Lecture 2
Sequence Alignment

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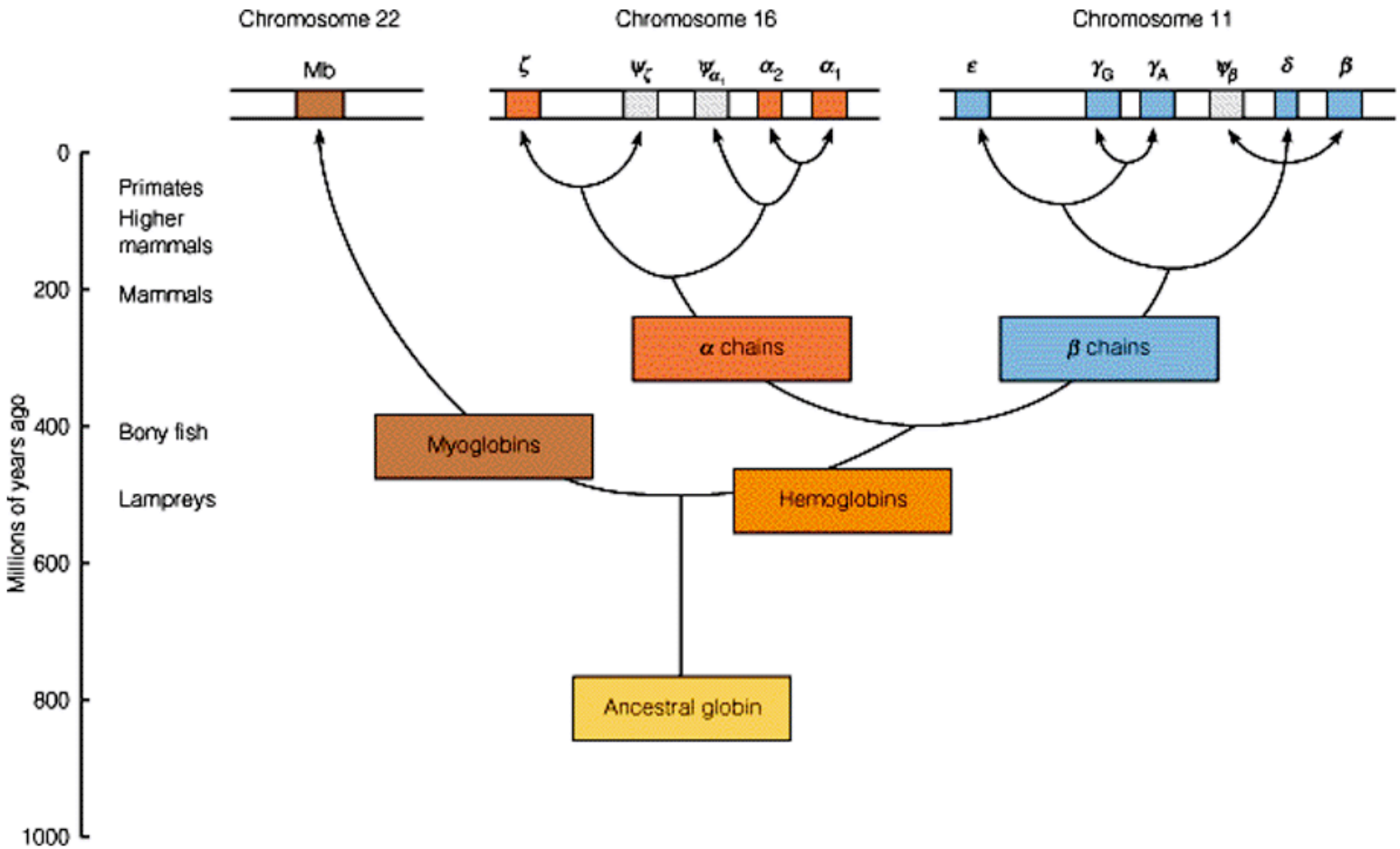
Sequence Alignment: Task Definition

- given:
  - a pair of sequences (DNA or protein)
  - a method for scoring a candidate alignment
- do:
  - determine the correspondences between substrings in the sequences such that the similarity score is maximized

Why Do Alignment?

