Comparative Modeling

Methods and Applications

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The Rockefeller University

Summary

- ✓ What is comparative modeling and why is it useful?
- ✓ Steps in CM (overview + some details)
- ✓ Accuracy of comparative models
- ✓ Target-Template alignment
- ✓ Loop modeling
- ✓ CM and Structural Genomics

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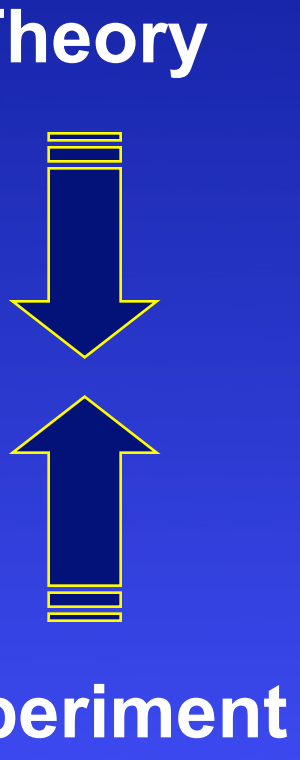
Why protein structure prediction?

	Y 2001	Y 2005
Sequences	700,000	millions
Structures	16,000	50,000

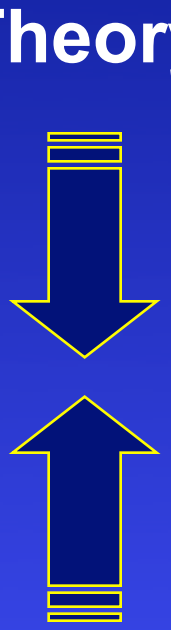
Why protein structure prediction?

	Y 2001
Sequences	700,000
Structures	15,000



Why protein structure prediction?

	Y 2001
Sequences	700,000
Structures	300,000



http://pipe.rockefeller.edu/modbase/

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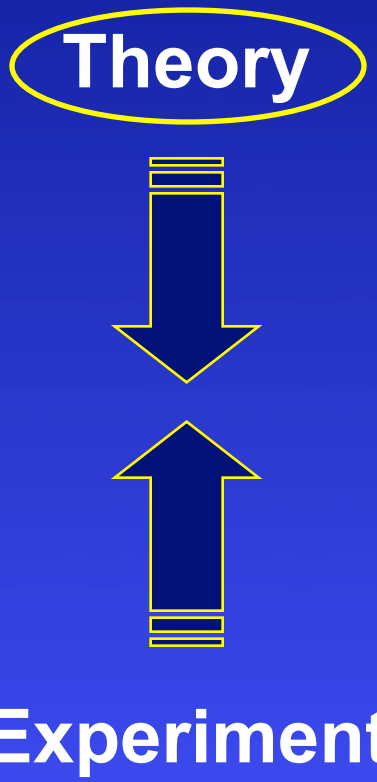
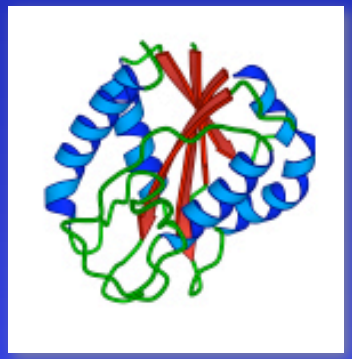


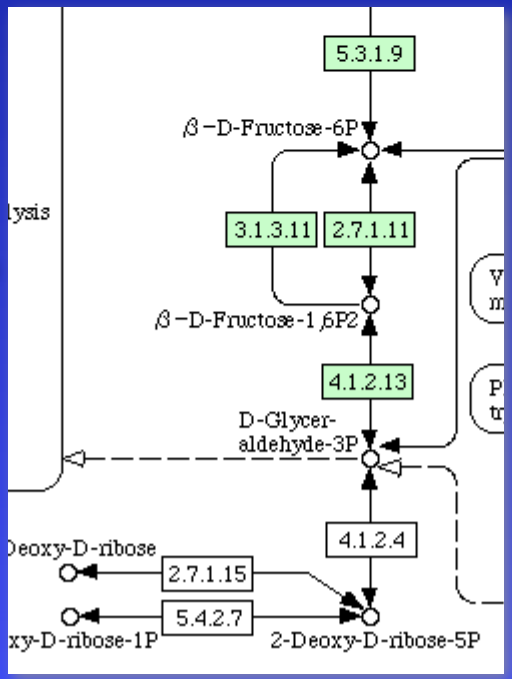
http://pipe.rockefeller.edu/modbase/

Function *via* Structure

Sequence → Structure → Function

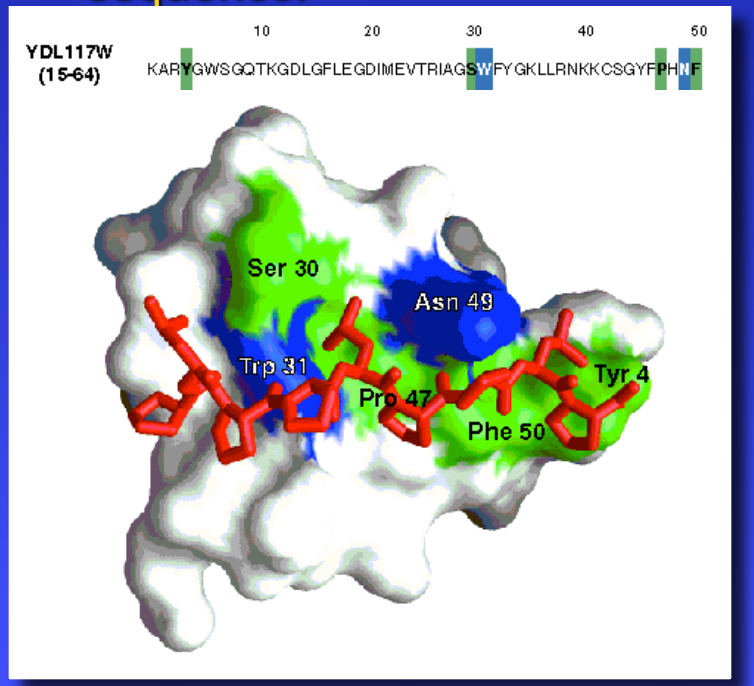
GFCHIKAYTRLIM…





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

Principles of Protein Structure

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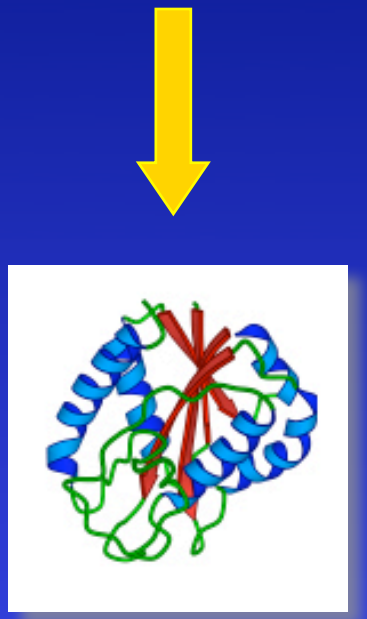

Folding

Ab initio prediction

Principles of Protein Structure

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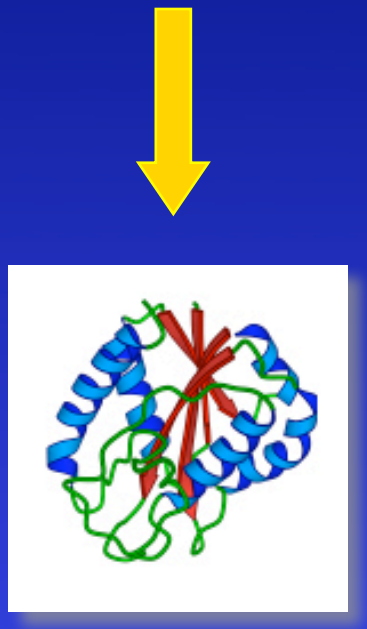

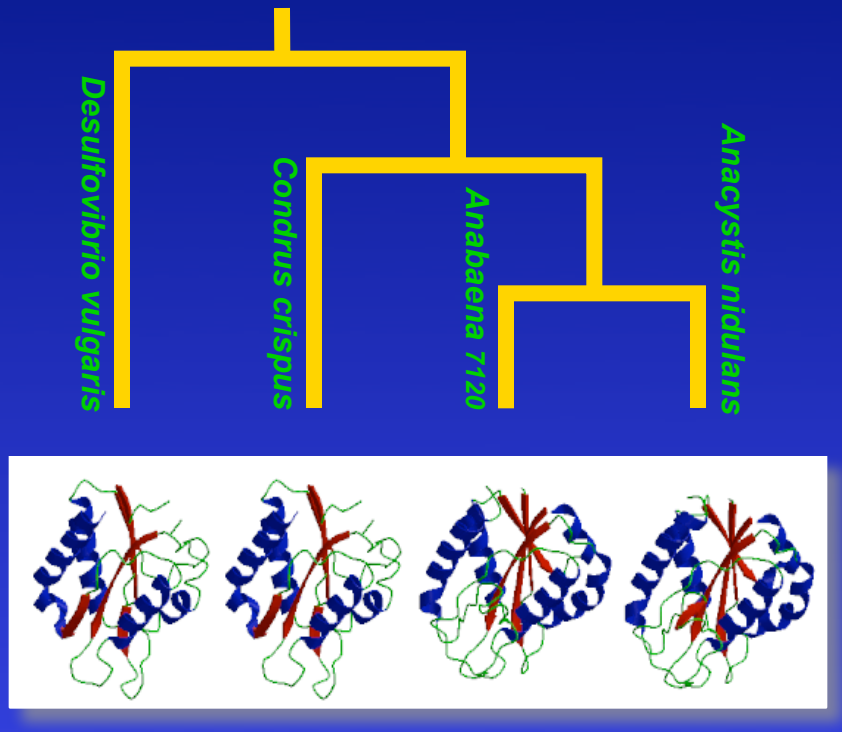

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Evolution

Threading
Comparative Modeling

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Steps in Comparative Protein Structure Modeling

START

TARGET

ASILPKRLFGNCEQTSDEGLK
IERTPLVPHISAQNVCLKIDD
VPERLIPERASFQWMNDK

A. Šali, *Curr. Opin. Biotech.* 6, 437, 1995.
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Steps in Comparative Protein Structure Modeling

START

Template Search

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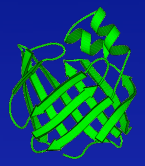
ASILPKRLFGNCEQTSDEGLK
IERTPLVPHISAQNVCLKIDD
VPERLIPERASFQWMNDK

TEMPLATE



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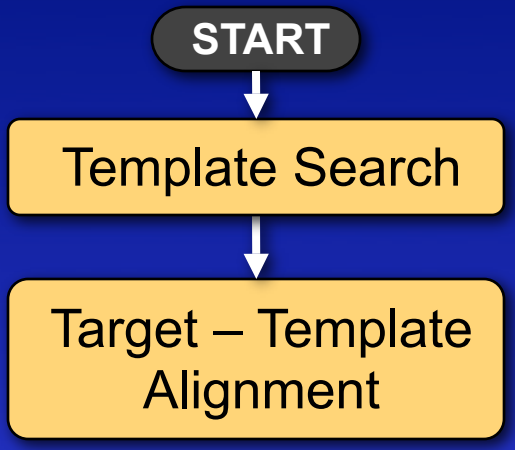

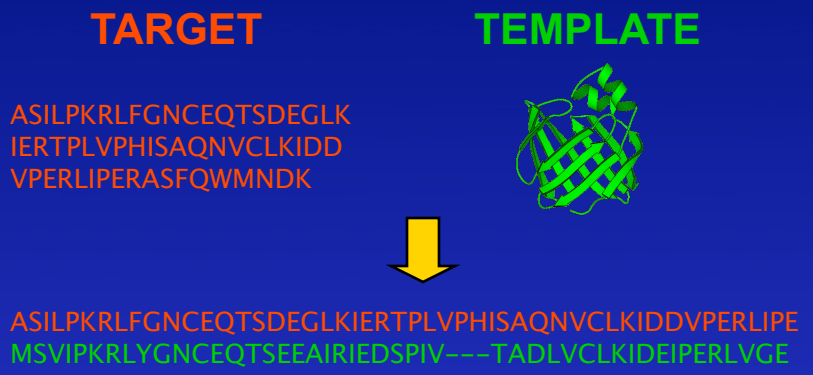

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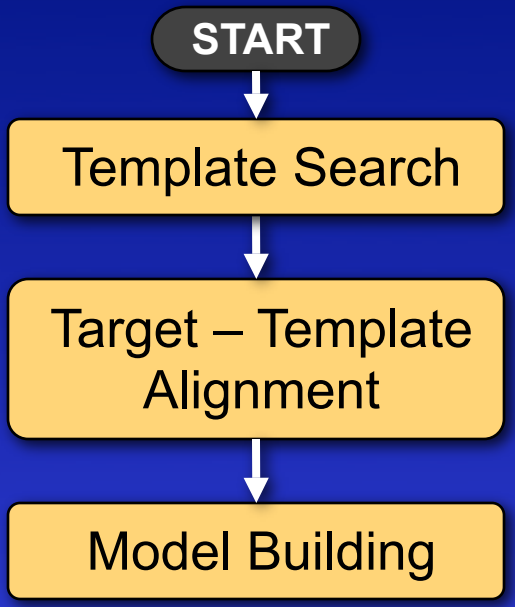

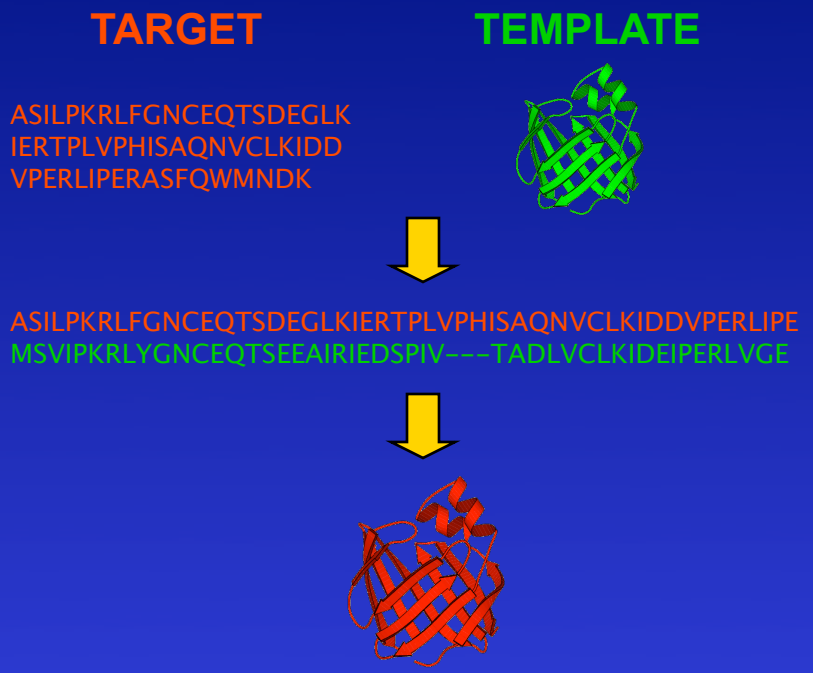

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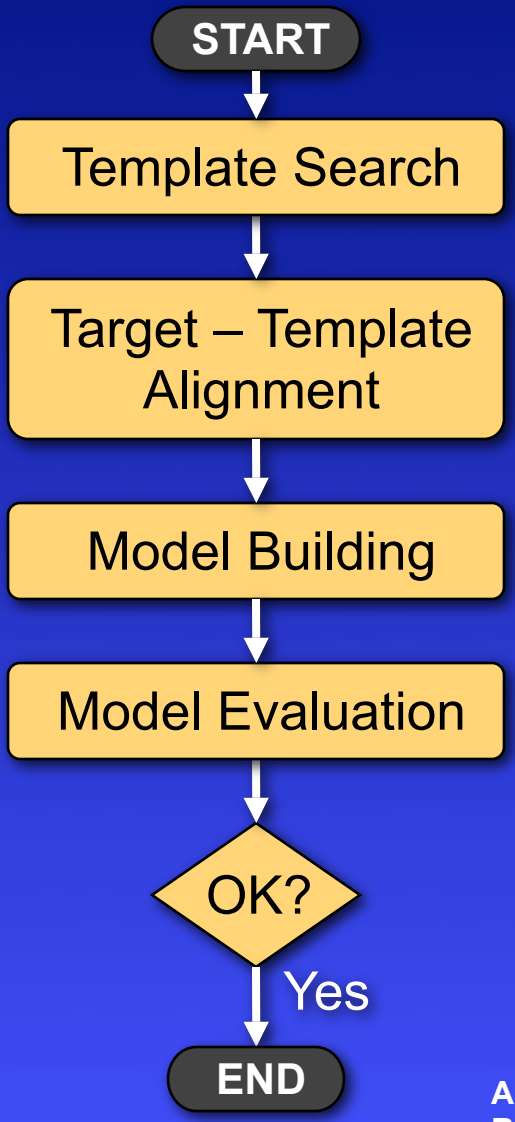

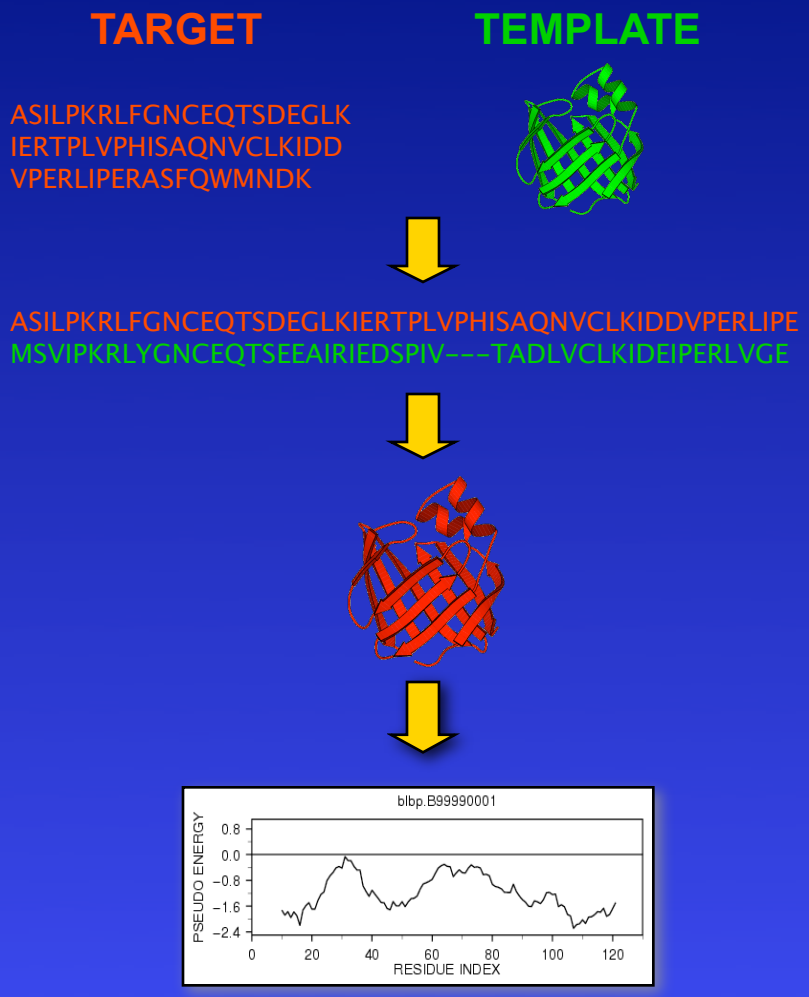

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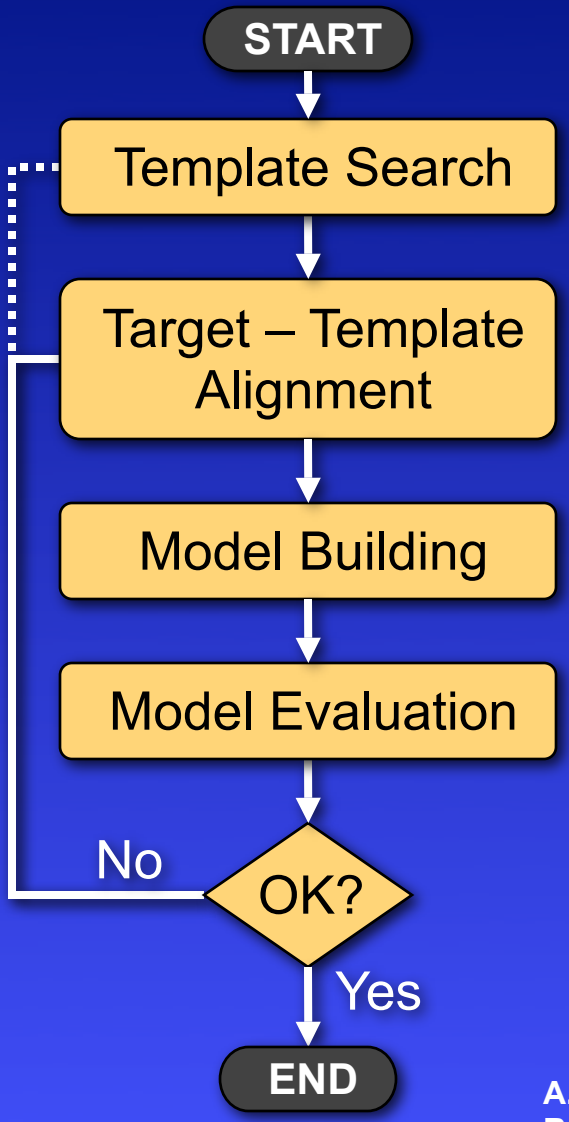

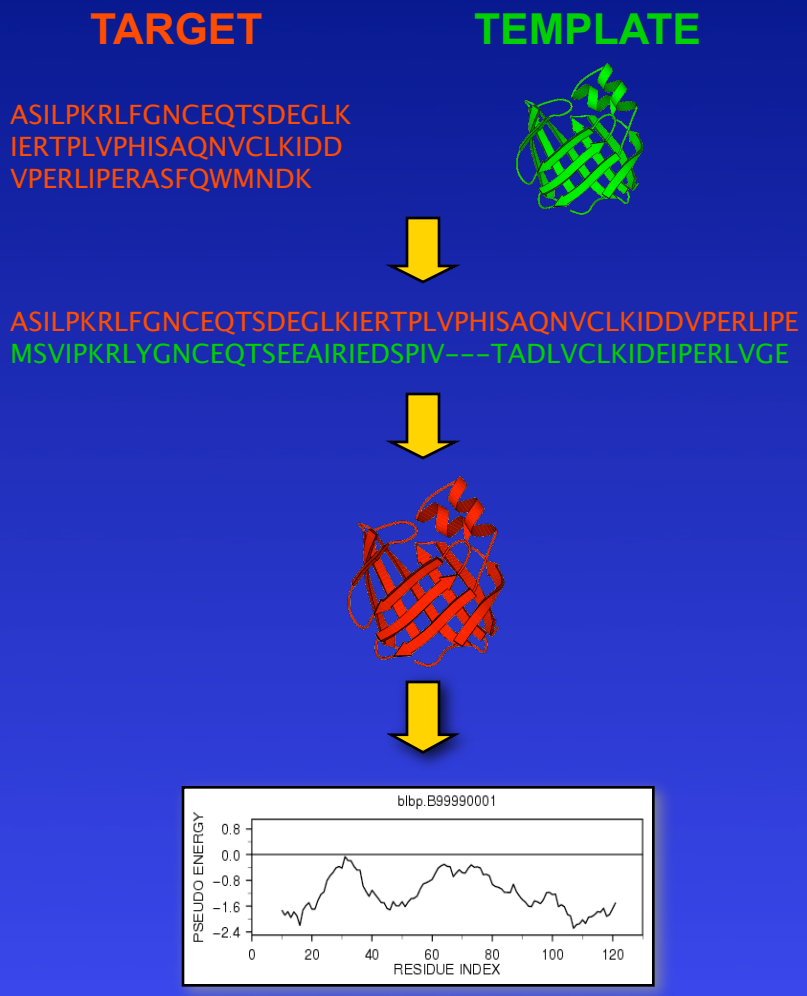

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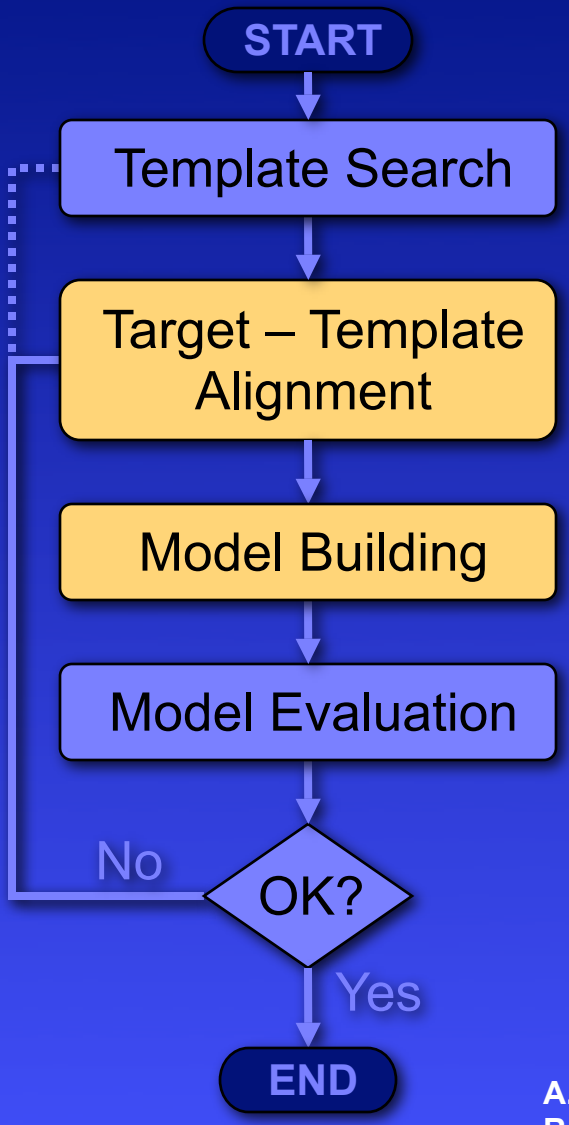

