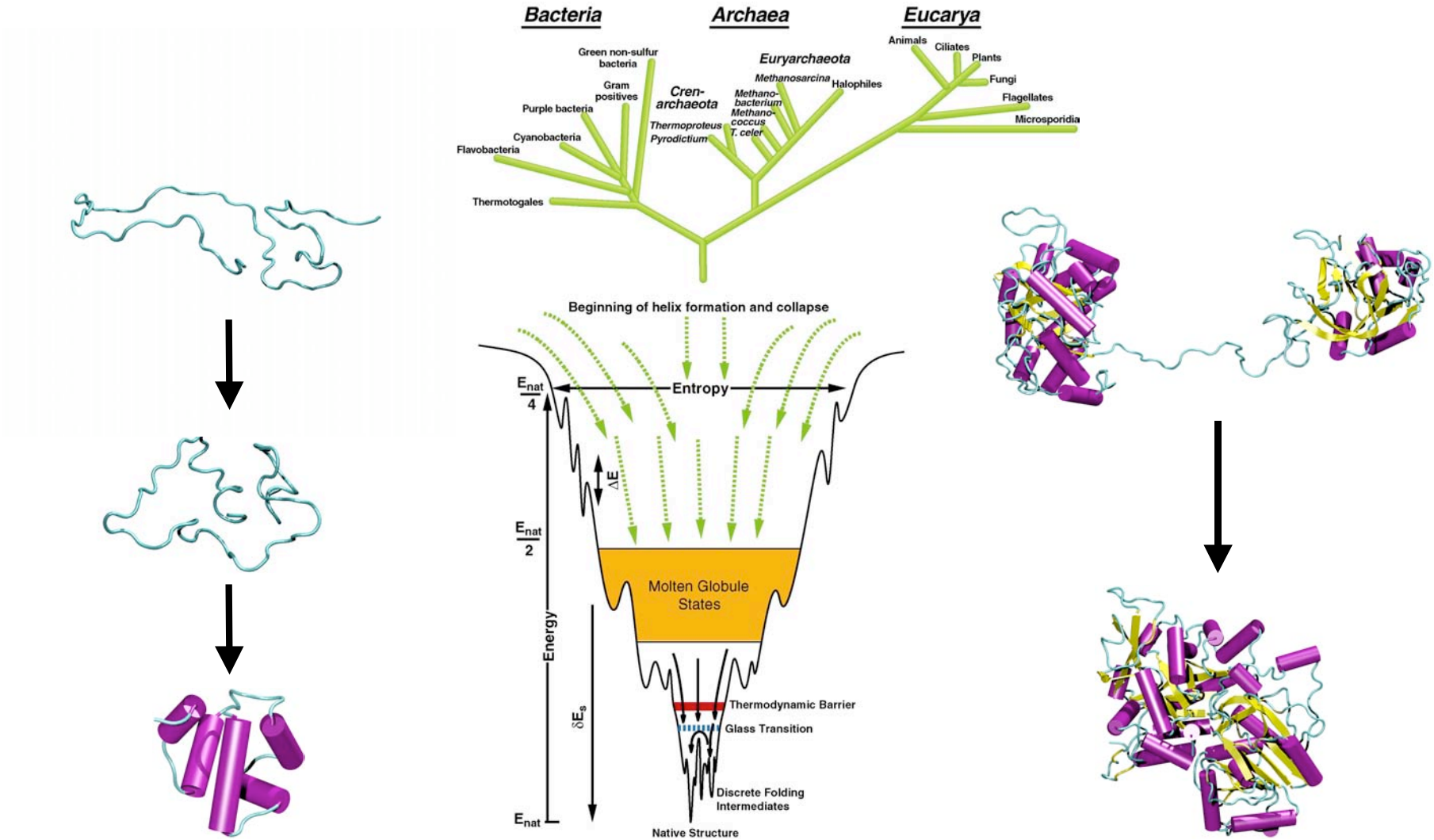
Bioinformatics I - Sequence and Structure Alignments
Z. Luthey-Schulten, UIUC



Perth, 2004

Sequence-Sequence Alignment (P)

- Smith-Watermann
- Needleman-Wunsch

Seq. 1: $a_1\ a_2\ a_3$ - - $a_4\ a_5 \ldots a_n$

Seq. 2: c_1 - $c_2\ c_3\ c_4\ c_5$ - $\ldots c_m$

Sequence-Structure Alignment (MS)

- Threading
- Hidden Markov

Profile 1: $A_1\ A_2\ A_3$ - - $A_4\ A_5 \ldots A_n$

Profile 2: C_1 - $C_2\ C_3\ C_4\ C_5$ - $\ldots C_m$

Structure-Structure Alignment (MS)

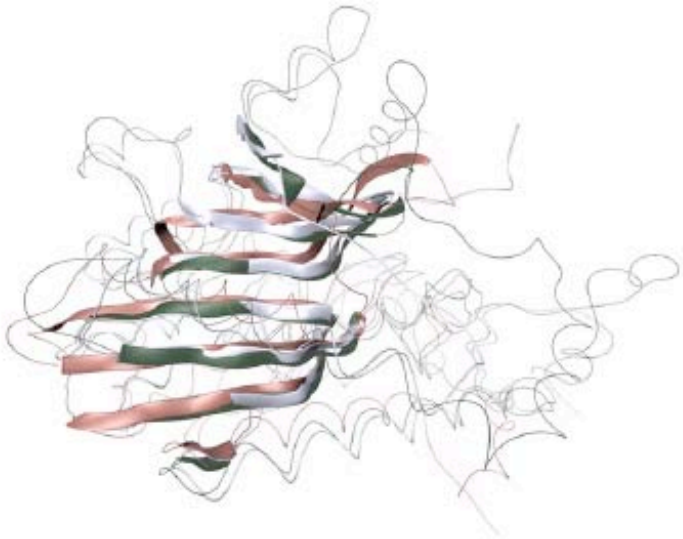
- STAMP - Barton and Russell
- CE - Bourne et al.

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



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June 2004

Sequence Alignment & Dynamic Programming

Seq. 1: $a_1\ a_2\ a_3$ - - $a_4\ a_5 \ldots a_n$

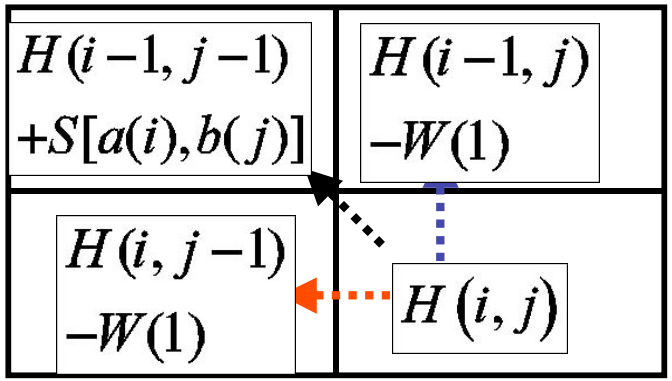
Seq. 2: c_1 - $c_2\ c_3\ c_4\ c_5$ - $\ldots c_m$

number of possible alignments:

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

Smith-Waterman alignment algorithm

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



S : substitution matrix

	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	-2	-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	-3	-2	-5	-1	-1	-6	-4	-2	-2	-3	-2	C
Q	0	2	1	-1	-4	8	2	-2	0	-3	-2	1	-1	-4	-2	1	-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	-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	-1	0	-1	-2	0	0	-2	-1	-1	-1	-1	-1	X

Score Matrix H: Traceback

AWGHE
AW--HE

		H	E	A	G	A	W	G	H	E	E
	0	0	0	0	0	0	0	0	0	0	0
P	0	0	0	0	0	0	0	0	0	0	0
A	0	0	0	5	0	5	0	1	0	0	0
W	0	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	0
P	0	0	0	0	0	0	0	0	0	0	0
A	0	0	0	5	0	5	0	1	0	0	0
W	0	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

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

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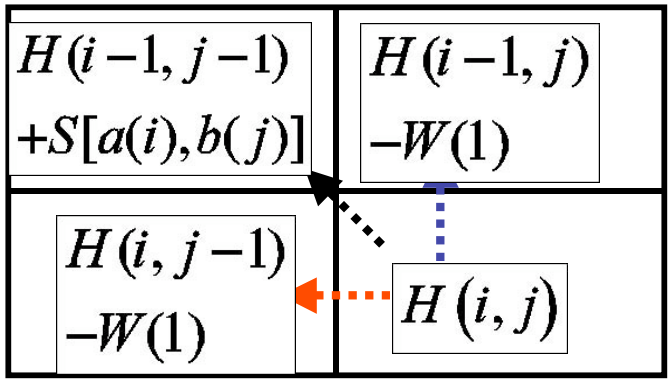
$$H(i,j) = MAX\begin{cases} H(i-1,j-1) + S[a(i),b(j)] \\ H(i,j-k) - W(k), \\ H(i-m,j) - W(m) \end{cases}$$



