Computational modeling of protein structures

Yinghao Wu
Department of Systems and Computational Biology
Albert Einstein College of Medicine
Fall 2014

EINSTEIN
Albert Einstein College of Medicine
OF YESHIVA UNIVERSITY

Outline

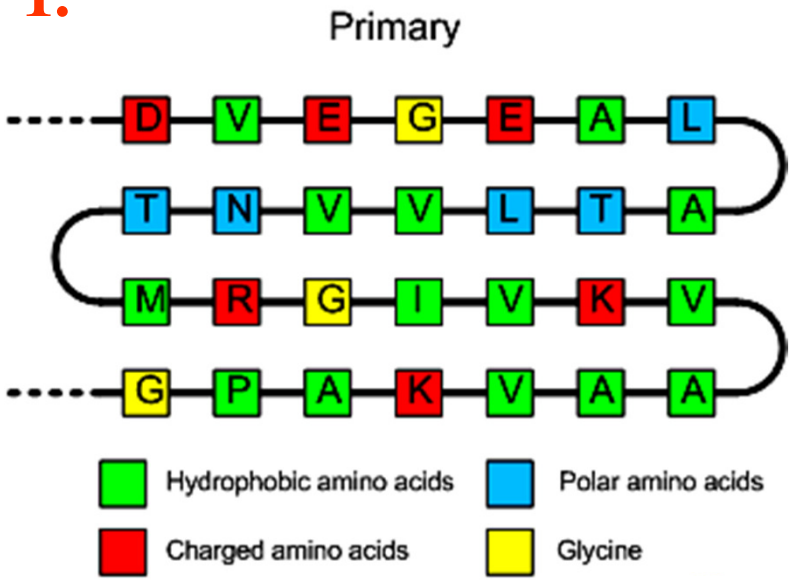
- Introduction to protein structures
- Protein structure classification
- Protein structure comparison
- Protein structure prediction

Outline

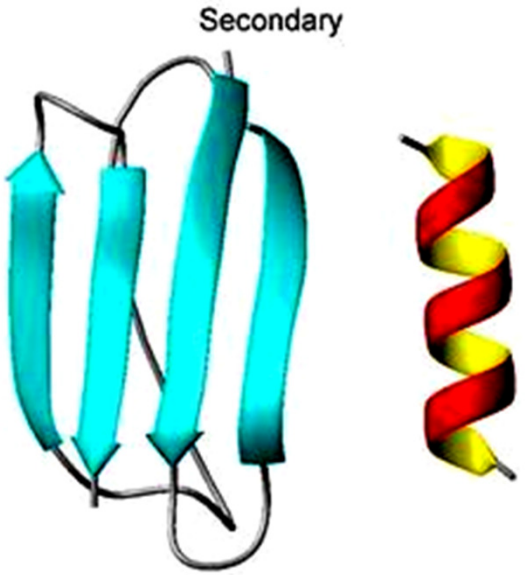
- Introduction to protein structures
- Protein structure classification
- Protein structure comparison
- Protein structure prediction

HIERARCHY OF PROTEIN STRUCTURE

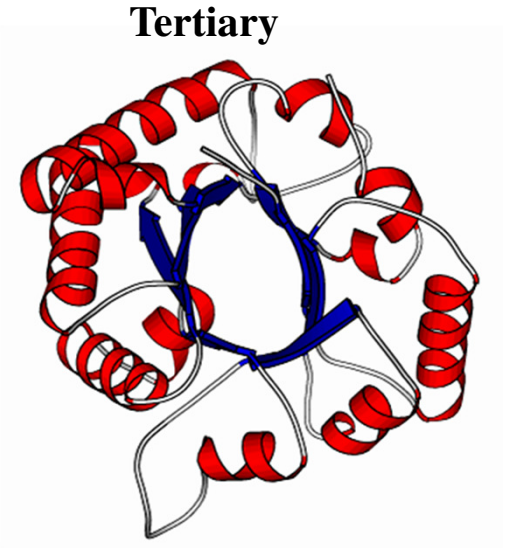
1.



2.



3.



4.

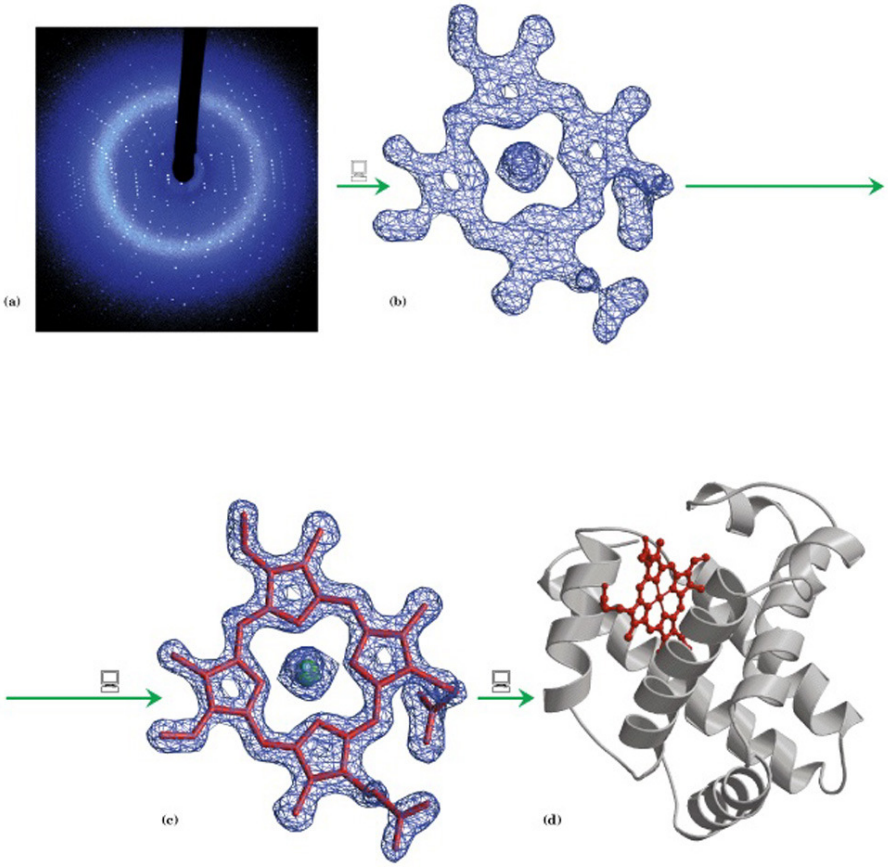
EINSTEIN
Albert Einstein College of Medicine
OF YESHIVA UNIVERSITY

X-Ray Crystallography

- **crystallize and immobilize single, perfect protein**
- **bombard with X-rays, record scattering diffraction patterns**
- **determine electron density map from scattering and phase via Fourier transform:**

$$F(\Delta k) = V \int_{x=0}^{x=1} \int_{y=0}^{y=1} \int_{z=0}^{z=1} \rho(x,y,z)\, e^{i\Delta \mathbf{k}\cdot(xa+yb+zc)}\, dxdydz$$

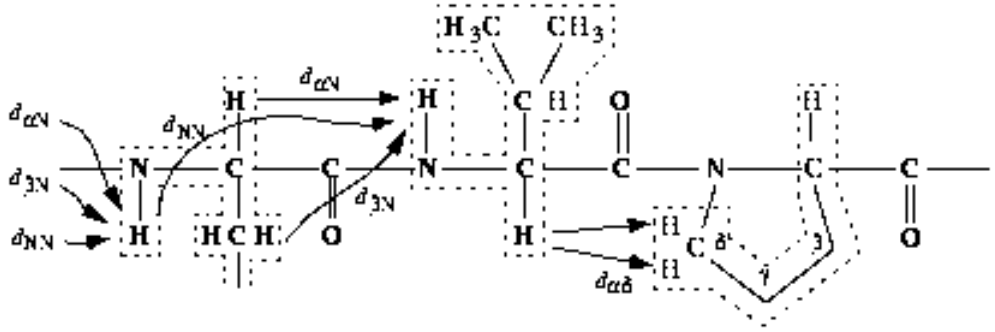
- **use electron density and biochemical knowledge of the protein to refine and determine a model**



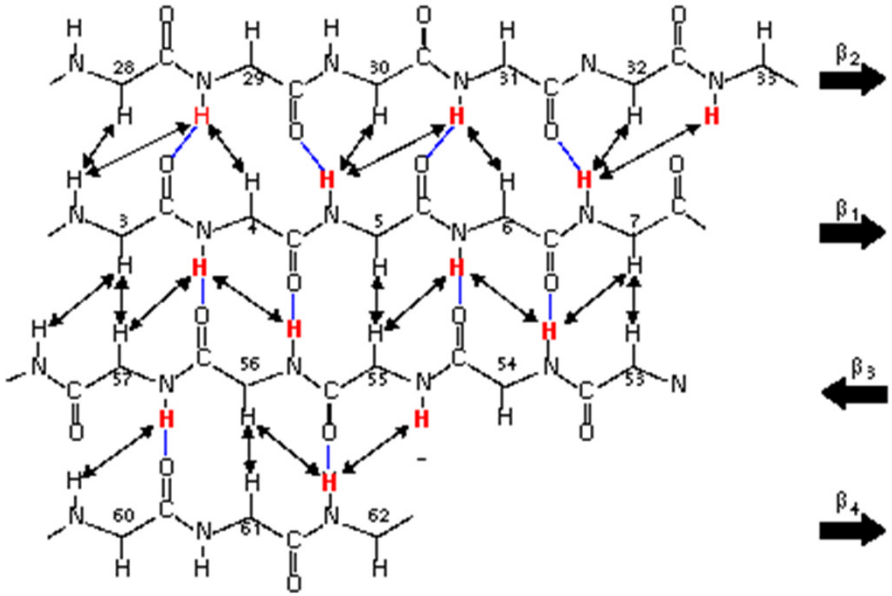
"**All crystallographic models are *not* equal.** ... The brightly colored stereo views of a protein model, which are in fact more akin to cartoons than to molecules, endow the model with a concreteness that exceeds the intentions of the thoughtful crystallographer. It is impossible for the crystallographer, with vivid recall of the massive labor that produced the model, to forget its shortcomings. It is all too easy for users of the model to be unaware of them. It is also all too easy for the user to be unaware that, through temperature factors, occupancies, undetected parts of the protein, and unexplained density, crystallography reveals more than a single molecular model shows."

- Rhodes, "Crystallography Made Crystal Clear" p. 183.

NMR Spectroscopy



determining constraints



using constraints to determine secondary structure

protein in aqueous solution, motile and tumbles/vibrates with thermal motion

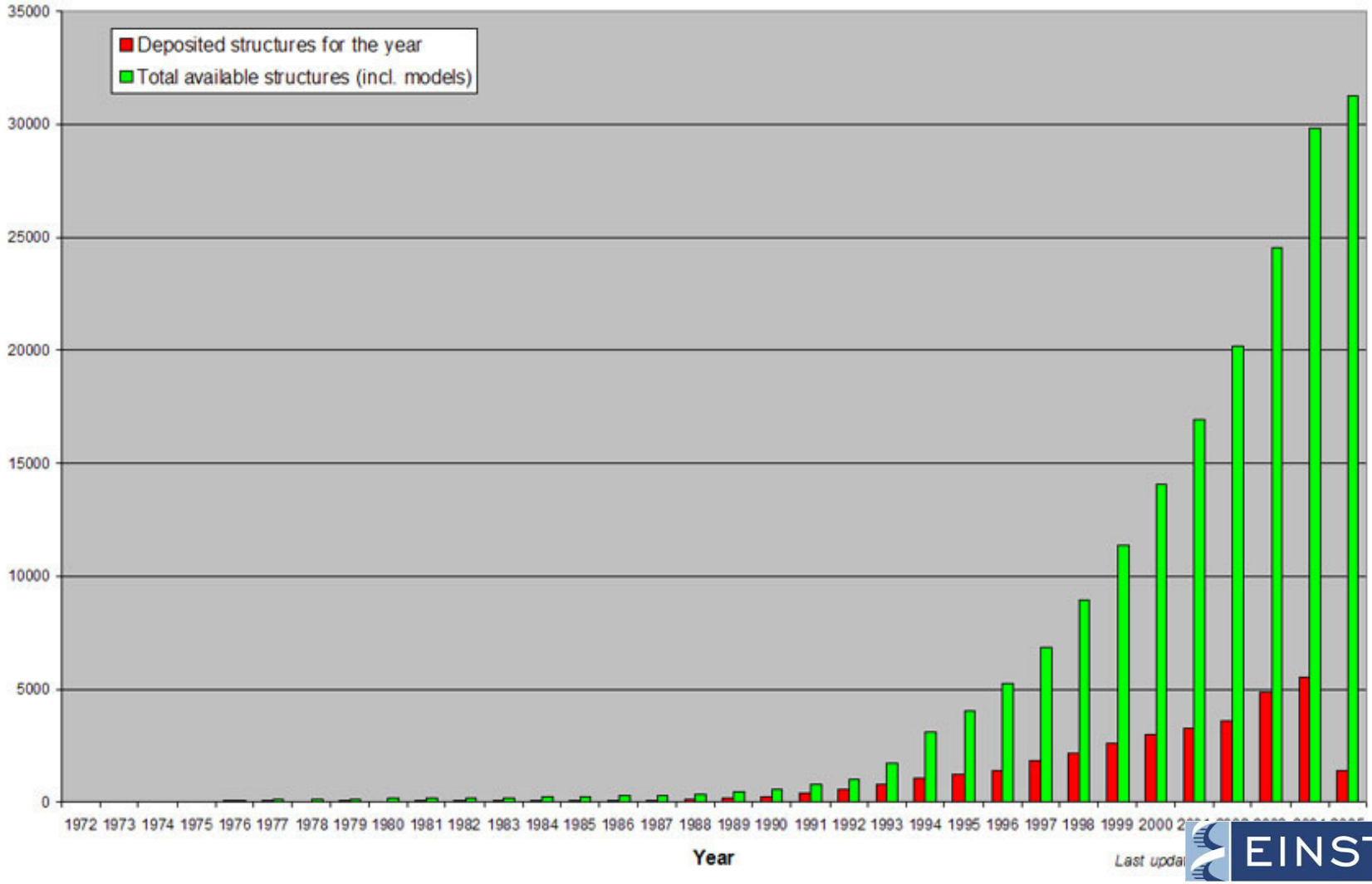
NMR detects chemical shifts of atomic nuclei with non-zero spin, shifts due to electronic environment nearby