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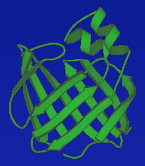
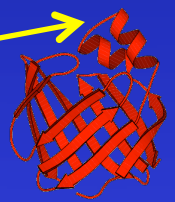
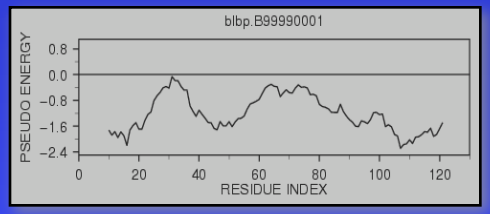


ASILPKRLFGNCEQTSDEGLKIERTPLVPHISAQNVCLKIDDVPERLIPE
MSVIPKRLYGNCEQTSEEAIRIEDSPIV---TADLVCLKIDEIPERLVGE

Loop modeling





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Template Search Methods

- ✓ Sequence similarity searches
  - ✓ BLAST [http://www.ncbi.nlm.nih.gov/]
  - ✓ 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/]
- ✓ Structure based threading
  - ✓ THREADER [http://bioinf.cs.ucl.ac.uk/]
  - ✓ PROFIT [http://www.came.sbg.ac.at/]

Target – Template Alignment Methods

- Dynamic Programming Pairwise Alignment
  - ALIGN [http://guitar.rockefeller.edu/modeller/]
- Multiple Alignments,
  - Psi-Blast [http://www.ncbi.nlm.nih.gov/]
  - HMM [http://www.cse.ucsc.edu/research/compbio/HMM-apps/]
  - ALIGN4D [http://guitar.rockefeller.edu/modeller/]
- Structure based approaches
  - Threading [http://bioinf.cs.ucl.ac.uk/]

Model Building Methods

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

Comparative Modeling by Satisfaction of Spatial Restraints (MODELLER)

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

A. Šali & T. Blundell. *J. Mol. Biol.* **234**, 779, 1993.
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http://guitar.rockefeller.edu/

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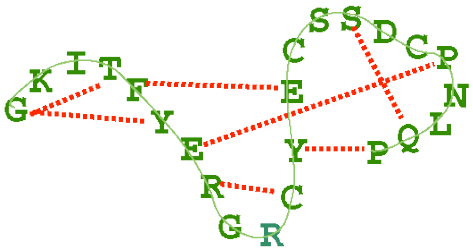
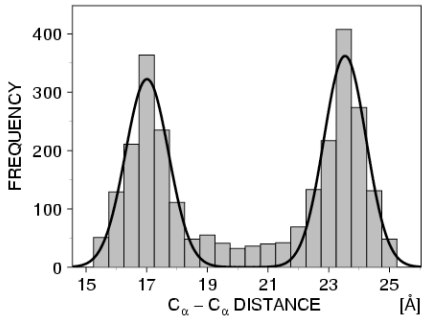
1. Extract spatial restraints





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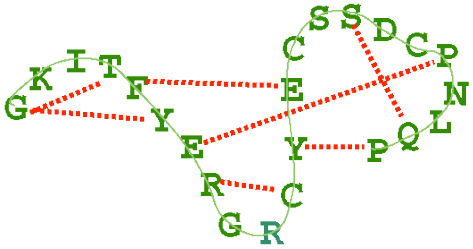
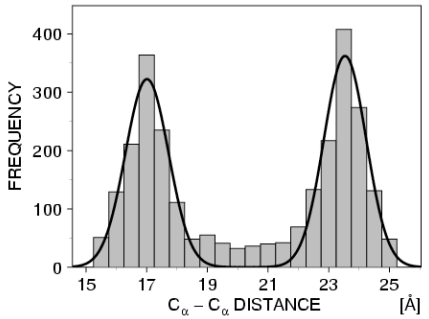
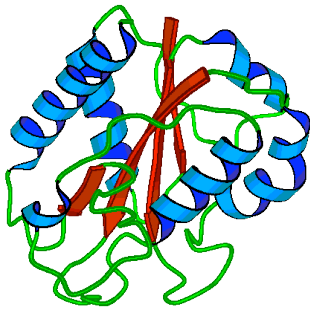




2. Satisfy spatial restraints



$$F(\mathbf{R}) = \prod_{i} p_i(f_i/I)$$

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http://guitar.rockefeller.edu/

Model Evaluation methods

- ✓ Stereochemistry
  - ✓ PROCHECK [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/]

http://guitar.rockefeller.edu/bioinformatics_resources.shtml

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Typical Errors in Comparative Models

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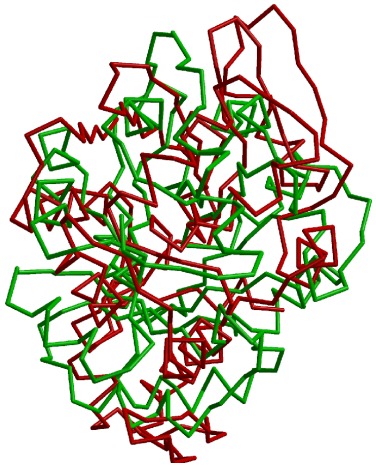
MODEL

X-RAY

TEMPLATE

Incorrect template



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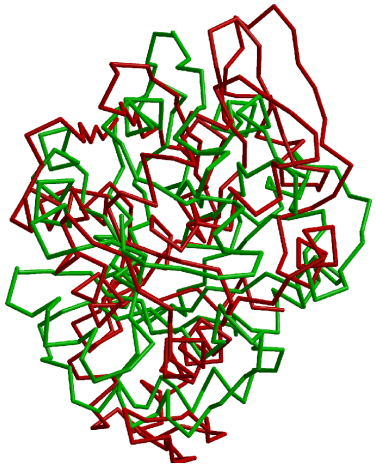
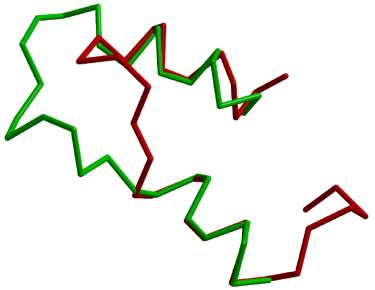
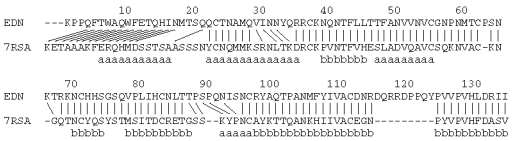


Misalignment





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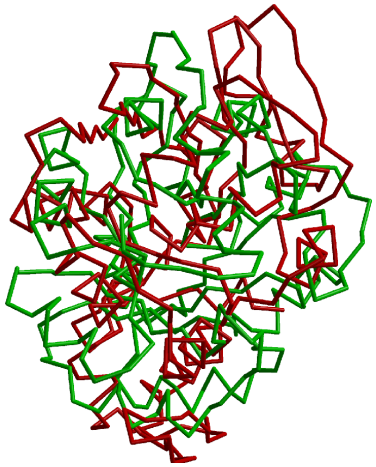

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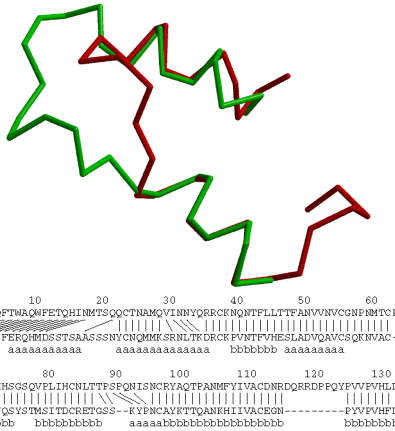
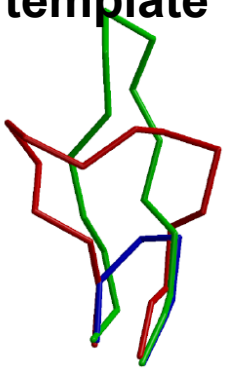


Region without a template



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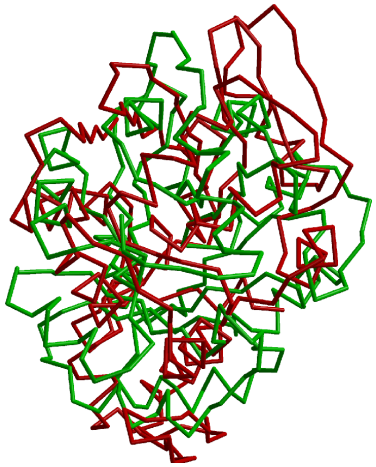

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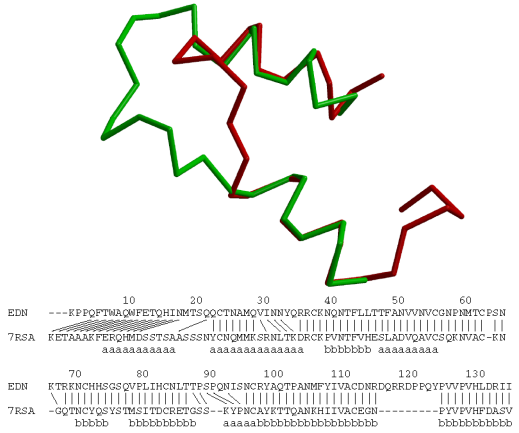

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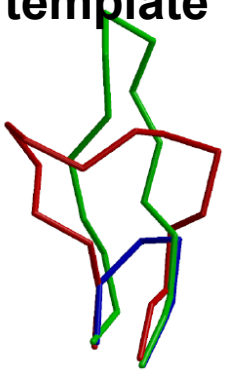
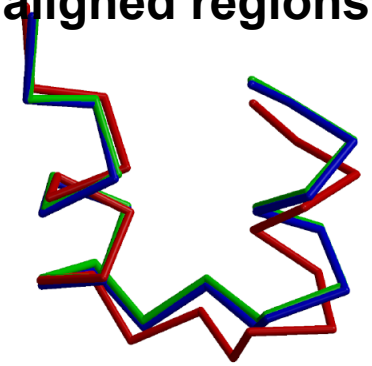


Distortion in correctly aligned regions



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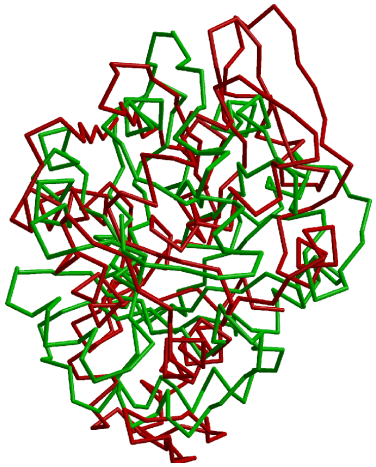

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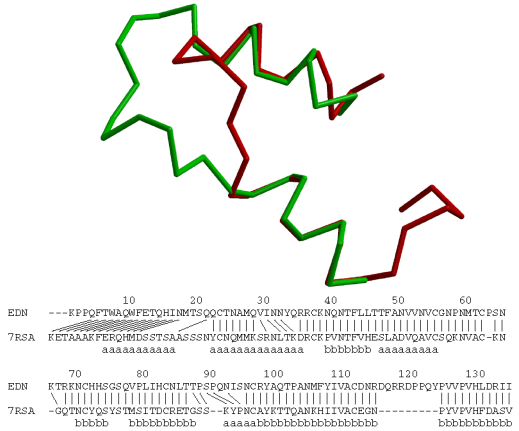
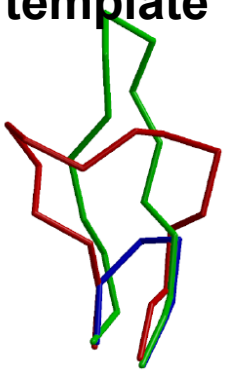
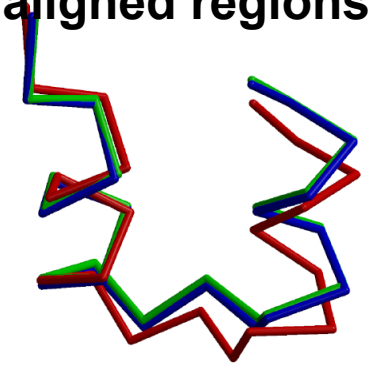


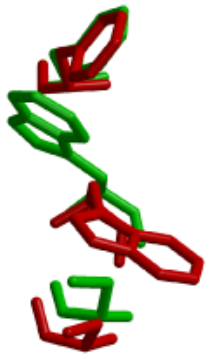
Region without a template



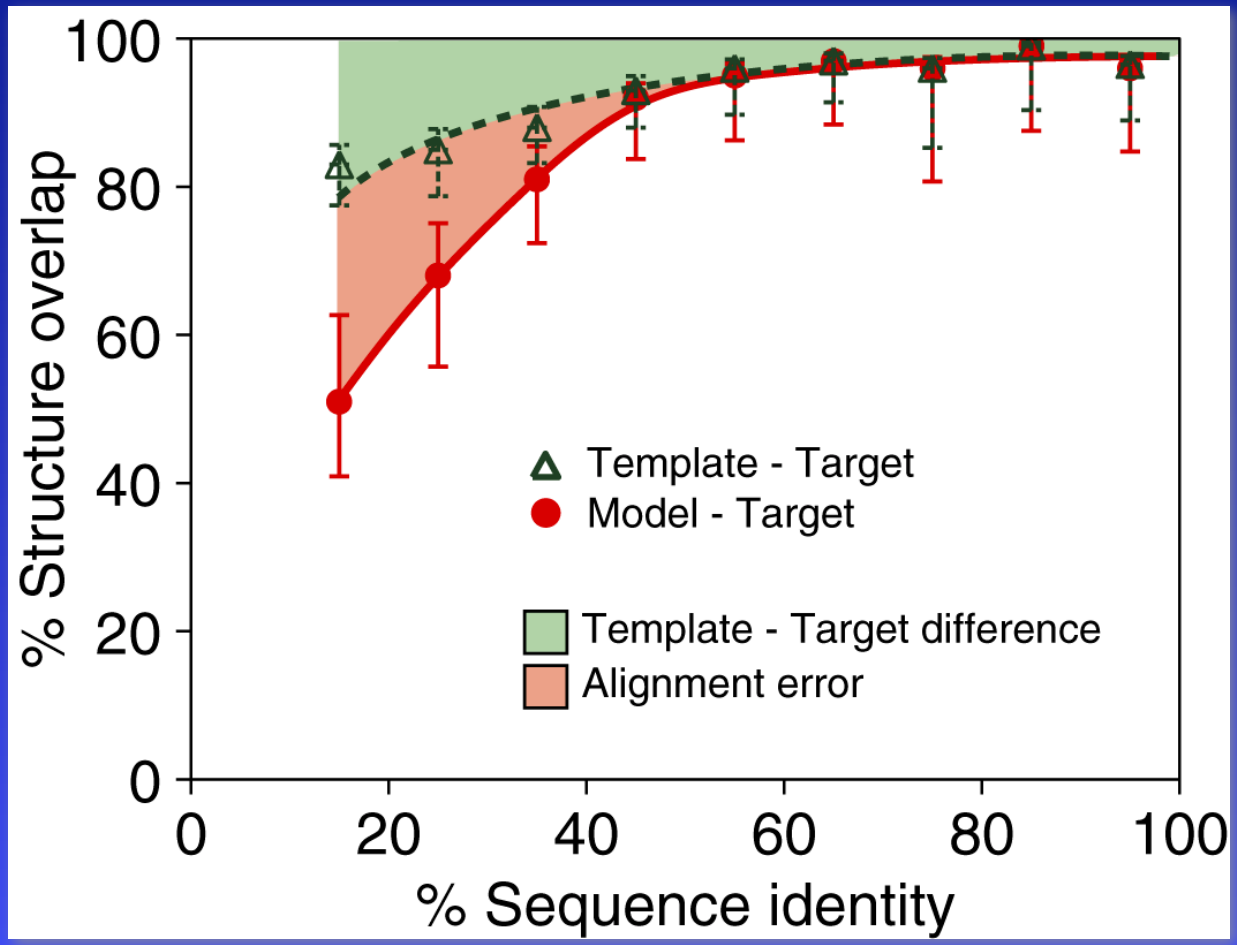
Distortion in correctly aligned regions



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).

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

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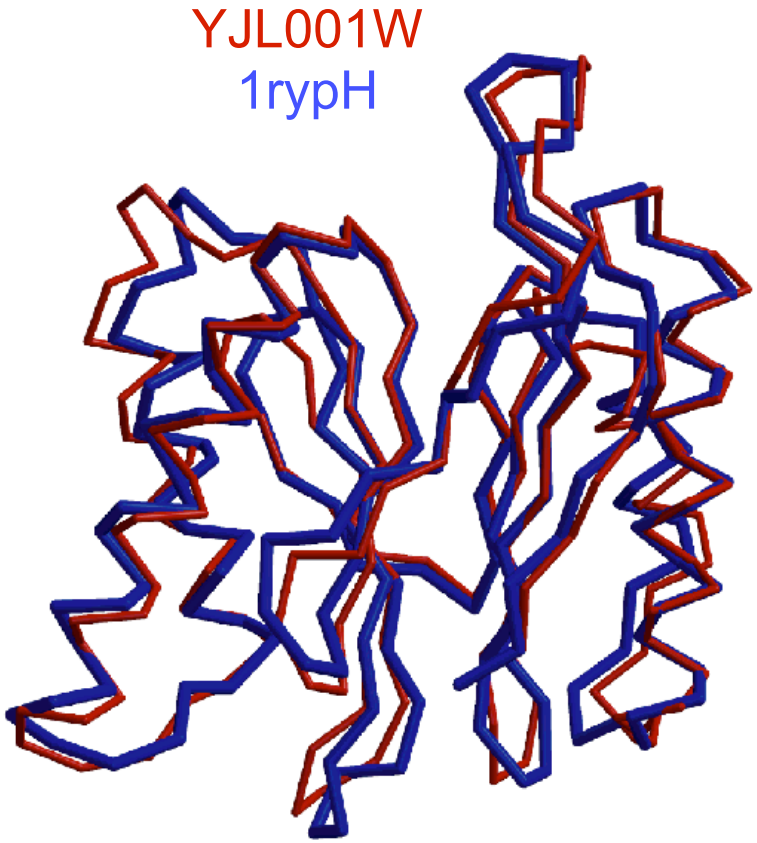
24% sequence identity



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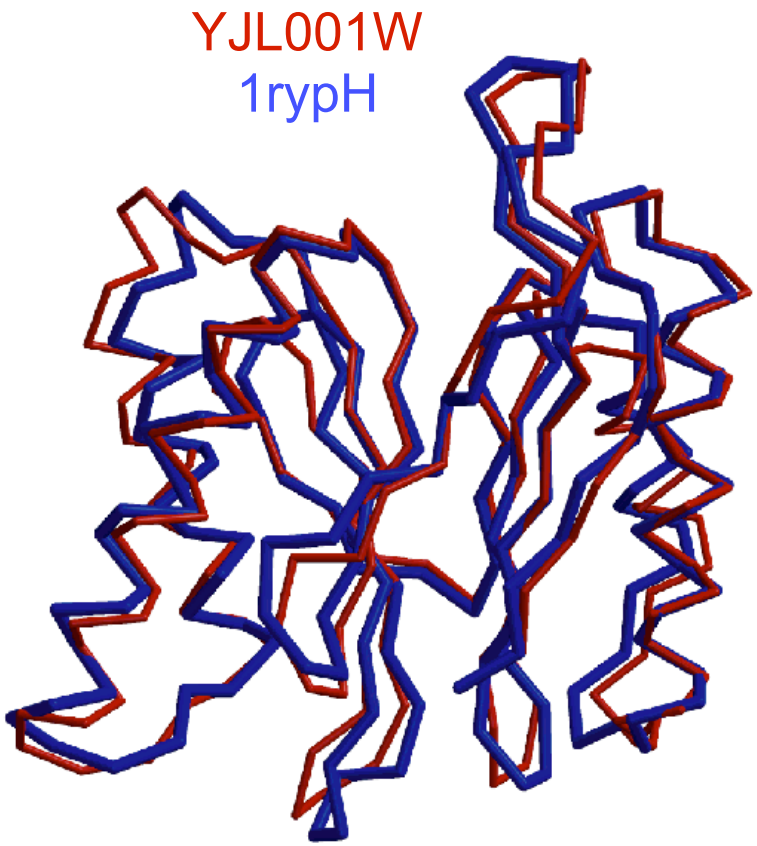
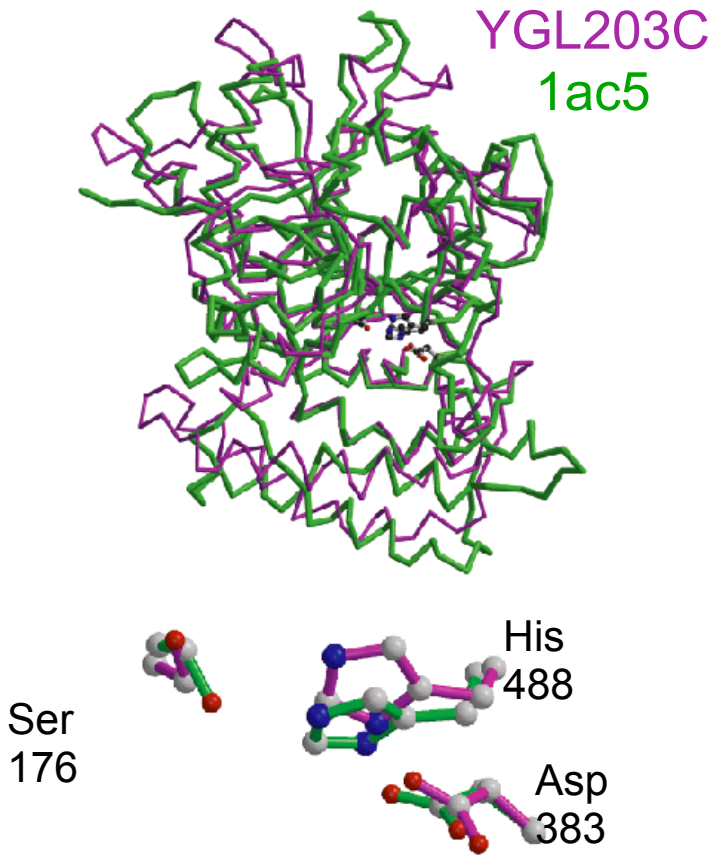


25% sequence identity



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.
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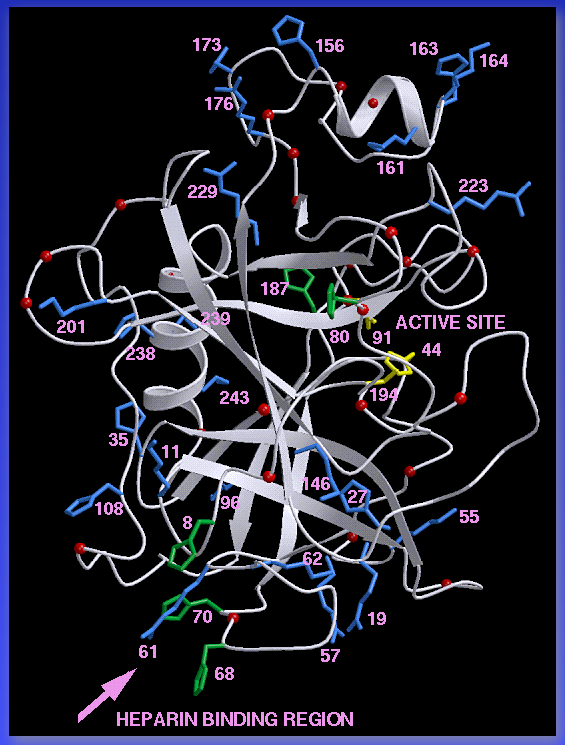

