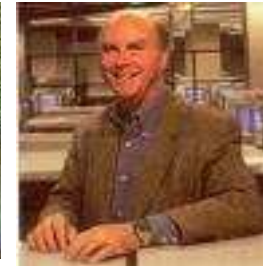
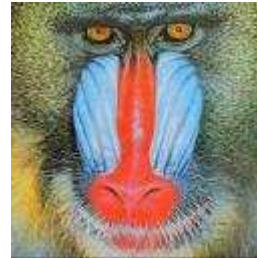


Multiple Alignment

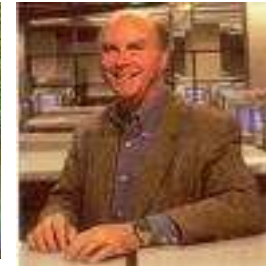
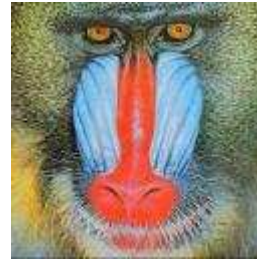
Multiple Alignment versus Pairwise Alignment

- **Up until now we have only tried to align two sequences.**



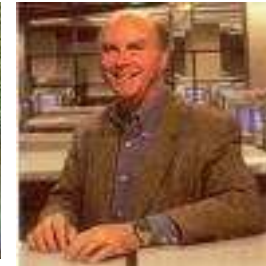
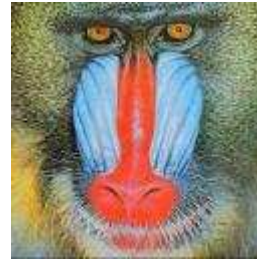
Multiple Alignment versus Pairwise Alignment

- Up until now we have only tried to align two sequences.
- What about more than two?
And what for?



Multiple Alignment versus Pairwise Alignment

- Up until now we have only tried to align two sequences.
- What about more than two? And what for?
- A faint similarity between two sequences becomes significant if present in many
- Multiple alignments can reveal subtle similarities that pairwise alignments do not reveal



Generalizing the Notion of Pairwise Alignment

- Alignment of 2 sequences is represented as a 2-row matrix
- In a similar way, we represent alignment of 3 sequences as a 3-row matrix

A	T	_	G	C	G	_
A	_	C	G	T	_	A
A	T	C	A	C	_	A

- Score: more conserved columns, better alignment

Alignments = Paths in...

- Align 3 sequences: ATGC, AATC, ATGC

	A	--	T	G	C
--	---	----	---	---	---

	A	A	T	--	C
--	---	---	---	----	---

	--	A	T	G	C
--	----	---	---	---	---

Alignment Paths

- Align 3 sequences: ATGC, AATC, ATGC

0	1	1	2	3	4
	A	--	T	G	C

x coordinate

	A	A	T	--	C
--	---	---	---	----	---

	--	A	T	G	C
--	----	---	---	---	---

Alignment Paths

- Align 3 sequences: ATGC, AATC, ATGC

0	1	1	2	3	4
---	---	---	---	---	---

x coordinate

	A	--	T	G	C
--	---	----	---	---	---

0	1	2	3	3	4
---	---	---	---	---	---

y coordinate

	A	A	T	--	C
--	---	---	---	----	---

	--	A	T	G	C
--	----	---	---	---	---

Alignment Paths

- Align 3 sequences: ATGC, AATC, ATGC

0	1	1	2	3	4
	A	--	T	G	C
0	1	2	3	3	4
	A	A	T	--	C
0	0	1	2	3	4
	--	A	T	G	C

x coordinate

y coordinate

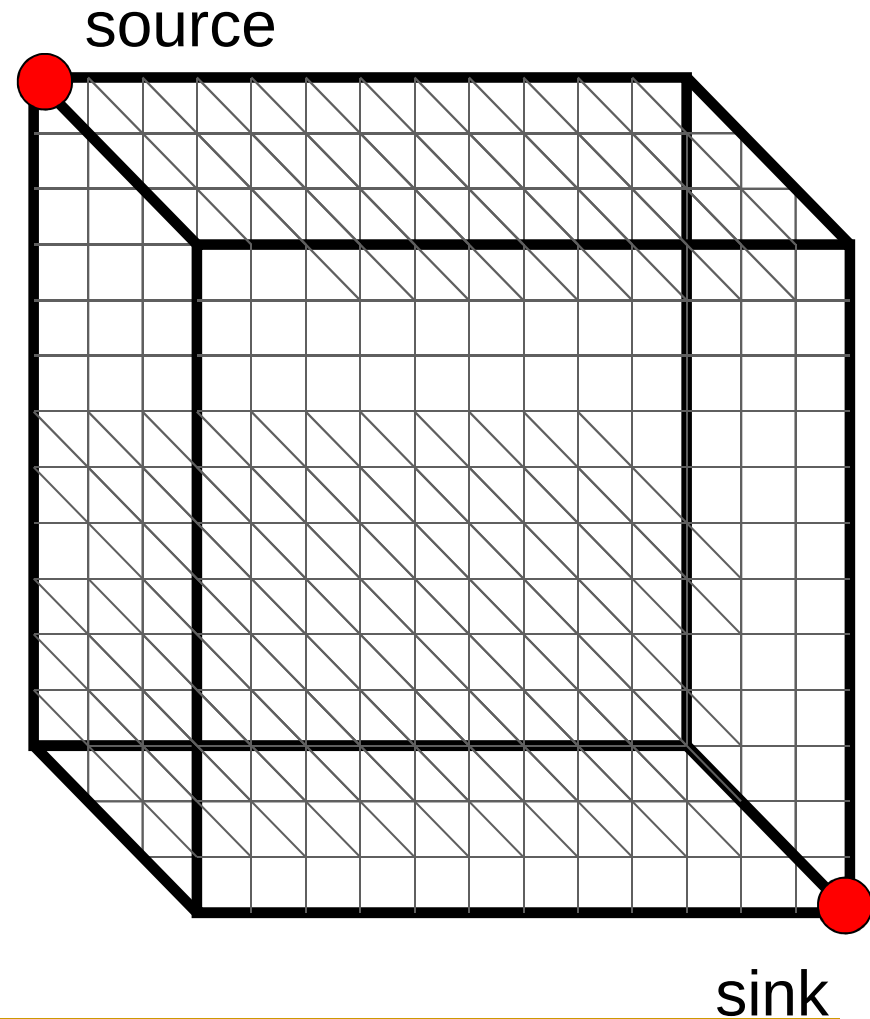
z coordinate

- Resulting path in (x,y,z) space:

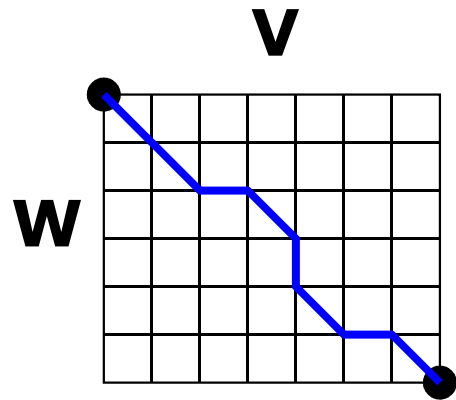
$(0,0,0) \rightarrow (1,1,0) \rightarrow (1,2,1) \rightarrow (2,3,2) \rightarrow (3,3,3) \rightarrow (4,4,4)$

