Structural Bioinformatics

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Structural Bioinformatics

David Dufour

- Estructura y biofísica de ácidos nucleicos y proteínas **25 febrero (DD)**
- Bases de datos de estructura de proteínas, ácidos nucleicos y pequeñas moléculas **11 marzo (DD)**
- Alineamiento y clasificación de estructura **25 marzo (DD)**
- Predicción de estructura tridimensional de ácidos nucleicos y proteínas **15 abril (DD)**

Francisco Martínez

- Docking de pequeñas moléculas en la superficie de estructura de proteínas **29 abril (FM)**
- Aplicaciones para el desarrollo de nuevos fármacos **13 mayo (FM)**

Outline...

COMPARATIVE MODELING

EXAMPLES

THE TROPICAL DISEASE INITIATIVE

Nomenclature

Homology: Sharing a common ancestor, may have similar or dissimilar functions

Similarity: Score that quantifies the degree of relationship between two sequences.

Identity: Fraction of identical aminoacids between two aligned sequences (case of similarity).

Target: Sequence corresponding to the protein to be modeled.

Template: 3D structure/s to be used during protein structure prediction.

Model: Predicted 3D structure of the target sequence.

Nomenclature

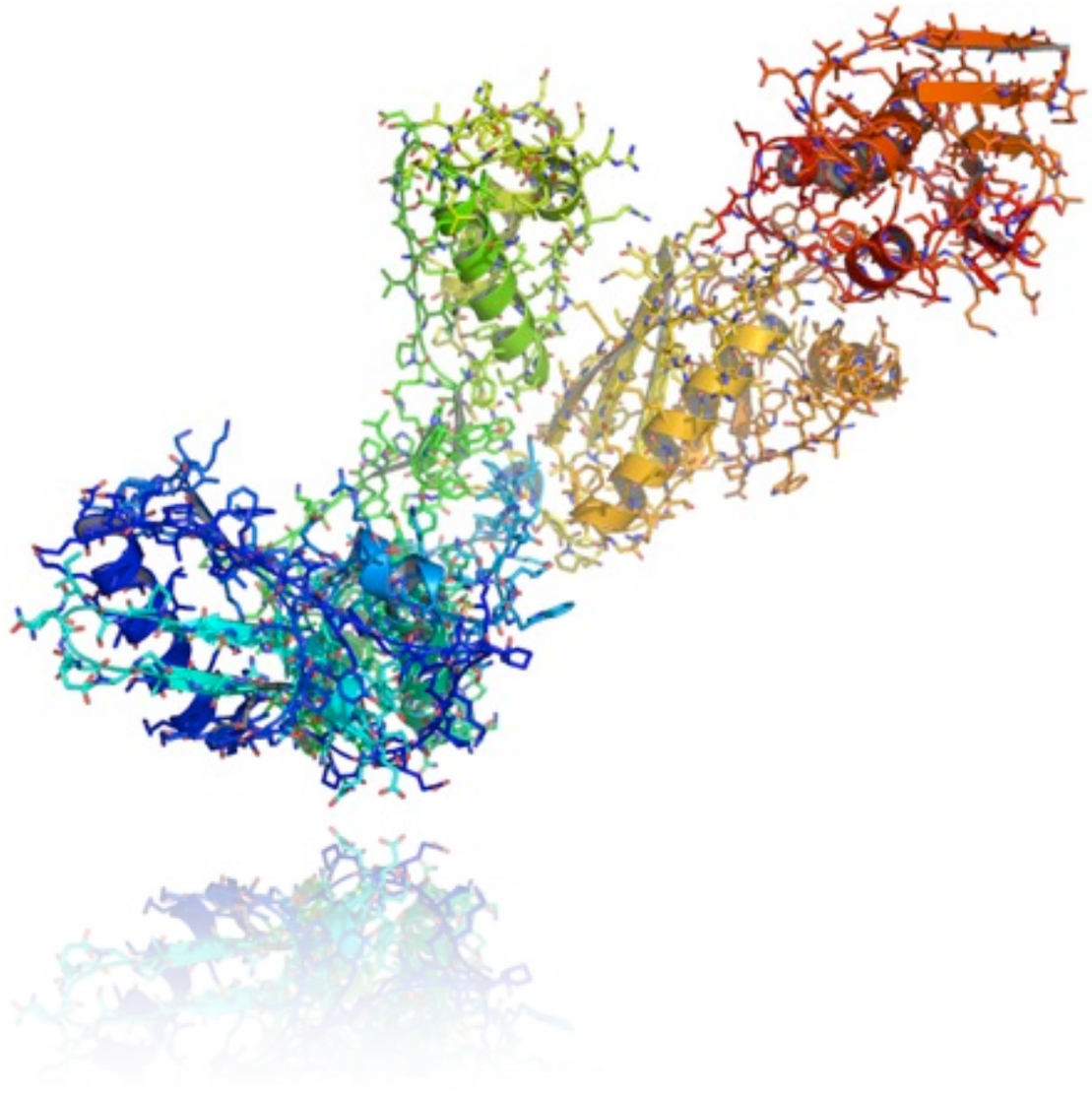
Fold: Three dimensional conformation of a protein sequence (usually at domain level).

Domain: Structurally globular part of a protein, which may independently fold.

Secondary Structure: Regular sub-domain structures composed by alpha-helices, beta-sheets and coils (or loops).

Backbone: Protein structure skeleton composed by the carbon, nitrogen and oxygen atoms.

Side-Chain: Specific atoms identifying each of the 20 residues types.



protein prediction .vs. protein determination

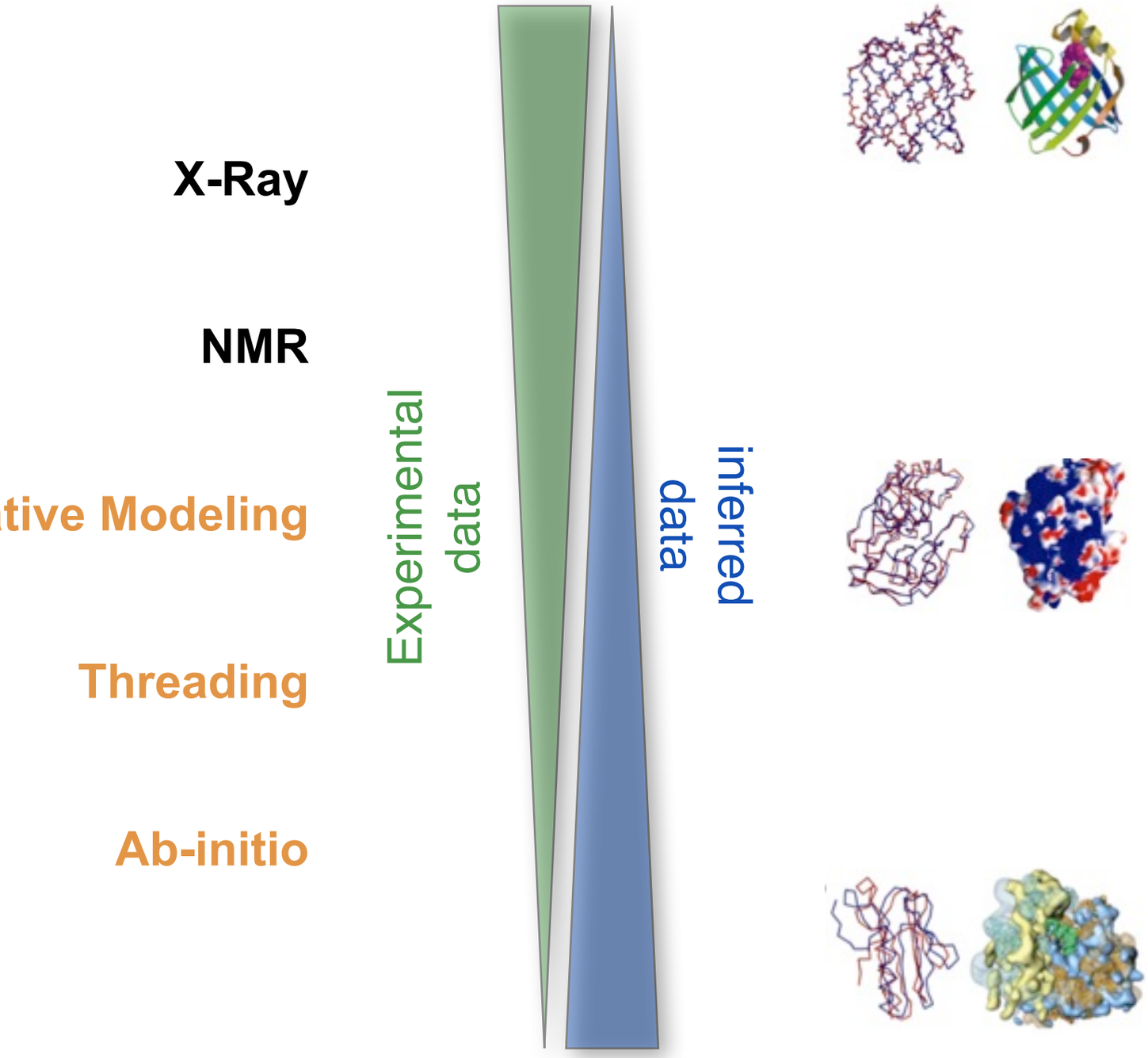
X-Ray

NMR

Comparative Modeling

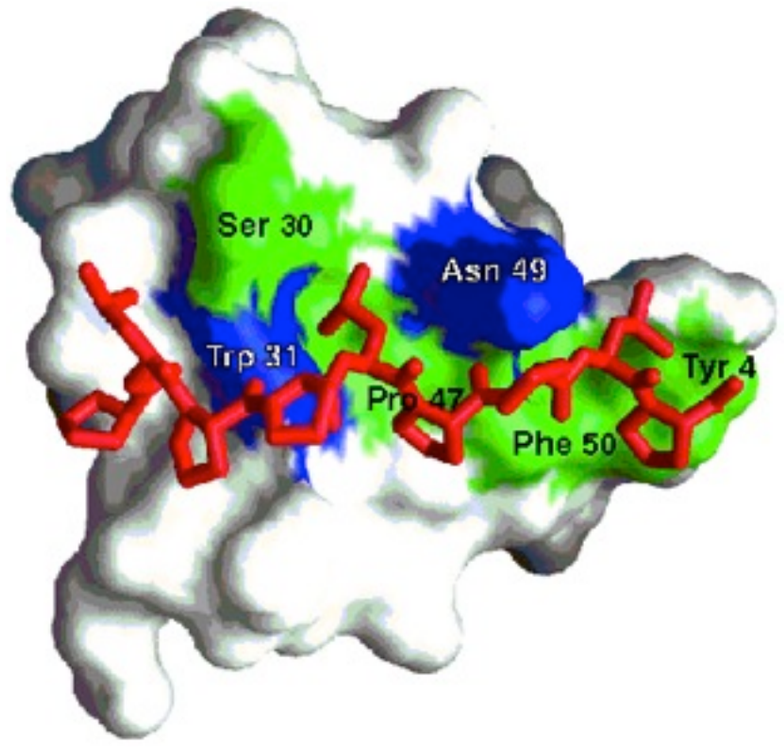
Threading

Ab-initio



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

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

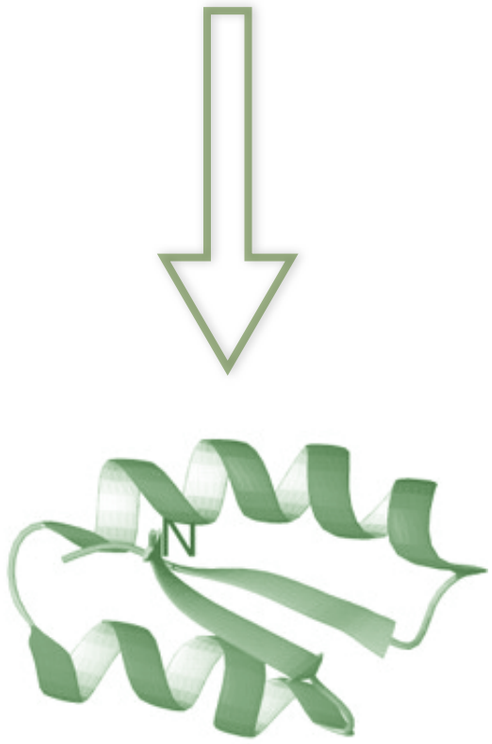


In addition, since evolution tends to conserve function and function depends more directly on structure than on sequence, **structure is more conserved in evolution than sequence**.

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

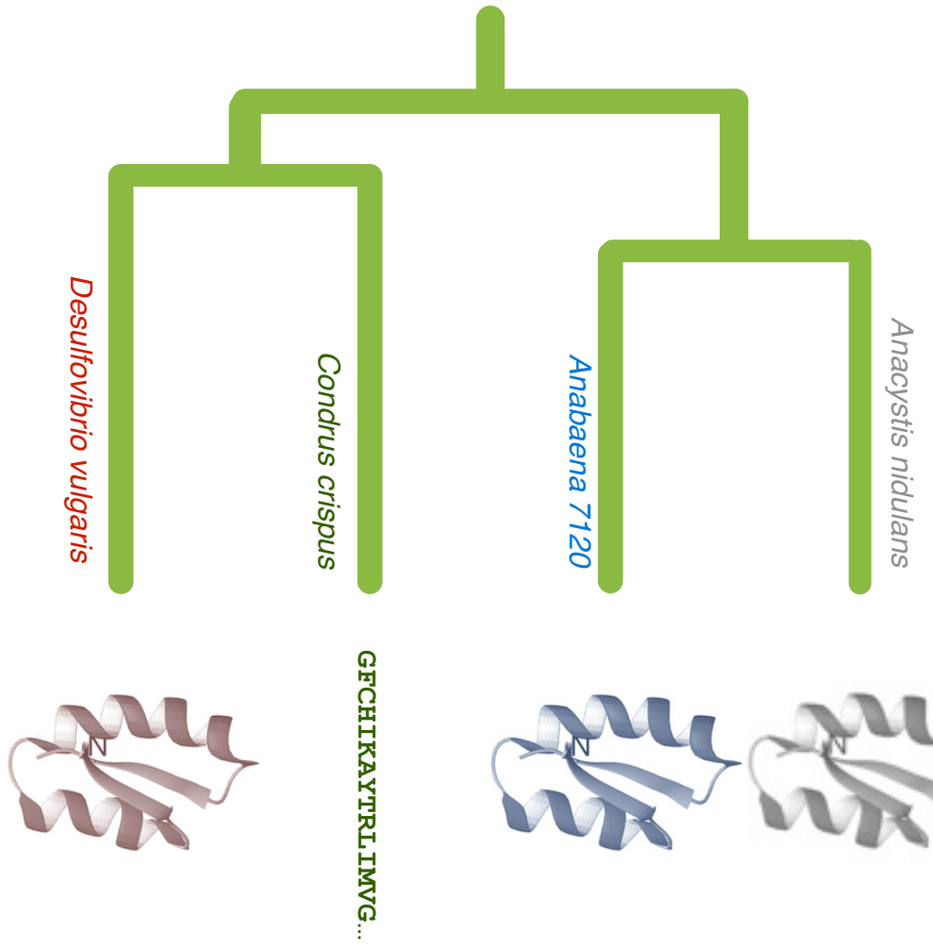
Principles of protein structure

GFCHIKAYTRLIMVG...



Folding (physics)

Ab initio prediction

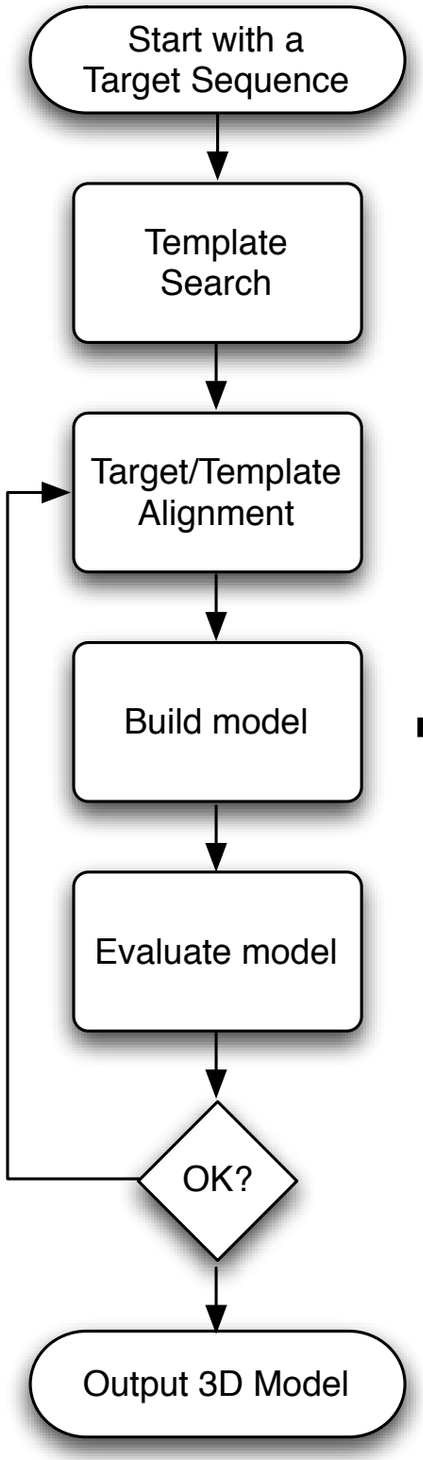


Evolution (rules)

Threading
Comparative Modeling

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

Comparative modeling by satisfaction of spatial restraints

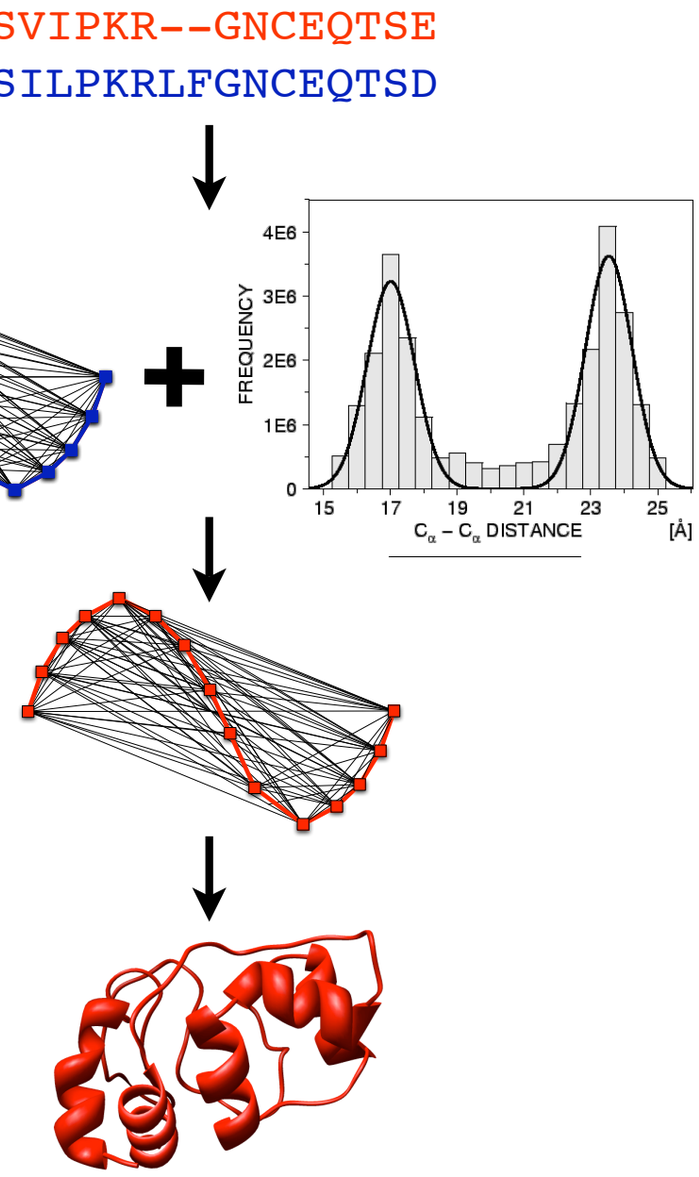


Given an alignment...

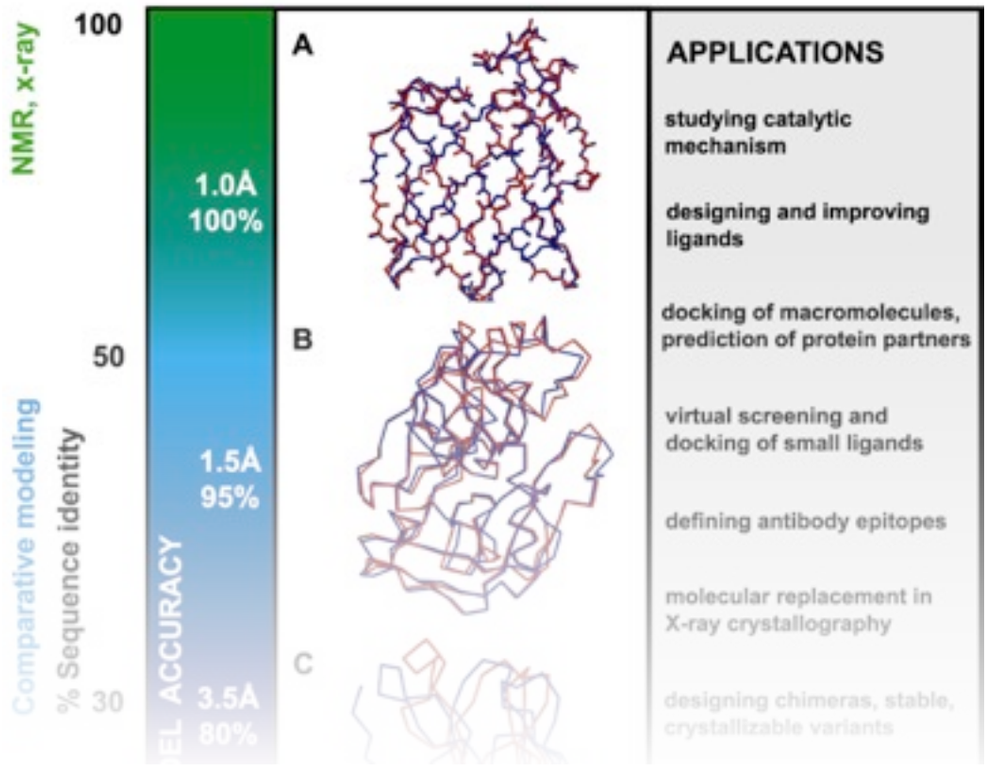
extract spatial features from the template(s) and statistics from known structures

apply these features as restraints on your target sequence

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

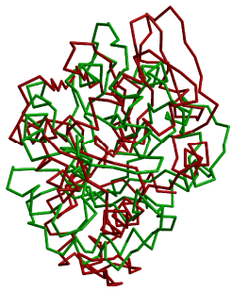


A. Šali & T. Blundell. J. Mol. Biol. 234, 779, 1993.
J.P. Overington & A. Šali. Prot. Sci. 3, 1582, 1994.
A. Fiser, R. Do & A. Šali, Prot. Sci., 9, 1753, 2000.

