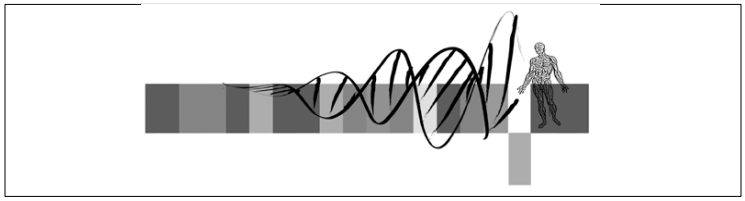


Bioinformatics for Biologists

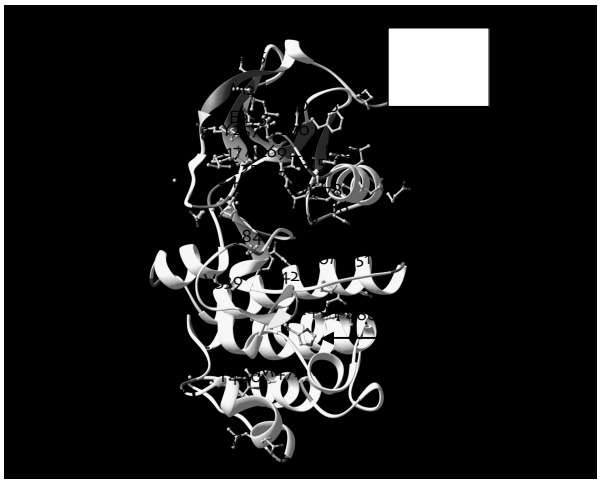
Comparative Protein Analysis:
Part III. Protein Structure Prediction and Comparison

Robert Latek, PhD
Sr. Bioinformatics Scientist
Whitehead Institute for Biomedical Research

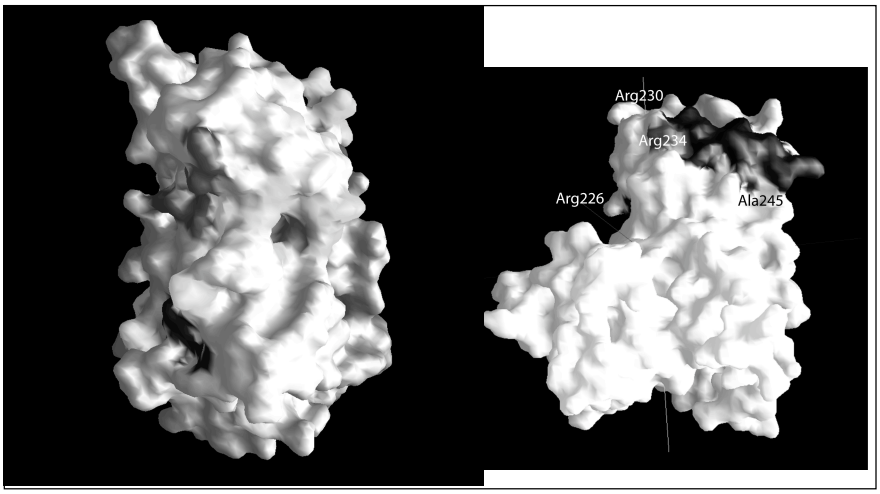
Protein Structure

Why is protein structure information useful?

