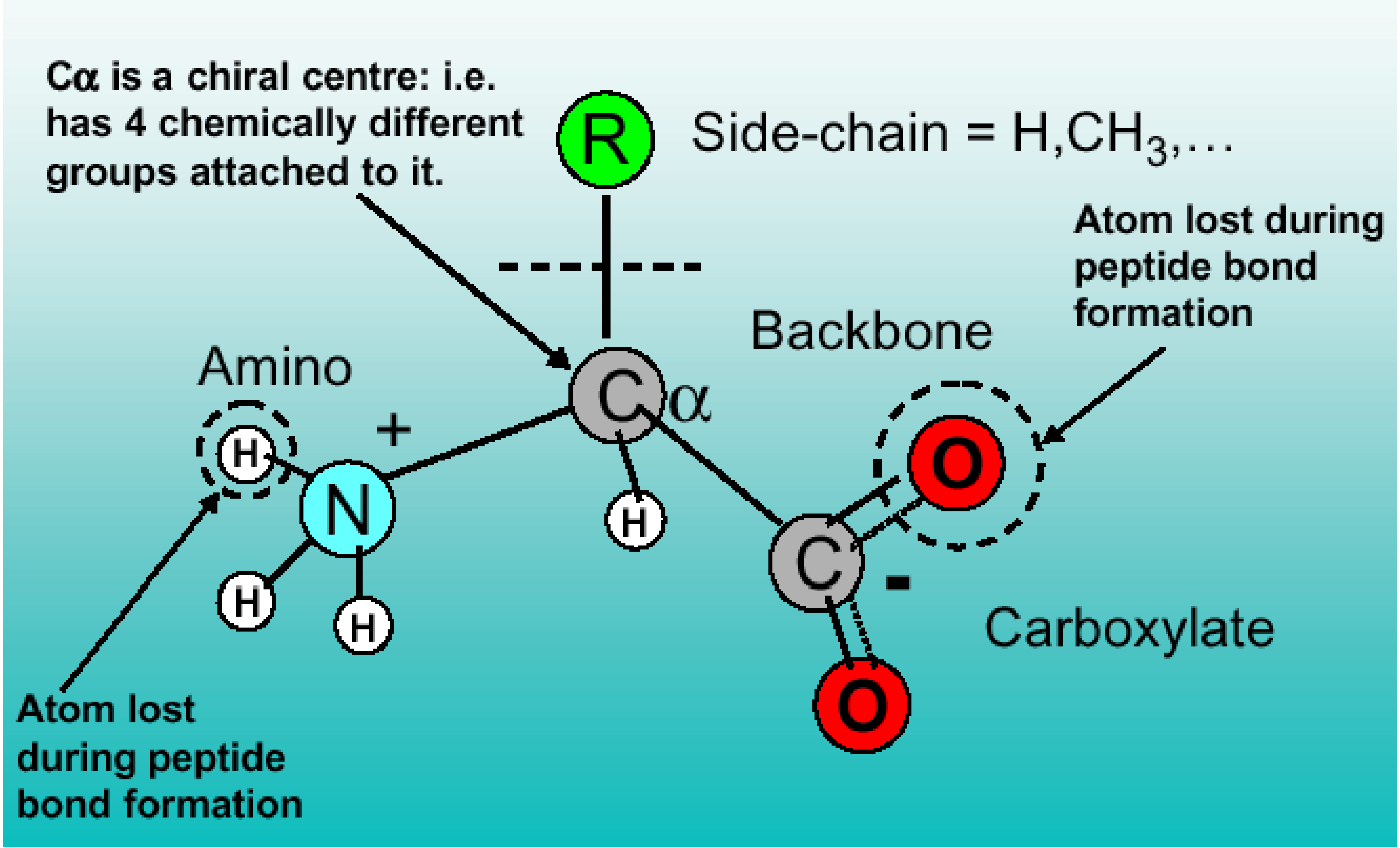


Red: Total
Blue: Yearly

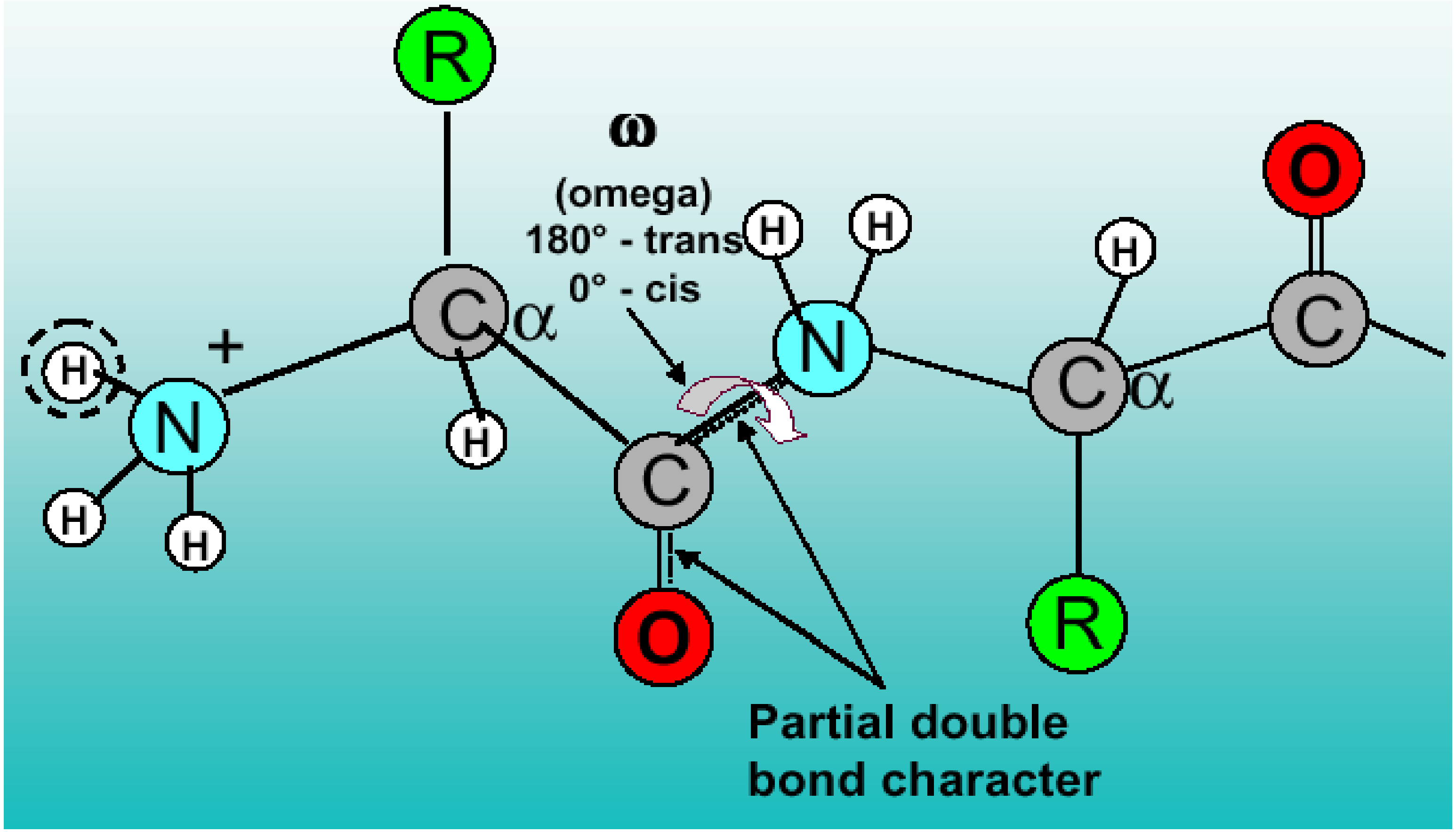
The basics of proteins

- Proteins are linear heteropolymers: one or more polypeptide chains
- Building blocks: 20 types of amino acids.
- Range from a few 10s-1000s
- Three-dimensional shapes (“fold”) adopted vary enormously.

Common structure of Amino Acid

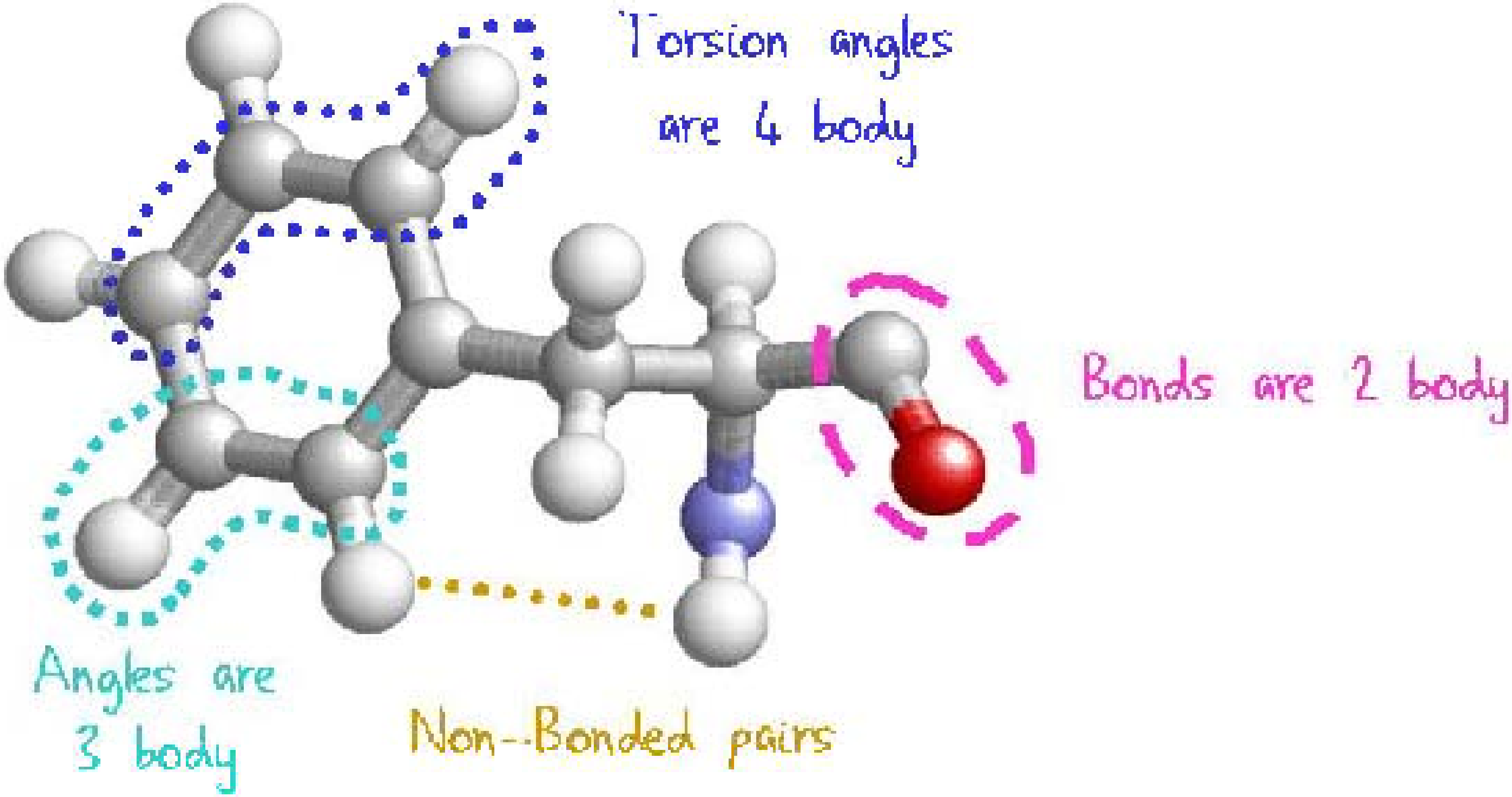

Cα is a chiral centre: i.e. has 4 chemically different groups attached to it.
R
Side-chain = H,CH$_3$,…
Atom lost during peptide bond formation
Backbone
Amino
+
H
N
H
H
C
α
H
O
C
-
O
Carboxylate
Atom lost during peptide bond formation

Formation of polypeptide chain



Basic Measurements for protein structure

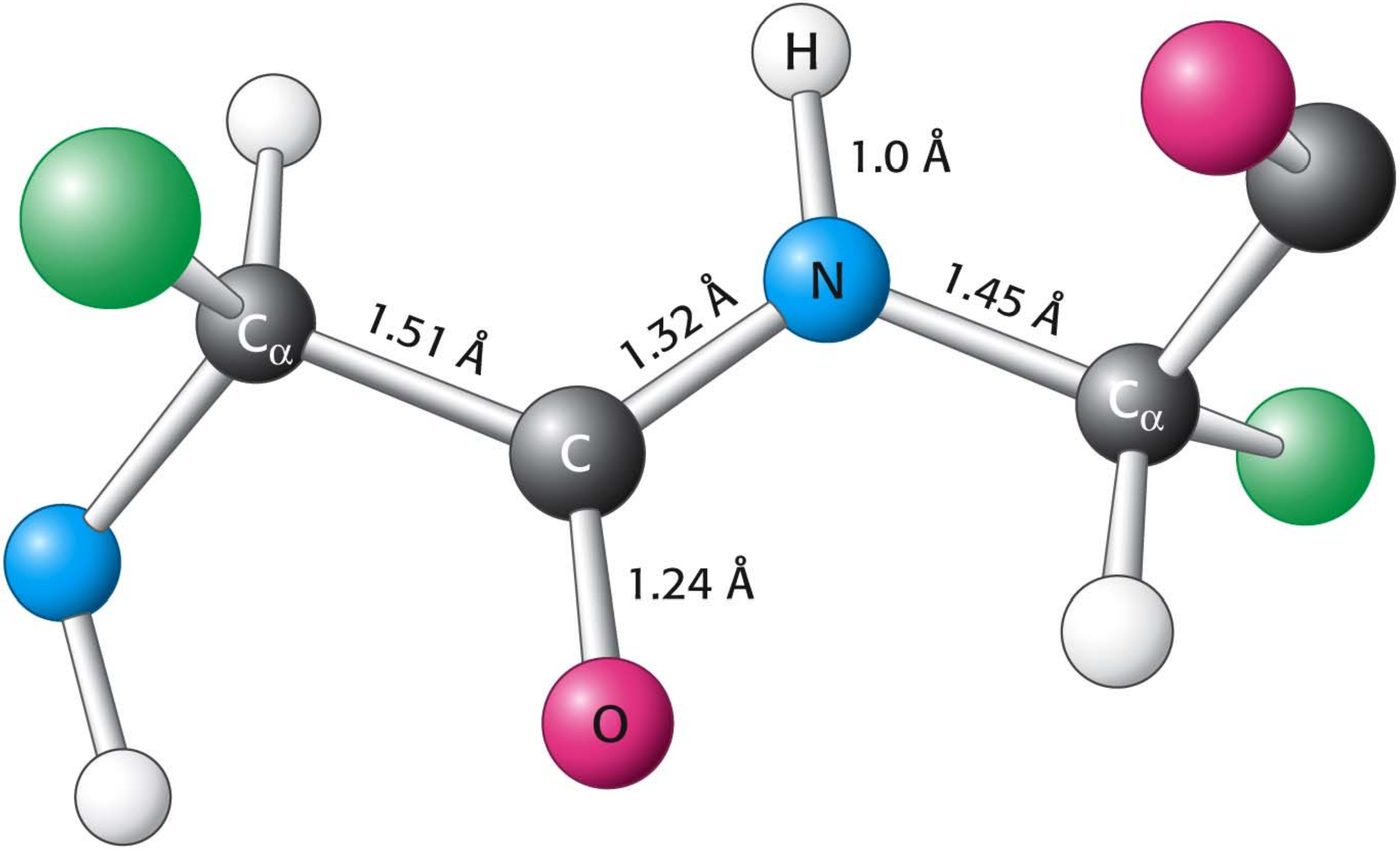
- Bond lengths
- Bond angles
- Dihedral (torsion) angles


Torsion angles are 4 body
Bonds are 2 body
Angles are 3 body
Non-Bonded pairs

Bond Length

- The distance between bonded atoms is constant
- Depends on the “type” of the bond
- Varies from 1.0 Å(C-H) to 1.5 Å(C-C)
- BOND LENGTH IS A FUNCTION OF THE POSITIONS OF TWO ATOMS.

Bond Length



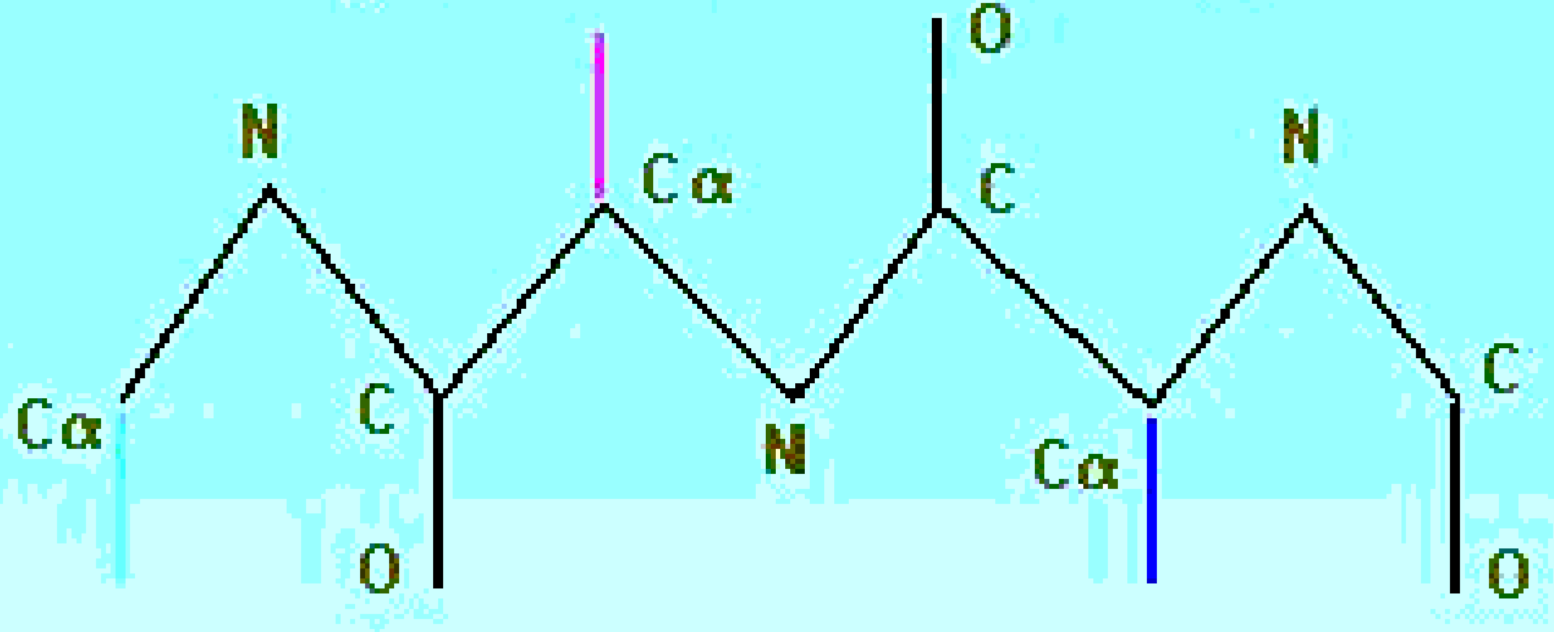
Bond Angles

- All bond angles are determined by chemical makeup of the atoms involved, and are constant.
- Depends on the type of atom, and number of electrons available for bonding.
- Ranges from 100° to 180°
- BOND ANGLES IS A FUNCTION OF THE POSITION OF THREE ATOMS.

Dihedral Angles

- These are usually variable
- Range from 0-360° in molecules
- Most famous are ϕ, ψ, ω and χ
- DIHEDRAL ANGLES ARE A FUNCTION OF THE POSITION OF FOUR ATOMS.