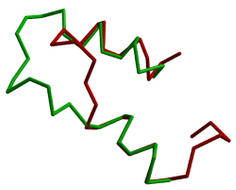


Accuracy and applicability of comparative models

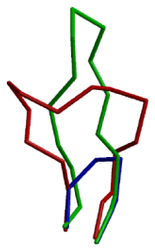
Comparative modeling by satisfaction of spatial restraints Types of errors and their impact



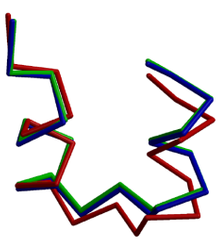
Wrong fold



Miss alignments

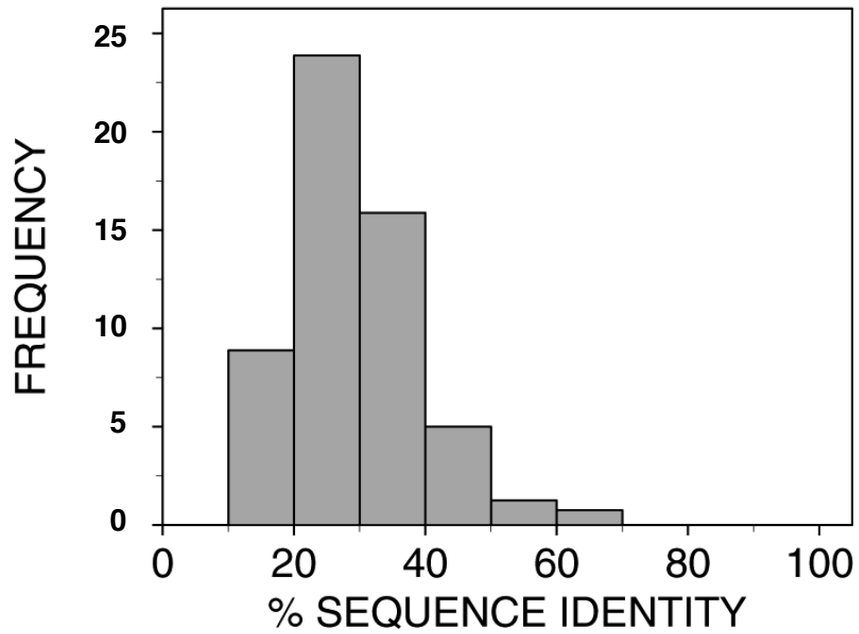


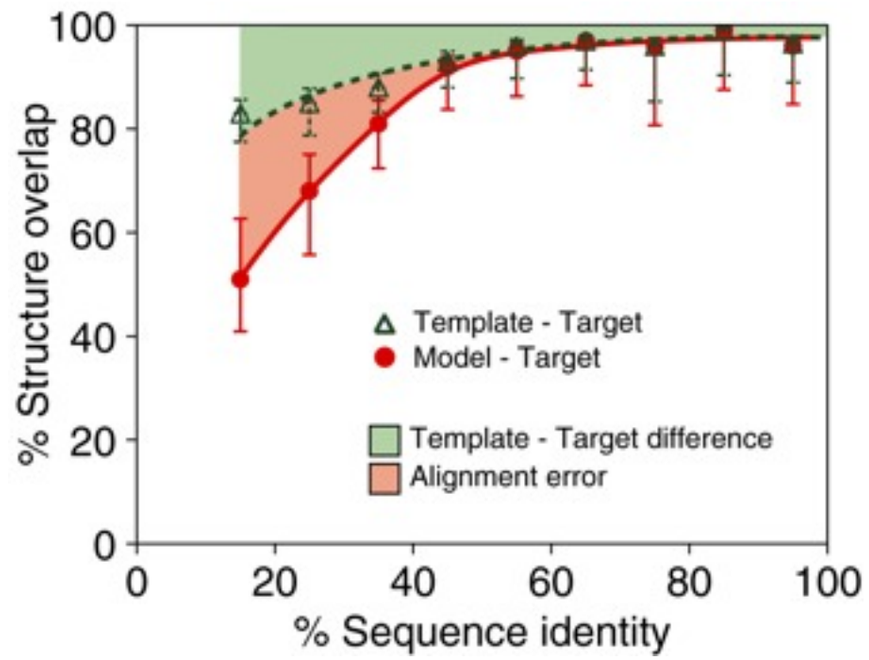
Loop regions



Rigid body distortions

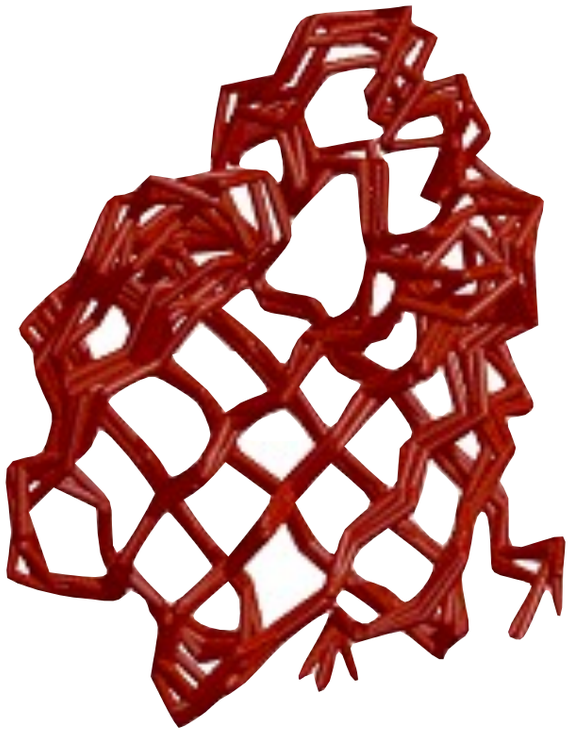
Side-chain packing





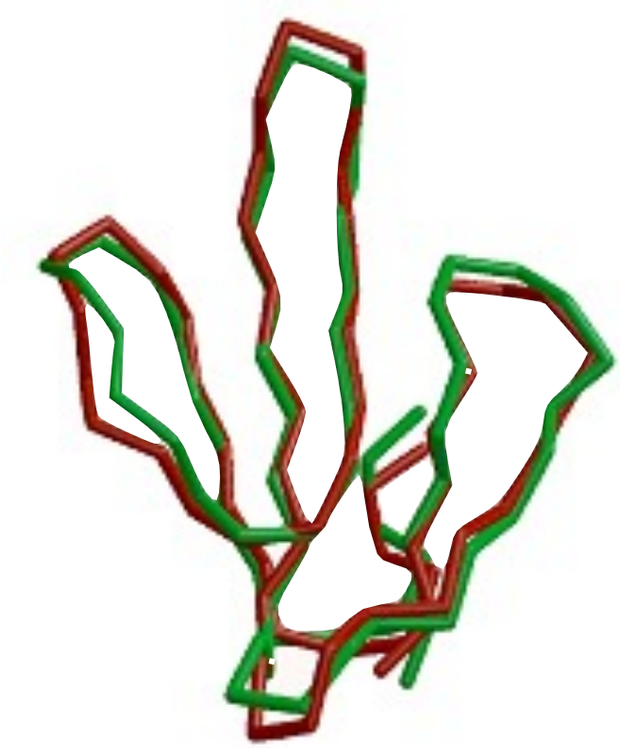
Marti-Renom etal. Ann Rev Biophys Biomol Struct (2000) 29, 291

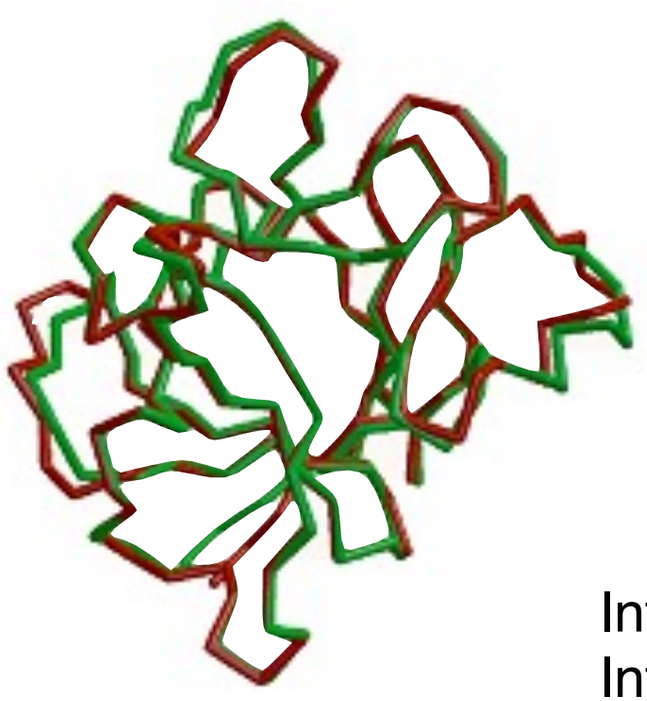
“Biological” significance of modeling errors



NMR
Ileal lipid-binding protein
1eal

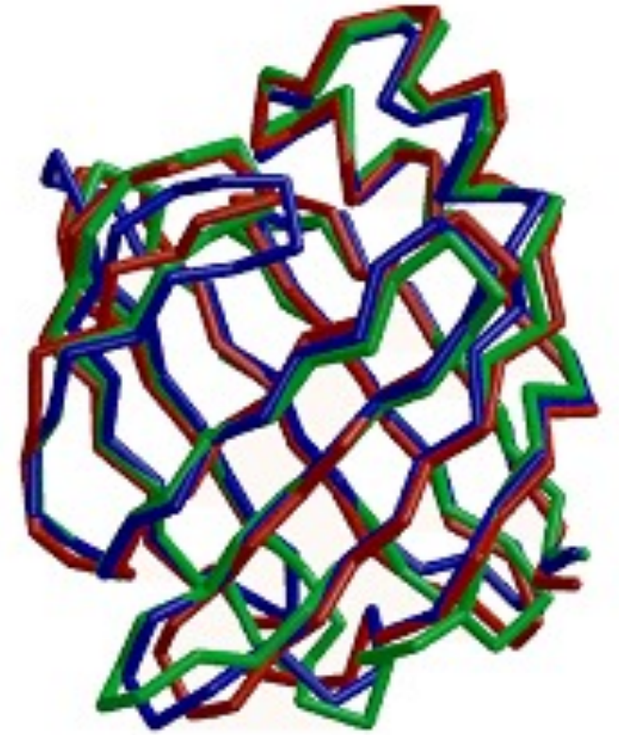
NMR – X-RAY
Erabutoxin 3ebx
Erabutoxin 1era





X-RAY
Interleukin 1β 41bi (2.9Å)
Interleukin 1β 2mib (2.8Å)

CRABPII 1opbB
FABP 1ftpA
ALBP 1lib
40% seq. id.



Model Accuracy

HIGH ACCURACY

NM23 Seq id 77%

Cα equiv 147/148
RMSD 0.41Å

Sidechains
Core backbone
Loops

MEDIUM ACCURACY

CRABP Seq id 41%

Cα equiv 122/137
RMSD 1.34Å

Sidechains
Core backbone
Loops
Alignment

LOW ACCURACY

EDN Seq id 33%

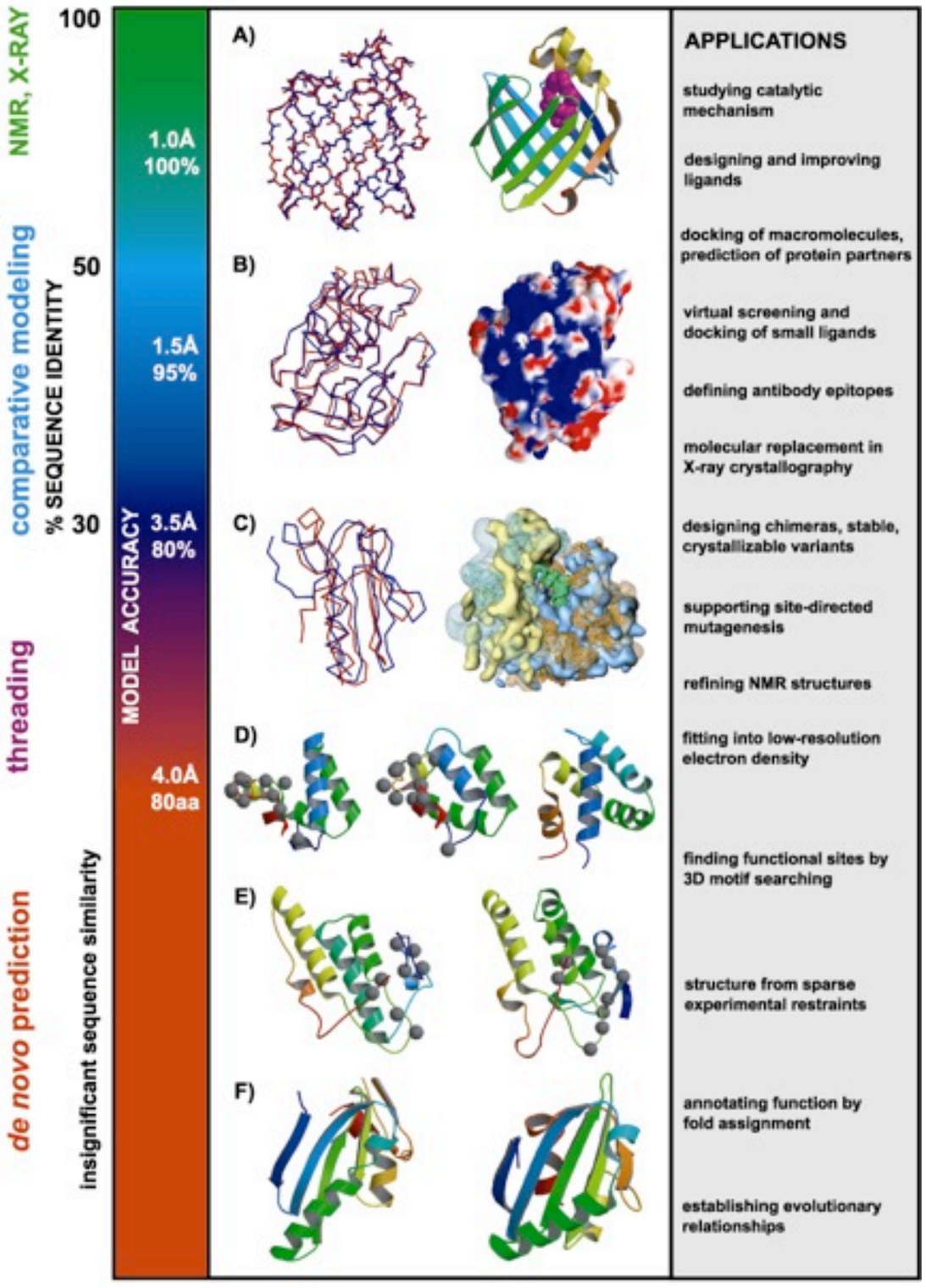
Cα equiv 90/134
RMSD 1.17Å

Sidechains
Core backbone
Loops
Alignment
Fold assignment

X-RAY / **MODEL**

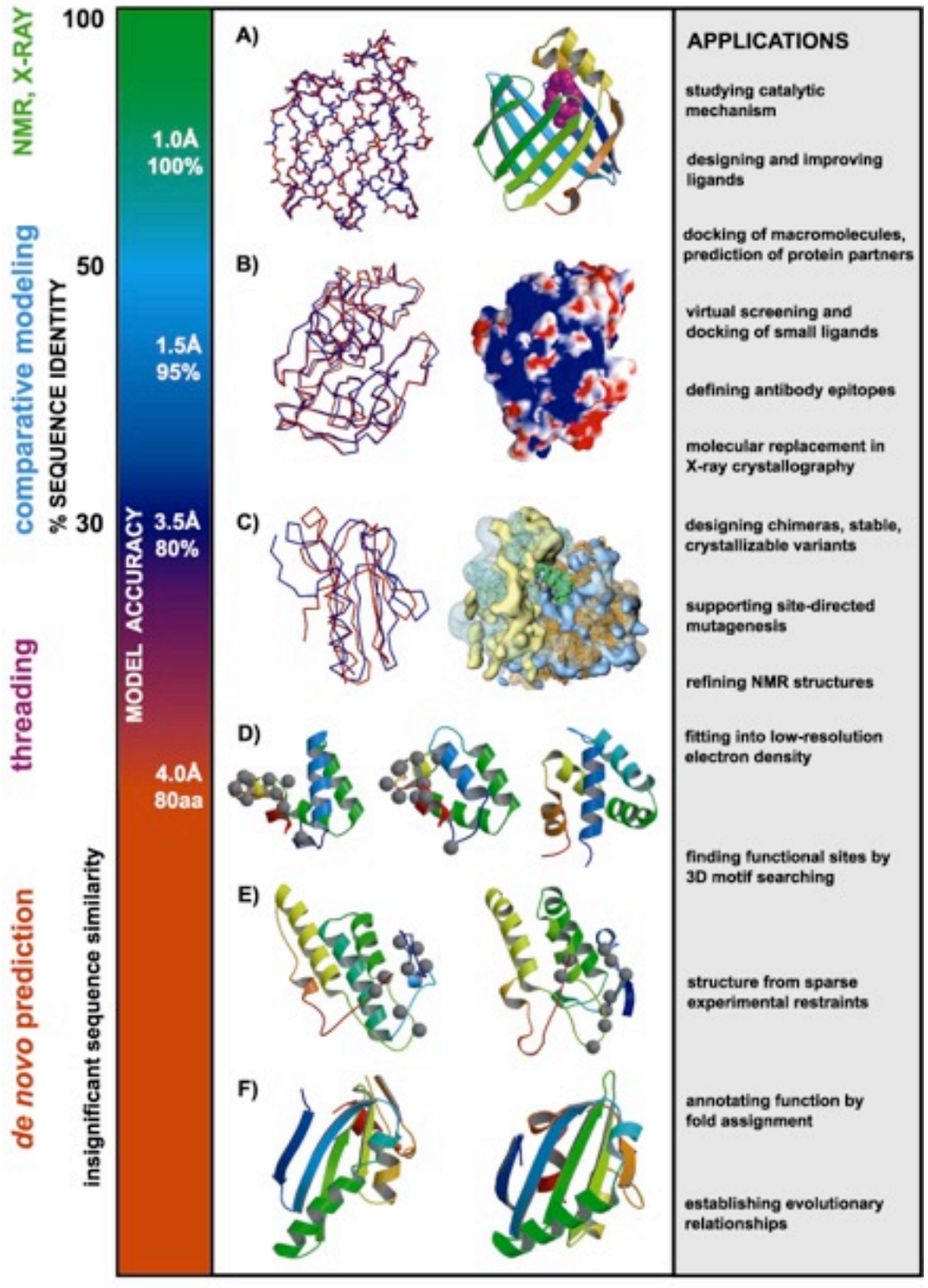
Marti-Renom et al. Annu.Rev.Biophys.Biomol.Struct. 29, 291-325, 2000.

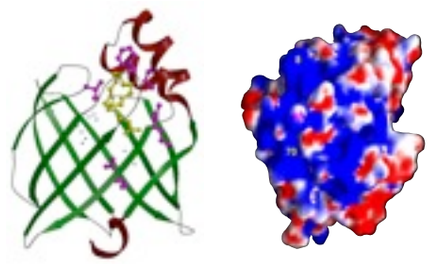
Utility of protein structure models, despite errors

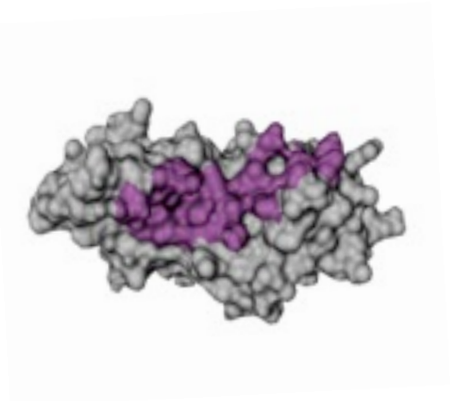


D. Baker & A. Sali. Science 294, 93, 2001.

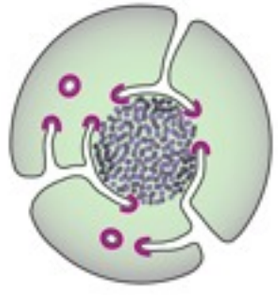
Can we use models to infer function?







