Comparative protein structure modeling of genes, genomes and complexes

Marc A. Marti-Renom

Department of Biopharmaceutical Sciences
University of California, San Francisco

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Modelat de gens, genomes i complexos

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Modelat de gens, genomes i complexos

Modelado estructural de genes, genomas y complejos. Aplicaciones biomédicas y biotecnológicas.

Ya po', modelando ene estructuras de genes, genomas y complejos… cachai?

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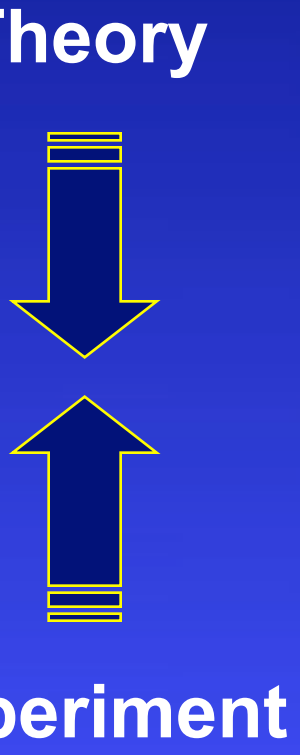
Modelado por homología…

Why protein structure prediction?

	Y 2003	Y 2005
Sequences	1,000,000	millions
Structures	18,000	50,000

Why protein structure prediction?

	Y 2003
Sequences	1,000,000
Structures	18,000



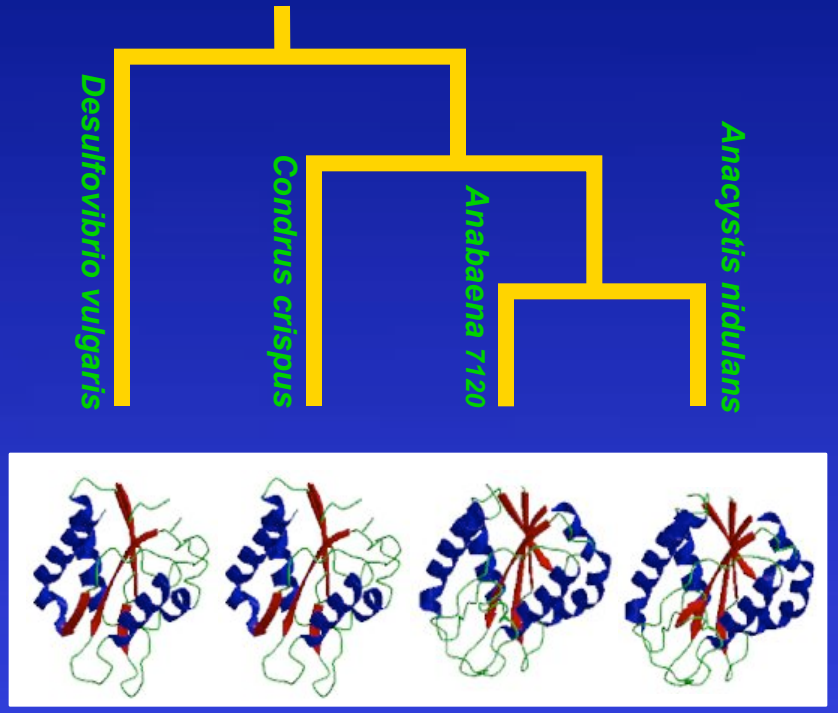
Why protein structure prediction?

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

http://salilab.org/modbase

Principles of Protein Structure

GFCHIKAYTRLIMVG…



Folding

Ab initio prediction

Evolution

Threading
Comparative Modeling

Comparative Modeling by Satisfaction of Spatial Restraints (MODELLER)

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

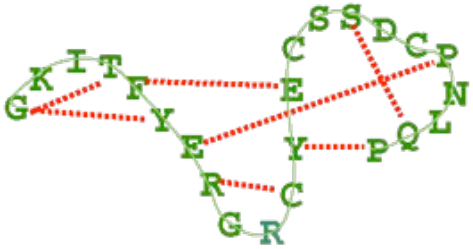
A. Šali & T. Blundell. *J. Mol. Biol.* **234**, 779, 1993.
J.P. Overington & A. Šali. *Prot. Sci*. **3**, 1582, 1994.
A. Fiser, R. Do & A. Šali. *Prot Sci*. **9**, 1753, 2000.

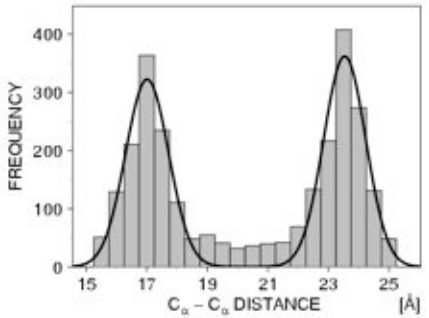
http://salilab.org/modeller

Comparative Modeling by Satisfaction of Spatial Restraints (MODELLER)

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

1. Extract spatial restraints





A. Šali & T. Blundell. *J. Mol. Biol.* **234**, 779, 1993.
J.P. Overington & A. Šali. *Prot. Sci.* **3**, 1582, 1994.
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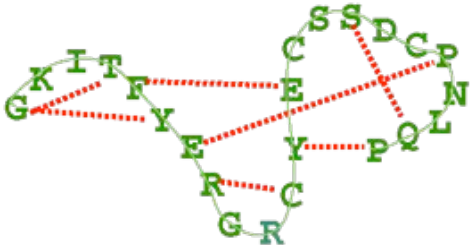
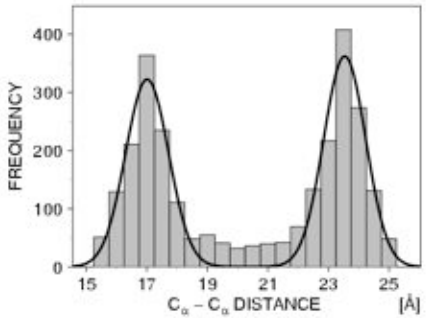
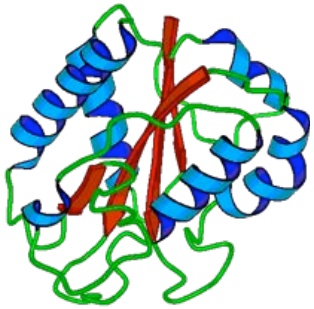
3D GKITFYERGFQGHCYESDC–NLQP…
SEQ GKITFYERG–––RCYESDCPNLQP…

1. Extract spatial restraints





2. Satisfy spatial restraints



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

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://salilab.org/modeller

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.
M. A. Martí-Renom *et al. Ann. Rev. Biophys. Biomolec. Struct.*, 29, 291, 2000.

Steps in Comparative Protein Structure Modeling

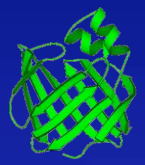
START

Template Search

TARGET

ASILPKRLFGNCEQTSDEGLK
IERTPLVPHISAQNVCLKIDD
VPERLIPERASFQWMNDK

TEMPLATE



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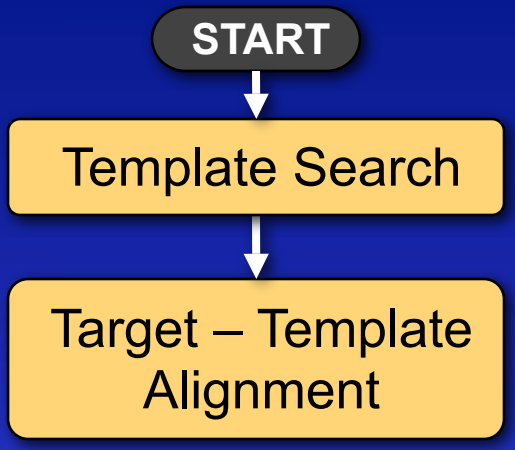
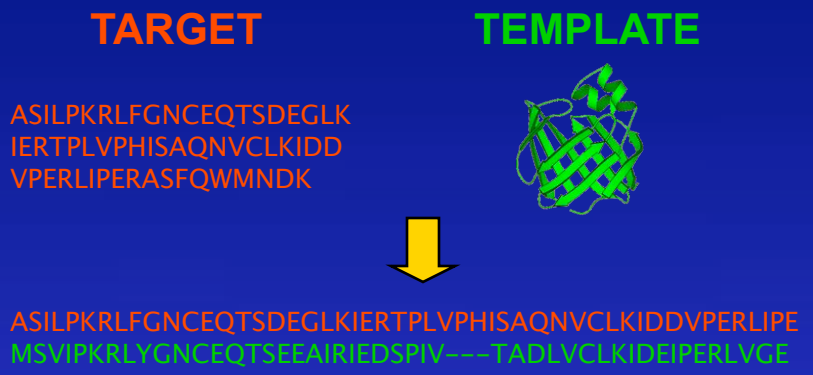
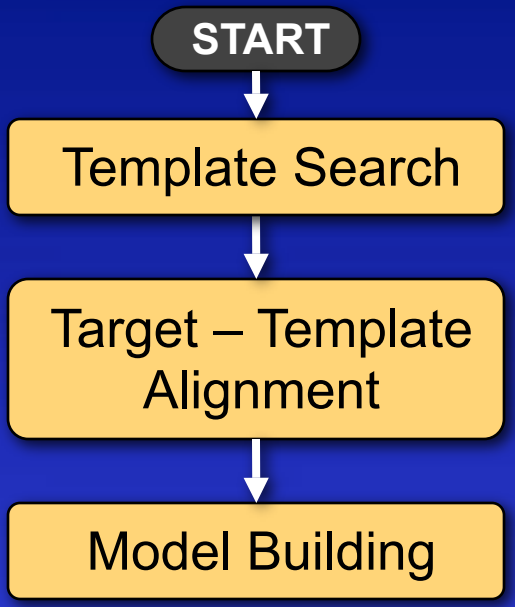
Steps in Comparative Protein Structure Modeling

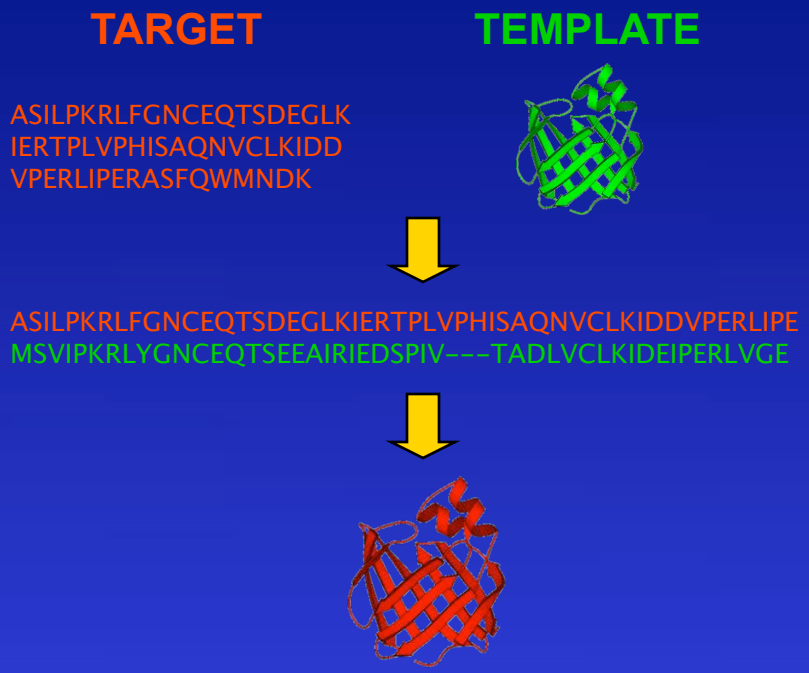




A. Šali, *Curr. Opin. Biotech.* 6, 437, 1995.
R. Sánchez & A. Šali, *Curr. Opin. Str. Biol.* 7, 206, 1997.
M. A. Martí-Renom *et al. Ann. Rev. Biophys. Biomolec. Struct.*, 29, 291, 2000.

Steps in Comparative Protein Structure Modeling





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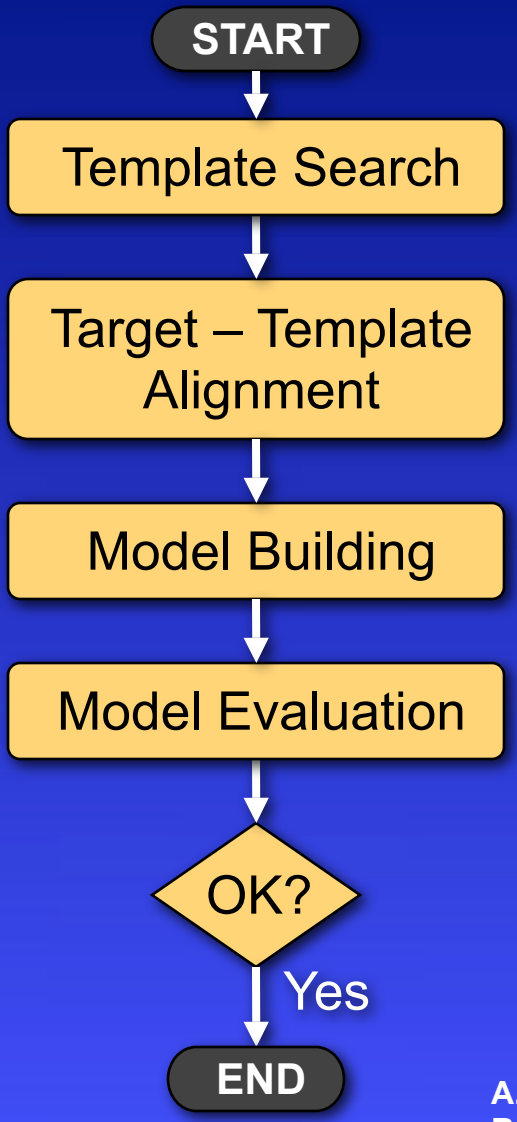
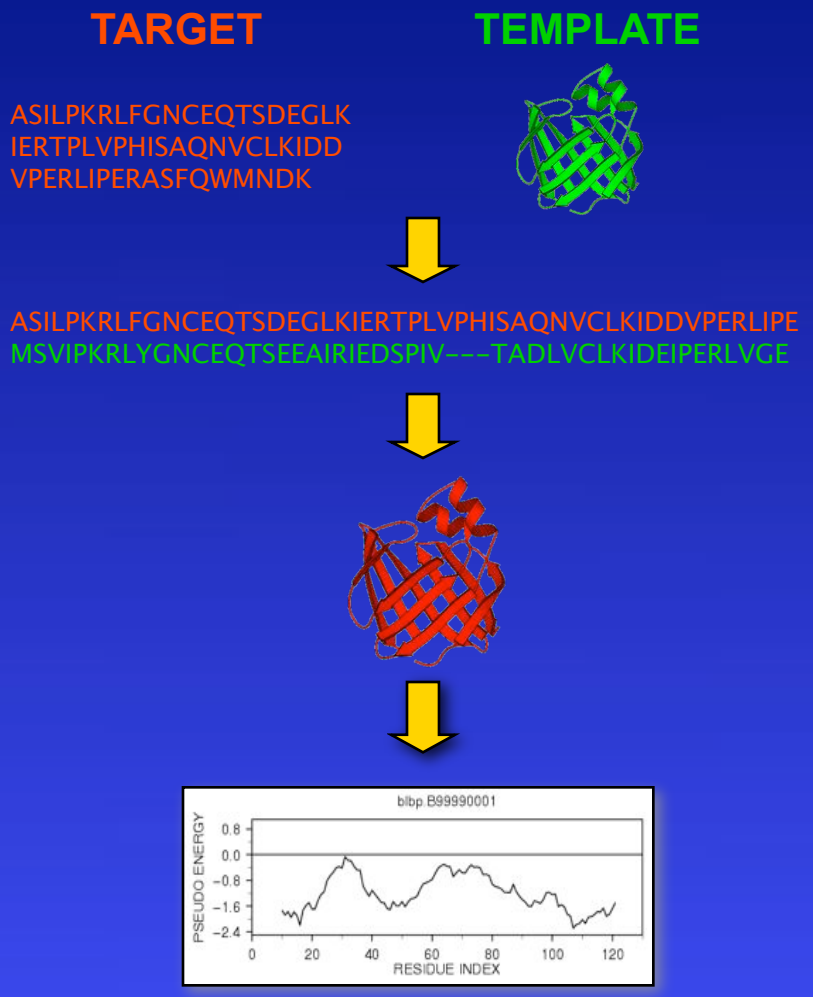
Steps in Comparative Protein Structure Modeling





