Lecture 3

Protein Structure Prediction

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Department of Biopharmaceutical Sciences

March 23rd, 2004

References

Protein Structure Prediction:

Marti-Renom *el al. Annu. Rev. Biophys. Biomol. Struct.* 29, 291-325, 2000.
Baker & Sali. *Science* 294, 93-96, 2001.

Comparative Modeling:

Marti-Renom *el al. Annu. Rev. Biophys. Biomol. Struct.* 29, 291-325, 2000.
Marti-Renom *el al. Current Protocols in Protein Science* 1, 2.9.1-2.9.22, 2002.

MODELLER:

Sali & Blundell. *J. Mol. Biol.* 234, 779-815, 1993.

Structural Genomics:

Sali. *Nat. Struct. Biol*. **5**, 1029, 1998.
Burley *et al*. *Nat. Genet*. **23**, 151, 1999.
Sali & Kuriyan. *TIBS* **22**, M20, 1999.
Sanchez *et al. Nat. Str. Biol.* 7, 986, 2000.
Baker & Sali. Science 294, 93-96, 2001.
Vitkup *et al*. *Nat. Struct. Biol*. **8**, 559, 2001.

Nomenclature

- Homology: Sharing a common ancestor, may have similar or dissimilar functions
- Similarity: Score that quantifies the degree of relationship between two sequences.
- Identity: Fraction of identical aminoacids between two aligned sequences (case of similarity).
- Target: Sequence corresponding to the protein to be modeled.
- Template/s: 3D structure/s to be used during protein structure prediction.
- Model: Predicted 3D structure of the target sequence.

Summary

- Protein Structure Prediction and why is it useful?
- Methods in Protein Structure Prediction
- Comparative Modeling
  - ✓ Steps in CM (overview + resources)
  - ✓ Accuracy/Applications of comparative models
  - ✓ Case example in MODELLER (*)
  - ✓ CM and Structural Genomics

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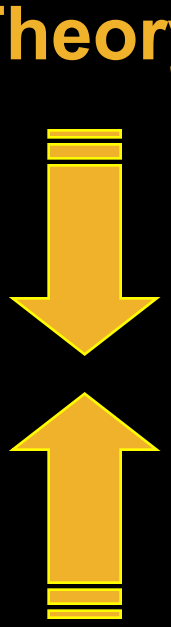
Why protein structure prediction?

	Y 2003	Y 2005
Sequences	1,500,000	millions
Structures	28,000	50,000

Why protein structure prediction?

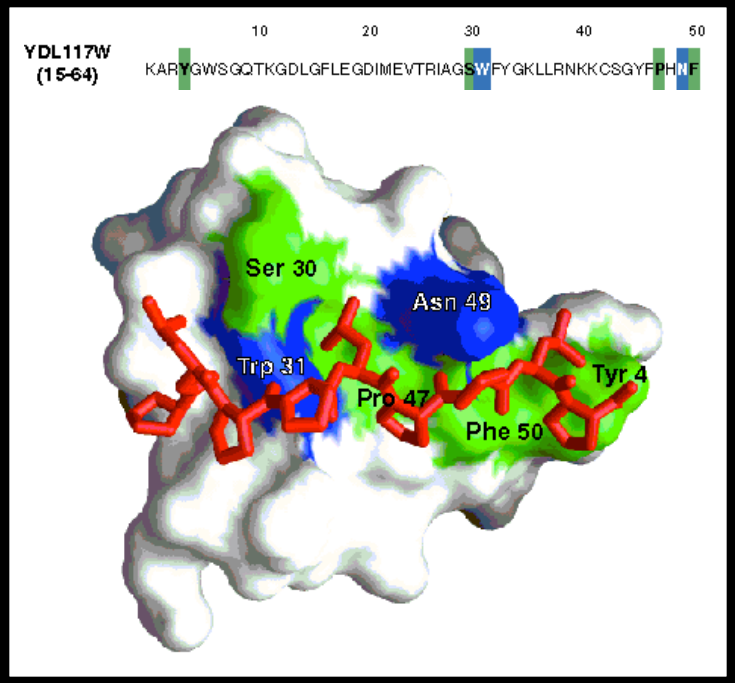
	Y 2003
Sequences	1,500,000
Structures	400,000



http://salilab.org/modbase/

Why is it useful to know the structure of a protein, not only its sequence?

- The biochemical function (activity) of a protein is defined by its interactions with other molecules.
- The biological function is in large part a consequence of these interactions.
- The 3D structure is more informative than sequence because interactions are determined by residues that are close in space but are frequently distant in sequence.

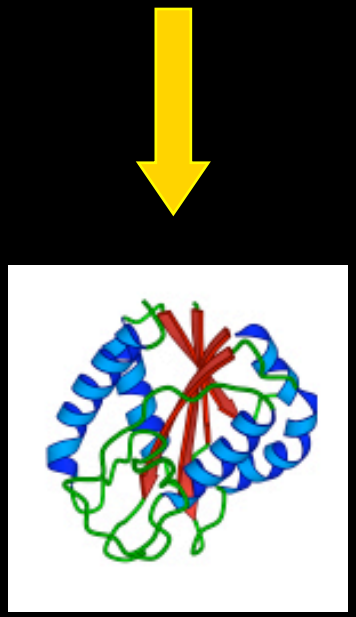


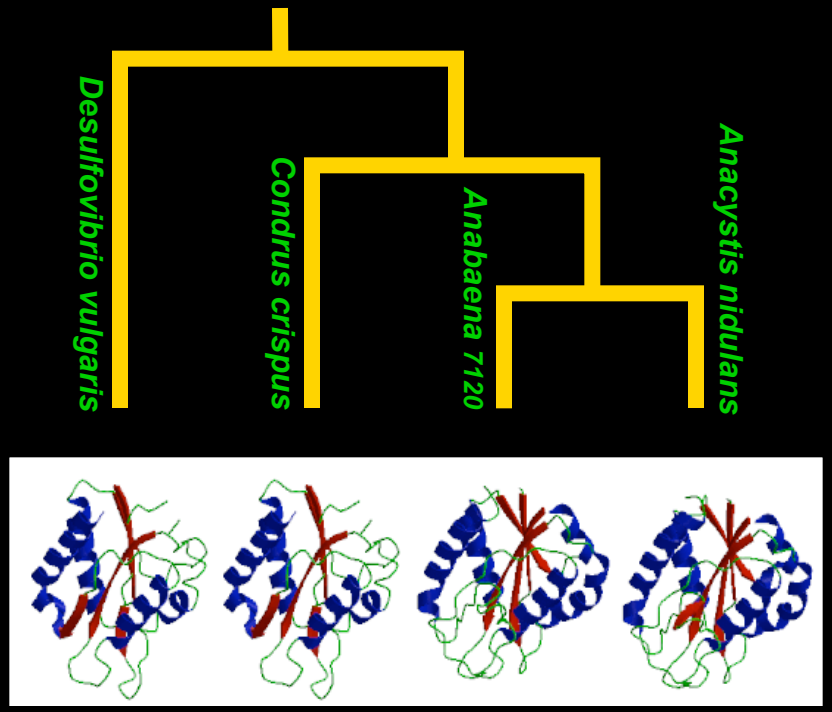
In addition, since evolution tends to conserve function and function depends more directly on structure than on sequence, structure is more conserved in evolution than sequence

The net result is that patterns in space are frequently more recognizable than patterns in sequence.

Principles of Protein Structure

GFCHIKAYTRLIMVG…





Folding

Ab initio prediction

Evolution

Threading
Comparative Modeling

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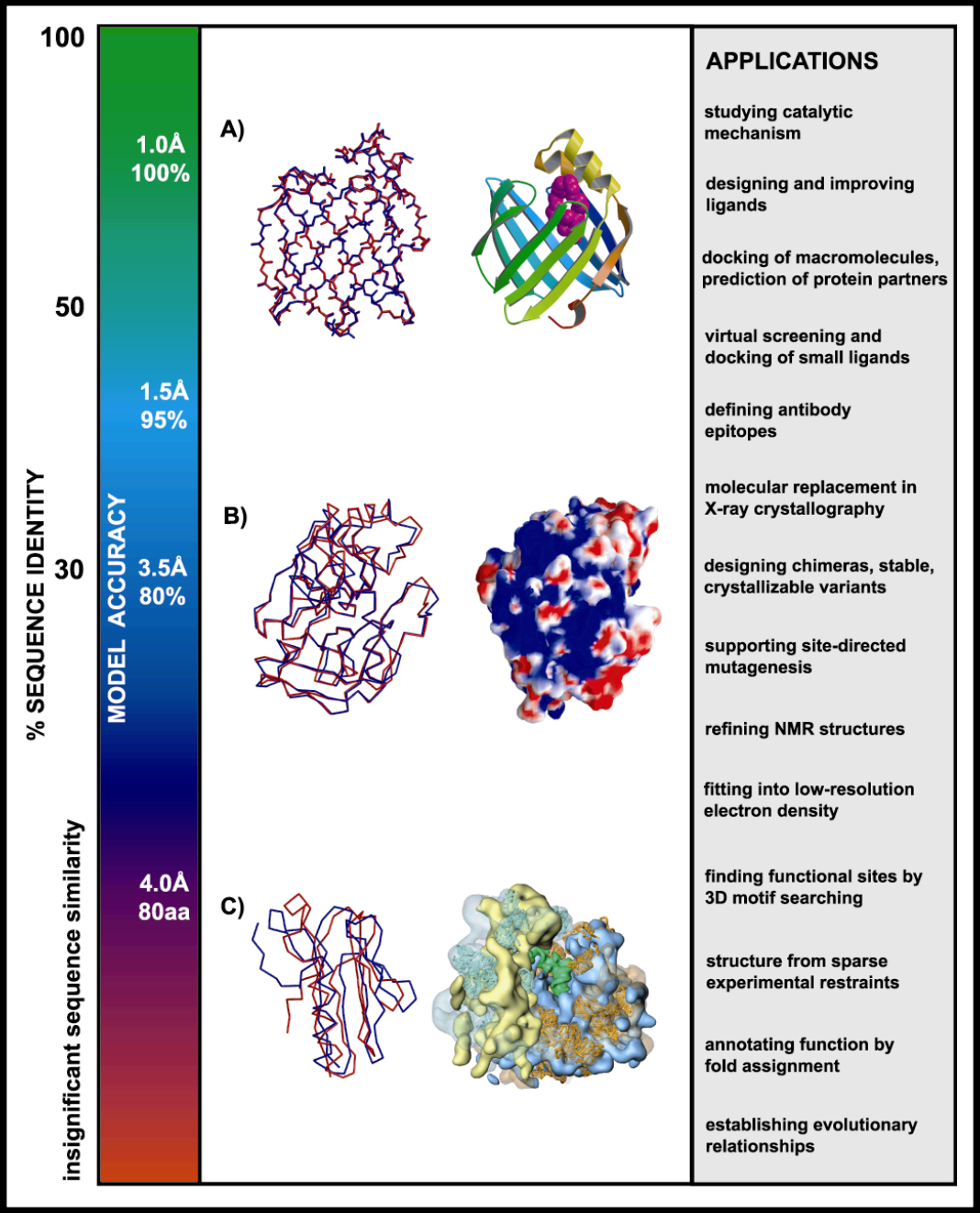
Methods for Protein Structure Prediction

- *Ab Initio*
  - *ROSETTA* [http://depts.washington.edu/bakerpg/]
- *Threading – Fold assignment*
  - *mGenTHREADER* [http://bioinf.cs.ucl.ac.uk/psipred/]
- *Comparative Modeling*
  - *MODELLER*

    [http://www.salilab.org/modeller]

Methods ←→ Resolution



A. Šali & J. Kuriyan.
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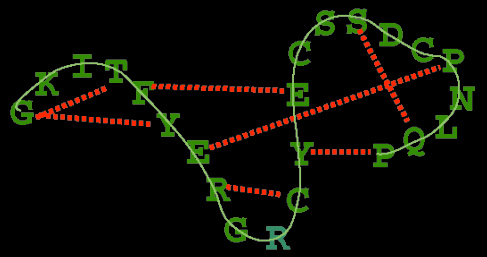
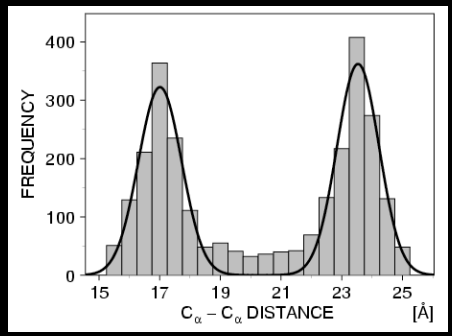
Comparative Modeling by Satisfaction of Spatial Restraints (MODELLER)

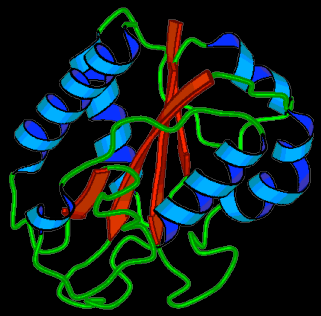
3D GKITFYERGFQGHCYESDC-NLQP…
SEQ GKITFYERG---RCYESDCPNLQP…

1. Extract spatial restraints





2. Satisfy spatial restraints



$$F(\mathbf{R}) = \prod_i p_i(f_i/l)$$

A. Šali & T. Blundell. *J. Mol. Biol.* **234**, 779, 1993.
J.P. Overington & A. Šali. *Prot. Sci.* **3**, 1582, 1994.
A. Fiser, R. Do & A. Šali, *Prot. Sci.*, 9, 1753, 2000.

http://www.salilab.org/

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Steps in Comparative Protein Structure Modeling

START

TARGET

ASILPKRLFGNCEQTSDEGLK
IERTPLVPHISAQNVCLKIDD
VPERLIPERASFQWMNDK

A. Šali, *Curr. Opin. Biotech.* 6, 437, 1995.
R. Sánchez & A. Šali, *Curr. Opin. Str. Biol.* 7, 206, 1997.
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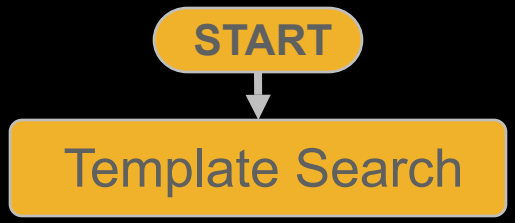
Steps in Comparative Protein Structure Modeling



TARGET

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TEMPLATE

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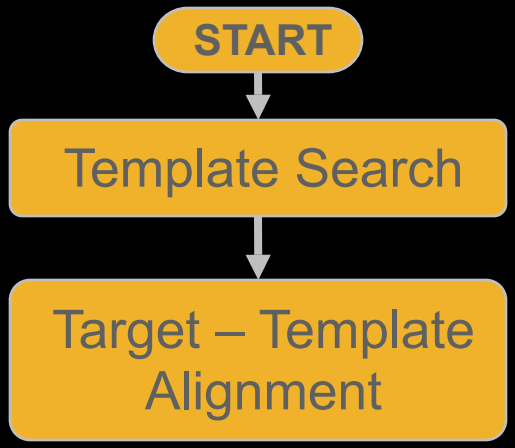

