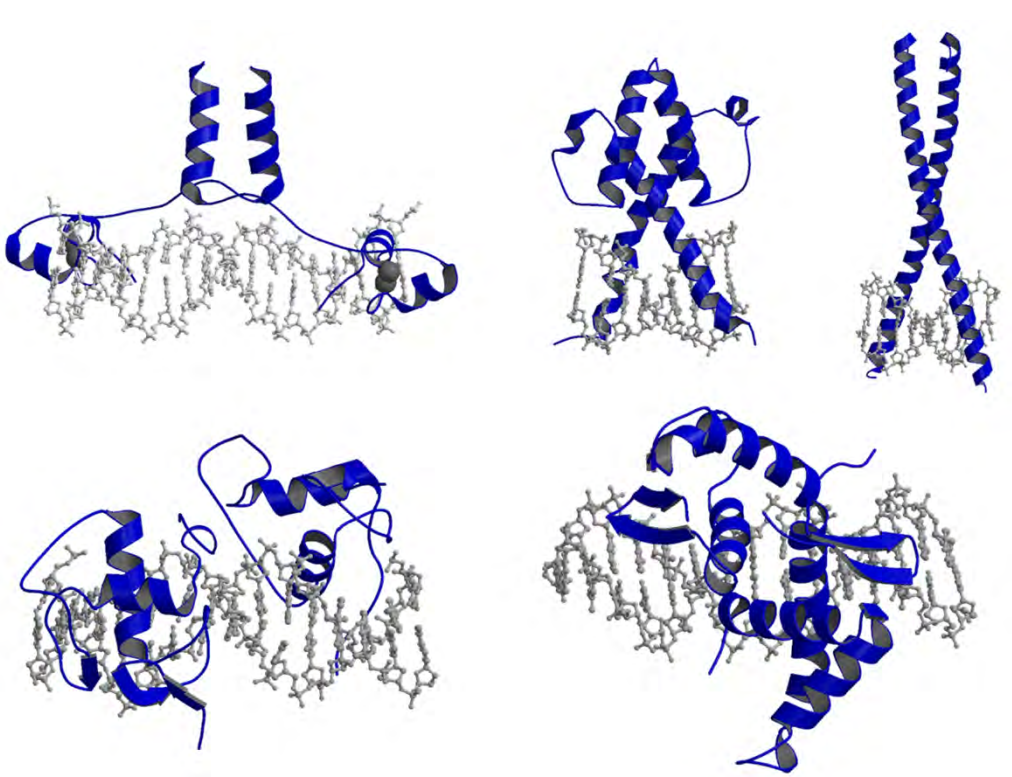
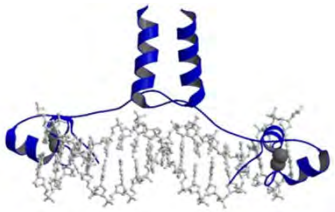
Transcription Factor Sequence Motifs





