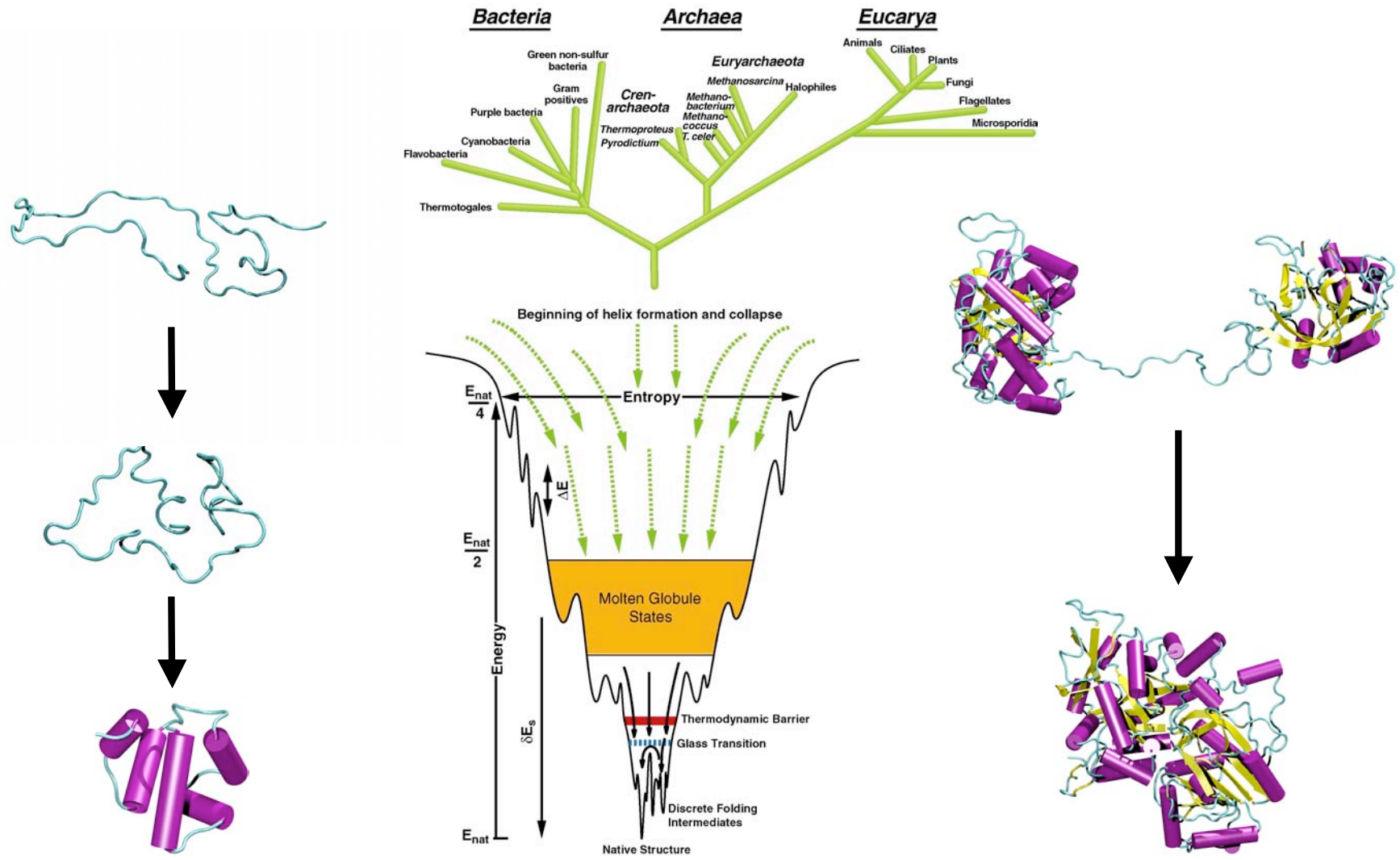


Bioinformatics I - Sequence and Structure Alignments

Z. Luthey-Schulten, UIUC



Sequence-Sequence Alignment (P)

- Smith-Watermann Seq. 1: $a_1 a_2 a_3 - - a_4 a_5 \dots a_n$
- Needleman-Wunsch Seq. 2: $c_1 - c_2 c_3 c_4 c_5 - \dots c_m$

Sequence-Structure Alignment (MS)

- Threading Profile 1: $A_1 A_2 A_3 - - A_4 A_5 \dots A_n$
- Hidden Markov Profile 2: $C_1 - C_2 C_3 C_4 C_5 - \dots C_m$

Structure-Structure Alignment (MS)

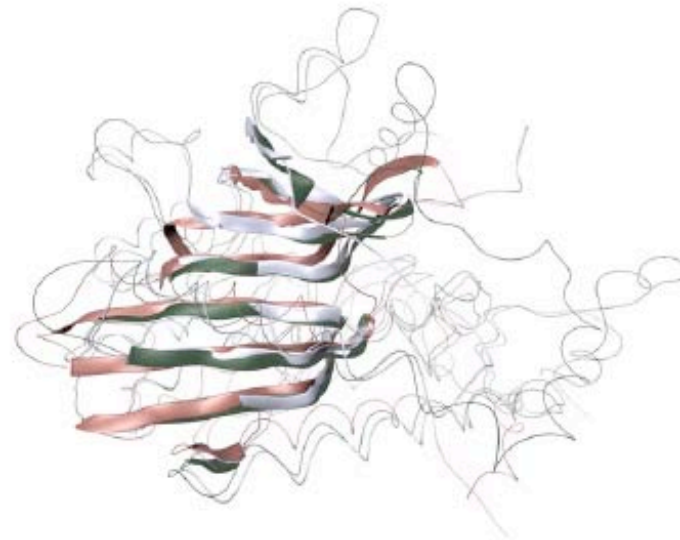
- STAMP - Barton and Russell
- CE - Bourne et al.

Sequence Database Searches (MS)

- Blast and Psi-Blast

University of Illinois at Urbana-Champaign
Luthey-Schulten Group
Theoretical and Computational Biophysics Group
Summer School 2004 - University of Western Australia, Perth

Sequence Alignment Algorithms



Rommie Amaro
Felix Autenrieth
Brijeet Dhaliwal
Barry Isralewitz

Zaida Luthey-Schulten
Anurag Sethi
Taras Pogorelov

June 2004

Sequence Alignment & Dynamic Programming

Seq. 1: $a_1 a_2 a_3 - - a_4 a_5 \dots a_n$
 Seq. 2: $c_1 - c_2 c_3 c_4 c_5 - \dots c_m$

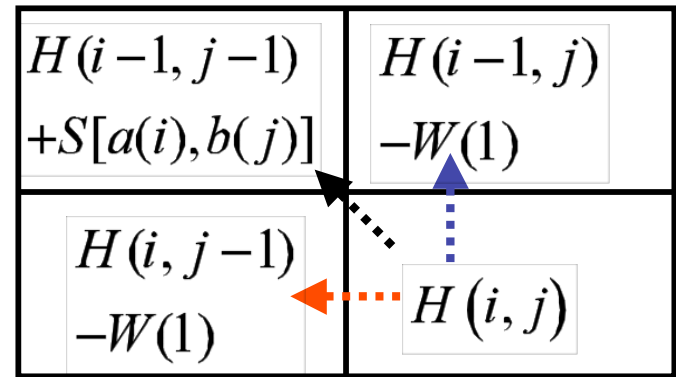


number of possible alignments:

$$= \binom{2n}{n} = 2^{2n} (\sqrt{n\pi})^{-1}$$

Smith-Waterman alignment algorithm

$$H(i, j) = \text{MAX} \begin{cases} H(i-1, j-1) + S[a(i), b(j)] \\ H(i, j-k) - W(k), \\ H(i-m, j) - W(m), 0 \end{cases}$$