Important Dihedral Angles

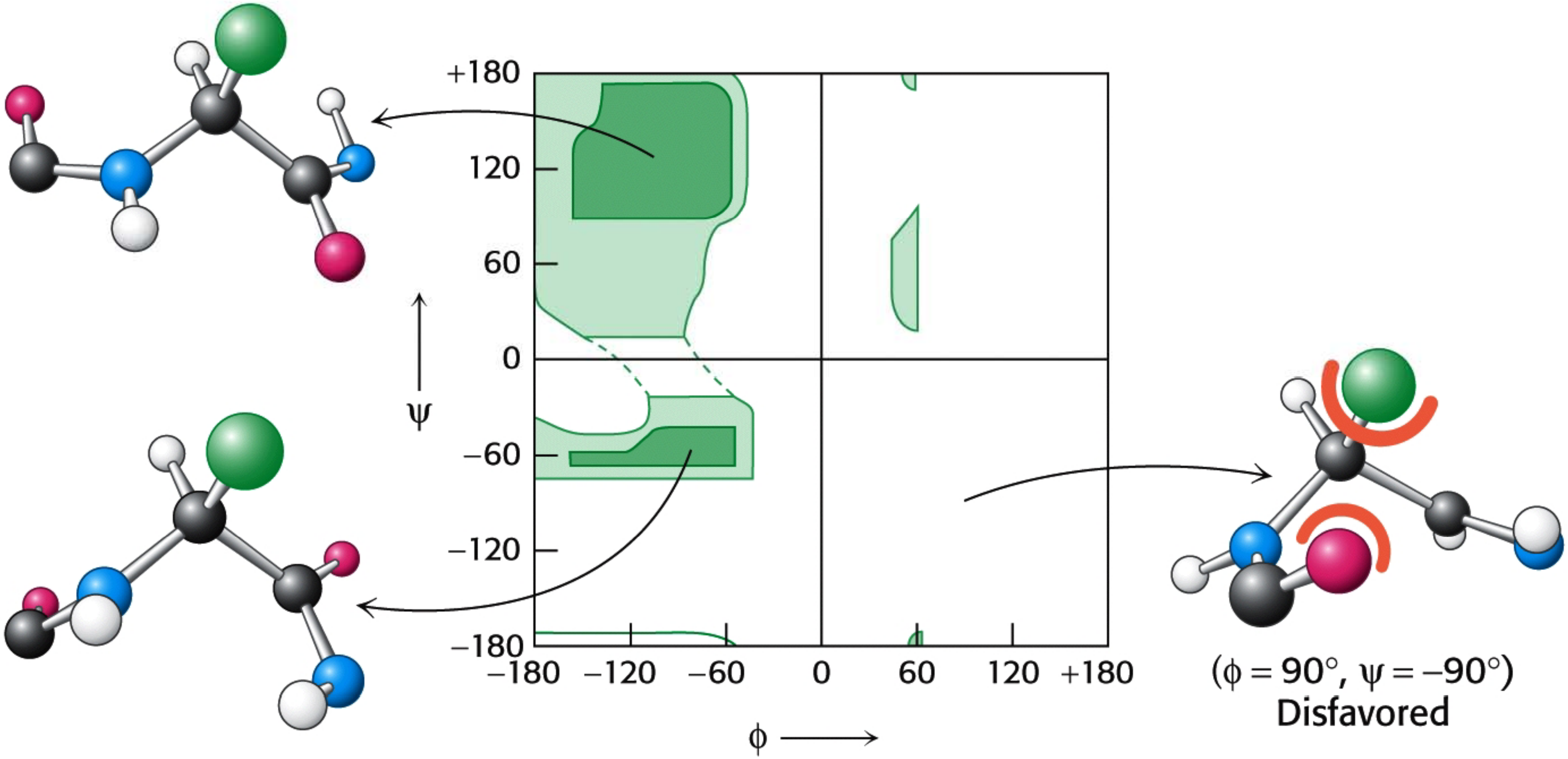


ω (omega) = Cα to Cα ⟷

ψ (psi) = N to N ⟷

ϕ (phi) = C to C ⟷

Ramachandran plot



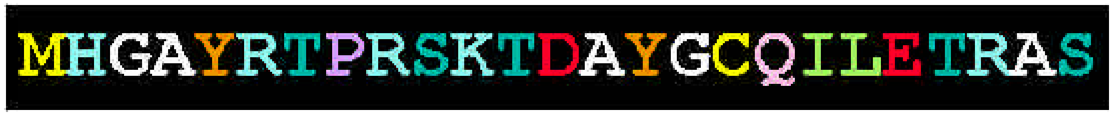
(ϕ = 90°, ψ = −90°)
Disfavored

Levels of protein structure

- Primary structure
- Secondary structure
- Tertiary structure
- Quaternary structure

Primary structure

- This is simply the amino acid sequences of polypeptides chains (proteins).

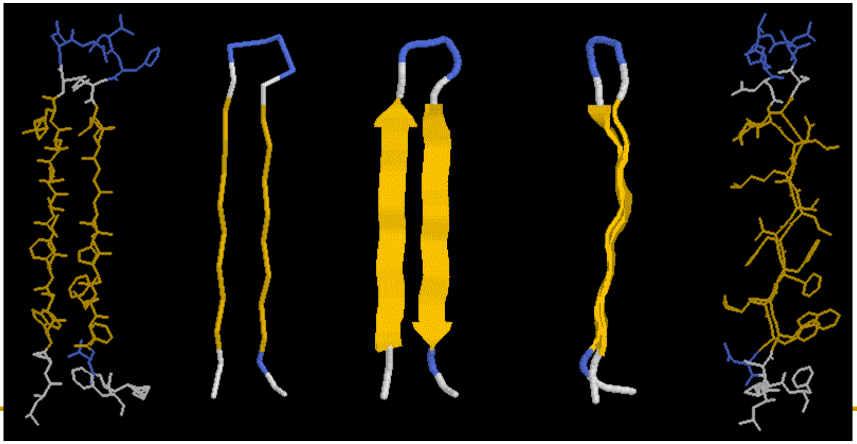


MHGAYRTPRSKTDAYGCQILETRAS

Secondary structure

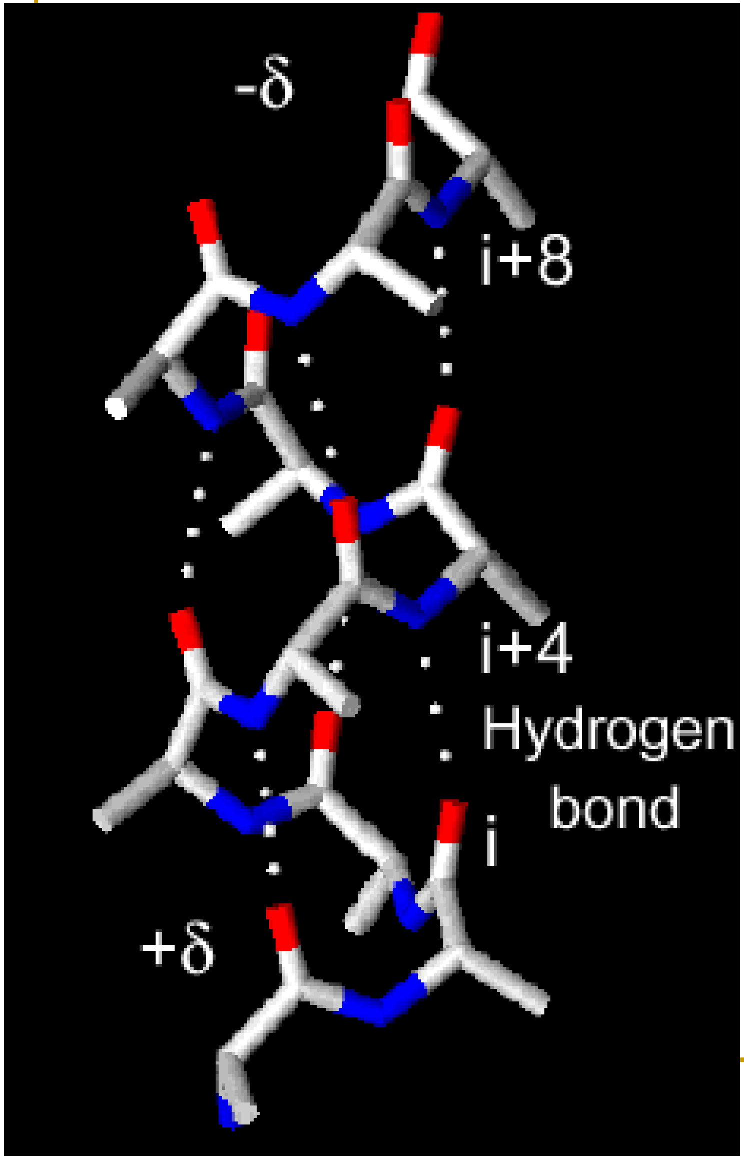
- Local organization of protein backbone: α-helix, β-strand (groups of β-strands assemble into β-sheet), turn and interconnecting loop.

an α-helix



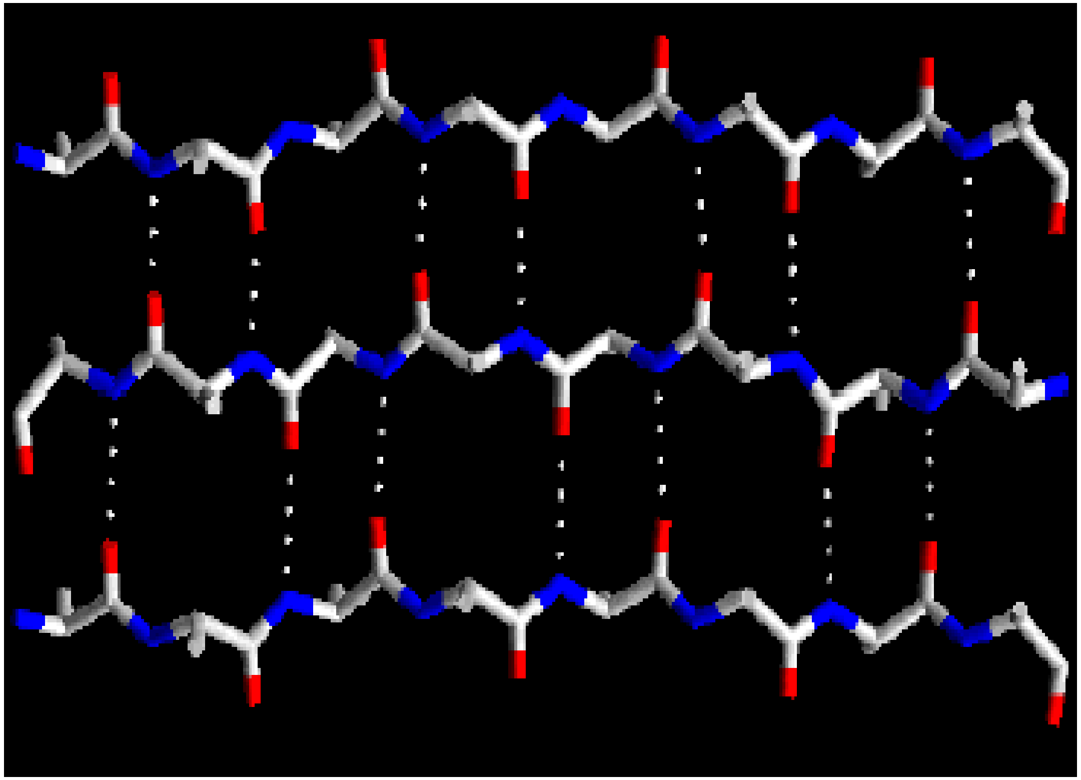
various representations and orientations of a two stranded β-sheet.

The α-helix



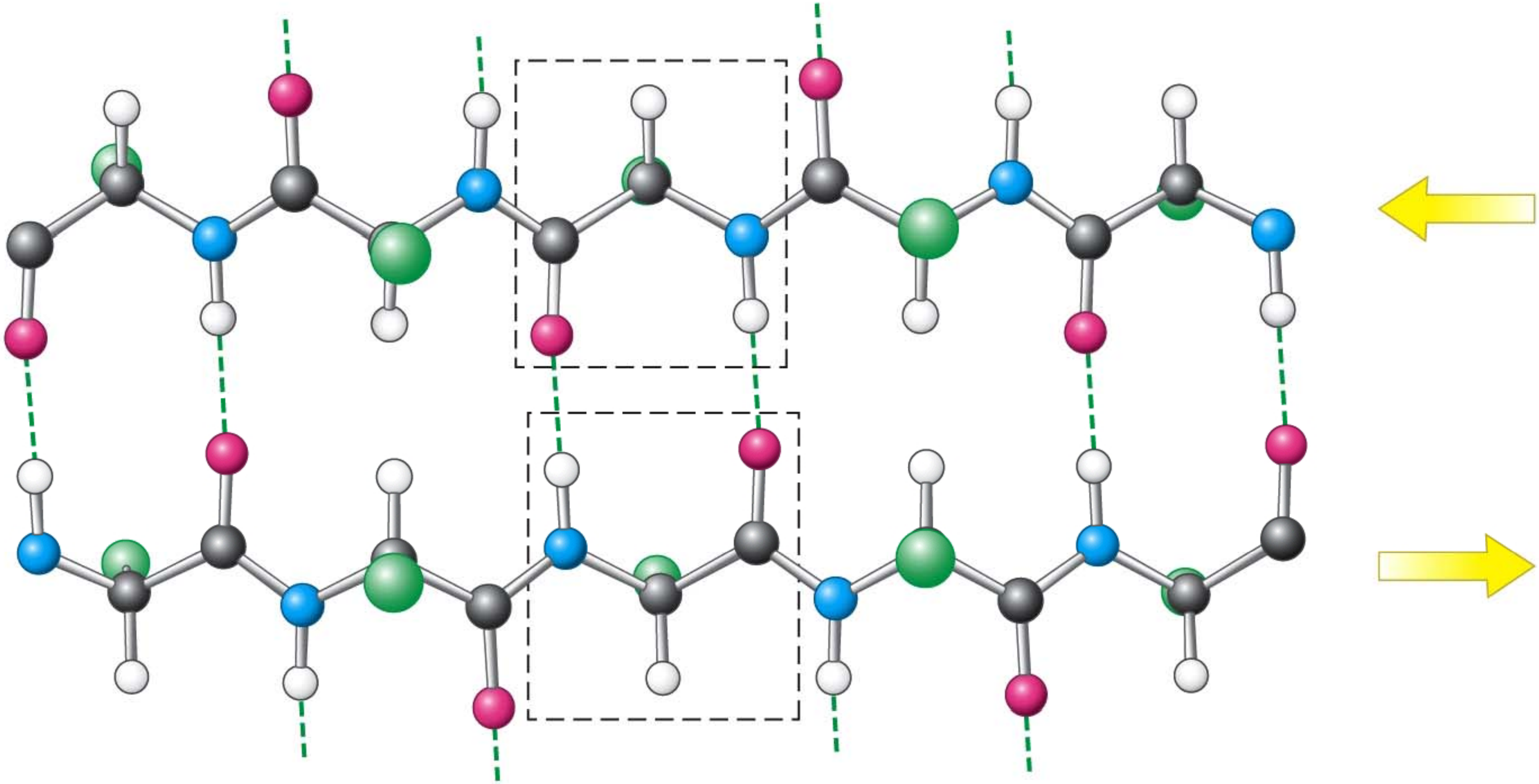
- One of the most closely packed arrangement of residues.
- Turn: 3.6 residues
- Pitch: 5.4 Å/turn

The β-sheet

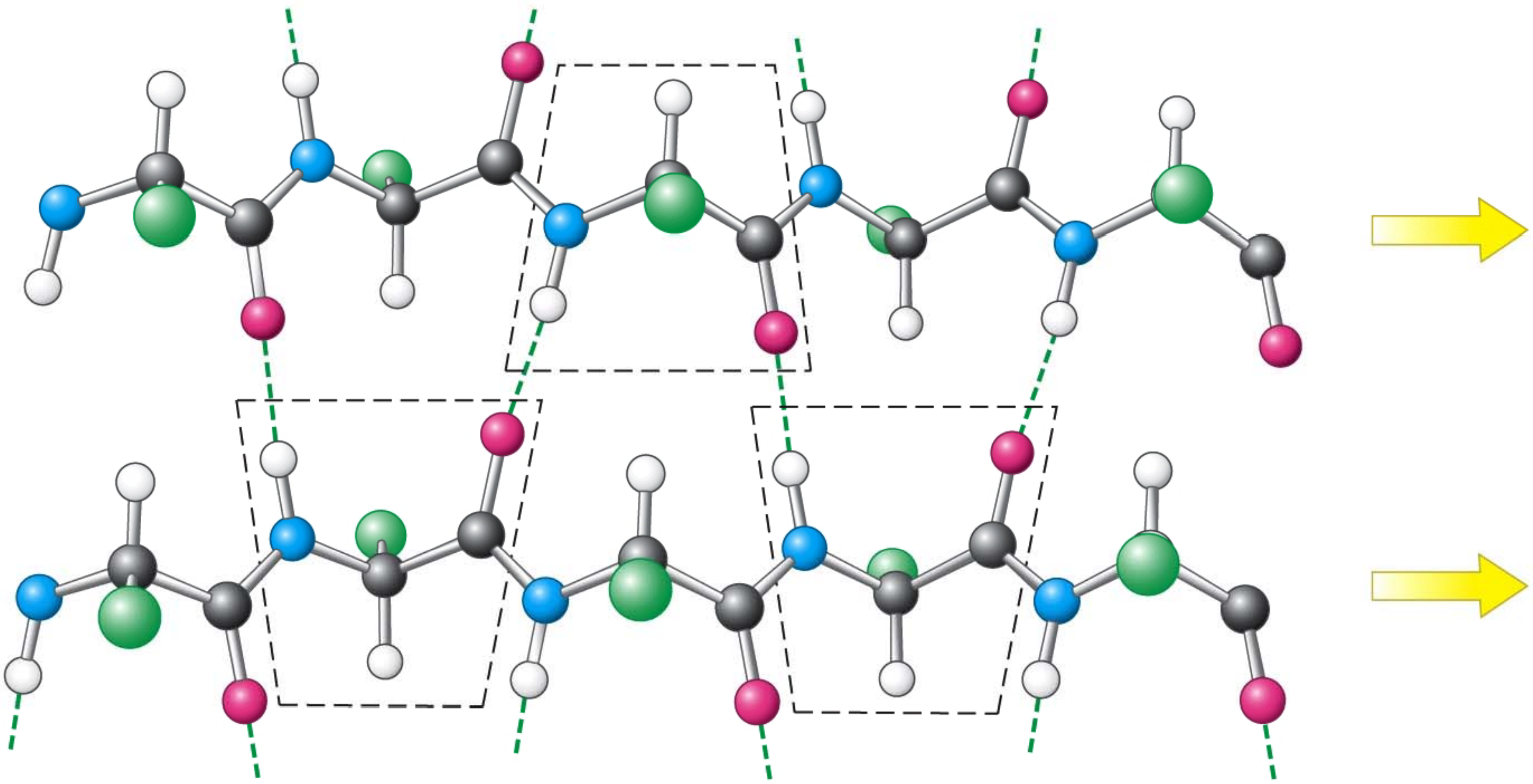


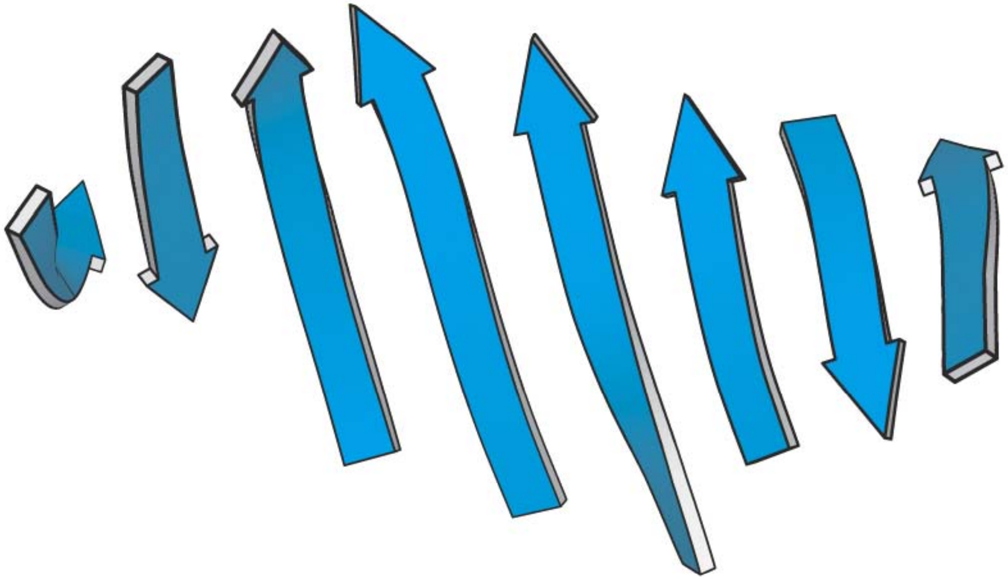
- Backbone almost fully extended, loosely packed arrangement of residues.

