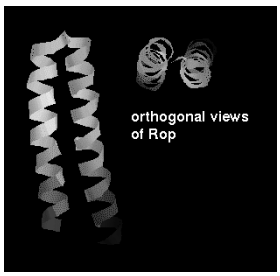
Protein Structure Analysis & Protein-Protein Interactions



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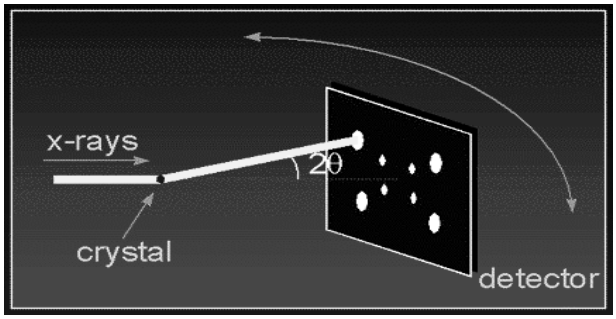
Much Ado About Structure

- **Structure ⟷ Function**
- **Structure ⟷ Mechanism**
- **Structure ⟷ Origins/Evolution**
- **Structure-based Drug Design**
- **Solving the Protein Folding Problem**

Routes to 3D Structure

- X-ray Crystallography (the best)
- NMR Spectroscopy (close second)
- Cryoelectron microsocopy (distant 3rd)
- Homology Modelling (sometimes VG)
- Threading (sometimes VG)
- Ab initio prediction (getting better)

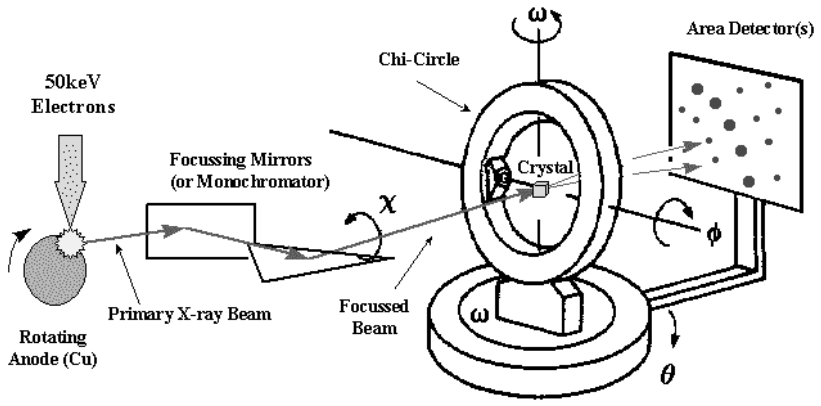
X-ray Crystallography



X-ray Crystallography

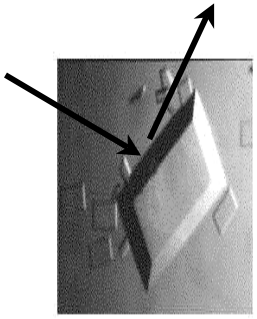
- Crystallization
- Diffraction Apparatus
- Diffraction Principles
- Conversion of Diffraction Data to Electron Density
- Resolution
- Chain Tracing

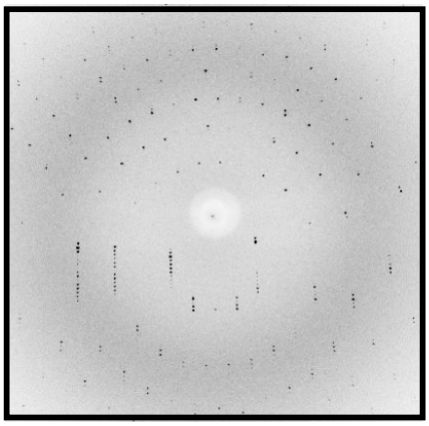
Diffraction Apparatus



4-Circle Goniometer (Eulerian or Kappa Geometry)