T. cruzi



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

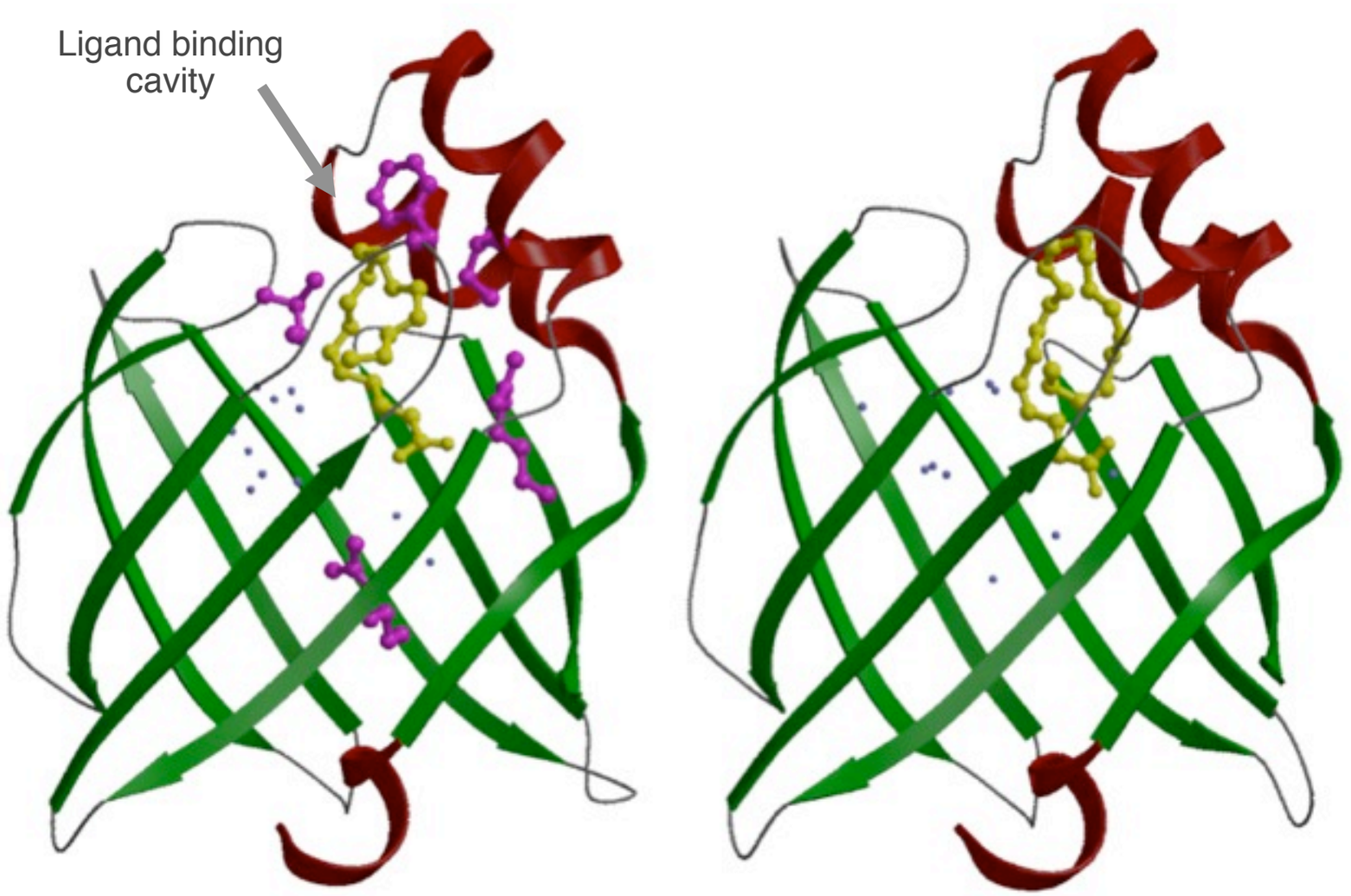
Predicting features of a model that are not present in the template

BLBP/oleic acid

Cavity is not filled

BLBP/docosahexaenoic acid

Cavity is filled



1. BLBP binds fatty acids.

2. Build a 3D model.

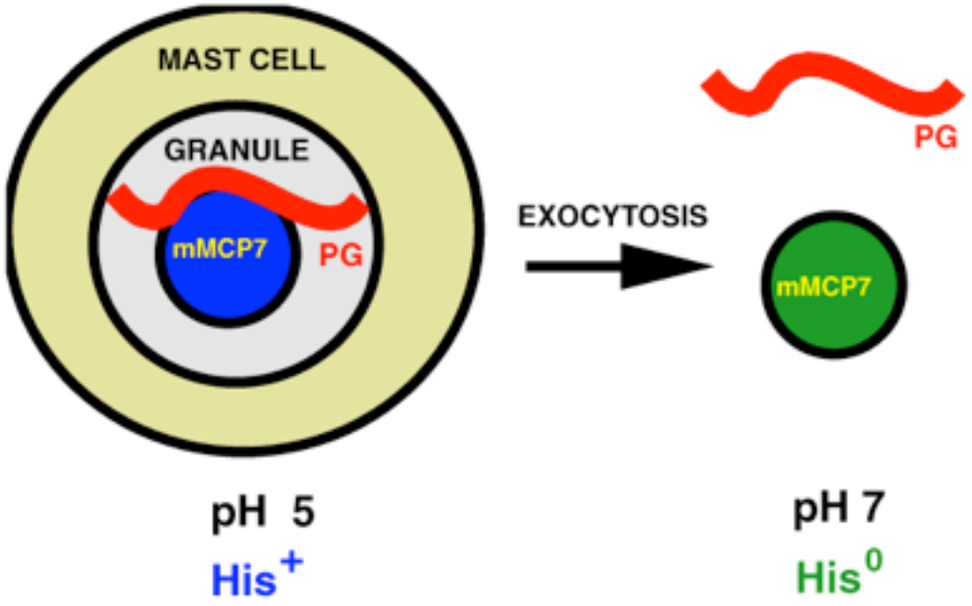
3. Find the fatty acid that fits most snuggly into the ligand binding cavity.

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

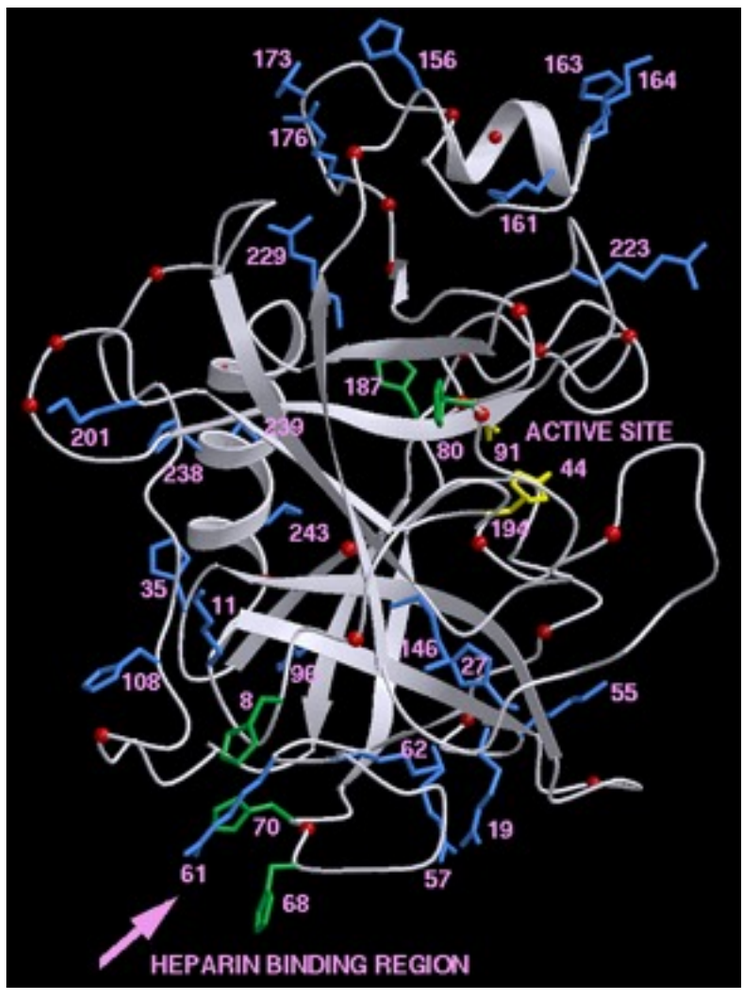
Do mast cell proteases bind proteoglycans? Where? When?

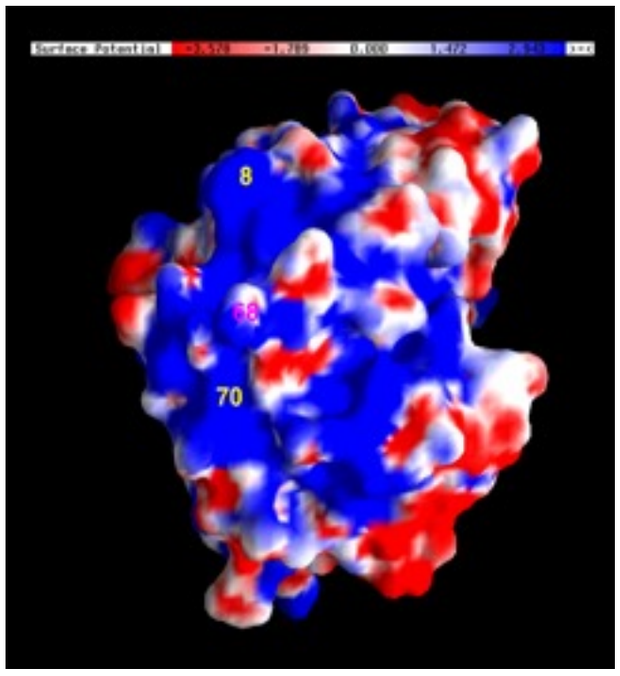
Predicting features of a model that are not present in the template

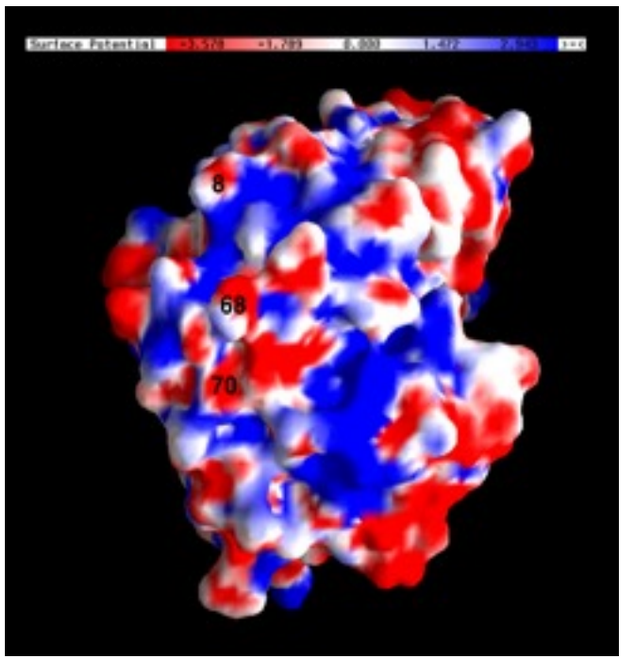
1. mMCPs bind negatively charged proteoglycans through electrostatic interactions
2. Comparative models used to find clusters of positively charged surface residues.
3. Tested by site-directed mutagenesis.



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.







Structural analysis of missense mutations in human BRCA1 BRCT domains

Mirkovic et al. Structure-based assessment of missense mutations in human BRCA1: implications for breast and ovarian cancer predisposition. Cancer Res (2004) vol. 64 (11) pp. 3790-7

[CANCER RESEARCH 64, 3790–3797, June 1, 2004]

Structure-Based Assessment of Missense Mutations in Human BRCA1: Implications for Breast and Ovarian Cancer Predisposition

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[1]Laboratory of Molecular Biophysics, Pels Family Center for Biochemistry and Structural Biology, Rockefeller University, New York, New York; [2]Departments of Biopharmaceutical Sciences and Pharmaceutical Chemistry, and California Institute for Quantitative Biomedical Research, University of California at San Francisco, San Francisco, California; [3]Abramson Family Cancer Research Institute, University of Pennsylvania, Philadelphia, Pennsylvania; [4]Strang Cancer Prevention Center, New York, New York; and [5]Department of Cell and Developmental Biology, Weill Medical College of Cornell University, New York, New York

ABSTRACT

The *BRCA1* gene from individuals at risk of breast and ovarian cancers can be screened for the presence of mutations. However, the cancer association of most alleles carrying missense mutations is unknown, thus creating significant problems for genetic counseling. To increase our ability to identify cancer-associated mutations in *BRCA1*, we set out to use the principles of protein three-dimensional structure as well as the correlation between the cancer-associated mutations and those that abolish transcriptional activation. Thirty-one of 37 missense mutations of known impact on the transcriptional activation function of BRCA1 are readily rationalized in structural terms. Loss-of-function mutations involve nonconservative changes in the core of the BRCA1 C-terminus (BRCT) fold or are localized in a groove that presumably forms a binding site involved in the transcriptional activation by BRCA1; mutations that do not abolish transcriptional activation are either conservative changes in the core or are on the surface outside of the putative binding site. Next, structure-based rules for predicting functional consequences of a given missense mutation were applied to 57 germ-line BRCA1 variants of unknown cancer association. Such a structure-based approach may be helpful in an integrated effort to identify mutations that predispose individuals to cancer.

INTRODUCTION

Many germ-line mutations in the human *BRCA1* gene are associated with inherited breast and ovarian cancers (1, 2). This information has allowed clinicians and genetic counselors to identify individuals at high risk for developing cancer. However, the disease association of over 350 missense mutations remains unclear, primarily because their relatively low frequency and ethnic specificity limit the usefulness of the population-based statistical approaches to identifying cancer-causing mutations. To address this problem, we use here the three-dimensional structure of the human BRCA1 BRCT domains to assess the transcriptional activation functions of BRCA1 mutants. Our study is made possible by the recently determined sequences (3–6) and three-dimensional structures of the BRCA1 homologs (7, 8). In addition, we benefited from prior studies that attempted to rationalize and predict functional effects of mutations in various proteins (9–12), including those of BRCA1 (13, 14).

BRCA1 is a nuclear protein that activates transcription and facilitates DNA damage repair (15, 16). The tandem BRCT domains at the COOH-terminus of BRCA1 are involved in several of its functions, including modulation of the activity of several transcription factors (15), binding to the RNA polymerase II holoenzyme (17), and activating transcription of a reporter gene when fused to a heterologous DNA-binding domain (18, 19). Importantly, cancer-associated mutations in the BRCT domains, but not benign polymorphisms, inactivate transcriptional activation and binding to RNA polymerase II (18–21). These observations suggest that abolishing the transcriptional activation function of BRCA1 leads to tumor development and provides a genetic framework for characterization of BRCA1 BRCT variants.

MATERIALS AND METHODS

The multiple sequence alignment (MSA) of orthologous BRCA1 BRCT domains from seven species, including *Homo sapiens* (GenBank accession number U14680), *Pan troglodytes* (AF207822), *Mus musculus* (U68174), *Rattus norvegicus* (AF036760), *Gallus gallus* (AF355273), *Canis familiaris* (U50709), and *Xenopus laevis* (AF416868), was obtained by using program ClustalW (22) and contains only one gapped position (Supplementary Fig. 1). According to PSI-BLAST (23), the latter six sequences are the only sequences in the nonredundant protein sequence database at National Center for Biotechnology Information that have between 30% and 90% sequence identity to the human BRCA1 BRCT domains (residues 1649–1859).

The multiple structure-based alignment of the native structures of the BRCT-like domains was obtained by the SALIGN command in MODELLER (Supplementary Fig. 2). It included the experimentally determined structures of the two human BRCA1 BRCT domains (Protein Data Bank code 1JNX; Refs. 8, 24), rat BRCA1 BRCT domains (1L0B; Ref. 7), human p53-binding protein (1KZY; Ref. 7), human DNA-ligase IIIα (1IMO; Ref. 25), and human XRCC1 protein (1CDZ; Ref. 13). Structure variability was defined by the root-mean-square deviation among the superposed Cα positions, as calculated by the COMPARE command of MODELLER. The purpose of these calculations was to gain insight into the variability of surface-exposed residues (*left panel* in Fig. 2). In conjunction with observed mutation clustering, these data may point to putative functional site(s) on the surface of BRCT repeats.

Comparative protein structure modeling by satisfaction of spatial restraints, implemented in the program MODELLER-6 (26), was used to produce a three-dimensional model for each of the 94 mutants. The crystallographic structure of the human wild-type BRCA1 BRCT domains was used as the template for modeling (8). The four residues missing in the crystallographic structure (1694 and 1817–1819) were modeled *de novo* (27). All of the models are available in the BRCA1 model set deposited in our ModBase database of comparative protein structure models (28).[6]

For the native structure of the human BRCT tandem repeat and each of the 94 mutant models, a number of sequence and structure features were calculated. These features were used in the classification tree in Fig. 3 (values for all 94 mutations are given in Supplementary Tables 1 and 2).

Buriedness. Accessible surface area of an amino acid residue was calculated by the program DSSP (29) and normalized by the maximum accessible surface area for the corresponding amino acid residue type. A residue was considered exposed if its accessible surface area was larger than 40Å^2 and if its relative accessible surface area was larger than 9% and buried otherwise. A mutation of a more exposed residue is less likely to change the structure and therefore its function.

Received 9/24/03; revised 1/30/04; accepted 3/15/04.

Grant support: This work was supported by Lee Kaplan Foundation, the Fashion Footwear Association of New York/QVC; United States Army award DAMD17-99-1-9389 and NIH CA92309 (A. N. A. M.); the Mathers Foundation, Sandler Family Supporting Foundation, Sun, IBM and Intel (A. S.); and NIH GM 54762 GM61390 (A. S.); and the Breast Cancer Research Foundation (B. L. W.). M. A. M-R. is a Rockefeller University Presidential Postdoctoral Fellow; A. S. is an Irma T. Hirschl Trust Career Scientist; and B. L. W. is an Abramson Investigator.

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Note: The authors declare that they have no competing financial interests. Supplemental data for this article are available at Cancer Research Online (http: cancerres@aacrjournals.org).

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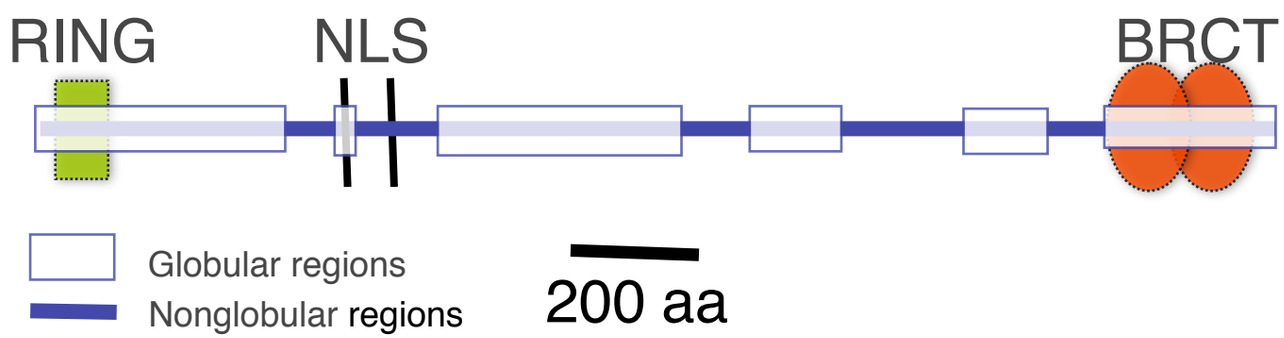
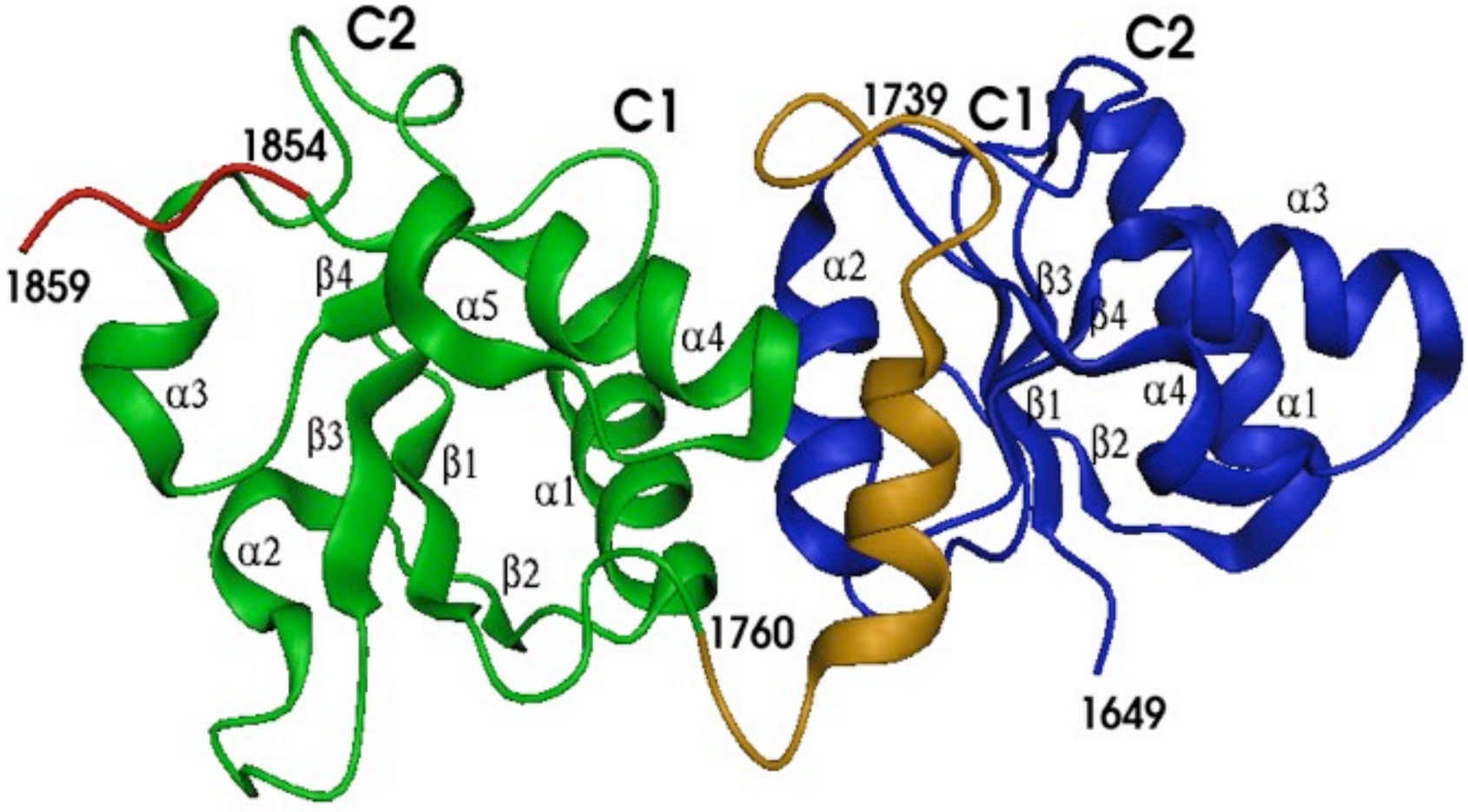
[6] http://salilab.org/modbase/.