Anti-parallel beta sheet



Parallel beta sheet

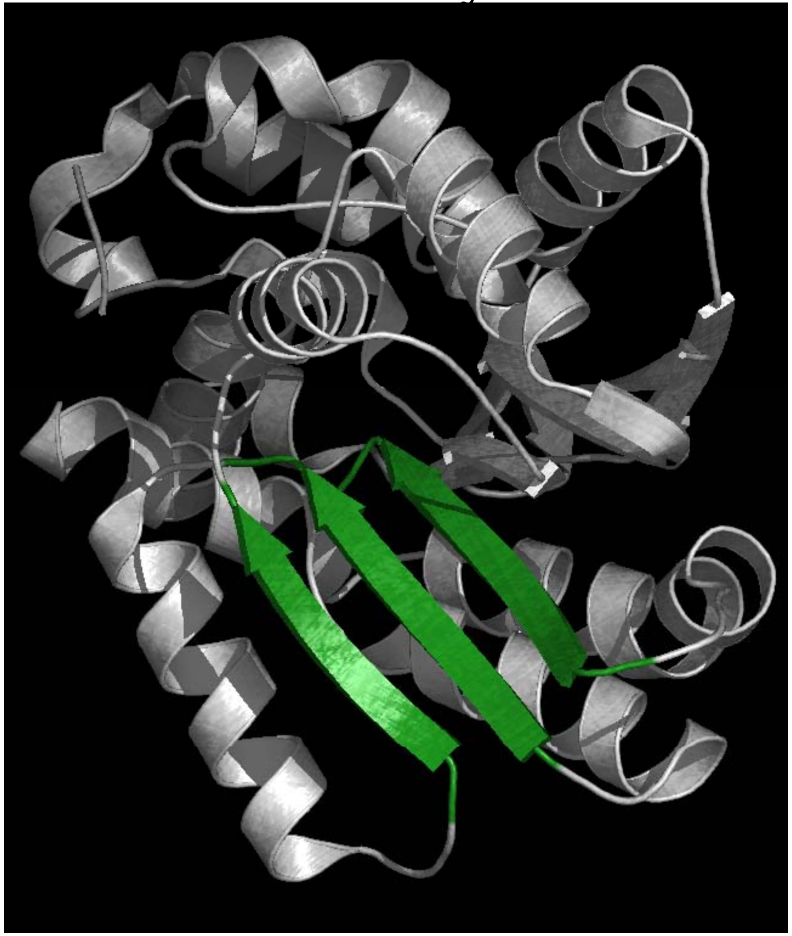




β-Sheet (parallel)

All strands run in the same direction

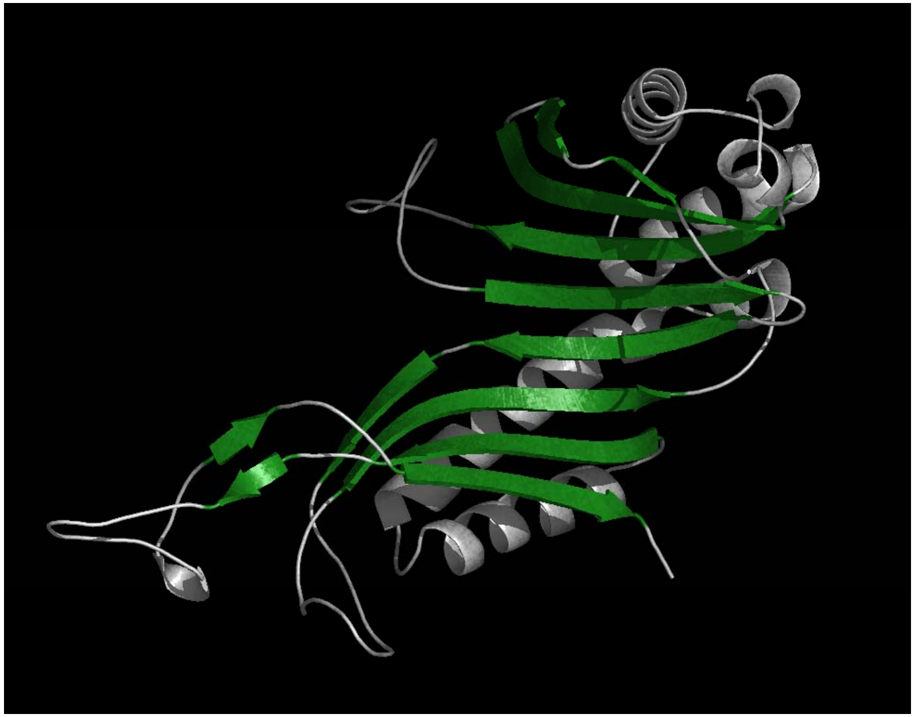
Catechol O-Methyltransferase



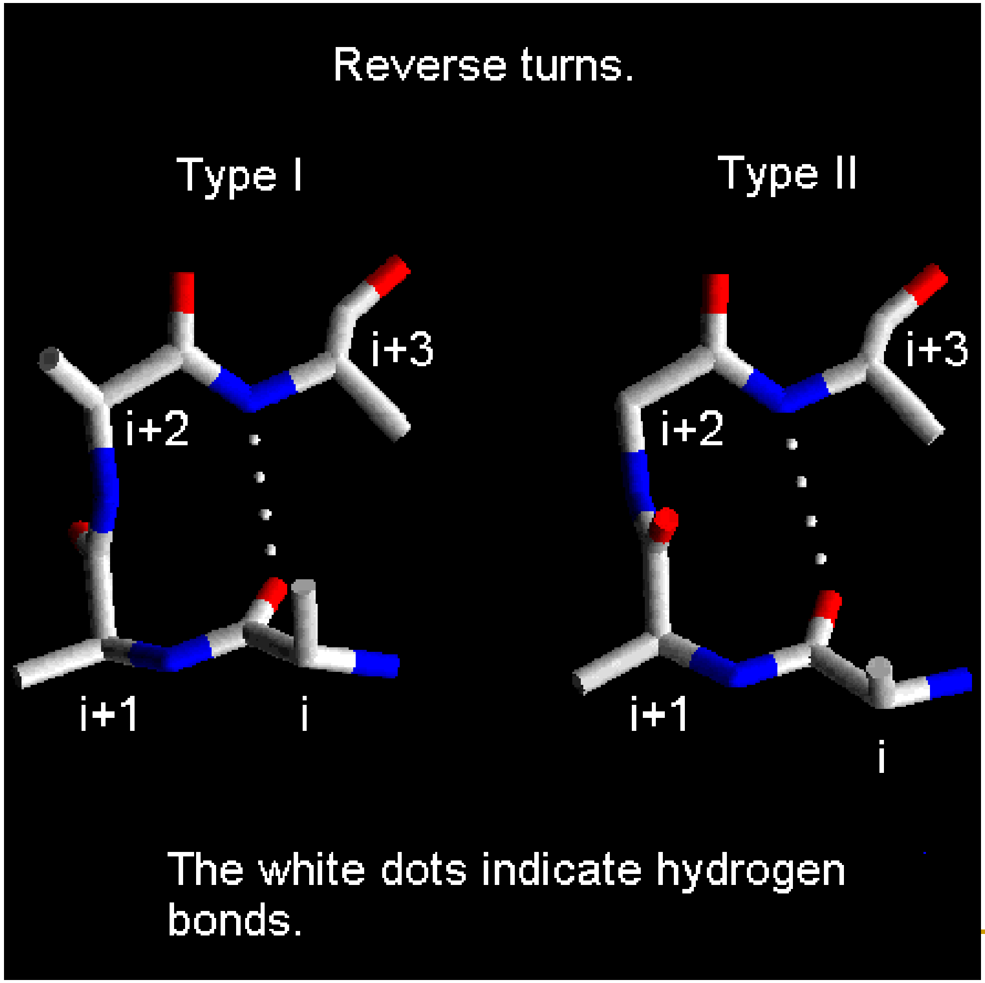
β-Sheet (antiparallel)

All strands run in the opposite direction, more stable

Urate oxidase



Loops and Turns



The white dots indicate hydrogen bonds.

Loops: often contain hydrophilic residue on the surface of proteins

Turns: loops with less than 5 residues and often contain G, P

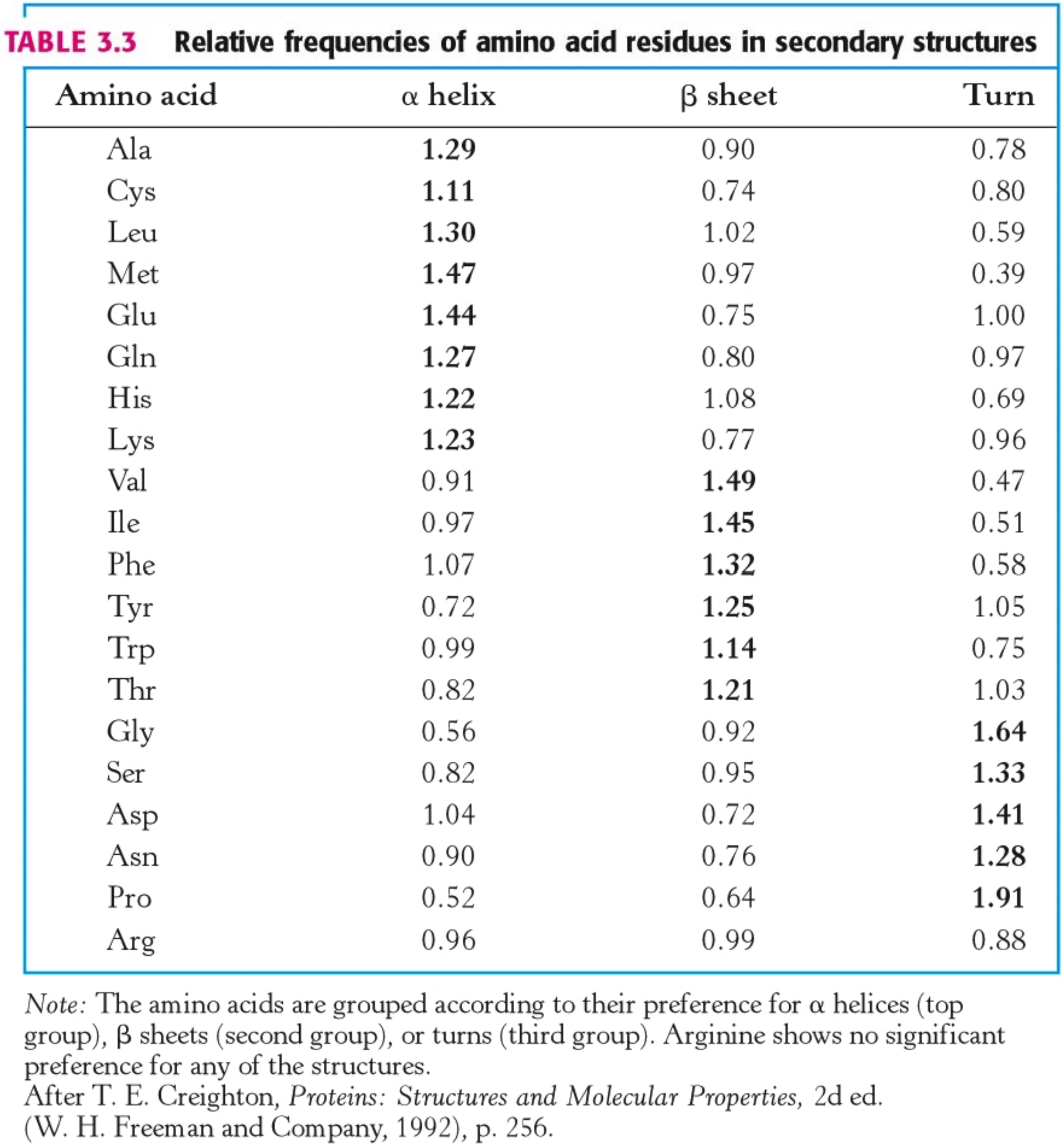


TABLE 3.3 **Relative frequencies of amino acid residues in secondary structures**

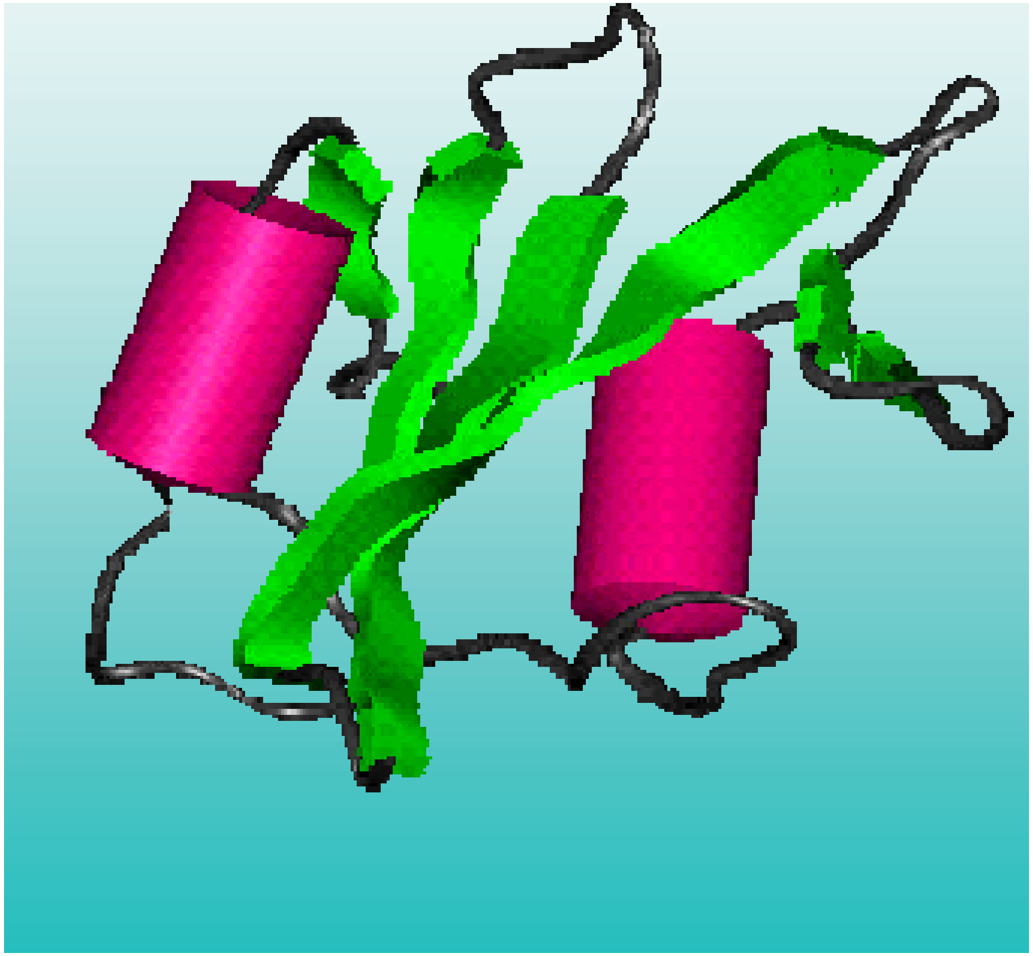
Amino acid	α helix	β sheet	Turn
Ala	**1.29**	0.90	0.78
Cys	**1.11**	0.74	0.80
Leu	**1.30**	1.02	0.59
Met	**1.47**	0.97	0.39
Glu	**1.44**	0.75	1.00
Gln	**1.27**	0.80	0.97
His	**1.22**	1.08	0.69
Lys	**1.23**	0.77	0.96
Val	0.91	**1.49**	0.47
Ile	0.97	**1.45**	0.51
Phe	1.07	**1.32**	0.58
Tyr	0.72	**1.25**	1.05
Trp	0.99	**1.14**	0.75
Thr	0.82	**1.21**	1.03
Gly	0.56	0.92	**1.64**
Ser	0.82	0.95	**1.33**
Asp	1.04	0.72	**1.41**
Asn	0.90	0.76	**1.28**
Pro	0.52	0.64	**1.91**
Arg	0.96	0.99	0.88

Note: The amino acids are grouped according to their preference for α helices (top group), β sheets (second group), or turns (third group). Arginine shows no significant preference for any of the structures.
After T. E. Creighton, *Proteins: Structures and Molecular Properties,* 2d ed.
(W. H. Freeman and Company, 1992), p. 256.

Tertiary structure

- Description of the type and location of SSEs is a chain's *secondary structure.*
- Three-dimensional coordinates of the atoms of a chain is its *tertiary structure*.
- *Quaternary structure*: describes the spatial packing of several folded polypeptides

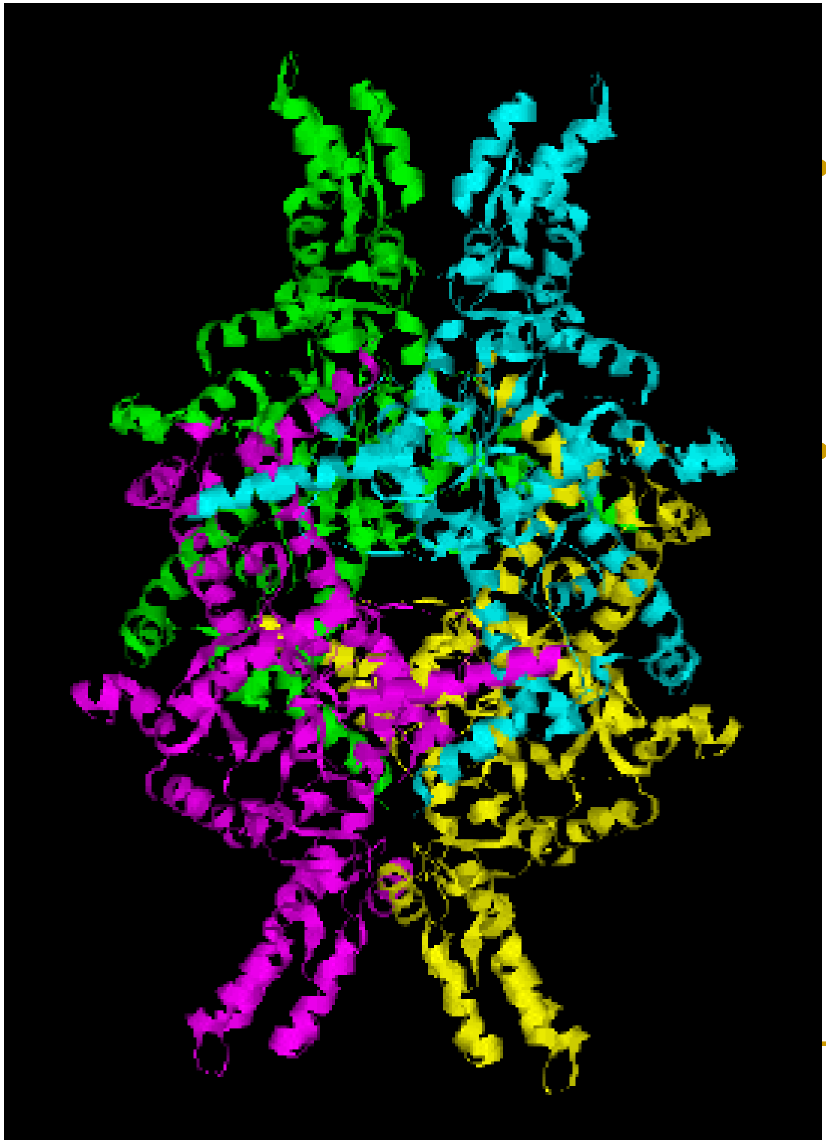
Tertiary structure



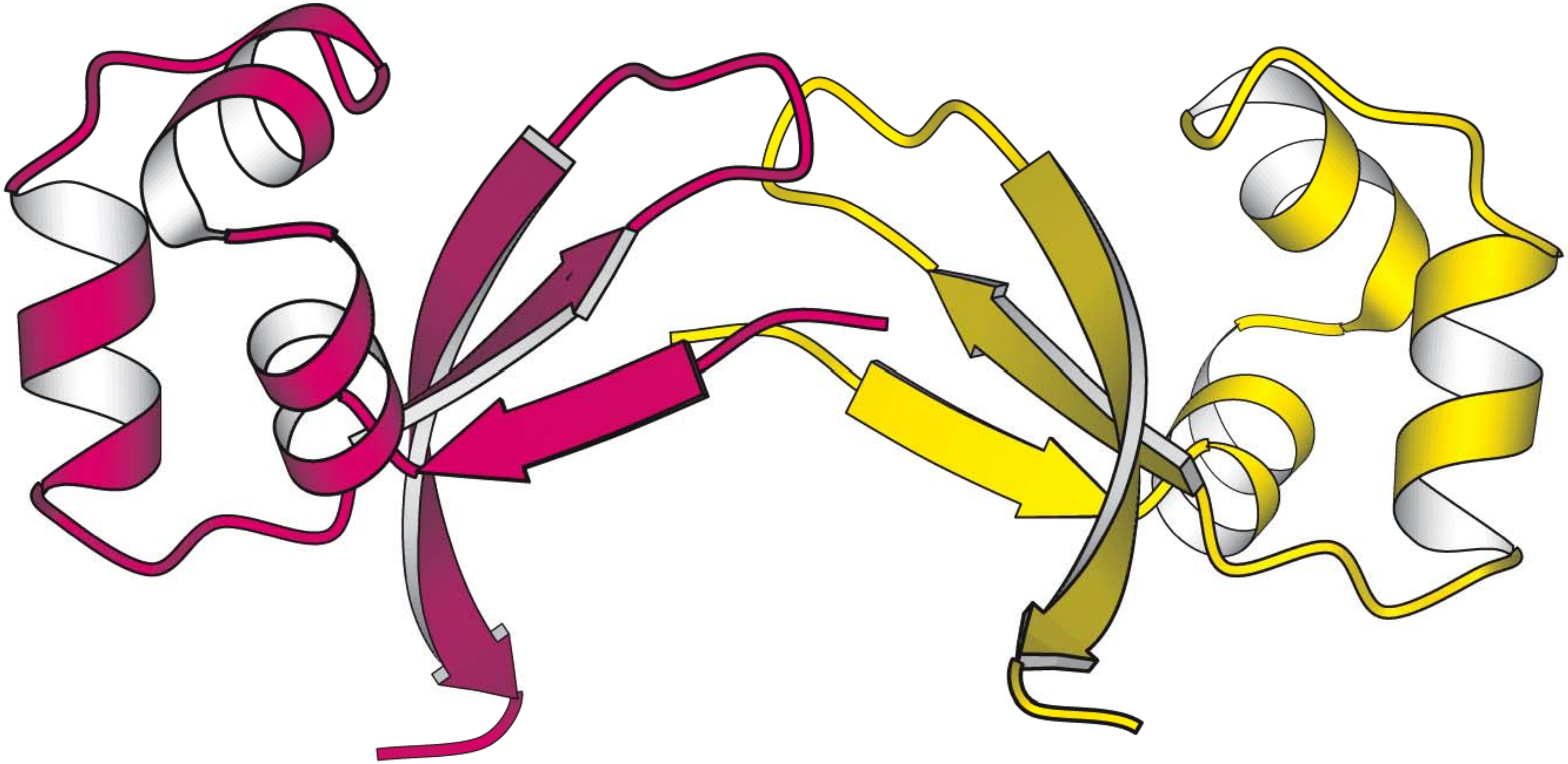
Packing the secondary structure elements into a compact spatial unit

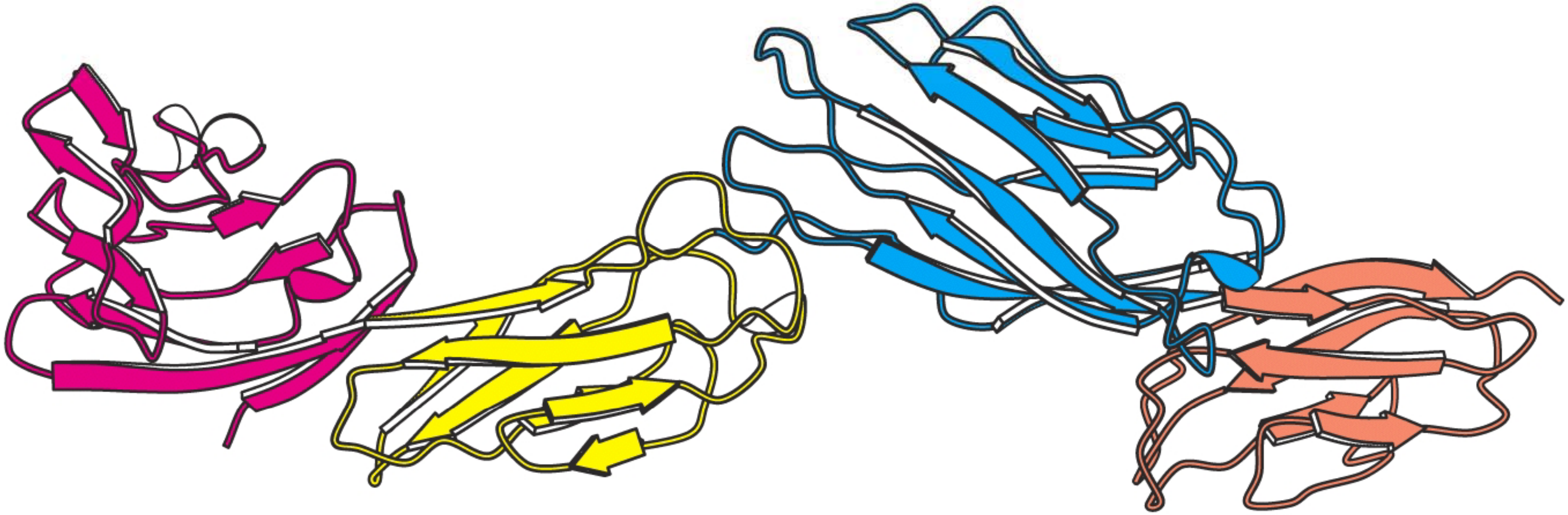
“Fold” or domain– this is the level to which structure prediction is currently possible.