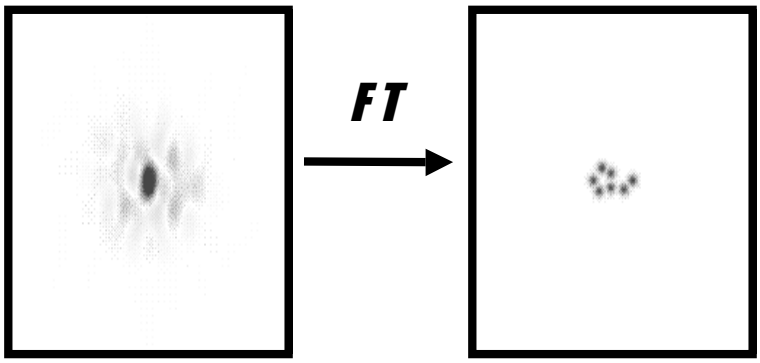
Protein Crystal Diffraction



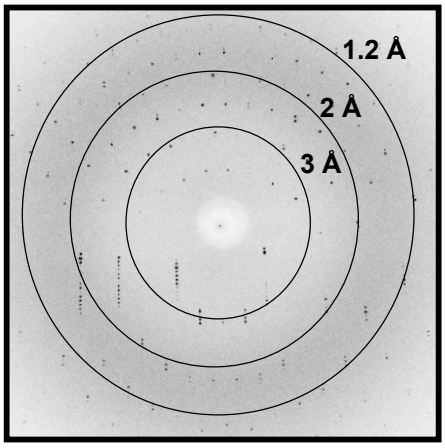


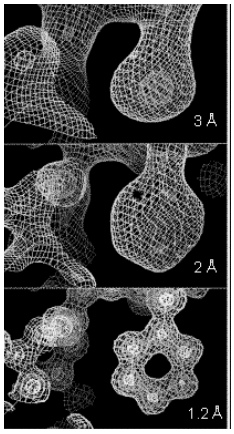
Diffraction Pattern

Converting Diffraction Data to Electron Density



Resolution





The Final Result

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ORIGX3      0.000000  0.000000  1.000000        0.00000                  2TRX 148
SCALE1      0.011173  0.000000  0.004858        0.00000                  2TRX 149
SCALE2      0.000000  0.019585  0.000000        0.00000                  2TRX 150
SCALE3      0.000000  0.000000  0.018039        0.00000                  2TRX 151
ATOM      1  N   SER A   1      21.389  25.406  -4.628  1.00 23.22       2TRX 152
ATOM      2  CA  SER A   1      21.628  26.691  -3.983  1.00 24.42       2TRX 153
ATOM      3  C   SER A   1      20.937  26.944  -2.679  1.00 24.21       2TRX 154
ATOM      4  O   SER A   1      21.072  28.079  -2.093  1.00 24.97       2TRX 155
ATOM      5  CB  SER A   1      21.117  27.770  -5.002  1.00 28.27       2TRX 156
ATOM      6  OG  SER A   1      22.276  27.925  -5.861  1.00 32.61       2TRX 157
ATOM      7  N   ASP A   2      20.173  26.028  -2.163  1.00 21.39       2TRX 158
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ATOM      9  C   ASP A   2      20.264  26.214   0.297  1.00 20.89       2TRX 160
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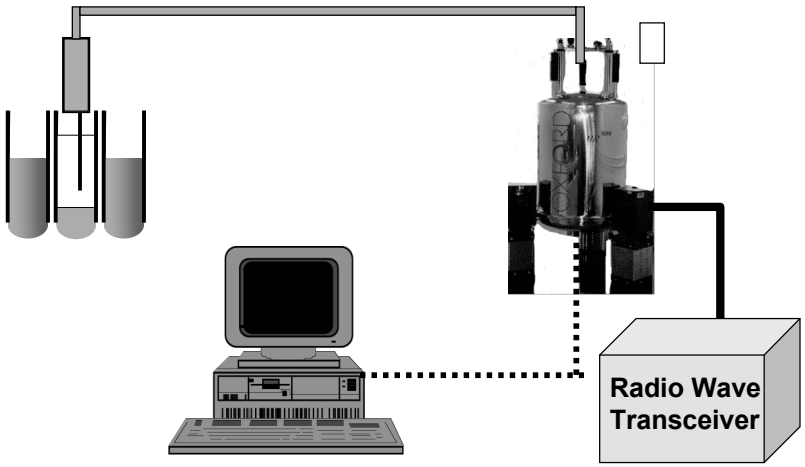
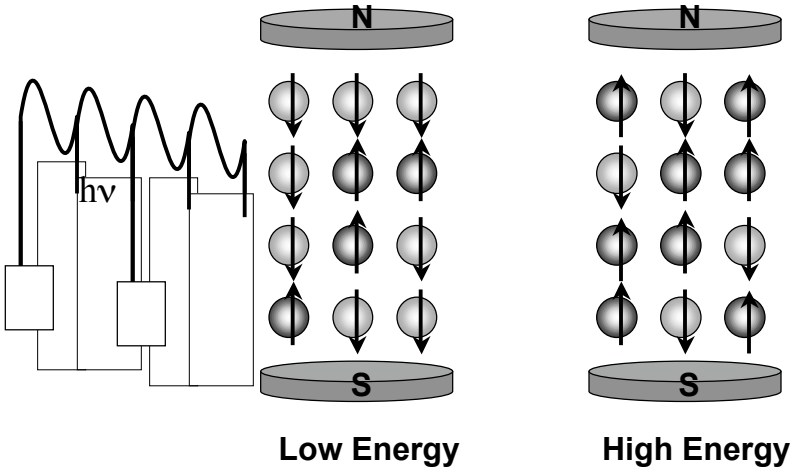
ATOM     11  CB  ASP A   2      18.439  24.914  -0.856  1.00 22.14       2TRX 162
```

http://www-structure.llnl.gov/Xray/101index.html

NMR Spectroscopy

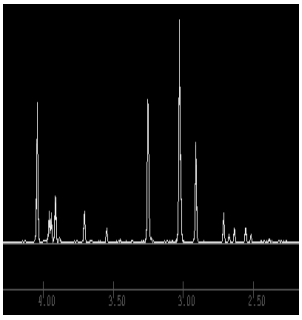


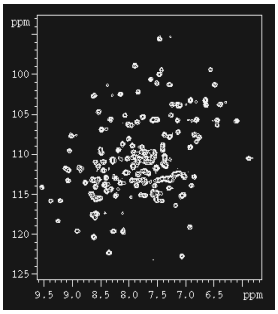
Principles of NMR

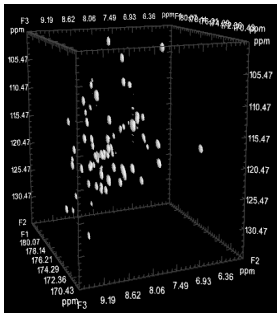


Multidimensional NMR

1D **2D** **3D**





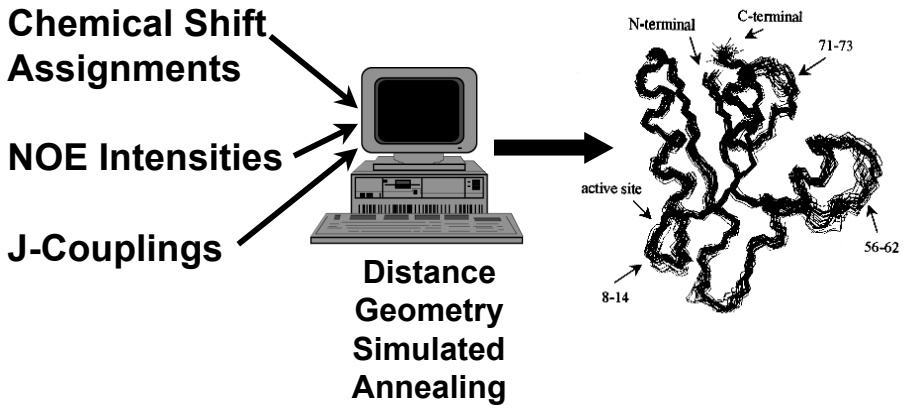


MW ~ 500 **MW ~ 10,000** **MW ~ 30,000**

The NMR Process

- Obtain protein sequence
- Collect TOCSY & NOESY data
- Use chemical shift tables and known sequence to assign TOCSY spectrum
- Use TOCSY to assign NOESY spectrum
- Obtain inter and intra-residue distance information from NOESY data
- Feed data to computer to solve structure

NMR Spectroscopy



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ATOM      3  C   SER A   1      20.937  26.944  -2.679  1.00 24.21         2TRX 154
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ATOM      6  OG  SER A   1      22.276  27.925  -5.861  1.00 32.61         2TRX 157
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ATOM     11  CB  ASP A   2      18.439  24.914  -0.856  1.00 22.14         2TRX 162
```

X-ray Versus NMR

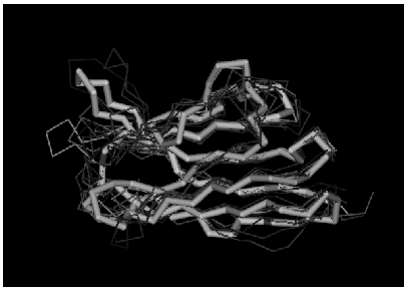
X-ray

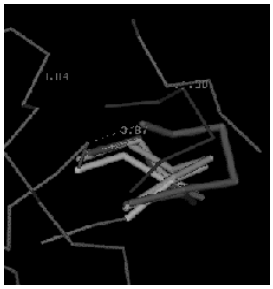
- Producing enough protein for trials
- Crystallization time and effort
- Crystal quality, stability and size control
- Finding isomorphous derivatives
- Chain tracing & checking

NMR

- Producing enough labeled protein for collection
- Sample "conditioning"
- Size of protein
- Assignment process is slow and error prone
- Measuring NOE's is slow and error prone

Comparative (Homology) Modelling





```
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ASDEYAHLRILDPQRSTVAYAYE--KSFAPPGSFKWEYEAHADS
MCDEYAHIRLMNPERSTVAGGHQWERT----GSFKEWYAAHADD
```

Homology Modelling

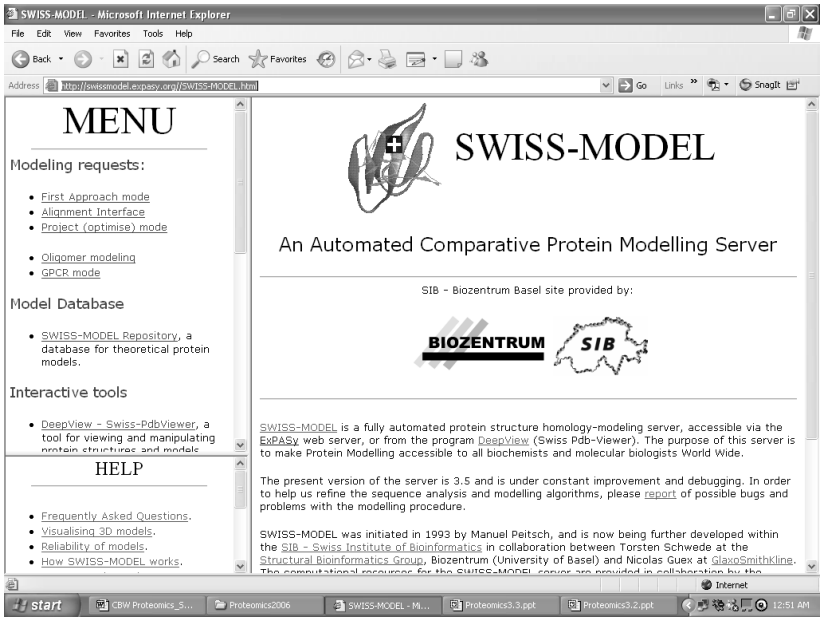
- Offers a method to "Predict" the 3D structure of proteins for which it is not possible to obtain X-ray or NMR data
- Can be used in understanding function, activity, specificity, etc.
- Of interest to drug companies wishing to do structure-aided drug design
- A keystone of Structural Proteomics

Homology Modelling

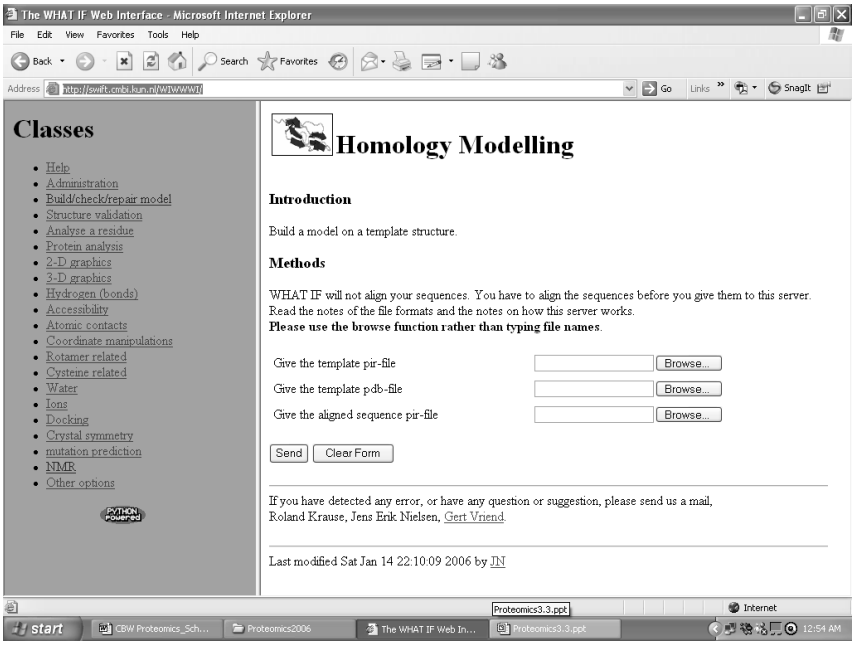
- Identify homologous sequences in PDB
- Align query sequence with homologues
- Find Structurally Conserved Regions (SCRs)
- Identify Structurally Variable Regions (SVRs)
- Generate coordinates for core region
- Generate coordinates for loops
- Add side chains (Check rotamer library)
- Refine structure using energy minimization
- Validate structure

