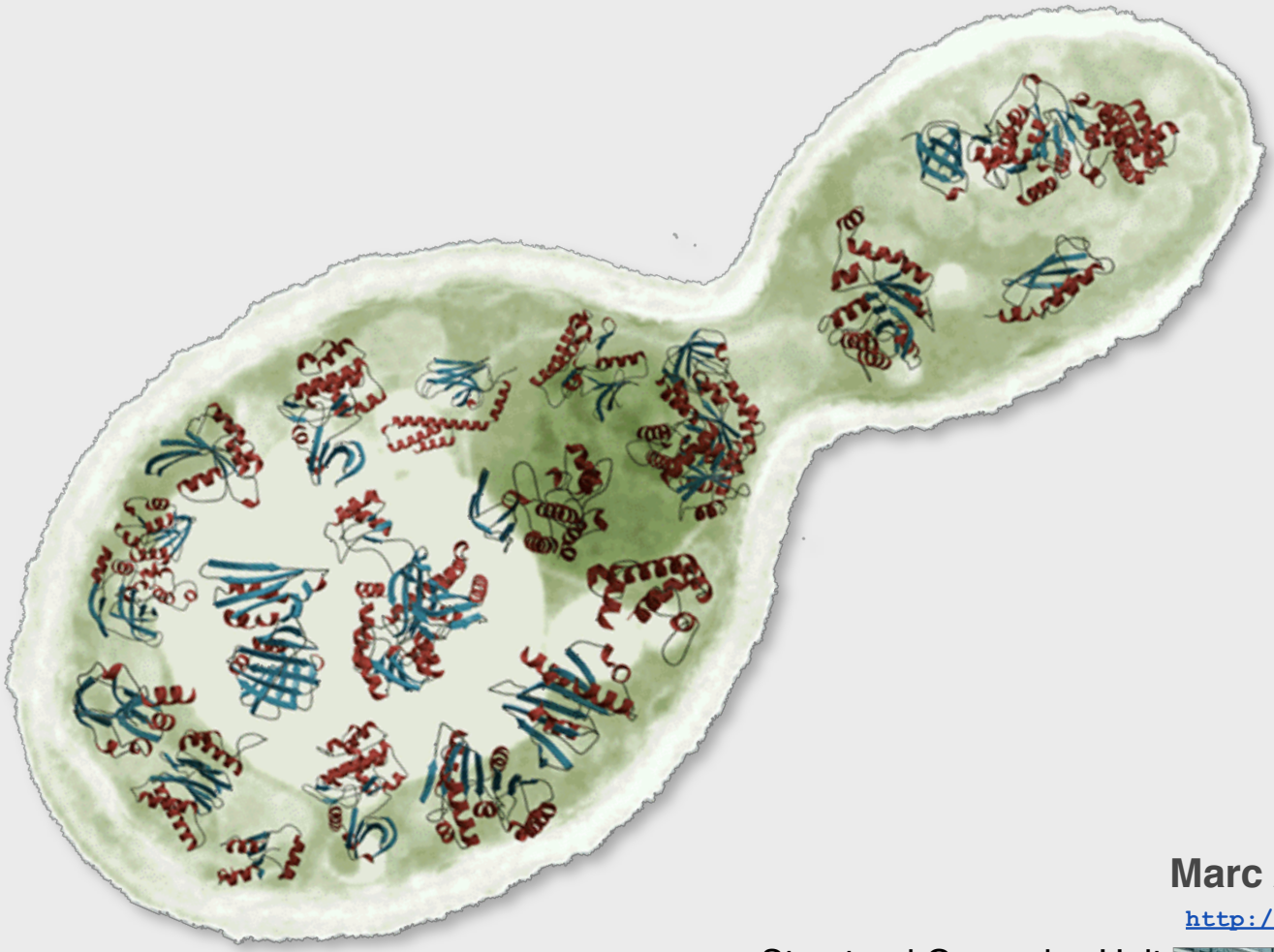
The Tropical Disease Initiative

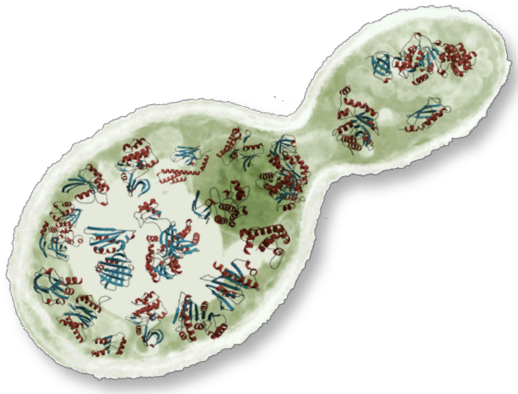
Comparative docking on protein structure models from ten tropical disease genomes.



Marc A. Marti-Renom

http://sgu.bioinfo.cipf.es

Structural Genomics Unit
Bioinformatics Department
Centro de Investigación Príncipe Felipe (CIPF), Valencia, Spain

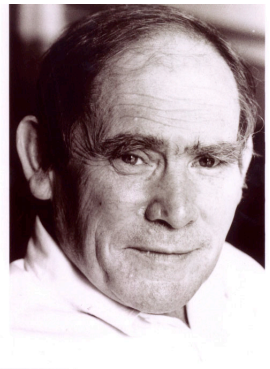


Bioinformatics @ CIPF
Introduction to CM
Modeling genomes
Modeling Genes
Tropical Disease Initiative

Data in the post-genomic era

Progress in science depends on new techniques, new discoveries and new ideas, probably in that order.

Sydney Brenner, 1980



The introduction and popularization of high-throughput techniques has drastically changed the way in which biological problems can be addressed and hypotheses can be tested.

But not necessarily the way in which we really address or test them…

From genotype to phenotype.

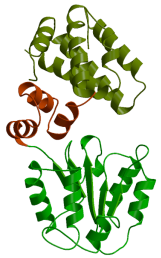
Genes in the DNA...

…code for proteins...

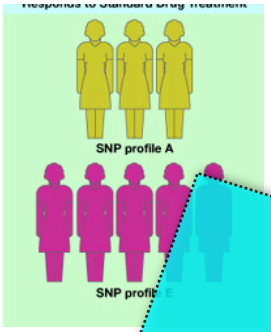
>protein kunase

acctgttgatggcgacagggactgtatgctg
atctatgctgatgcatgcatgctgactactgat

…whose structure accounts



…plus the environment...



…produces the final phenotype

From genotype to phenotype.

(post-genomics scenario)

Genes in the DNA...

Now: 22240 (NCBI build 35 12/04)

50-70% display alternative splicing

25%-60% unknown

Transfrags

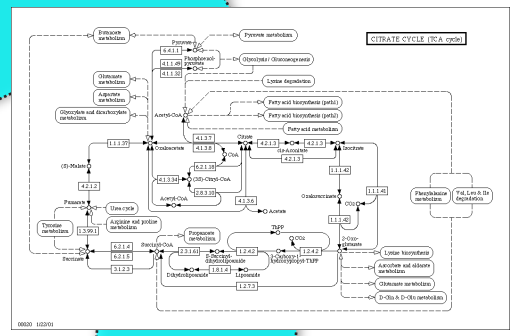
>protein kunase

acctgttgatggcgacagggactgtatgctgatctat
gctgatgcatgcatgctgactactgatgtgggggcta
ttgacttgatgtctatc....

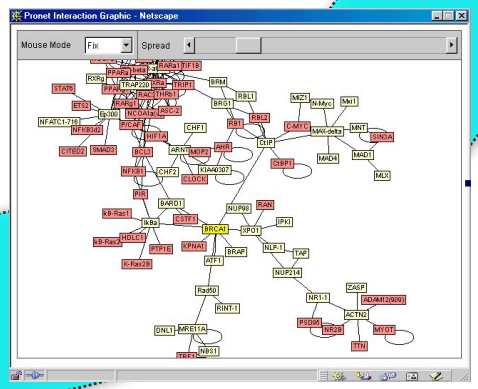
...which can be different because of the variability.

10 million SNPs

...whose final effect configures the phenotype...



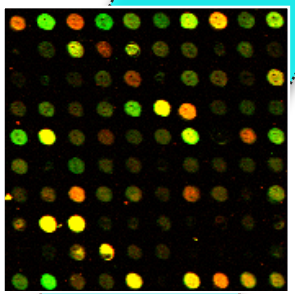
...conforming complex interaction networks...



...in cooperation with other proteins...

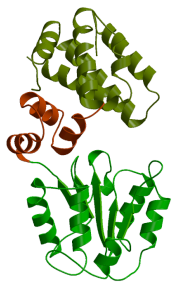
Each protein has an average of 8 interactions

...when expressed in the proper moment and place...



A typical tissue is expressing among 5000 and 10000 genes

...code for proteins...



That undergo post-translational modifications, somatic recombination...

100K-500K proteins

...whose structures account for function...

Similar things behave similar...

genes

Information

Information Databases

interactions

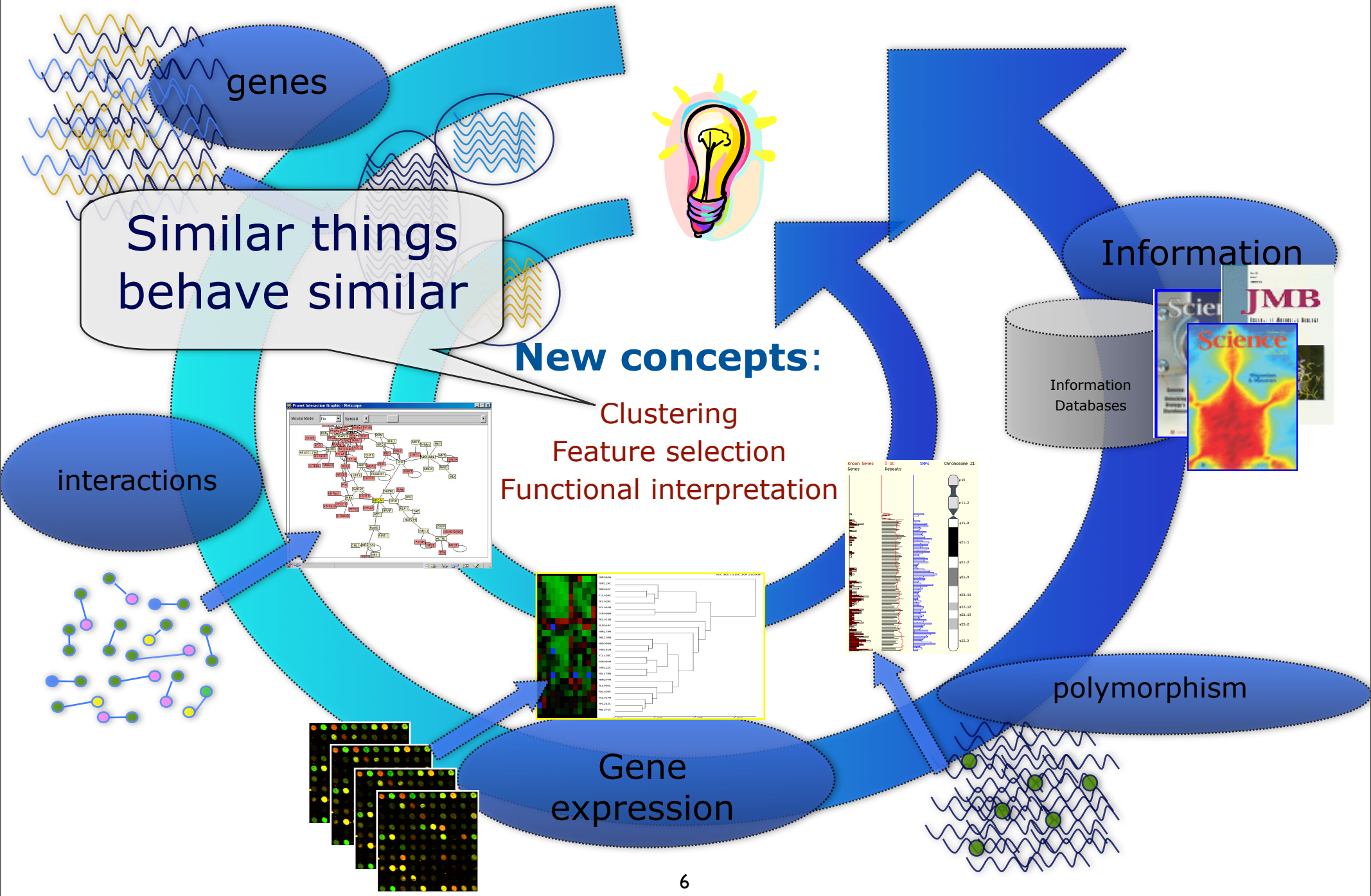
New concepts:

Clustering
Feature selection
Functional interpretation

polymorphism

Gene expression

Similar things behave similar...



