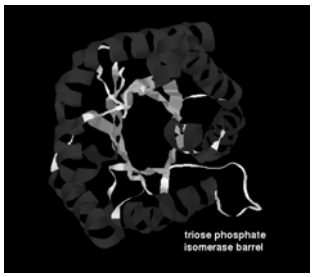
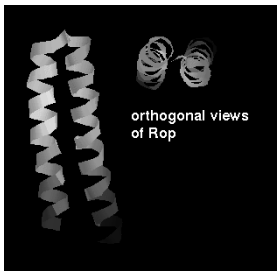
Protein Structure Analysis & Protein-Protein Interactions





David Wishart
University of Alberta, Edmonton, Canada
david.wishart@ualberta.ca

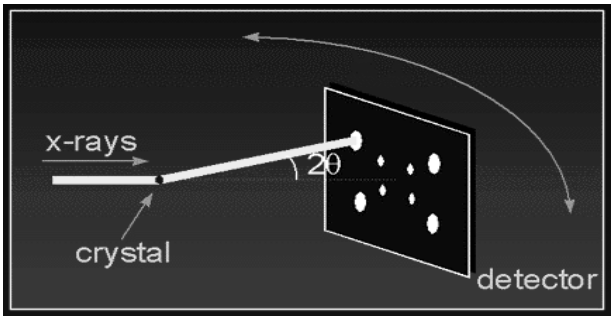
Much Ado About Structure

- **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)

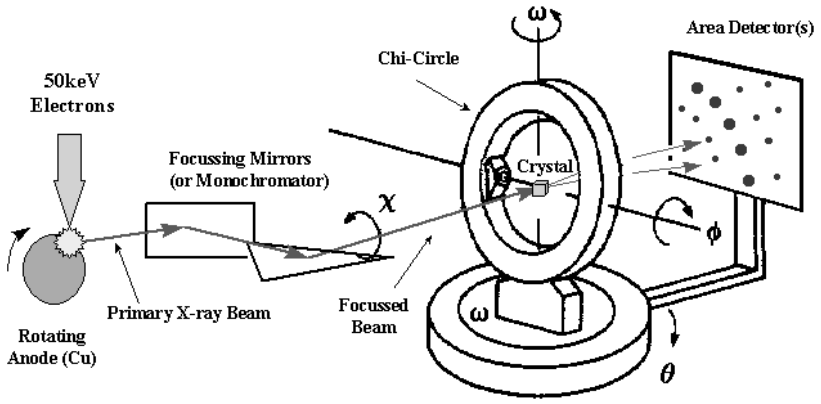
X-ray Crystallography



X-ray Crystallography

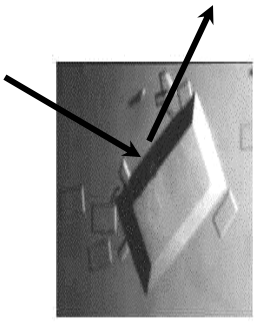
- Crystallization
- Diffraction Apparatus
- Diffraction Principles
- Conversion of Diffraction Data to Electron Density
- Resolution
- Chain Tracing

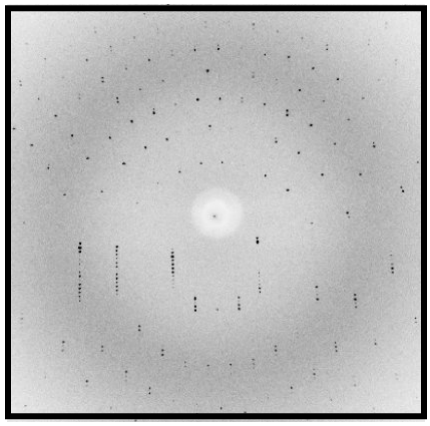
Diffraction Apparatus



4-Circle Goniometer (Eulerian or Kappa Geometry)