Modelling on the Web

- Prior to 1998 homology modelling could only be done with commercial software or command-line freeware
- The process was time-consuming and labor-intensive
- The past few years has seen an explosion in automated web-based homology modelling servers
- Now anyone can homology model!



http://swissmodel.expasy.org//SWISS-MODEL.html



http://swift.cmbi.kun.nl/WIWWWI/

The Final Result

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ORIGX2      0.000000  1.000000  0.000000        0.00000                  2TRX 147
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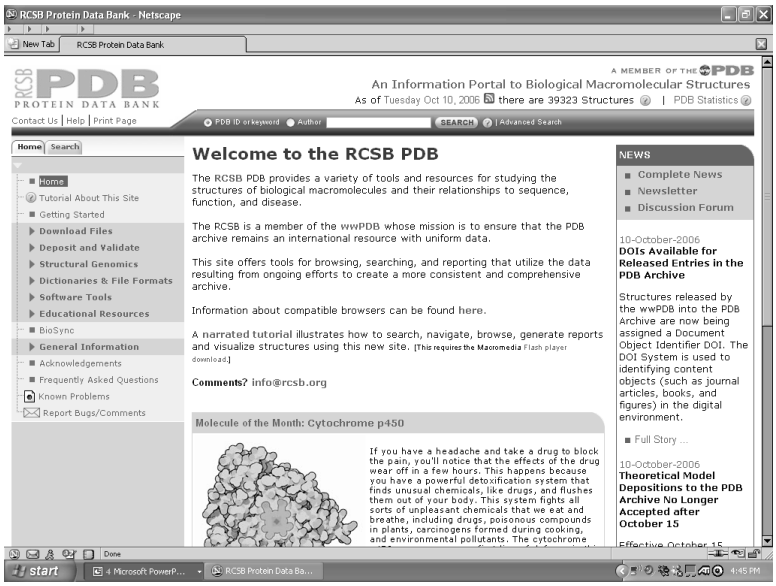
ATOM     10  O   ASP A   2      19.760  26.575   1.371  1.00 21.49       2TRX 161
ATOM     11  CB  ASP A   2      18.439  24.914  -0.856  1.00 22.14       2TRX 162
```

The PDB

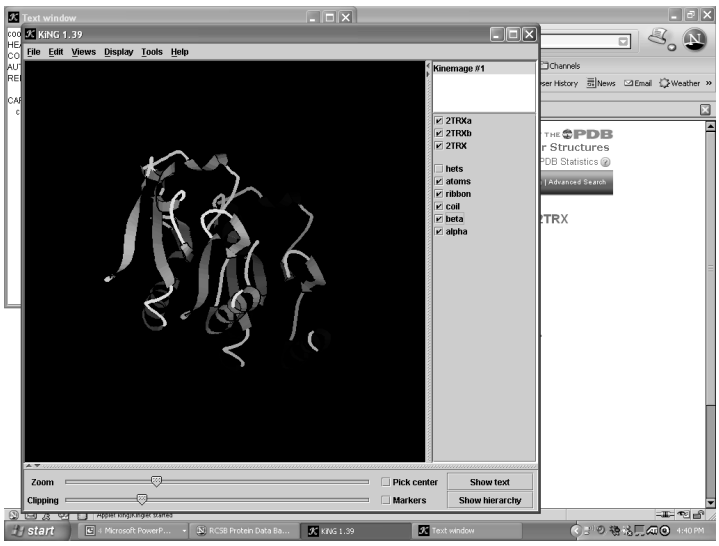
- PDB - Protein Data Bank
- Established in 1971 at Brookhaven National Lab (7 structures)
- Primary archive for macromolecular structures (proteins, nucleic acids, carbohydrates – now 40,000 structrs)
- Moved from BNL to RCSB (Research Collaboratory for Structural Bioinformatics) in 1998



http://www.rcsb.org/pdb/

Viewing 3D Structures

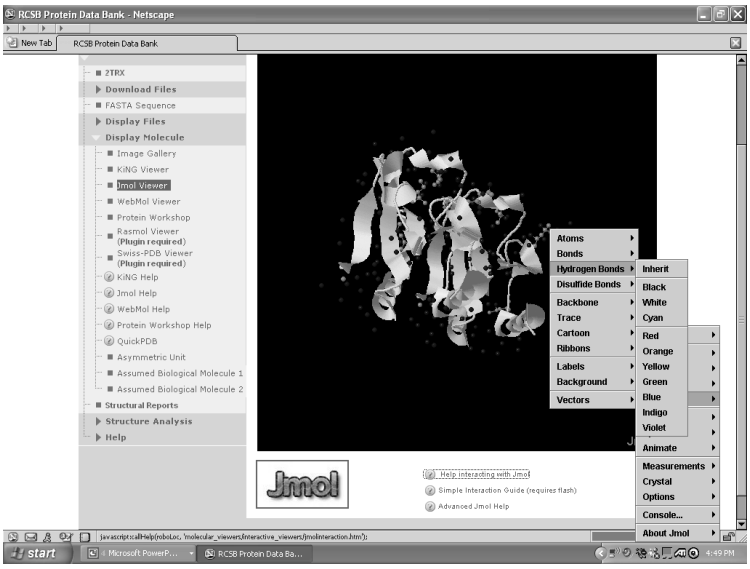
KiNG (Kinemage) 1.39



KiNG (Kinemage)

- Both a (signed) Java Applet and a downloadable application
- Application is compatible with most Operating systems
- Compatible with most Java (1.3+) enabled browsers including:
  - Internet Explorer (Win32)
  - Mozilla/Firefox (Win32, OSX, *nix)
  - Safari (Mac OS X) and Opera 7.5.4

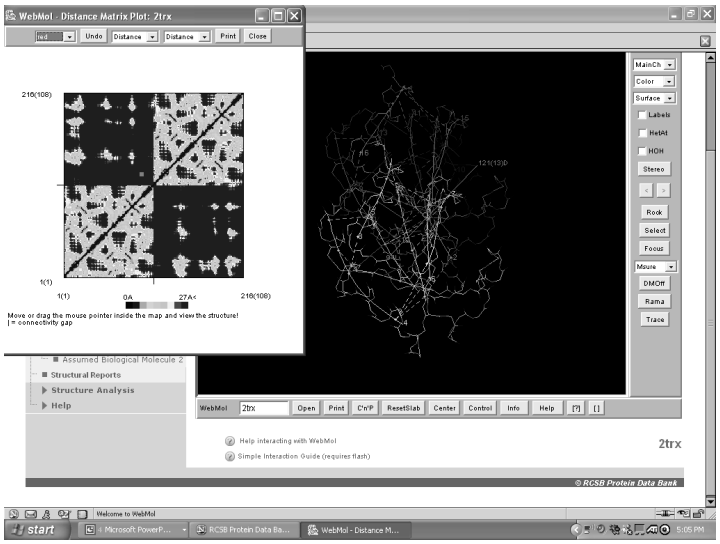
JMol Applet



JMol

- Java-based program
- Open source applet and application
  - Compatible with Linux, MacOS, Windows
- Menus access by clicking on Jmol icon on lower right corner of applet
- Supports all major web browsers
  - Internet Explorer (Win32)
  - Mozilla/Firefox (Win32, OSX, *nix)
  - Safari (Mac OS X) and Opera 7.5.4

WebMol



WebMol

- Both a Java Applet and a downloadable application
- Offers many tools including distance, angle, dihedral angle measurements, detection of steric conflicts, interactive Ramachandran plot, diff. distance plot
- Compatible with most Java (1.3+) enabled browsers including:
  - Internet Explorer 6.0 on Windows XP
  - Safari on Mac OS 10.3.3
