CSE 427 Comp Bio

Sequence Alignment

Sequence Alignment

What

Why

A Dynamic Programming Algorithm

Sequence Alignment

Goal: position characters in two strings to "best" line up identical/similar ones with one another

We can do this via Dynamic Programming

What is an alignment?

Compare two strings to see how “similar” they are

E.g., maximize the # of identical chars that line up

ATGTTAT vs ATCGTAC

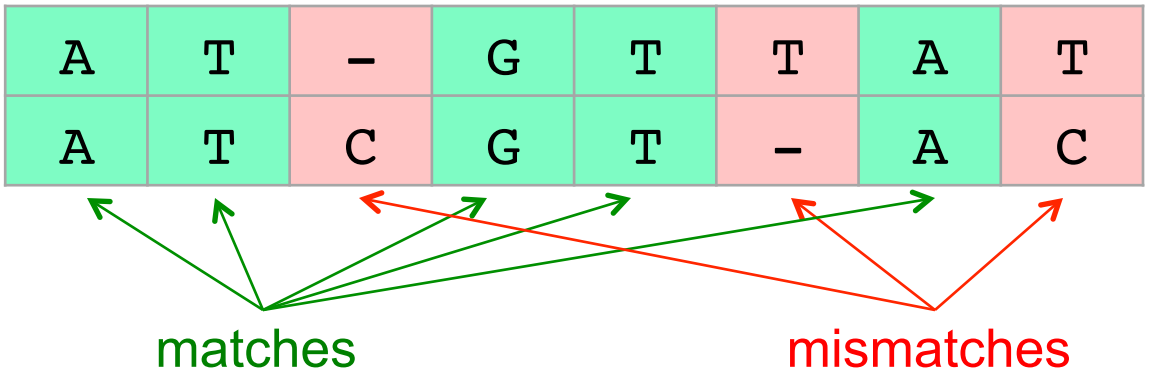
A	T	-	G	T	T	A	T
A	T	C	G	T	-	A	C

What is an alignment?

Compare two strings to see how “similar” they are

E.g., maximize the # of identical chars that line up

ATGTTAT vs ATCGTAC



Sequence Alignment: Why

Biology

- Among most widely used comp. tools in biology
- DNA sequencing & assembly
- New sequence always compared to data bases
- **Similar sequences often have similar origin and/or function**
- Recognizable similarity after 10^8 –10^9 yr

Other

- spell check/correct, diff, svn/git/…, plagiarism, …

BLAST Demo

http://www.ncbi.nlm.nih.gov/blast/

Taxonomy Report

```
root ..............................    64 hits    16 orgs
. Eukaryota .......................    62 hits    14 orgs [cellular organisms]
```

Try it!

pick any protein, e.g. hemoglobin, insulin, exportin,… BLAST to find distant relatives.

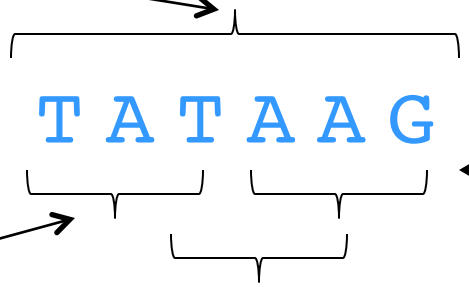
Alternate demo:

- go to http://www.uniprot.org/uniprot/O14980 "**Exportin-1**"
- find "BLAST" button about ½ way down page, under "Sequences", just above big grey box with the amino sequence of this protein
- click "go" button
- after a minute or 2 you should see the 1st of 10 pages of "hits" – matches to similar proteins in other species
- you might find it interesting to look at the species descriptions and the "identity" column (generally above 50%, even in species as distant from us as fungus -- extremely unlikely by chance on a 1071 letter sequence over a 20 letter alphabet)
- Also click any of the colored "alignment" bars to see the actual alignment of the human XPO1 protein to its relative in the other species – in 3-row groups (query 1st, the match 3rd, with identical letters highlighted in between)

```
` mphocystis disease virus .........     1 hits     1 orgs [Viruses; dsDNA viruses, no RNA …]
```

Terminology

string
ordered list of letters



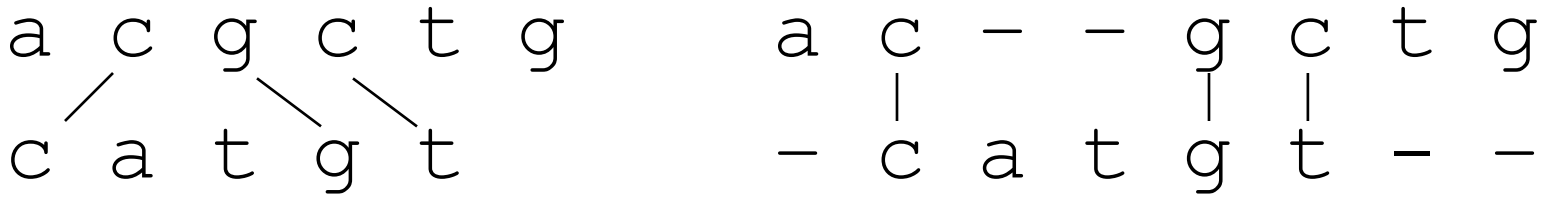
suffix
consecutive letters from back

prefix
consecutive letters from front

substring
consecutive letters from anywhere

subsequence
any ordered, nonconsecutive letters,
i.e. `AAA` , `TAG`

Formal definition of an alignment



An alignment of strings S, T is a pair of strings S', T' with dash characters "–" inserted, so that

1. |S'| = |T'|, and (|S| = "length of S")
2. Removing dashes leaves S, T

Consecutive dashes are called "a gap."

(Note that this is a definition for a general alignment, not optimal.)