- *homology*: similarity due to descent from a common ancestor
- often we can infer homology from similarity
- thus we can sometimes infer structure/function from sequence similarity

Homology Example: Evolution of the Globins



Homology

- homologous sequences can be divided into two groups
  - *orthologous sequences*: sequences that differ because they are found in different species (e.g. human α -globin and mouse α-globin)
  - *paralogous sequences*: sequences that differ because of a gene duplication event (e.g. human α-globin and human β-globin, various versions of both)

Issues in Sequence Alignment

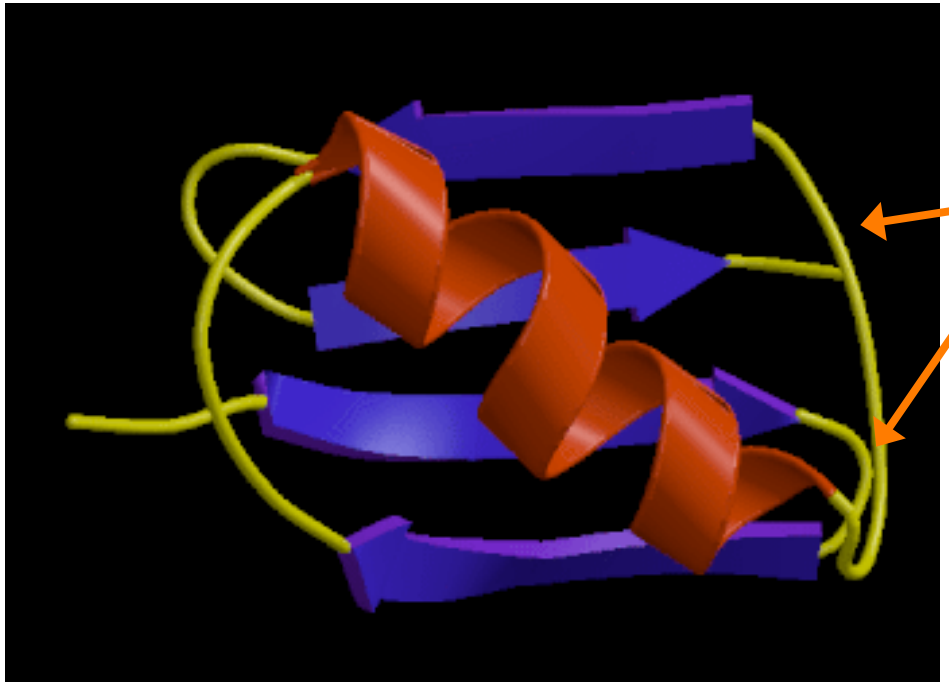
- the sequences we're comparing probably differ in length
- there may be only a relatively small region in the sequences that match
- we want to allow partial matches (i.e. some amino acid pairs are more substitutable than others)
- variable length regions may have been inserted/deleted from the common ancestral sequence

Sequence Variations

- sequences may have diverged from a common ancestor through various types of mutations:
  - substitutions (A**C**GA → A**G**GA)
  - insertions (ACGA → AC**CGGA**GA)
  - deletions (A**CGGA**GA → AGA)
- the latter two will result in *gaps* in alignments

Insertions, Deletions and Protein Structure

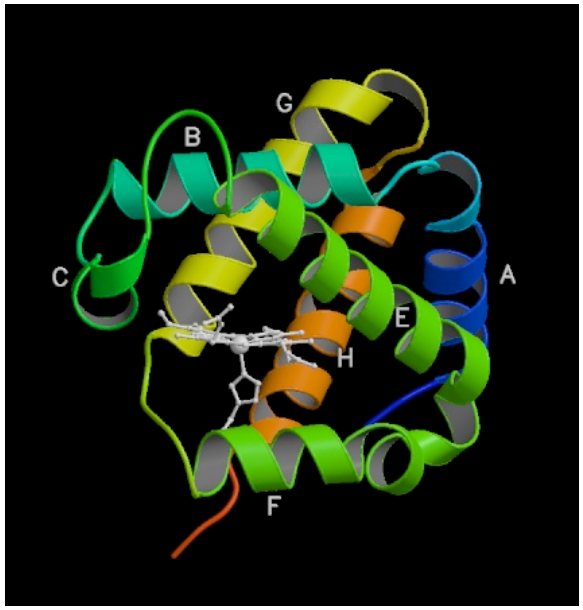
- Why is it that two "similar" sequences may have large insertions/deletions?
  - some insertions and deletions may not significantly affect the structure of a protein