- determine distances between specific pairs of atoms based on shifts, "constraints"
- use constraints and biochemical knowledge of the protein to determine an ensemble of models

Outline

- Introduction to protein structures
- Protein structure classification
- Protein structure comparison
- Protein structure prediction

PDB Growth



EINSTEIN
Albert Einstein College of Medicine
OF YESHIVA UNIVERSITY

Only a few folds are found in nature

PROTEINS

One thousand families for the molecular biologist

Cyrus Chothia

NATURE · VOL 357 · 18 JUNE 1992

How many families of proteins are there? By putting together the information to be found in papers published over the past few months we can make an initial estimate, and my calculation suggests that the large majority of proteins come from no more than one thousand families.

Proteins are clustered into families

lies, crystallography, NMR and molecular modelling will produce, at least in outline, structures for most proteins in time for the completion of the genome projects. □

TABLE 2 Genome projects

Species	Approximate number of genes	Tentative date of completion
Escherichia coli	4,000	1995–98
Yeast	7,000	2000
Caenorhabditis elegans	15,000	2000
Human	50–100,000	2015

TABLE 1 New gene sequences that are related to previously determined sequences

Genome projects

Source	Total number of genes	Genes related to those previously determined	Ref.
Caenorhabditis elegans chromosome III (part)	32	14 (44%)	1
Yeast chromosome III	182	52–66 (29–36%)	2
chromosome IX (part)	46	15 (33%)	*

Large libraries of expressed genes

Source	Total number of clones	Clones related to previously determined protein sequences	
Human brain	~1,400†	406 (~30%)	3
Caenorhabditis elegans			
St Louis–Cambridge	1,517	512 (34%)	
NIH	585	210 (36%)	

EINSTEIN
Albert Einstein College of Medicine
OF YESHIVA UNIVERSITY

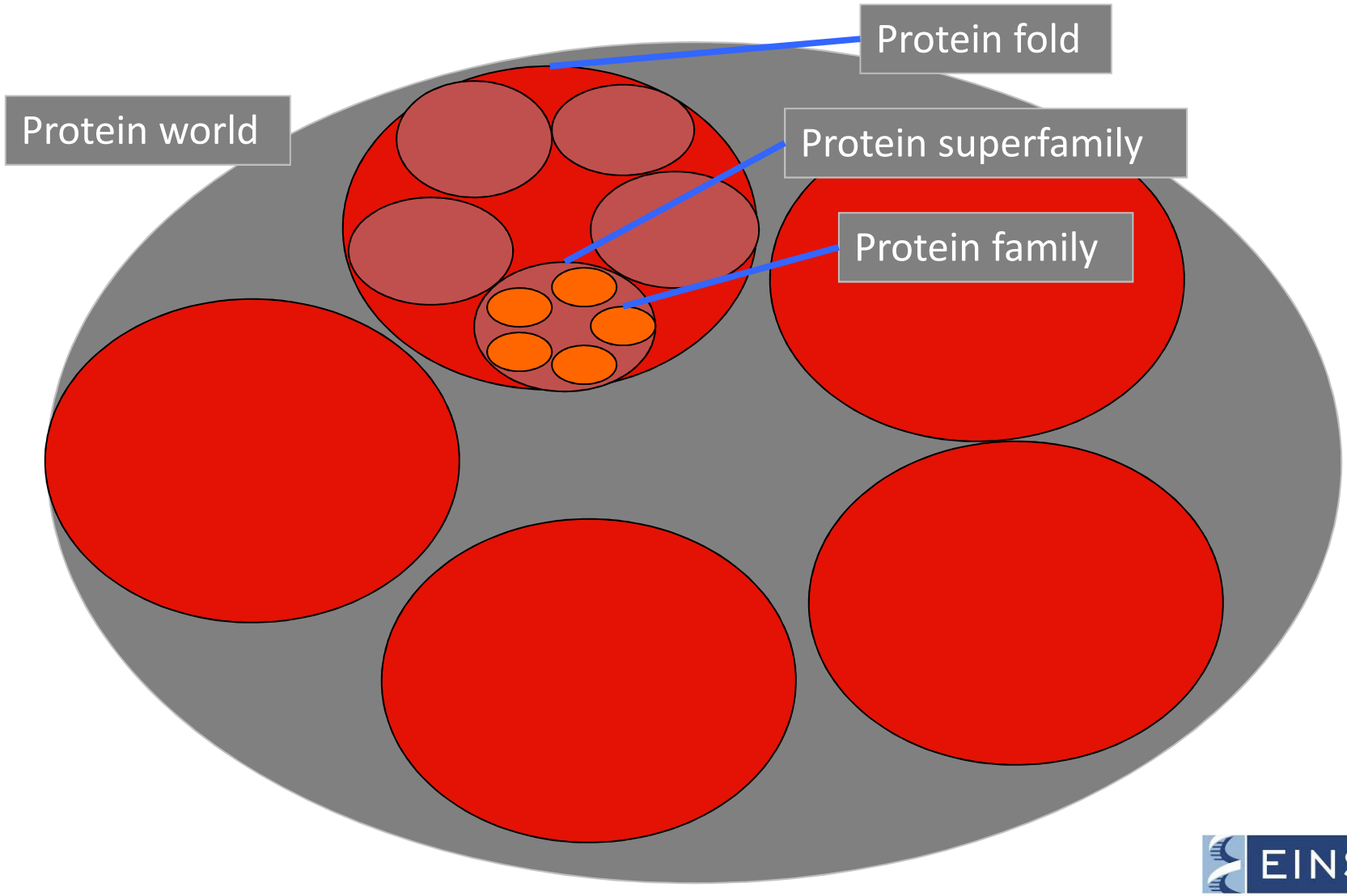
Protein classification

- Number of protein sequences grows exponentially
- Number of solved structures grows exponentially
- Number of new folds identified very small (and close to constant)
- Protein classification can
  - Generate overview of structure types
  - Detect similarities (evolutionary relationships) between protein sequences
  - Help predict 3D structure of new protein sequences

Structure Classification Databases

- SCOP
  - Manual classification (A. Murzin)
  - scop.berkeley.edu
- CATH
  - Semi manual classification (C. Orengo)
  - www.biochem.ucl.ac.uk/bsm/cath
- FSSP
  - Automatic classification (L. Holm)
  - www.ebi.ac.uk/dali/fssp/fssp.html

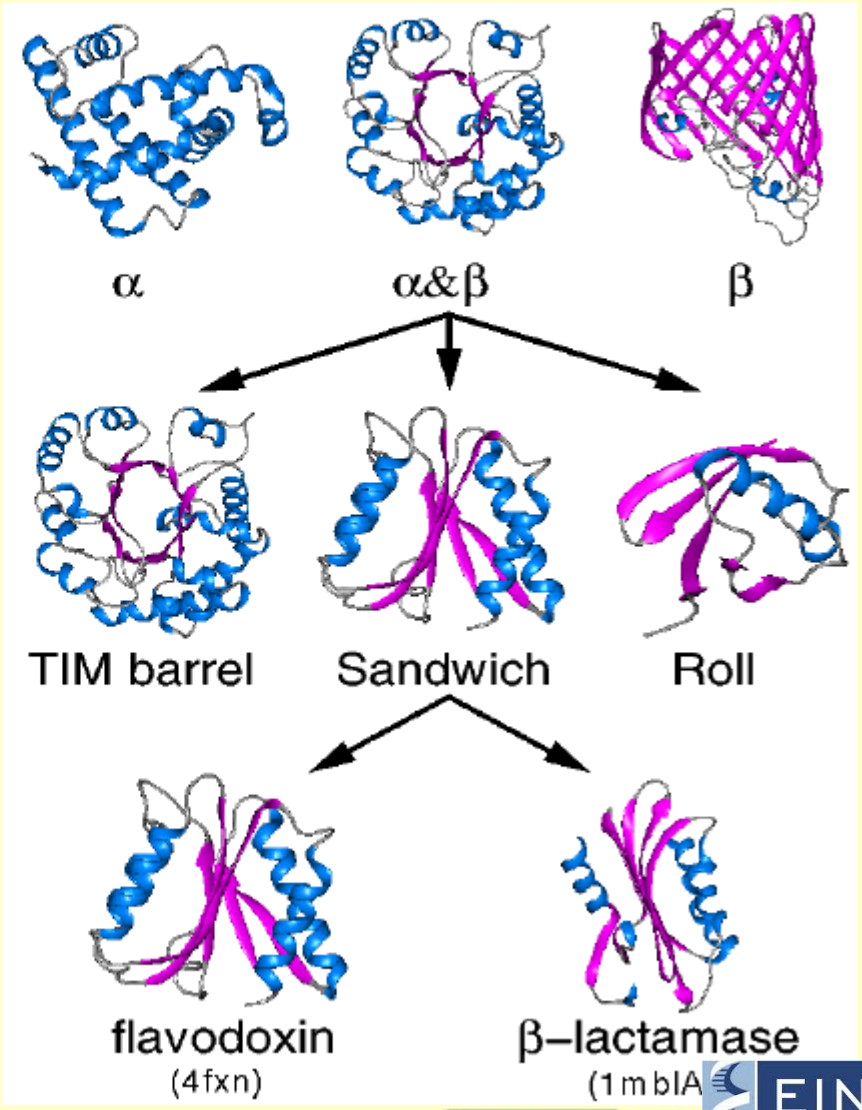
Protein structure classification: SCOP



Structural Classification of Proteins (SCOP)

http://scop.berkeley.edu/

- Class
  - Similar secondary structure content
  - All α, all β, alternating α/β etc
- Fold (Architecture)
  - Major structural similarity
  - SSE's in similar arrangement
- Superfamily (Topology)
  - Probable common ancestry
  - HMM family membership
- Family
  - Clear evolutionary relationship
  - Pairwise sequence similarity > 25%

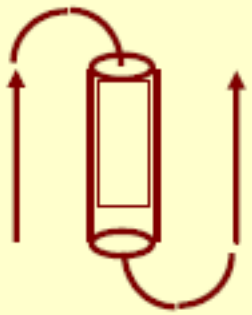


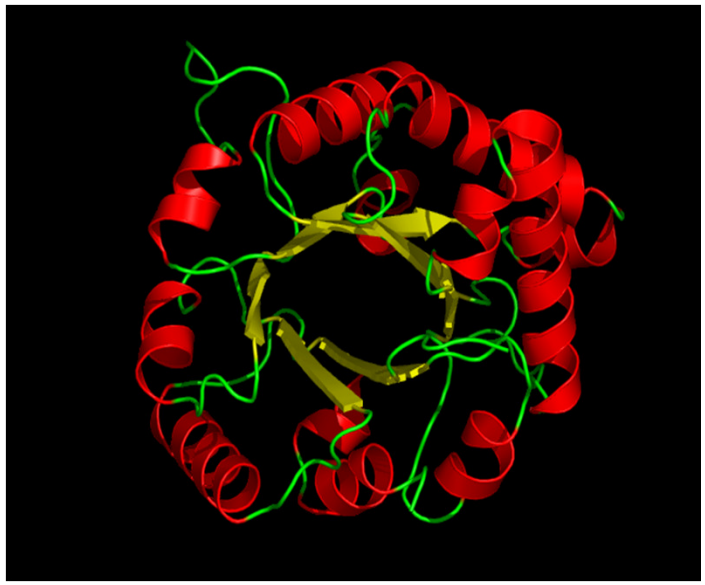
Major classes in SCOP

- **Classes**
  - All α proteins
  - All β proteins
  - α and β proteins (α/β)
  - α and β proteins ($\alpha+\beta$)
  - Multi-domain proteins
  - Membrane and cell surface proteins
  - Small proteins
  - Coiled coil proteins