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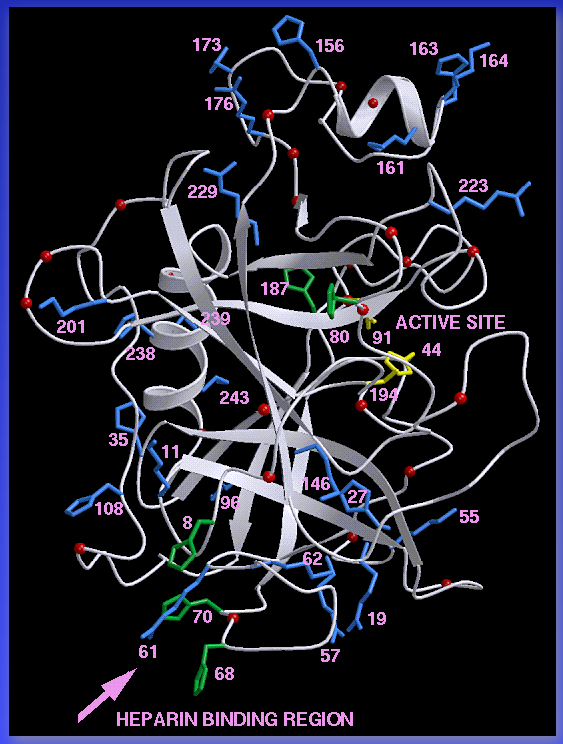
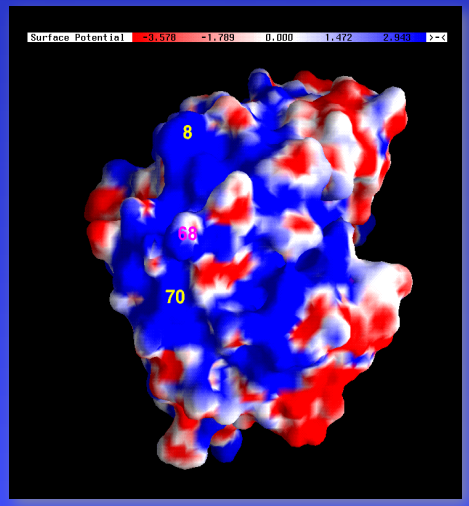
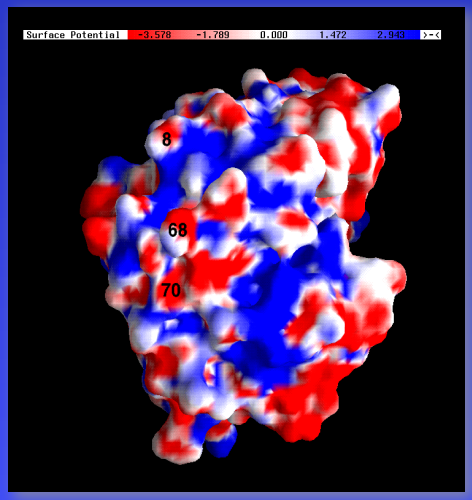




Native mMCP-7 at *p*H=5 (His^{+})



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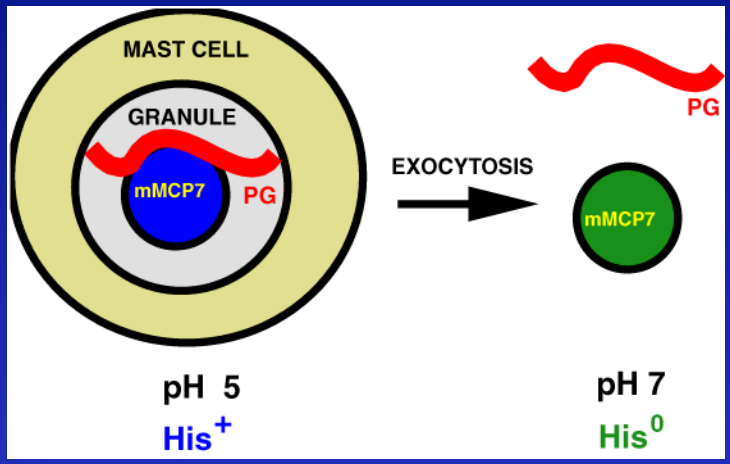
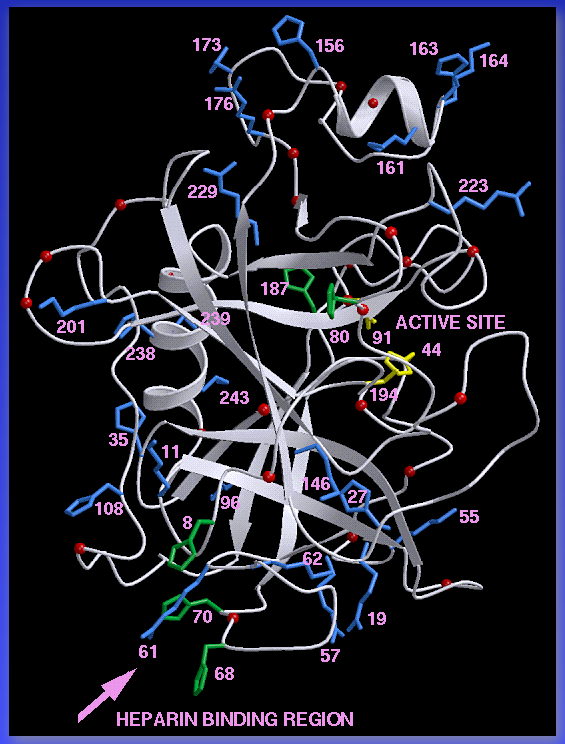
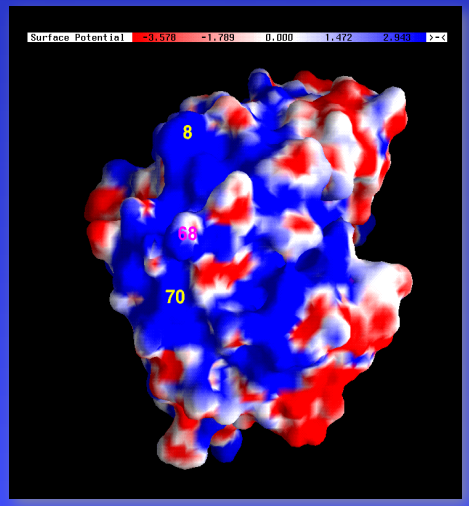


Huang *et al*. *J. Clin. Immunol*. **18**,169,1998.
Matsumoto *et al*. *J.Biol.Chem*. **270**,19524,1995.
Šali *et al*. *J. Biol. Chem*. **268**, 9023, 1993.





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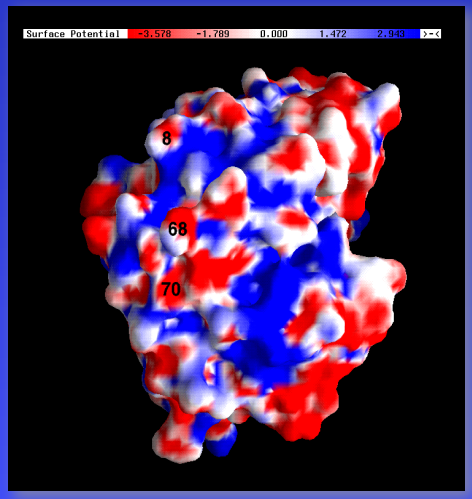

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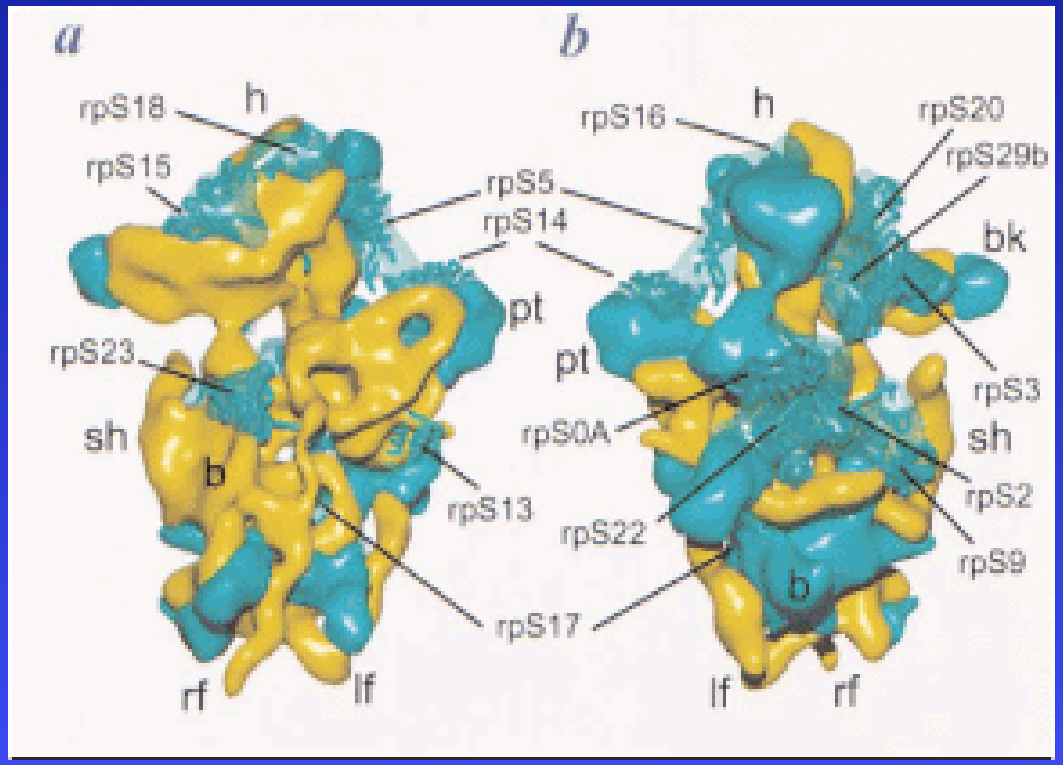
Some Models Can Be Used in Docking to Density Maps
(Yeast Ribosomal 40S subunit)

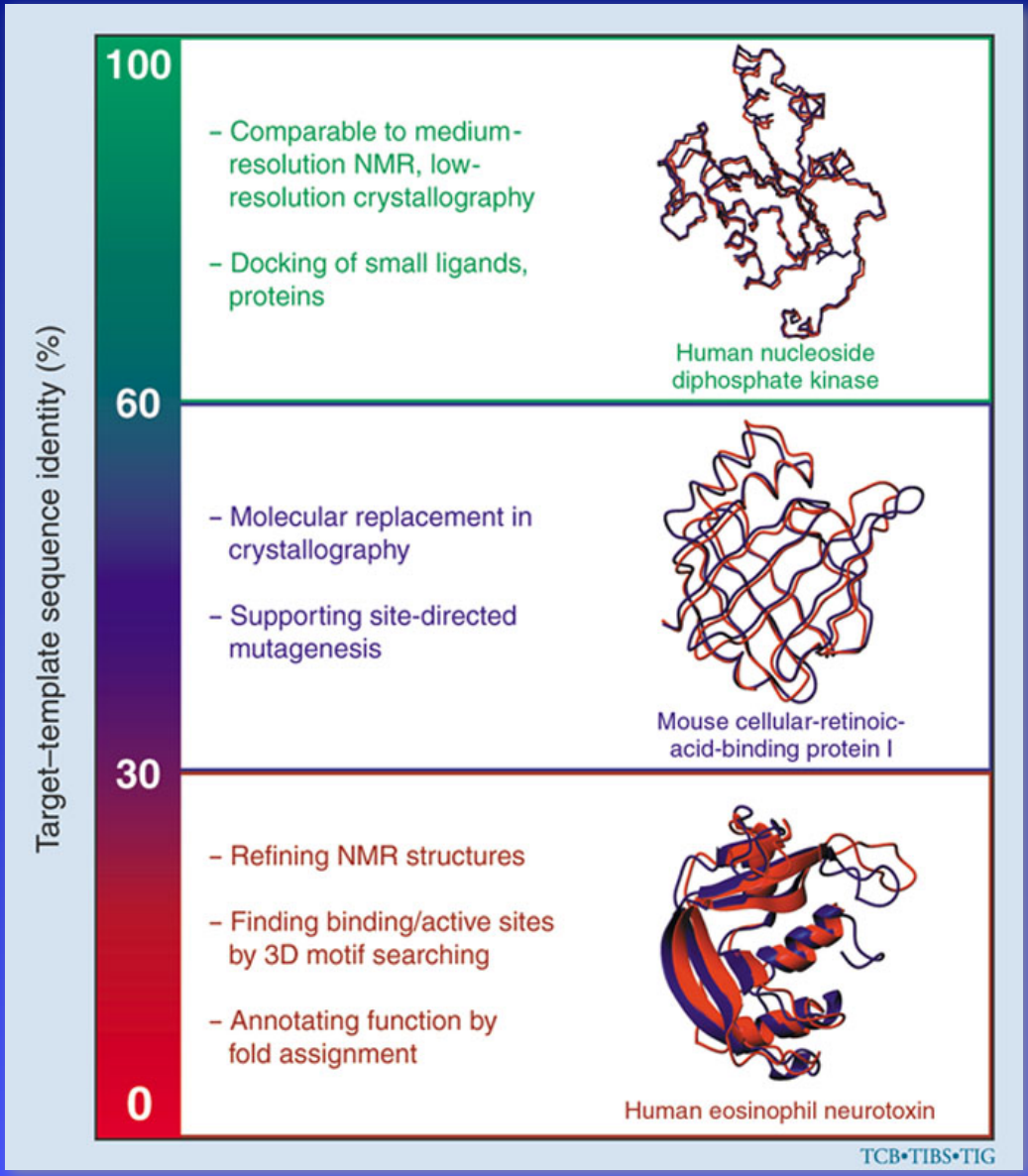


Small 30S subunit from *Thermus thermophilus*
Large 50S subunit from *Haloarcula marismortui*

Docking of comparative models into the cryo-EM map.

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

Applications of Comparative Models



Šali & Kuriyan.
TIBS **22**, M20, 1999.

Summary

- ✓ What is comparative modeling and why is it useful?
- ✓ Steps in CM (overview + some details)
- ✓ Accuracy of comparative models
- ✓ Target-Template alignment
- ✓ Loop modeling
- ✓ CM and Structural Genomics

Experiment (*in silico*)

- **Benchmarking the best alignment methods.**
- **New alignment method.**
- **Projected gains.**

Methods: Reference set

CE alignments with **387**

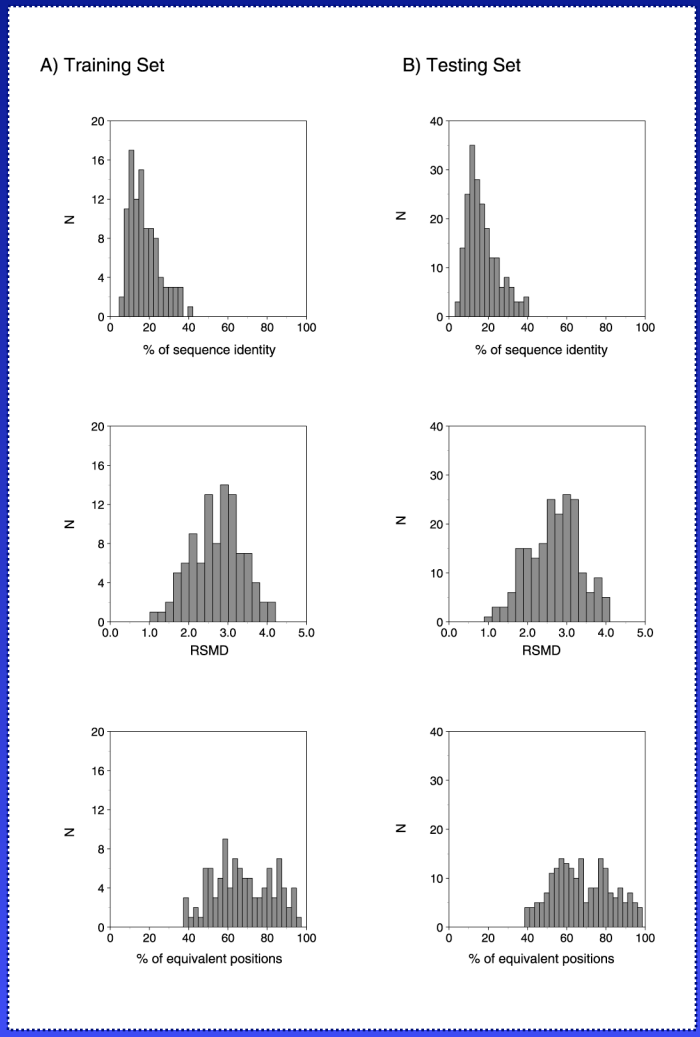
- < 40% sequence identity
- > 100 EqPos
- > 50% EqPos
- > 90% coverage for one chain

Filter: MAMMOTH alignments with **300**

- > 50% EqPos

100 Training set

200 Testing set



Methods: Evaluated methods

Sequence A: AGHLAHTRCELKLPTCRGNMSSRFC

Sequence B: AGHLRHTRRCLRLPTAGNARFC

Seq.-Seq.

ALIGN: DP pairwise method

BLAST2SEQ: Local method

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PSI-BLAST: Local search method that uses multiple sequence information for one of the sequences.

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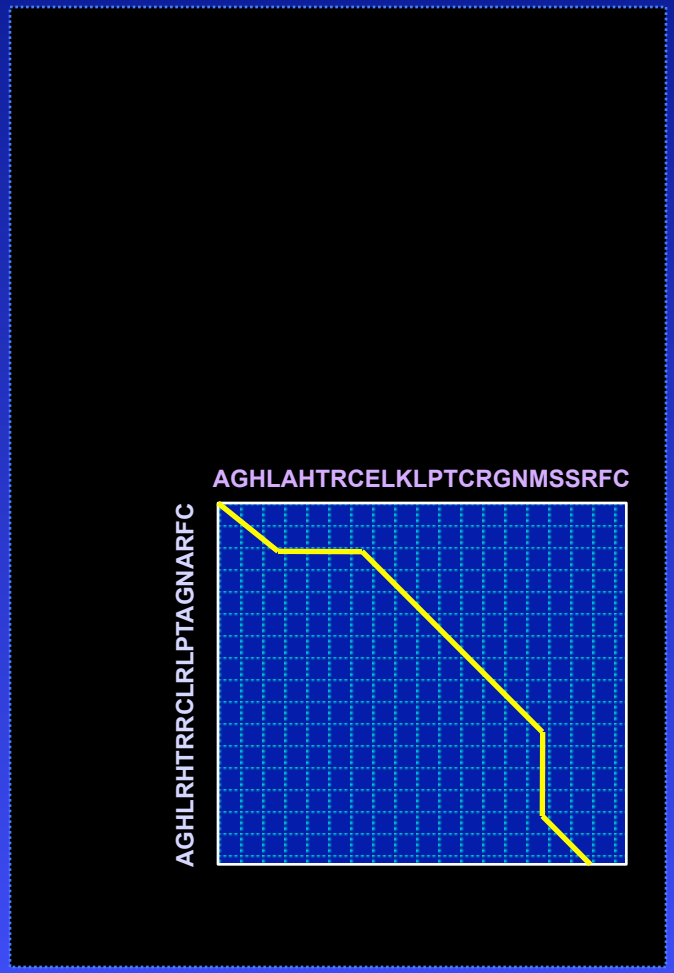

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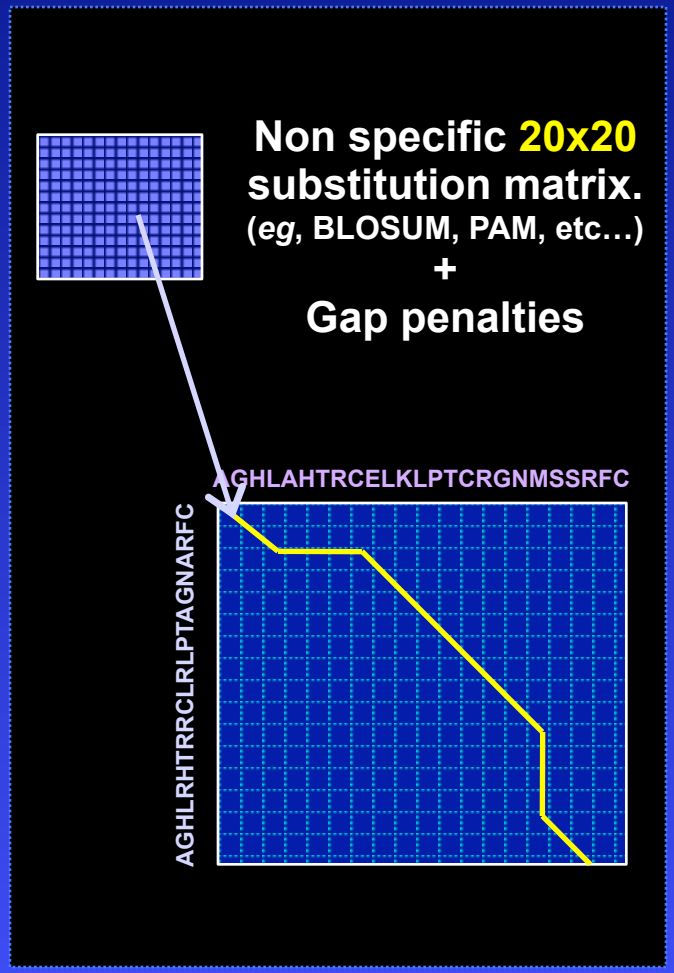

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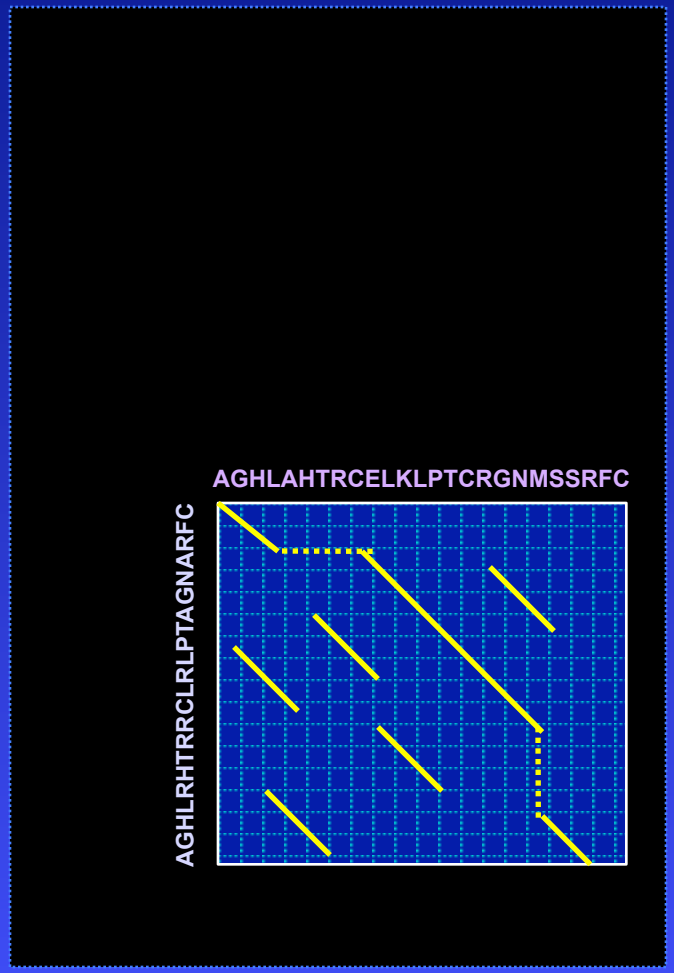

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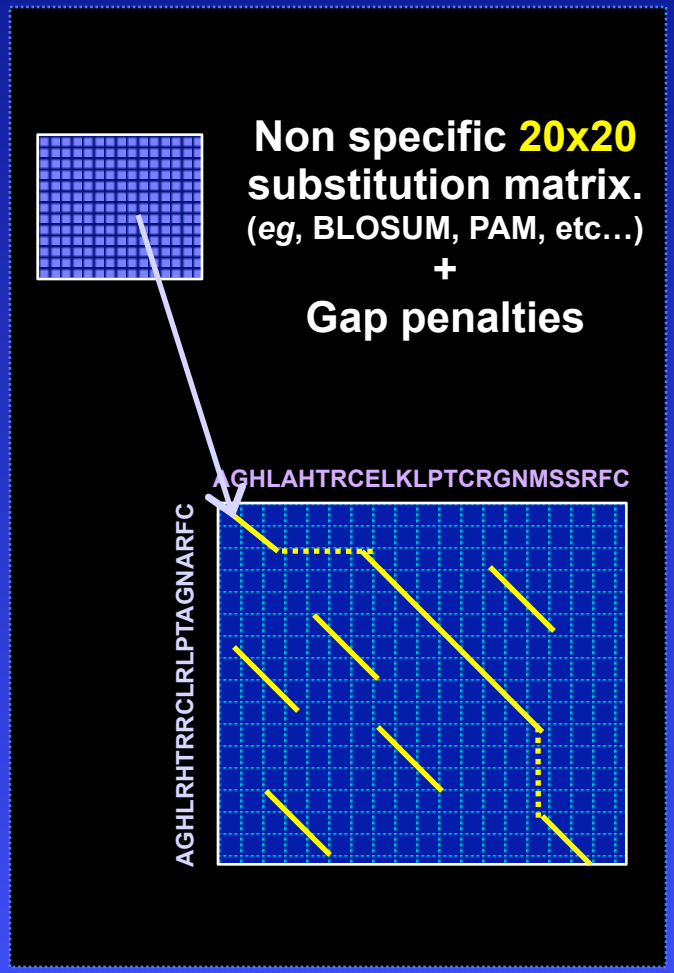