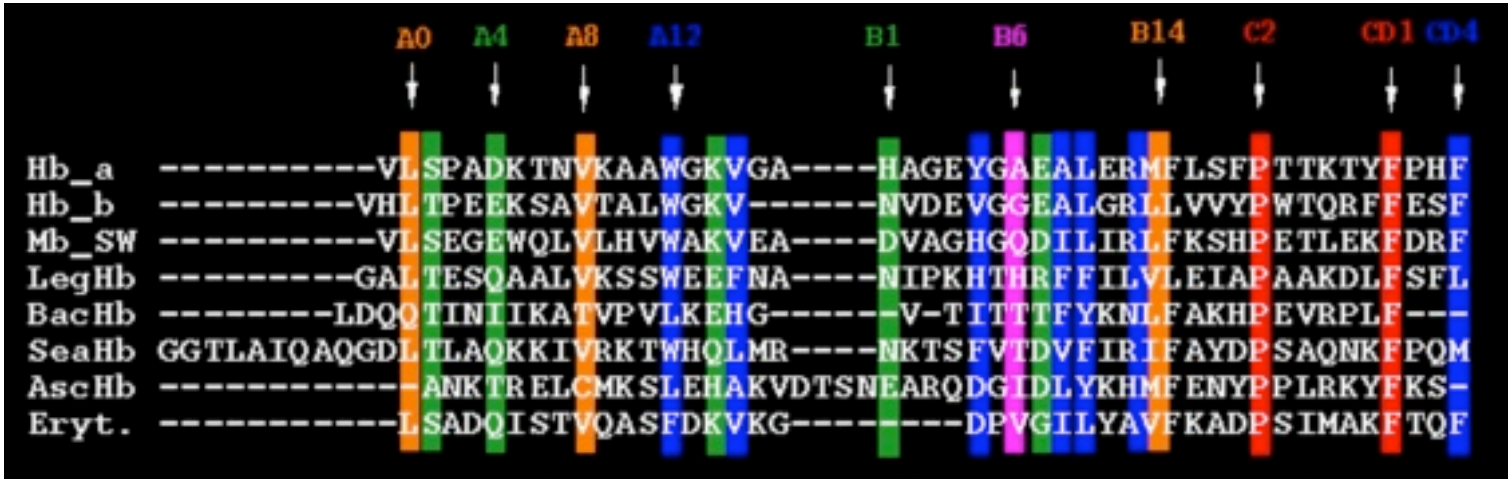
loop structures: insertions/deletions here not so significant

Example Alignment: Globins

- figure at right shows prototypical structure of globins



- figure below shows part of alignment for 8 globins (-'s indicate gaps)



Three Key Questions

- Q1: what do we want to align?
- Q2: how do we “score” an alignment?
- Q3: how do we find the “best” alignment?

Q1: What Do We Want to Align?

- *global alignment*: find best match of both sequences in their entirety
- *local alignment*: find best subsequence match
- *semi-global alignment*: find best match without penalizing gaps on the ends of the alignment

The Space of Global Alignments

- some possible global alignments for `ELV` and `VIS`

```
ELV        -ELV        --ELV        ELV-
VIS        VIS-        VIS--        -VIS


E-LV        ELV--        EL-V
VIS-        --VIS        -VIS
```

Q2: How Do We Score Alignments?

- gap penalty function
  - $w(k)$ indicates cost of a gap of length k

- substitution matrix
  - $s(a,b)$ indicates score of aligning character a with character b

Linear Gap Penalty Function

- different gap penalty functions require somewhat different dynamic programming algorithms
- the simplest case is when a linear gap function is used

$$w(k) = g \times k$$

where g is a constant

- we'll start by considering this case

Scoring an Alignment

- the score of an alignment is the sum of the scores for pairs of aligned characters plus the scores for gaps
- example: given the following alignment

```
VAHV---D--DMPNALSALSDLHAHKL
AIQLQVTGVVVTDATLKNLGSVHVSKG
```

- we would score it by

$$s(\mathrm{V,A}) + s(\mathrm{A,I}) + s(\mathrm{H,Q}) + s(\mathrm{V,L}) + 3g + s(\mathrm{D,G}) + 2g \ldots$$

Q3: How Do We Find the Best Alignment?