α/β alternating

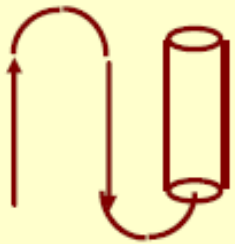
- Parallel β sheets, β-α-β units

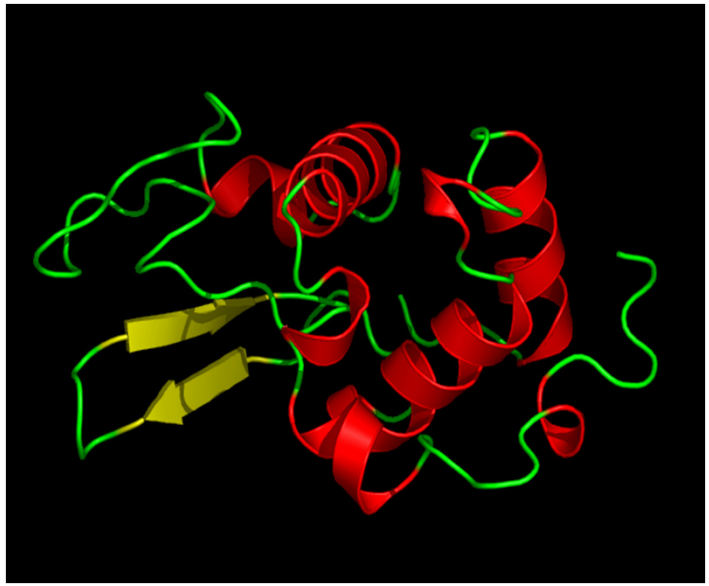




α +β

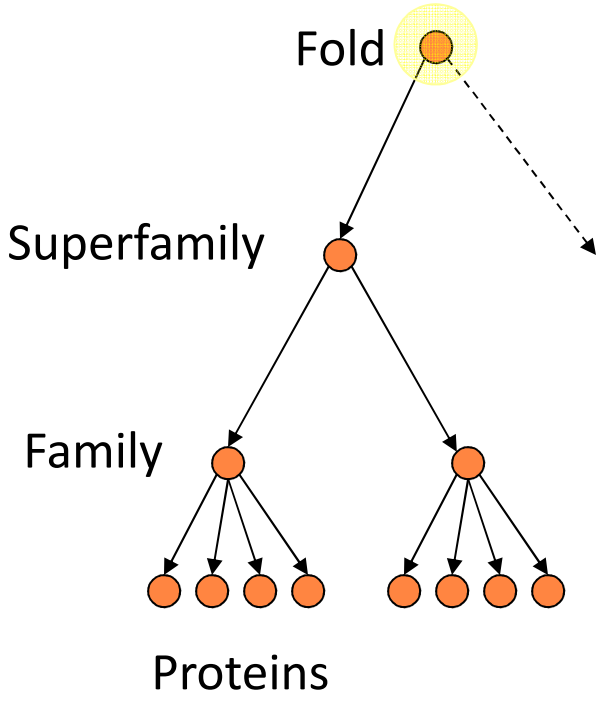
- Anti-parallel β sheets, segregated α and β regions
- helices mostly on one side of sheet





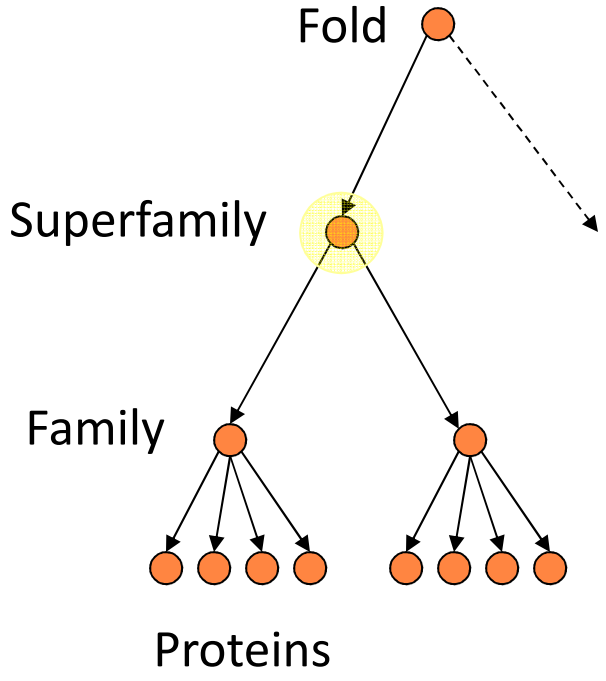
Folds

- >~50% secondary structure elements arranged in the same order in sequence and in 3D
- No evolutionary relation



Superfamilies

- Proteins which are (remotely) evolutionarily related
  - Sequence similarity low
  - Share function
  - Share special structural features
- Relationships between members of a superfamily may not be readily recognizable from the sequence alone



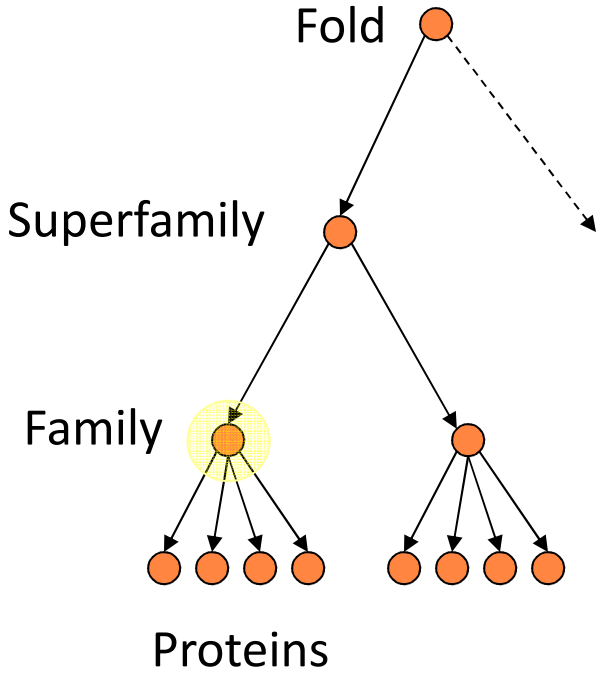
Families

- Proteins whose evolutionarily relationship is readily recognizable from the sequence

  (>~25% sequence identity)

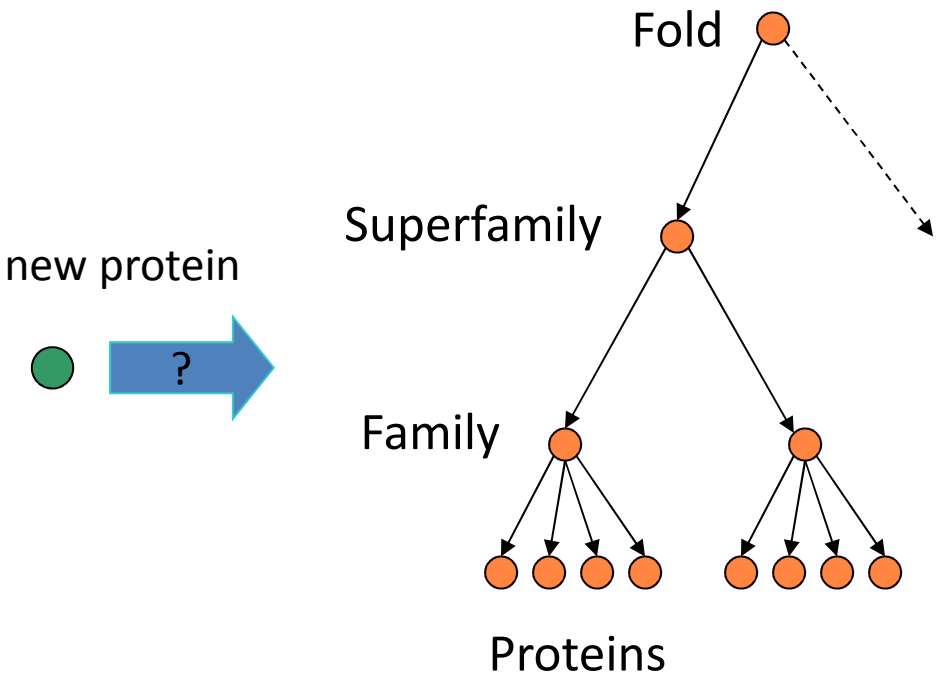
- Families are further subdivided into Proteins

- Proteins are divided into Species
  - The same protein may be found in several species



Protein Classification

- Given a new protein **sequence**, can we place it in its "correct" position within an existing protein hierarchy?



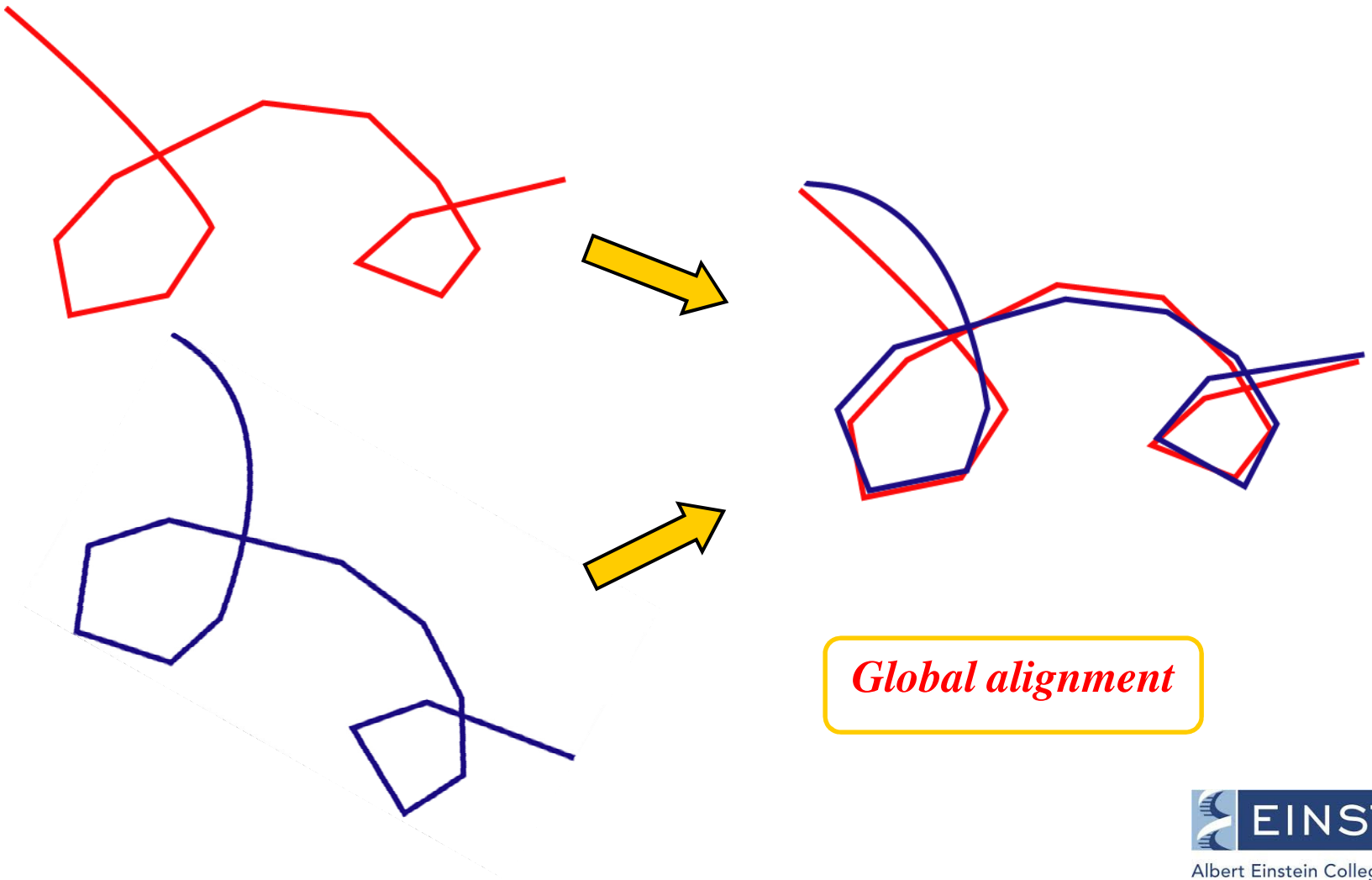
Outline

- Introduction to protein structures
- Protein structure classification
- Protein structure comparison
- Protein structure prediction