S : substitution matrix

Score Matrix H: Traceback

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V	B	Z	X	
A	5	-2	-1	-1	-2	0	-1	1	-2	-1	-2	-1	-1	-3	-2	1	0	-3	-2	0	-1	-1	0	A
R	-2	9	0	-1	-3	2	-1	-3	0	-3	-2	3	-1	-2	-3	-1	-2	-3	-1	-2	-1	0	-1	R
N	-1	0	8	2	-2	1	-1	0	1	-2	-3	0	-2	-3	-2	1	0	-4	-2	-3	4	0	-1	N
D	-1	-1	2	9	-2	-1	2	-2	0	-4	-3	0	-3	-4	-2	0	-1	-5	-3	-3	6	1	-1	D
C	-2	-3	-2	-2	16	-4	-2	-3	-4	-4	-2	-3	-4	-2	-3	-2	-5	-1	-6	-4	-2	-2	-3	C
Q	0	2	1	-1	-4	8	2	-2	0	-3	-2	1	-1	-4	-2	1	-1	-1	-3	0	4	-1	Q	
E	-1	-1	-1	2	-2	2	7	-3	0	-4	-2	1	-2	-3	0	0	-1	-2	-2	-3	1	5	-1	E
G	1	-3	0	-2	-3	-2	-3	8	-2	-4	-4	-2	-2	-3	-1	0	-2	-2	-3	-4	-1	-2	-1	G
H	-2	0	1	0	-4	0	0	-2	13	-3	-2	-1	1	-2	-2	-1	-2	-5	2	-4	0	0	-1	H
I	-1	-3	-2	-4	-4	-3	-4	-4	-3	6	2	-3	1	1	-2	-2	-1	-3	0	4	-3	-4	-1	I
L	-2	-2	-3	-3	-2	-2	-2	-4	-2	2	6	-2	3	2	-4	-3	-1	-1	0	2	-3	-2	-1	L
K	-1	3	0	0	-3	1	1	-2	-1	-3	-2	6	-1	-3	-1	0	0	-2	-1	-2	0	1	-1	K
M	-1	-1	-2	-3	-3	-1	-2	-2	1	1	3	-1	7	0	-2	-2	-1	-2	1	1	-3	-2	0	M
F	-3	-2	-3	-4	-2	-4	-3	-3	-2	1	2	-3	0	9	-4	-2	-1	1	4	0	-3	-4	-1	F
P	-2	-3	-2	-2	-5	-2	0	-1	-2	-2	-4	-1	-2	-4	11	-1	0	-4	-3	-3	-2	-1	-2	P
S	1	-1	1	0	-1	1	0	0	-1	-2	-3	0	-2	-2	-1	5	2	-5	-2	-1	0	0	0	S
T	0	-2	0	-1	-1	-1	-1	-2	-2	-1	-1	0	-1	-1	0	2	6	-4	-1	1	0	-1	0	T
W	-3	-2	-4	-5	-6	-1	-2	-2	-5	-3	-1	-2	1	-4	-5	-4	19	3	-3	-4	-2	-2	W	
Y	-2	-1	-2	-3	-4	-1	-2	-3	2	0	0	-1	1	4	-3	-2	-1	3	9	-1	-3	-2	-1	Y
V	0	-2	-3	-3	-2	-3	-3	-4	-4	4	2	-2	1	0	-3	-1	1	-3	-1	5	-3	-3	-1	V
B	-1	-1	4	6	-2	0	1	-1	0	-3	-3	0	-3	-3	-2	0	0	-4	-3	-3	5	2	-1	B
Z	-1	0	0	1	-3	4	5	-2	0	-4	-2	1	-2	-4	-1	0	-1	-2	-2	-3	2	5	-1	Z
X	0	-1	-1	-1	-2	-1	-1	-1	-1	-1	-1	0	-1	-2	0	0	-2	-1	-1	-1	-1	-1	-1	X

AWGHE
 AW--HE

	H	E	A	G	A	W	G	H	E	E	
	0	0	0	0	0	0	0	0	0	0	
P	0	0	0	0	0	0	0	0	0	0	
A	0	0	0	5	0	5	0	1	0	0	
W	0	0	0	3	0	24	16	8	0	0	
H	0	13	5	0	0	1	16	22	29	21	13
E	0	5	20	12	4	0	8	14	22	36	28
A	0	0	12	25	17	9	1	9	14	28	35
E	0	0	7	17	22	15	7	1	9	21	35

Smith-Waterman Local Alignment Score Matrix

	H	E	A	G	A	W	G	H	E	E
	0	0	0	0	0	0	0	0	0	0
P	0	0	0	0	0	0	0	0	0	0
A	0	0	0	5	0	5	0	1	0	0
W	0	0	0	0	3	0	24	16	8	0
H	0	13	5	0	0	1	16	22	29	13
E	0	5	20	12	4	0	8	14	22	36
A	0	0	12	25	17	9	1	9	14	28
E	0	0	7	17	22	15	7	1	9	21

AWGHE

AW--HE

Blosum 40 Substitution Matrix

A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V	B	Z	X	
5	-2	-1	-1	-2	0	-1	1	-2	-1	-2	-1	-1	-3	-2	1	0	-3	-2	0	-1	-1	0	A
-2	9	0	-1	-3	2	-1	-3	0	-3	-2	3	-1	-2	-3	-1	-2	-2	-1	-2	-1	0	-1	R
-1	0	8	2	-2	1	-1	0	1	-2	-3	0	-2	-3	-2	1	0	-4	-2	-3	4	0	-1	N
-1	-1	2	9	-2	-1	2	-2	0	-4	-3	0	-3	-4	-2	0	-1	-5	-3	-3	6	1	-1	D
-2	-3	-2	-2	16	-4	-2	-3	-4	-4	-2	-3	-3	-2	-5	-1	-1	-6	-4	-2	-2	-3	-2	C
0	2	1	-1	-4	8	2	-2	0	-3	-2	1	-1	-4	-2	1	-1	-1	-1	-3	0	4	-1	Q
-1	-1	-1	2	-2	2	7	-3	0	-4	-2	1	-2	-3	0	0	-1	-2	-2	-3	1	5	-1	E
1	-3	0	-2	-3	-2	-3	8	-2	-4	-4	-2	-2	-3	-1	0	-2	-2	-3	-4	-1	-2	-1	G
-2	0	1	0	-4	0	0	-2	13	-3	-2	-1	1	-2	-2	-1	-2	-5	2	-4	0	0	-1	H
-1	-3	-2	-4	-4	-3	-4	-4	-3	6	2	-3	1	1	-2	-2	-1	-3	0	4	-3	-4	-1	I
-2	-2	-3	-3	-2	-2	-2	-4	-2	2	6	-2	3	2	-4	-3	-1	-1	0	2	-3	-2	-1	L
-1	3	0	0	-3	1	1	-2	-1	-3	-2	6	-1	-3	-1	0	0	-2	-1	-2	0	1	-1	K
-1	-1	-2	-3	-3	-1	-2	-2	1	1	3	-1	7	0	-2	-2	-1	-2	1	1	-3	-2	0	M
-3	-2	-3	-4	-2	-4	-3	-3	-2	1	2	-3	0	9	-4	-2	-1	1	4	0	-3	-4	-1	F
-2	-3	-2	-2	-5	-2	0	-1	-2	-2	-4	-1	-2	-4	11	-1	0	-4	-3	-3	-2	-1	-2	P
1	-1	1	0	-1	1	0	0	-1	-2	-3	0	-2	-2	-1	5	2	-5	-2	-1	0	0	0	S
0	-2	0	-1	-1	-1	-1	-2	-2	-1	-1	0	-1	-1	0	2	6	-4	-1	1	0	-1	0	T
-3	-2	-4	-5	-6	-1	-2	-2	-5	-3	-1	-2	-2	1	-4	-5	-4	19	3	-3	-4	-2	-2	W
-2	-1	-2	-3	-4	-1	-2	-3	2	0	0	-1	1	4	-3	-2	-1	3	9	-1	-3	-2	-1	Y
0	-2	-3	-3	-2	-3	-3	-4	-4	4	2	-2	1	0	-3	-1	1	-3	-1	5	-3	-3	-1	V
-1	-1	4	6	-2	0	1	-1	0	-3	-3	0	-3	-3	-2	0	0	-4	-3	-3	5	2	-1	B
-1	0	0	1	-3	4	5	-2	0	-4	-2	1	-2	-4	-1	0	-1	-2	-2	-3	2	5	-1	Z
0	-1	-1	-1	-2	-1	-1	-1	-1	-1	-1	-1	0	-1	-2	0	0	-2	-1	-1	-1	-1	-1	X

Sequence Alignment & Dynamic Programming

Seq. 1: $a_1 a_2 a_3 - - a_4 a_5 \dots a_n$
 Seq. 2: $c_1 - c_2 c_3 c_4 c_5 - \dots c_m$



number of possible alignments:

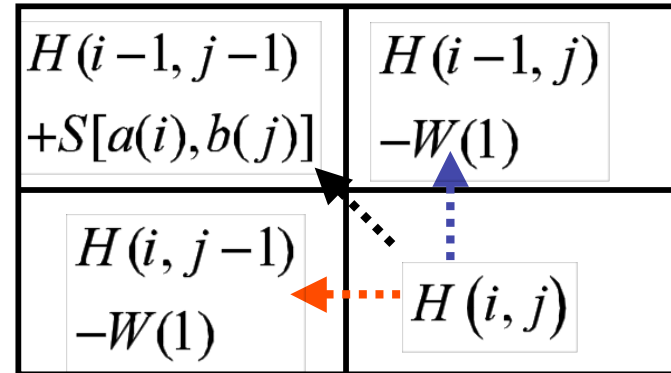
$$= \binom{2n}{n} = 2^{2n} (\sqrt{n\pi})^{-1}$$

Needleman-Wunsch alignment algorithm

$$H(i, j) = \text{MAX} \begin{cases} H(i-1, j-1) + S[a(i), b(j)] \\ H(i, j-k) - W(k), \\ H(i-m, j) - W(m) \end{cases}$$