Score Matrix H: Traceback

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	
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	-2	-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	-3	-2	-5	-1	-1	-6	-4	-2	-2	-3	-2	C
Q	0	2	1	-1	-4	8	2	-2	0	-3	-2	1	-1	-4	-2	1	-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	-4	-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	-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	-1	0	-1	-2	0	0	-2	-1	-1	-1	-1	-1	X

??? Tutorial: W=d

Needleman-Wunsch Global Alignment

Similarity Values

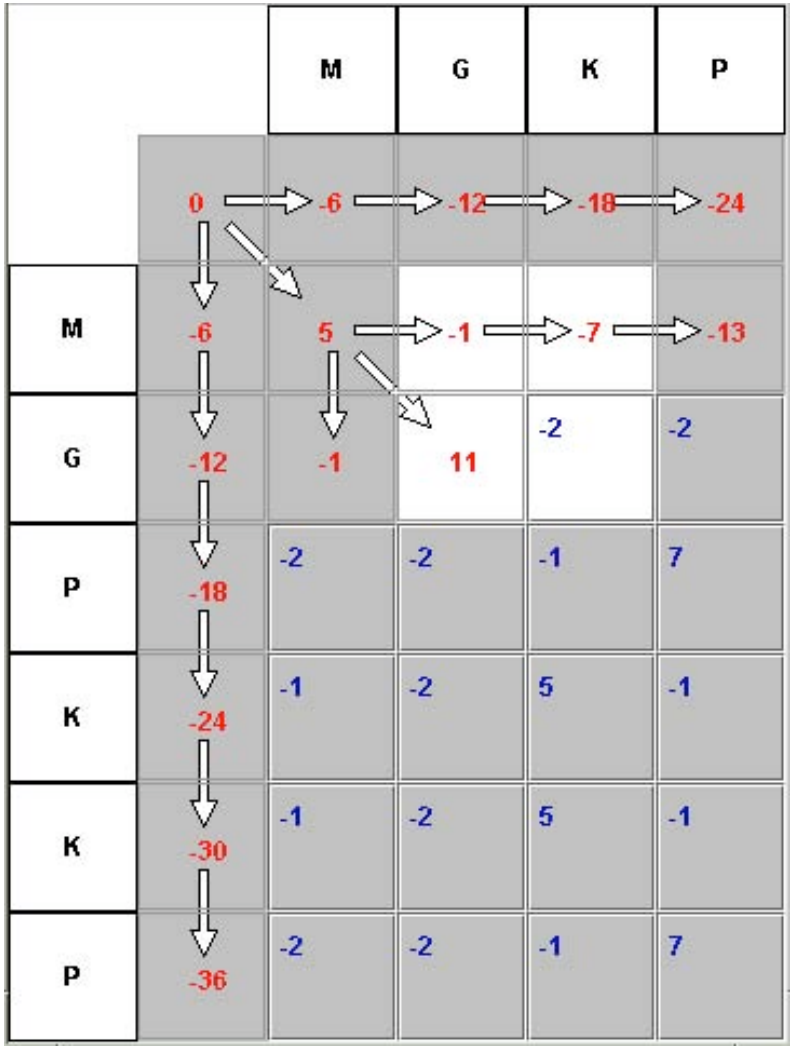
		M	G	K	P
M		5	-3	-1	-2
G		-3	6	-2	-2
P		-2	-2	-1	7
K		-1	-2	5	-1
K		-1	-2	5	-1
P		-2	-2	-1	7

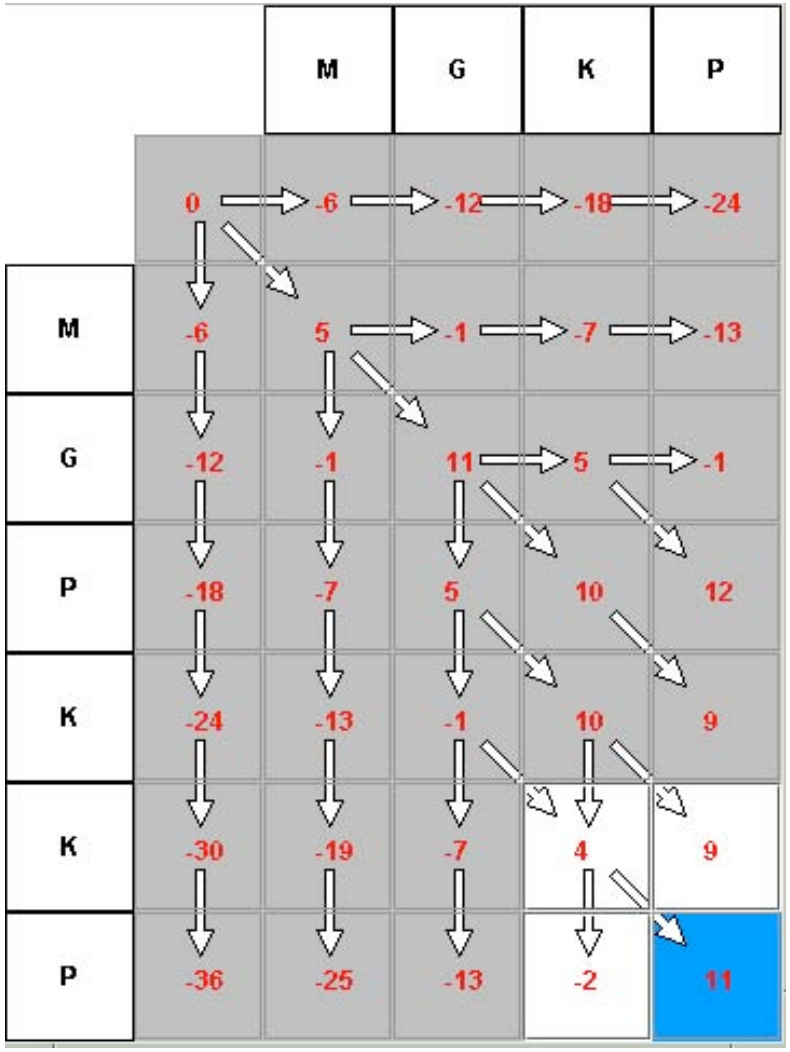
Initialization of Gap Penalties

		M	G	K	P
	0	-6	-12	-18	-24
M	-6	5	-3	-1	-2
G	-12	-3	6	-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