Scoring an arbitrary alignment

Define a score for *pairs* of aligned chars, e.g.

$$\sigma(x, y) = \begin{cases} \text{match} & 2 \\ \text{mismatch} & -1 \end{cases}$$

(Toy scores for examples in slides)

Apply that *per column*, then *add*.

```
a  c  -  -  g  c  t  g
   |        |  |
-  c  a  t  g  t  -  -
```

```
-1 +2 -1 -1 +2 -1 -1 -1
```

Total Score = -2

More Realistic Scores: BLOSUM 62

(the "σ" scores)

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

Optimal Alignment: A Simple Algorithm

for all subseqs A of S, B of T s.t. |A| = |B| **do**
  align A[i] with B[i], 1 ≤ i ≤ |A|
  align all other chars to spaces
  compute its value
  retain the max
end
output the retained alignment

S = agct A = ct
T = wxyz B = xz

```
-agc-t   a-gc-t
w--xyz   -w-xyz
```

Analysis

Assume |S| = |T| = n

Cost of evaluating one alignment: ≥ n

How many alignments are there: $\geq \binom{2n}{n}$

pick n chars of S,T together

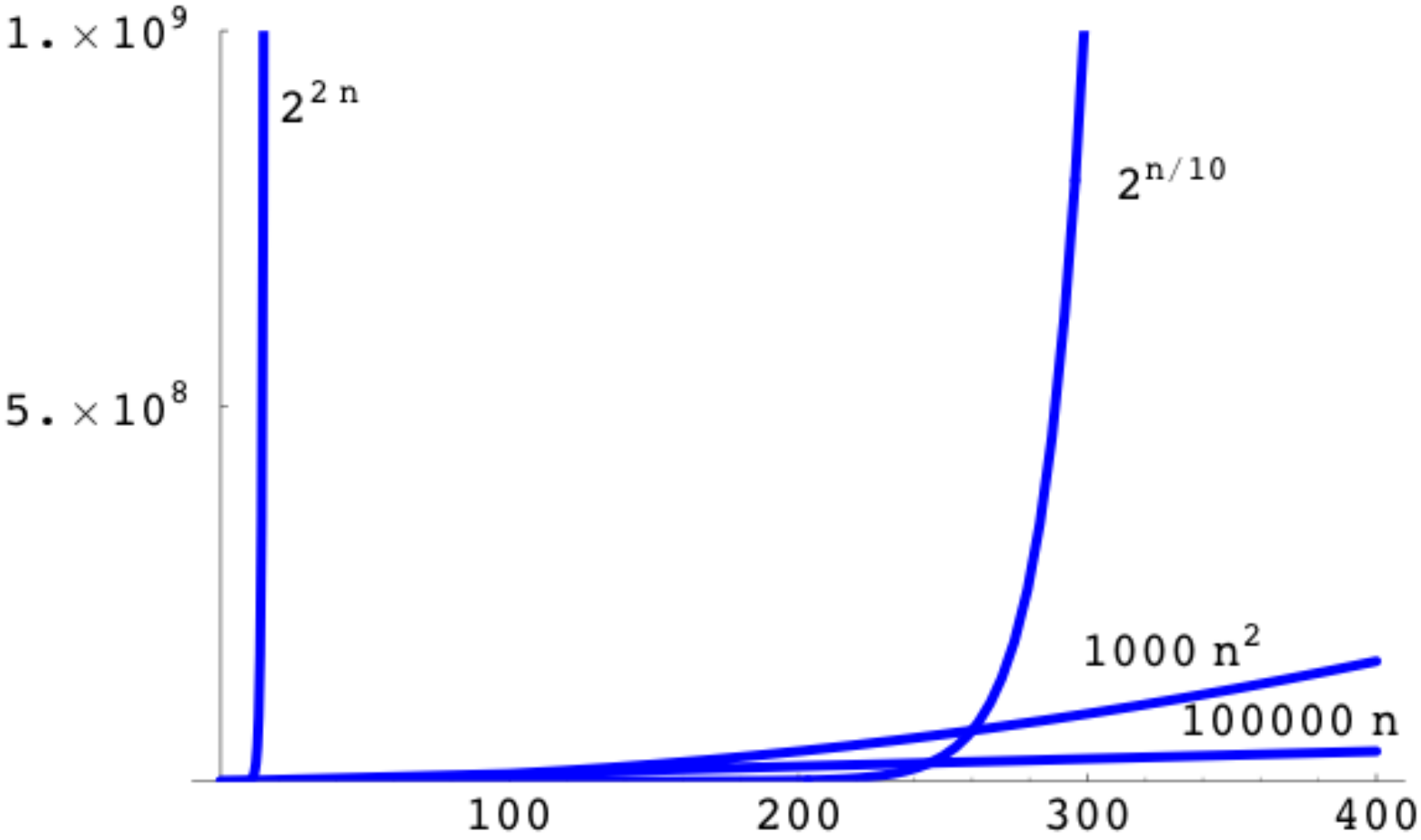
say k of them are in S

match these k to the k *un*picked chars of T

Total time: $\geq n\binom{2n}{n} > 2^{2n}$, for $n > 3$

E.g., for n = 20, time is > 2^{40} operations

Polynomial vs Exponential Growth



Fibonacci Numbers (recursion)