  - Mozilla 1.6 on Linux (Redhat 8.0)

Analyzing and Assessing 3D Structures

Why Assess Structure?

- A structure can (and often does) have mistakes
- A poor structure will lead to poor models of mechanism or relationship
- Unusual parts of a structure may indicate something important (or an error)

Famous “bad” structures

- Azobacter ferredoxin (wrong space group)
- Zn-metallothionein (mistraced chain)
- Alpha bungarotoxin (poor stereochemistry)
- Yeast enolase (mistraced chain)
- Ras P21 oncogene (mistraced chain)
- Gene V protein (poor stereochemistry)

How to Assess Structure?

- Assess experimental fit (look at R factor {X-ray} or rmsd {NMR})
- Assess correctness of overall fold (look at disposition of hydrophobes, location of charged residues)
- Assess structure quality (packing, stereochemistry, bad contacts, etc.)

A Good Protein Structure..

X-ray structure	NMR structure
R = 0.59 random chain	rmsd = 4 Å random
R = 0.45 initial structure	rmsd = 2 Å initial fit
R = 0.35 getting there	rmsd = 1.5 Å OK
R = 0.25 typical protein	rmsd = 0.8 Å typical
R = 0.15 best case	rmsd = 0.4 Å best case
R = 0.05 small molecule	rmsd = 0.2 Å dream on

Cautions...

- A low R factor or a good RMSD value does not guarantee that the structure is "right"
- Differences due to crystallization conditions, crystal packing, solvent conditions, concentration effects, etc. can perturb structures substantially
- Long recognized need to find other ways to ID good structures from bad (not just assessing experimental fit)

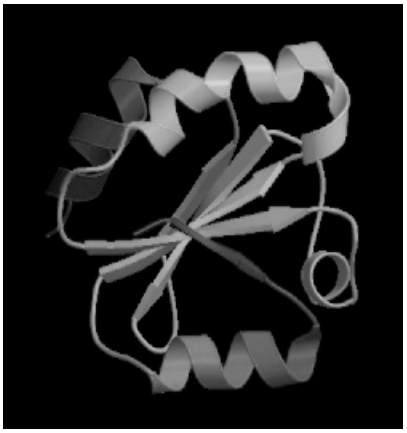
Structure Variability

X-ray to X-ray
Interleukin 1β
(41bi vs 2mlb)

NMR to X-ray
Erabutoxin
(3ebx vs 1era)

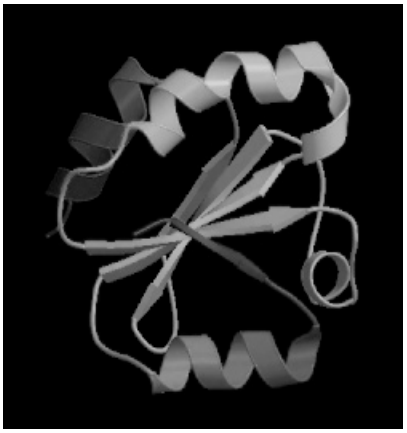
A Good Protein Structure..

- Minimizes disallowed torsion angles
- Maximizes number of hydrogen bonds
- Maximizes buried hydrophobic ASA
- Maximizes exposed hydrophilic ASA
- Minimizes interstitial cavities or spaces



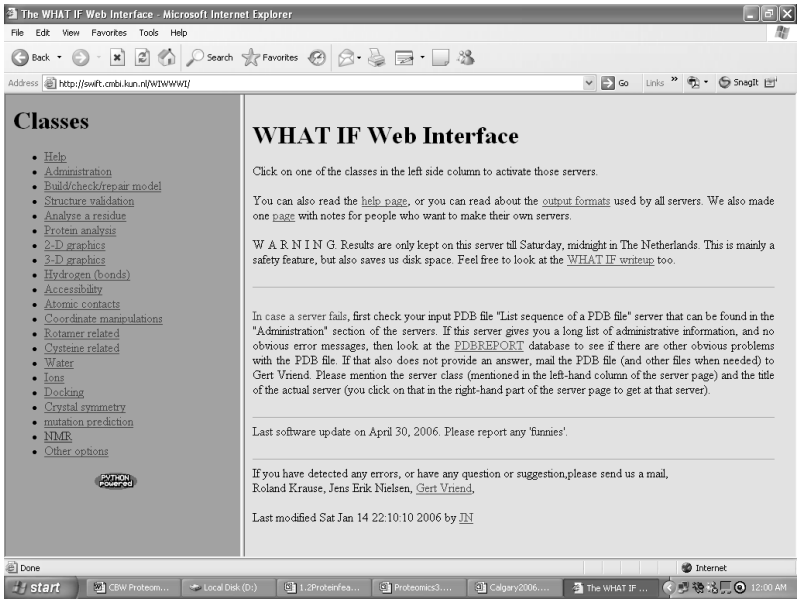
A Good Protein Structure..

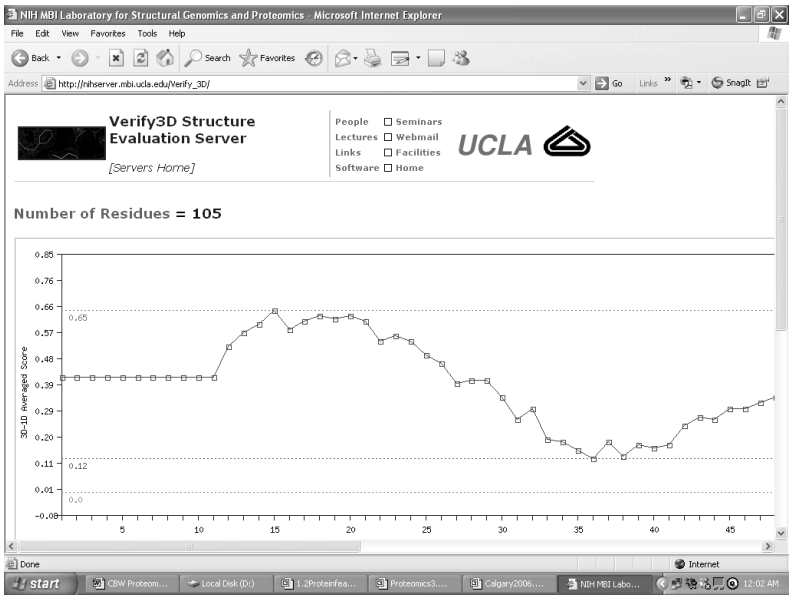
- Minimizes number of “bad” contacts
- Minimizes number of buried charges
- Minimizes radius of gyration
- Minimizes covalent and noncovalent (van der Waals and coulombic) energies



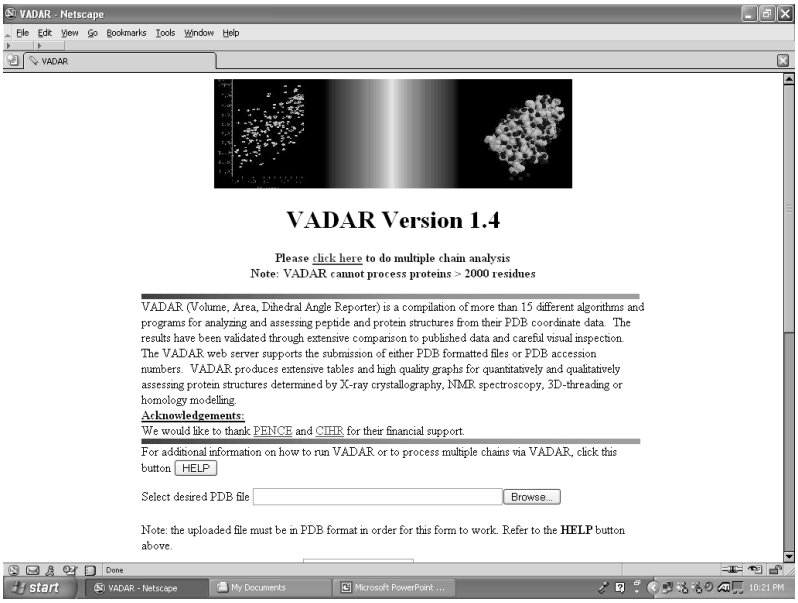
Structure Validation Servers

- **Whatlf Web Server -**
