CHAPTER 5: PAIRWISE SEQUENCE ALIGNMENT AND DATABASE SEARCHING

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Alignment algorithms (preview)

- Needleman-Wunsch (1970) and variations:
  - for aligning two sequences
  - uses dynamic programming to "consider" all possible alignments (10^{600} for two sequences of length 1000!)
- FASTA: uses a heuristic method for efficient searches (though not guaranteed to find the optimal solution)
  - Creates dictionary of *k*-tuples for the query sequence which is checked against sequences in the database
  - A local alignment algorithm is used to complete the alignment
- BLAST (Basic Local Alignment Search Tool): also fast and uses a heuristic
  - Finds short matches (which do not have to be perfect)
  - Then uses local alignment to complete the alignment

Needleman and Wunsch Dynamic Programming method

- Dynamic programming method
  - The problem can be divided into smaller parts
- Consider the following alignment of the sequences

x = THISLINE and ***y*** = ISALIGNED

X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}
T	H	**I**	**S**	–	**L**	**I**	–	**N**	**E**	–
–	–	**I**	**S**	A	**L**	**I**	G	**N**	**E**	D
Y_1	Y_2	Y_3	Y_4	Y_5	Y_6	Y_7	Y_8	Y_9	Y_{10}	Y_{11}

Dynamic Programming

X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}
T	H	**I**	**S**	–	**L**	**I**	–	**N**	**E**	–
–	–	**I**	**S**	A	**L**	**I**	G	**N**	**E**	D
Y_1	Y_2	Y_3	Y_4	Y_5	Y_6	Y_7	Y_8	Y_9	Y_{10}	Y_{11}

In general, the alignment is

$$\boxed{\begin{matrix} X_1 \cdots X_u \\ Y_1 \cdots Y_u \end{matrix}}\ \boxed{\begin{matrix} X_{u+1} \cdots X_v \\ Y_{u+1} \cdots Y_v \end{matrix}}\ \boxed{\begin{matrix} X_{v+1} \cdots X_L \\ Y_{v+1} \cdots Y_L \end{matrix}}$$

where X_u and Y_v correspond to alignment *positions.*

The score of the optimal global alignment from positions 1→ L is the sum of the partial alignment scores from positions 1→ *u*, *u*+1 → *v*, and *v*+1 → *L*

Dynamic Programming Algorithm

Let $S_{i,j}$ be the score of the optimal alignment of all characters up to x_i of sequence ***x*** and y_j of sequence ***y***.

Then there are three possibilities for this alignment

$$\begin{array}{l} \cdots\ x_{i-1}\ x_i \\ \cdots\ y_{j-1}\ y_j \end{array} \qquad \begin{array}{l} \cdots\ x_i\ - \\ \cdots\ y_{j-1}\ y_j \end{array} \qquad \begin{array}{l} \cdots\ x_{i-1}\ x_i \\ \cdots\ y_j\ - \end{array}$$

$S_{i-1,j-1}$ $\qquad$ $S_{i,j-1}$ $\qquad$ $S_{i-1,j}$

The optimal alignment score $S_{i,j}$ is the maximum of the following

$S_{i-1,\,j-1} + s(x_i, y_j)$ $\qquad$ $S_{i,\,j-1} + g$ $\qquad$ $S_{i-1,\,j} + g$

Where $s(x_i, y_j)$ is the substitution (or match/mismatch score) for character x_i aligned with y_j, and g is a constant gap penalty

Example:

x = THISLINE and ***y*** = ISALIGNED

```
T  H  I  S  -  L  I  -  N  E  -
-  -  I  S  A  L  I  G  N  E  D
```

Assume a constant (linear) gap penalty $g = 4$, and $s(x_i, y_j)$ calculated using the BLOSUM-62 scoring matrix.