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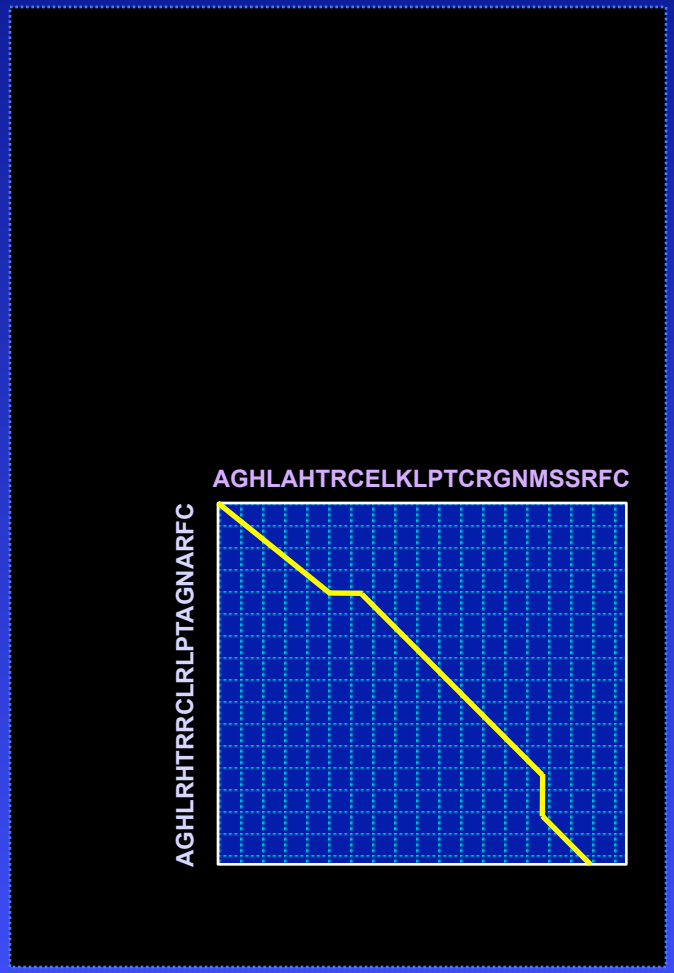

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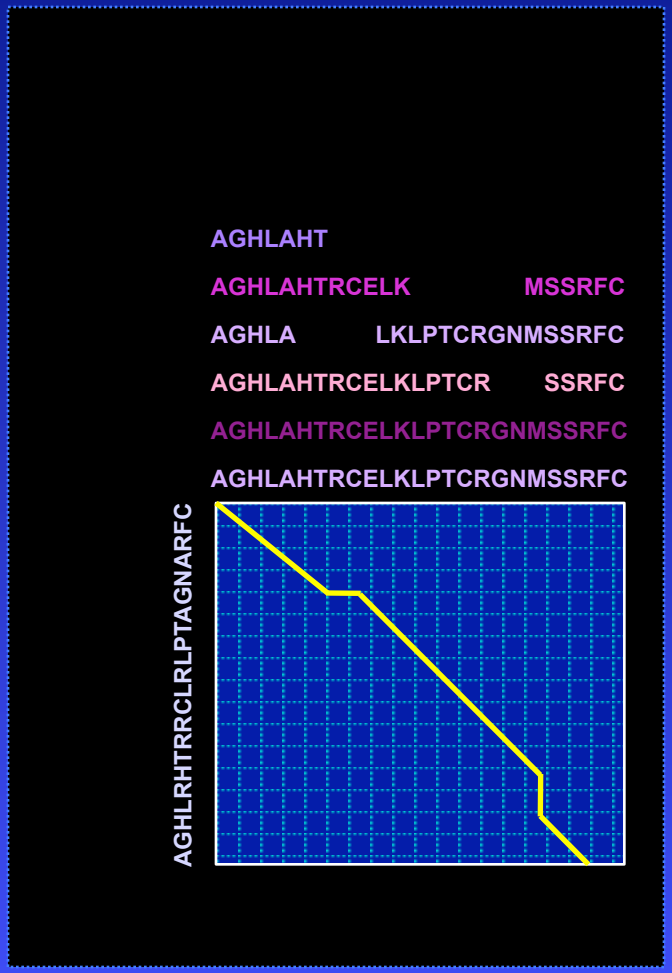

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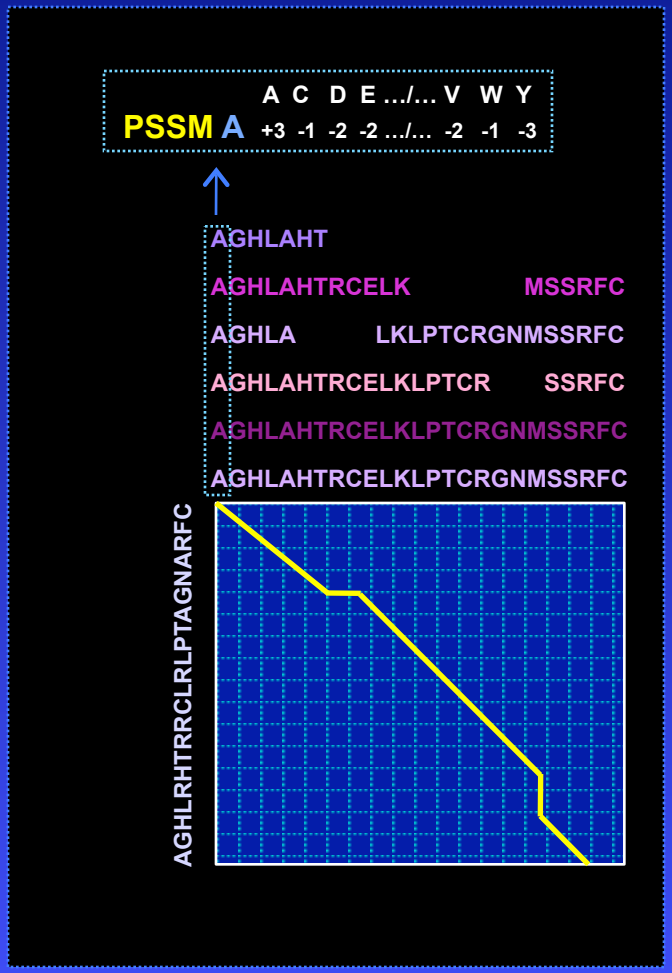

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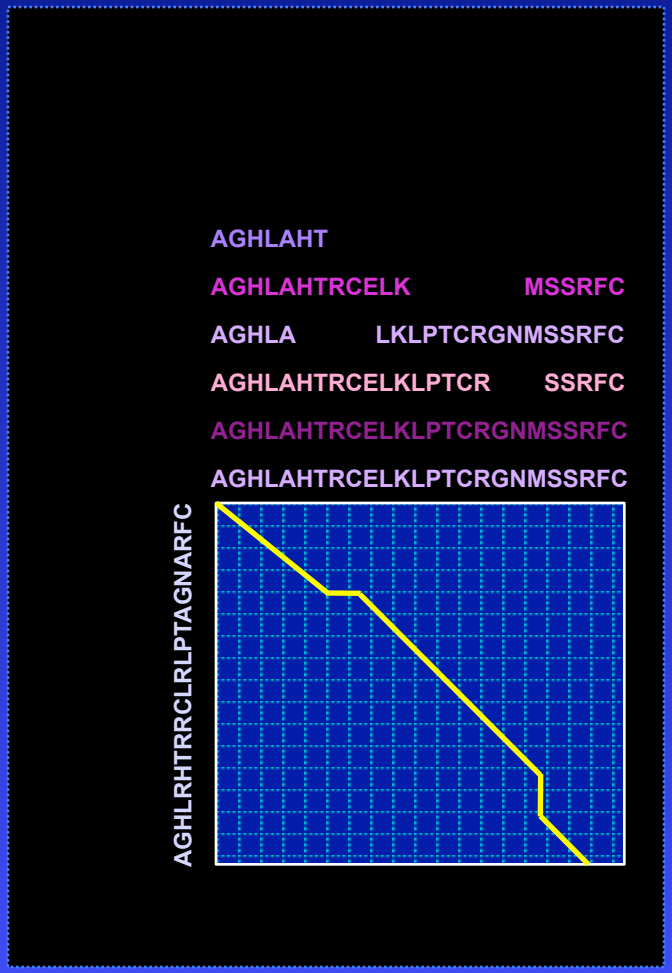

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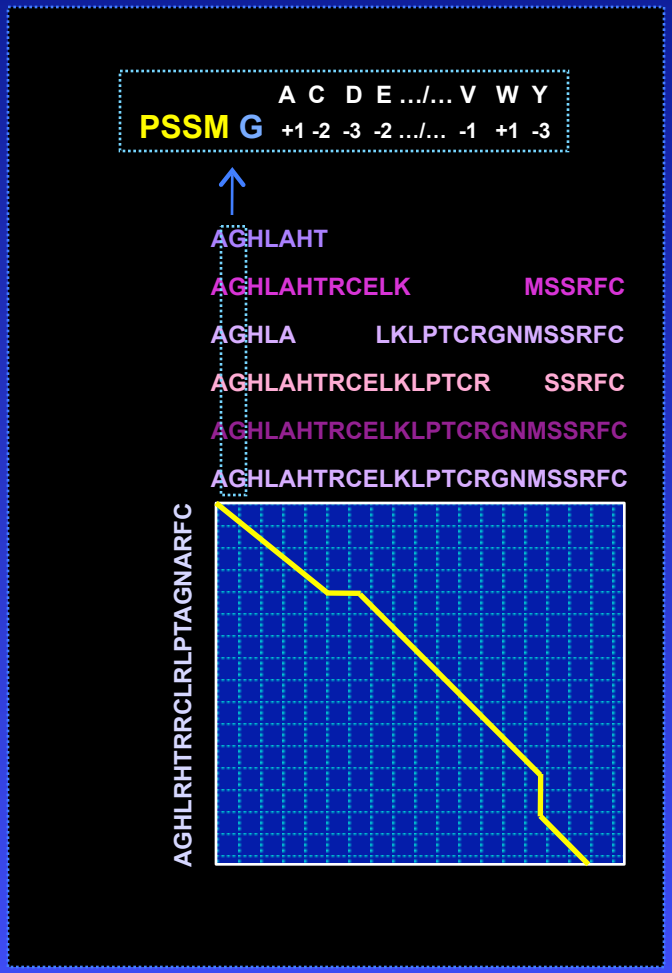

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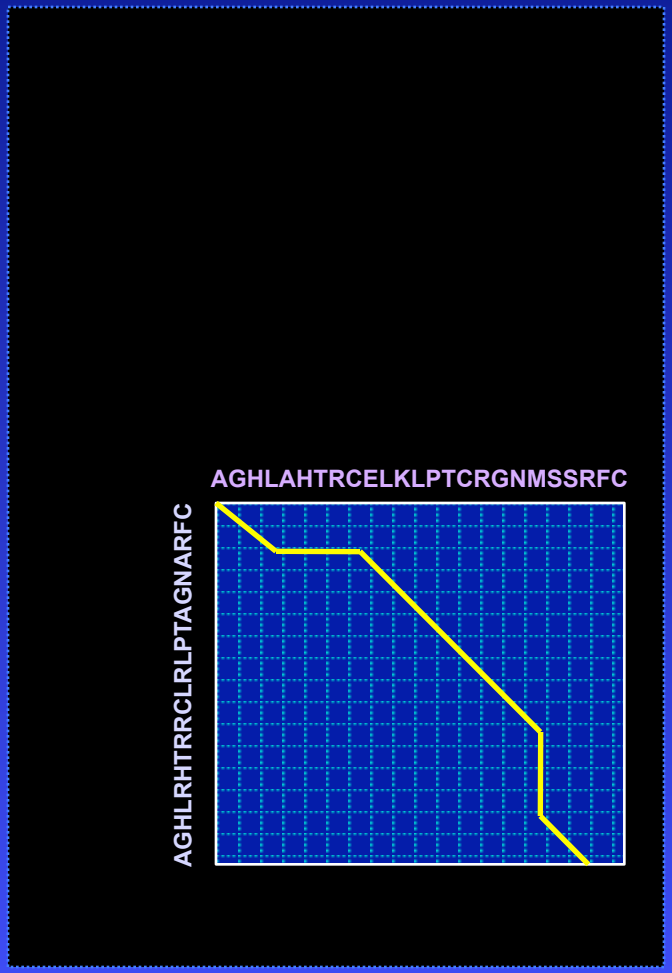

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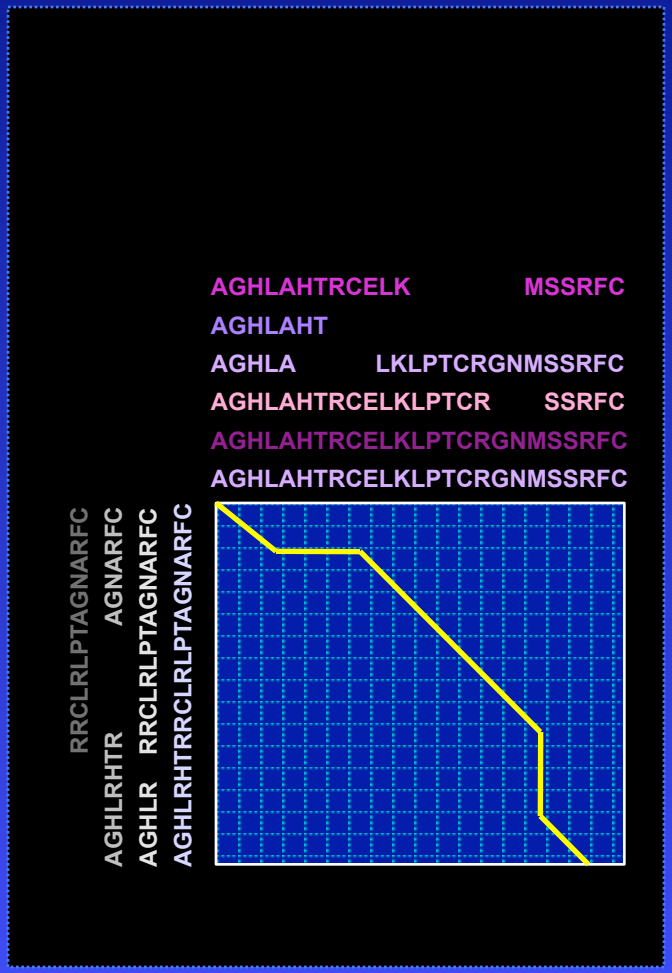
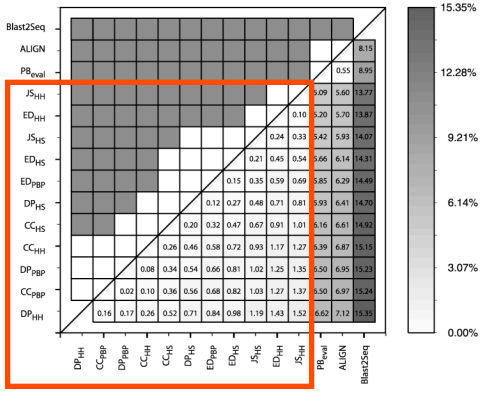


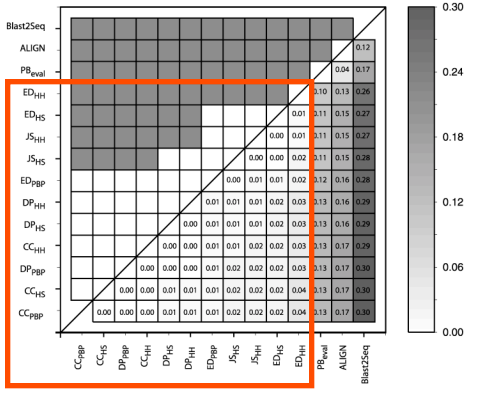
Results: Comparison of alignment dependent measures

ALIGN4D protocol	% of Correct SeqA	% of Correct SeqB	Shift Score
CC_{PBP}	55.34 [8.00 - 100.00]	55.49 [7.00 - 100.00]	0.61 [0.08 - 1.00]
CC_{HH}	54.96 [8.00 - 100.00]	55.30 [7.00 - 100.00]	0.61 [-0.07 - 1.00]
CC_{HS}	54.48 [6.00 - 100.00]	54.80 [7.00 - 100.00]	0.61 [0.04 - 1.00]
ED_{PBP}	54.22 [6.00 - 99.00]	54.17 [7.00 - 99.00]	0.60 [-0.07 - 0.99]
ED_{HH}	52.90 [8.00 - 100.00]	53.01 [7.00 - 100.00]	0.58 [-0.07 - 1.00]
ED_{HS}	53.70 [9.00 - 100.00]	53.89 [7.00 - 100.00]	0.59 [-0.07 - 1.00]
DP_{PBP}	55.02 [7.00 - 100.00]	55.47 [7.00 - 100.00]	0.61 [0.00 - 1.00]
DP_{HH}	55.50 [7.00 - 100.00]	55.81 [9.00 - 100.00]	0.61 [-0.06 - 1.00]
DP_{HS}	54.07 [6.00 - 100.00]	54.41 [7.00 - 100.00]	0.61 [0.01 - 1.00]
JS_{HH}	52.56 [6.00 - 100.00]	52.82 [7.00 - 100.00]	0.59 [0.03 - 1.00]
JS_{HS}	53.24 [6.00 - 100.00]	53.48 [7.00 - 100.00]	0.60 [-0.01 - 1.00]
ALIGN	41.55 [6.00 - 94.00]	41.84 [5.00 - 94.00]	0.44 [-0.07 - 0.96]
BLAST2SEQ	26.09 [0.00 - 92.00]	26.07 [0.00 - 93.00]	0.32 [-0.08 - 0.95]
PB (e-val)	42.95 [0.00 - 96.00]	43.11 [0.00 - 95.00]	0.48 [-0.12 - 0.98]

A) % of correctly aligned positions.



B) Shift Score.



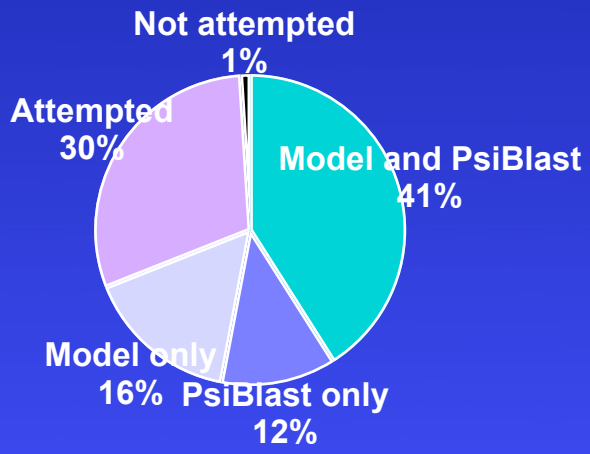
Results: Comparison of success rates

Method	% of alignments at 1Å	% of alignments at 2Å	% of alignments at 3Å	% of alignments at average
CE	20.50	82.50	100.00	82.50
ALIGN	8.50	23.00	35.00	21.00
BLAST2SEQ	8.00	21.50	30.00	20.00
PB (e-val)	8.00	31.00	45.50	29.50
CC_{PBP}	11.50	37.00	55.50	35.50
DP_{PBP}	11.00	37.50	53.50	35.50

Results. Turn over.

Mycoplasma genitalium MODPIPE Models

Number of ORFs	479
Average ORF length	364



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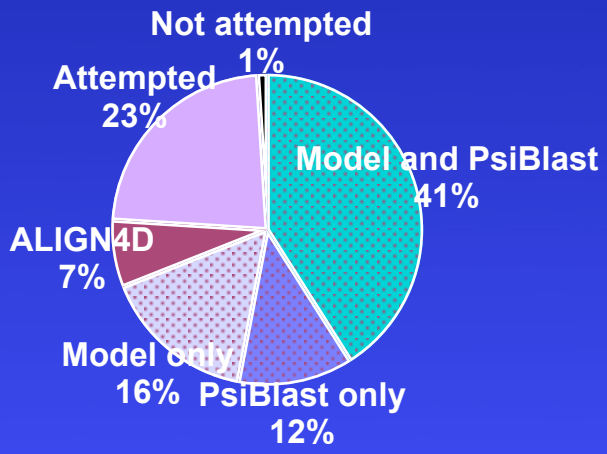

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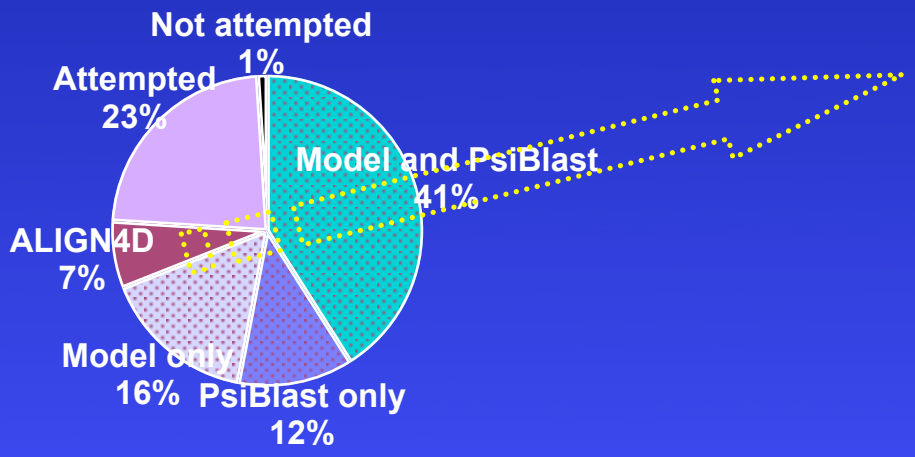


~ 34 extra accurate models for *M. g.* genome.

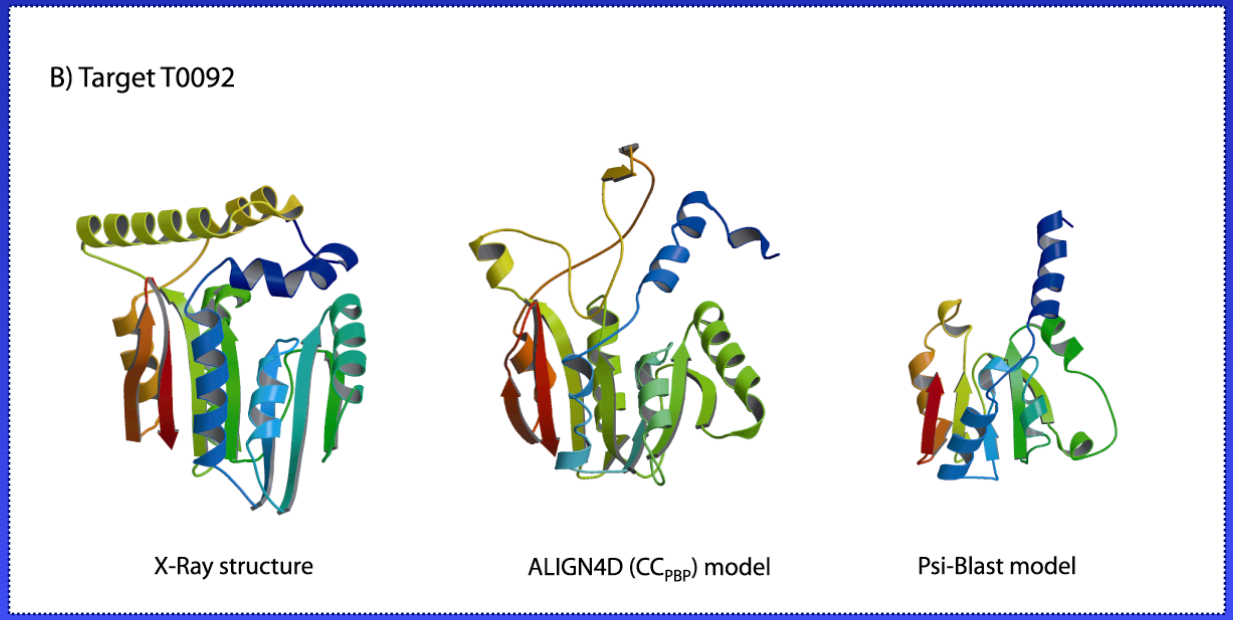
~ 50,000 models for TrEMBL-SP "genome".

Examples: T0092 model

- **Target T0092 at CASP4:**
  - Hypothetical protein HI0319
  - *Haemophilus influenzae*
  - Parent: **1d2cA** (Methyltransferase)
  - **ALIGN4D** alignment at **8.4%** seq id.

Method	RMSD Å	% of EqPos
ALIGN4D CC_{PBP}	5.9	67.84
PSI-BLAST	4.9	31.72
Best predictions at CASP4	6.0	65.20

Data from CASP4, Asilomar, CA, December 2000.