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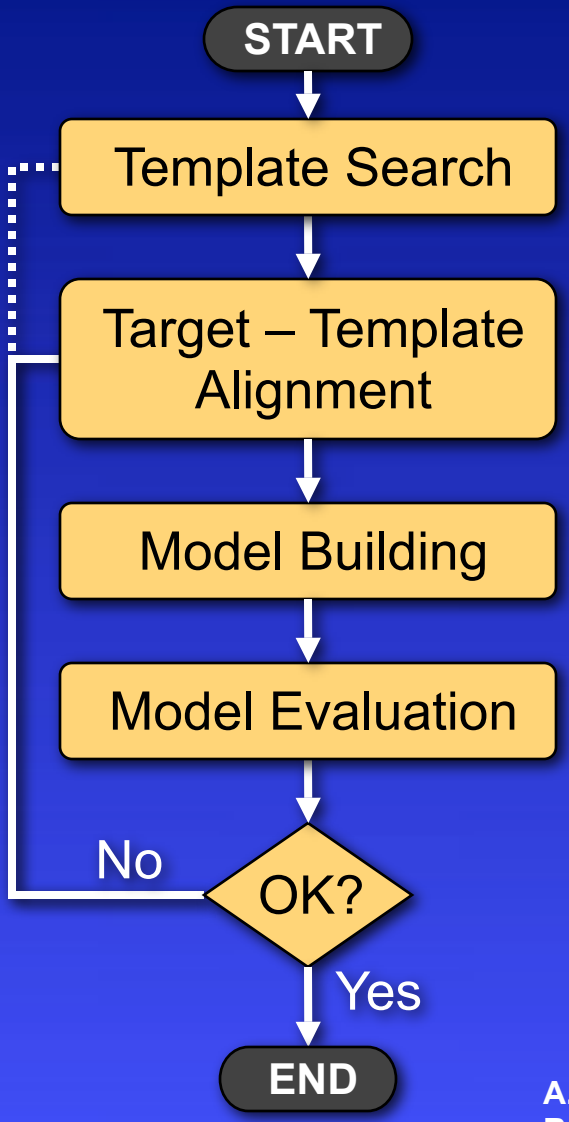
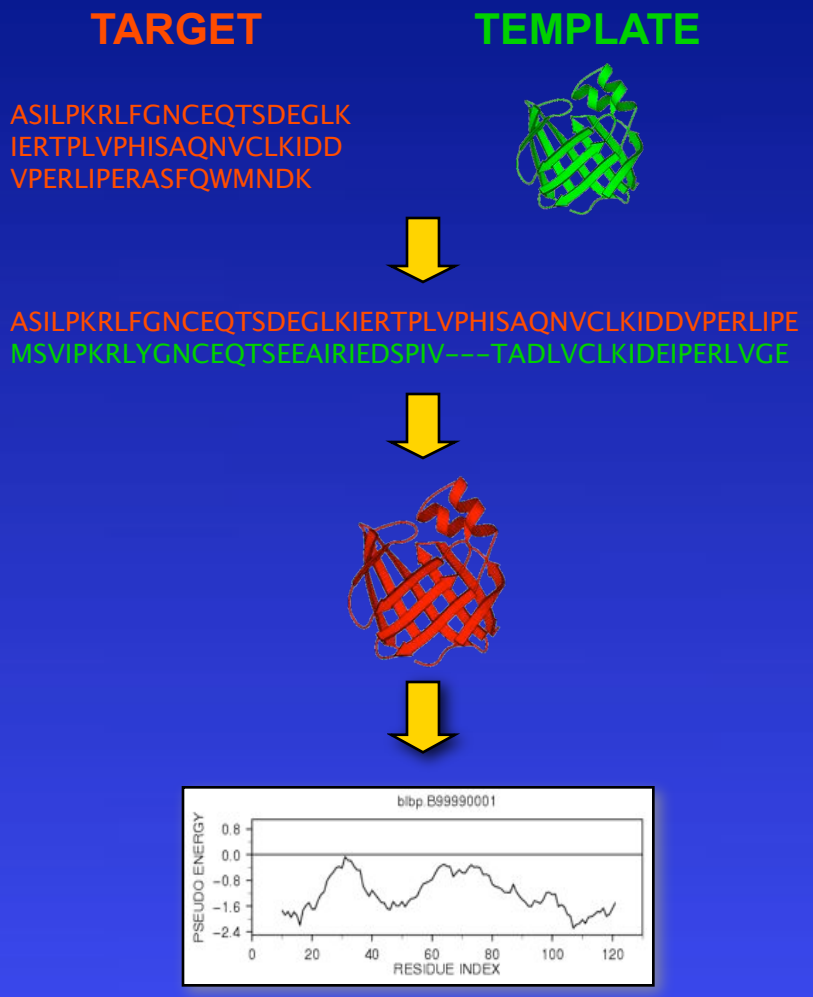
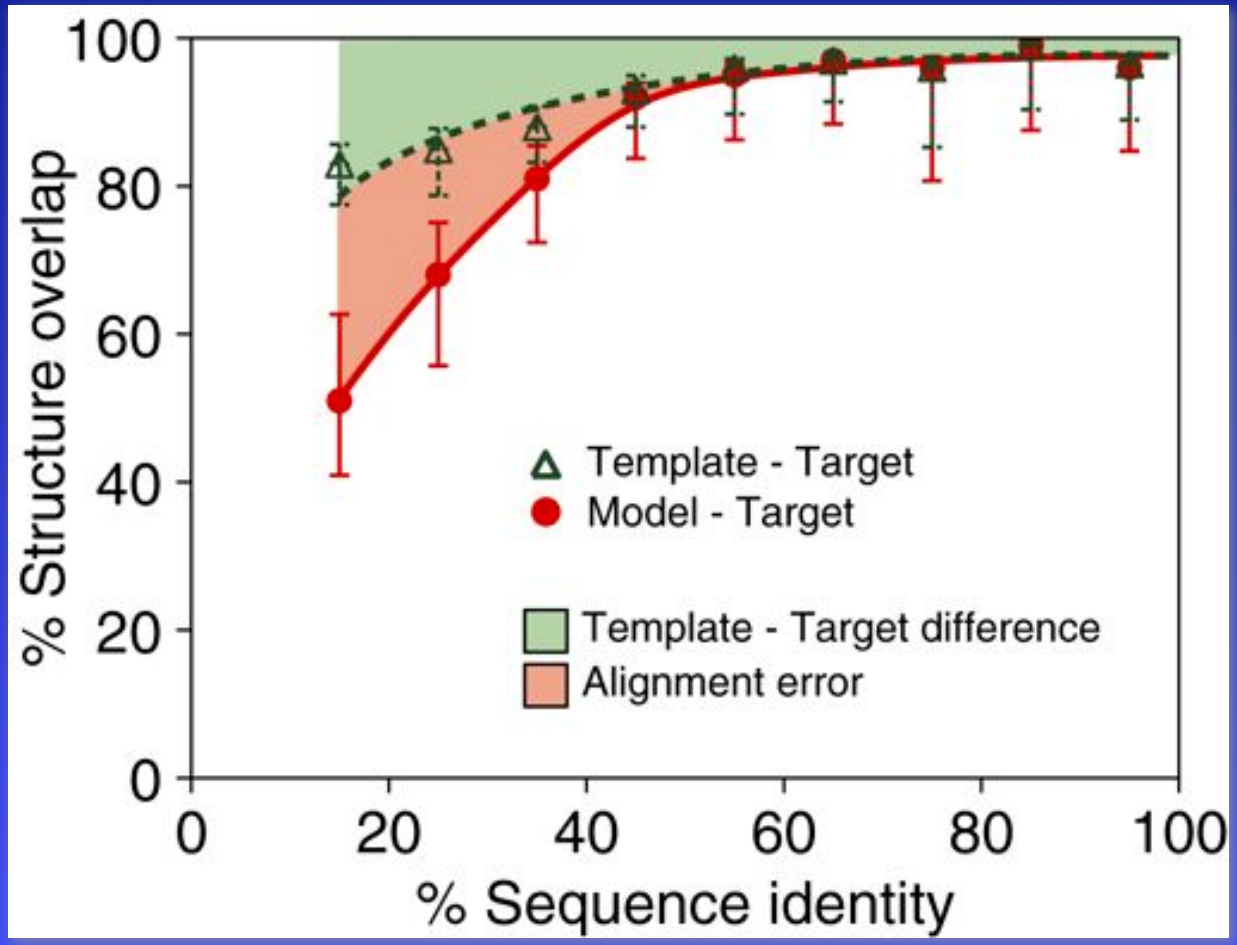
Steps in Comparative Protein Structure Modeling





A. Šali, *Curr. Opin. Biotech.* 6, 437, 1995.
R. Sánchez & A. Šali, *Curr. Opin. Str. Biol.* 7, 206, 1997.
M. A. Martí-Renom *et al. Ann. Rev. Biophys. Biomolec. Struct.*, 29, 291, 2000.