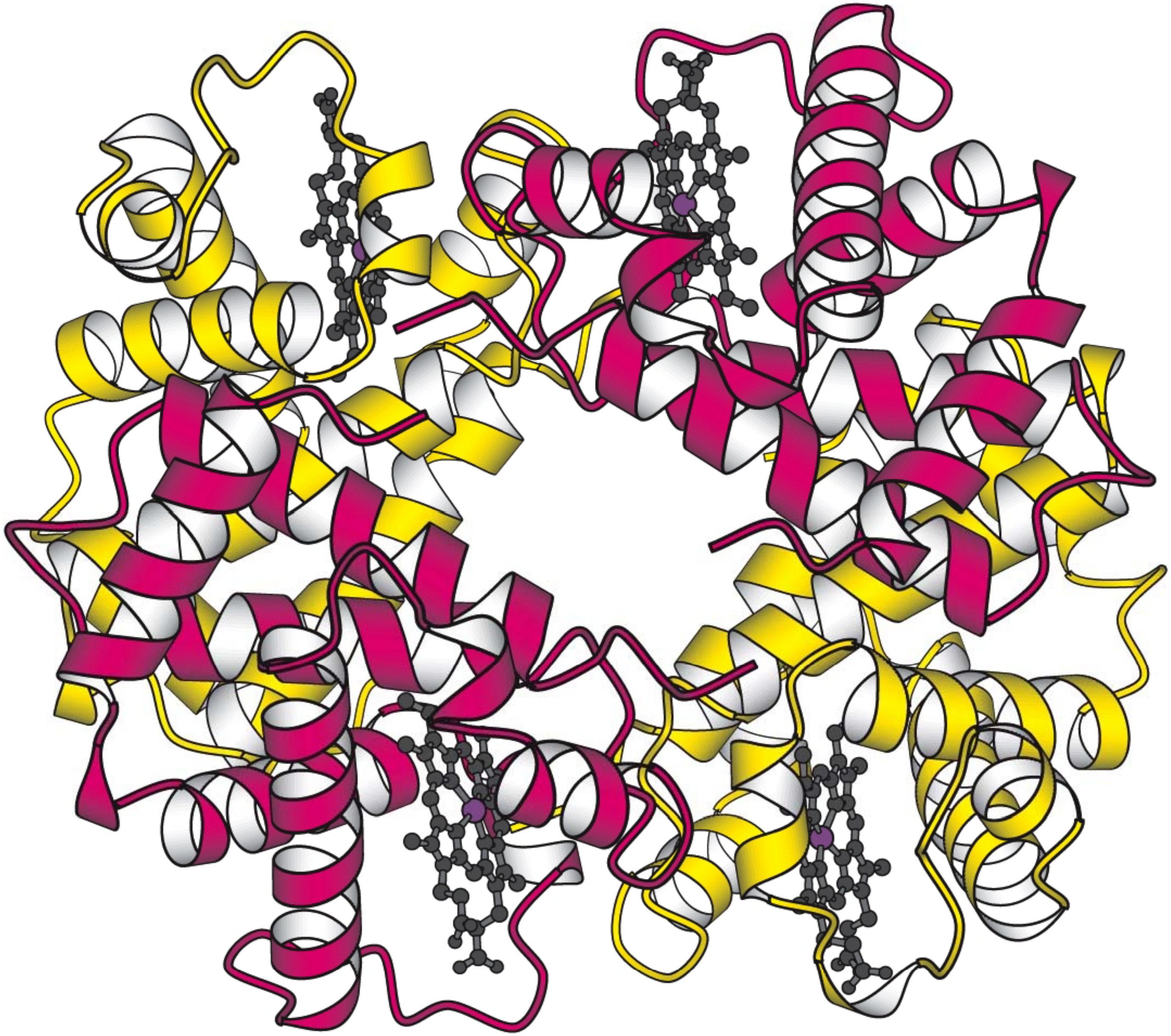
Quaternary structure



- Assembly of homo or heteromeric protein chains.
- Usually the functional unit of a protein, especially for enzymes







- Primary and secondary structure are ONE-dimensional; Tertiary and quaternary structure are THREE-dimensional.

- “structure” usually refers to 3-D structure of protein.

PDB Files: the “header”

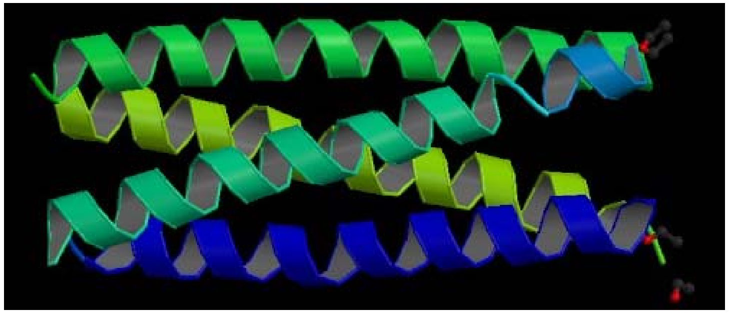
```
HEADER   OXIDOREDUCTASE(SUPEROXIDE ACCEPTOR)     13-JUL-94
COMPND   MANGANESE SUPEROXIDE DISMUTASE (E.C.1.15.1.1) COMPLEXED
COMPND   2 WITH AZIDE
OURCE    (THERMUS THERMOPHILUS, HB8)
AUTHOR   M.S.LAH,M.DIXON,K.A.PATTRIDGE,W.C.STALLINGS,J.A.FEE,
AUTHOR   2 M.L.LUDWIG
REVDAT   2   15-MAY-95
REVDAT   1   15-OCT-94
JRNL     AUTH   M.S.LAH,M.DIXON,K.A.PATTRIDGE,W.C.STALLINGS,
JRNL     AUTH 2 J.A.FEE,M.L.LUDWIG
JRNL     TITL   STRUCTURE-FUNCTION IN E. COLI IRON SUPEROXIDE
JRNL     TITL 2 DISMUTASE: COMPARISONS WITH THE MANGANESE ENZYME
JRNL     TITL 3 FROM T. THERMOPHILUS
JRNL     REF    TO BE PUBLISHED
REMARK   1  AUTH   M.L.LUDWIG,A.L.METZGER,K.A.PATTRIDGE,W.C.STALLINGS
REMARK   1  TITL   MANGANESE SUPEROXIDE DISMUTASE FROM THERMUS
REMARK   1  TITL 2 THERMOPHILUS.  A STRUCTURAL MODEL REFINED AT 1.8
REMARK   1  TITL 3 ANGSTROMS RESOLUTION
REMARK   1  REF    J.MOL.BIOL.                  V. 219   335 1991
REMARK   1  REFN   ASTM JMOBAK  UK ISSN 0022-2836
REMARK   1 REFERENCE 2
REMARK   1  AUTH   W.C.STALLINGS,C.BULL,J.A.FEE,M.S.LAH,M.L.LUDWIG
REMARK   1  TITL   IRON AND MANGANESE SUPEROXIDE DISMUTASES:
REMARK   1  TITL 2 CATALYTIC INFERENCES FROM THE STRUCTURES
```

PDB Files: the coordinates

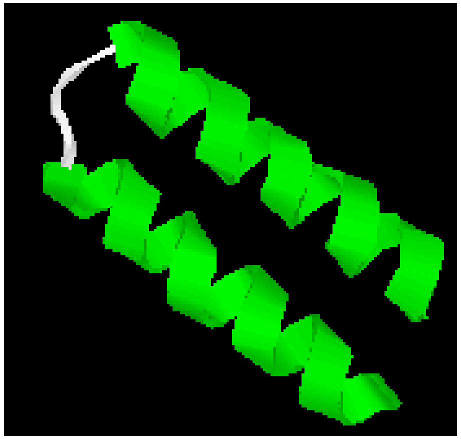
Atom & Residue **XYZ Coordinates**

```
ATOM      1  N   PRO A   1      10.846  26.225 -13.938  1.00 30.15      1MNG 192
ATOM      2  CA  PRO A   1      12.063  25.940 -14.715  1.00 28.55      1MNG 193
ATOM      3  C   PRO A   1      12.061  26.809 -15.946  1.00 26.55      1MNG 194
ATOM      4  O   PRO A   1      11.151  27.612 -16.176  1.00 26.17      1MNG 195
ATOM      5  CB  PRO A   1      12.010  24.474 -15.162  1.00 30.21      1MNG 196
ATOM      6  CG  PRO A   1      11.044  23.902 -14.231  1.00 31.38      1MNG 197
ATOM      7  CD  PRO A   1       9.997  25.028 -14.008  1.00 31.86      1MNG 198
ATOM      8  N   TYR A   2      13.050  26.576 -16.777  1.00 23.36      1MNG 199
ATOM      9  CA  TYR A   2      13.197  27.328 -17.983  1.00 22.11      1MNG 200
ATOM     10  C   TYR A   2      12.083  27.050 -19.032  1.00 21.02      1MNG 201
ATOM     11  O   TYR A   2      11.733  25.895 -19.264  1.00 21.68      1MNG 202
ATOM     12  CB  TYR A   2      14.579  26.999 -18.523  1.00 20.16      1MNG 203
ATOM     13  CG  TYR A   2      14.905  27.662 -19.832  1.00 19.42      1MNG 204
ATOM     14  CD1 TYR A   2      14.516  27.092 -21.038  1.00 18.28      1MNG 205
ATOM     15  CD2 TYR A   2      15.610  28.864 -19.875  1.00 19.69      1MNG 206
ATOM     16  CE1 TYR A   2      14.813  27.696 -22.233  1.00 19.13      1MNG 207
ATOM     17  CE2 TYR A   2      15.924  29.465 -21.070  1.00 19.25      1MNG 208
ATOM     18  CZ  TYR A   2      15.515  28.863 -22.251  1.00 19.25      1MNG 209
ATOM     19  OH  TYR A   2      15.857  29.417 -23.448  1.00 21.67      1MNG 210
ATOM     20  N   PRO A   3      11.583  28.094 -19.731  1.00 19.90      1MNG 211
ATOM     21  CA  PRO A   3      11.912  29.520 -19.665  1.00 18.36      1MNG 212
```

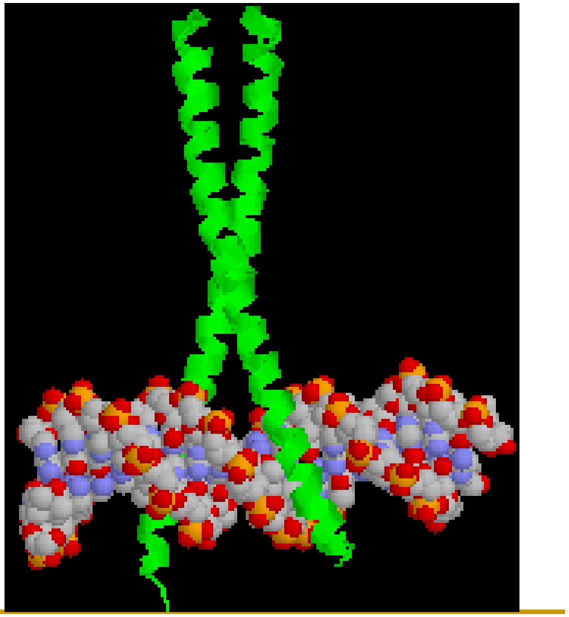
Motifs



Four helix bundle



Helix-loop-helix



Coiled coil

Secondary structure prediction

- Given a protein sequence (primary structure)

GHWIATRGQLIREAYEDYRHFSSECPFIP

- Predict its secondary structure content

  (C=coils H=Alpha Helix E=Beta Strands)

CEEEEECHHHHHHHHHHHCCCHHCCCCCC

Why Secondary Structure Prediction?

- Easier problem than 3D structure prediction (more than 40 years of history).
- Accurate secondary structure prediction can be an important information for the tertiary structure prediction
- Improving sequence alignment accuracy
- Protein function prediction
- Protein classification
- Predicting structural change

Prediction Methods

- Statistical methods
  - Chou-Fasman method, GOR I-IV
- Nearest neighbors
  - NNSSP, SSPAL
- Neural network
  - PHD, Psi-Pred, J-Pred
- Support vector machine

Assumptions

- The entire information for forming secondary structure is contained in the primary sequence.
- Side groups of residues will determine structure.
- Examining windows of 13 - 17 residues is sufficient to predict structure.

Chou-Fasman method

- Compute parameters for amino acids
  - Preference to be in
    - alpha helix: P(a)
    - beta sheet: P(b)
    - Turn: P(turn)
  - Frequencies with which the amino acid is in the 1st, 2nd, 3rd, and 4th position of a turn: f(i), f(i+1), f(i+2), f(i+3).
- Use a sliding window

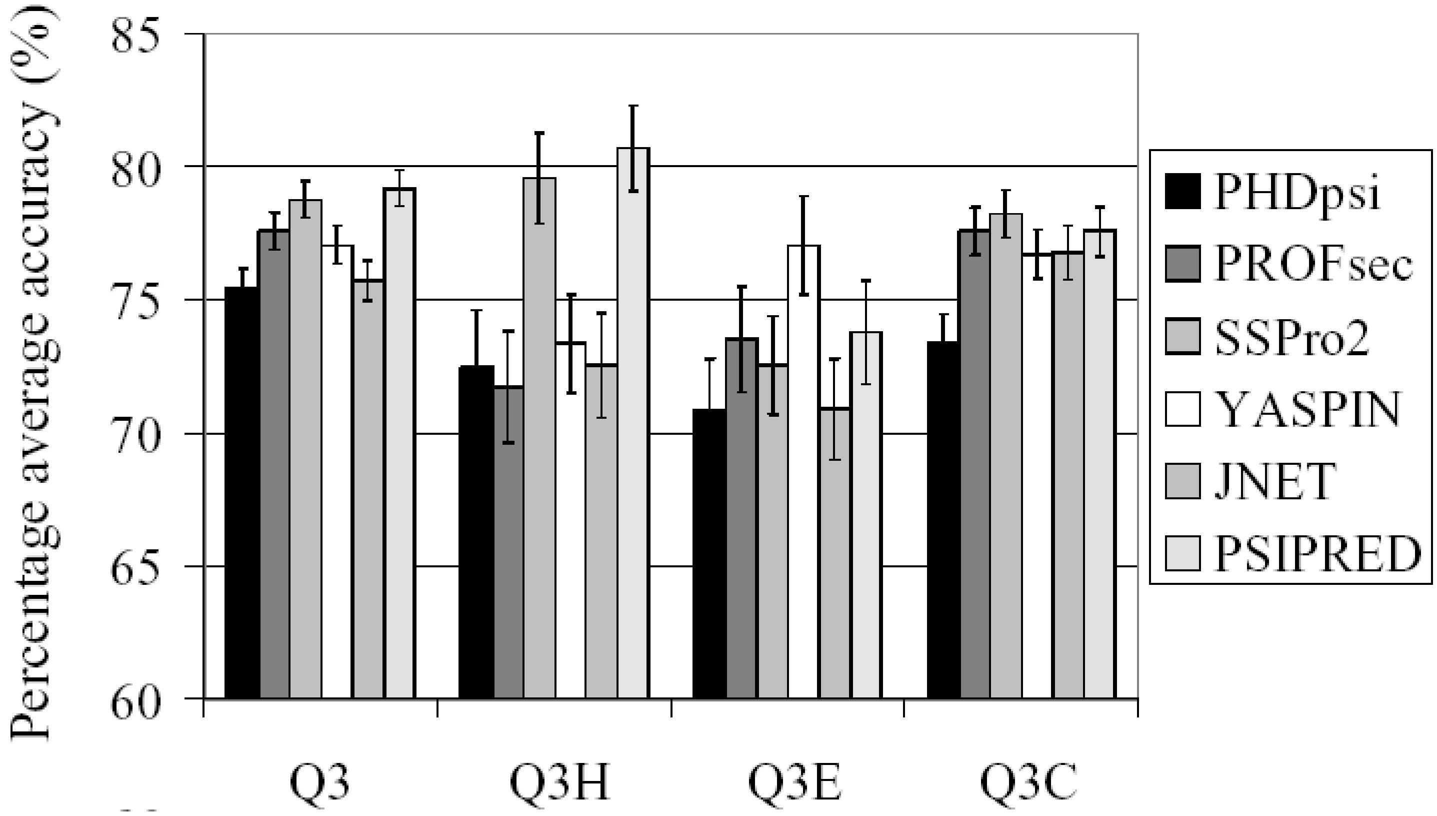
SSE prediction

- Alpha-helix prediction
  - Find all regions where 4 of the 6 amino acids in window have P(a) > 100.
  - Extend the region in both directions unless 4 consecutive residues have P(a) < 100.
  - If Σ P(a) > Σ P(b) then the region is predicted to be alpha-helix.
- Beta-sheet prediction is analogous.
- Turn prediction
  - Compute P(t) = f(i) + f(i+1) + f(i+2) + f(i+3) for 4 consecutive residues.
  - Predict a turn if
    - P(t) > 0.000075 (check)
    - The average P(turn) > 100
    - Σ P(turn) > Σ P(a) and Σ P(turn) > Σ P(b)

GOR method

- Use a sliding window of 17 residues
- Compute the frequencies with which each amino acid occupies the 17 positions in helix, sheet, and turn.
- Use this to predict the SSE probability of each residue.