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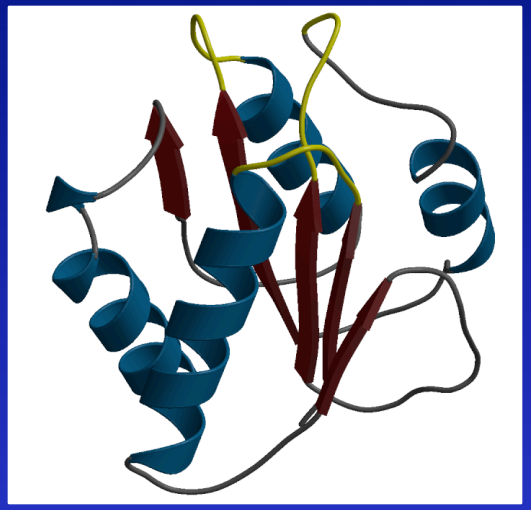
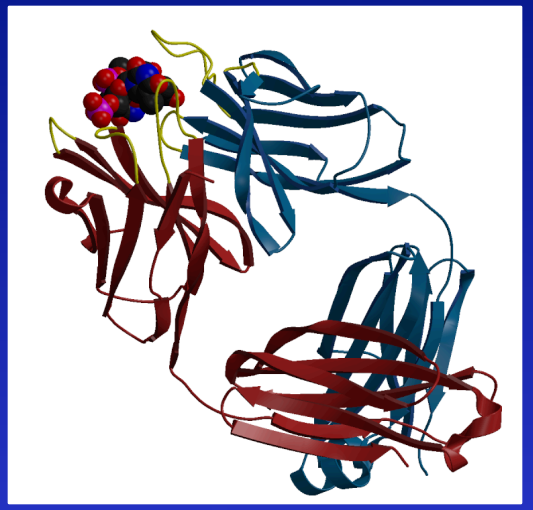
- ✓ CM and Structural Genomics

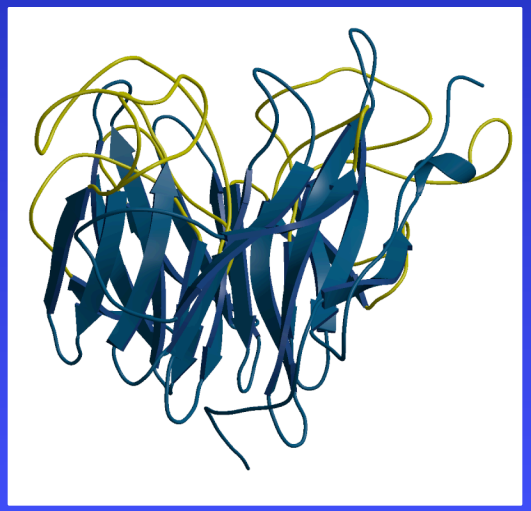
Loop Modeling in Protein Structures



α+β barrel: flavodoxin



IG fold: immunoglobulin



antiparallel β-barrel

A. Fiser, R. Do & A. Šali, *Prot. Sci.,* **9**, 1753, 2000

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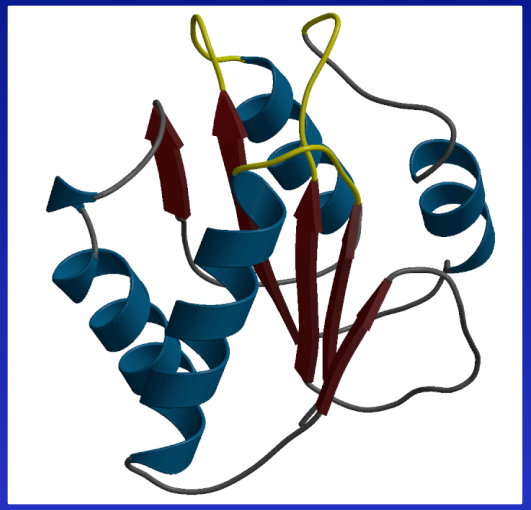

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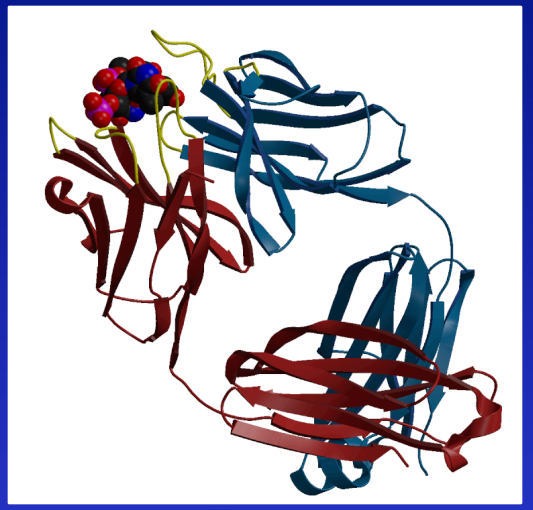

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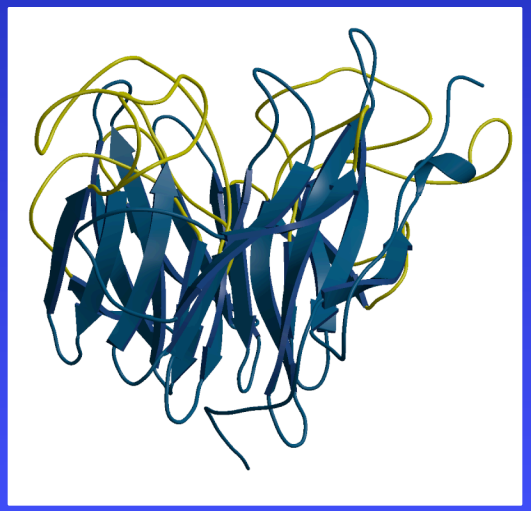

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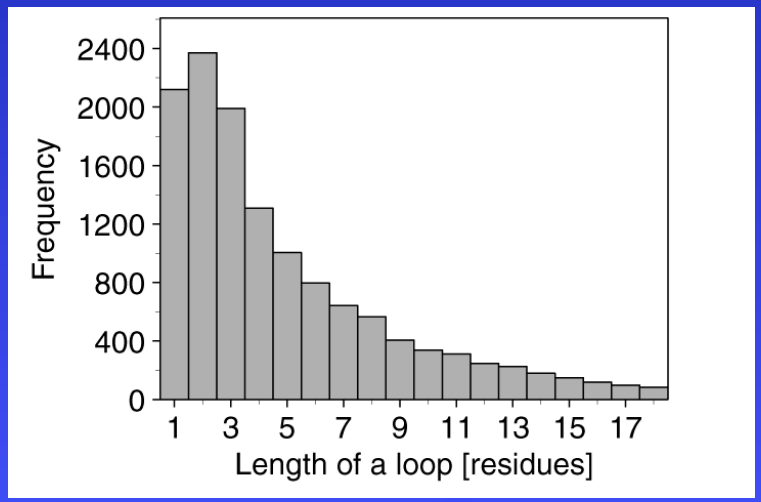

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Loop modeling strategies

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Database search Conformational search

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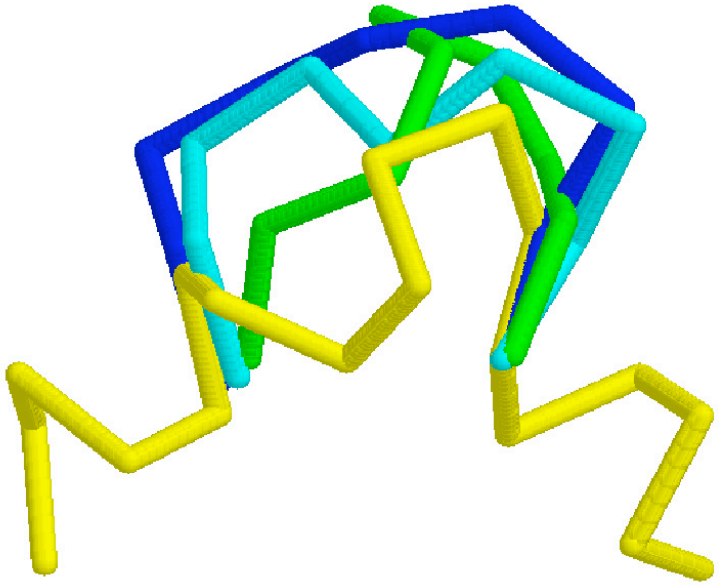

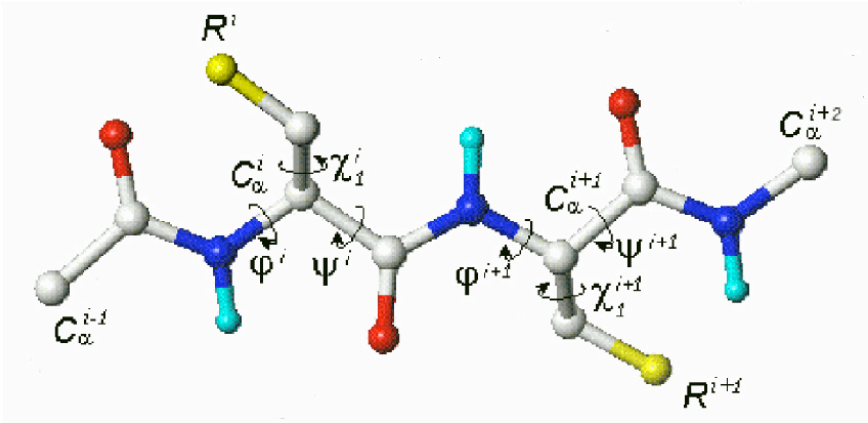

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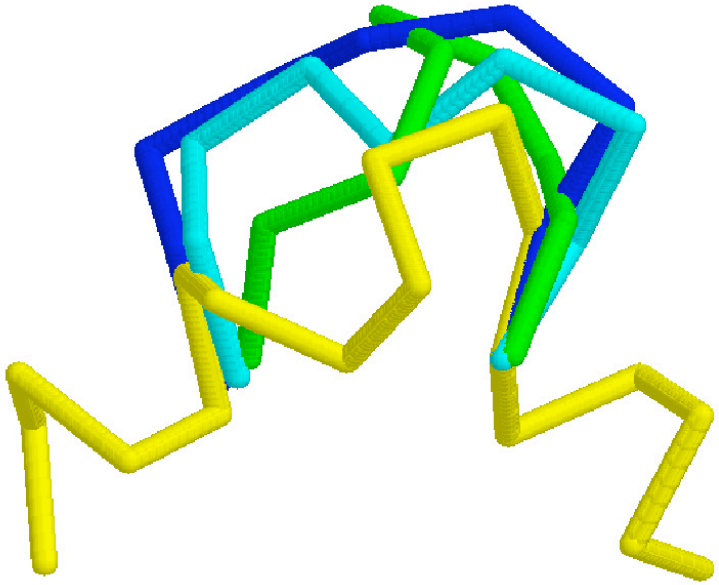

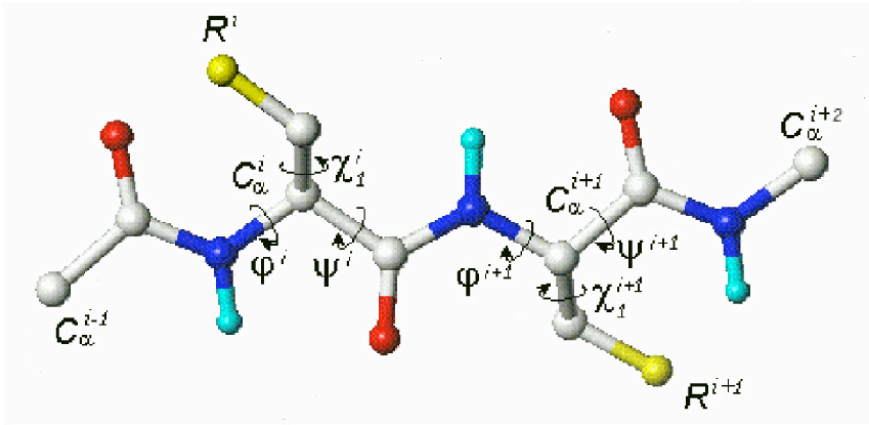

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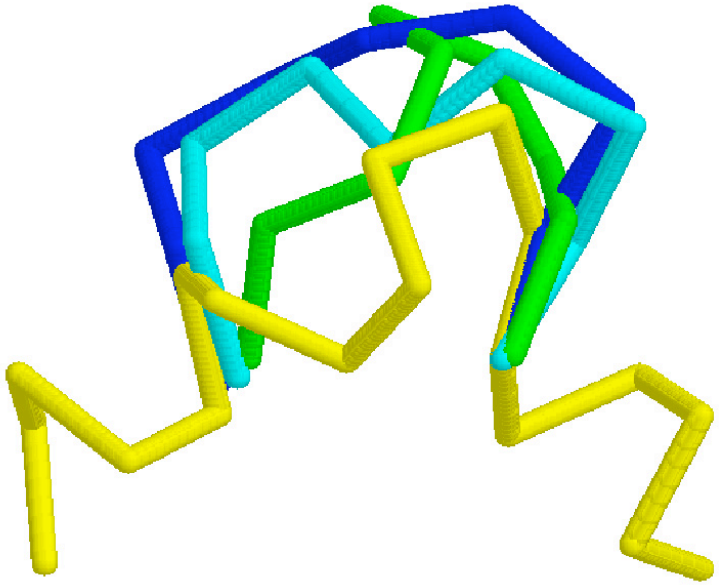

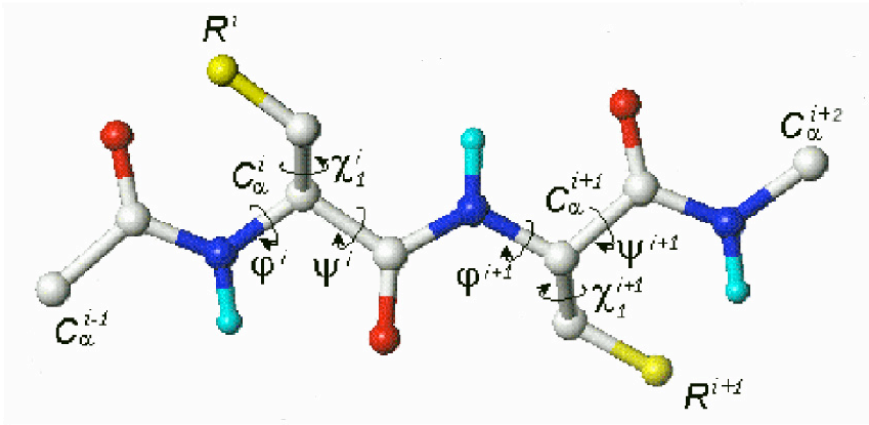

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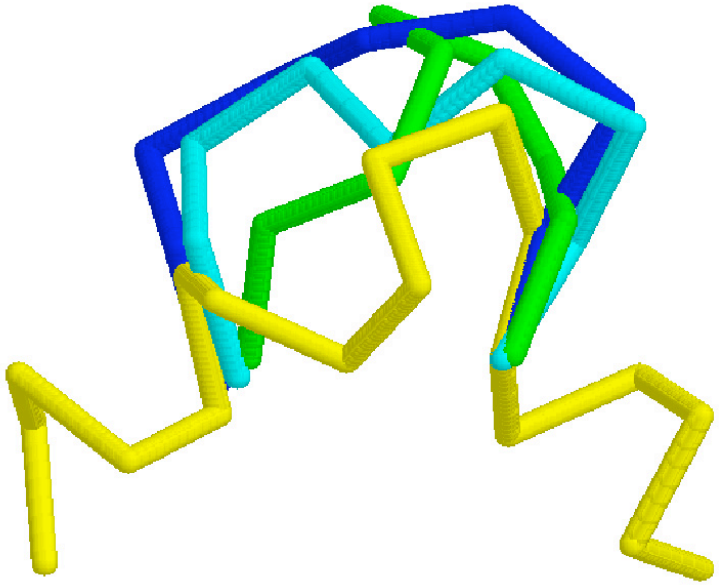

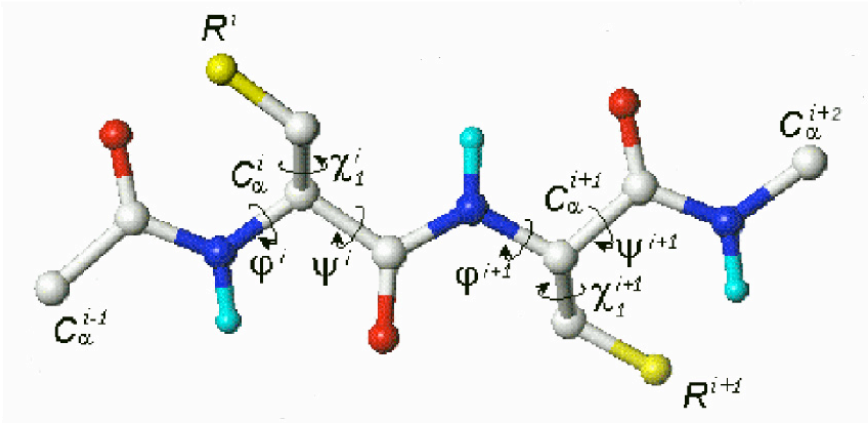

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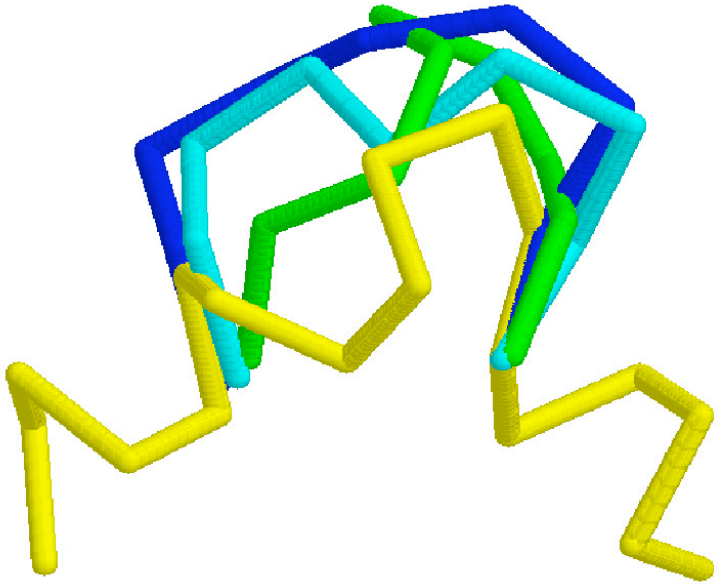

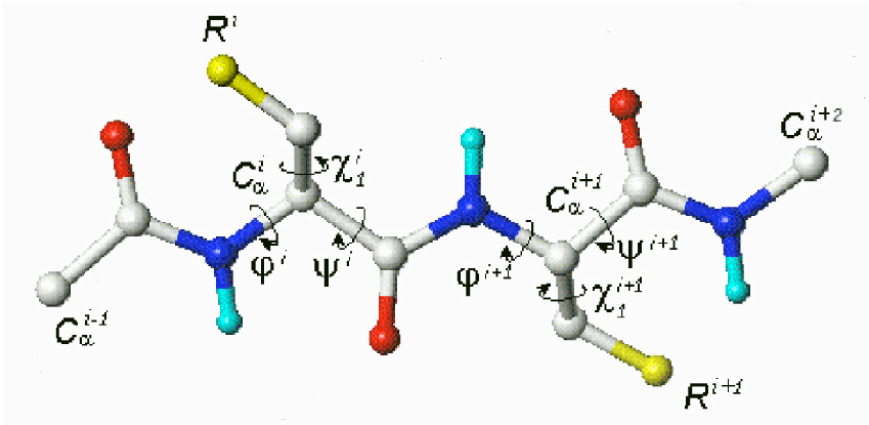

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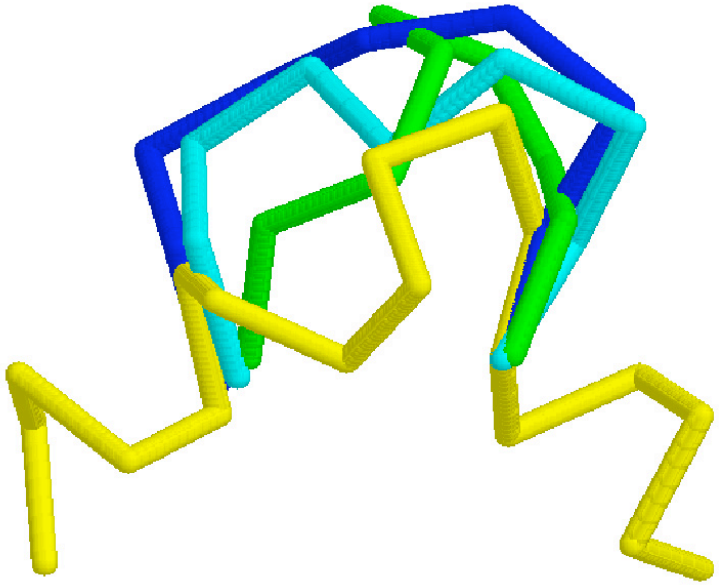

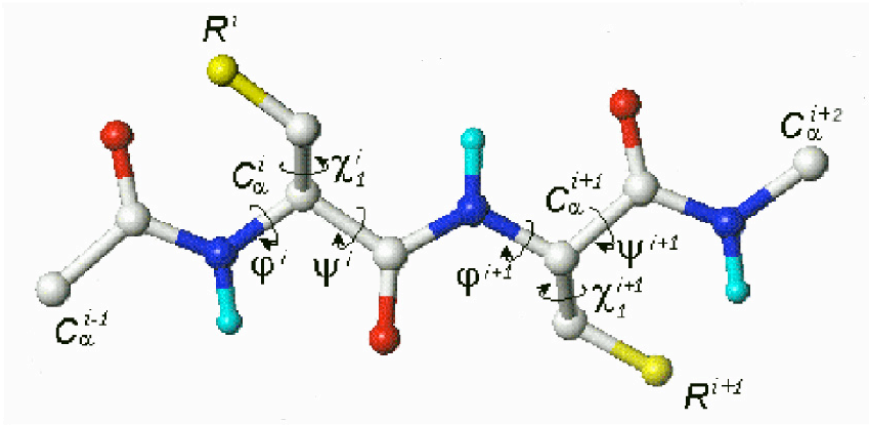

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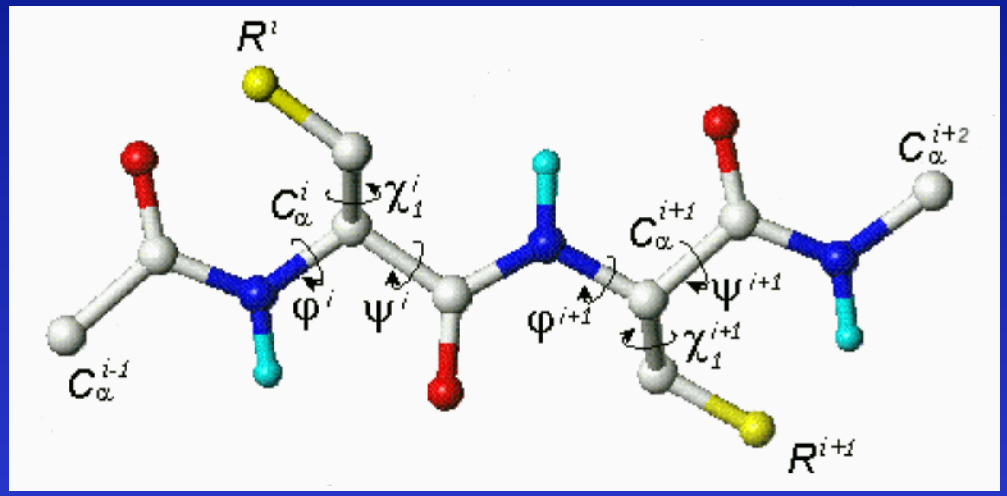
Loop Modeling by Conformational Search



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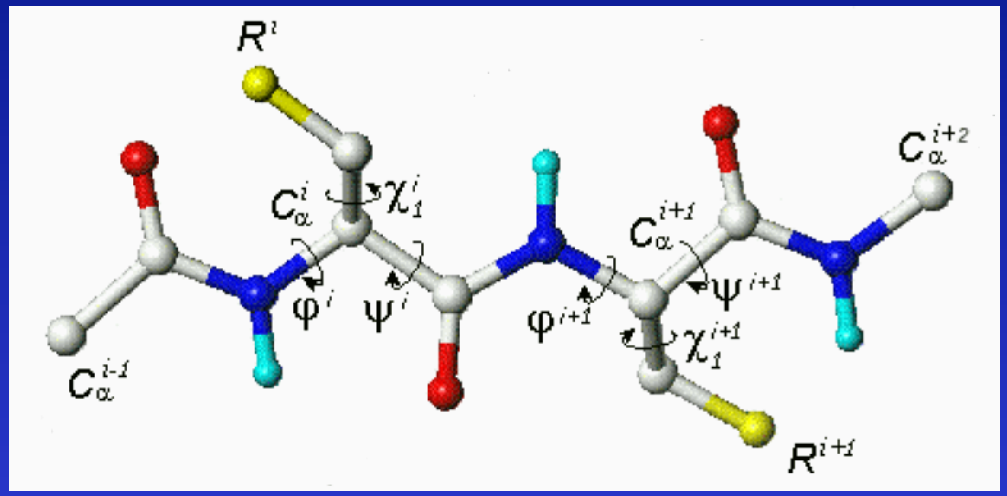

1. Protein representation.

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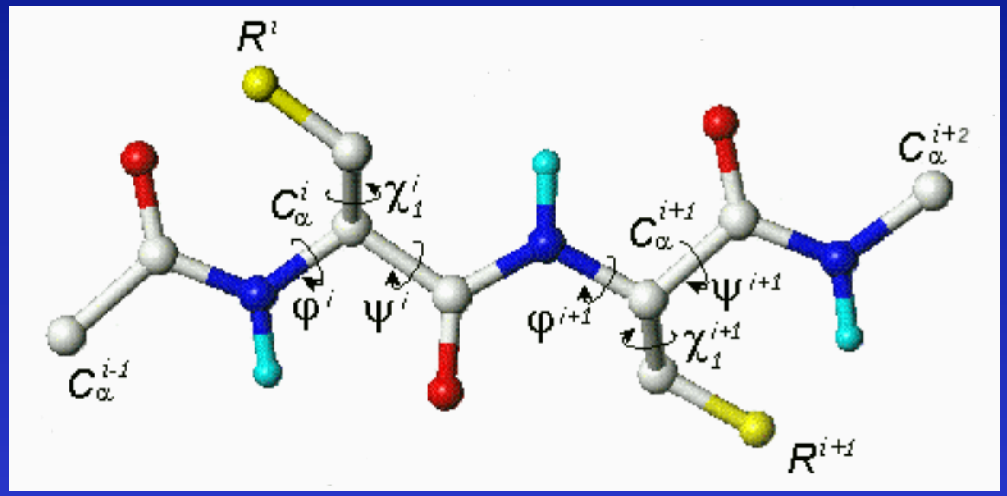

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2. Energy (scoring) function.