$S_{4,2}$ is the optimal score for aligning THIS with IS and is the maximum of

```
THI   S        THIS   -        THI   S
--I + S        --I- + S        IS- + -
```

$$\underbrace{-4 + 4}_{0} \qquad \underbrace{-8 + -4}_{-12} \qquad \underbrace{-6 + -4}_{-10}$$

Optimal alignment of THI with I

```
THI   S
--I + S
```

-4 + 4

0

Optimal alignment of THIS with I

```
THIS   -
--I- + S
```

-8 + -4

-12

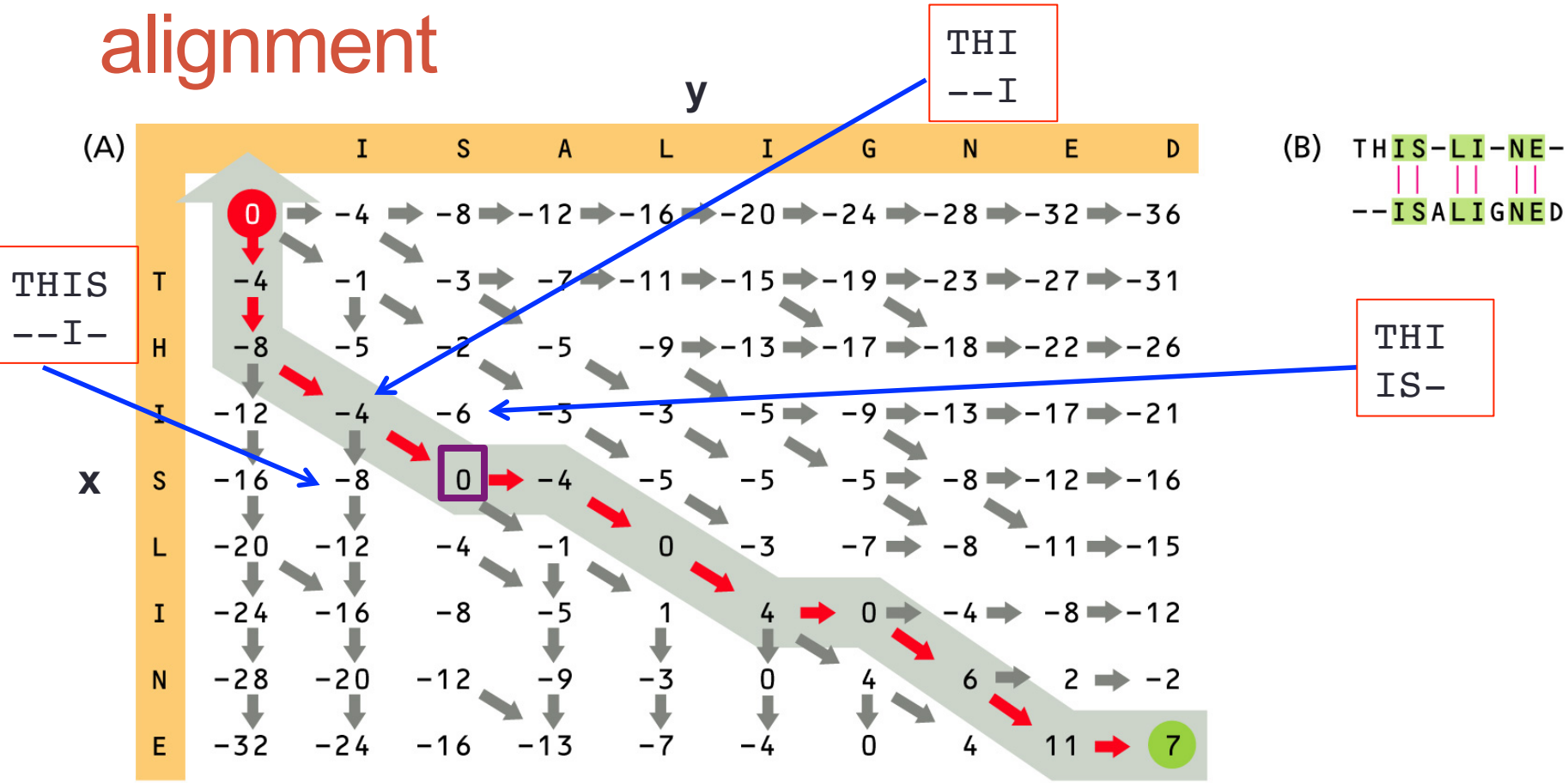
Optimal alignmentof THI with IS

```
THI   S
IS- + -
```