- simple approach: compute & score all possible alignments
- but there are

$$\binom{2n}{n} = \frac{(2n)!}{(n!)^2} \approx \frac{2^{2n}}{\sqrt{\pi n}}$$

possible global alignments for 2 sequences of length n

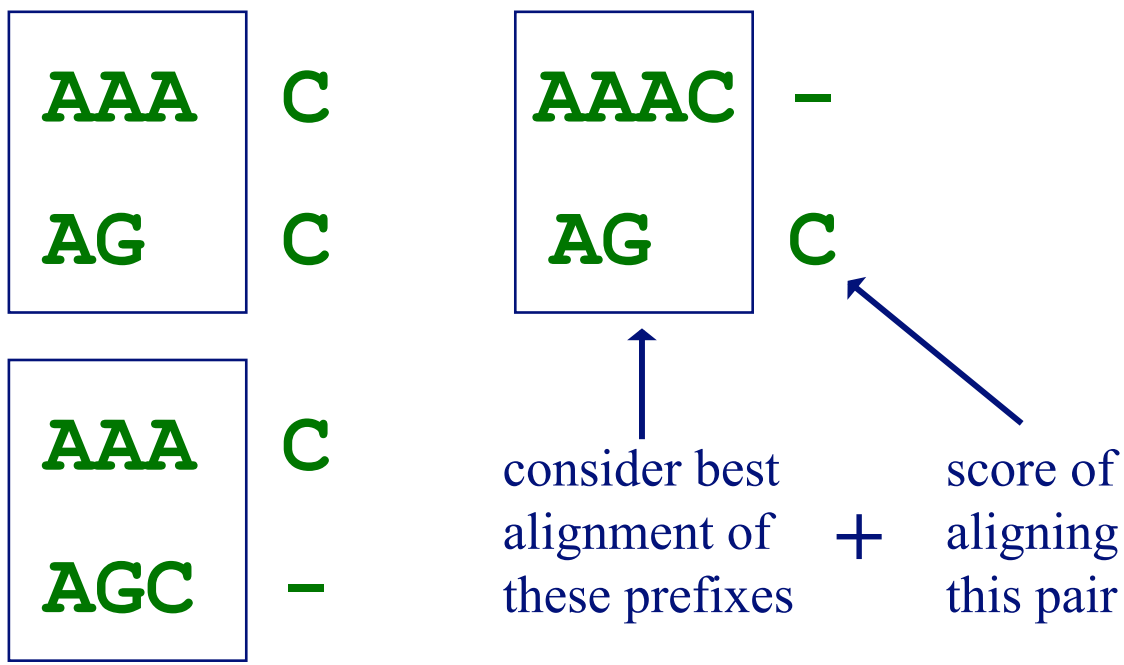
- e.g. two sequences of length 100 have $\approx 10^{77}$ possible alignments

Pairwise Alignment Via Dynamic Programming

- *dynamic programming*: solve an instance of a problem by taking advantage of solutions for subparts of the problem
  - reduce problem of best alignment of two sequences to best alignment of all prefixes of the sequences
  - avoid recalculating the scores already considered
    - example: Fibonacci sequence 1, 1, 2, 3, 5, 8, 13, 21, 34…
- first used in alignment by Needleman & Wunsch, *Journal of Molecular Biology*, 1970

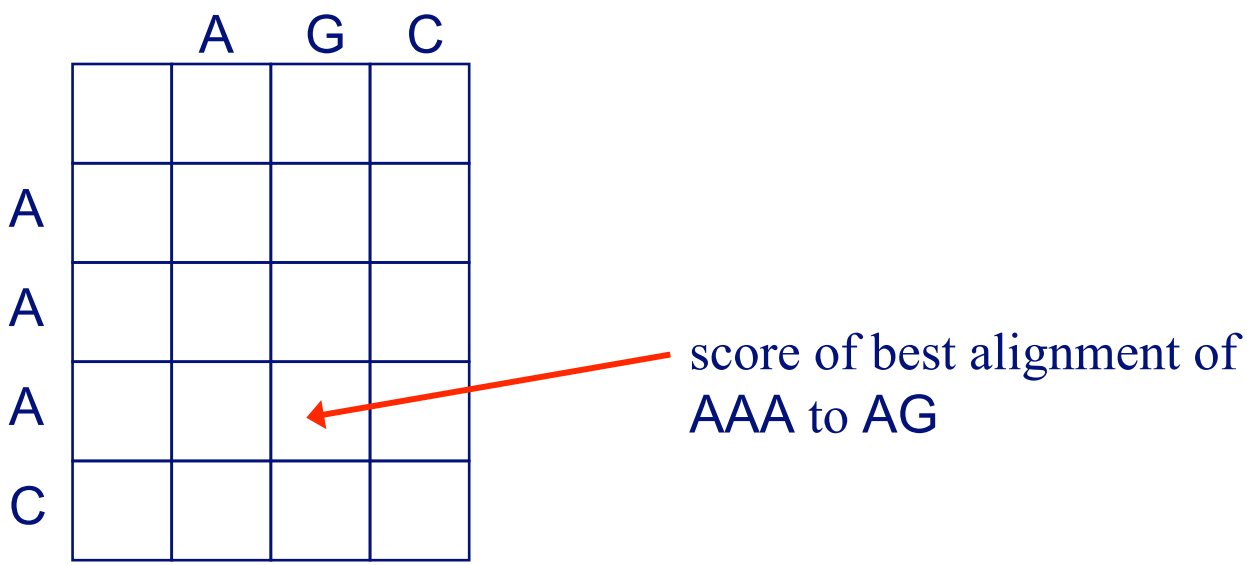
Dynamic Programming Idea

- consider last step in computing alignment of **AAAC** with **AGC**
- three possible options; in each we'll choose a different pairing for end of alignment, and add this to best alignment of previous characters