Similar things behave similar...

genes

Similar things behave similar

Not everything in the data is relevant

New concepts:

Clustering
Feature selection
Functional interpretation

Information Databases

interactions

polymorphism

Gene expression

Similar things behave similar...

genes

Similar things behave similar

Not everything in the data is relevant

New concepts:

Clustering
Feature selection
Functional interpretation

interactions

The data has to be transformed into biological information

Gene expression

polymorphism

Information Databases

Bioinformatics Department http://bioinfo.cipf.es

Bioinformatics Department http://bioinfo.cipf.es

Functional Genomics
Dr. Joaquín Dopazo

GEPAS

Pupa Suite

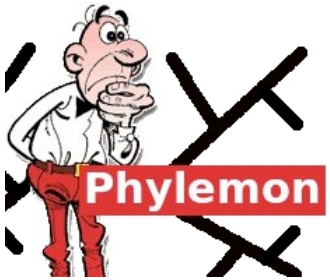
BABELOMICS

http://gepas.bioinfo.cipf.es
Gene Expression Pattern Analysis Suite

http://pupasuite.bioinfo.cipf.es
SNP Analysis Suite

http://babelomics.bioinfo.cipf.es
Functional Profiling Analysis Suite

Comparative Genomics
Pharmacogenomics
Dr. Hernán Dopazo



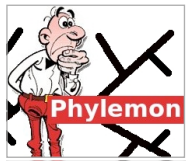
http://phylemon.bioinfo.cipf.es
Molecular Evolution Analysis Suite

Structural Genomics
Dr. Marc A. Marti-Renom

DBAli v2.0
Home

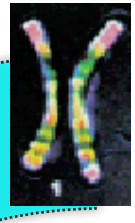
http://www.dbali.org
Structural Biology Analysis Suite

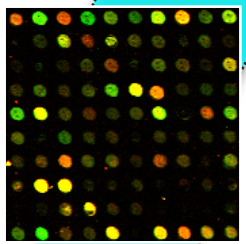
Bioinformatics Department http://bioinfo.cipf.es

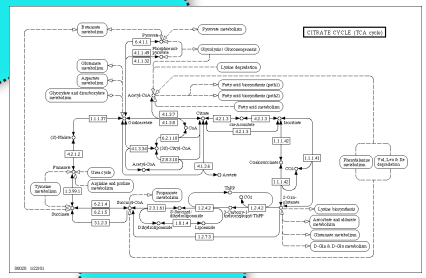


>protein kunase

acctgttgatggcgacagggactgtatgct
gatctatgctgatgcatgcatgctgactact

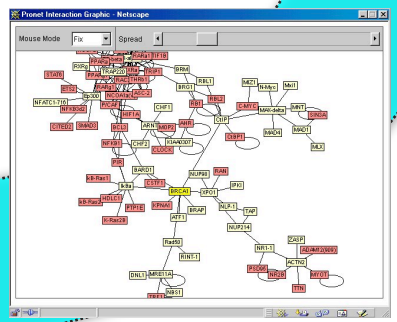






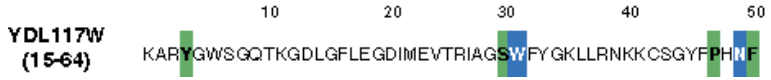
From genotype to phenotype.

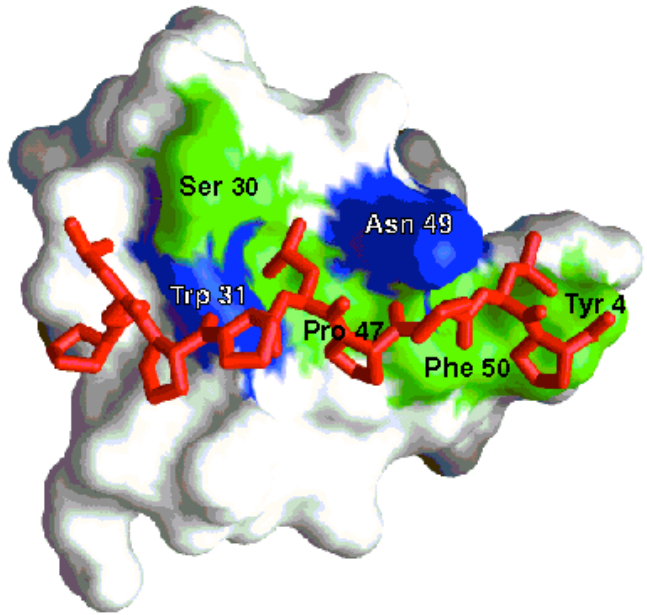
(post-genomics scenario)



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.

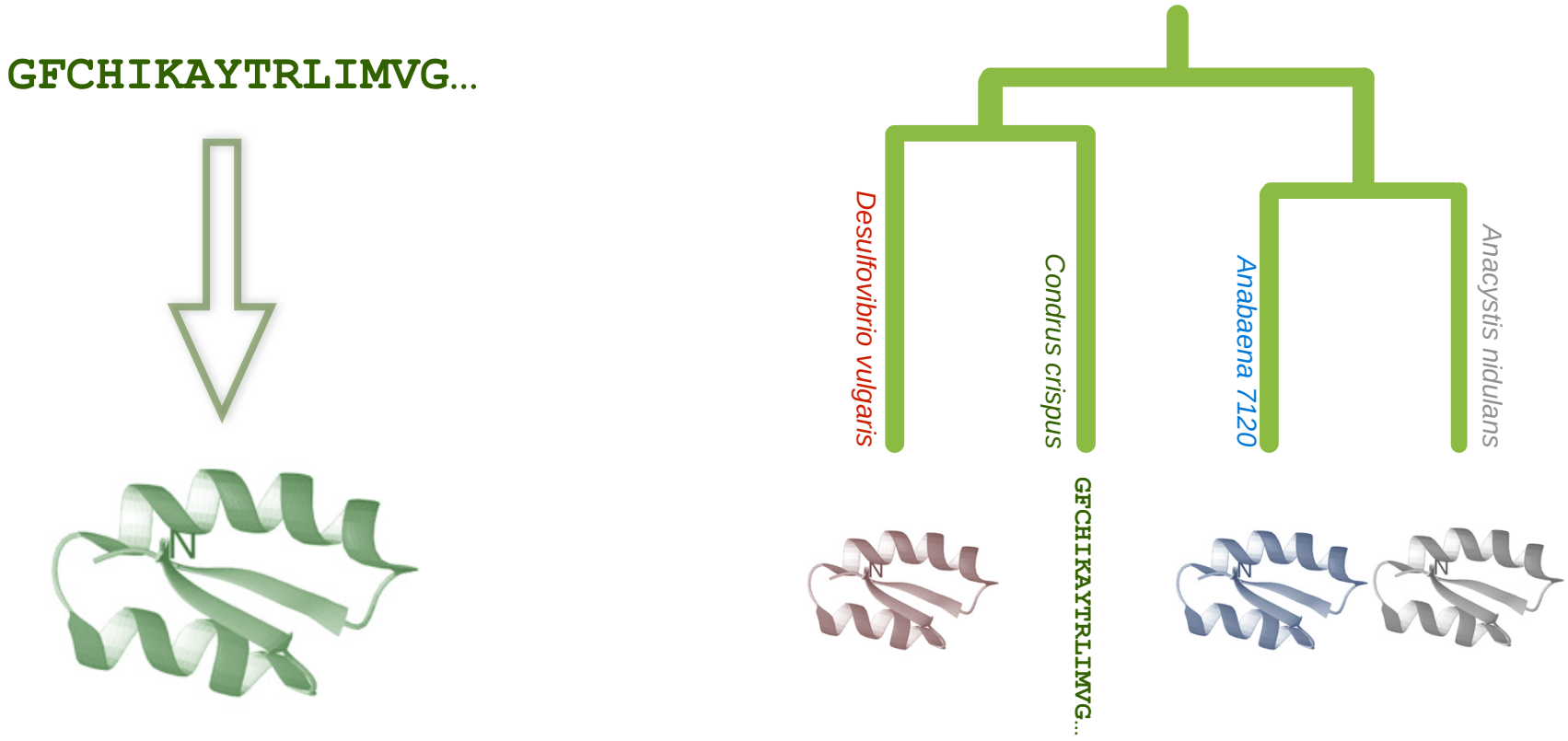




In addition, since evolution tends to conserve function and function depends more directly on structure than on sequence, **structure is more conserved in evolution than sequence**.

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

Principles of protein structure



Folding (physics)

Ab initio prediction

Evolution (rules)

Threading

Comparative Modeling

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

protein prediction vs protein determination

X-Ray

NMR

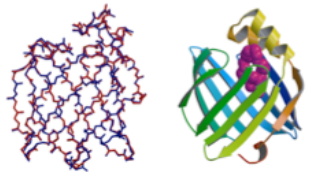
Comparative Modeling

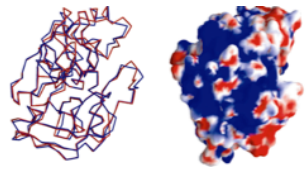
Threading

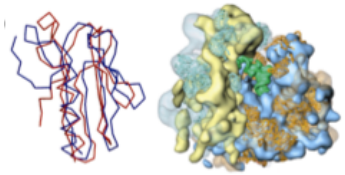
Ab-initio

Experimental data

inferred data

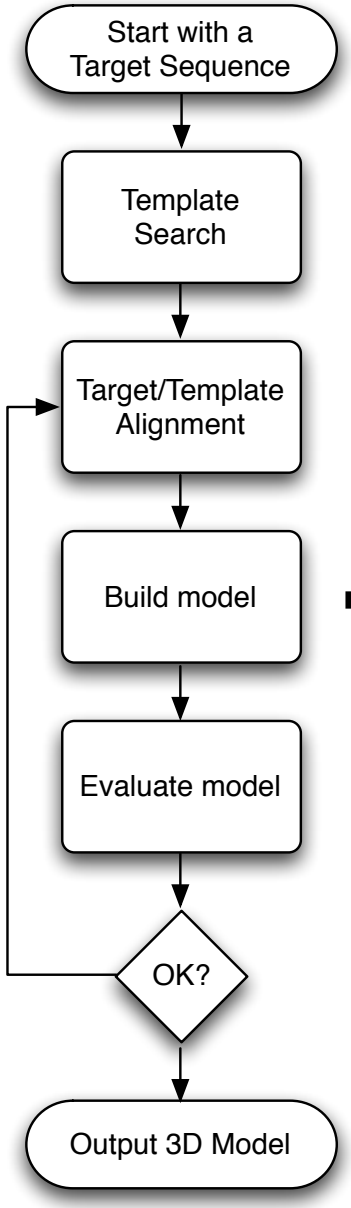






Comparative modeling by satisfaction of spatial restraints

MODELLER

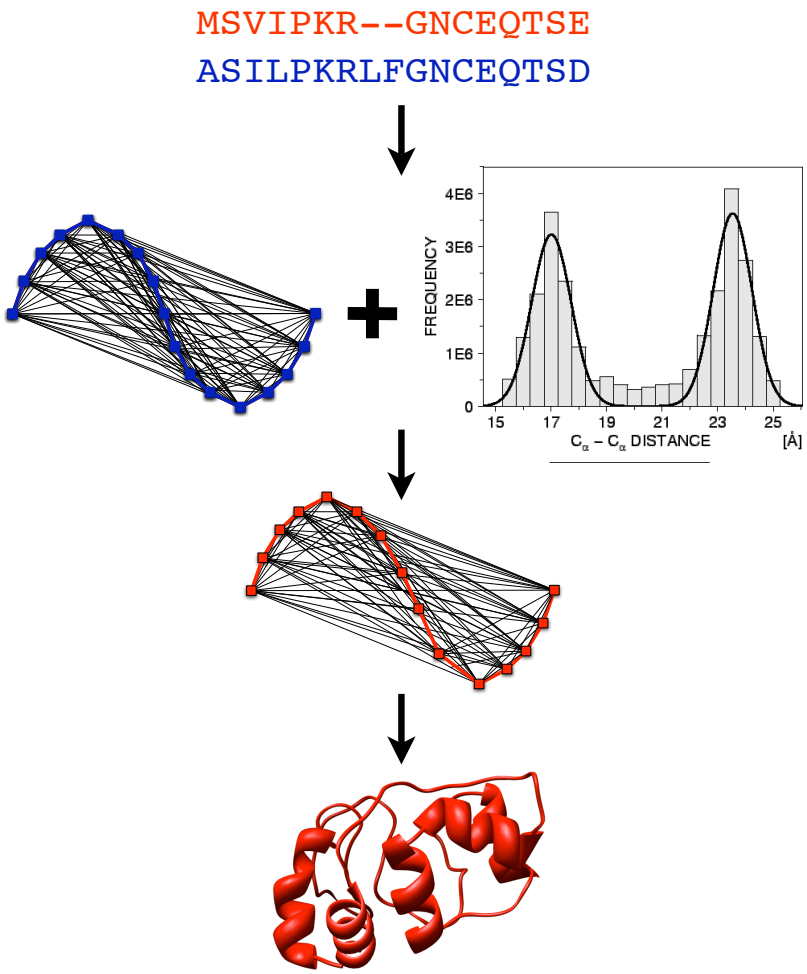


Given an alignment...

extract spatial features from the template(s) and statistics from known structures

apply these features as restraints on your target sequence

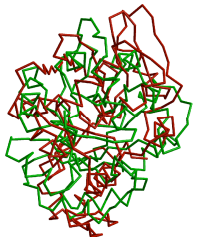
optimize to find the best solution for the restraints to produce your 3D model



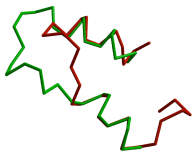
A. Šali & T. Blundell. J. Mol. Biol. 234, 779, 1993.
J.P. Overington & A. Šali. Prot. Sci. 3, 1582, 1994.
A. Fiser, R. Do & A. Šali, Prot. Sci., 9, 1753, 2000.