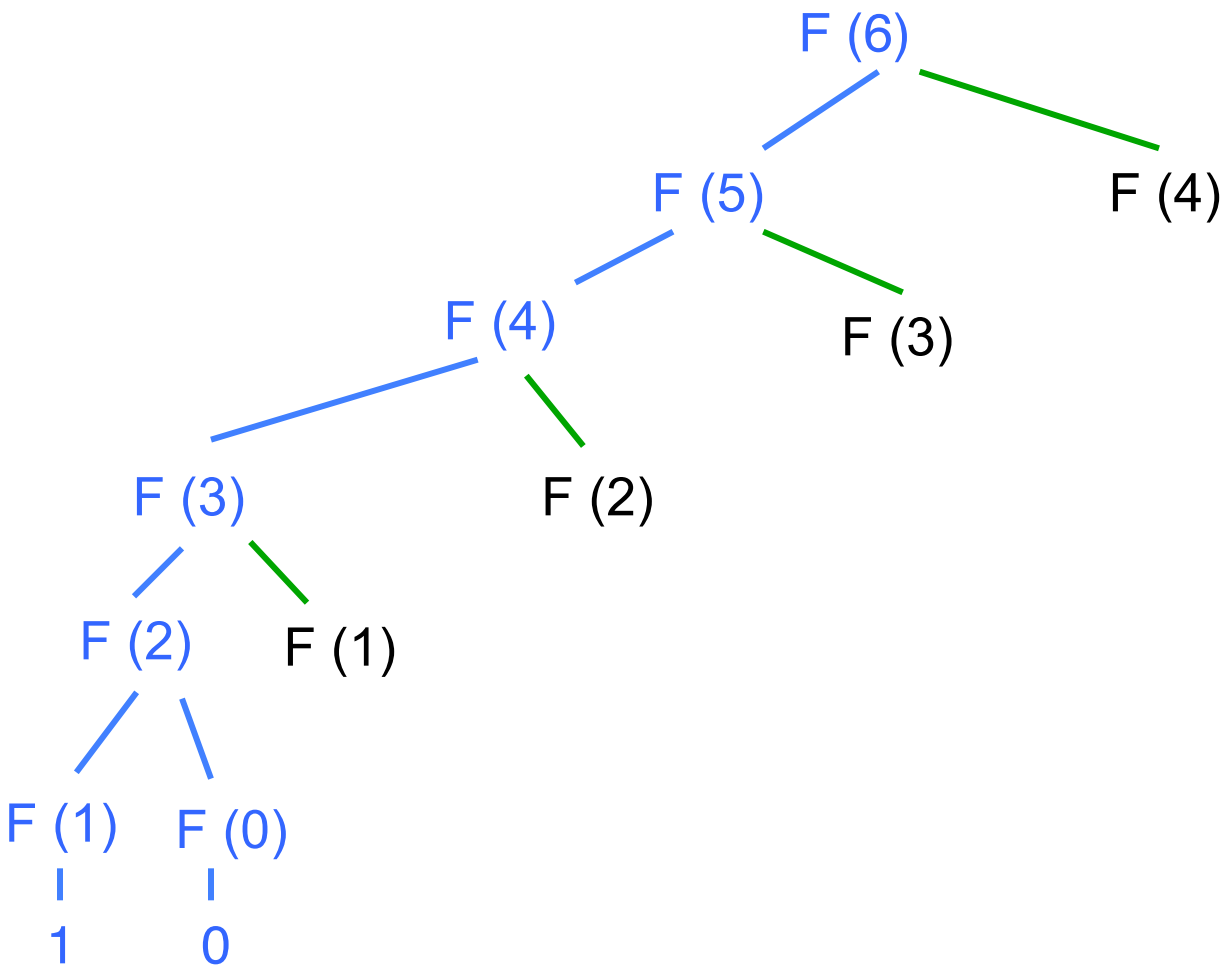
```
fibr(n) {
  if (n <= 1) {
    return 1;
  } else {
    return fibr(n-1) + fibr(n-2);
  }
}
```

Simple recursion, but many repeated subproblems!!