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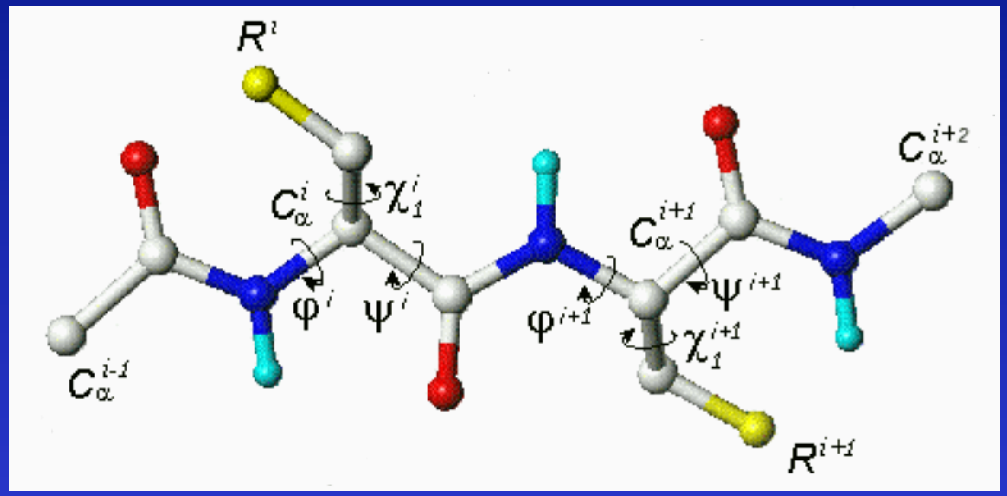

1. Protein representation.
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3. Optimization algorithm.

Energy Function for Loop Modeling

The energy function is a sum of many terms:

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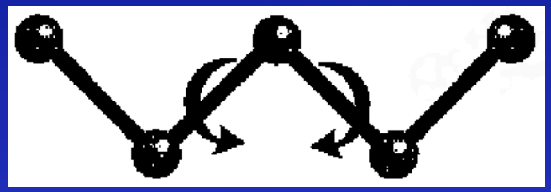

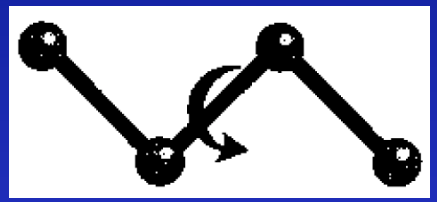

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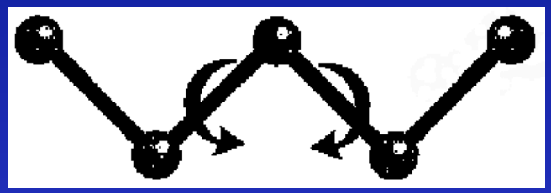
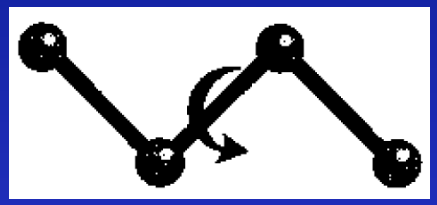
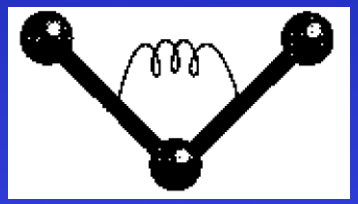
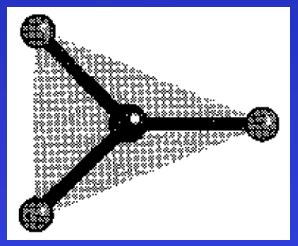
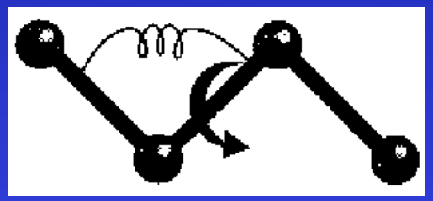




2) Restraints from the CHARMM-22 force field:







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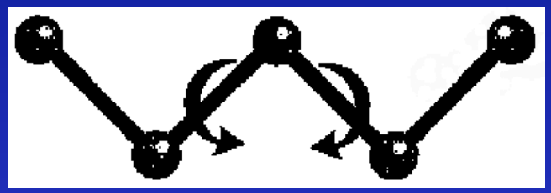

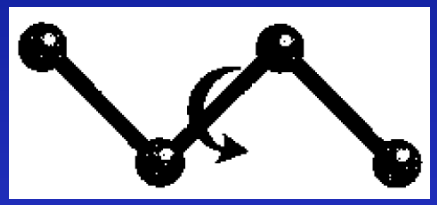

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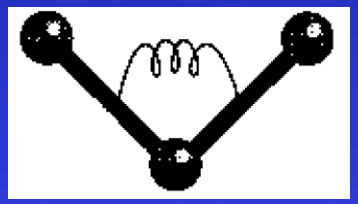
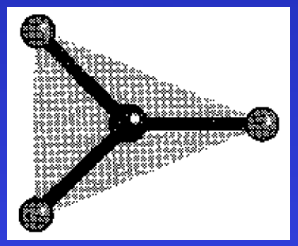
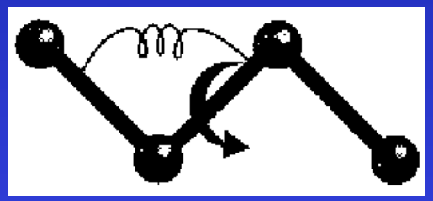
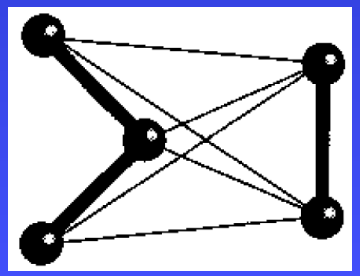






3) Statistical potential for non-bonded contacts:



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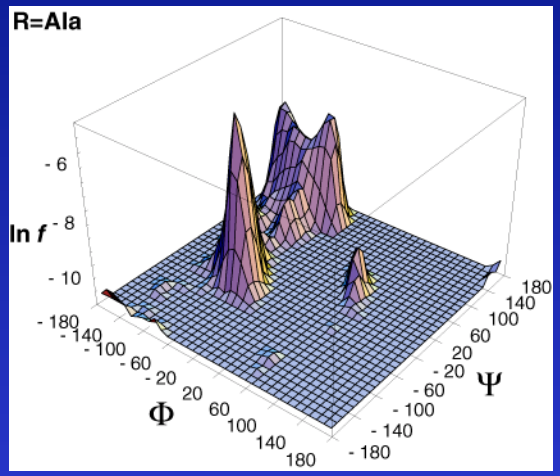

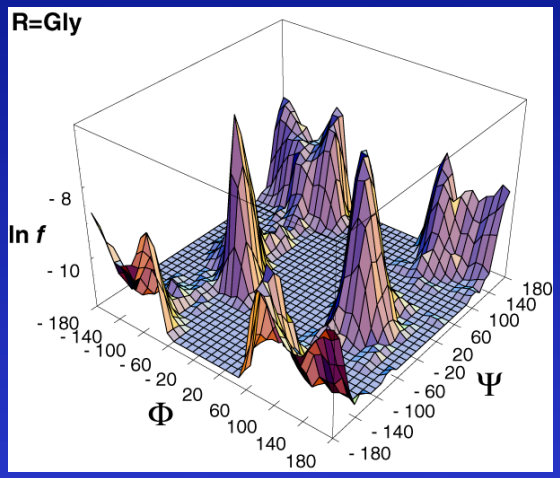

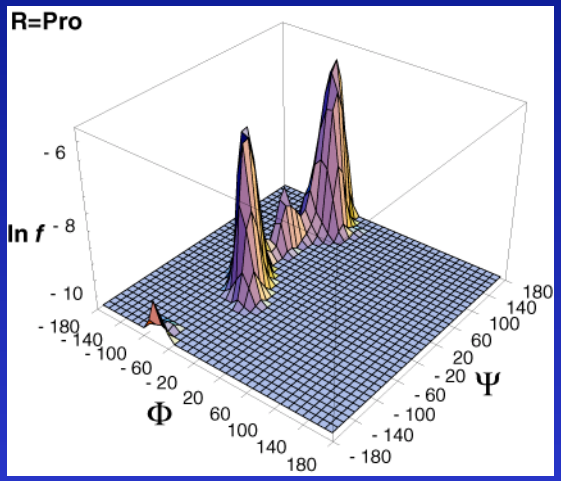

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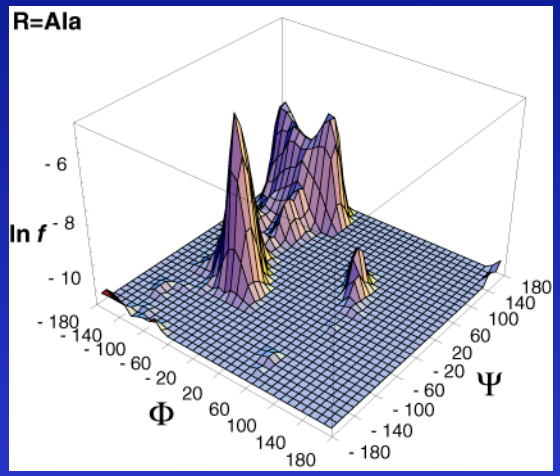
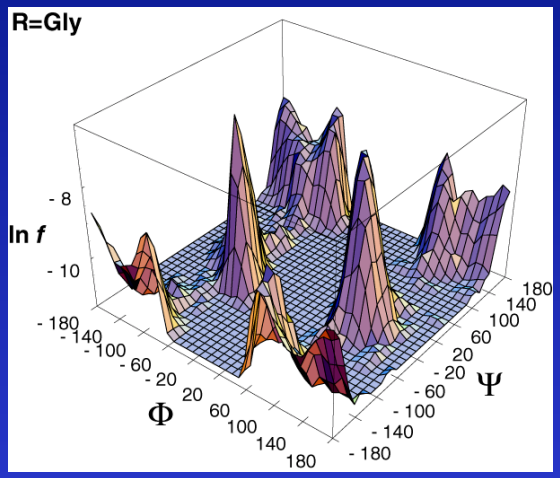
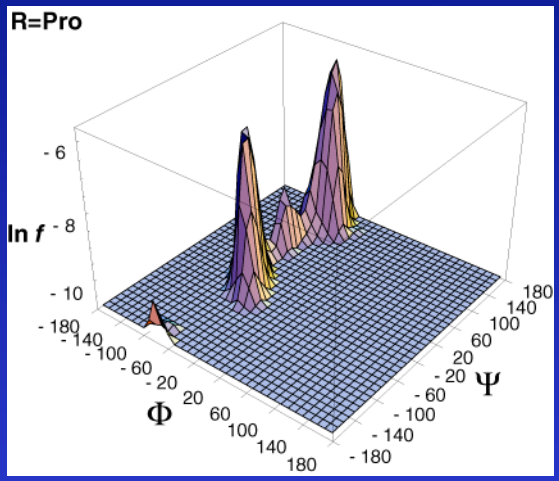
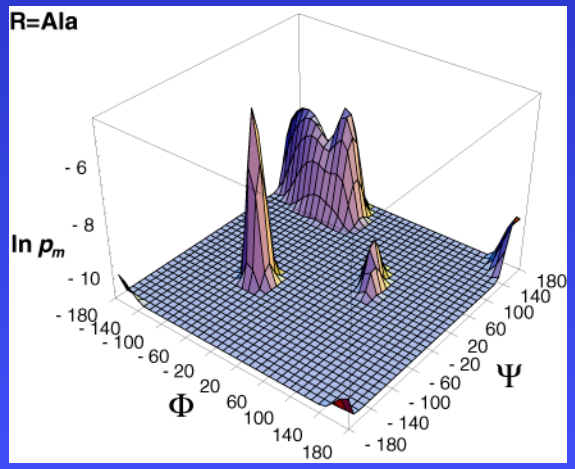
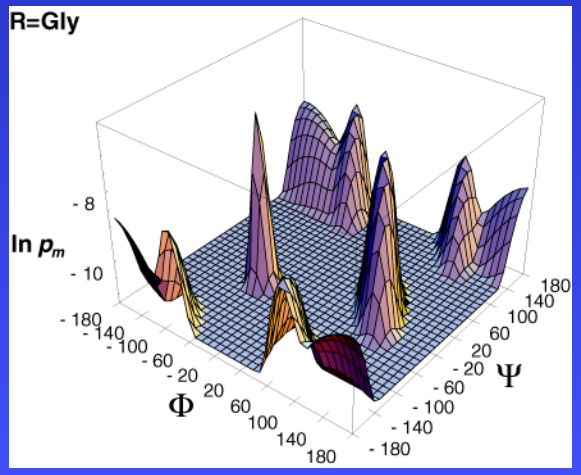
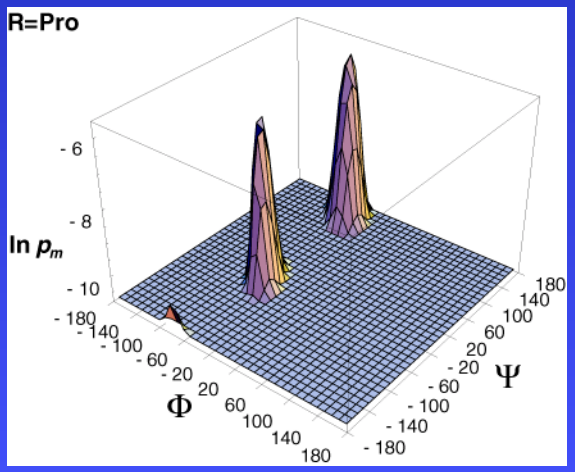






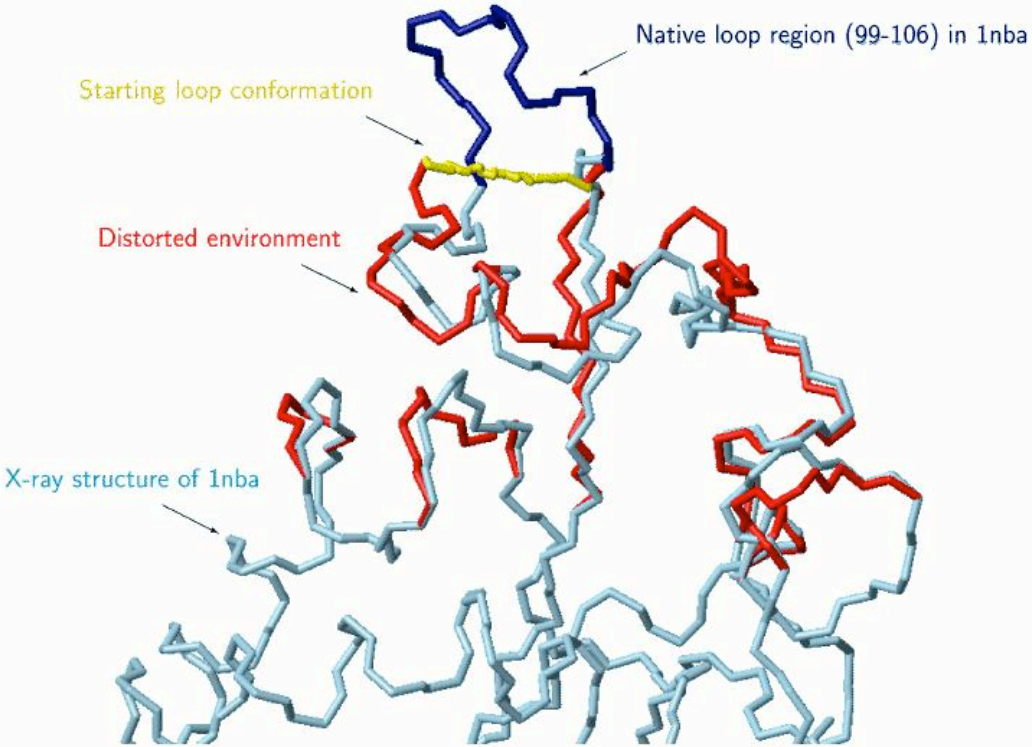






Optimization of Objective Function

- Test set: 40 randomly selected loops of known structures, for each length from 1 to 14 residues.
- Starting conformation: Loop atoms were spaced evenly on a line spanning the two anchor regions, then randomized by $\pm$ 5 Å.
- To simulate real comparative modeling situations, performance of the loop modeling problem was determined by predicting loops in only approximately correct environment.



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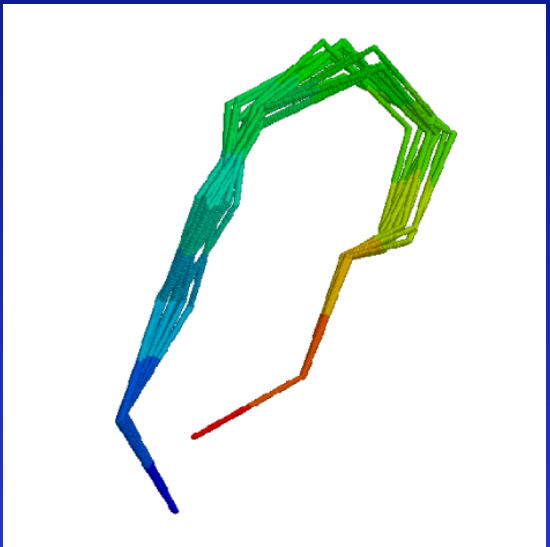

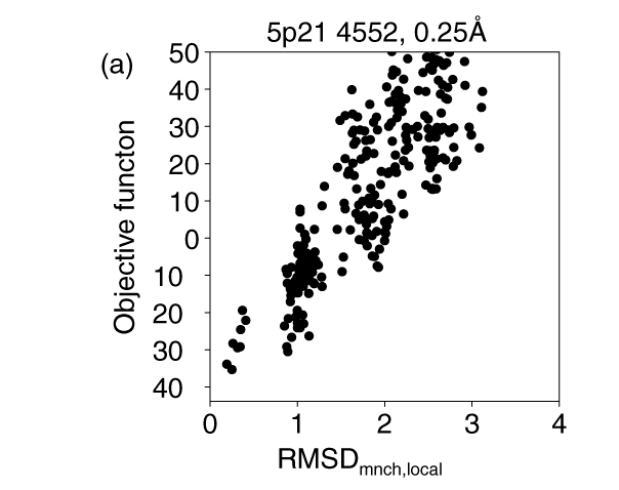

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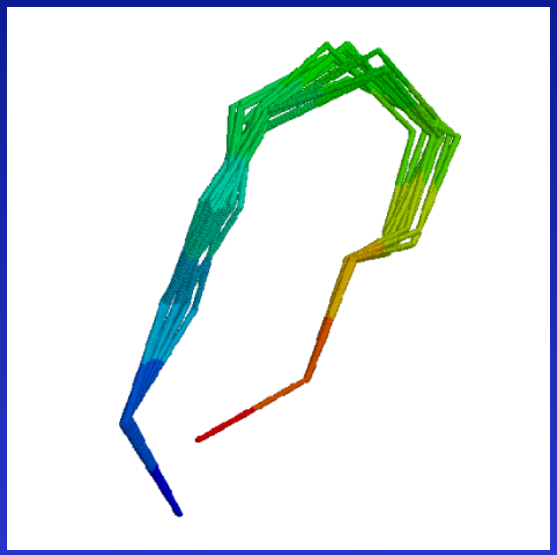

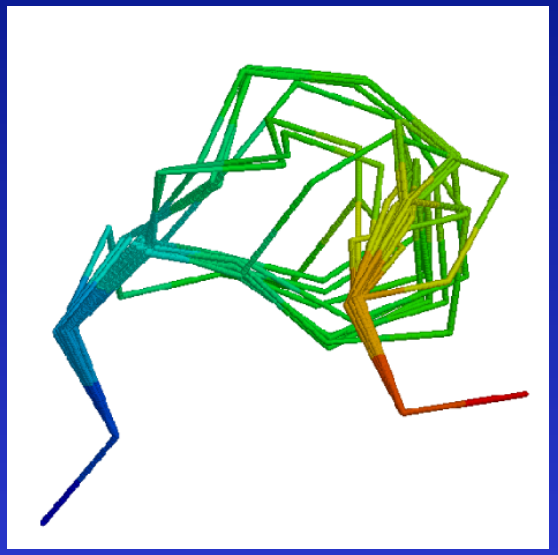

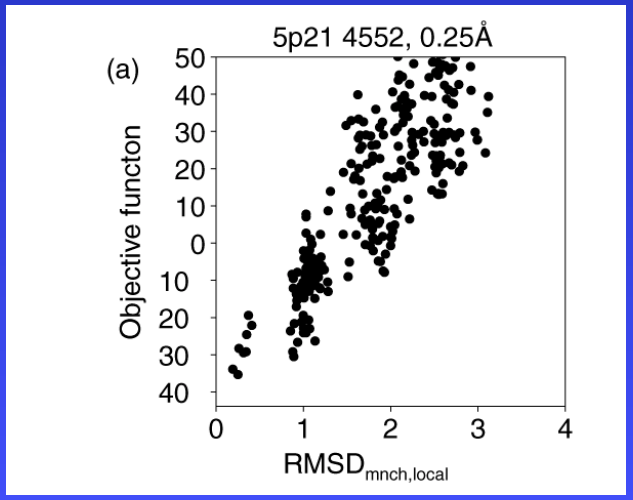

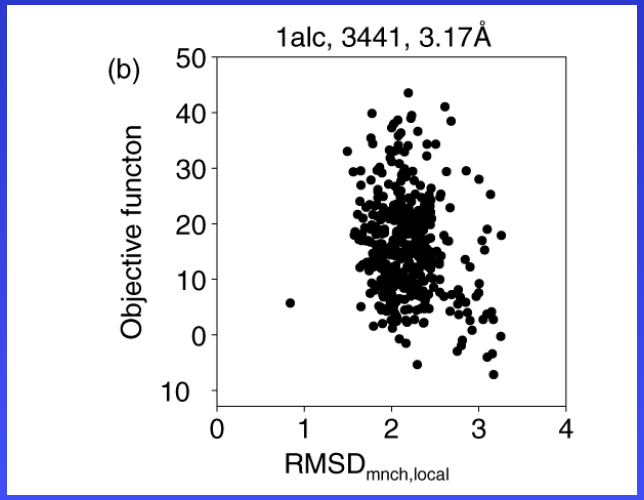

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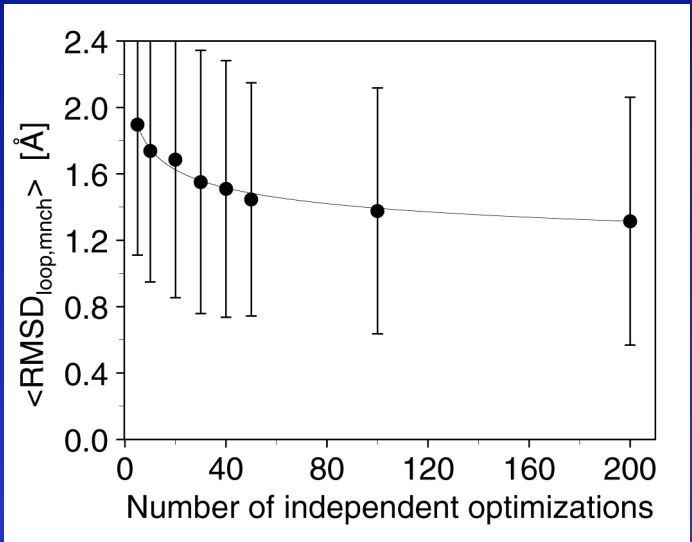