Dynamic Programming Idea

- given an n-character sequence x, and an m-character sequence y
- construct an $(n+1) \times (m+1)$ matrix F
- $F(i, j)$ = score of the best alignment of $x[1 \ldots i]$ with $y[1 \ldots j]$



Needleman-Wunch Algorithm

- one way to specify the DP is in terms of its recurrence relation:

match x_i with y_j

$$F(i,j) = \max \begin{cases} F(i-1, j-1) + s(x_i, y_j) \\ F(i-1, j) + g \\ F(i, j-1) + g \end{cases}$$

insertion in x

insertion in y

DP Algorithm Sketch: Global Alignment

- initialize first row and column of matrix
- fill in rest of matrix from top to bottom, left to right
- for each $F(i, j)$, save pointer(s) to cell(s) that resulted in best score
- $F(m, n)$ holds the optimal alignment score; trace pointers back from $F(m, n)$ to $F(0, 0)$ to recover alignment

Initializing Matrix

		A	G	C
	0	g	$2g$	$3g$
A	g			
A	$2g$			
A	$3g$			
C	$4g$			

Global Alignment Example

- suppose we choose the following scoring scheme:

$$s(x_i, y_i) = \begin{cases} +1 & \text{when } x_i = y_i \\ -1 & \text{when } x_i \neq y_i \end{cases}$$

g (penalty for aligning with a gap) = -2

Global Alignment Example

		A	G	C
A				
A				
A				
C				

$$s(x_i, y_i) =$$

$+1$ when $x_i = y_i$

-1 when $x_i \neq y_i$

$g = -2$

Global Alignment Example

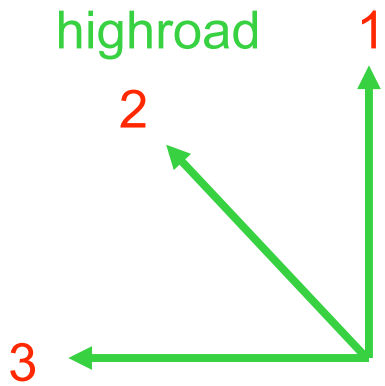
		A	G	C
	0	-2	-4	-6
A	-2	1	-1	-3
A	-4	-1	0	-2
A	-6	-3	-2	-1
C	-8	-5	-4	-1

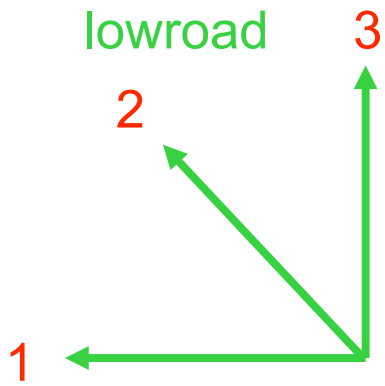
<u>one optimal alignment</u>

x:	A	A	A	C
y:	A	G	-	C

Equally Optimal Alignments

- many optimal alignments may exist for a given pair of sequences
- can use preference ordering over paths when doing traceback





- *highroad* and *lowroad* alignments show the two most different optimal alignments

Highroad & Lowroad Alignments

		A	G	C
	0	-2	-4	-6
A	-2	1	-1	-3
A	-4	-1	0	-2
A	-6	-3	-2	-1
C	-8	-5	-4	-1

<u>highroad alignment</u>

x:	A	A	A	C
y:	A	G	-	C

<u>lowroad alignment</u>

x:	A	A	A	C
y:	-	A	G	C

DP Comments

- works for either DNA or protein sequences, although the substitution matrices used differ
- finds an optimal alignment
- the exact algorithm (and computational complexity) depends on gap penalty function (we'll come back to this)

Local Alignment

- so far we have discussed *global alignment*, where we are looking for best match between sequences from one end to the other
- more commonly, we will want a *local alignment*, the best match between <u>subsequences</u> of x and y

Local Alignment Motivation

- useful for comparing protein sequences that share a common *motif* (conserved pattern) or *domain* (independently folded unit) but differ elsewhere
- useful for comparing DNA sequences that share a similar *motif* but differ elsewhere
- useful for comparing protein sequences against *genomic DNA sequences* (long stretches of uncharacterized sequence)
- more sensitive when comparing highly diverged sequences

Local Alignment DP Algorithm

- original formulation: Smith & Waterman, *Journal of Molecular Biology*, 1981

- interpretation of array values is somewhat different
  - $F(i, j)$ = score of the best alignment of <u>a suffix of</u> $x[1 \ldots i]$ and <u>a suffix of</u> $y[1 \ldots j]$

Local Alignment DP Algorithm

- the recurrence relation is slightly different than for global algorithm

$$F(i,j) = \max \begin{cases} F(i-1, j-1) + s(x_i, y_j) \\ F(i-1, j) + g \\ F(i, j-1) + g \\ 0 \end{cases}$$