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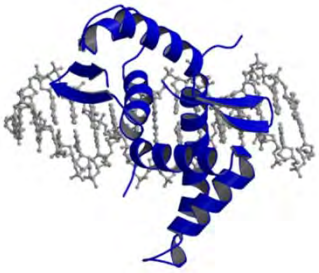
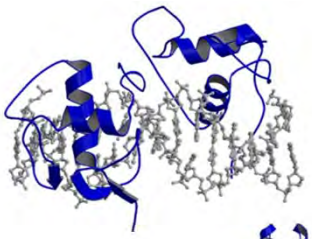
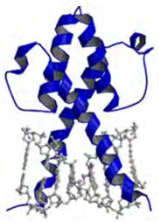
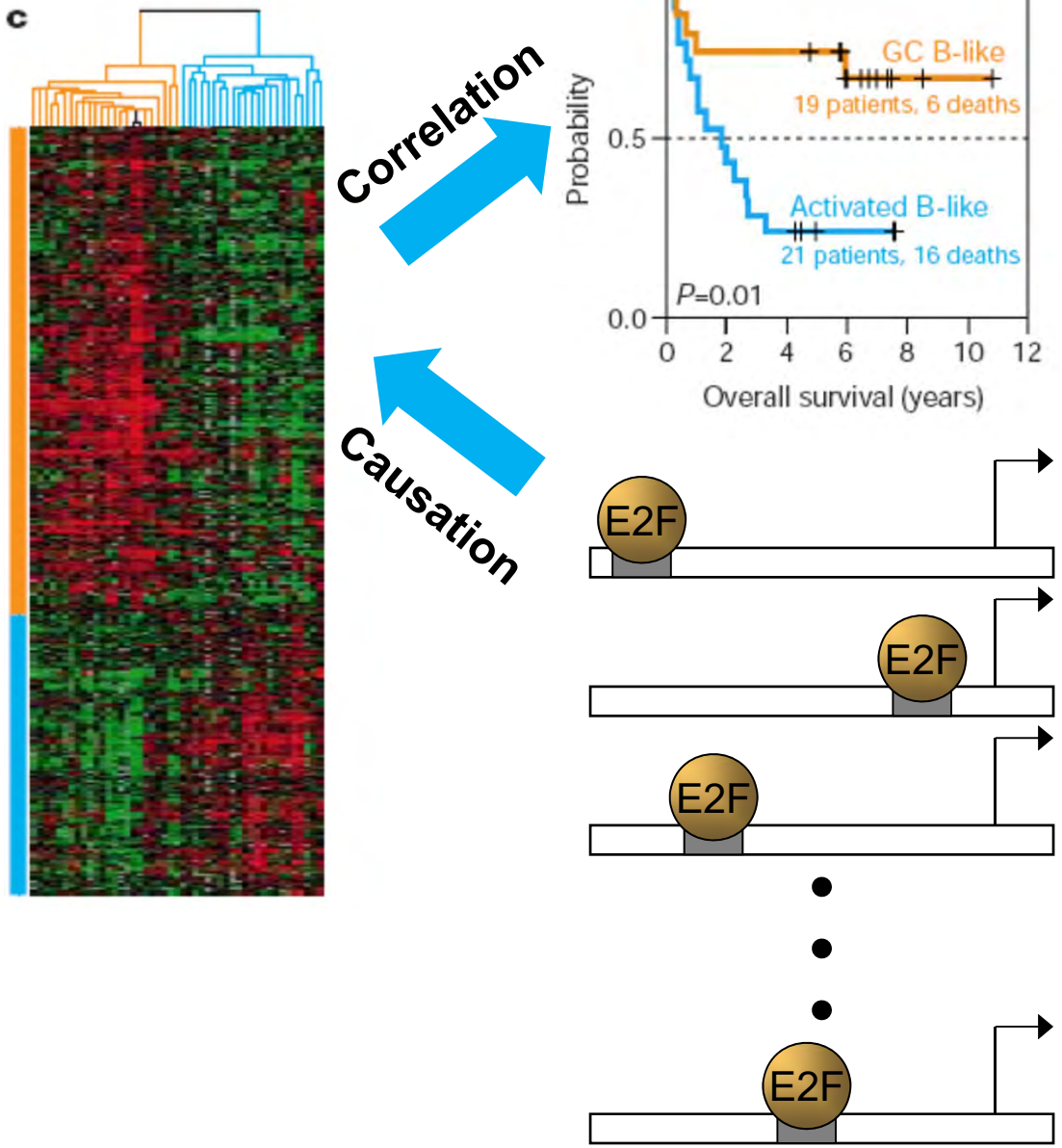


Outline

- Why look for TF binding sites?
- What is a motif?
- How do I compute a motif from bound sites?
- Is a motif enriched?
- How do you find a motif from unaligned seqs?