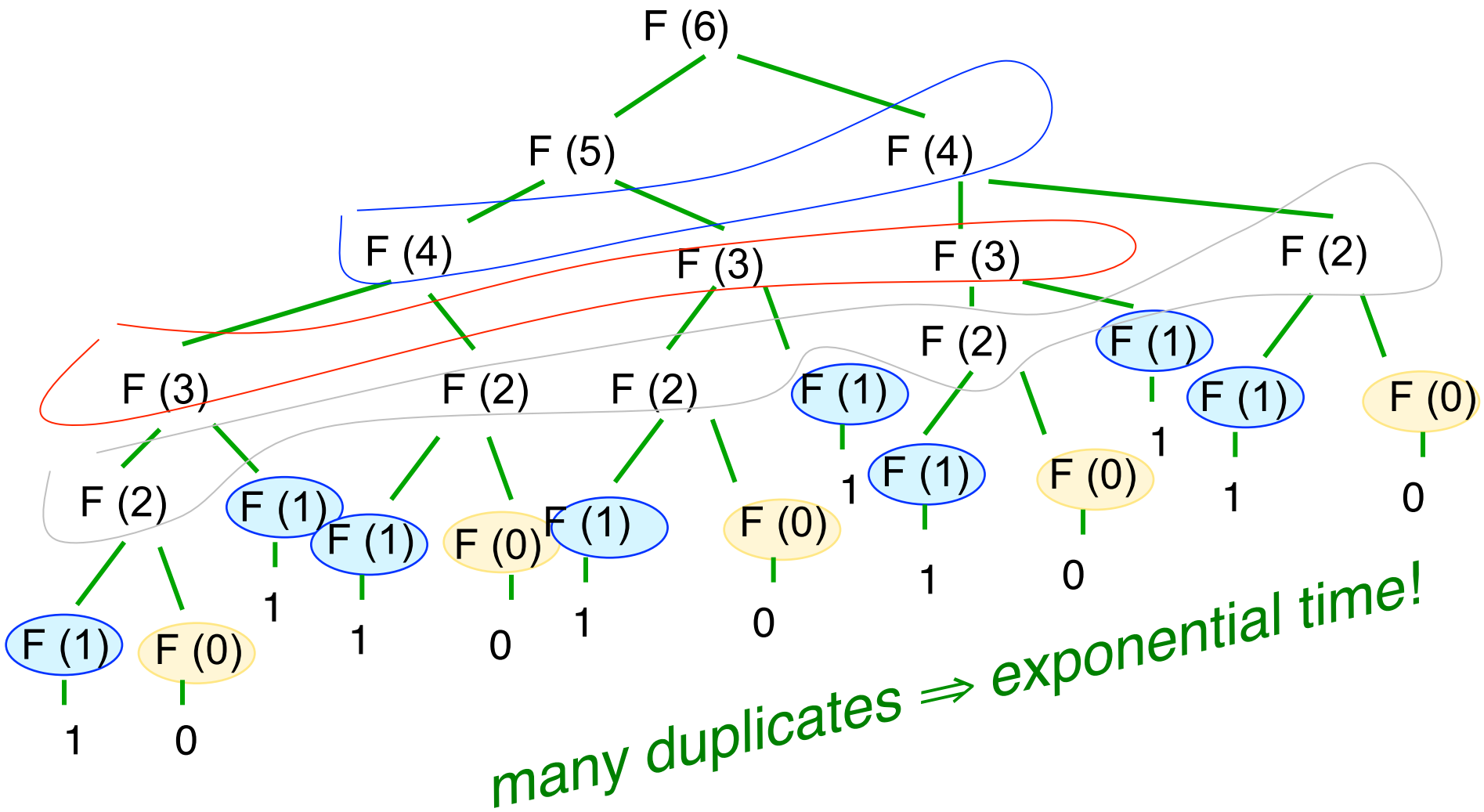
$\Rightarrow$

Time = $\Omega(1.61^n)$

Call tree - start



Full call tree



Fibonacci, II
(dynamic programming)

```
int fibd[n];
fibd[0] = 1;
fibd[1] = 1;
for(i=2; i<=n; i++) {
  fibd[i] = fibd[i-1] + fibd[i-2];
}
return fibd[n];
```

Avoid repeated subproblems by tabulating their solutions

$\Rightarrow$

Time = O(n)

(in this case)

Can we use Dynamic Programming?

1. Can we decompose into **subproblems?**

   E.g., can we align smaller substrings (say, prefix/ suffix in this case), then combine them somehow?

2. Do we have **optimal substructure?**

   I.e., is optimal solution to a subproblem *independent of context?* E.g., is appending two optimal alignments also be optimal? Perhaps, but some changes at the interface might be needed?

Optimal Substructure
(In More Detail)

Optimal alignment *ends* in 1 of 3 ways:

- last chars of S & T aligned with each other
- last char of S aligned with dash in T
- last char of T aligned with dash in S

(never align dash with dash; $\sigma(-, -) < 0$)

In each case, the rest of S & T should be optimally aligned to each other

Optimal Alignment in $O(n^2)$ via "Dynamic Programming"

Input: S, T, |S| = n, |T| = m

Output: value of optimal alignment

Easier to solve a "harder" problem:

V(i,j) = value of optimal alignment of

S[1], …, S[i] with T[1], …, T[j]

for all $0 \le i \le n$, $0 \le j \le m$.

Base Cases

V(i,0): first i chars of S all match dashes

$$V(i,0) = \sum_{k=1}^{i} \sigma(S[k], -)$$

V(0,j): first j chars of T all match dashes

$$V(0,j) = \sum_{k=1}^{j} \sigma(-, T[k])$$

General Case

Opt align of S[1], …, S[i] vs T[1], …, T[j]:

$$\begin{bmatrix} \sim\sim\sim\sim & S[i] \\ \sim\sim\sim\sim & T[j] \end{bmatrix}, \quad \begin{bmatrix} \sim\sim\sim\sim & S[i] \\ \sim\sim\sim\sim & - \end{bmatrix}, \text{ or } \begin{bmatrix} \sim\sim\sim\sim & - \\ \sim\sim\sim\sim & T[j] \end{bmatrix}$$

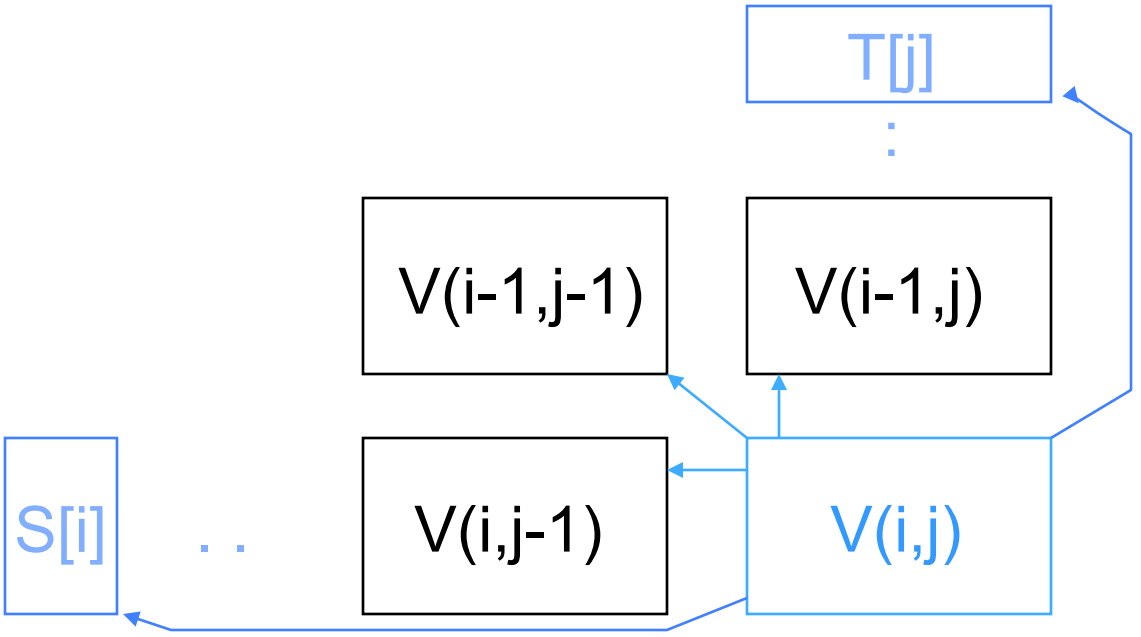
Opt align of $S_1 \ldots S_{i-1}$ & $T_1 \ldots T_{j-1}$

$$V(i,j) = \max \begin{cases} V(i\text{-}1,j\text{-}1) + \sigma(S[i],T[j]) \\ V(i\text{-}1,j) + \sigma(S[i], \text{ - }) \\ V(i,j\text{-}1) + \sigma(\text{ - }, T[j]) \end{cases},$$

for all $1 \le i \le n, \ 1 \le j \le m.$

Calculating One Entry

$$V(i,j) = \max \begin{cases} V(i\text{-}1,j\text{-}1) + \sigma(S[i],T[j]) \\ V(i\text{-}1,j) + \sigma(S[i], \text{ - }) \\ V(i,j\text{-}1) + \sigma(\text{ - }, T[j]) \end{cases}$$



