BMI-206

Structure-Structure comparisons
Sequence-Structure comparisons

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February 3rd, 2005

How to use this lectures

- Ask!
- Outline
  - Basic introduction
  - Theory (representation-scoring-optimization)
  - Available programs
  - Application
- Assignment
  - *The POM152 sequence. Modeling exercise.*

Structure-Structure comparison

- Outline
  - Before we start…
    - Some theory
    - Coverage .vs. Accuracy
  - How can we compare structures…
    - SALIGN (properties comparison)
    - VAST (vector alignment)
    - CE (local heuristic comparison)
    - MAMMOTH (vector alignment)
  - How we classify the structural space…
    - SCOP (manual)
    - CATH (semi-automatic)
    - DBAli (fully automatic and comprehensive)

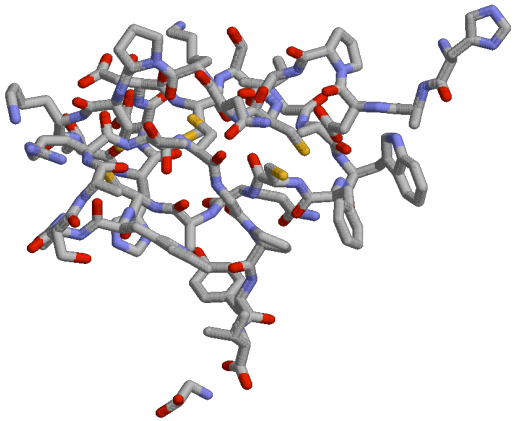
Structure-Structure alignments

As any other bioinformatics problem…

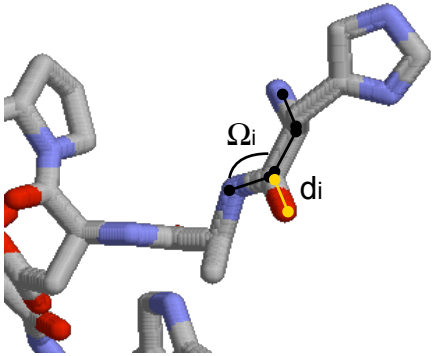
- **Representation**
- **Scoring**
- **Optimizer**

Representation

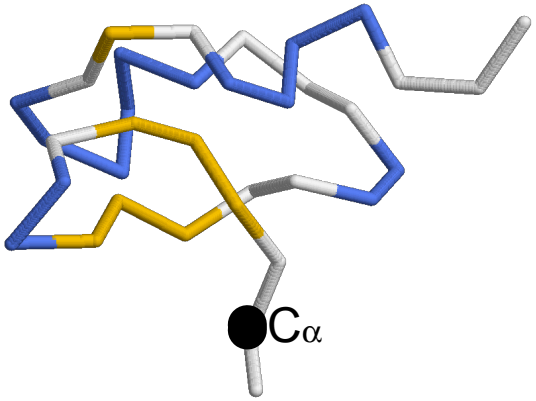
Structures



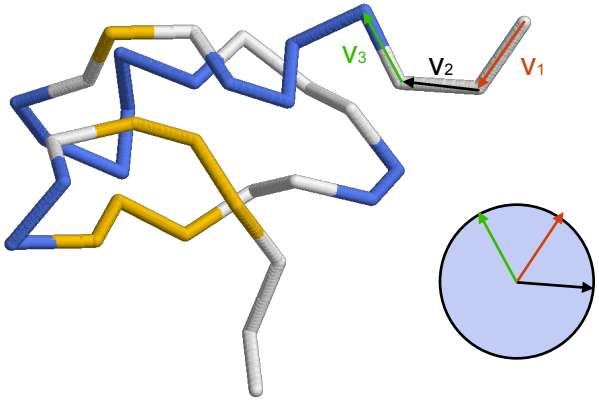
All atoms and coordinates



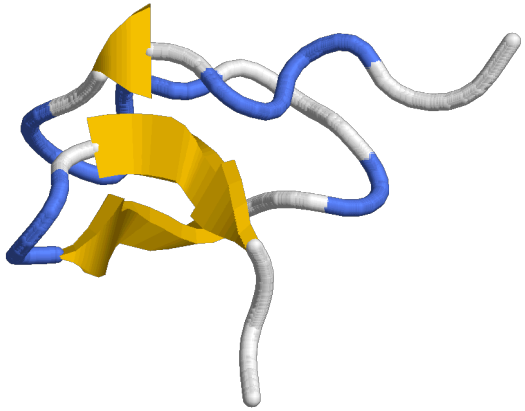
Dihedral space or distance space



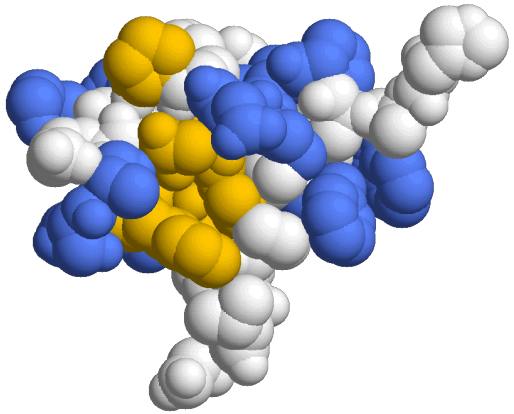
Reduced atom representation



Vector representation



Secondary Structure



Accessible surface (and others)

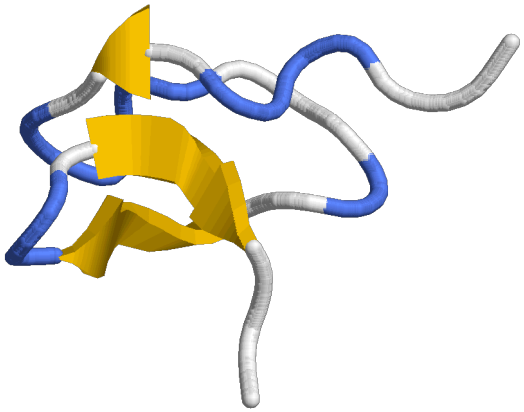
Scoring
Raw scores

	C	S	T	P	A	G	N	D	E	Q	H	R	K	M	I	L	V	F	Y	W
C	**9**	-1	-1	-3	0	-3	-3	-3	-4	-3	-3	-3	-3	-1	-1	-1	-1	-2	-2	-2
S	-1	**4**	1	-1	1	0	1	0	0	0	-1	-1	0	-1	-2	-2	-2	-2	-2	-3
T	-1	1	**4**	1	-1	1	0	1	0	0	0	-1	0	-1	-2	-2	-2	-2	-2	-3
P	-3	-1	1	**7**	-1	-2	-1	-1	-1	-1	-2	-2	-1	-2	-3	-3	-2	-4	-3	-4
A	0	1	-1	-1	**4**	0	-1	-2	-1	-1	-2	-1	-1	-1	-1	-1	-2	-2	-2	-3
G	-3	0	1	-2	0	**6**	-2	-1	-2	-2	-2	-2	-2	-3	-4	-4	0	-3	-3	-2
N	-3	1	0	-2	-2	0	**6**	1	0	0	-1	0	0	-2	-3	-3	-3	-3	-2	-4
D	-3	0	1	-1	-2	-1	1	**6**	2	0	-1	-2	-1	-3	-3	-4	-3	-3	-3	-4
E	-4	0	0	-1	-1	-2	0	2	**5**	2	0	0	1	-2	-3	-3	-3	-3	-2	-3
Q	-3	0	0	-1	-1	-2	0	0	2	**5**	0	1	1	0	-3	-2	-2	-3	-1	-2
H	-3	-1	0	-2	-2	-2	1	1	0	0	**8**	0	-1	-2	-3	-3	-2	-1	2	-2
R	-3	-1	-1	-2	-1	-2	0	-2	0	1	0	**5**	2	-1	-3	-2	-3	-3	-2	-3
K	-3	0	0	-1	-1	-2	0	-1	1	1	-1	2	**5**	-1	-3	-2	-3	-3	-2	-3
M	-1	-1	-1	-2	-1	-3	-2	-3	-2	0	-2	-1	-1	**5**	1	2	-2	0	-1	-1
I	-1	-2	-2	-3	-1	-4	-3	-3	-3	-3	-3	-3	-3	1	**4**	2	1	0	-1	-3
L	-1	-2	-2	-3	-1	-4	-3	-4	-3	-2	-3	-2	-2	2	2	**4**	3	0	-1	-2
V	-1	-2	-2	-2	0	-3	-3	-3	-2	-2	-3	-3	-2	1	3	1	**4**	-1	-1	-3
F	-2	-2	-2	-4	-2	-3	-3	-3	-3	-3	-1	-3	-3	0	0	0	-1	**6**	3	1
Y	-2	-2	-2	-3	-2	-3	-2	-3	-2	-1	2	-2	-2	-1	-1	-1	-1	3	**7**	2
W	-2	-3	-3	-4	-3	-2	-4	-4	-3	-2	-2	-3	-3	-1	-3	-2	-3	1	2	**11**

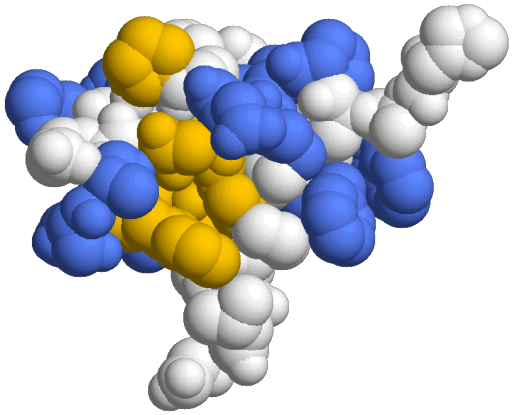
Aminoacid substitutions

$$RMSD = \sqrt{\sum \left(x_i - \overline{x} \right)^2}$$

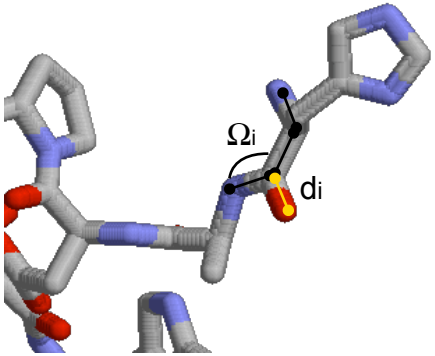
Root Mean Square Deviation



Secondary Structure (H,B,C)



Accessible surface (B,A [%])



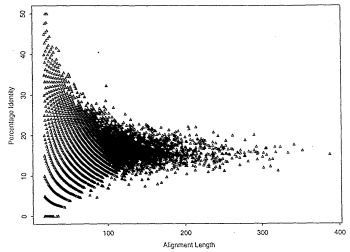
Angles or distances

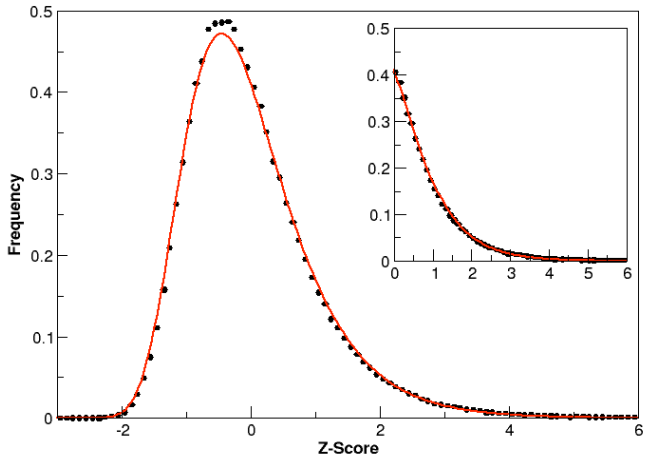
Significance of an alignment (score)

remember Patsy's class

Probability that the optimal alignment of two random sequences/structures of the same length and composition as the aligned sequences/structures have at least as good a score as the evaluated alignment.

Empirical





Sometimes approximated by Z-score (normal distribution).

Analytic

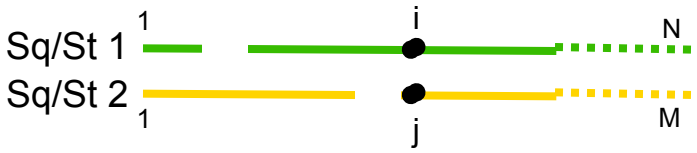
$$P(s) = e^{-\lambda (s-\mu)}$$

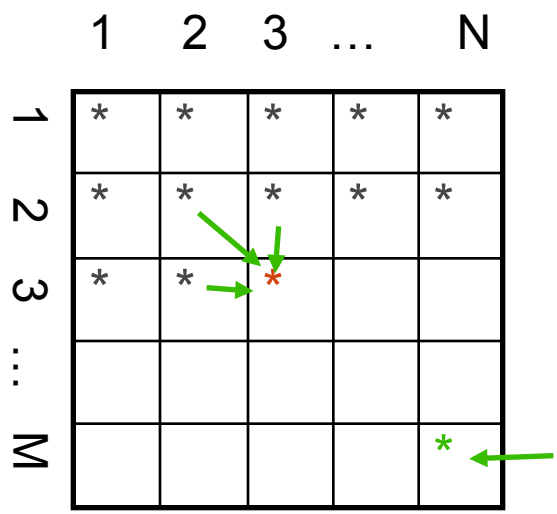
$$P(s \geq x) = 1 - \exp\left(e^{-\lambda (s-\mu)}\right)$$

Karlin and Altschul, 1990 *PNAS* **87**, pp2264

Global dynamic programming alignment

remember Patsy's class





Best alignment score

$$D_{ij} = \min \begin{cases} D_{i,j-1} + \text{Score}_{(\text{Ä},rj)} \\ D_{i-1,j-1} + \text{Score}_{(ri,rj)} \\ D_{i-1,j} + \text{Score}_{(ri,\text{Ä})} \\ 0 \end{cases}$$

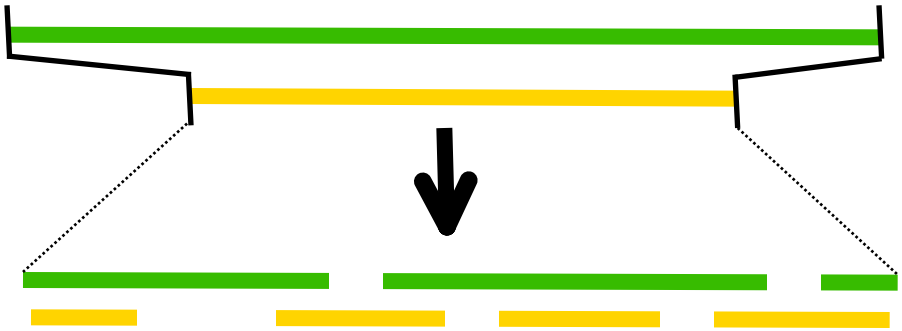
Backtracking to get the best alignment

Needleman and Wunsch (1970) *J. Mol Biol*, **3** pp443

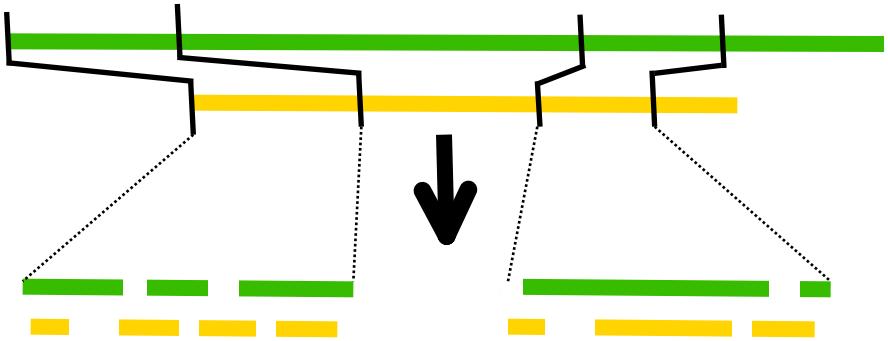
Optimizer

Global *.vs.* local alignment

remember Patsy's class



Global alignment



Local alignment

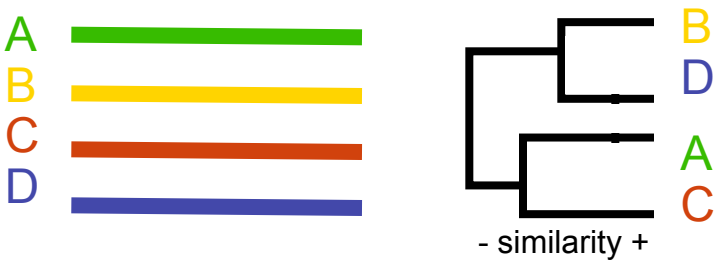
Optimizer

Multiple alignment

remember Patsy's class

Pairwise alignments

Example – 4 sequences A, B, C, D.



6 pairwise comparisons
then cluster analysis

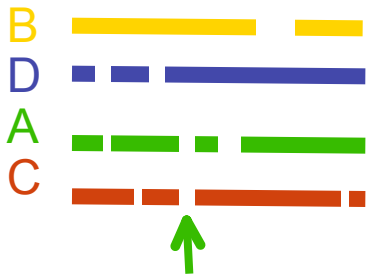
Multiple alignments

Following the tree from step 1

Align the most similar pair

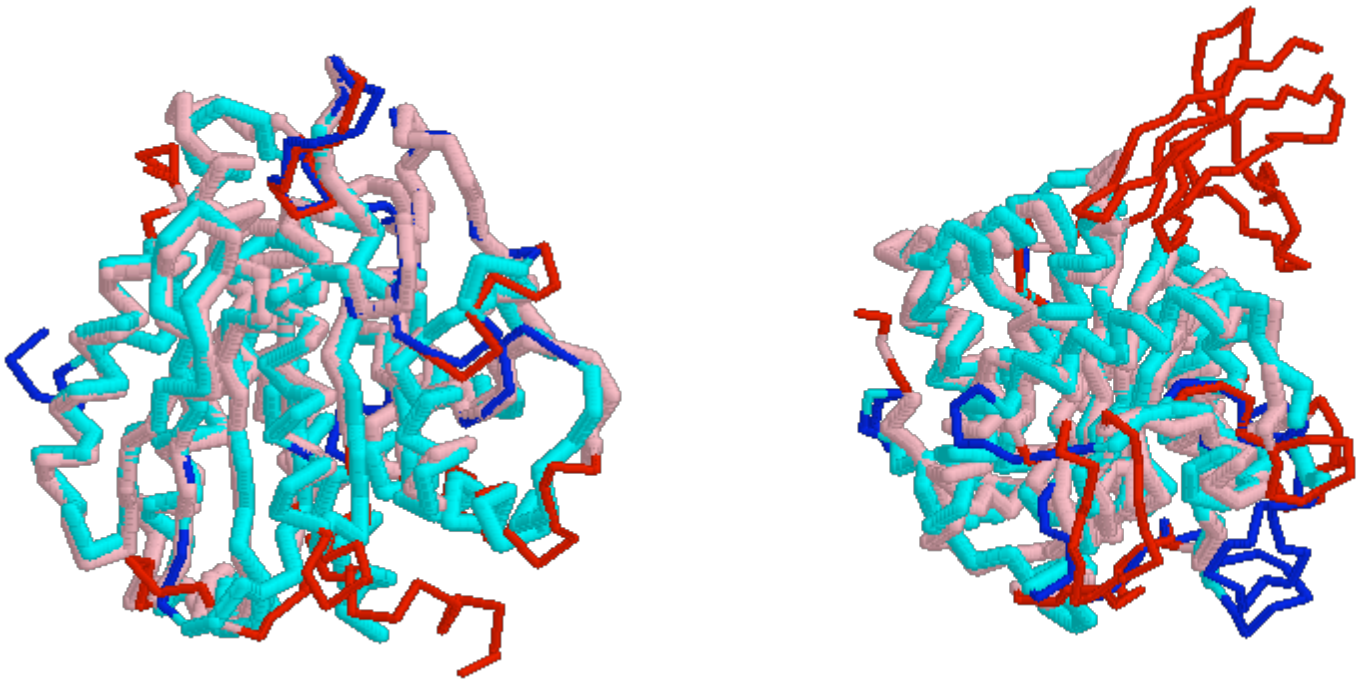
Align next most similar pair

Align B-D with A-C



New gap in A-C to optimize
its alignment with B-D

Coverage .vs. Accuracy

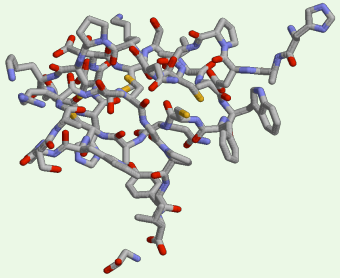


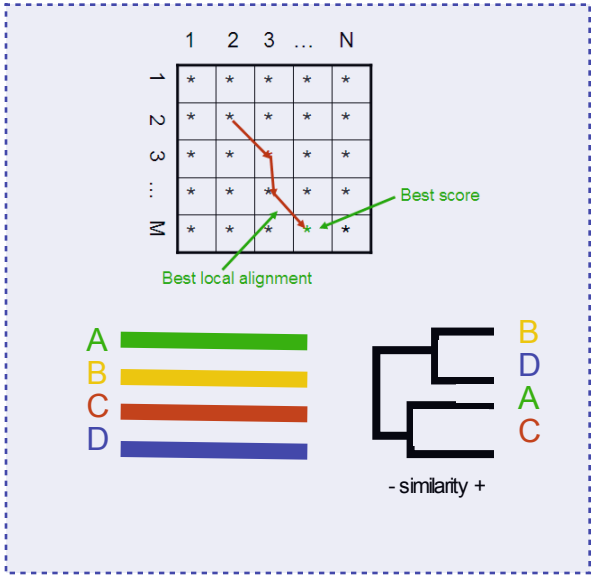
Same RMSD ~ 2.5Å

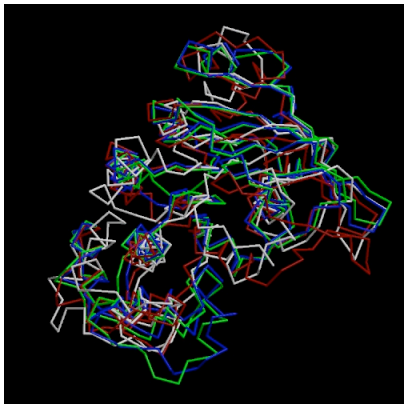
Coverage ~90% Cα

Coverage ~75% Cα

Sequence-Structure alignment by properties conservation (SALIGN-MODELLER)

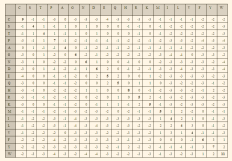


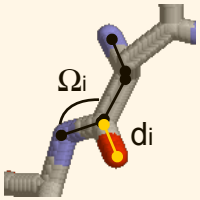


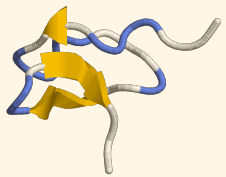


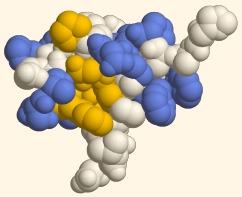
✓ Uses all available structural information
✓ Provides the optimal alignment

Computationally expensive









$$\text{RMSD} = \sqrt{\sum \left(x_i - \bar{x} \right)^2}$$

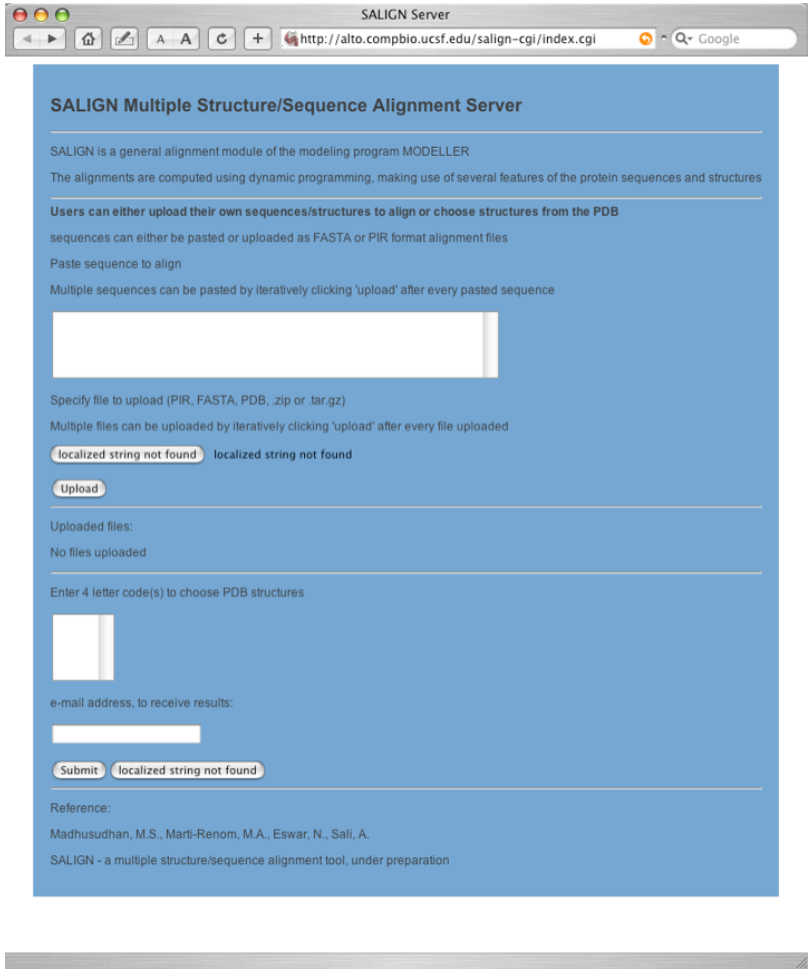
$R_{i,j}$ $D_{i(3),j(3)}$ $S_{i,j}$ $B_{i,j}$ $I_{i,j}$

$$Score_{i,j} = w_1 * R_{i,j} + w_2 * D_{i(a),j(a)} + w_3 * S_{i,j} + w_4 * B_{i,j} + w_5 * I_{i,j} + w_6 * X_{i,j}$$