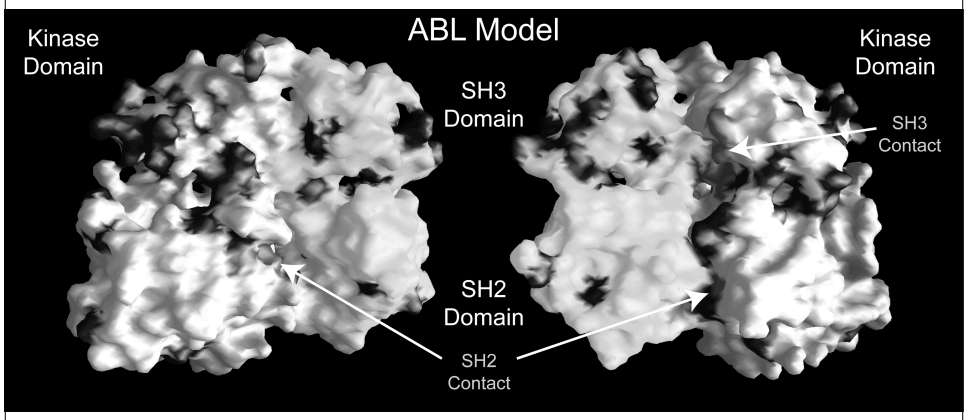
Predicting Important AAs



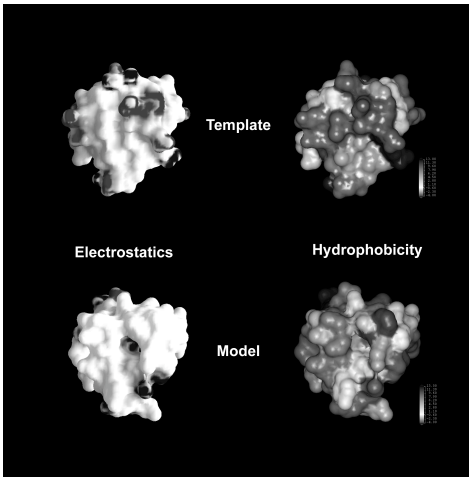
Surface Mapping



Protein Interfaces



Property Comparisons

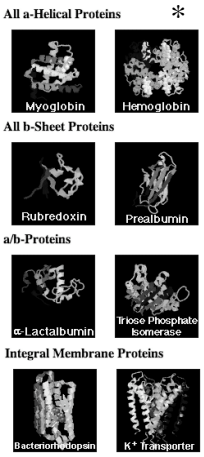


Syllabus

- **Protein Structure Classification**
- Structure Coordinate Files & Databases
- Comparing Protein Structures
  - Aligning 3D Structures
- Predicting Protein Structure
  - Specialized Structural Regions
  - Secondary Structure Prediction
  - Tertiary Structure Prediction
    - Threading
    - Modeling
- Structure Visualization

Structure Classification

- Proteins can adopt only a limited number of possible 3D conformations
  - Combinations of α helices, β sheets, loops, and coils
- Completely different sequences can fold into similar shapes
- Protein Structure Classes
  - Class α: bundles of α helices
  - Class β: anti-parallel β sheets (sandwiches and barrels)
  - Class α / β: parallel β sheets with intervening α helices
  - Class α + β: segregated α helices and anti-parallel β sheets
  - Multi-domain
  - Membrane/Cell surface proteins



*http://info.bio.cmu.edu/courses/03231/ProtStruc/ProtStruc2.htm

Structure Families

- Divide structures into the limited number of possible structure families
  - Homologous proteins can be identified by examining their respective structures for conserved fold patterns
  - Representative members can be used for modeling sequences of unknown structure

Structure Family Databases

- **SCOP**: Structural Classification Of Proteins
  - based on a definition of structural similarities. Hierarchical levels to reflect evolutionary and structural relationships
  - http://scop.mrc-lmb.cam.ac.uk/scop
- **CATH**: Classification by Class, Architecture, Topology, and Homology
  - classified first into hierarchical levels like SCOP
  - http://www.biochem.ucl.ac.uk/bsm/cath/
- **FSSP**: Fold classification based on Structure-structure alignment of proteins
  - based on structural alignment of all pair-wise combinations of proteins in PDB by DALI (used to id common folds and place into groups)
  - http://www2.embl-ebi.ac.uk/dali/fssp/fssp.html
- **MMDB**
  - Aligns 3D structures based on similar arrangements of secondary structural elements (VAST)
  - http://www.ncbi.nlm.nih.gov/Structure/MMDB/mmdb.shtml
- **SARF**
  - categorized on the basis of structural similarity, categories are similar to other dbs
  - http://123d.ncifcrf.gov/

Syllabus

- Protein Structure Classification
- **Structure Coordinate Files & Databases**
- Comparing Protein Structures
  - Aligning 3D Structures
- Predicting Protein Structure
  - Specialized Structural Regions
  - Secondary Structure Prediction
  - Tertiary Structure Prediction
    - Threading
    - Modeling
- Structure Visualization

Coordinates

- Coordinate Data: location of a molecule's atoms in space (XYZ triple)
- XYZ triple is labeled with an atom, residue, chain
  - Modified aa are labeled with X, H's not usually listed