Aligning Three Sequences

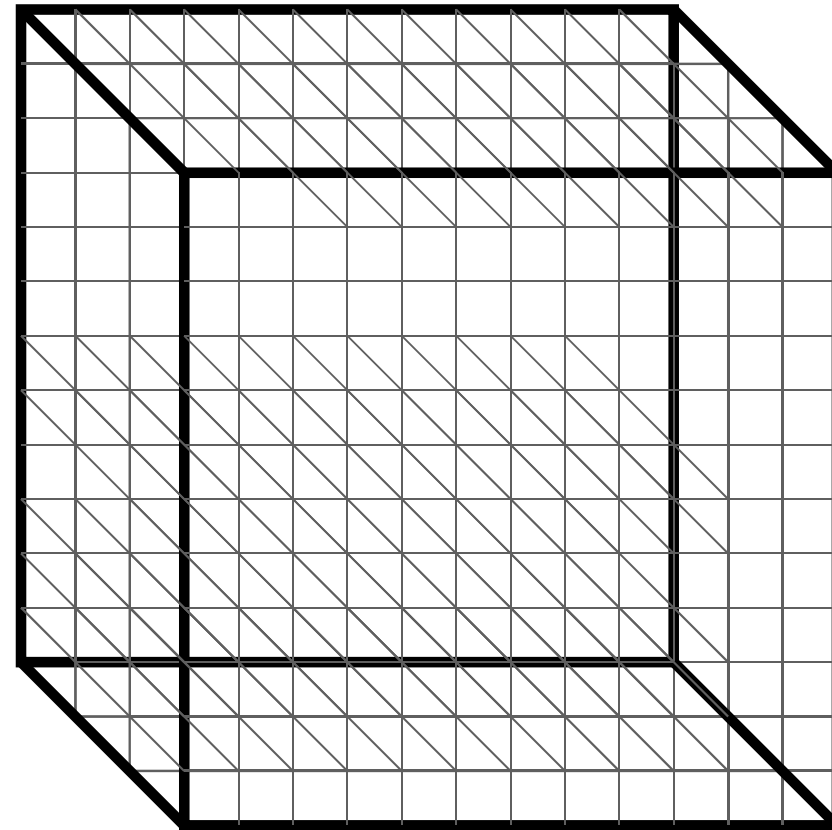
- Same strategy as aligning two sequences
- Use a 3-D “Manhattan Cube”, with each axis representing a sequence to align
- For global alignments, go from source to sink



2-D vs 3-D Alignment Grid

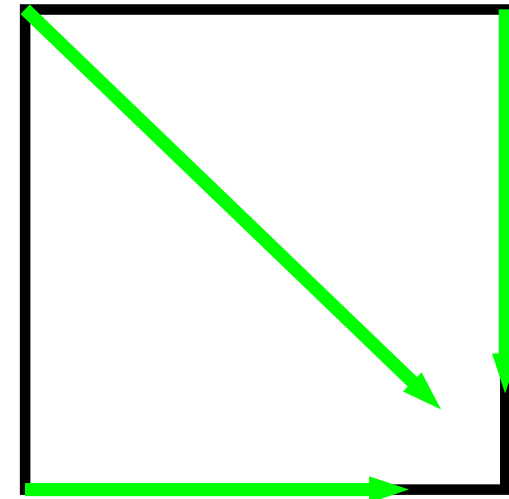
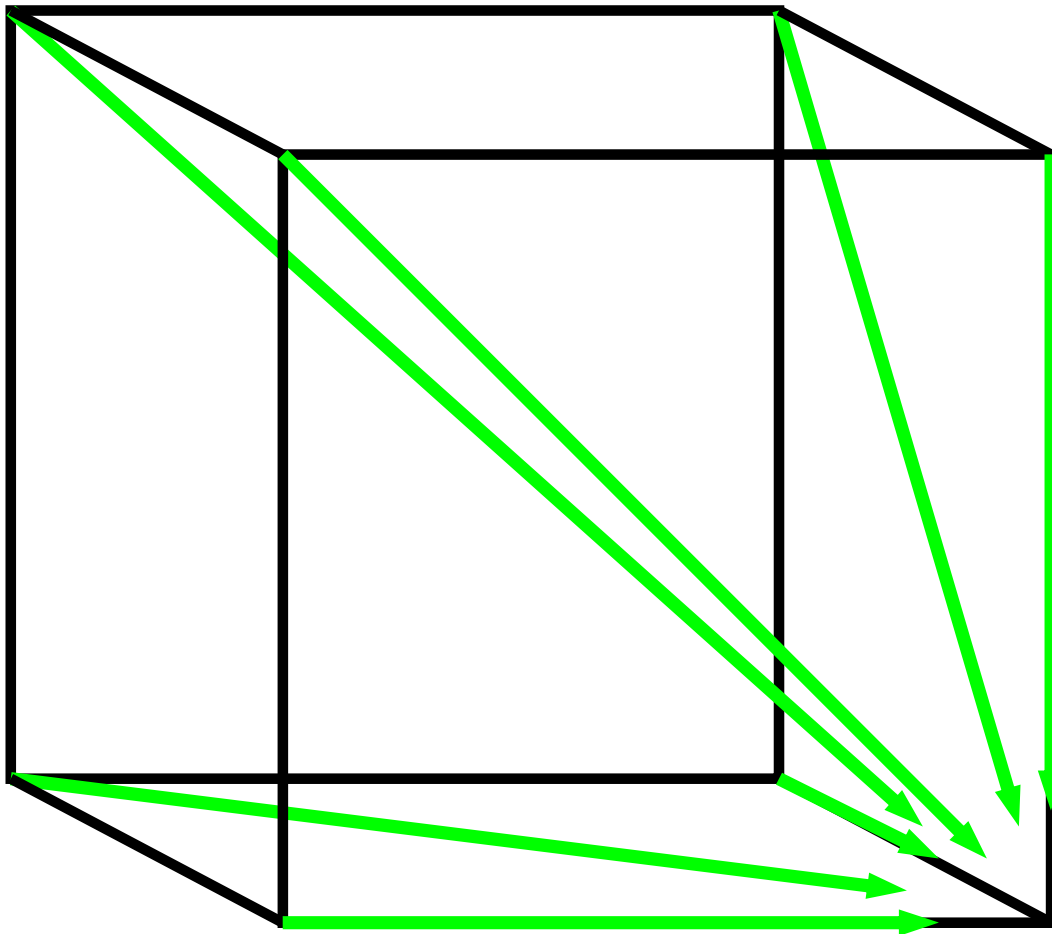