Protein Structure Comparison

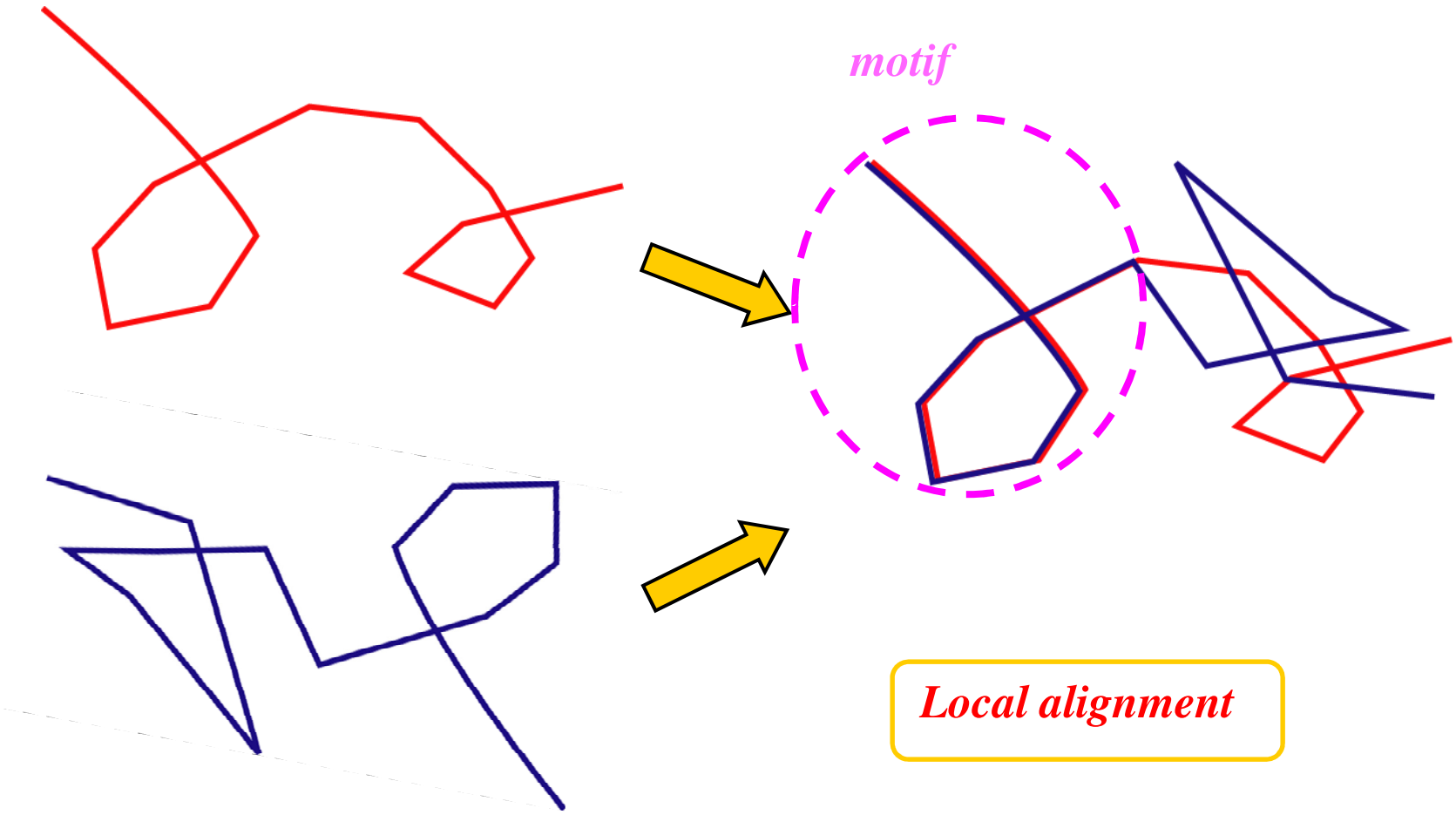
- Global versus local alignment
- Measuring protein shape similarity
- Protein structure superposition
- Protein structure alignment

Global versus Local



Global alignment

Global versus Local (2)



Measuring protein structure similarity

Given two "shapes" or structures A and B, we are interested in defining a distance, or similarity measure between A and B.

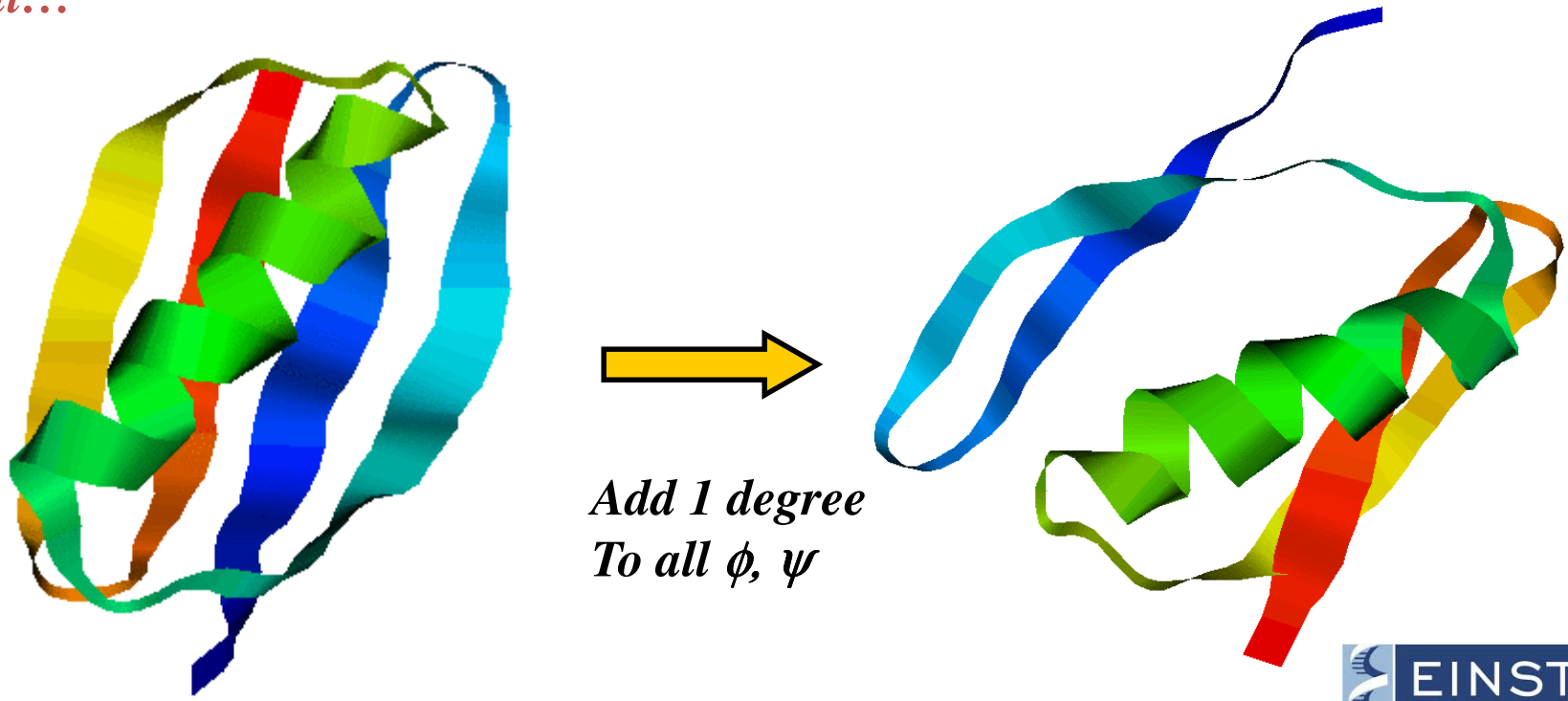
- *Visual comparison*
- *Dihedral angle comparison*
- *Distance matrix*
- *RMSD (root mean square distance)*

Comparing dihedral angles

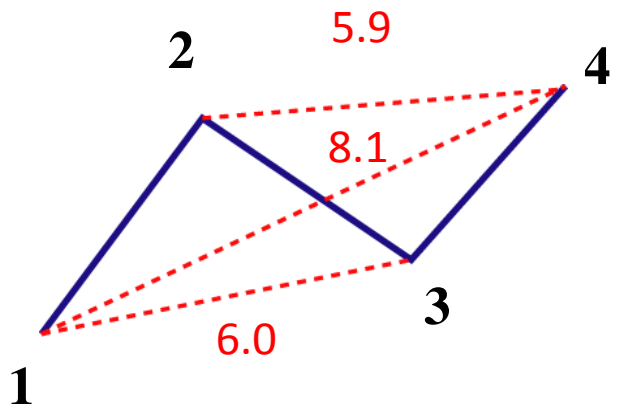
Torsion angles (ϕ,ψ) are:

- local by nature
- invariant upon rotation and translation of the molecule
- compact (O(n) angles for a protein of n residues)

But…



Distance matrix



	1	2	3	4
1	0	3.8	6.0	8.1
2	3.8	0	3.8	5.9
3	6.0	3.8	0	3.8
4	8.1	5.9	3.8	0

Distance matrix (2)

- *Advantages*

  - invariant with respect to rotation and translation

  - can be used to compare proteins

- *Disadvantages*

  - the distance matrix is O(n2) for a protein with n residues

  - comparing distance matrix is a hard problem

  - insensitive to chirality

Root Mean Square Distance (RMSD)

To compare two sets of points (atoms) A={a_1, a_2, …a_N} and B={b_1, b_2, …,b_N}:

-Define a 1-to-1 correspondence between A and B

for example, a_i corresponds to b_i, for all i in [1,N]

-Compute RMS as:

$$RMS(A,B) = \sqrt{\frac{1}{N}\sum_{i=1}^{N} d(a_i,b_i)^2}$$

$d(A_i,B_i)$ is the Euclidian distance between a_i and b_i.

Protein Structure Superposition

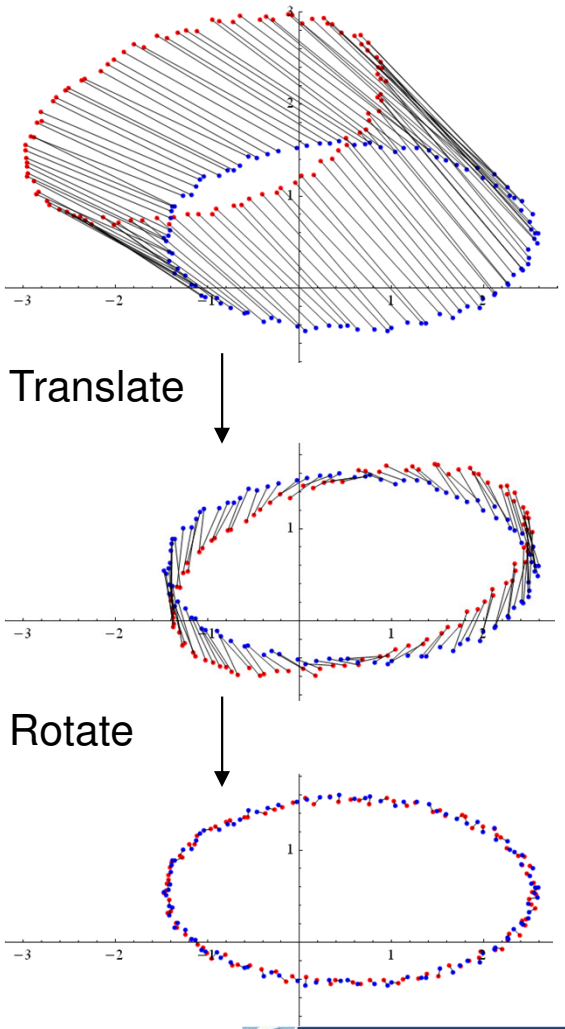
- Simplified problem: we know the correspondence between set A and set B
- We wish to compute the rigid transformation T that best align a_1 with b_1, a_2 with b_2, …, a_N with b_N
- The error to minimize is defined as:

$$\varepsilon = \min_{T} \sum_{i=1}^{N} \| T(a_i) - b_i \|^2$$

Protein Structure Superposition (2)

- A rigid-body transformation T is a combination of a translation t and a rotation R: $T(x) = Rx+t$
- The quantity to be minimized is:

$$\varepsilon = \min_{t,R} \sum_{i=1}^{N} \left\| Ra_i - b_i + t \right\|^2$$



The translation part

ε is minimum with respect to t when:

$$\frac{\partial \varepsilon}{\partial t} = 2\sum_{i=1}^{N}\left(Ra_i - b_i + t\right) = 0$$

Then:

$$t = -R\left(\sum_{i=1}^{N} a_i\right) + \sum_{i=1}^{N} b_i$$

If both data sets A and B have been centered on 0, then t = 0 !