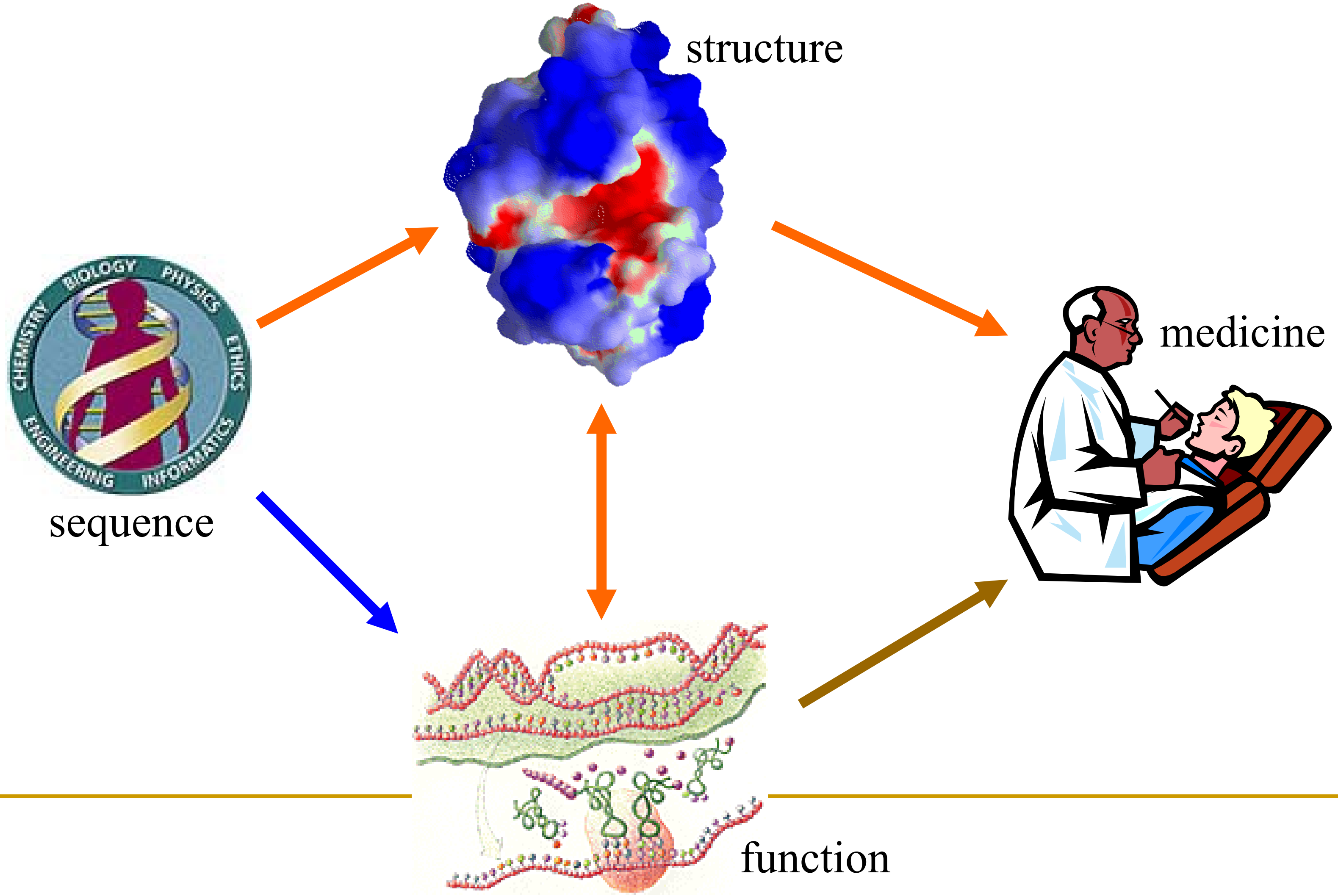
Performance of SSE prediction



Q3 and SOV are standards for computing errors

A Simple and Fast Secondary Structure Prediction Method using Hidden Neural Networks
Kuang Lin, Victor A. Simossis, Willam R. Taylor, Jaap Heringa,
Bioinformatics Advance Access published September 17, 2004

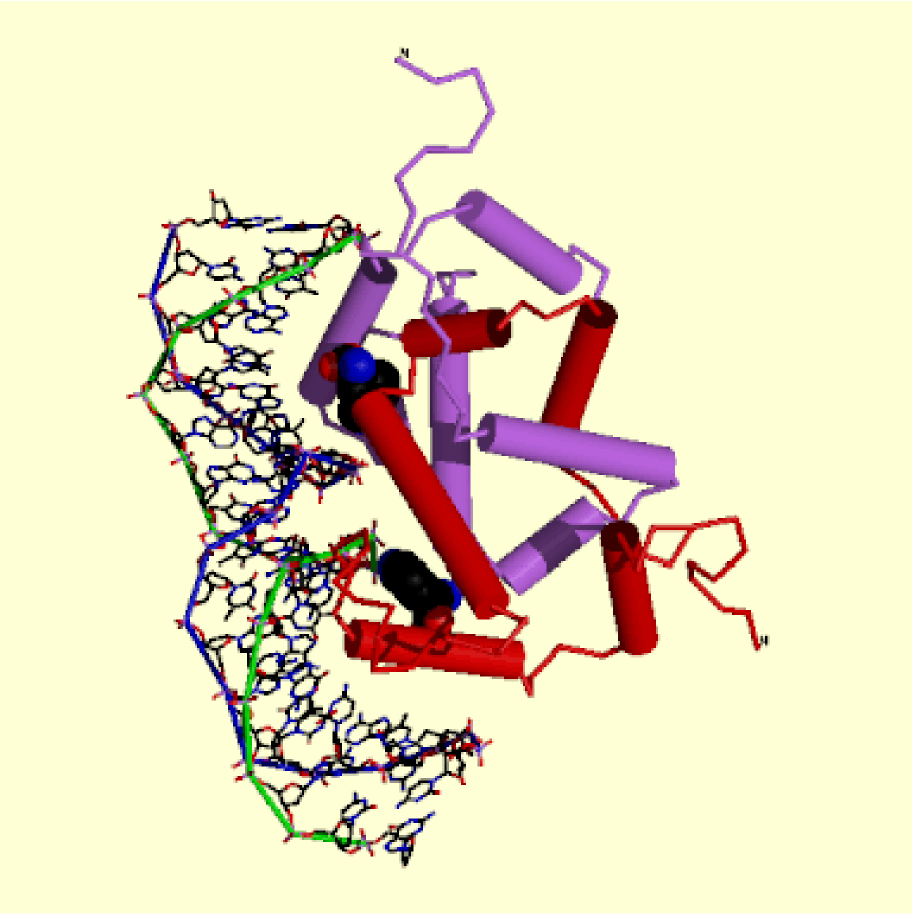
Relevance of Protein Structure in the Post-Genome Era



Structure-Function Relationship

Certain level of function can be found without structure. But a structure is a key to understand the detailed mechanism.

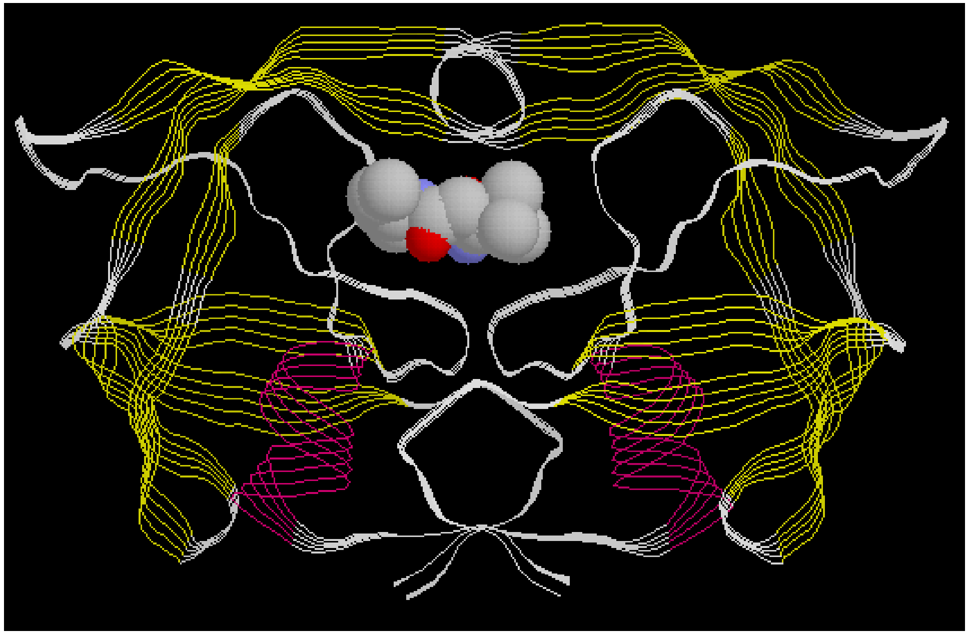
A predicted structure is a powerful tool for function inference.



Trp repressor as a function switch

Structure-Based Drug Design

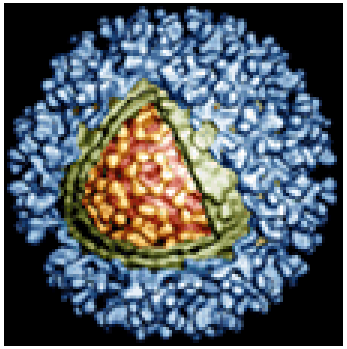
Structure-based rational drug design is a major method for drug discovery.



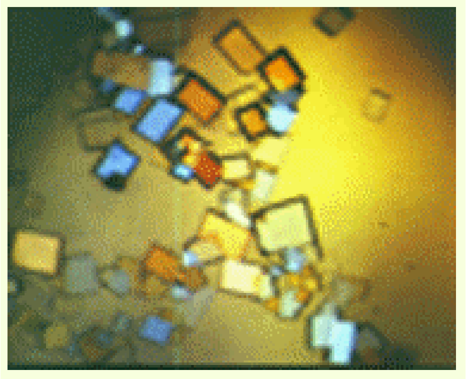
HIV protease inhibitor

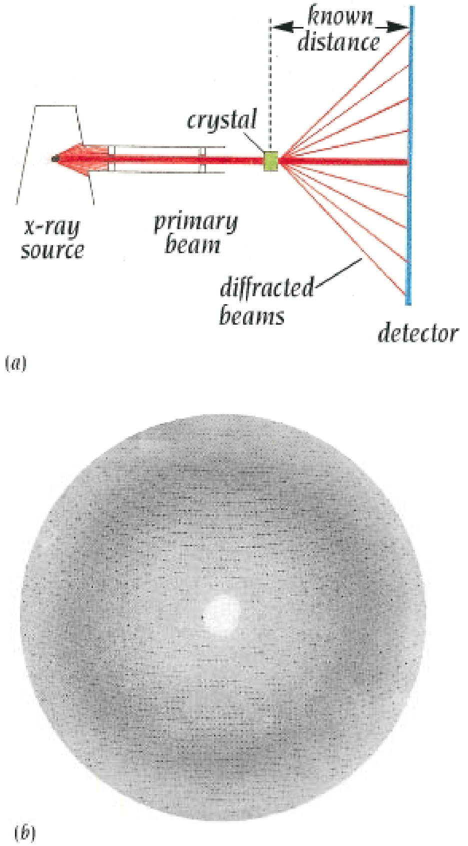
Experimental techniques for structure determination

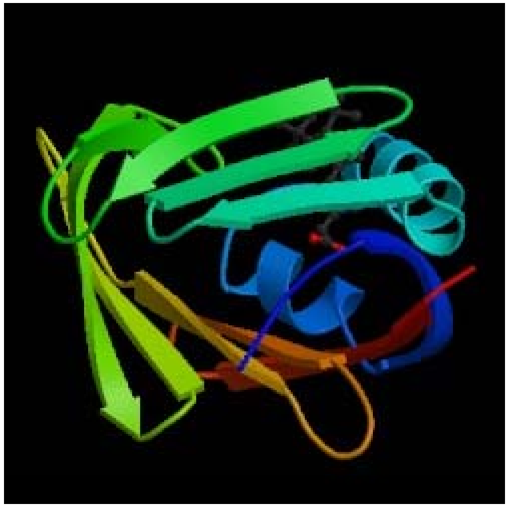
- X-ray Crystallography
- Nuclear Magnetic Resonance spectroscopy (NMR)
- Electron Microscopy/Diffraction
- Free electron lasers ?



X-ray Crystallography

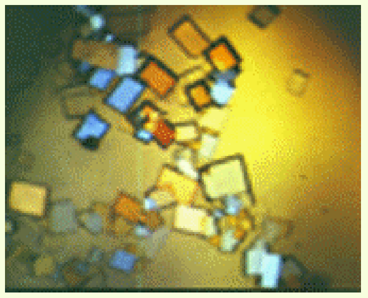






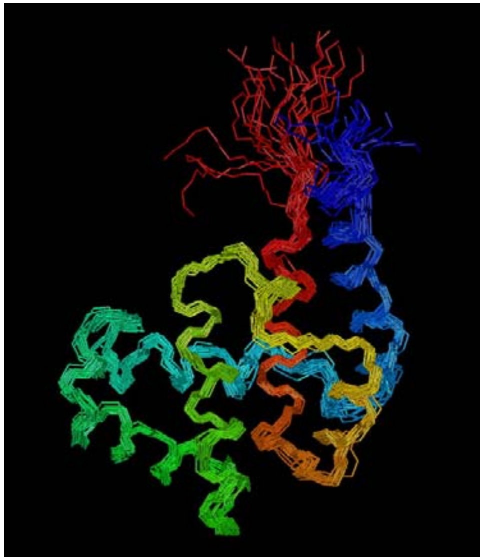
X-ray Crystallography..

- From small molecules to viruses
- Information about the positions of individual atoms
- Limited information about dynamics
- Requires crystals



NMR

- Limited to molecules up to ~50kDa (good quality up to 30 kDa)
- Information about distances between pairs of atoms
  - A 2-d resonance spectrum with off-diagonal peaks
- Requires soluble, non-aggregating material

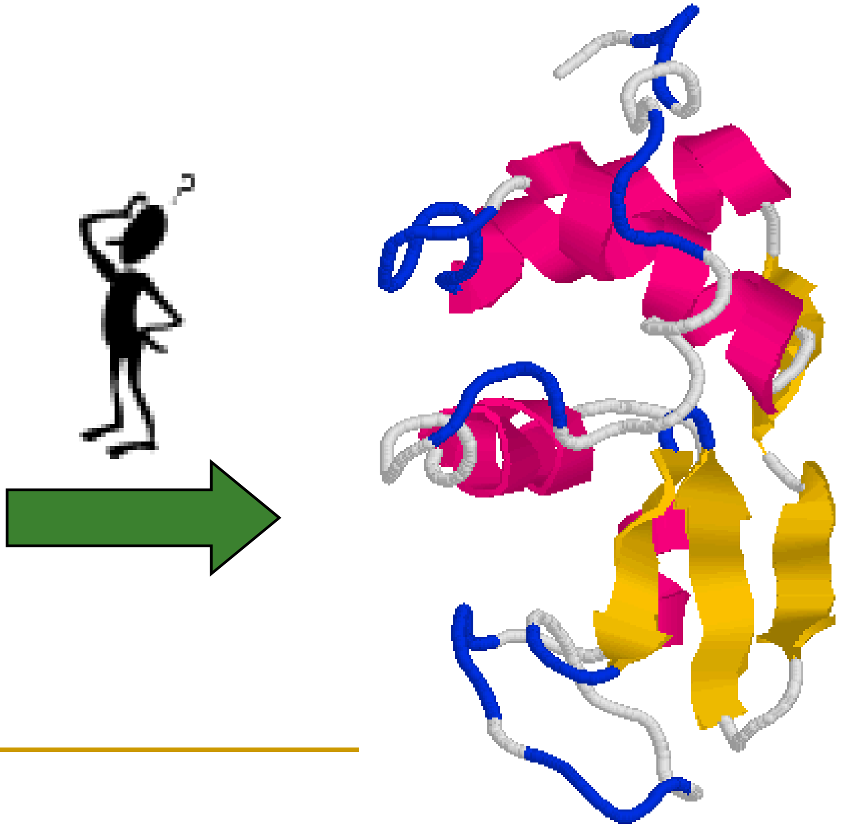


Protein Folding Problem

A protein folds into a unique 3D structure under the physiological condition: determine this structure

Lysozyme sequence:

```
KVFGRCELAA AMKRHGLDNY
RGYSLGNWVC AAKFESNFNT
QATNRNTDGS TDYGILQINS
RWWCNDGRTP GSRNLCNIPC
SALLSSDITA SVNCAKKIVS
DGNGMNAWVA WRNRCKGTDV
QAWIRGCRL
```



Levinthal’s paradox

- Consider a 100 residue protein. If each residue can take only 3 positions, there are $3^{100} = 5 \times 10^{47}$ possible conformations.
  - If it takes 10^{-13}s to convert from 1 structure to another, exhaustive search would take 1.6×10^{27} years!
- Folding must proceed by progressive stabilization of intermediates.

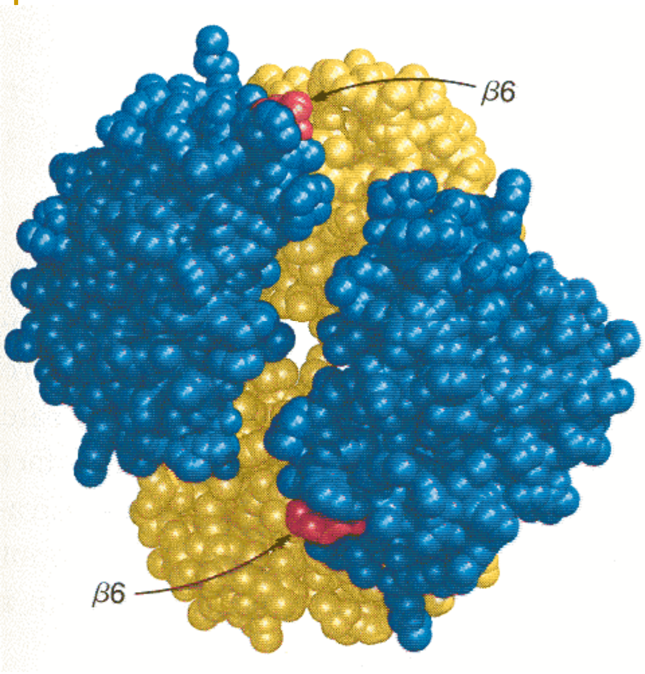
Forces driving protein folding

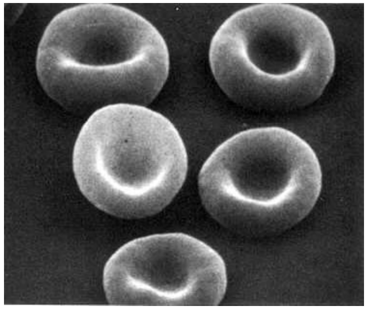
- It is believed that *hydrophobic collapse* is a key driving force for protein folding
  - Hydrophobic core
  - Polar surface interacting with solvent
- Minimum volume (no cavities)
- Disulfide bond formation stabilizes
- Hydrogen bonds
- Polar and electrostatic interactions

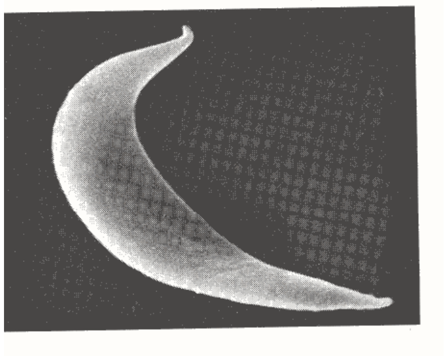
Effect of a single mutation

- Hemoglobin is the protein in red blood cells (erythrocytes) responsible for binding oxygen.
- The mutation E→V in the β chain replaces a charged Glu by a hydrophobic Val on the surface of hemoglobin
- The resulting "sticky patch" causes hemoglobin to agglutinate (stick together) and form fibers which deform the red blood cell and do not carry oxygen efficiently
- Sickle cell anemia was the first identified molecular disease

Sickle Cell Anemia







Sequestering hydrophobic residues in the protein core protects proteins from hydrophobic agglutination.

Protein Structure Prediction

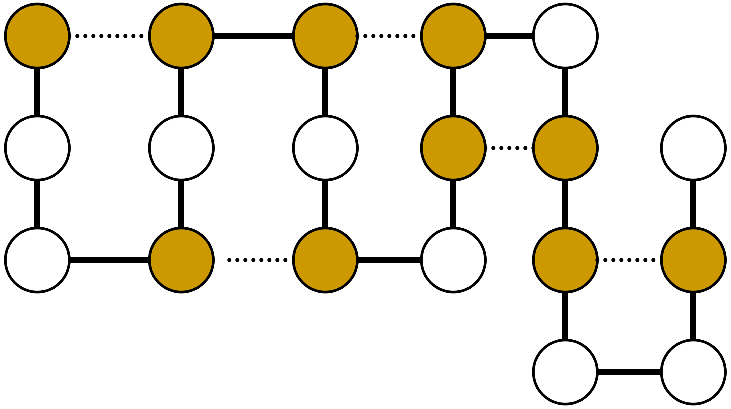
- *Ab-initio* techniques
- Homology modeling
  - Sequence-sequence comparison
- Protein threading
  - Sequence-structure comparison

Lattice models

- Simple lattice models (HP-models)
  - Two types of residues: hydrophobic and polar
  - 2-D or 3-D lattice
  - The only force is hydrophobic collapse
  - Score = number of H–H contacts

Scoring Lattice Models

- H/P model scoring: count hydrophobic interactions.



Score = 5

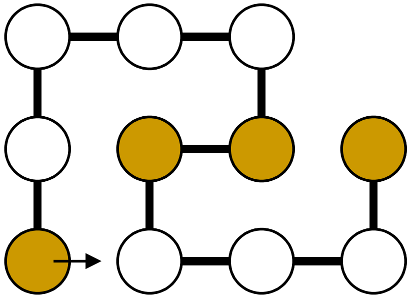
- Sometimes:
  - Penalize for buried polar or surface hydrophobic residues

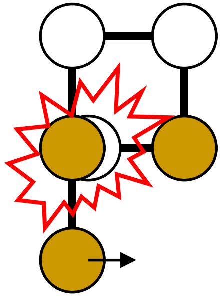
What can we do with lattice models?

- NP-complete
- For smaller polypeptides, exhaustive search can be used
  - Looking at the “best” fold, even in such a simple model, can teach us interesting things about the protein folding process
- For larger chains, other optimization and search methods must be used
  - Greedy, branch and bound
  - Evolutionary computing, simulated annealing
  - Graph theoretical methods

Representing a lattice model

- Absolute directions
  - UURRDLDRRU
- Relative directions
  - LFRFRRLLFL
  - Advantage, we can’t have UD or RL in absolute
  - Only three directions: LRF
- *What about bumps?* LFRRR
  - Give bad score to any configuration that has bumps



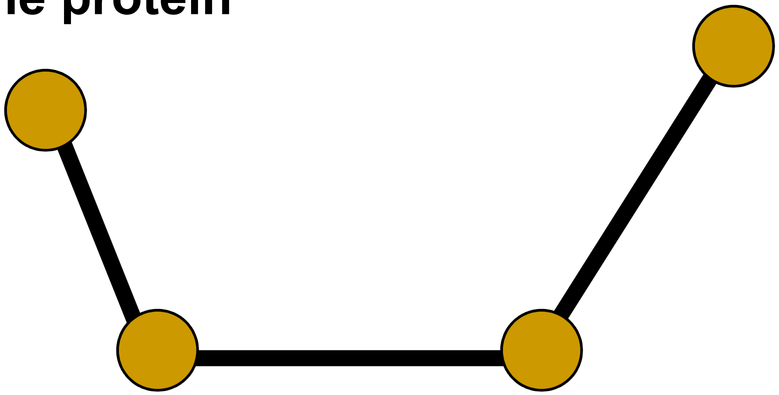


More realistic models

- Higher resolution lattices (45° lattice, etc.)
- Off-lattice models
  - Local moves
  - Optimization/search methods and ϕ/ψ representations
    - Greedy search
    - Branch and bound
    - EC, Monte Carlo, simulated annealing, etc.