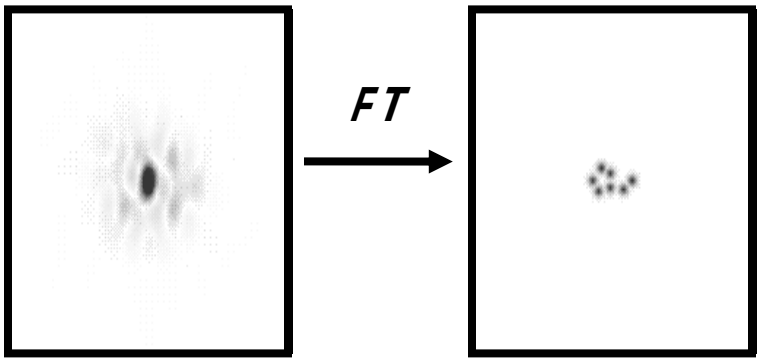
Protein Crystal Diffraction



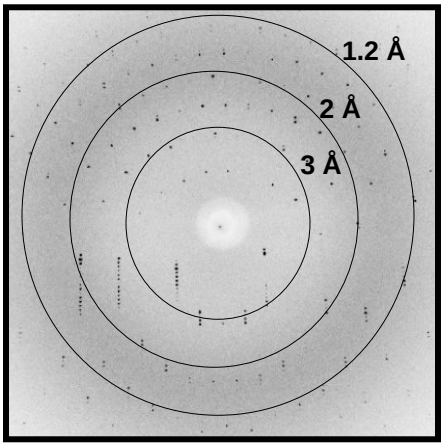


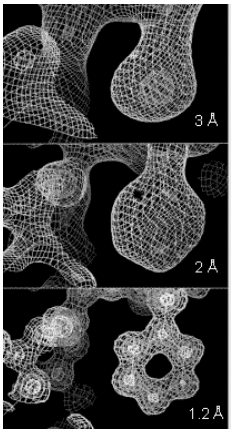
Diffraction Pattern

Converting Diffraction Data to Electron Density



Resolution





The Final Result

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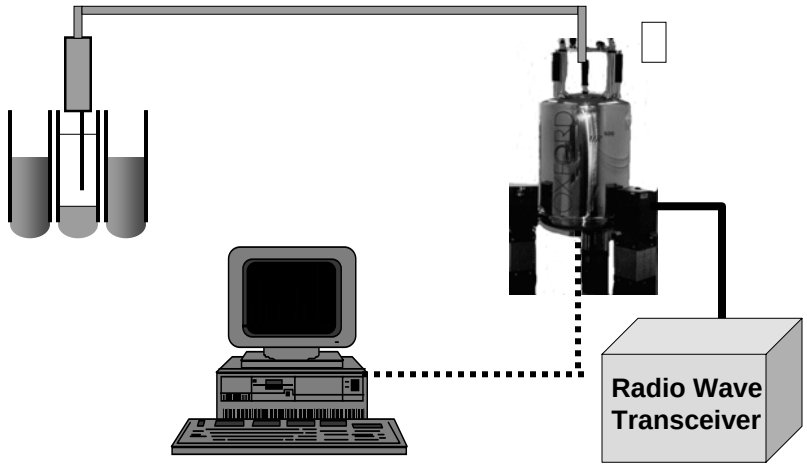
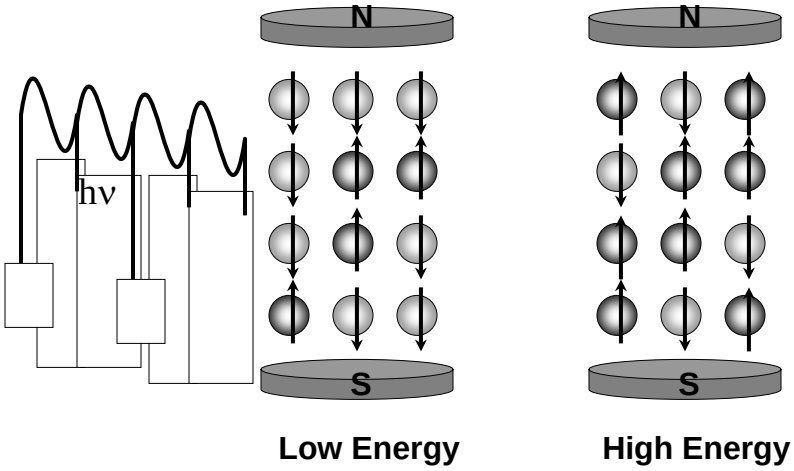
ORIGX2      0.000000  1.000000  0.000000        0.00000                  2TRX 147
ORIGX3      0.000000  0.000000  1.000000        0.00000                  2TRX 148
SCALE1      0.011173  0.000000  0.004858        0.00000                  2TRX 149
SCALE2      0.000000  0.019585  0.000000        0.00000                  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
ATOM      8  CA  ASP A   2      19.395  26.125  -0.949  1.00 21.57       2TRX 159
ATOM      9  C   ASP A   2      20.264  26.214   0.297  1.00 20.89       2TRX 160
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
```

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

NMR Spectroscopy

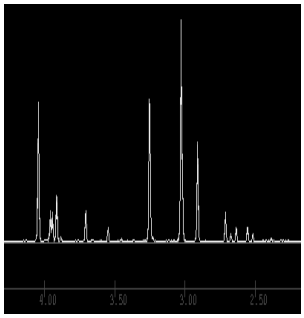


Principles of NMR



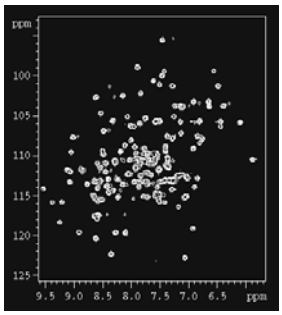
Multidimensional NMR

1D



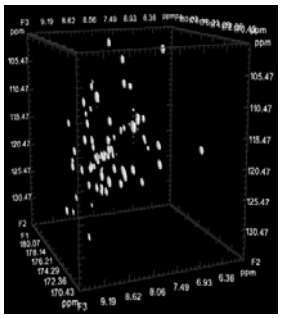
MW ~ 500

2D



MW ~ 10,000

3D

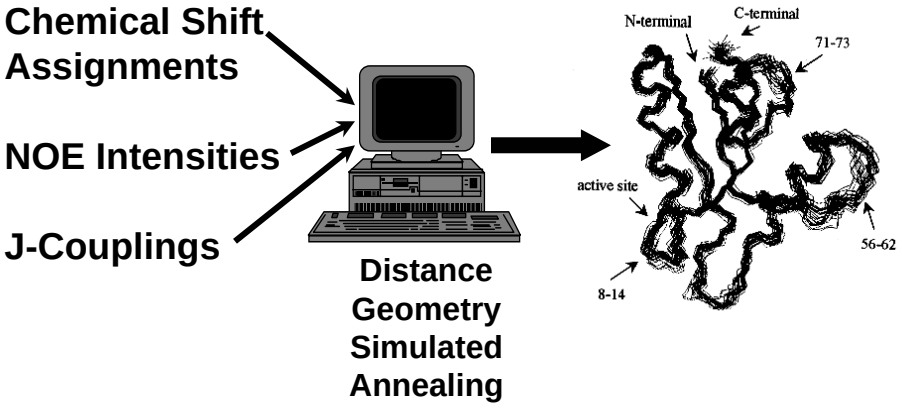


MW ~ 30,000

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



The Final Result

```
ORIGX2      0.000000  1.000000  0.000000        0.00000                  2TRX 147
ORIGX3      0.000000  0.000000  1.000000        0.00000                  2TRX 148
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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
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ATOM      8  CA  ASP A   2      19.395  26.125  -0.949  1.00 21.57       2TRX 159
ATOM      9  C   ASP A   2      20.264  26.214   0.297  1.00 20.89       2TRX 160
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
```

X-ray Versus NMR

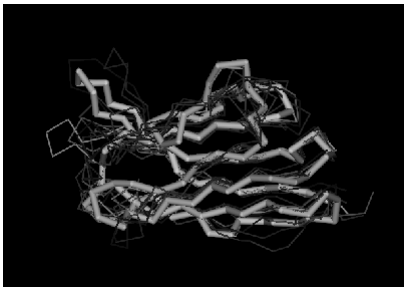
X-ray

- 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

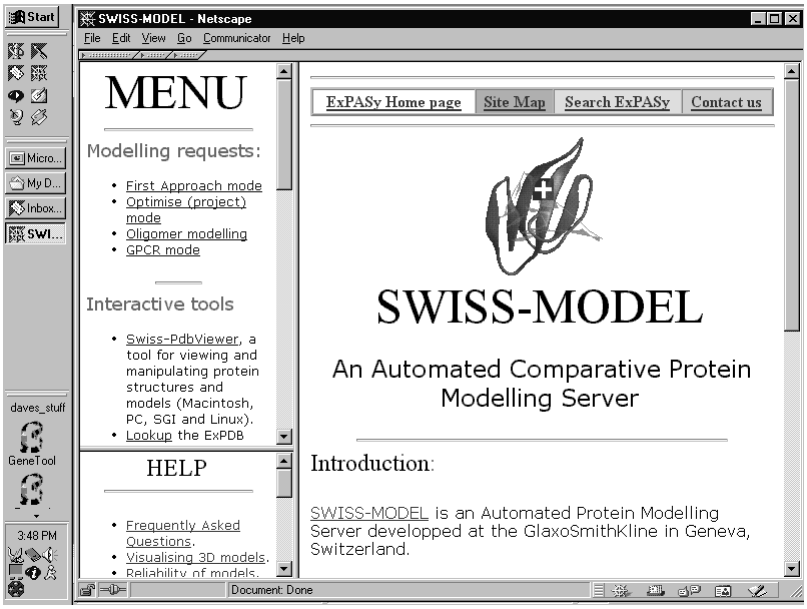
- Offers a method to "Predict" the 3D structure of proteins for which it is not possible to obtain X-ray or NMR data
- Can be used in understanding function, activity, specificity, etc.
- Of interest to drug companies wishing to do structure-aided drug design
- A keystone of Structural Proteomics

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://www.expasy.ch/swissmod/SWISS-MODEL.html

The Final Result