Step 1: Translate point sets A and B such that their centroids coincide at the origin of the framework

The rotation part (1)

Let μ_A and μ_B be the centers of A and B, and A' and B' the matrices containing the coordinates of the points of A and B centered on O:

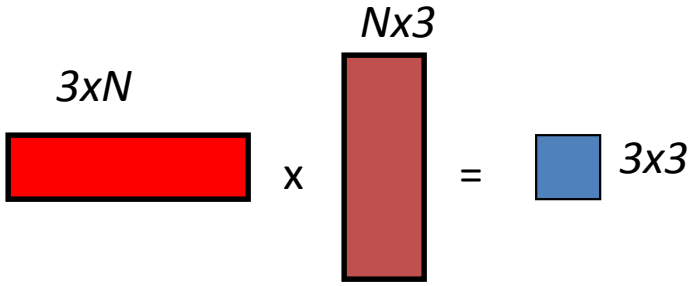
$$\mu_A = \frac{1}{N}\sum_{i=1}^{N} a_i$$

$$\mu_B = \frac{1}{N}\sum_{i=1}^{N} b_i$$

$$A = [a_1 - \mu_A \quad a_2 - \mu_A \quad \dots \quad a_N - \mu_A]$$

$$B = [b_1 - \mu_B \quad b_2 - \mu_B \quad \dots \quad b_N - \mu_B]$$

Build covariance matrix: $C = AB^T$



The rotation part (2)

Compute SVD (Singular Value Decomposition) of C:

$$C = UDV^T$$

U and V are orthogonal matrices, and D is a diagonal matrix containing the singular values.
U, V and D are 3x3 matrices

Define S by:

$$S = \begin{cases} I & \text{if } \det(C) > 0 \\ diag\{1,1,-1\} & \text{otherwise} \end{cases}$$

Then

$$R = USV^T$$

The algorithm

1. Center the two point sets A and B

2. Build covariance matrix:

$$C = AB^T$$

3. Compute SVD (Singular Value Decomposition) of C:

$$C = UDV^T$$

4. Define S:

$$S = \begin{cases} I & \text{if } \det(C) > 0 \\ diag\{1,1,-1\} & \text{otherwise} \end{cases}$$

5. Compute rotation matrix

$$R = USV^T$$

6. Compute RMSD:

$$RMSD = \sqrt{\frac{\sum_{i=1}^{N} a'^2_i + \sum_{i=1}^{N} b'^2_i - 2\sum_{i=1}^{3} d_i s_i}{N}}$$

Example: NMR structures

Superposition of NMR Models

1AW6

Protein Structure Alignment

The Problem:

Given two sets of points *A=(a1, a2, …, an)* and *B=(b1,b2,…bm)* in 3D space, find the **optimal** subsets *A(P)* and *B(Q)* with |A(P)|=|B(Q)|, and find the **optimal** rigid body transformation between the two subsets *A(P)* and *B(Q)* that minimizes a given distance metric D over all possible rigid body transformation *G*, i.e.

$$\min_{G}\{D(A(P)-G(B(Q)))\}$$

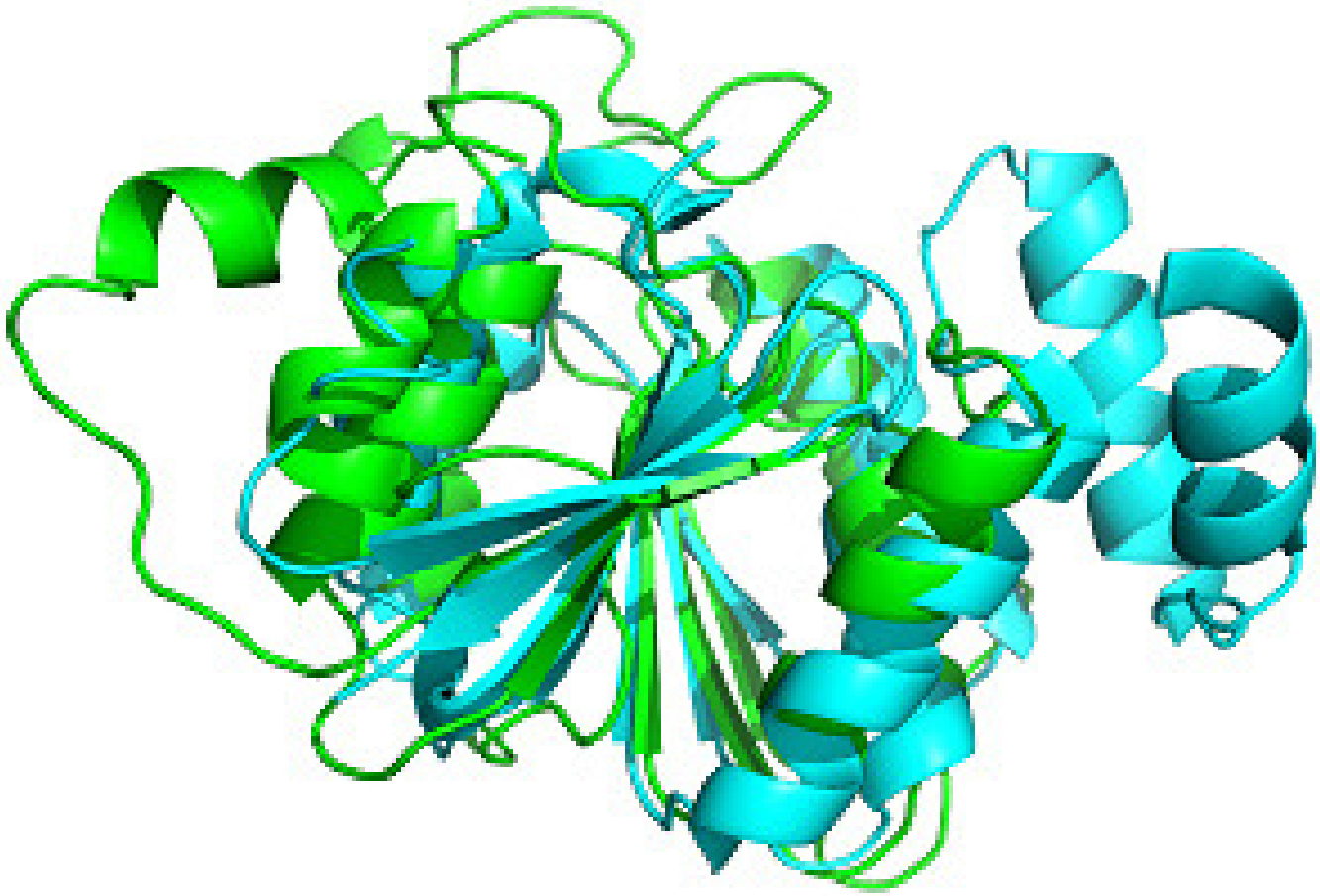
The two subsets *A(P)* and *B(Q)* define a "correspondence", and $p = |A(P)|=|B(Q)|$ is called the correspondence length.

Protein Structure Alignment

Iterate N times:

1. Set Correspondence C to a **seed** correspondence set (small set sufficient to generate an alignment transform)
2. Compute the alignment transform G for C and apply G to the second protein B
3. Update C to include all pairs of features that are close apart
4. If C has changed, then return to Step 2

Protein Structure Alignment: Example



Existing Software

- DALI (Holm and Sander, 1993)
- SSAP (Orengo and Taylor, 1989)
- STRUCTAL (Levitt et al, 1993)
- VAST [Gibrat et al., 1996]
- LOCK [Singh and Brutlag, 1996]
- CE [Shindyalov and Bourne, 1998]
- SSM [Krissinel and Henrik, 2004]
- …

Outline

- Introduction to protein structures
- Protein structure classification
- Protein structure comparison
- Protein structure prediction

Why do we need computational approaches?

- The goal of research in the area of structural genomics is to provide the means to characterize and identify the large number of protein sequences that are being discovered

- Knowledge of the three-dimensional structure
  - helps in the rational design of site-directed mutations
  - can be of great importance for the design of drugs
  - greatly enhances our understanding of how proteins function and how they interact with each other , for example, explain antigenic behaviour, DNA binding specificity, etc

- Structural information from x-ray crystallographic or NMR results
  - obtained much more slowly.
  - techniques involve elaborate technical procedures
  - many proteins fail to crystallize at all and/or cannot be obtained or dissolved in large enough quantities for NMR measurements
  - The size of the protein is also a limiting factor for NMR

- ***With a better computational method this can be done extremely fast.***

Computational methods for Protein Structure Prediction

- Homology or Comparative Modeling
- Fold Recognition or threading Methods
- Ab initio methods that utilize knowledge-based information
- Ab initio methods without the aid of knowledge-based information

Homology Modeling Process

- Template recognition
- Alignment
- Determining structurally conserved regions
- Backbone generation
- Building loops or variable regions
- Conformational search for side chains
- Refinement of structure
- Validating structures

Template Recognition

- First we search the related proteins sequence(templates) to the target sequence in any structural database of proteins
- The accuracy of model depends on the selection of proper template
- FASTA and BLAST from EMBL-EBI and NCBI can be used
- This gives a probable set of templates but the final one is not yet decided
- After intial aligments and finding structurally conserved regions among templates, we choose the final template

Alignment in Homology Modeling

Sequence alignment is central technique in homology modeling

- Used in determining which areas of the reference proteins are conserved in sequence
- Hence suggesting where the reference proteins may also be structurally conserved
- After SCRs are found,it is used to establish one to one correspondence between the amino acids of reference proteins and the target in SCRs
- Thus providing basis of the transforming of coordinates from the reference to the model

The First Developed Algorithm

- Needleman and Wunch algorithm for pairwaise sequence alignment
- It is based on Dynamic Programming Algorithm
- Its a Global Alignment approach

Dynamic Programming Algorithm

- A dynamic programming algorithm solves a problem by combining solutions to **sub-problems** that are computed once and saved in a **table or matrix.**
- The basic idea behind dynamic programming is the **organization of work** in order to avoid repetition of work already done.
- DPAs are typically used when a problem has many possible solutions and an optimal one has to be found.