http://www.dkfz-heidelberg.de/tbi/bioinfo/PracticalSection/AliApplet/index.html

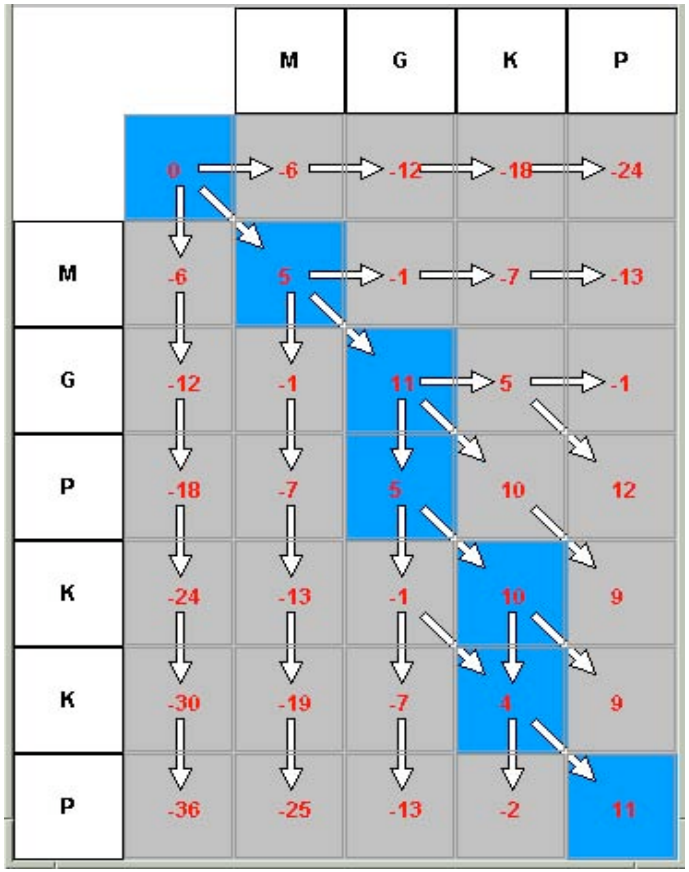
Filling out the Score Matrix H





http://www.dkfz-heidelberg.de/tbi/bioinfo/PracticalSection/AliApplet/index.html

Traceback and Alignment



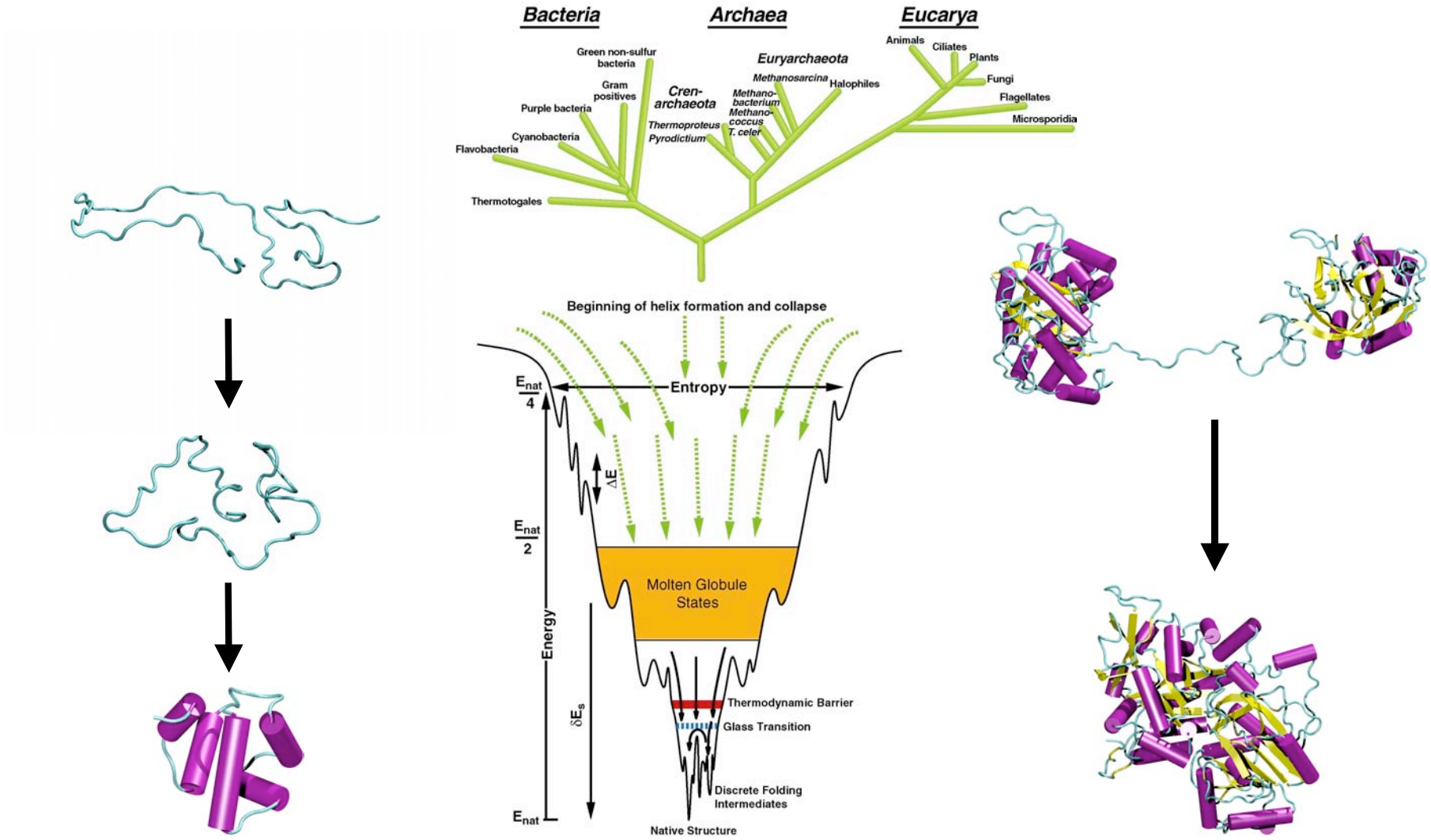
The Alignment

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

Traceback (blue) from optimal score

http://www.dkfz-heidelberg.de/tbi/bioinfo/PracticalSection/AliApplet/index.html

Energy Landscape Theory of Structure Prediction



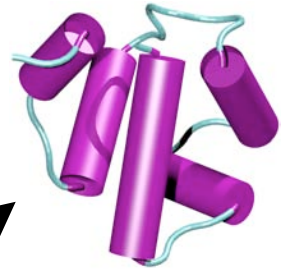
Protein Structure Prediction

1-D protein sequence

3-D protein structure

SISSIRVKSKRIQLG….

Ab Initio protein folding



Seq-Str Alignment

Target protein of unknown structure → SISSRVKSKRIQLGLNQAELAQKV------GTTQ···

Homologous/analogous protein of **known** structure → QFANEFKVRRIKLGYTQTNVGEALAAVHGS···

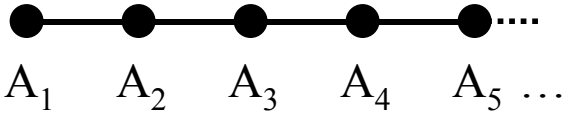
Sequence -Structure Alignment: the Energy Function

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

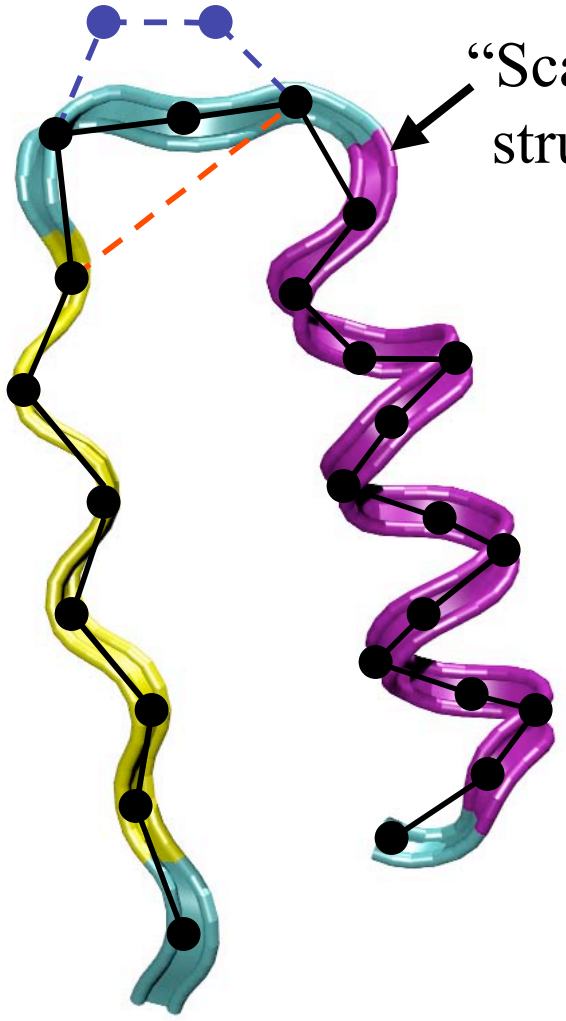
$E_{match} =$

Threading: Sequence-Structure Alignment

Target sequence



threading alignment
between target and scaffold



Threading Energy Function

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

$$E_{profile} = \sum_{i}^{n} \gamma^{(p)}\left(A_i, SS_i, SA_i\right)$$

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

$$E_{gap}(r,l) = \gamma_g \log\left(P_g\right)$$

R. Goldstein, Z. Luthey-Schulten, P. Wolynes (1992, PNAS)

Gap Penalties

Distribution of Gaps

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

Sequence-Structure Gap Energy

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

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

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

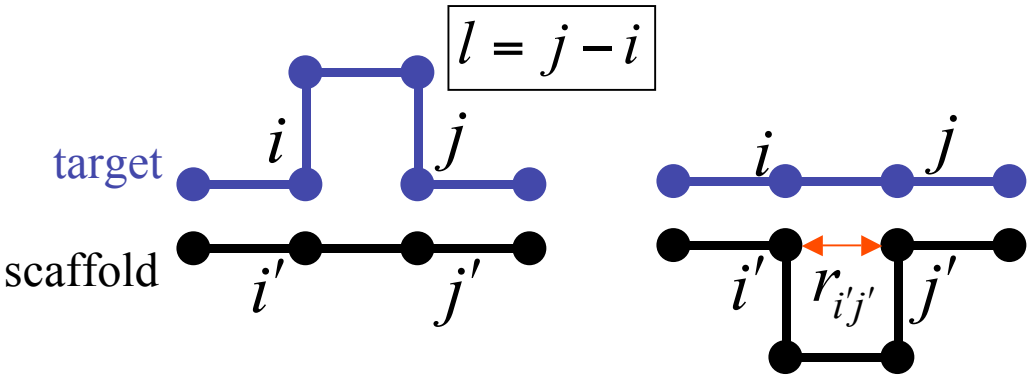
range $\Rightarrow 3.0\,\text{Å} < r < 7.5\,\text{Å}$