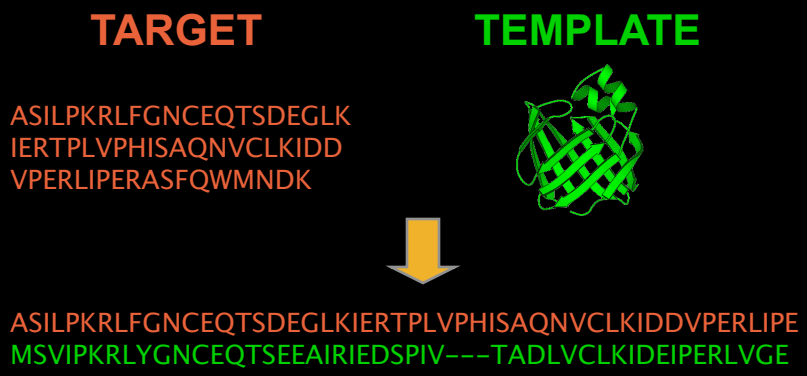

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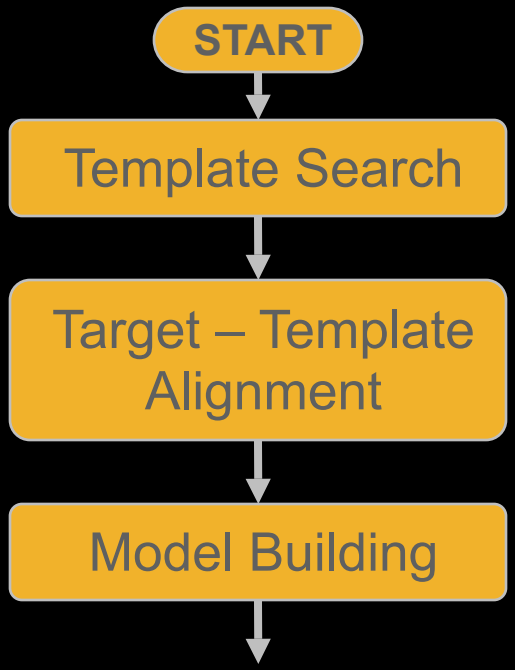

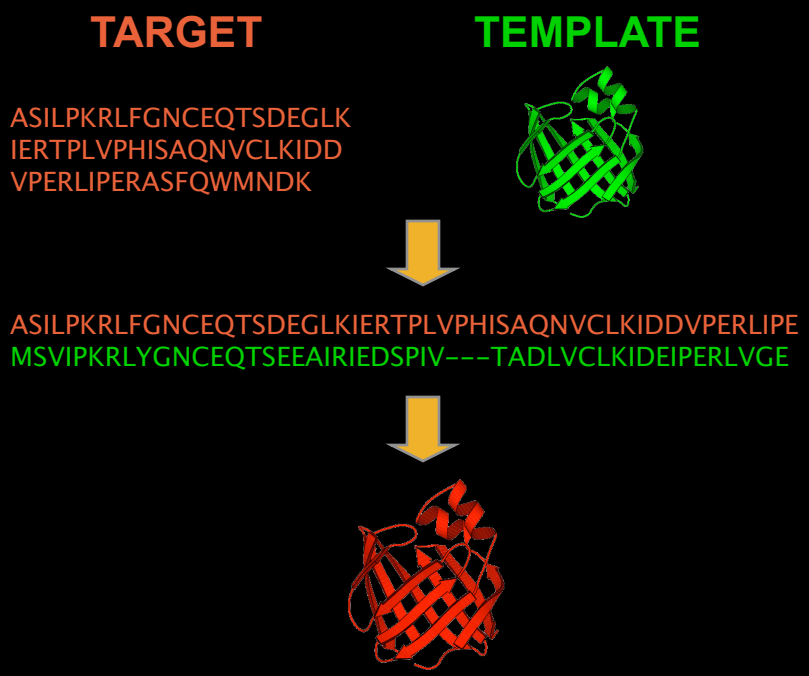

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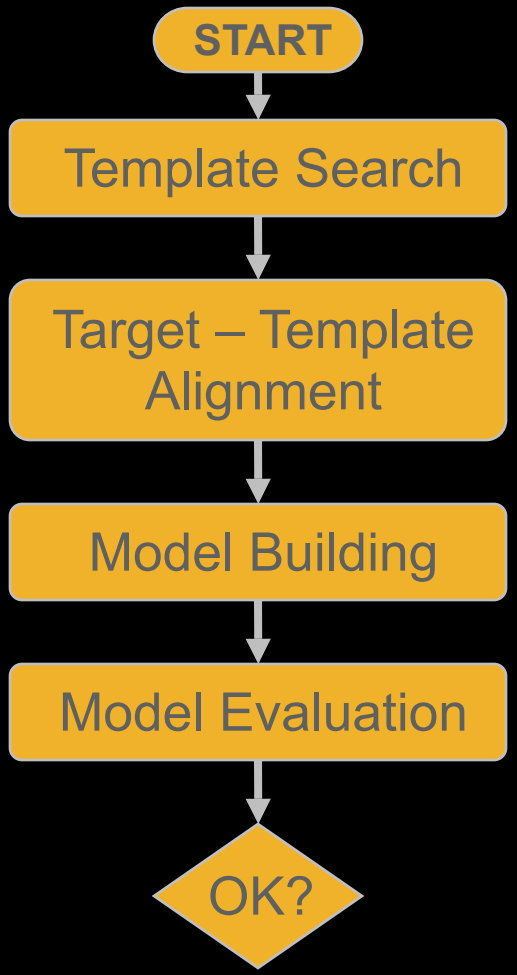

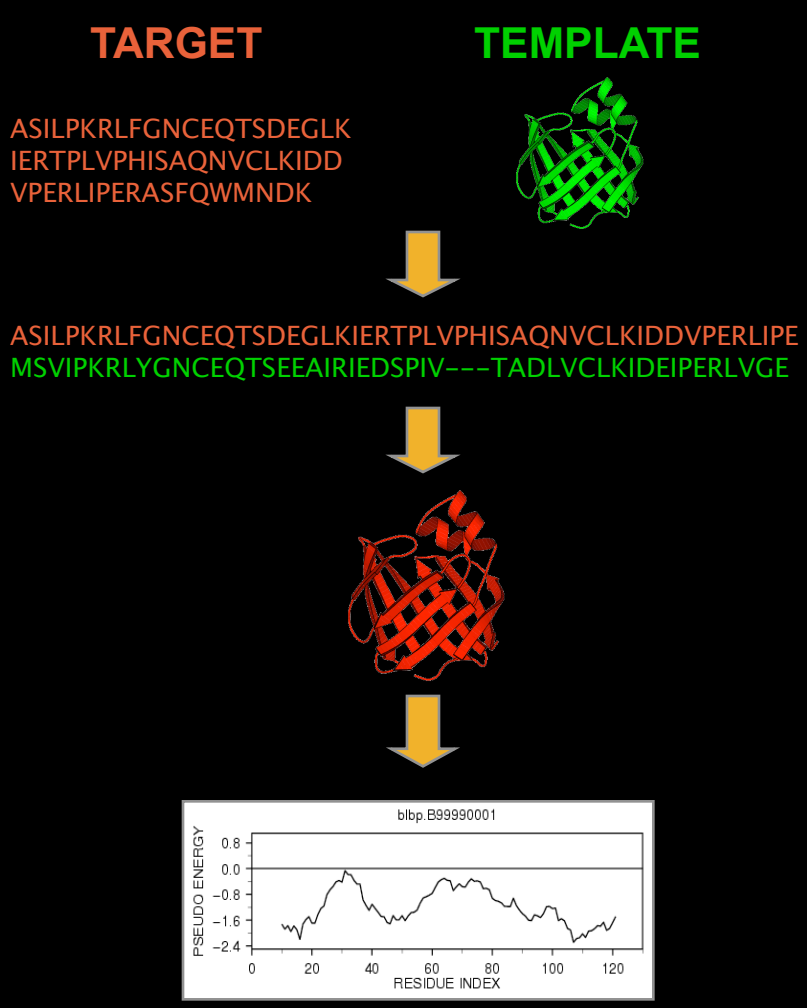

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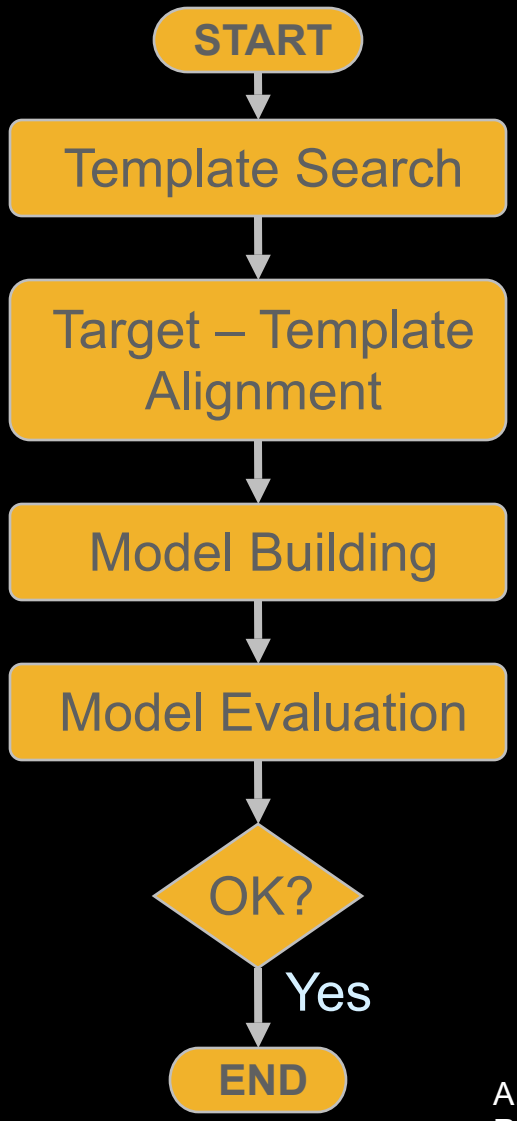
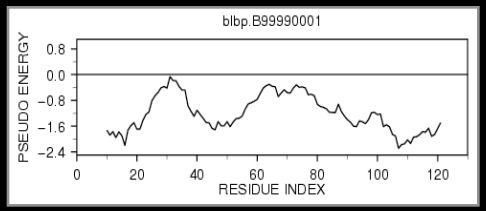


TARGET **TEMPLATE**

ASILPKRLFGNCEQTSDEGLK
IERTPLVPHISAQNVCLKIDD
VPERLIPERASFQWMNDK

ASILPKRLFGNCEQTSDEGLKIERTPLVPHISAQNVCLKIDDVPERLIPE
MSVIPKRLYGNCEQTSEEAIRIEDSPIV---TADLVCLKIDEIPERLVGE



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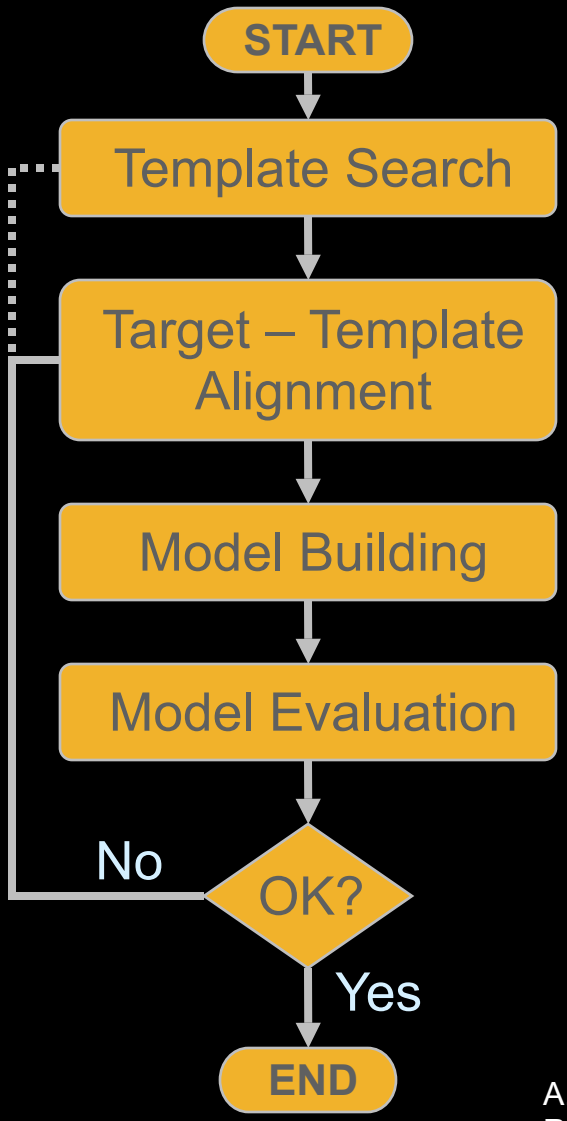

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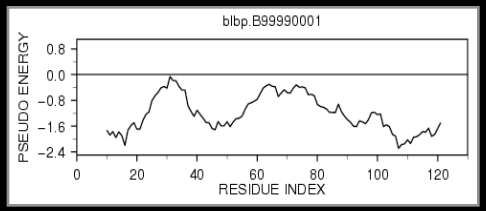
MSVIPKRLYGNCEQTSEEAIRIEDSPIV---TADLVCLKIDEIPERLVGE



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Template Search Methods

- Sequence similarity searches
  - BLAST [http://www.ncbi.nlm.nih.gov/BLAST/]
  - FastA program [http://www.ebi.ac.uk/fasta33/]
- Profile and iterative methods
  - HMMs [http://www.cse.ucsc.edu/research/compbio/HMM-apps/]
  - PSI-BLAST [http://www.ncbi.nlm.nih.gov/BLAST/]
- Structure based threading
  - mGenTHREADER [http://bioinf.cs.ucl.ac.uk/psipred/]
  - PROFIT [http://www.came.sbg.ac.at/]

Target – Template Alignment Methods

- Dynamic Programming Pairwise Alignment
  - ALIGN [http://www.salilab.org/modeller/]
- Multiple Alignments,
  - Psi-Blast [http://www.ncbi.nlm.nih.gov/BLAST/]
  - HMM [http://www.cse.ucsc.edu/research/compbio/HMM-apps/]
  - SALIGN [http://www.salilab.org/modeller/]
  - CLUSTALW [http://www.ebi.ac.uk/clustalw/]
  - SALIGN [http://www.salilab.org/modeller/]
- Structure based approaches
  - Threading [http://bioinf.cs.ucl.ac.uk/threader/]

Model Building Methods

- Rigid Body Assembly
  - COMPOSER [http://www-cryst.bioc.cam.ac.uk/]
- Segment Matching
  - SEGMOD
- Satisfaction of Spatial Restraints
  - MODELLER [http://www.salilab.org/modeller/]

Model Evaluation methods

- Stereochemistry
  - PROCHECK/ WHAT-IF [http://www.biochem.ucl.ac.uk/~roman/procheck/procheck.html]
- Environment
  - VERIFY3D [http://www.doe-mbi.ucla.edu/Services/Verify_3D/]
- Statistical potentials based methods
  - PROSAII [http://www.came.sbg.ac.at/]
  - ANOLEA [http://protein.bio.puc.cl/cardex/servers/index.html]

http://www.salilab.org/bioinformatics_resources.shtml

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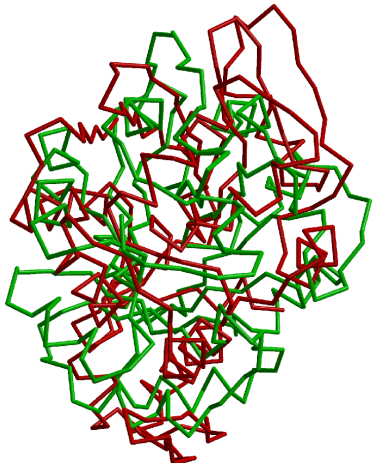
Typical Errors in Comparative Models