  http://swift.cmbi.kun.nl/WIWWWI/
- **Biotech Validation Suite -**
  http://biotech.ebi.ac.uk:8400/cgi-bin/sendquery
- **Verify3D -**
  http://www.doe-mbi.ucla.edu/Services/Verify_3D/
- **VADAR - http://redpoll.pharmacy.ualberta.ca**





High scores = good Low scores = bad

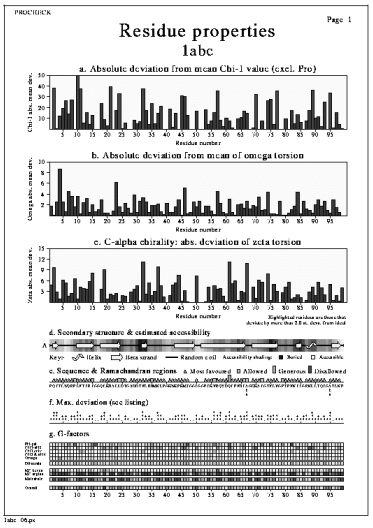


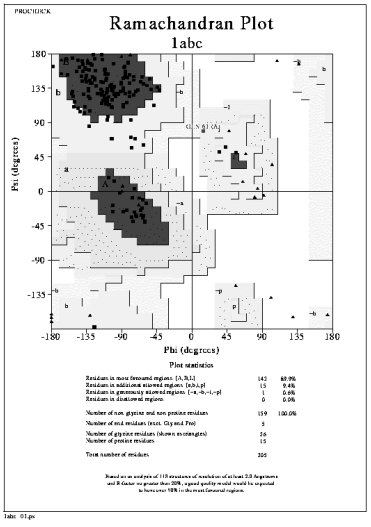
http://redpoll.pharmacy.ualberta.ca

Structure Validation Programs

- **PROCHECK -**
  http://www.biochem.ucl.ac.uk/~roman/procheck/procheck.html
- **PROSA II -**
  http://lore.came.sbg.ac.at/People/mo/Prosa/prosa.html
- **VADAR -**
  http://www.pence.ualberta.ca/ftp/vadar/
- **DSSP -**
  http://www.embl-heidelberg.de/dssp/

Procheck

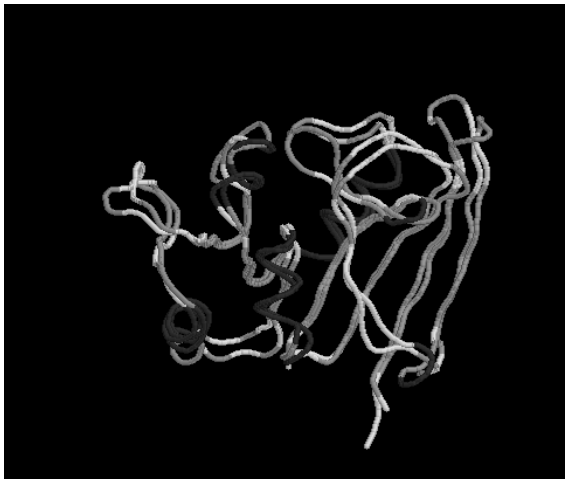




Comparing 3D Structures

Qualitative vs. Quantitative

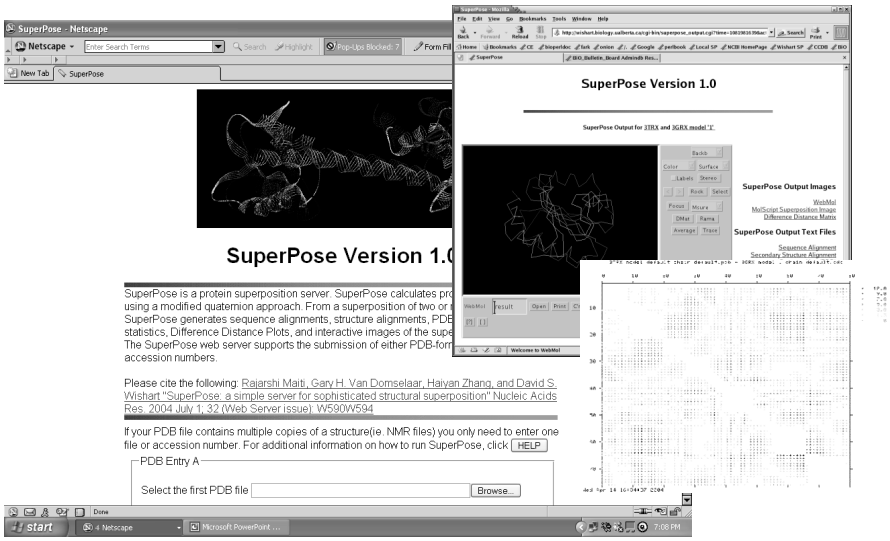
Rigid Body Superposition



Superposition

- Objective is to match or overlay 2 or more similar objects
- Requires use of translation and rotation operators (matrices/vectors)
- Least squares or conjugate gradient minimization (McLachlan/Kabsch)
- Lagrangian multipliers
- Quaternion-based methods (*fastest*)

SuperPose Web Server

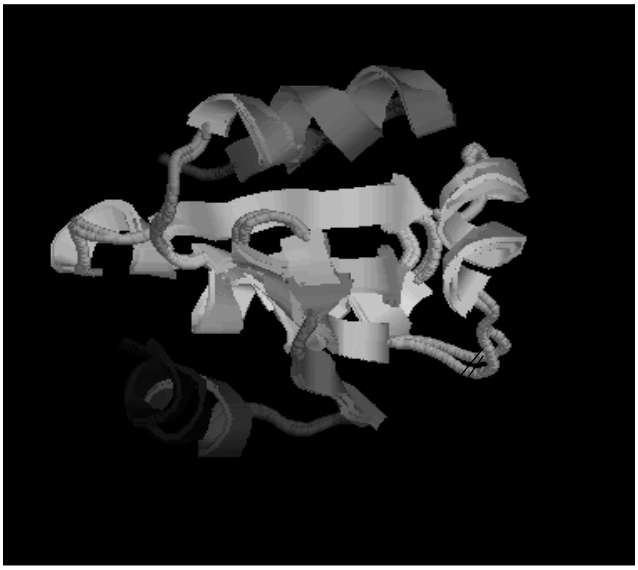


http://wishart.biology.ualberta.ca/SuperPose/

Superposition - Applications

- Ideal for comparing or overlaying two or more protein structures
- Allows identification of structural homologues (CATH and SCOP)
- Allows loops to be inserted or replaced from loop libraries (comparative modelling)
- Allows side chains to be replaced or inserted with relative ease

Measuring Superpositions



RMSD - Root Mean Square Deviation

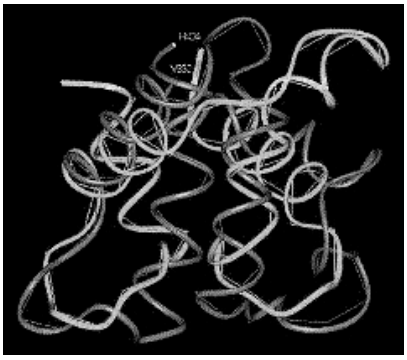
- Method to quantify structural similarity - same as standard deviation
- Requires 2 superimposed structures (designated here as "a" & "b")
- N = number of atoms being compared

$$\text{RMSD} = \frac{\sqrt{\sum_i (x_{ai} - x_{bi})^2 + (y_{ai} - y_{bi})^2 + (z_{ai} - z_{bi})^2}}{\sqrt{N}}$$

RMSD

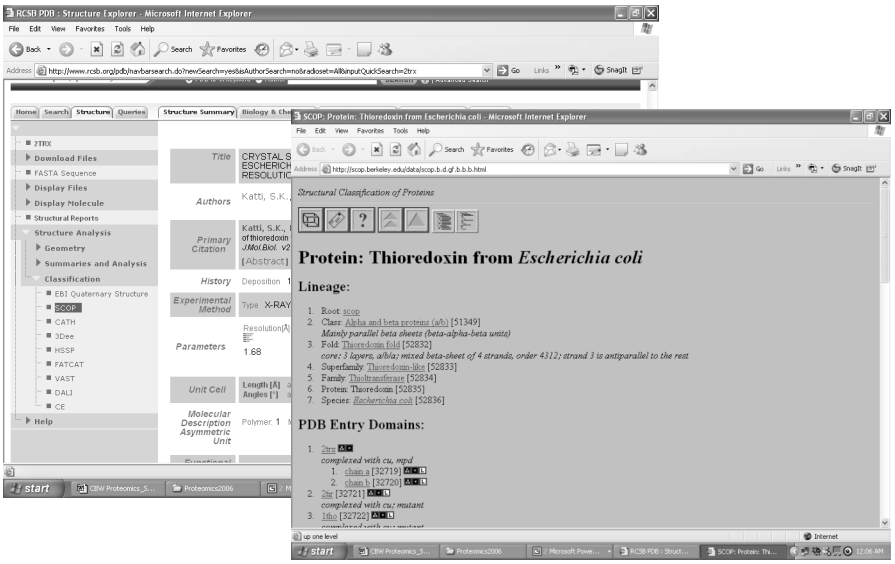
- 0.0-0.5 Å → Essentially Identical
- <1.5 Å → Very good fit
- < 5.0 Å → Moderately good fit
- 5.0-7.0 Å → Structurally related
- > 7.0 Å → Dubious relationship
- > 12.0 Å → Completely unrelated

Detecting Unusual Relationships

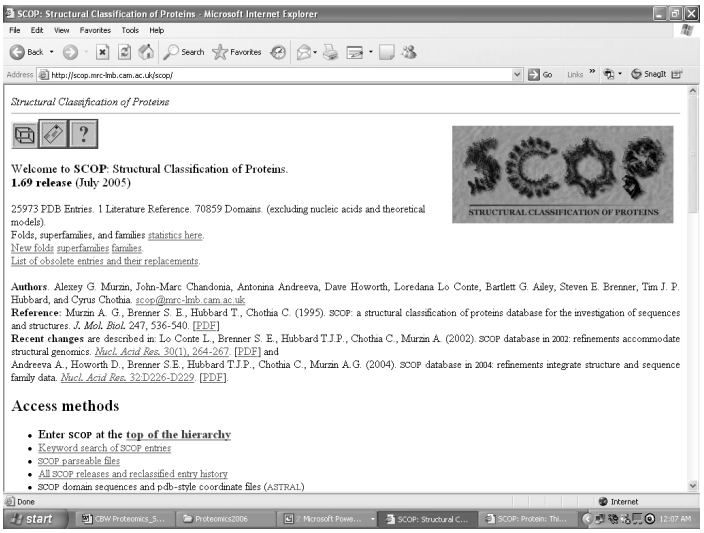