Local Alignment DP Algorithm

- initialization: first row and first column initialized with 0's
- traceback:
  - find maximum value of $F(i, j)$; can be <u>anywhere</u> in matrix
  - stop when we get to a cell with value 0

Local Alignment Example

$s(x_i, y_i) =$

$+1$ when $x_i = y_i$

-1 when $x_i \neq y_i$

$g = -2$

		A	A	G	A
T					
T					
A					
A					
G					

Local Alignment Example

x: A A G

y: A A G

		A	A	G	A
	0	0	0	0	0
T	0	0	0	0	0
T	0	0	0	0	0
A	0	1	1	0	1
A	0	1	2	0	1
G	0	0	0	3	1

More On Gap Penalty Functions

- a gap of length k is more probable than k gaps of length 1
  - a gap may be due to a single mutational event that inserted/deleted a stretch of characters
  - separated gaps are probably due to distinct mutational events
- a linear gap penalty function treats these cases the same
- it is more common to use an *affine* gap penalty function, which involves two terms:
  - a penalty h associated with <u>opening</u> a gap
  - a smaller penalty g for <u>extending</u> the gap

Gap Penalty Functions

- linear

$$w(k) = gk$$

- affine

$$w(k) = \begin{cases} h + gk, & k \geq 1 \\ 0, & k = 0 \end{cases}$$

Dynamic Programming for the Affine Gap Penalty Case

- to do in $O(n^2)$ time, need 3 matrices instead of 1

$M(i, j)$	best score given that $x[i]$ is aligned to $y[j]$
$I_x(i, j)$	best score given that $x[i]$ is aligned to a gap
$I_y(i, j)$	best score given that $y[j]$ is aligned to a gap

Global Alignment DP for the Affine Gap Penalty Case

$$M(i,j) = \max \begin{cases} M(i-1,j-1) + s(x_i, y_j) & \text{match } x_i \text{ with } y_j \\ I_x(i-1,j-1) + s(x_i, y_j) & \text{insertion in } x \\ I_y(i-1,j-1) + s(x_i, y_j) & \text{insertion in } y \end{cases}$$

$$I_x(i,j) = \max \begin{cases} M(i-1,j) + h + g & \text{open gap in } x \\ I_x(i-1,j) + g & \text{extend gap in } x \end{cases}$$

$$I_y(i,j) = \max \begin{cases} M(i,j-1) + h + g & \text{open gap in } y \\ I_y(i,j-1) + g & \text{extend gap in } y \end{cases}$$

Global Alignment DP for the Affine Gap Penalty Case

- initialization

  $M(0,0) = 0$

  $I_x(i,0) = h + g \times i$

  $I_y(0,j) = h + g \times j$

  other cells in top row and leftmost column $= -\infty$

- traceback
  - start at largest of $M(m,n), I_x(m,n), I_y(m,n)$
  - stop at any of $M(0,0), I_x(0,0), I_y(0,0)$
  - note that pointers may traverse all three matrices

Global Alignment Example

(Affine Gap Penalty)

h = -3, g = -1

		A	C	A	C	T
	M : 0 I_x : -3 I_y : -3	-∞ -∞ -4	-∞ -∞ -5	-∞ -∞ -6	-∞ -∞ -7	-∞ -∞ -8
A	-∞ -4 -∞	1 -∞ -∞	-5 -∞ -3	-4 -∞ -4	-7 -∞ -5	-8 -∞ -6
A	-∞ -5 -∞	-3 -3 -∞	0 -9 -7	-2 -8 -4	-5 -11 -5	-6 -12 -6
T	-∞ -6 -∞	-6 -4 -∞	-4 -4 -10	-1 -6 -8	-3 -9 -5	-4 -10 -6

Global Alignment Example (Continued)

		A	C	A	C	T
	M : 0 I_x : -3 I_y : -3	-∞ -∞ -4	-∞ -∞ -5	-∞ -∞ -6	-∞ -∞ -7	-∞ -∞ -8
A	-∞ -4 -∞	1 -∞ -∞	-5 -∞ -3	-4 -∞ -4	-7 -∞ -5	-8 -∞ -6
A	-∞ -5 -∞	-3 -3 -∞	0 -9 -7	-2 -8 -4	-5 -11 -5	-6 -12 -6
T	-∞ -6 -∞	-6 -4 -∞	-4 -4 -10	-1 -6 -8	-3 -9 -5	-4 -10 -6

three optimal alignments:

```
ACACT      ACACT      ACACT
AA--T      A--AT      --AAT
```