2-D edit graph



3-D edit graph

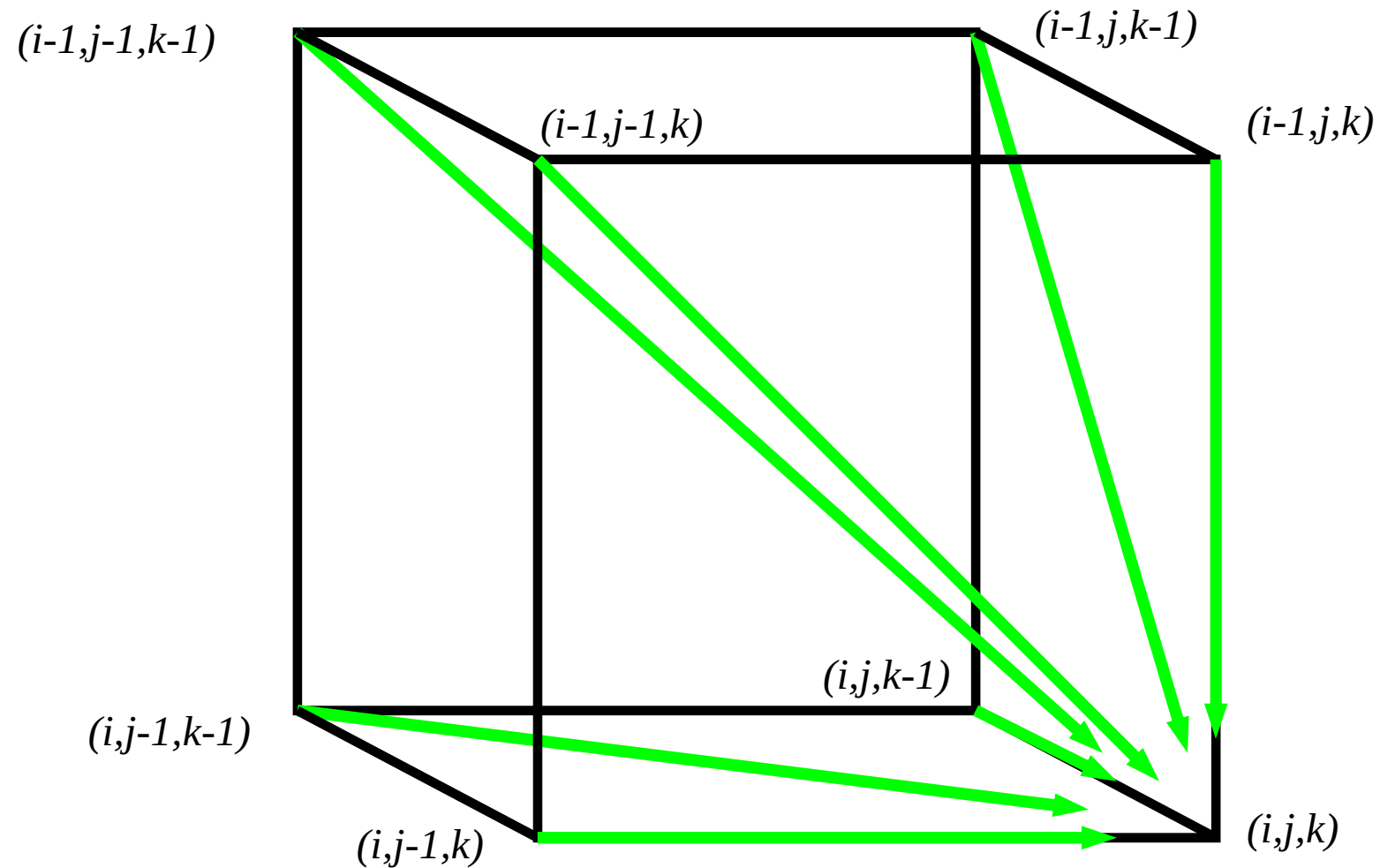
2-D cell versus 2-D Alignment Cell



In **2-D**, 3 edges
in each unit
square

In **3-D**, 7 edges
in each unit cube

Architecture of 3-D Alignment Cell



Multiple Alignment: Dynamic Programming

- $s_{i,j,k} = \max \left\{ \begin{array}{ll} s_{i-1,j-1,k-1} + \delta(v_i, w_j, u_k) & \text{cube diagonal:} \\ s_{i-1,j-1,k} + \delta(v_i, w_j, _) & \text{no indels} \\ s_{i-1,j,k-1} + \delta(v_i, _, u_k) & \text{face diagonal:} \\ s_{i,j-1,k-1} + \delta(_, w_j, u_k) & \text{one indel} \\ s_{i-1,j,k} + \delta(v_i, _, _) & \text{edge diagonal:} \\ s_{i,j-1,k} + \delta(_, w_j, _) & \text{two indels} \\ s_{i,j,k-1} + \delta(_, _, u_k) & \end{array} \right.$
- $\delta(x, y, z)$ is an entry in the 3-D scoring matrix

Multiple Alignment: Running Time

- For 3 sequences of length n , the run time is $7n^3$; $O(n^3)$
- For k sequences, build a k -dimensional Manhattan, with run time $(2^k-1)(n^k)$; $O(2^k n^k)$
- Conclusion: dynamic programming approach for alignment between two sequences is easily extended to k sequences but it is impractical due to exponential running time

Multiple Alignment Induces Pairwise Alignments

Every multiple alignment induces pairwise alignments

x: AC - GCGG - C
y: AC - GC - GAG
z: GCCGC - GAG

Induces:

x: ACGCGG - C; **x:** AC - GCGG - C; **y:** AC - GCGAG
y: ACGC - GAC; **z:** GCCGC - GAG; **z:** GCCGCGAG

Reverse Problem: Constructing Multiple Alignment from Pairwise Alignments

Given 3 **arbitrary** pairwise alignments:

x: ACGCTGG-C; **x:** AC-GCTGG-C; **y:** AC-GC-GAG
y: ACGC--GAC; **z:** GCCGCA-GAG; **z:** GCCGCAGAG

can we construct a multiple alignment that induces them?

Reverse Problem: Constructing Multiple Alignment from Pairwise Alignments

Given 3 **arbitrary** pairwise alignments:

x: ACGCTGG-C; **x:** AC-GCTGG-C; **y:** AC-GC-GAG
y: ACGC--GAC; **z:** GCCGCA-GAG; **z:** GCCGCAGAG

can we construct a multiple alignment that induces them?