Atom	Residue	Chain	X	Y	Z
54	ALA	C	35.4	-9.3	102.5

- Data Representation
  - Chemistry Rules Approach: connect the dots utilizing a standard rules base to specify bond distances (not consistent among applications)
  - Explicit Bonding Approach: explicit bonding information is specified in the file (very consistent)

Coordinate File Formats

- **MMDB** "Molecular Modeling DataBank" Format
  - ASN.1 standard data description language (explicit bond information)
- **mmCIF** "Chemical Interchange Format"
  - (relational db format)
- **PDB** "Protein DataBank" Format
  - Column oriented, "flexible format" (chemistry rules)

Example PDB File

tag	Residue	Atom#	Atom type	Chain	Residue#	X	Y	Z	Structure scores
ATOM	1432	N	ALA	A	259	15.711	12.486	46.370	1.00 28.54
ATOM	1433	CA	ALA	A	259	17.047	12.953	46.726	1.00 27.48
ATOM	1434	C	ALA	A	259	17.029	14.459	46.979	1.00 25.31
ATOM	1435	O	ALA	A	259	17.787	15.207	46.367	1.00 25.19
ATOM	1436	CB	ALA	A	259	18.035	12.617	45.610	1.00 25.32
ATOM	1437	N	TRP	A	260	16.149	14.897	47.875	1.00 23.61
ATOM	1438	CA	TRP	A	260	16.033	16.312	48.210	1.00 21.03
ATOM	1439	C	TRP	A	260	17.121	16.700	49.211	1.00 20.94
ATOM	1440	O	TRP	A	260	17.917	17.601	48.957	1.00 19.84

Coordinate Databases

- **RCSB** (Research Collaboratory for Structural Bioinformatics) http://www.rcsb.org/
  - Formally know as the Protein Data Bank at Brookhaven National Laboratories
  - Structure Explorer PDB search engine
    - Text and PDB ID (4 letter code) searching
- **MMDB** (Molecular Modeling Database @NCBI)
  - Compilation of structures represented in multiple formats
  - Provides structure summaries
  - BLAST sequences to search for available structures

Syllabus

- Protein Structure Classification
- Structure Coordinate Files & Databases
- **Comparing Protein Structures**
  - Aligning 3D Structures
- Predicting Protein Structure
  - Specialized Structural Regions
  - Secondary Structure Prediction
  - Tertiary Structure Prediction
    - Threading
    - Modeling
- Structure Visualization

Sequence & Structure Homology

- **Sequence**
  - Identify relationships between sets of linear protein sequences
- **Structure**
  - Categorize related structures based on 3D shapes
    - Structure families do not necessarily share sequence homology

Structure Comparison

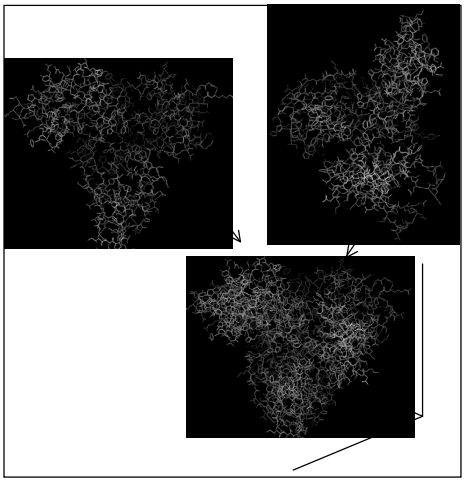
- Compare Structures that are:
  - **Identical**
    - Similarity/difference of independent structures, x-ray vs. nmr, apo vs. holo forms, wildtype vs. mutant
  - **Similar**
    - Predict function, evolutionary history, important domains
  - **Unrelated**
    - Identify commonalities between proteins with no apparent common overall structure - focus on active sites, ligand binding sites
- Superimpose Structures by 3D Alignment for Comparison

Structural Alignment

- Structure alignment forms relationships in **3D space**
  - similarity can be redundant for multiple sequences
- **Considerations**
  - Which atoms/regions between two structure will be compared
  - Will the structures be compared as rigid or flexible bodies
  - Compare all atoms including side chains or just the backbone/C□
  - Try to maximize the number of atoms to align or focus on one localized region (biggest differences usually in solvent-exposed loop structures)
  - How does the resolution of each structure affect comparison