Model Accuracy as a Function of Target-Template Sequence Identity



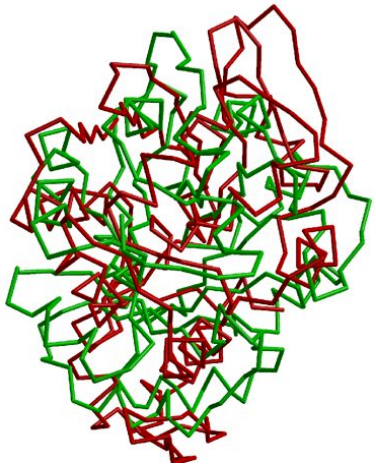
Typical Errors in Comparative Models

MODEL

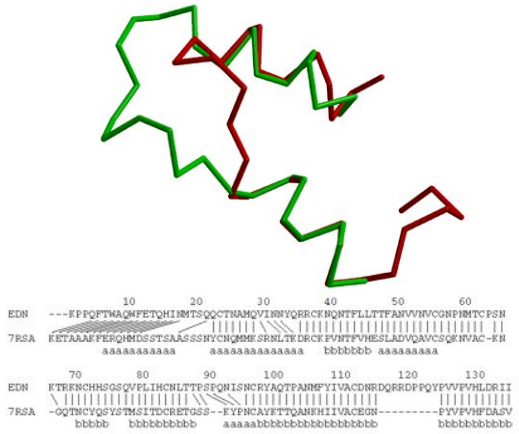
X-RAY

TEMPLATE

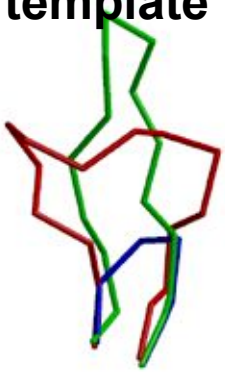
Incorrect template



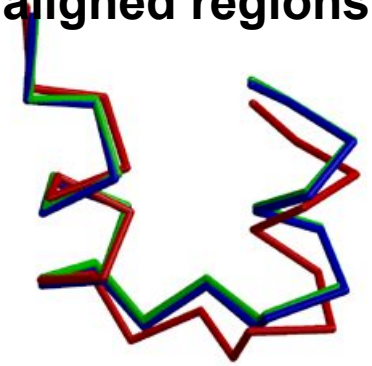
Misalignment



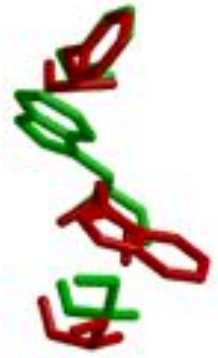
Region without a template



Distortion in correctly aligned regions



Sidechain packing

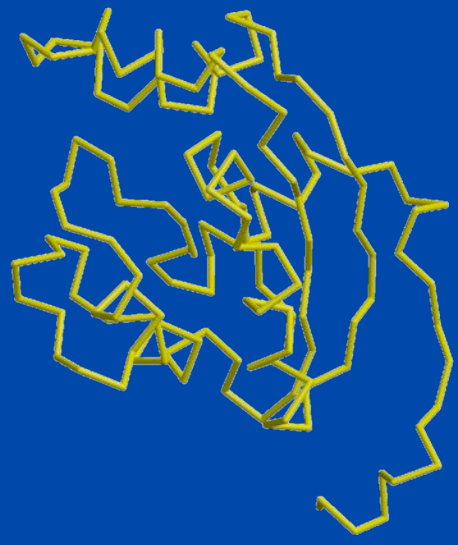


Model Accuracy

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

HIGH ACCURACY

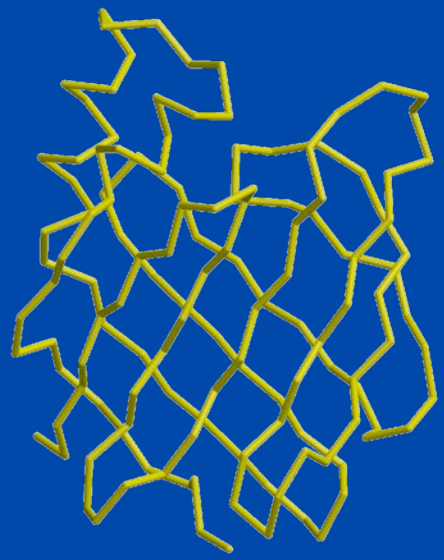
NM23
Seq id 77%



X-RAY

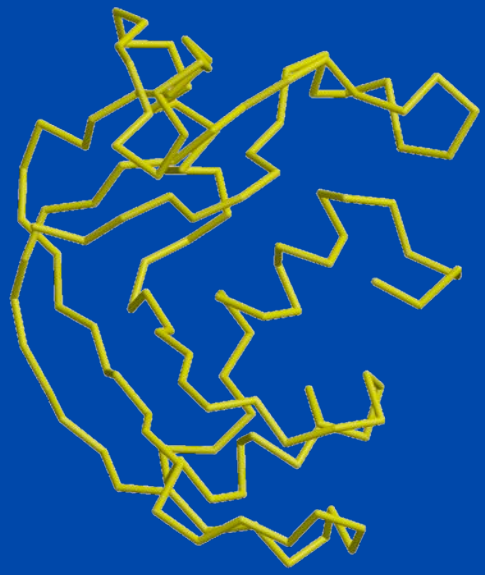
MEDIUM ACCURACY

CRABP
Seq id 41%



LOW ACCURACY

EDN
Seq id 33%

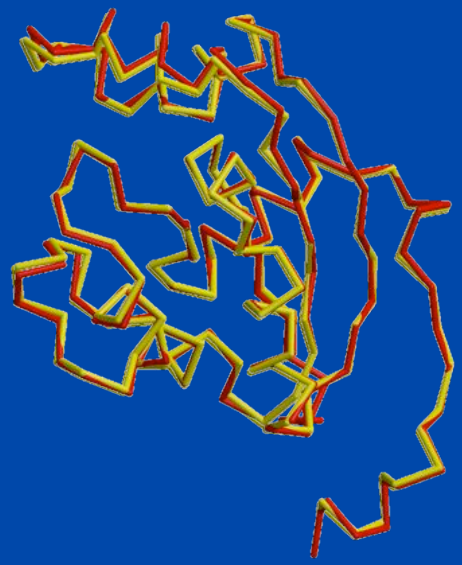


Model Accuracy

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

HIGH ACCURACY

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

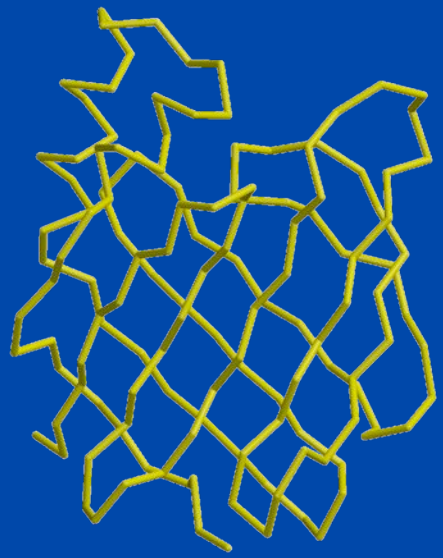


Sidechains
Core backbone
Loops

X-RAY / MODEL

MEDIUM ACCURACY

CRABP
Seq id 41%



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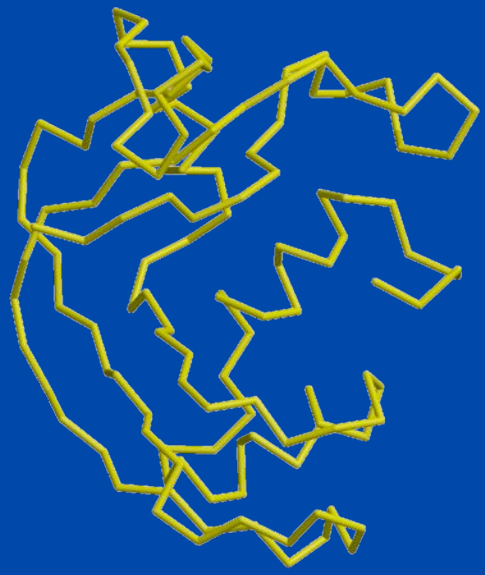
EDN
Seq id 33%



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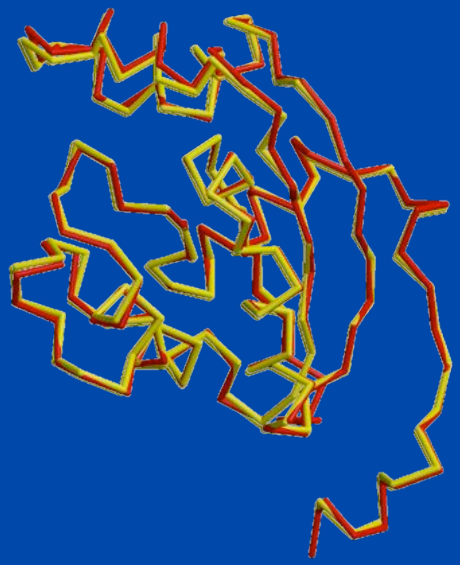

Sidechains
Core backbone
Loops

X-RAY / MODEL

MEDIUM ACCURACY

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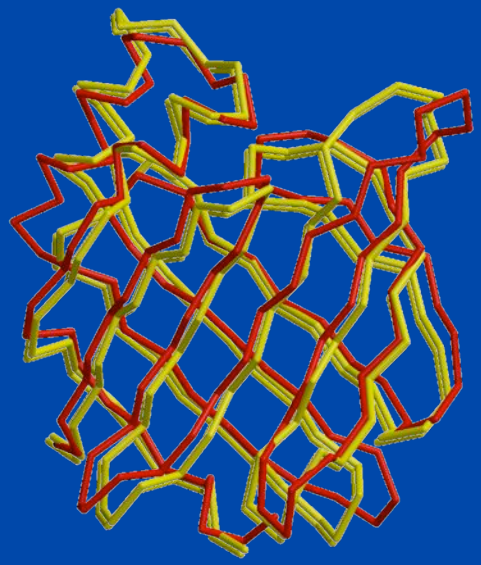
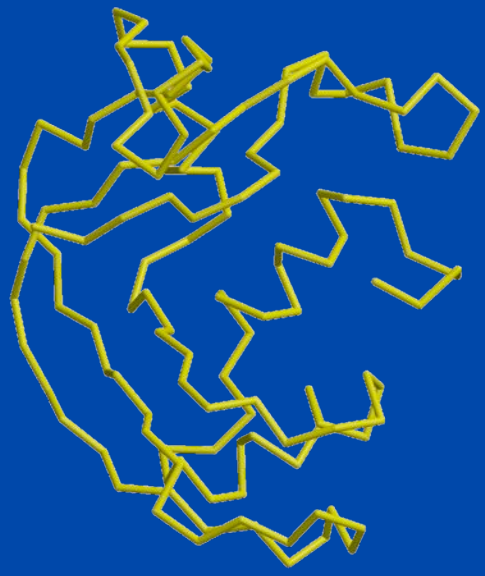
Seq id 41%
Cα equiv 122/137
RMSD 1.34Å



Sidechains
Core backbone
Loops
Alignment

LOW ACCURACY

EDN
Seq id 33%



Model Accuracy

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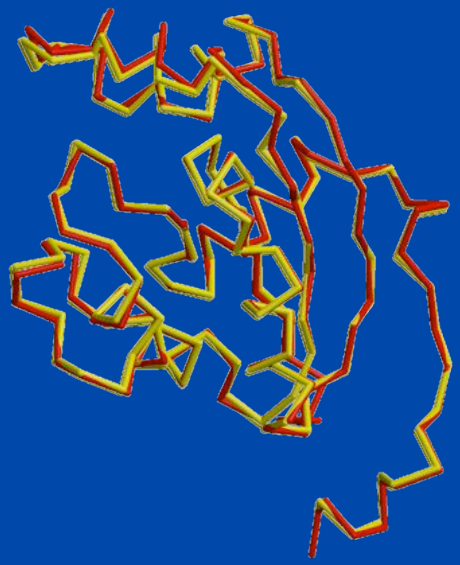
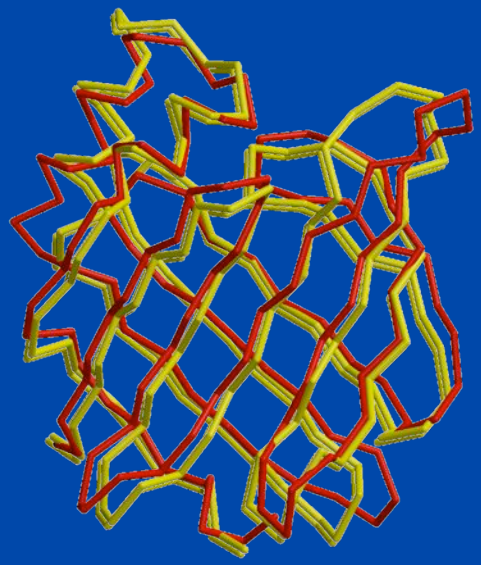


Sidechains
Core backbone
Loops

X-RAY / MODEL

MEDIUM ACCURACY

CRABP
Seq id 41%
Cα equiv 122/137
RMSD 1.34Å



Sidechains
Core backbone
Loops
Alignment

LOW ACCURACY

EDN
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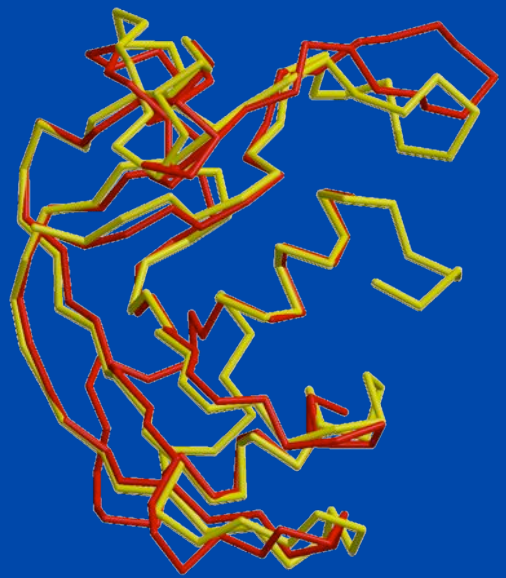
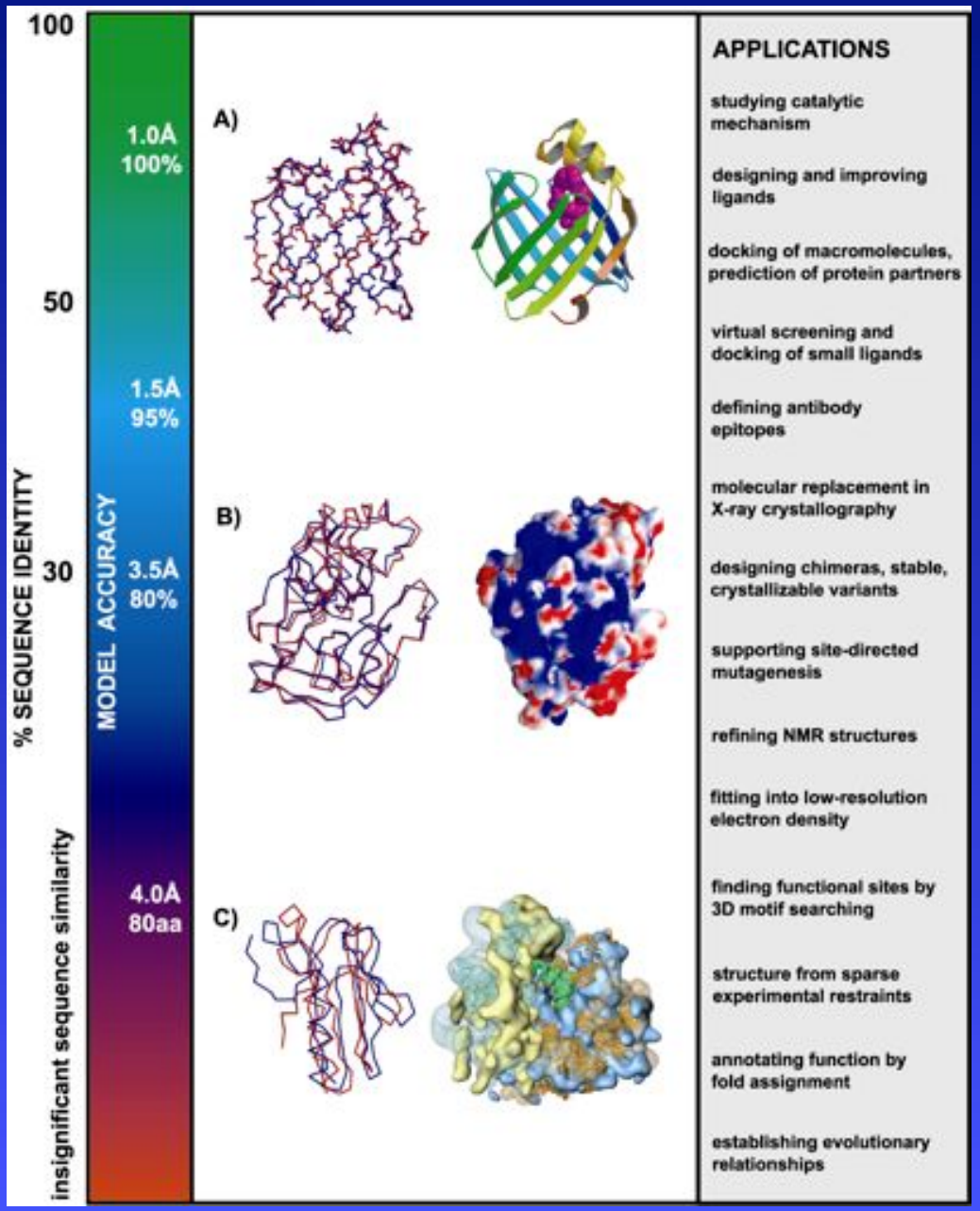


Sidechains
Core backbone
Loops
Alignment
Fold assignment

Applications of Comparative Models



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

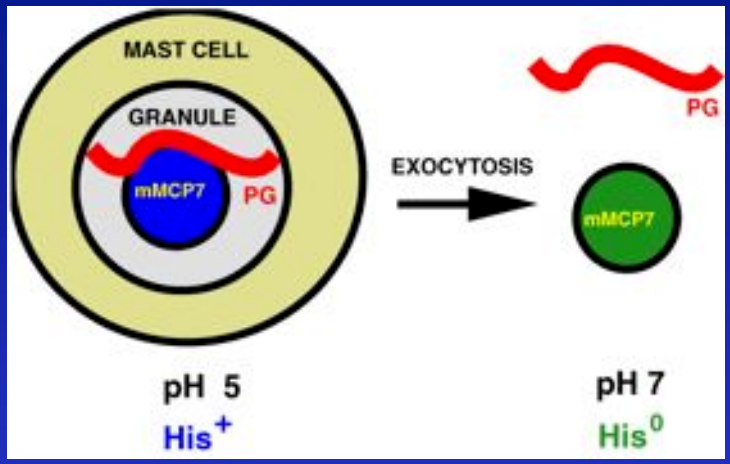
A. Sali & J. Kuriyan.
TIBS **22**, M20, 1999.

genes…

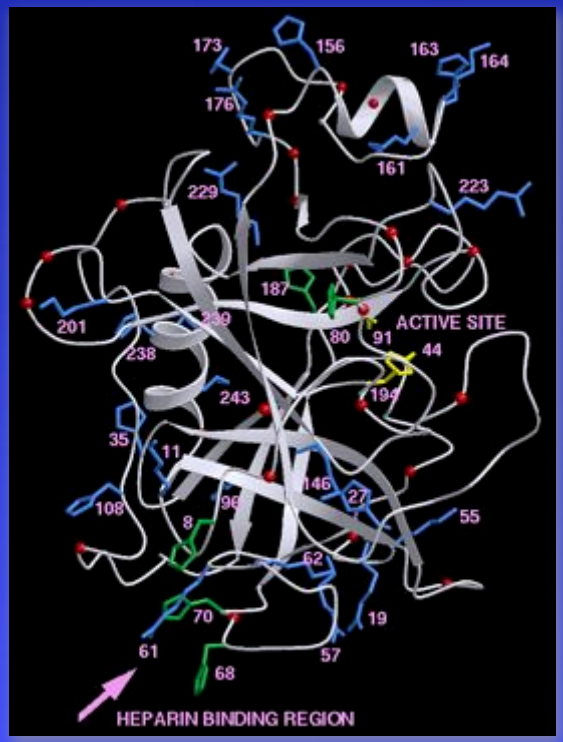
Do mast cell proteases bind proteoglycans? Where? When?