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

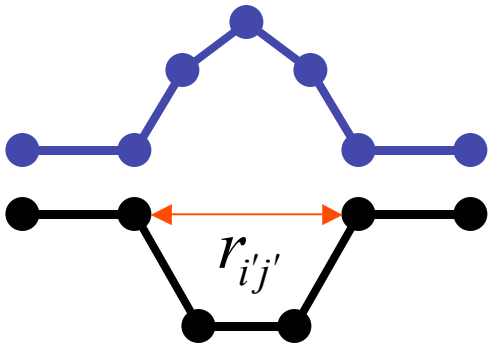
range $\Rightarrow r > 4.0\,\text{Å}$

Insertion

Deletion



Bulge



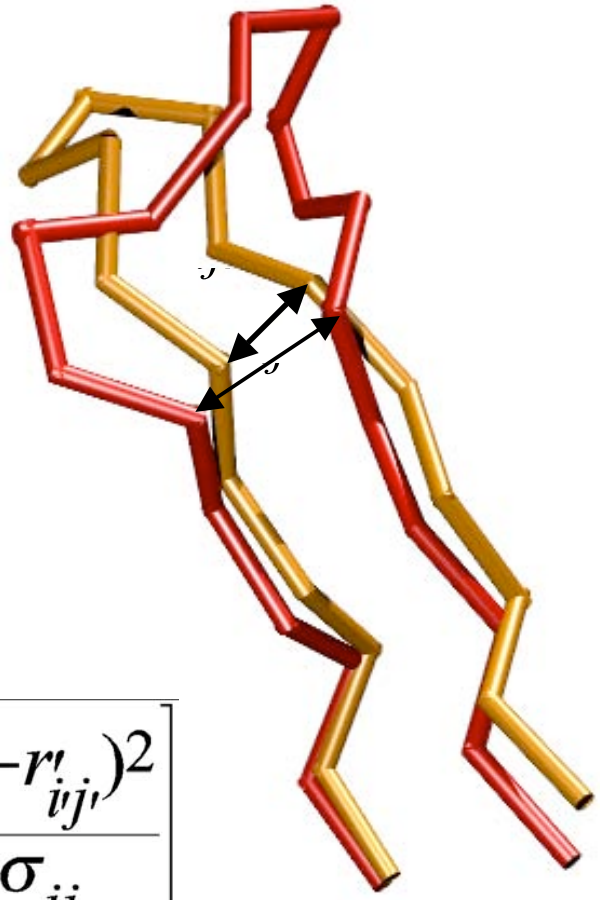
R. Goldstein, Z. Luthey-Schulten, P. Wolynes (1994) *Proc 27th Annu Hawaii Int Conf Sys Sci*:306.

Similarity Measures

Sequence Identity

fraction of identically matched residues

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



Q "Structural Identity"

fraction of native contacts

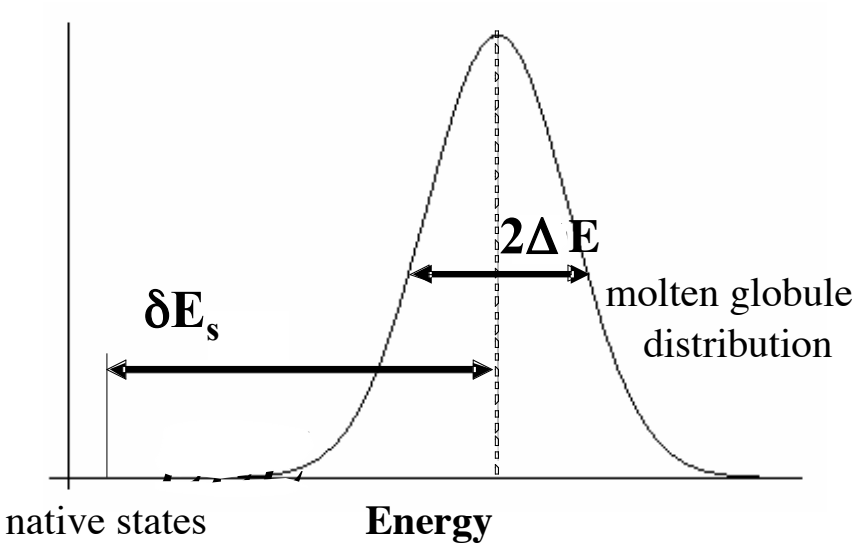
$$Q = \frac{2}{(N_{ALN}-1)(N_{ALN}-2)} \sum_{i<j-1} \exp\left[\frac{(r_{ij}-r'_{ij'})^2}{2\sigma_{ij}}\right]$$

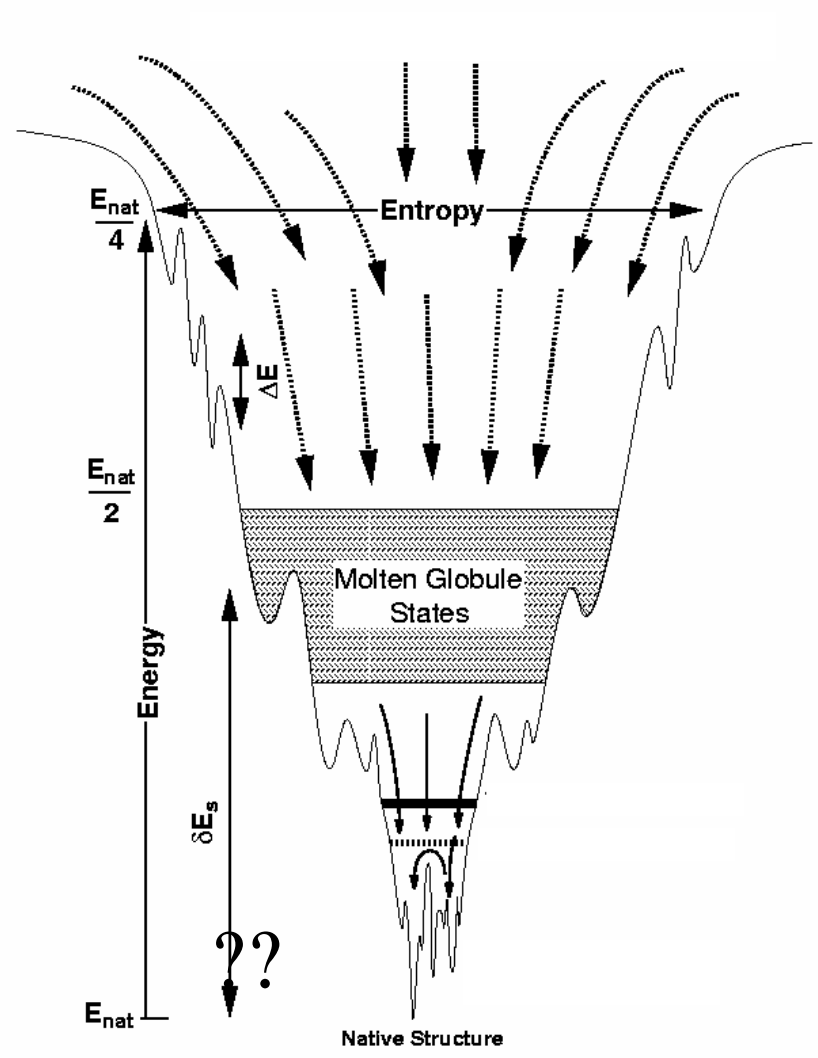
A summary of Energy Landscape Theory

Energy Landscape Theory

When $\langle \delta E_s/\Delta E \rangle$ is maximum
the energy landscape is **optimally funneled.**