-6 + -4

-10

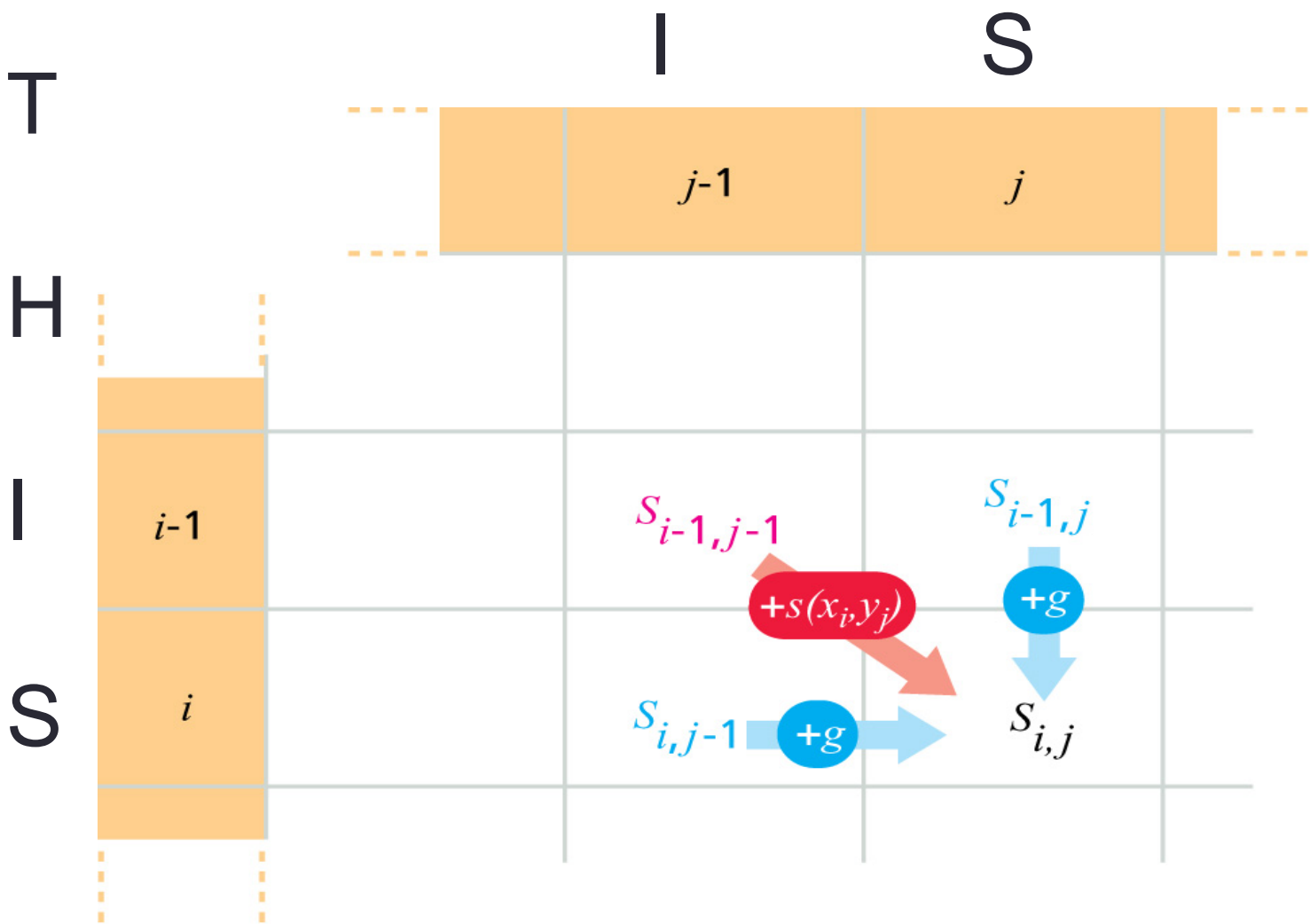
Dynamic programming matrix for global alignment



$S_{i,j}$ is the score of the optimal alignment of all characters up to x_i of sequence ***x*** and y_j of sequence ***y***.

The score of the optimal alignment of THIS with IS is $S_{4,2} = 0$.

