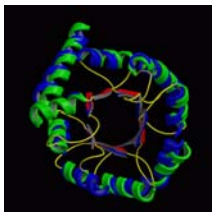
Protein threading

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

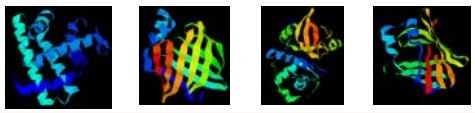
Concept of Threading

- 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

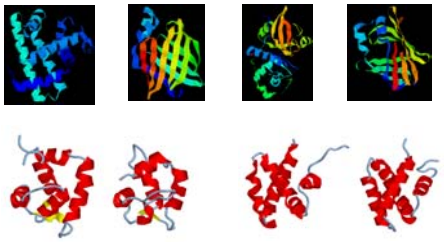
- Threading: Given a sequence, and a fold (template), compute the optimal alignment score between the sequence and the fold.
- If we can solve the above problem, then
  - Given a sequence, we can try each known fold, and find the best fold that fits this sequence.
  - Because there are only a few thousands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

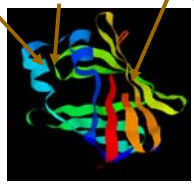
- Build a template database



Protein Threading – energy function

MTYKLILNGKTKGETTTEAVDAATAEKVFQYANDNGVDGEWTYTE

how preferable to put two particular residues nearby: E_p



how well a residue fits a structural environment: E_s

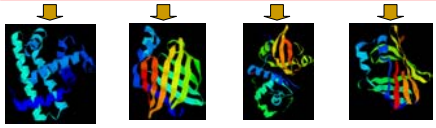
alignment gap penalty: E_g

total energy: E_p + E_s + E_g

find a sequence-structure alignment to minimize the energy function

Assessing Prediction Reliability

MTYKLILNGKTKGETTTEAVDAATAEKVFQYANDNGVDGEWTYTE



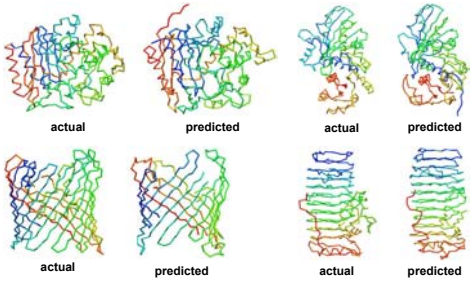
Score = -1500 Score = -720 Score = -1120 Score = -900

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

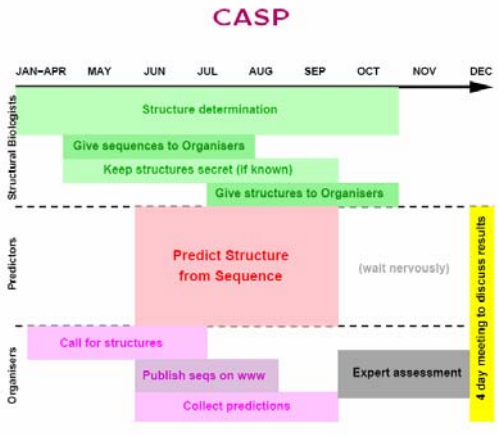


Prediction of Protein Structures

- 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/CAFASP

- CASP: Critical Assessment of Structure Prediction

CASP Predictor

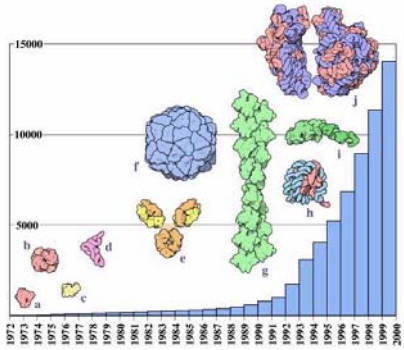
- CAFASP: Critical Assessment of Fully Automated Structure Prediction

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 will be held this year (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)

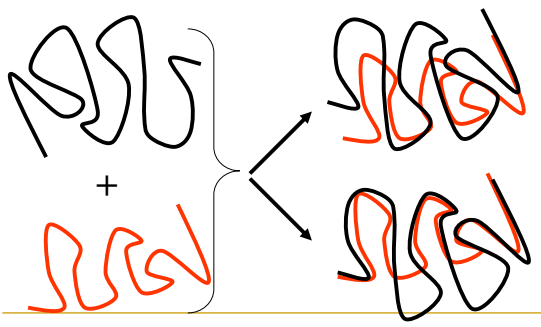
Structure Analysis – Basic Issues

- 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

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

Translation

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

Rotation

Structure Comparison

Scoring system to find optimal alignment

Answer: Root Mean Square Deviation (*RMSD*)

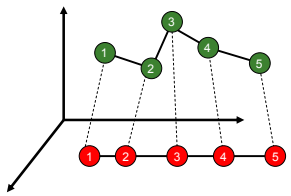
$$RMSD = \sqrt{\frac{\sum_i d_i^2}{n}}$$

n = number of atoms

d_i = distance between 2 corresponding atoms i in 2 structures

Root Mean Square Deviation

$$\mathbf{RMS} = \sqrt{\frac{\sum_{i=1}^{5} (X_{RED1} - X_{BLUE1})^2}{5}} \sim \frac{d_1 + d_2 + d_3 + d_4 + d_5}{5}$$



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)

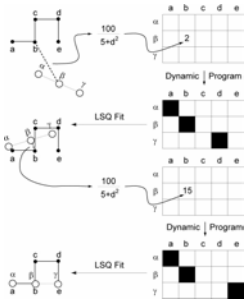
1. Start with arbitrary alignment of the points in two molecules A and B
2. Superimpose in order to minimize RMSD.
3. Compute a *structural alignment (SA) matrix* where entry (i,j) is the score for the structural similarity between the i^{th} point of A and the j^{th} point of B
4. Use DP to compute the next alignment.
   Gap cost = 0
5. Iterate steps 2--4 until the overall score converges
6. Repeat with a number of initial alignments

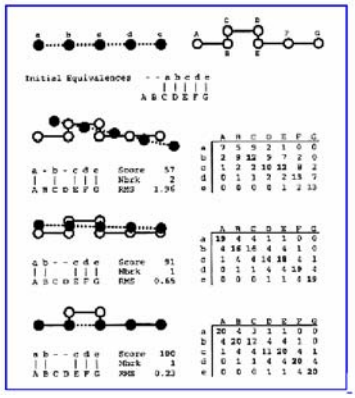
STRUCTAL

- 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
Score 57
Mark 2
RMS 1.96
Score 91
Mark 1
RMS 0.66
Score 100
Mark 1
RMS 0.23