Mismatch = -1
Match = 2

Example

	j	0	1	2	3	4	5	
i			c	a	t	g	t	←T
0		0	-1	-2	-3	-4	-5	
1	a	-1						
2	c	-2						
3	g	-3						
4	c	-4						
5	t	-5						
6	g	-6						
	↑ S							

c
-

Score(c,-) = -1

Mismatch = -1
Match = 2

Example

	j	0	1	2	3	4	5	
i			c	a	t	g	t	←T
0		0	-1	-2	-3	-4	-5	
1	a	-1						
2	c	-2						
3	g	-3						
4	c	-4						
5	t	-5						
6	g	-6						
	↑ S							

-
a

Score(-,a) = -1

Mismatch = -1
Match = 2

Example

i	j	0	1 c	2 a	3 t	4 g	5 t
0		0	-1	-2	-3	-4	-5
1	a	-1					
2	c	-2					
3	g	-3					
4	c	-4					
5	t	-5					
6	g	-6					

←T

↑S

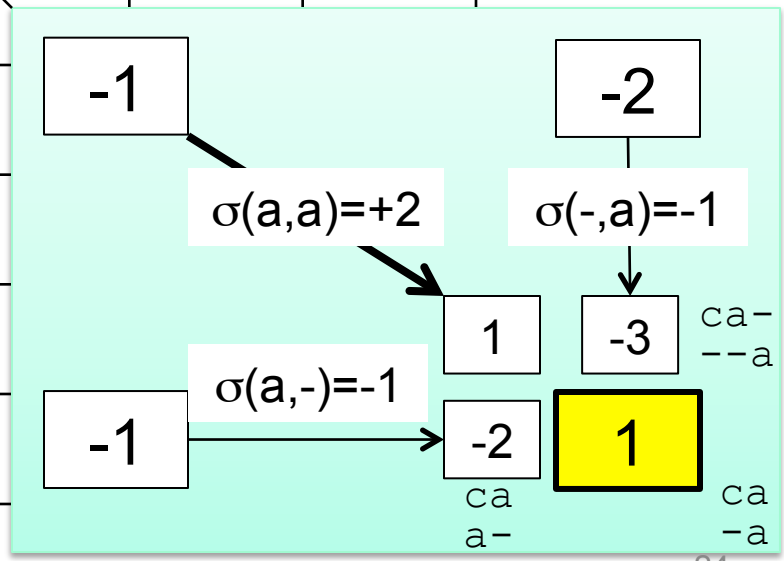
Mismatch = -1
Match = 2

Example

i	j	0	1 c	2 a	3 t	4 g	5 t
0		0	-1	-2	-3	-4	-5
1	a	-1	-1	1			
2	c	-2					
3	g	-3					
4	c	-4					
5	t	-5					
6	g	-6					

←T

S↑



Mismatch = -1
Match = 2

Example

	j	0	1	2	3	4	5
i			c	a	t	g	t
0		0	-1	-2	-3	-4	-5
1	a	-1	-1	1			
2	c	-2	1				
3	g	-3					
4	c	-4					
5	t	-5					
6	g	-6					

←T

Time = O(mn)