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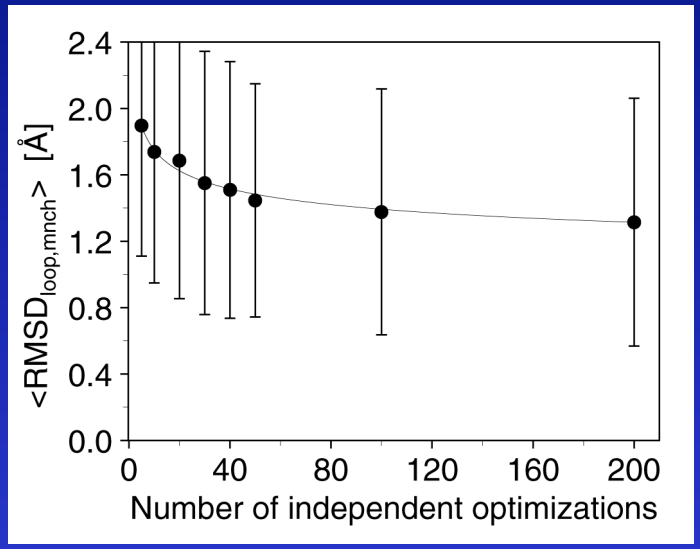
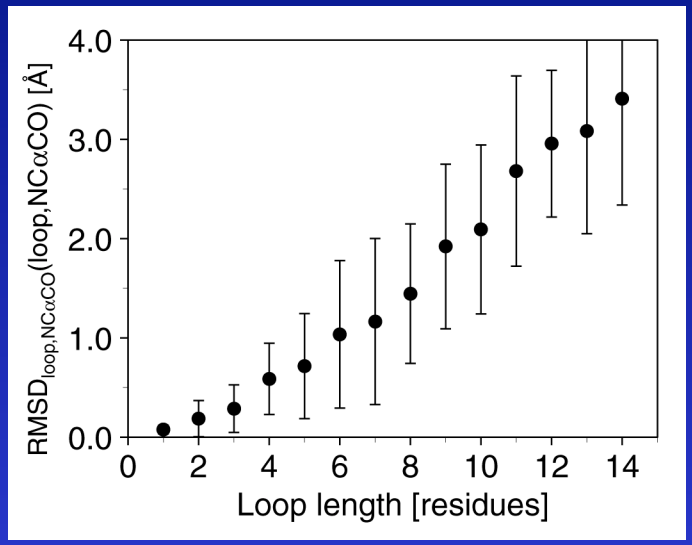
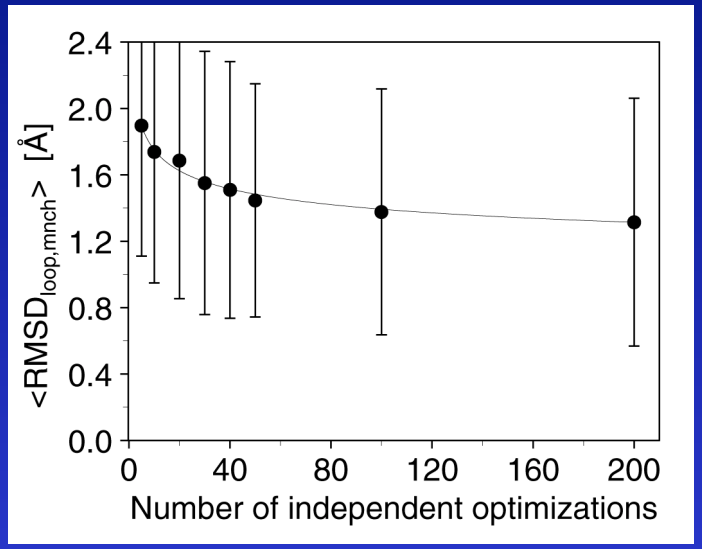
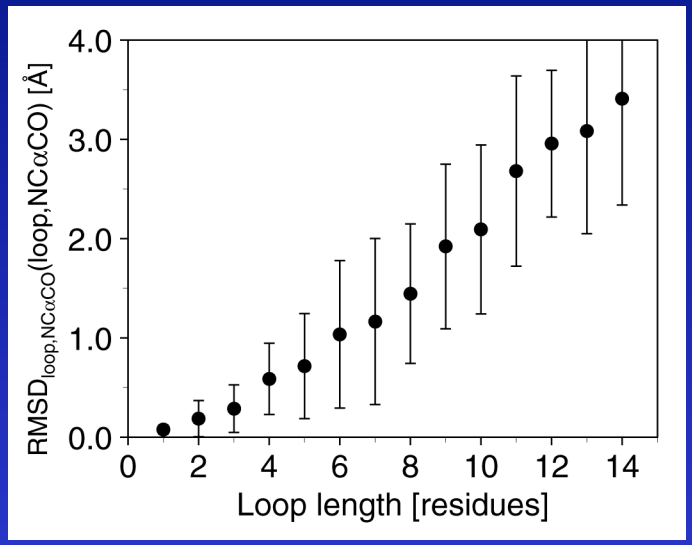
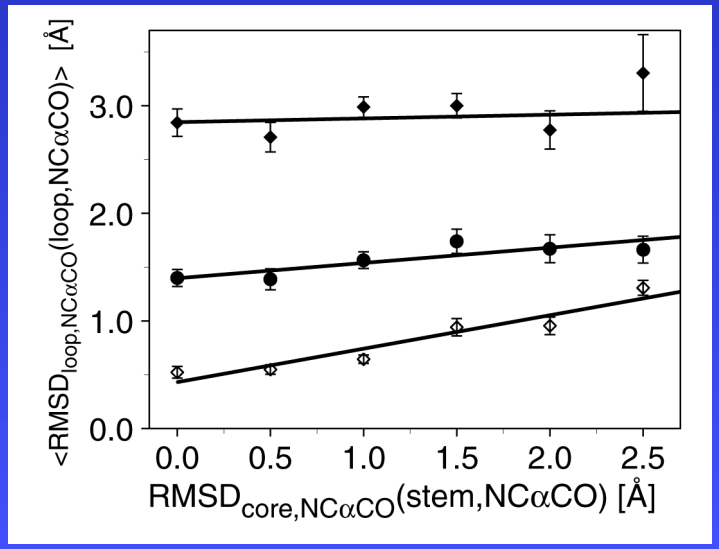




Accuracy of loop models



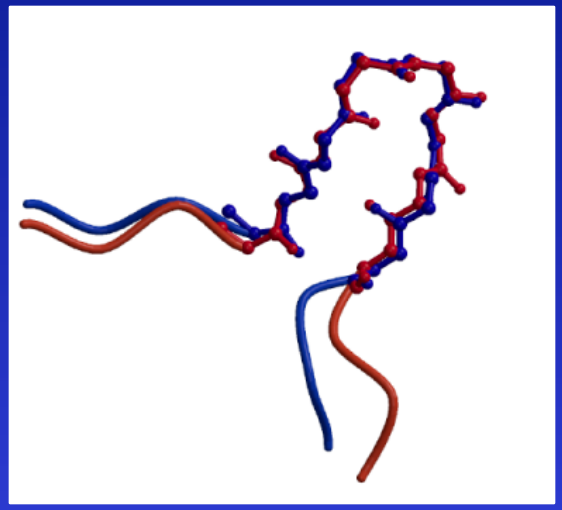




Accuracy of Loop Modeling

A. Fiser, R. Do & A. Šali, *Prot. Sci.*, **9**, 1537, 2000

Accuracy of Loop Modeling



HIGH ACCURACY (<1Å)

50% (30%) of 8-residue loops

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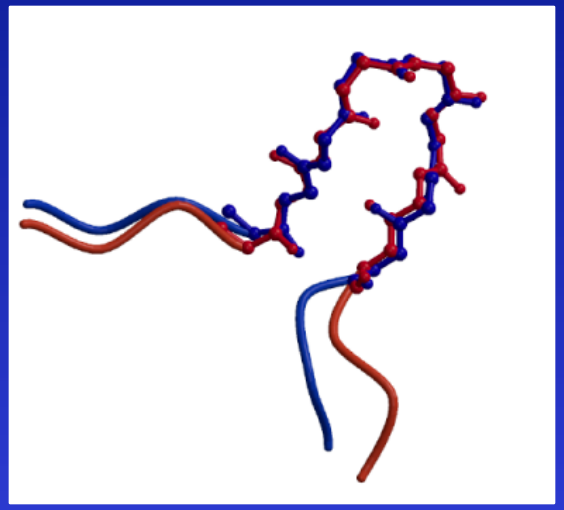
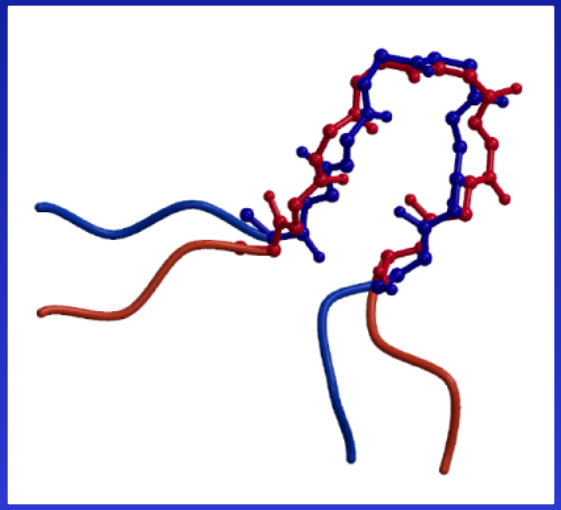




HIGH ACCURACY (<1Å)

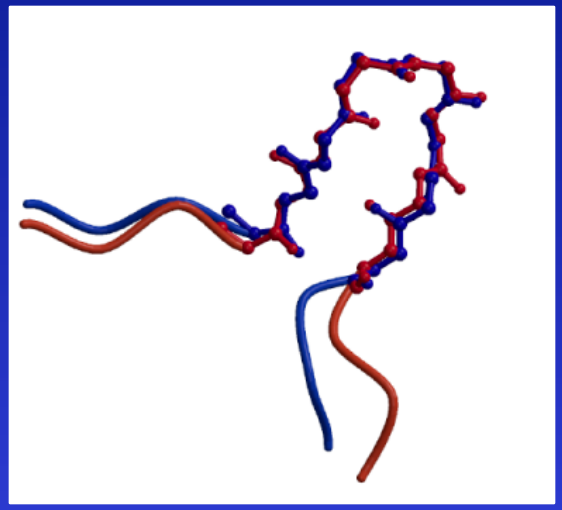
50% (30%) of 8-residue loops

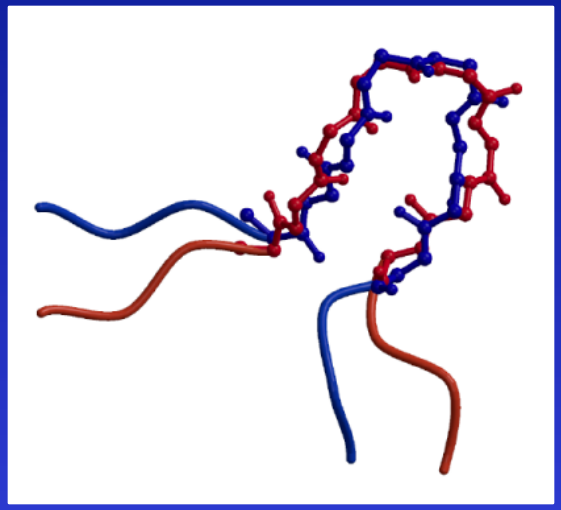
MEDIUM ACCURACY (<2Å)

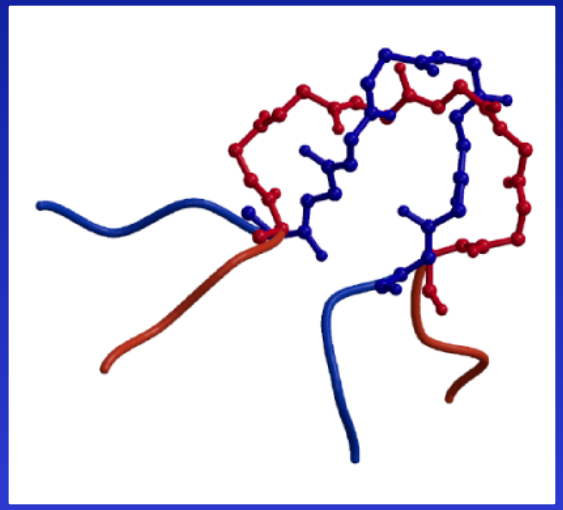
40% (48%) of 8-residue loops

A. Fiser, R. Do & A. Šali, *Prot. Sci.*, **9**, 1537, 2000

Accuracy of Loop Modeling







HIGH ACCURACY (<1Å)

50% (30%) of 8-residue loops

MEDIUM ACCURACY (<2Å)

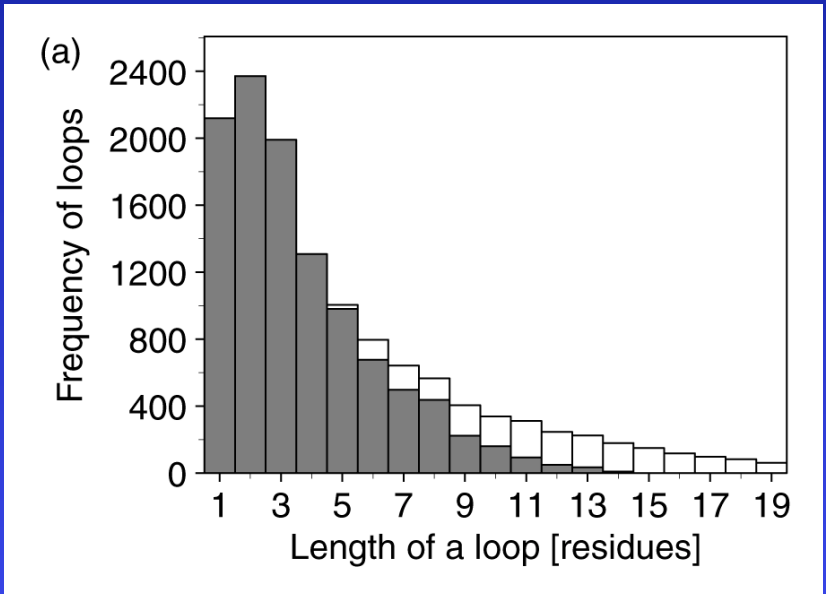
40% (48%) of 8-residue loops

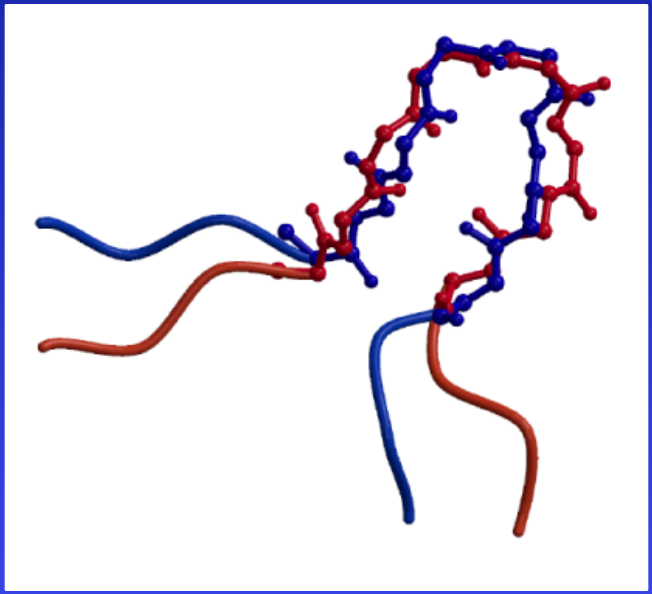
LOW ACCURACY (>2Å)

10% (22%) of 8-residue loops

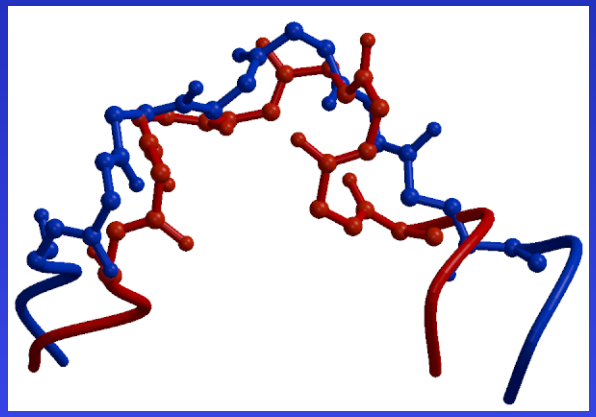
A. Fiser, R. Do & A. Šali, *Prot. Sci.*, **9**, 1537, 2000

Fraction of Loops Modeled With at Least Medium Accuracy

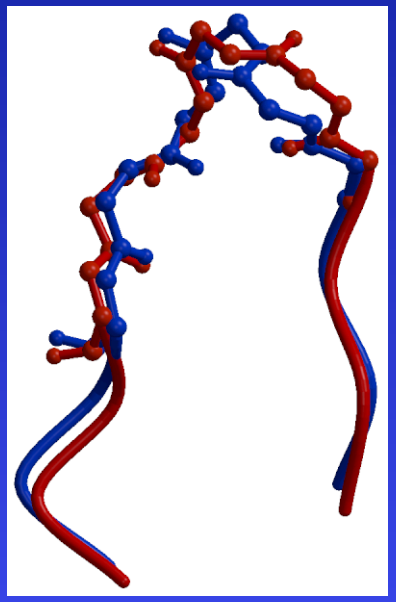




Problems in Practical Loop Modeling



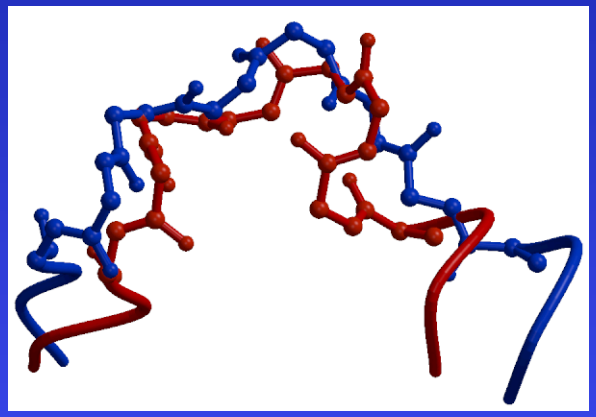
T0076: 46-53
$RMSD_{mnch}$ loop = 1.37 Å
$RMSD_{mnch}$ anchors = 1.52 Å



T0058: 80-85
$RMSD_{mnch}$ loop = 1.09 Å
$RMSD_{mnch}$ anchors = 0.29 Å

Problems in Practical Loop Modeling

1. Decide which regions to model as loops.



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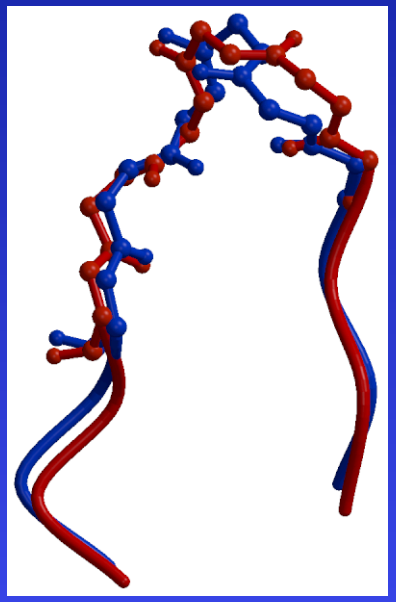

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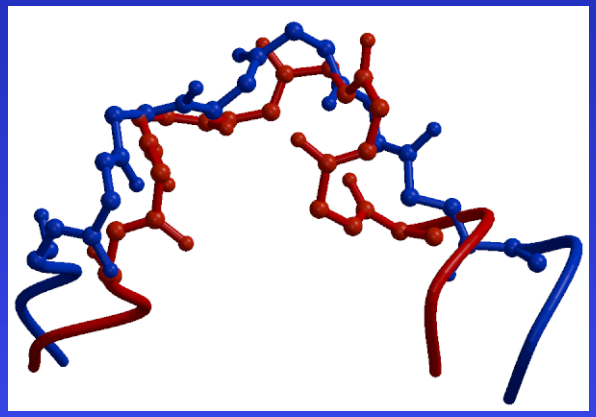
Problems in Practical Loop Modeling

1. Decide which regions to model as loops.
2. Correct alignment of anchor regions & environment.



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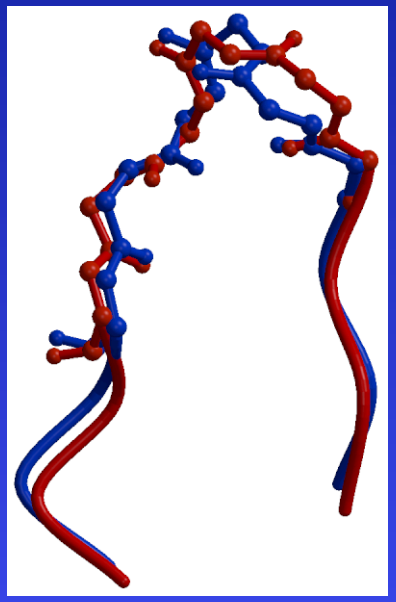

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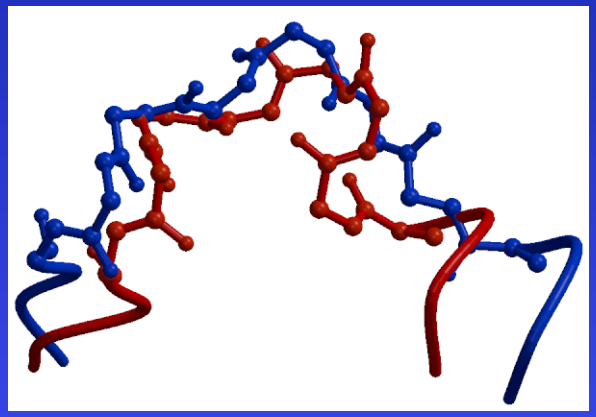
3. Modeling of a loop.



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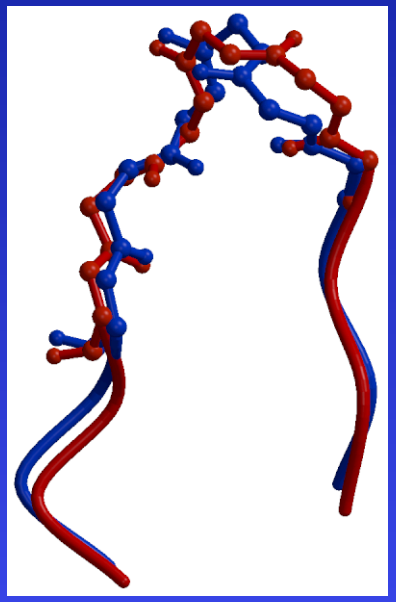

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Summary

- ✓ What is comparative modeling and why is it useful?
- ✓ Steps in CM (overview + some details)
- ✓ Accuracy of comparative models
- ✓ Loop modeling
- ✓ CM and Structural Genomics

Structural Genomics

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

- ✓ **Definition:**

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

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

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Šali & Kuriyan. *TIBS* **22**, M20, 1999.

Burley *et al*. *Nat. Genet*. **23**, 151, 1999.
Sanchez *et al. Nat. Str. Biol.* 7, 986, 2000

How can Comparative Modeling be used in Structural Genomics?