Dynamic programming matrix illustrating possible optimal alignments for subsequences $x_1, \ldots x_i$ and $y_1, \ldots y_j$



Algorithm for finding the optimal global alignment

- For two sequences, $x_1, \ldots x_m$ and $y_1, \ldots y_n$
- Construct an $(m+1)$ by $(n+1)$ matrix (starting with element $S_{0,0}$ at the top left)
- For each i, the element $S_{i,0}$ corresponds to the alignment

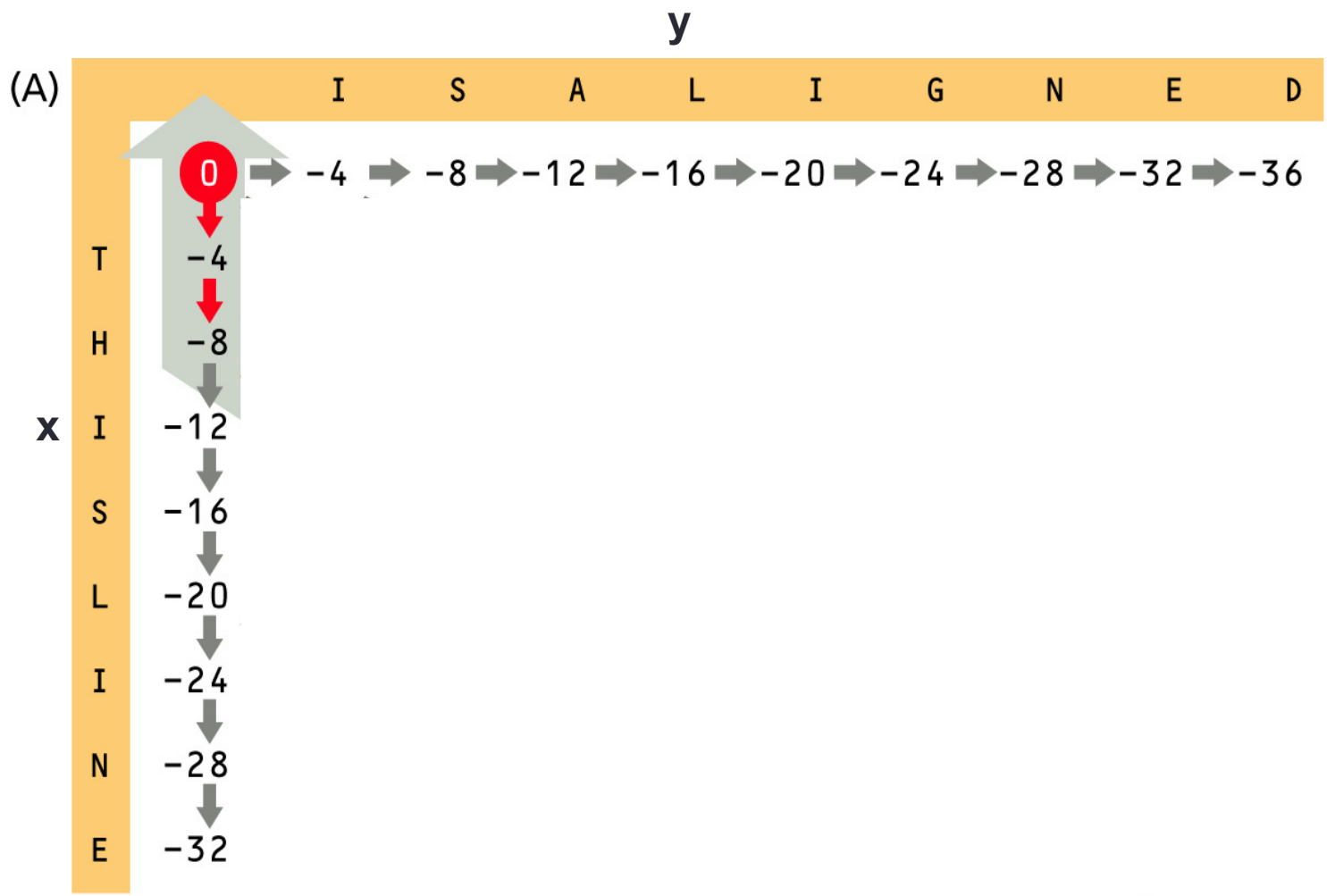
$$\begin{matrix} x_1 & x_2 & \ldots & x_i \\ - & - & \ldots & - \end{matrix}$$