Optimization over an Ensemble of Folds

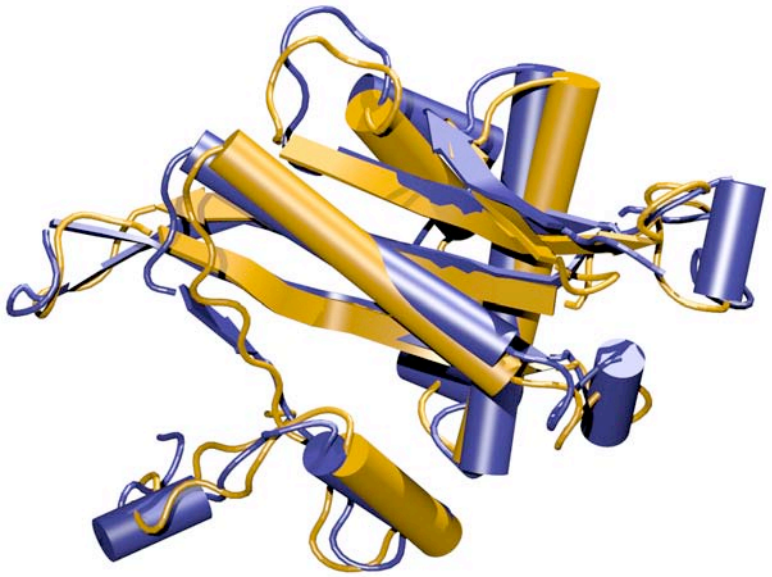


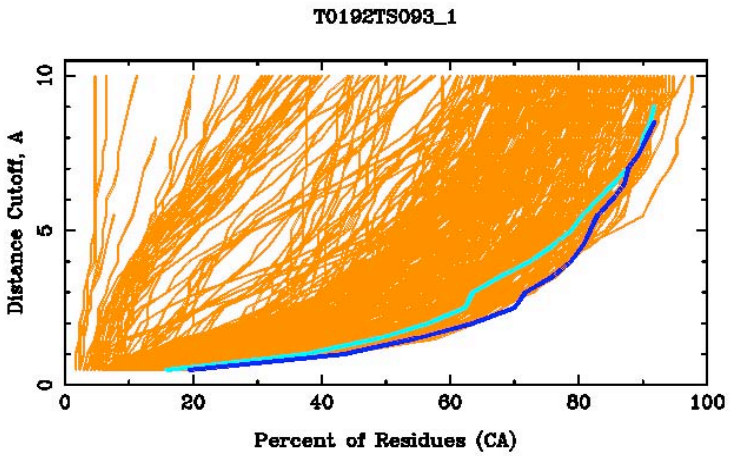


Onuchic , Luthey -Schulten, Wolynes (**1997**) *Annu . Rev. Phys. Chem.* 48:545 -600.
Koretke , Luthey -schulten,Wolynes(**1996)** *Prot. Sci***.** 5:1043

Homology Modeling - Threading

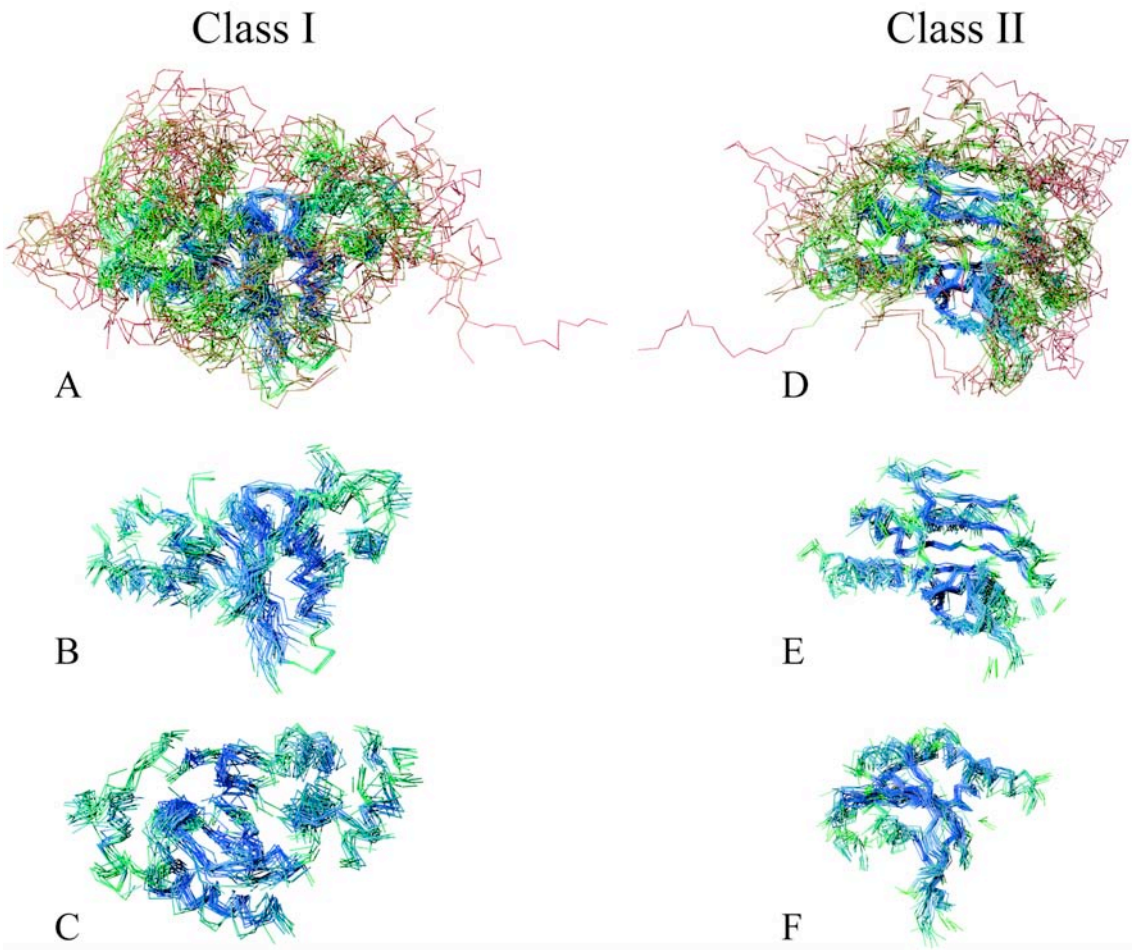
Single Sequence to Single Structure





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 bewteen 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.

R. Russell, G. Barton (1992) *Proteins* **14:** 309.

Multiple Structural Alignments

STAMP – cont'd

2. Refine Initial Alignment & Produce Multiple Structural Alignment

Alignment score:

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

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

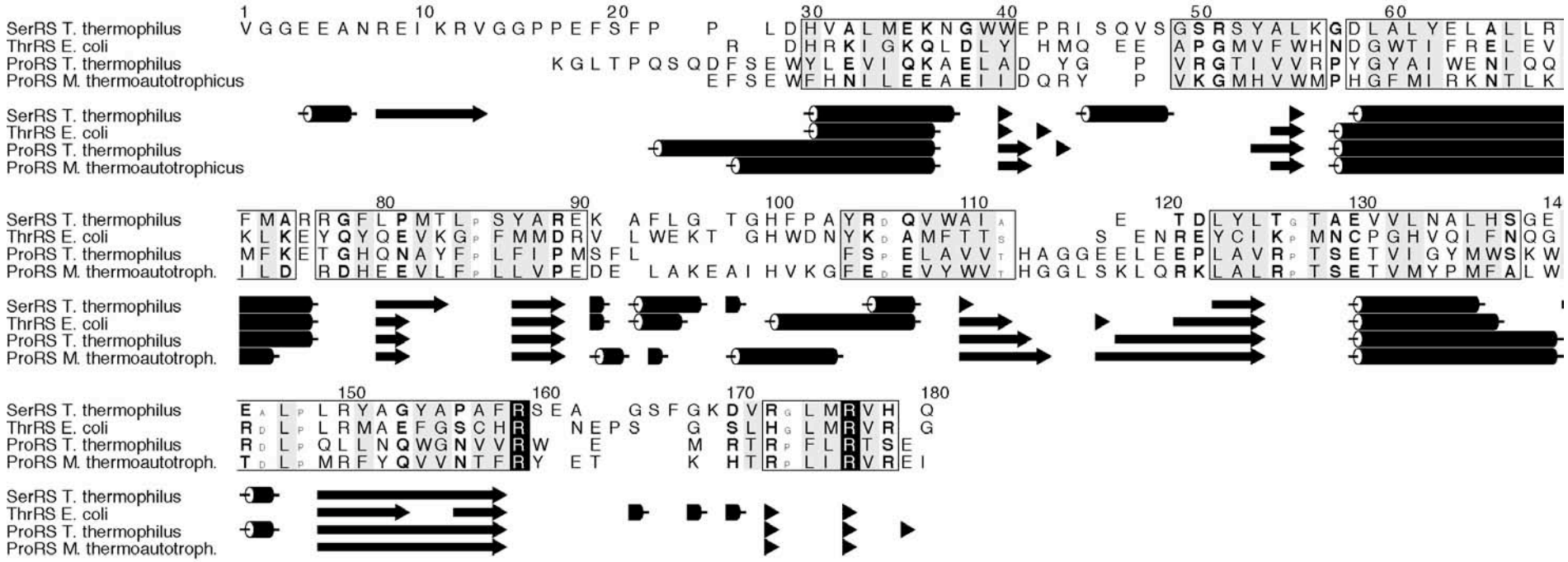
L_p, L_A, L_B – length of alignment, sequence A, sequence B