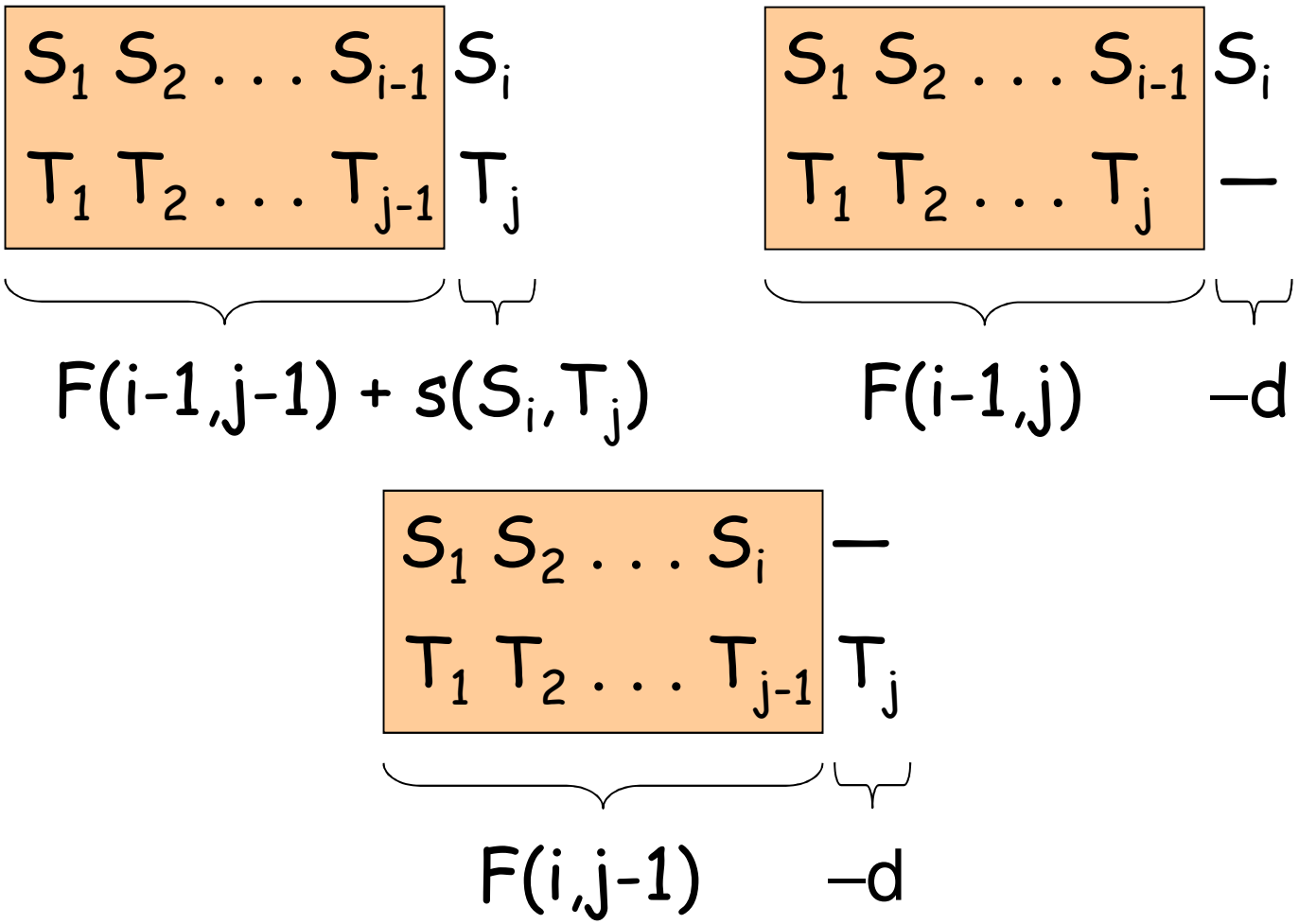
Dynamic Programming Algorithm

Mathematical formulation

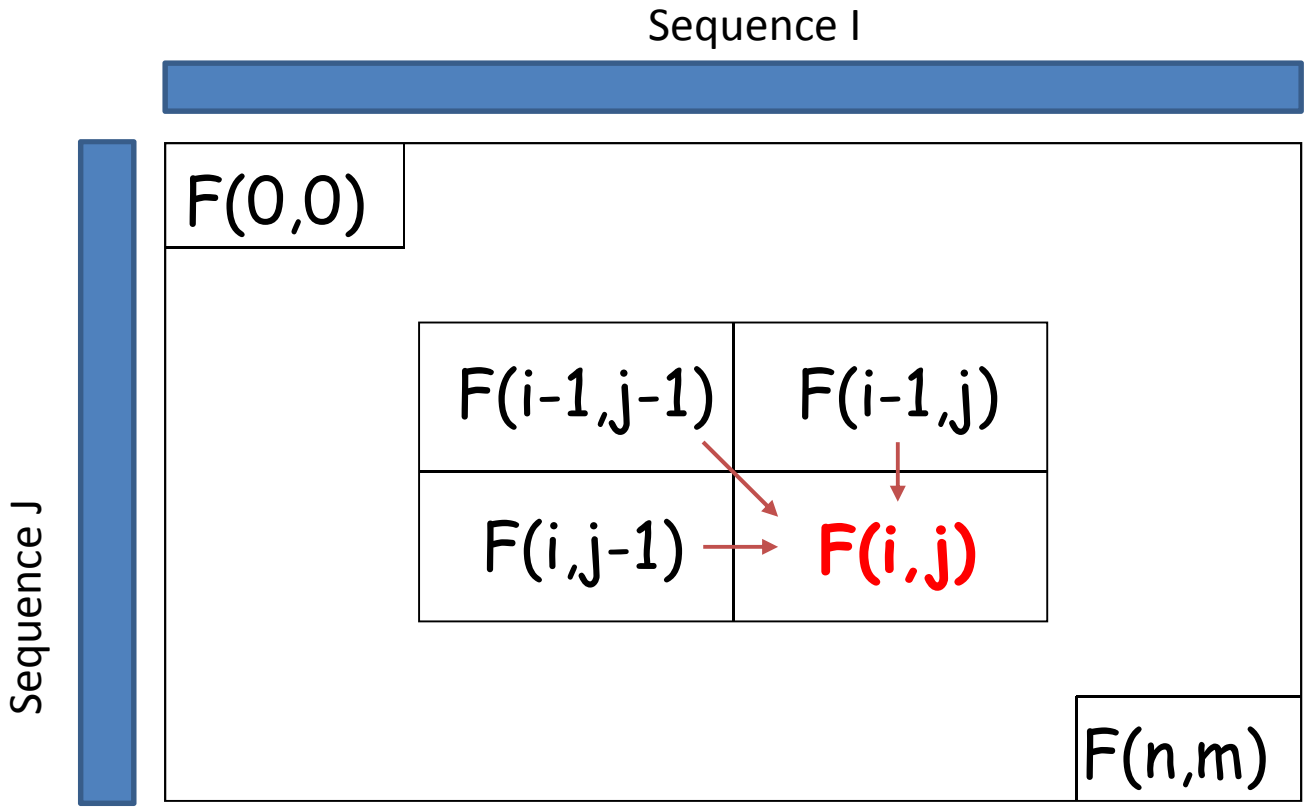
$$
\begin{aligned}
F(i,j) = \text{MAX} \{ & \\
& F(i-1,\ j-1) + s(x_i,\ y_j), \\
& F(i-1,\ j) - d, \\
& F(i,\ j-1) - d \\
& \}
\end{aligned}
$$

Where F(i,j) is the value in cell (i,j); s is the score for that match in the table; d is the gap penalty

Dynamic Programming Algorithm



Dynamic Programming Algorithm



$$\mathrm{F}(i,\,j) = \max\left\{\mathrm{F}(i-1,\,j-1) + s(S_i, T_j),\; F(i-1,\,j) - d,\; F(i,\,j-1) - d\right\}$$

Dynamic Programming Algorithm

Example alignment:
ELVIS LIVES?

		E	L	V	I	S
L						
I						
V						
E						
S						

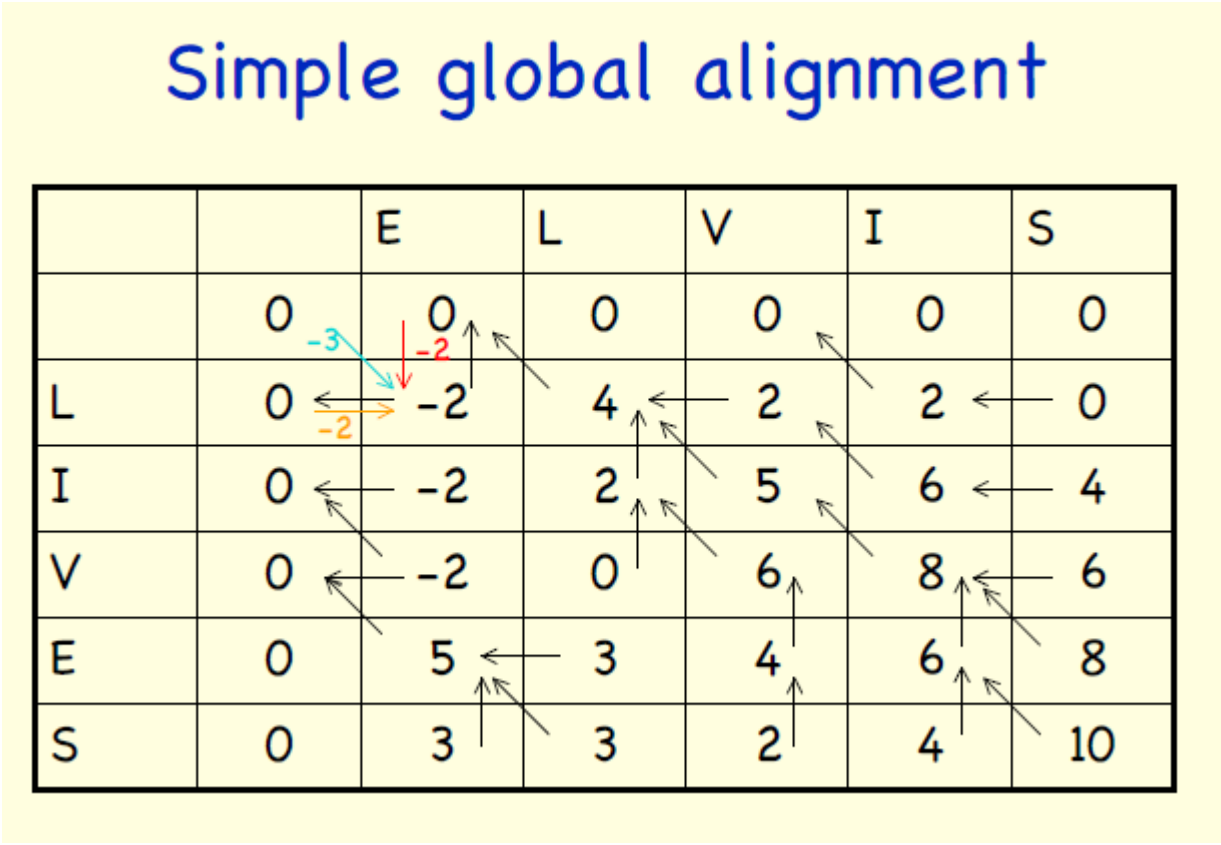
Dynamic Programming Algorithm

BLOSUM62 substitution matrix

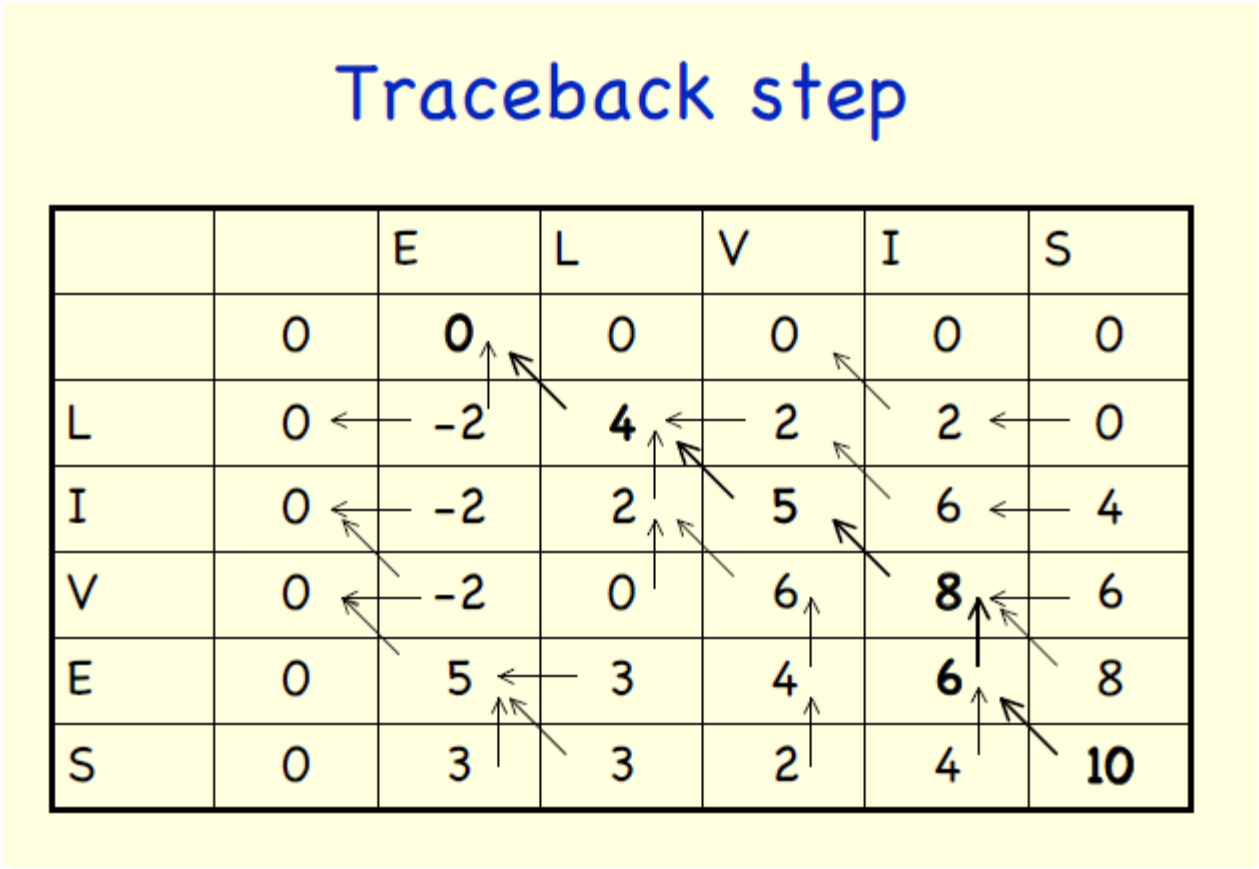
	C	S	T	P	A	G	N	D	E	Q	H	R	K	M	I	L	V	F	Y	W
C	**9**	-1	-1	-3	0	-3	-3	-3	-4	-3	-3	-3	-3	-1	-1	-1	-1	-2	-2	-2
S	-1	**4**	1	-1	1	0	1	0	0	0	-1	-1	0	-1	-2	-2	-2	-2	-2	-3
T	-1	1	**4**	1	-1	1	0	1	0	0	0	-1	0	-1	-2	-2	-2	-2	-2	-3
P	-3	-1	1	**7**	-1	-2	-1	-1	-1	-1	-2	-2	-1	-2	-3	-3	-2	-4	-3	-4
A	0	1	-1	-1	**4**	0	-1	-2	-1	-1	-2	-1	-1	-1	-1	-1	-2	-2	-2	-3
G	-3	0	1	-2	0	**6**	-2	-1	-2	-2	-2	-2	-2	-3	-4	-4	0	-3	-3	-2
N	-3	1	0	-2	-2	0	**6**	1	0	0	-1	0	0	-2	-3	-3	-3	-3	-2	-4
D	-3	0	1	-1	-2	-1	1	**6**	2	0	-1	-2	-1	-3	-3	-4	-3	-3	-3	-4
E	-4	0	0	-1	-1	-2	0	2	**5**	2	0	0	1	-2	-3	-3	-3	-3	-2	-3
Q	-3	0	0	-1	-1	-2	0	0	2	**5**	0	1	1	0	-3	-2	-2	-3	-1	-2
H	-3	-1	0	-2	-2	-2	1	1	0	0	**8**	0	-1	-2	-3	-3	-2	-1	2	-2
R	-3	-1	-1	-2	-1	-2	0	-2	0	1	0	**5**	2	-1	-3	-2	-3	-3	-2	-3
K	-3	0	0	-1	-1	-2	0	-1	1	1	-1	2	**5**	-1	-3	-2	-3	-3	-2	-3
M	-1	-1	-1	-2	-1	-3	-2	-3	-2	0	-2	-1	-1	**5**	1	2	-2	0	-1	-1
I	-1	-2	-2	-3	-1	-4	-3	-3	-3	-3	-3	-3	-3	1	**4**	2	1	0	-1	-3
L	-1	-2	-2	-3	-1	-4	-3	-4	-3	-2	-3	-2	-2	2	2	**4**	3	0	-1	-2
V	-1	-2	-2	-2	0	-3	-3	-3	-2	-2	-3	-3	-2	1	3	1	**4**	-1	-1	-3
F	-2	-2	-2	-4	-2	-3	-3	-3	-3	-3	-1	-3	-3	0	0	0	-1	**6**	3	1
Y	-2	-2	-2	-3	-2	-3	-2	-3	-2	-1	2	-2	-2	-1	-1	-1	-1	3	**7**	2
W	-2	-3	-3	-4	-3	-2	-4	-4	-3	-2	-2	-3	-3	-1	-3	-2	-3	1	2	**11**