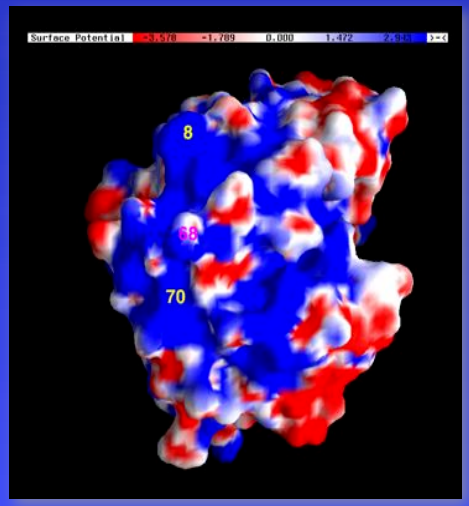
Predicting features of a model that are not present in the template

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.

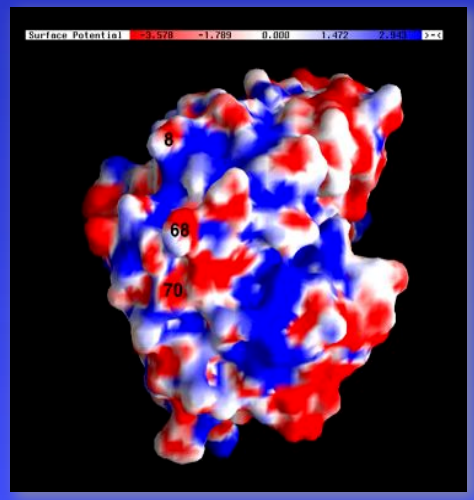


Huang *et al*. *J. Clin. Immunol*. **18**,169,1998.
Matsumoto *et al*. *J.Biol.Chem*. **270**,19524,1995.
Šali *et al*. *J. Biol. Chem*. **268**, 9023, 1993.





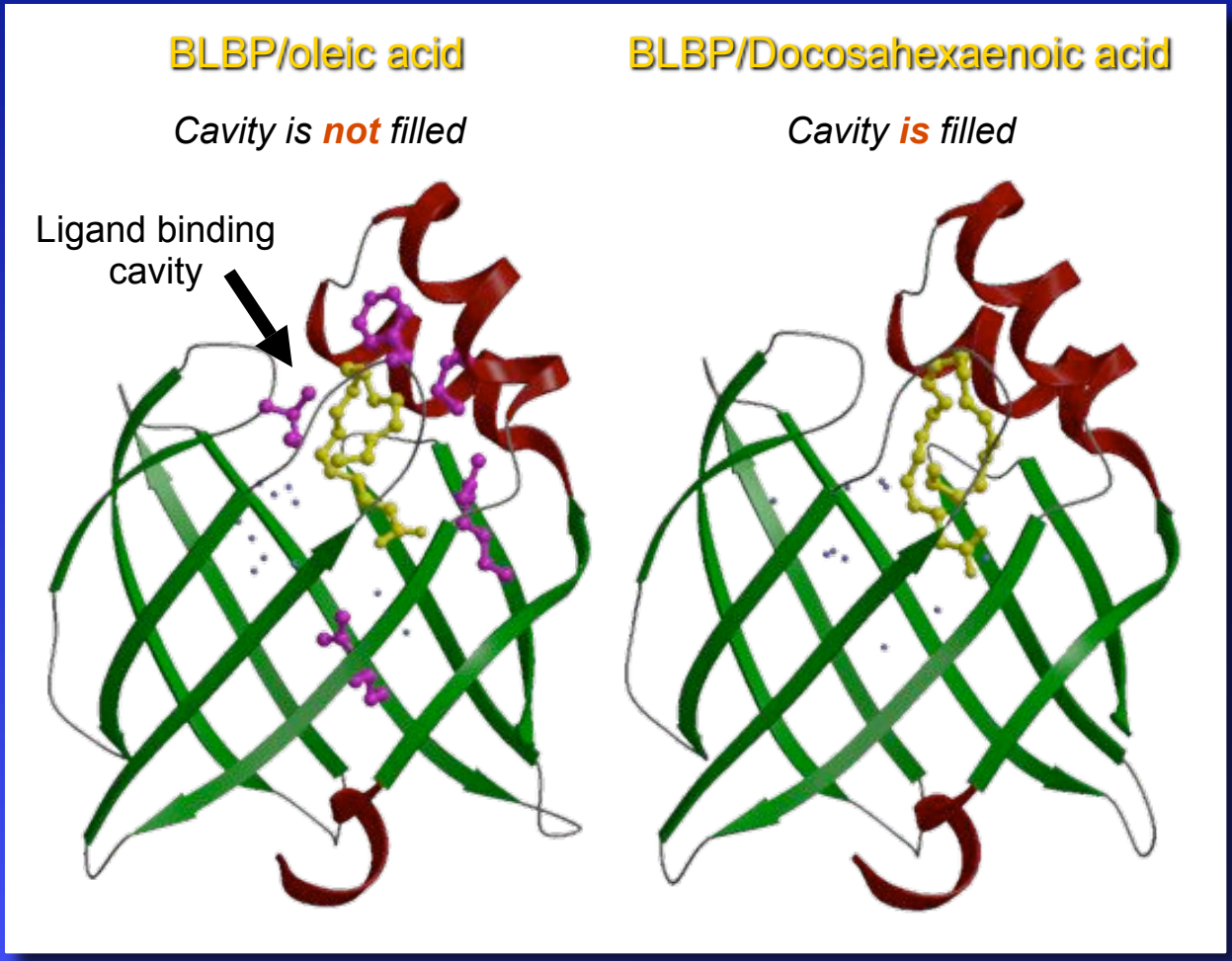
Native mMCP-7 at *p*H=5 (His^{+})



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

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

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

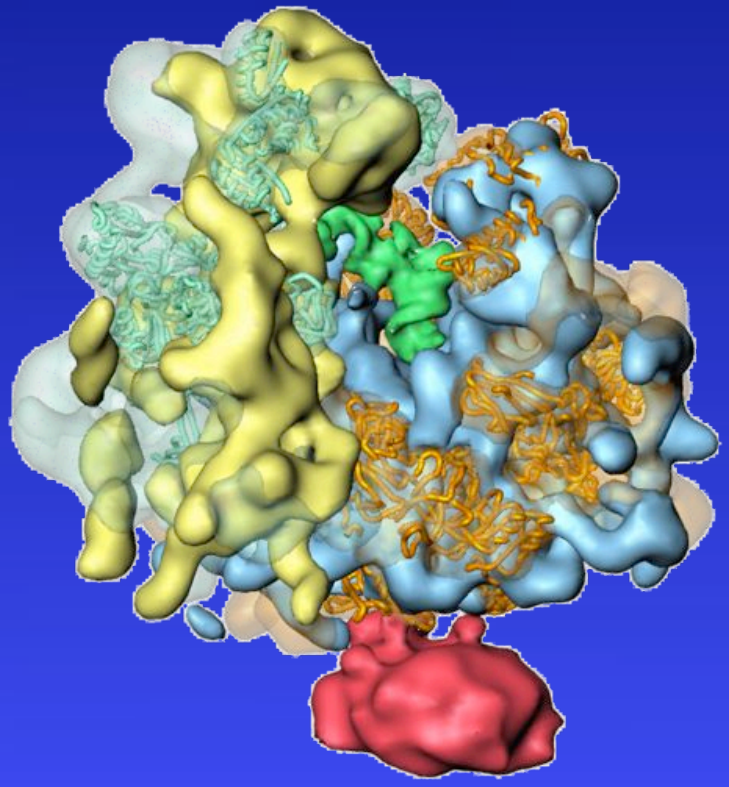


1. BLBP binds fatty acids.
2. Build a 3D model.
3. Find the fatty acid that fits most snuggly into the ligand binding cavity.

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

Some Models Can Be Used in Docking to Density Maps

(Yeast Ribosomal 40S subunit)

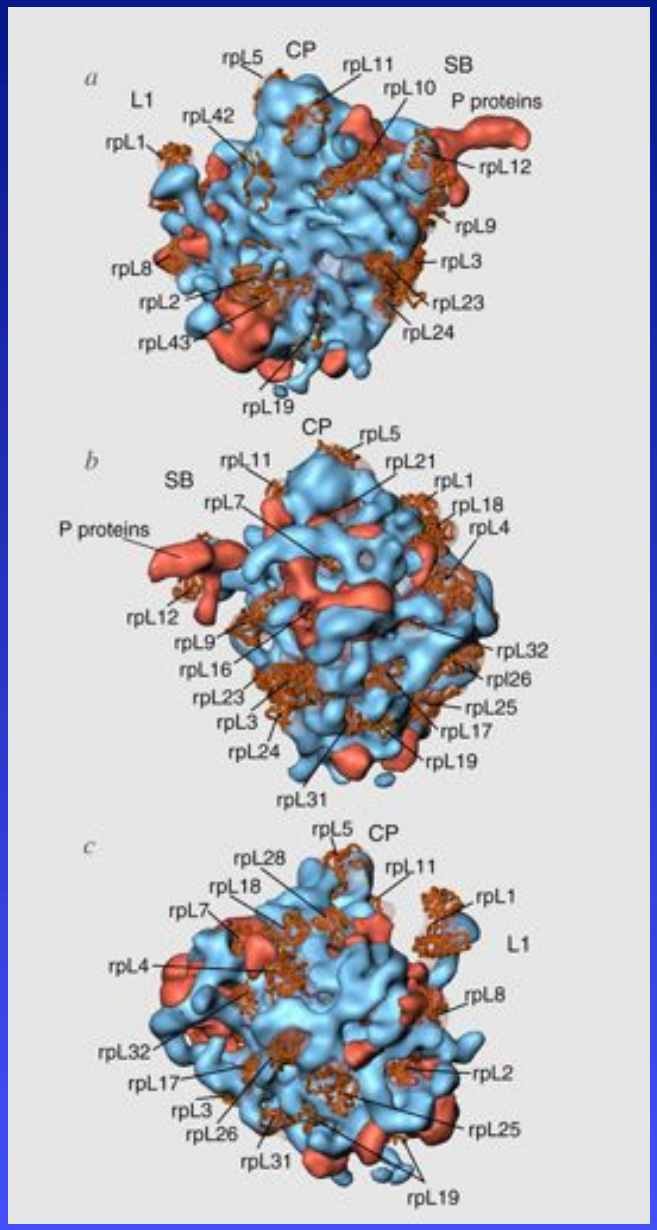


Small 30S subunit from *Thermus thermophilus*
Large 50S subunit from *Haloarcula marismortui*

Docking of comparative models into the cryo-EM map.

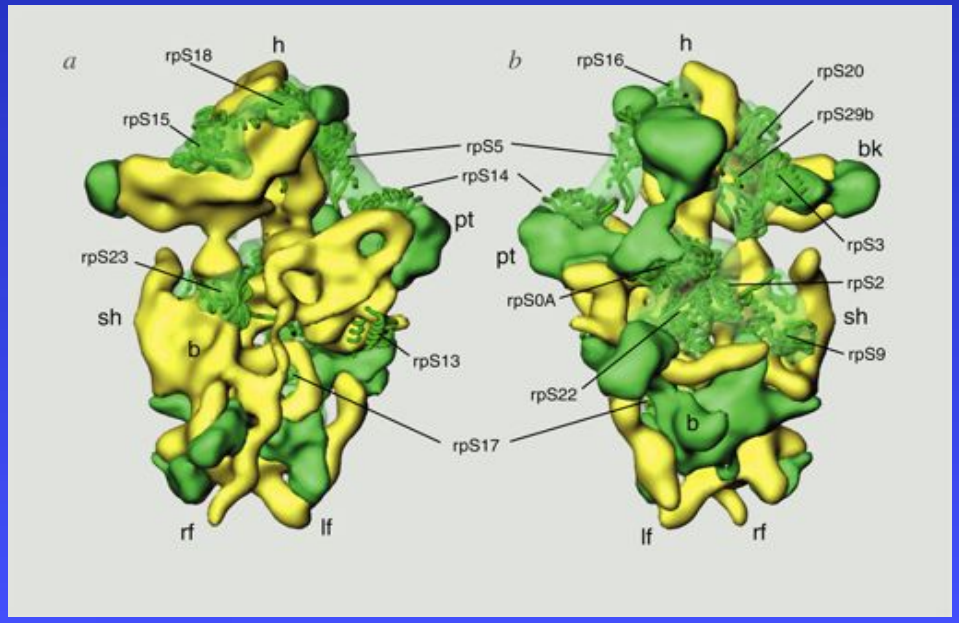
Spahn *et al.* 2001 Cell **107**:373-386

60S Subunit



- 43 proteins could be modeled on 20-56% seq.id. to a known structure.
- The coverage of the models ranges from 34-99%.
- Models were manually docked into the 15Å cryo-electron density map.
- The solid orange in the 60S subunit and the solid green in the 40S subunit correspond to proteins without known bacterial homologs.