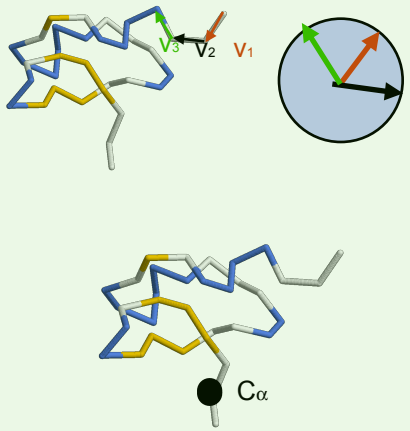
Madhusudhan et al. *in preparation*

Structural alignment by properties conservation (SALIGN-MODELLER)

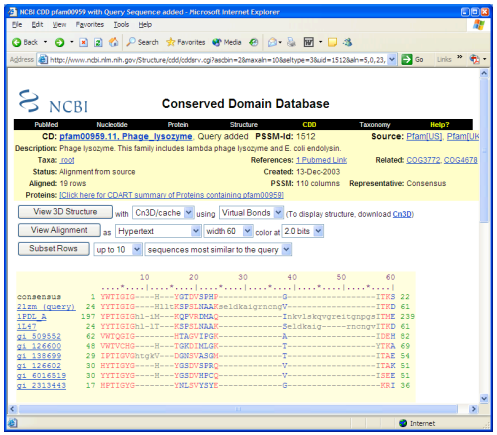
http://alto.compbio.ucsf.edu/salign-cgi/index.cgi



Vector Alignment Search Tool (VAST)

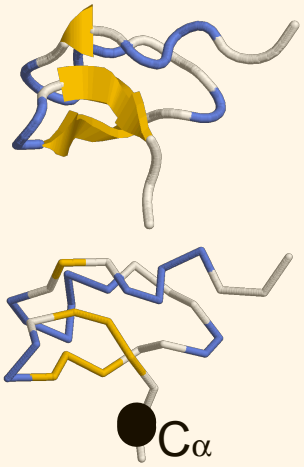


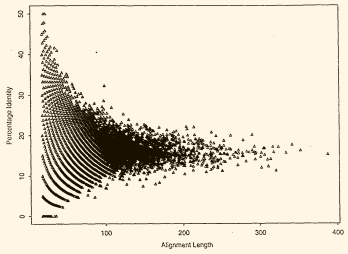
- Graph theory search of similar SSE
- Refining by Monte Carlo at all atom resolution



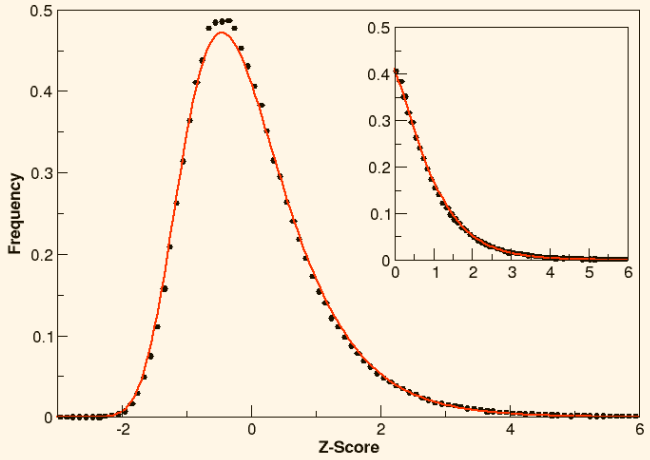
✓ **Good scoring system with significance**

Reduces the protein representation





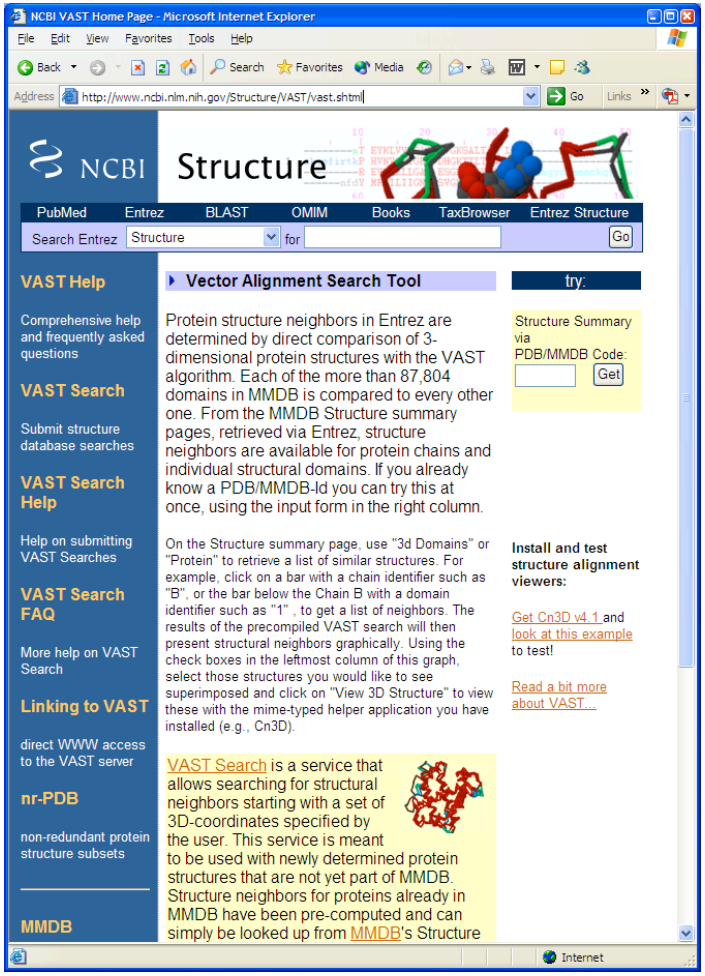
$$RMSD = \sqrt{\sum \left(x_i - \bar{x} \right)^2}$$



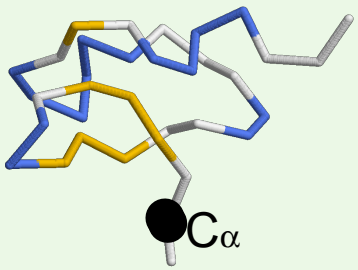
Gibrat JF et al. (1996) *Curr Opin Struct Biol* **3** pp377

Vector Alignment Search Tool (VAST)

http://www.ncbi.nlm.nih.gov/Structure/VAST/vast.shtml



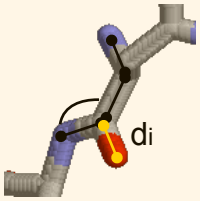
Incremental combinatorial extension (CE)



- Exhaustive combination of fragments
- Longest combination of AFPs
- Heuristic similar to PSI-BLAST

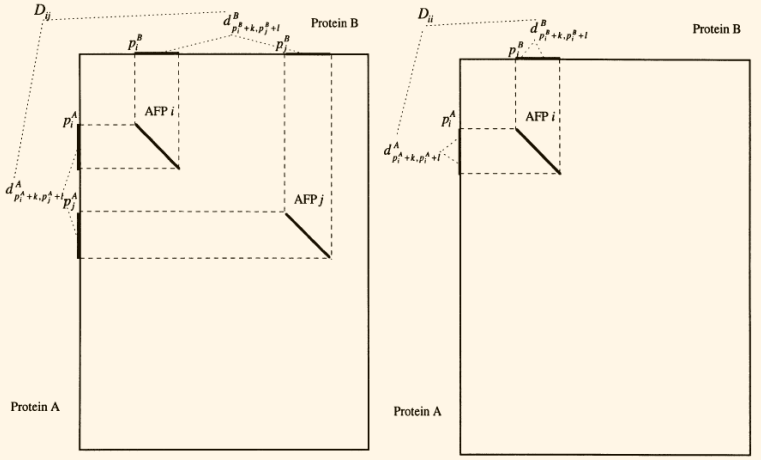
✓ **FAST!**
✓ **Good quality of local alignments**

Complicated scoring and heuristics



8 residues peptides

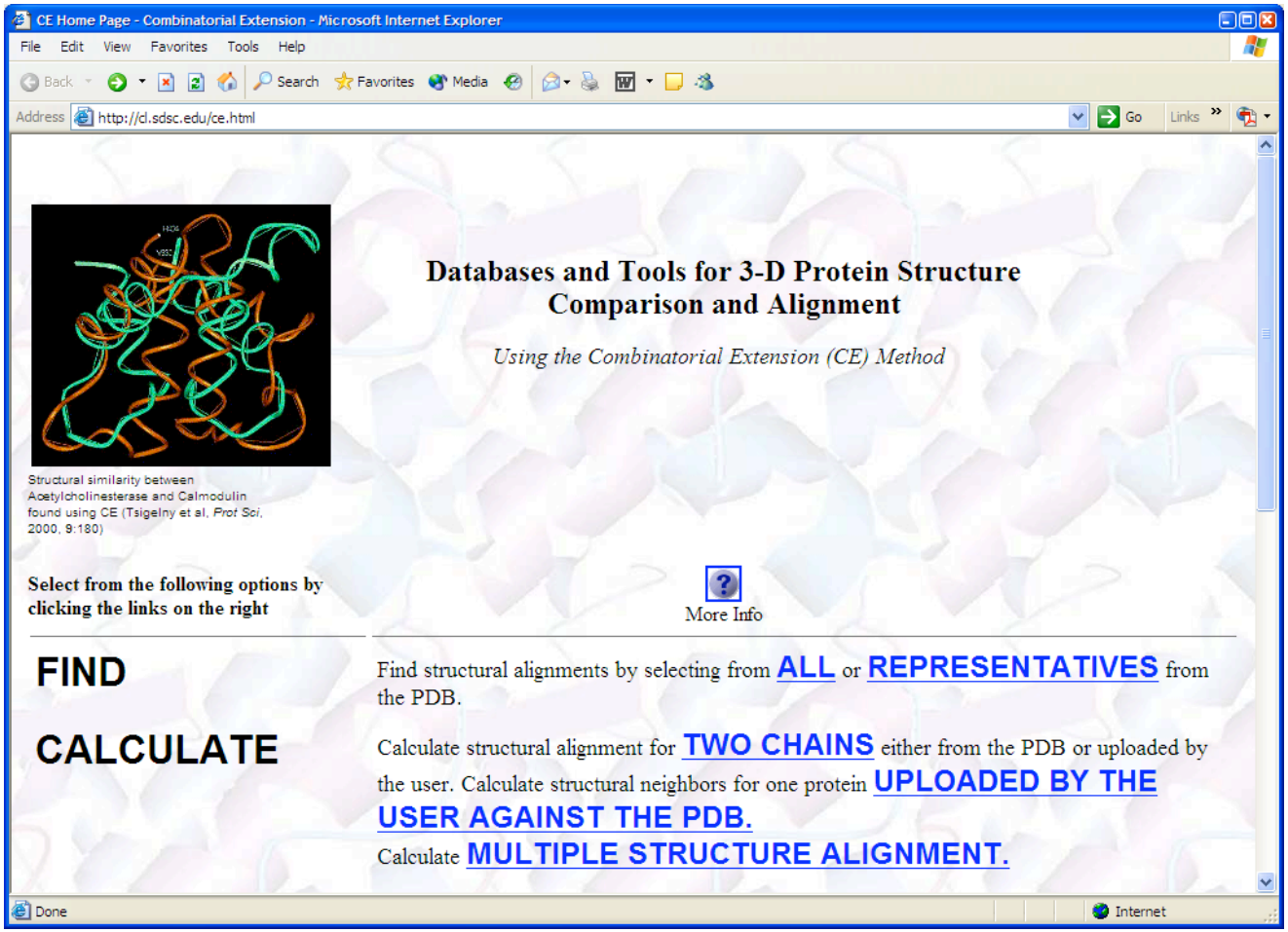
$$RMSD = \sqrt{\sum \left(x_i - \bar{x} \right)^2}$$



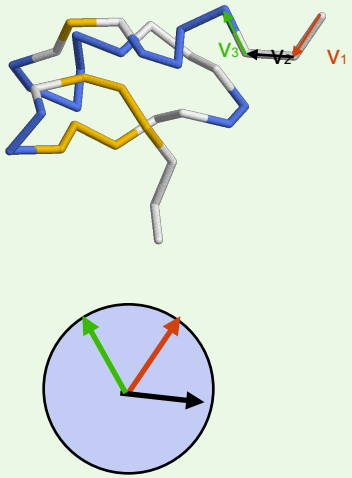
Shindyalov IN, amd Bourne PE. (1998) *Protein Eng.* **9** pp739

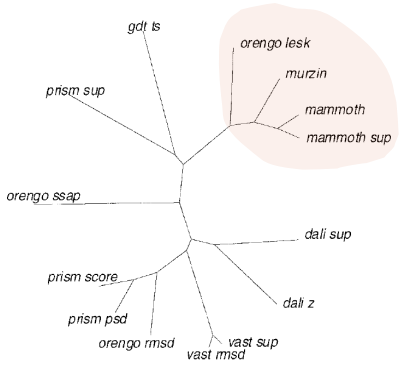
Incremental combinatorial extension (CE)

http://cl.sdsc.edu/ce.html



Matching molecular models obtained from theory (MAMMOTH)



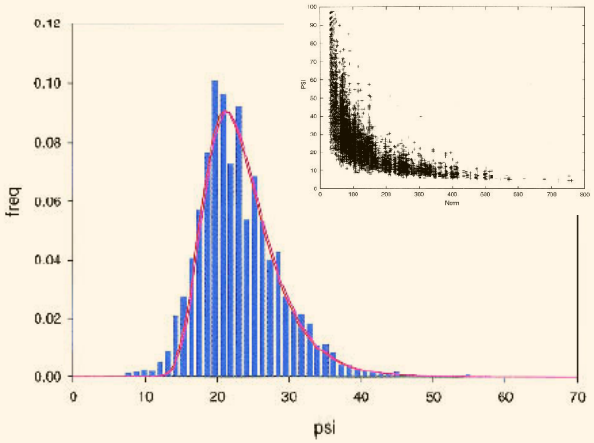


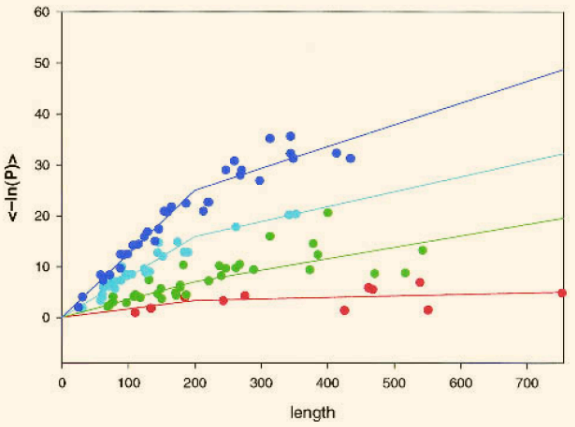
- ✓ **VERY FAST!**
- ✓ **Good scoring system with significance**

Reduces the protein representation

$$URMS^{R} = \sqrt{2.0 - \frac{2.84}{\sqrt{n}}}$$

$$S_{AB} = \frac{(URMS^{R} - URMS^{AB})D}{URMS^{R}}$$

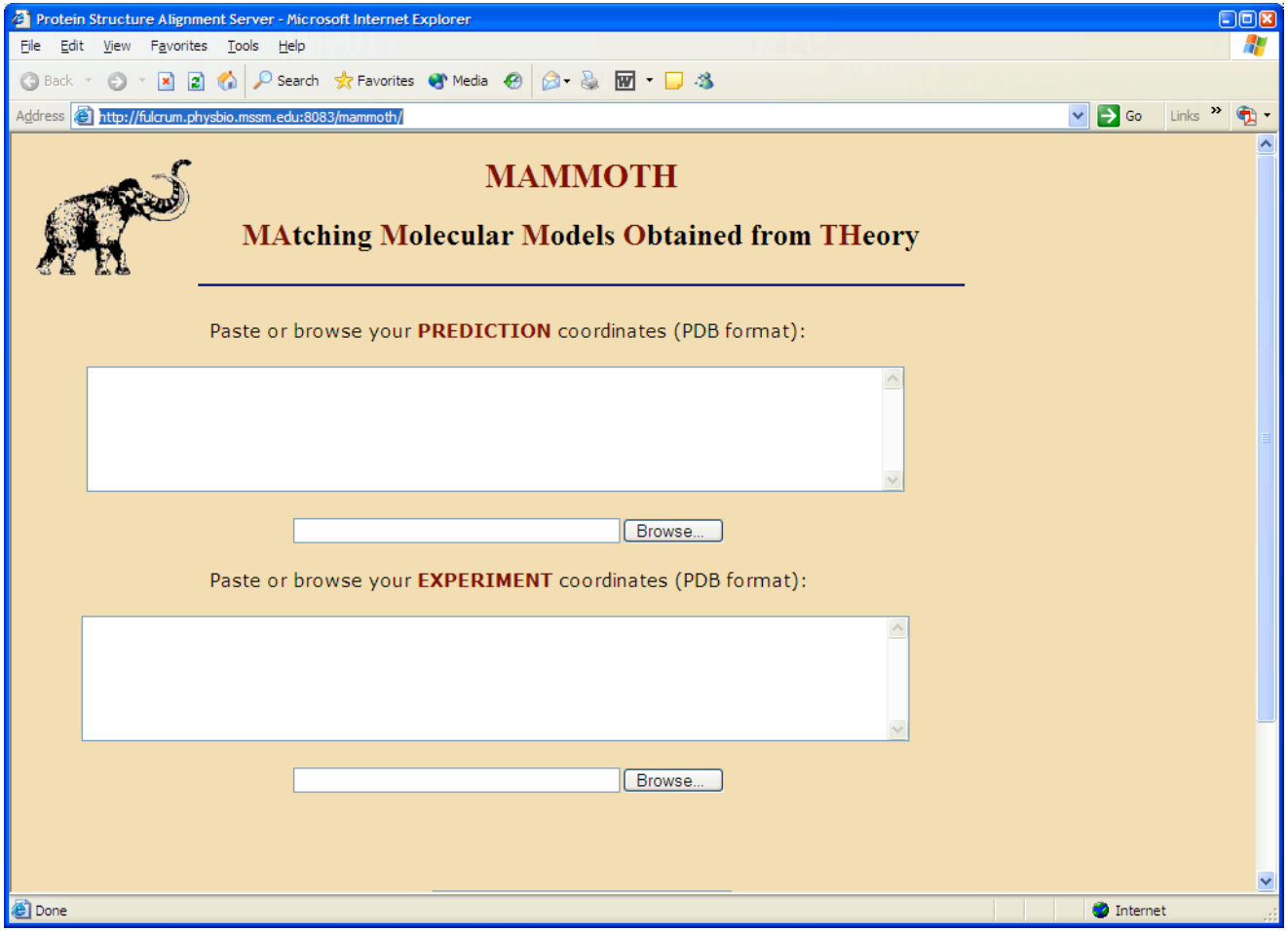




Ortiz AR, (2002) *Protein Sci.* **11** pp2606

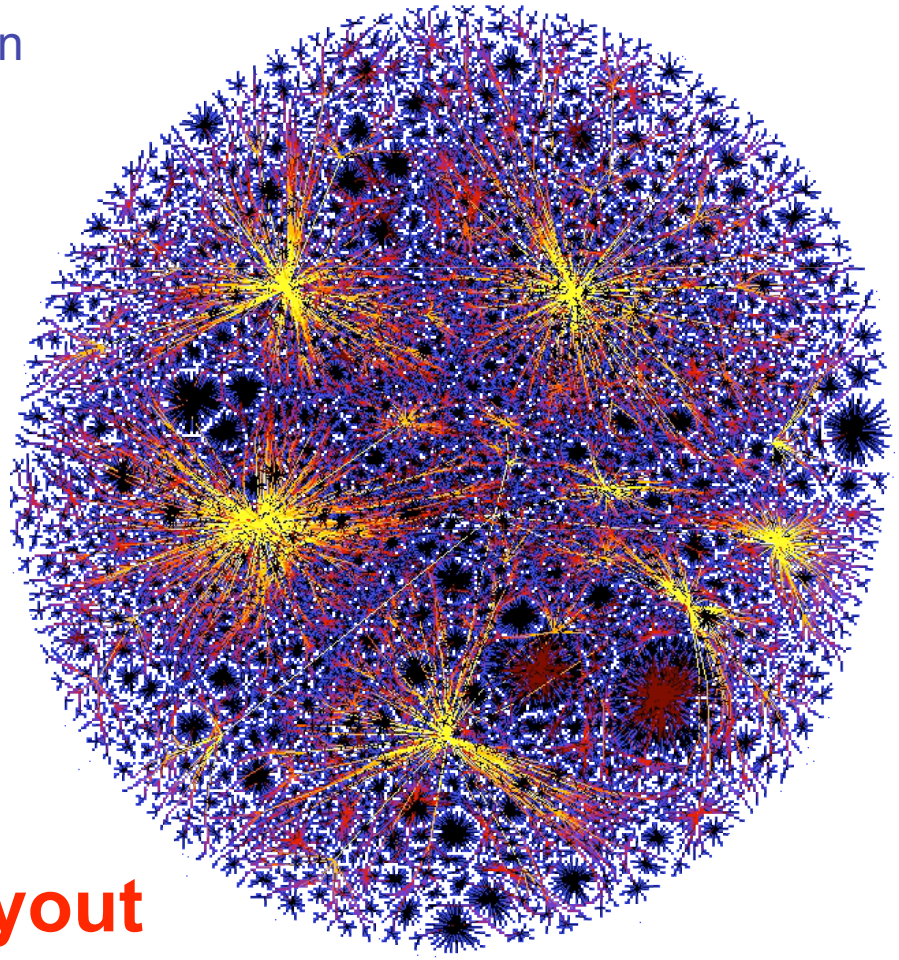
Matching molecular models obtained from theory (MAMMOTH)

http://fulcrum.physbio.mssm.edu:8083/



Classification of the structural space

SCOP classification



Large Graph Layout

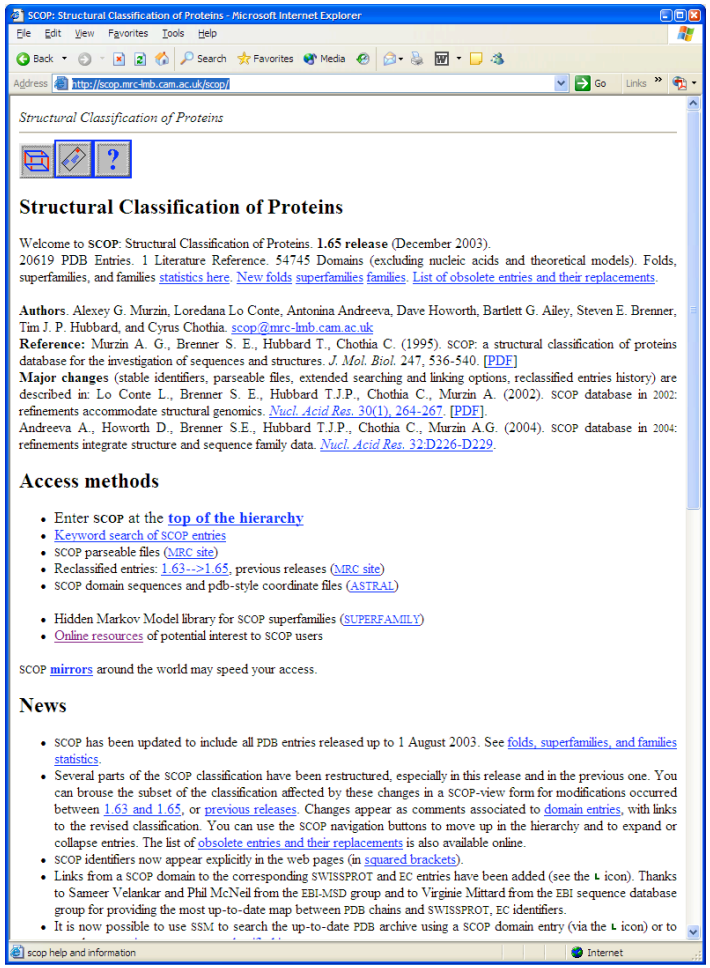
Alex Adai

Adai AT, Date SV, Wieland S, Marcotte EM. J Mol Biol. 2004 Jun 25;340(1):179-90

http://bioinformatics.icmb.utexas.edu/lgl/

SCOP$_{1.65}$ database

http://scop.mrc-lmb.cam.ac.uk/scop/



- ✓ Largely recognized as “standard of gold”
- ✓ Manually classification
- ✓ Clear classification of structures in:
  - CLASS
  - FOLD
  - SUPER-FAMILY
  - FAMILY
- ✓ Some large number of tools already available

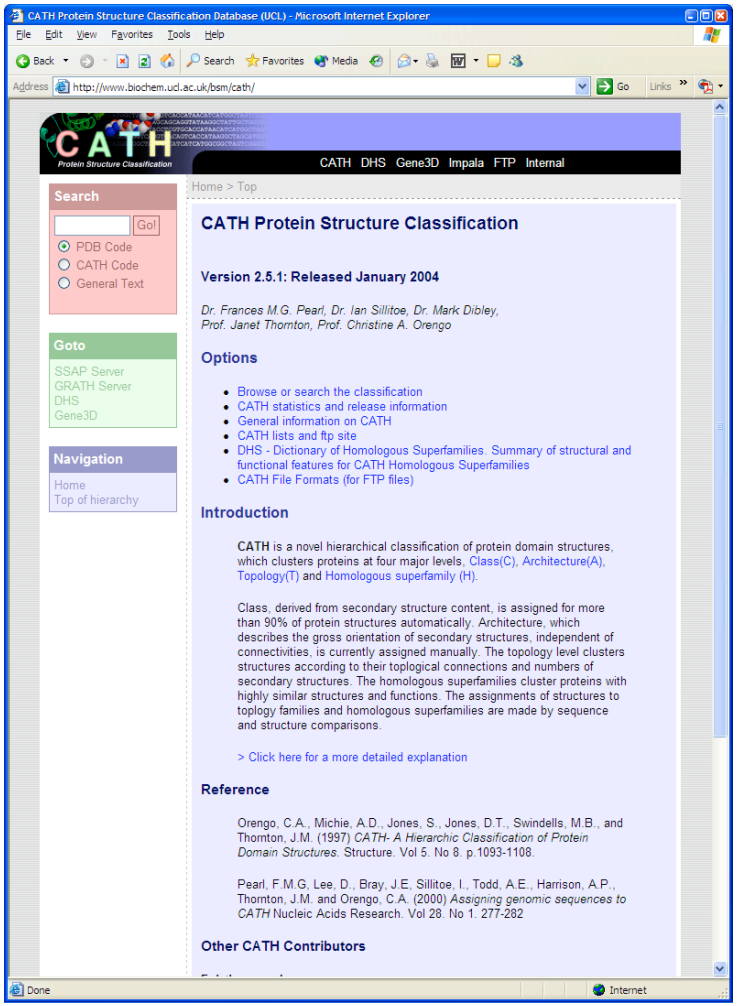
Manually classification
Not 100% up-to-date
Domain boundaries definition

Class	Number of folds	Number of superfamilies	Number of families
All alpha proteins	179	299	480
All beta proteins	126	248	462
Alpha and beta proteins (a/b)	121	199	542
Alpha and beta proteins (a+b)	234	349	567
Multi-domain proteins	38	38	53
Membrane and cell surface proteins	36	66	73
Small proteins	66	95	150
Total	800	1294	2327

Murzin A. G.,el at. (1995). *J. Mol. Biol.* **247**, 536-540.