S : substitution matrix

A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V	B	Z	X		
5	-2	-1	-1	-2	0	-1	1	-2	-1	-2	-1	-1	-3	-2	1	0	-3	-2	0	-1	-1	0	A	
-2	9	0	-1	-3	2	-1	-3	0	-3	-2	3	-1	-2	-3	-1	-2	-3	-1	-2	-1	-2	0	-1	R
-1	0	8	2	-2	1	-1	0	1	-2	-3	0	-2	-3	-2	1	0	-4	-2	-3	4	0	-1	-1	N
-1	-1	2	9	-2	-1	2	-2	0	-4	-3	0	-3	-4	-2	0	-1	-5	-3	-3	6	1	-1	-1	D
-2	-3	-2	-2	16	-4	-2	-3	-4	-4	-2	-3	-3	-2	-5	-1	-1	-6	-4	-2	-2	-3	-2	-2	C
0	2	1	-1	-4	8	2	-2	0	-3	-2	1	-1	-4	-2	1	-1	-1	-1	-3	0	4	-1	-1	Q
-1	-1	-1	2	-2	2	7	-3	0	-4	-2	1	-2	-3	0	0	-1	-2	-2	-3	1	5	-1	-1	E
1	-3	0	-2	-3	-2	-3	8	-2	-4	-4	-2	-2	-3	-1	0	-2	-2	-3	-4	-1	-2	-1	-1	G
-2	0	1	0	-4	0	0	-2	13	-3	-2	-1	1	-2	-2	-1	-2	-5	2	-4	0	0	-1	-1	H
-1	-3	-2	-4	-4	-3	-4	-4	-3	6	2	-3	1	1	-2	-2	-1	-3	0	4	-3	-4	-1	-1	I
-2	-2	-3	-3	-2	-2	-2	-4	-2	2	6	-2	3	2	-4	-3	-1	-1	0	2	-3	-2	-1	-1	L
-1	3	0	0	-3	1	1	-2	-1	-3	-2	6	-1	-3	-1	0	0	-2	-1	-2	0	1	-1	-1	K
-1	-1	-2	-3	-3	-1	-2	-2	1	1	3	-1	7	0	-2	-2	-1	-2	1	1	-3	-2	0	0	M
-3	-2	-3	-4	-2	-4	-3	-3	-2	1	2	-3	0	9	-4	-2	-1	1	4	0	-3	-4	-1	-1	F
-2	-3	-2	-2	-5	-2	0	-1	-2	-2	-4	-1	-2	-4	11	-1	0	-4	-3	-3	-2	-1	-2	-2	P
1	-1	1	0	-1	1	0	0	-1	-2	-3	0	-2	-2	-1	5	2	-5	-2	-1	0	0	0	0	S
0	-2	0	-1	-1	-1	-1	-2	-2	-1	-1	0	-1	-1	0	2	6	-4	-1	1	0	-1	0	-1	T
-3	-2	-4	-5	-6	-1	-2	-2	-5	-3	-1	-2	-2	1	-4	-5	-4	19	3	-3	-4	-2	-2	-2	W
-2	-1	-2	-3	-4	-1	-2	-3	2	0	0	-1	1	4	-3	-2	-1	3	9	-1	-3	-2	-1	-1	Y
0	-2	-3	-3	-2	-3	-3	-4	-4	4	2	-2	1	0	-3	-1	1	-3	-1	5	-3	-3	-1	-1	V
-1	-1	4	6	-2	0	1	-1	0	-3	-3	0	-3	-3	-2	0	0	-4	-3	-3	5	2	-1	-1	B
-1	0	0	1	-3	4	5	-2	0	-4	-2	1	-2	-4	-1	0	-1	-2	-2	-3	2	5	-1	-1	Z
0	-1	-1	-1	-2	-1	-1	-1	-1	-1	-1	0	-1	-2	0	0	-2	-1	-1	-1	-1	-1	-1	-1	X



Score Matrix H: Traceback

??? Tutorial: $W=d$

Needleman-Wunsch Global Alignment

Similarity Values

		M	G	K	P
M		5	-3	-1	-2
G		-3	6	-2	-2
P		-2	-2	-1	7
K		-1	-2	5	-1
K		-1	-2	5	-1
P		-2	-2	-1	7

Initialization of Gap Penalties

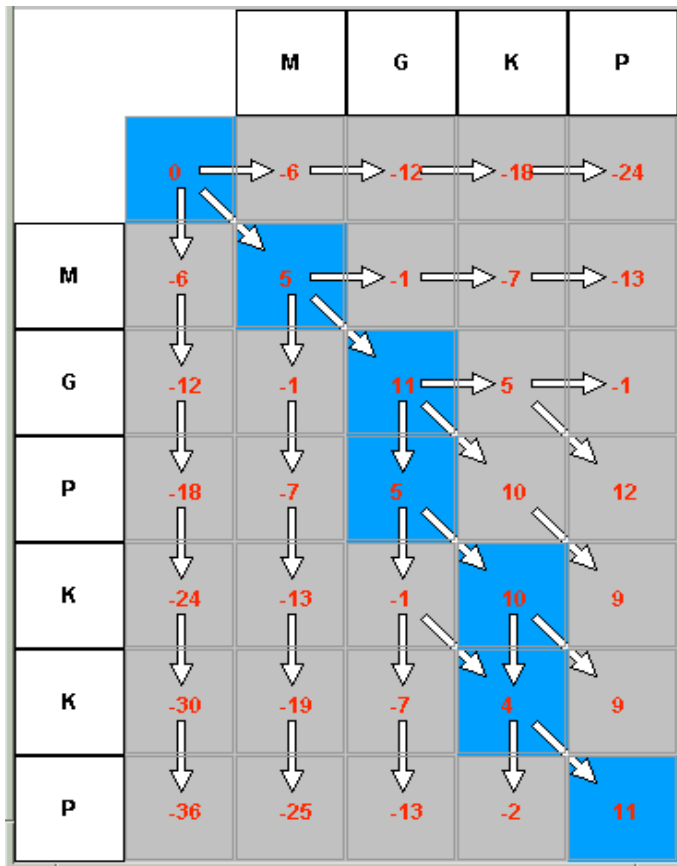
		M	G	K	P
		0			
		-6			
M		5	-3	-1	-2
G		-3	6	-2	-2
P		-2	-2	-1	7
K		-1	-2	5	-1
K		-1	-2	5	-1
P		-2	-2	-1	7

Filling out the Score Matrix H

	M	G	K	P	
	0	-6	-12	-18	-24
M	-6	5	-1	-7	-13
G	-12	-1	11	-2	-2
P	-18	-2	-2	-1	7
K	-24	-1	-2	5	-1
K	-30	-1	-2	5	-1
P	-36	-2	-2	-1	7

	M	G	K	P	
	0	-6	-12	-18	-24
M	-6	5	-1	-7	-13
G	-12	-1	11	5	-1
P	-18	-7	5	10	12
K	-24	-13	-1	10	9
K	-30	-19	-7	4	9
P	-36	-25	-13	-2	11