Similarity between Calmodulin and Acetylcholinesterase

Classifying Protein Folds



SCOP Database

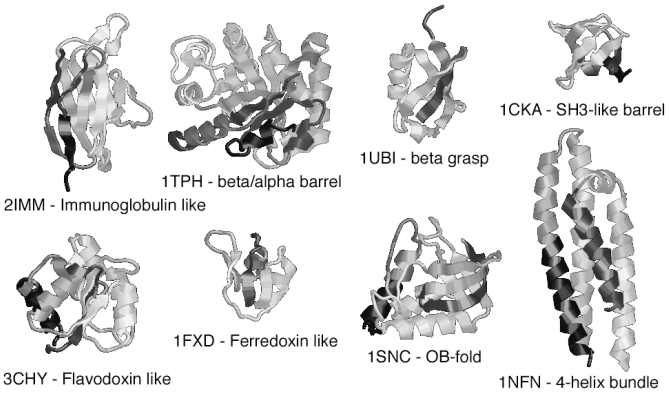


http://scop.mrc-lmb.cam.ac.uk/scop

SCOP

- Class folding class derived from secondary structure content
- Fold derived from topological connection, orientation, arrangement and # 2° structures
- Superfamily clusters of low sequence ID but related structures & functions
- Family clusers of proteins with seq ID > 30% with v. similar struct. & function

SCOP Structural Classification



The eight most frequent SCOP superfolds

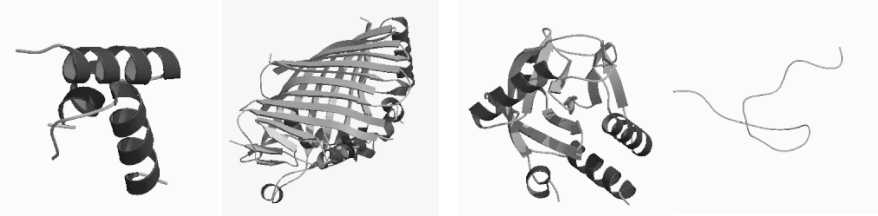
The CATH Database

http://www.cathdb.info/latest/index.html

CATH

- Class [C] derived from secondary structure content (automatic)
- Architecture (A) derived from orientation of 2° structures (manual)
- Topology (T) derived from topological connection and # 2° structures
- Homologous Superfamily (H) clusters of similar structures & functions

CATH - Class



Class 1: Mainly Alpha

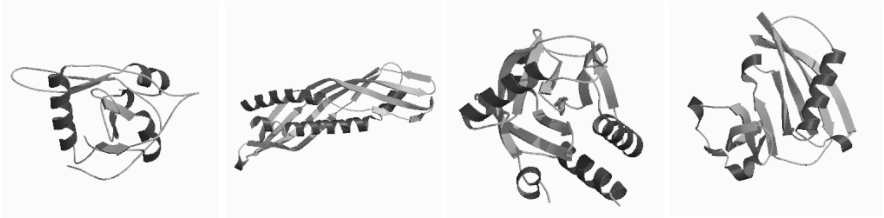
Class 2: Mainly Beta

Class 3: Mixed Alpha/Beta

Class 4: Few Secondary Structures

Secondary structure content (automatic)

CATH - Architecture



Roll | Super Roll | Barrel | 2-Layer Sandwich

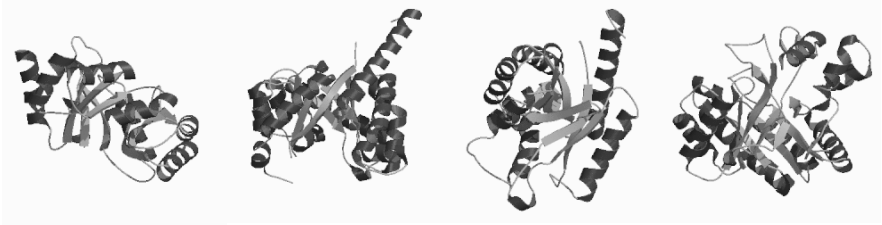
Orientation of secondary structures (manual)

CATH - Topology

L-fucose Isomerase | Serine Protease | Aconitase, domain 4 | TIM Barrel

Topological connection and number of secondary structures

CATH - Homology



Alanine racemase | Dihydropteroate (DHP) synthetase | FMN dependent fluorescent proteins | 7-stranded glycosidases

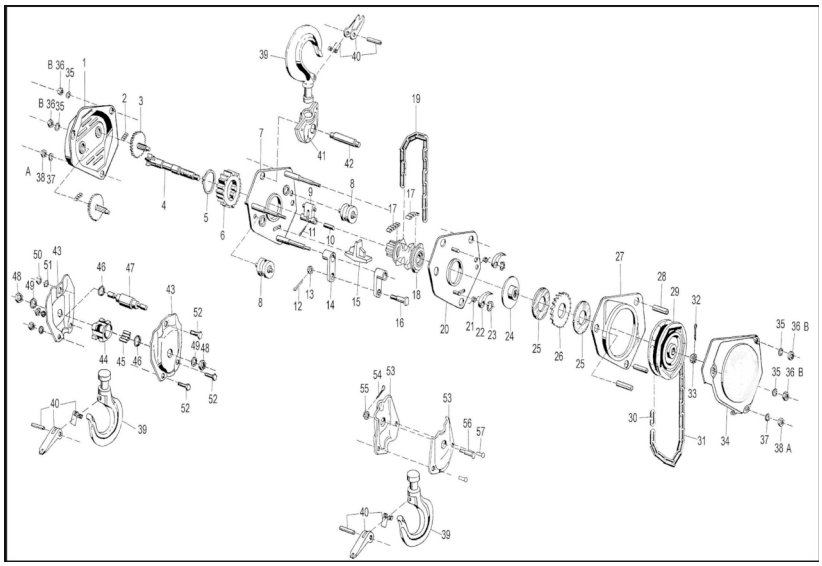
Superfamily clusters of similar structures & functions

Other Servers/Databases

- **Dali** - http://www.ebi.ac.uk/dali/
- **VAST** - www.ncbi.nlm.nih.gov/Structure/VAST/vast.shtml
- **CE** - http://cl.sdsc.edu/ce.html
- **FSSP** - http://www.ebi.ac.uk/dali/fssp/fssp.html
- **PDBsum** - www.biochem.ucl.ac.uk/bsm/pdbsum/

Protein Interactions

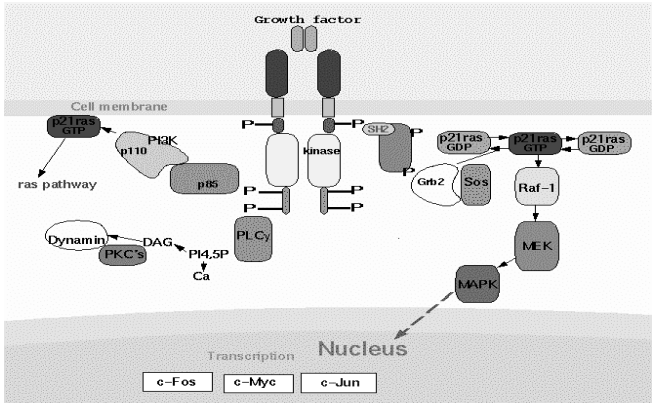
The Protein Parts List



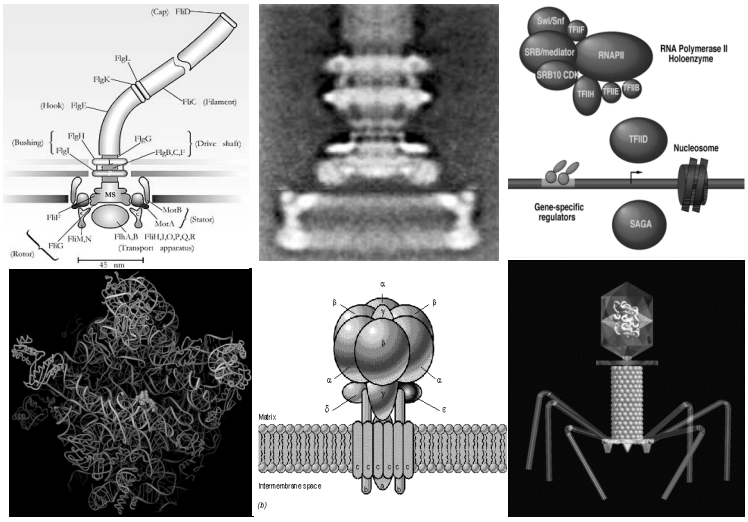
The Parts List

- Sequencing gives "serial number"
- Sequence alignment gives a name
- Microarrays give # of parts
- X-ray and NMR give a picture