40S Subunit



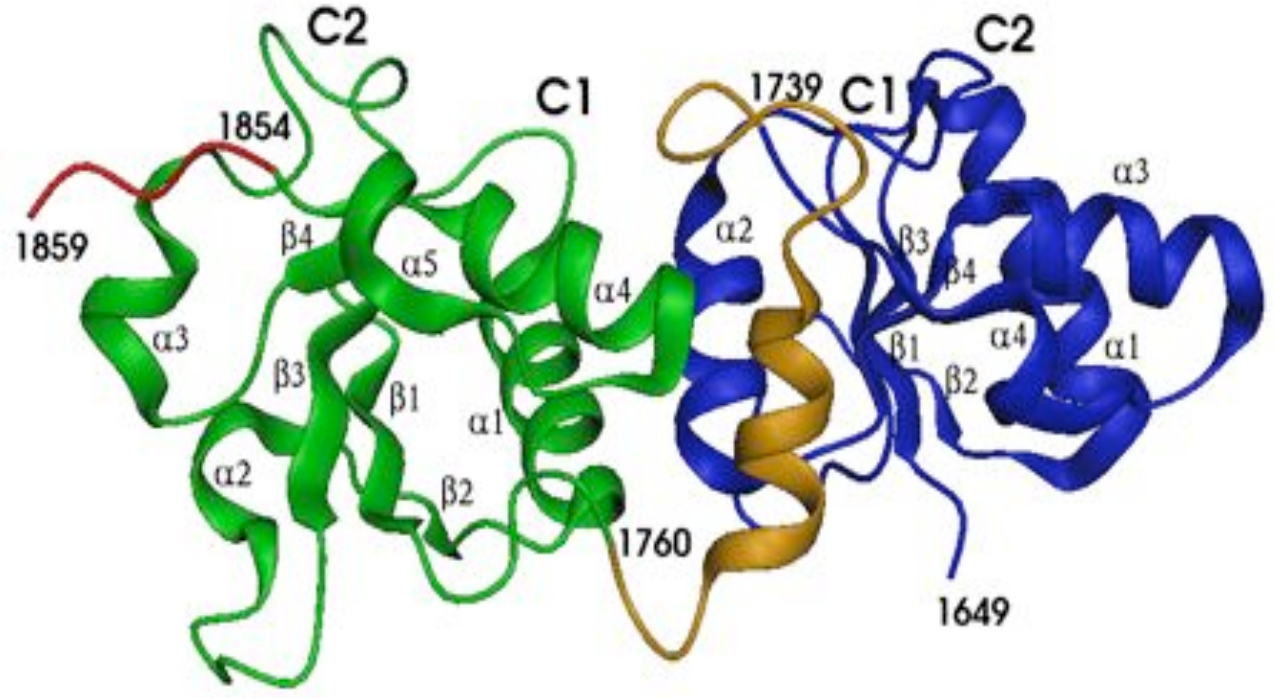
Structural analysis of missense mutations in human BRCA1 BRCT domains

Nebojsa Mirkovic, Marc A. Marti-Renom, Andrej Sali

Alvaro N.A. Monteiro (Sprang Center, Cornell U.)

Human BRCA1 and its two BRCT domains

BRCA1 BRCT repeats, 1jnx



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

CONFIDENTIAL

BRACAnalysis™
Comprehensive BRCA1-BRCA2 Gene Sequence Analysis Result

	SPECIMEN	PATIENT
Strang Cancer Prevention Center 428 E 72nd St New York, NY 10021	Specimen Type: Blood Draw Date: n/a Accession Date: Oct 27, 2000 Report Date: Nov 17, 2000	Name: Date of Birth: Feb 02, 1953 Patient ID: Gender: Female Accession #: 00010999 Requisition #: 38894

Physician

Test Result

Gene Analyzed	Specific Genetic Variant
BRCA2	H2116R
BRCA1	None Detected

Interpretation

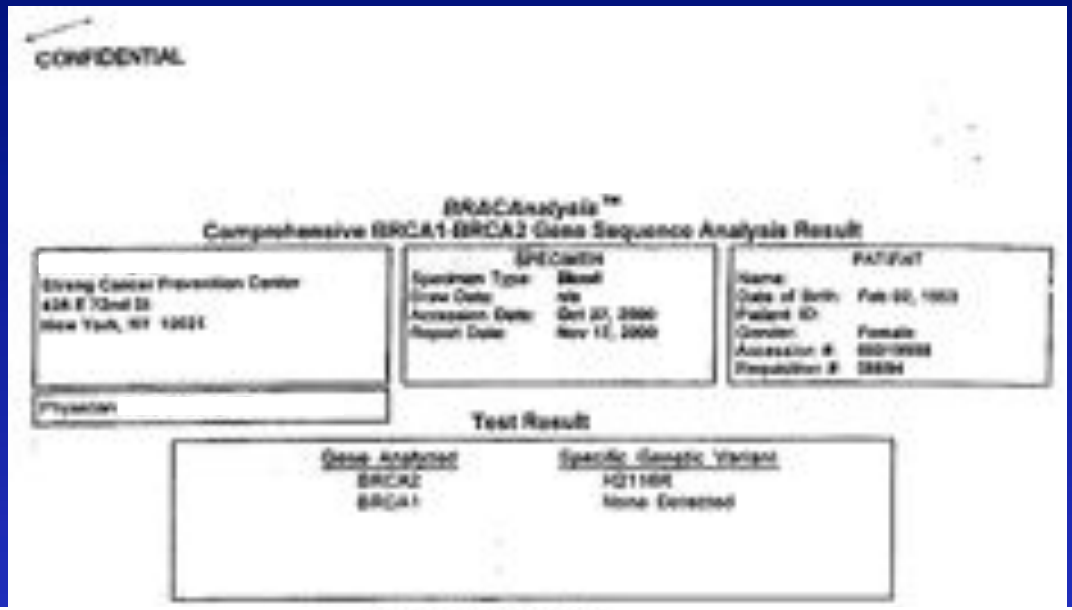
GENETIC VARIANT OF UNCERTAIN SIGNIFICANCE

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:

Laboratory Director   Medical Director

[illegible]



CONFIDENTIAL

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[illegible]

SPECIMEN
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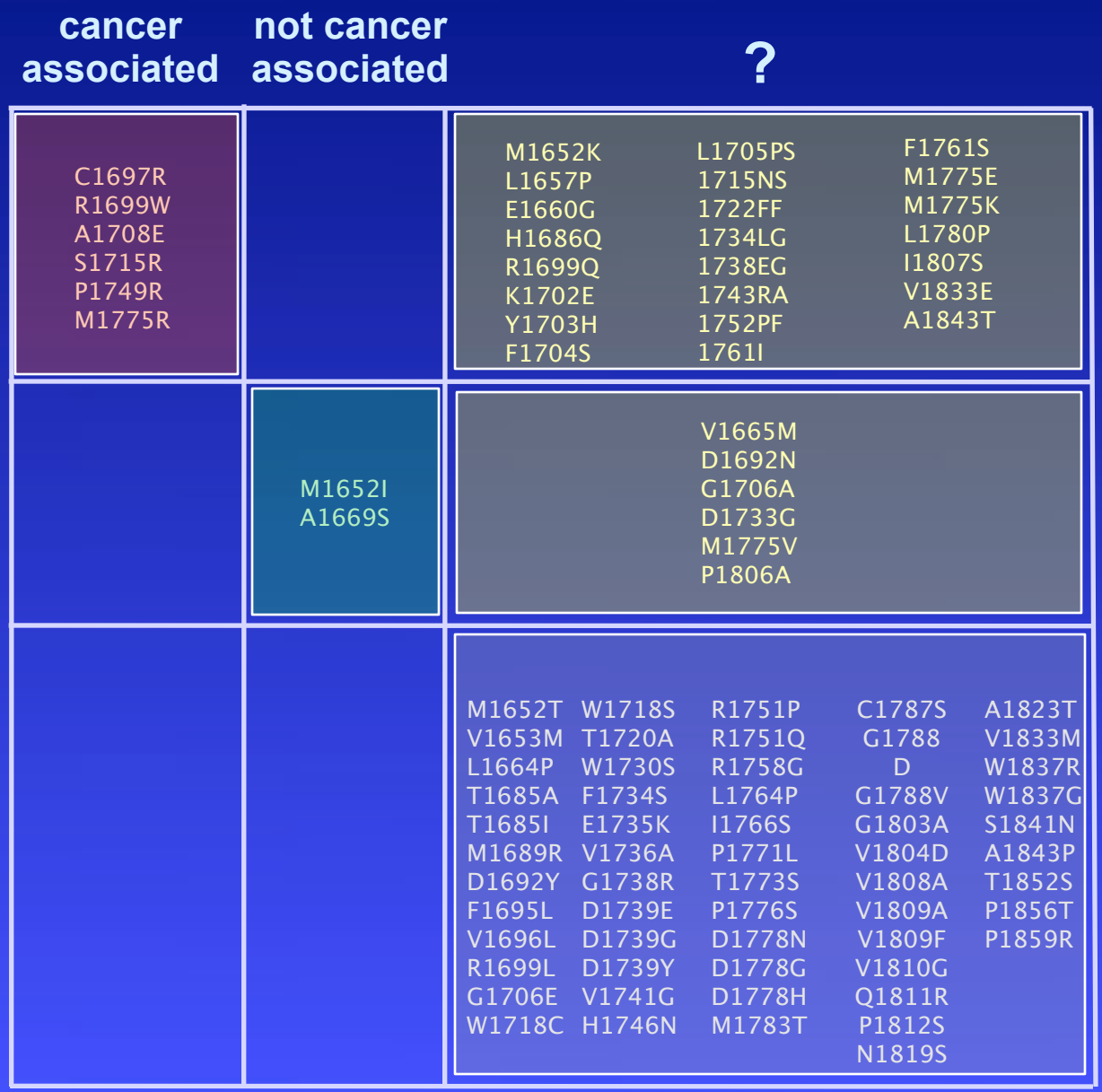
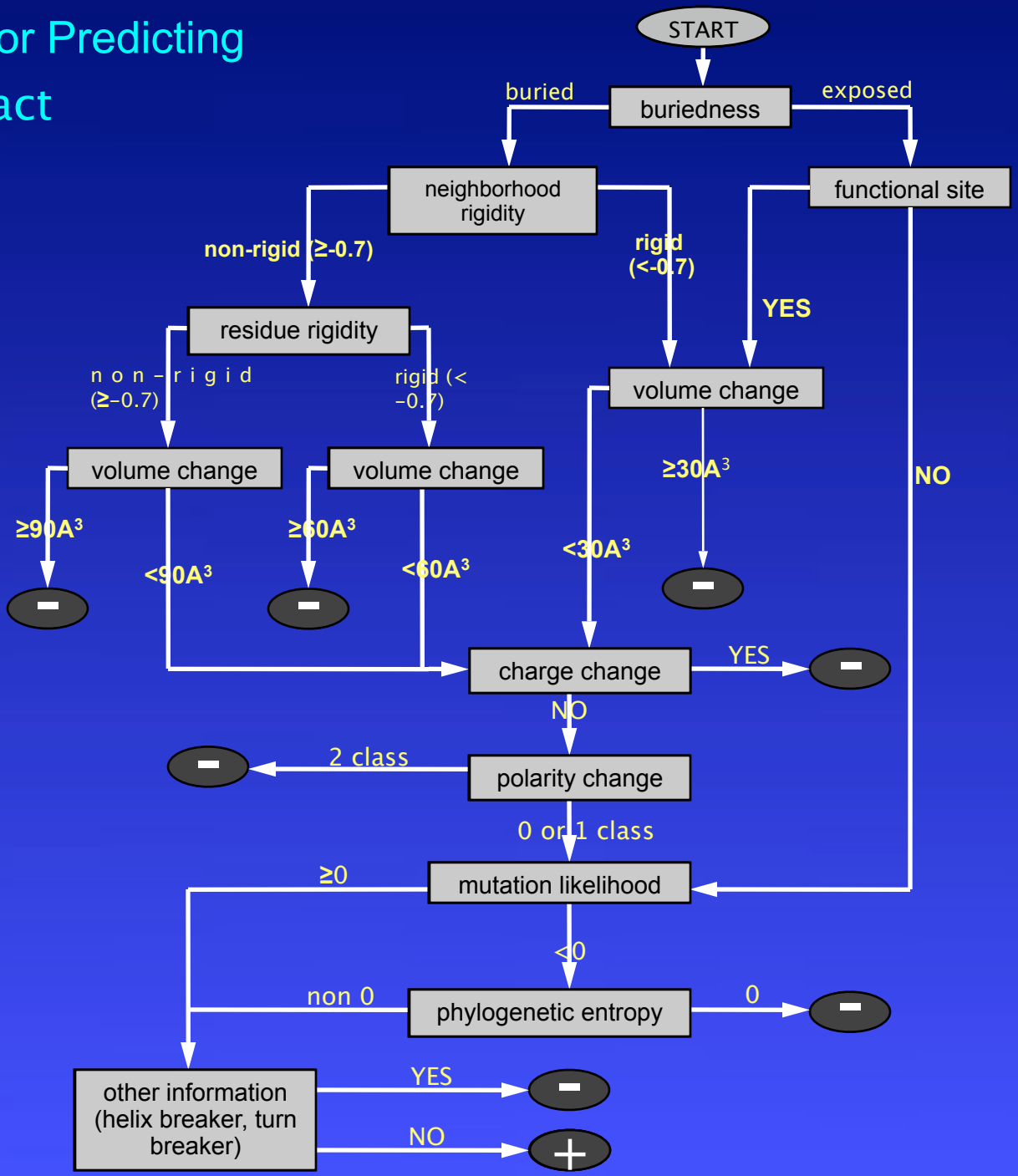
[illegible]

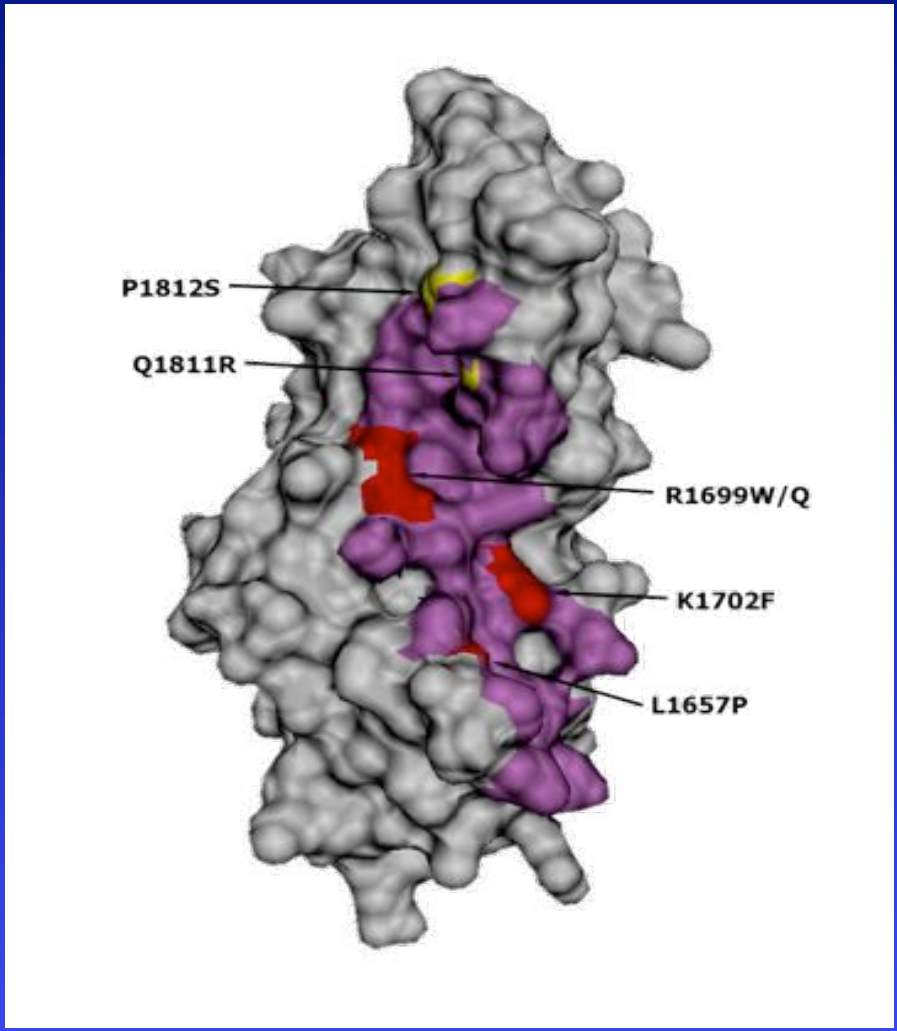
Missense Mutations in BRCT Domains by Function