Human BRCA1 and its two BRCT domains



BRCA1 BRCT repeats, 1jnx



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

CONFIDENTIAL

MYRIAD

BRACAnalysis™

Comprehensive BRCA1-BRCA2 Gene Sequence Analysis Result

Niecee Singer, MS Strang Cancer Prevention Center 428 E 72nd St New York, NY 10021	SPECIMEN Specimen Type: Blood Draw Date: n/a Accession Date: Oct 27, 2000 Report Date: Nov 17, 2000	PATIENT Name: Date of Birth: Feb 02, 1953 Patient ID: Gender: Female Accession #: 00019998 Requisition #: 56594
Physician: Fred Gilbert, MD		

Test Result

Gene Analyzed	Specific Genetic Variant
BRCA2	H2116R
BRCA1	None Detected

Interpretation

GENETIC VARIANT OF UNCERTAIN SIGNIFICANCE

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

Authorized Signature:

Brian E. Ward, Ph.D.
Laboratory Director

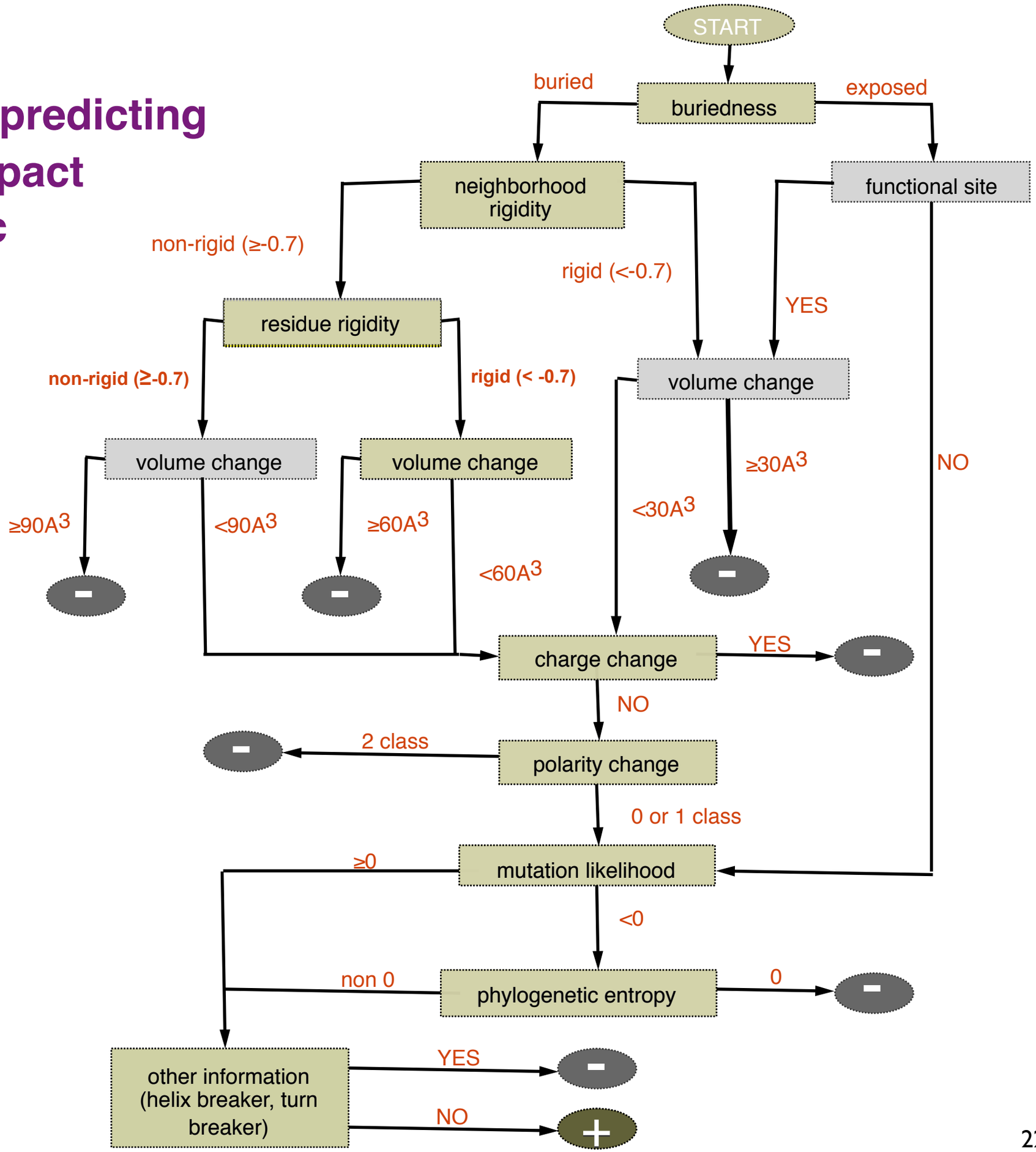
Thomas S. Frank, M.D.
Medical Director

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

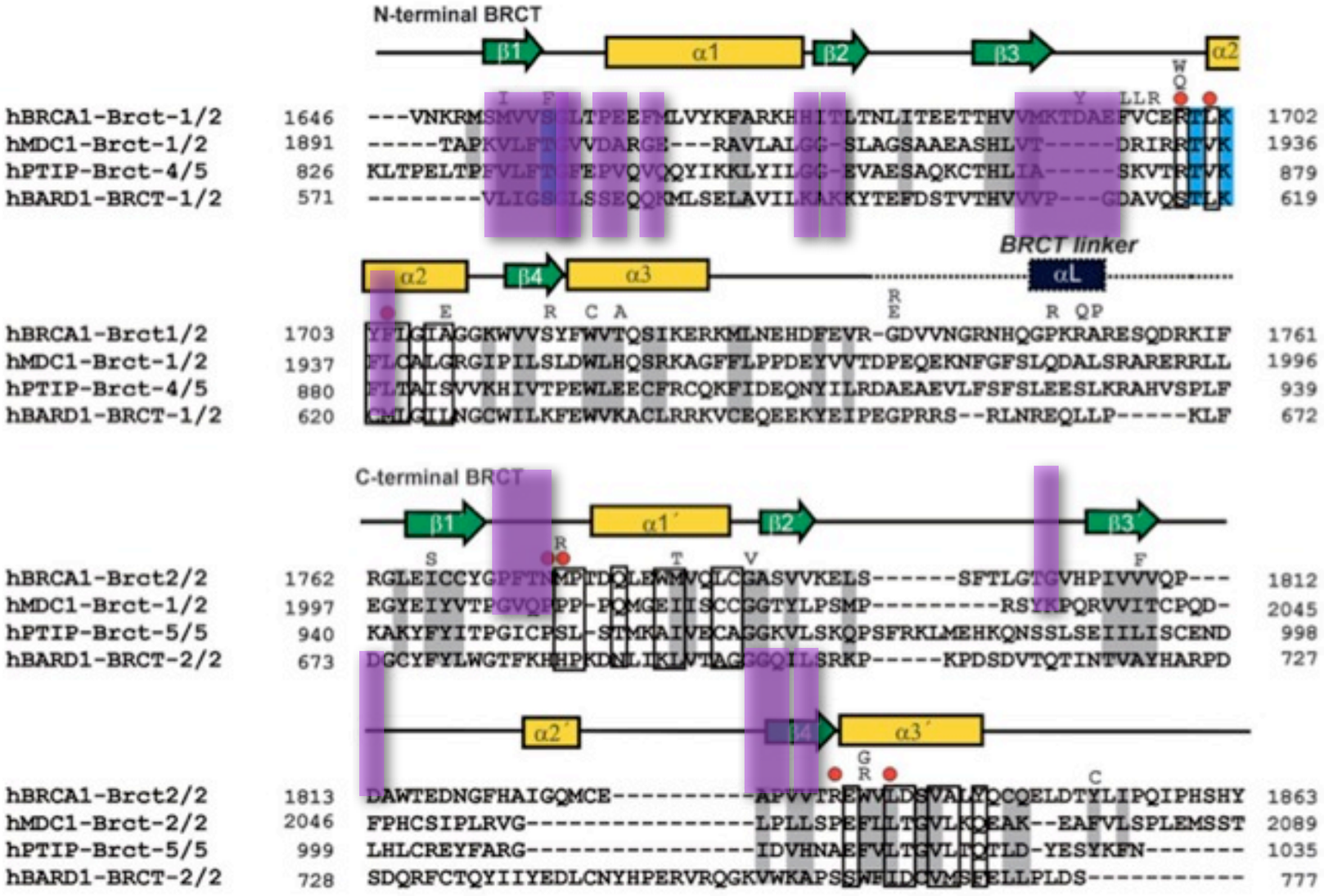
Missense mutations in BRCT domains by function

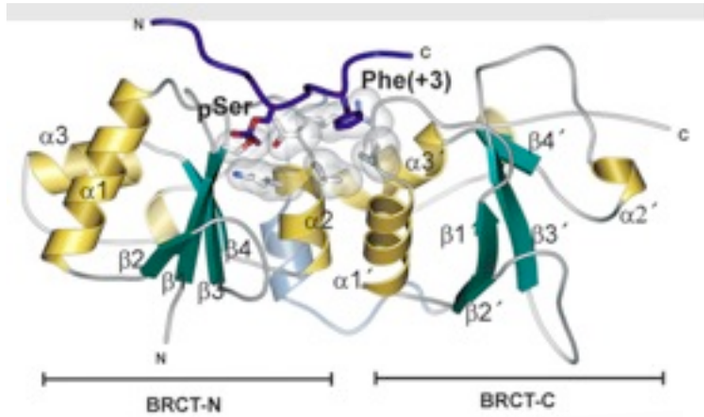
	cancer associated	not cancer associated	?
no transcription activation	C1697R R1699W A1708E S1715R P1749R M1775R		M1652K L1657P E1660G H1686Q R1699Q K1702E Y1703HF 1704S L1705PS 1715NS1 722FF17 34LG173 8EG1743 RA1752P F1761I F1761S M1775E M1775K L1780P I1807S V1833E A1843T
transcription activation		M1652I 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

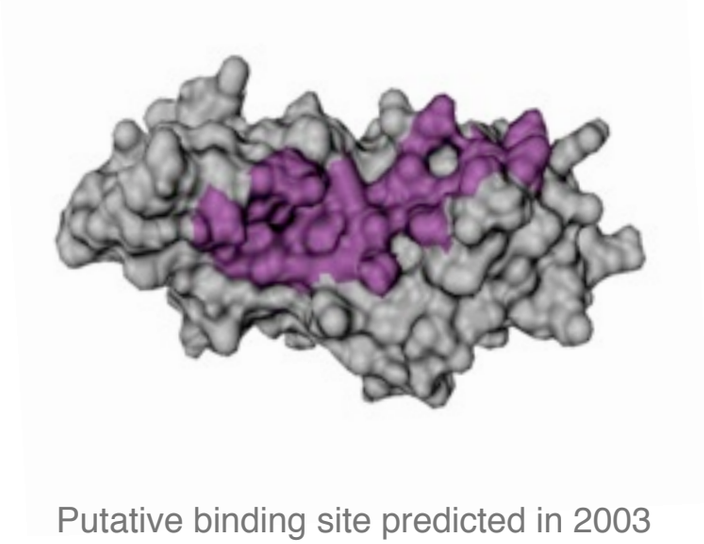
"Decision" tree for predicting functional impact of genetic variants



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

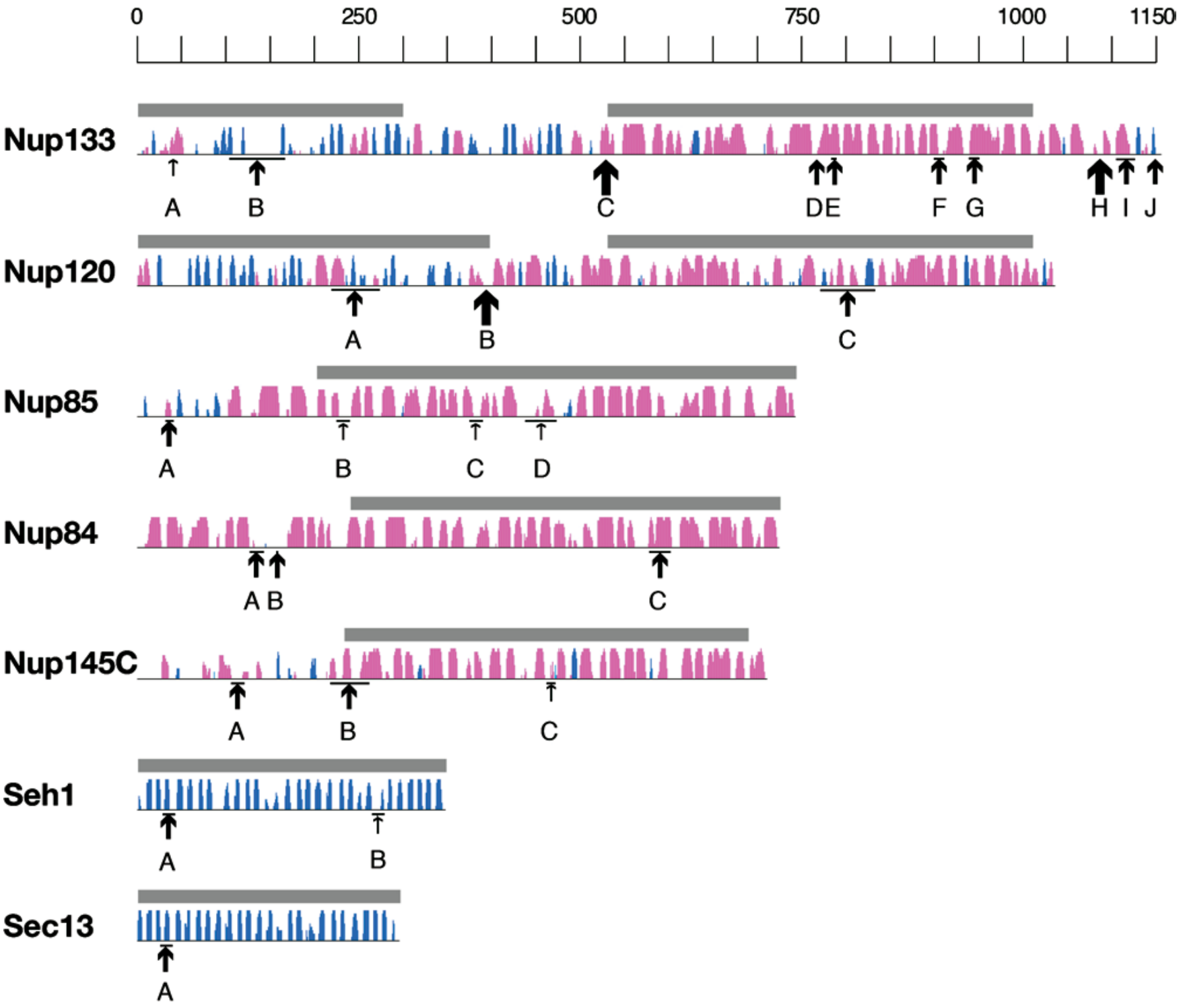
Mirkovic *et al.* 2004 Cancer Research. **June 2004 64**:3790

Common Evolutionary Origin of Coated Vesicles and Nuclear Pore Complexes

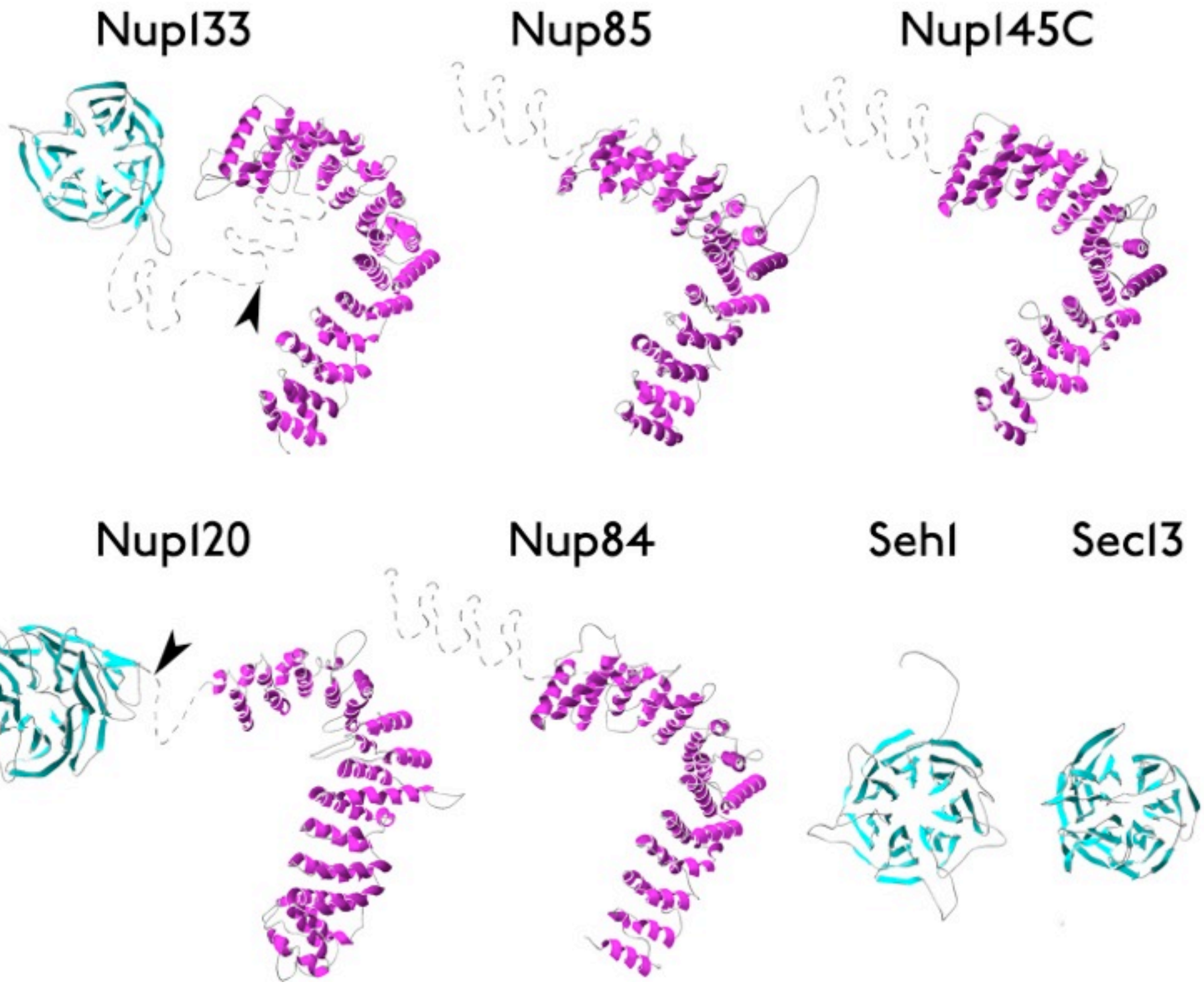
mGenThreader + SALIGN + MOULDER

D. Devos, S. Dokudovskaya, F. Alber, R. Williams, B.T. Chait, A. Sali, M.P. Rout.
Components of Coated Vesicles and Nuclear Pore Complexes Share a Common Molecular Architecture.
PLOS Biology **2(12):**e380, 2004

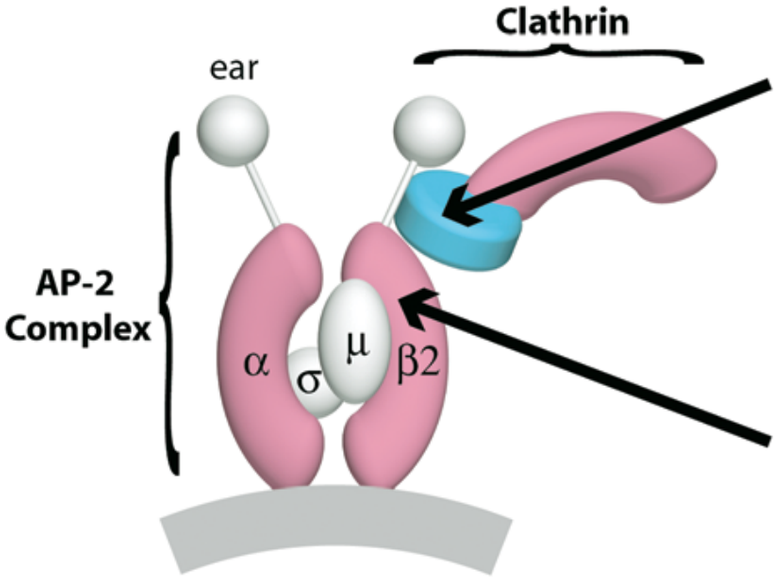
yNup84 complex proteins

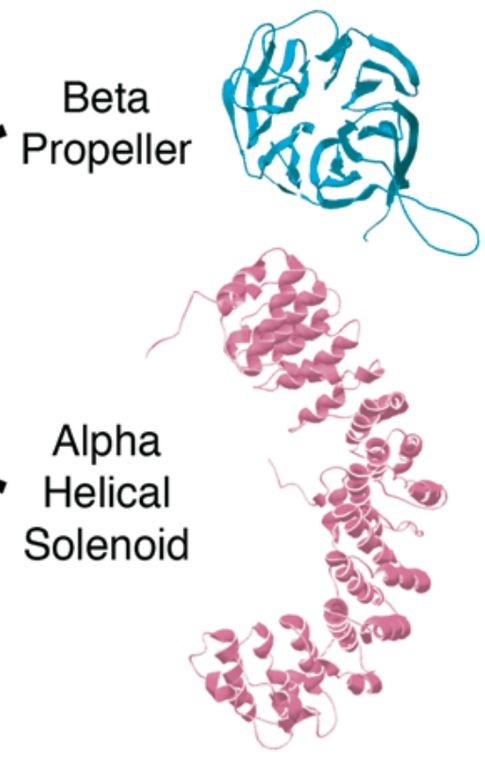


All Nucleoporins in the Nup84 Complex are Predicted to Contain β-Propeller and/or α-Solenoid Folds