Traceback and Alignment

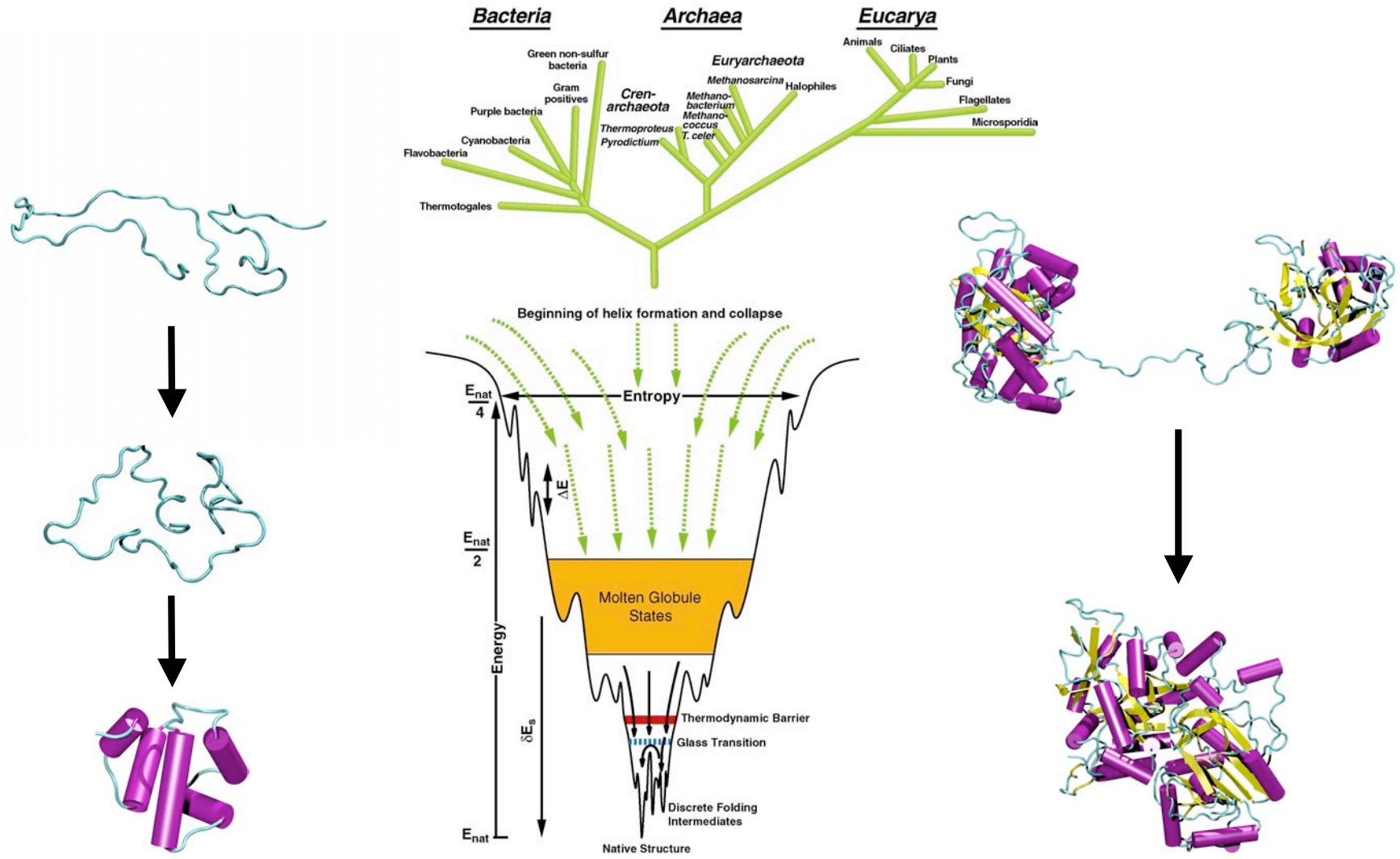


The Alignment

M	G	-	K	-	P
:	:		:		:
M	G	P	K	K	P

Traceback (blue) from optimal score

Energy Landscape Theory of Structure Prediction



Protein Structure Prediction

1-D protein sequence

SISSIRVKS KRIQLG...

Homology Modeling/ FR

$$E = E_{match} + E_{gap}$$

Target Sequence

SISSRVKSKRIQLGLNQAELAQKV-----GTTQ...
 QFANEFKVRRIKLGYTQ-----TNVGEALAAVHGS...

Known structure(s)

3-D protein structure



Ab Initio protein folding

$$E_{AM} = - \sum_{\mu=1}^{N_{\mu}} \sum_{i,j} \left\{ \gamma_{AM} [P_i, P_j, P_i^{\mu}, P_j^{\mu}] \right\}$$

$$X \exp \left[\frac{-(r_{ij} - r_{ij}^{\mu})^2}{2\sigma_{ij}^2} \right]$$

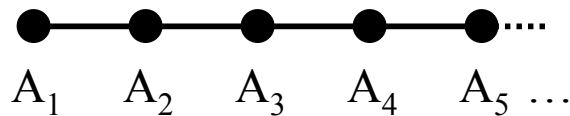
Eastwood, Hardin, Luthey-Schulten, Wolynes (2001)

IBM. J.RES.&DEV.45:475-497

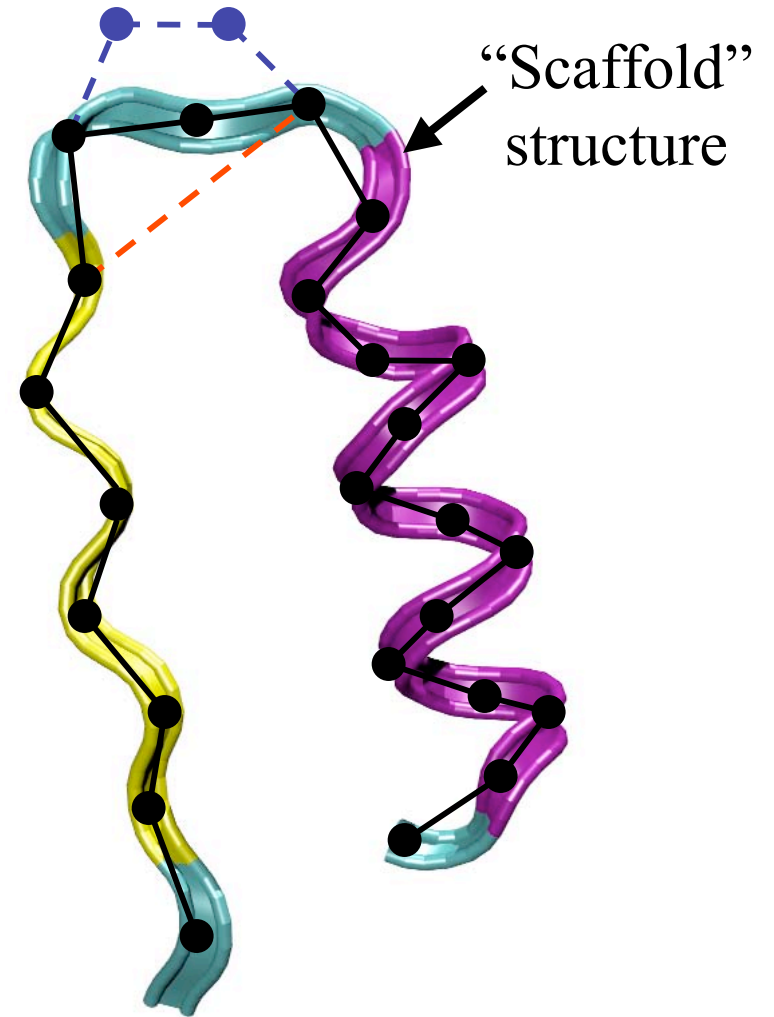
Papoian, et.al. PNAS (2004)

Sequence-Structure Alignment

Target sequence



Alignment between
target(s) and scaffold(s)



1. Energy Based Threading*

$$H = E_{contact} + E_{profile} + E_{H-bonds} + E_{gap}$$

$$E_{profile} = \sum_i^n \gamma^{(p)}(A_i, SS_i, SA_i)$$

$$E_{contact} = \sum_{i,j} \sum_{k=1}^2 \gamma_k^{(ct)}(A_i, A_j) * U(r_k - r_{ij})$$

2. Sequence – Structure Profile Alignments

Clustal, Hidden Markov (HMMER, PSSM)
with position dependent gap penalties

*R. Goldstein, Z. Luthey-Schulten, P. Wolynes (1992, PNAS), K. Koretke et.al. (1996, Proteins)

Gap Penalties

$$E_{gap} = kT \log(P_g)$$

Distribution of Gaps

Sequence-Structure Gap Energy

$$H = E_{contact} + E_{profile} + E_{H-bonds} + E_{gap}$$

$$P_{insertion}(l) = a_1 * \exp(-b_1 l)$$

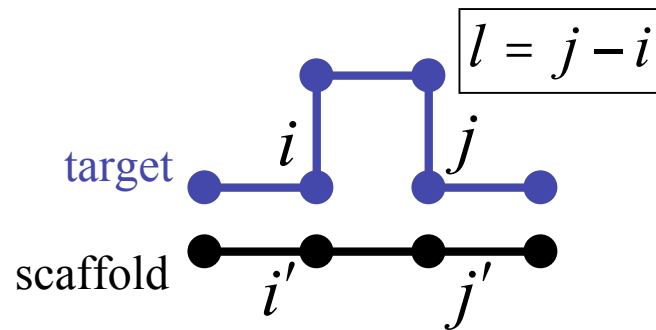
$$P_{deletion}(r) = a_2 * \exp\left(-\frac{(r - b_2)^2}{2\sigma_2^2}\right)$$

$$\text{range} \Rightarrow 3.0 \text{ \AA} < r < 7.5 \text{ \AA}$$

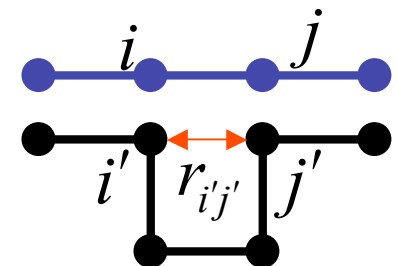
$$P_{bulge}(l, r) = \frac{a_3}{(\sigma_3 l)^{3/2}} * r^2 * \exp\left(-c_3 l - \frac{r^2}{\sigma_3 l}\right)$$