Local Alignment DP for the Affine Gap Penalty Case

$$M(i,j) = \max \begin{cases} M(i-1,j-1) + s(x_i, y_j) \\ I_x(i-1,j-1) + s(x_i, y_j) \\ I_y(i-1,j-1) + s(x_i, y_j) \\ 0 \end{cases}$$

$$I_x(i,j) = \max \begin{cases} M(i-1,j) + h + g \\ I_x(i-1,j) + g \end{cases}$$

$$I_y(i,j) = \max \begin{cases} M(i,j-1) + h + g \\ I_y(i,j-1) + g \end{cases}$$

Local Alignment DP for the Affine Gap Penalty Case

- initialization

  $M(0,0) = 0$

  $M(i,0) = 0$

  $M(0,j) = 0$

  cells in top row and leftmost column of $I_x, I_y = -\infty$

- traceback
  - start at largest $M(i,j)$
  - stop at $M(i,j) = 0$

Gap Penalty Functions

- linear: $w(k) = gk$

- affine:

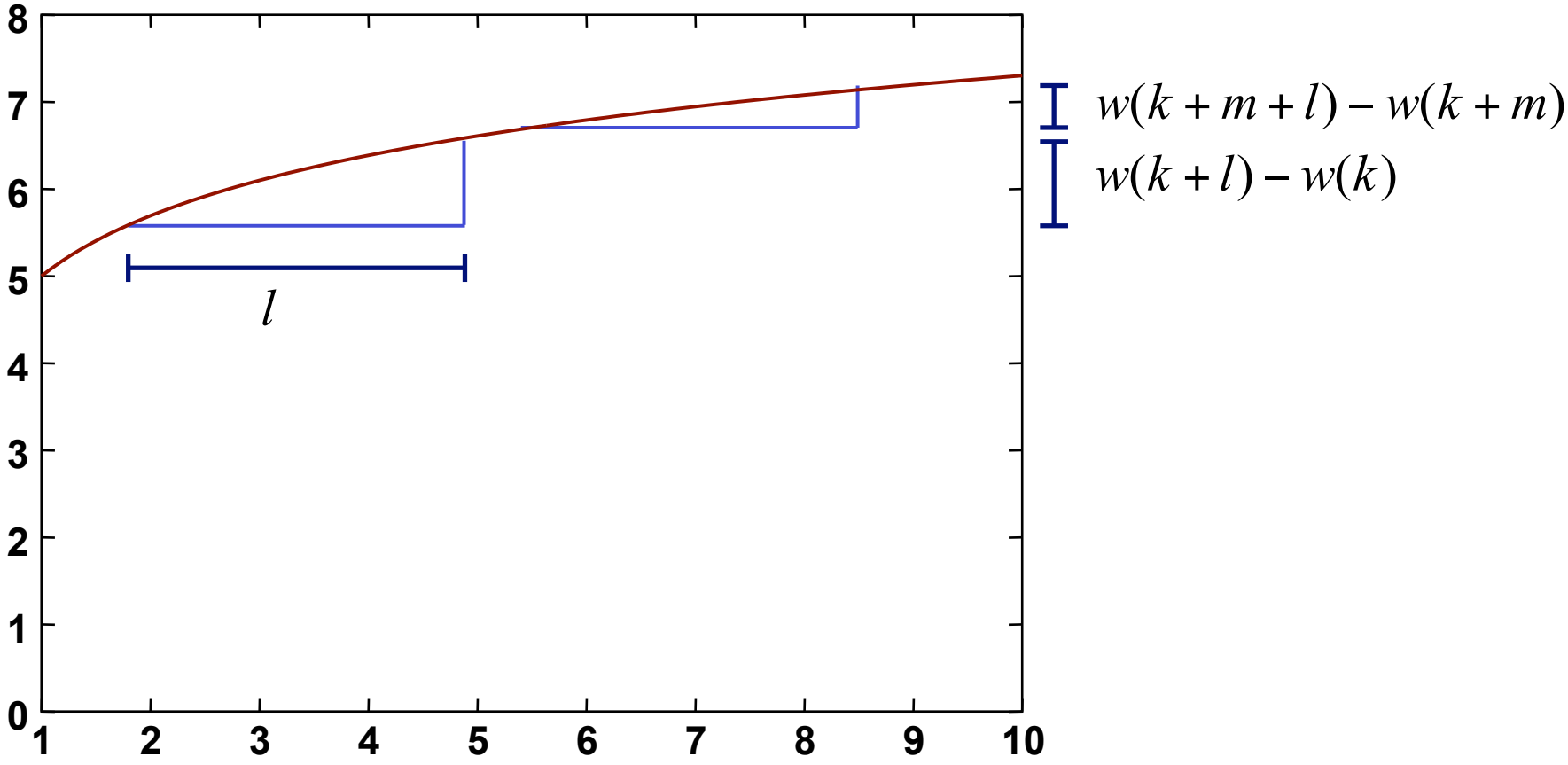
$$w(k) = \begin{cases} h + gk, & k \geq 1 \\ 0, & k = 0 \end{cases}$$