Comparative modeling by satisfaction of spatial restraints

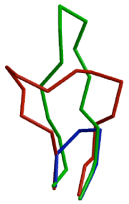
Types of errors and their impact



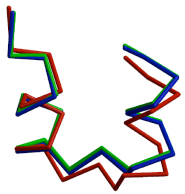
Wrong fold



Miss alignments

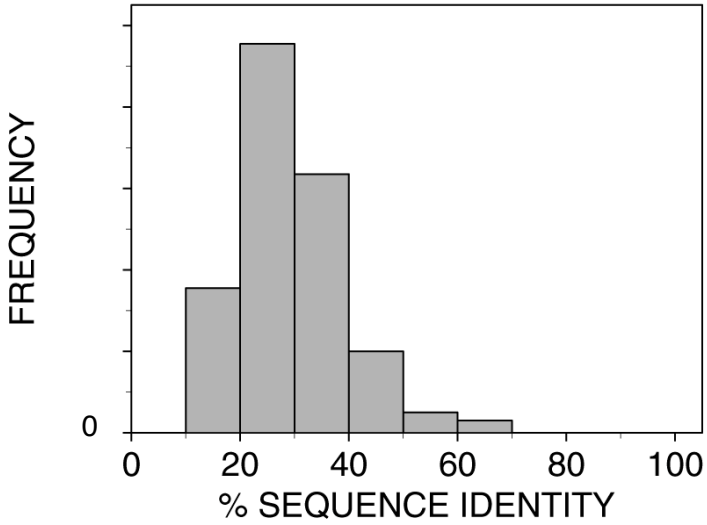


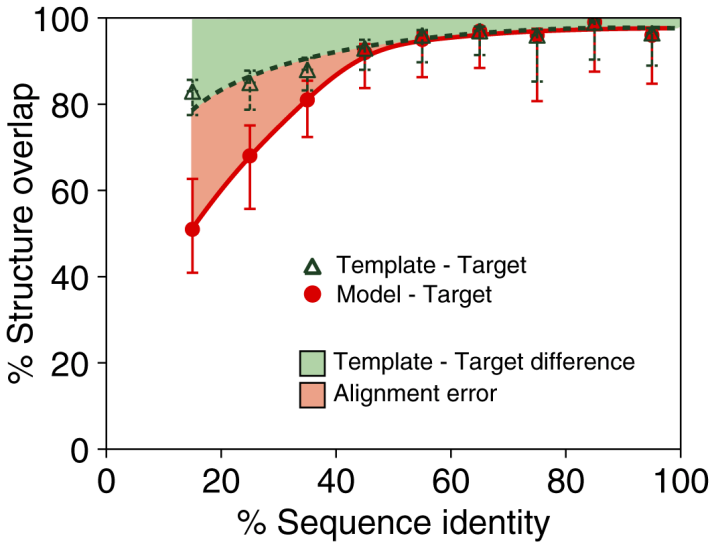
Loop regions



Rigid body distortions

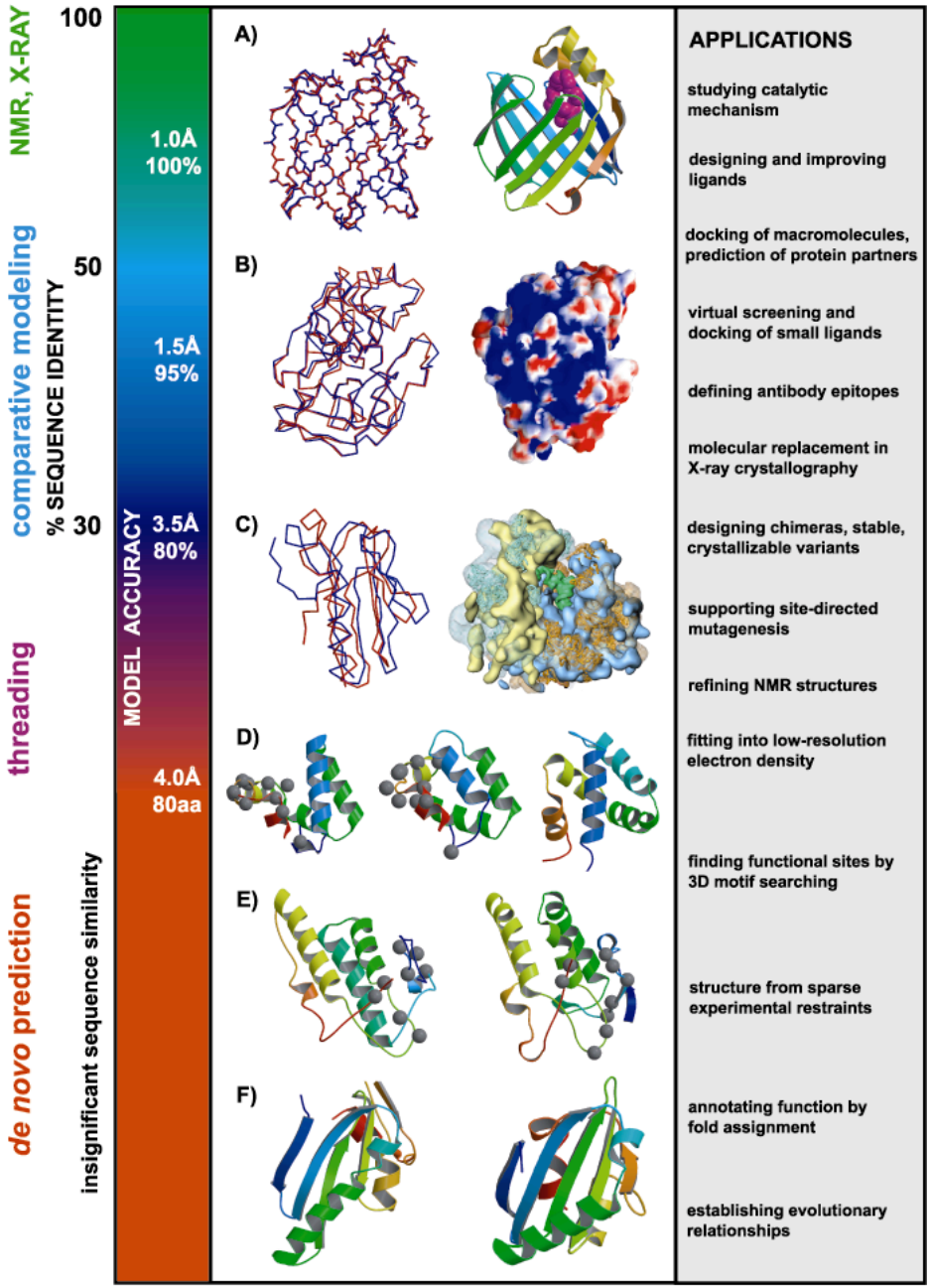
Side-chain packing





Marti-Renom etal. Ann Rev Biophys Biomol Struct (2000) 29, 291

Utility of protein structure models, despite errors

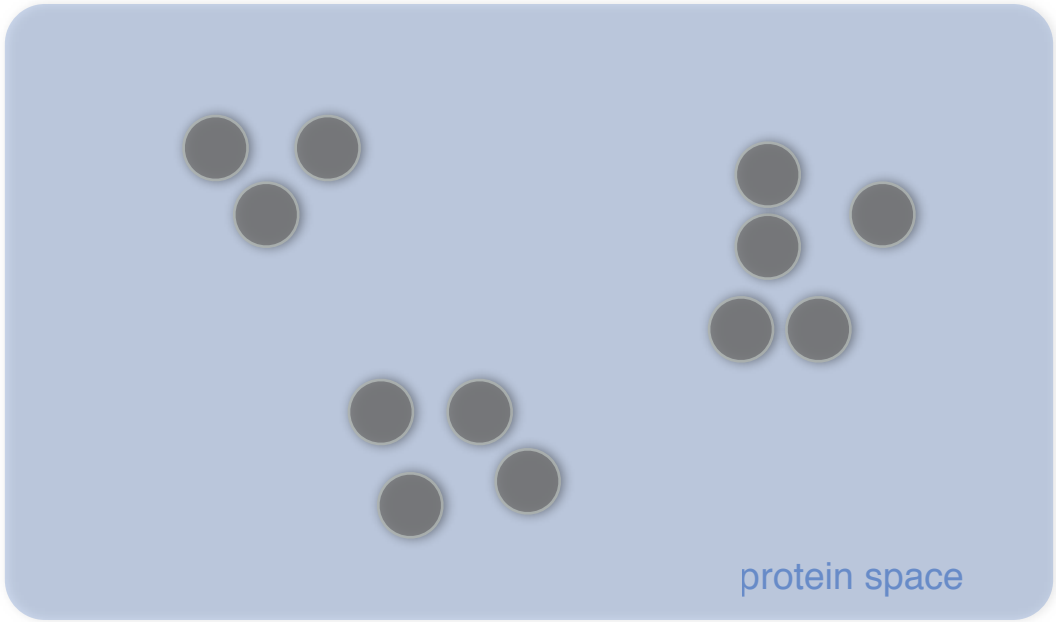


D. Baker & A. Sali. Science 294, 93, 2001.

Structural Genomics

Characterize most protein sequences based on related known structures

1. The number of "**families**" is much **smaller** than the number of proteins.
2. **Any one** of the members of a family is **fine**.



There are **~16,000** families (90%)
@ 30% sequence identity cutoff

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.*
Vitkup et al. Nat. Struct. Biol. ***8****, 559, 2001*

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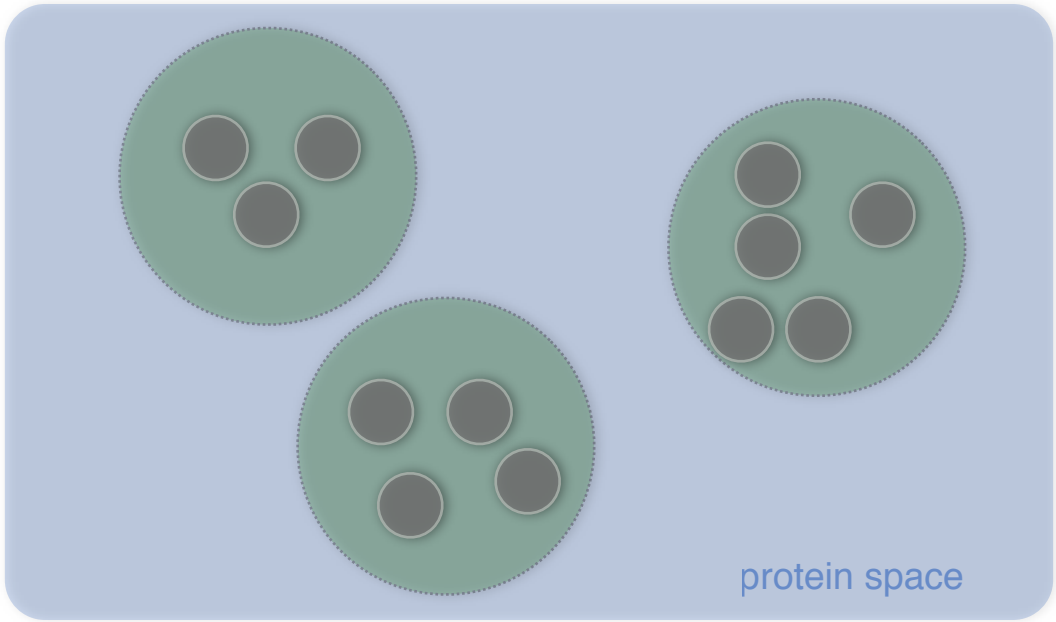

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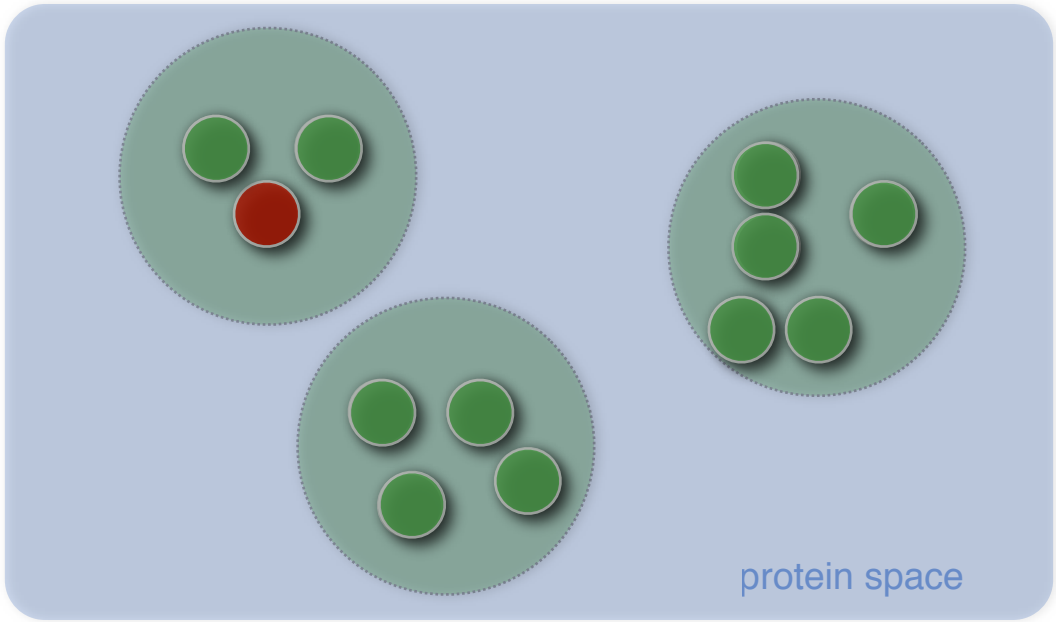