$$\text{range} \Rightarrow r > 4.0 \text{ \AA}$$

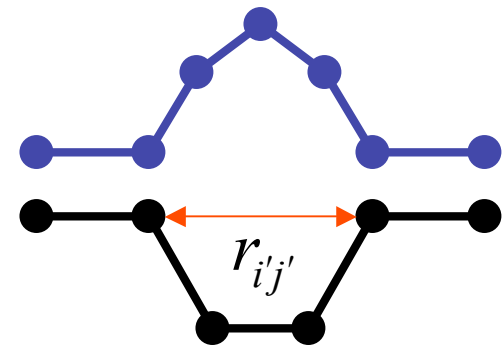
Insertion



Deletion



Bulge



Similarity Measures

Sequence Identity

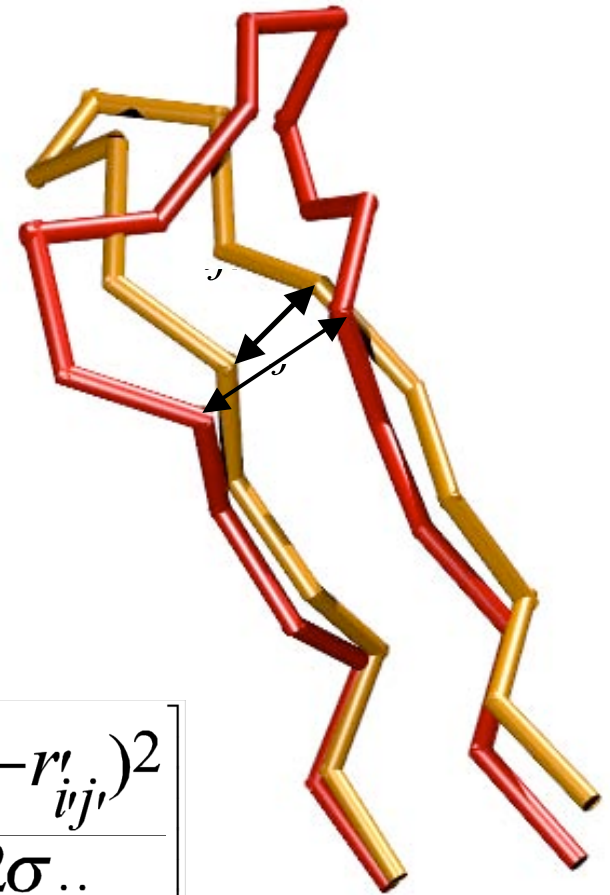
fraction of identically
matched residues

$$S = \frac{N_{match}}{N_{sequence\ length}}$$

Q “Structural Identity”

fraction of native contacts

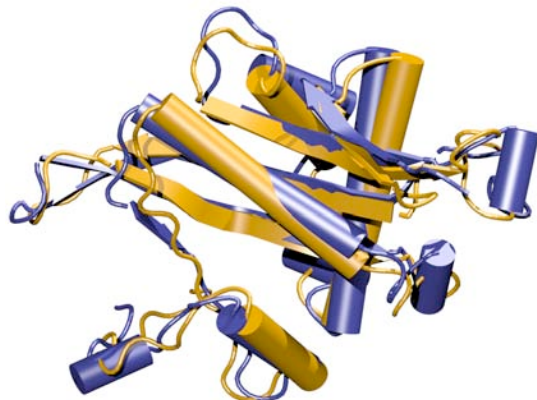
$$Q = \frac{2}{(N_{ALN} - 1)(N_{ALN} - 2)} \sum_{i < j - 1} \exp \left[-\frac{(r_{ij} - r'_{ij})^2}{2\sigma_{ij}} \right]$$



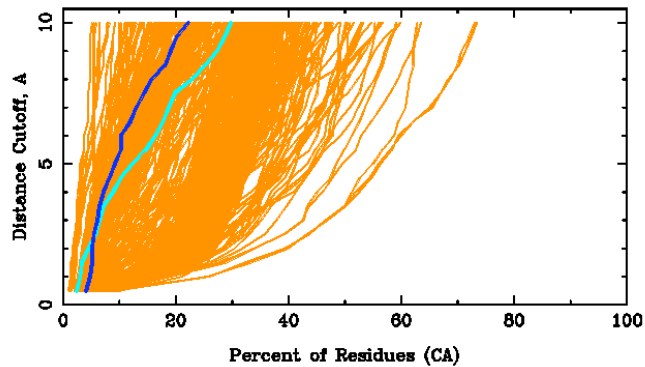
CASP5

Fold Recognition/Threading

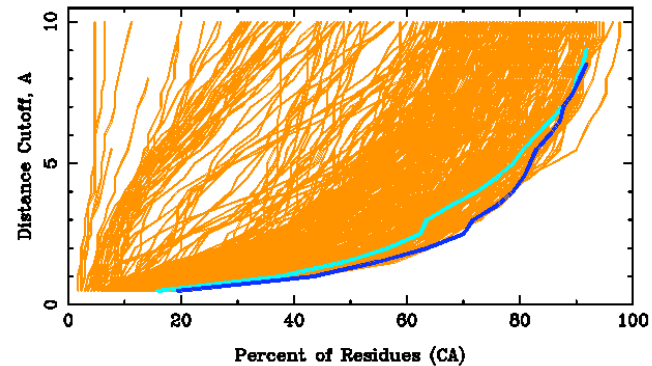
Schulten-Wolynes Group



T017ZTS093_1



T019ZTS093_1



The prediction is never better than the scaffold.

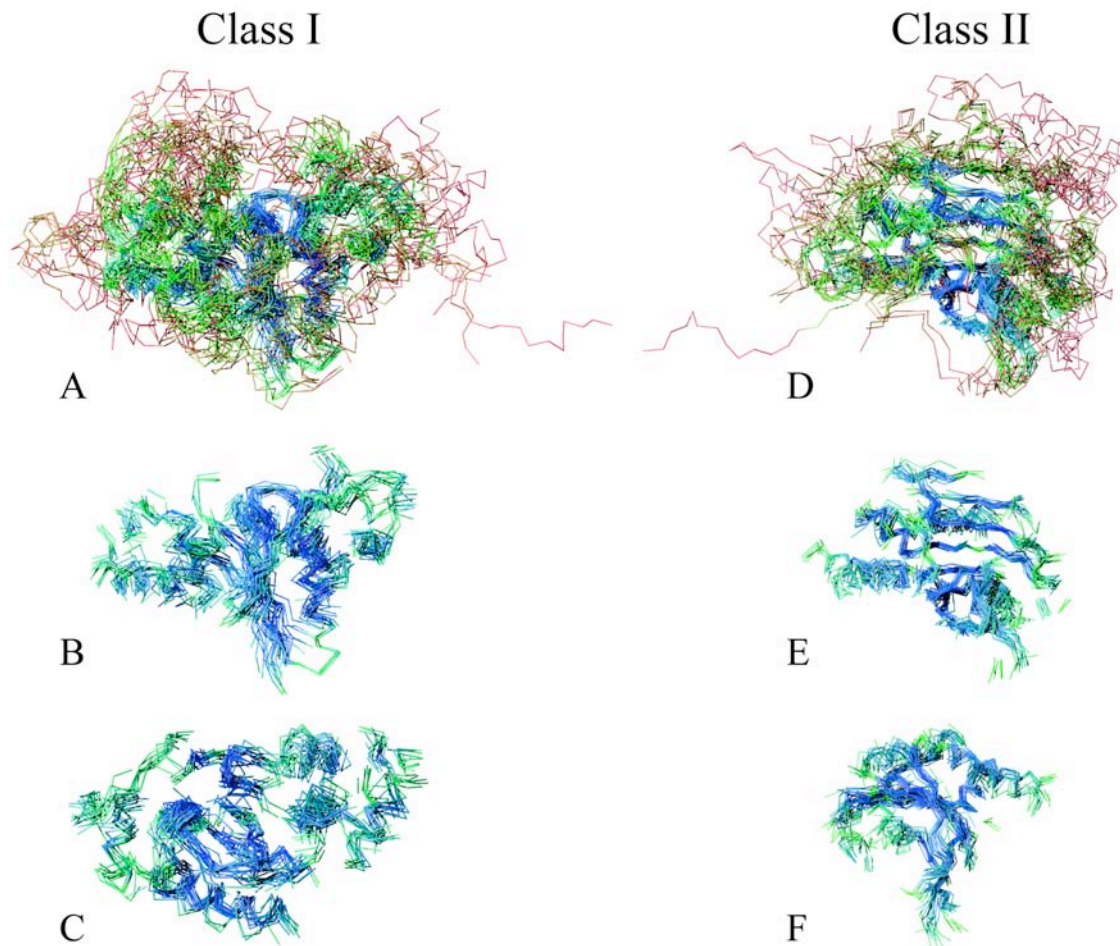
Threading energy function/profiles requires improvement.

Structural Profiles

1. Adding more structural information
2. Structure more conserved than sequences!!! Similar structures at the Family and Superfamily levels
3. Which structures and sequences to include?