NPC and Coated Vesicles Share the β-**Propeller and** α-**Solenoid** Folds and Associate with Membranes



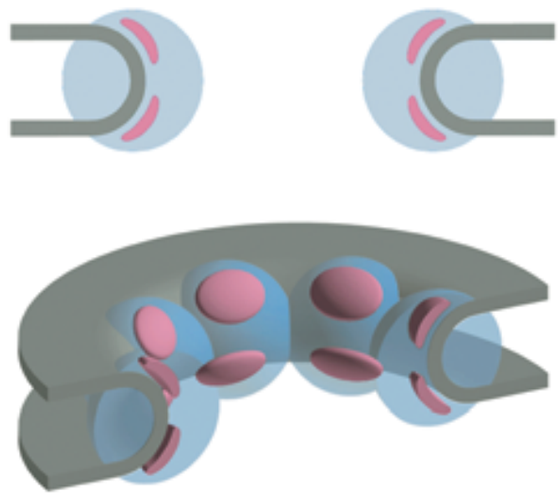


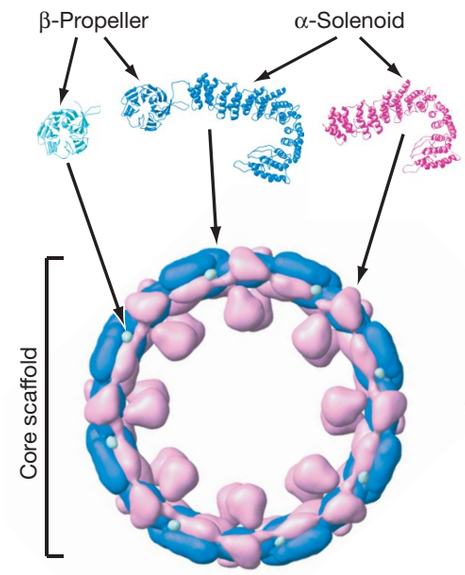
EXAMPLE	FOLDS	VARIANTS			
Clathrin/AP-2 Complex		*CCVs*	*COPI*	*COPII*	*NPC*
	Beta Propeller	Clathrin-N	α-subunit-N β'-subunit-N	Sec13 (Seh1) Sec31-N	Sec13 Seh1 Nup133-N Nup120-N
	Alpha Helical Solenoid	Clathrin-C α/γ/δ/ε-subunit β-subunits	α-subunit-C β'-subunit-C β-subunit γ-subunit	Sec31-C	Nup133-C Nup120-C Nup85 Nup84 Nup145C

NPC and Coated Vesicles Both Associate with Membranes

Coated Vesicle

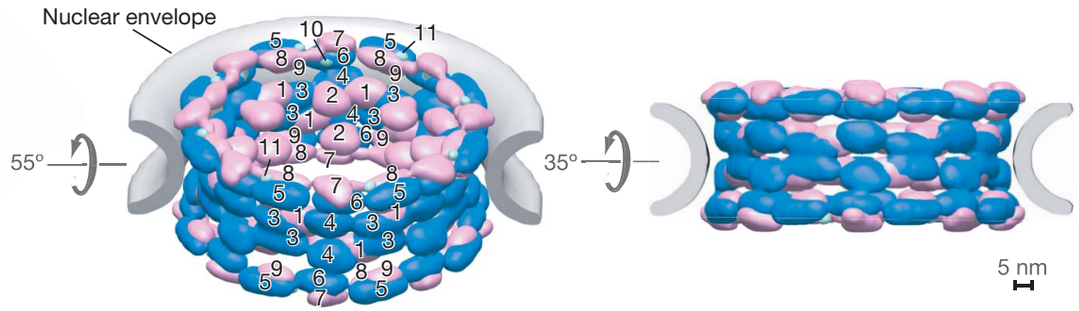
NPC model





Nup 84 complex

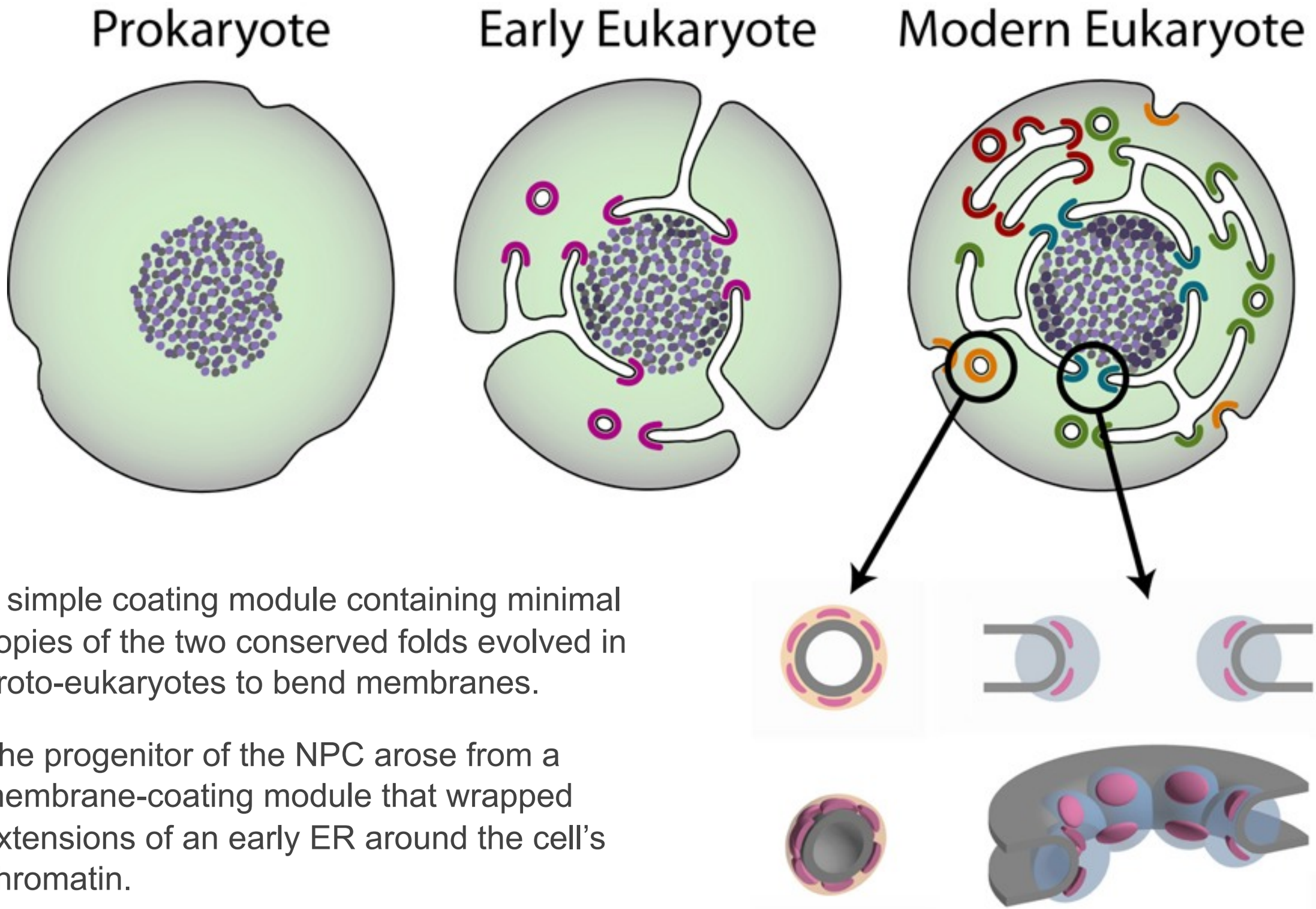
1 Nup192, 2 Nup188, 3 Nup170, 4 Nup157, 5 Nup133,
6 Nup120, 7 Nup85, 8 Nup84, 9 Nup145C, 10 Seh1, 11 Sec13



Alber et al. The molecular architecture of the nuclear pore complex. Nature (2007) vol. 450 (7170) pp. 695-701

A Common Evolutionary Origin for Nuclear Pore Complexes and Coated Vesicles?

The proto-coatomer hypothesis

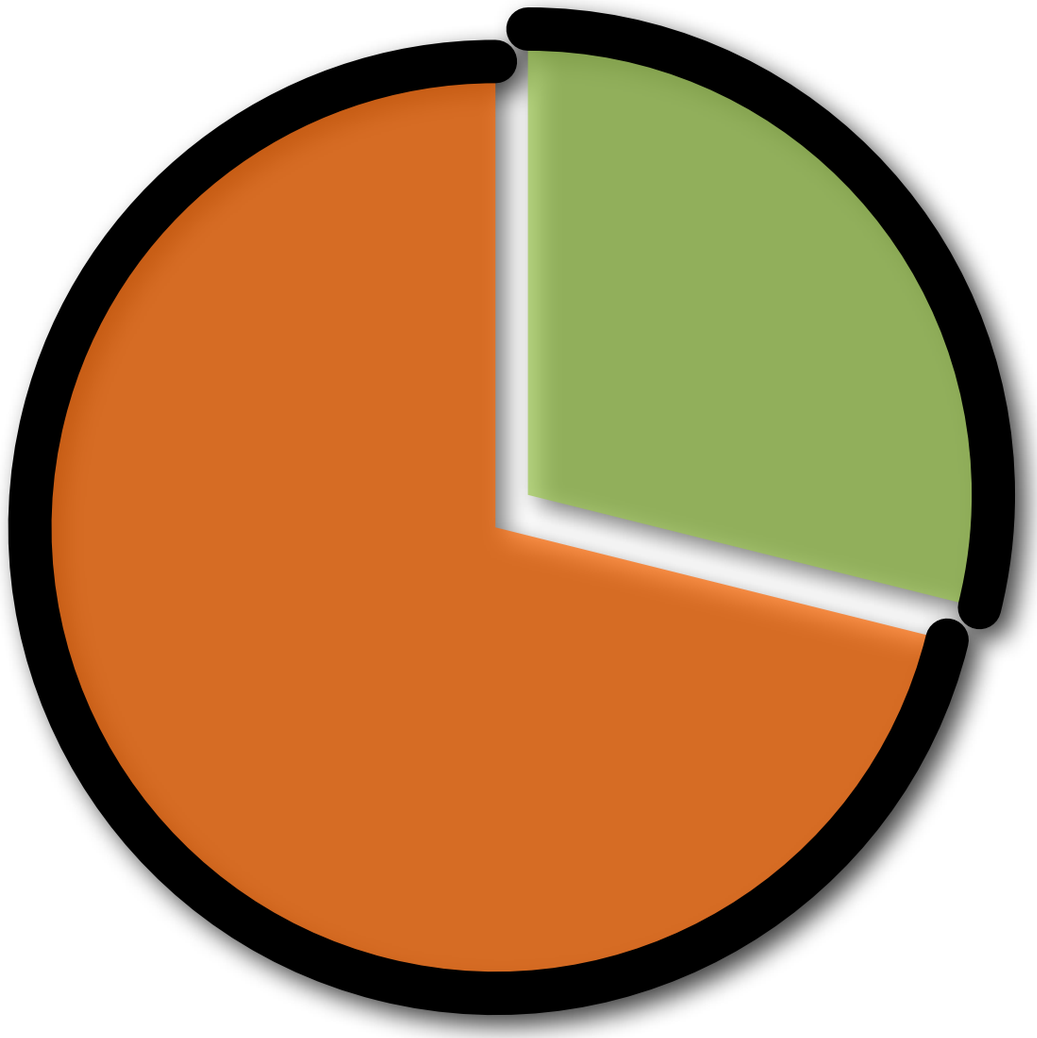


A simple coating module containing minimal copies of the two conserved folds evolved in proto-eukaryotes to bend membranes.

The progenitor of the NPC arose from a membrane-coating module that wrapped extensions of an early ER around the cell's chromatin.

Tropical Disease Initiative (TDI)

Predicting binding sites in protein structure models.



http://www.tropicaldisease.org

TDI *a story*

2004

.Steve Maurer (Berkeley) and Arti Rai (Duke)
.PLoS Medicine, Dec. 2004. Vol 1(3):e56

2005

.TDI web site `http://TropicalDisease.org`
.Ginger Taylor and The Synaptic Leap

2006

.Maurer and Sali 41th in “50 Who Matter”
.TSL web site `http://TheSynapticLeap.org`

2008

.TDI kernel `http://TropicalDisease.org/kernel`

Initial feed-back...

14 Mar 2005

I think TDI is a unique and very interesting project. I w
it...

So, where are we going? What's happening? Wha

I still trust in open s

Luca Brivio

16 Feb 2005

Hi,

10 Feb 2005

Hello,
My name is Adam Huber and I am a medical student at UNSW in Sydney Australia. I am interested in beginning research focused on tropical and infectious disease for underserved populations (A mission that seemingly matches TDI). I am,

bottlenecks are?
otential avenues to explore,
n!

9 Mar 2005
I'm a programmer, not a bioinformatician, but I stumbled across your site and thought I'd say something to keep the list active :)

GNU started with RMS. He gave us programming/administration tools to play with.
Linux started with Linus. He released an operating system for us to play with.
You need someone great in the field to release something for everyone to 'play with'. Then people start sending patches...

I know this is chicken-egg, but someone needs to point this out, since I haven't seen this brought up in the papers or the website.

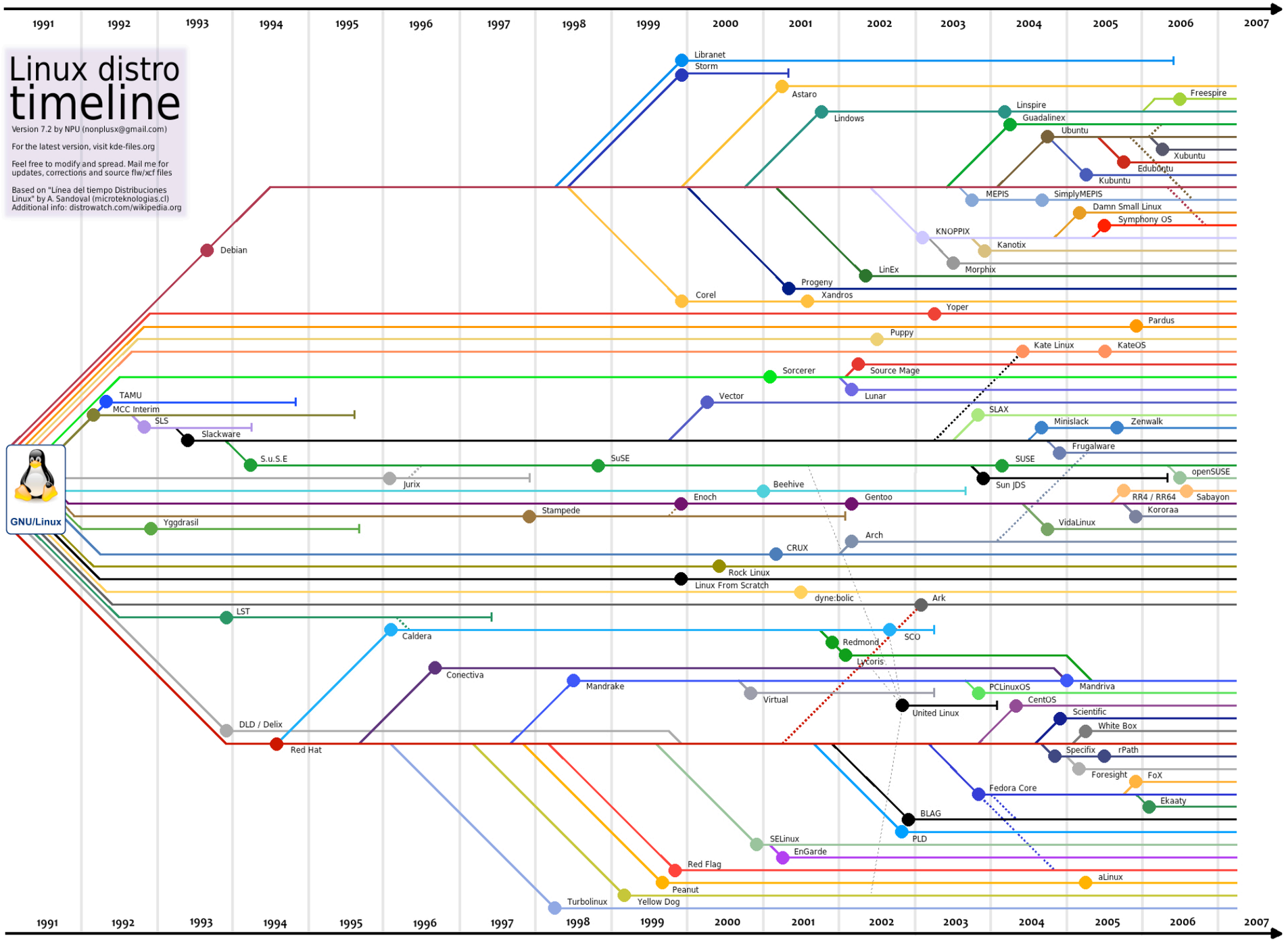
And you might consider merging into the bios.net effort mentioned already. Together, you just might reach the critical mass for things to take off. Consider this like when people jumped off the HURD project to come together and make linux work.

Daniel Amelang

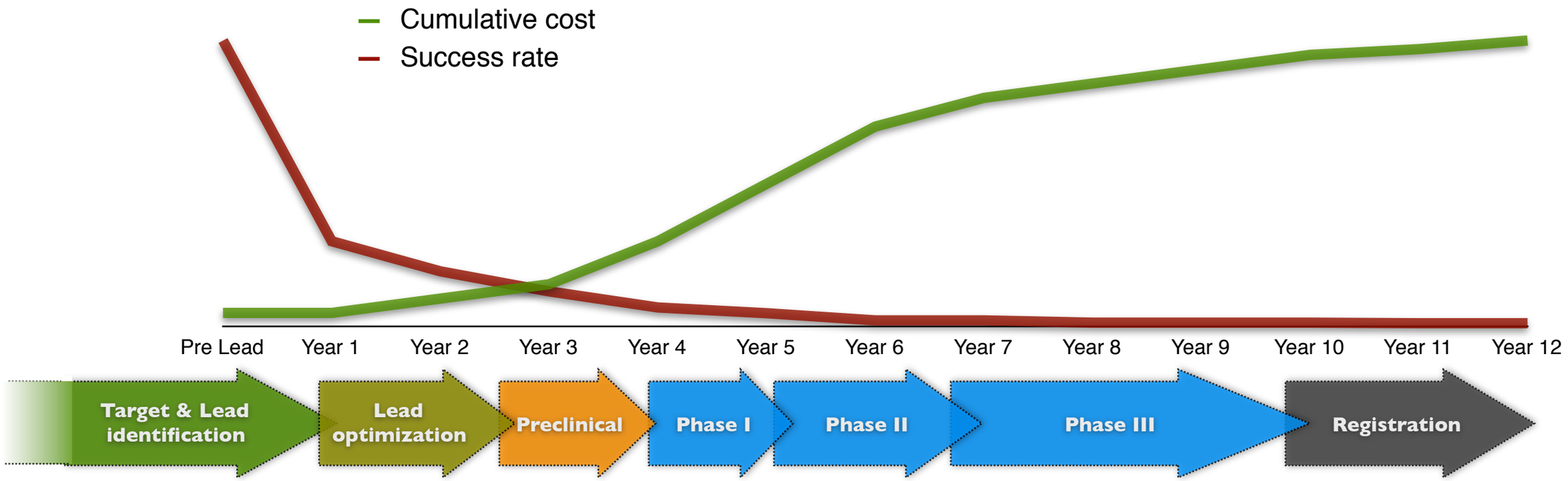
stic that the rest

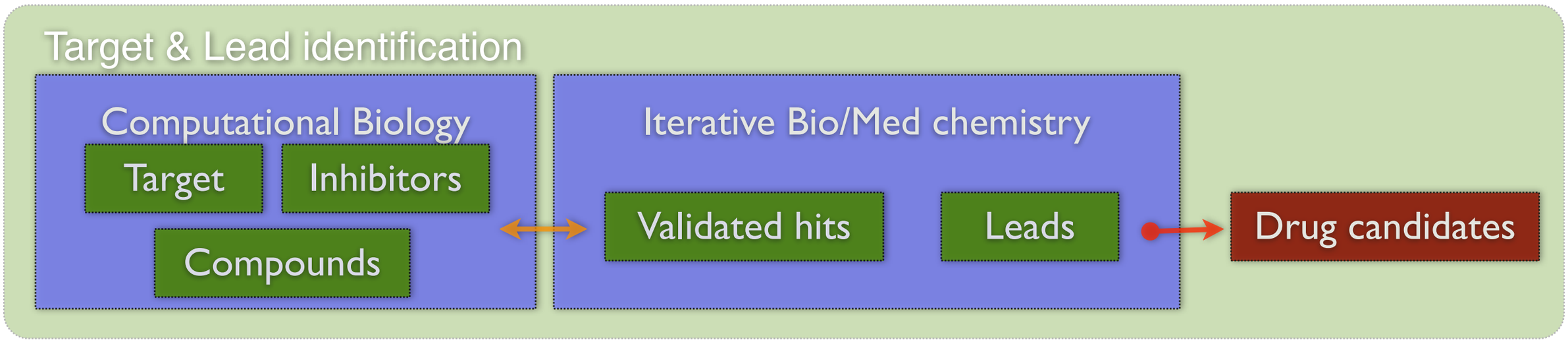
Stephen Mark Maurer

Open Source without a Kernel?