NOT ALWAYS

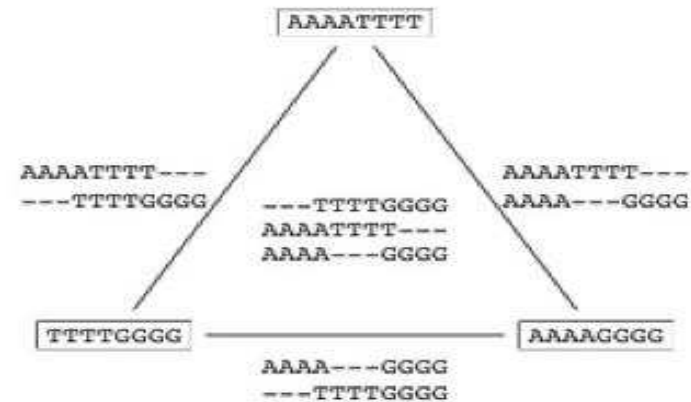
Pairwise alignments may be inconsistent

Inferring Multiple Alignment from Pairwise Alignments

- From an optimal multiple alignment, we can infer pairwise alignments between all pairs of sequences, but they are not necessarily optimal
 - It is difficult to infer a “good” multiple alignment from optimal pairwise alignments between all sequences
-

Combining Optimal Pairwise Alignments into Multiple Alignment

Can combine pairwise alignments into multiple alignment



(a) Compatible pairwise alignments

Can **not** combine pairwise alignments into multiple alignment



(b) Incompatible pairwise alignments

Profile Representation of Multiple Alignment

	-	A	G	G	C	T	A	T	C	A	C	C	T	G
T	A	G	-	C	T	A	C	C	A	-	-	-	-	G
C	A	G	-	C	T	A	C	C	A	-	-	-	-	G
C	A	G	-	C	T	A	T	C	A	C	-	-	G	G
C	A	G	-	C	T	A	T	C	G	C	-	-	G	G
A		1				1			.8					
C	.6			1			.4	1		.6	.2			
G			1	.2					.2			.4	1	
T	.2				1	.6						.2		
-	.2		.8							.4	.8	.4		

Profile Representation of Multiple Alignment

	-	A	G	G	C	T	A	T	C	A	C	C	T	G
T	A	G	-	C	T	A	C	C	A	-	-	-	-	G
C	A	G	-	C	T	A	C	C	A	-	-	-	-	G
C	A	G	-	C	T	A	T	C	A	C	-	-	G	G
C	A	G	-	C	T	A	T	C	G	C	-	-	G	G
A		1				1			.8					
C	.6				1			.4	1		.6	.2		
G			1	.2					.2				.4	1
T	.2					1		.6					.2	
-	.2			.8							.4	.8	.4	

In the past we were aligning a **sequence against a sequence**

Can we align a **sequence against a profile?**

Can we align a **profile against a profile?**

Aligning alignments

- Given two alignments, can we align them?

```
x GGGCACTGCAT
y GGTACGTC--
z GGGAACTGCAG
```

Alignment 1

```
w GGACGTACC--
v GGACCT-----
```

Alignment 2

Aligning alignments

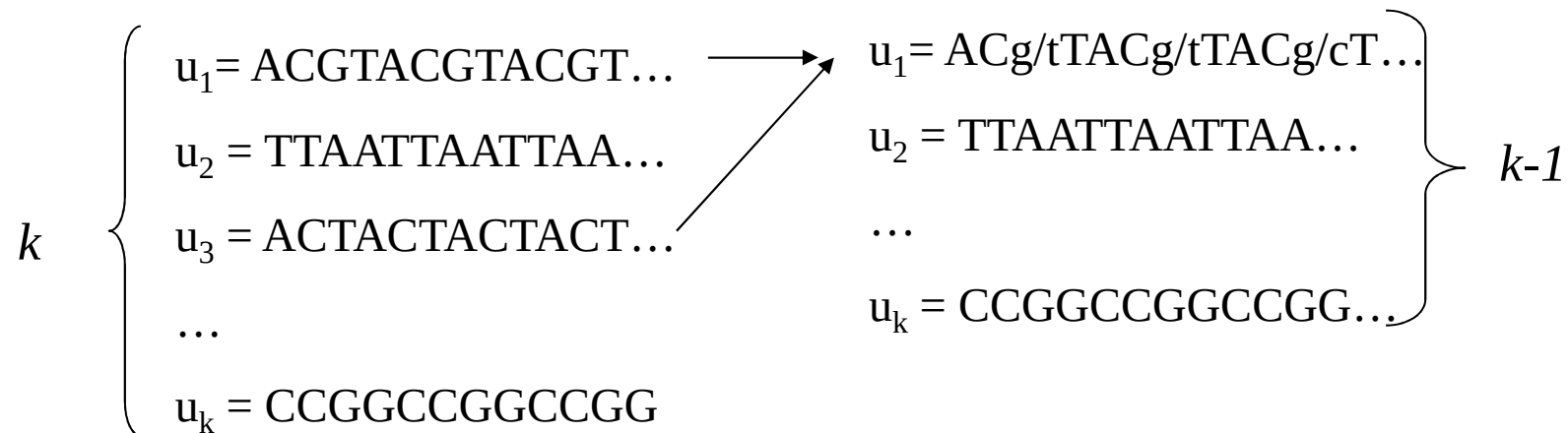
- Given two alignments, can we align them?
- Hint: use alignment of corresponding profiles

```
x GGGCACTGCAT
y GGTACGTC - -
z GGGAACTGCAG
w GGACGTACC - -
v GGACCT - - - -
```

Combined Alignment

Multiple Alignment: Greedy Approach

- Choose most similar pair of strings and combine into a profile, thereby reducing alignment of k sequences to an alignment of $k-1$ sequences/profiles. **Repeat**
- This is a heuristic greedy method



Greedy Approach: Example

- Consider these 4 sequences

s1 GATTCA

s2 GTCTGA

s3 GATATT

s4 GTCAGC

Greedy Approach: Example (cont'd)

- There are $\binom{4}{2} = 6$ possible alignments

s2 **GTCTGA**
s4 **GTCAGC** (score = 2)

s1 **GATTCA--**
s4 **G-T-CAGC**(score = 0)

s1 **GAT-TCA**
s2 **G-TCTGA** (score = 1)

s2 **G-TCTGA**
s3 **GATAT-T** (score = -1)

s1 **GAT-TCA**
s3 **GATAT-T** (score = 1)

s3 **GAT-ATT**
s4 **G-TCAGC** (score = -1)

Greedy Approach: Example (cont'd)

s_2 and s_4 are closest; combine:

s_2	GTCTGA	}	$s_{2,4}$ (profile)	GTCt/aGa/cA
s_4	GTCAGC			

new set of 3 sequences:

s_1	GATTCA
s_3	GATATT
$s_{2,4}$	GTCt/aGa/c

Progressive Alignment

- *Progressive alignment* is a variation of greedy algorithm with a somewhat more intelligent strategy for choosing the order of alignments.
 - Progressive alignment works well for close sequences, but deteriorates for distant sequences
 - Gaps in consensus string are permanent
 - Use profiles to compare sequences
-

ClustalW

- Popular multiple alignment tool today
 - ‘W’ stands for ‘weighted’ (different parts of alignment are weighted differently).
 - Three-step process
 - 1.) Construct pairwise alignments
 - 2.) Build Guide Tree
 - 3.) Progressive Alignment guided by the tree
-

Step 1: Pairwise Alignment

- Aligns each sequence against each other giving a similarity matrix
- Similarity = exact matches / sequence length (percent identity)

	V_1	V_2	V_3	V_4
V_1	-			
V_2	.17	-		
V_3	.87	.28	-	
V_4	.59	.33	.62	-

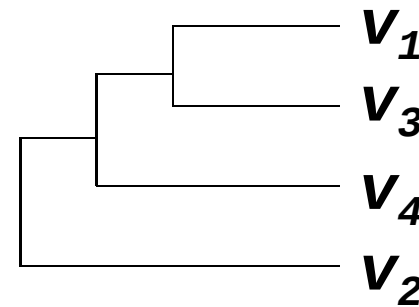
(.17 means 17 % identical)

Step 2: Guide Tree

- Create Guide Tree using the similarity matrix
 - ClustalW uses the neighbor-joining method
 - Guide tree roughly reflects evolutionary relations
-

Step 2: Guide Tree (cont'd)

	V_1	V_2	V_3	V_4
V_1	-			
V_2	.17	-		
V_3	.87	.28	-	
V_4	.59	.33	.62	-



calculate:

$$\begin{aligned}
 V_{1,3} &= \text{alignment}(V_1, V_3) \\
 V_{1,3,4} &= \text{alignment}((V_{1,3}), V_4) \\
 V_{1,2,3,4} &= \text{alignment}((V_{1,3,4}), V_2)
 \end{aligned}$$

- Start by aligning the two most similar sequences
- Following the guide tree, add in the next sequences, aligning to the existing alignment
- Insert gaps as necessary

Dots and stars show how well-conserved a column is.