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



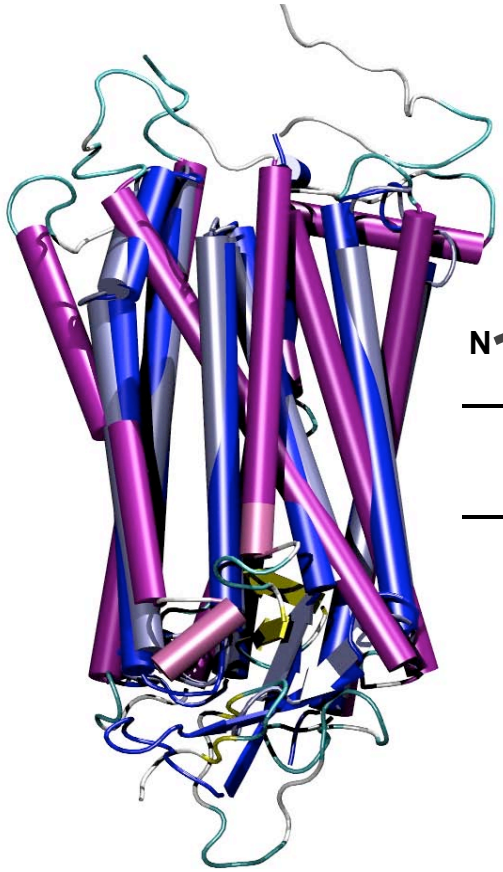
O'Donoghue and Luthey-Schulten, UIUC 2003

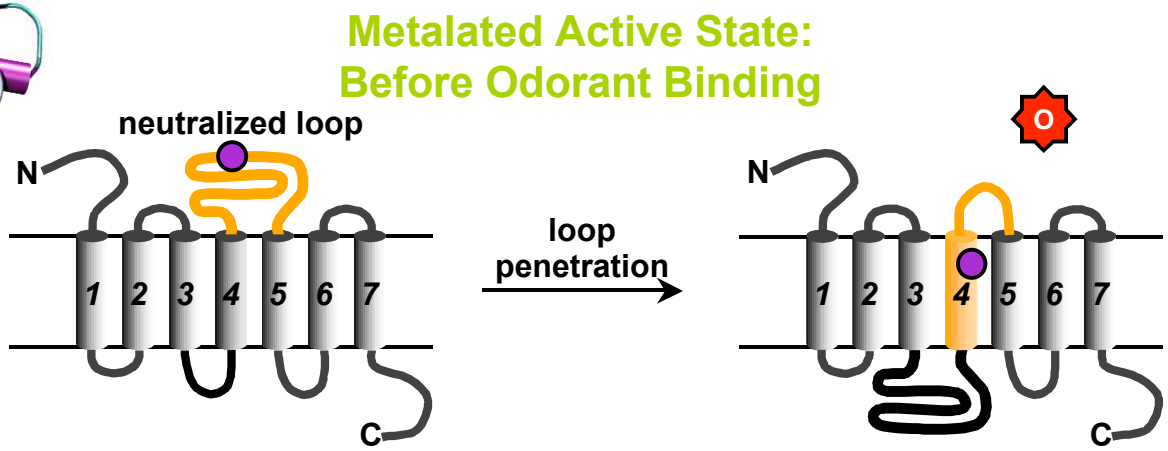
Stamp Output/Clustal Format

```
SerRS-T_thermophilus           VGGEEANREIKRVGGPPEFSFP--P--LDHVALMEKNGWWEPRISQVSGSRSYALKGDLA
ThrRS-E_coli                   -------------------------R--DHRKIGKQLDLY-HMQ-EE-APGMVFWHNDGW
ProRS-T_thermophilus           ----------------KGLTPQSQDFSEWYLEVIQKAELAD-YG--P-VRGTIVVRPYGY
ProRS-M_thermoautotrophicus    ------------------------EFSEWFHNILEEAEIIDQRY--P-VKGMHVWMPHGF
space                          ------------------------------------------------------------
SerRS-T_thermophilus           --SGGG-EEEEEES-----SS--------HHHHHHHHT-B-TTHHHHH-SS---B-THHH
ThrRS-E_coli                   ----------------------------HHHHHHHTT-E-E---TT-STT--EE-HHHH
ProRS-T_thermophilus           --------------------HHHHHHHHHHHHHHHHTTSEE-E---S-STT-EEE-HHHH
ProRS-M_thermoautotrophicus    ------------------------HHHHHHHHHHHTT-EE-----S-STT--EE-HHHH

SerRS-T_thermophilus           LYELALLRFAMDFMARRGFLPMTLPSYAREK-AFLG-TGHFPAYRDQVWAIA-----E--
ThrRS-E_coli                   TIFRELEVFVRSKLKEYQYQEVKGPFMMDRV-LWEKT-GHWDNYKDAMFTTS----S-EN
ProRS-T_thermophilus           AIWENIQQVLDRMFKETGHQNAYFPLFIPMSFL----------FSPELAVVTHAGGEELE
ProRS-M_thermoautotrophicus    MIRKNTLKILRRILD-RDHEEVLFPLLVPEDE-LAKEAIHVKGFEDEVYWVTHGGLSKLQ
space                          ------------------------------------------------------------
SerRS-T_thermophilus           HHHHHHHHHHHHHHHHTT-EEEE--SEEEHH-HHHH-HT-TTTTGGGS-B-T-----T--
ThrRS-E_coli                   HHHHHHHHHHHHHHHHHTT-EE----SEEEHH-HHHTT-THHHHHGGG--EEE----E-TT
ProRS-T_thermophilus           HHHHHHHHHHHHHHHHHTT-EE----SEEESTT-------------TT--EEEE-SSSEEE
ProRS-M_thermoautotrophicus    HHHHHHHHHHHHHHT-TT-EE----SEEEHHH-HTTSHHHHHHTTTT--EEEEETTEEEE

SerRS-T_thermophilus           TDLYLTGTAEVVLNALHSGEILPYEALPLRYAGYAPAFRSEA--GSFGKDVRGLMRVH-Q
ThrRS-E_coli                   REYCIKPMNCPGHVQIFNQGLKSYRDLPLRMAEFGSCHR--NEPS--G-SLHGLMRVR-G
ProRS-T_thermophilus           EPLAVRPTSETVIGYMWSKWIRSWRDLPQLLNQWGNVVRW--E----M-RTRPFLRTSE-
ProRS-M_thermoautotrophicus    RKLALRPTSETVMYPMFALWVRSHTDLPMRFYQVVNTFRY-ET----K-HTRPLIRVREI
space                          ------------------------------------------------------------
SerRS-T_thermophilus           SSEEE-S-THHHHHHHTTT-EEEGGG-SEEEEEEEEE------S--SSTTTTTTS-S-E
ThrRS-E_coli                   EEEEE-S-SHHHHHHHHTSS--BTTT-SEEEEE--EEE------G--G-G-BTTTB-S-E
ProRS-T_thermophilus           EEEEE-S-SHHHHHHHHHHH--BGGG--EEEEEEEEEE---------S-S-BTTTB-SE-
ProRS-M_thermoautotrophicus    EEEEE-SSSHHHHHHHHHHH--BTTT--EEEEEEEEEE---------S---BTTTB-SEE
```