- concave: a function for which the following holds for all $k, l, m \geq 0$

$$w(k + m + l) - w(k + m) \leq w(k + l) - w(k)$$

e.g. $w(k) = h + g \times \log(k)$

Concave Gap Penalty Functions



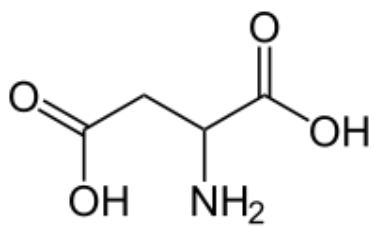
$$w(k+m+l)-w(k+m) \le w(k+l)-w(k)$$

More On Scoring Matches

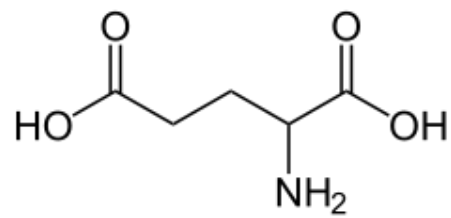
- so far, we've discussed multiple gap penalty functions, but only one match-scoring scheme:

$$s(x_i, y_i) = \begin{cases} +1 & \text{when } x_i = y_i \\ -1 & \text{when } x_i \neq y_i \end{cases}$$

- for protein sequence alignment, some amino acids have similar structures and can be substituted in nature:



aspartic acid (D)



glutamic acid (E)

Substitution Matrices

- two popular sets of matrices for protein sequences
  - PAM matrices [Dayhoff *et al.,* 1978]
  - BLOSUM matrices [Henikoff & Henikoff, 1992]

- both try to capture the the relative substitutability of amino acid pairs in the context of evolution

BLOSUM62 Matrix

BLOSUM62

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

Positive for chemically similar substitution

Common amino acids have low weights

Rare amino acids have high weights

Heuristic Methods

- the algorithms we learned today take $O(nm)$ time to align sequences, which is too slow for searching large databases
  - imagine an internet search engine, but where queries and results are protein sequences
- heuristic methods do fast approximation to dynamic programming
  - example: BLAST [Altschul *et al*., 1990; Altschul et al., 1997]
  - break sequence into small (e.g. 3 base pair) "words"
  - scan database for word matches
  - extend all matches to seek high-scoring alignments
  - tradeoff: sensitivity for speed

Multiple Sequence Alignment

- we've only discussed aligning 2 sequences, but we may want to do more
- discover common motifs in a set of sequences (e.g. DNA sequences that bind the same protein)
- characterize a set of sequences (e.g. a protein family)
- much more complex

```
GGWWRGdy.ggkkqLWFPSNYV
IGWLNGynettgerGDFPGTYV
PNWWEGql..nnrrGIFPSNYV
DEWWQArr..deqiGIVPSK--
GEWWKAqs..tgqeGFIPFNFV
GDWWLArs..sgqtGYIPSNYV
GDWWDAel..kgrrGKVPSNYL
-DWWEArslssghrGYVPSNYV
GDWWYArslitnseGYIPSTYV
GEWWKArslatrkeGYIPSNYV
GDWWLArslvtgreGYVPSNFV
GEWWKAkslsskreGFIPSNYV
GEWCEAqt.kngq.GWVPSNYI
SDWWRVvnlttrqeGLIPLNFV
LPWWRArd.kngqeGYIPSNYI
RDWWEFrsktvytpGYYESGYV
EHWWKVkd.algnvGYIPSNYV
IHWWRVqd.rngheGYVPSSYL
KDWWKVev..ndrqGFVPAAYV
VGWMPGlnertrqrGDFPGTYV
PDWWEGel..ngqrGVFPASYV
ENWWNGei..gnrkGIFPATYV
EEWLEGec..kgkvGIFPKVFV
GGWWKGdy.gtriqQYFPSNYV
DGWWRGsy..ngqvGWFPSNYV
QGWWRGei..ygrvGWFPANYV
GRWWKArr.angetGIIPSNYV
GGWTQGel.ksgqkGWAPTNYL
GDWWEArsn.tgenGYIPSNYV
NDWWTGrt..ngkeGIFPANYV
```

Figure from A. Krogh, An Introduction to Hidden Markov Models for Biological Sequences

Next Time…

- basic molecular biology
- sequence alignment
- **probabilistic sequence models**
- gene expression analysis
- protein structure prediction
  - by Ameet Soni