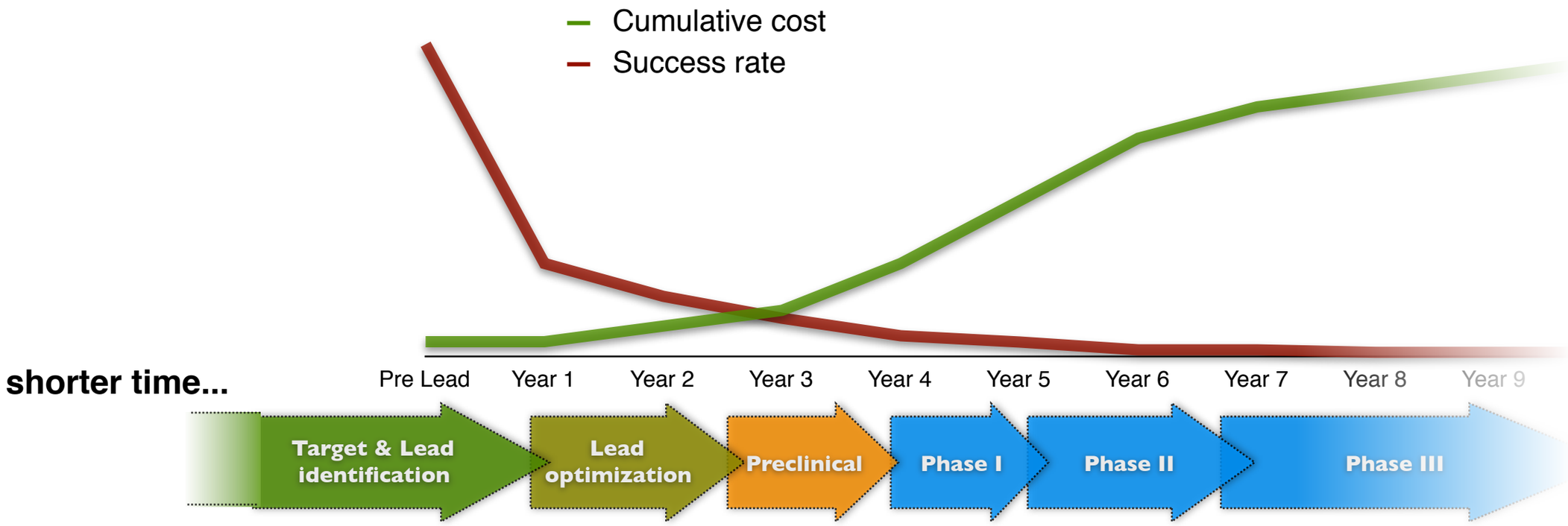
Drug Discovery pipeline





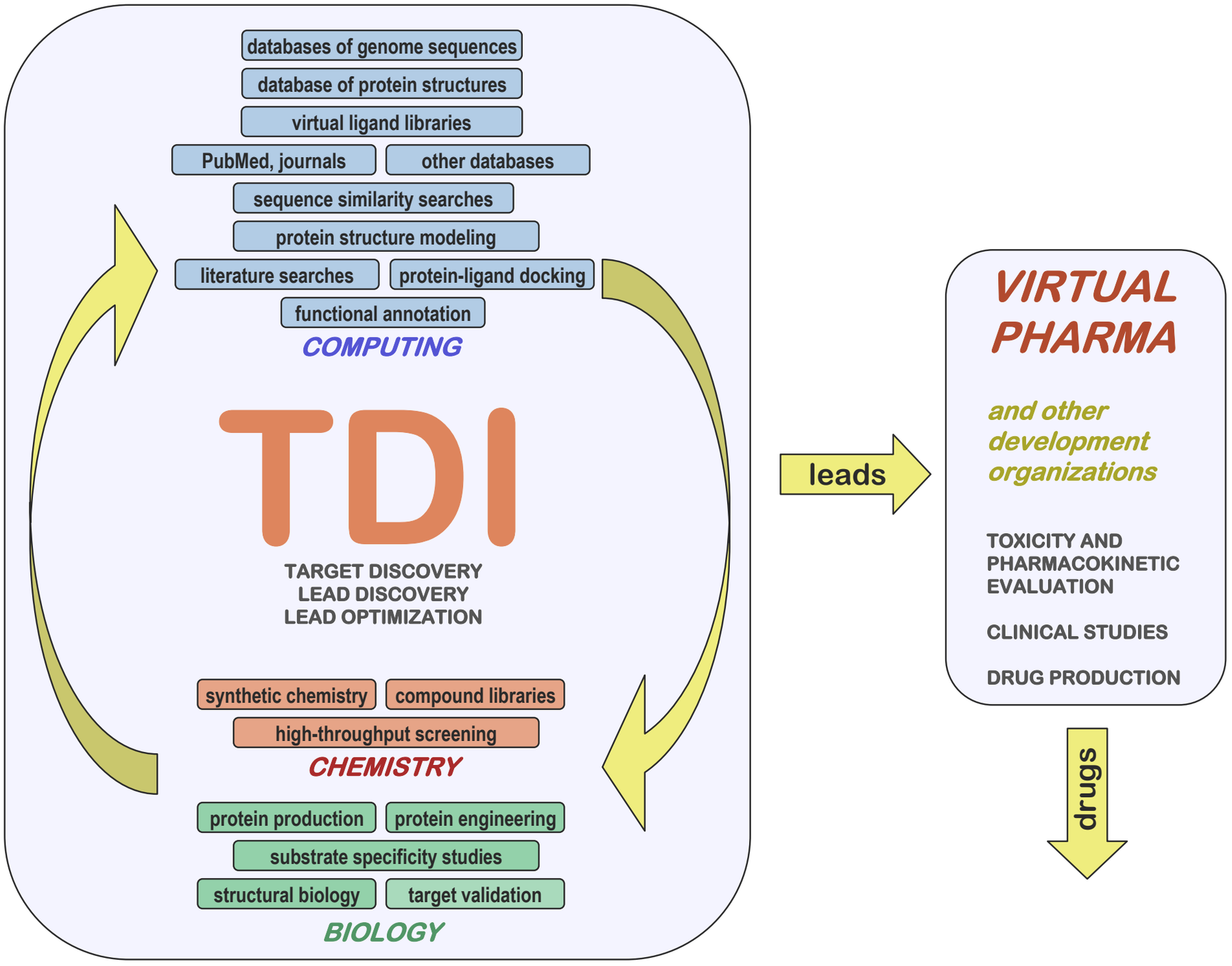
Adapted from: - Nwaka & Ridley. (2003) *Nature Reviews. Drug Discovery*. **2**:919
- Austin, Brady, Insel & collins. (2004) *Science*. **306**:1138

Drug Discovery pipeline



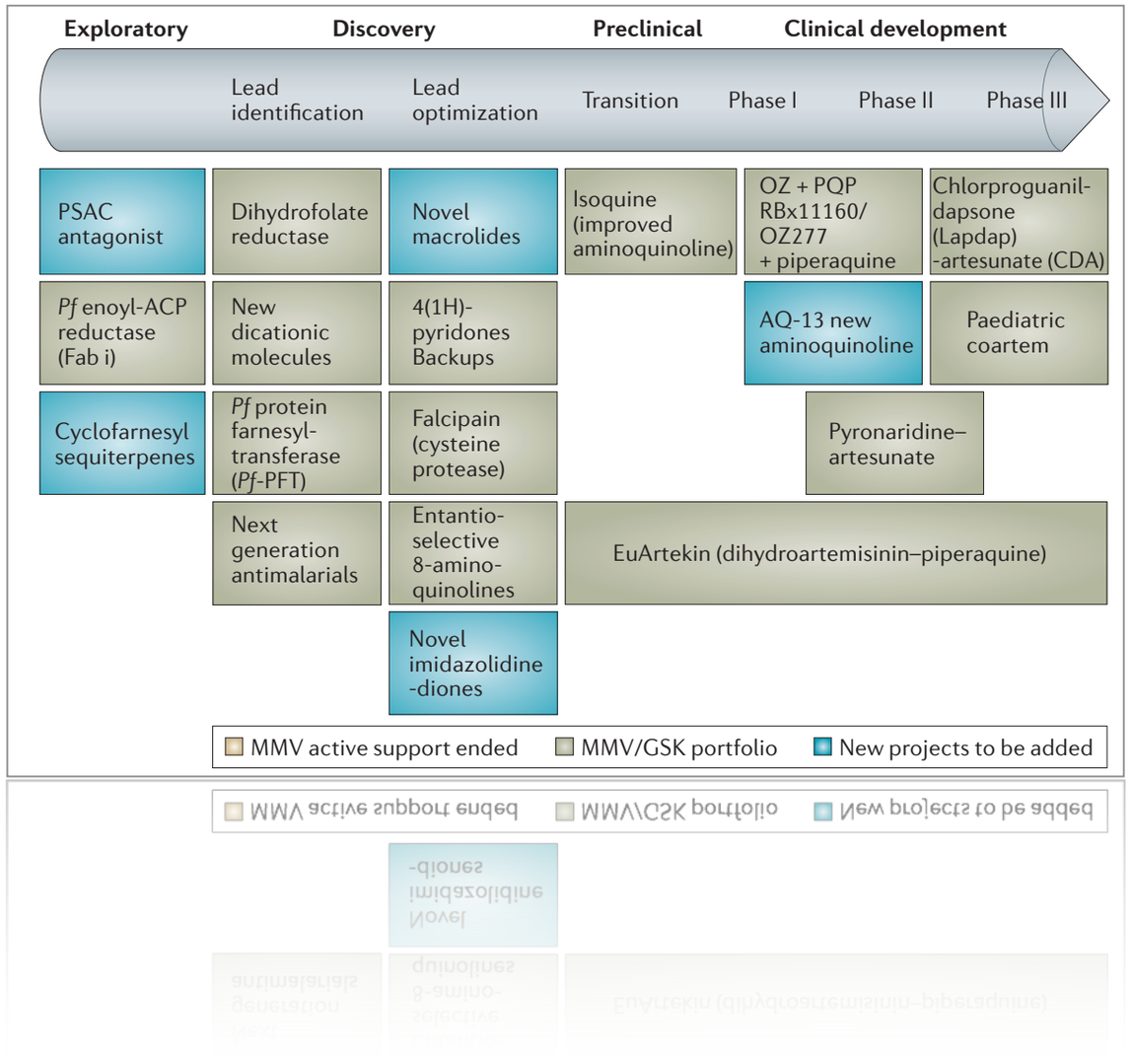
+ Completeness of genome projects (*eg*, Malaria)
+ New and more complete biological databases
+ New software and computers (cheaper and faster)
+ Internet == more people == less cost

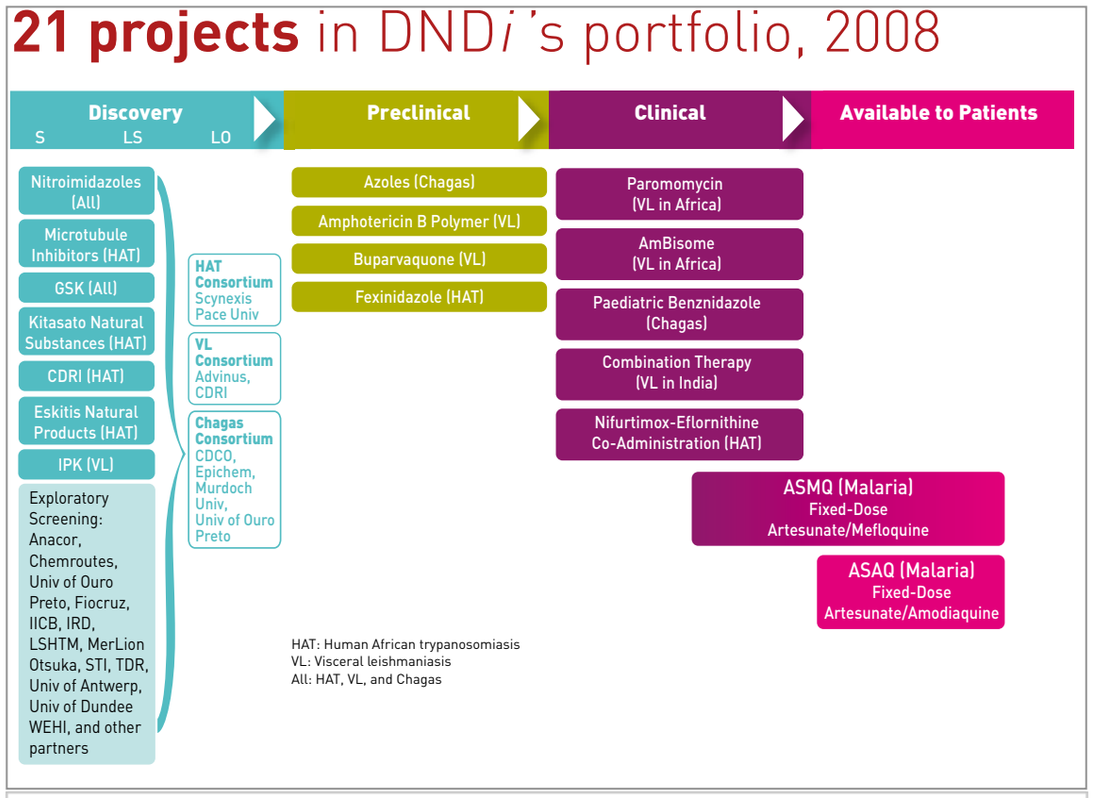
TDI flowchart



Non-Profit organizations

Open-Source + Out-Source = low cost business model



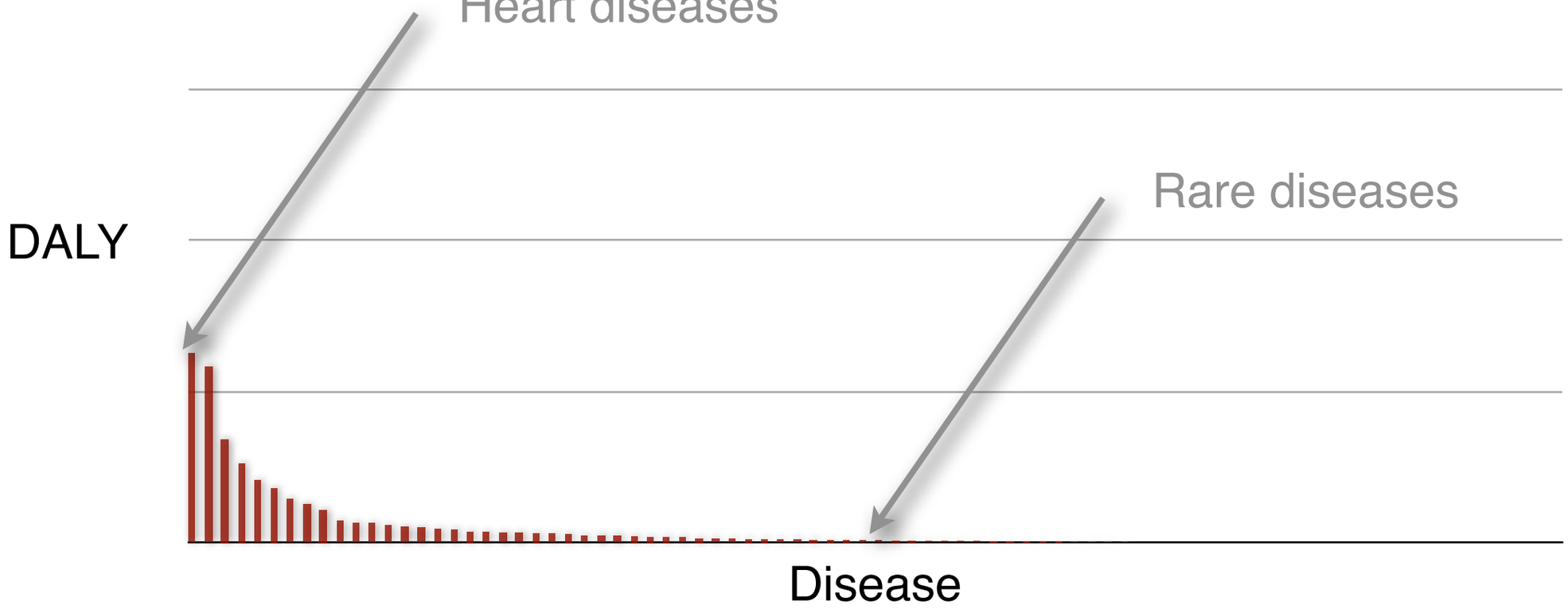


Munos (2006) Nature Reviews. Drug Discovery.

Need is High in the Tail

DALY Burden Per Disease in Developed Countries

DALY Burden Per Disease in Developing Countries



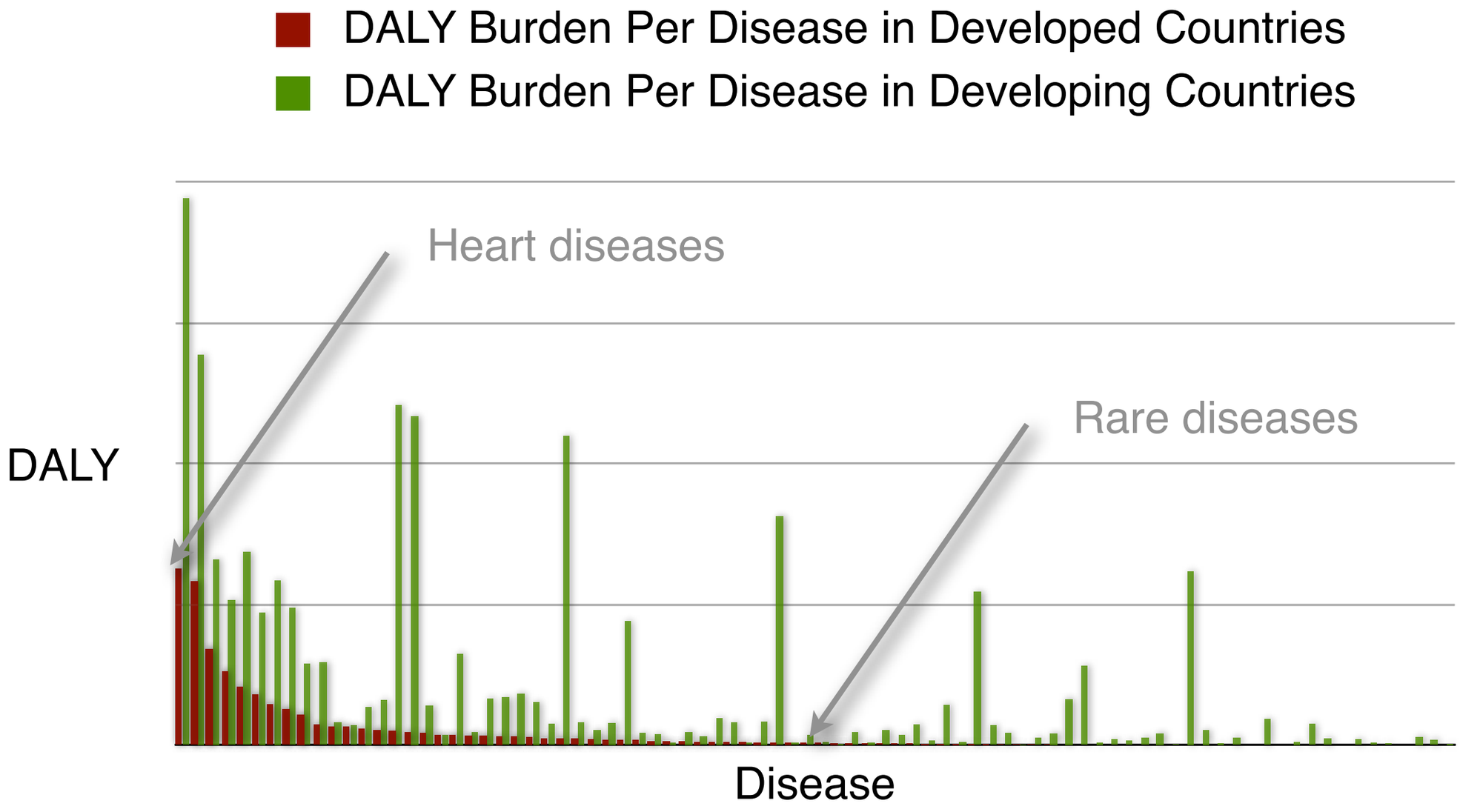
Disease data taken from WHO, *World Health Report 2004*