MODEL

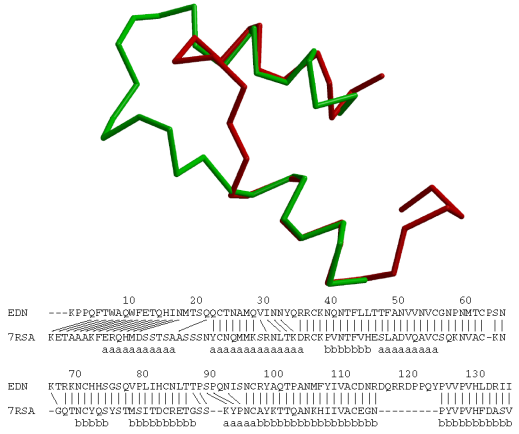
X-RAY

TEMPLATE

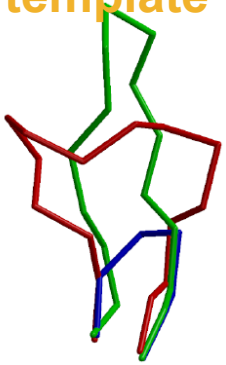
Incorrect template



Misalignment

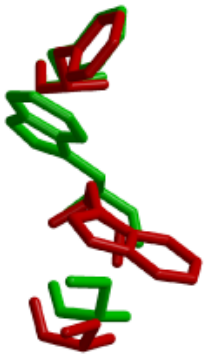


Region without a template

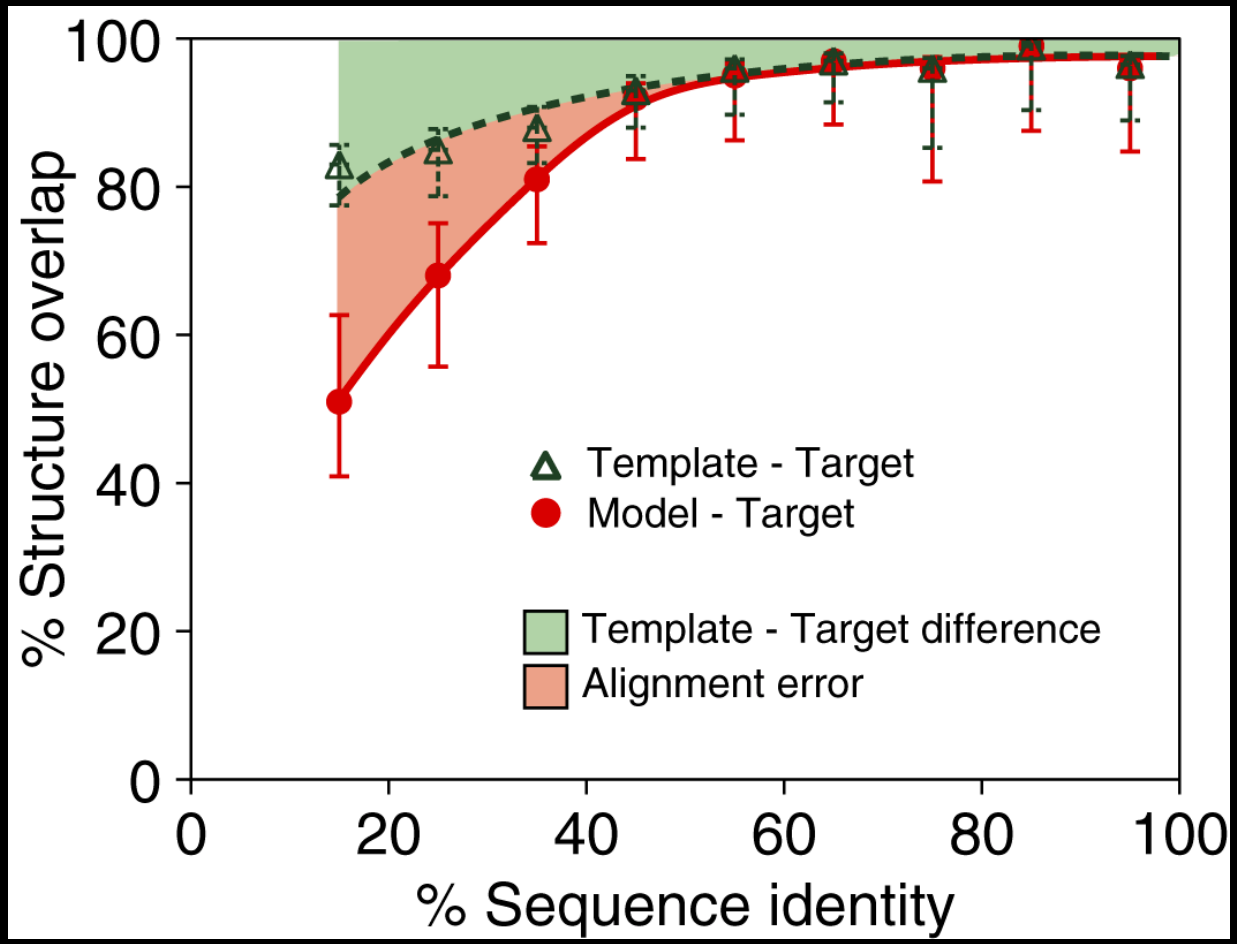


Distortion in correctly aligned regions

Sidechain packing



Model Accuracy as a Function of Target-Template Sequence Identity



Sánchez, R., Šali, A. *Proc Natl Acad Sci U S A.* 95 pp13597-602. (1998).

Model Accuracy

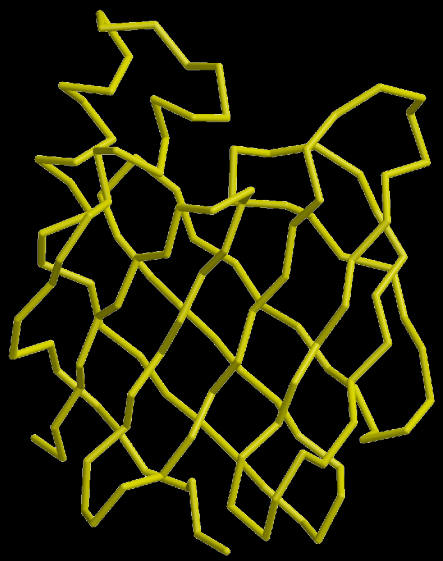
Marti-Renom *et al.* Annu.Rev.Biophys.Biomol.Struct. **29**, 291-325, 2000.

HIGH ACCURACY

NM23
Seq id 77%

MEDIUM ACCURACY

CRABP
Seq id 41%



LOW ACCURACY

EDN
Seq id 33%

X-RAY

Model Accuracy

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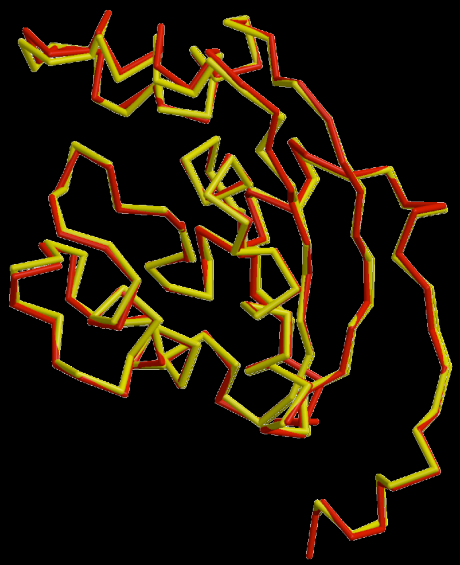
HIGH ACCURACY

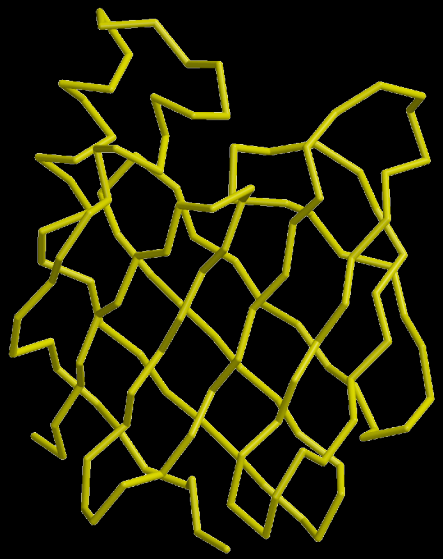
NM23
Seq id 77%
Cα equiv 147/148
RMSD 0.41Å



Sidechains

MEDIUM ACCURACY

CRABP
Seq id 41%



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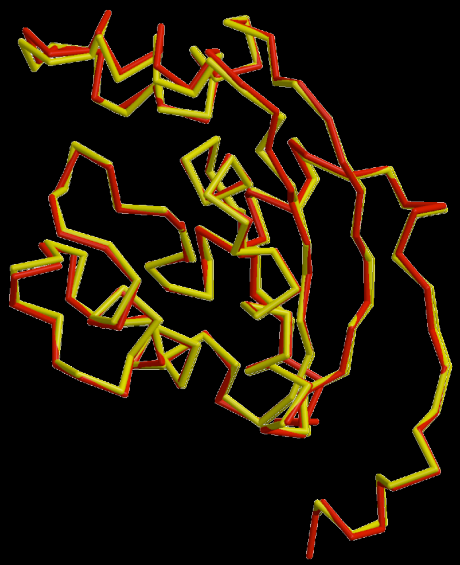

Sidechains

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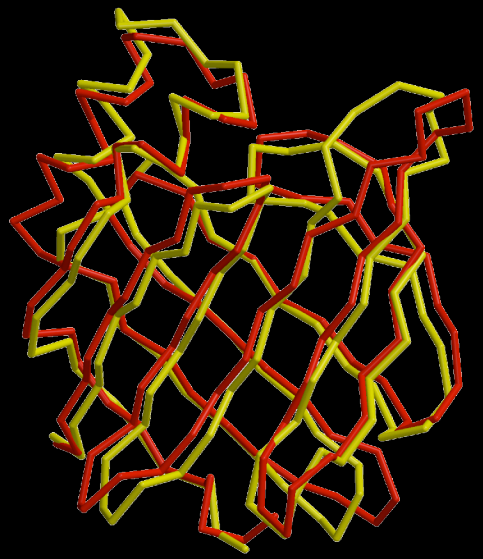

Sidechains
Loops

LOW ACCURACY

EDN
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X-RAY **MODEL**

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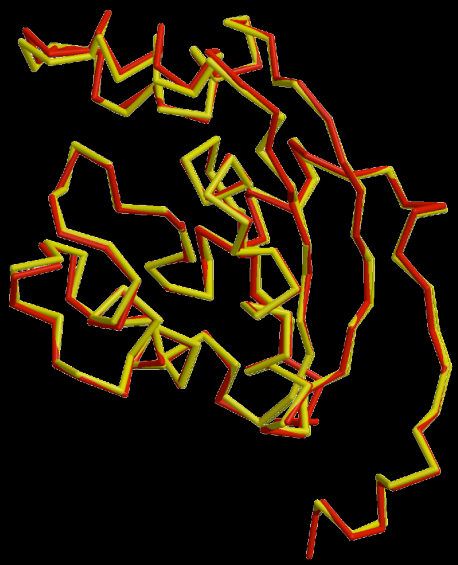

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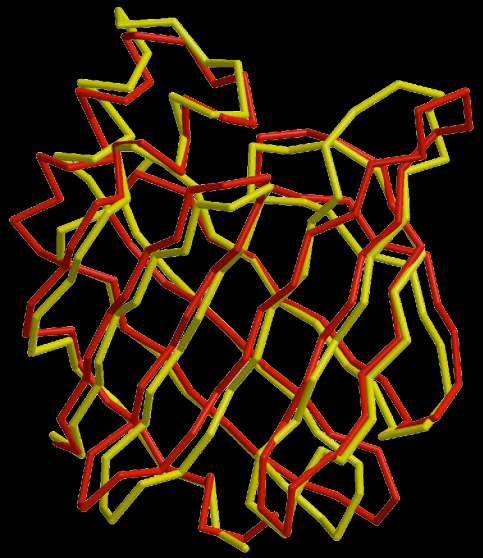

Sidechains
Loops

LOW ACCURACY

EDN
Seq id 33%
Cα equiv 90/134
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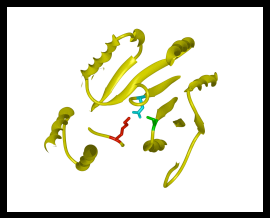
Sidechains
Core backbone
Loops
Alignment

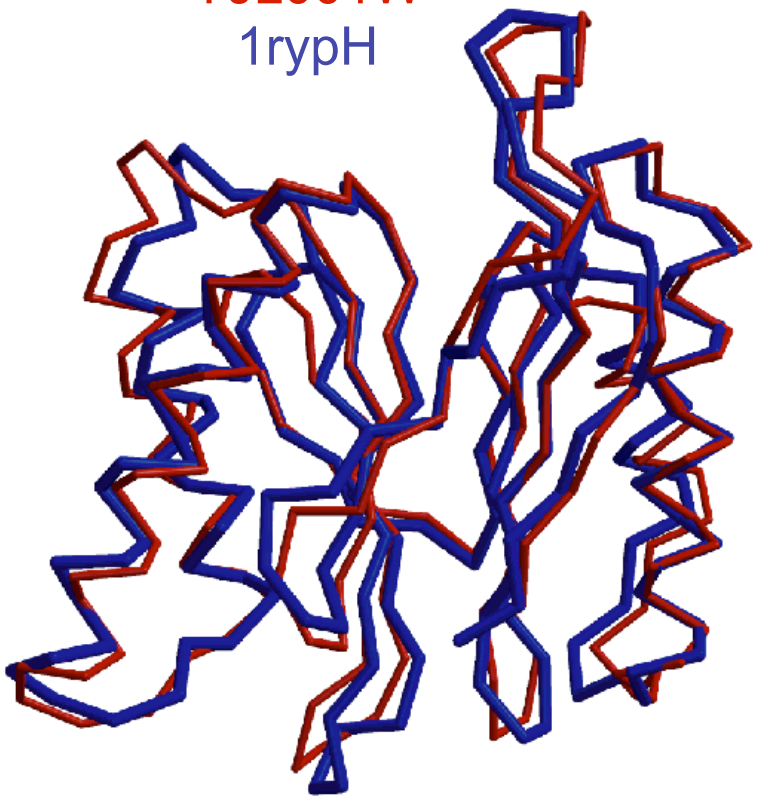
X-RAY **MODEL**

Some Models Can Be Surprisingly Accurate
(in Some Core or Active Site Regions)