"Decision" Tree for Predicting Functional Impact of Genetic Variants



Putative Binding Site on BRCA1



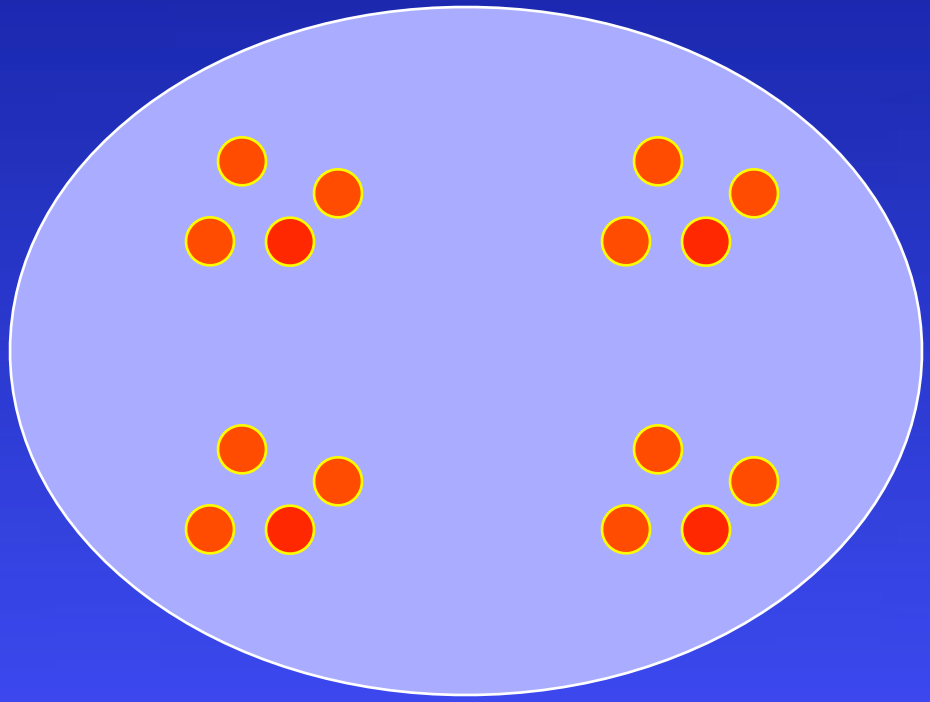
RMSMVVSGLTPEEFMLVYKFARKHHITLTNLITEETTHVVMKTDAEFVCERTLKYFLGIAGGKWVVSYFWVTQSIKERK
MLNEHDFEVRGDVVNGRNHQGPKRARESQDRKIFRGLEICCYGPFTNMPTDQLEWMVQLCGASVVKELSSFTLGTGVHP
IVVVQPDAWTEDNGFHAIGQMCEAPVVTREWVLDSVALYQCQELDTYLIPQIP

genomas…

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**.**

Characterize most protein sequences based on related known structures.



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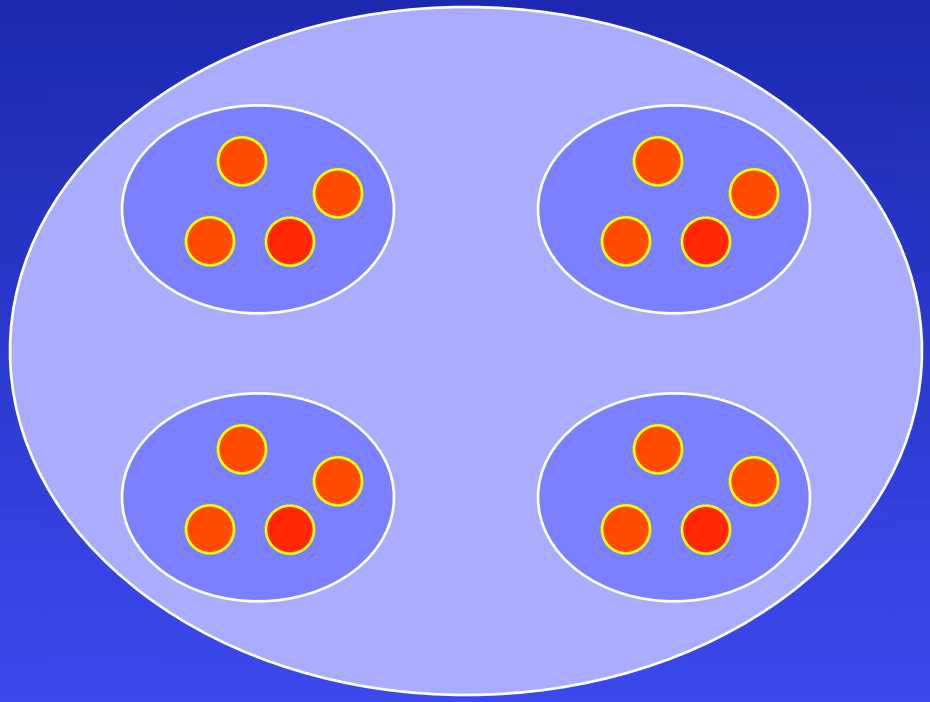

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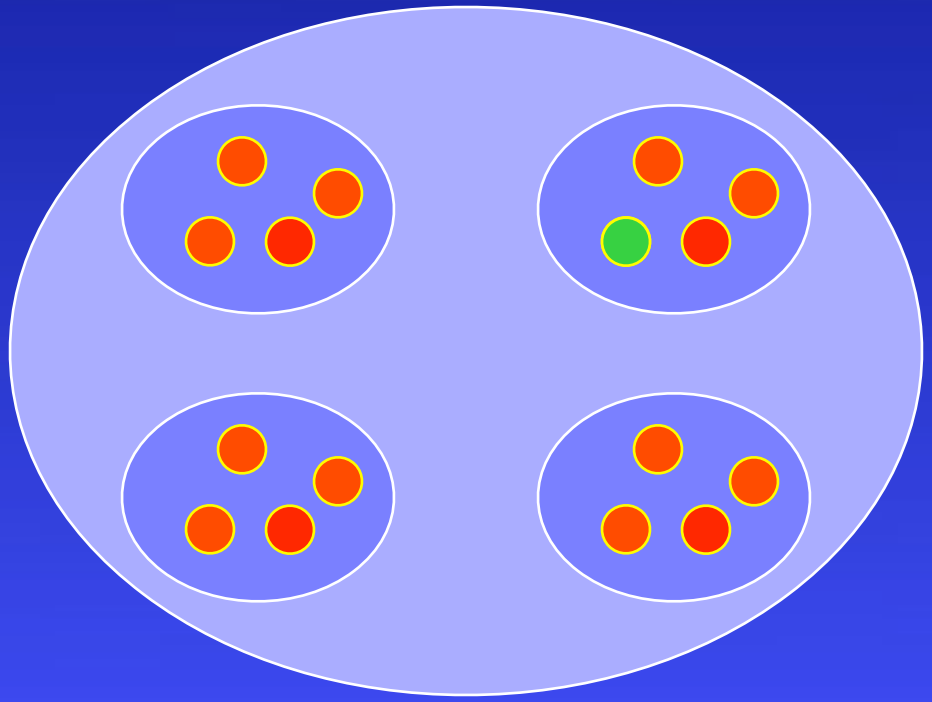

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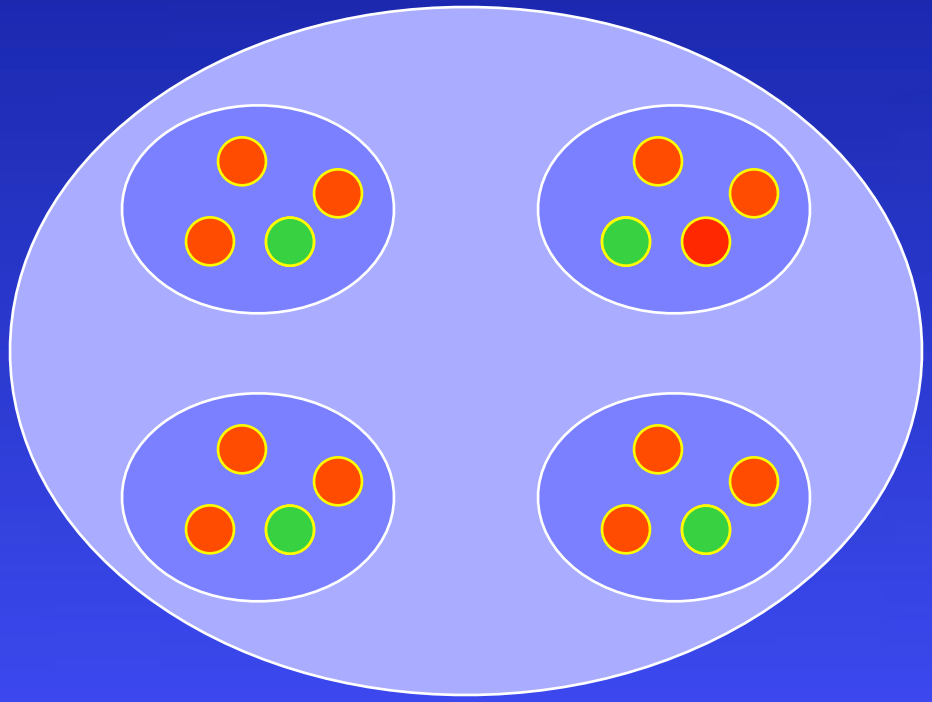

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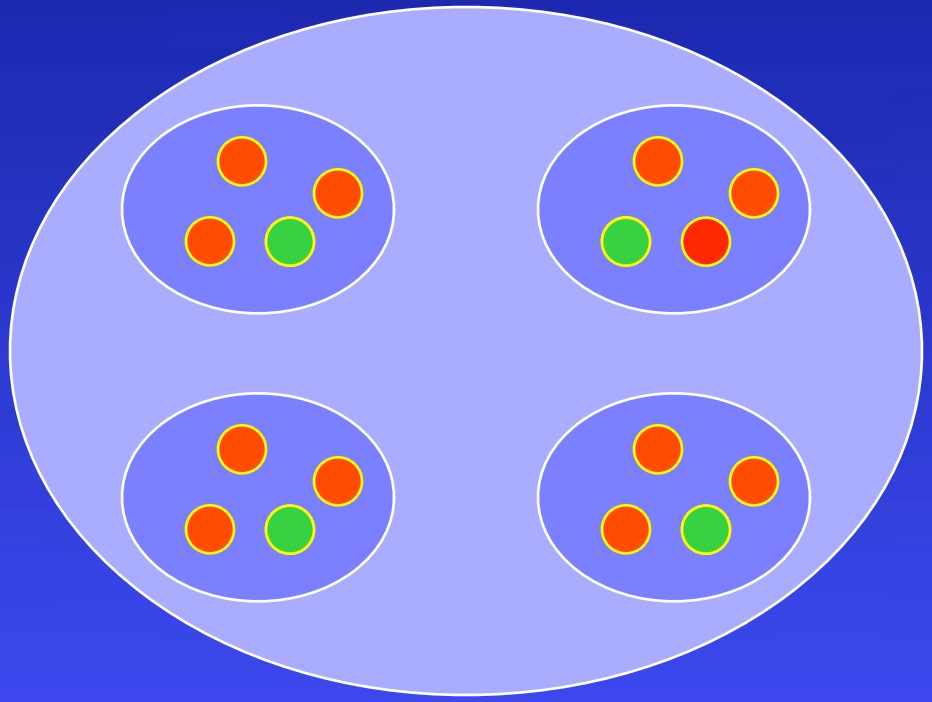

The number of “families” is much smaller than the number of proteins.

Any one of the members of a family is fine.

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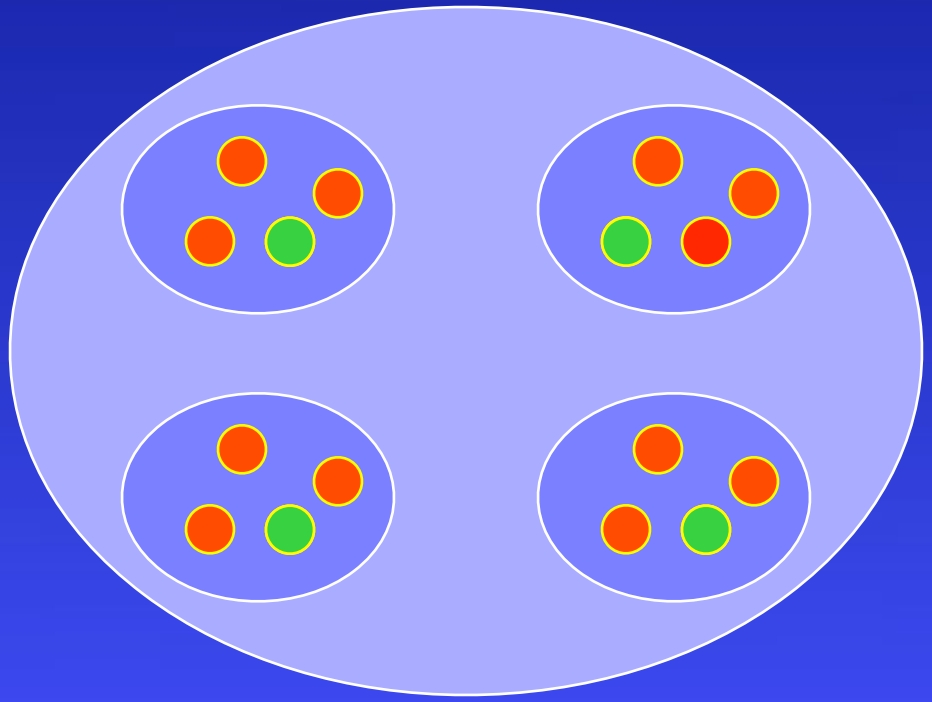

The number of "families" is much smaller than the number of proteins.