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



Disease data taken from WHO, *World Health Report 2004*

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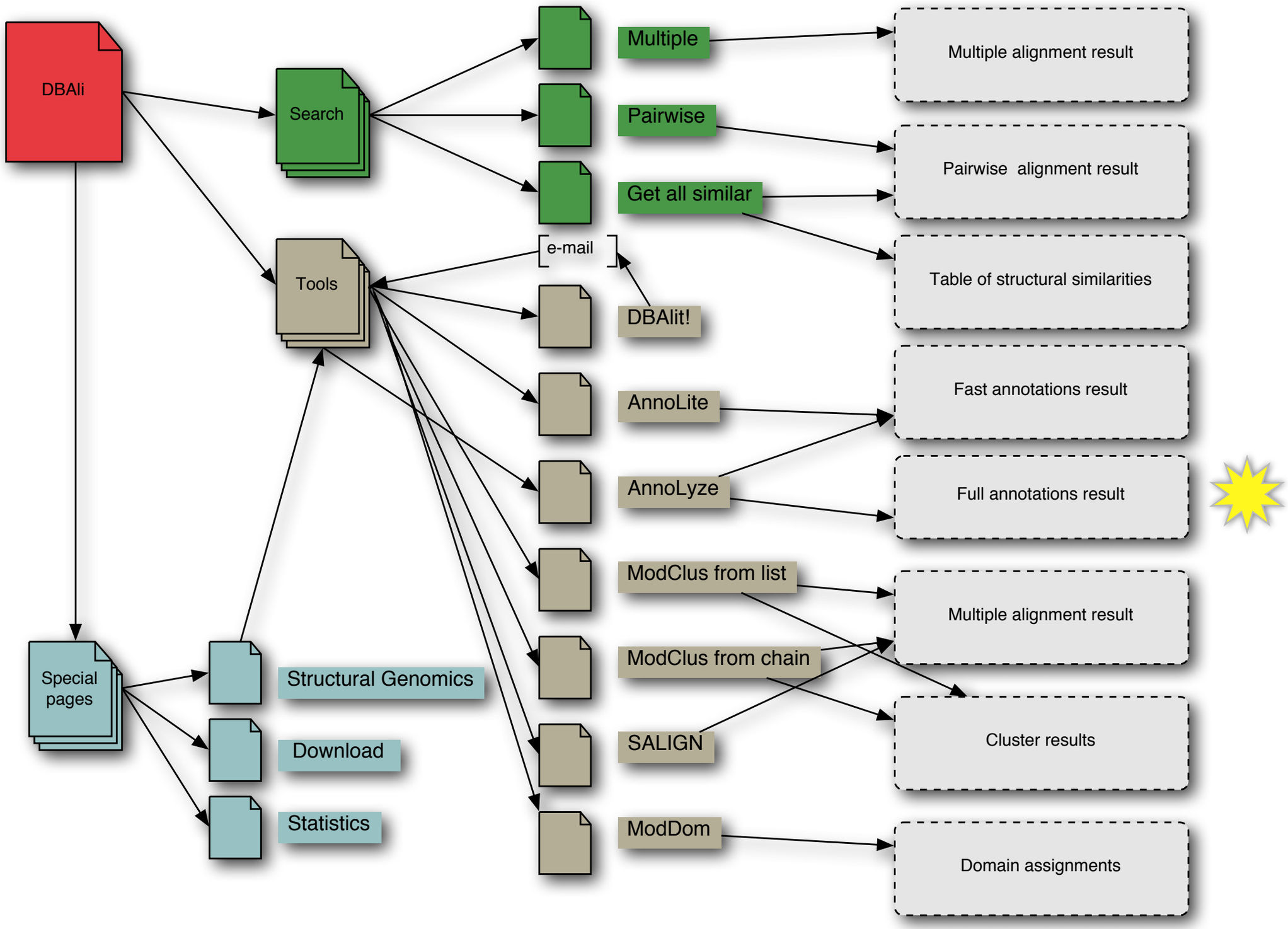
“Unprofitable” Diseases and Global DALY (in 1000’s)

Malaria*	**46,486**	Trichuriasis	1,006
Tetanus	7,074	Japanese encephalitis	709
Lymphatic filariasis*	**5,777**	**Chagas Disease***	**667**
Syphilis	4,200	**Dengue***	**616**
Trachoma	2,329	**Onchocerciasis***	**484**
Leishmaniasis*	**2,090**	**Leprosy***	**199**
Ascariasis	1,817	Diphtheria	185
Schistosomiasis*	**1,702**	Poliomyelitise	151
Trypanosomiasis*	**1,525**	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.

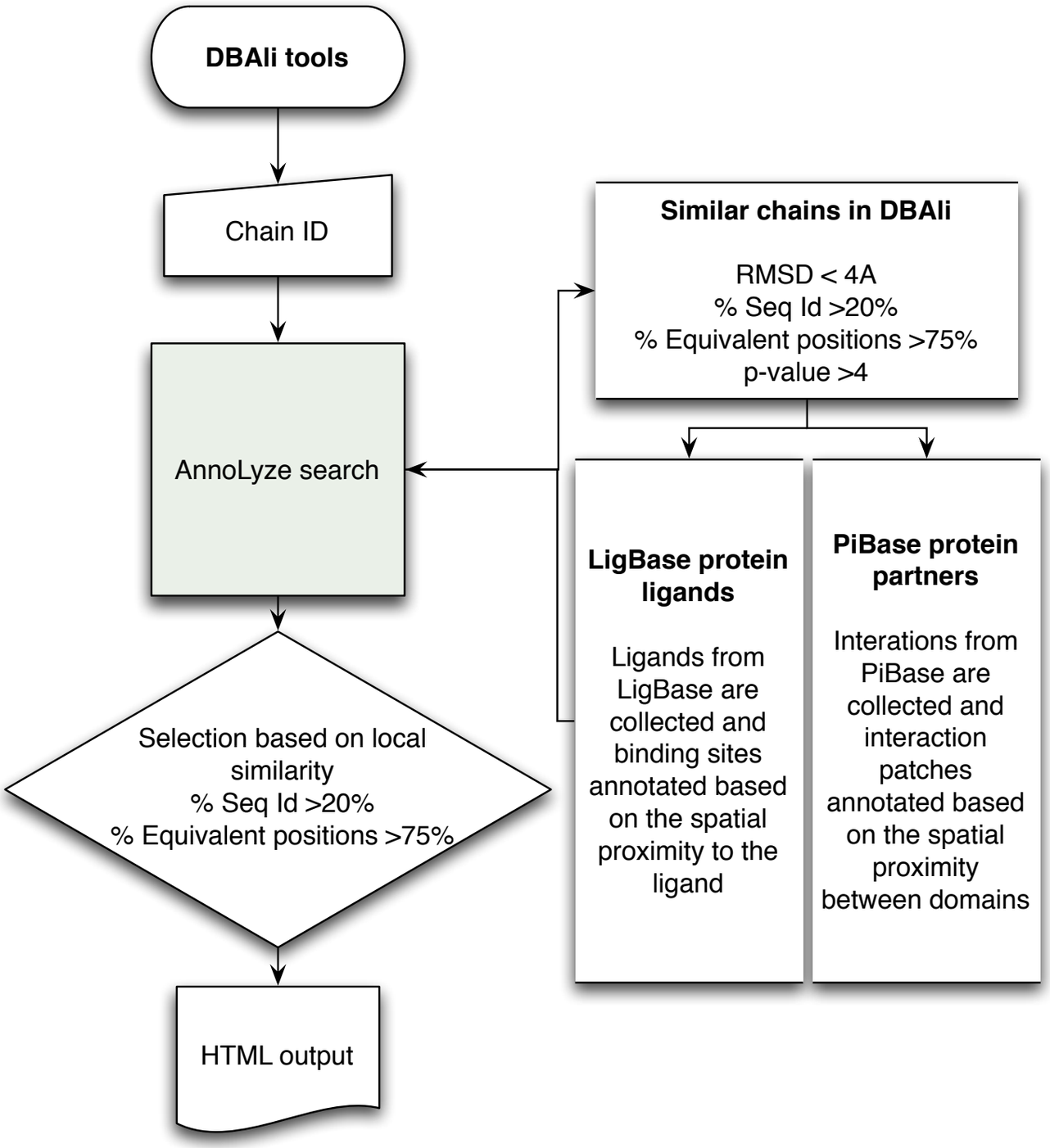
DBAli$_{v2.0}$ database

http://www.dbali.org



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

Method

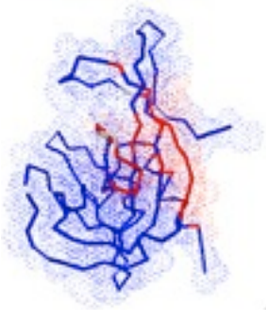


Inherited ligands: 4

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

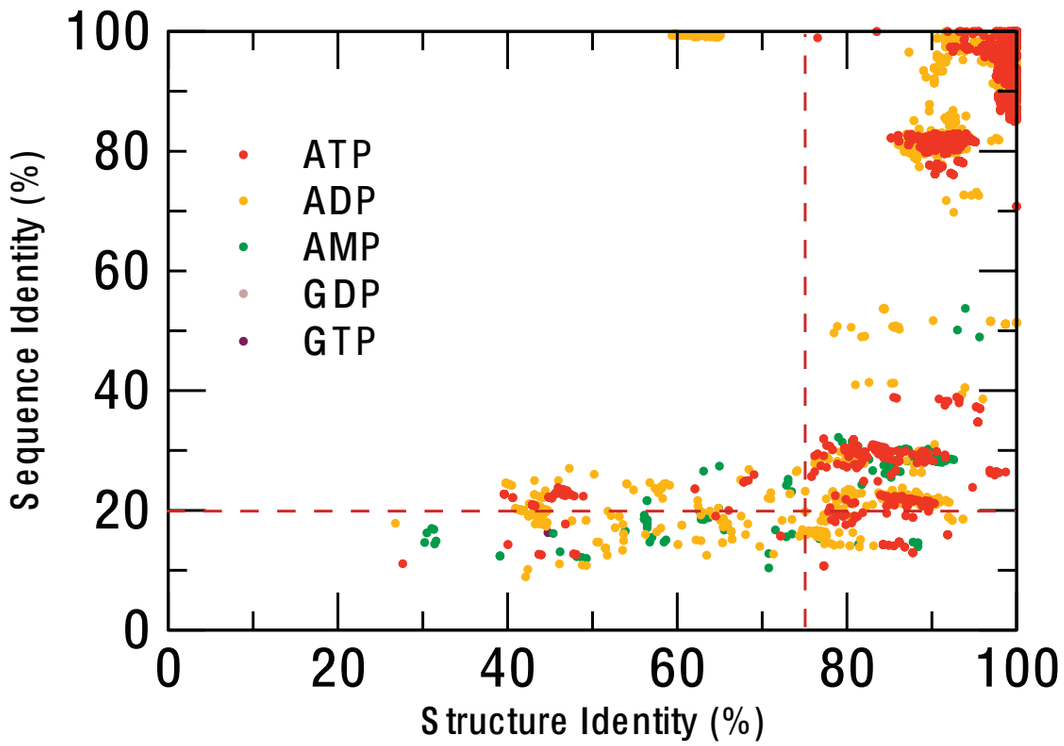
Inherited partners:1

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

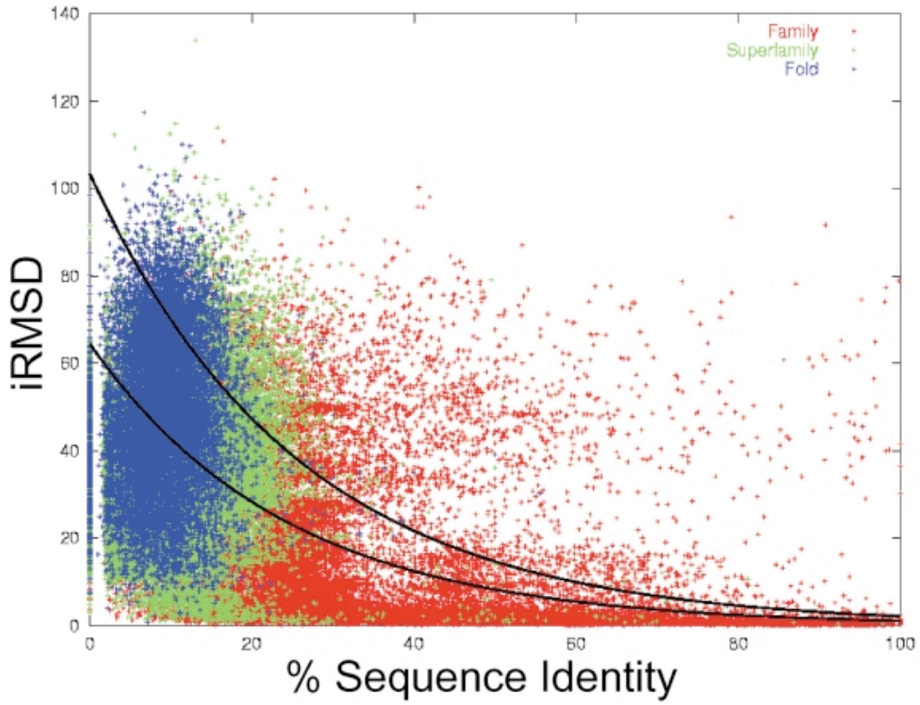


Scoring function

Ligands



Partners



Aloy *et al.* (2003) J.Mol.Biol. 332(5):989-98.

Benchmark

	Number of chains
Initial set*	78,167
LigBase**	30,126
Non-redundant set***	4,948 (8,846 ligands)

*all PDB chains larger than 30 aminoacids in length (8th of August, 2006)
**annotated with at least one ligand in the LigBase database
***not two chains can be structurally aligned within 3A, superimposing more than 75% of their Cα atoms, result in a sequence alignment with more than 30% identity, and have a length difference inferior to 50aa

	Number of chains
Initial set*	78,167
πBase**	30,425
Non-redundant set***	4,613 (11,641 partnerships)

*all PDB chains larger than 30 aminoacids in length (8th of August, 2006)
**annotated with at least one partner in the πBase database
***not two chains can be structurally aligned within 3A, superimposing more than 75% of their Cα atoms, result in a sequence alignment with more than 30% identity, and have a length difference inferior to 50aa

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

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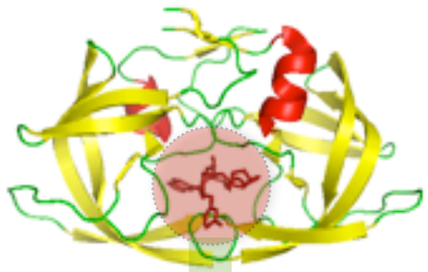
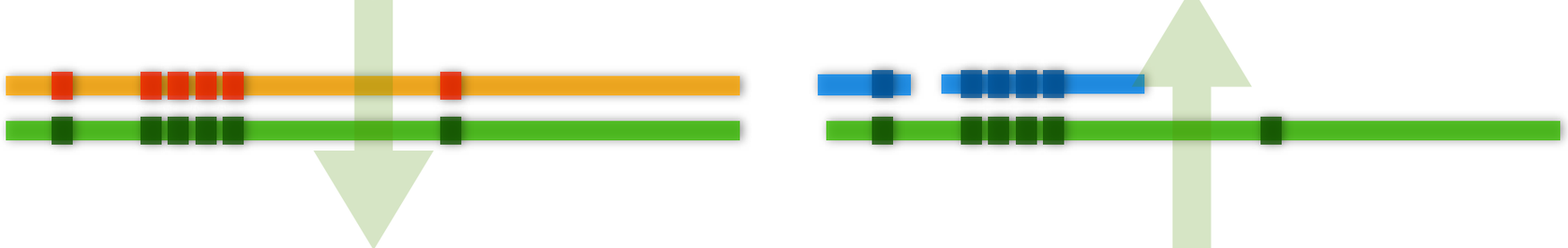
Comparative docking

Expansion

co-crystalized protein/ligand

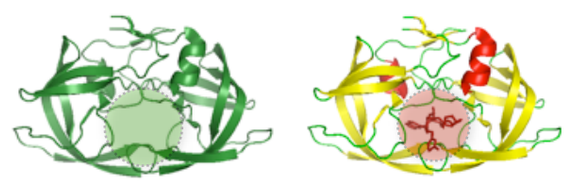




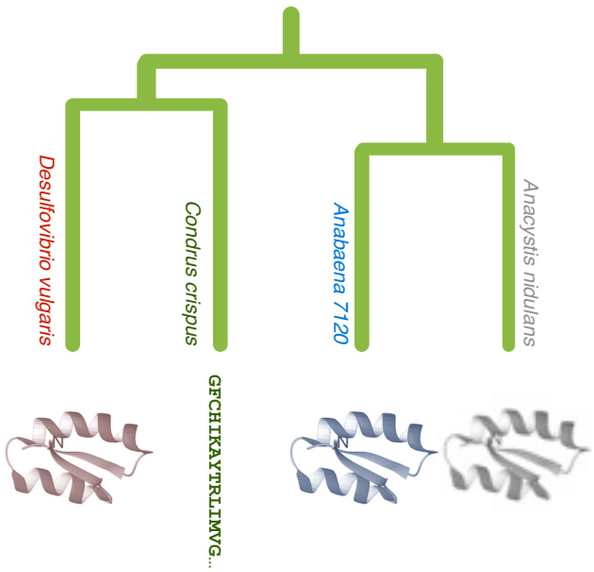
crystalized protein

2. Inheritance

model



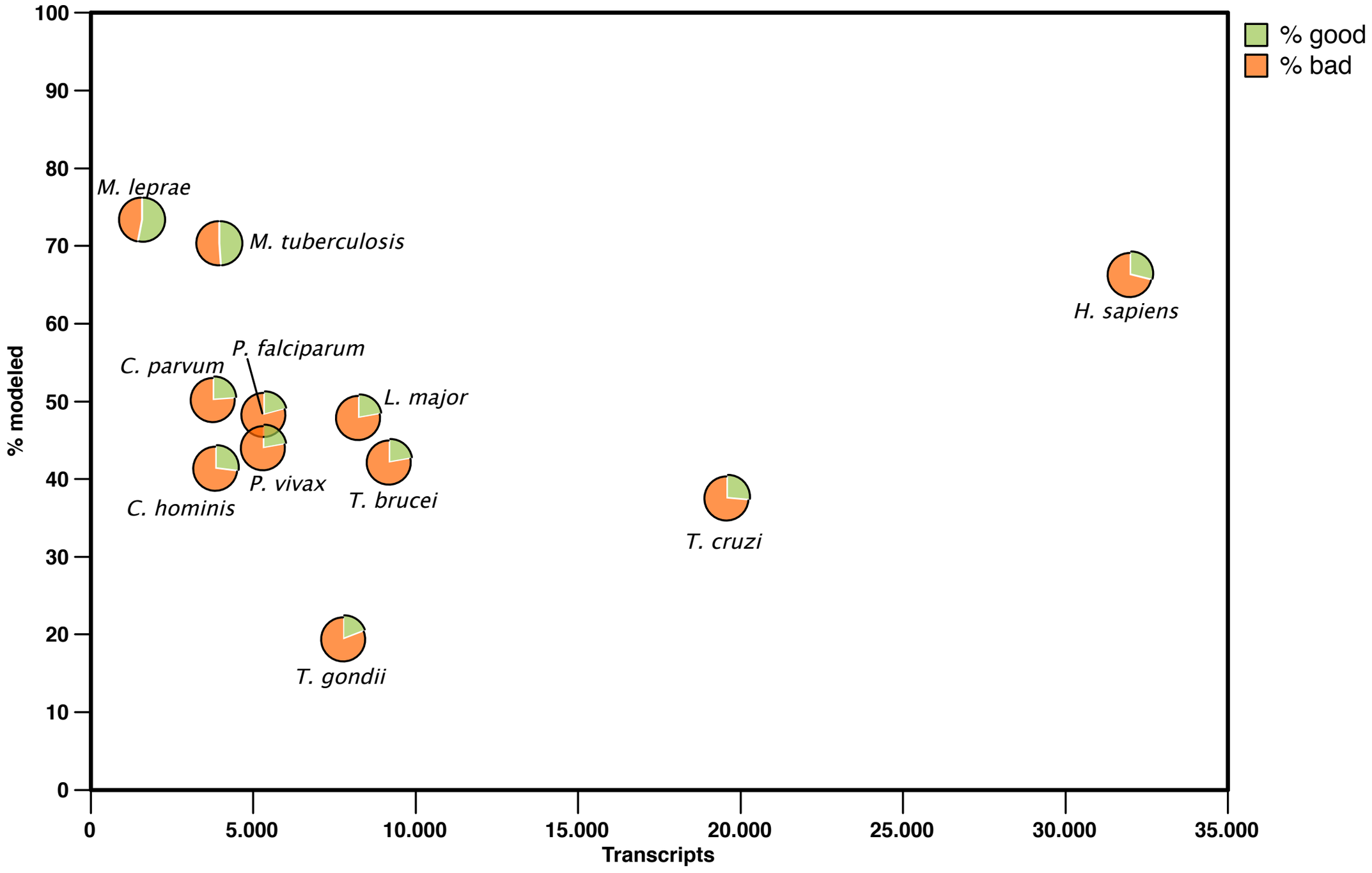
template



1. Modeling

Modeling Genomes

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



A good model has MPQS of 1.0 or higher

Summary table

models with inherited ligands

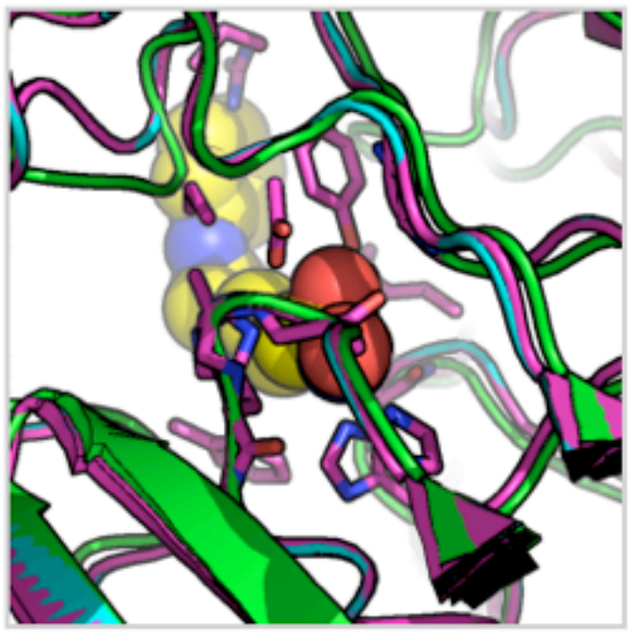
29,271 targets with good models, 297 inherited a ligand/substance similar to a known drug in DrugBank

	Transcripts	Modeled targets	Selected models	Inherited ligands	Similar to a drug	Drugs
C. hominis	3,886	1,614	666	197	20	13
C. parvum	3,806	1,918	742	232	24	13
L. major	8,274	3,975	1,409	478	43	20
M. leprae	1,605	1,178	893	310	25	6
M. tuberculosis	3,991	2,808	1,608	365	30	10
P. falciparum	5,363	2,599	818	284	28	13
P. vivax	5,342	2,359	822	268	24	13
T. brucei	7,793	1,530	300	138	13	6
T. cruzi	19,607	7,390	3,070	769	51	28
T. gondii	9,210	3,900	1,386	458	39	21
TOTAL	**68,877**	**29,271**	**11,714**	**3,499**	**297**	**143**

L. major Histone deacetylase 2 + Vorinostat

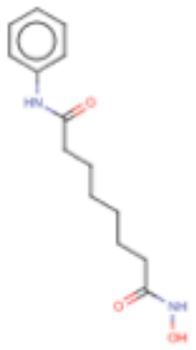
Template 1t64A a human HDAC8 protein.