```
ORIGX2      0.000000  1.000000  0.000000        0.00000                 2TRX 147
ORIGX3      0.000000  0.000000  1.000000        0.00000                 2TRX 148
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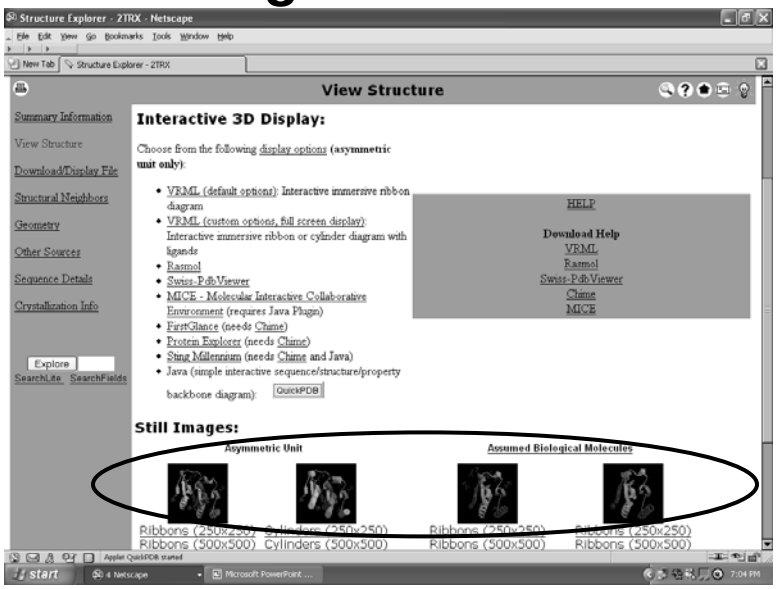
ATOM     11  CB  ASP A   2      18.439  24.914  -0.856  1.00 22.14      2TRX 162
```

The PDB

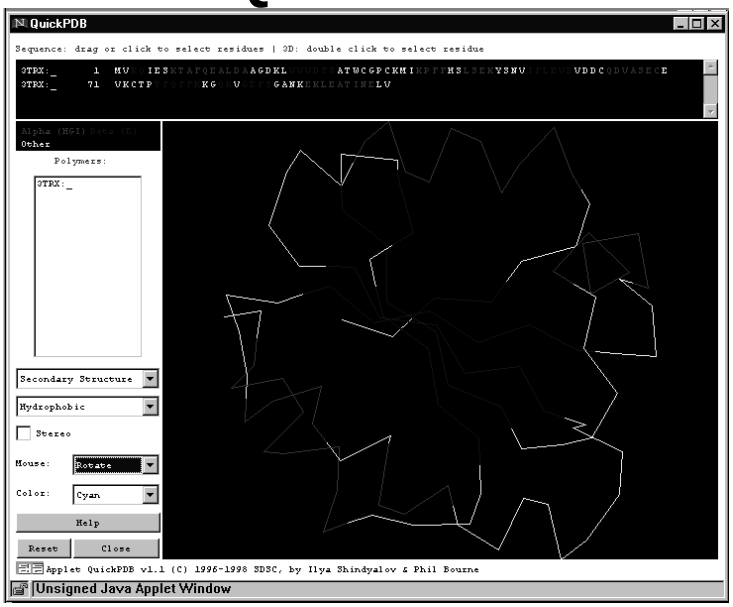
- **PDB - Protein Data Bank**
- **Established in 1971 at Brookhaven National Lab (7 structures)**
- **Primary archive for macromolecular structures (proteins, nucleic acids, carbohydrates – now 30,000 structrs)**
- **Moved from BNL to RCSB (Research Collaboratory for Structural Bioinformatics) in 1998**

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

Viewing 3D Structures



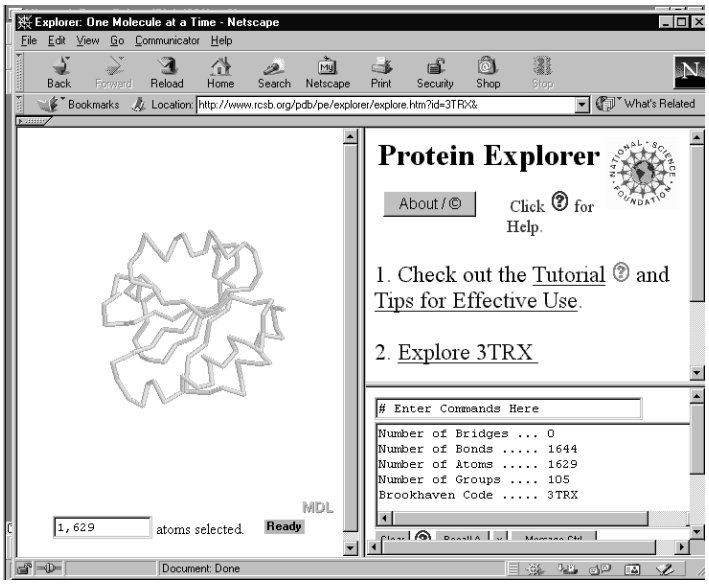
QuickPDB



Quick PDB

- **http://www.sdsc.edu/pb/Software.html**
- **Very simple viewing program with limited manipulation and very limited rendering capacity -- Very fast**
- **Java Applet (Source code available)**
- **Compatible with most browsers and computer platforms**

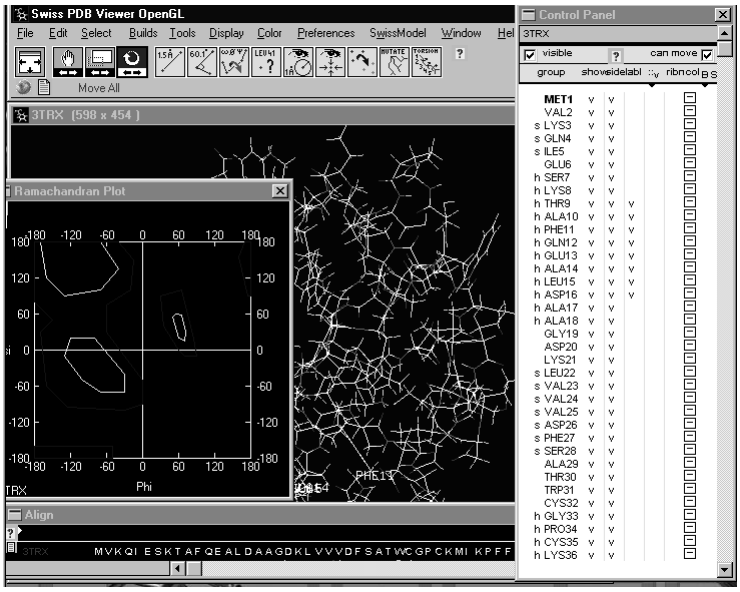
Protein Explorer (Chime)



Protein Explorer

- http://www.umass.edu/microbio/chime/explorer/
- **Uses Chime & Rasmol for its back-end**
- **Very flexible, user friendly, well documented, offers morphing, sequence structure interface, comparisons, context-dependent help, smart zooming, off-line**
- **Browser Plug-in (Like PDF reader)**
- **Compatible with Netscape (Mac & Win)**

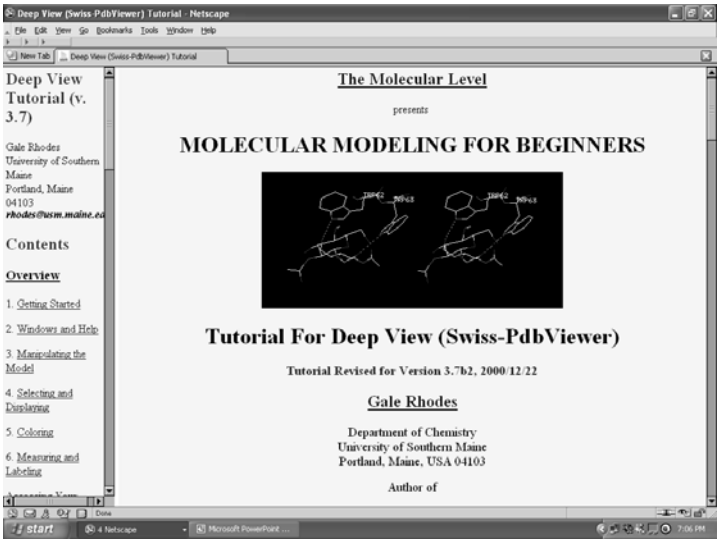
Swiss PDB Viewer



Swiss PDB Viewer

- http://www.expasy.ch/spdbv/
- Among most sophisticated molecular rendering, manipulation and modelling packages (commercial or freeware)
- Supports threading, hom. Modelling, energy minimization, seq/struc interface
- Stand-alone version only
- Compatible on Mac, Win, Linux, SGI

Swiss PDB Tutorial

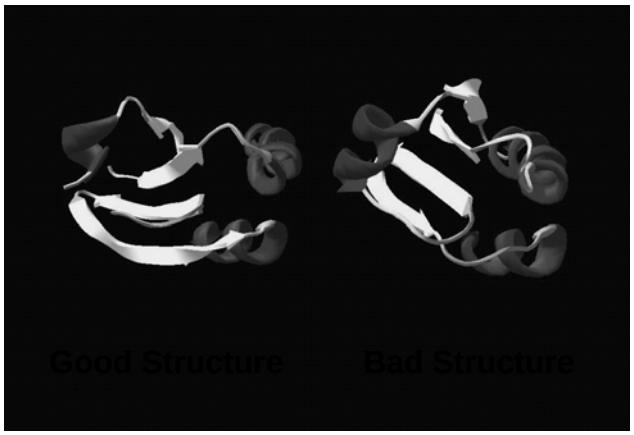


http://www.usm.maine.edu/~rhodes/SPVTut/

Summary

	Mac	Win	Unix	Rendr	SeqView	Super	E Min	Modeling
Rasmol	+	+	+	++	-	-	-	-
Chime	+	+	-	+	-	-	-	-
Prot. Expl.	+	+	-	++	+	+	-	-
Quick PDB	+	+	+	+	+	-	-	-
Biomer	+	+	+	++	-	+	+	+