Profile - Multiple Structural Alignments

Representative Profile of AARS Family



STAMP - Multiple Structural Alignments

1. Initial Alignment Inputs

- Multiple Sequence alignment
- Ridged Body “Scan”

2. Refine Initial Alignment & Produce Multiple Structural Alignment

$$P_{ij} = \left\{ e^{-d_{ij}^2/2E_1} \right\} \left\{ e^{-s_{ij}^2/2E_2} \right\}$$

probability that residue i on structure A is equivalent to residue j on structure B.

d_{ij} -- distance between i & j

s_{ij} -- conformational similarity; function of rms between $i-1, i, i+1$ and $j-1, j, j+1$.

- Dynamic Programming (Smith-Waterman) through P matrix gives optimal set of equivalent residues.
- This set is used to re-superpose the two chains. Then iterate until alignment score is unchanged.
- This procedure is performed for all pairs.

Multiple Structural Alignments

STAMP – cont'd

2. Refine Initial Alignment & Produce Multiple Structural Alignment

Alignment score:

$$S_C = \frac{S_P}{L_P} \frac{L_P - i_A}{L_A} \frac{L_P - i_B}{L_B}$$

$$S_P = \sum_{aln.path} P_{ij}$$

L_P, L_A, L_B -- length of alignment, sequence A, sequence B

i_A, i_B -- length of gaps in A and B.

Multiple Alignment:

- Create a dendrogram using the alignment score.
- Successively align groups of proteins (from branch tips to root).
- When 2 or more sequences are in a group, then average coordinates are used.

Variation in Secondary Structure STAMP Output



Stamp Output/Clustal Format

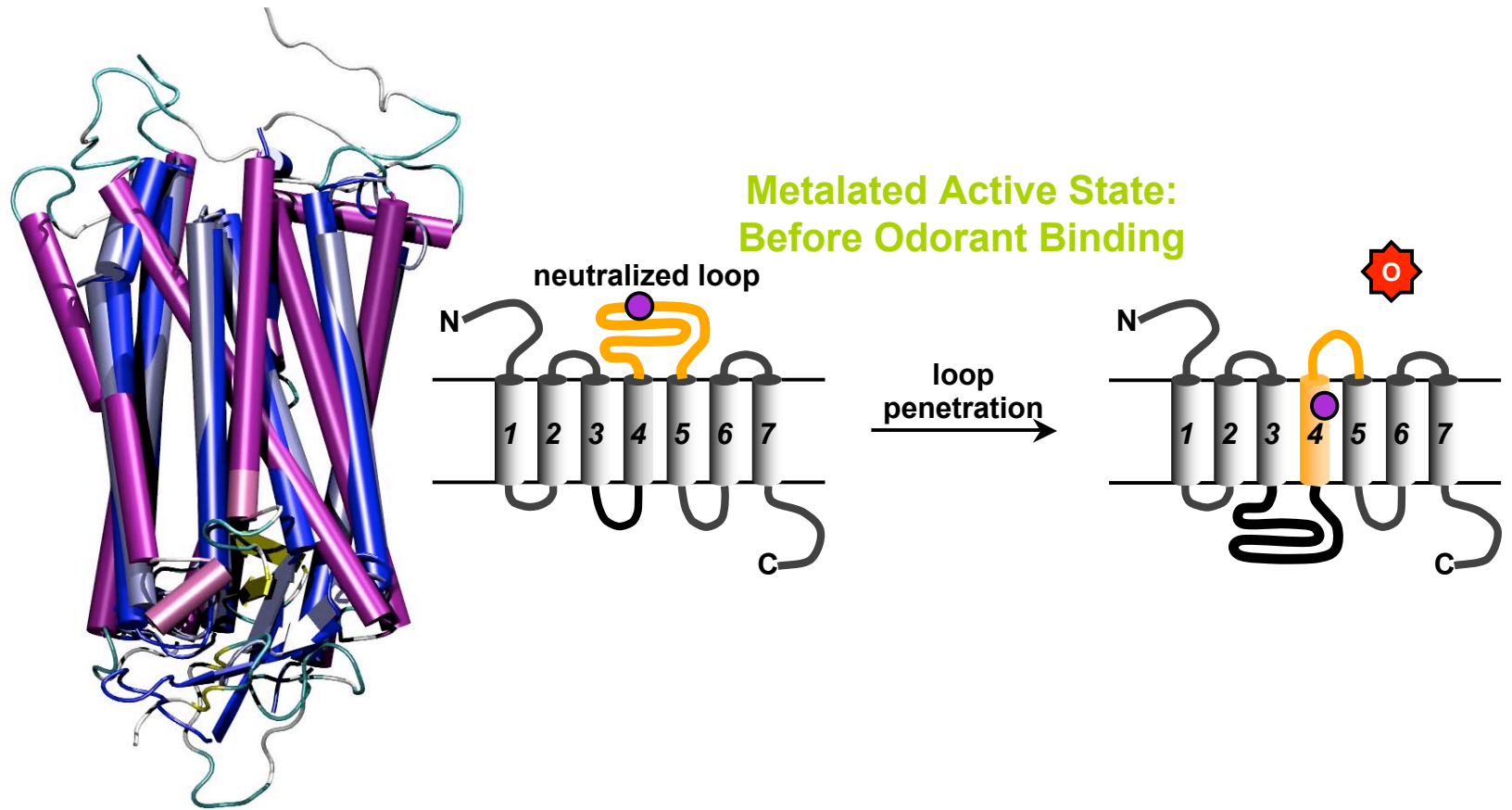
```
SerRS-T_thermophilus      VGGEENREIKRVGGPPEFSFP--P--LDHVALMEKNGWWEPRISQVSGSRSYALKGDLA
ThrRS-E_coli              -----R--DHRKIGKQLDLY-HMQ-EE-APGMVFWHNDGW
ProRS-T_thermophilus      -----KGLTPQSQDFSEWYLEVIQKAEALAD-YG--P-VRGTIVVRPYGY
ProRS-M_thermoautotrophic space -----EFSEWFHNILEEAEIIDQRY--P-VKGMHVWMPHGF
SerRS-T_thermophilus      --SGGG-EEEEES-----SS-----HHHHHHHHT-B-TTHHHH-SS---B-THHH
ThrRS-E_coli              -----HHHHHHHHT-E-E---TT-STT--EE-HHHH
ProRS-T_thermophilus      -----HHHHHHHHHHHHHHTTSEE-E---S-STT-EEE-HHHH
ProRS-M_thermoautotrophic space -----HHHHHHHHHHHHTT-EE-----S-STT--EE-HHHH

SerRS-T_thermophilus      LYELALLRFAMDFMARRGFLPMTLPSYAREK-AFLG-TGHFPAYRDQVWAI-----E--
ThrRS-E_coli              TIFRELEVFVRSKLKEYQYQEVKGPFFMDRV-LWEKT-GHWDNYKDAMFTTS----S-EN
ProRS-T_thermophilus      AIWENIQQVLDRMFKETGHQNAFYPLFIPMSFL-----FSPELAVVTHAGGEELE
ProRS-M_thermoautotrophic space -----MIRKNTLKILRRILD-RDHEEVLFPLLVPEDE-LAKEAIHVKGFEDEVYVWVTHGGLSKLQ
SerRS-T_thermophilus      HHHHHHHHHHHHHHHTT-EEEE--SEEEHH-HHHH-HT-TTTGGGS-B-T-----T--
ThrRS-E_coli              HHHHHHHHHHHHHHHTT-EE----SEEEHH-HHHT-THHHHGGG--EEE----E-TT
ProRS-T_thermophilus      HHHHHHHHHHHHHHHTT-EE----SEESTT-----TT--EEEE-SSSEEE
ProRS-M_thermoautotrophic space -----HHHHHHHHHHHHTT-TT-EE----SEEEHH-HHTSHHHHHHTTTT--EEEEETEEEE

SerRS-T_thermophilus      TDLYLTGTAEVVLNALHSGEILPYEALPLRYAGYAPAFRSEA--GSFGKDVRGLMRVH-Q
ThrRS-E_coli              REYCIKPMNCPGHVQIFNQGLKSYRDLPLRMAEFGSCHR--NEPS--G-SLHGLMRVR-G
ProRS-T_thermophilus      EPLAVRPTSETVIGYMWSKWIRSWRDLPLLNQWGNVVRW--E----M-RTRPFLRTSE-
ProRS-M_thermoautotrophic space -----RKLALRPTSETVMYPMFALWVRSHDLPMPFYQVNTFRY-ET----K-HTRPLIRVREI
SerRS-T_thermophilus      SEEEE-S-THHHHHHHTTT-EEEGGG-SEEEEEEEEE-----S--SSTTTTTTS-S-E
ThrRS-E_coli              EEEEE-S-SHHHHHHHTSS--BTTT-SEEEEE--EEE-----G--G-G-BTTTB-S-E
ProRS-T_thermophilus      EEEEE-S-SHHHHHHHHHH--BGGG--EEEEEEEE-----S-S-BTTTB-SE-
ProRS-M_thermoautotrophic space -----EEEE-SSSHHHHHHHHH--BTTT--EEEEEEEE-----S--BTTTB-SEE
```

From multiple structure alignment compute position probabilities for amino acids and gaps!!!!