Translation and Rotation

- **Alignment**
  - Translate center of mass to a common origin
  - Rotate to find a suitable superposition
- **Algorithms**
  - Identify equivalent pairs (3) of atoms between structures to seed alignment
    - Iterate translation/rotation to maximize the number of matched atom pairs
  - Examine all possible combinations of alignments and identify the optimal solution



Alignment Methods

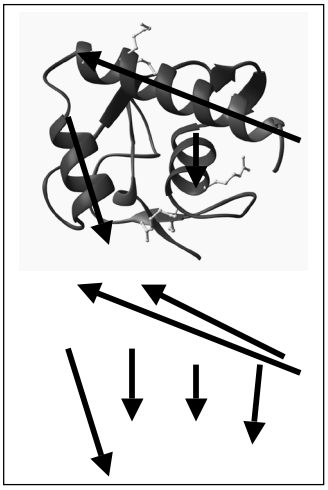
- Initially examine secondary structural elements and Cα-Cα distances to identify folds and the ability to align
- Gap penalties for structures that have discontinuous regions that do not align (alignment-gap-alignment)
  - Anticipate that two different regions may align separately, but not in the same alignment
- Proceed with alignment method:
  - Fast, Secondary Structure-Based
  - Dynamic Programming
  - Distance Matrix

Fast Alignment by SS

- Secondary structure elements can be represented by a vector starting at the beginning of the element
  - Position & length
- Compare the arrangement of clustered vectors between two structures to identify common folds
- Sometimes supplement vectors with information about the arrangement of the side chains (burial/exposure)
- Significance of alignment
  - Likelihood that a cluster of secondary structural elements would be expected between unrelated structures

VAST and SARF

- Implement automatic methods to assign secondary structure



- VAST

http://www.ncbi.nlm.nih.gov:80/Structure/VAST/vastsearch.html

- SARF

http://123d.ncifcrf.gov/

Exhaustive Alignment

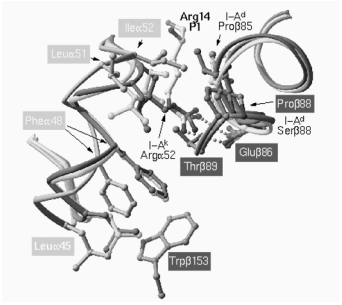
- **Dynamic Programming**
  - Local environment defined in terms of Interatomic distances, bond angles, side chain identity, side chain burial/exposure
  - Align structures by matching local environments - for example, draw vectors representing each Cα-Cα bond, superimpose vectors
- **Distance Matrix**
  - Graphic procedure similar to a dot matrix alignment of two sequences to identify atoms that lie most closely together in a 3D structure (based on Cα distances)
  - Similar structures have super-imposable graphs

DALI Distance Alignment

- DALI - http://www2.embl-ebi.ac.uk/dali/
- Aligns two structures
- Determines if a new structure is similar to one already in database (for classification)

Alignment Quality

- Calculate deviation between two aligned structures
- **RMSD** (Root Mean Square Deviation)
  - Goodness of fit between two sets of coordinates
  - Best if < 3 Å
  - Calculate Cα-Cα distances, sum square of distances, divide by the number of pairs, square root

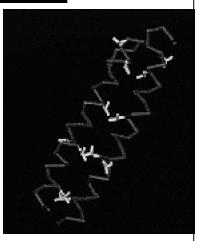


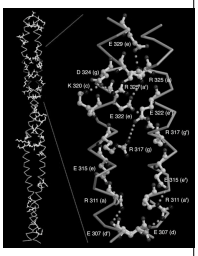
$$RMSD = \sqrt{\frac{N}{\Sigma D_i^2/N}}$$

Syllabus

- Protein Structure Classification
- Structure Coordinate Files & Databases
- Comparing Protein Structures
  - Aligning 3D Structures
- **Predicting Protein Structure**
  - Specialized Structural Regions
  - Secondary Structure Prediction
  - Tertiary Structure Prediction
    - Threading
    - Modeling
- Structure Visualization