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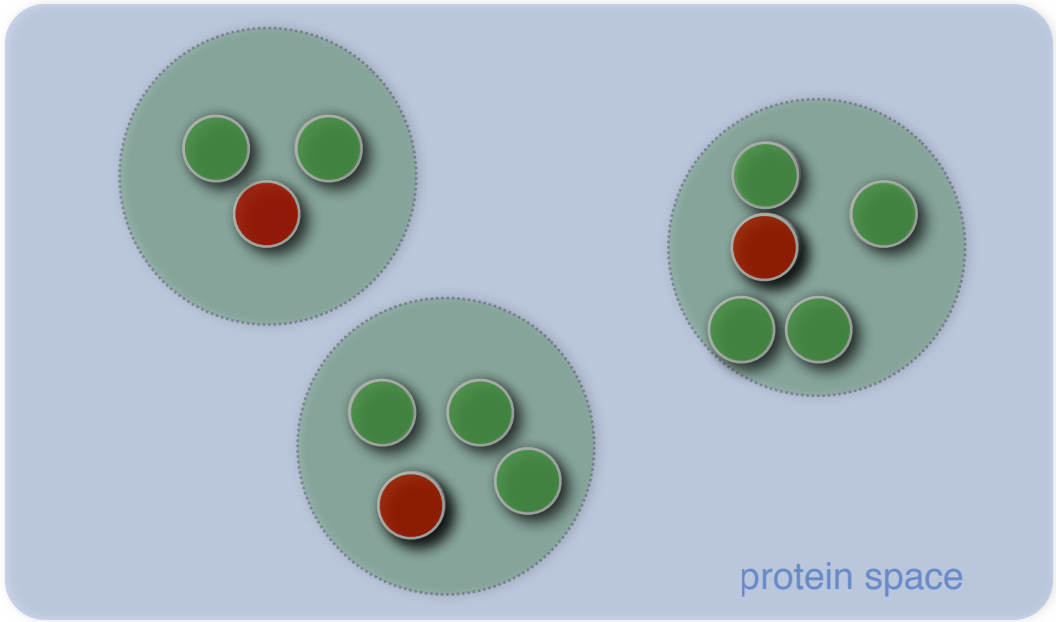

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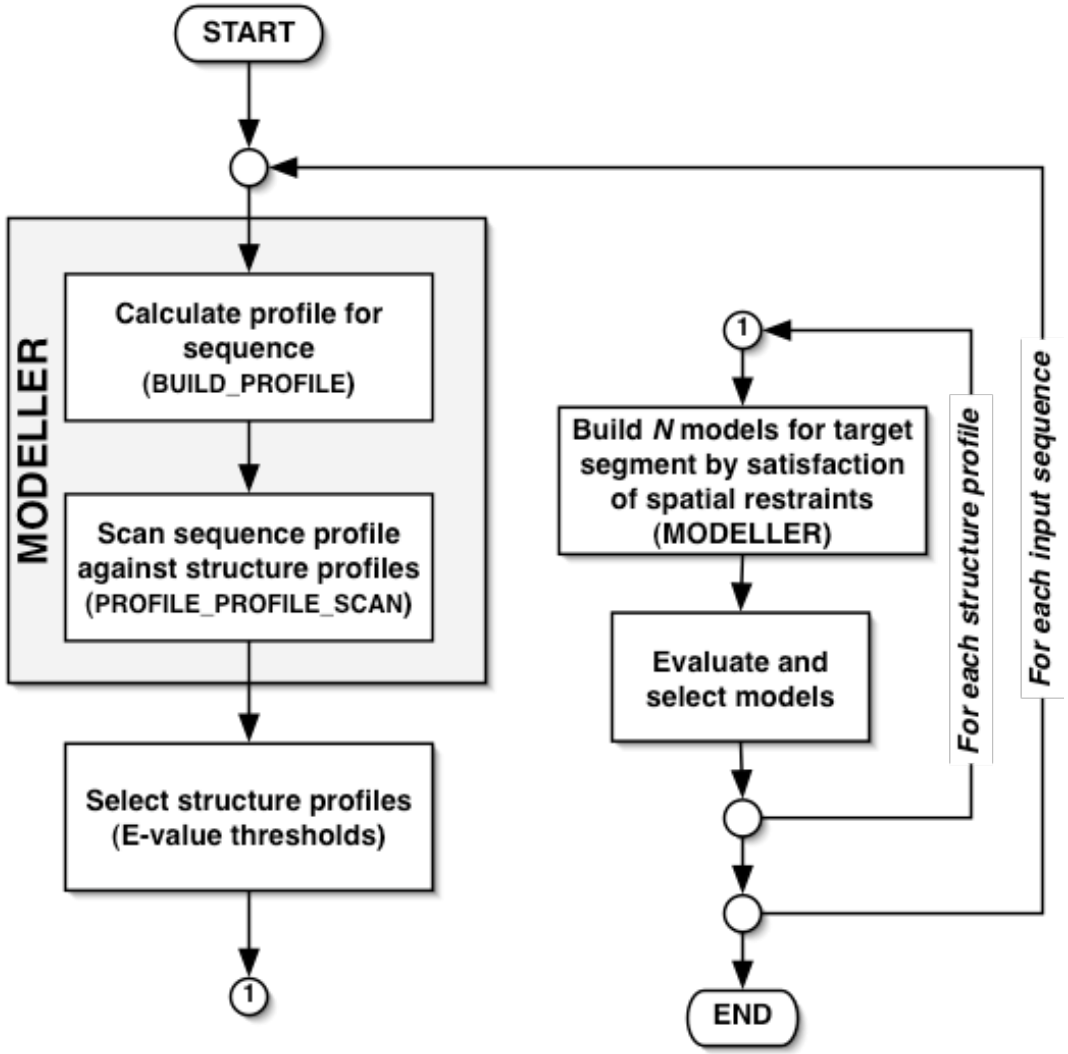
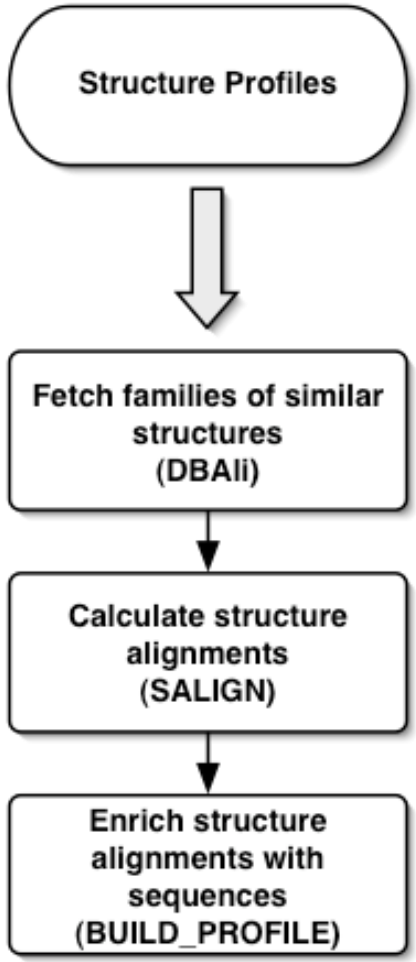
Sali. Nat. Struct. Biol. ***5****, 1029, 1998.*
Sali et al. Nat. Struct. Biol., ***7****, 986, 2000.*
Sali. Nat. Struct. Biol. ***7****, 484, 2001.*
Baker & Sali. Science ***294,*** *93, 2001.*
Vitkup et al. Nat. Struct. Biol. ***8****, 559, 2001*

MODPIPE2.0

Large-Scale Protein Structure Modeling





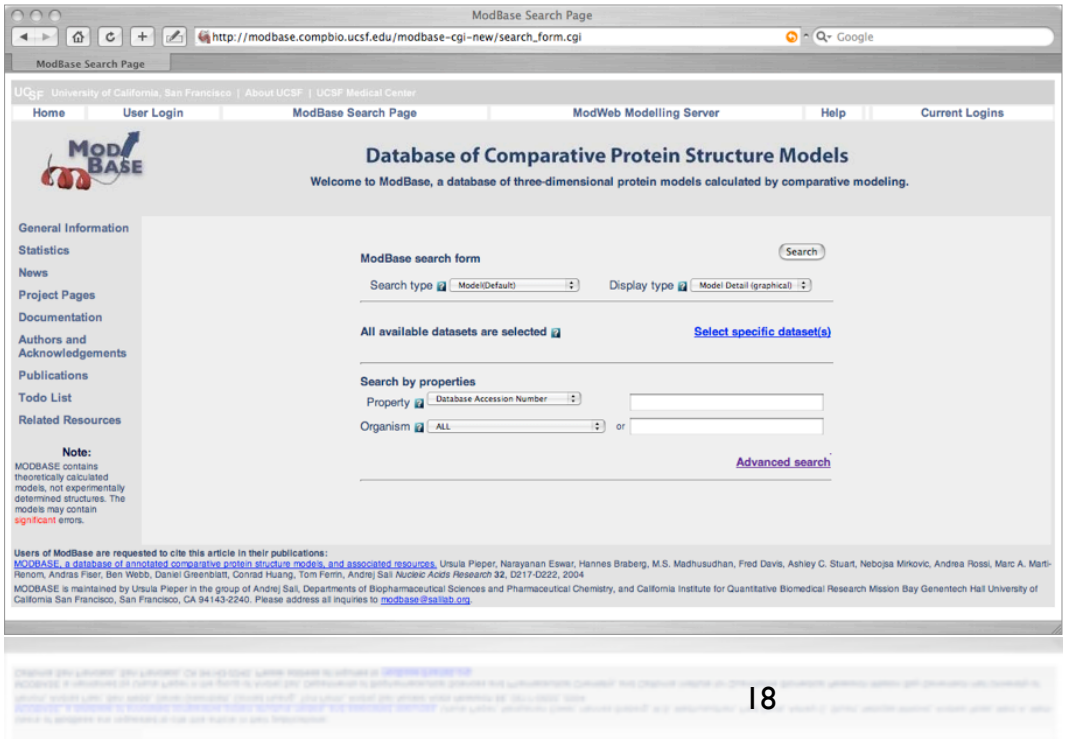
Eswar et.al., (2003) Nucl.Acids.Res. 31(13)

ModBase Statistics

Large-scale modeling of the TrEMBL-SWISSPROT databases

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

Sequences (total)	2,186,210
Sequences (modeled)	1,340,687
Models	4,284,570



UCSF
University of California
San Francisco

Pieper et al. NAR 34, D291 (2006)