Hidden Markov Models of Transmembrane Proteins



Bacteriorhodopsin/Rhodopsins

Olfactory Receptor/Bovine Rhodopsin

J. Wang, Z. Luthey-Schulten, K. Suslick (2003) *PNAS* 100(6):3035-9

Stamp Profile

```
d119ha_3 MNGTEGPNFYVPFSNKTGVVRSPPFEAPQYYLAEPWQFSMLAAYNF[L]GFP[N]FLTLYVTVQH
d1e12a -----R-ENALLS[SLW]NVALAG[IL]FV[NGRT]--IR
d1jgja_1 -----MVGL[LFW]GA[G]GTLAFA[AGRD]--AG

d119ha_3 KKLRTPLNYIL[NLA]ADLFM[FG]TTTLYTSLHG YFV-F-----GPTGCNL
d1e12a PG---RPRLI[GAT]IPL[S]-SSYLG[L]----S--G[TVGM]EMPAGHALA[EMVR]--SQWG
d1jgja_1 S----GERRY[TL]GISG[AA-V]YAV[A]----L--G[GWVP]-----[ERT]--VFVP

d119ha_3 EGF FAT[GGE]A[W-SL]AIERYVVVCKPMSNFRFGENHA[MG]FTWV[A]CAAPPLVGV
d1e12a RY[TWA]STP[I]LA-LGL[A]-----D----[D]GS[FTVIAAD]CTG--LA
d1jgja_1 RY[DWI]LTP[I]SYF-LGL[A]-----G----[DSREF]IVIT[NTV]M[AG]--FA

d119ha_3 SRYIPEGMQCSCGIDYY-[PHEETNNE]FVIYMFVVF[II]PLIV[FF-CY]-QLVFTVKEAAAAT
d1e12a [A]-----M--TTE[AL]--[FRNAF]V[SCA-F]S[LSALVTDW]-[ASA-S]-----
d1jgja_1 [A]-----M--VP-[A]--[ERIAL]G[AV-A]I[G]Y[YL]VGPM-TE[SA-S]-----

d119ha_3 TQKAEKE[TR]V[I]V[A]F[C]LPVAGVAF-Y-IFTHQG[D-FGPIFM]IPAF[AK-T]AVYNP
d1e12a --SA--GTA[E]D[TLR]LTVV[L]G[PIVWA]GVE--G--AL[Q]VGAT[WAYSVLD]FAKYV[F]
d1jgja_1 --QRSSG[K]S[RLRN]LTVV[L]A[IPF]W[L]GPP--G--AL[PTVD]VALIV[L]D[V]KVGF

d119ha_3 V[Y]M-[NKQFR]NCMVTTLCCGKNPLGDST--TVSKTETSQV-APA-----
d1e12a F[LLR]W[AN]-----NERT-----VAV-----
d1jgja_1 F[ALDA-AA]-----
```


Building HMM HMM.982259 ..

Selected Option for HMM Model HMM.982259: build

```
HMMER2.0 [2.2g]
NAME inclustal
LENG 370
ALPH Amino
RF no
CS no
MAP yes
COM /usr/local/bin/hmmbuild /bio/tmp/inclustal.982259.hmm /bio/tmp/inclustal.982259
NSEQ 3
DATE Sun Jun 8 18:12:11 2003
CKSUM 1057
XT      -8455      -4  -1000  -1000  -8455      -4  -8455      -4
NULT      -4  -8455
NULE      595  -1558      85   338  -294   453  -1158   197   249   902  -1085  -142
HMM       A      C      D      E      F      G      H      I      K      L      M      N
          m->m  m->i  m->d  i->m  i->i  d->m  d->d  b->m  m->e
          -567      *  -1622
1  -1029  -1038  -2200  -1928  -323  -2073  -1373   319  -1471   569  4218  -1777
-   -149   -500   233    43   -381   399   106  -626   210  -466  -720   275
-    -31  -6105  -7147  -894  -1115  -701  -1378  -567      *
2  -706  -1410   -63   -215  -1846  -1134  -697  -2058  -581  -2198  -1604  3525
-   -149   -500   233    43   -381   399   106  -626   210  -466  -720   275
-    -31  -6105  -7147  -894  -1115  -701  -1378      *      *
3  -855  -1188  -1421  -1605  -2567  3376  -1671  -2629  -1846  -2761  -2202  -1433
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-    -31  -6105  -7147  -894  -1115  -701  -1378      *      *
4  -101   -603  -1245  -1194  -1643  -916  -1116  -943  -1033  -1432  -944  -909
-   -149   -500   233    43   -381   399   106  -626   210  -466  -720   275
-    -31  -6105  -7147  -894  -1115  -701  -1378      *      *
```

State transition Probabilities (a)

i	$M_i \rightarrow M_{i+1}$	$M_i \rightarrow D_i$	$M_i \rightarrow I_i$
1	0.67	0.33	0
2	0.67	0	0.33
3	1	0	0

Protein X : A B - B A
Protein Y : A - - B A
Protein Z : A A B A A

State π : M_1 M_2 I_2 M_3 M_4
 D_1

M_i - i^{th} Match State
 I_i - i^{th} Insert State
 D_i - i^{th} Delete State

$$\begin{aligned}
 P(x_j, t) &= P(x_j \mid \pi_j = t) \times P(\pi_j = t) \\
 &= e(x_j \mid \pi_j = t) \times a(\pi_j = t \mid \pi_{j-1} = s)
 \end{aligned}$$

New protein aligned to profile with Verterbi
 (Dynamic Programming) algorithm -
 Maximum probability path through state
 transitions.

**Position dependent amino acid
(Emission) Probabilities (e) - PSSM**

i - M	$e(A M)$	$e(B M)$
1	1	0
2	0.5	0.5
3	0.33	0.67
4	1	0

Amino acid probabilities at insert states is background
 probability of occurrence of the corresponding amino acid.

$$P(A|I) = 0.72$$

$$P(B|I) = 0.28$$

Leads to affine gap penalty.

$$P(-|D) = 1.$$

HMMer Profile-Profile Alignment

```
d119ha_3 MNGTEGPNFYVPPFSNKTGVVRSPPFEAPQYYAEPWQFSMLAAYMFLIMLGFPWFFLTYVTVQH
d1e12a -----R-ENALLSSSLWNYVALAGILFVYNGRT--IR
d1at9_1 -----A--Q--TGRPEVIWLALGTALMGLGTLYFVKGMG-VSD
d1jgja_1 -----MVGLTLFWLGAIGMLGTLFAFAWAGRD-A-G

d119ha_3 -KLRTPLNWYILLNLAADLFMFGSTTLYTSLHGYYF-F-----GPTGCN
d1e12a PG-----PRLIAGATIPLE--SYLGLL-----SGTGMEMPAGHALGEMVR--SQW
d1at9_1 P-D---A--FYAITTTPAIAF--MYLML-----LGYGTMVPP-----GEQNP--W
d1jgja_1 S-G---ERYYVTLEGISGIAA-VYAVMA-----LGGWVVP-----AERT--V

d119ha_3 LEGFFATLGGELAW-SL--SAIERVYVVKPMSNFRFGENHAMG--FTWYMAACAAPPLG
d1e12a RYTWALTP--LLA-LGL--A-----D---DGGFTVIADGMCVTG-L-
d1at9_1 RYADWFTTPL--LLD-LGL--V-----D---ADQGLA--ADGINGTG-L-
d1jgja_1 PRYDWLTTPL--LYF-LGL--A-----G---DSREFIVITLTVVLAG-F-

d119ha_3 WSRYPPEGMQCSCGIDYYPHEETNNEFVIYMFVVHFIPLIVFFCYG-QLV-FTVKEAAAA
d1e12a A--A-----M-TTAL--LRFAFAISCA-FF--LSALVTD--AAS--AS-----
d1at9_1 VGA-----L-T-KVY--SRVFAISTA-AMVLYVLFFG--TSK--A-----
d1jgja_1 AGA-----M-V-P-G--IERAL--GAV-AFIG--YYLVGPH--TES--AS-----

d119ha_3 TTQ-KAEKEVTRV--VIAF--CLPAGVAF-Y-IFTHQC-D-FGPIFMTPAFFAK--AVY
d1e12a ---SA--GTAEFDTLRLVTVVLELTPVVA--GVE--G--ALVQV--GATVA--SVLDVFAKYV
d1at9_1 ---ESMRPEVASTFKLRN--TVVLS--YVWLGSE--GA--V-PLN--TL--VLDV--AKVG
d1jgja_1 ---Q-RSSG--KSL--RLRNLTVVLELTPVWLGPP--G--AL--PT--VALI--YLD--VTKVG

d119ha_3 NPVY--M--NKFRNCMVTTLCGKNPLGDS--TTVSKTETSQVAPA
d1e12a F--LL--RW--AM-----ERTV-----AV--
d1at9_1 FGL--LLRSRA-I-F-----G-----
d1jgja_1 FGF--ALD--A-A-A-----
```