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

Modeling with NY-SGRC structures

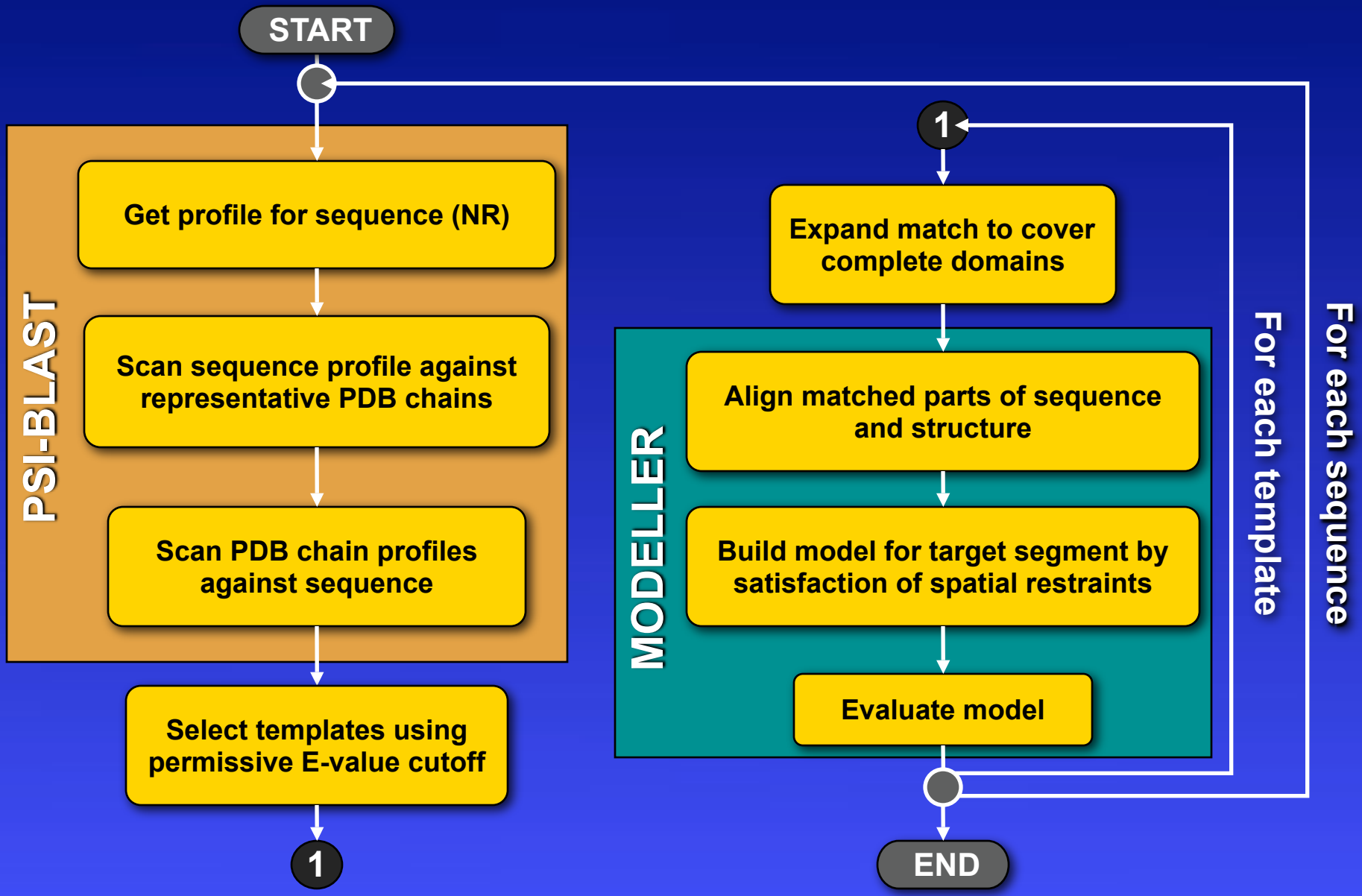
http://salilab.org/modweb/

June 2001

Protein Name	GI or Swissprot Code	Length	# Acceptable Models	Min. Seq. ID	Max. Seq. ID	# Models >50% Seq. ID	# Models 30-50% Seq. ID	# Models <30% Seq. ID
Yeast hypothetical protein	P38187	230	46	21	45	0	35	11
PNP oxidase	P38075	205	43	18	58	1	33	9
Yeast hypothetical protein	P40354	271	234	15	54	4	40	190
T. maritima L-threonine acetaldehyde-lyase	GI 4982822	342	2069	9	51	2	27	2069
Hypothetical esterase	P40363	288	157	8	48	0	13	144
Hypothetical protein	P40155	214	35	19	86	1	6	28
mevalonate diphosphate decarboxylase	GI 1292880	391	121	10	68	2	18	101
yeast glutathione synthetase	GI 2100534	419	26	30	42	0	26	0

Bonanno *et al*. *Proc.Natl.Acad.Sci.USA* **98**, 12896, 2001.
Chance *et al*. Protein Science **11**, 723, 2002.

MODPIPE: Large-Scale Comparative Protein Structure Modeling



R. Sánchez & A. Šali, *Proc. Natl. Acad. Sci. USA* **95**, 13597, 1998.
N. Eswar, M. Marti-Renom, M.S. Madhusudhan, B. John, A. Fiser, R. Sánchez, F. Melo, N. Mirkovic, A. Šali.

Comparative modeling of the TrEMBL database

Unique sequences processed: 733,239

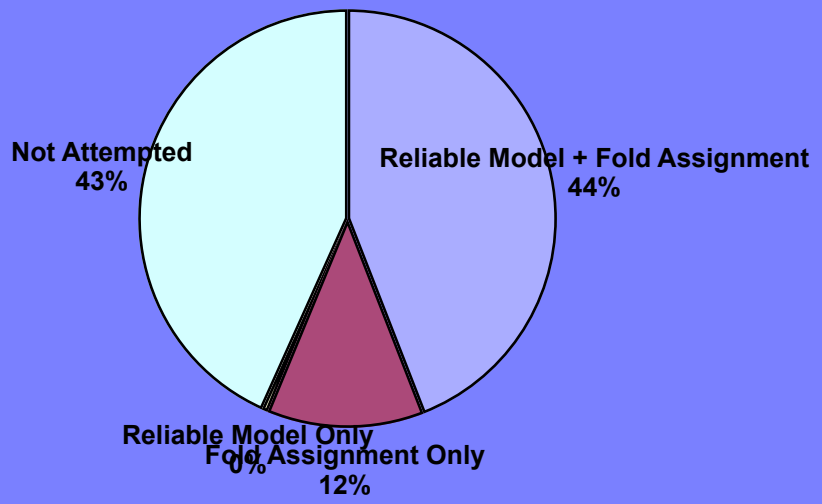
Sequences with fold assignments or models: 415,937 (57%)

70% of models based on <30% sequence identity to template.

On average, only a domain per protein is modeled
(an “average” protein has 2.5 domains of 175 aa).

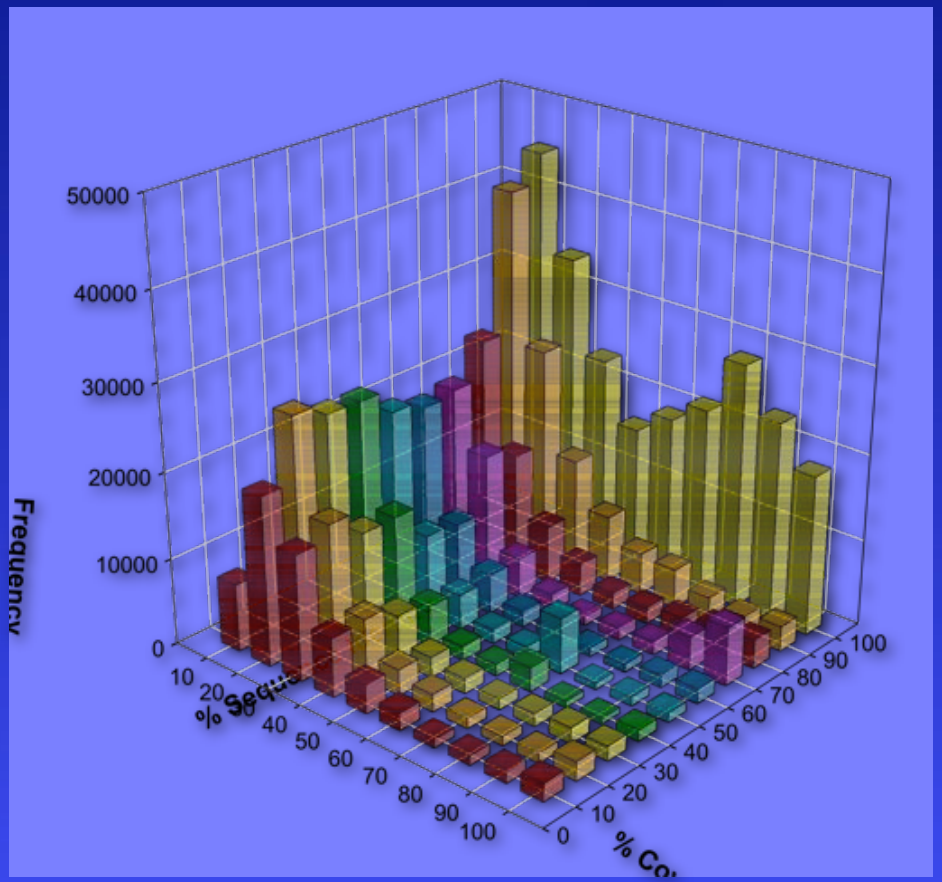
4/03/02 ~4 weeks on 500 Pentium III CPUs

Modeling Coverage of the Sequence Space



Fold assignment: PSI-BLAST E-value $\leq 10^{-4}$

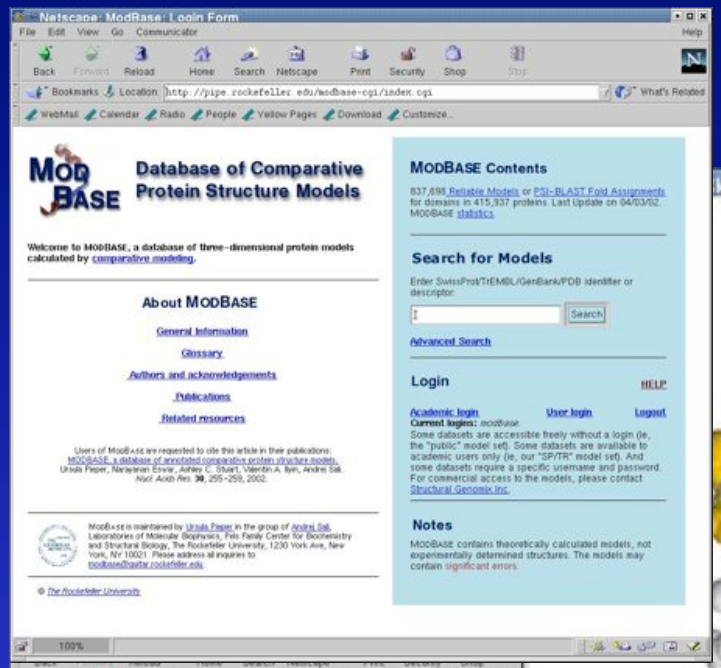
Reliable Model: Model Score ≥ 0.7

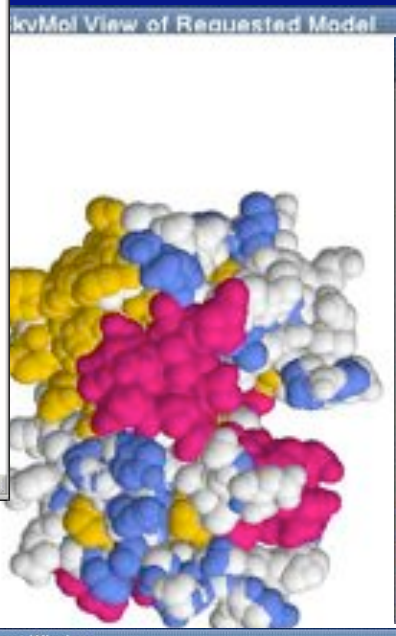


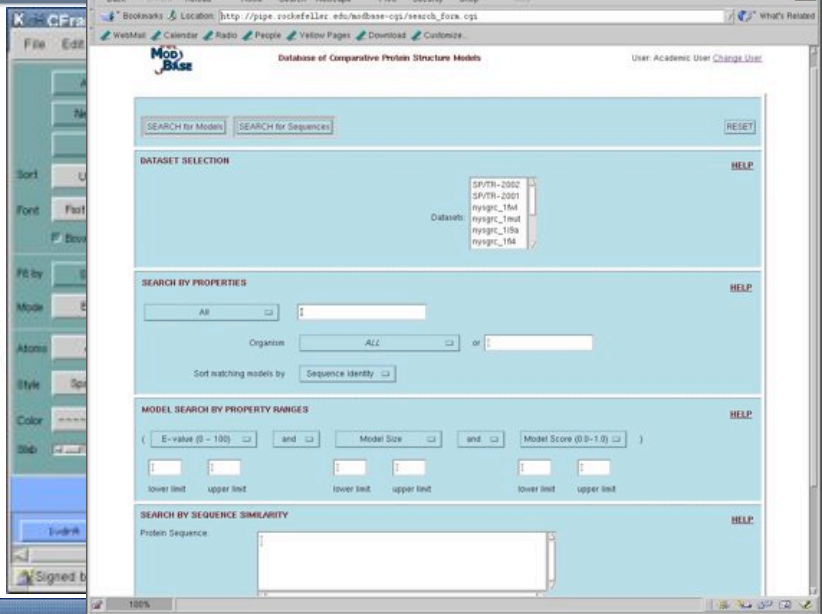
http://salilab.org/modbase

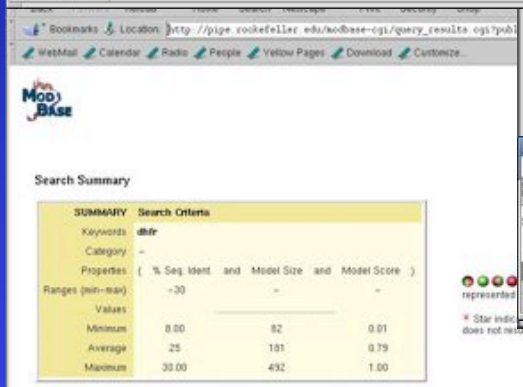
Pieper *et al.*, Nucl. Acids Res. 2002.