SwP Viewer	+	+	+	+++	+	+	+	+
MolMol	-	+	+	+++	-	+	-	+

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)

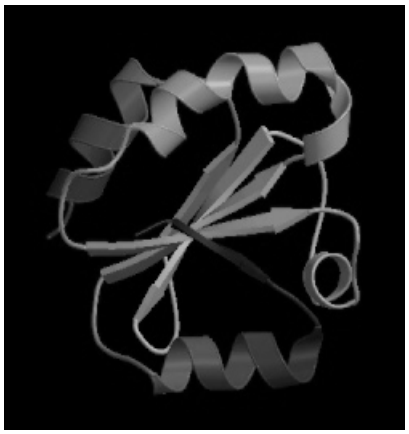
Structure Variability

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

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

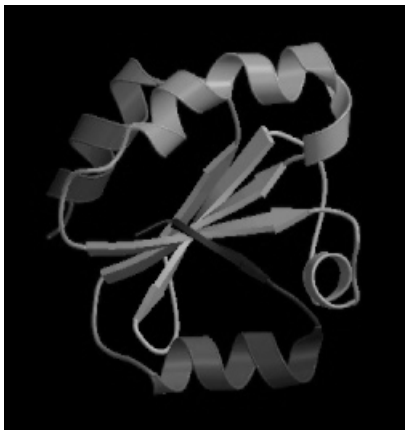
A Good Protein Structure..

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



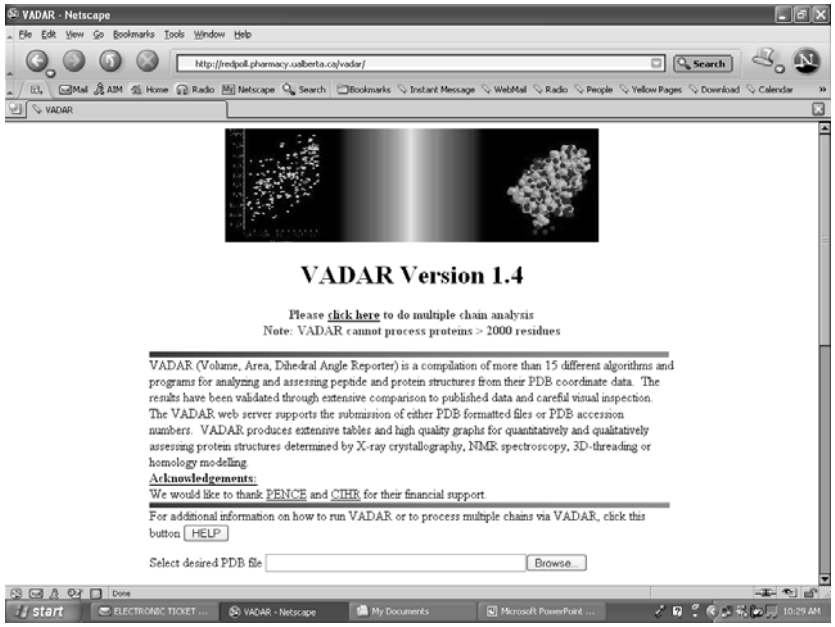
A Good Protein Structure..

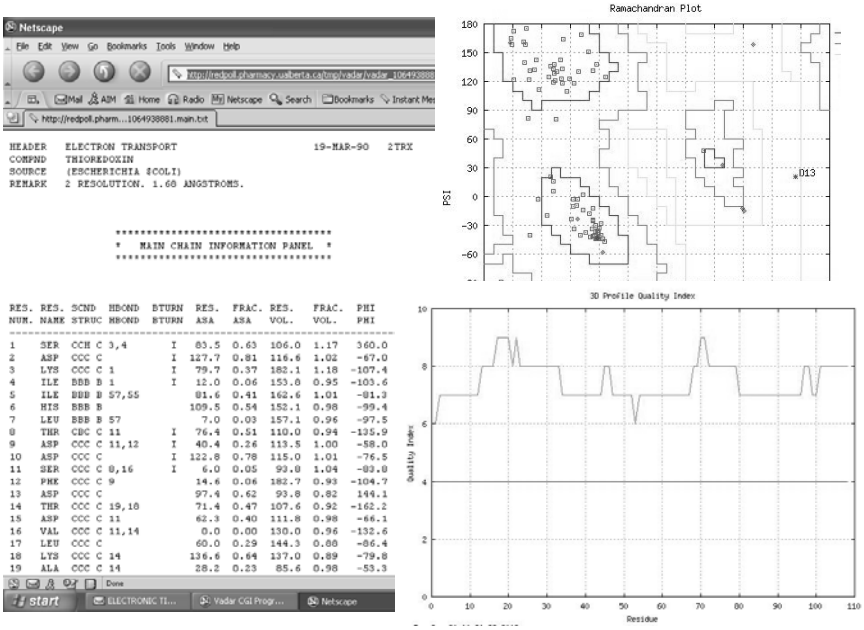
- Minimizes number of "bad" contacts
- Minimizes number of buried charges
- Minimizes radius of gyration
- Minimizes covalent and noncovalent (van der Waals and coulombic) energies



Structure Validation Servers

- **WhatIf Web Server -**
  http://www.cmbi.kun.nl:1100/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

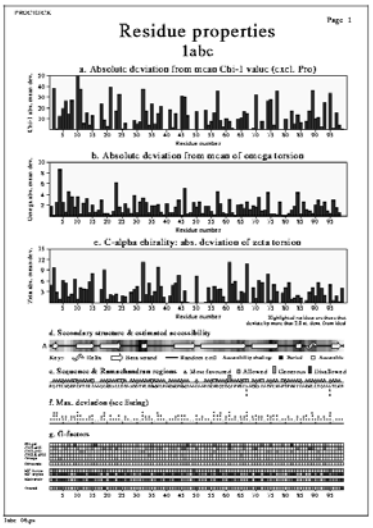

VADAR - Netscape
http://redpoll.pharmacy.ualberta.ca/vadar/
VADAR Version 1.4
Please click here to do multiple chain analysis
Note: VADAR cannot process proteins > 2000 residues
VADAR (Volume, Area, Dihedral Angle Reporter) is a compilation of more than 15 different algorithms and programs for analyzing and assessing peptide and protein structures from their PDB coordinate data. The results have been validated through extensive comparison to published data and careful visual inspection. The VADAR web server supports the submission of either PDB formatted files or PDB accession numbers. VADAR produces extensive tables and high quality graphs for quantitatively and qualitatively assessing protein structures determined by X-ray crystallography, NMR spectroscopy, 3D-threading or homology modelling.
Acknowledgements:
We would like to thank PENCE and CIHR for their financial support.
For additional information on how to run VADAR or to process multiple chains via VADAR, click this button HELP
Select desired PDB file
Browse...

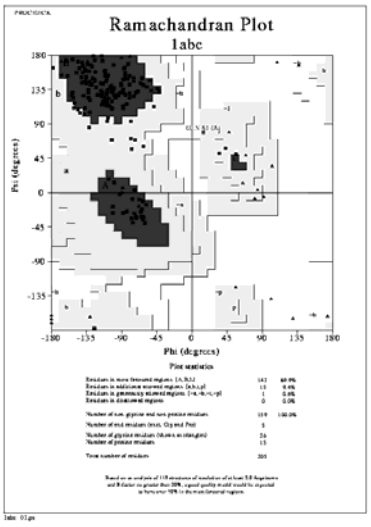

Netscape
HEADER ELECTRON TRANSPORT 19-MAR-90 2TRX
COMPND THIOREDOXIN
SOURCE (ESCHERICHIA SCOLI)
REMARK 2 RESOLUTION. 1.68 ANGSTROMS.
MAIN CHAIN INFORMATION PANEL
Ramachandran Plot
PSI
3D Profile Quality Index
Quality Index
Residue