Multiple Alignments: Scoring

- Number of matches (multiple longest common subsequence score)
 - Entropy score
 - Sum of pairs (SP-Score)
-

Multiple LCS Score

- A column is a “match” if all the letters in the column are the same

AAA
AAA
AAT
ATC

- Only good for very similar sequences

Entropy

- Define frequencies for the occurrence of each letter in each column of multiple alignment
 - $p_A = 1, p_T=p_G=p_C=0$ (1st column)
 - $p_A = 0.75, p_T = 0.25, p_G=p_C=0$ (2nd column)
 - $p_A = 0.50, p_T = 0.25, p_C=0.25, p_G=0$ (3rd column)
- Compute entropy of each column

$$- \sum_{X = A, T, G, C} p_X \log p_X$$

AAA
AAA
AAT
ATC

Entropy: Example

$$\text{entropy} \begin{pmatrix} A \\ A \\ A \\ A \end{pmatrix} = 0 \quad \text{Best case}$$

$$\text{Worst case} \quad \text{entropy} \begin{pmatrix} A \\ T \\ G \\ C \end{pmatrix} = -\sum \frac{1}{4} \log \frac{1}{4} = -4 \left(\frac{1}{4} * -2 \right) = 2$$

Multiple Alignment: Entropy Score

Entropy for a multiple alignment is the sum of entropies of its columns:

$$\sum_{\text{over all columns}} \sum_{X=A,T,G,C} p_X \log p_X$$

Entropy of an Alignment: Example

column entropy:

$$-(p_A \log p_A + p_C \log p_C + p_G \log p_G + p_T \log p_T)$$

A	A	A
A	C	C
A	C	G
A	C	T

- Column 1 = $-[1 \cdot \log(1) + 0 \cdot \log 0 + 0 \cdot \log 0 + 0 \cdot \log 0]$
= 0

- Column 2 = $-[(1/4) \cdot \log(1/4) + (3/4) \cdot \log(3/4) + 0 \cdot \log 0 + 0 \cdot \log 0]$
= $-[(1/4) \cdot (-2) + (3/4) \cdot (-.415)] = +0.811$

- Column 3 = $-[(1/4) \cdot \log(1/4) + (1/4) \cdot \log(1/4) + (1/4) \cdot \log(1/4) + (1/4) \cdot \log(1/4)]$
= $4 \cdot -[(1/4) \cdot (-2)] = +2.0$

- Alignment Entropy = $0 + 0.811 + 2.0 = +2.811$

Multiple Alignment Induces Pairwise Alignments

Every multiple alignment induces pairwise alignments

x: AC - GCGG - C
y: AC - GC - GAG
z: GCCGC - GAG

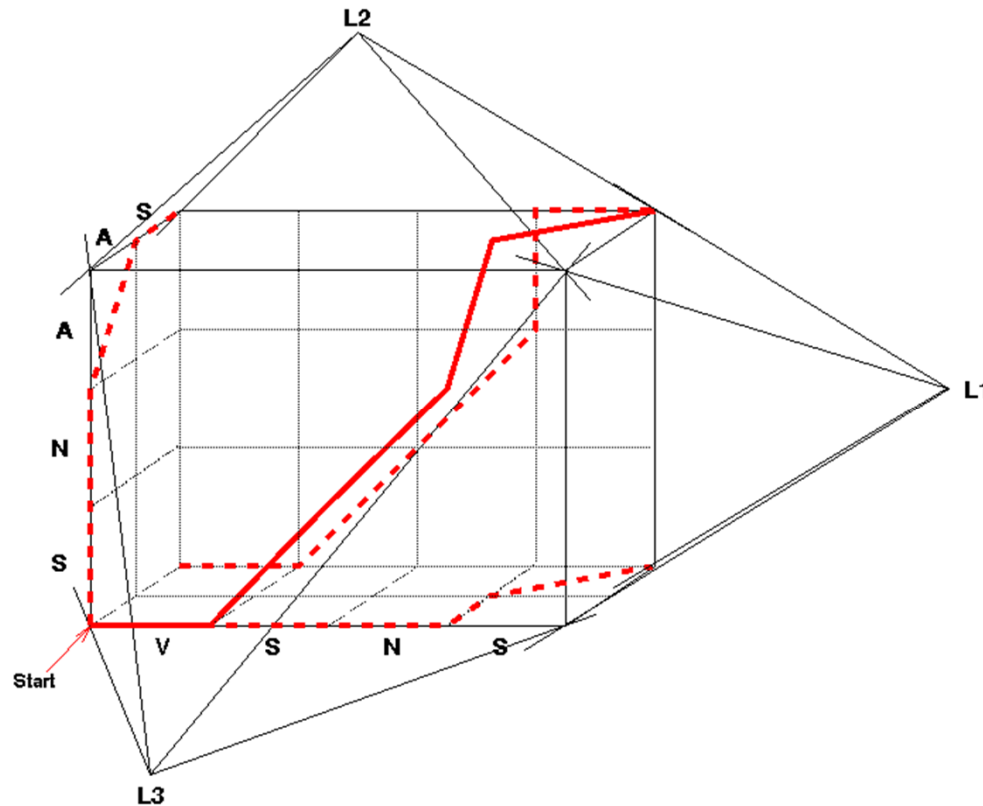
Induces:

x: ACGCGG - C; **x:** AC - GCGG - C; **y:** AC - GCGAG
y: ACGC - GAC; **z:** GCCGC - GAG; **z:** GCCGCGAG

Inferring Pairwise Alignments from Multiple Alignments

- From a multiple alignment, we can infer pairwise alignments between all sequences, but they are not necessarily optimal
 - This is like projecting a 3-D multiple alignment path on to a 2-D face of the cube
-

Multiple Alignment Projections



A 3-D alignment can be projected onto the 2-D plane to represent an alignment between a pair of sequences.