↑S

Mismatch = -1
Match = 2

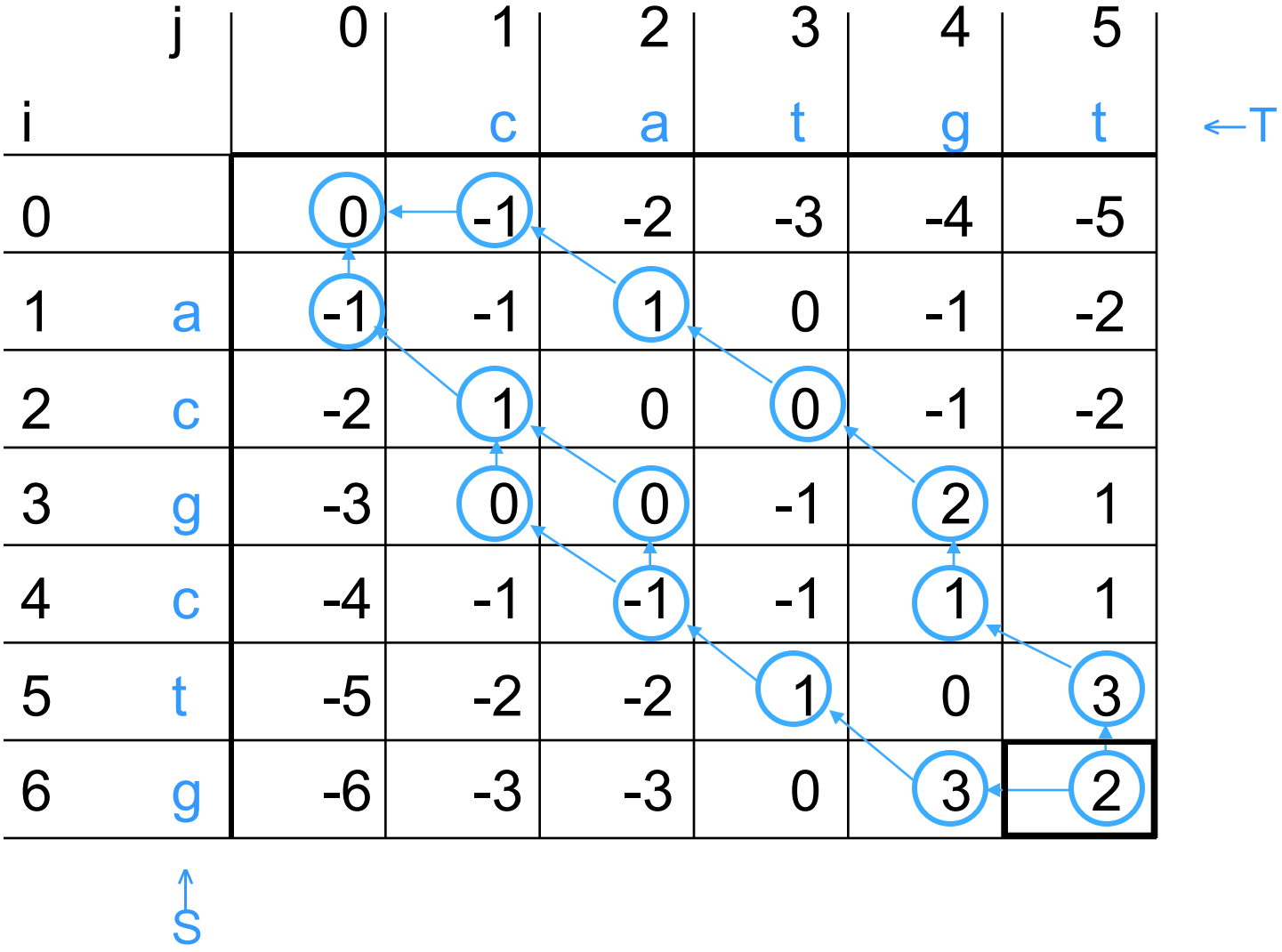
Example

i	j	0	1 c	2 a	3 t	4 g	5 t	←T
0		0	-1	-2	-3	-4	-5	
1	a	-1	-1	1	0	-1	-2	
2	c	-2	1	0	0	-1	-2	
3	g	-3	0	0	-1	2	1	
4	c	-4	-1	-1	-1	1	1	
5	t	-5	-2	-2	1	0	3	
6	g	-6	-3	-3	0	3	2	

↑S

Finding Alignments: Trace Back

Arrows = (ties for) max in V(i,j); 3 LR-to-UL paths = 3 optimal alignments



Ex: what are the 3 alignments? C.f. slide 12.

Complexity Notes

Time = O(mn), (value and alignment)

Space = O(mn)

Easy to get value in Time = O(mn) and Space = O(min(m,n))

Possible to get value *and alignment* in Time = O(mn) and Space =O(min(m,n))

Sequence Alignment

Part II

Local alignments & gaps

Variations

- Local Alignment
  - Preceding gives *global* alignment, i.e. full length of both strings;
  - Might well miss strong similarity of part of strings amidst dissimilar flanks
- Gap Penalties
  - 10 adjacent spaces cost 10 x one space?
- Many others
- Similarly fast DP algs often possible

Local Alignment: Motivations

"Interesting" (evolutionarily conserved, functionally related) segments may be a small part of the whole

- "Active site" of a protein
- Scattered genes or exons amidst "junk", e.g. retroviral insertions, large deletions
- Don't have whole sequence

Global alignment might miss them if flanking junk outweighs similar regions

Local Alignment

Optimal *local alignment* of strings S & T: Find substrings A of S and B of T having max value global alignment

S = abcxdex A = c x d e

T = xxxcde B = c - d e value = 5

Local Alignment: "Obvious" Algorithm

for all substrings A of S and B of T:
    Align A & B via dynamic programming
    Retain pair with max value
end ;

Output the retained pair

Time: $O(n^2)$ choices for A, $O(m^2)$ for B, $O(nm)$ for DP, so $O(n^3m^3)$ total.

[Best possible? Lots of redundant work…]

Local Alignment in O(nm) via Dynamic Programming

Input: S, T, |S| = n, |T| = m

Output: value of optimal local alignment

Better to solve a "harder" problem
for all $0 \le i \le n$, $0 \le j \le m$:

V(i,j) = max value of opt (global)
alignment of a suffix of S[1], …, S[i]
with a suffix of T[1], …, T[j]

Report best i,j

Base Cases

Assume $\sigma(x,-) \leq 0$, $\sigma(-,x) \leq 0$

V(i,0): some suffix of first i chars of S; all match spaces in T; best suffix is empty

$V(i,0) = 0$

V(0,j): similar

$V(0,j) = 0$

General Case Recurrences

Opt suffix align S[1], …, S[i] vs T[1], …, T[j]:

$$\left[\begin{matrix}\sim\sim\sim\sim & S[i]\\ \sim\sim\sim\sim & T[j]\end{matrix}\right],\ \left[\begin{matrix}\sim\sim\sim\sim & S[i]\\ \sim\sim\sim\sim & -\end{matrix}\right],\ \left[\begin{matrix}\sim\sim\sim\sim & -\\ \sim\sim\sim\sim & T[j]\end{matrix}\right],\ \text{or}\ \left[\begin{matrix} & \\ & \end{matrix}\right]$$

Opt align of suffix of $S_1 \ldots S_{i-1}$ & $T_1 \ldots T_{j-1}$

$$V(i,j) = \max\begin{cases} V(i\text{-}1,j\text{-}1) + \sigma(S[i],T[j]) \\ V(i\text{-}1,j) + \sigma(S[i], \text{ - }) \\ V(i,j\text{-}1) + \sigma(\text{ - }, T[j]) \\ 0 \end{cases},$$

$$\text{for all } 1 \le i \le n,\ 1 \le j \le m.$$

opt suffix alignment has: 2, 1, 1, 0 chars of S/T

Scoring Local Alignments

i	j	0	1 x	2 x	3 x	4 c	5 d	6 e
0		0	0	0	0	0	0	0
1	a	0						
2	b	0						
3	c	0						
4	x	0						
5	d	0						
6	e	0						
7	x	0						

←T

↑
S

Finding Local Alignments

Again, arrows follow max *term* (not max neighbor)

i	j		0	1 x	2 x	3 x	4 c	5 d	6 e
0			0	0	0	0	0	0	0
1	a		0	0	0	0	0	0	0
2	b		0	0	0	0	0	0	0
3	c		0	0	0	0	2	1	0
4	x		0	2	2	2	1	1	0
5	d		0	1	1	1	1	3	2
6	e		0	0	0	0	0	2	5
7	x		0	2	2	2	1	1	4
		↑ S							

←T

One align-ment is:

```
c-de
cxde
```

What's the other?