CATH$_{2.5.1}$ database

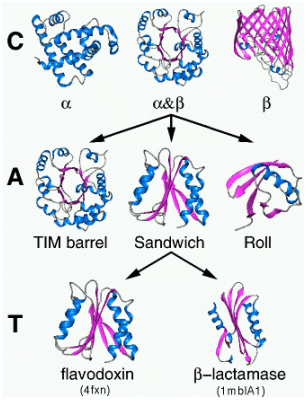
http://www.biochem.ucl.ac.uk/bsm/cath/



Uses FSSP for superimposition

- ✓ **Recognized as "standard of gold"**
- ✓ **Semi-automatic classification**
- ✓ **Clear classification of structures in:**
  - **CLASS**
  - **ARCHITECTURE**
  - **TOPOLOGY**
  - **HOMOLOGOUS SUPERFAMILIES**
- ✓ **Some large number of tools already available**
- ✓ **Easy to navigate**

Semi-automatic classification
Domain boundaries definition



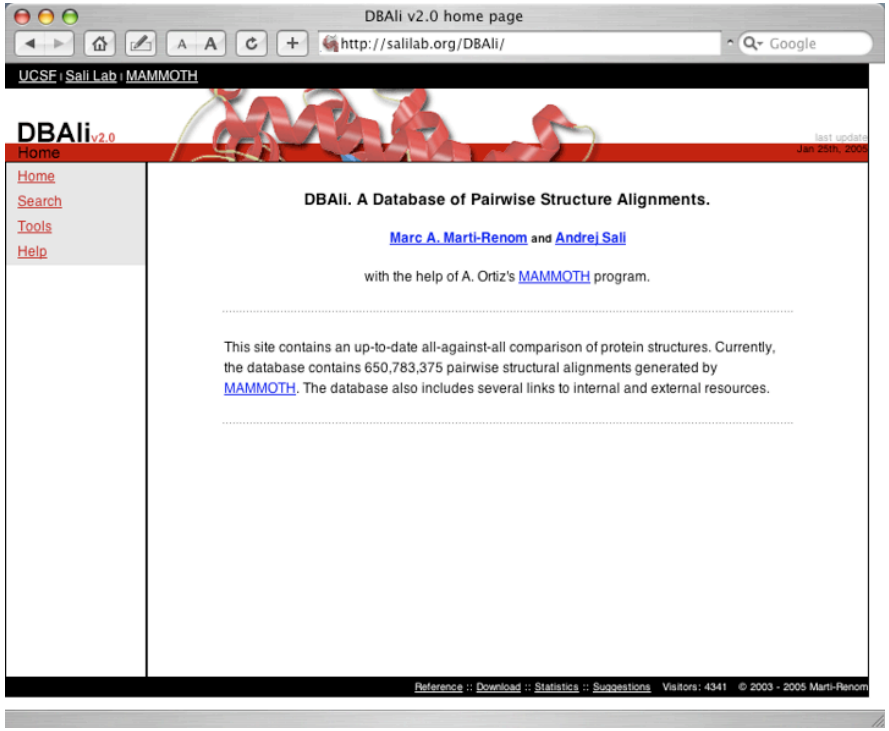
Version	2.5.1
Date	28-01-2004

	A	T	H	S	N	I	D
Mainly Alpha	5	227	428	948	1713	3946	10155
Mainly Beta	19	139	292	951	2344	5011	14259
Alpha Beta	12	368	648	2010	3631	8639	23025
Few Secondary Structures	1	86	91	114	225	378	952
Multi-domain chains	1	1053	1057	1071	2186	5801	12471
Preliminary single domain assigments	1	371	374	422	479	789	1663
Multi-domain domains	2	31	31	49	67	139	287
CATH-35 Sequence families	1	997	997	997	1108	2154	3431
Fragments from multi-chain domains	1	28	28	30	33	56	106

Orengo, C.A., et al. (1997) *Structure*. **5**. 1093-1108.

DBAli$_{v2.0}$ database

http://salilab.org/DBAli/



Uses MAMMOTH for superimposition

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

Does not provide (yet) a stable classification

Last updated:
January 25th, 2005

Number of chains in database:
60,656

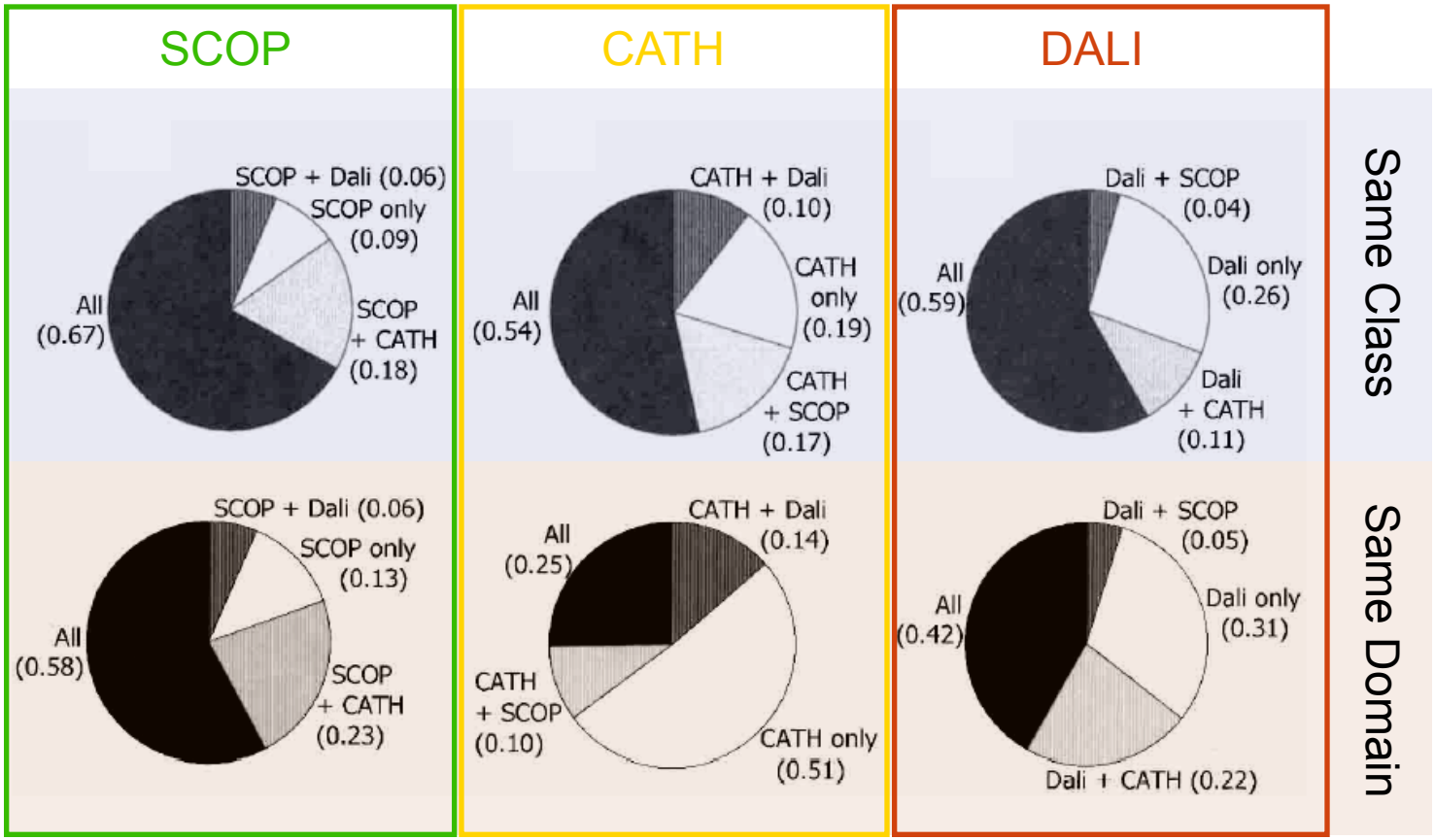
Number of structure-structure comparisons:
650,783,375

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

Classification of the structural space

Not an easy task!

Domain definition AND domain classification



Day, et al. (2003) *Protein Sciences*, **12** pp2150

Sequence-Structure comparison

- Outline
  - Before we start…
    - Some theory…
    - Domain boundaries
  - Structural predictions from sequence…
    - SALIGN (gap penalties and substitution matrices)
    - mGenThreader (SSE prediction and alignment/potential scores)
    - Fugue (gap penalties and substitution matrices)
    - 3D-Jury (as a meta server example)

General overview (Threading)

- Matches sequences to 3D structures
  - Requires a scoring function to asses the fit of a sequence to a given fold
  - Scoring functions deried from known structures and include atom contact and solvation terms evaluated in a pairwise fashion
  - May include secondary structure terms, multiple alignments…

- Threading servers available using several different approaches
  - Fold recognition server at Imperial College, UK
    http://www.sbg.bio.ic.ac.uk/~3dpssm/
  - PredictProtein server at EMBL
    http://www.embl-heidelberg.de/predictprotein/predictprotein.html
  - Protein sequence-structure threading at NCBI
    http://www.ncbi.nlm.nih.gov/Structure/RESEARCH/threading.shtml

Template comparison methods

- Uses 3D “templates” for searching structural databases
  - active site or binding site templates generated to reflect functionally important structural signatures
- Available software/servers
  - Template Search and Superposition (TESS), Thornton Group
    http://www.biochem.ucl.ac.uk/bsm/PROCAT/PROCAT.html
    Wallace AC; Borkakoti N; Thornton JM. (1997) *Protein Science* **6** pp2308
  - “Fuzzy Functional Forms” , Skolnick - commercial availability
    Fetrow, Js and Skolnick, J (1998) *J. Mo. Biol* **281** pp949
  - Spatial Arrangements of Side-chain and Main-chain (SPASM), Kleywegt, Univ. of Uppsala
    http://portray.bmc.uu.se/cgi-bin/dennis/spasm.pl
    Kleywegt GJ (1999). *J. Mol. Biol.* **285** pp1887

Empirical energy functions (PMF)

Idea: **energy leads to structure, thus it should be possible to infer energy from many known structures**

To be used in: **model refinement and assessment**

Properties needed:

- Deep minimum at correct state (native)
- Smooth (energy landscape)
- Simple (CPU calculation)

Types:

- Contact potential
- Distance potentials
- Surface potentials

Approximations/Limitations in PMFs

Database size.

PMF versus Energy (additive/higher order terms).

Reference state.

Physical origin.

Finkelstein et al. (1995) *Proteins* **23**, pp142

Sequence-Structure alignments

As any other bioinformatics problem…

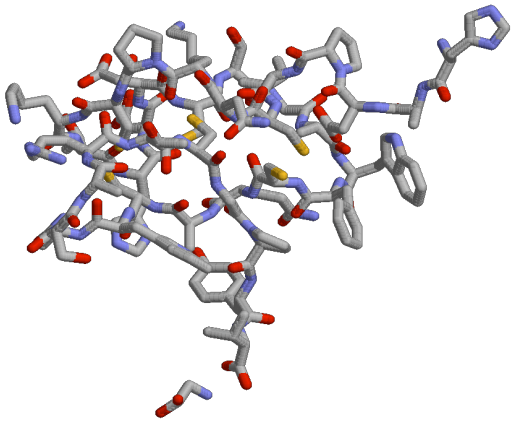
- **Representation**
- **Scoring**
- **Optimizer**

Representation

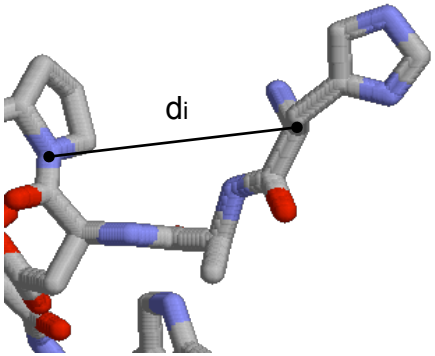
Sequence/Structures

```
>gi42541361
MDIRSVSSLRGLLCLPPSWPRR
```

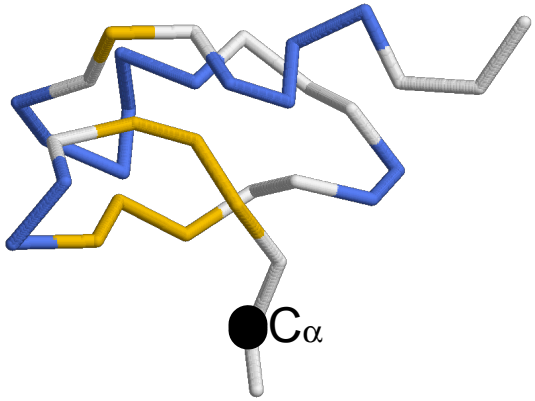
Primary sequence



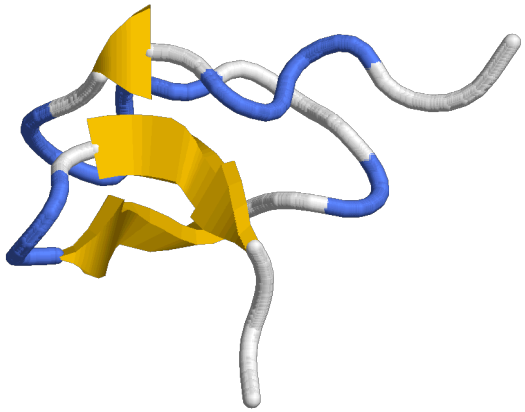
All atoms and coordinates



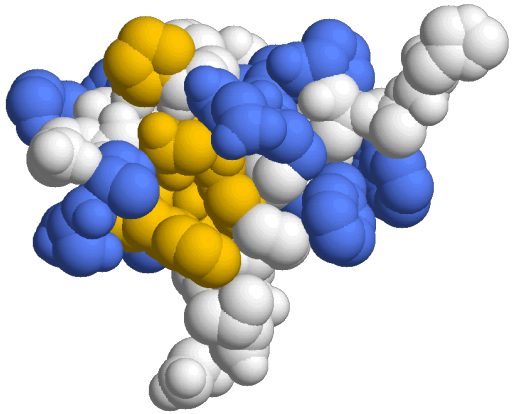
Distance space



Reduced atoms representation



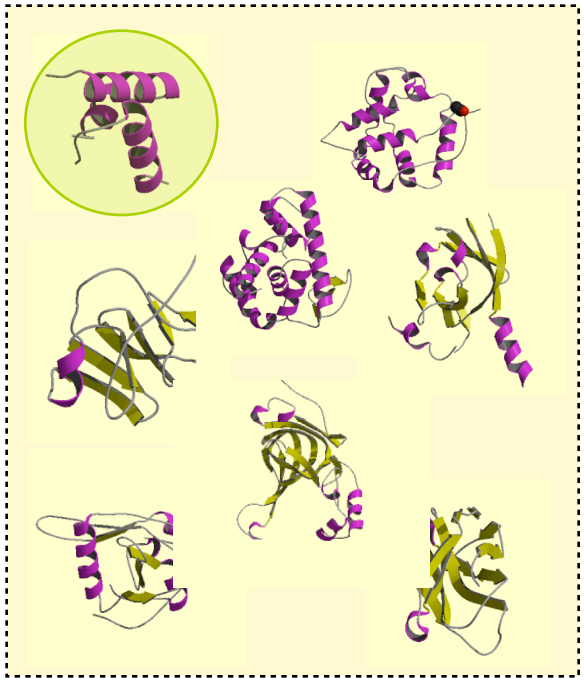
Secondary Structure



Accessible surface

Statistical Potentials (background)

Structural space



Sequence space

MKLLIVLTCISLCSCICTVVQRCASNKPHVLEDPCKVQH

HLSVNQCVLLPQCCPKSCKICTHLISIEVVLTCRAVDKM

MHVNCVEQCSLQDCIKIAPRVLKTCILCVLKPCLTSVSH

VHLVQPTSCCCKKNCICHVEIRSLDILTKSVQLACLVPM

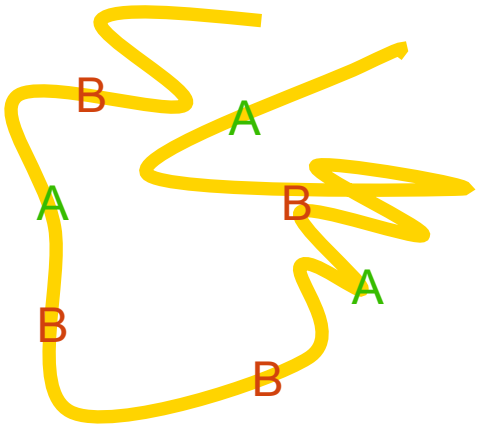
⋮

MQCCRVQKICDLLAVELCKLHISTPSCKILCVVTSVPHN

Statistical Potential (inspiration)

$$K = \frac{[AB]}{[A]\cdot[B]}$$

$$\Delta G = -RT\ln(K) = -RT\ln\frac{[AB]}{[A]\cdot[B]}$$



From statistical physics, we know that energy difference between two states (ΔE) and the ratio of their occupancies (N_1:N_2) are related [9]:

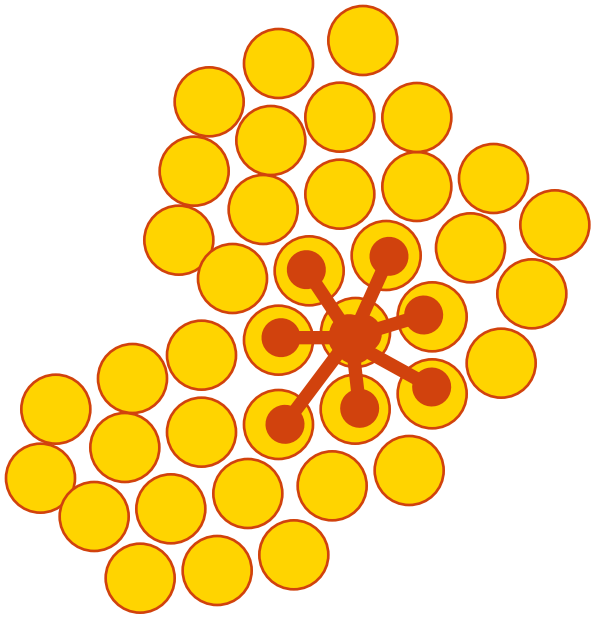
$$\Delta E = -kT \ln\left(\frac{N_1}{N_2}\right) \qquad (1)$$

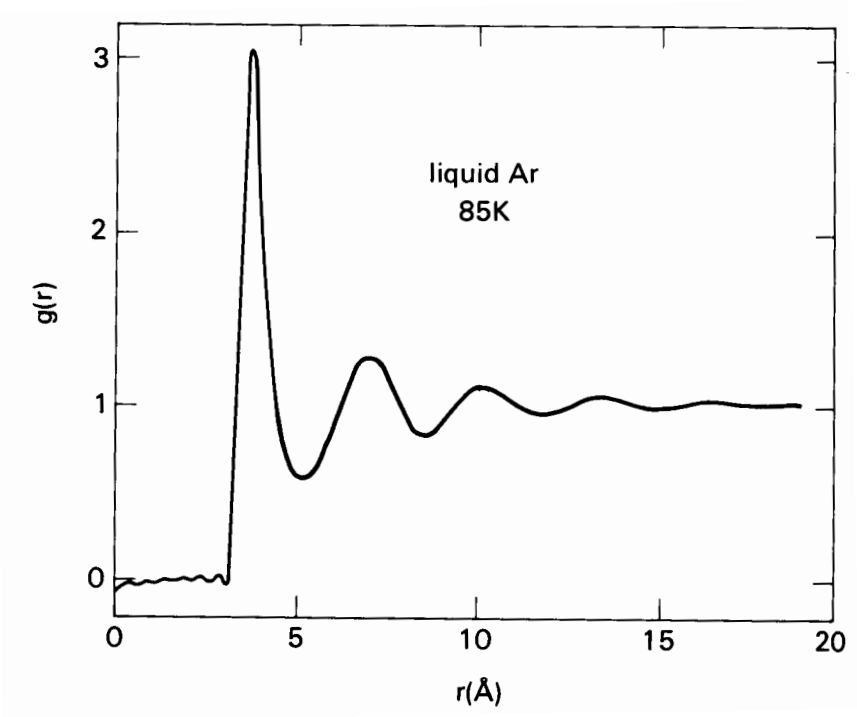
in which T is the absolute temperature and k is the Boltzmann's constant. As we are interested in an interaction energy between two amino acid side chains, it would seem natural to define N_1 as the number of interactions between these two residues types in a group of real protein structures, a number which is readily available from simple database analysis. But this number must be compared with the number of interactions in some other system, N_2, to obtain the energy difference between them.

Tanaka and Sheraga (1975) *PNAS*, **72** pp3802
Sippl, (1990) J.Mo.Biol. **213** pp859
Godzik, (1996) *Structure* **15** pp363

Scoring

Statistical Potential (reference state)

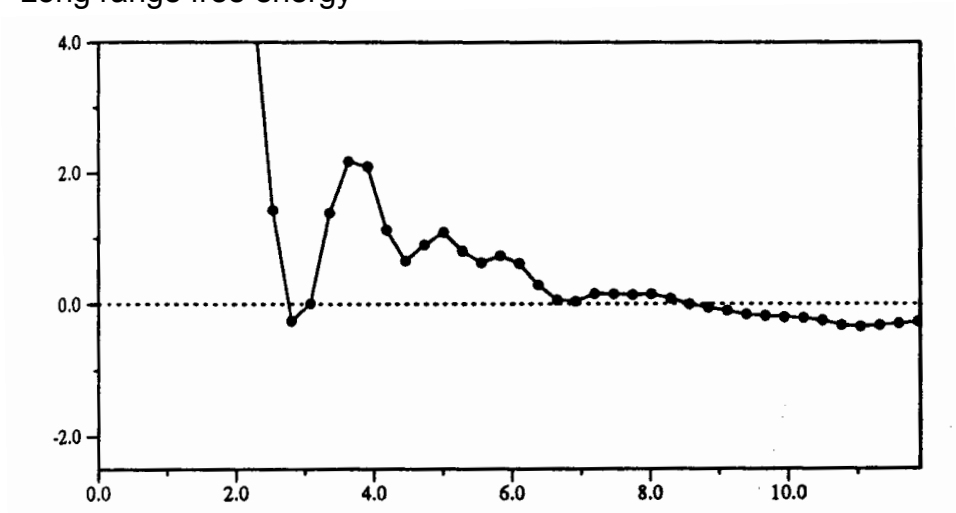




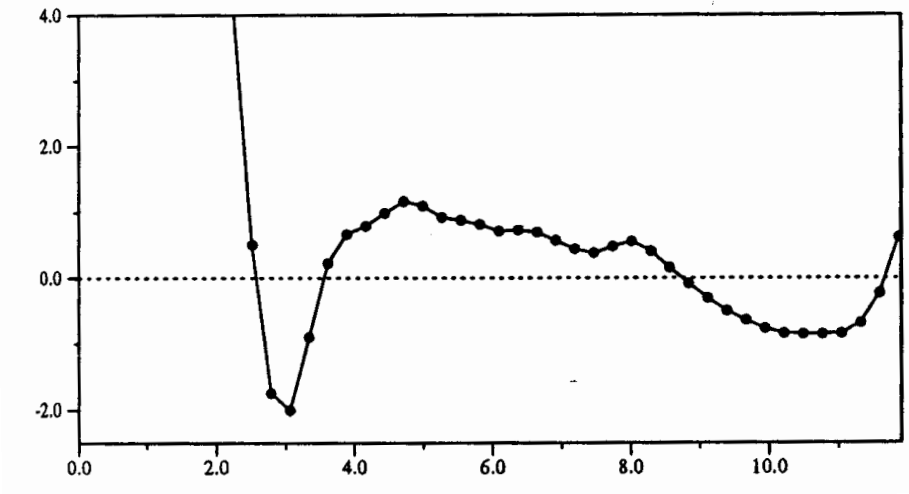
Theory of simple liquids 2nd edition JP Hansen and IR McDonald, Academic Press.