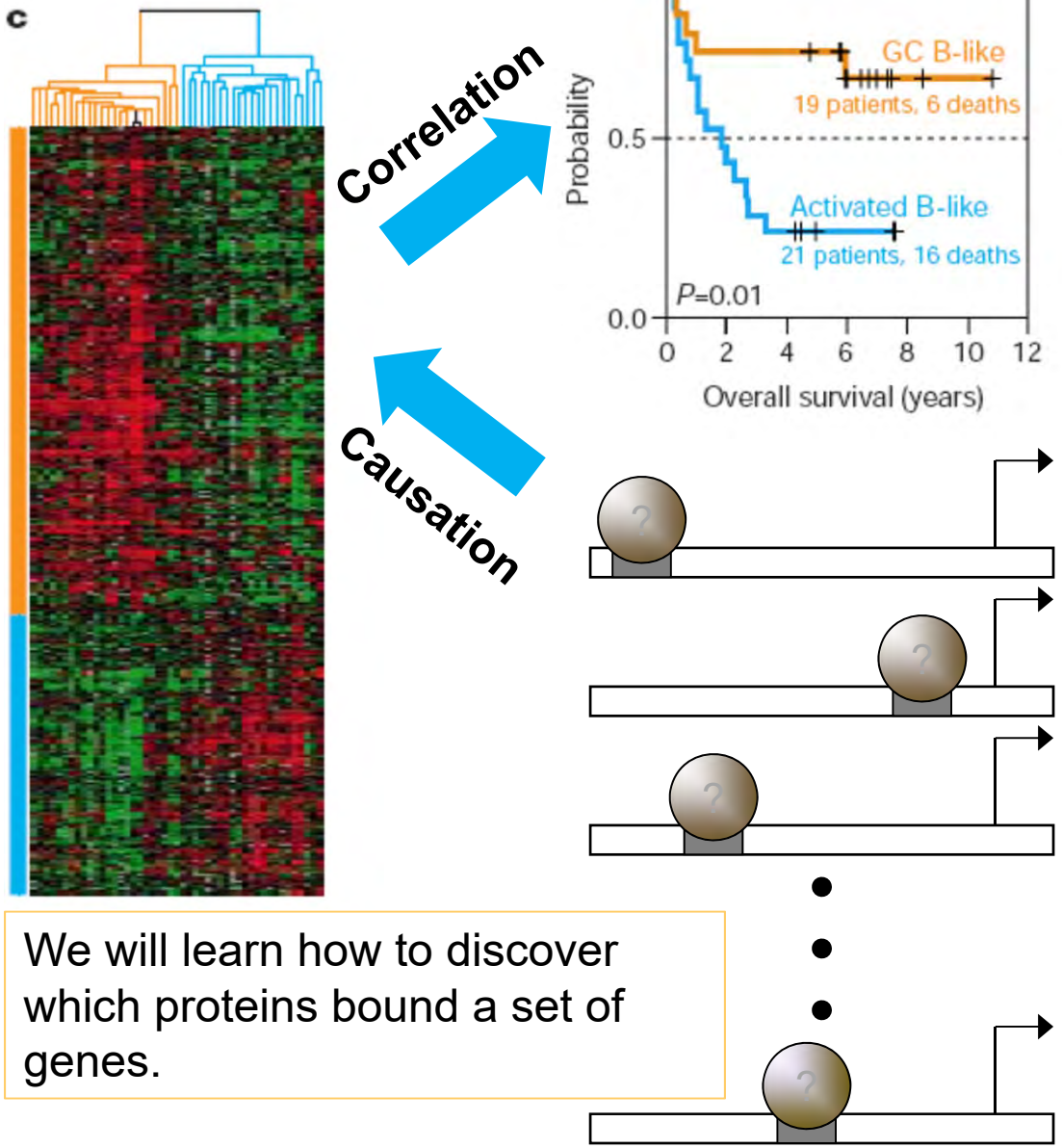




Why do these genes predict outcome?

The are regulated by the E2F family of cell-cycle regulatory proteins.