All 3 Pairwise Projections of the Multiple Alignment

Sum of Pairs Score(SP-Score)

- Consider pairwise alignment of sequences
 a_i and a_j
imposed by a multiple alignment of k sequences
- Denote the score of this suboptimal (not necessarily optimal) pairwise alignment as
 $s^*(a_i, a_j)$
- Sum up the pairwise scores for a multiple alignment:

$$s(a_1, \dots, a_k) = \sum_{i,j} s^*(a_i, a_j)$$

Computing SP-Score

Aligning 4 sequences: 6 pairwise alignments

Given a_1, a_2, a_3, a_4 :

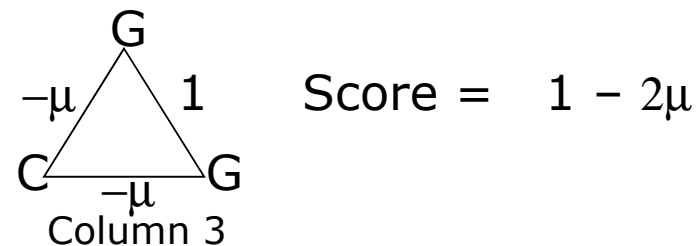
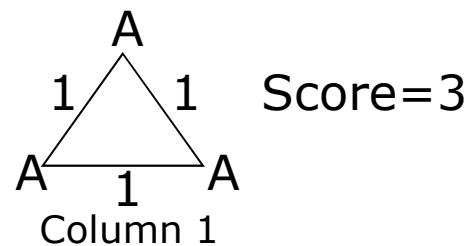
$$\begin{aligned} s(a_1 \dots a_4) = \sum s^*(a_i, a_j) = & s^*(a_1, a_2) + s^*(a_1, a_3) \\ & + s^*(a_1, a_4) + s^*(a_2, a_3) \\ & + s^*(a_2, a_4) + s^*(a_3, a_4) \end{aligned}$$

SP-Score: Example

a_1 ATG-C-AAT
 . A-G-CATAT
 a_k ATCCCATT

To calculate each column:

$$s(a_1 \dots a_k) = \sum_{i,j} s^*(a_i, a_j) \leftarrow \binom{n}{2} \text{ Pairs of Sequences}$$



Multiple Alignment: History

1975 Sankoff

Formulated multiple alignment problem and gave dynamic programming solution

1988 Carrillo-Lipman

Branch and Bound approach for MSA

1990 Feng-Doolittle

Progressive alignment

1994 Thompson-Higgins-Gibson-ClustalW

Most popular multiple alignment program

1998 Morgenstern et al.-DIALIGN

Segment-based multiple alignment

2000 Notredame-Higgins-Heringa-T-coffee

Using the library of pairwise alignments

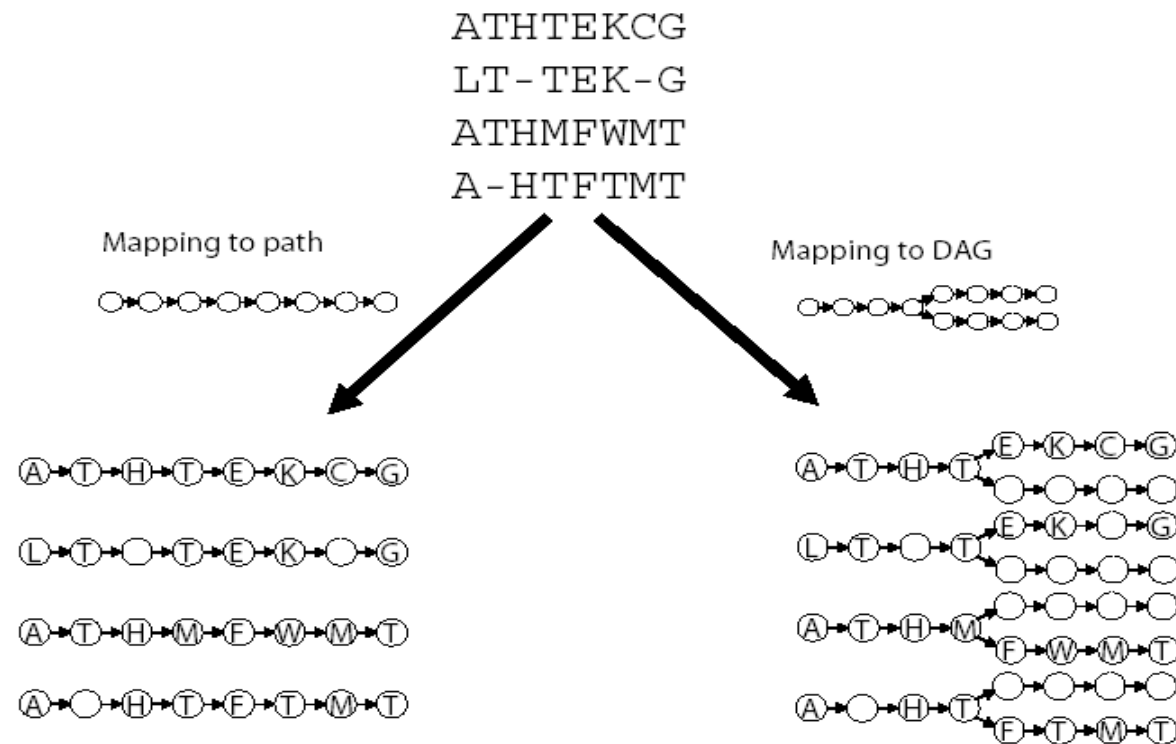
2004 MUSCLE

What's next?

Problems with Multiple Alignment

- Multidomain proteins evolve not only through point mutations but also through domain duplications and domain recombinations
 - Although MSA is a 30 year old problem, there were no MSA approaches for aligning **rearranged** sequences (i.e., multi-domain proteins with shuffled domains) prior to 2002
 - Often impossible to align all protein sequences throughout their entire length
-

POA vs. Classical Multiple Alignment Approaches



Some content from Chris Lee, POA, UCLA

http://www.bioinformatics.ucla.edu/poa/Poa_Tutorial.html

Alignment as a Graph

A

.	.	P	K	M	I	V	R	P	Q	K	N	E	T	V	.
T	H	.	K	M	L	V	R	.	.	.	N	E	T	I	M

- Conventional Alignment

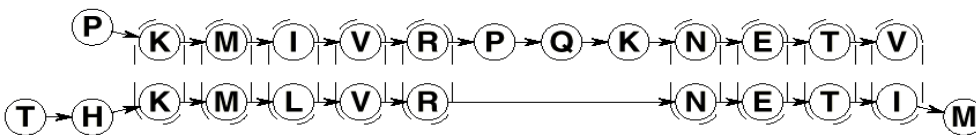
B



```
graph LR; P((P)) --> K((K)); K --> M((M)); M --> I((I)); I --> V((V)); V --> R((R)); R --> P((P)); P --> Q((Q)); Q --> K((K)); K --> N((N)); N --> E((E)); E --> T((T)); T --> V((V))
```