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

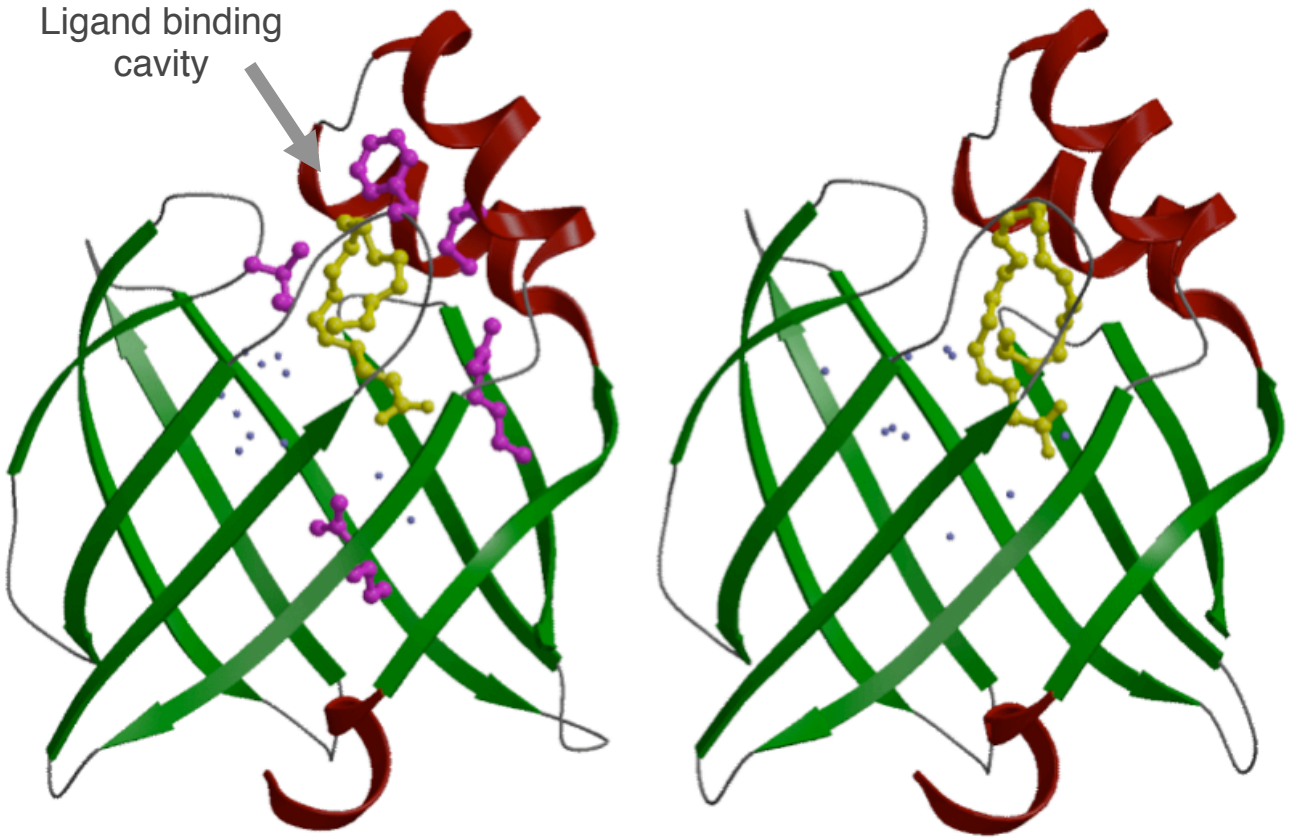
Predicting features of a model that are not present in the template

BLBP/oleic acid

Cavity is not filled

BLBP/docosahexaenoic acid

Cavity is filled



1. BLBP binds fatty acids.

2. Build a 3D model.

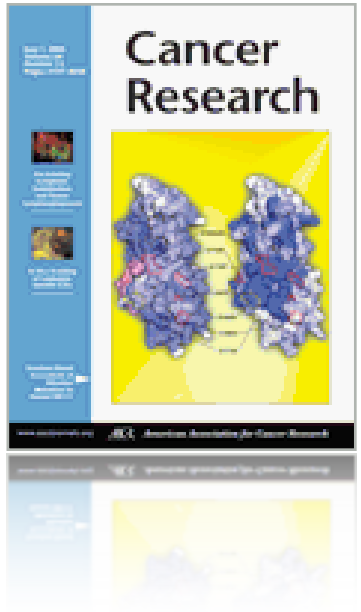
3. Find the fatty acid that fits most snuggly into the ligand binding cavity.

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

Structural analysis of missense mutations in human BRCA1 BRCT domains

Nebojsa Mirkovic, Marc A. Marti-Renom, Barbara L. Weber, Andrej Sali and Alvaro N.A. Monteiro

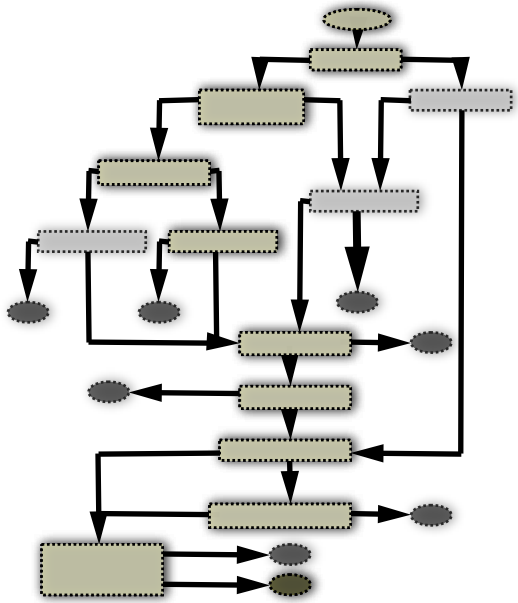
Cancer Research (June 2004). 64:3790-97



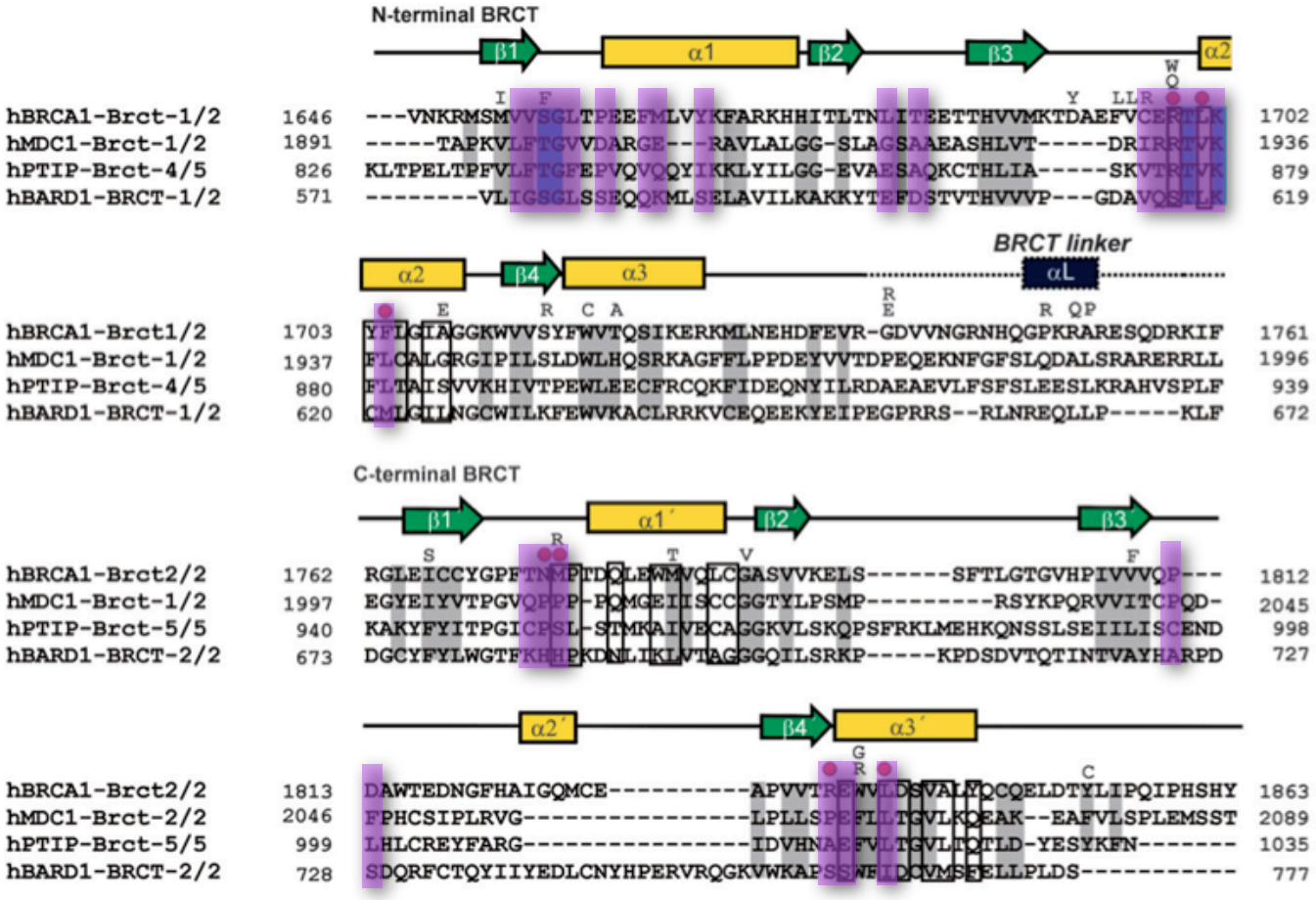
Cannot measure the functional impact of every possible SNP at all positions in each protein! Thus, prediction based on general principles of protein structure is needed.

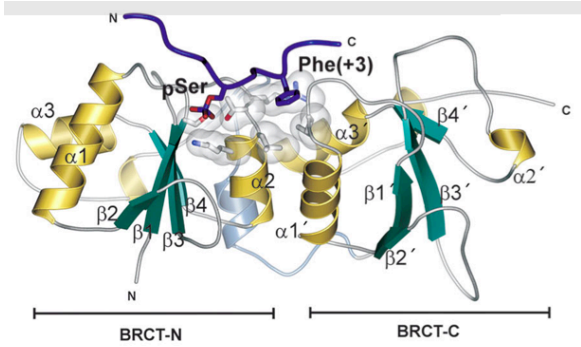
Missense mutations in BRCT domains by function

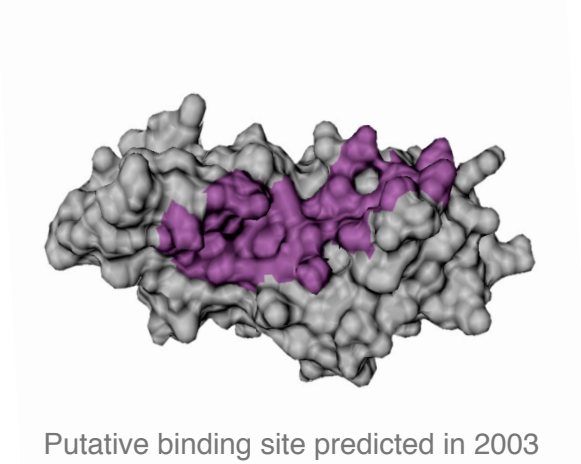
	cancer associated	not cancer associated	?
no transcription activation	C1697R R1699W A1708E S1715R P1749R M1775R		M1652K L1657P E1660G H1686Q R1699Q K1702E Y1703HF1 704S L1705PS1 715NS172 2FF1734L G1738EG 1743RA1 752PF176 1I F1761S M1775E M1775K L1780P I1807S V1833E A1843T
transcription activation		M1652I A1669S	V1665M D1692N G1706A D1733G M1775V P1806A
?			M1652T V1653M L1664P T1685A T1685I M1689R D1692Y F1695L V1696L R1699L G1706E W1718C W1718S T1720A W1730S F1734S E1735K V1736A G1738R D1739E D1739G D1739Y V1741G H1746N R1751P R1751Q R1758G L1764P I1766S P1771L T1773S P1776S D1778N D1778G D1778H M1783T C1787S G1788D G1788V G1803A V1804D V1808A V1809A V1809F V1810G Q1811R P1812S N1819S A1823T V1833M W1837R W1837G S1841N A1843P T1852S P1856T P1859R



Putative binding site on BRCA1





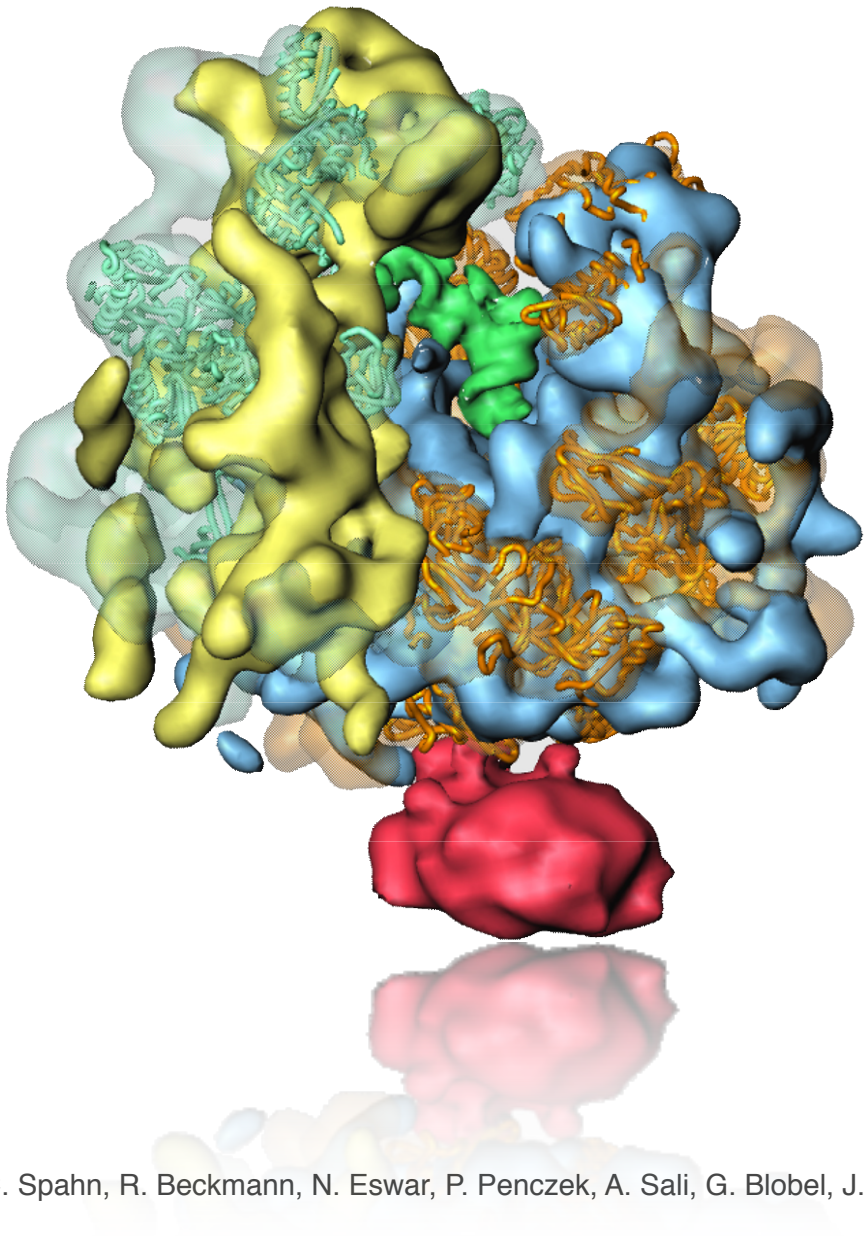


Putative binding site predicted in 2003 and accepted for publication on March 2004.