For each j, the element $S_{0,j}$ corresponds to the alignment

$$\begin{matrix} - & - & \ldots & - \\ y_1 & y_2 & \ldots & y_i \end{matrix}$$

Fill in the matrix for all $S_{i,0}$ and $S_{0,j}$ appropriately

Assignment of $S_{i,0}$ and $S_{0,j}$ when a linear gap penalty of 4 is used



Algorithm for finding the optimal global alignment (con't)

- For $i = 1, \ldots, m$ and $j = 1, \ldots, n$

$$\text{set } S_{i,j} = \max \begin{cases} S_{i-1,j-1} + s(x_i, x_j) & \searrow \\ S_{i-1,j} + g & \downarrow \\ S_{i,j-1} + g & \rightarrow \end{cases}$$

and draw an arrow to $S_{i,j}$ from the cell where the score was derived ($S_{i-1,j-1}$, $S_{i-1,j}$, *or* $S_{i,j-1}$)

Global alignment: Dynamic programming matrix using linear gap penalty of 4

(A)

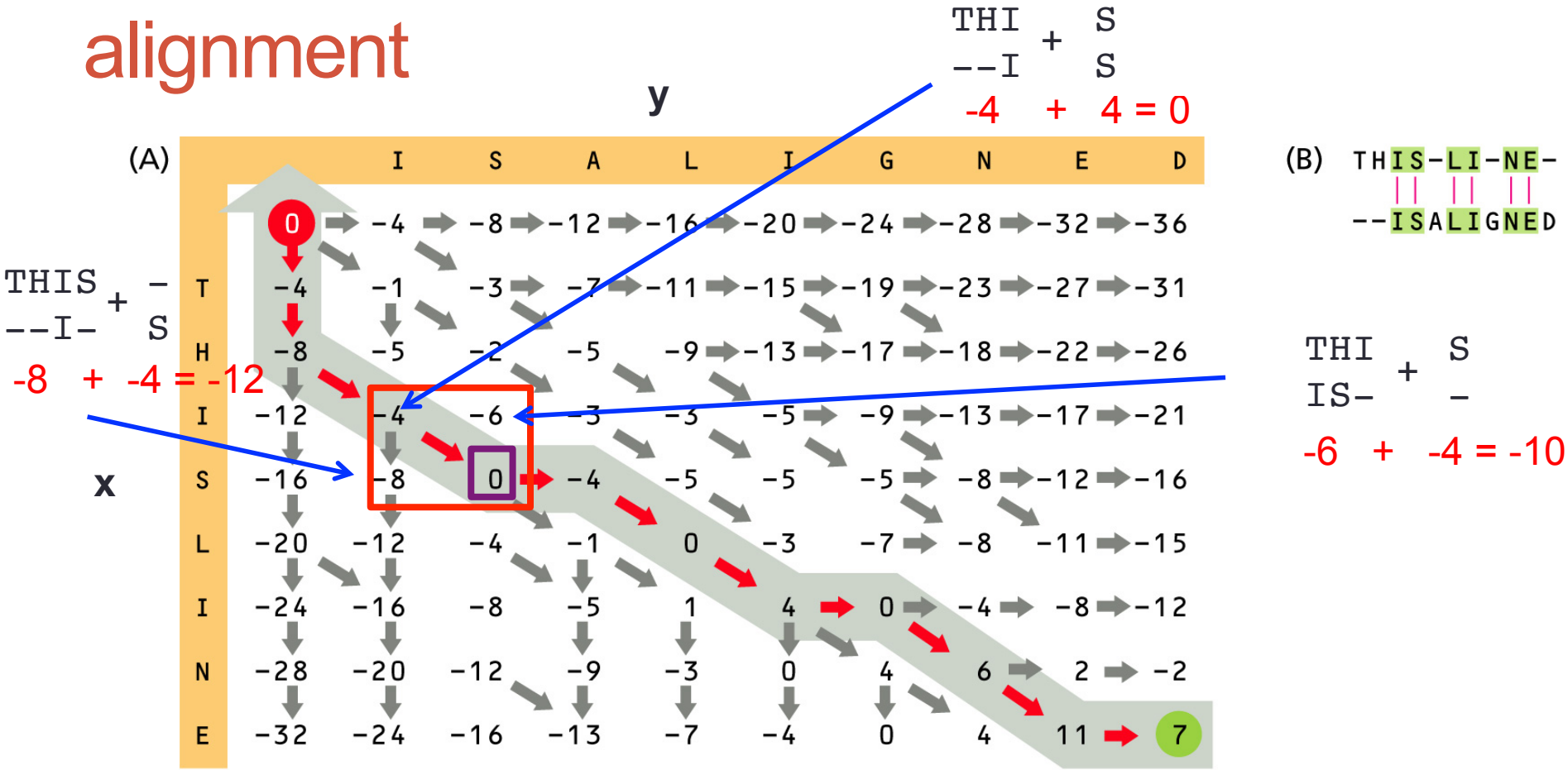
x \ y		I	S	A	L	I	G	N	E	D
	0	-4	-8	-12	-16	-20	-24	-28	-32	-36
T	-4	-1	-3	-7	-11	-15	-19	-23	-27	-31
H	-8	-5	-2	-5	-9	-13	-17	-18	-22	-26
I	-12	-4	-6	-3	-3	-5	-9	-13	-17	-21
S	-16	-8	0	-4	-5	-5	-5	-8	-12	-16
L	-20	-12	-4	-1	0	-3	-7	-8	-11	-15
I	-24	-16	-8	-5	1	4	0	-4	-8	-12
N	-28	-20	-12	-9	-3	0	4	6	2	-2
E	-32	-24	-16	-13	-7	-4	0	4	11	7