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

Hidden Markov Models of Transmembrane Proteins



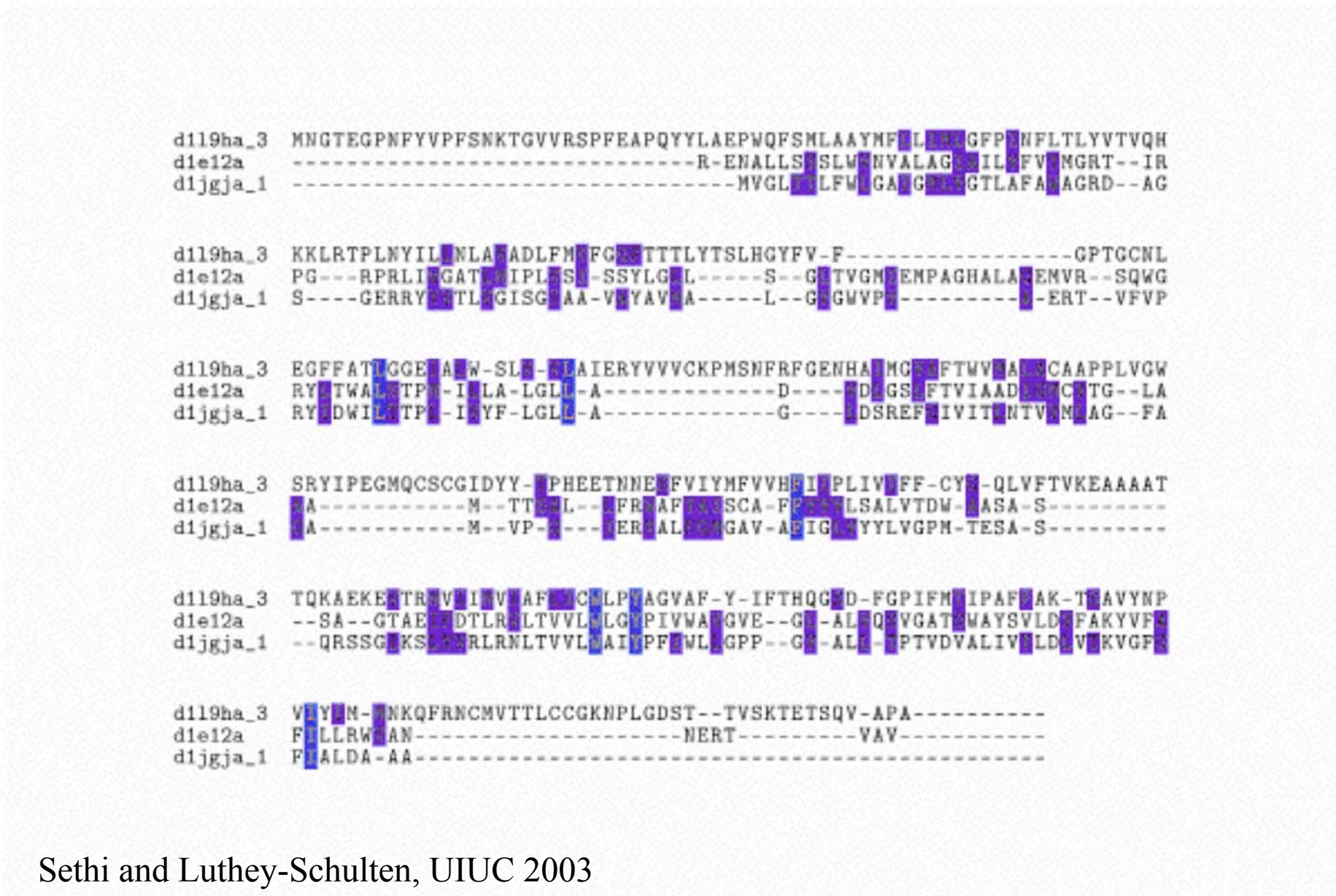


Bacteriorhodpsin/Rhodopsins

Olfactory Receptor/Bovine Rhodopsin

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

Stamp Profile



Sethi and Luthey-Schulten, UIUC 2003

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
     -   -149   -500    233     43   -381    399    106   -626    210   -466   -720    275
     -    -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      *      *
```

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

State π   :     M1   M2   I2   M3   M4
                      D1
```

$\mathbf{M}_i$ – i^{th} Match State
$\mathbf{I}_i$ – i^{th} Insert State
$\mathbf{D}_i$ – i^{th} Delete State

$$P(x_j, t) = P\left(x_j \mid \pi_j = t\right) \times P\left(\pi_j = t\right)$$
$$= e\left(x_j \mid \pi_j = t\right) \times a\left(\pi_j = t \mid \pi_{j-1} = s\right)$$

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

State transition Probabilities (a)

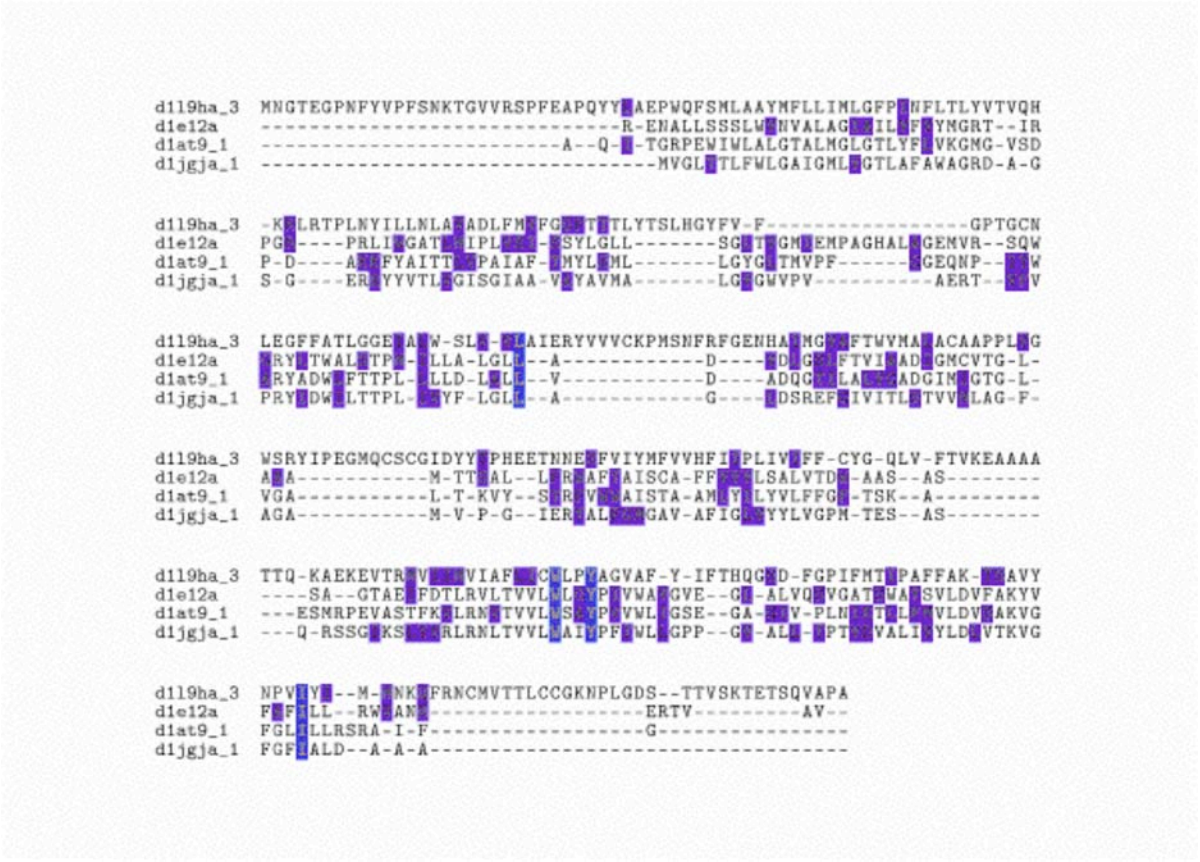
i	$\mathbf{M}_i \longrightarrow \mathbf{M}_{i+1}$	$\mathbf{M}_i \longrightarrow \mathbf{D}_i$	$\mathbf{M}_i \longrightarrow \mathbf{I}_i$
1	0.67	0.33	0
2	0.67	0	0.33
3	1	0	0