Notes

Time and Space = O(mn)

Space O(min(m,n)) possible with time O(mn), but finding alignment is trickier

Local alignment: “Smith-Waterman”

Global alignment: “Needleman-Wunsch”

Significance of Alignments

Is “42” a good score?

Compared to what?

Usual approach: compared to a specific “null model”, such as “random sequences”

More on this later; a taste now, for use in next HW

Overall Alignment Significance, II
Empirical (via randomization)

You just searched with x, found "good" score for x:y

Generate N random "y-like" sequences (say N = 10^3 - 10^6)

Align x to each & score

If k of them have score than better or equal to that of x to y, then the (empirical) probability of a chance alignment as good as observed x:y alignment is (k+1)/(N+1)

e.g., if 0 of 99 are better, you can say "estimated p ≤ .01"

How to generate "random y-like" seqs? Scores depend on:

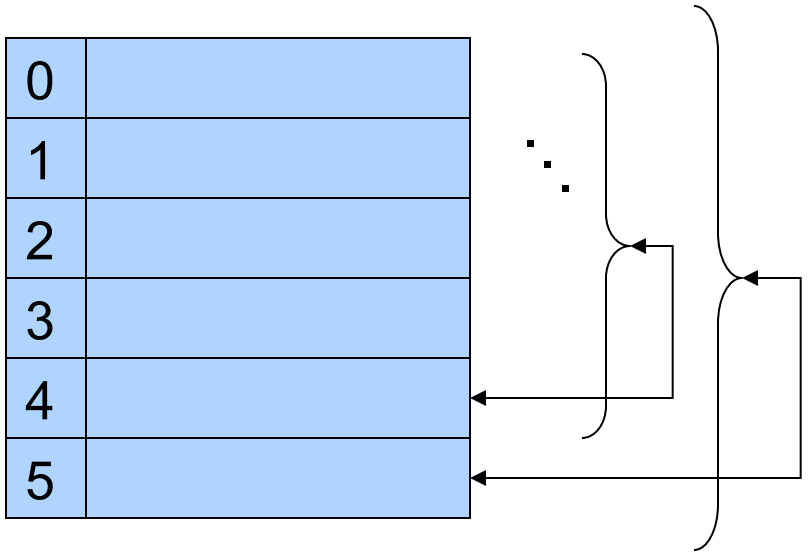
Length, so use same length as y

Sequence composition, so uniform 1/20 or 1/4 is a bad idea; even background p_i can be dangerous (if *y* unusual)

Better idea: *permute* y N times

Generating Random Permutations

```
for (i = n-1; i > 0; i--){
    j = random(0..i);
    swap X[i] <-> X[j];
}
```



All n! permutations of the original data equally likely: A specific element will be last with prob 1/n; given that, another specific element will be next-to-last with prob 1/(n-1), …; overall: 1/(n!)

C.f. http://en.wikipedia.org/wiki/Fisher–Yates_shuffle and (for subtle way to go wrong) http://www.codinghorror.com/blog/2007/12/the-danger-of-naivete.html

Alignment With Gap Penalties

Gap: maximal run of dashes in S' or T'

```
ag--ttc-t        2 gaps in S'
a---ttcgt        1 gap in T'
```

Motivations, e.g.:

- mutation might insert/delete several or even many residues at once
- matching mRNA (no introns) to genomic DNA (exons and introns)
- some parts of proteins less critical

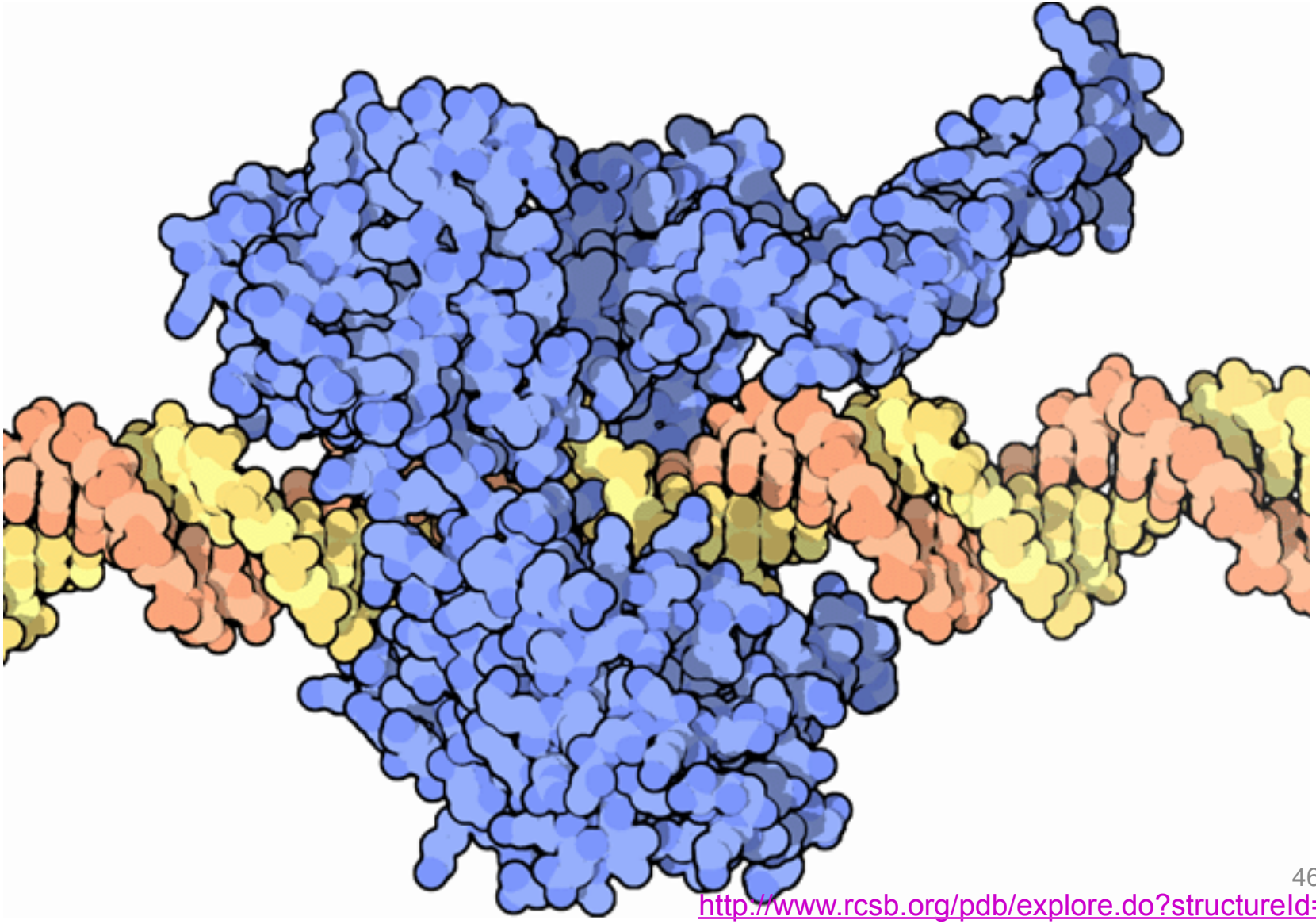
A Protein Structure: (Dihydrofolate Reductase)