Statistical Potential… Hydrogen Bonds

Long range free energy



Short range free energy



Free energy of the protein backbone hydrogen bond N · · · O compiled from a database of 289 X-ray structures

$$\rho_{NO}(r) = \sum_{ij} \delta(r - r_{ij})$$

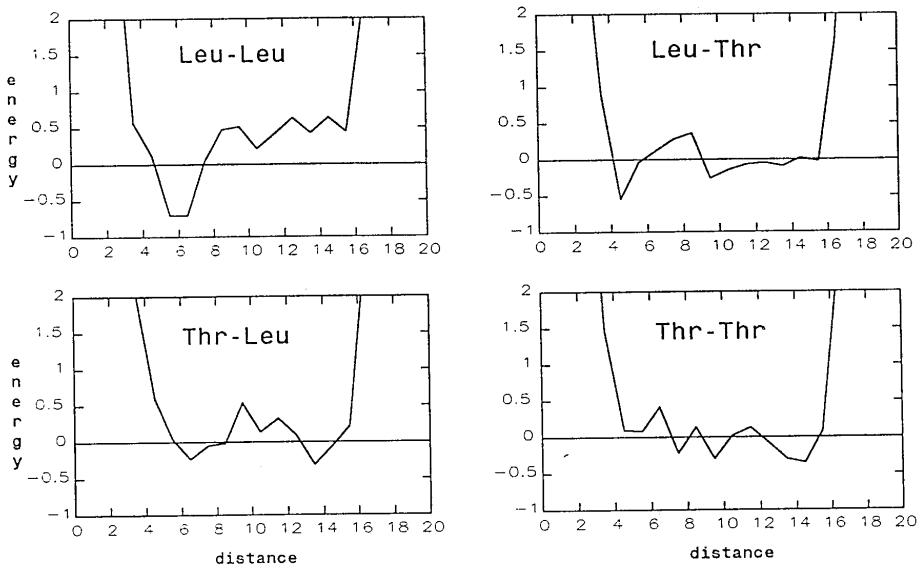
$$g_{NO}(r) = \frac{\rho_{NO}(r)}{\rho^2}$$

$$w_{NO}(r) = -kT \ln\left(g_{NO}(r)\right)$$

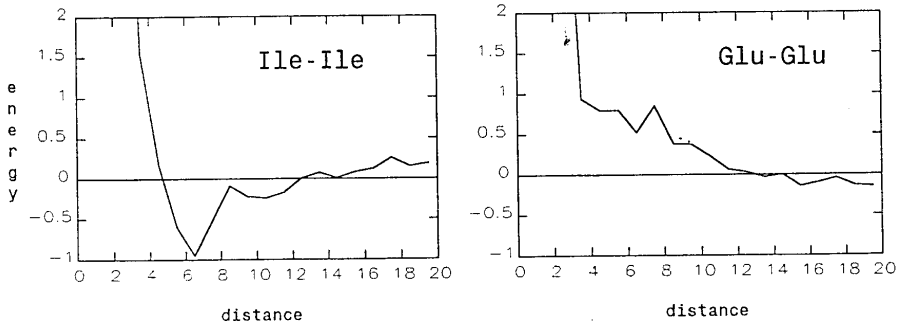
Sippl (1996). *JMB* **260** pp644

Statistical Potential… Distance Potentials

Long range free energy



Short range free energy



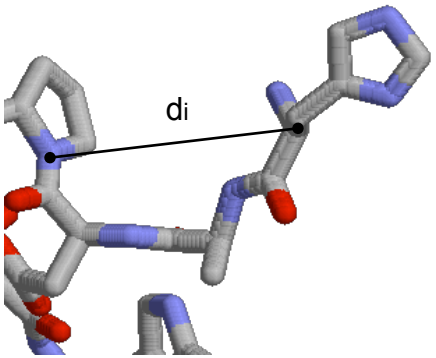
Sippl (1993). *JCAM* **7** pp473

Scoring

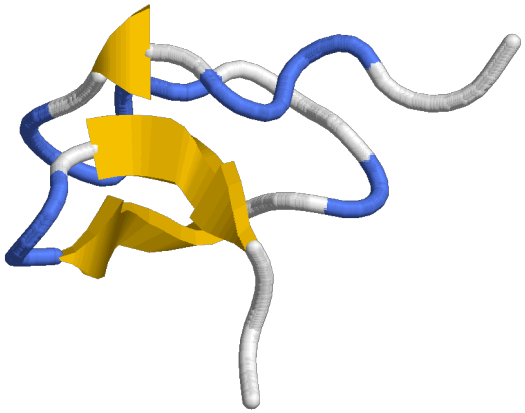
Raw scores of an alignment

	C	S	T	P	A	G	N	D	E	Q	H	R	K	M	I	L	V	F	Y	W
C	**9**	-1	-1	-3	0	-3	-3	-3	-4	-3	-3	-3	-3	-1	-1	-1	-1	-2	-2	-2
S	-1	**4**	1	-1	1	0	1	0	0	0	-1	-1	0	-1	-2	-2	-2	-2	-2	-3
T	-1	1	**4**	1	-1	1	0	1	0	0	0	-1	0	-1	-2	-2	-2	-2	-2	-3
P	-3	-1	1	**7**	-1	-2	-1	-1	-1	-1	-2	-2	-1	-2	-3	-3	-2	-4	-3	-4
A	0	1	-1	-1	**4**	0	-1	-2	-1	-1	-2	-1	-1	-1	-1	-1	-2	-2	-2	-3
G	-3	0	1	-2	0	**6**	-2	-1	-2	-2	-2	-2	-2	-3	-4	-4	0	-3	-3	-2
N	-3	1	0	-2	-2	0	**6**	1	0	0	-1	0	0	-2	-3	-3	-3	-3	-2	-4
D	-3	0	1	-1	-2	-1	1	**6**	2	0	-1	-2	-1	-3	-3	-4	-3	-3	-3	-4
E	-4	0	0	-1	-1	-2	0	2	**5**	2	0	0	1	-2	-3	-3	-3	-3	-2	-3
Q	-3	0	0	-1	-1	-2	0	0	2	**5**	0	1	1	0	-3	-2	-2	-3	-1	-2
H	-3	-1	0	-2	-2	-2	1	1	0	0	**8**	0	-1	-2	-3	-3	-2	-1	2	-2
R	-3	-1	-1	-2	-1	-2	0	-2	0	1	0	**5**	2	-1	-3	-2	-3	-3	-2	-3
K	-3	0	0	-1	-1	-2	0	-1	1	1	-1	2	**5**	-1	-3	-2	-3	-3	-2	-3
M	-1	-1	-1	-2	-1	-3	-2	-3	-2	0	-2	-1	-1	**5**	1	2	-2	0	-1	-1
I	-1	-2	-2	-3	-1	-4	-3	-3	-3	-3	-3	-3	-3	1	**4**	2	1	0	-1	-3
L	-1	-2	-2	-3	-1	-4	-3	-4	-3	-2	-3	-2	-2	2	2	**4**	3	0	-1	-2
V	-1	-2	-2	-2	0	-3	-3	-3	-2	-2	-3	-3	-2	1	3	1	**4**	-1	-1	-3
F	-2	-2	-2	-4	-2	-3	-3	-3	-3	-3	-1	-3	-3	0	0	0	-1	**6**	3	1
Y	-2	-2	-2	-3	-2	-3	-2	-3	-2	-1	2	-2	-2	-1	-1	-1	-1	3	**7**	2
W	-2	-3	-3	-4	-3	-2	-4	-4	-3	-2	-2	-3	-3	-1	-3	-2	-3	1	2	**11**

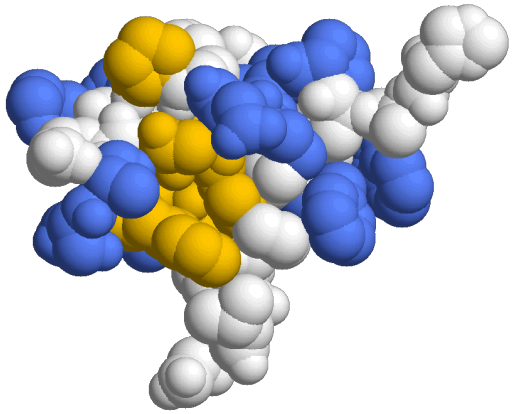
Aminoacid substitutions



Distance space



Secondary Structure (H,B,C)

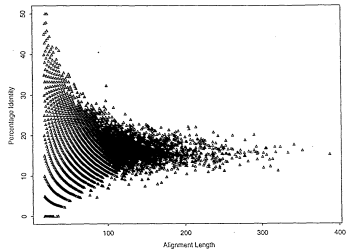


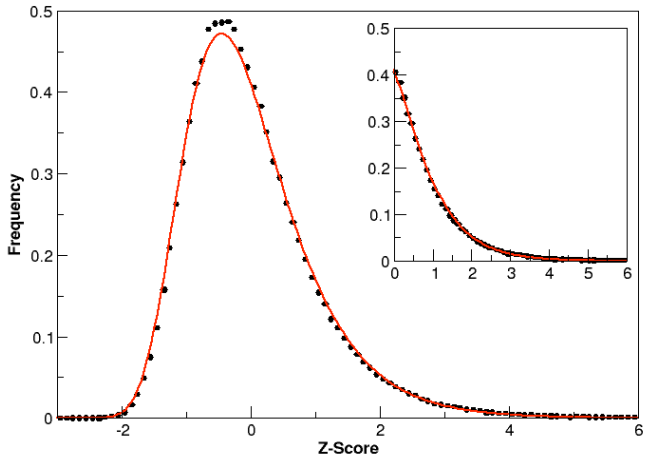
Accessible surface (B,A [%])

Significance of an alignment (score)

Probability that the optimal alignment of two random sequences/structures of the same length and composition as the aligned sequences/structures have at least as good a score as the evaluated alignment.

Empirical





Sometimes approximated by Z-score (normal distribution).

$$P(s) = e^{-\lambda (s-\mu)}$$

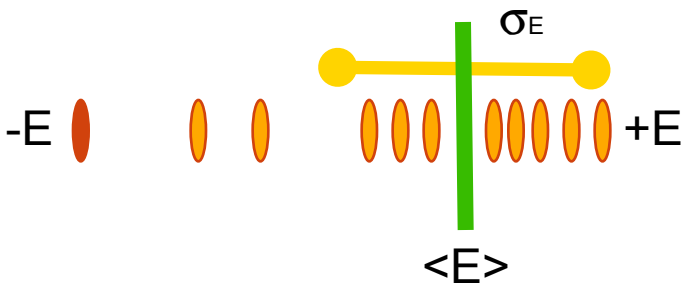
Analytic

$$P(s \geq x) = 1 - \exp\left(e^{-\lambda (s-\mu)}\right)$$

Karlin and Altschul, 1990 *PNAS* **87**, pp2264

Significance of an alignment (score)

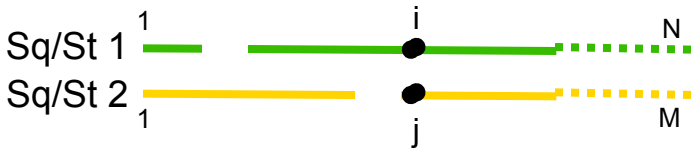
Energy Z-score the model with respect the energy of random models (or rest of decoys).

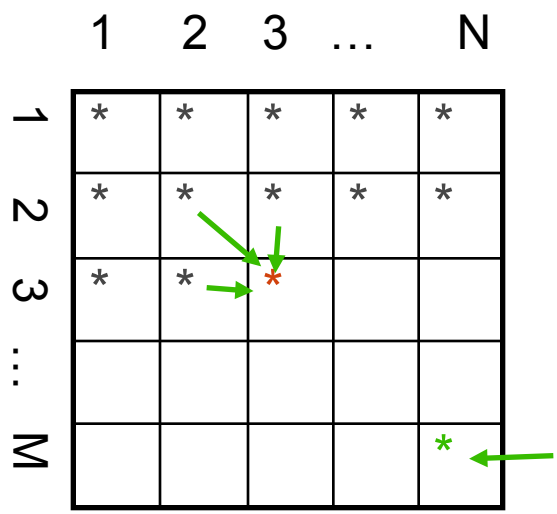


$$Zscore = \frac{(\langle E \rangle - E_m)}{\sigma_E}$$

Global dynamic programming alignment

remember Patsy's class





Best alignment score

$$D_{ij} = \min \begin{cases} D_{i,j-1} + \text{Score}_{(\text{Ä},rj)} \\ D_{i-1,j-1} + \text{Score}_{(ri,rj)} \\ D_{i-1,j} + \text{Score}_{(ri,\text{Ä})} \\ 0 \end{cases}$$

Backtracking to get the best alignment

Needleman and Wunsch (1970) *J. Mol Biol*, **3** pp443

Applications of PMFs

Model assessment.

Ab initio folding simulations.

Sequence-structure matching (threading).

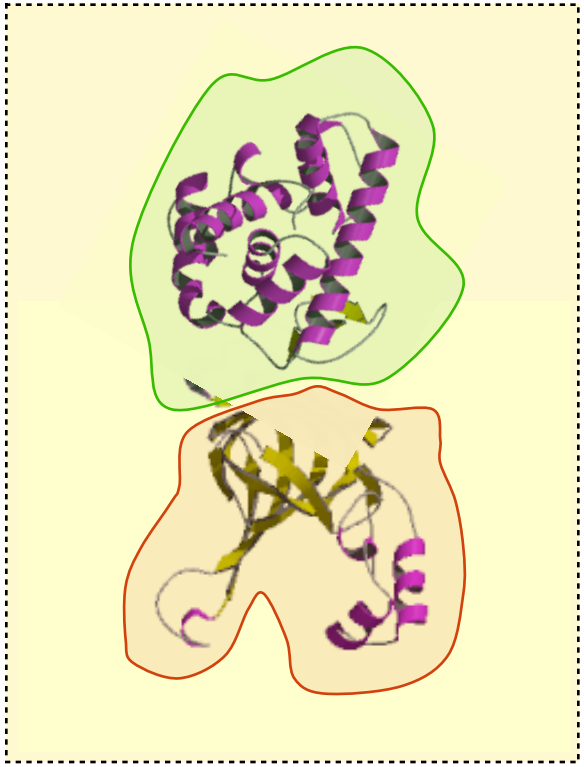
Comparative protein structure modeling (loops, sidechains, …).

Secondary structure prediction, *etc.*

Finkelstein et al. (1995) *Proteins* **23**, pp142

Domain boundaries from sequence

VERY DIFFICULT!!!!



MENFEIWVEKYRPRTLDEVVGQDEVIQRLKGYVERKNIPHLLFSGPPGTGKTATAIALARDLFGENWRDN
FIEMNASDERGIDVVRHKIKEFARTAPIGGAPFKIIFLDEADALTADAQAALRRTMEMYSKSCRFILSCN
YVSRIIEPIQSRCAVFRFKPVPKEAMKKRLLEICEKEGVKITEDGLEALIYISGGDFRKAINALQGAAAI
GEVVDADTIYQITATARPEEMTELIQTALKGNFMEARELLDRLMVEYGMSGEDIVAQLFREIISMPIKDS
LKVQLIDKLGEVDFRLTEGANERIQLDAYLAYLSTLAKK

Domain boundaries from sequence (SnapDragon)

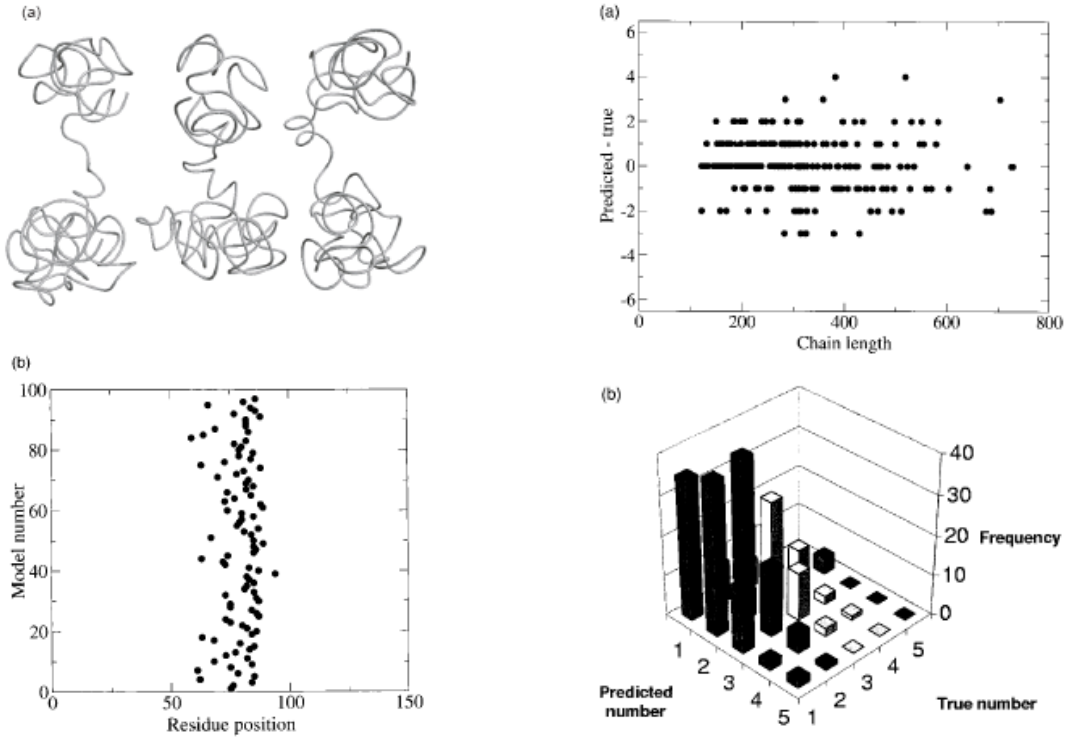


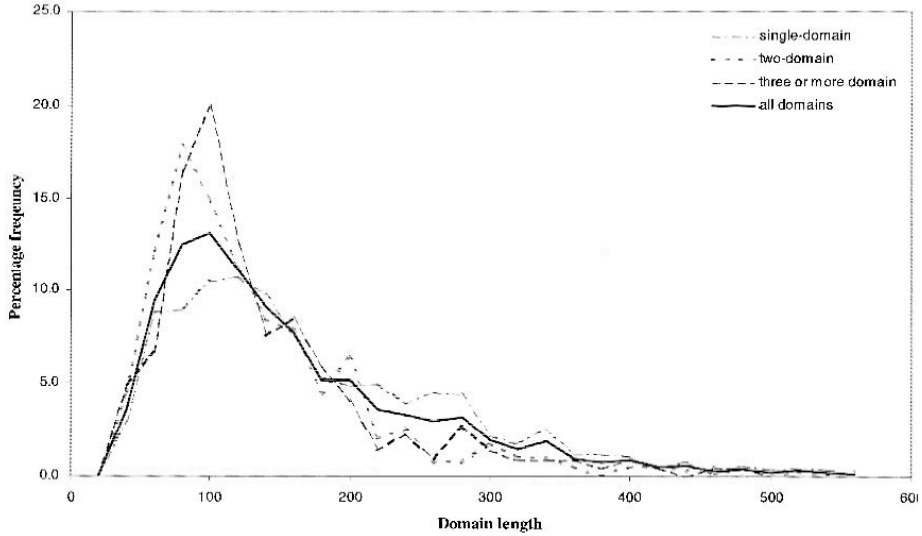
Table 2. Average accuracy percentages of linker prediction over 57 proteins

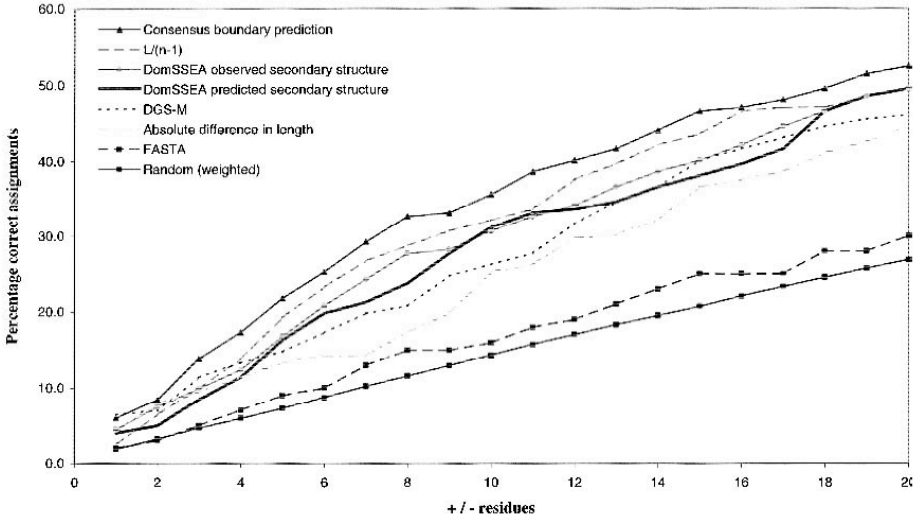
		Continuous set	Discontinuous set	Full set
Randomised background Z-score >2	Coverage	63.3	43.6	54.8
	Success	27.2	31.1	28.9
Self-normalised Z-score >1	Coverage	64.7	39.5	53.5
	Success	26.6	31.7	28.9
Self-normalised Z-score >2	Coverage	48.7	24.3	38.7
	Success	41.3	28.3	29.9

Georgea and Heringa (2002) *J. Mol. Biol.* **316** pp839

Domain boundaries from sequence and predicted SSE (DomSSEA)

Methods	% Correctly assigned	
	All chains	Multidomain chains
DomSSEA observed secondary structure	70.2	24.7
DomSSEA predicted & consensus	68.6	24.0
DomSSEA predicted & L/(N-1)	68.0	24.0
DomSSEA predicted secondary structure	68.7	23.6
Absolute difference in length	62.0	8.4
Average domain length & DGS-M	66.6	6.1
FASTA alignment	57.9	2.3
Random (weighted)	58.3	1.1
DGS-M	76.6	0.0
DGS-W	76.6	0.0



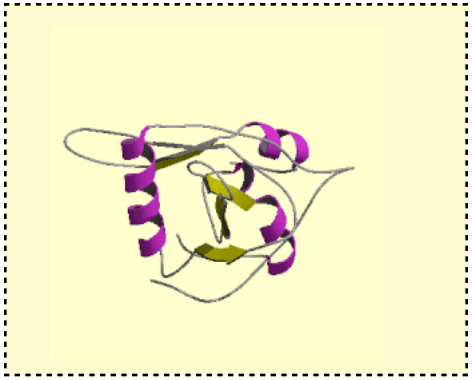


Dersden *et al.* (2003) *Prot. Science* **11** pp2014

Prediction of Secondary Structure (PSI-PRED)

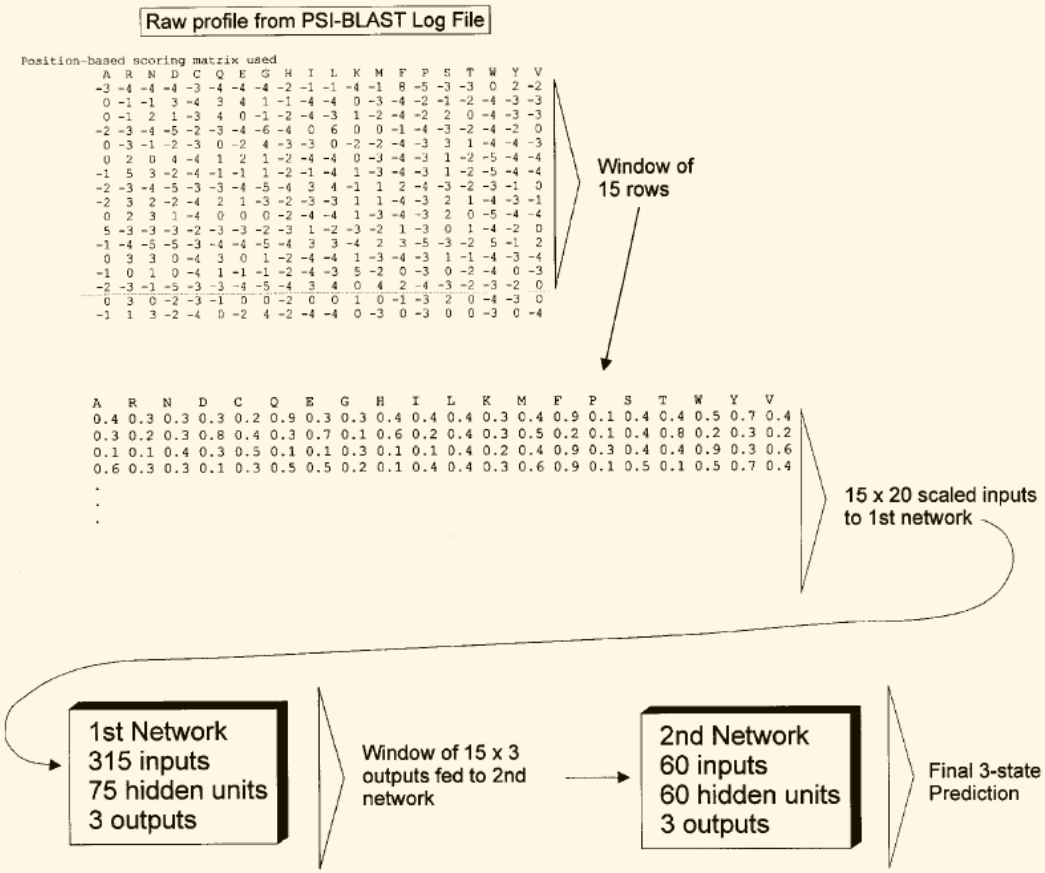
```
>gi42541361
MDIRSVSSLRGLLCLPPSWPRR
```