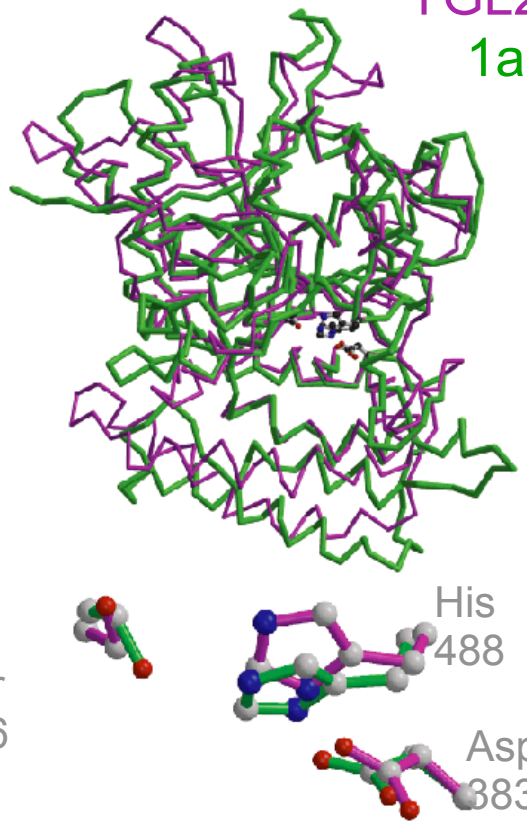
Multicatalytic Proteinase
24% sequence identity

YJL001W
1rypH



Carboxypeptidase
25% sequence identity

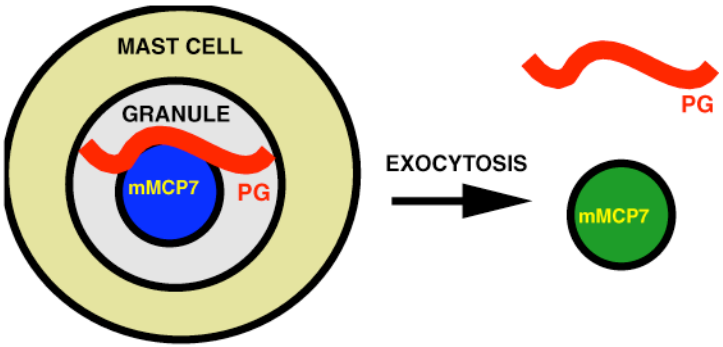
YGL203C
1ac5



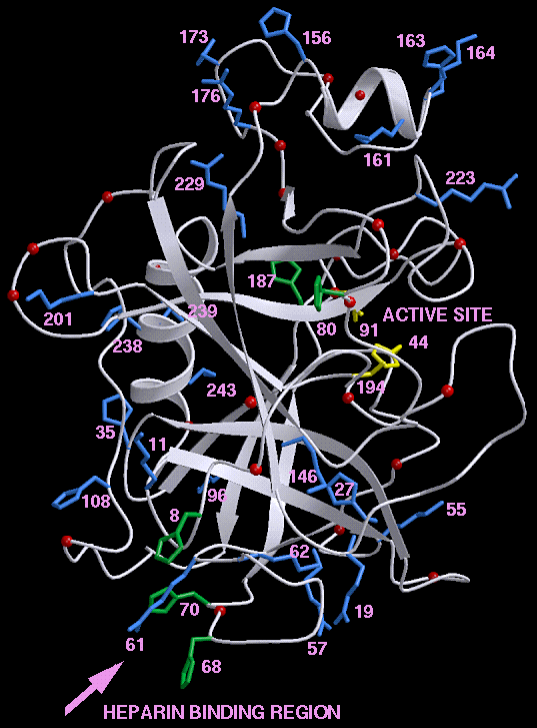
Do mast cell proteases bind proteoglycans? Where? When?

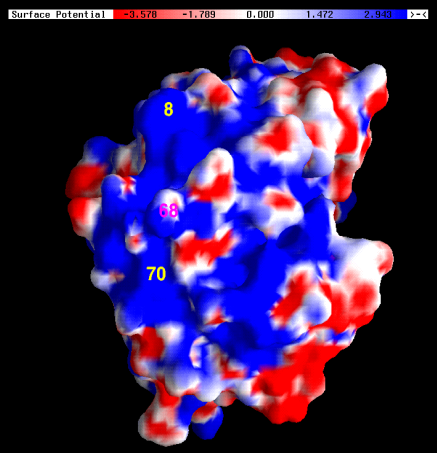
Predicting features of a model that are not present in the template

1. mMCPs bind negatively charged proteoglycans through electrostatic interactions
2. Comparative models used to find clusters of positively charged surface residues.
3. Tested by site-directed mutagenesis.

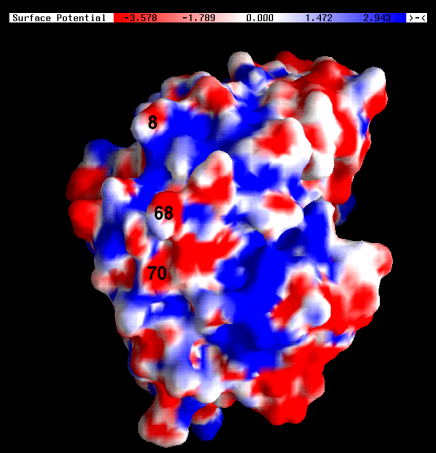


Huang *et al*. *J. Clin. Immunol*. **18**,169,1998.
Matsumoto *et al*. *J.Biol.Chem*. **270**,19524,1995.
Šali *et al*. *J. Biol. Chem*. **268**, 9023, 1993.





Native mMCP-7 at *p*H=5 (His^+)

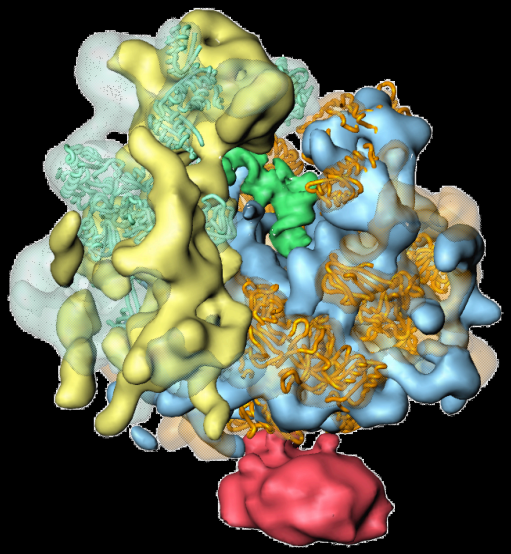


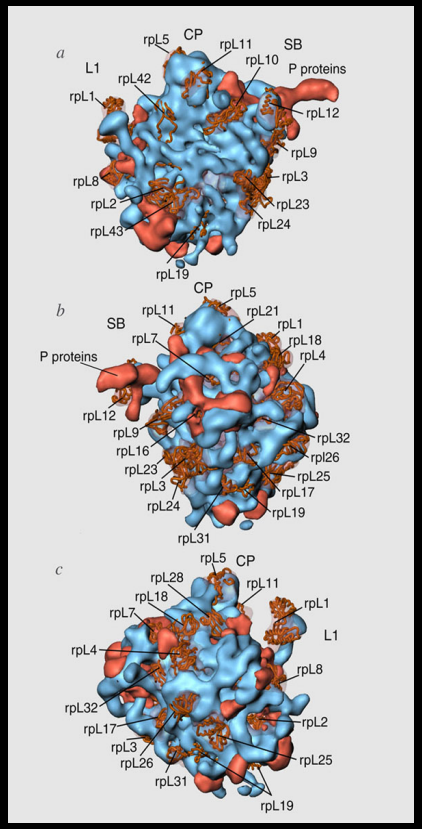
Native mMCP-7 at *p*H=7 (His^0)

BayGenomics

Some Models Can Be Used in Docking to Density Maps

(Yeast Ribosomal 40S subunit)





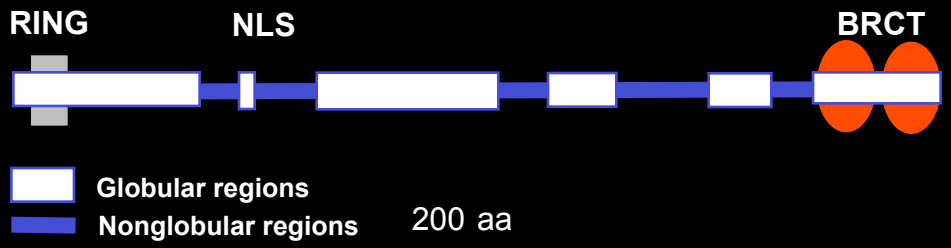
Docking of comparative models into the cryo-EM map.

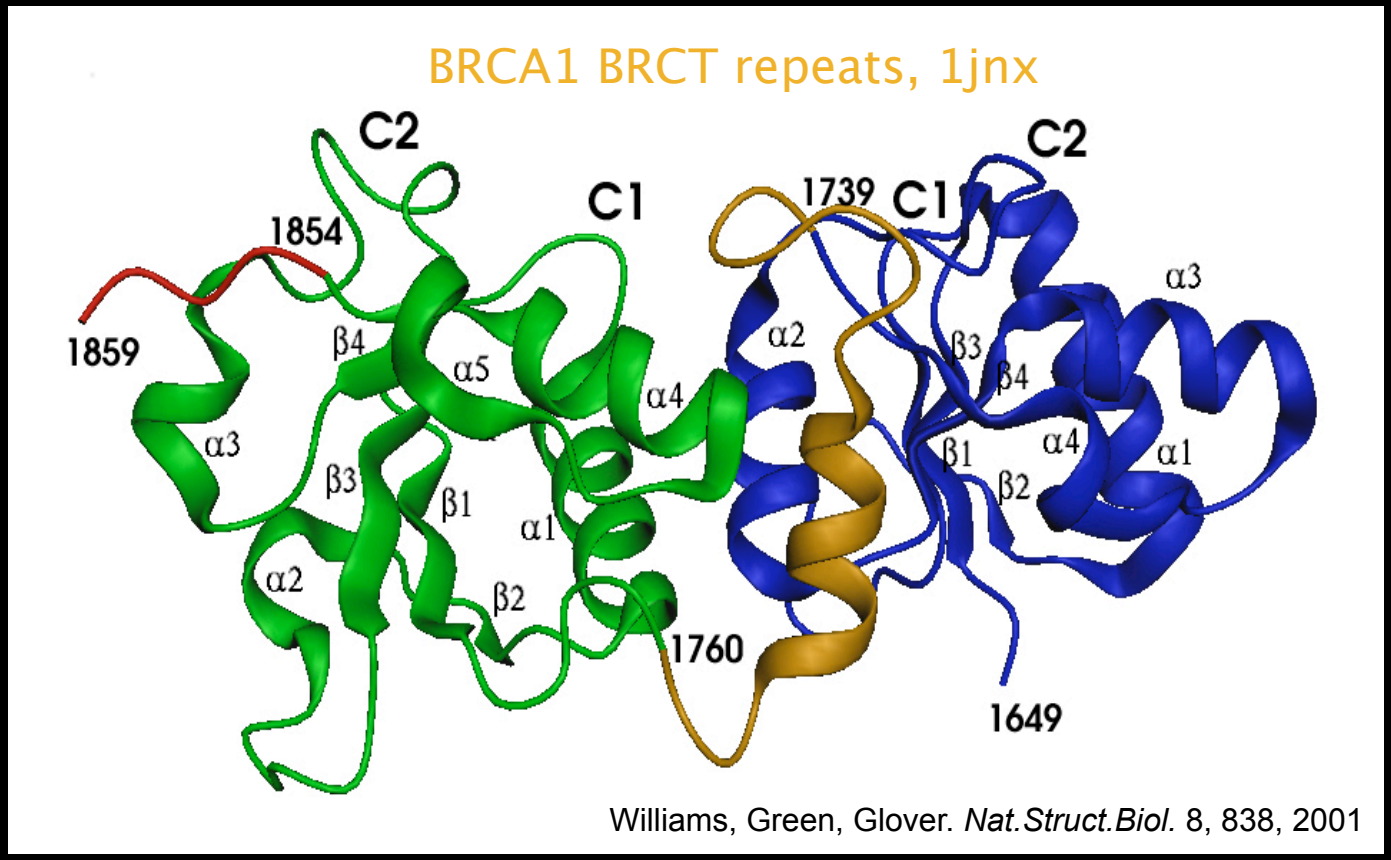
Spahn *et al.* 2001 Cell **107**:373-386

Small 30S subunit from *Thermus thermophilus*
Large 50S subunit from *Haloarcula marismortui*

Human BRCA1 and its two BRCT domains

(structural analysis of missense mutations SNPs)





Williams, Green, Glover. *Nat.Struct.Biol.* 8, 838, 2001

CONFIDENTIAL

MYRIAD

BRACAnalysis™

Comprehensive BRCA1-BRCA2 Gene Sequence Analysis Result

Niecee Singer, MS Strang Cancer Prevention Center 428 E 72nd St New York, NY 10021	SPECIMEN Specimen Type: Blood Draw Date: n/a Accession Date: Oct 27, 2000 Report Date: Nov 17, 2000	PATIENT Name: Date of Birth: Feb 02, 1953 Patient ID: Gender: Female Accession #: 00019998 Requisition #: 56694

Physician: Fred Gilbert, MD

Test Result

Gene Analyzed	Specific Genetic Variant
BRCA2	H2116R
BRCA1	None Detected

Interpretation

GENETIC VARIANT OF UNCERTAIN SIGNIFICANCE

The BRCA2 variant H2116R results in the substitution of arginine for histidine at amino acid position 2116 of the BRCA2 protein. Variants of this type may or may not affect BRCA2 protein function. Therefore, the contribution of this variant to the relative risk of breast or ovarian cancer cannot be established solely from this analysis. The observation by Myriad Genetic Laboratories of this particular variant in an individual with a deleterious truncating mutation in BRCA2, however, reduces the likelihood that H2116R is itself deleterious.

Authorized Signature:

Brian E. Ward, Ph.D.
Laboratory Director

Thomas S. Frank, M.D.
Medical Director

These test results should only be used in conjunction with the patient's clinical history and any previous analysis of appropriate family members. It is strongly recommended that these results be communicated to the patient in a setting that includes appropriate counseling. The accompanying Technical Specifications summary describes the analysis, method, performance characteristics, nomenclature, and interpretive criteria of this test. This test may be considered investigational by some states. This test was developed and its performance characteristics determined by Myriad Genetic Laboratories. It has not been reviewed by the U.S. Food and Drug Administration. The FDA has determined that such clearance or approval is not necessary.