(B)

```
THIS-LI-NE-
  ||  ||  ||
--ISALIGNED
```

The score for the optimal alignment of THIS with IS is highlighted

Dynamic programming matrix for global alignment



$S_{i,j}$ is the score of the optimal alignment of all characters up to x_i of sequence ***x*** and y_j of sequence ***y***.

The score of the optimal alignment of THIS with IS is $S_{4,2} = 0$.

Algorithm for finding the optimal global alignment (con't)

- After the dynamic programming matrix is filled in, $S_{m,n}$ contains the score of the optimal global alignment
- A process called ***traceback*** is then used to recover the alignment itself, based on the path used to generate the optimal alignment score (based on the arrows), starting with $S_{m,n}$ and working backwards to $S_{0,0}$
- If the score $S_{i,j}$ was derived
  - diagonally from $S_{i-1,j-1}$, then align x_i with y_j

    … x_i
    … y_j

  - vertically from $S_{i-1,j}$, then align a gap with y_j

    … x_i
    … –

  - horizontally from $S_{i,j-1}$, then align x_i with a gap

    … –
    … y_j

Global alignment: Dynamic programming matrix using linear gap penalty of 4

(A)

x \ y		I	S	A	L	I	G	N	E	D
	0	-4	-8	-12	-16	-20	-24	-28	-32	-36
T	-4	-1	-3	-7	-11	-15	-19	-23	-27	-31
H	-8	-5	-2	-5	-9	-13	-17	-18	-22	-26
I	-12	-4	-6	-3	-3	-5	-9	-13	-17	-21
S	-16	-8	0	-4	-5	-5	-5	-8	-12	-16
L	-20	-12	-4	-1	0	-3	-7	-8	-11	-15
I	-24	-16	-8	-5	1	4	0	-4	-8	-12
N	-28	-20	-12	-9	-3	0	4	6	2	-2
E	-32	-24	-16	-13	-7	-4	0	4	11	7