Structure Validation Programs

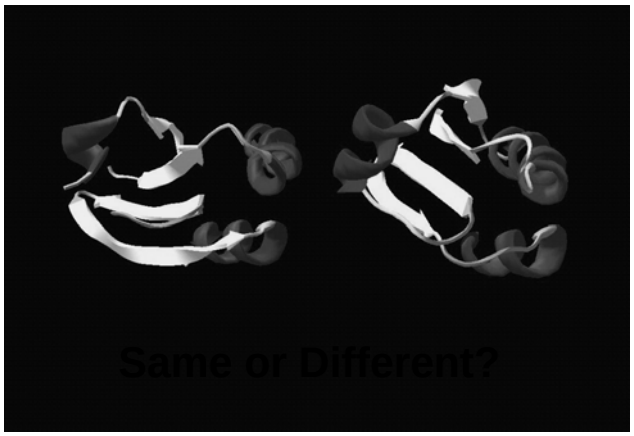
- **PROCHECK -**
  http://www.biochem.ucl.ac.uk/~roman/procheck/procheck.html
- **PROSA II -**
  http://lore.came.sbg.ac.at/People/mo/Prosa/prosa.html
- **VADAR -**
  http://www.pence.ualberta.ca/ftp/vadar/
- **DSSP -**
  http://www.embl-heidelberg.de/dssp/

Procheck



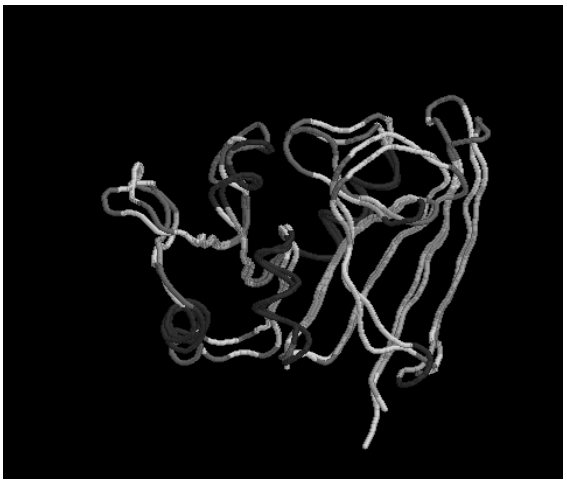


Comparing 3D Structures



Qualitative vs. Quantitative

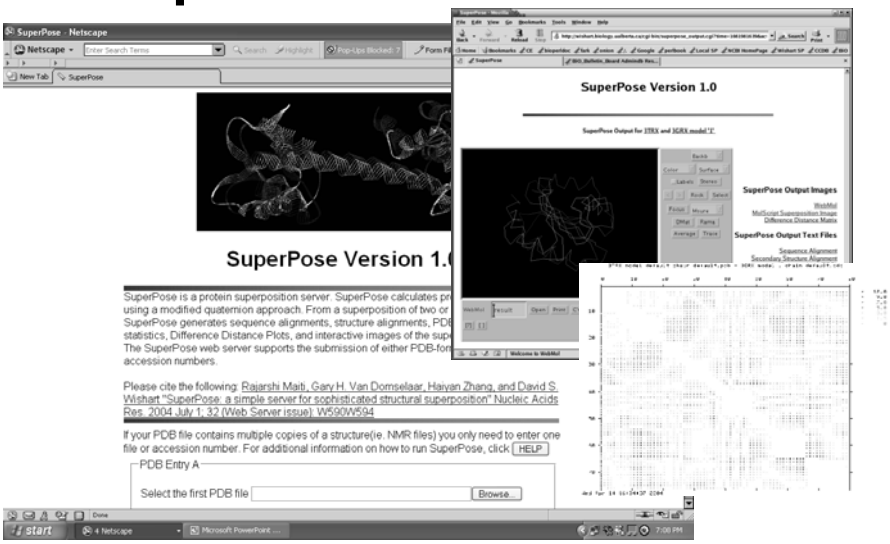
Rigid Body Superposition



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

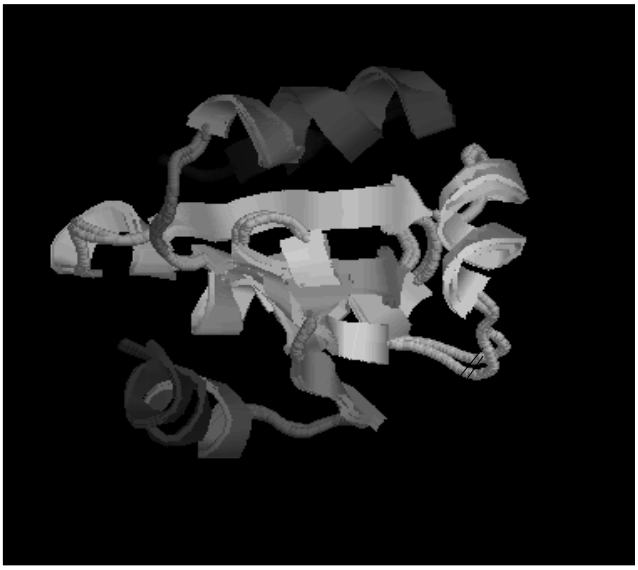


http://wishart.biology.ualberta.ca/SuperPose/

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

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

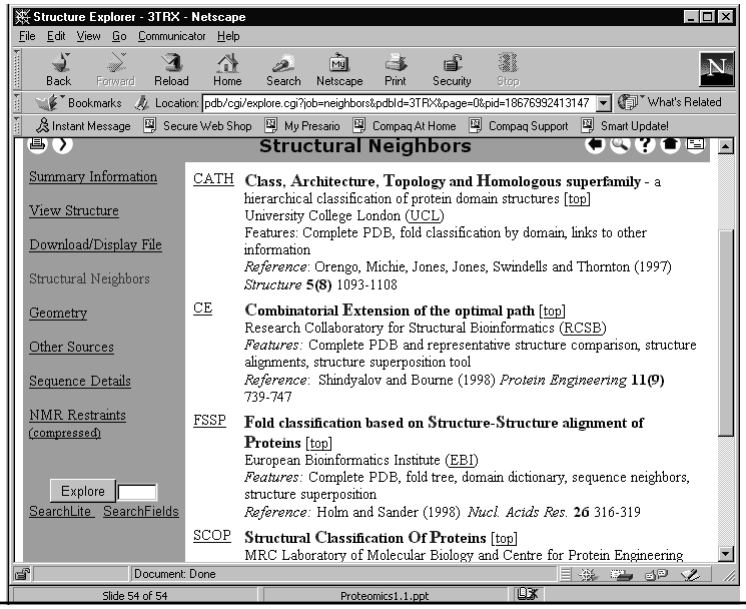
RMSD

- 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

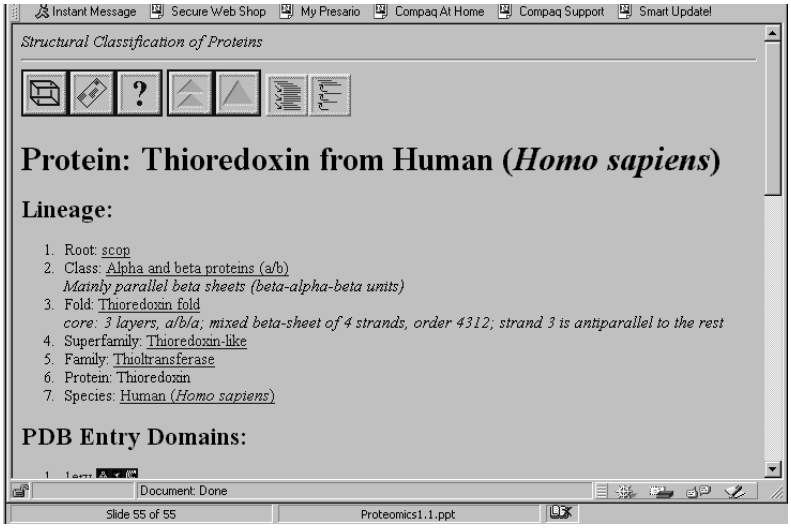
Detecting Unusual Relationships

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 # 2º structures**
- **Superfamily clusters of low sequence ID but related structures & functions**
- **Family clusers of proteins with seq ID > 30% with v. similar struct. & function**

Different Folding Classes

Lactate Dehydrogenase: Mixed α / β	Immunoglobulin Fold: β	Hemoglobin B Chain: α

CATH Database

http://www.biochem.ucl.ac.uk/bsm/cath/

CATH

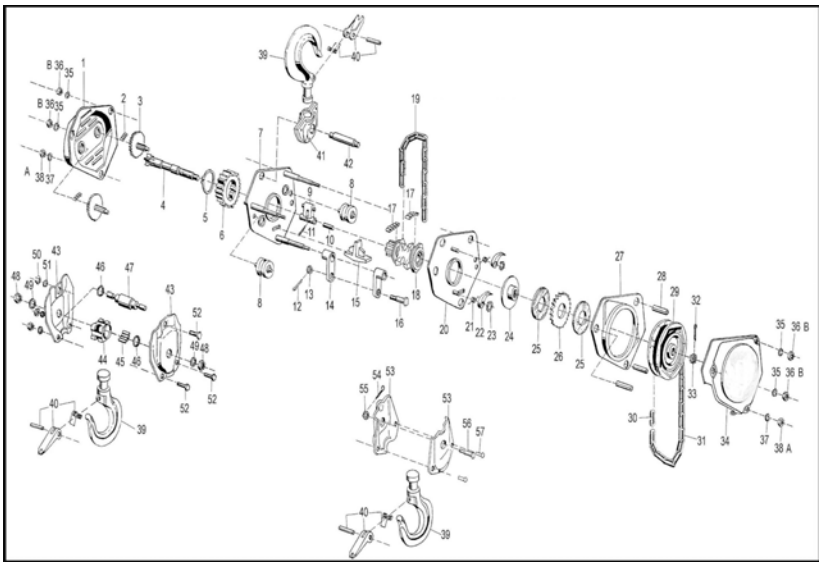
- Class [C] derived from secondary structure content (automatic)
- Architecture (A) derived from orientation of 2° structures (manual)
- Topology (T) derived from topological connection and # 2° structures
- Homologous Superfamily (H) clusters of similar structures & functions

Other Servers/Databases

- **Dali -** http://www.ebi.ac.uk/dali/
- **VAST -** http://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

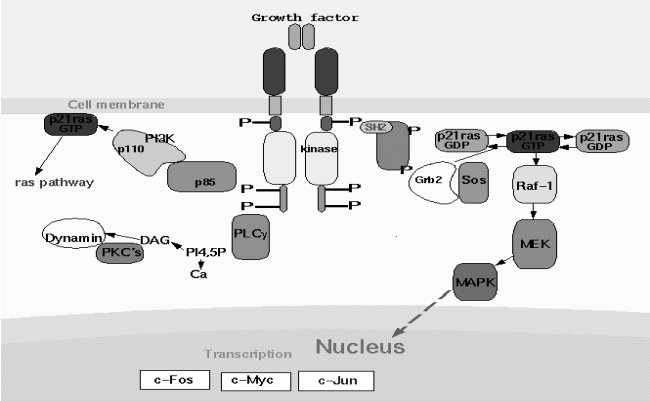
The Protein Parts List



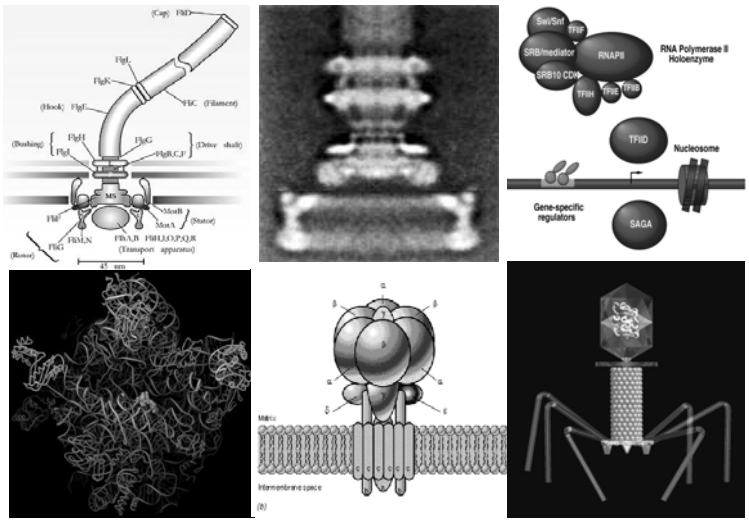
The Parts List

- 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*

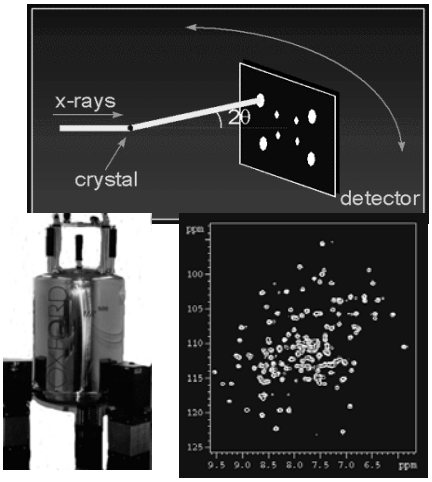


Types of Interactions

- **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**

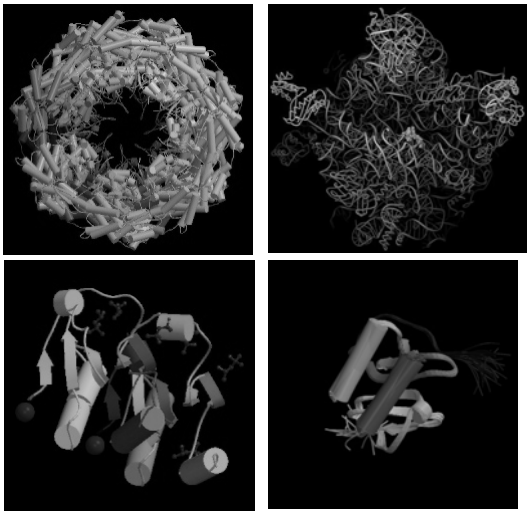