- However, having a collection of parts and names doesn't tell you how to put something together or how things connect -- *this is biology*

Remember: *Proteins Interact*



Proteins *Assemble*

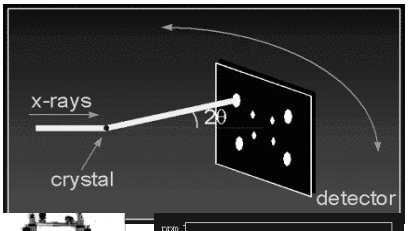


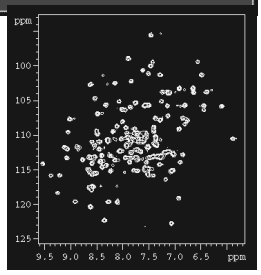
Types of Interactions

- **Permanent (quaternary structure, formation of stable complexes)**
- **Transient (brief interactions, signaling events, pathways)**
- **About 1/4 to 1/3 of all proteins form complexes (dimers → multimers)**
- **Each protein may transiently interact with ~3 other proteins**

Protein Interaction Tools and Techniques - Experimental Methods

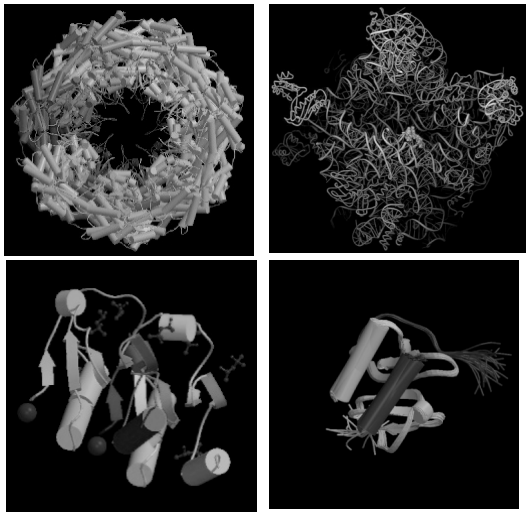
3D Structure Determination





- **X-ray crystallography**
  - **grow crystal**
  - **collect diffract. data**
  - **calculate e- density**
  - **trace chain**
- **NMR spectroscopy**
  - **label protein**
  - **collect NMR spectra**
  - **assign spectra & NOEs**
  - **calculate structure using distance geom.**

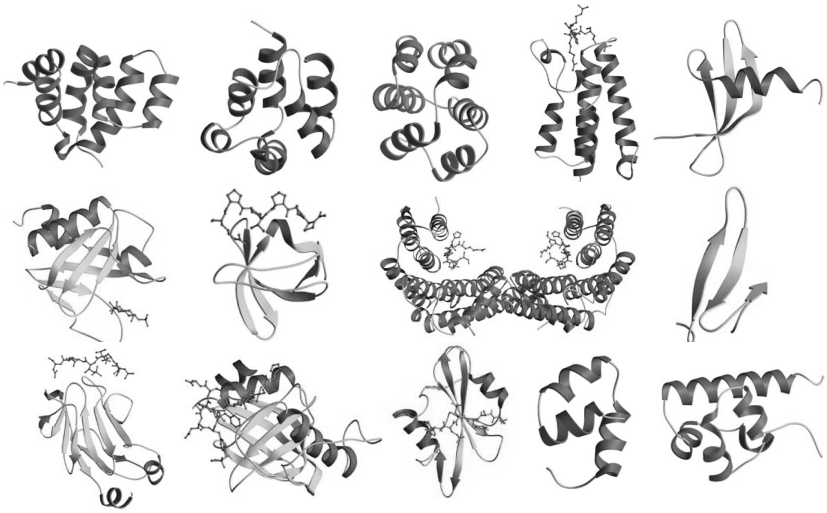
Quaternary Structure



Some interactions are real

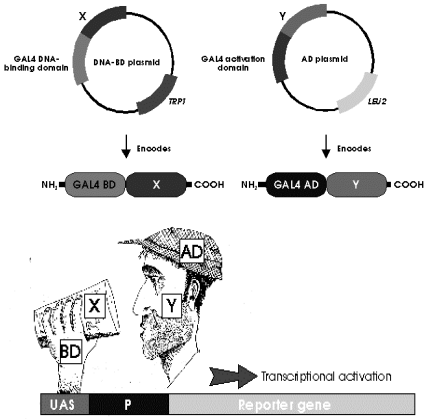
Others are not

Protein Interaction Domains



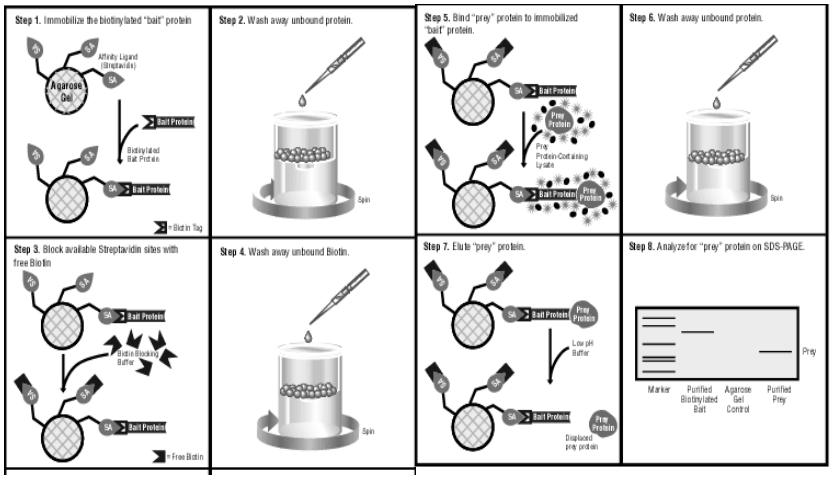
http://www.mshri.on.ca/pawson/domains.html

Yeast Two-Hybrid Analysis

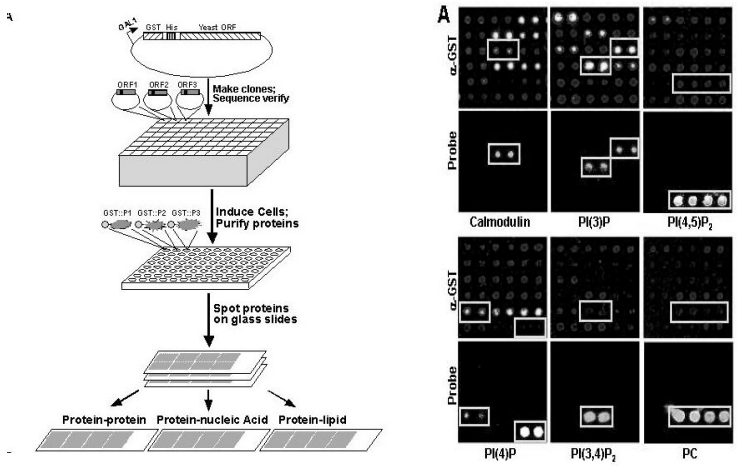


- Yeast two-hybrid experiments yield information on protein protein interactions
- GAL4 Binding Domain
- GAL4 Activation Domain
- X and Y are two proteins of interest
- If X & Y interact then reporter gene is expressed

Affinity Pull-down



Protein Arrays



A Flood of Data

- High throughput techniques are leading to more and more data on protein interactions
- Very high level of false positives – need tools to sort and rationalize
- This is where bioinformatics can play a key role
- Some suggest that this is the "future" for bioinformatics

Interaction Databases

- **BIND**
  - **http://www.bind.ca/**
- **DIP**
  - **http://dip.doe-mbi.ucla.edu/**
- **MINT**
  - **http://160.80.34.4/mint/**
- **IntAct**
  - **http://www.ebi.ac.uk/intact/index.jsp**

More Protein Interaction Databases
http://www.hgmp.mrc.ac.uk/GenomeWeb/prot-interaction.html

Reliability of HT Interaction Data (Patil & Nakamura, BMC Bioinf. 6:100, 2005)

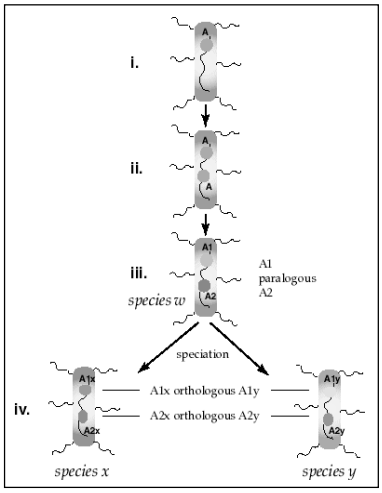
- **Assessed reliability using known interacting Pfam domains, Gene Ontology annotations and sequence homology**