- Protein sequence as a path

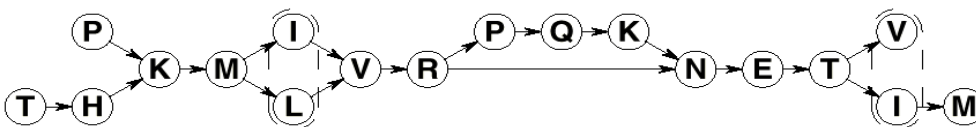
C



```
graph LR; P1((P)) --> K1((K)); K1 --> M1((M)); M1 --> I1((I)); I1 --> V1((V)); V1 --> R1((R)); R1 --> P2((P)); P2 --> Q1((Q)); Q1 --> K2((K)); K2 --> N1((N)); N1 --> E1((E)); E1 --> T1((T)); T1 --> V1; T2((T)) --> H((H)); H --> K2; K2 --> M2((M)); M2 --> L((L)); L --> V2((V)); V2 --> R2((R)); R2 --> N2((N)); N2 --> E2((E)); E2 --> T2((T)); T2 --> I((I)); I --> M((M))
```

- Two paths

D

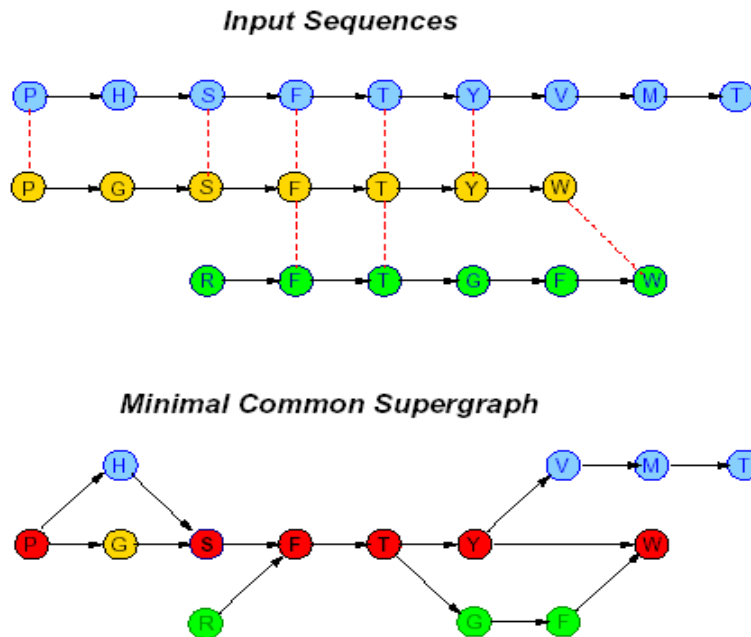


```
graph LR; P((P)) --> K((K)); K --> M((M)); M --> I((I)); I --> V((V)); V --> R((R)); R --> P((P)); P --> Q((Q)); Q --> K((K)); K --> N((N)); N --> E((E)); E --> T((T)); T --> V((V)); T2((T)) --> H((H)); H --> K((K)); K --> M((M)); M --> L((L)); L --> V((V)); V --> R((R)); R --> N((N)); N --> E((E)); E --> T((T)); T --> I((I)); I --> M((M))
```

- Combined graph (partial order) of both sequences

Solution: Representing Sequences as Paths in a Graph

Each protein sequence is represented by a path. Dashed edges connect “equivalent” positions; vertices with identical labels are fused.



Partial Order Multiple Alignment

Two objectives:

- Find a graph that represents domain structure
- Find mapping of each sequence to this graph

Solution

- ***PO-MSA (Partial Order Multiple Sequence Alignment)*** – for a set of sequences S is a graph such that every sequence in S is a path in G .
-

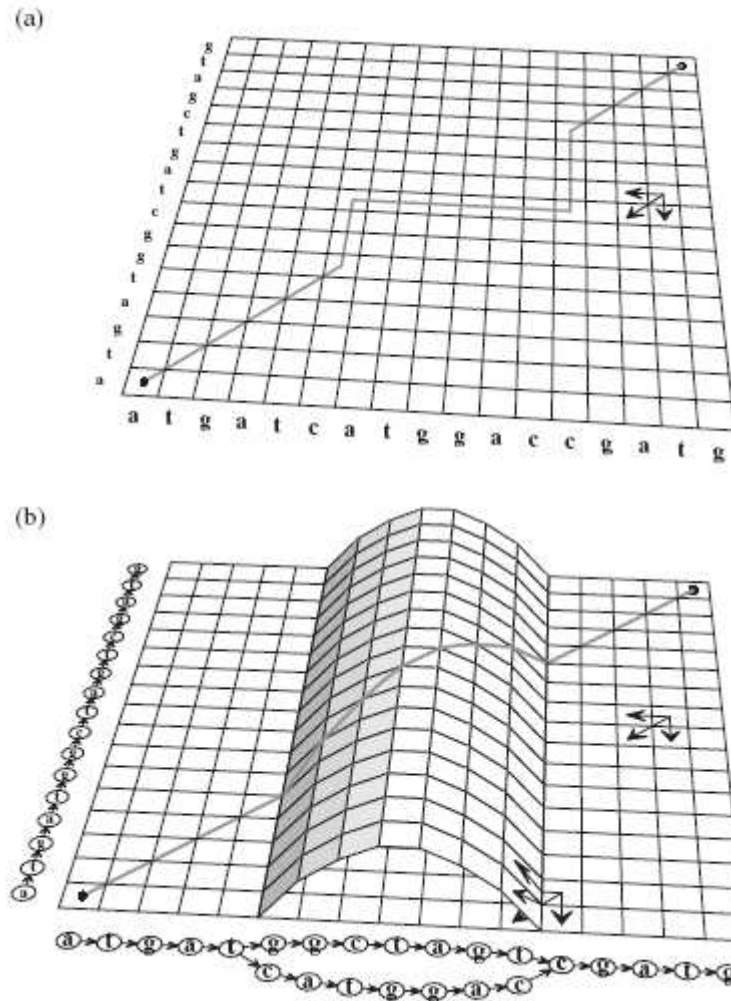
Partial Order Alignment (POA) Algorithm

Aligns sequences onto a directed acyclic graph (DAG)

1. Guide Tree Construction
 2. Progressive Alignment Following Guide Tree
 3. Dynamic Programming Algorithm to align two PO-MSAs (PO-PO Alignment).
-

PO-PO Alignment

- We learned how to align one sequence (*path*) against another sequence (*path*).
- We need to develop an algorithm for aligning a directed *graph* against a directed *graph*.