Williams *et al.* 2004 Nature Structure Biology. **June 2004 11**:519

Mirkovic *et al.* 2004 Cancer Research. **June 2004 64**:3790

S. cerevisiae ribosome



Fitting of comparative models into 15Å cryo-electron density map.

43 proteins could be modeled on 20-56% seq.id. to a known structure.

The modeled fraction of the proteins ranges from 34-99%.

C. Spahn, R. Beckmann, N. Eswar, P. Penczek, A. Sali, G. Blobel, J. Frank. Cell 107, 361-372, 2001.

Tropical Disease Initiative (TDI)

Predicting binding sites in protein structure models.

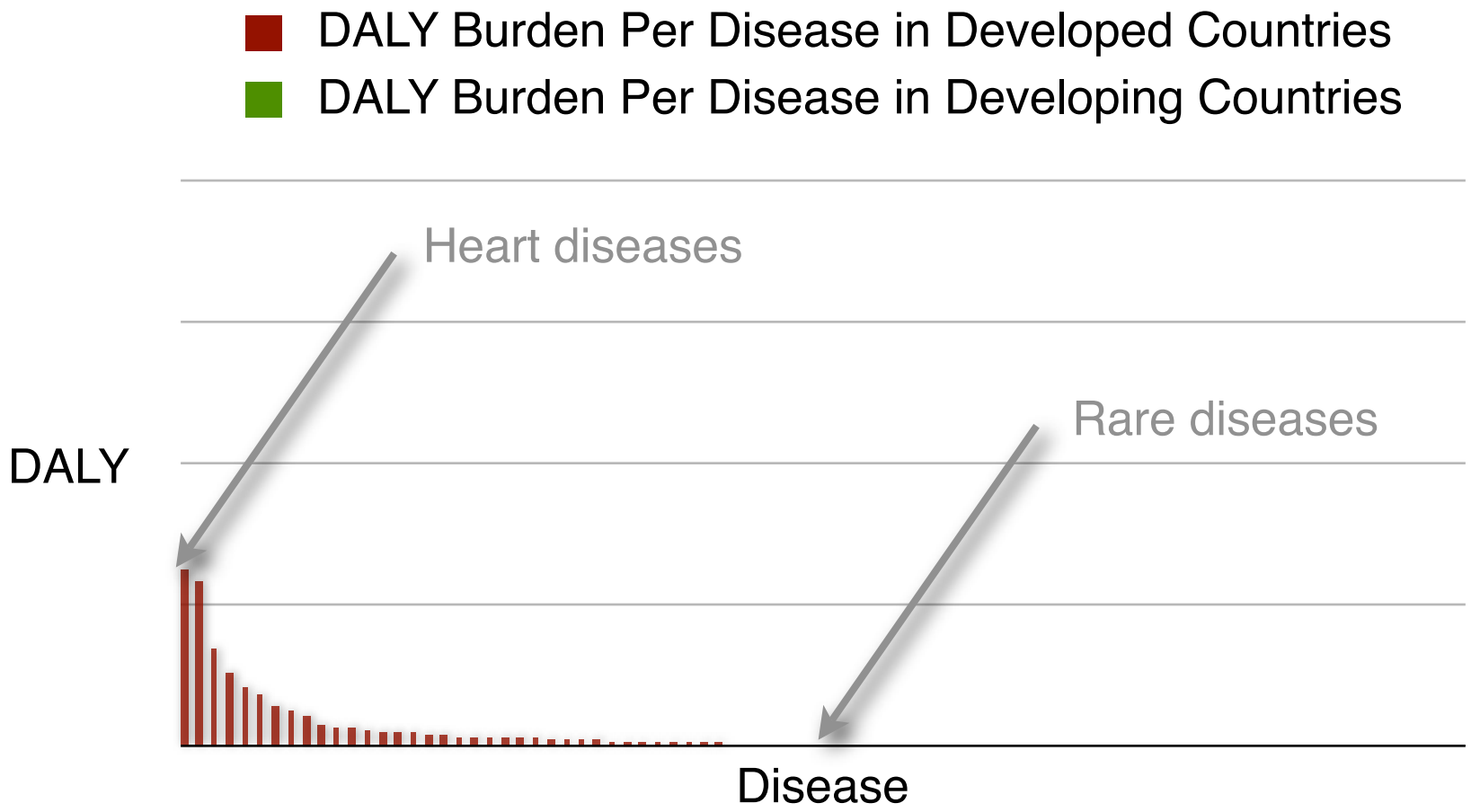
UCSF

Duke
UNIVERSITY

PRINCIPE FELIPE
CENTRO DE INVESTIGACION

http://www.tropicaldisease.org

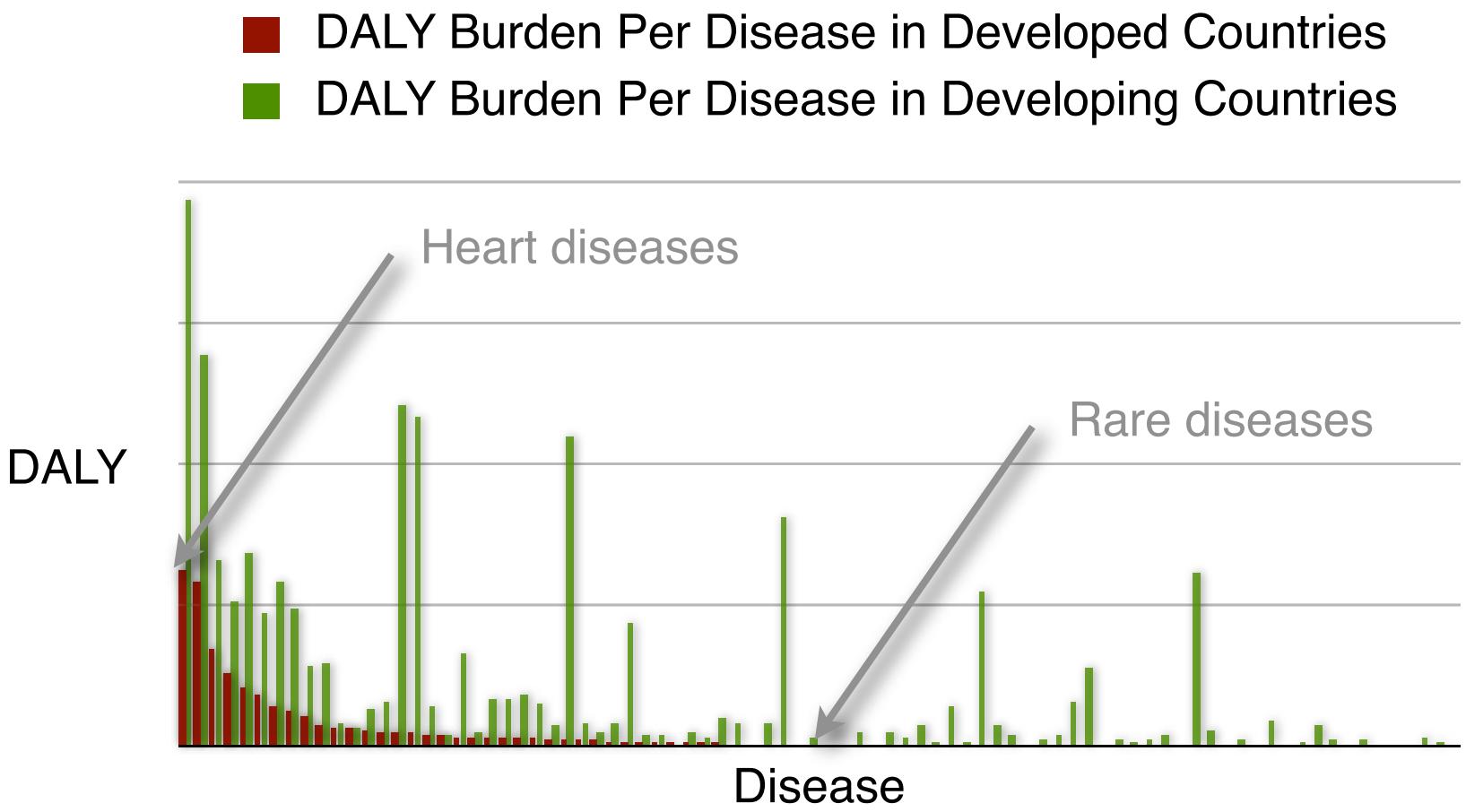
Need is High in the Tail



Disease data taken from WHO, *World Health Report 2004*
DALY - Disability adjusted life years
DALY is not a perfect measure of market size, but is certainly a good measure for importance.

DALYs for a disease are the sum of the years of life lost due to premature mortality (YLL) in the population and the years lost due to disability (YLD) for incident cases of the health condition. The DALY is a health gap measure that extends the concept of potential years of life lost due to premature death (PYLL) to include equivalent years of 'healthy' life lost in states of less than full health, broadly termed disability. One DALY represents the loss of one year of equivalent full health.

Need is High in the Tail



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"Unprofitable" Diseases and Global DALY (in 1000's)

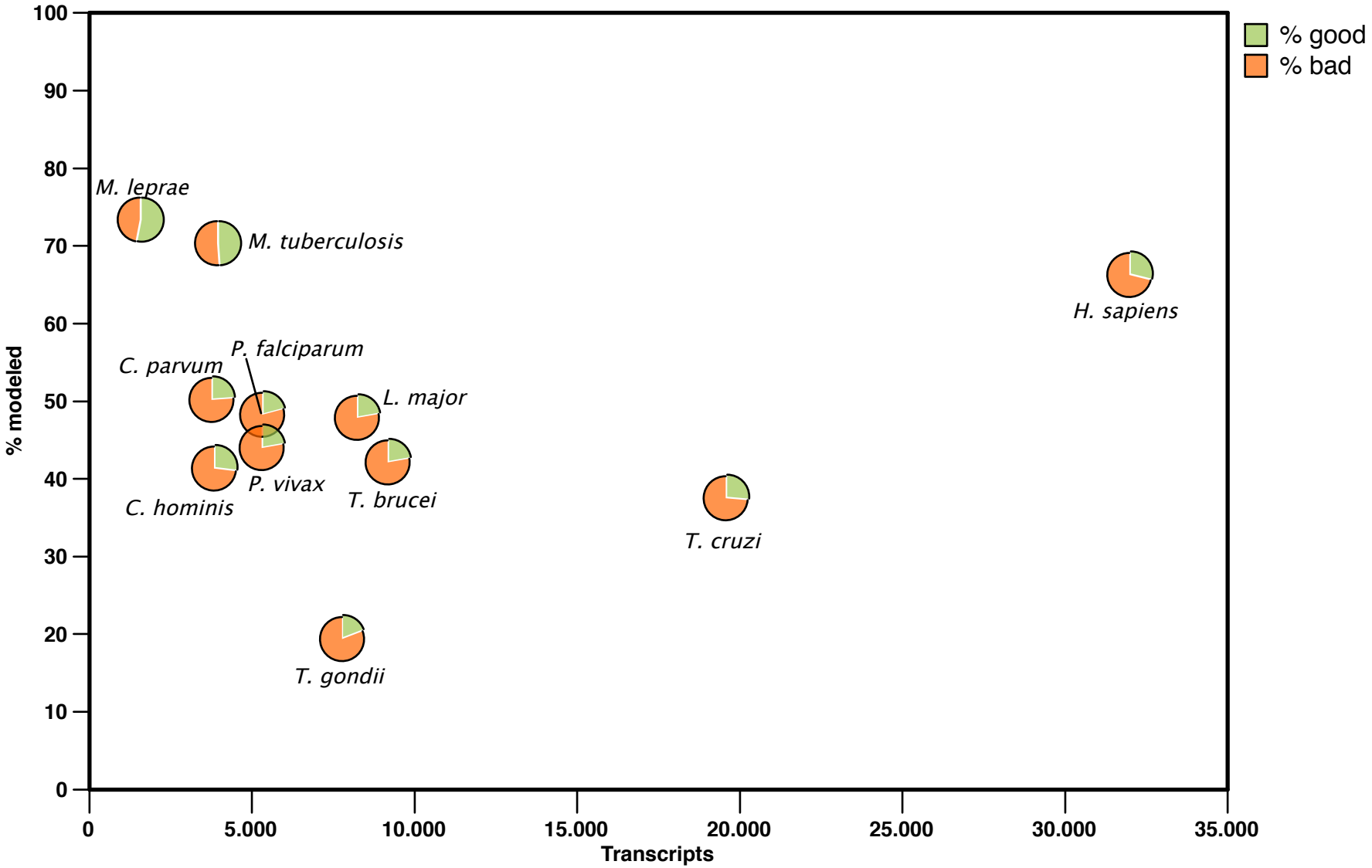
Malaria*	**46,486**
Tetanus	7,074
Lymphatic filariasis*	**5,777**
Syphilis	4,200
Trachoma	2,329
Leishmaniasis*	**2,090**
Ascariasis	1,817
Schistosomiasis*	**1,702**
Trypanosomiasis*	**1,525**

Trichuriasis	1,006
Japanese encephalitis	709
Chagas Disease*	**667**
Dengue*	**616**
Onchocerciasis*	**484**
Leprosy*	**199**
Diphtheria	185
Poliomyelitise	151
Hookworm disease	59

Disease data taken from WHO, *World Health Report 2004*
DALY - Disability adjusted life year in 1000's.
* Officially listed in the WHO Tropical Disease Research disease portfolio.