CONFIDENTIAL

MYRIAD

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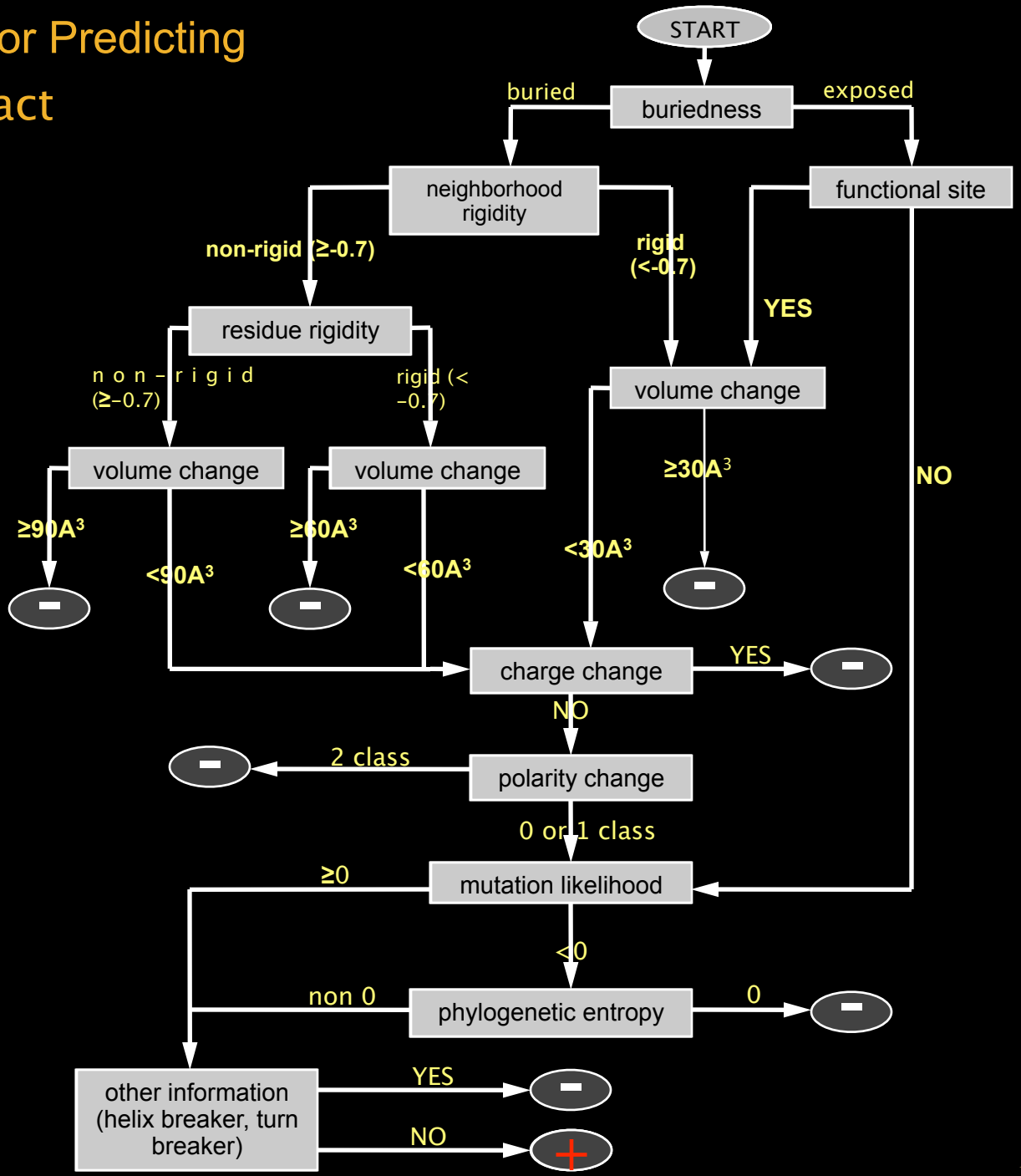
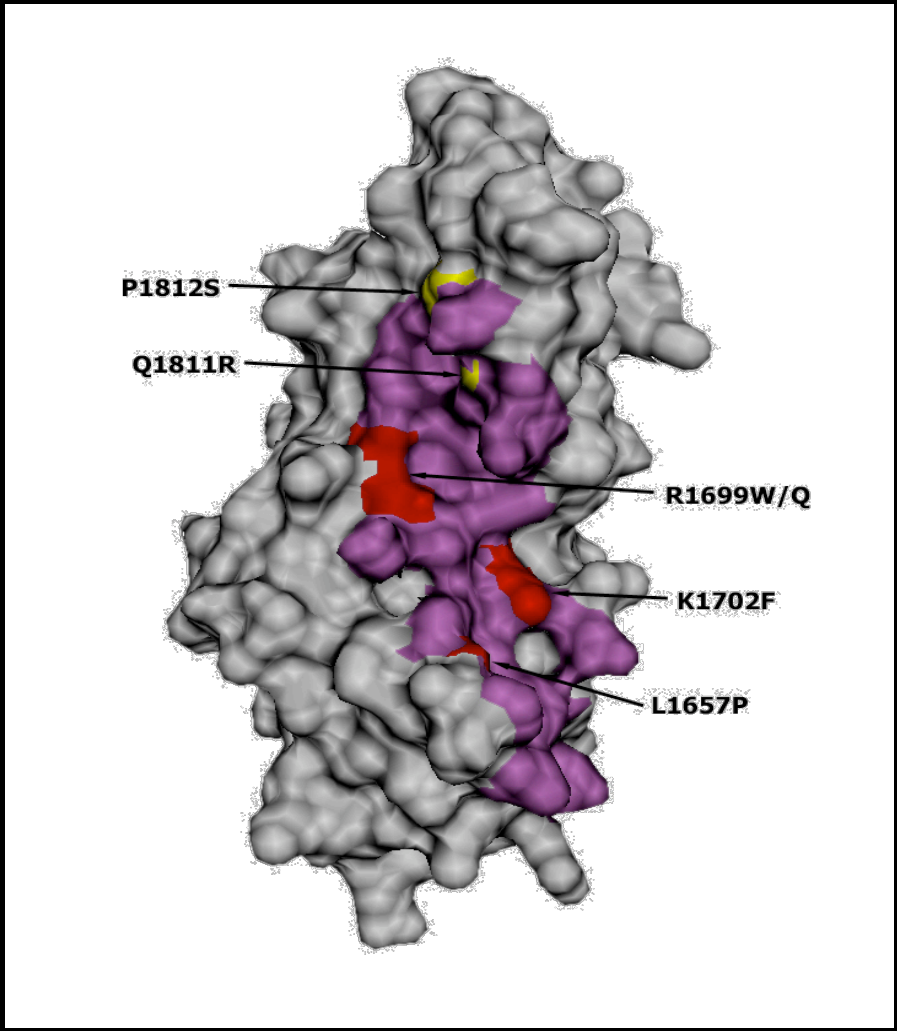
Missense Mutations in BRCT Domains by Function

	cancer associated	not cancer associated	?
no transcription activation	C1697R R1699W A1708E S1715R P1749R M1775R		M1652K L1657P E1660G H1686Q R1699Q K1702E Y1703H F1704S L1705PS 1715NS 1722FF 1734LG 1738EG 1743RA 1752PF 1761I F1761S M1775E M1775K L1780P I1807S V1833E A1843T
transcription activation		M1652I A1669S	V1665M D1692N G1706A D1733G M1775V P1806A
?			M1652T V1653M L1664P T1685A T1685I M1689R D1692Y F1695L V1696L R1699L G1706E W1718C W1718S T1720A W1730S F1734S E1735K V1736A G1738R D1739E D1739G D1739Y V1741G H1746N R1751P R1751Q R1758G L1764P I1766S P1771L T1773S P1776S D1778N D1778G D1778H M1783T C1787S G1788 D G1788V G1803A V1804D V1808A V1809A V1809F V1810G Q1811R P1812S N1819S A1823T V1833M W1837R W1837G S1841N A1843P T1852S P1856T P1859R

"Decision" Tree for Predicting Functional Impact of Genetic Variants

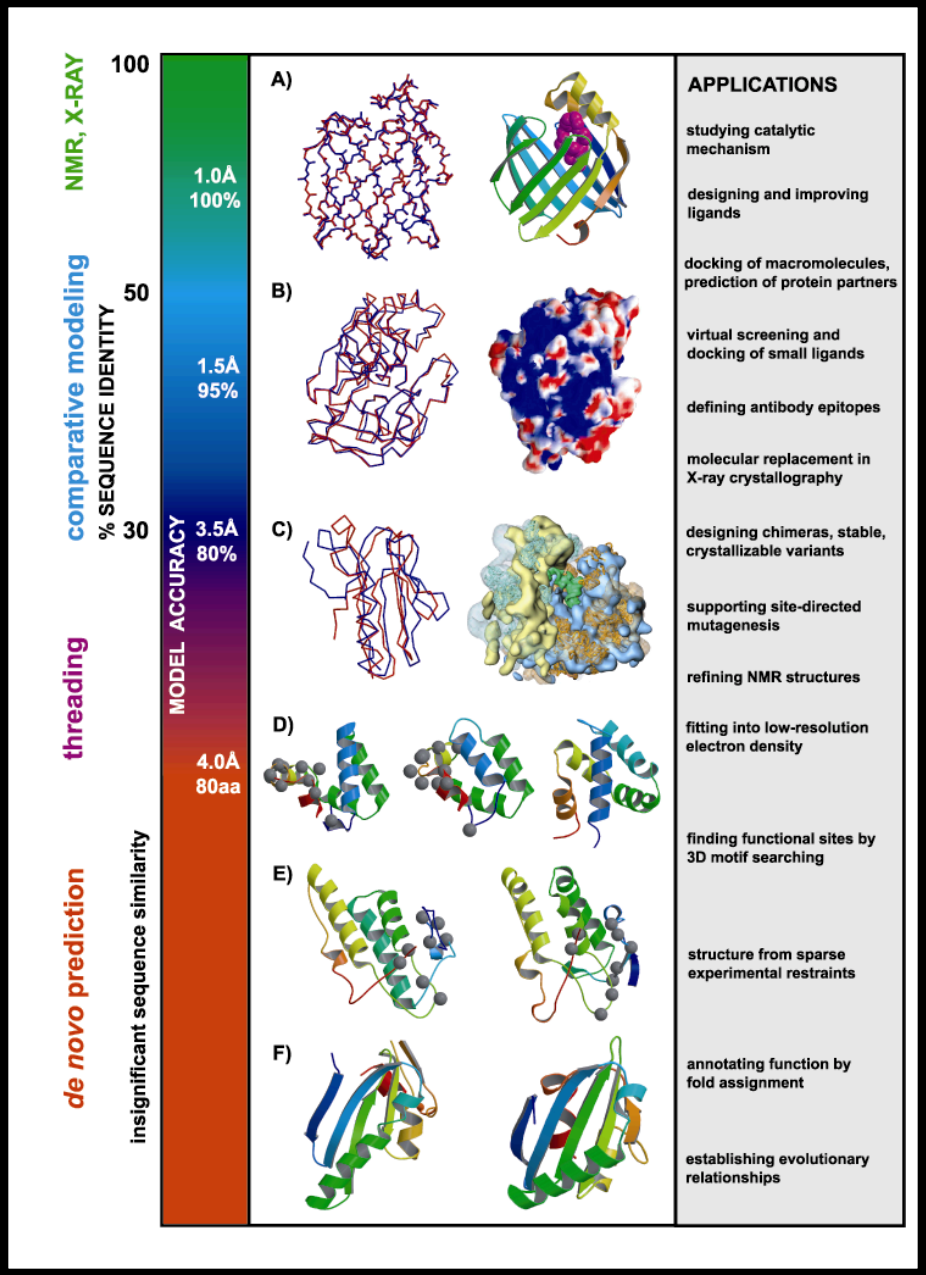


Putative Binding Site on BRCA1



RMSMVVSGLTPEEFMLVYKFARKHHITLTNLITEETTHVVMKTDAEFVCERTLKYFLGIAGGKWVVSYFWVTQSIKERK
MLNEHDFEVRGDVVNGRNHQGPKRARESQDRKIFRGLEICCYGPFTNMPTDQLEWMVQLCGASVVKELSSFTLGTGVHP
IVVVQPDAWTEDNGFHAIGQMCEAPVVTREWVLDSVALYQCQELDTYLIPQIP

Applications of Protein Structure Models



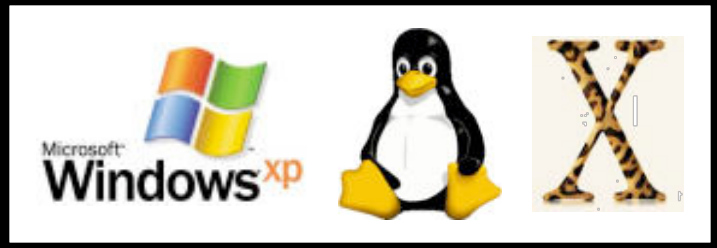
Baker & Sali
Science **294**,
93-96, 2001

Summary

- Protein Structure Prediction and why is it useful?
- Methods in Protein Structure Prediction
- Comparative Modeling
  - ✓ Steps in CM (overview + resources)
  - ✓ Accuracy/Applications of comparative models
  - ✓ Case example in MODELLER (*)
  - ✓ CM and Structural Genomics

Obtaining MODELLER and related information

- MODELLER (6v2) web page
- `http://www.salilab.org/modeller/`
  - Download Software (Linux/Windows/Mac)
  - HTML Manual
  - Join Mailing List



Using MODELLER

- No GUI! ☹
- Controlled by command file (script) ☹☹
- Script is written in TOP language ☹☹☹
- TOP language is simple ☺☺☺☺

Using MODELLER

- INPUT:
  - Target Sequence (FASTA/PIR format)
  - Template Structure (PDB format)
  - TOP command file
- OUTPUT:
  - Target-Template Alignment
  - Model in PDB format
  - Other data

Example 1: Modeling of BLBP

Input

- ✓ Target: Brain lipid-binding protein (BLBP)
- ✓ BLBP sequence in PIR (MODELLER) format:

```
>P1;blbp
sequence:blbp:::::::
VDAFCATWKLTDSQNFDEYMKALGVGFATRQVGNVTKPTVIISQEGGKVVIRTQCTFKNTEINFQLGEEFEETSI
DDRNCKSVVRLDGDKLIHVQKWDGKETNCTREIKDGKMVVTLTFGDIVAVRCYEKA*
```

- PSI-BLAST template search: Template: PDB file 1HMS:_

Example 1: Modeling of BLBP

STEP 1: Align blbp and 1hms sequences

TOP script for target-template alignment

```
READ_MODEL FILE = '1hms.pdb'
SEQUENCE_TO_ALI ALIGN_CODES = '1hms'
READ_ALIGNMENT FILE = 'blbp.seq', ALIGN_CODES = 'blbp', ADD_SEQUENCE = on
ALIGN
WRITE_ALIGNMENT FILE='blbp-1hms.ali', ALIGNMENT_FORMAT = 'PIR'
WRITE_ALIGNMENT FILE='blbp-1hms.pap', ALIGNMENT_FORMAT = 'PAP'
```

Run by typing `mod align.top` directory where you have the TOP file.
MODELLER will produce a `align.log` file

Example 1: Modeling of BLBP

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```

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Example 1: Modeling of BLBP

STEP 1: Align blbp and 1hms sequences

Output

```
>P1;1hms
structureX:1hms:    1 : : 131 : :undefined:undefined:-1.00:-1.00
VDAFLGTWKLVDSKNFDDYMKSLGVGFATRQVASMTKPTTIIEKNGDILTLKTHSTFKNTEISFKLGVEFDETTA
DDRKVKSIVTLDGGKLVHLQKWDGQETTLVRELIDGKLILTLTHGTAVCTRTYEKE*
>P1;blbp
sequence:blbp:      : :      : : : : 0.00: 0.00
VDAFCATWKLTDSQNFDEYMKALGVGFATRQVGNVTKPTVIISQEGGKVVIRTQCTFKNTEINFQLGEEFEETSI
DDRNCKSVVRLDGDKLIHVQKWDGKETNCTREIKDGKMVVTLTFGDIVAVRCYEKA*
```

Example 1: Modeling of BLBP

STEP 1: Align blbp and 1hms sequences

Output

```
>P1;1hms
structureX:1hms:    1 : : 131 : :undefined:undefined:-1.00:-1.00
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DDRKVKSIVTLDGGKLVHLQKWDGQETTLVRELIDGKLILTLTHGTAVCTRTYEKE*
>P1;blbp
sequence:blbp:      : :      : : : : 0.00: 0.00
VDAFCATWKLTDSQNFDEYMKALGVGFATRQVGNVTKPTVIISQEGGKVVIRTQCTFKNTEINFQLGEEFEETSI
DDRNCKSVVRLDGDKLIHVQKWDGKETNCTREIKDGKMVVTLTFGDIVAVRCYEKA*
```

Example 1: Modeling of BLBP

STEP 1: Align blbp and 1hms sequences

Output

```
 _aln.pos         10        20        30        40        50        60
1hms       VDAFLGTWKLVDSKNFDDYMKSLGVGFATRQVASMTKPTTIIEKNGDILTLKTHSTFKNT
blbp       VDAFCATWKLTDSQNFDEYMKALGVGFATRQVGNVTKPTVIISQEGGKVVIRTQCTFKNT
 _consrvd ****  **** ** *** *** **********   **** **   *      *  *****

 _aln.pos         70        80        90       100       110       120
1hms       EISFKLGVEFDETTADDRKVKSIVTLDGGKLVHLQKWDGQETTLVRELIDGKLILTLTHG
blbp       EINFQLGEEFEETSIDDRNCKSVVRLDGDKLIHVQKWDGKETNCTREIKDGKMVVTLTFG
 _consrvd ** * ** ** **  ***  ** * *** ** * ***** **   **  ***   *** *

 _aln.pos        130
1hms       TAVCTRTYEKE
blbp       DIVAVRCYEKA
 _consrvd   *  * ***
```

Example 1: Modeling of BLBP

STEP 2: Model the blbp structure using the alignment from step 1.