http://www.rcsb.org/pdb/explore/jmol.do?structureId=5CC9&bionumber=1

Alignment of 5 Dihydrofolate reductase proteins

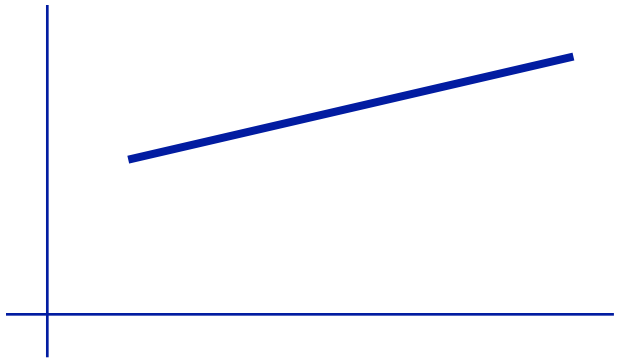
CLUSTAL W (1.82) multiple sequence alignment
http://pir.georgetown.edu/cgi-bin/multialn.pl
2/11/2013

Topoisomerase I



http://www.rcsb.org/pdb/explore.do?structureId=1a36

Affine Gap Penalties



gap open penalty

gap extend penalty

Gap penalty = g + e*(gaplen-1), g ≥ e ≥ 0

Note: no longer suffices to know just the *score* of best subproblem(s) – *state* matters: do they end with '-' or not.

Global Alignment with Affine Gap Penalties

V(i,j) = value of opt alignment of
S[1], …, S[i] with T[1], …, T[j]

G(i,j) = …, s.t. last pair matches S[i] & T[j]

F(i,j) = …, s.t. last pair matches S[i] & –

E(i,j) = …, s.t. last pair matches – & T[j]

S	T
x/–	x/–
x	x
x	–
–	x

Time: O(mn) [calculate all, O(1) each]

Affine Gap Algorithm

gap open penalty

gap extend penalty

Gap penalty = g + e*(gaplen-1), g ≥ e ≥ 0

V(i,0) = E(i,0) = V(0,i) = F(0,i) = -g-(i-1)*e

V(i,j) = max(G(i,j), F(i,j), E(i,j))

G(i,j) = V(i-1,j-1) + σ(S[i],T[j])

F(i,j) = max(F(i-1,j)-e , V(i-1,j)-g)

E(i,j) = max(E(i,j-1)-e , V(i,j-1)-g)

old gap new gap

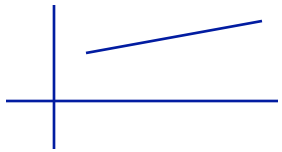
S	T
x/–	x/–
x	x
x	–
–	x

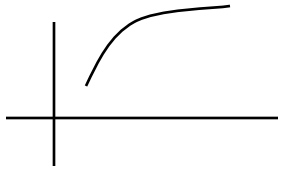
Q. Why is the "V" case a "new gap" when V includes E & F?

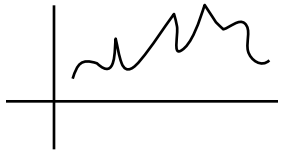
Other Gap Penalties

Score = f(gap length)

Kinds, & best known alignment time

☞ affine  $O(n^2)$ [really, $O(mn)$]

convex  $O(n^2 \log n)$

general  $O(n^3)$

Summary: Alignment

Functionally similar proteins/DNA often have recognizably similar sequences even after eons of divergent evolution

Ability to find/compare/experiment with “same” sequence in other organisms is a huge win

Surprisingly simple scoring works well in practice: score positions separately & add, usually w/ fancier affine gap model

Simple dynamic programming algorithms can find *optimal* alignments under these assumptions in poly time (product of sequence lengths)

This, and heuristic approximations to it like BLAST, are workhorse tools in molecular biology, and elsewhere.

Summary: Dynamic Programming

Keys to D.P. are to

a) identify the subproblems (usually repeated/overlapping)

b) solve them in a careful order so all small ones solved before they are needed by the bigger ones, and

c) build table with solutions to the smaller ones so bigger ones just need to do table lookups (*no* recursion, despite recursive formulation implicit in (a))

d) Implicitly, optimal solution to whole problem devolves to optimal solutions to subproblems

A *really* important algorithm design paradigm