- Neural Network



✓ **Very simple idea**
✓ **Simple scoring**

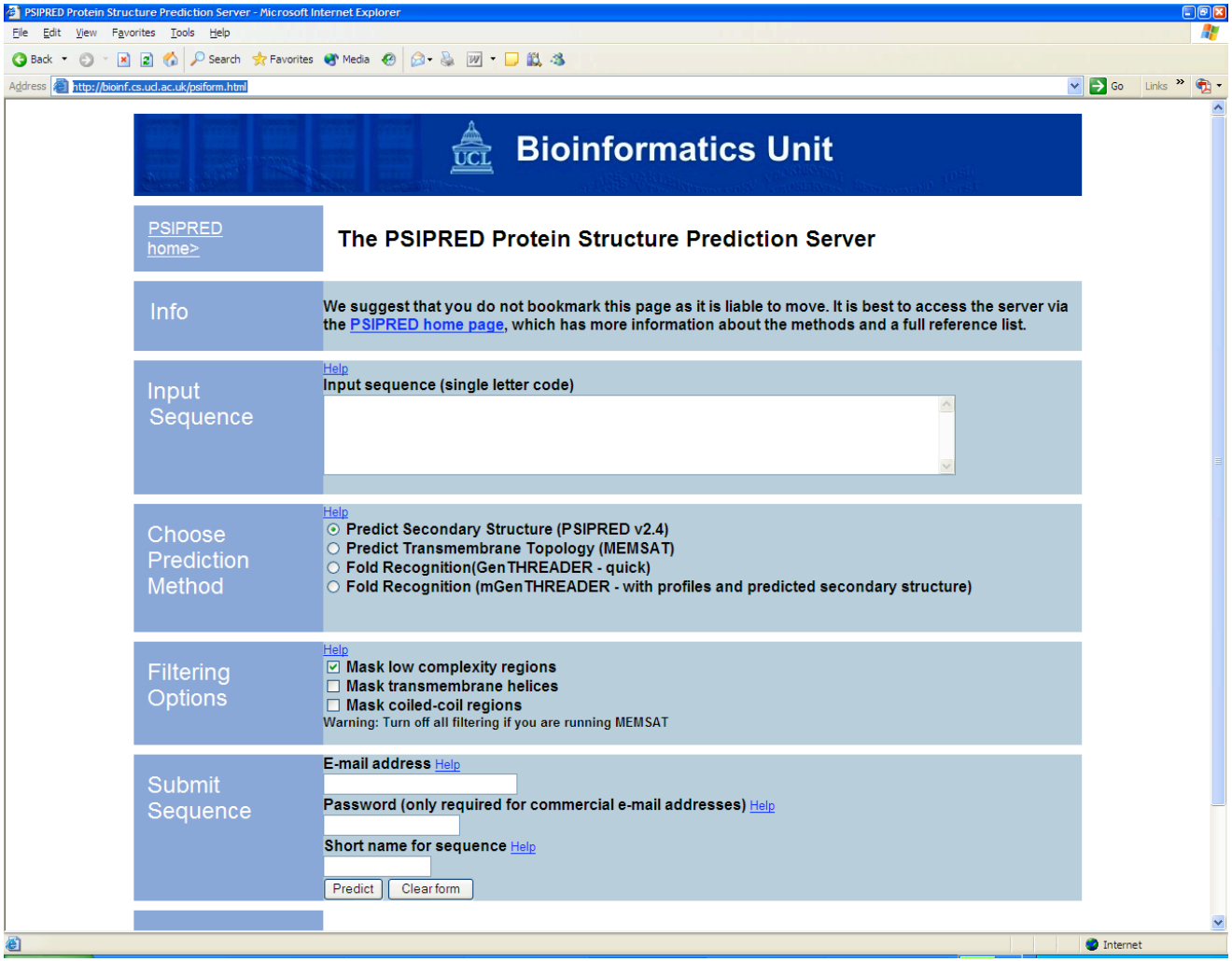
Obscure optimizer



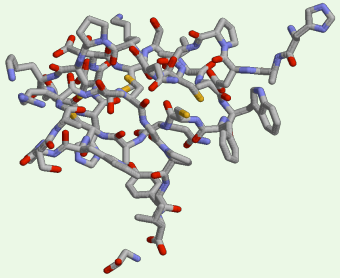
Jones DT. (1999) *J. Mol. Biol.* **292** pp195

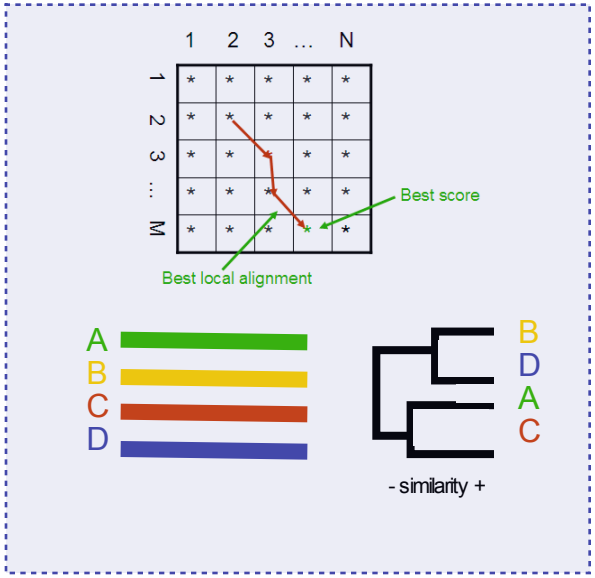
Prediction of Secondary Structure (PSI-PRED)

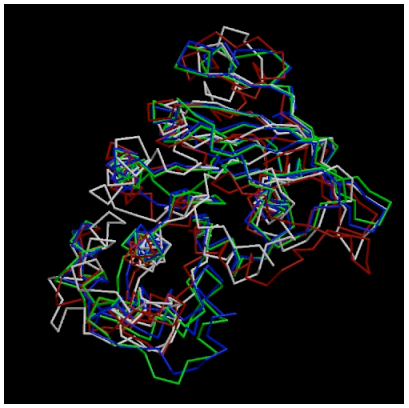
http://bioinf.cs.ucl.ac.uk/psiform.html



Sequence-Structure alignment by properties conservation (SALIGN-MODELLER)

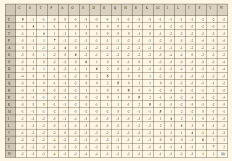


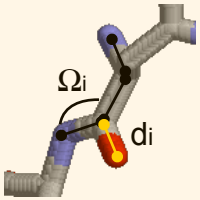


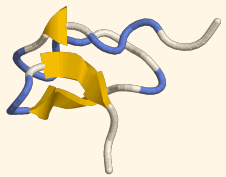


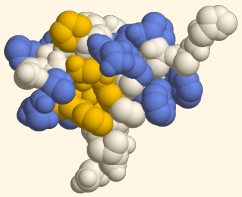
✓ Uses all available structural information
✓ Provides the optimal alignment

Computationally expensive









$$\text{RMSD} = \sqrt{\sum \left(x_i - \bar{x} \right)^2}$$

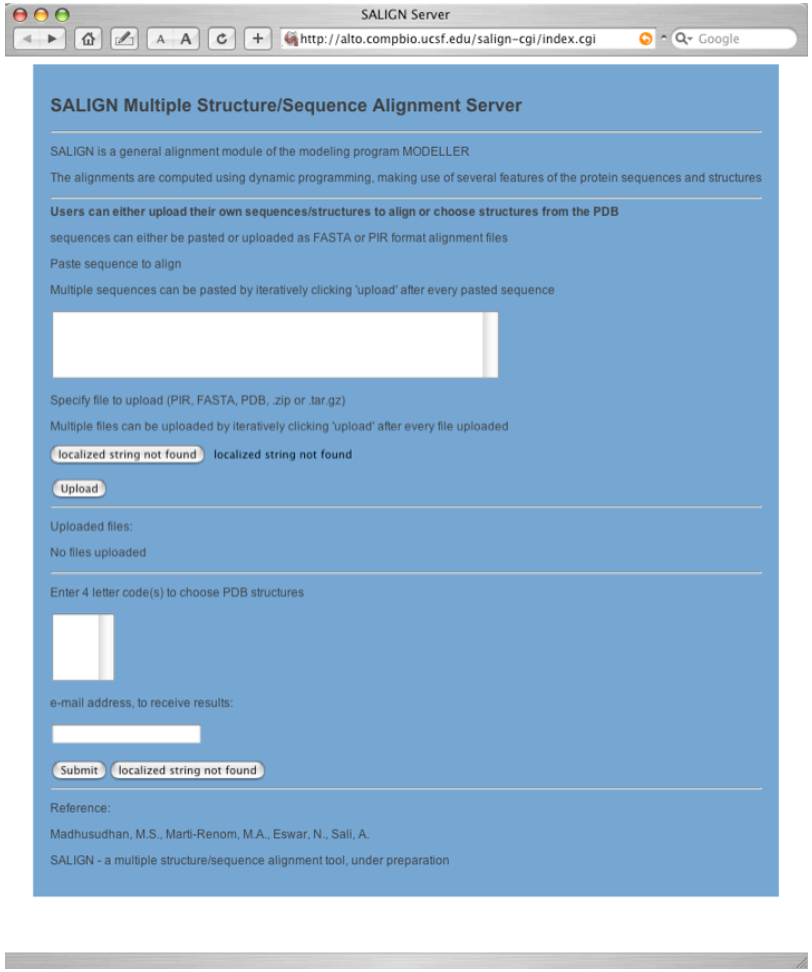
$R_{i,j}$ $D_{i(3),j(3)}$ $S_{i,j}$ $B_{i,j}$ $I_{i,j}$

$$Score_{i,j} = w_1 * R_{i,j} + w_2 * D_{i(a),j(a)} + w_3 * S_{i,j} + w_4 * B_{i,j} + w_5 * I_{i,j} + w_6 * X_{i,j}$$

Madhusudhan et al. *in preparation*

Sequence-Structure alignment by properties conservation (SALIGN-MODELLER)

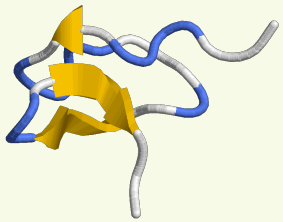
http://alto.compbio.ucsf.edu/salign-cgi/index.cgi

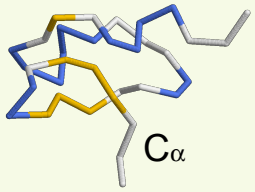


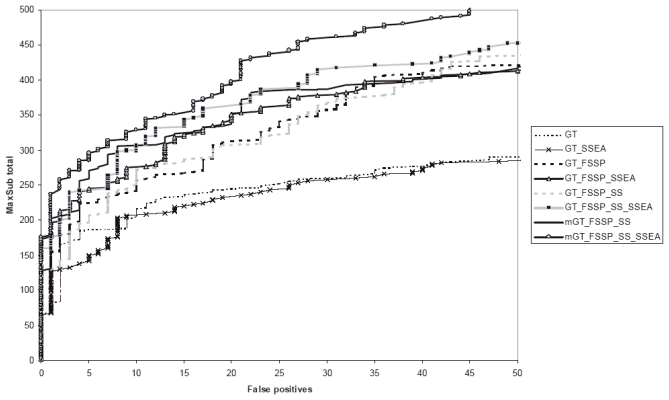
Threading (mGenThreader)

>gi42541361
MDIRSVSSLRGLLCLPPSWPRR

- Neural Network

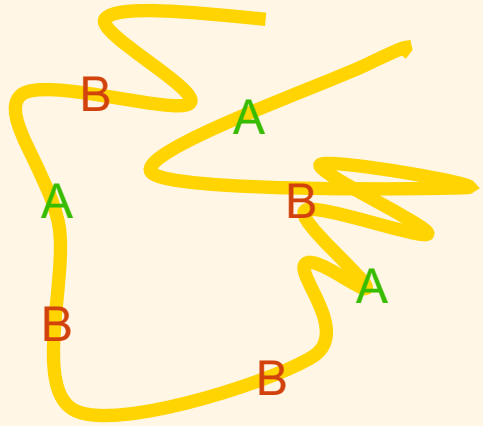


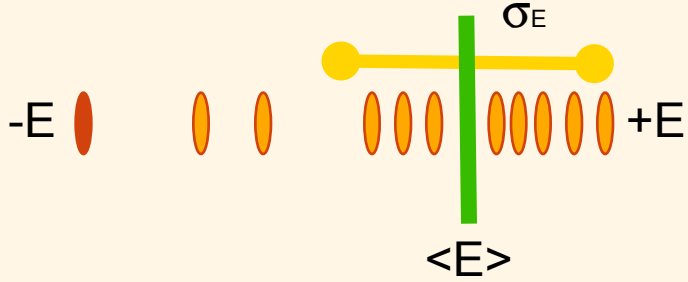




✓ **Good row and significance scoring**

Obscure optimizer



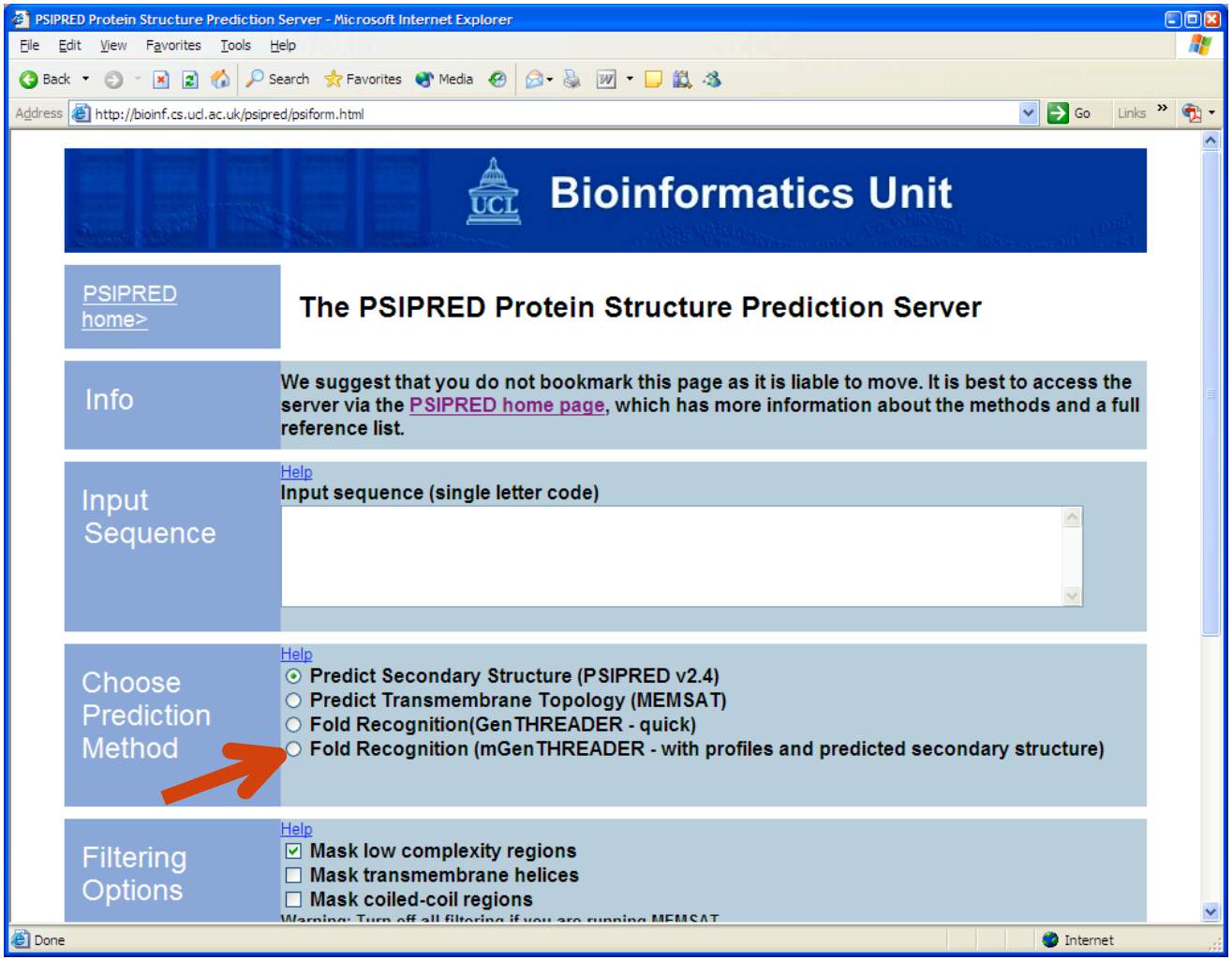


$$Zscore = \frac{(\langle E \rangle - E_m)}{\sigma_E}$$

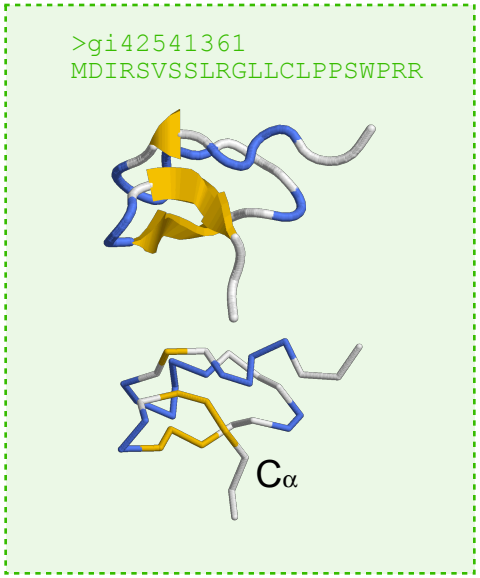
McGuffin LJ, Jones DT. (2003) *Bioinformatics*, **19**, pp874

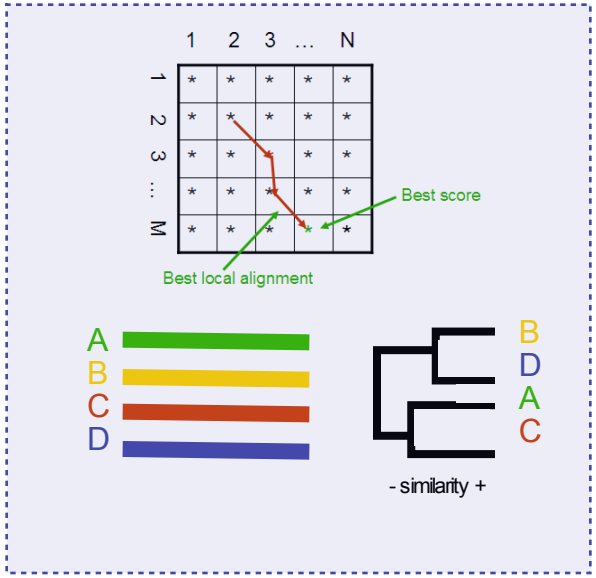
Threading (mGenThreader)

http://bioinf.cs.ucl.ac.uk/psiform.html



Remote homology detection (FUGUE)

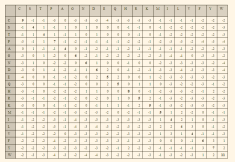




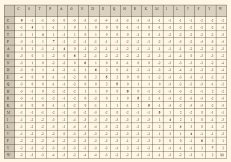
FUGUE

- ✓ Uses most of the structural information
- ✓ Easy to access either locally and on the web
- ✓ Good row and significance scoring

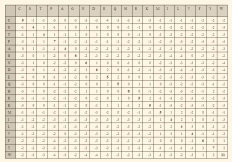
Does not uses multiple sequence information



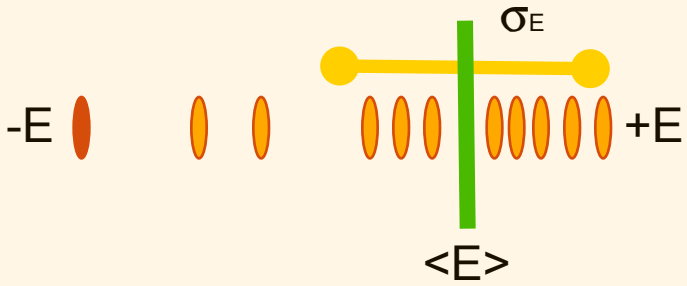
$R^{H}_{i,j}$



$R^{B}_{i,j}$



$R^{C}_{i,j}$

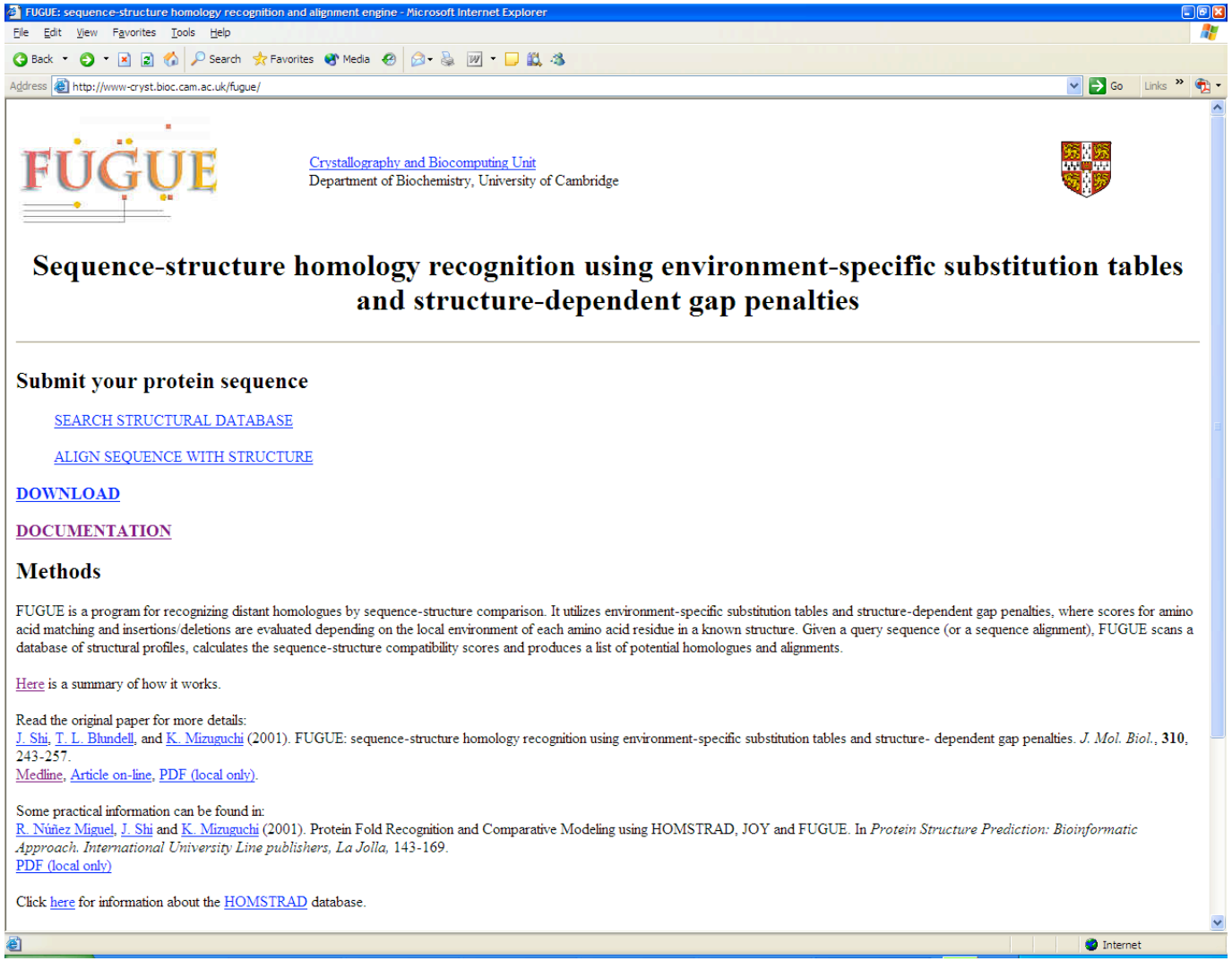


$$Zscore = \frac{(\langle E \rangle - E_m)}{\sigma_E}$$

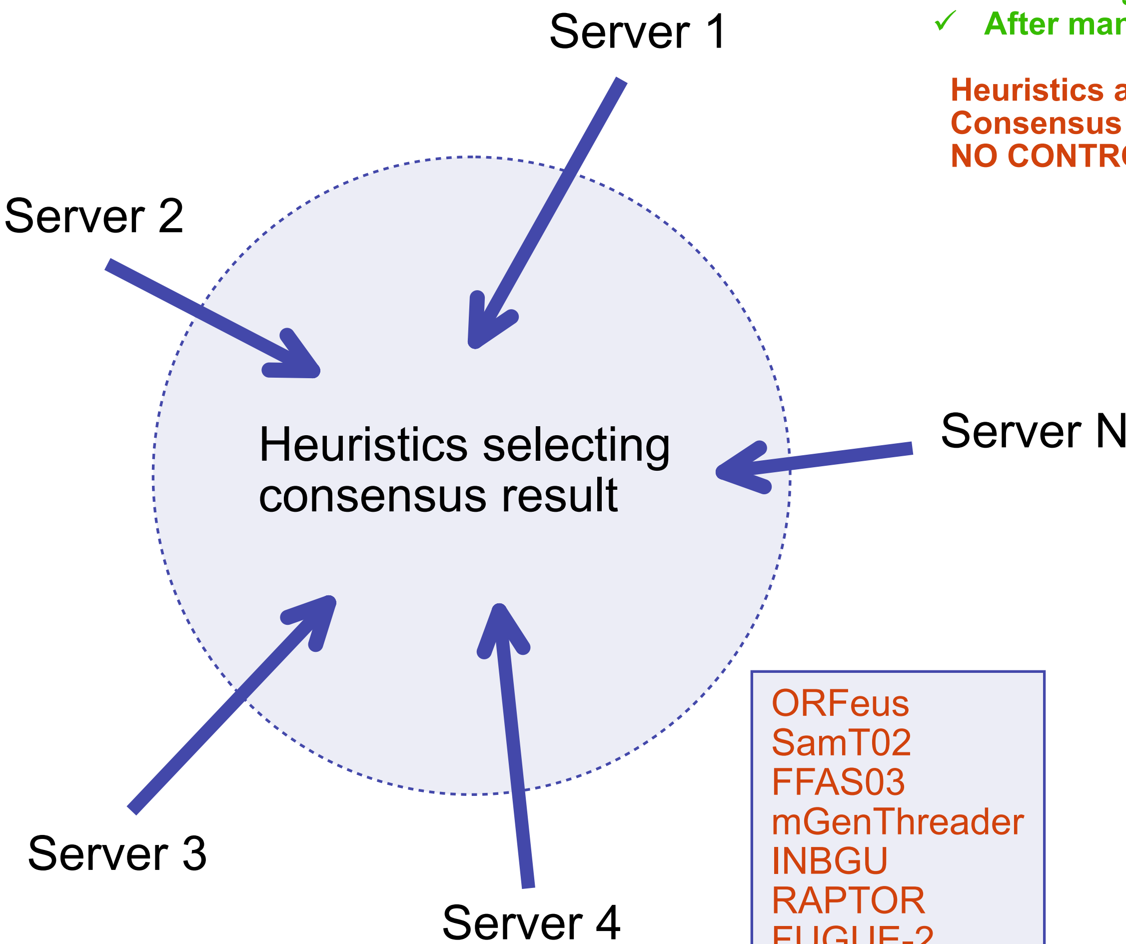
Shi et al. (2001) *J. Mol. Biol* **310** pp241

Remote homology detection (FUGUE)

http://www-cryst.bioc.cam.ac.uk/fugue/



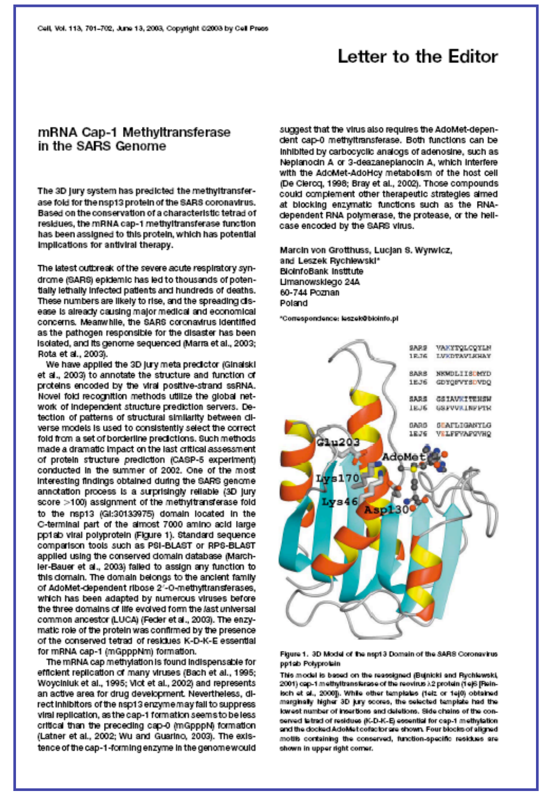
Meta-Servers (3D-Jury)



- ✓ Collecting several results
- ✓ After manual analysis… good results

Heuristics and complicated scoring
Consensus results
NO CONTROL OF DATA GENERATION or SERVERS!