PDB		Template		Model		Ligand	Exact	SupStr	SubStr	Similar
1c3sA	83.33/80.00	1t64A	36.00/1.47	LmjF21.0680.1.pdb	90.91/100.00	SHH	DB02546	DB02546	DB02546	DB02546



DB02546 Vorinostat

Small Molecule; Approved; Investigational



Drug categories:

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

Drug indication:

For the treatment of cutaneous manifestations in patients with cutaneous T-cell lymphoma who have progressive, persistent or recurrent disease on or following two systemic therapies.

L. major Histone deacetylase 2 + Vorinostat

Literature

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)

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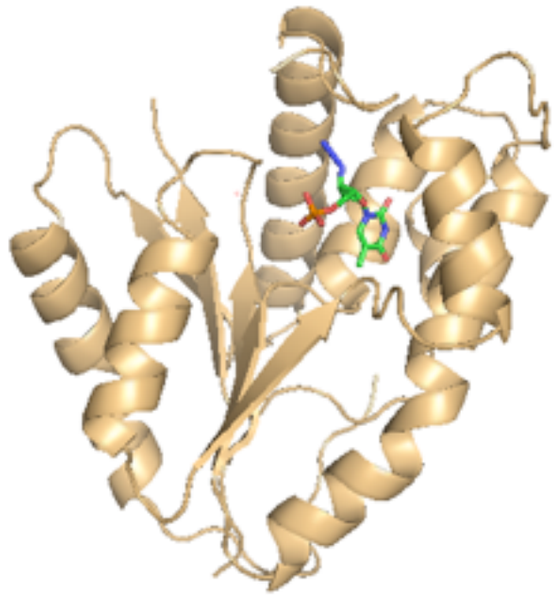
ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 2004, p. 1435–1436 Vol. 48, No. 4
0066-4804/04/$08.00+0 DOI: 10.1128/AAC.48.4.1435–1436.2004

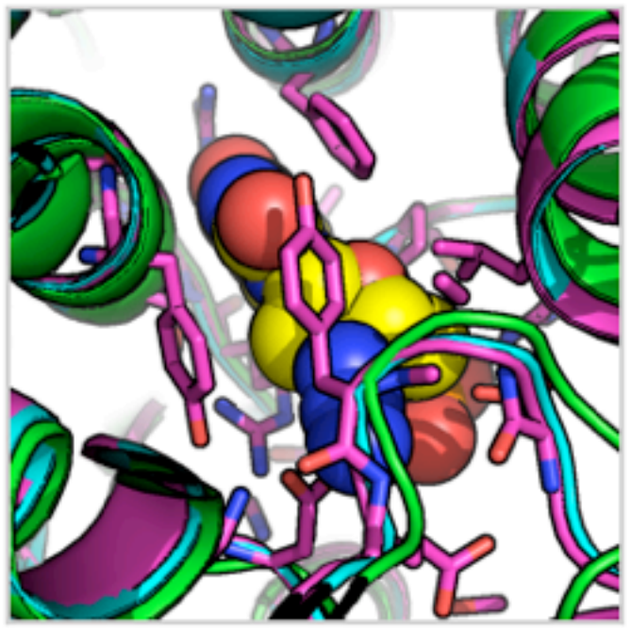
Antimalarial and Antileishmanial Activities of Aroyl-Pyrrolyl-Hydroxyamides, a New Class of Histone Deacetylase Inhibitors

P. falciparum tymidylate kinase + zidovudine

Template 3tmkA a yeast tymidylate kinase.



PDB		Template		Model		Ligand	Exact	SupStr	SubStr	Similar
2tmkB	100.00/100.00	3tmkA	41.00/1.49	PFL2465c.2.pdb	82.61/100.00	ATM		DB00495		DB00495

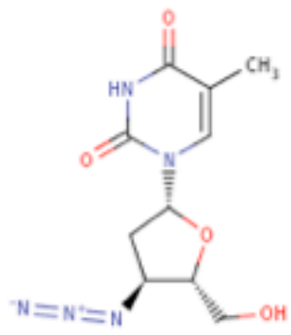


DB00495 Zidovudine

Small Molecule; Approved

Drug categories:

Anti-HIV Agents
Antimetabolites
Nucleoside and Nucleotide Reverse Transcriptase Inhibitors

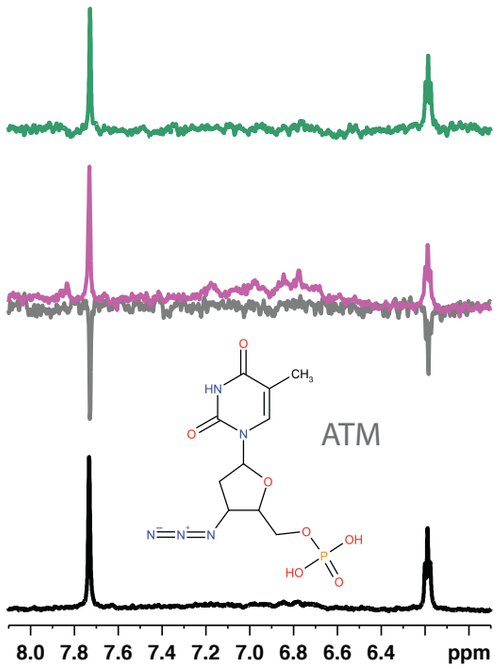


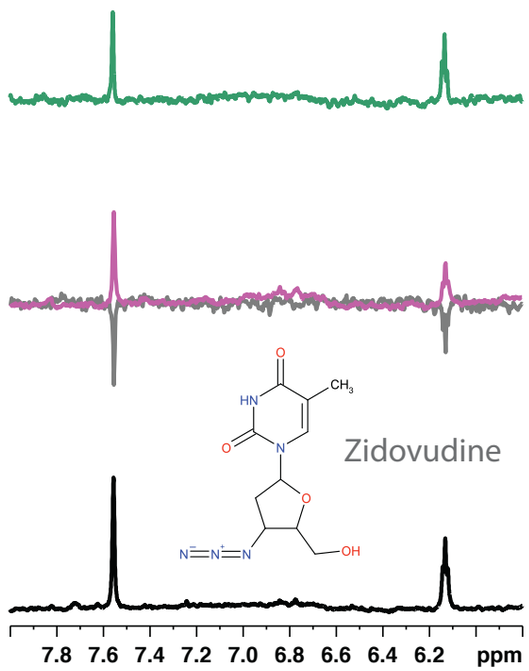
Drug indication:

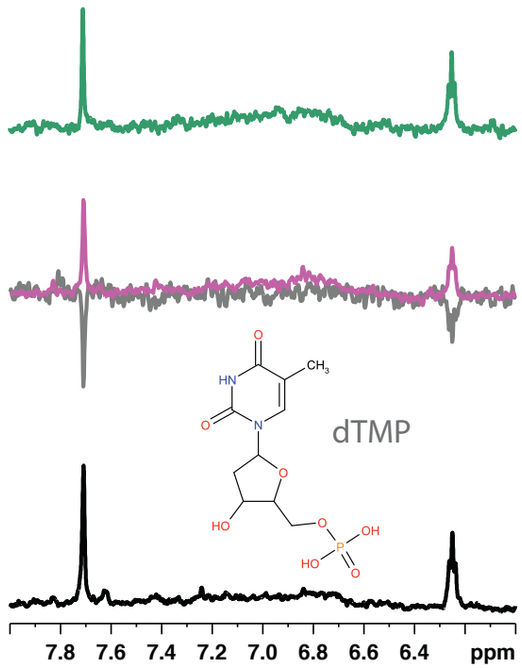
For the treatment of human immunovirus (HIV) infections.

P. falciparum tymydilate kinase + zidovudine

NMR Water-LOGSY and STD experiments



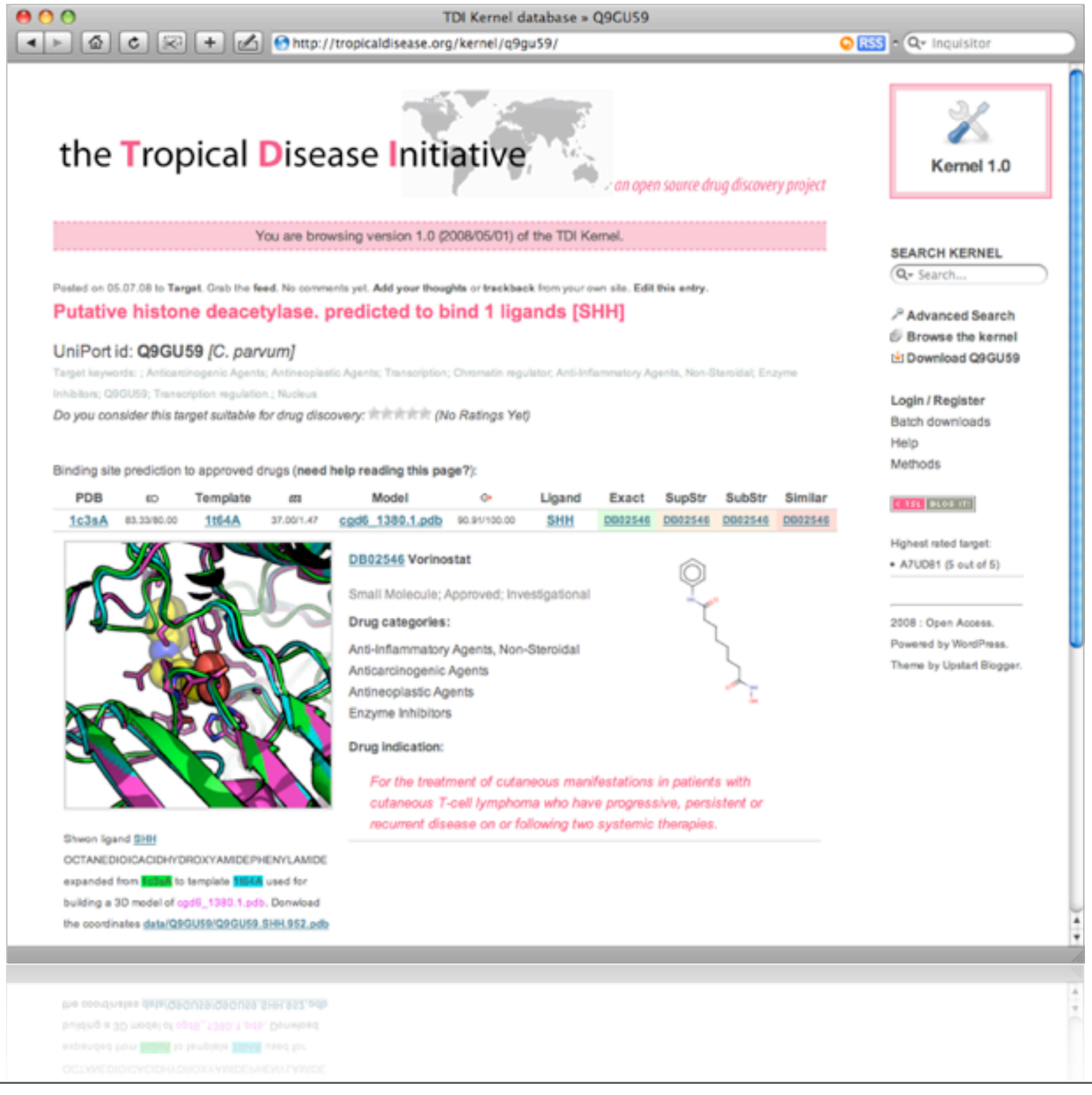




Leticia Ortí, Rodrigo J. Carbajo, and Antonio Pineda-Lucena

TDI's kernel

http://tropicaldisease.org/kernel



TDI's kernel

http://tropicaldisease.org/kernel

L. Orti *et al.*, *Nat Biotechnol* **27**, 320 (Apr, 2009).

CORRESPONDENCE

A kernel for the Tropical Disease Initiative

To the Editor:

Identifying proteins that are good drug targets and finding drug leads that bind to them is generally a challenging problem. It is particularly difficult for neglected tropical diseases, such as malaria and tuberculosis, where research resources are relatively scarce[1]. Fortunately, several developments improve our ability to deal with drug discovery for neglected diseases: first, the sequencing of many complete genomes of organisms that cause tropical diseases; second, the determination of a large number of protein structures; third, the creation of compound libraries, including already-approved drugs; and fourth, the availability of improved bioinformatics analysis, including methods for comparative protein structure modeling, binding site identification, virtual ligand screening and drug design. Therefore, we are now in a position to increase the odds of identifying high-quality drug targets and drug leads for neglected tropical diseases. Here we encourage a collaboration among scientists to engage in drug discovery for tropical diseases by providing a 'kernel' for the Tropical Disease Initiative (TDI, http://www.tropicaldisease.org/)[2]. As the Linux kernel did for open source code development, we suggest that the TDI kernel may help overcome a major stumbling block, in this case, for open source drug discovery: the absence of a critical mass of preexisting work that volunteers can build on incrementally. This kernel complements several other initiatives on neglected tropical diseases[3–5], including collaborative web portals (e.g., http://www.thesynapticleap.org/), public-private partnerships (e.g., http://www.mmv.org/) and private foundations (e.g., http://www.gatesfoundation.org/); for an updated list of initiatives, see the TDI website above.

The TDI kernel was derived with our software pipeline[6,7] for predicting structures of protein sequences by comparative modeling, localizing small-molecule binding sites on the surfaces of the models and predicting ligands that bind to them. Specifically, the pipeline linked 297 proteins from ten pathogen genomes with already approved drugs that were developed for treating other diseases (**Table 1**). Such links, if proven experimentally, may significantly increase the efficiency of target identification, target validation, lead discovery, lead optimization and clinical trials. Two of the kernel targets were tested for their binding to a known drug by NMR spectroscopy, validating one of our predictions (**Fig. 1** and **Supplementary Data** online). It is difficult to assess the accuracy of our computational predictions based on this limited experimental testing. Thus, we encourage other investigators to donate their expertise and facilities to test additional predictions. We hope the testing will occur within the open source context, where results are made available with limited or no restrictions.

A freely downloadable version of the TDI kernel is available in accordance with the Science Commons protocol for implementing open access data (http://sciencecommons.org/projects/publishing/open-access-data-protocol/), which prescribes standard academic attribution and facilitates tracking of work but imposes no other restrictions. We do not seek intellectual property rights in the actual discoveries based on the TDI kernel, in the hope of reinvigorating drug discovery for neglected tropical diseases[8]. By minimizing restrictions on the data, including viral terms that would be inherited by all derivative works, we hope to attract as many eyeballs as we possibly can to use and improve the kernel. Although many of the drugs in the kernel are proprietary under diverse types of rights, we believe that the existence of public domain pairs of targets and compounds will reduce the royalties that patent owners can charge and sponsors must pay. This should decrease the large sums of money governments and

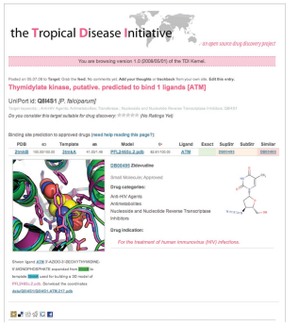


Figure 1 TDI kernel snapshot of the web page for the *Plasmodium falciparum* thymidylate kinase target (http://tropicaldisease.org/kernel/q8i4s1/). Our computational pipeline predicted that thymidylate kinase from *P. falciparum* binds ATM (3′-azido-3′-deoxythymidine-5′-monophosphate), a suprastructure of the zidovudine drug approved for the treatment of HIV infection. The binding of this ligand to a site on the kinase was experimentally validated by one-dimensional Water-LOGSY[9] and saturation transfer difference[10] NMR experiments.

Table 1 TDI kernel genomes

Organism[a]	Transcripts[b]	Modeled targets[c]	Similar[d]	Exact[e]
Cryptosporidium hominis	3,886	666	20	13
Cryptosporidium parvum	3,806	742	24	13
Leishmania major	8,274	1,409	43	20
Mycobacterium leprae	1,605	893	25	6
Mycobacterium tuberculosis	3,991	1,608	30	10
Plasmodium falciparum	5,363	818	28	13
Plasmodium vivax	5,342	822	24	13
Toxoplasma gondii	7,793	300	13	6
Trypanosoma cruzi	19,607	3,070	51	28
Trypanosoma brucei	9,210	1,386	39	21
Total	68,877	11,714	297	143

[a]Organisms in bold are included in the World Health Organization (Geneva) Tropical Disease portfolio. [b]Number of transcripts in each genome. [c]Number of targets with at least one domain accurately modeled (that is, MODPIPE quality score of at least 1.0). [d]Number of modeled targets with at least one predicted binding site for a molecule with a Tanimoto score[11] of at least 0.9 to a drug in DrugBank[12]. [e]Number of modeled targets with at least one predicted binding site for a molecule in DrugBank.

L. Orti *et al.*, *PLoS Negl Trop Dis* **3**, e418 (2009).

OPEN ACCESS Freely available online

PLoS NEGLECTED TROPICAL DISEASES

A Kernel for Open Source Drug Discovery in Tropical Diseases

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Abstract

Background: Conventional patent-based drug development incentives work badly for the developing world, where commercial markets are usually small to non-existent. For this reason, the past decade has seen extensive experimentation with alternative R&D institutions ranging from private–public partnerships to development prizes. Despite extensive discussion, however, one of the most promising avenues—open source drug discovery—has remained elusive. We argue that the stumbling block has been the absence of a critical mass of preexisting work that volunteers can improve through a series of granular contributions. Historically, open source software collaborations have almost never succeeded without such "kernels".

Methodology/Principal Findings: Here, we use a computational pipeline for: (i) comparative structure modeling of target proteins, (ii) predicting the localization of ligand binding sites on their surfaces, and (iii) assessing the similarity of the predicted ligands to known drugs. Our kernel currently contains 143 and 297 protein targets from ten pathogen genomes that are predicted to bind a known drug or a molecule similar to a known drug, respectively. The kernel provides a source of potential drug targets and drug candidates around which an online open source community can nucleate. Using NMR spectroscopy, we have experimentally tested our predictions for two of these targets, confirming one and invalidating the other.

Conclusions/Significance: The TDI kernel, which is being offered under the Creative Commons attribution share-alike license for free and unrestricted use, can be accessed on the World Wide Web at http://www.tropicaldisease.org. We hope that the kernel will facilitate collaborative efforts towards the discovery of new drugs against parasites that cause tropical diseases.

Citation: Ortí L, Carbajo RJ, Pieper U, Eswar N, Maurer SM, et al. (2009) A Kernel for Open Source Drug Discovery in Tropical Diseases. PLoS Negl Trop Dis 3(4): e418. doi:10.1371/journal.pntd.0000418

Editor: Timothy G. Geary, McGill University, Canada

Received December 29, 2008; **Accepted** March 23, 2009; **Published** April 21, 2009

Funding: MAM-R acknowledges the support from a Spanish Ministerio de Educación y Ciencia grant (BIO2007/66670). AS acknowledges the support from the Sandler Family Supporting Foundation and the National Institutes of Health (R01 GM54762, U54 GM074945, P01 AI035707, and P01 GM71790). AP-L acknowledges the support from a Spanish Ministerio de Ciencia e Innovación grant (SAF2008-01845). RJC acknowledges the support from the Ramon y Cajal Program of the Spanish Ministerio de Educación y Ciencia. We are also grateful for computer hardware gifts to AS from Ron Conway, Mike Homer, Intel, IBM, Hewlett-Packard, and NetApp. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

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Introduction

There is a lack of high-quality protein drug targets and drug leads for neglected diseases [1,2]. Fortunately, many genomes of organisms that cause tropical diseases have already been sequenced and published. Therefore, we are now in a position to leverage this information by identifying potential protein targets for drug discovery. Atomic-resolution structures can facilitate this task. In the absence of an experimentally determined structure, comparative modeling can provide useful models for sequences that are detectably related to known protein structures [3,4]. Approximately half of known protein sequences contain domains that can be currently predicted by comparative modeling [5,6]. This coverage will increase as the number of experimentally determined structures grows and modeling software improves. A protein model can facilitate at least four important tasks in the early stages of drug discovery [7]: prioritizing protein targets for drug discovery [8], identifying binding sites for small molecules [9,10], suggesting drug leads [11,12], and optimizing these leads [13–15].

Here, we address the first three tasks by assembling our computer programs into a software pipeline that automatically and on large-scale predicts protein structures, their ligand binding sites, and known drugs that interact with them. As a proof of principle, we applied the pipeline to the genomes of ten organisms that cause tropical diseases ("target genomes"). We also experimentally tested two predicted drug-target interactions using Nuclear Magnetic

Acknowledgments

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COMPARATIVE MODELING
Andrej Sali
M. S. Madhusudhan
Narayanan Eswar
Min-Yi Shen
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MODEL ASSESSMENT
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Min-Yi Shen
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FUNCTIONAL ANNOTATION
Andrea Rossi (Rinat-Pfizer)
Fred Davis (Janelia Fram)

FUNDING
CNAG
MINECO
Era-Net Pathogenomics
HFSP

MODEL ASSESSMENT
Francisco Melo (CU)
Alejandro Panjkovich (CU)

NMR
Antonio Pineda-Lucena
Leticia Ortí
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3D Genomes
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Tropical Disease Initiative
Stephen Maurer (UC Berkeley)
Arti Rai (Duke U)
Andrej Sali (UCSF)
Ginger Taylor (TSL)
Matthew Todd (U Sydney)

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