$$s(S_i, T_j)$$

Dynamic Programming Algorithm



Dynamic Programming Algorithm

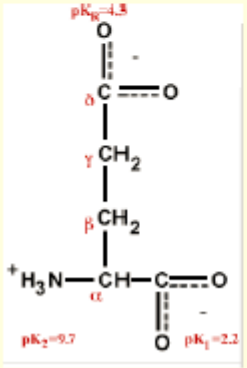


Dynamic Programming Algorithm

Resulting alignment

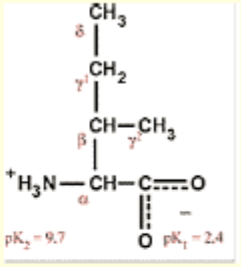
~~EL-VIS~~
~~-LIVES~~

ELVI-S
-LIVES

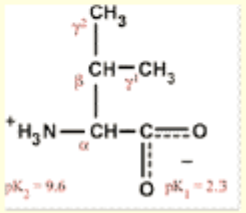


Glutamic acid (E)

Score:
-2+4+3+3-2+4
=10



Isoleucine (I)



Valine (V)

The Modified Version Of Needleman Wunch

- Smith Waterman algorithm is modified Needleman Wunch
- It is for local alignment
- Locate the best local alignment between two sequences
- What is Global and Local Alignment
- In global, we try to find similarity in whole sequence
- In local, we try to find small similar segments within sequences

Local Alignment

- Comparing sequences of different length
- Proteins are from different protein families

Tools based on local alignment

- BLAST & FASTA – alignment against databases
- LALIGN & EMBOSS align – alignment of two sequences
- Infact there are more tools, these are the widely used

Dynamic Programming Algorithm

Local alignment

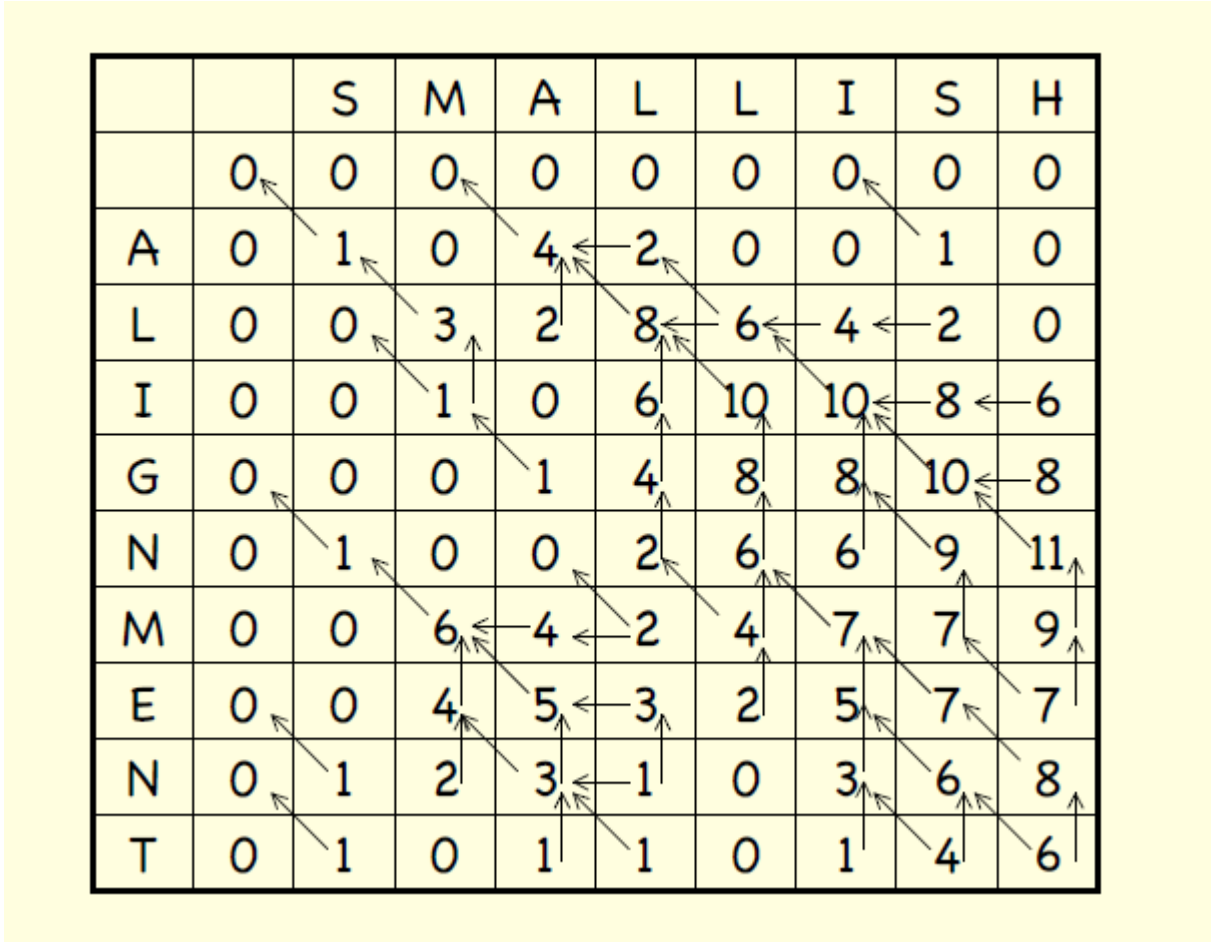
- Differences from global alignments
  - Minimum value allowed = 0
    - Corresponds to starting a new alignment
  - The alignment can end anywhere in the matrix, not just the bottom right corner
    - To find the best alignment, find the best score and trace back from there
  - Expected score for a random match MUST be negative

Dynamic Programming Algorithm

Local alignment formula

$$
\begin{aligned}
F(i,j) = \mathrm{MAX}\ \{ & \\
& 0, \\
& F(i-1,\ j-1) + s(x_i,\ y_j), \\
& F(i-1,\ j) - d, \\
& F(i,\ j-1) - d \\
& \}
\end{aligned}
$$

Dynamic Programming Algorithm



		S	M	A	L	L	I	S	H
	0	0	0	0	0	0	0	0	0
A	0	1	0	4	2	0	0	1	0
L	0	0	3	2	8	6	4	2	0
I	0	0	1	0	6	10	10	8	6
G	0	0	0	1	4	8	8	10	8
N	0	1	0	0	2	6	6	9	11
M	0	0	6	4	2	4	7	7	9
E	0	0	4	5	3	2	5	7	7
N	0	1	2	3	1	0	3	6	8
T	0	1	0	1	1	0	1	4	6

EINSTEIN
Albert Einstein College of Medicine
OF YESHIVA UNIVERSITY

Dynamic Programming Algorithm

		S	M	A	L	L	I	S	H
	0	0	0	0	0	0	0	0	0
A	0	1	0	4	2	0	0	1	0
L	0	0	3	2	8	6	4	2	0
I	0	0	1	0	6	10	10	8	6
G	0	0	0	1	4	8	8	10	8
N	0	1	0	0	2	6	6	9	11
M	0	0	6	4	2	4	7	7	9
E	0	0	4	5	3	2	5	7	7
N	0	1	2	3	1	0	3	6	8
T	0	1	0	1	1	0	1	4	6

Dynamic Programming Algorithm

Resulting alignments

ALLISH	**ALLISH**
AL-IGN	**A-LIGN**
Score:	Score:
4+4-2+4+0+1	4-2+4+4+0+1
=11	=11

Dynamic Programming Algorithm

Alignment with affine gap scores

- Affine gap scores
  - "affine" means that the penalty for a gap is computed as a linear function of its length
  - Have a gap opening penalty
  - Also have a less prohibitive gap extension penalty
- Have to keep track of multiple values for each pair of residue coefficients i and j in place of the single value F(i,j)
  - We keep two matrices, M and I

Dynamic Programming Algorithm

Affine gap score formula

$$
\begin{aligned}
M(i,j) = \mathrm{MAX}\ \{ & \\
& M(i-1,\ j-1) + s(x_i, y_j), \\
& I(i-1,\ j-1) + s(x_i, y_j) \\
& \} \\
I(i,j) = \mathrm{MAX}\ \{ & \\
& M(i,\ j-1) - d, \\
& I(i,\ j-1) - e, \\
& M(i-1,\ j) - d, \\
& I(i-1,\ j) - e \\
& \}
\end{aligned}
$$

Comparison Of Different Algorithms

- Traditional algorithms
  - Find optimal alignment under a specific scoring criterian that includes the scoring matrix and gap penalties
  - Optimal alignment is quite often not the true biological alignment(Argos et al, 1991,Agarwal and States,1996)
- Heuristic algorithms
  - Heuristic search tools find the optimal alignment with high probability and are less computationaly expensive
  - HMM based search methods improve both the sensitivity and selectivity of sequence database searches,using position dependent scores to characterize and build a model for an entire family of sequences
  - Probabilistic Smith-waterman is based on HMM for a single sequence, more accurate from others for complete length protein query sequences in large protein family but poor when used with partial length query sequence

Multiple Sequence Alignment

- This is all about pairwise alignment
- In general homology modeling, we would like to include more than two protein references for the template protein
- It helps in finding conserved domains among similar reference proteins
- Therefore providing more information about structurally conserved domains in sequences
- **Multiple Sequence Alignment- Methods**
  - Multiple alignment is more difficult than pairwise alignment because the number of possible alignments increases exponentially with the number of sequences to be aligned
  - No ideal method exists, several heuristic algorithms are being used
  - Simple way is to use Needleman and Wunch algorithm for pairwise alignment in multidimensional space
  - Disadvantage of this is exponetial increase of memory usage and time consumpiton

Determining Structurally Conserved Regions (SCRs)

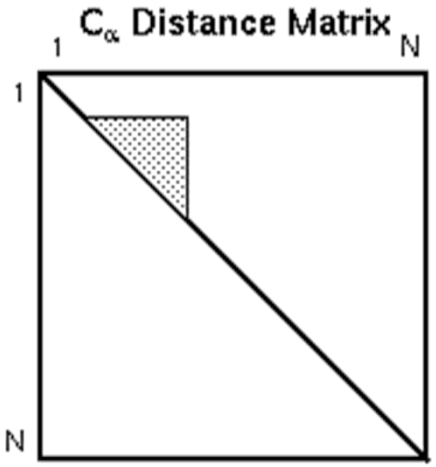
- When two or more reference protein structures are available
- Establish structural guidelines for the family of proteins under consideration
- First step in building a model protein by homology is determining what regions are structurally conserved or constant among all the reference proteins
- Target protein is supposed to assume the same conformation in conserved regions