TOP script for model building

```
INCLUDE
SET ALNFILE = 'blbp-1hms.ali'
SET KNOWNS = '1hms'
SET SEQUENCE = 'blbp'
SET STARTING_MODEL = 1
SET ENDING_MODEL = 1
CALL ROUTINE = 'model'
```

Run by typing `mod model.top`.

Check file `model.log`

Example 1: Modeling of BLBP

STEP 2: Model the blbp structure using the alignment from step 1.

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```
INCLUDE
SET ALNFILE = 'blbp-1hms.ali'
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Example 1: Modeling of BLBP

STEP 2: Model the blbp structure using the alignment from step 1.

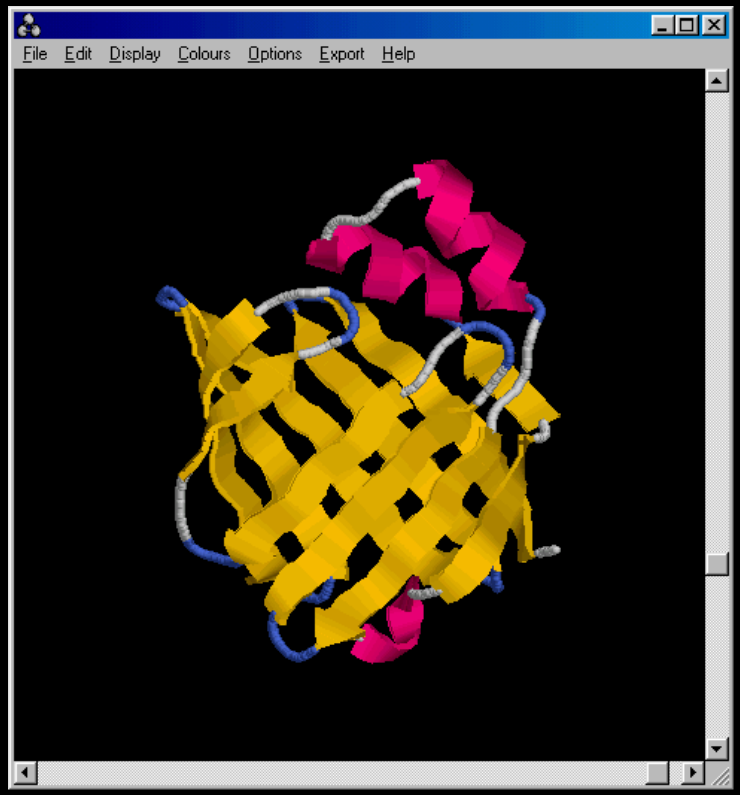
Output coordinates file

Model file → `blbp.B99990001`

- PDB file
- Can be viewed with Chimera [http://www.cgl.ucsf.edu/chimera/]

Rasmol

[http://www.bernstein-plus-sons.com/software/rasmol/]

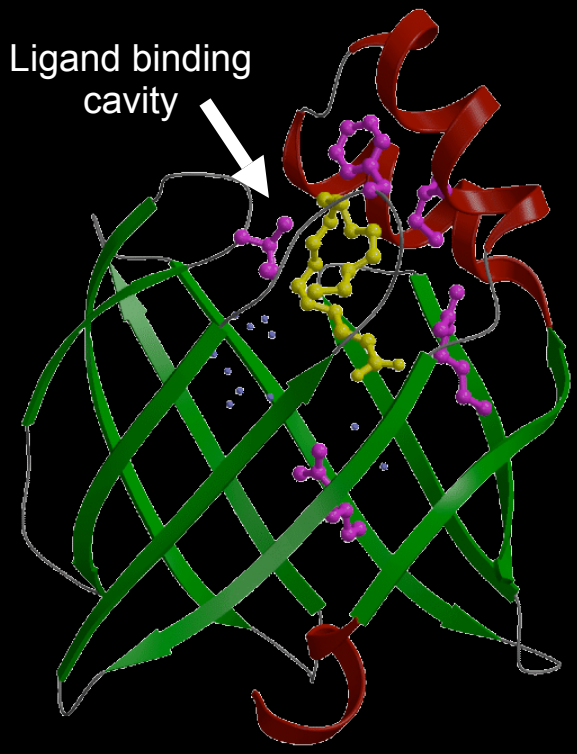


What is the physiological ligand of Brain Lipid-Binding Protein?

Predicting features of a model that are not present in the template

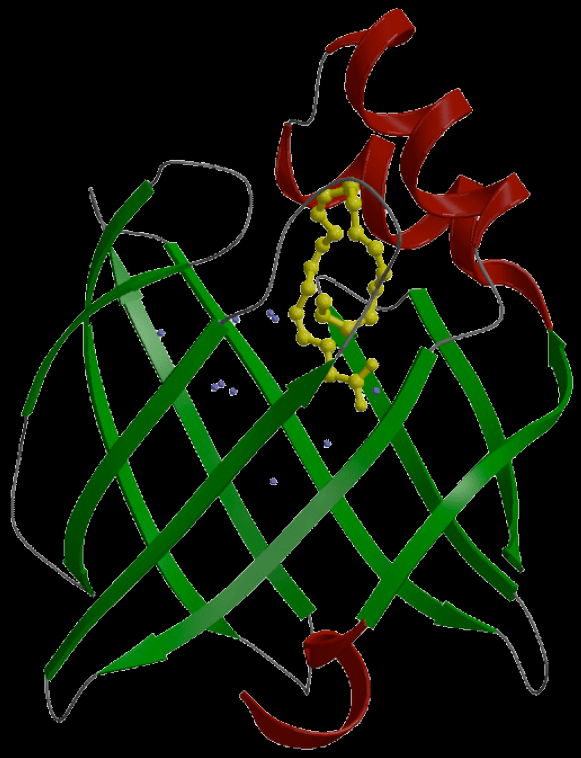
BLBP/oleic acid

*Cavity is **not** filled*



BLBP/Docosahexaenoic acid

*Cavity **is** filled*



1. BLBP binds fatty acids.
2. Build a 3D model.
3. Find the fatty acid that fits most snuggly into the ligand binding cavity.

L. Xu, R. Sánchez, A. Šali, N. Heintz, *J. Biol. Chem.* **271**, 24711, 1996.

Summary

- Protein Structure Prediction and why is it useful?
- Methods in Protein Structure Prediction
- Comparative Modeling
  - ✓ Steps in CM (overview + some details)
  - ✓ Accuracy of comparative models
  - ✓ Case example in MODELLER
  - ✓ CM and Structural Genomics

Structural Genomics

(3D genome project)

- **Definition:**
  - The aim of structural genomics is to put every protein sequence within a modeling distance of a known protein structure.

- **Size of the problem:**
  - There are a few thousand domain fold families.
  - There are ~16,000 sequence families (30% sequence id).

- **Solution:**
  - Determine many protein structures.
  - Increase modeling distance.

Šali. *Nat. Struct. Biol*. **5**, 1029, 1998.
Šali & Kuriyan. *TIBS* **22**, M20, 1999.
Burley *et al*. *Nat. Genet*. **23**, 151, 1999.
Sanchez *et al. Nat. Str. Biol.* 7, 986, 2000

Structural Genomics

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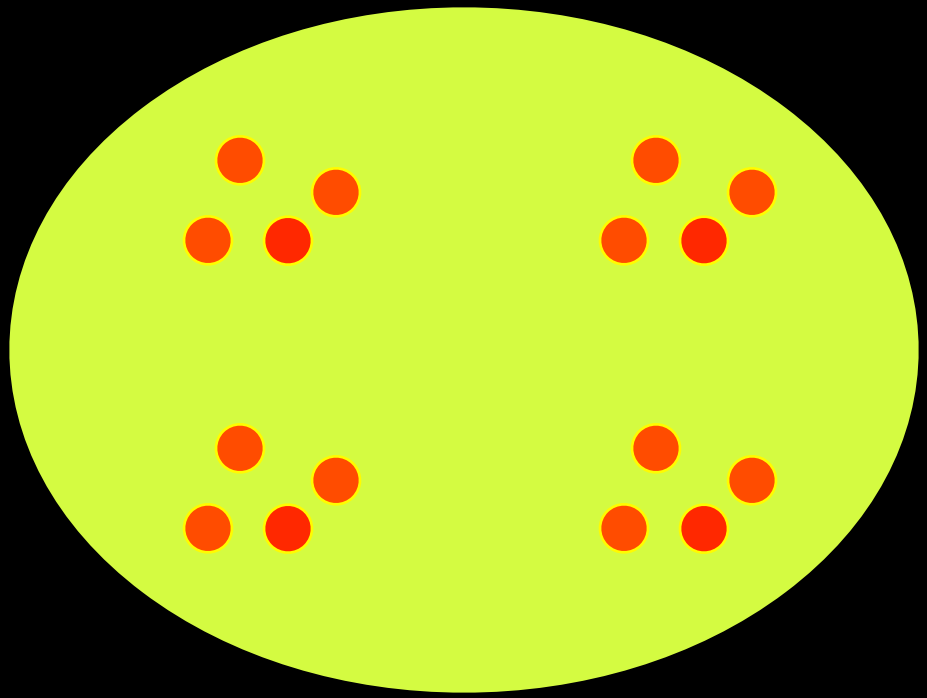
Baker & Sali. *Science* **294,** 93, 2001.

Characterize most protein sequences based on related known structures.



Structural Genomics

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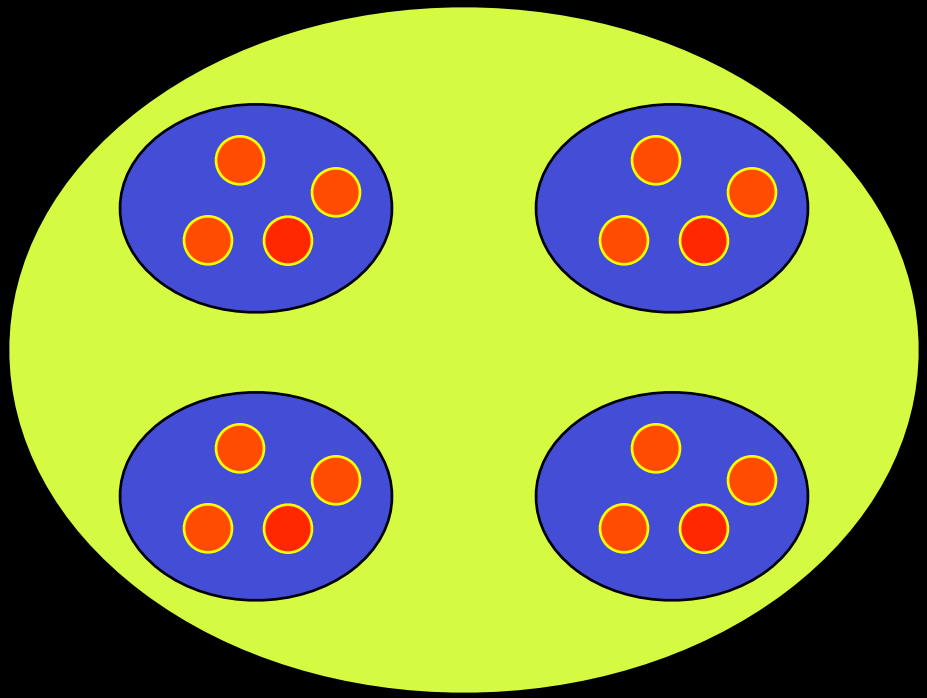

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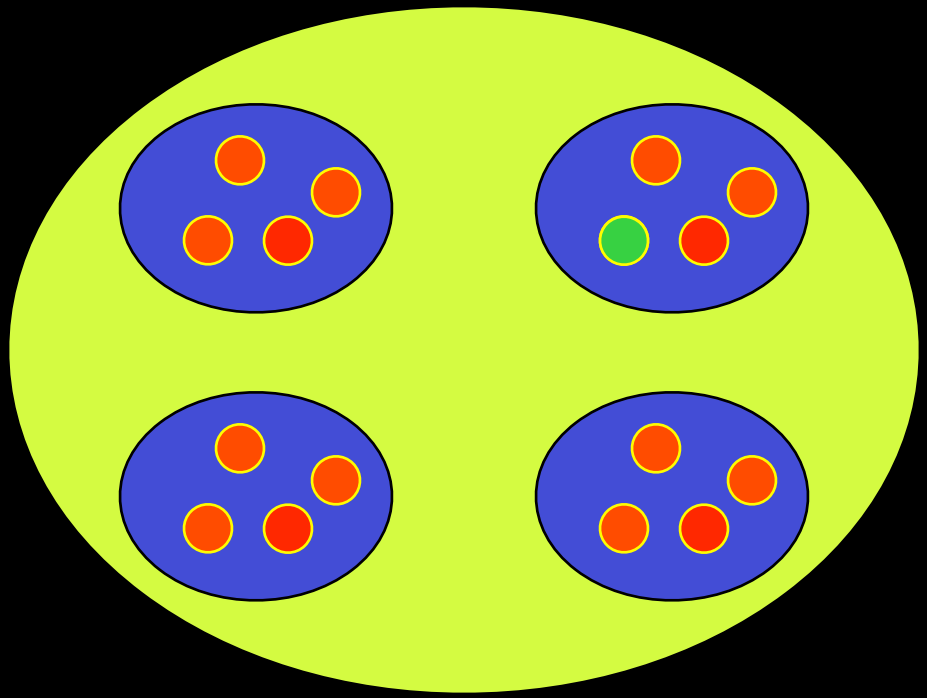