Predicting Specialized Structures

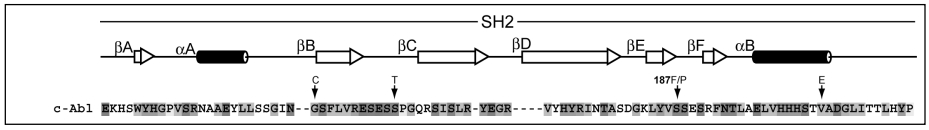




- **Leucine Zippers**
  - Antiparallel helices held together by interactions between L residues spaced at ever 7th position
- **Coiled Coils**
  - 2 or three a helices coiled around each other in a left-handed supercoil
  - Multicoil http://jura.wi.mit.edu/cgi-bin/multicoil/multicoil.pl
  - COILS2 http://www.ch.embnet.org/software/COILS_form.html
- **Transmembrane Regions**
  - 20-30aa domains with strong hydrophobicity
  - PHDhtm, PHDtopology, TMpred (TMbase)
  - http://www.embl-heidelberg.de/predictprotein/predictprotein.html

Predicting Secondary Structure

- Recognizing Potential Secondary Structure
  - 50% of a sequence is usually alpha helices and beta sheet structures
  - Helices: 3.6 residues/turn, N+4 bonding
  - Strands: extended conformation, interactions between strands, disrupted by beta bulges
  - Coils: A,G,S,T,P are predominant
  - Sequences with >45% sequence identity should have similar structures
- Databases of sequences and accompanying secondary structures (DSSP)



SS Prediction Algorithms Chou-Fasman/GOR

- Analyze the **frequency** of each of the 20 aa in every secondary structure (Chou, 1974)
- A,E,L,M prefer □ helices; P,G break helices
- Use a 4-6aa examination window to predict probability of □ helix, 3-5aa window for beta strands
  - Extend regions by moving window along sequence
- 50-60% effective (Higgins, 2000)
- GOR method assumes that residues flanking the central window/core also influence secondary structure

SS Prediction Algorithms Neural Networks

- Examine patterns in secondary structures by **computationally learning to recognize combinations of aa** that are prevalent within a particular secondary structure
- Program is trained to distinguish between patterns located in a secondary structure from those that are not usually located in it
- PHDsec (Profile network from HeiDelberg)
  - ~ 70% correct predictions

http://www.embl-heidelberg.de/predictprotein/submit_def.html

SS Prediction Algorithms Nearest Neighbor

- Generate an iterated list of peptide fragments by sliding a fixed-size window along sequence
- Predict structure of aa in center of the window by examining its k neighbors (Yi, 1993)
  - Propensity of center position to adopt a structure within the context of the neighbors
- Method relies on an initial training set to teach it how neighbors influence secondary structure
- NNSSP http://bioweb.pasteur.fr/seqanal/interfaces/nnssp-simple.html

SS Prediction Tools

- **NNpredict** - 65 % effective*, outputs H,E,-
  - http://www.cmpharm.ucsf.edu/~nomi/nnpredict.html
- **PredictProtein** - query sequence examined against SWISS-PROT to find homologous sequences
  - MSA of results given to PHD for prediction
  - 72% effective*
  - http://www.embl-heidelberg.de/predictprotein/submit_def.html
- **Jpred** - integrates multiple structure prediction applications and returns a consensus, 73% effective*
  - http://www.compbio.dundee.ac.uk/~www-jpred/submit.html

Tertiary Structure Prediction

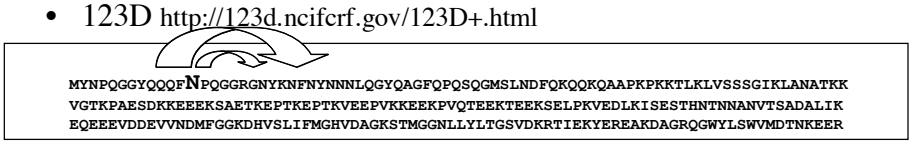
- Goal
  - Build a model to use for comparison with other structures, identify important residues/interactions, determine function
- Challenges
  - Reveal interactions that occur between residues that are distant from each other in a linear sequence
  - Slight changes in local structure can have large effects on global structure
- Methods
  - **Sequence Homology** - use a homologous sequence as a template
  - **Threading** - search for structures that have similar fold configurations without any obvious sequence similarity