Energy functions

- **An energy function to describe the protein**
  - bond energy
  - bond angle energy
  - dihedral angel energy
  - van der Waals energy
  - electrostatic energy



- **Minimize the function and obtain the structure.**
- **Not practical in general**
  - Computationally too expensive
  - Accuracy is poor
- Empirical force fields
  - Start with a database
  - Look at neighboring residues – similar to known protein folds?

Difficulties

Why is structure prediction and especially *ab initio* calculations hard?

- Many degrees of freedom / residue. Computationally too expensive for realistic-sized proteins.

- Remote non-covalent interactions

- Nature does not go through all conformations

- Folding assisted by enzymes & chaperones

Protein Structure Prediction

- *Ab-initio* techniques
- Homology modeling
  - Sequence-sequence comparison
- Protein threading
  - Sequence-structure comparison

Homology modeling steps

1. Identify a set of template proteins (with known structures) related to the target protein. This is based on sequence homology (BLAST, FASTA) with sequence identity of 30% or more.
2. Align the target sequence with the template proteins. This is based on multiple alignment (CLUSTALW). Identify conserved regions.
3. Build a model of the protein backbone, taking the backbone of the template structures (conserved regions) as a model.
4. Model the loops. In regions with gaps, use a loop-modeling procedure to substitute segments of appropriate length.
5. Add sidechains to the model backbone.
6. Evaluate and optimize entire structure.

Homology Modeling

- Servers
  - SWISS-MODEL
  - ESyPred3D

Protein Structure Prediction

- *Ab-initio* techniques
- Homology modeling
- Protein threading
  - Sequence-structure comparison

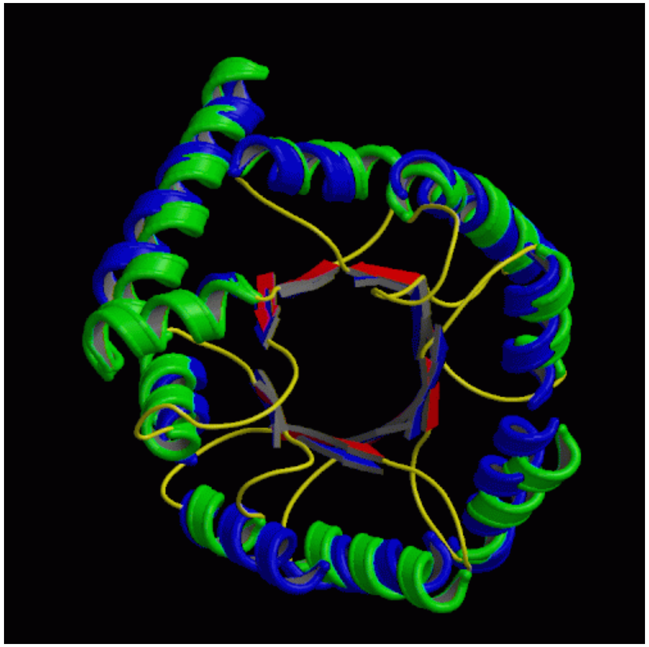
Protein threading

Structure is better conserved than sequence

Structure can adopt a wide range of mutations.

Physical forces favor certain structures.

Number of folds is limited.
Currently ~700
Total: 1,000 ~10,000



TIM barrel

Protein Threading

- Basic premise

The number of unique structural (domain) folds in nature is fairly small (possibly a few thousand)

- Statistics from Protein Data Bank (~35,000 structures)

90% of new structures submitted to PDB in the past three years have similar structural folds in PDB

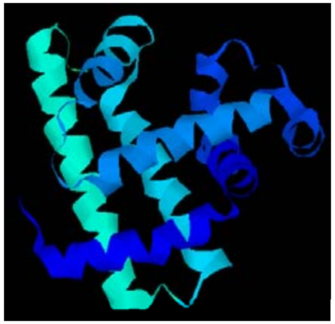
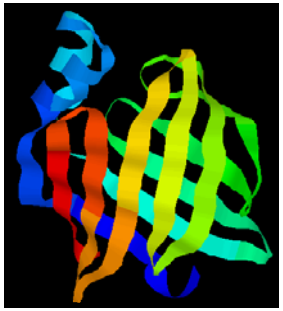
Concept of Threading

- Thread (*align* or *place)* a query protein sequence onto a template structure in “optimal” way
- Good alignment gives approximate backbone structure

Query sequence

MTYKLILNGKTKGETTTEAVDAATAEKVFQYANDNGVDGEWTYTE

Template set

Threading problem

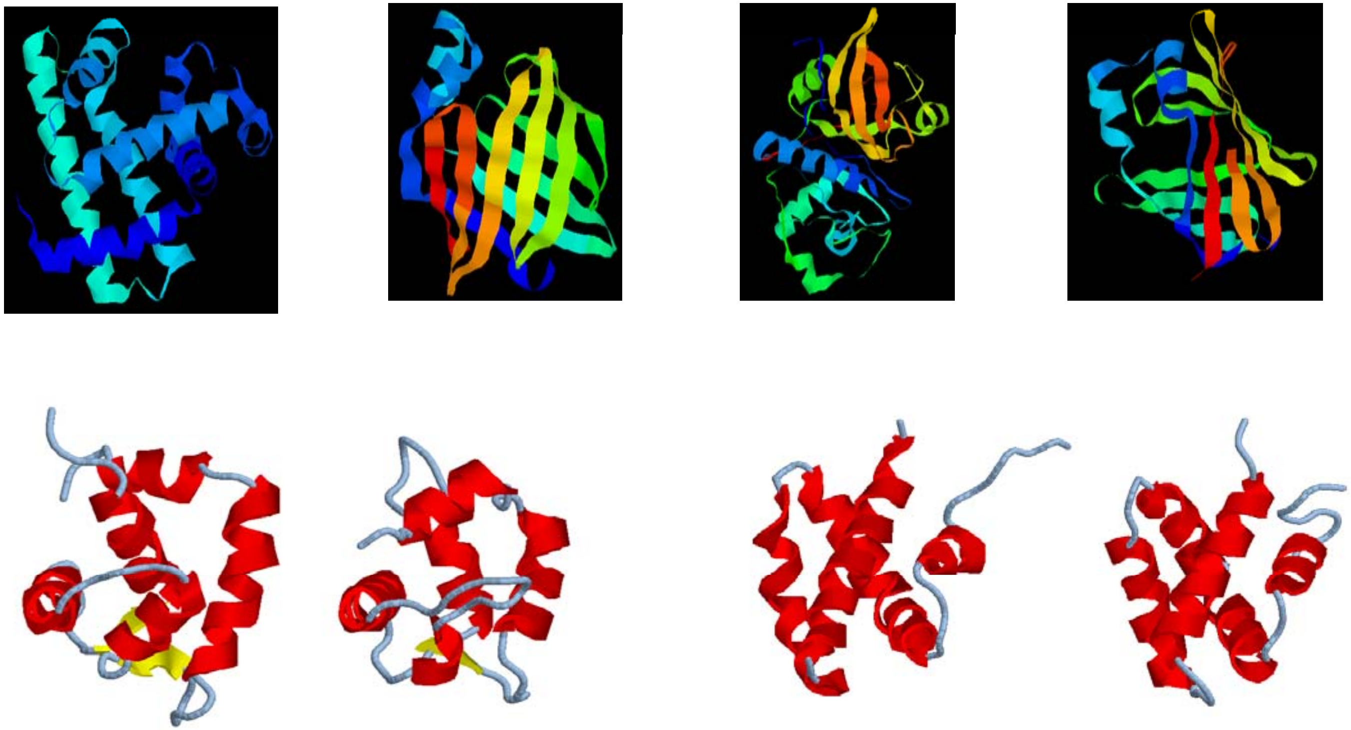
- Threading: Given a sequence, and a fold (template), compute the optimal alignment score between the sequence and the fold.
- If we can solve the above problem, then
  - Given a sequence, we can try each known fold, and find the best fold that fits this sequence.
  - Because there are only a few thousands folds, we can find the correct fold for the given sequence.
- Threading is NP-hard.

Components of Threading

- Template library
  - Use structures from DB classification categories (PDB)
- Scoring function
  - Single and pairwise energy terms
- Alignment
  - Consideration of pairwise terms leads to NP-hardness
  - heuristics
- Confidence assessment
  - Z-score, P-value similar to sequence alignment statistics
- Improvements
  - Local threading, multi-structure threading

Protein Threading – structure database

- Build a template database



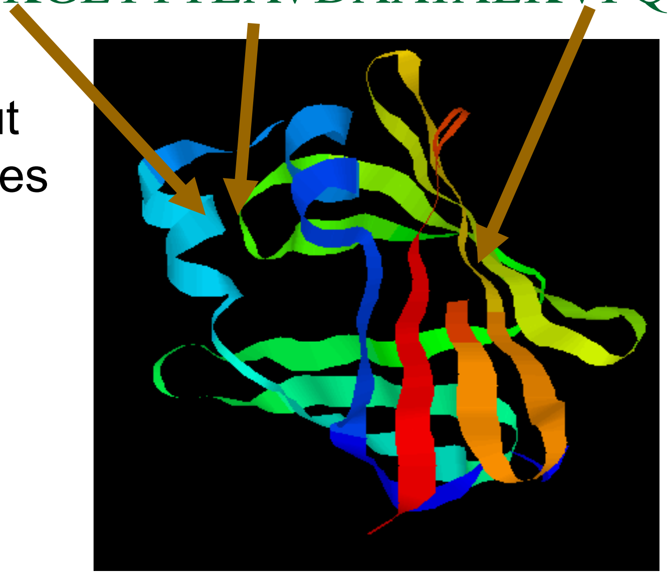
Protein Threading – energy function

MTYKLILNGKTKGETTTEAVDAATAEKVFQYANDNGVDGEWTYTE

how preferable to put two particular residues

nearby: E_p

alignment gap penalty: E_g



how well a residue fits a structural environment: E_s

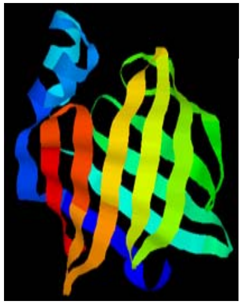
total energy: E_p + E_s + E_g

find a sequence-structure alignment to minimize the energy function

Assessing Prediction Reliability

MTYKLILNGKTKGETTTEAVDAATAEKVFQYANDNGVDGEWTYTE

Score = -1500



Score = -720

Score = -1120

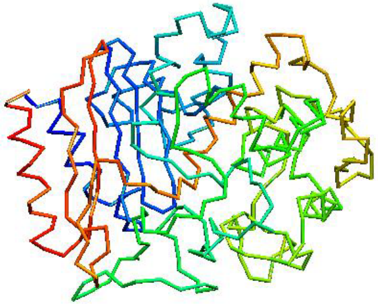
Score = -900

Which one is the correct structural fold for the target sequence if any?

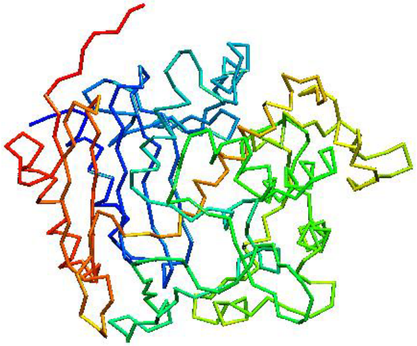
The one with the highest score ?

Prediction of Protein Structures

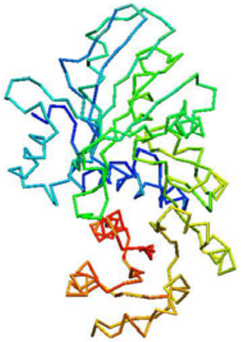
- Examples – a few good examples



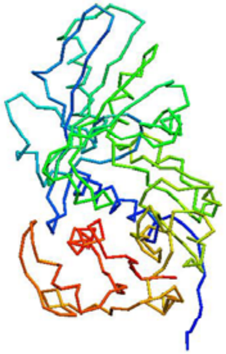
actual



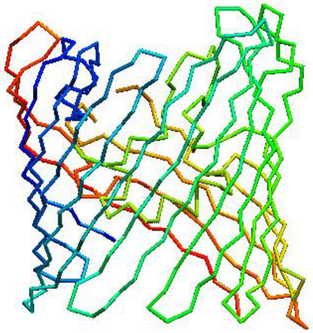
predicted



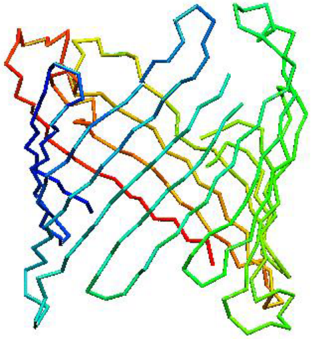
actual



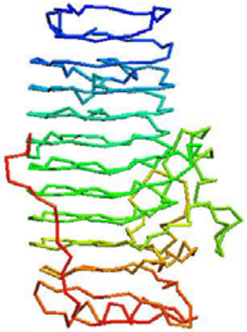
predicted



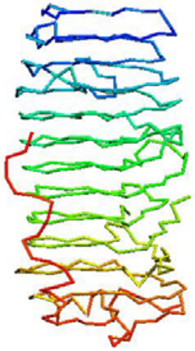
actual



predicted



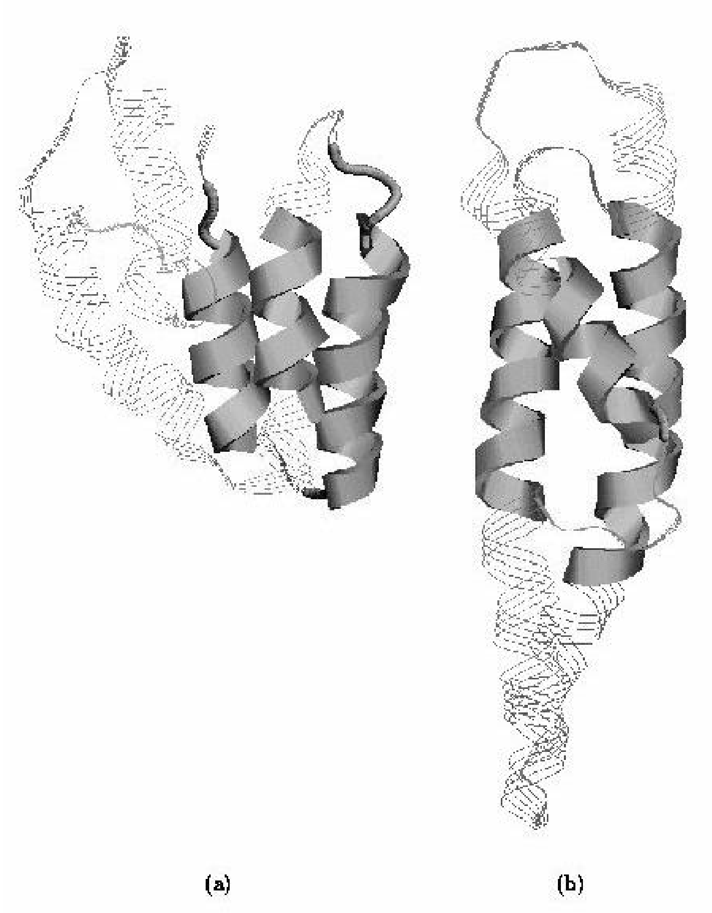
actual



predicted

Prediction of Protein Structures

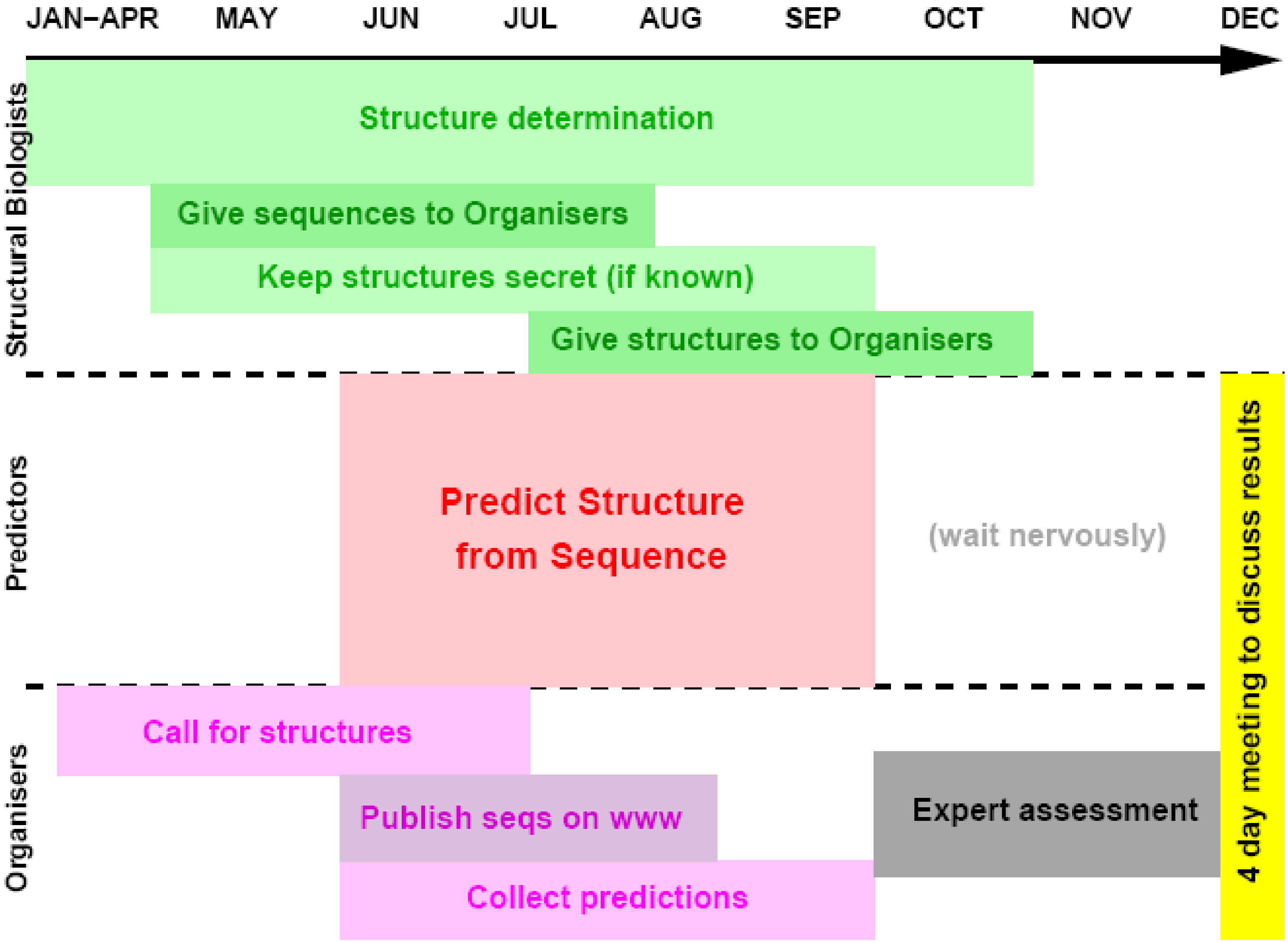
- Not so good example



Existing Prediction Programs

- PROSPECT
  - https://csbl.bmb.uga.edu/protein_pipeline
- FUGU
  - http://www-cryst.bioc.cam.ac.uk/~fugue/prfsearch.html
- THREADER
  - http://bioinf.cs.ucl.ac.uk/threader/

CASP

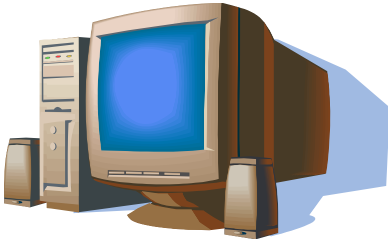

JAN–APR
MAY
JUN
JUL
AUG
SEP
OCT
NOV
DEC
Structural Biologists
Structure determination
Give sequences to Organisers
Keep structures secret (if known)
Give structures to Organisers
Predictors
Predict Structure from Sequence
(wait nervously)
Organisers
Call for structures
Publish seqs on www
Collect predictions
Expert assessment
4 day meeting to discuss results