Modeling Genomes

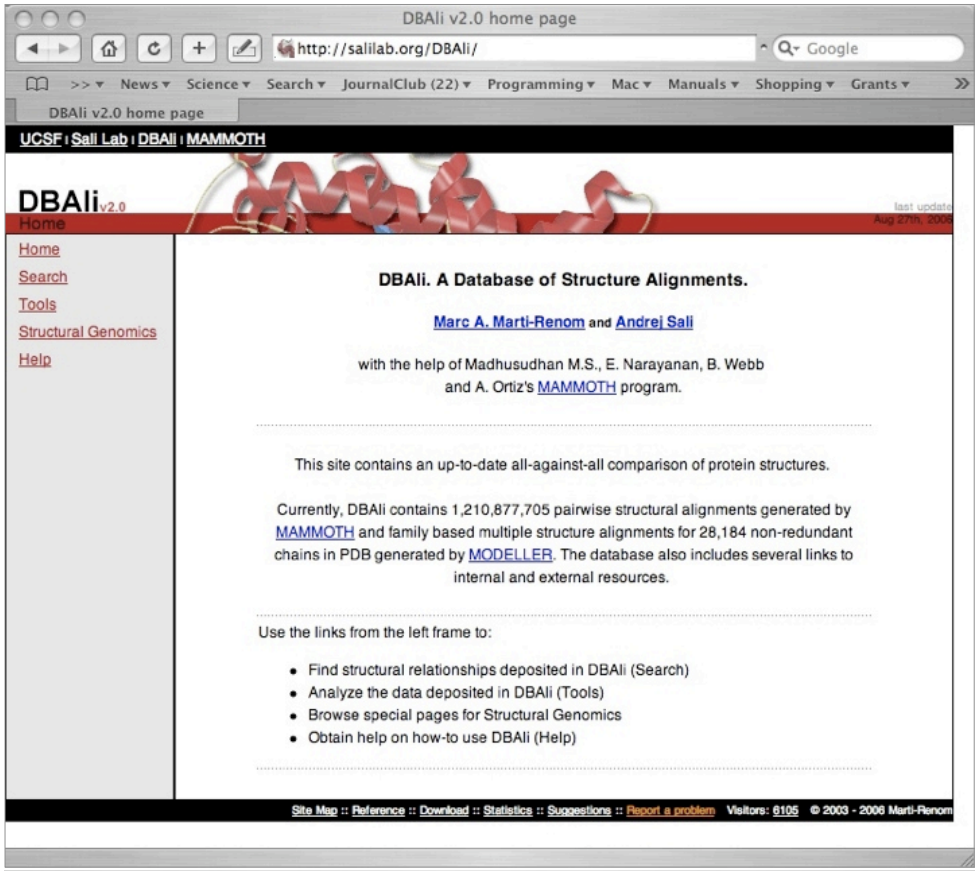
data from models generated by ModPipe (Eswar, Pieper & Sali)



A good model has MPQS of 1.1 or higher

DBAli$_{v2.0}$ database

http://www.dbali.org



- ✓ Fully-automatic
- ✓ Data is kept up-to-date with PDB releases
- ✓ Tools for "on the fly" classification of families.
- ✓ Easy to navigate
- ✓ Provides tools for structure analysis

Does not provide a stable classification similar to that of CATH or SCOP

Pairwise structure alignments	
Last update:	July 5th, 2007
Number of chains:	93,307
Number of structure-structure comparisons:*	1,617,719,157
Multiple structure alignments	
Last update:	March 22nd, 2007
Number of representative chains:	31,848
Number of families:	11,900

Uses MAMMOTH for similarity detection

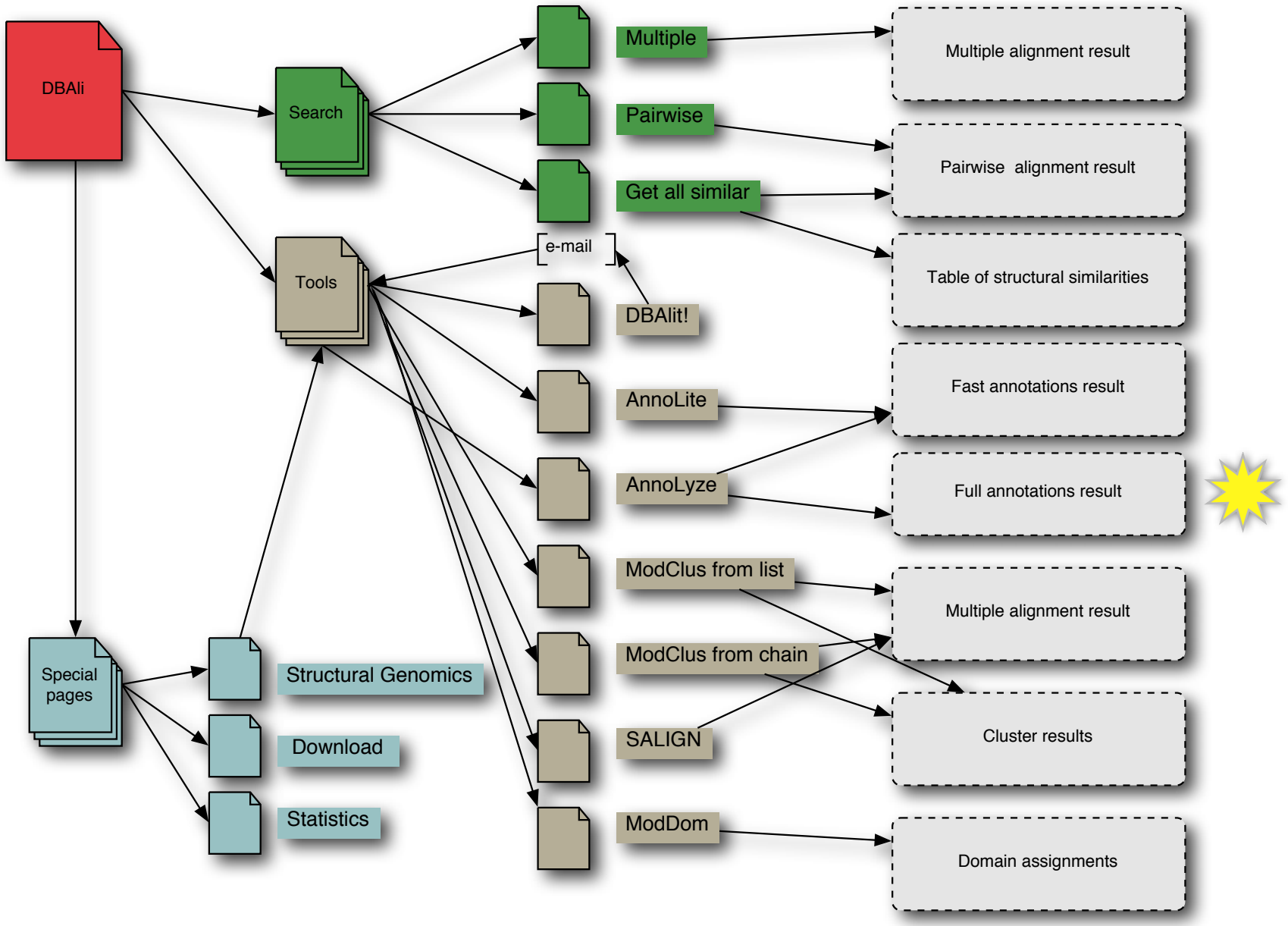
- ✓ VERY FAST!!!
- ✓ Good scoring system with significance

Ortiz AR, (2002) Protein Sci. 11 pp2606

Marti-Renom et al. 2001. Bioinformatics. 17, 746

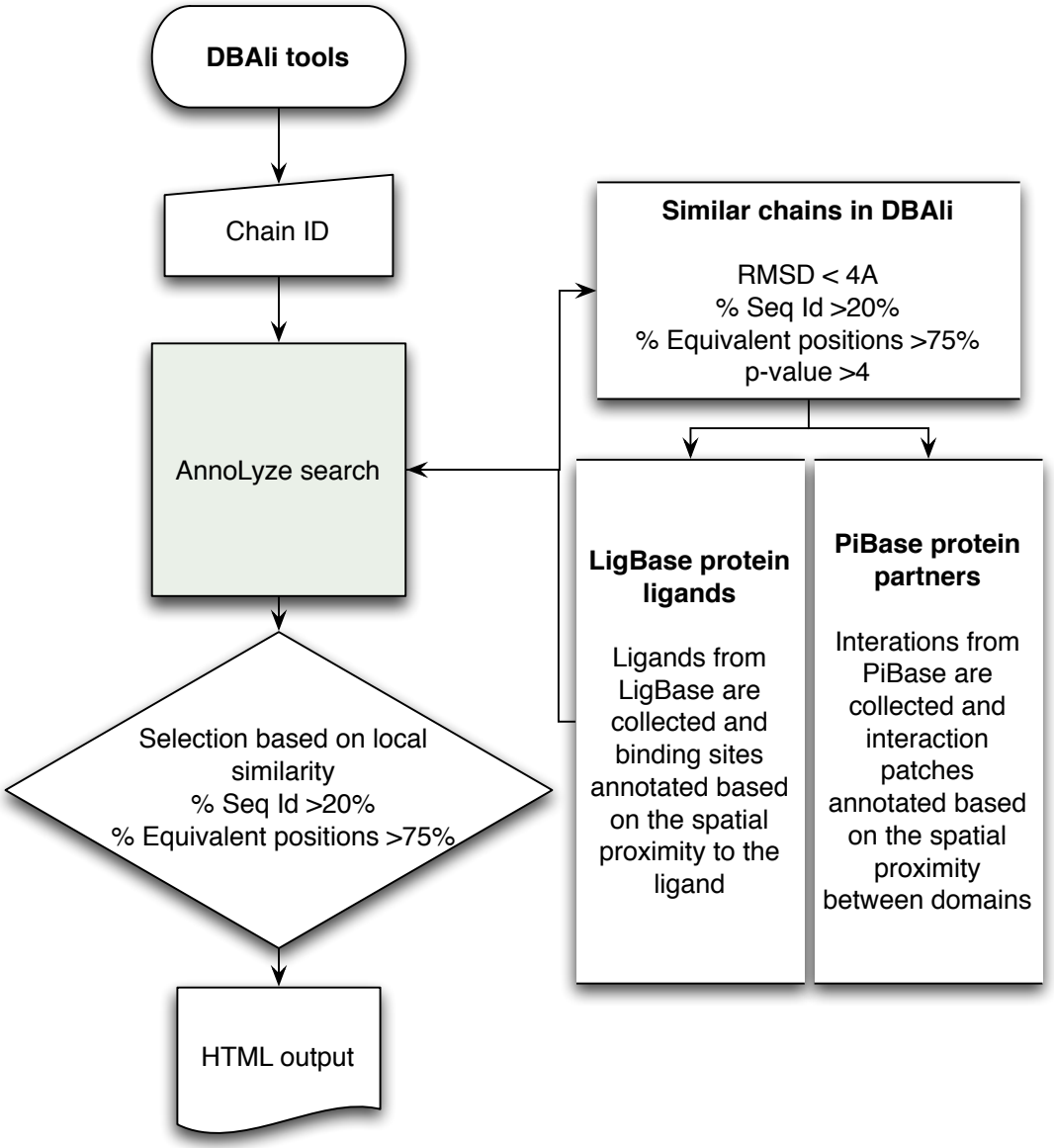
DBAli$_{v2.0}$ database

http://www.dbali.org



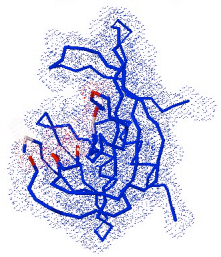
Marti-Renom et al. BMC Bioinformatics (2007) Volume 8. Suppl S4