Tumors that divide more rapidly express more of these proteins and are more dangerous



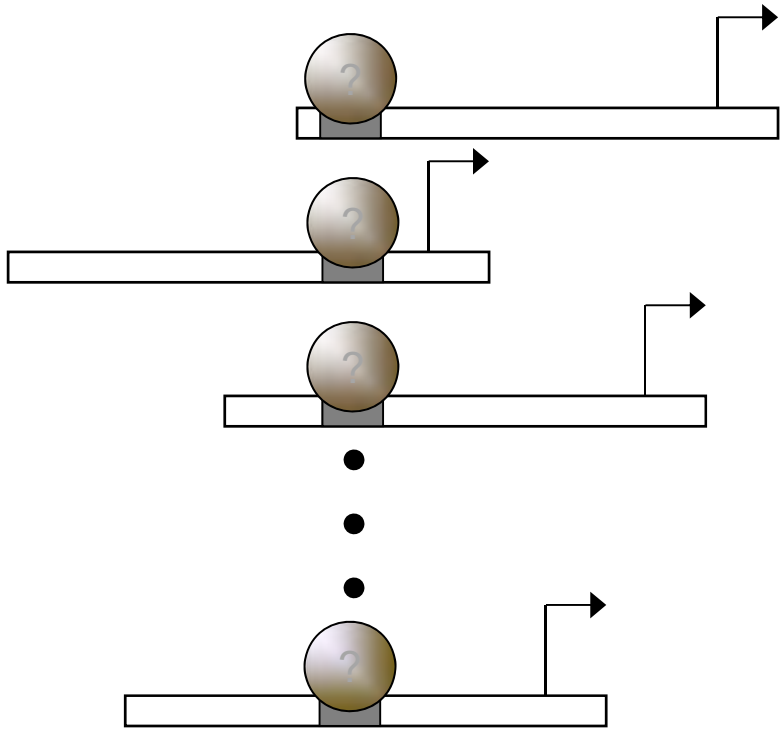
We will learn how to discover which proteins bound a set of genes.

Why do these genes predict outcome?

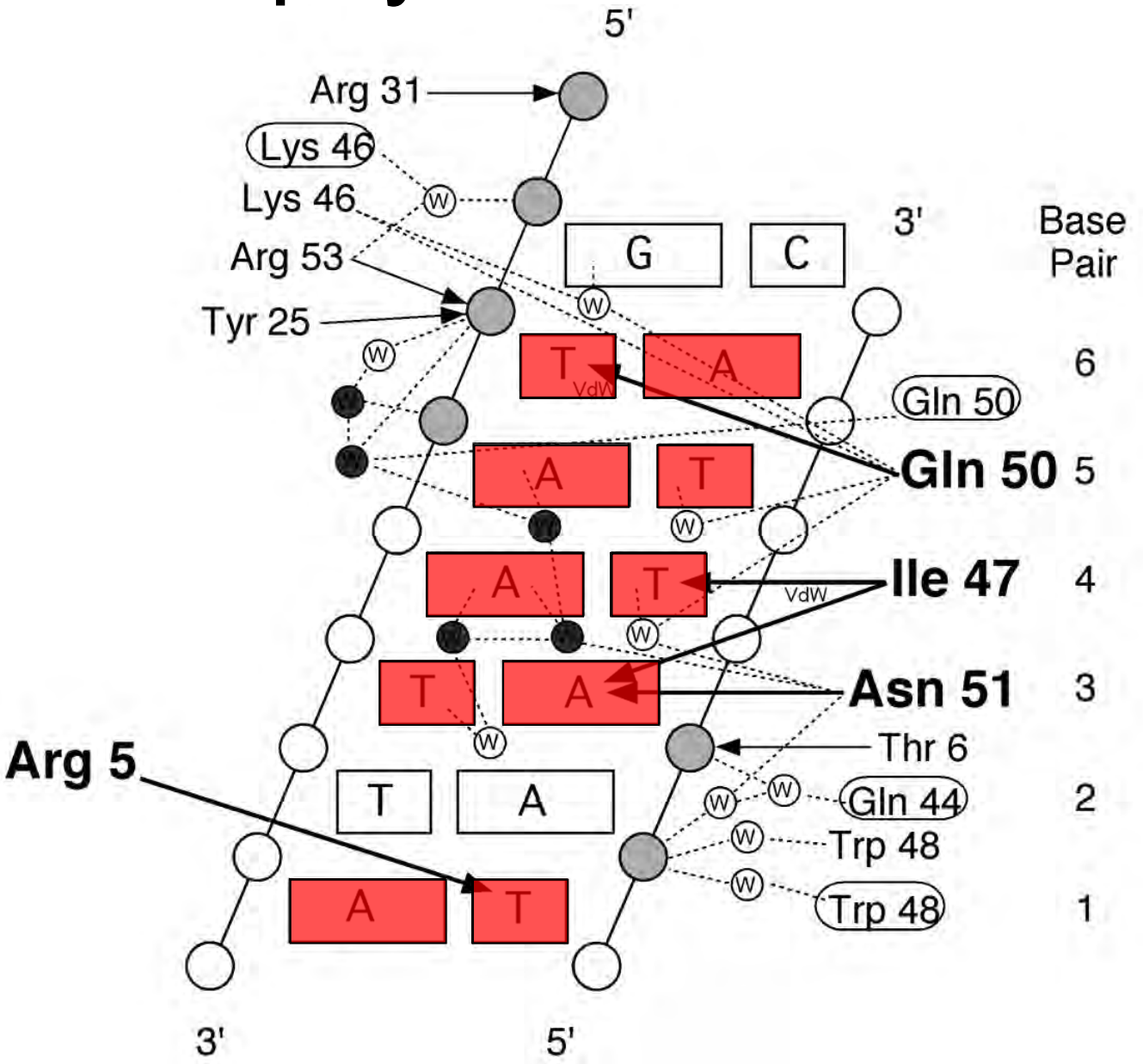
The are regulated by the E2F family of cell-cycle regulatory proteins.