ORFeus
SamT02
FFAS03
mGenThreader
INBGU
RAPTOR
FUGUE-2
3D-PSSM


Cell, Vol. 113, 701–702, June 13, 2003, Copyright ©2003 by Cell Press

Letter to the Editor

mRNA Cap-1 Methyltransferase in the SARS Genome

The 3D jury system has predicted the methyltransferase fold for the nsp13 protein of the SARS coronavirus. Based on the conservation of a characteristic tetrad of residues, the mRNA cap-1 methyltransferase function has been assigned to this protein, which has potential implications for antiviral therapy.

The latest outbreak of the severe acute respiratory syndrome (SARS) epidemic has led to thousands of potentially lethally infected patients and hundreds of deaths. These numbers are likely to rise, and the spreading disease is already causing major medical and economical concerns. Meanwhile, the SARS coronavirus identified as the pathogen responsible for the disaster has been isolated, and its genome sequenced (Marra et al., 2003; Rota et al., 2003).

We have applied the 3D jury meta predictor (Ginalski et al., 2003) to annotate the structure and function of proteins encoded by the viral positive-strand ssRNA. Novel fold recognition methods utilize the global network of independent structure prediction servers. Detection of patterns of structural similarity between diverse models is used to consistently select the correct fold from a set of borderline predictions. Such methods made a dramatic impact on the last critical assessment of protein structure prediction (CASP-5 experiment) conducted in the summer of 2002. One of the most interesting findings obtained during the SARS genome annotation process is a surprisingly reliable (3D jury score >100) assignment of the methyltransferase fold to the nsp13 (GI:30133975) domain located in the C-terminal part of the almost 7000 amino acid large pp1ab viral polyprotein (Figure 1). Standard sequence comparison tools such as PSI-BLAST or RPS-BLAST applied using the conserved domain database (Marchler-Bauer et al., 2003) failed to assign any function to this domain. The domain belongs to the ancient family of AdoMet-dependent ribose 2′-O-methyltransferases, which has been adapted by numerous viruses before the three domains of life evolved form the last universal common ancestor (LUCA) (Feder et al., 2003). The enzymatic role of the protein was confirmed by the presence of the conserved tetrad of residues K-D-K-E essential for mRNA cap-1 (mGpppNm) formation.

The mRNA cap methylation is found indispensable for efficient replication of many viruses (Bach et al., 1995; Woyciniuk et al., 1995; Vict et al., 2002) and represents an active area for drug development. Nevertheless, direct inhibitors of the nsp13 enzyme may fail to suppress viral replication, as the cap-1 formation seems to be less critical than the preceding cap-0 (mGpppN) formation (Latner et al., 2002; Wu and Guarino, 2003). The existence of the cap-1-forming enzyme in the genome would suggest that the virus also requires the AdoMet-dependent cap-0 methyltransferase. Both functions can be inhibited by carbocyclic analogs of adenosine, such as Neplanocin A or 3-deazaneplanocin A, which interfere with the AdoMet-AdoHcy metabolism of the host cell (De Clercq, 1998; Bray et al., 2002). Those compounds could complement other therapeutic strategies aimed at blocking enzymatic functions such as the RNA-dependent RNA polymerase, the protease, or the helicase encoded by the SARS virus.

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BioinfoBank Institute
Limanowskiego 24A
60-744 Poznan
Poland

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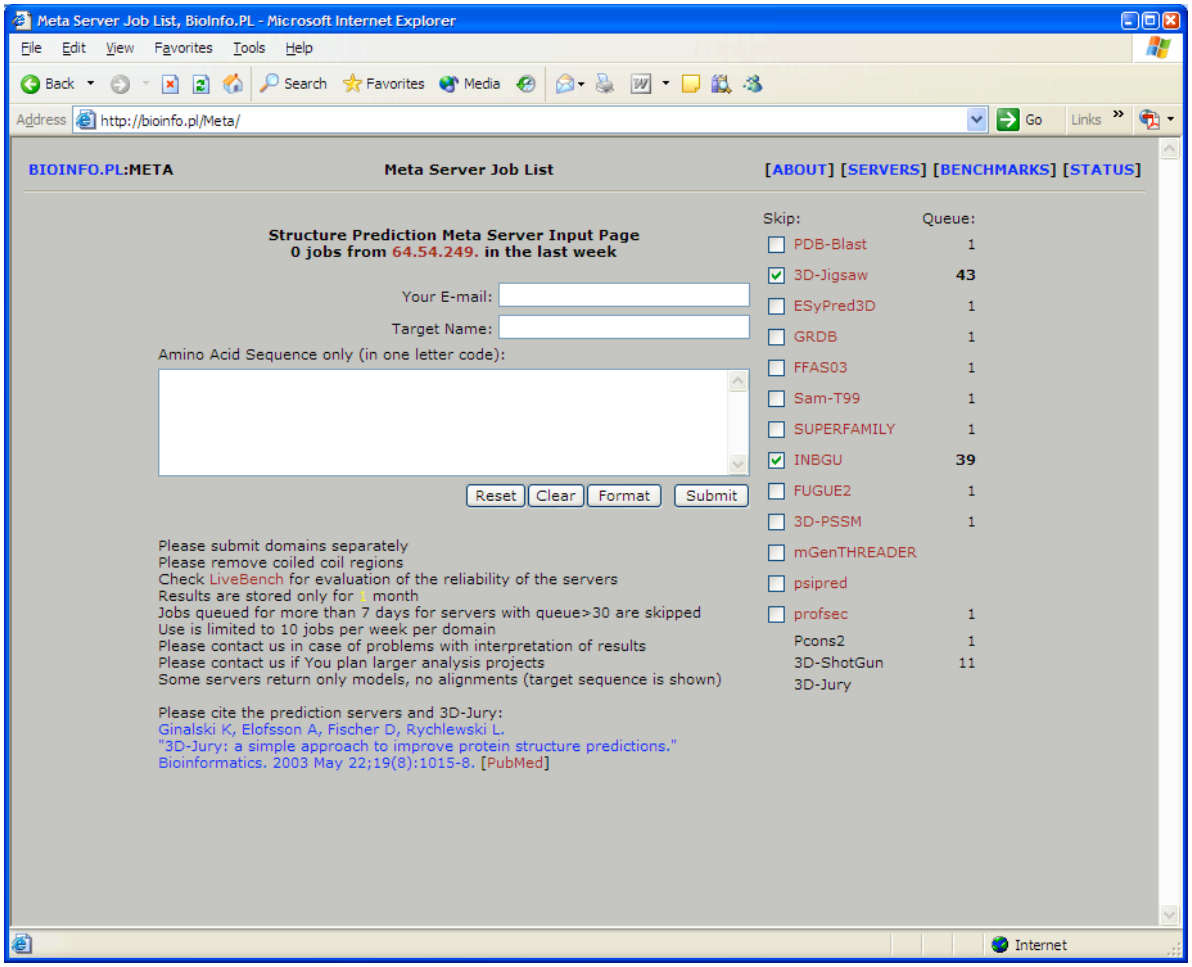
Figure 1. 3D Model of the nsp13 Domain of the SARS Coronavirus pp1ab Polyprotein

This model is based on the reassigned (Bujnicki and Rychlewski, 2001) cap-1 methyltransferase of the reovirus λ2 protein (1ej6 [Reinisch et al., 2000]). While other templates (1eiz or 1ej0) obtained marginally higher 3D jury scores, the selected template had the lowest number of insertions and deletions. Side chains of the conserved tetrad of residues (K-D-K-E) essential for cap-1 methylation and the docked AdoMet cofactor are shown. Four blocks of aligned motifs containing the conserved, function-specific residues are shown in upper right corner.

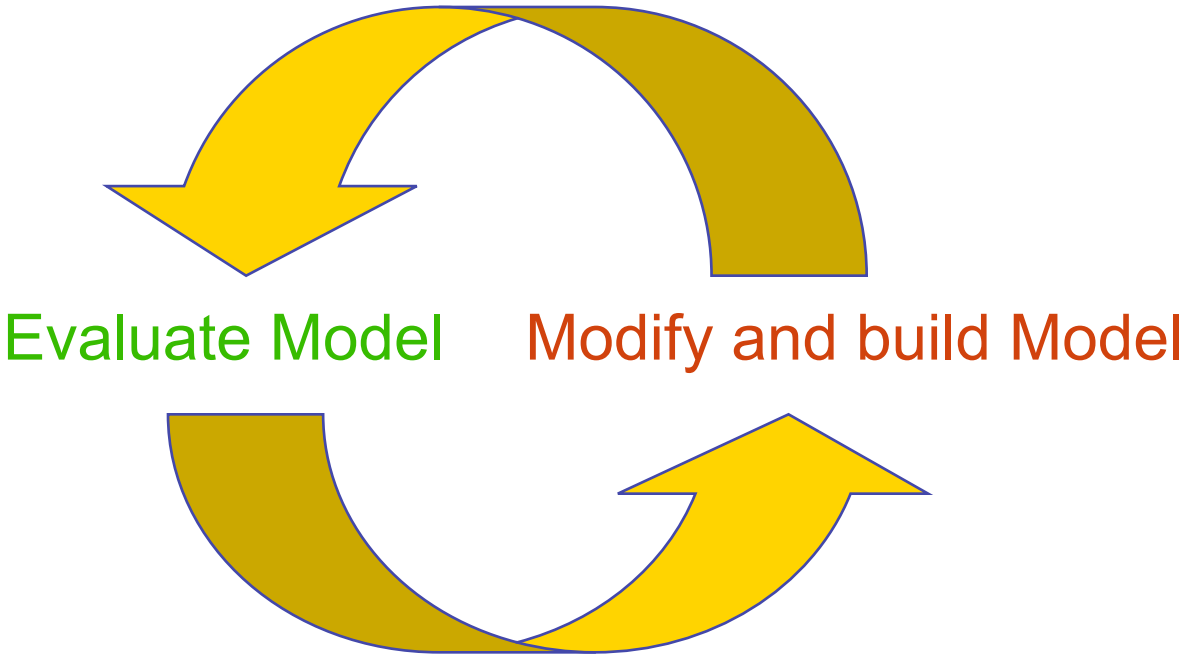
Ginalski K, et al. (2003) *Bioinformatics* **19** pp1015

Meta-Servers (3D-Jury)

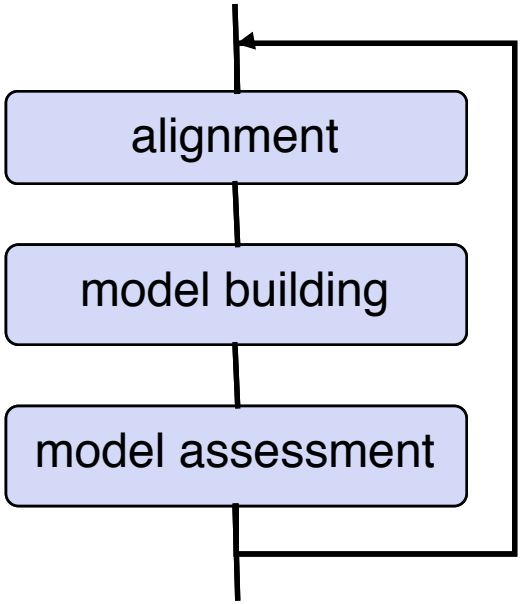
http://bioinfo.pl/Meta/

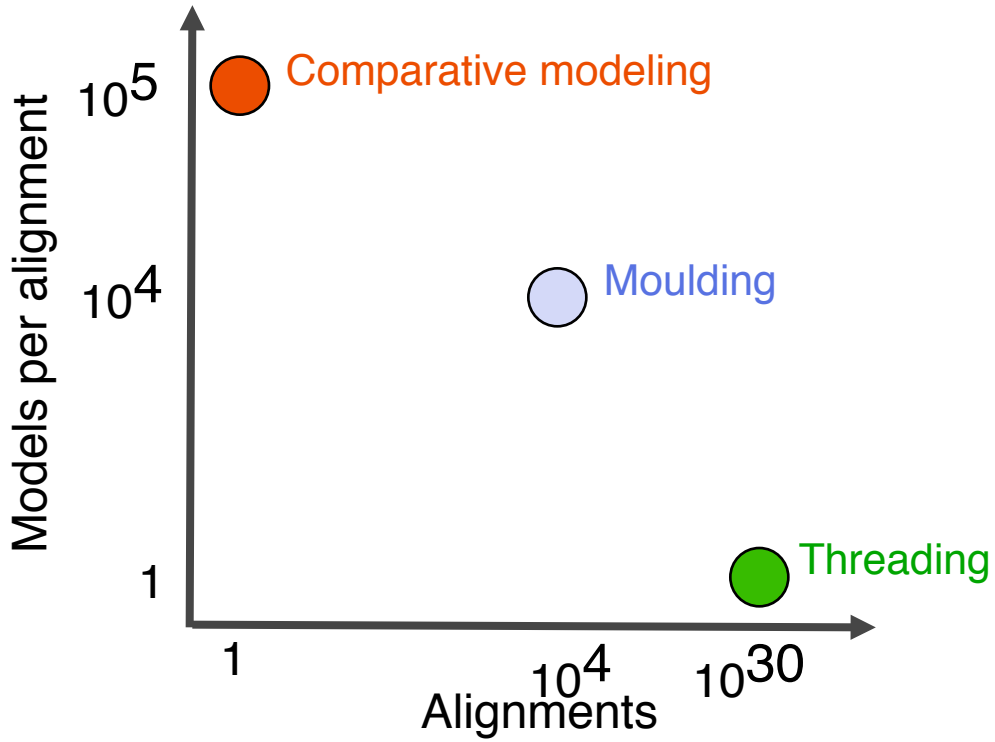


Iterative process… better models(?)

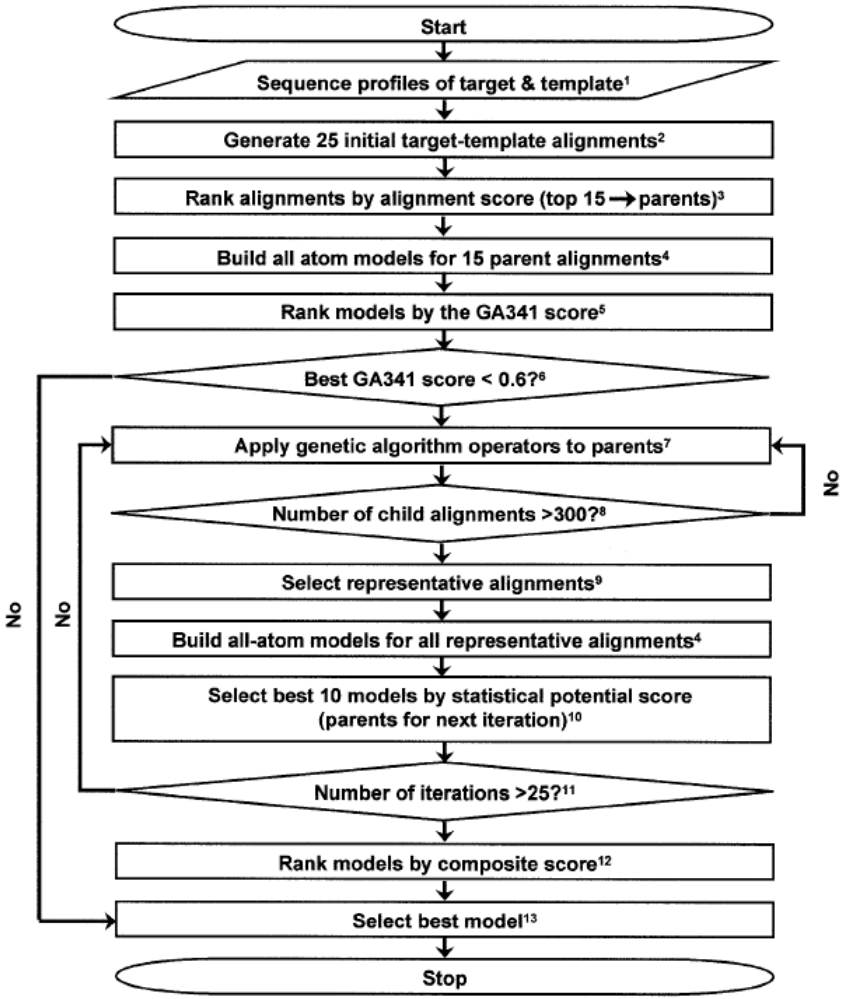


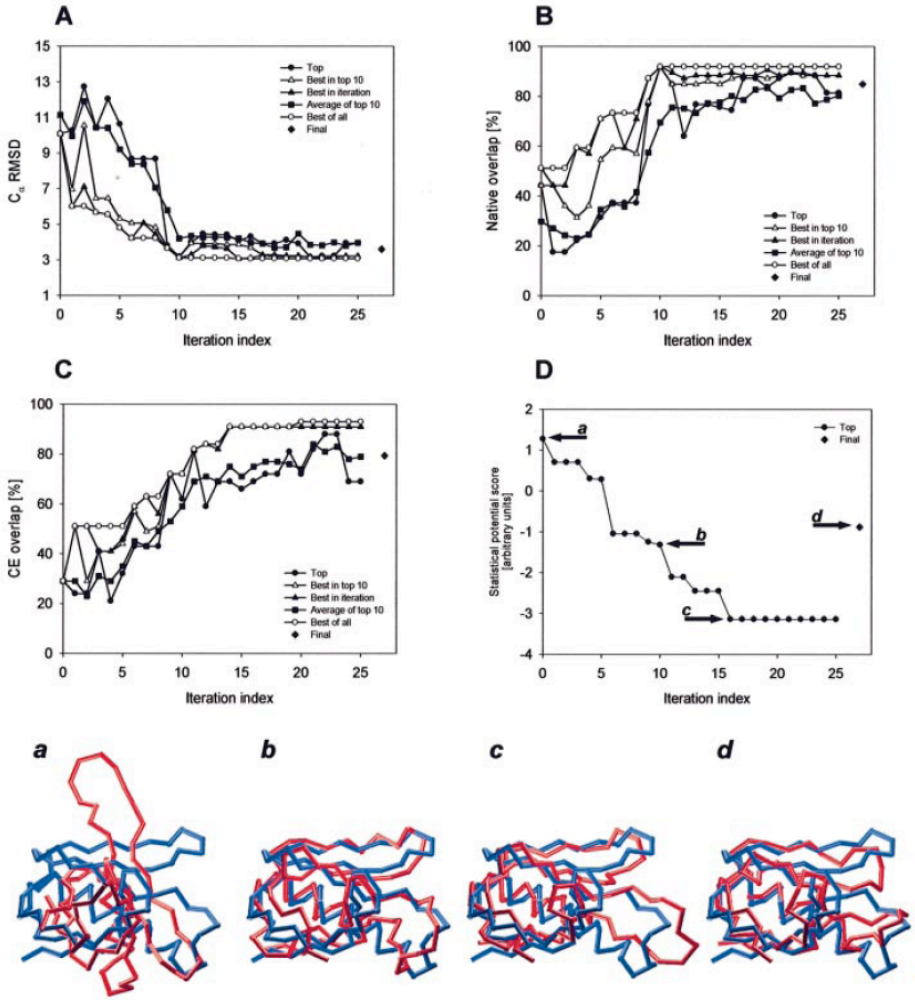
Moulding: iterative alignment, model building, model assessment





Iterative process… MOULDER

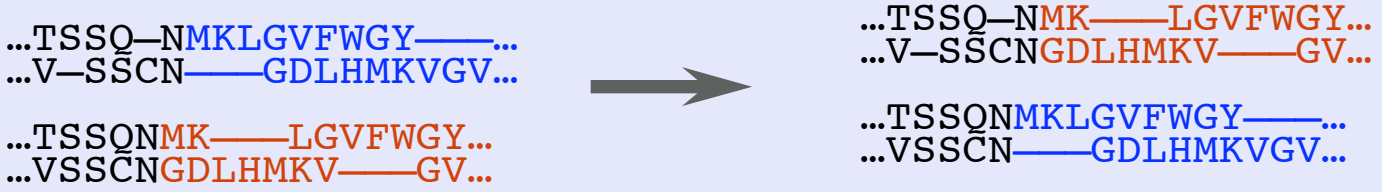




John & Sali (2003). *NAR* **31** pp3982

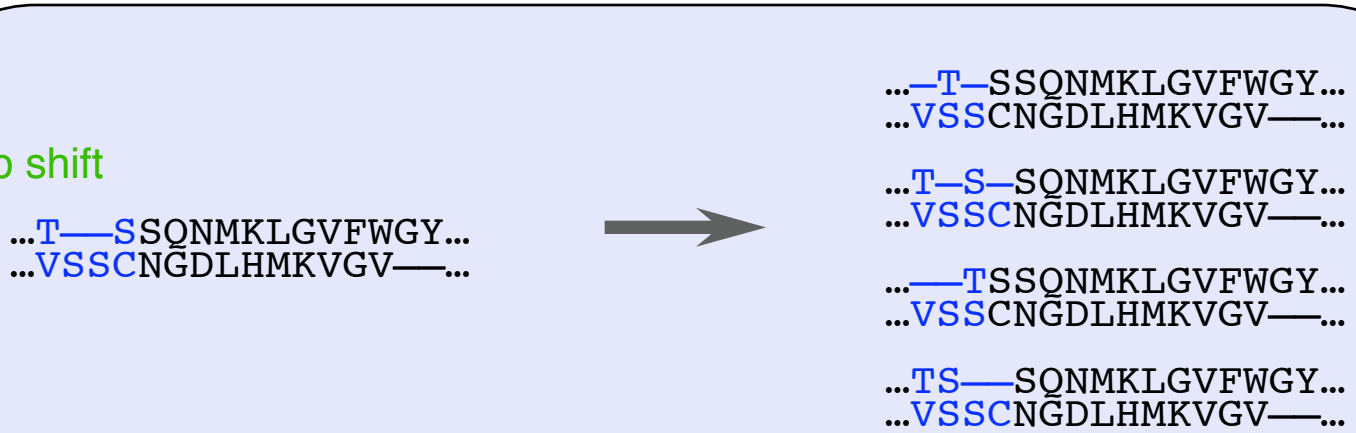
Genetic algorithm operators

Single point cross-over



Gap insertion

Gap shift



Also, “two point crossover” and “gap deletion”.