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



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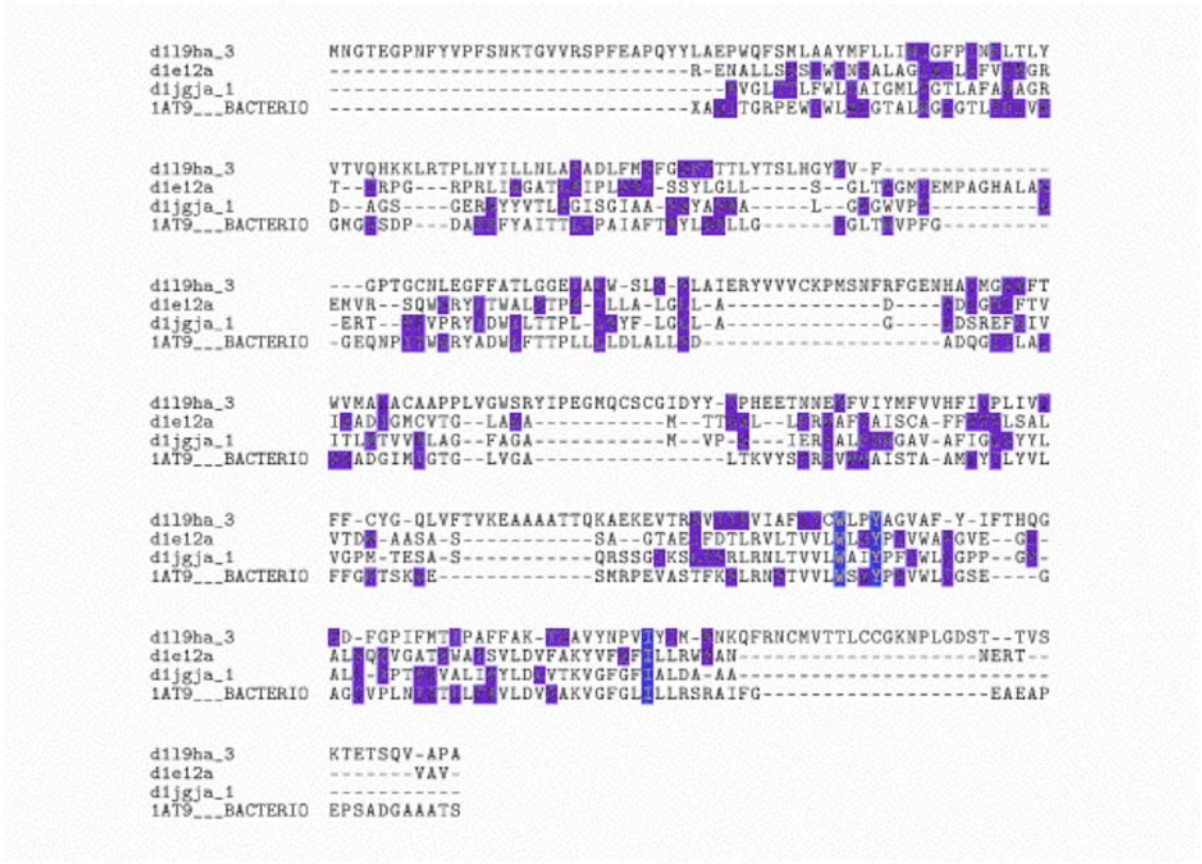
Clustal Profile-Profile Alignment



Sethi and Luthey-Schulten, 2003

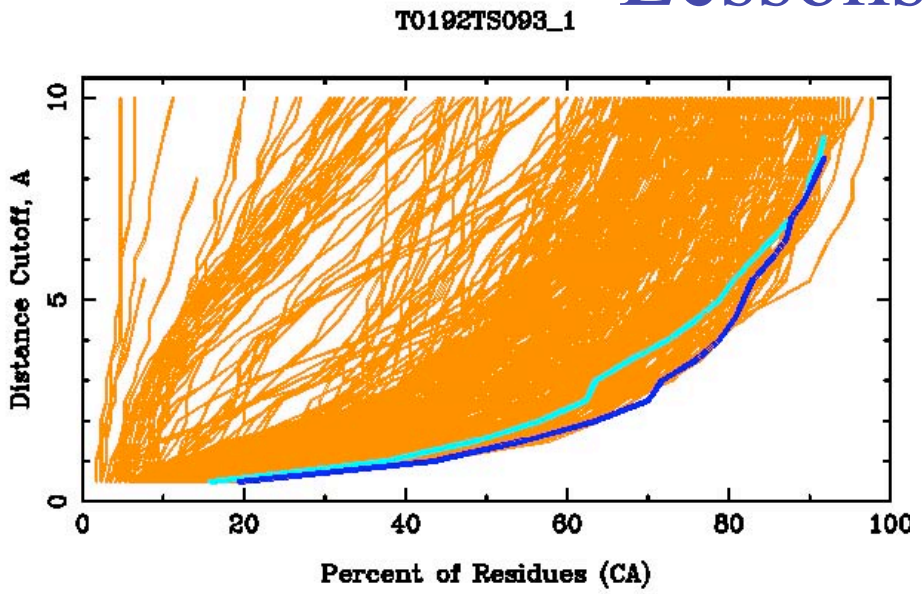
Refine Structure Prediction with Modeller 6.2

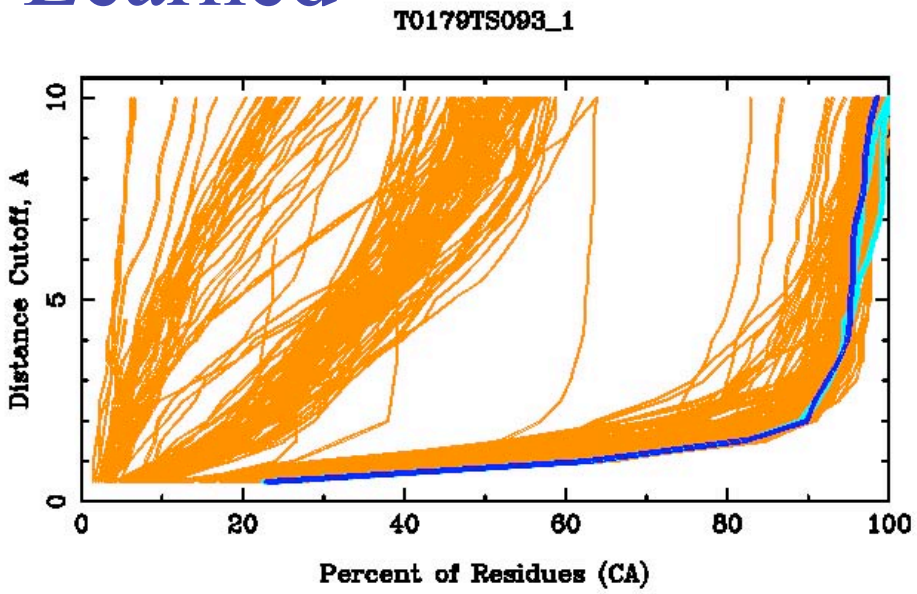
Sethi and Luthey-Schulten, UIUC 2003

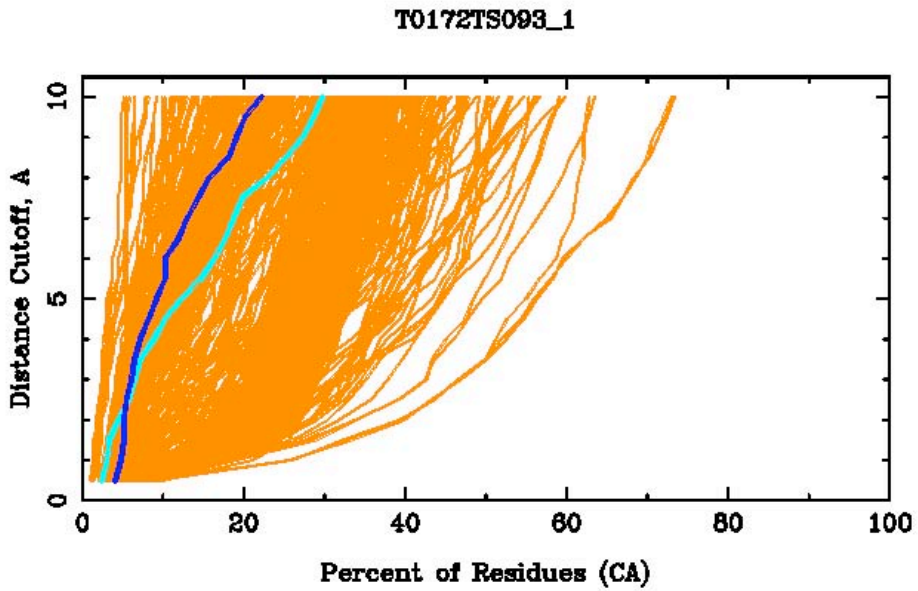
Modeller 6.2 A. Sali, et al.

CM/Fold Recognition Results from CASP5

Lessons Learned







The prediction is never better than the scaffold.

Threading Energy Function and Profiles need improvement.

We need non-redundant, evolutionary profiles! True representative sets of protein sequences and structures from which to draw correct statistical inferences. Structure more conserved than sequence!!!! You are now entering the twilight zone of sequence identity.

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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Algorithms

Mike Heath (UIUC)
Rob Russell (EMBL) **STAMP**

Protein Structure Prediction

Peter Wolynes, Jose Onuchic,
Ken Suslick

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