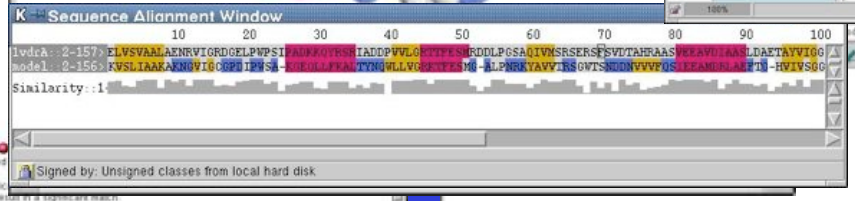
8/9/02

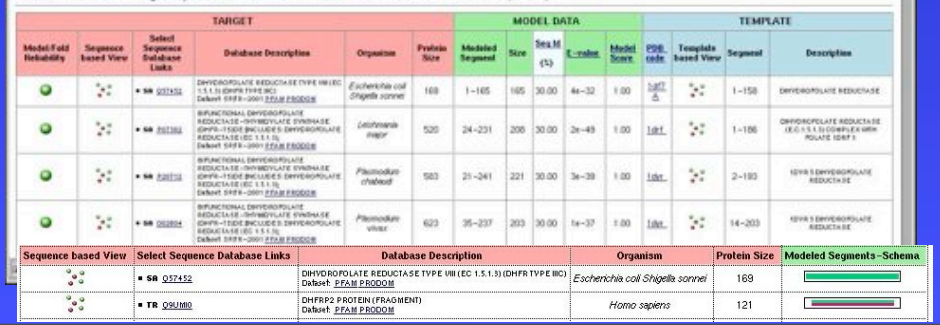


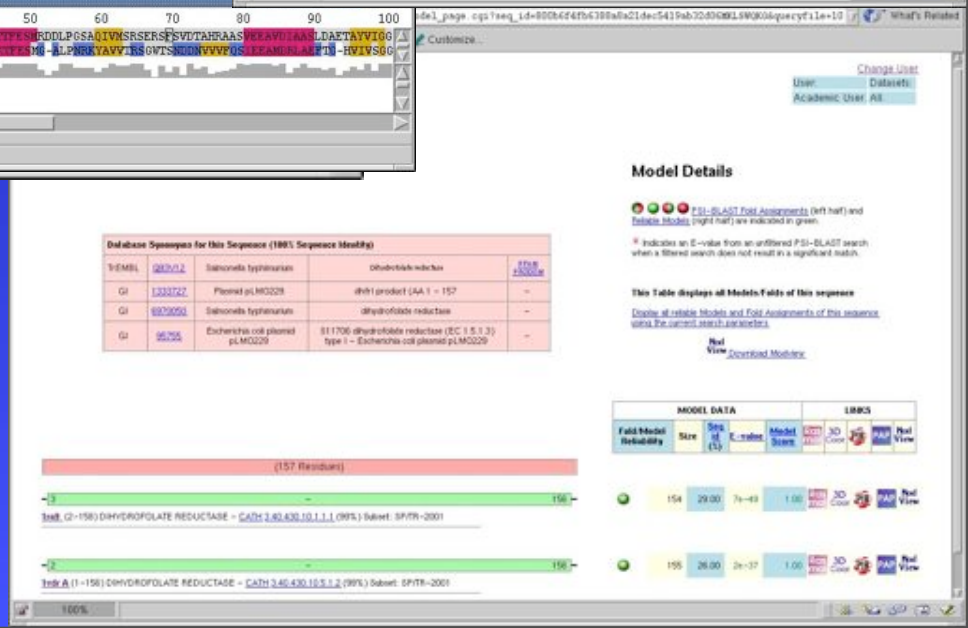






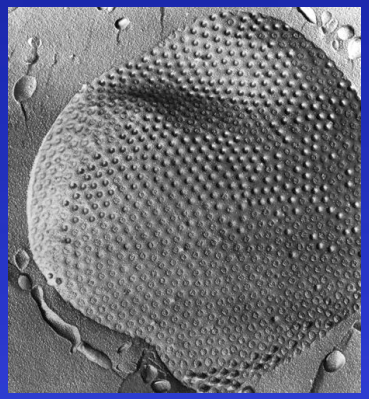
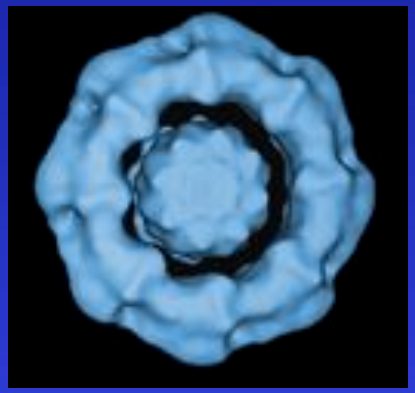
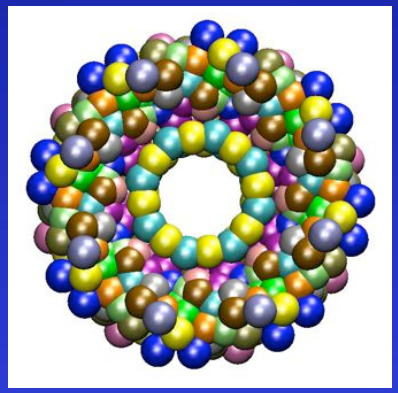






complejos…

Modeling the Yeast Nuclear Pore complex by satisfaction of spatial restraints

Andrej Sali
Frank Alber, Damien Devos

Depts. Of Biopharmaceutical Sciences and Pharmaceutical Chemistry
California Institute for Quantitative Biomedical Research
University of California at San Francisco

UCSF

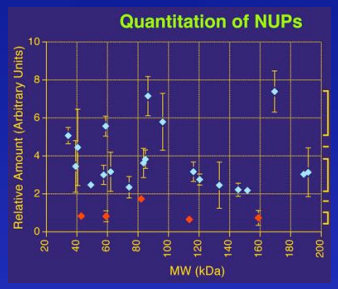
http://salilab.org/

Mike Rout,
T. Suprapto, J. Kipper, L. Veenhoff,
S. Dokudovskaya

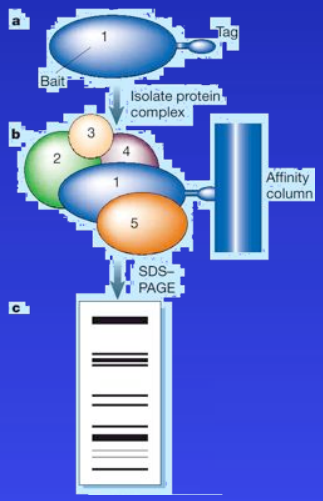
Brian Chait,
W. Zhang

The Rockefeller University,
1230 York Avenue, New York

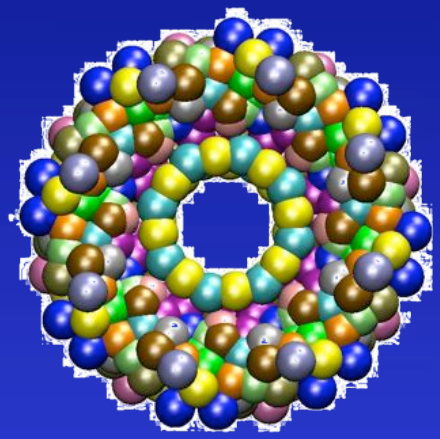
Integrate spatial information



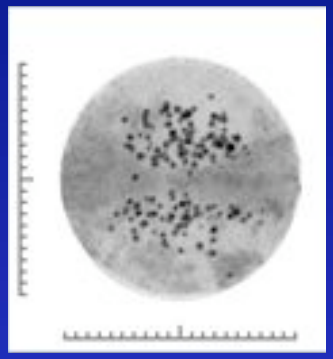
NUP Stoichiometry



NUP– NUP Interactions



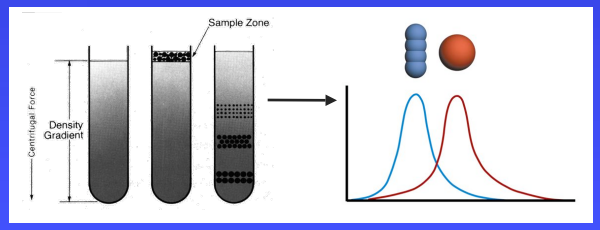
NUP Localization



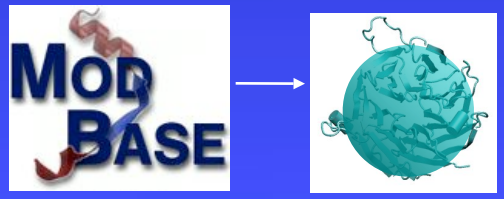
Symmetry Global shape

NUP Shape

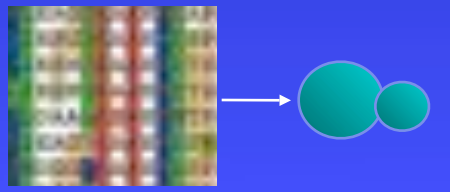
1)



2)

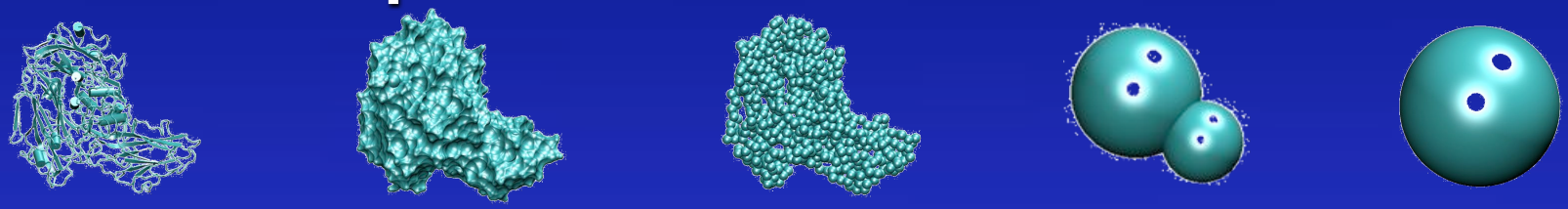


3)



Modeling scheme

- **Protein representation**



- **Scoring Function: A sum of spatial restraints**

- Excluded volume of proteins
- Symmetry of NPC (EM).
- Radial and axial localization of proteins (IEM)
- Protein-protein contacts (immuno-purification).

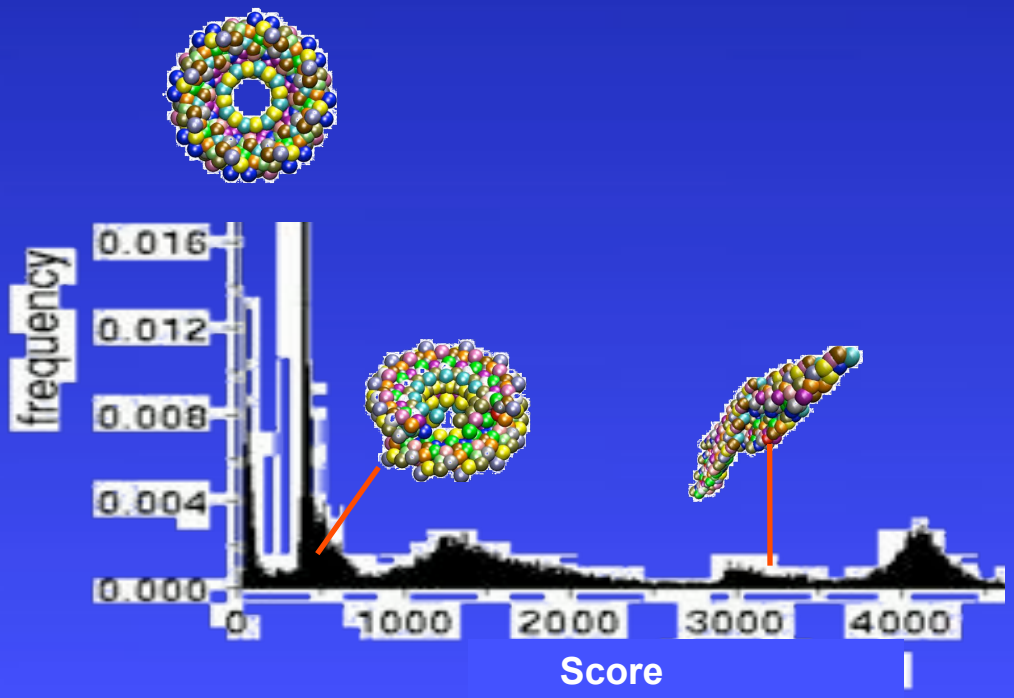
- **Optimization**

Minimize violations of input restraints by conjugate gradients and molecular dynamics with simulated annealing.

Obtain an “ensemble” (~100,000) of many independently calculated models, starting from random configuration of protein centers.

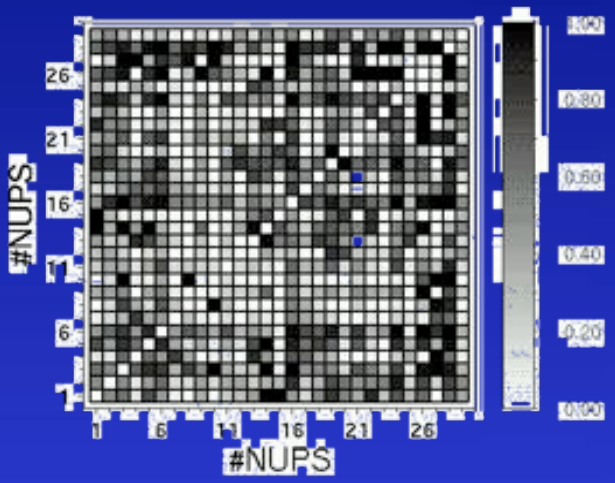
Analysis

- Assessing the well scoring models
  - How similar are the models to each other?
  - Do the models make sense given other data?
  - Using “toy” models as benchmarks.

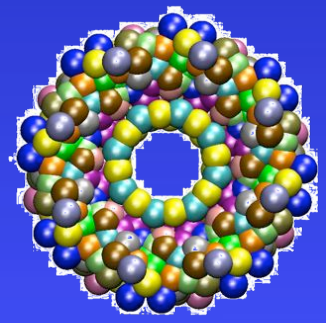


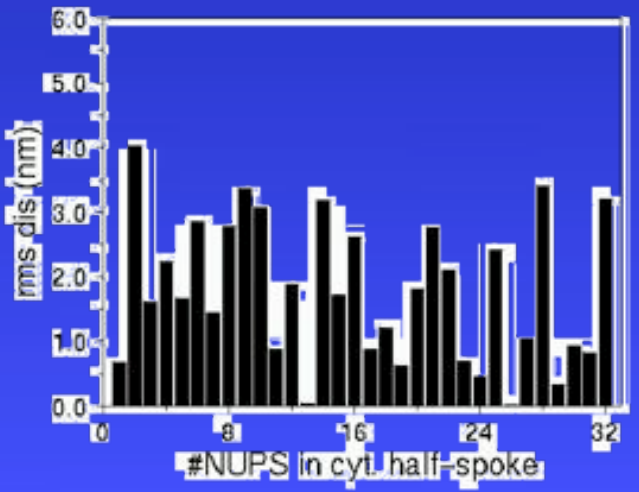
Analysis

- Search conservation of :
  - Protein-protein contacts



  - Structural features





Conclusiones

- ✓ Hoy en día se pueden modelar alrededor de el 55% de las secuencias de proteínas (un ~25% de los dominios de proteínas).
- ✓ Aplicación a problemas biológicos.
- ✓ La Genómica Estructural intenta determinar o predecir el mayor número posible de estructuras de proteínas.
- ✓ Los modelos de baja resolución de complejos nos ayudan a entender las interacciones de proteínas las células.

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

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