Protein Interaction Tools and Techniques - Experimental Methods

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.**

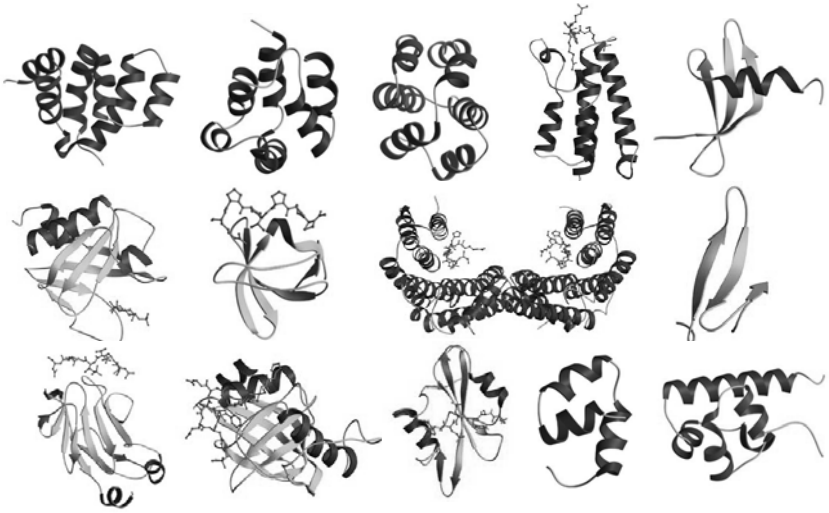
Quaternary Structure



Some interactions are real

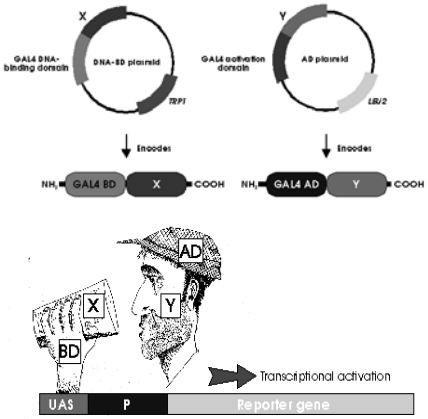
Others are not

Protein Interaction Domains



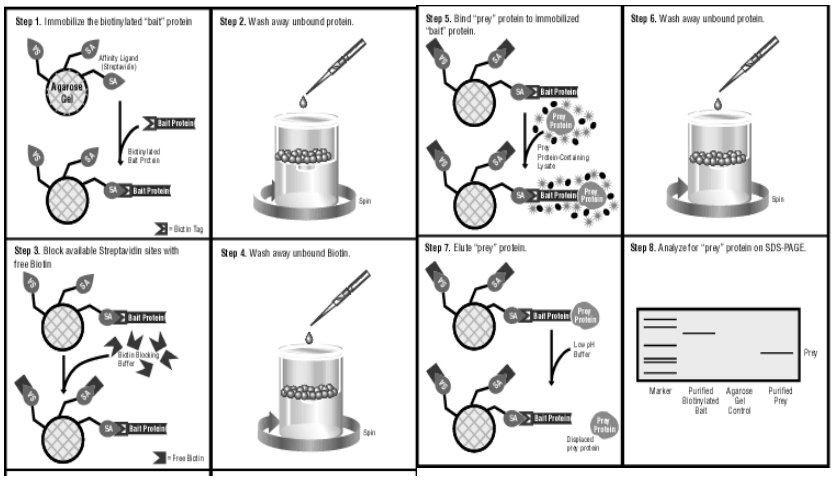
http://www.mshri.on.ca/pawson/domains.html

Yeast Two-Hybrid Analysis

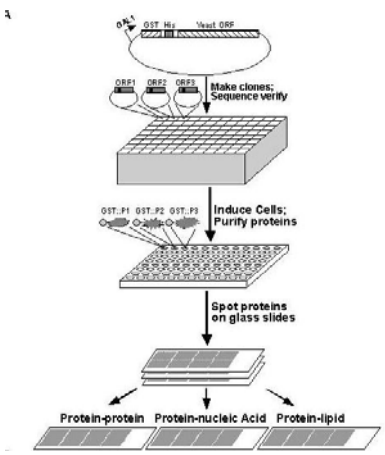


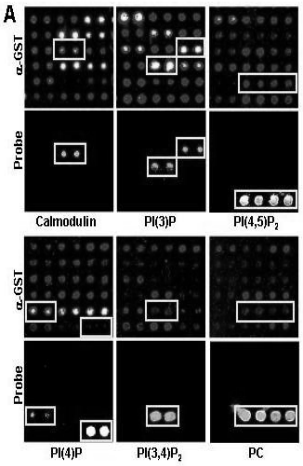
- Yeast two hybrid experiments yield information on protein protein interactions
- GAL4 Binding Domain
- GAL4 Activation Domain
- X and Y are two proteins of interest
- If X & Y interact then reporter gene is expressed

Affinity Pull-down



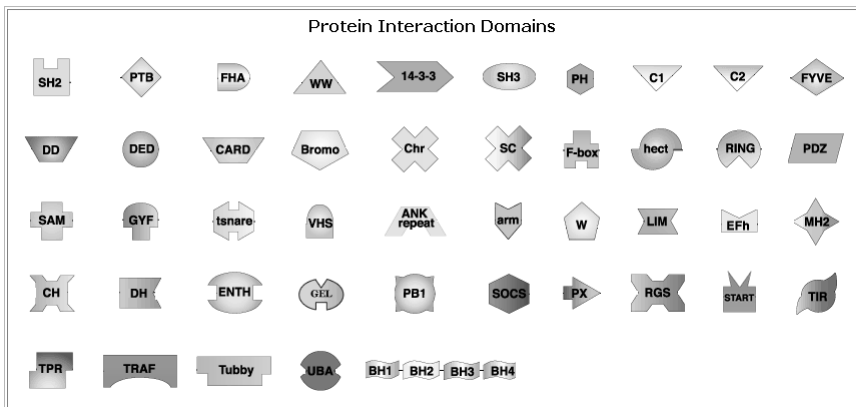
Protein Arrays





Protein Interaction Tools and Techniques - Computational Methods

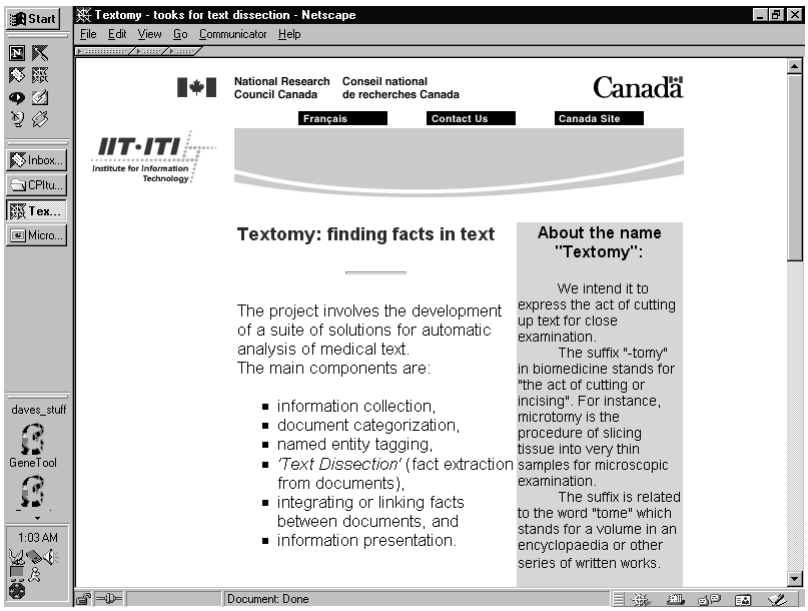
Sequence Searching Against Known Domains



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

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**
- **Sometimes called “Textomy”**



http://textomy.iit.nrc.ca/

Pre-BIND

- ***Donaldson et al. BMC Bioinformatics 2003 4:11***
- **Used Support Vector Machine (SVM) to scan literature for protein interactions**
- **Precision, accuracy and recall of 92% for correctly classifying PI abstracts**
- **Estimated to capture 60% of all abstracted protein interactions for a given organism**

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:

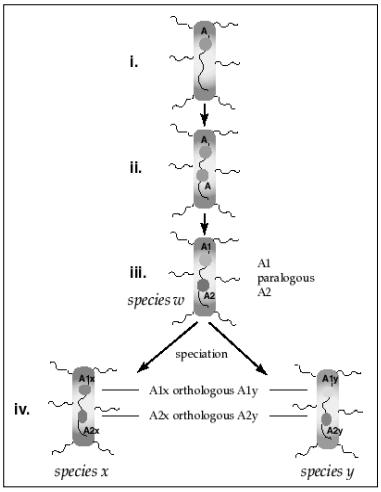
- fused in invertebrates
- form a heterocomplex in mammals

Interologs, Homologs, Paralogs...

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

YM2

- **Interolog**
  - **Protein - Protein Interaction**



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

The BIND Database

- BIND - Biomolecular Interaction Network Database
- Designed to capture almost all interactions between biomolecules (large and small)
- Largest database of its kind -- 135,000 interactions recorded to date

BIND Can Encode...

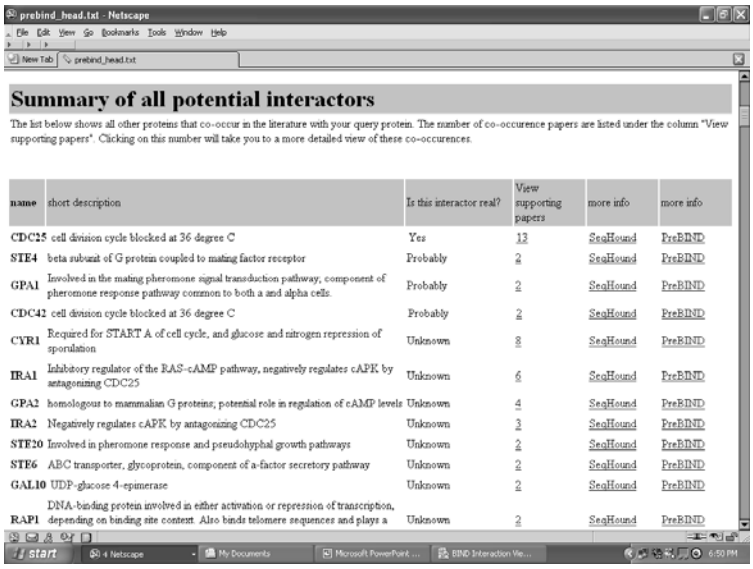
- Simple binary interactions
- Enzymes, substrates and conformational changes
- Restriction enzymes