(B)

```
THIS-LI-NE-
  ||  ||  ||
--ISALIGNED
```

Global alignment: Dynamic programming matrix using linear gap penalty of 4

(A)

y

x

		I	S	A	L	I	G	N	E	D
	0	-4	-8	-12	-16	-20	-24	-28	-32	-36
T	-4	-1	-3	-7	-11	-15	-19	-23	-27	-31
H	-8	[illegible]	-2	-5	-9	-13	-17	-18	-22	-26
I	-12	-4	[illegible]	[illegible]	-3	-5	-9	-13	-17	-21
S	-16	-8	0	-4	[illegible]	[illegible]	-5	-8	-12	-16
L	-20	-12	-4	-1	0	[illegible]	[illegible]	[illegible]	-11	-15
I	-24	-16	-8	-5	1	4	0	[illegible]	-8	-12
N	-28	-20	-12	-9	-3	0	4	6	[illegible]	[illegible]
E	-32	-24	-16	-13	-7	-4	0	4	11	7

T –
H –
I I
S S
– A
L L
I I
– G
N N
E E
– D

(B)

```
THIS-LI-NE-
  ||  ||  ||
--ISALIGNED
```

Global alignment: Dynamic Programming Matrix using linear gap penalty of 8

(A)

		I	S	A	L	I	G	N	E	D
	0	-8	-16	-24	-32	-40	-48	-56	-64	-72
T	-8	-1	-7	-15	-23	-31	-39	-47	-55	-63
H	-16	-9	-2	-9	-17	-25	-33	-40	-47	-55
I	-24	-12	-10	-3	-7	-13	-21	-29	-37	-45
S	-32	-20	-8	-9	-5	-9	-13	-20	-28	-35
L	-40	-28	-16	-9	-5	-3	-11	-16	-23	-31
I	-48	-36	-24	-17	-7	-1	-7	-14	-19	-26
N	-56	-44	-32	-25	-15	-9	-1	-1	-9	-17
E	-64	-52	-40	-33	-23	-17	-9	-1	4	-4

(B)

```
THISLINE-
  ||
ISALIGNED
```

Modification of algorithm for semiglobal alignments

- A ***semiglobal alignment*** does not penalize for gaps at the beginning and end of an alignment
- Appropriate for global alignments of sequences that are not the same length
- Modifications to algorithm for semiglobal alignment:
  - Set $S_{i,0}$ and $S_{0,j}$ to 0 for all *i* and *j*
  - Start the traceback from the highest scoring element in the bottom row or last column

Semiglobal alignment: Dynamic Programming Matrix using a linear gap penalty of 8

(A)

		I	S	A	L	I	G	N	E	D
	0	0	0	0	0	0	0	0	0	0
T	0	-1	1	0	-1	-1	-2	0	-1	-1
H	0	-3	-2	-1	-3	-4	-3	-1	0	-2
I	0	-4	-5	-3	1	1	-7	-6	-4	-3
S	0	-2	8	0	-5	-1	1	-6	-6	-4
L	0	2	0	7	4	-3	-5	-2	-9	-10
I	0	4	0	-1	9	8	0	-8	-5	-12
N	0	-3	5	-2	1	6	8	6	-2	-4
E	0	-3	-3	4	-4	-2	4	8	11	3

(B)

```
THIS-LI-NE-
 ||  ||  ||
--ISALIGNED
```

Modification of algorithm for local alignments (1st proposed by Smith-Watterman)

- A ***local alignment*** aligns *regions* of two sequences, will not necessarily span the length of each sequence
- Appropriate for identifying functional domains of a protein
- Modifications to algorithm for local alignment:
  - set $S_{i,j} = \max \begin{cases} S_{i-1,j-1} + s(x_i, x_j) \\ S_{i-1,j} + g \\ S_{i,j-1} + g \\ 0 \end{cases}$
  - assumes that the expected alignment score is negative for random sequences and is positive for similar sequences
- Traceback starts from highest scoring matrix element and ends at 0

Local alignment: Dynamic Programming Matrix using a linear gap penalty of 8

(A)

		I	S	A	L	I	G	N	E	D
	0	0	0	0	0	0	0	0	0	0
T	0	0	1	0	0	0	0	0	0	0
H	0	0	0	0	0	0	0	1	0	0
I	0	4	0	0	2	4	0	0	0	0
S	0	0	8	1	0	0	4	1	0	0
L	0	2	0	7	5	2	0	1	0	0
I	0	4	0	0	9	9	1	0	0	0
N	0	0	5	0	1	6	9	7	0	1
E	0	0	0	4	0	0	4	9	12	4

(B)

```
SLI-NE
 ||  ||
ALIGNE
```