Dynamic Programming for Aligning Two Directed Graphs

$$S(n, o) = \max_{\substack{p \rightarrow n \\ q \rightarrow o}} \begin{cases} S(p, q) + s(n, o) \\ S(p, o) + \Delta(n) \\ S(n, q) + \Delta(o) \end{cases}$$

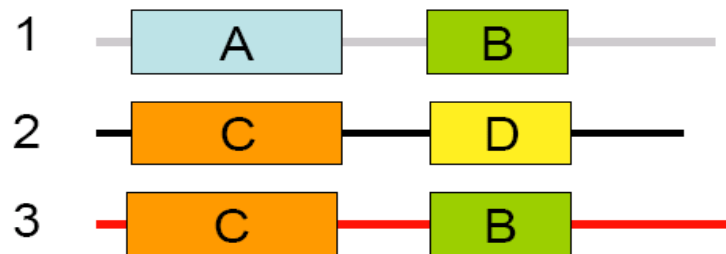
- $S(n, o)$ – the optimal score
- n : node in G
- o : node in G'

Scoring:

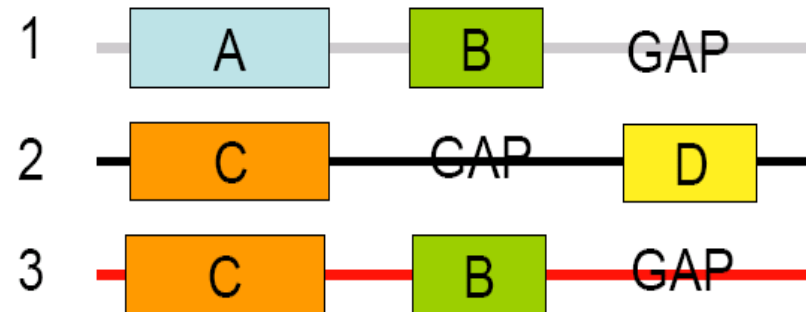
- *match/mismatch*: aligning two nodes with score $s(n, o)$
- *deletion/insertion*:
 - omitting node n from the alignment with score $\Delta(n)$
 - omitting node o with score $\Delta(o)$

Row-Column Alignment

Input Sequences



Row-column alignment



POA Advantages

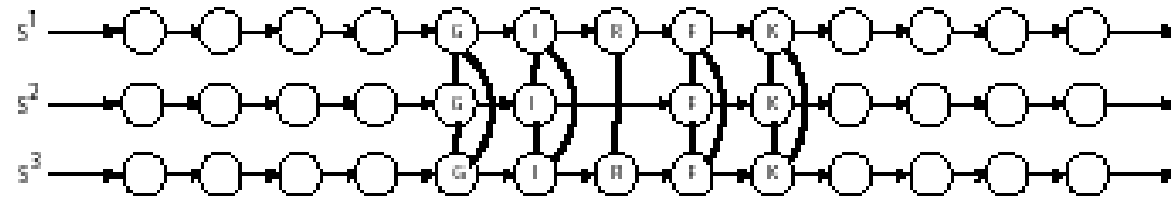
- POA is more flexible: standard methods force sequences to align linearly
 - PO-MSA representation handles gaps more naturally and retains (and uses) all information in the MSA (unlike linear profiles)
-

A-Bruijn Alignment (ABA)

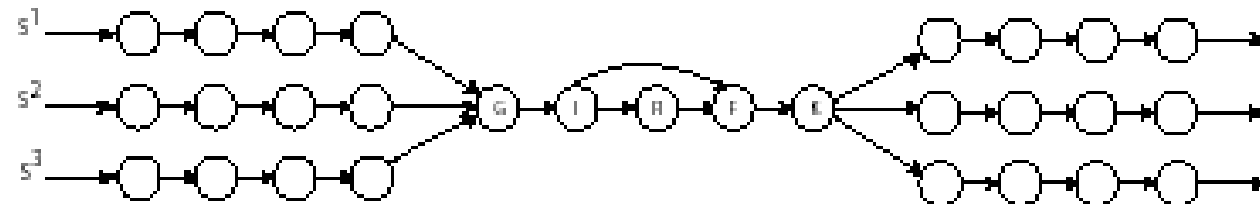
- POA- represents alignment as directed graph; no cycles
 - ABA - represents alignment as directed graph that may contains cycles
-

ABA

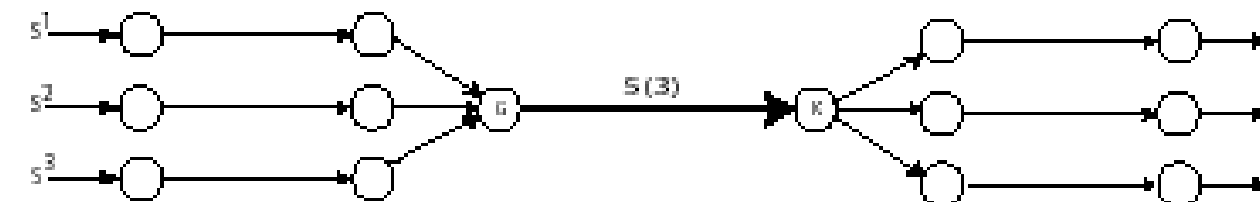
(a)



(b)

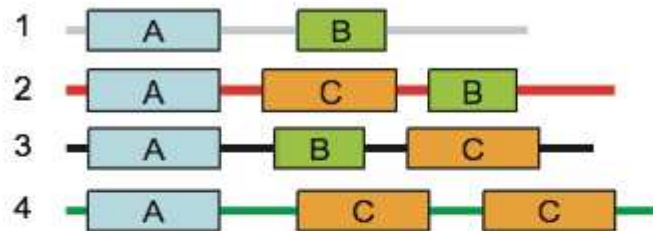


(c)

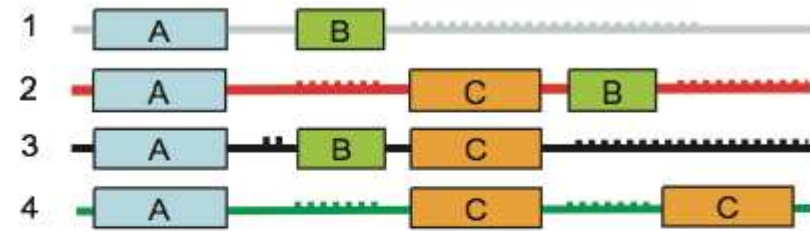


ABA vs. POA vs. MSA

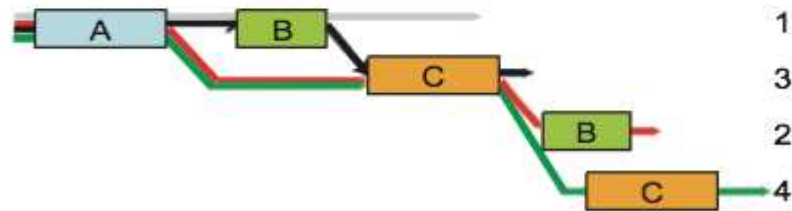
(a)



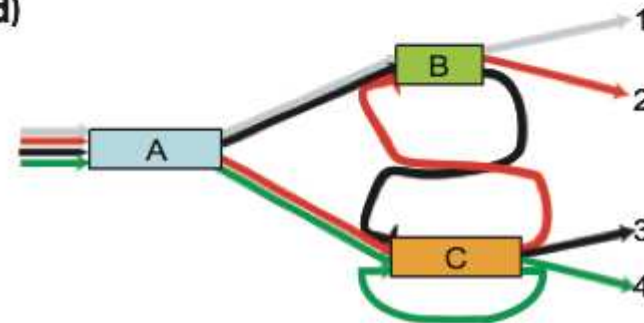
(b)



(c)



(d)



Advantages of ABA

- ABA more flexible than POA: allows larger class of evolutionary relationships between aligned sequences
 - ABA can align proteins that contain duplications and inversions
 - ABA can align proteins with shuffled and/or repeated domain structure
 - ABA can align proteins with domains present in multiple copies in some proteins
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ABA: multiple alignments of protein

ABA handles:

- Domains not present in all proteins
- Domains present in different orders in different proteins