CASP/CAFASP

- CASP: Critical Assessment of Structure Prediction

CASP Predictor

- CAFASP: Critical Assessment of Fully Automated Structure Prediction

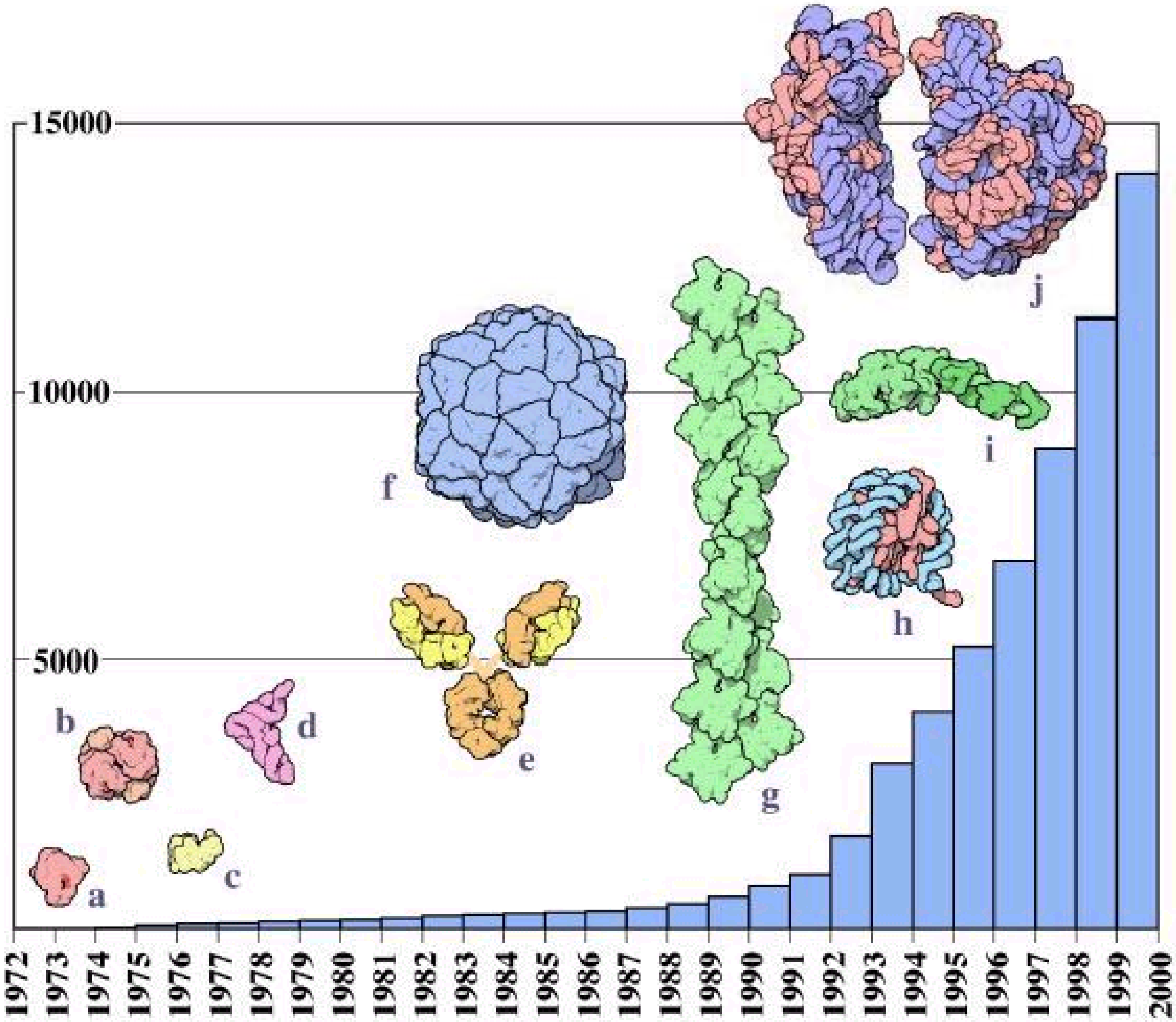


CAFASP Predictor

1. Won't get tired
2. High-throughput

CASP6/CAFASP4

- 64 targets
- Resources for predictors
  - No X-ray, NMR machines (of course)
  - CAFASP4 predictors: no manual intervention
  - CASP6 predictors: anything (servers, google,…)
- Evaluation:
  - CASP6 Assessed by experts+computer
  - CAFASP4 evaluated by a computer program.
  - Predicted structures are superimposed on the experimental structures.
- CASP7 was held last November



(a) myoglobin (b) hemoglobin (c) lysozyme (d) transfer RNA
(e) antibodies (f) viruses (g) actin (h) the nucleosome
(i) myosin (j) ribosome

Courtesy of David Goodsell, TSRI

Protein structure databases

- PDB
  - 3D structures
- SCOP
  - Murzin, Brenner, Hubbard, Chothia
  - Classification
    - Class (mostly alpha, mostly beta, alpha/beta (interspersed), alpha+beta (segregated), multi-domain, membrane)
    - Fold (similar structure)
    - Superfamily (homology, distant sequence similarity)
    - Family (homology and close sequence similarity)

The SCOP Database

Structural Classification Of Proteins

FAMILY: proteins that are >30% similar, or >15% similar and have similar known structure/function

SUPERFAMILY: proteins whose families have some sequence and function/structure similarity suggesting a common evolutionary origin

COMMON FOLD: superfamilies that have same secondary structures in same arrangement, probably resulting by physics and chemistry

CLASS: alpha, beta, alpha–beta, alpha+beta, multidomain

Protein databases

- CATH
  - Orengo et al
  - Class (alpha, beta, alpha/beta, few SSEs)
  - Architecture (orientation of SSEs but ignoring connectivity)
  - Topology (orientation and connectivity, based on SSAP = fold of SCOP)
  - Homology (sequence similarity = superfamily of SCOP)
    - S level (high sequence similarity = family of SCOP)
  - SSAP alignment tool (dynamic programming)

Protein databases

- FSSP
  - DALI structure alignment tool (distance matrix)
    - Holm and Sander
- MMDB
  - VAST structure comparison (hierarchical)
    - Madej, Bryant et al

Protein structure comparison

- Levels of structure description
  - Atom/atom group
  - Residue
  - Fragment
  - Secondary structure element (SSE)
- Basis of comparison
  - Geometry/architecture of coordinates/relative positions
  - sequential order of residues along backbone, ...
  - physio-chemical properties of residues, …

How to compare?

- **Key problem**: find an optimal correspondence between the arrangements of atoms in two molecular structures (say A and B) in order to align them in 3D
- Optimality of the alignment is determined using a root mean square measure of the distances between corresponding atoms in the two molecules
- **Complication**: It is not known a priori which atom in molecule B corresponds to a given atom in molecule A (the two molecules may not even have the same number of atoms)

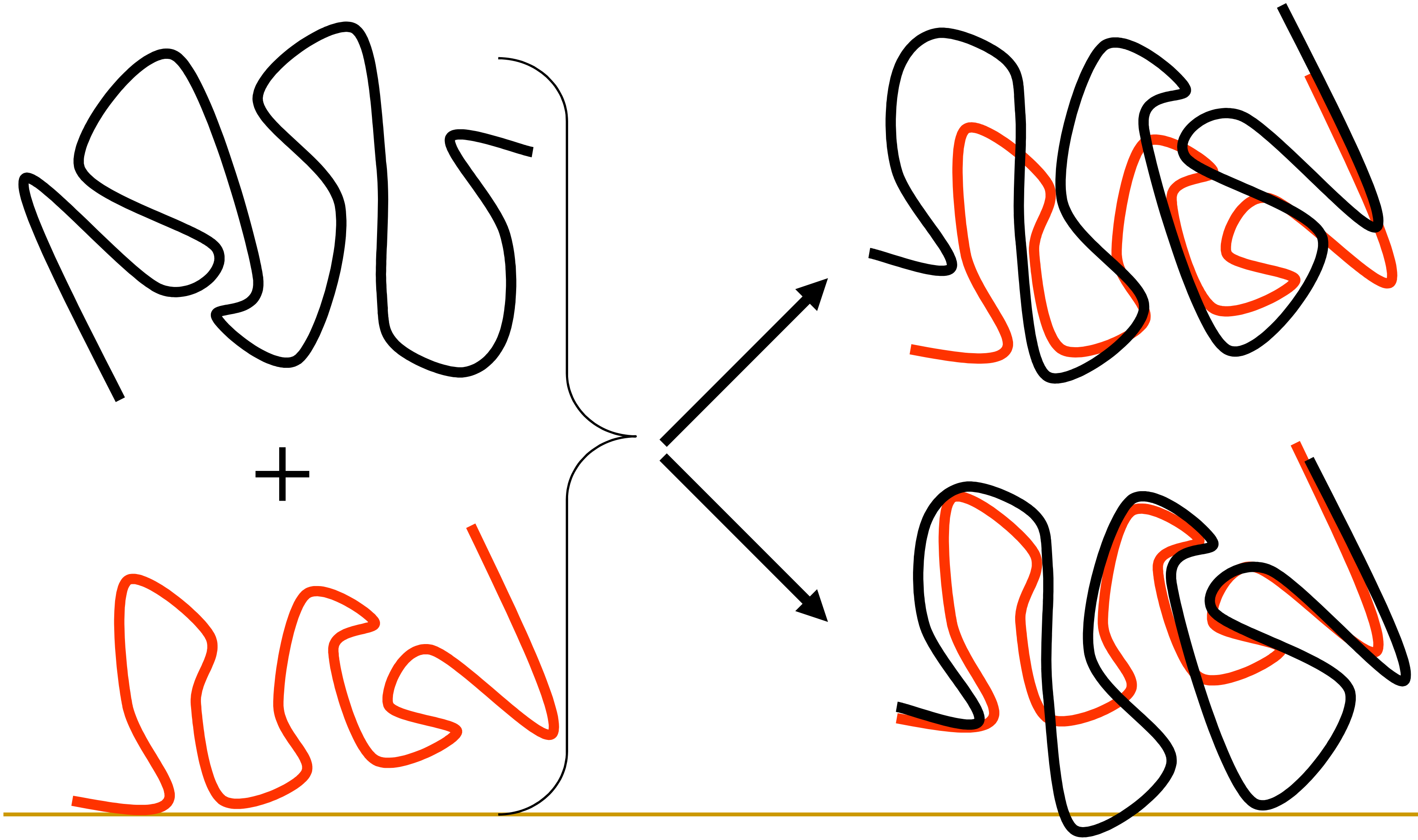
Structure Analysis – Basic Issues

- Coordinates for representing 3D structures
  - Cartesian
  - Other (e.g. dihedral angles)
- Basic operations
  - Translation in 3D space
  - Rotation in 3D space
  - Comparing 3D structures
    - Root mean square distances between points of two molecules are typically used as a measure of how well they are aligned
    - Efficient ways to compute minimal RMSD once correspondences are known (O(n) algorithm)
      - Using eigenvalue analysis of correlation matrix of points
- Due to the high computational complexity, practical algorithms rely on heuristics

Structure Analysis – Basic Issues

- Sequence order dependent approaches
  - Computationally this is easier
  - Interest in motifs preserving sequence order
- Sequence order independent approaches
  - More general
  - Active sites may involve non-local AAs
  - Searching with structural information

Find the optimal alignment



Optimal Alignment

- Find the highest number of atoms aligned with the lowest RMSD (Root Mean Squared Deviation)
- Find a balance between local regions with very good alignments and overall alignment

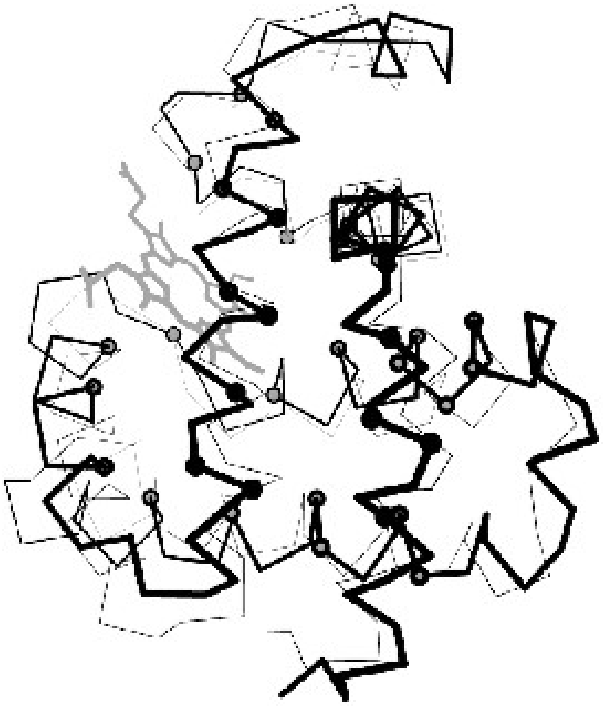
Structure Comparison

Which atom in structure A corresponds to which atom in structure B ?

```
THESESENTENCESALIGN--NICELY
|||  ||  |||| |||||  ||||||
THE--SEQUENCE-ALIGNEDNICELY
```

Structural Alignment

Structural Alignment of Two Globins



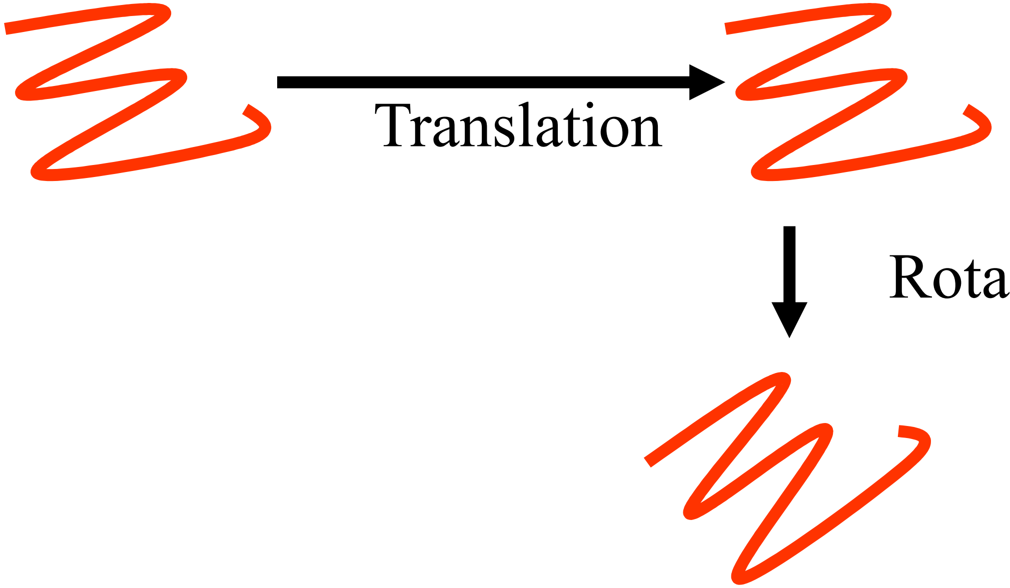
An optimal superposition of myoglobin and beta-hemoglobin, which are structural neighbors. However, their sequence homology is only 8.5%

Structure Comparison

Methods to superimpose structures

by translation and rotation

$$\begin{pmatrix} x_1, y_1, z_1 \\ x_2, y_2, z_2 \\ x_3, y_3, z_3 \end{pmatrix}$$



$$\begin{pmatrix} x_1 + d, y_1, z_1 \\ x_2 + d, y_2, z_2 \\ x_3 + d, y_3, z_3 \end{pmatrix}$$

Structure Comparison

Scoring system to find optimal alignment

Answer: Root Mean Square Deviation (*RMSD*)

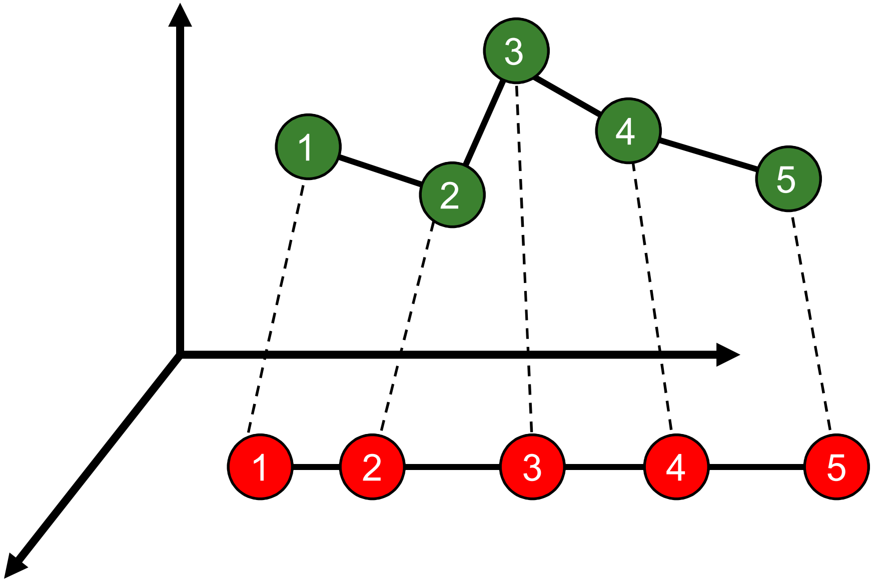
$$RMSD = \sqrt{\frac{\sum_i d_i^2}{n}}$$

n = number of atoms

d_i = distance between 2 corresponding atoms i in 2 structures

Root Mean Square Deviation

$$\mathbf{RMS} = \sqrt{\frac{\sum_{i=1}^{5}(\mathbf{X}_{\mathbf{RED1}} - \mathbf{X}_{\mathbf{BLUE1}})^2}{\mathbf{5}}} \sim \frac{\mathbf{d}_1 + \mathbf{d}_2 + \mathbf{d}_3 + \mathbf{d}_4 + \mathbf{d}_5}{\mathbf{5}}$$



RMSD

Unit of RMSD => e.g. Ångstroms

- identical structures => *RMSD* = “0”
- similar structures => *RMSD* is small (1 – 3 Å)
- distant structures => *RMSD* > 3 Å

Pitfalls of RMSD

- all atoms are treated equally

  (*e.g. residues on the surface have a higher degree of freedom than those in the core*)

- best alignment does not always mean minimal RMSD

- significance of RMSD is size dependent

Alternative RMSDs

- aRMSD = best root-mean-square distance calculated over all aligned alpha-carbon atoms
- bRMSD = the RMSD over the highest scoring residue pairs
- wRMSD = weighted RMSD

Source: W. Taylor(1999), *Protein Science*, *8*: 654-665.

Structural Alignment Methods

- **Distance based methods**
  - DALI (Holm and Sander, 1993): Aligning 2-dimensional distance matrices
  - STRUCTAL (Subbiah 1993, Gerstein and Levitt 1996): Dynamic programming to minimize the RMSD between two protein backbones.
  - SSAP (Orengo and Taylor, 1990): Double dynamic programming using intra-molecular distance;
  - CE (Shindyalov and Bourne, 1998): Combinatorial Extension of best matching regions
- **Vector based methods**
  - VAST (Madej et al., 1995): Graph theory based SSE alignment;
  - 3dSearch (Singh and Brutlag, 1997) and 3D Lookup (Holm and Sander, 1995): Fast SSE index lookup by geometric hashing.
  - TOP (Lu, 2000): SSE vector superpositioning.
  - TOPSCAN (Martin, 2000): Symbolic linear representation of SSE vectors.
- **Both vector and distance based**
  - LOCK (Singh and Brutlag, 1997): Hierarchically uses both secondary structures vectors and atomic distances.

Basic DP (STRUCTAL)

1. Start with arbitrary alignment of the points in two molecules A and B
2. Superimpose in order to minimize RMSD.
3. Compute a *structural alignment (SA) matrix* where entry (i,j) is the score for the structural similarity between the i^{th} point of A and the j^{th} point of B
4. Use DP to compute the next alignment.