Clustal Profile-Profile Alignment

```
d119ha_3      NNGTEGPNFYVPPFSNKTGVVRSPPFEAPQYYLAEPWQFSNLAAYMFLLEGGFMMLTLY
die12a      -----R-ENALLSSEWENALAGLFLFVGR
d1jgja_1     -----VGLLFWLAIGMLGTLAFAAGR
IAT9__BACTERIO -----XATGRPEWVWLTALGGLTLEVV

d119ha_3      VTVQHKLRTPLNYYILLNLAADLFNFGSSTLYTSLNGYV-F-----
die12a      T--RPG---RPRLIGATIPLE---SSYLGLL-----S--GLTGMEMPAGHALA
d1jgja_1     D--AGS---GERYYVTLGISGIAA---YAA---L--GCWVP-----
IAT9__BACTERIO GNGSDP---DA---FYAITT---PAIAFTVLLG-----GLTVVFP-----

d119ha_3      ---GPTGCNLEGGFATLGGEA---W-SL---LAIERYVVVCKPMSNFRFGENHAMG---FT
die12a      ENVR--SQWRYTWALTP---LLA-LG---L-A-----D---DGLFTV
d1jgja_1     -ERT--FVPRYDWTTP---GYF-LG---L-A-----G---DSREFIV
IAT9__BACTERIO -GEQNP---WRYADWFTTPLL---LDLALLD-----ADQQLA

d119ha_3      WVMAACAAPPLVGSRYIPEGNQCSGIDYY---PHEETMNEFVIYMFVVHFIPLIV
die12a      IADGMCVTG--LA---M--TTGL--LRRAF--AISCA--FF--LSAL
d1jgja_1     ITLTVVLAG--FAGA-----M--VP---IERAL---GAV-AFIG---YYL
IAT9__BACTERIO ADGIMGTG--LVGA-----LTKVYSRVAISTA-AM---LYVL

d119ha_3      FF-CYG-QLVFTVKEAAAATTQKAEKEVTRV---VIAF---CLPAGVAF-Y-IFTHQG
die12a      VTD--AASA-S-----SA--GTAEFDTLRVLTVVLLVFWVA---GVE--G-
d1jgja_1     VGPM-TESA-S-----QRSSGKSLRLRNLTVVLAIPFFWL---GPP--G-
IAT9__BACTERIO FFGTSKLE-----SMRPEVASTFKLRN---TVVLSNPFVWL---GSE---G

d119ha_3      D-FGPIFMTPAFFAK---AVYNPVY---M---NKQFRNCMVTTLCCGKNPLGDST--TVS
die12a      ALQVGATVA---SVLDVFAKYVFF---LLRW---AH-----NERT--
d1jgja_1     AL---PT---VALIYLDVTKVGFGEALDA-AA-----
IAT9__BACTERIO AGVPLN---L---VLDYAKVGFGL---LLRSRAIFG-----EAEAP

d119ha_3      KTETSQV-APA
die12a      -----YAV-
d1jgja_1     -----
IAT9__BACTERIO EPSADGAAATS
```

Refine Structure Prediction with Modeller 6.2



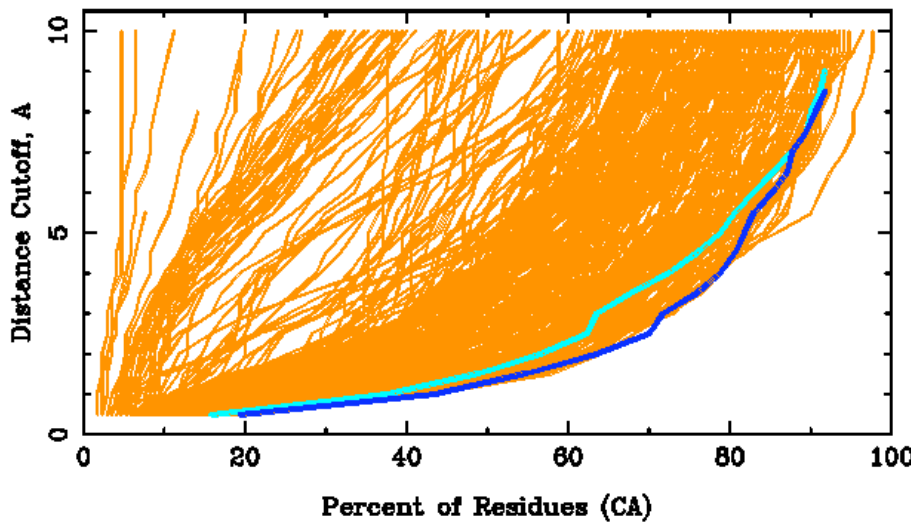
Sethi and Luthey-Schulten, UIUC 2003

Modeller 6.2 A. Sali, et al.

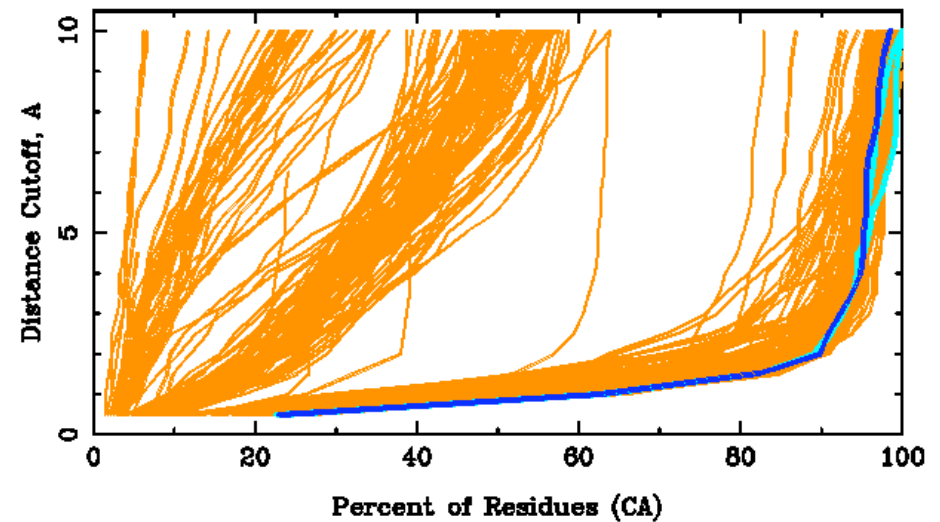
CM/Fold Recognition Results from CASP5

Lessons Learned

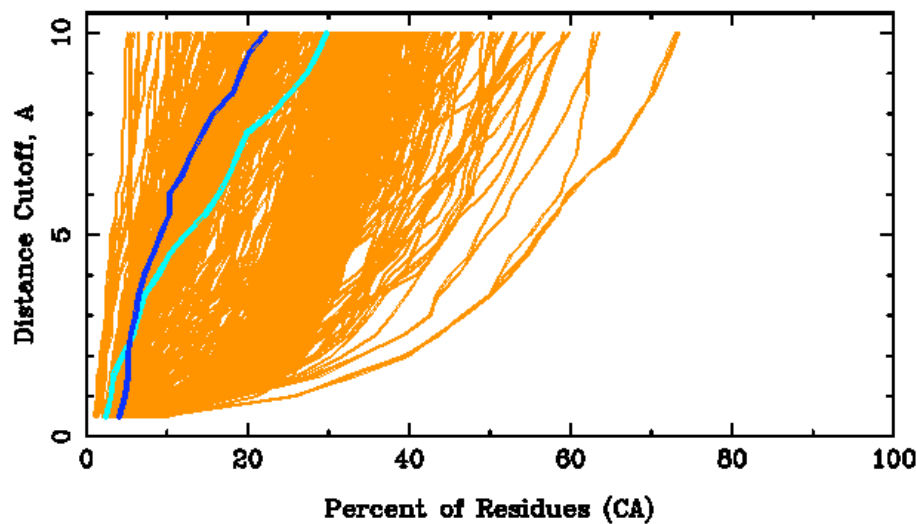
T0192TS093_1



T0179TS093_1



T0172TS093_1



The prediction is never better than the scaffold.

Threading Energy Function and Profiles need improvement.

Evolutionary Profiles

We need non-redundant, evolutionary profiles! True representative sets of protein sequences and structures from which to draw correct statistical inferences. You are now entering the twilight zone of sequence identity. Only structures and evolution can help you!!!

Watch for Bioinformants!!!

Profiles – Evolution Revisited

- “What molecular sequences taught us in the 1960’s was that the genealogical history of an organism is written to one extent or another into the sequences of each of its genes, an insight that became the central tenet of a new discipline, molecular evolution”

- Woese (PNAS, 2000)

Pauling (1965)

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Collaborators

Evolutionary Studies

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Algorithms

Mike Heath (UIUC)

Rob Russell (EMBL) **STAMP**

Protein Structure Prediction

Peter Wolynes, Jose Onuchic,
Ken Suslick