- **56% of HT data for yeast are reliable**
- **27% of HT data for C. elegans are reliable**
- **18% of HT data for D. melanogaster are reliable**
- **68% of HT data for H. sapiens are reliable**

Protein Interaction Tools and Techniques - Computational Methods

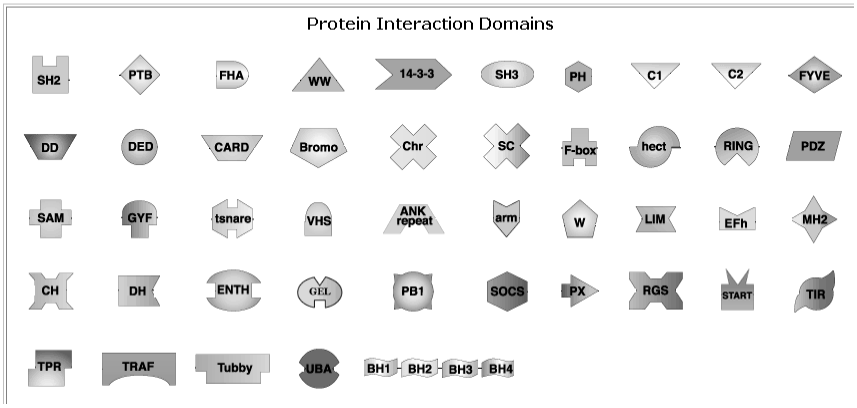
Interologs, Homologs, Paralogs...

- **Homolog**
  - Common Ancestors
  - Common 3D Structure
  - Common Active Sites
- **Ortholog**
  - Derived from Speciation
- **Paralog**
  - Derived from Duplication
- **Interolog**
  - Protein-Protein Interaction

YM2



Sequence Searching Against Known Domains



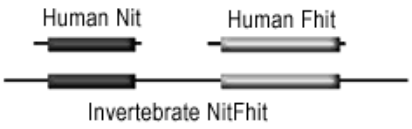
http://www.mshri.on.ca/pawson/domains.html

Rosetta Stone Method

Monomeric proteins that are fused in other organisms tend to be functionally related and physically interacting.

For example, using the Rosetta Stone™ method, it was found that human Nit and Fhit proteins are:

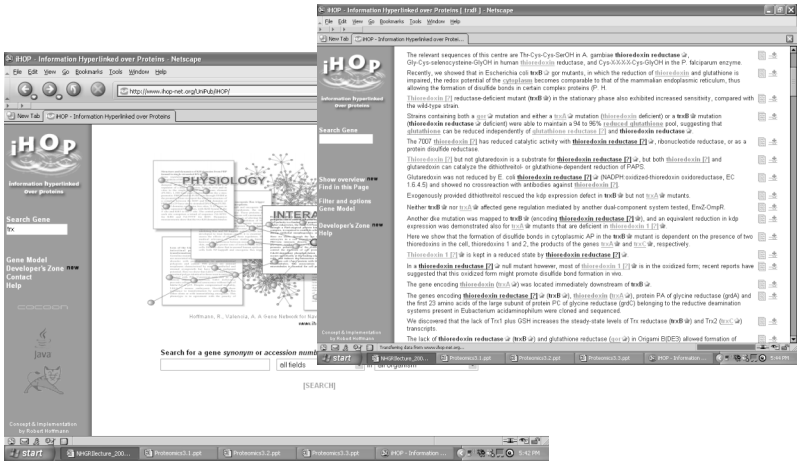
- fused in invertebrates
- form a heterocomplex in mammals



Text Mining

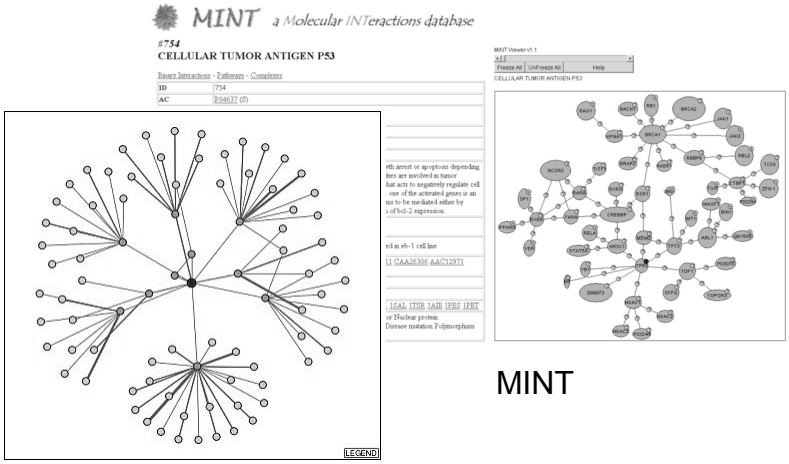
- Searching Medline or Pubmed for words or word combinations
- “X binds to Y”; “X interacts with Y”; “X associates with Y” etc. etc.
- Requires a list of known gene names or protein names for a given organism (a protein/gene thesaurus)

iHOP (Information hyperlinked over proteins)



http://www.ihop-net.org/UniPub/iHOP/

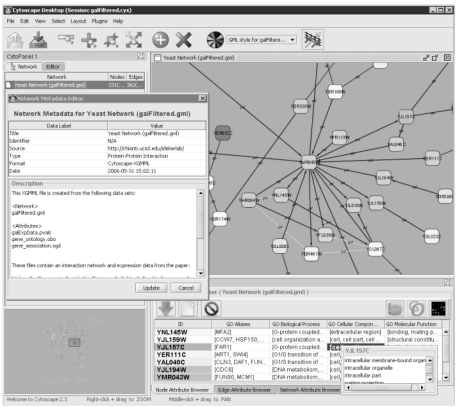
Visualizing Interactions

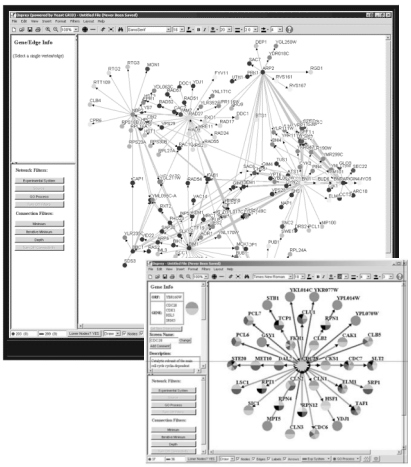


MINT

DIP

Visualizing Interactions

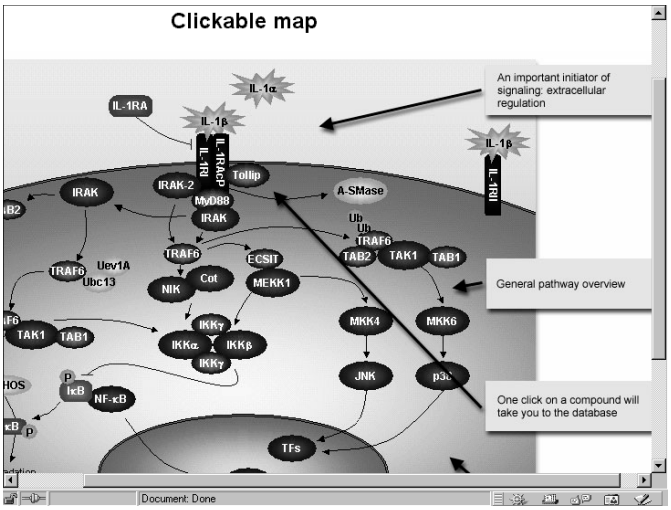




Cytoscape (www.cytoscape.org)

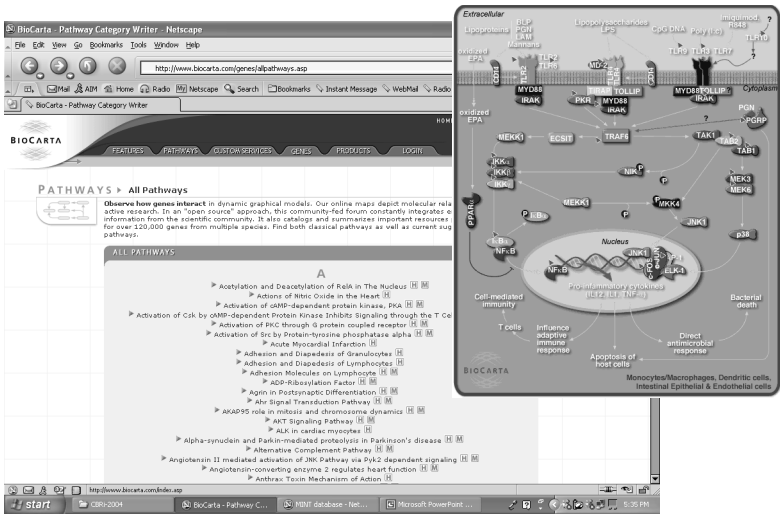
Osprey http://biodata.mshri.on.ca/osprey/servlet/Index

Pathway Visualization with TRANSPATH



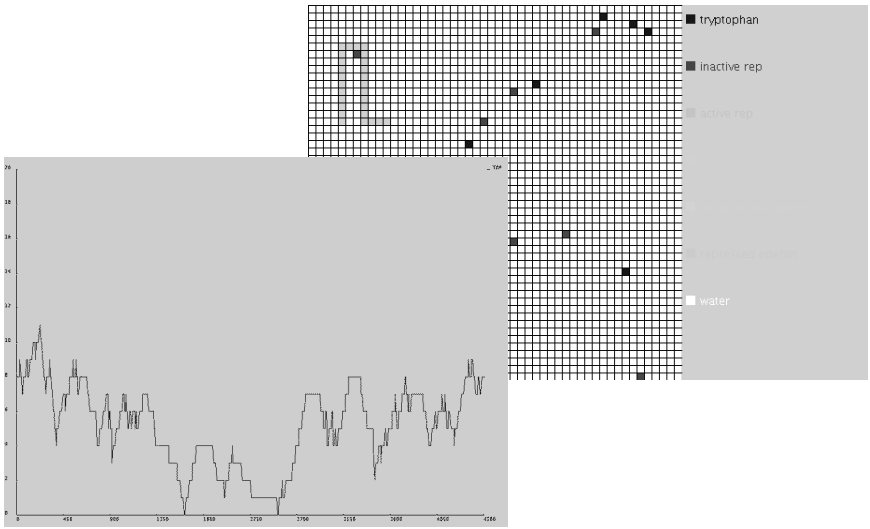
http://www.biobase.de/pages/products/transpath.html

Pathway Visualization with BioCarta



www.biocarta.com

Dynamic Simulations using SimCell



Summary

- First application of bioinformatics was probably in protein structure (the PDB)
- Structural biology continues to be a rich source for bioinformatics innovation and bioinformaticians
- Next “big” step in bioinformatics is to go from the “parts list” to figuring out how to put it all together