- Limited proteolysis
- Phosphorylation (reversible)
- Glycosylation
- Intron splicing
- Transcriptional factors

BIND

BIND Queries

- Users may search PreBIND by
  - Protein name, organism, protein accession # or PubMed ID
- Users may search BIND by
  - Accession or GI #, GO ID, PDB ID, PubMed ID, taxonomy, author, journal, Entrez GeneID, or more than 20 different database identifier tags
  - Sequence (via BINDBlast)

PreBIND Query (Ras1 & Yeast)



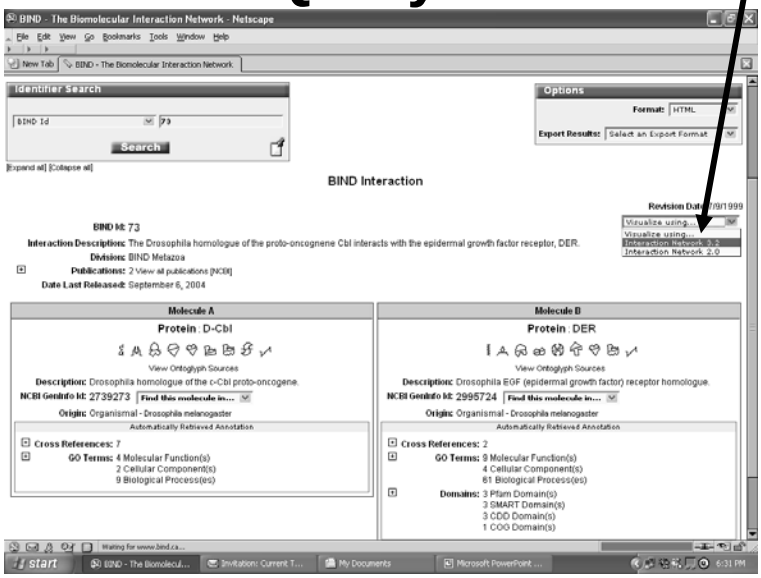
Summary of all potential interactors

The list below shows all other proteins that co-occur in the literature with your query protein. The number of co-occurence papers are listed under the column "View supporting papers". Clicking on this number will take you to a more detailed view of these co-occurences.

name	short description	Is this interactor real?	View supporting papers	more info	more info
CDC25	cell division cycle blocked at 36 degree C	Yes	13	SeqHound	PreBIND
STE4	beta subunit of G protein coupled to mating factor receptor	Probably	2	SeqHound	PreBIND
GPA1	Involved in the mating pheromone signal transduction pathway, component of pheromone response pathway common to both a and alpha cells.	Probably	2	SeqHound	PreBIND
CDC42	cell division cycle blocked at 36 degree C	Probably	2	SeqHound	PreBIND
CYR1	Required for START A of cell cycle, and glucose and nitrogen repression of sporulation	Unknown	8	SeqHound	PreBIND
IRA1	Inhibitory regulator of the RAS-cAMP pathway, negatively regulates cAPK by antagonizing CDC25	Unknown	6	SeqHound	PreBIND
GPA2	homologous to mammalian G proteins; potential role in regulation of cAMP levels	Unknown	4	SeqHound	PreBIND
IRA2	Negatively regulates cAPK by antagonizing CDC25	Unknown	3	SeqHound	PreBIND
STE20	Involved in pheromone response and pseudohyphal growth pathways	Unknown	2	SeqHound	PreBIND
STE6	ABC transporter, glycoprotein, component of a-factor secretory pathway	Unknown	2	SeqHound	PreBIND
GAL10	UDP-glucose 4-epimerase	Unknown	2	SeqHound	PreBIND
RAP1	DNA-binding protein involved in either activation or repression of transcription, depending on binding site context. Also binds telomere sequences and plays a	Unknown	2	SeqHound	PreBIND