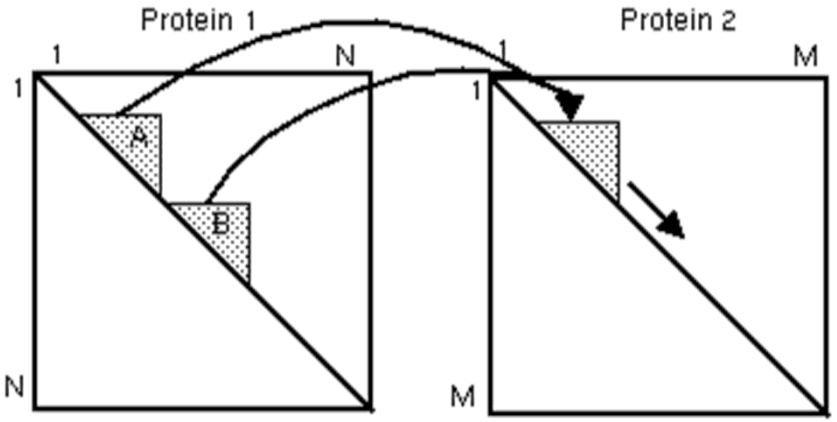
Structurally Conserved Regions

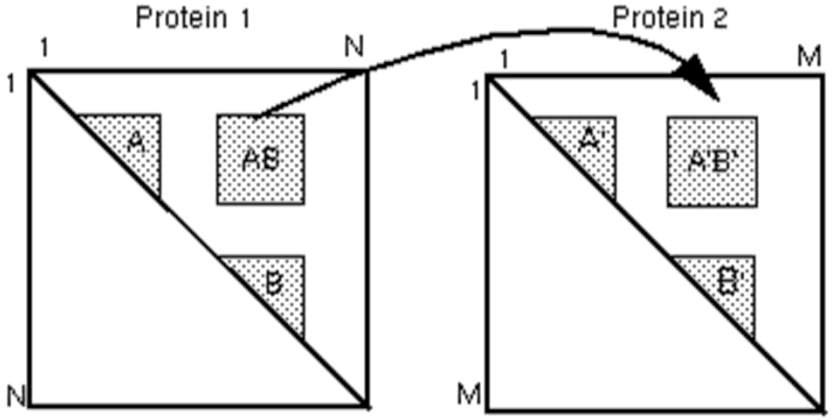
- SCRs are region in all proteins of a particular family that are nearly identical in structures.
- Tend to be at inner cores of the proteins
- Usually contains alpha-helices and beta sheets

There are generally two main approaches

- Constructing c-alpha distance matrix
- Aligning vectors of secondary structure units







Alignment of Target Protein with SCR

- After doing alignments and finding SCRs
- We align the unknown sequence with the aligned reference proteins with the knowledge of SCRs
- Assignment of coordinates within conserved regions is done
- SCR cant contain insertions and deletions

Assignment of coordinates within conserved region

- Once the correspondence between amino acids in the reference and model sequences has been made, the coordinates for an SCR can be assigned
- The reference proteins' coordinates are used as a basis for this assignment

- Where the side chains of the reference and model proteins are the same at corresponding locations along the sequence, all the coordinates for the amino acid are transferred

- Where they differ, the backbone coordinates are transferred , but the side chain atoms are automatically replaced to preserve the model protein's residue types

Assignment of coordinates in loop or variable region

Two main methods

- Finding similar peptide segments in other proteins
- Generating a segment de-novo

Assignment of coordinates in loop or variable region

Finding similar peptide segment in other proteins

- Advantage: all loops found are guaranteed to have reasonable internal geometries and conformations

- Disadvantage: may not fit properly into the given model protein's framework

In this case, de-novo method is advisable

Selection Of Loops

- Check the loops on the basis of steric overlaps
- A specified degree of overlap can be tolerated
- Check the atoms within the loop against each other
- Then check loop atoms against rest of the protein's atoms

Side Chain Conformation Search

- With bond lengths, bond angles and two rotable backbone bonds per residue ϕ and ψ, its very difficult to find the best conformation of a side chain
- In addition, side chains of many residues have one or more degree of freedom.
- Hence Side chain conformational search in loop regions is must
- Side chain residues replaced during coordinate transformations should also be checked

Selection Of Good Rotamer

- Fortunately, statstical studies show side chain adopt only a small number of many possible conformations
- The correct rotamer of a particular residue is mainly determined by local environment
- Side chain generally adopt conformations where they are closely packed

Selection Of Good Rotamer … Contd

It is observed that:

- In homologous proteins, corresponding residues virtually retain the same rotameric state (Ponder and Richards 1987, Benedetti et al. 1983)
- Within a range of χ values, 80% of the identical residues and 75% of the mutated residues have the same conformations(Summers et al. 1987)
- Certain rotamers are almost always associated with certain secondary structure(McGregor et al. 1987).

Refinement of model using Molecular Mechanics

Many structural artifacts can be introduced while the model protein is being built

- Substitution of large side chains for small ones
- Strained peptide bonds between segments taken from difference reference proteins
- Non optimum conformation of loops

Optimisation Approaches

- Energy Minimisation is used to produce a chemically and conformationally reasonable model of protein structure

Two mainly used optimisation algorithms are

- Steepest Descent
- Conjugate Gradients

Model Validation

- Every homology model contains errors.Two main reasons
  - % sequence identity between reference and model
  - The number of errors in templates
- Hence it is essential to check the correctness of overall fold/ structure, errors of localized regions and stereochemical parameters: bond lengths, angles, geometries

Challenges

- To model proteins with lower similarities(eg < 30% sequence identity)
- To increase accuracy of models and to make it fully automated
- Improvements may include simulataneous optimization techniques in side chain modeling and loop modeling
- Developing better optimizers and potential function, which can lead the model structure away from template towards the correct structure
- Although comparative modelling needs significant improvement, it is already a mature technique that can be used to address many practical problems

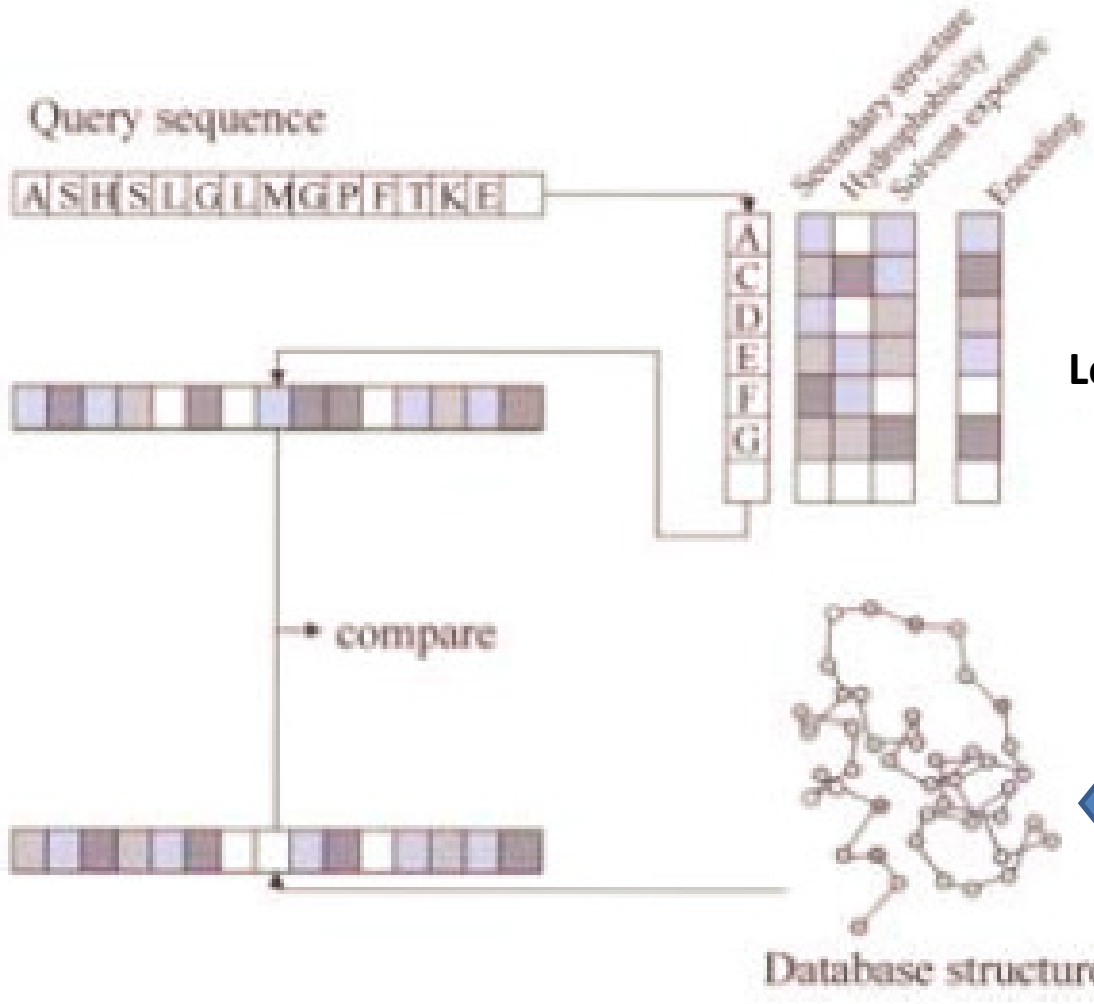
Fold Recognition Methods

- Profile-based methods
- Threading-based methods

Profile-based methods

- **Physico-chemical properties of the amino acids of the target protein must "fit" with the environment in which they are placed in the modeled structure.**

Profile-based methods



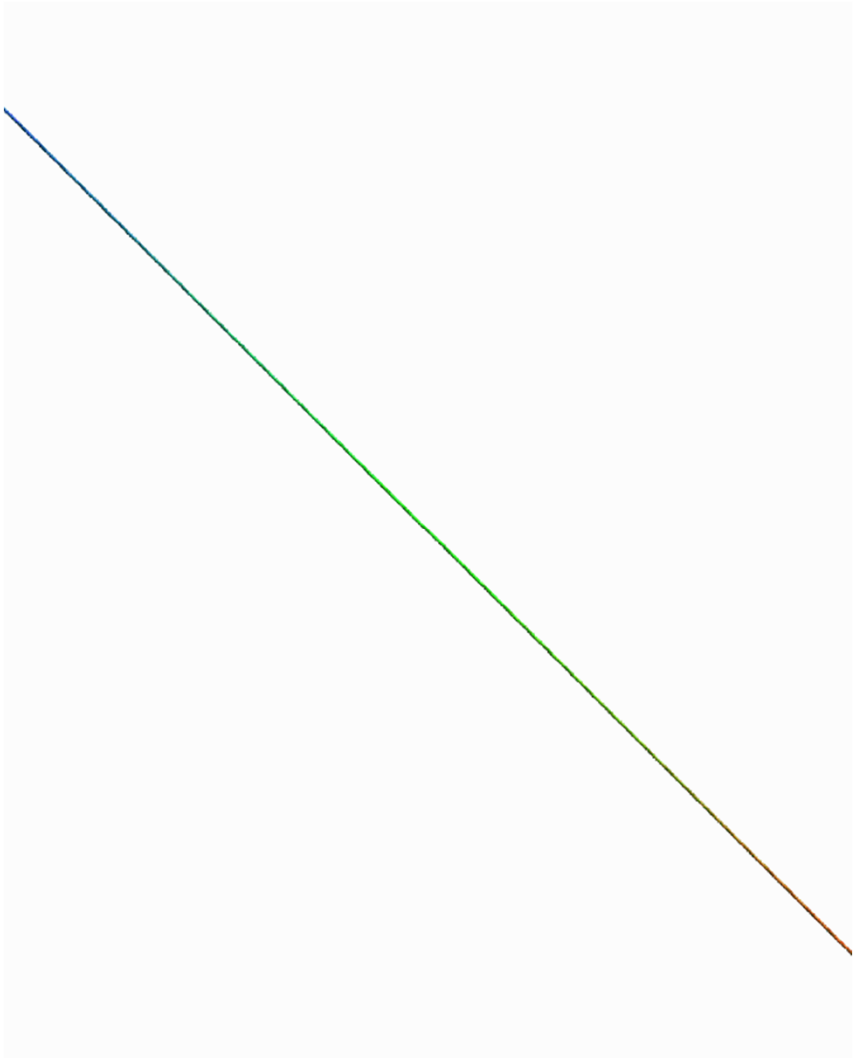
Look-up Table

Template Library

Threading-based methods

- A library of different protein folds is derived from the database of protein structures.
- Each fold is considered as a chain tracing through space; the original sequence being ignored completely.
- The test sequence is then optimally fitted to each library fold, allowing for relative insertions and deletions in loop regions.
- The 'energy' of each possible fit (or threading) is calculated by summing the proposed pairwise interactions and the solvation energy.
- The library of folds is then ranked in ascending order of total energy, with the lowest energy fold being taken as the most probable match.

Ab initio methods



Summary

- Introduction to protein structures
- Protein structure classification
- Protein structure comparison
- Protein structure prediction