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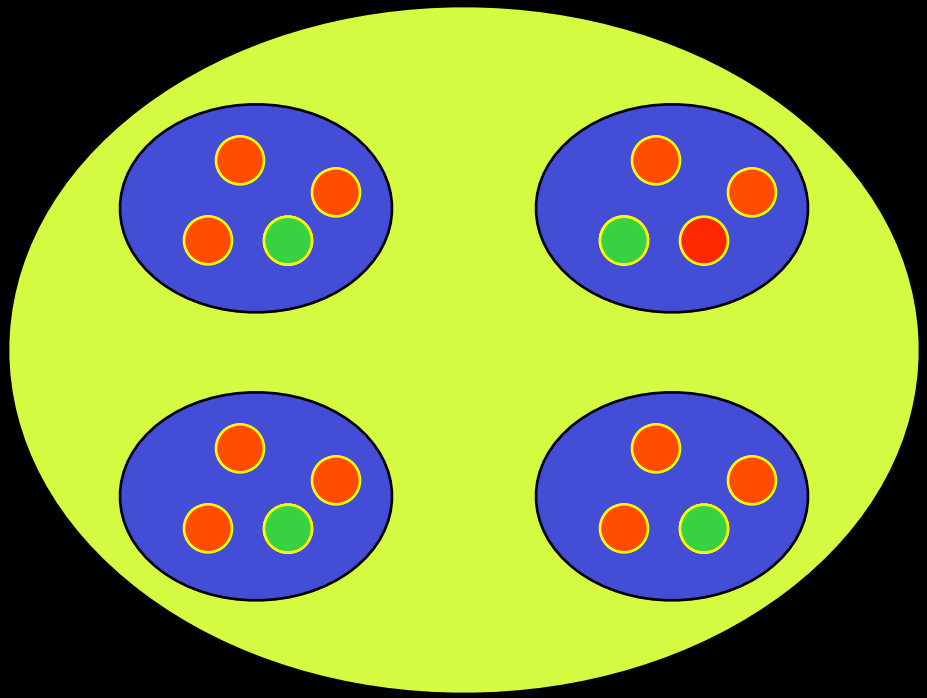

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Baker & Sali. *Science* **294,** 93, 2001.

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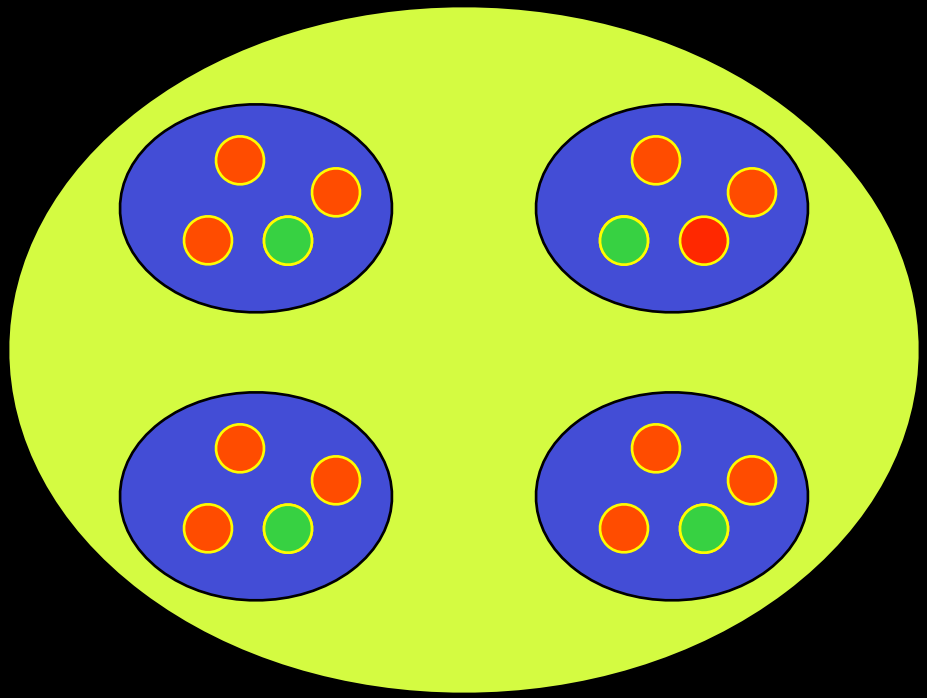

The number of "families" is much smaller than the number of proteins.

Any one of the members of a family is fine.

Structural Genomics

Sali. *Nat. Struct. Biol.* **5**, 1029, 1998.
Sali *et al. Nat. Struct. Biol.*, **7**, 986, 2000.
Sali. *Nat. Struct. Biol.* **7**, 484, 2001.
Baker & Sali. *Science* **294,** 93, 2001.

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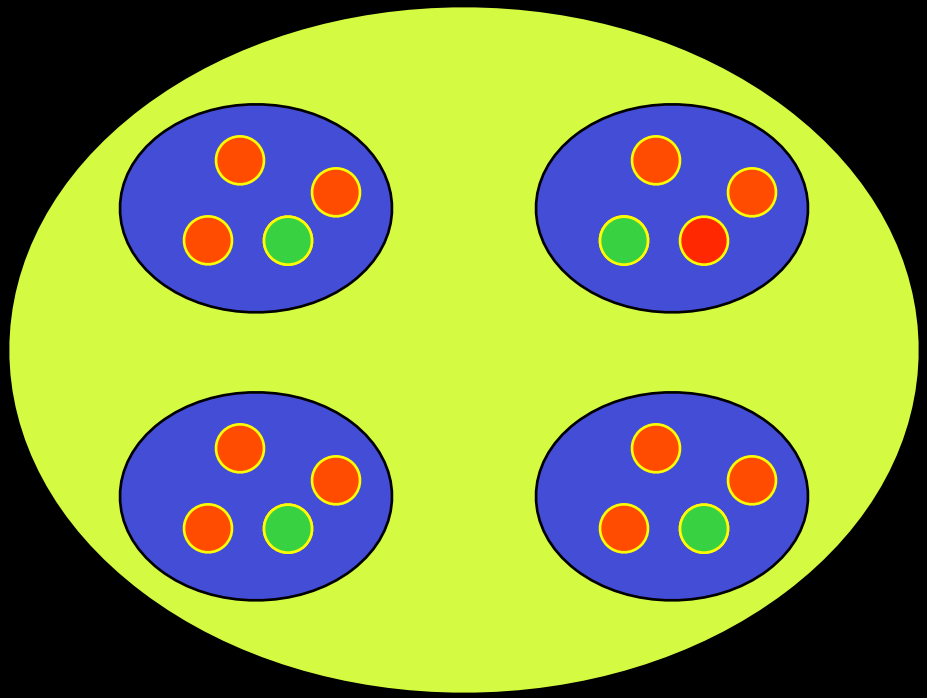


The number of "families" is much smaller than the number of proteins.

Any one of the members of a family is fine.

There are ~16,000 30% seq id families (90%)
(Vitkup *et al. Nat. Struct. Biol.* **8**, 559, 2001)

How can Comparative Modeling be used in Structural Genomics?

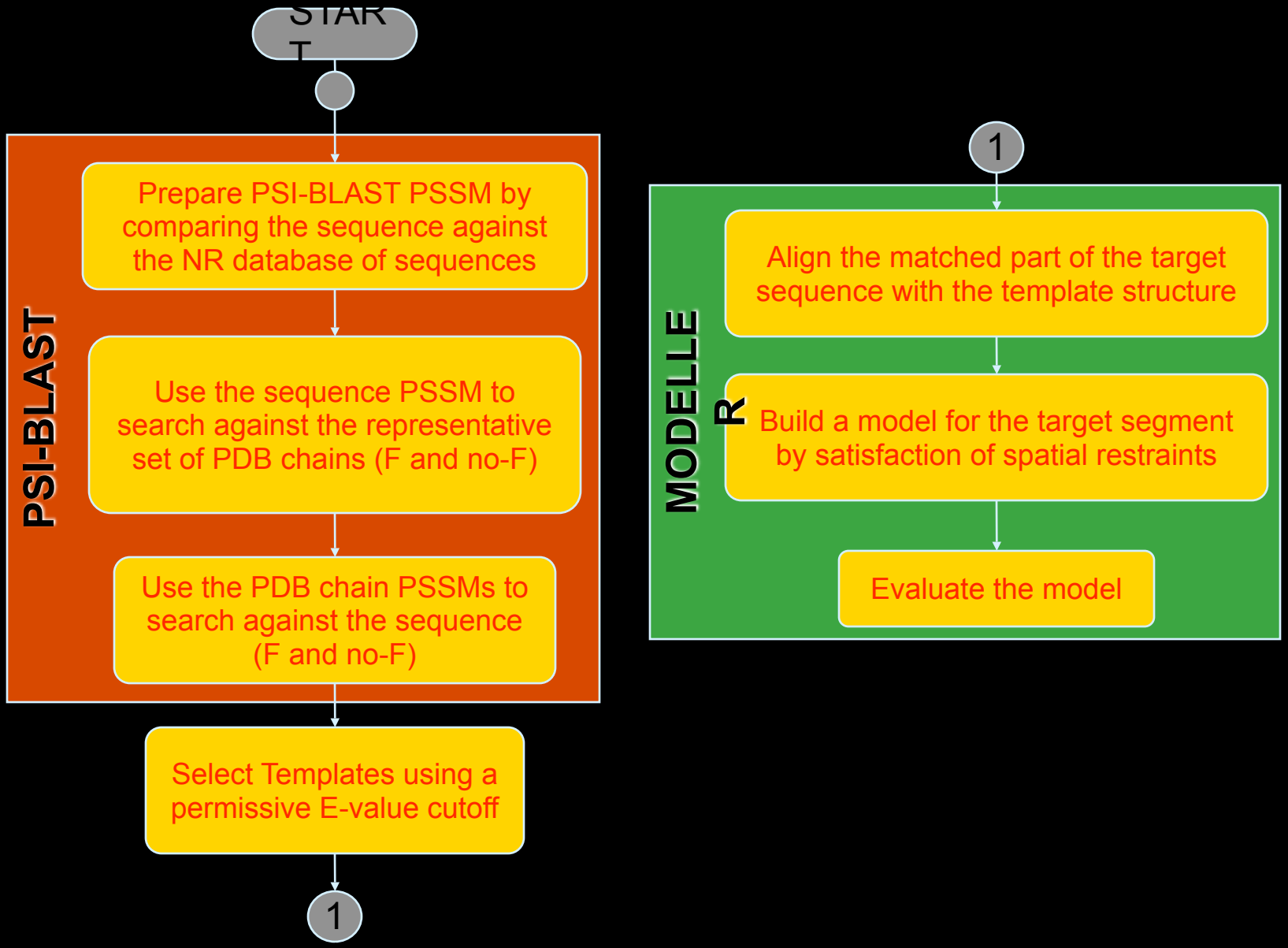
- Target Selection

  How many structures need to be solved?
  Which structures should we solve first?