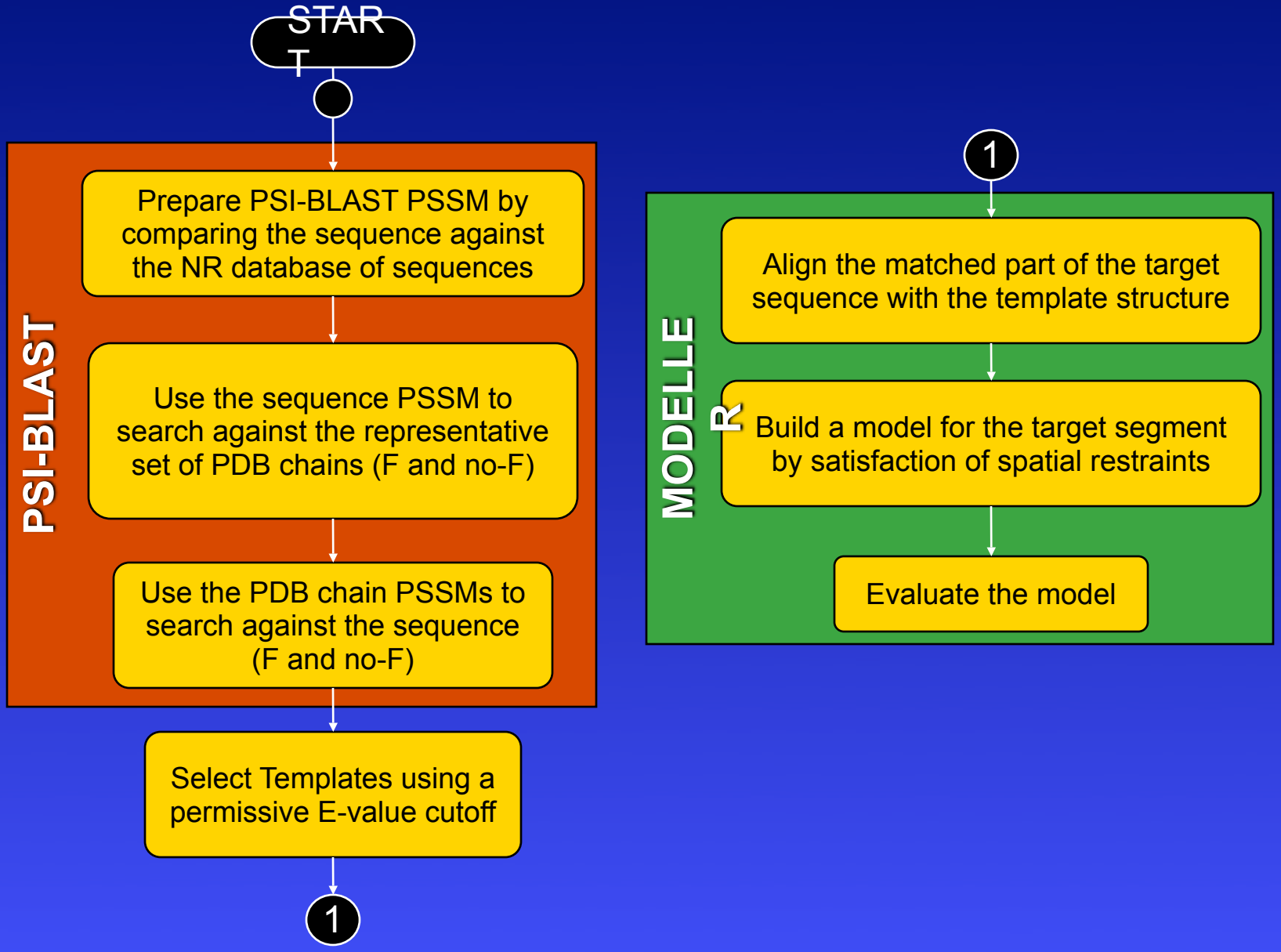
- Target Selection

  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



R. Sánchez & A. Šali, *Proc. Natl. Acad. Sci. USA* **95**, 13597, 1998
R. Sánchez, F. Melo, N. Mirkovic, A. Šali, in preparation

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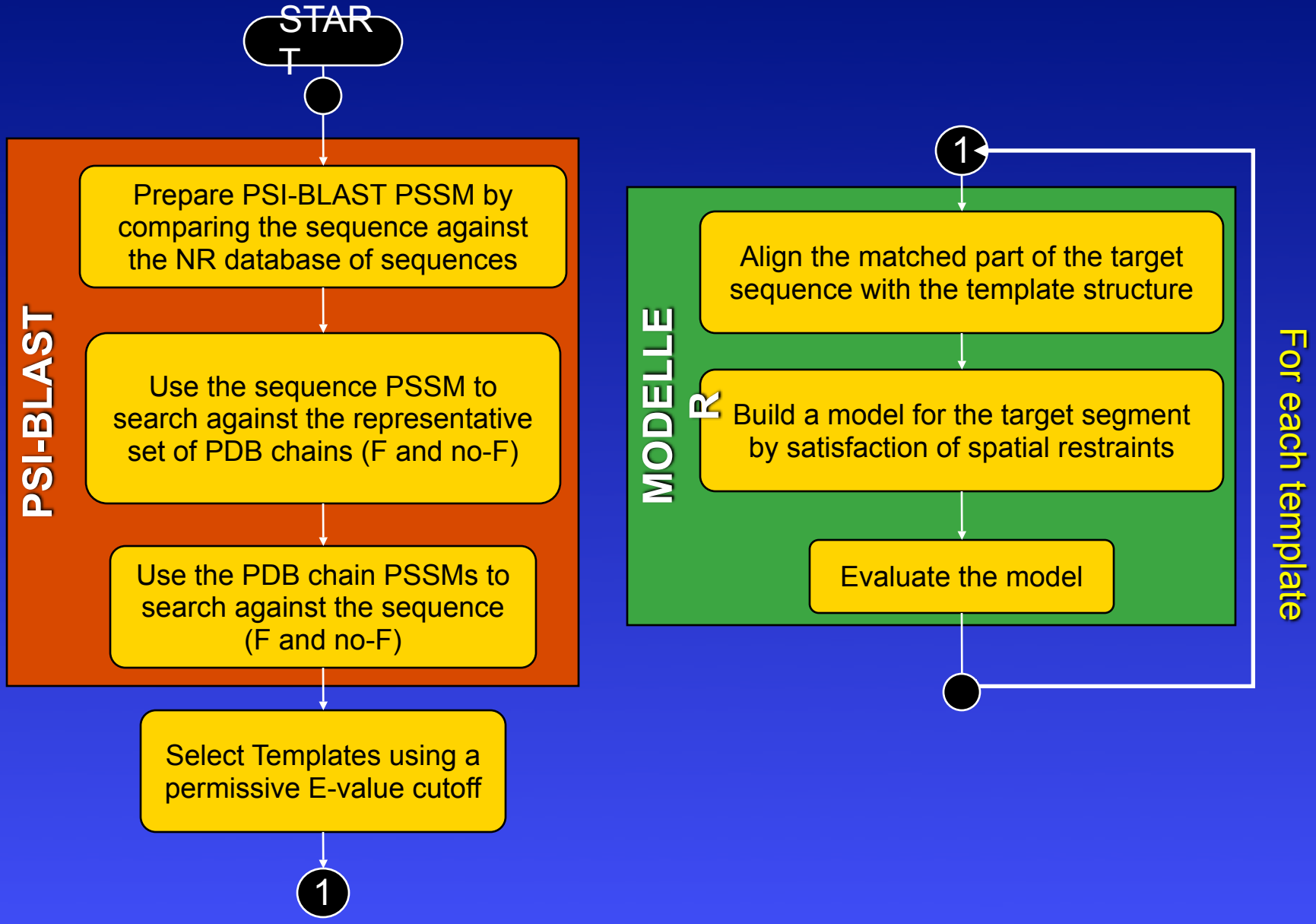

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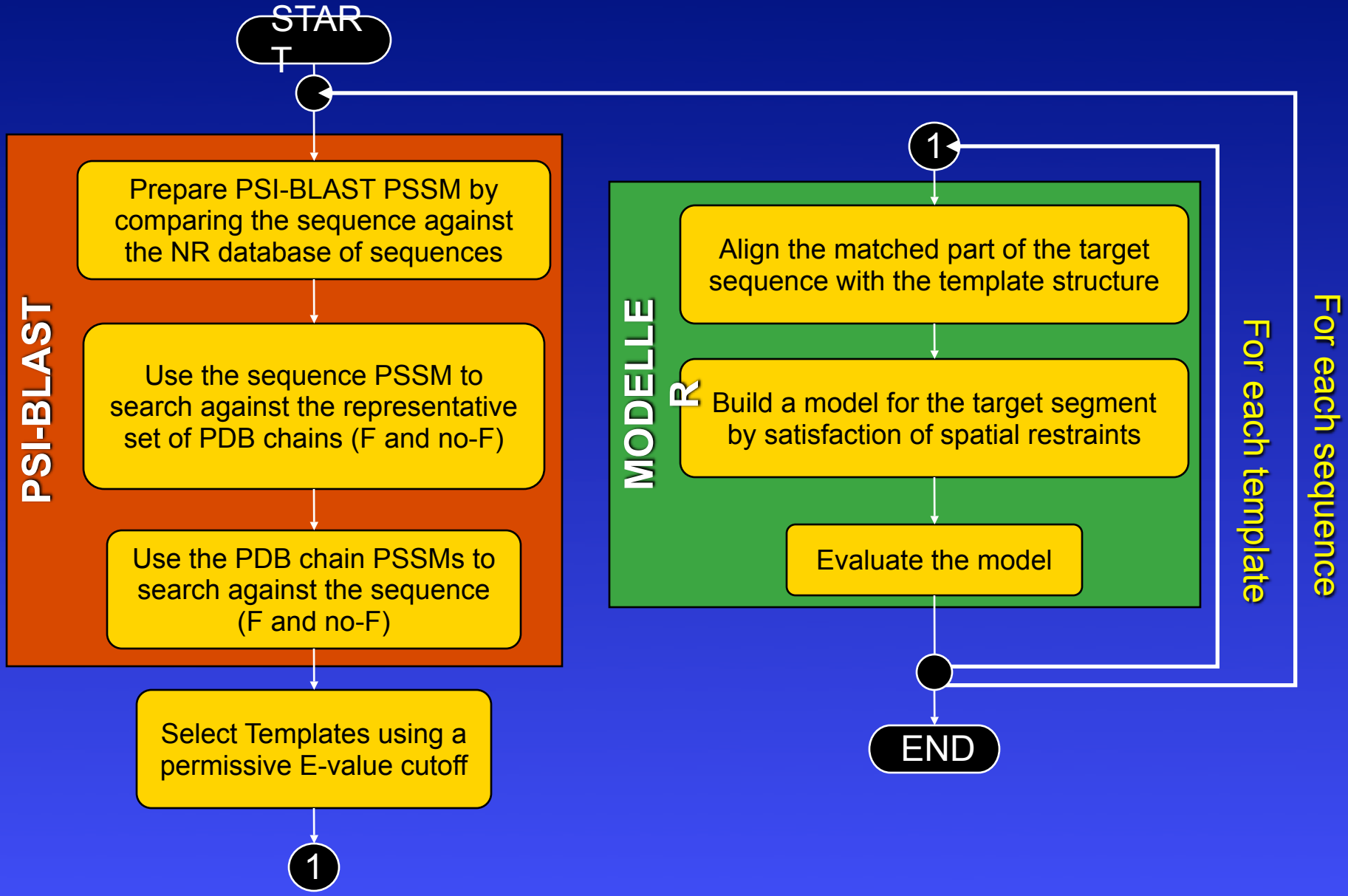

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Comparative modeling of the TrEMBL database

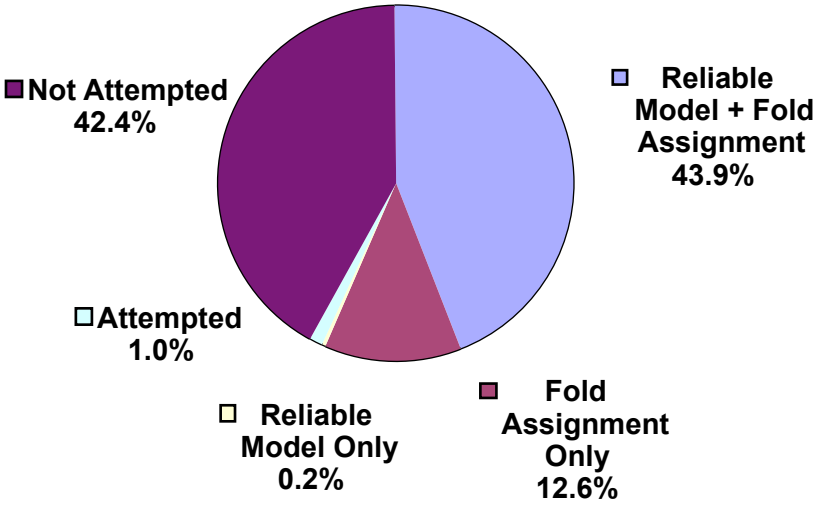
Fold Assignments

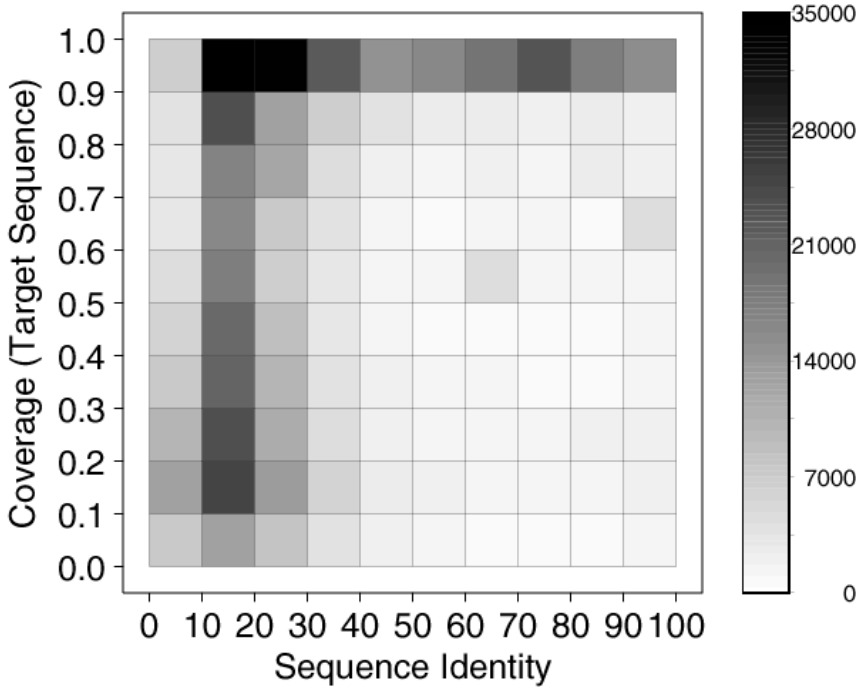
- ✓Reliable fold assignments: 620,370
- ✓Sequences with reliable folds: 304,517 (56%)
- ✓Average length of queries: 514 amino acids
- ✓Average length of folds: 226 amino acids

Comparative Models

- ✓Reliable models: 392,397
- ✓Sequences with reliable models: 237,143 (44%)
- ✓Structures used as templates: 5523 (90%)

Modeling Coverage Of The Sequence Space





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

Organism Statistics

Top 10 organism by number of models

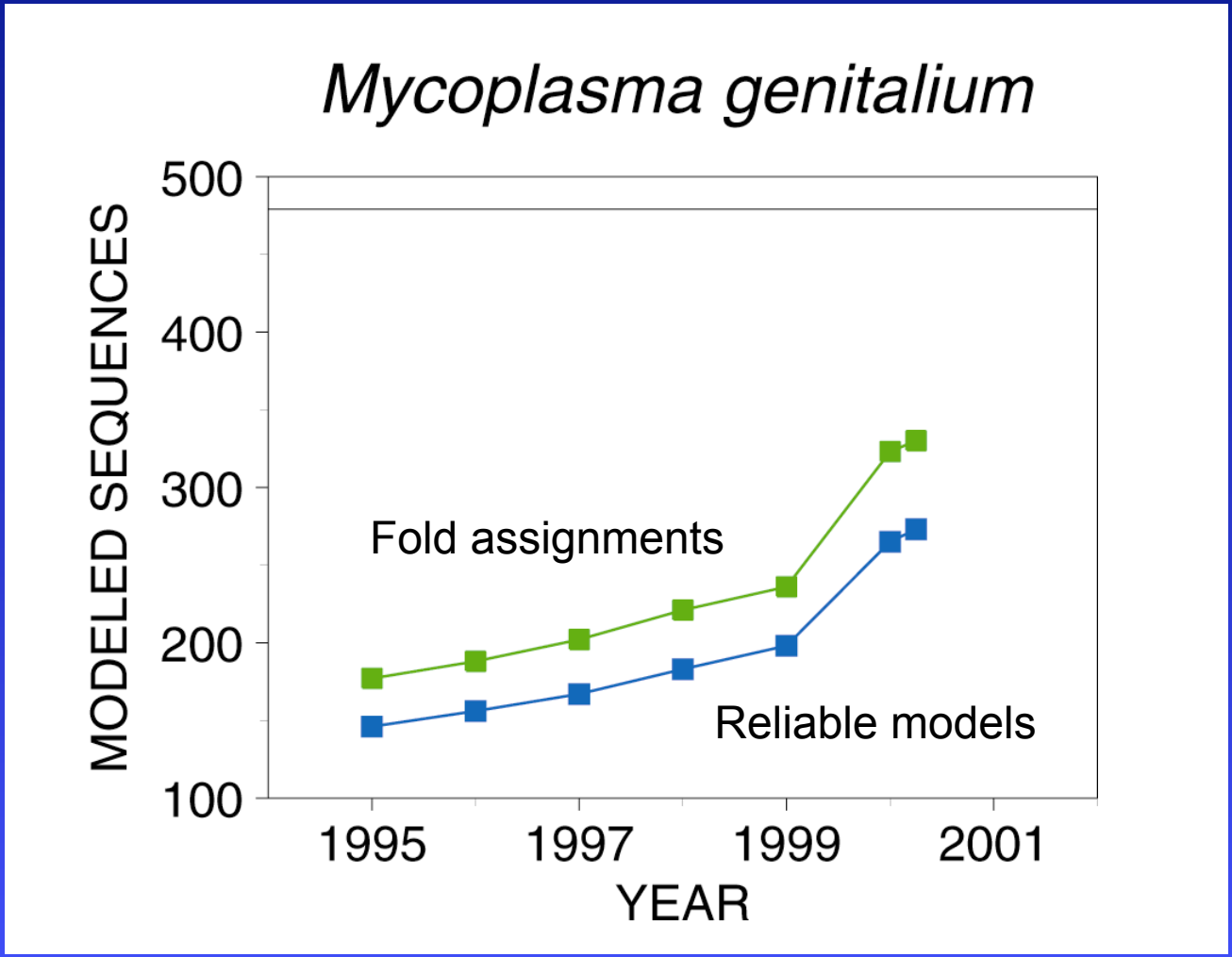
Organism	# sequences	# models	models/ seq#	# CATH folds
Homo sapiens	13,785	37,638	2.73	315
HIV type 1	25,654	33,180	1.29	12
D. melanogaster	8,248	25,314	3.06	299
C. elegans	7,260	20,095	2.76	289
A. thaliana	8,852	18,695	2.11	294
Mus musculus	6,232	17,248	2.76	271
R. norvegicus	3,586	9,299	2.59	246
S. cerevisiae	2,580	5,749	2.22	237
S. Pombe	2,315	4,497	1.94	221
E. coli	2,862	4,333	1.51	259

Organism Statistics

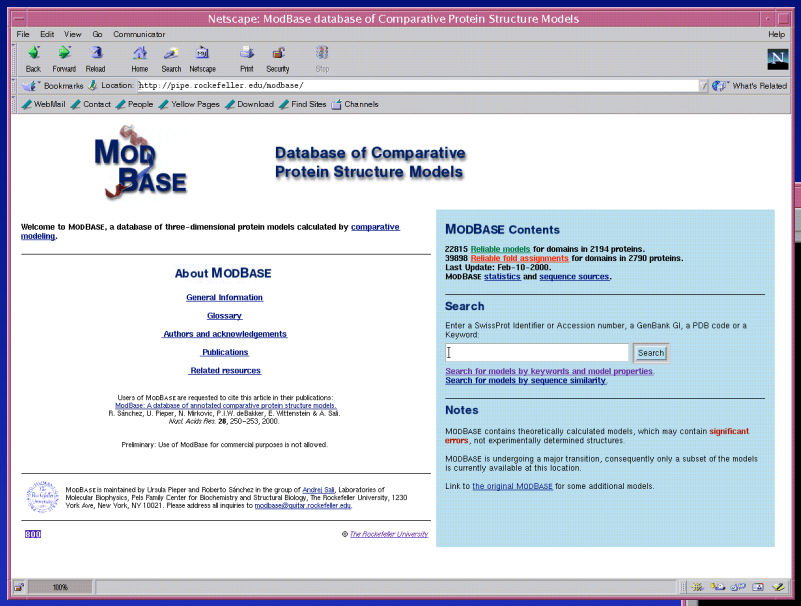
Top 10 organism by number of models

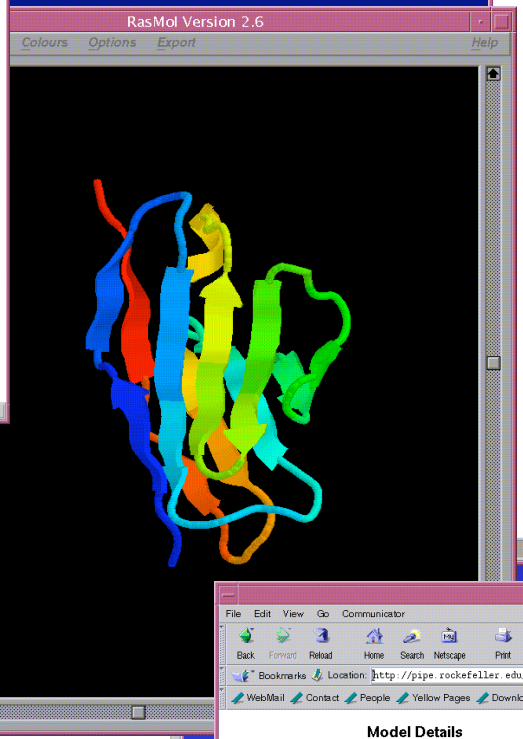
Organism	Avg. seq. length	Avg. model length	Avg. Sequence coverage	"Organism" coverage
Homo sapiens	517	191	0.55	0.36
HIV type 1	165	124	0.84	0.75
D. melanogaster	634	209	0.47	0.32
C. elegans	563	209	0.50	0.37
A. thaliana	480	218	0.55	0.45
Mus musculus	510	191	0.53	0.37
R. norvegicus	511	207	0.57	0.40
S. cerevisiae	590	255	0.55	0.43
S. Pombe	527	247	0.58	0.46
E. coli	367	248	0.75	0.67

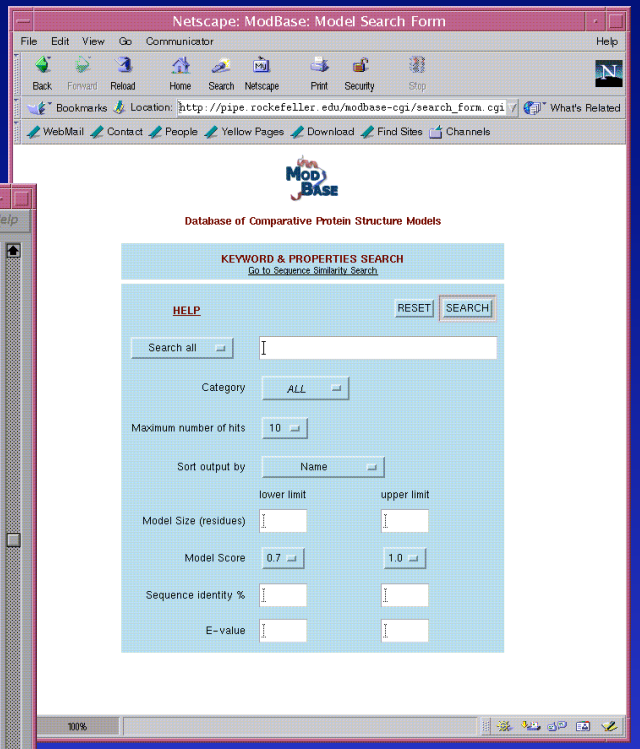
Factors affecting coverage: PDB growth

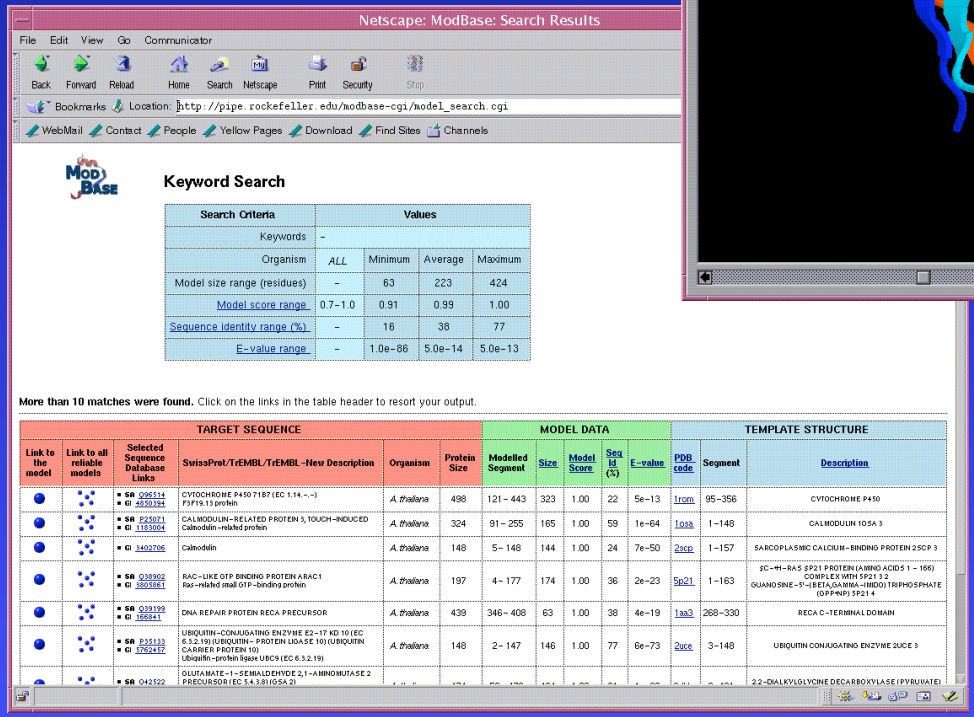


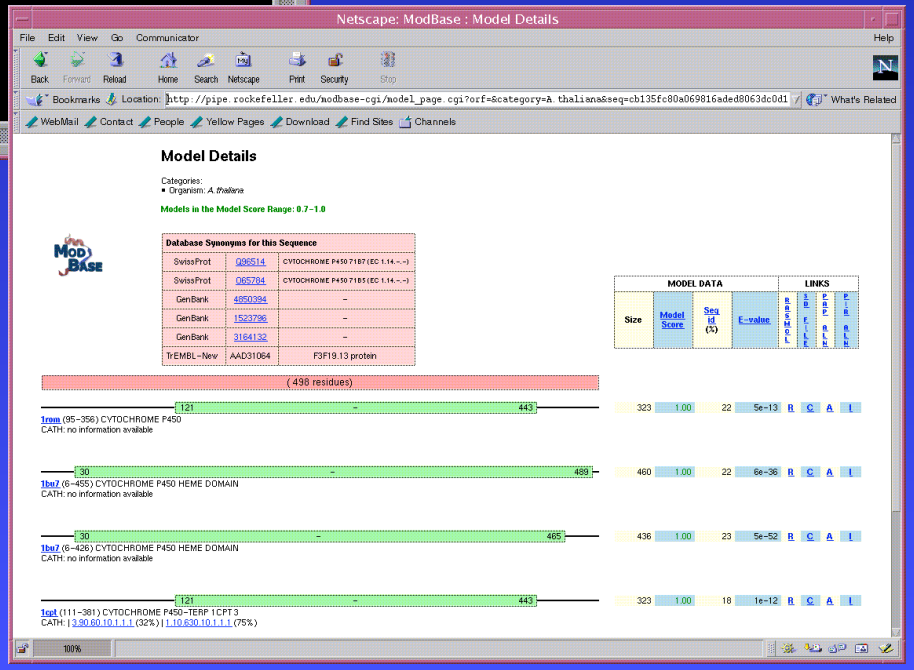
MODBASE











http://pipe.rockefeller.edu/modbase/

Conclusions

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- ✓ 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.

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- ✓ 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

Burroughs Wellcome Fund

Andrej Šali
Frank Alber
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Narayanan Eswar
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Nebojša Mirković
Ursula Pieper
Andrea Rossi

http://guitar.rockefeller.edu