Threading - Approaches

- Sequence is compared for its compatibility (structural similarity) with existing structures
- Approaches to determine compatibility
  - **Environmental Template**: environment of ea. aa in a structure is classified into one of 18 types, evaluate ea. position in query sequence for how well it fits into a particular type (Mount, 2001)
  - **Contact Potential Method**: analyze the closeness of contacts between aa in the structure, determine whether positions within query sequence could produce similar interactions (find most energetically favorable) (Mount, 2001)

Threading Process

- Sequence moved **position-by-position** through a structure
- Protein fold modeled by pair-wise inter-atomic calculations to align a sequence with the backbone of the template
  - Comparisons between local and non-local atoms
  - Compare position i with every other position j and determine whether interactions are feasible
- Optimize model with pseudo energy minimizations - most energetically stable alignment assumed to be most favorable
- Thread the smallest segment reasonable! Computationally intensive.
- 123D http://123d.ncifcrf.gov/123D+.html



Model Building

- Perform automated model constructions
  - SWISS-MODEL
    - Compare sequence to ExPdb to find a template (homology)
    - Define your own templates (from threading)
    - http://www.expasy.ch/swissmod/SWISS-MODEL.html
  - GENO3D
    - PSI-BLAST to identify homologs possessing structures to be used as templates
    - http://geno3d-pbil.ibcp.fr

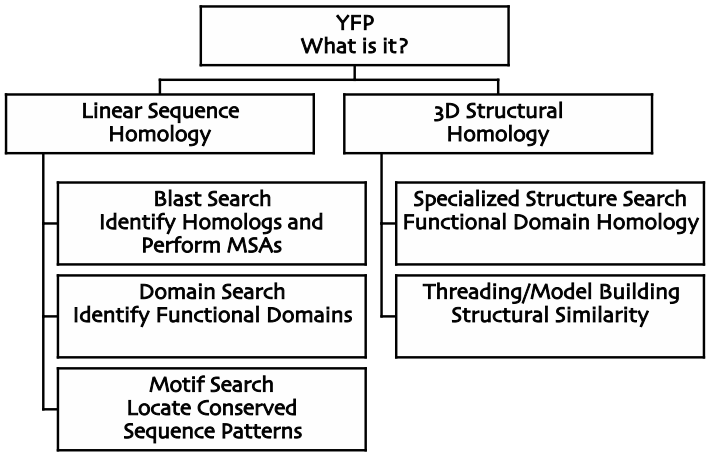
Model Evaluation

- Manually examine model and alignments
- Find similar structures through database searches
  - DALI
- How does the model compare to other structures with the template family?
- Remember, it's only a MODEL (but even models can be useful)

Structure Visualization

- Different representations of molecule
  - wire, backbone, space-filling, ribbon
- NMR ensembles
  - Models showing dynamic variation of molecules in solution
- VIEWERS
  - **RasMol** (Chime is the Netscape plug-in)
    - http://www.umass.edu/microbio/rasmol/index2.html
  - **Cn3D** MMDB viewer (See in 3D) with explicit bonding
    - http://www.ncbi.nlm.nih.gov/Structure
  - **SwissPDB** Viewer
    - http://www.expasy.ch/spdbv/mainpage.html
  - **iMol**
    - http://www.pirx.com/iMol

Pulling It All Together



Demonstration

- Thread sequence to identify template
  - Web-based: 123D
    http://123d.ncifcrf.gov/123D+.html
- Model sequence with template
  - http://www.expasy.ch/swissmod/SWISS-MODEL.html
- Visualization

References

Bioinformatics: Sequence and genome Analysis. David W. Mount. CSHL Press, 2001.

Bioinformatics: A Practical Guide to the Analysis of Genes and Proteins. Andreas D. Baxevanis and B.F. Francis Ouellete. Wiley Interscience, 2001.

Bioinformatics: Sequence, structure, and databanks. Des Higgins and Willie Taylor. Oxford University Press, 2000.

Chou, P.Y. and Fasman, G. D. (1974). Biochemistry, 13, 211.

Yi, T-M. and Lander, E.S.(1993) J. Mol. Biol., 232,1117.