BIND Query Result

click



BIND Interaction

BIND Id: 73

Interaction Description: The Drosophila homologue of the proto-oncogene Cbl interacts with the epidermal growth factor receptor, DER.

Division: BIND Metazoa

Publications: 2 View all publications [NCBI]

Date Last Released: September 6, 2004

Molecule A — Protein: D-Cbl

Description: Drosophila homologue of the c-Cbl proto-oncogene.

NCBI GenInfo Id: 2739273

Origin: Organismal - Drosophila melanogaster

Cross References: 7

GO Terms: 4 Molecular Function(s); 2 Cellular Component(s); 9 Biological Process(es)

Molecule B — Protein: DER

Description: Drosophila EGF (epidermal growth factor) receptor homologue.

NCBI GenInfo Id: 2995724

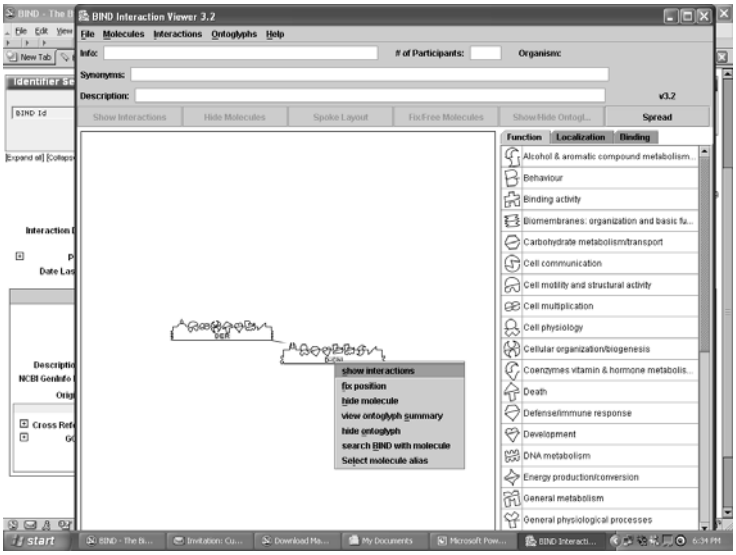
Origin: Organismal - Drosophila melanogaster

Cross References: 2

GO Terms: 9 Molecular Function(s); 4 Cellular Component(s); 61 Biological Process(es)

Domains: 3 Pfam Domain(s); 3 SMART Domain(s); 3 CDD Domain(s); 1 COG Domain(s)

BIND Details



Ontoglyphs

Function	Localization	Binding
Alcohol & aromatic compound metabolism...	Actin cytoskeleton	Antigen binding
Behaviour	Axon or dendrite	ATP binding
Binding activity	Biological membrane	Coenzyme binding
Biomembranes: organization and basic fu...	Cell periphery	Calmodulin binding
Carbohydrate metabolism/transport	Cytoplasm	Carbohydrate binding
Cell communication	Cytoplasmic vesicle	Cytokine binding
Cell motility and structural activity	Endoplasmic reticulum	Cytoskeletal protein binding
Cell multiplication	Endosome	DNA binding
Cell physiology	Extracellular /cell surface	Double stranded DNA binding
Cellular organization/biogenesis	Flagellum /cilium	Guanyl nucleotide binding
Coenzymes vitamin & hormone metabolis...	Golgi apparatus	Lipid binding
Death	Lipid particle	Metal ion binding
Defense/immune response	Microtubule cytoskeleton	mRNA binding
Development	Mitochondrion	Nucleic acid binding
DNA metabolism	Nuclear periphery	Nucleotide binding
Energy production/conversion	Nucleolus	Oxygen binding
General metabolism	Nucleus	Protein binding
General physiological processes	Peroxisome	Adenyl nucleotide binding

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