   Gap cost = 0
5. Iterate steps 2--4 until the overall score converges
6. Repeat with a number of initial alignments

STRUCTAL

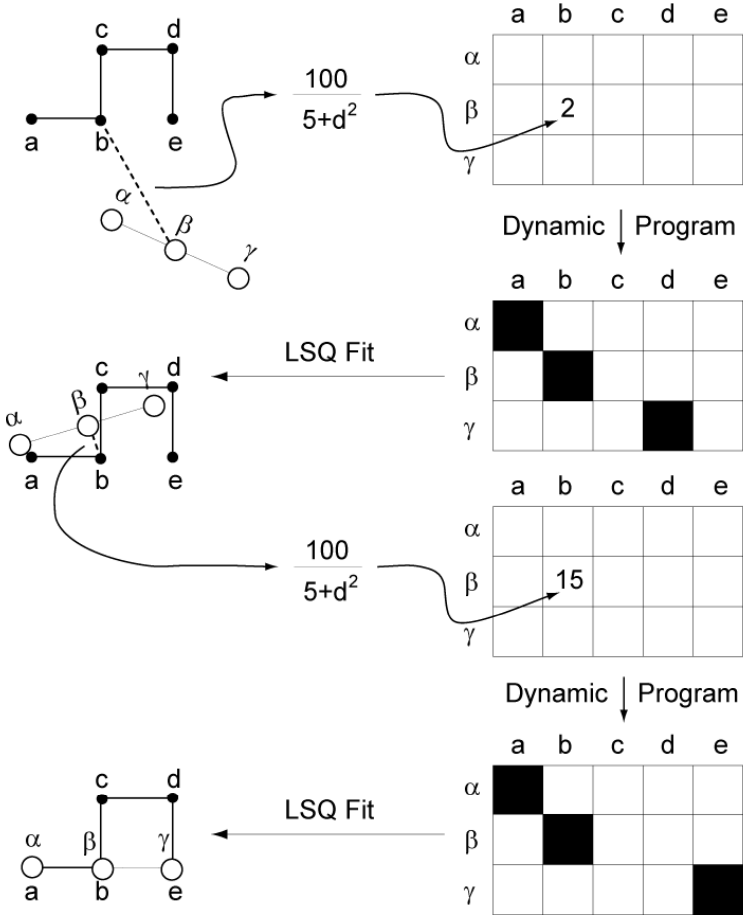
- Given
  2 Structures (A & B),
  2 Basic Comparison Operations

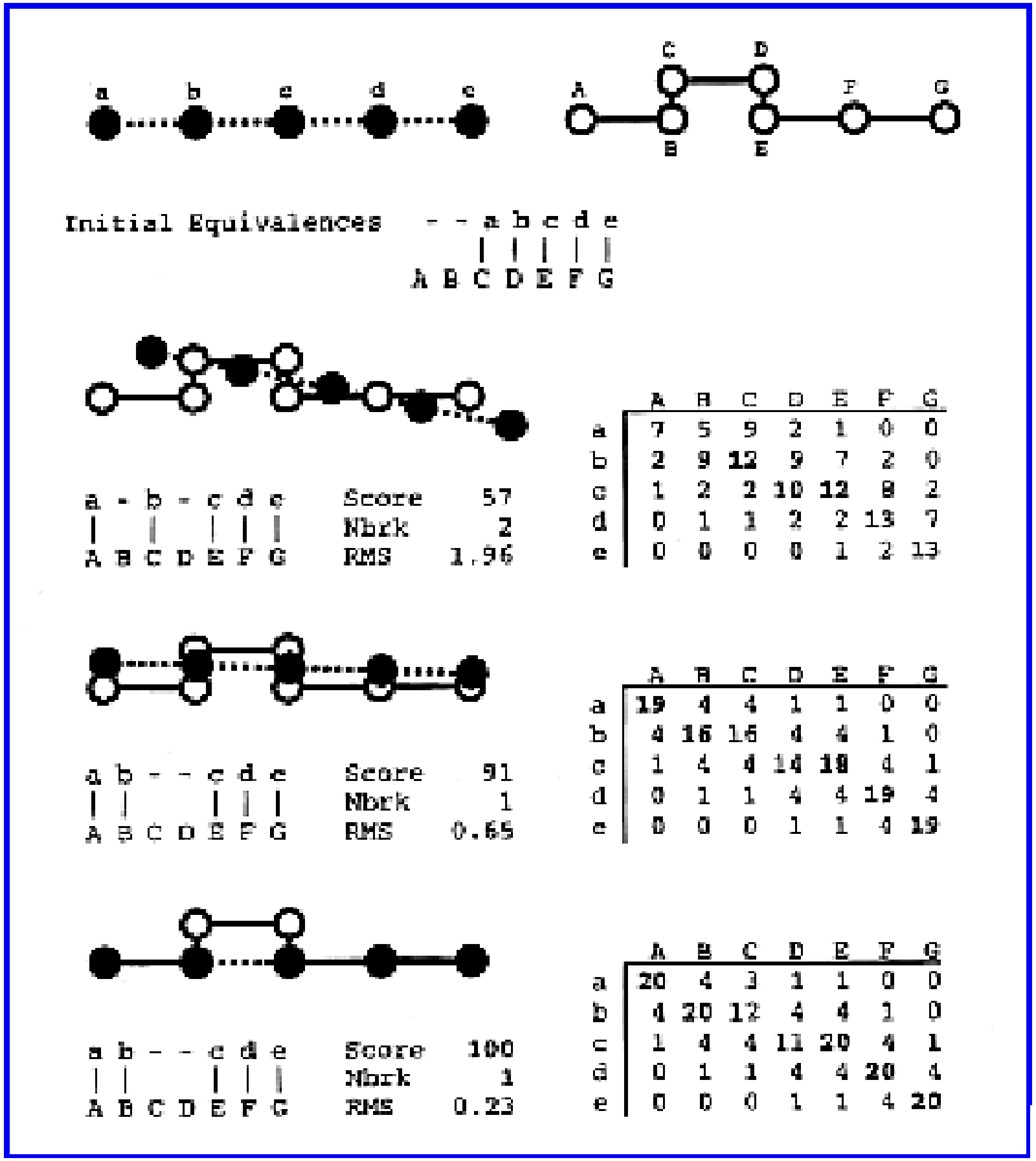
1. Given an alignment optimally **SUPERIMPOSE** A onto B

2. **Find an Alignment** between A and B based on their 3D coordinates

$$S_{ij} = M/[1+(d_{ij}/d_0)^2]$$

M and d_0 are constants





```
Initial Equivalences     - - a b c d e
                             | | | | |
                         A B C D E F G
```

```
a - b - c d e      Score      57
|   |   | | |      Nbrk        2
A B C D E F G      RMS      1.96
```

	A	B	C	D	E	F	G
a	7	5	9	2	1	0	0
b	2	9	12	9	7	2	0
c	1	2	2	10	12	8	2
d	0	1	1	2	2	13	7
e	0	0	0	0	1	2	13

```
a b - - c d e      Score      91
| |     | | |      Nbrk        1
A B C D E F G      RMS      0.65
```

	A	B	C	D	E	F	G
a	19	4	4	1	1	0	0
b	4	16	16	4	4	1	0
c	1	4	4	14	18	4	1
d	0	1	1	4	4	19	4
e	0	0	0	1	1	4	19

```
a b - - c d e      Score     100
| |     | | |      Nbrk        1
A B C D E F G      RMS      0.23
```

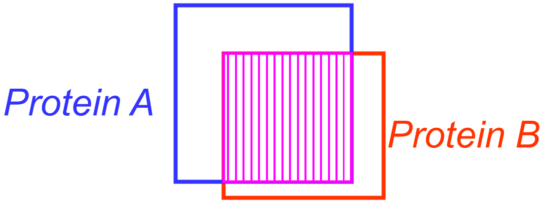
	A	B	C	D	E	F	G
a	20	4	3	1	1	0	0
b	4	20	12	4	4	1	0
c	1	4	4	11	20	4	1
d	0	1	1	4	4	20	4
e	0	0	0	1	1	4	20

DALI Method

- Distance mAtrix aLIgnment
- Liisa Holm and Chris Sander, “Protein structure comparison by alignment of distance matrices”, *Journal of Molecular Biology Vol. 233*, 1993.
- Liisa Holm and Chris Sander, “Mapping the protein universe”, *Science Vol. 273*, 1996.
- Liisa Holm and Chris Sander, “Alignment of three-dimensional protein structures: network server for database searching”, *Methods in Enzymology Vol. 266*, 1996.

How DALI Works?

- Based on fact: similar 3D structures have similar intra-molecular distances.
- Background idea
  - Represent each protein as a 2D matrix storing intra-molecular distance.
  - Place one matrix on top of another and slide vertically and horizontally – until a common the sub-matrix with the best match is found.

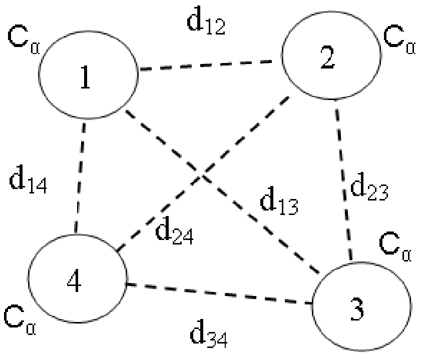


- Actual implementation
  - Break each matrix into small sub-matrices of fixed size.
  - Pair-up similar sub-matrices (one from each protein).
  - Assemble the sub-matrix pairs to get the overall alignment.

Structure Representation of DALI

- 3D shape is described with a *distance matrix* which stores all *intra-molecular distances* between the C_α atoms.
- Distance matrix is independent of coordinate frame.
- Contains enough information to re-construct the 3D coordinates.

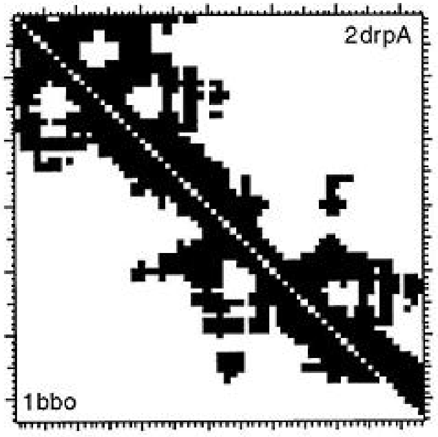
Protein A



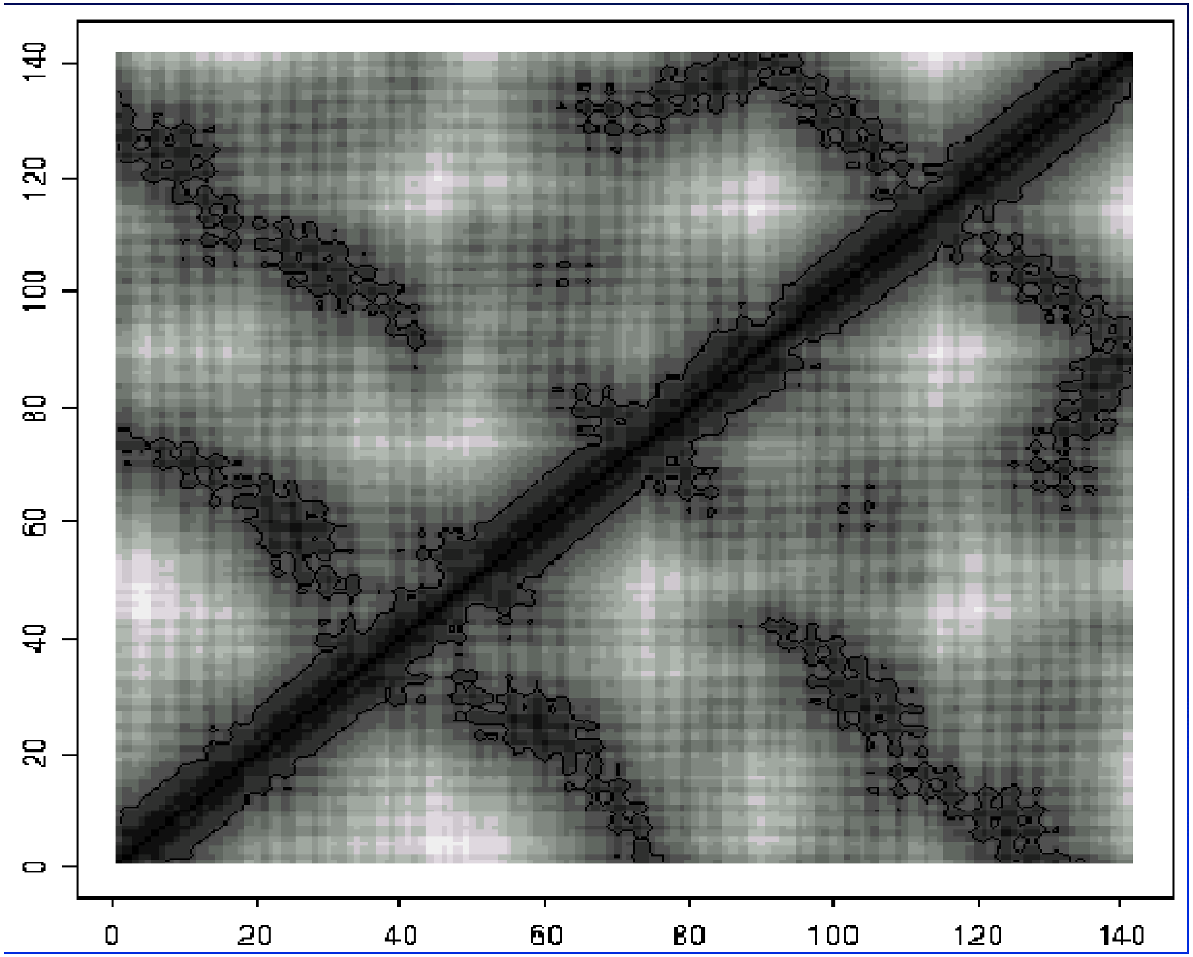
Distance matrix for Protein A

	1	2	3	4
1	0	d_{12}	d_{13}	d_{14}
2	d_{12}	0	d_{23}	d_{24}
3	d_{13}	d_{23}	0	d_{34}
4	d_{14}	d_{24}	d_{34}	0

Distance matrix for 2drpA and 1bbo



Intra-molecular distance for myoglobin



DALI Algorithm

1. Decompose distance matrix into elementary *contact patterns* (sub-matrices of fixed size)
   - Use hexapeptide-hexapeptide contact patterns.
2. Compare contact patterns (pair-wise), and store the matching pairs in *pair list.*
3. Assemble pairs in the correct order to yield the overall alignment.

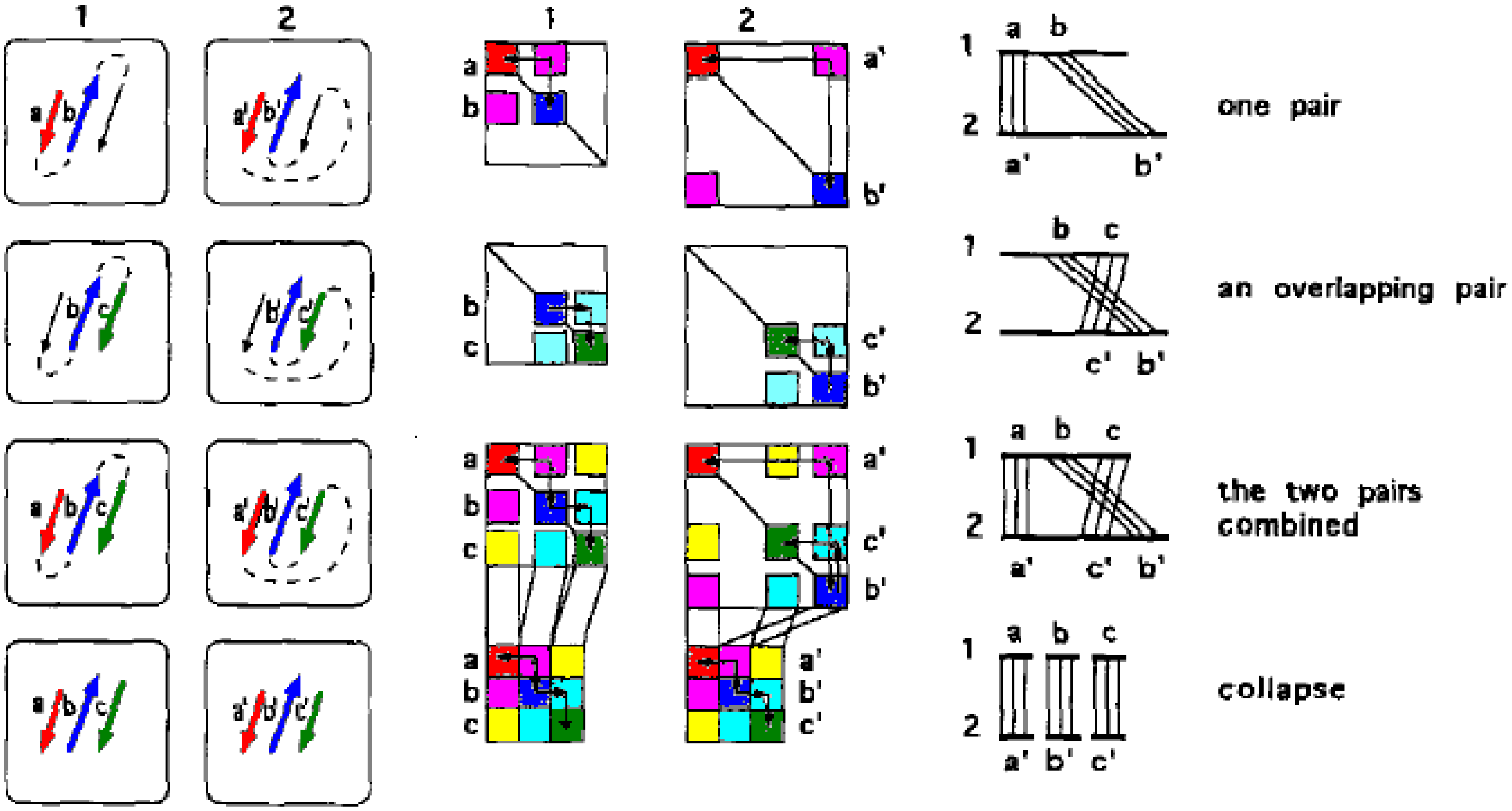
Assembly of Alignments

- Non-trivial combinatory problem.
- Assembled in the manner (AB) – (A’B’), (BC) – (B’C’), . . . (i.e., having one overlapping segment with the previous alignment)
- Available Alignment Methods:
  - *Monte Carlo* optimization
  - Brach-and-bound
  - Neighbor walk

Schematic View of DALI Algorithm

3D (Spatial) **2D (Distance Matrix)** **1D**

(Sequence)

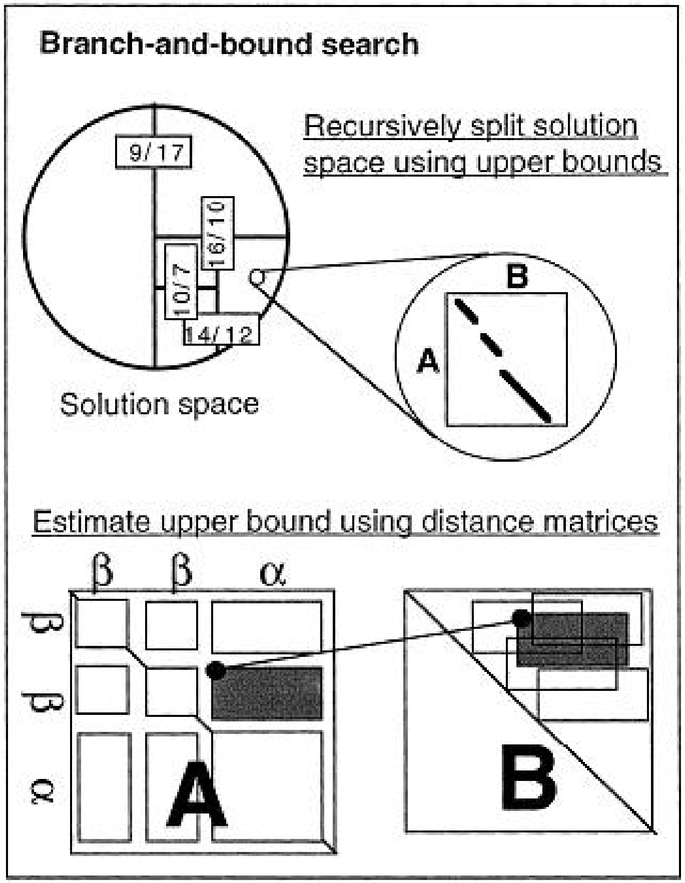


Monte Carlo Optimization

- Used in the earlier versions of DALI.
- Algorithm
  - Compute a similarity score for the current alignment.
  - Make a random trial change to the current alignment (adding a new pair or deleting an existing pair).
  - Compute the change in the score (ΔS).
  - If Δ*S* > 0, the move is always accepted.
  - If Δ*S* <= 0, the move may be accepted by the probability exp(β * Δ*S*), where β is a parameter.
  - Once a move is accepted, the change in the alignment becomes permanent.
  - This procedure is iterated until there is no further change in the score, i.e., the system is converged.

Branch-and-bound method

- Used in the later versions of DALI.
- Based on *Lathrop and Smith's* (1996) *threading* (sequence-structure alignment) algorithm.
- *Solution space* consists of all possible placements of residues in protein A relative to the segment of residues of protein B.
- The algorithm recursively split the solution space that yields the highest upper bound of the similarity score until there is a single alignment trace left.


Branch-and-bound search
9/17
16/10
10/7
14/12
Recursively split solution space using upper bounds
B
A
Solution space
Estimate upper bound using distance matrices
β
β
α
β
β
α
A
B

LOCK

- Uses a hierarchical approach
- Larger secondary structures such as helixes and strands are represented using vectors and dealt with first
- Atoms are dealt with afterwards
- Assumes large secondary structures provide most stability and function to a protein, and are most likely to be preserved during evolution

LOCK (Contd.)

- Key algorithm steps:
  1. Represent secondary structures as vectors
  2. Obtain initial superposition by computing local alignment of the secondary structure vectors (using dynamic programming)
  3. Compute atomic superposition by performing a greedy search to try to minimize *root mean square deviation* (a RMS distance measure) between pairs of nearest atoms from the two proteins
  4. Identify “core” (well aligned) atoms and try to improve their superposition (possibly at the cost of degrading superposition of non-core atoms)
- Steps 2, 3, and 4 require iteration at each step

Alignment of SSEs

- Define an orientation-dependent score and an orientation-independent score between SSE vectors.
- For every pair of query vectors, find all pairs of vectors in database protein that align with a score above a threshold. Two of these vectors must be adjacent. Use orientation independent scores.
- For each set of four vectors from previous step, find the transformation minimizing rmsd. Apply this transformation to the query.
- Run dynamic programming using both orientation-dependent and orientation-independent scores to find the best local alignment.
- Compute and apply the transformation from the best local alignment.
- Superpose in order to minimize rmsd.

Atomic superposition

- Loop
  - find matching pairs of C_α atoms
  - use only those within 3 A
  - find best alignment
- until rmsd does not change

Core identification

- Loop
  - find the best core (symmetric nns) and align; remove the rest
- until rmsd does not change

VAST

- Begin with a set of nodes (a,x) where SSEs a and x are of the same type
- Add an edge between (a,x) and (b,y) if angle and distance between (a,b) is same as between (x,y)
- Find the maximal clique in this graph; this forms the initial SSE alignment
- Extend the initial alignment to C_α atoms using Gibbs sampling
- Report statistics on this match

Quality of a structure match

- Statistical theory similar to BLAST
- Compare the likelihood of a match as compared to a random match
- Less agreement regarding score matrix
  - z-scores of CE, DALI, and VAST may not be compatible