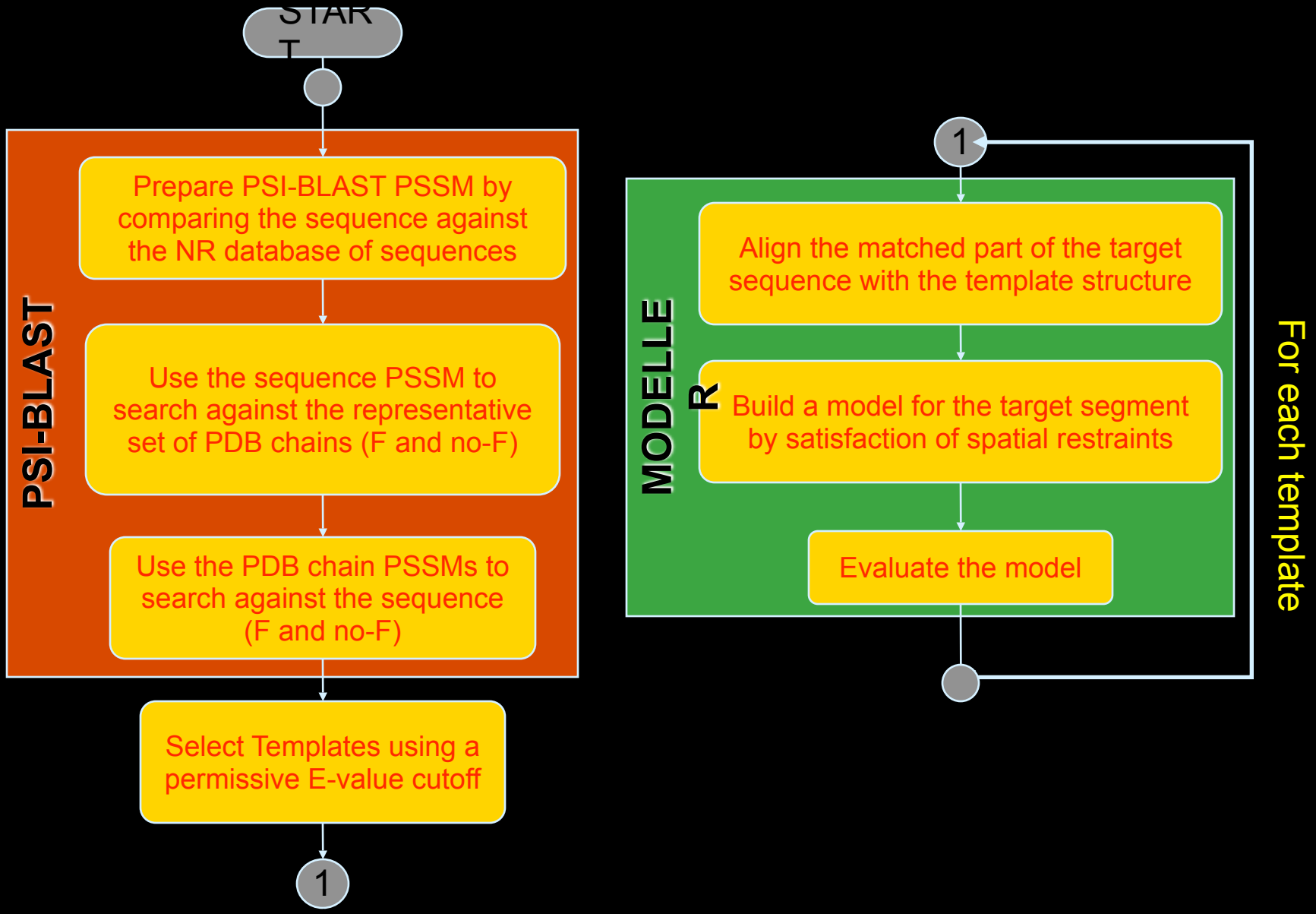
- Target Amplification

  How much of the sequence space is covered by:
  a new structure
  all structures

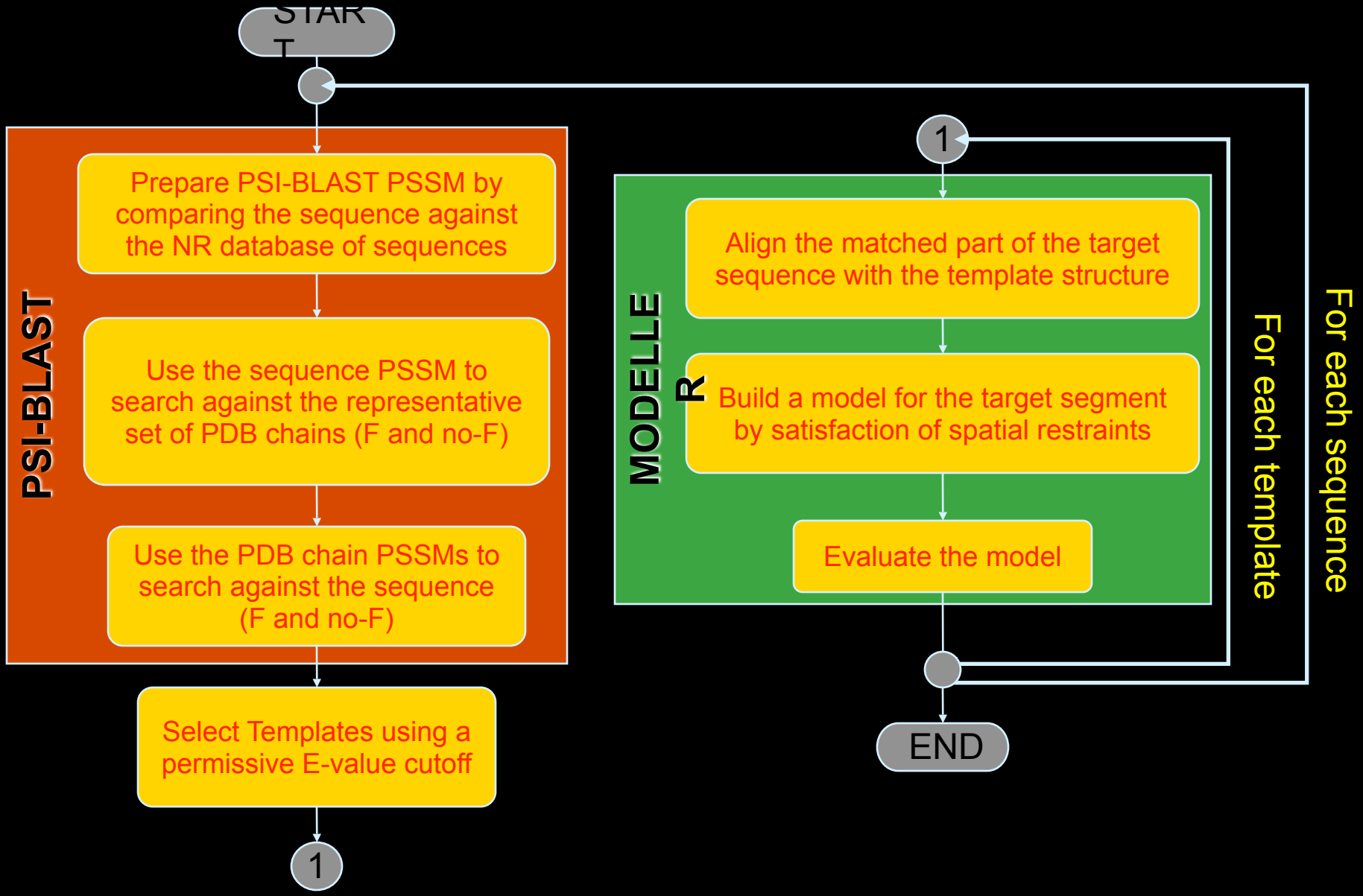
MODPIPE: Large-Scale Comparative Protein Structure Modeling



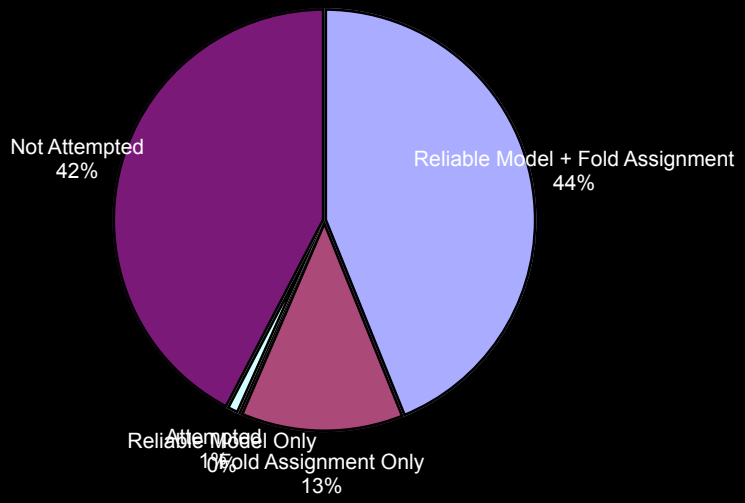
MODPIPE: Large-Scale Comparative Protein Structure Modeling

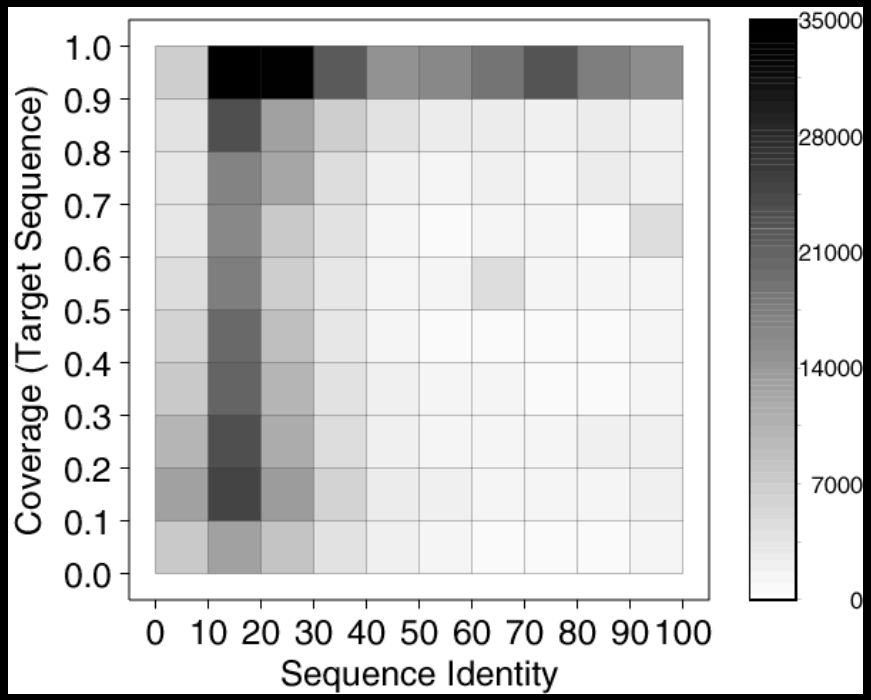


MODPIPE: Large-Scale Comparative Protein Structure Modeling



Modeling Coverage Of The Sequence Space





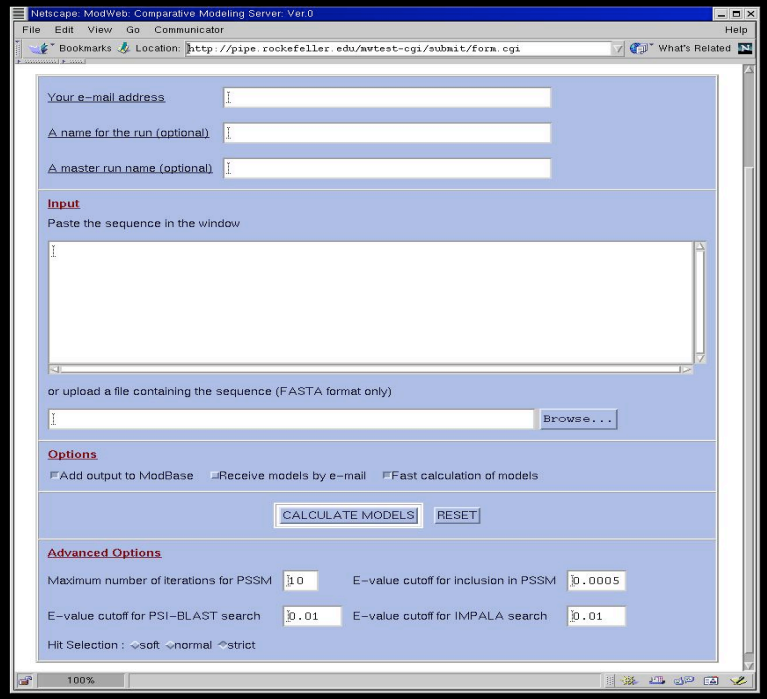
Fold assignment: PSI-BLAST E-value $\leq 1e^{-4}$
Reliable Model: Model Score ≥ 0.7

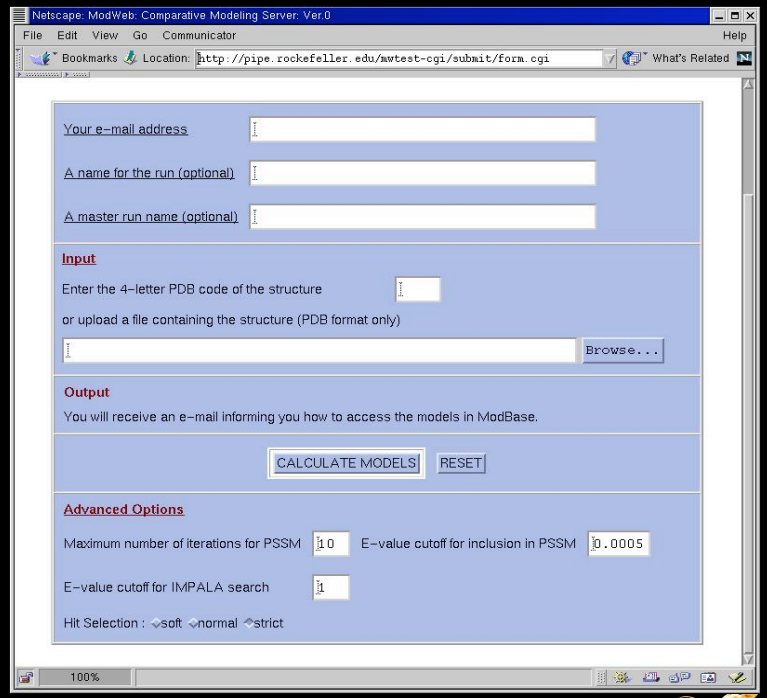
TWO WEB SITES…

ModWeb

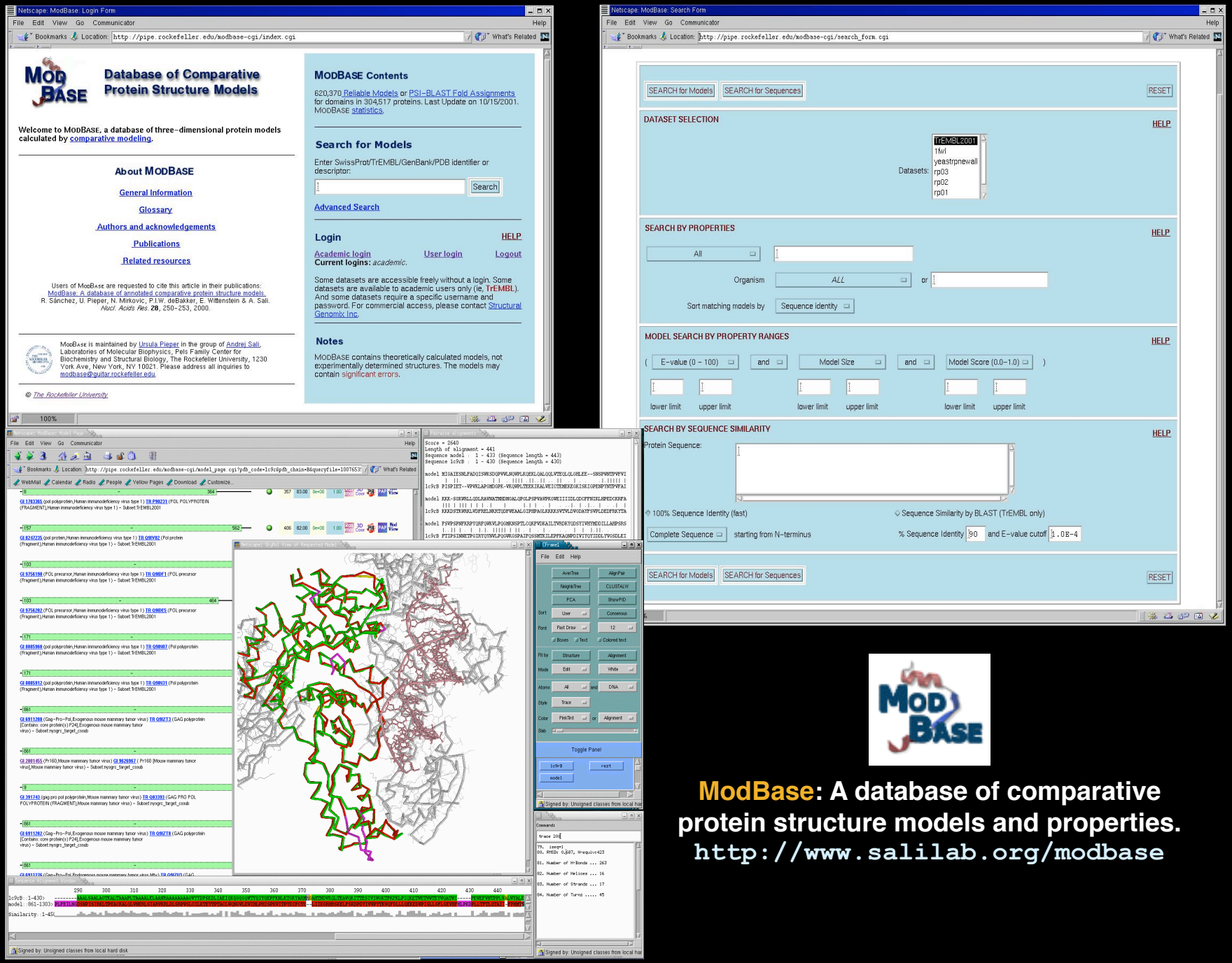
A Server for Comparative Protein Structure Modeling

http://www.salilab.org/modweb





BayGenomics



ModBase: A database of comparative protein structure models and properties.

`http://www.salilab.org/modbase`

Conclusions

- ✓ Comparative models help to understand protein's function:
  - ✓ Detecting remote structural (functional?) relationships.
  - ✓ Revealing features that are not present in the templates.
  - ✓ Revealing features that are not recognizable from the sequence.

- ✓ Currently, useful 3D models can be obtained for domains in approximately 50% of the proteins (30% of domains), because of the improved **methods** and because of the many **known protein structures** and **sequences**.

- ✓ We will be able to calculate useful models for most globular domains soon after the completion of the genome projects, because of **structural genomics**.

Acknowledgments

Andrej Sali

Frank Alber
Fred Davis
Damien Devos
Narayanan Eswar
Rachel Karchin
Libusha Kelly
Michael F. Kim
Dmitry Korkin
M. S. Madhusudhan
Nebosja Mirkovic
Ursula Pieper
Andrea Rossi
Min-yi Shen
Maya Topf
Ben Webb

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