Method



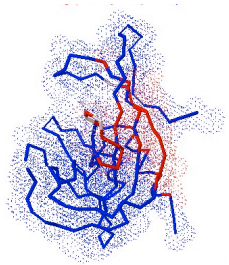
Inherited ligands: 4

Ligand	Av. binding site seq. id.	Av. residue conservation	Residues in predicted binding site (size proportional to the local conservation)
MO2	59.03	0.185	48 49 52 62 63 66 67 113 116
CRY	20.00	0.111	23 29 31 37 44 48 49 83 85 94 96 103 121
8OG	20.00	0.111	19 20 21 48 49 51 96 98 136
ACY	15.87	0.163	23 29 31 37 44 45 81 83 85 94 96 98 103 121 135



Inherited partners:1

Partner	Av. binding site seq. id.	Av. residue conservation	Residues in predicted binding site (size proportional to the local conservation)
d.113.1.1	23.68	0.948	19 20 50 51 52 53 54 55 56 57 58 77 78 79 80 81 82 83 84 85 93 95 97 99 134 135 138 142 145



Sensitivity .vs. Precision

	Optimal cut-off	Sensitivity (%) Recall or TPR	Precision (%)
Ligands	30%	71.9	13.7

$$\text{Sensitivity} = \frac{TP}{TP + FN} \qquad \text{Precision} = \frac{TP}{TP + FP}$$

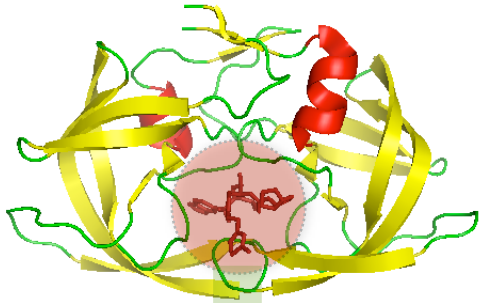
~90-95% of residues correctly predicted

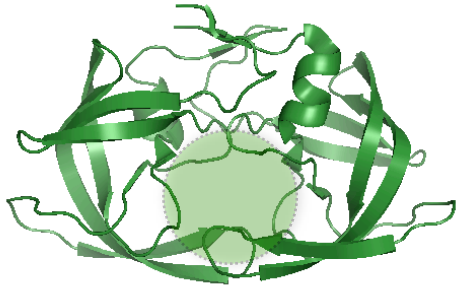
Marti-Renom et al. BMC Bioinformatics (2007) Volume 8. Suppl S4

Comparative docking

1. Expansion

co-crystalized protein/ligand

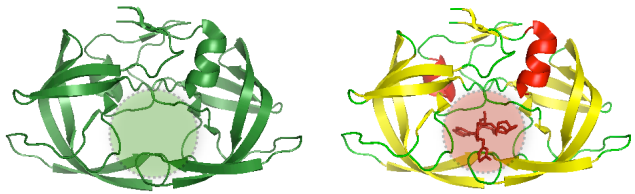




crystalized protein

2. Inheritance

model



template

Summary table

models with inherited ligands

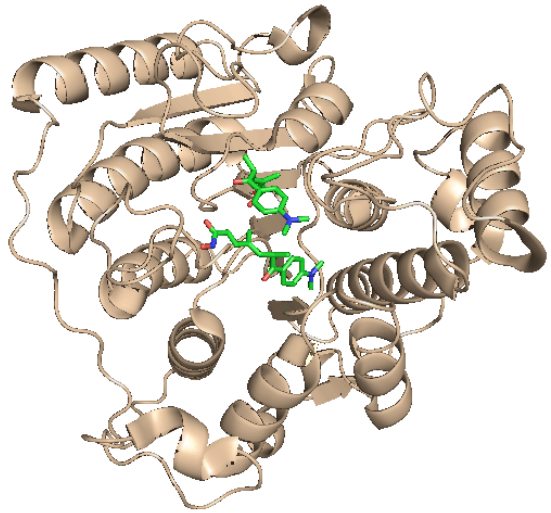
from 16,284 good models, 295 inherited a ligand/substance with at least one compound already approved by FDA and ready to be used from ZINC

	Transcripts	Good	Ligands	Lipinski	Lipinski+ZINC	FDA+ZINC
C. hominis	3,886	886	183	131	28	12 (10)
C. parvum	3,806	949	219	145	30	12 (10)
L. major	8,274	1,845	488	334	84	44 (34)
M. leprae	1,605	1,321	286	189	39	29 (25)
M. tuberculosis	3,991	2,887	404	285	71	44 (37)
P. falciparum	5,363	1,057	271	191	48	20 (16)
P. vivax	5,342	1,042	267	177	37	18 (15)
T. brucei	921	1,795	440	309	94	46 (36)
T. cruzi	19,607	3,915	730	493	127	62 (52)
T. gondii	7,793	587	174	124	28	8 (7)
TOTAL	**60,588**	**16,284**	**3,462**	**2,378**	**586**	**295 (242)**

Example of inheritance (expansion)

LmjF21.0680 from L. major *"Histone deacetylase 2" (model 1)*

Template 1t64A a human HDAC8 protein.



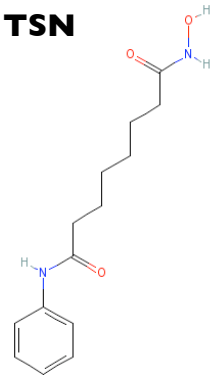
	Origen	Formula	Name	Cov.	Seq, Id. (%)
ZN	X-ray	Zn^{2+}	Zinc ion	--	--
NA	X-ray	Na^{+}	Sodium ion	--	--
CA	X-ray	Ca^{2+}	Calcium ion	--	--
TSN	X-ray	$C_{17}H_{22}N_2O_3$	Trichostatin A	--	--
SHH	Expanded	$C_{14}H_{20}N_2O_3$	Octadenioic acid hudroxyamide phenylamide	100.00	83.8

Example of inheritance (inheritance)

LmjF21.0680 from L. major *"Histone deacetylase 2" (model 1)*

	Formula	Name	Cov.	Seq, Id. (%)	Residues
TSN	C_{17} H_{22} N_2 O_3	Trichostatin A	100.00	90.9	90 131 132 140 141 167 169 256 263 293 295
SHH	C_{14} H_{20} N_2 O_3	Octadenioic acid hudroxyamide phenylamide	100.00	90.9	

TSN



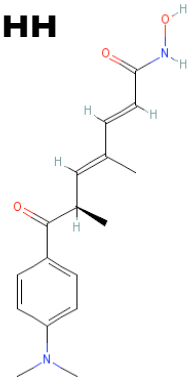
suberoylanilide hydroxamic acid

Pharmacological Action:

- Anti-Inflammatory Agents, Non-Steroidal
- Antineoplastic Agents
- Enzyme Inhibitors
- Anticarcinogenic Agents

Inhibits histone deacetylase I and 3

SHH



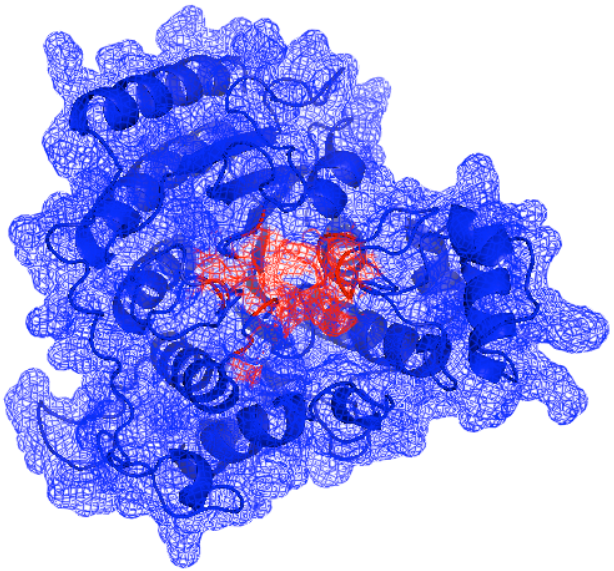
trichostatin A

Pharmacological Action:

- Antibiotics, Antifungal
- Enzyme Inhibitors
- Protein Synthesis Inhibitors

chelates zinc ion in the active site of histone deacetylases, resulting in preventing histone unpacking so DNA is less available for transcription

	LmjF21.0680.1.pdb
Template	1t64A
Seq. Id (%)	**38.00**
MPQS	1.47



Example of inheritance (CDD-Roos-literature)

LmjF21.0680 from L. major *"Histone deacetylase 2" (model 1)*

Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 13143–13147, November 1996
Medical Sciences

Apicidin: A novel antiprotozoal agent that inhibits parasite histone deacetylase

(cyclic tetrapeptide/Apicomplexa/antiparasitic/malaria/coccidiosis)

SANDRA J. DARKIN-RATTRAY*†, ANNE M. GURNETT*, ROBERT W. MYERS*, PAULA M. DULSKI*, TAMI M. CRUMLEY*, JOHN J. ALLOCCO*, CHRISTINE CANNOVA*, PETER T. MEINKE‡, STEVEN L. COLLETTI‡, MARIA A. BEDNAREK‡, SHEO B. SINGH§, MICHAEL A. GOETZ§, ANNE W. DOMBROWSKI§, JON D. POLISHOOK§, AND DENNIS M. SCHMATZ*

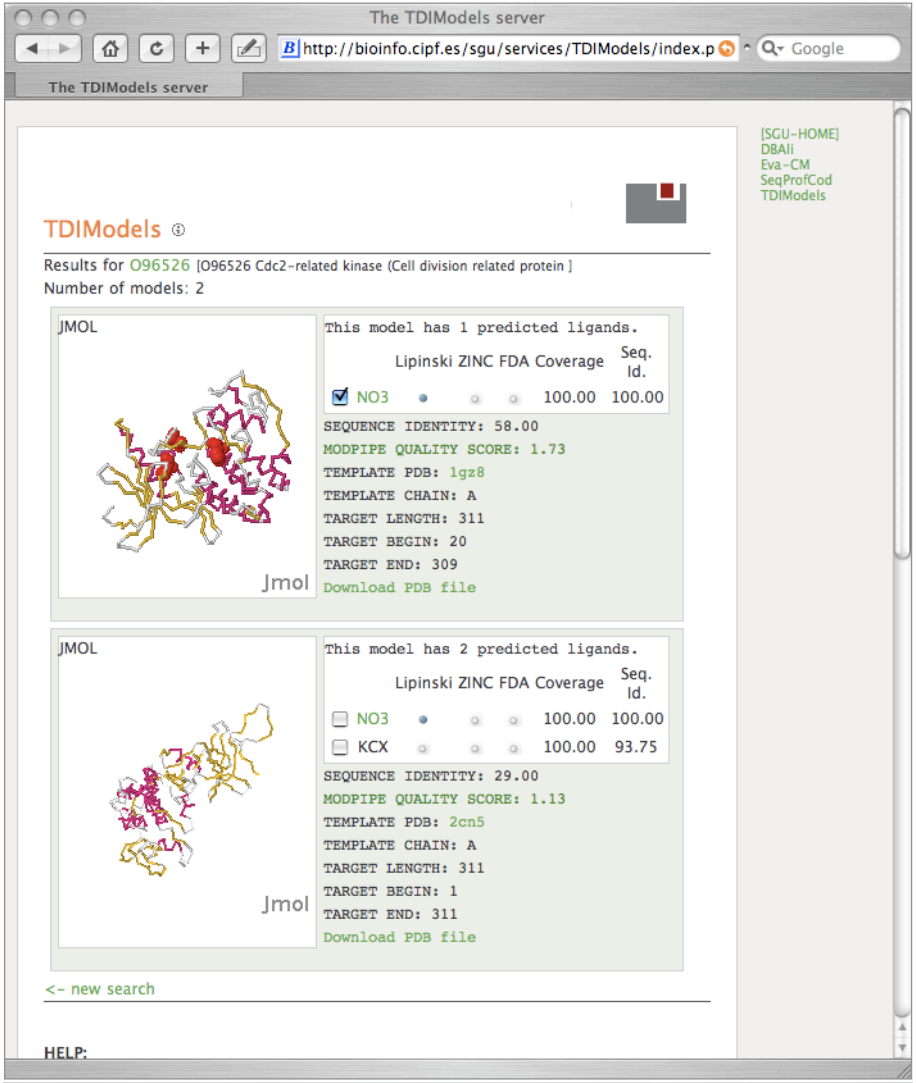
Departments of *Parasite Biochemistry and Cell Biology, ‡Medicinal Chemistry, and §Natural Products Drug Discovery, Merck Research Laboratories, P.O. Box 2000, Rahway, NJ 07065

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 2004, p. 1435–1436 Vol. 48, No. 4
0066-4804/04/$08.00+0 DOI: 10.1128/AAC.48.4.1435–1436.2004

Antimalarial and Antileishmanial Activities of Aroyl-Pyrrolyl-Hydroxyamides, a New Class of Histone Deacetylase Inhibitors

Models database

http://bioinfo.cipf.es/sgu/services/TDIModels/



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Ginger Taylor (TSL)
Barri Bunin (CDD)

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John Kuriyan (UCB)
NY-SGXRC

MAMMOTH
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Alvaro Monteiro (Cornell U)
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Joaquin Dopazo

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M. S. Madhusudhan
Narayanan Eswar
Min-Yi Shen
Ursula Pieper
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Maya Topf

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Fred Davis

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