Composite model assessment score

Weighted linear combination of several scores:

- Pair (P_p) and surface (P_s) statistical potentials;
- Structural compactness (S_c);
- Harmonic average distance score (H_a);
- Alignment score (A_s).

$$Z = 0.17\ Z(P_P) + 0.02\ Z(P_S) + 0.10\ Z(S_C) + 0.26\ Z(H_a) + 0.45\ (A_S)$$

$Z(\text{score}) = (\text{score} - \mu)/\sigma$

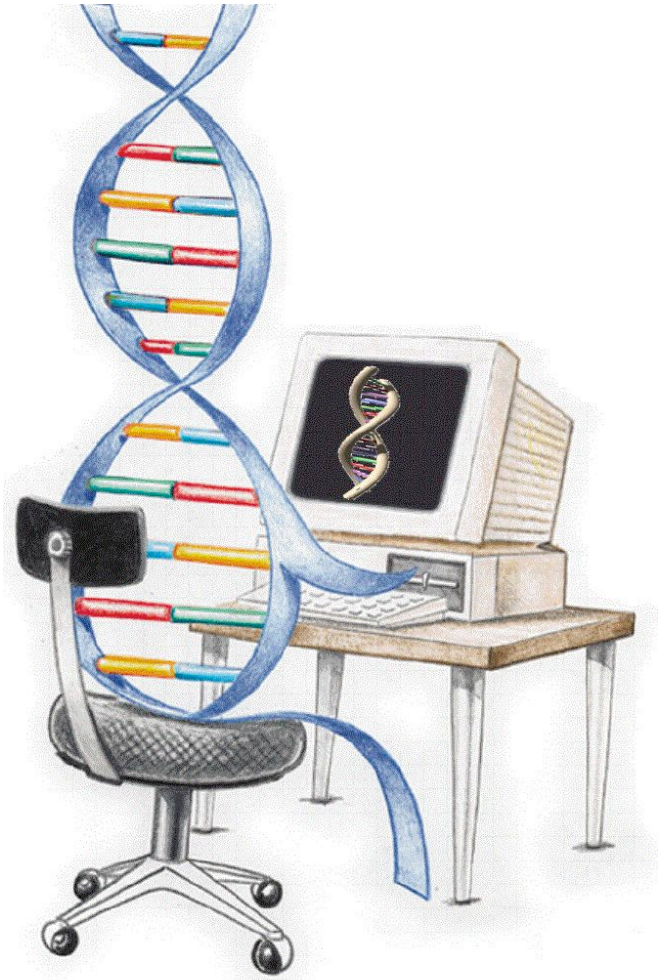
μ … average score of all models

σ … standard deviation of the scores

Benchmark with the “very difficult” test set

D. Fischer threading test set of 68 structural pairs (a subset of 19)

Target -template	Sequence identity [%]	Coverage [% aa]	Initial prediction		Final prediction		Best prediction	
			Cα RMSD [Å]	CE overlap [%]	Cα RMSD [Å]	CE overlap [%]	Cα RMSD [Å]	CE overlap [%]
1ATR-1ATN	13.8	94.3	19.2	20.2	18.8	20.2	17.1	24.6
1BOV-1LTS	4.4	83.5	10.1	29.4	3.6	79.4	3.1	92.6
1CAU-1CAU	18.8	96.7	11.7	15.6	10.0	27.4	7.6	47.4
1COL-1CPC	11.2	81.4	8.6	44.0	5.6	58.6	4.8	59.3
1LFB-1HOM	17.6	75.0	1.2	100.0	1.2	100.0	1.1	100.0
1NSB-2SIM	10.1	89.2	13.2	20.2	13.2	20.1	12.3	26.8
1RNH-1HRH	26.6	91.2	13.0	21.2	4.8	35.4	3.5	57.5
1YCC-2MTA	14.5	55.1	3.4	72.4	5.3	58.4	3.1	75.0
2AYH-1SAC	8.8	78.4	5.8	33.8	5.5	48.0	4.8	64.9
2CCY-1BBH	21.3	97.0	4.1	52.4	3.1	73.0	2.6	77.0
2PLV-1BBT	20.2	91.4	7.3	58.9	7.3	58.9	6.2	60.7
2POR-2OMF	13.2	97.3	18.3	11.3	11.4	14.7	10.5	25.9
2RHE-1CID	21.2	61.6	9.2	33.7	7.5	51.1	4.4	71.1
2RHE-3HLA	2.4	96.0	8.1	16.5	7.6	9.4	6.7	43.5
3ADK-1GKY	19.5	100.0	13.8	26.6	11.5	37.7	7.7	48.1
3HHR-1TEN	18.4	98.9	7.3	60.9	6.0	66.7	4.9	79.3
4FGF-81IB	14.1	98.6	11.3	24.0	9.3	30.6	5.4	41.2
6XIA-3RUB	8.7	44.1	10.5	14.5	10.1	11.0	9.0	34.3
9RNT-2SAR	13.1	88.5	5.8	41.7	5.1	51.2	4.8	69.0
AVERAGE	**14.2**	**85.2**	**9.6**	**36.7**	**7.7**	**44.8**	**6.3**	**57.8**



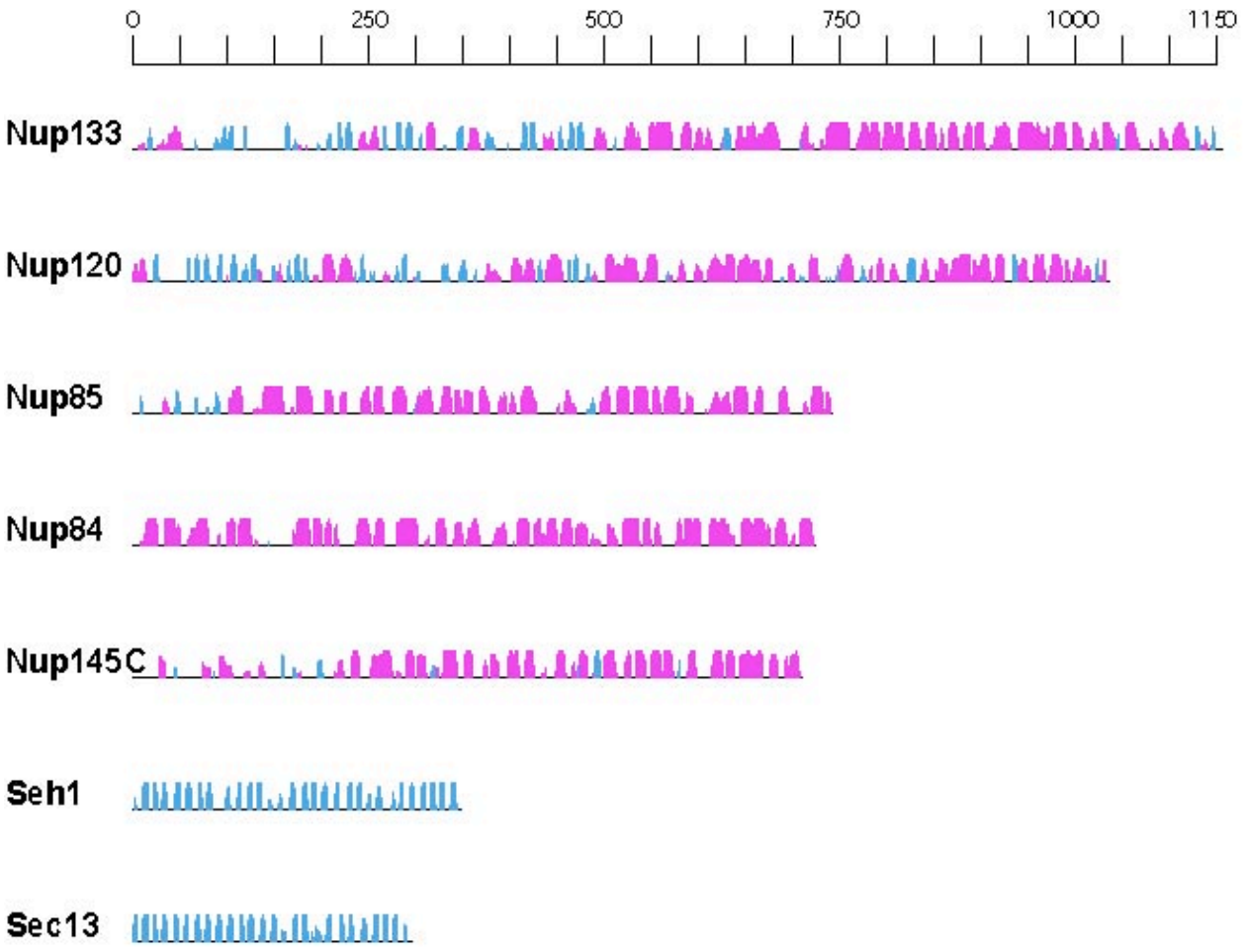
some biology? please...

Common Evolutionary Origin of Coated Vesicles and Nuclear Pore Complexes

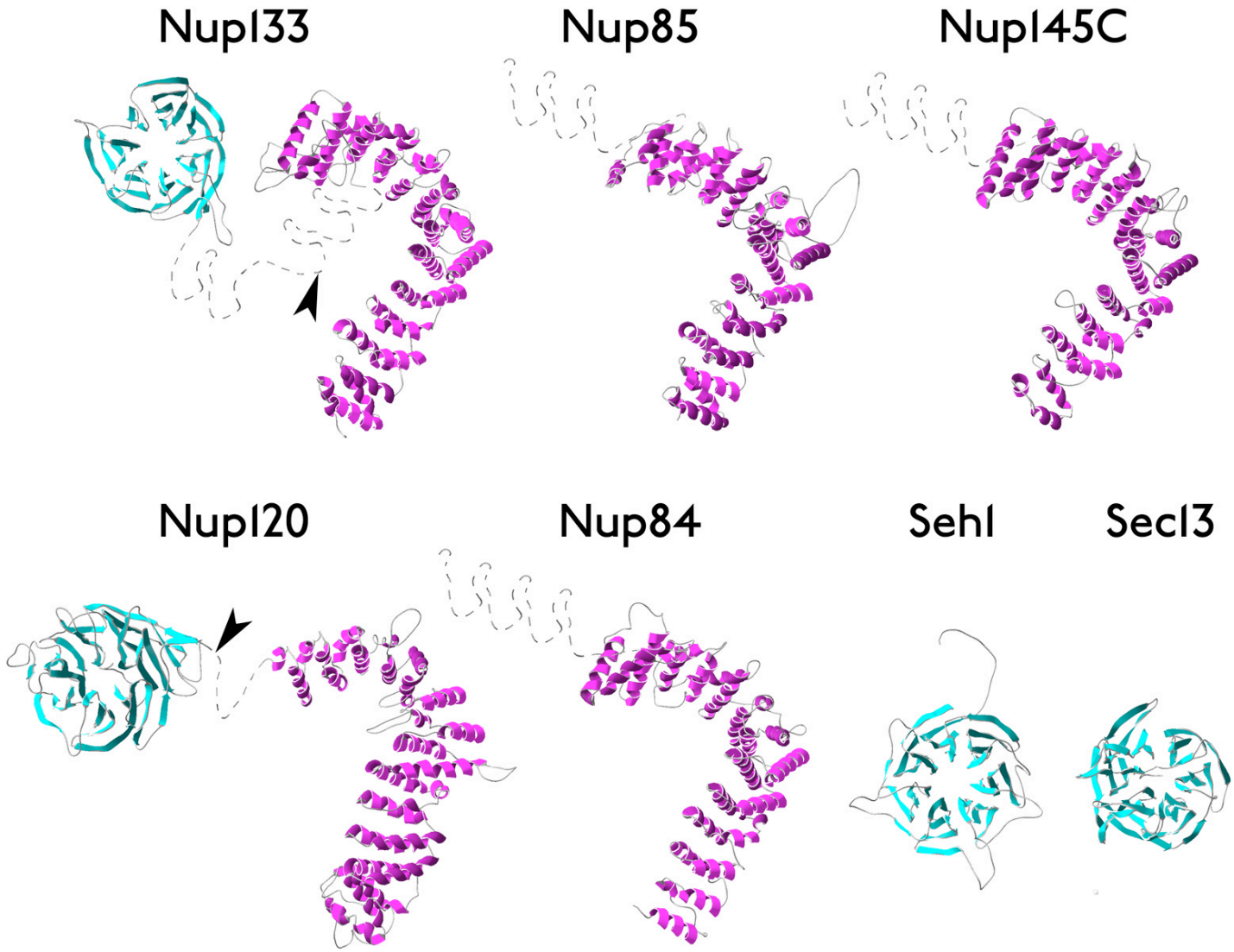
mGenThreader + SALIGN + MOULDER

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

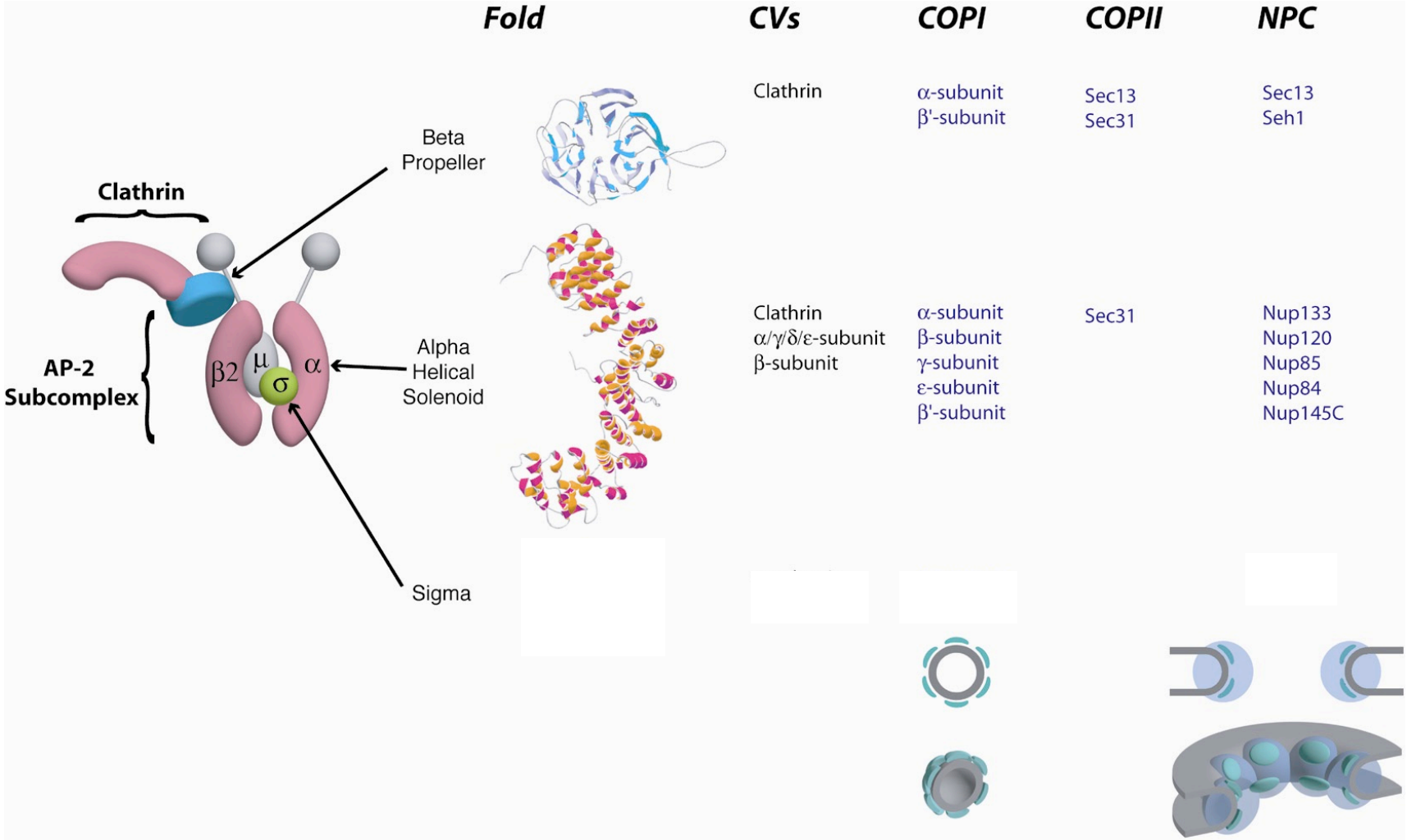
yNup84 complex proteins



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



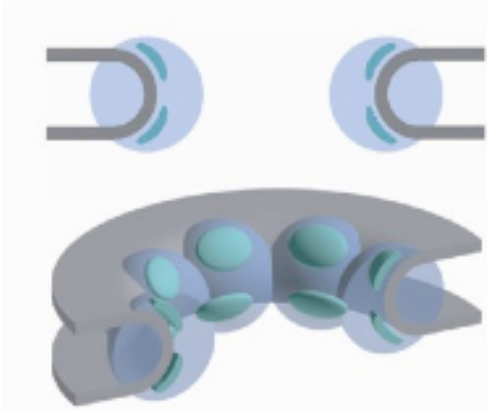
NPC and Coated Vesicles Share the β-**Propeller and** α-**Solenoid** Folds and Associate with Membranes



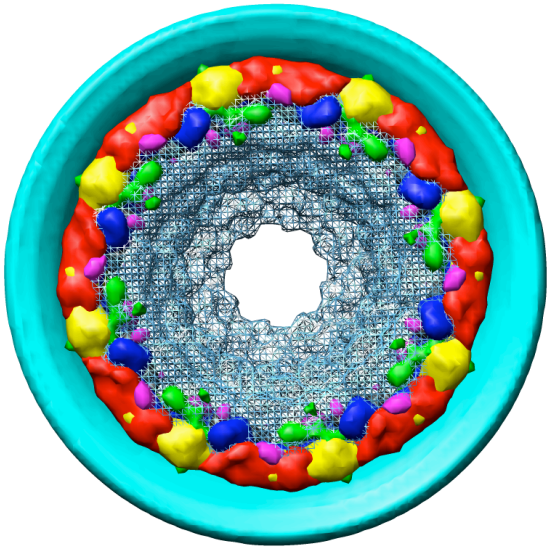
NPC and Coated Vesicles Both Associate with Membranes

Coated Vesicle

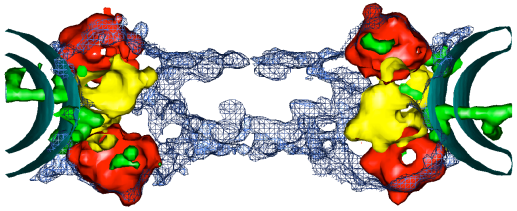
NPC model



top view

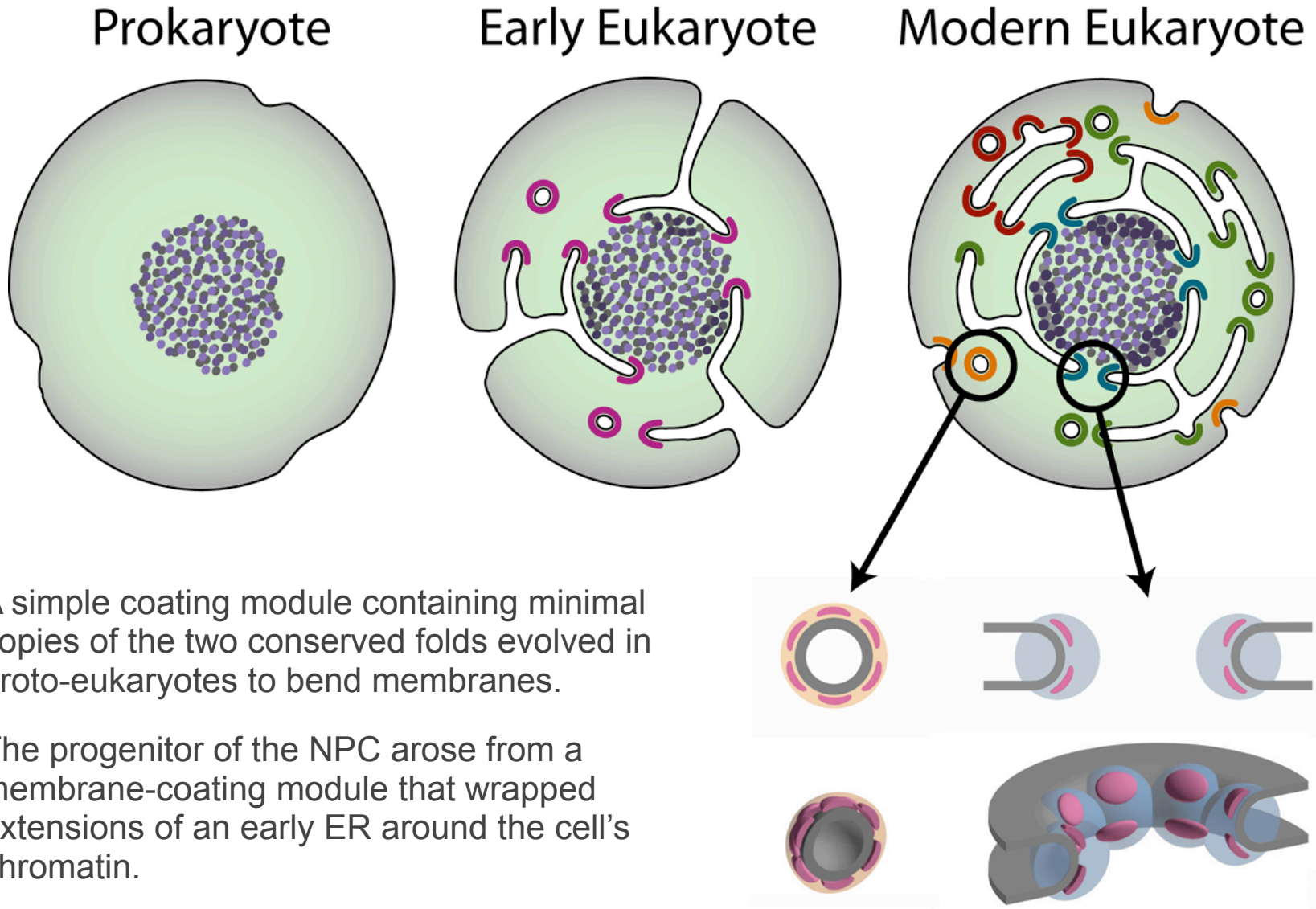


side view



Nup 84 complex

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.

Course assignment
The POM152 nucleoporin protein

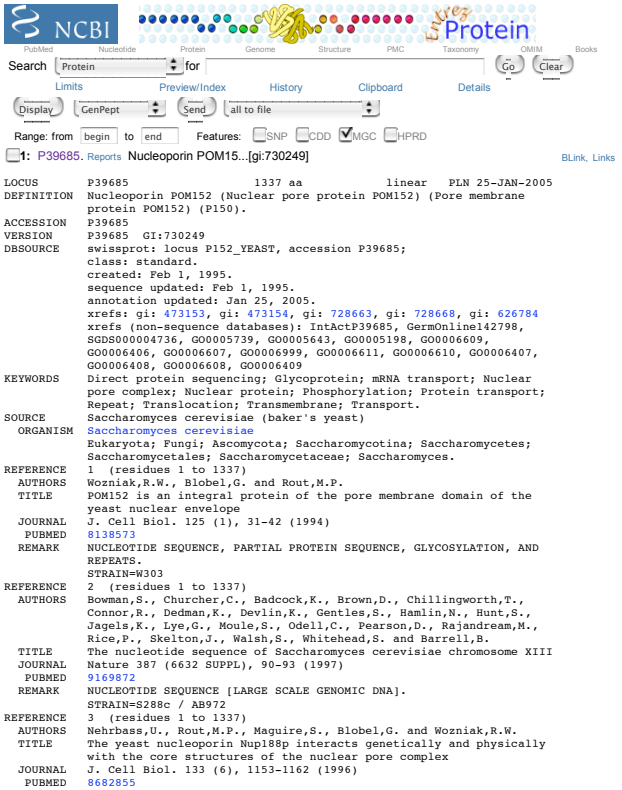
Course assignment
The POM152 nucleoporin protein

Introduction

The POM152 protein functions as a component of the nuclear pore complex (NPC). NPC components, collectively referred to as nucleoporins (NUPs), can play the role of both NPC structural components and of docking or interaction partners for transiently associated nuclear transport factors. POM152 is important for the de novo assembly of NPCs.
The nuclear pore complex (NPC) constitutes the exclusive means of nucleocytoplasmic transport. NPCs allow the passive diffusion of ions and small molecules and the active, nuclear transport receptor-mediated bidirectional transport of macromolecules such as proteins, RNAs, ribonucleoparticles (RNPs), and ribosomal subunits across the nuclear envelope. The 55-60 MDa NPC is composed of at least 31 different subunits. Due to its 8-fold rotational symmetry, all subunits are present with 8 copies or multiples thereof. POM152 is known to interact with NUP188.

Assignment

1. Predict the domain boundaries for the POM152 sequence
2. Search for a suitable template/s for the POM152 domains
3. Align the sequences of POM152 domains against the template/s sequences
4. Build a 3D-models of the POM152 domains
5. Evaluate the models
6. Indicate possible applications of the models



GRADING: The entire assignment is worth 20 points.

http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=protein&val=730249

BMI 206

MODELLER TUTORIAL

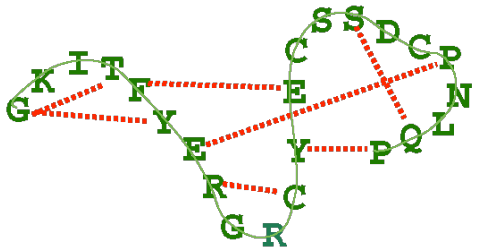
http://www.salilab.org/modeller/tutorial/

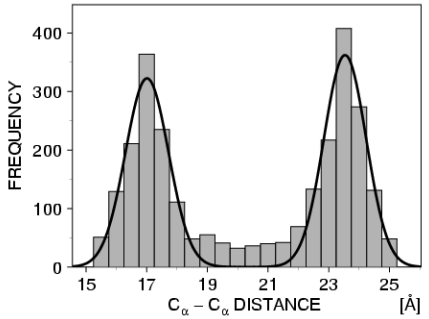
Marc A. Marti-Renom
Assistant Adjunct Professor
Department of Biopharmaceutical Sciences

Comparative Modeling by Satisfaction of Spatial Restraints (MODELLER)

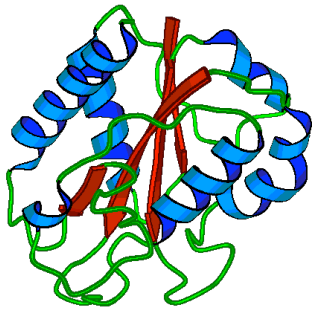
3D GKITFYERGFQGHCYESDC-NLQP…
SE GKITFYERG---RCYESDCPNLQP…

1. Extract spatial restraints





2. Satisfy spatial restraints

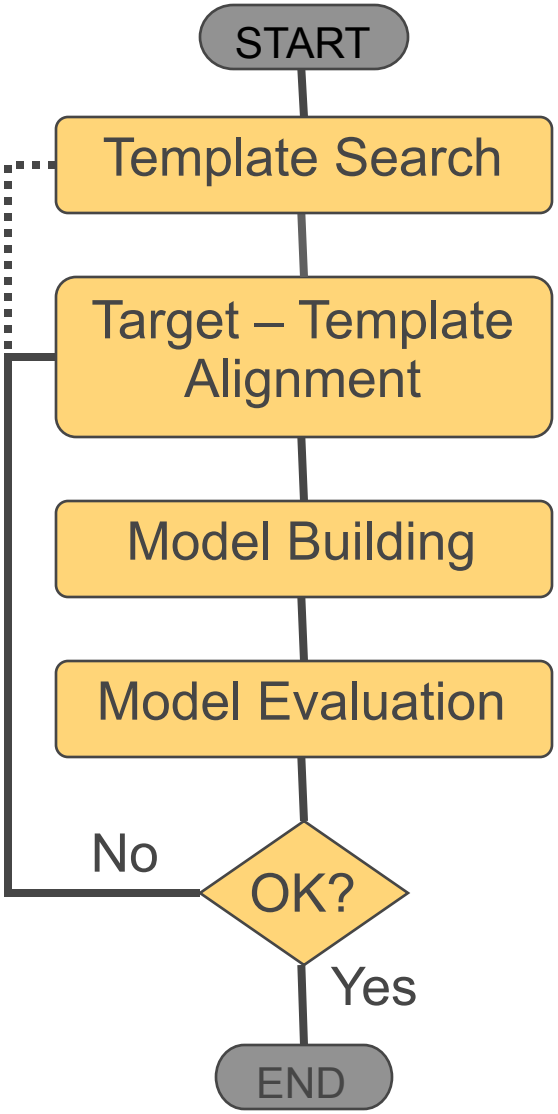


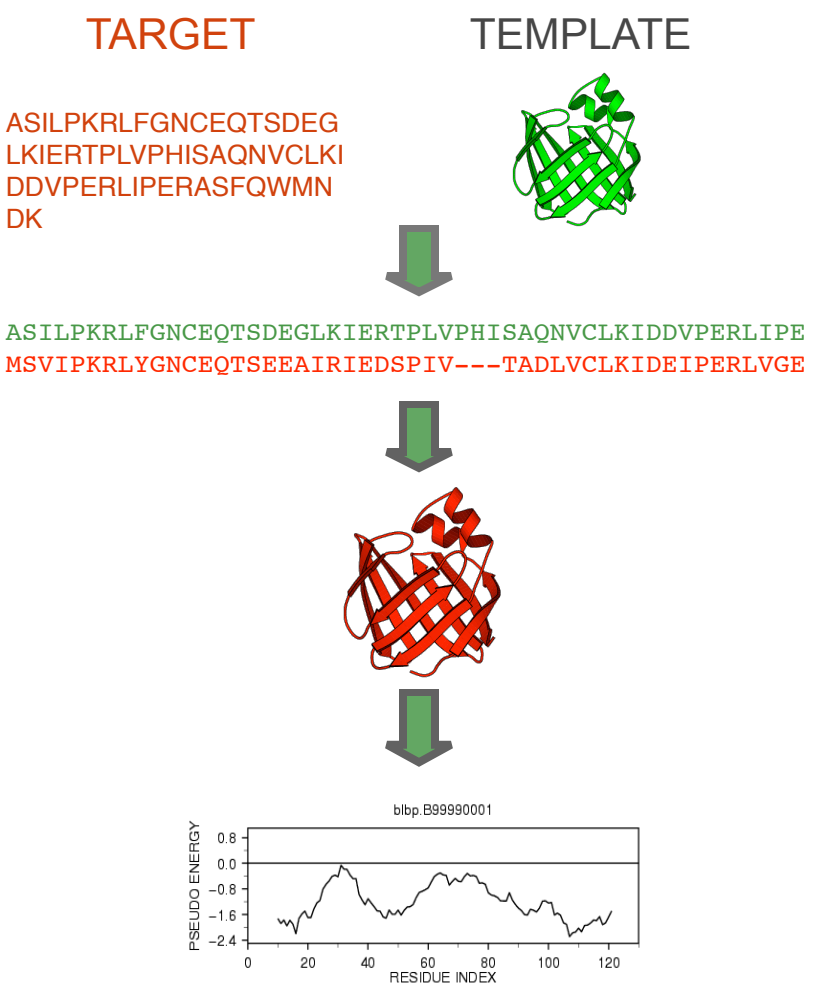
$$F(R) = \prod_i p_i(f_i/I)$$

http://www.salilab.org/modeller

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.

Steps in Comparative Protein Structure Modeling



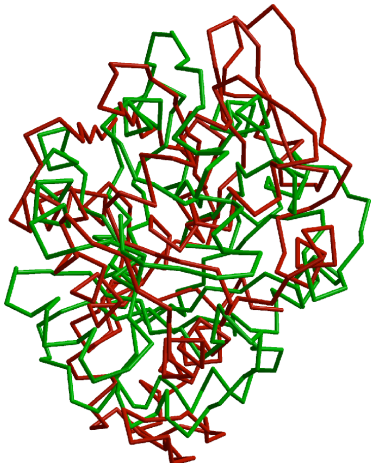


A. Šali, Curr. Opin. Biotech. 6, 437, 1995.
R. Sánchez & A. Šali, Curr. Opin. Str. Biol. 7, 206, 1997.
M. Marti et al. Ann. Rev. Biophys. Biomolec. Struct., 29, 291, 2000.

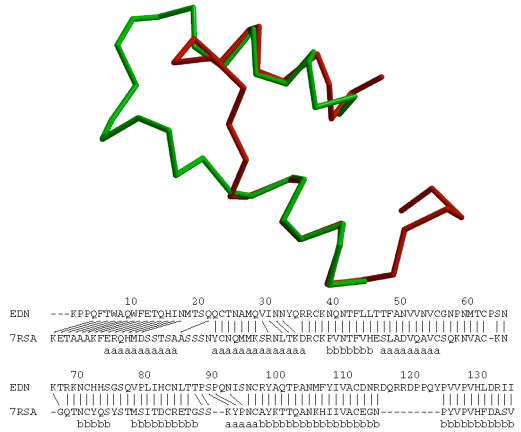
Typical errors in comparative models

MODEL
X-RAY
TEMPLATE

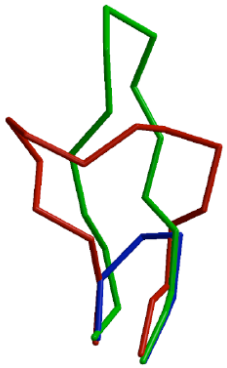
Incorrect template



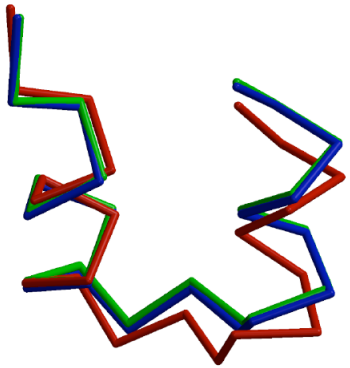
Misalignment



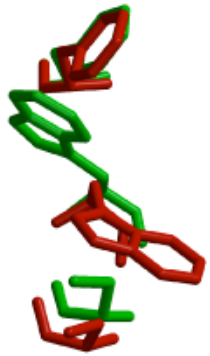
Region without a template



Distortion/shifts in aligned regions

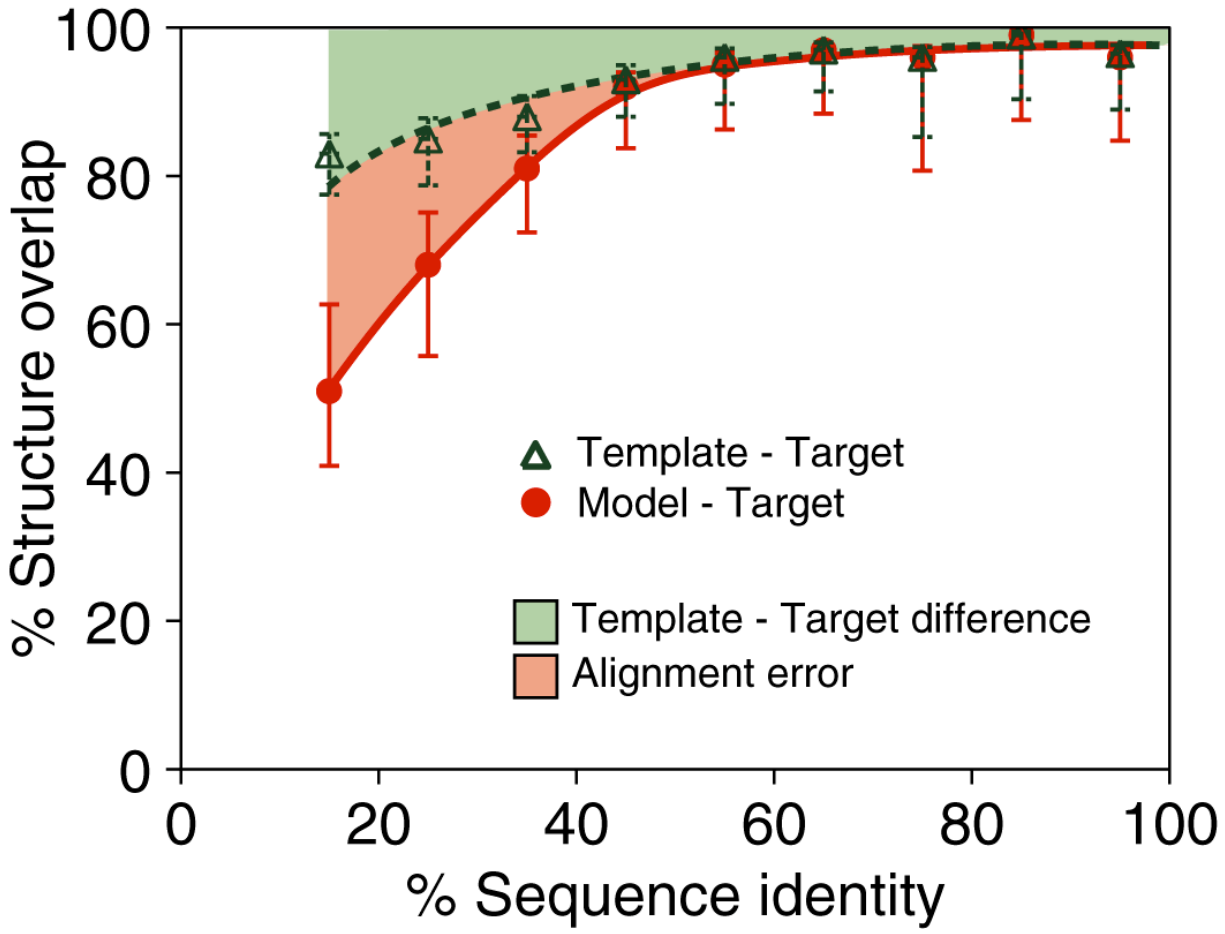


Sidechain packing



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

Model Accuracy as a Function of Target-Template Sequence Identity

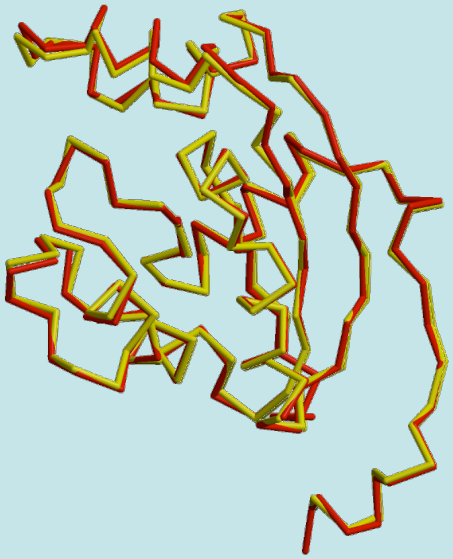


Sánchez, R., Šali, A. Proc Natl Acad Sci U S A. 95 pp13597-602. (1998).

Model Accuracy

HIGH ACCURACY

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

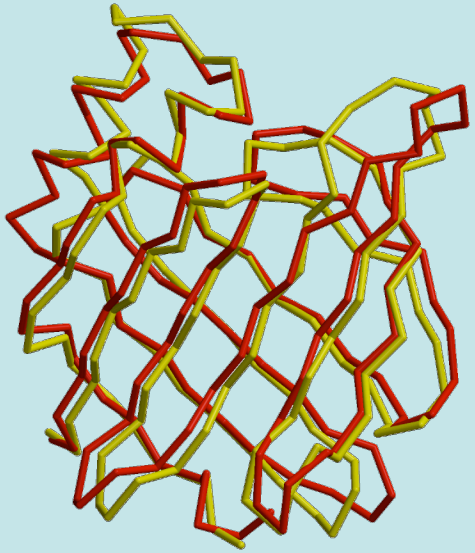


Sidechains
Core backbone
Loops

X-RAY / MODEL

MEDIUM ACCURACY

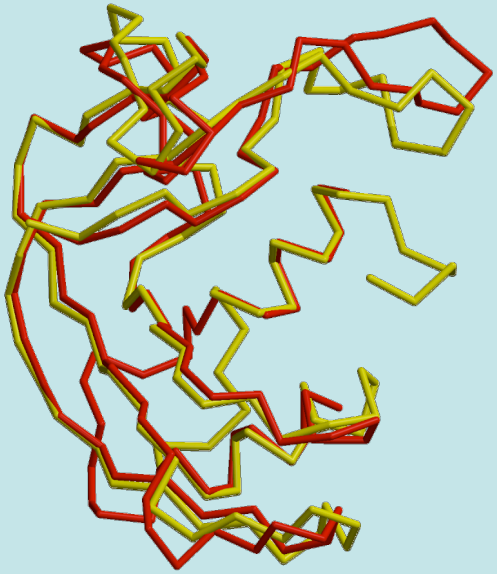
CRABP
Seq id 41%
Cα equiv 122/137
RMSD 1.34Å



Sidechains
Core backbone
Loops
Alignment

LOW ACCURACY

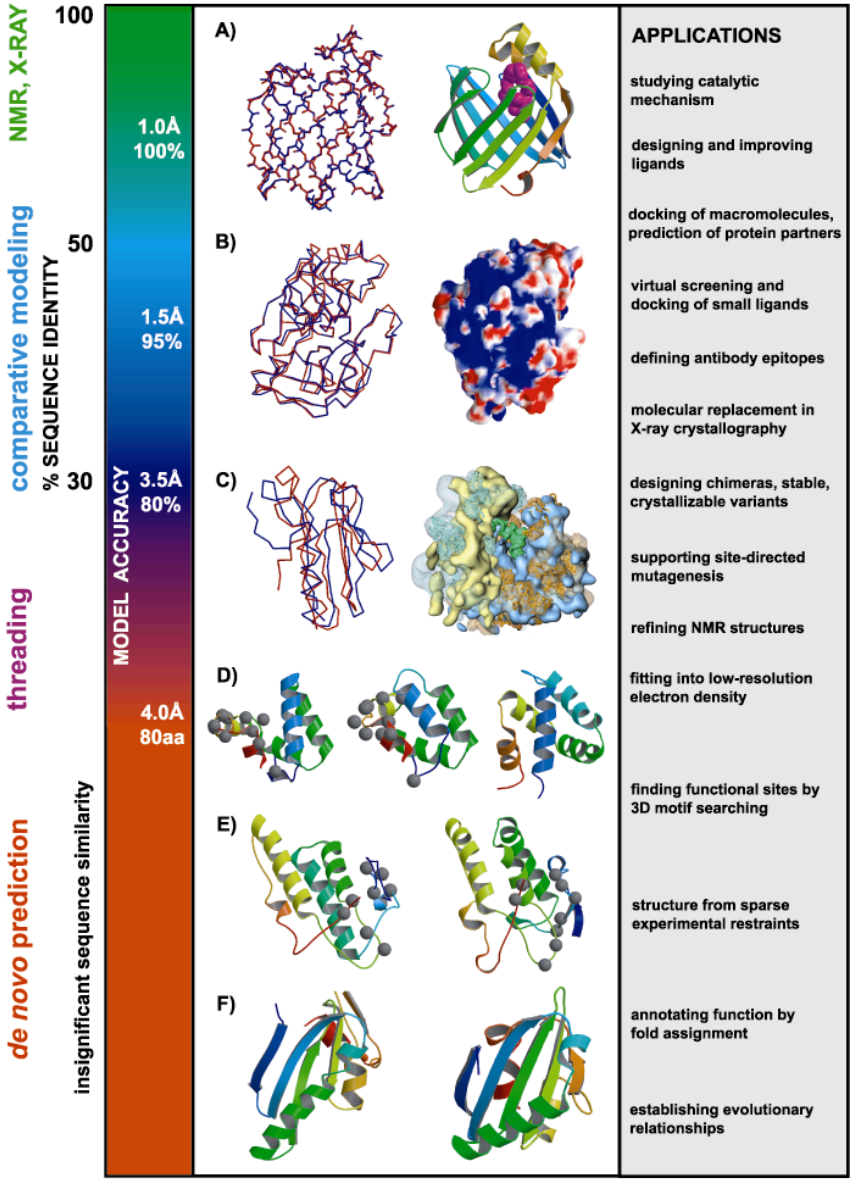
EDN
Seq id 33%
Cα equiv 90/134
RMSD 1.17Å



Sidechains
Core backbone
Loops
Alignment
Fold assignment

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

Applications of Protein Structure Models



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

Obtaining MODELLER and related information

- MODELLER (7v7) web page
- **`http://www.salilab.org/modeller/`**
  - Download Software (Linux/Windows/Mac/Solaris)
  - HTML Manual
  - Join Mailing List


Using MODELLER

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

Using MODELLER

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

Modeling of BLBP
Input

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

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

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

Modeling of BLBP

STEP 1: Align blbp and 1hms sequences

TOP script for target-template alignment

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

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

Modeling of BLBP

STEP 1: Align blbp and 1hms sequences

TOP script for target-template alignment

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

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

Modeling of BLBP

STEP 1: Align blbp and 1hms sequences

TOP script for target-template alignment

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

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

Modeling of BLBP

STEP 1: Align blbp and 1hms sequences

TOP script for target-template alignment

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

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

Modeling of BLBP

STEP 1: Align blbp and 1hms sequences

Output

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

Modeling of BLBP

STEP 1: Align blbp and 1hms sequences

Output

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

Modeling of BLBP

STEP 1: Align blbp and 1hms sequences

Output

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

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

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

Modeling of BLBP

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

TOP script for model building

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

Run by typing `mod model.top`.

Check file `model.log`

Modeling of BLBP

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

TOP script for model building

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

Run by typing `mod model.top`.

Check file `model.log`

Modeling of BLBP

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

TOP script for model building

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

Run by typing `mod model.top`

Check file `model.log`

Modeling of BLBP

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

Output coordinates file

Model file → `blbp.B99990001`

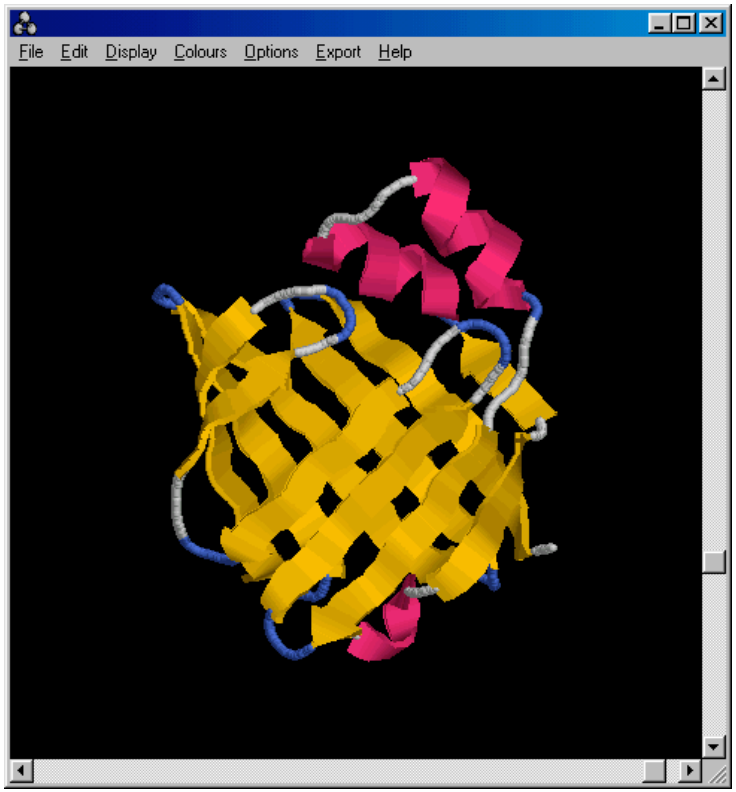
PDB file

Can be viewed with Chimera

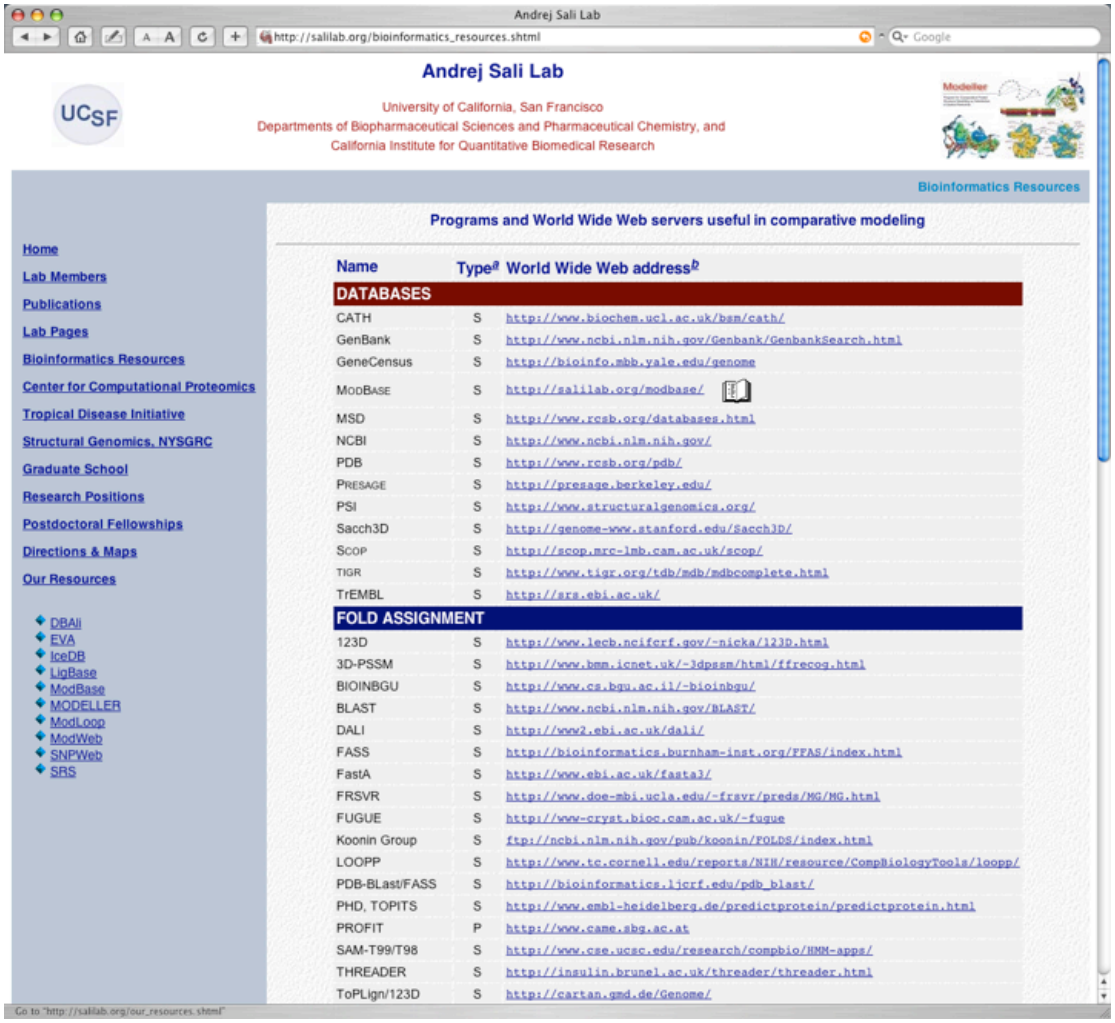
http://www.cgl.ucsf.edu/chimera/

Rasmol

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



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



References

Protein Structure Prediction:

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

Comparative Modeling:

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

MODELLER:

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

Structural Genomics:

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