Tumors that divide more rapidly express more of these proteins and are more dangerous

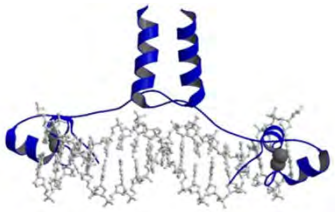
What would I expect if I lined up the sequences of binding sites?



Biophysics determines binding



Some base pairs are more critical than others



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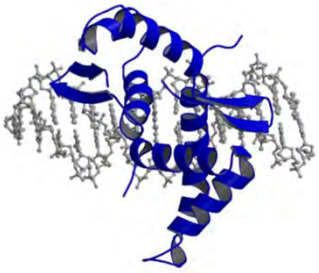

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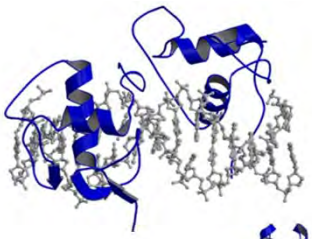

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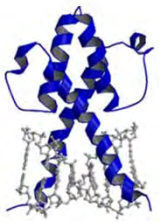


Is a motif enriched?

How do you find a motif from unaligned seqs?

Motifs

- Measure the *odds* that a sequence was *generated* by the motif model after compared to the *background* frequencies of each nucleotide

Is a region a valid binding site?

Match?

ACGTAGATCGATCCCCTGATCAAAATCGTGTTGAGCGCGCGTAATATCGCTAGCTAGCAAATTCCGATA

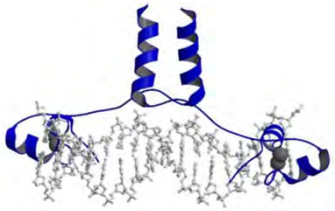
- Steps:
  1. Define a mathematical model for matching sequences
  2. Define a model for sequences that don't match
  3. Quantitatively compare the two hypotheses

$$odds = \frac{Probability_{binding\_site}}{Probability_{not\_a\_binding\_site}}$$

Define motif model → Define background model → Compare the models

Sequence Logos

- A letter's height indicates the information it contains (i.e. reduction in uncertainty)
- The top letter at each position can be read to obtain the *consensus sequence*



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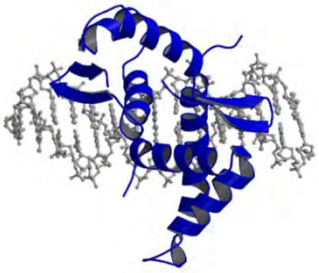

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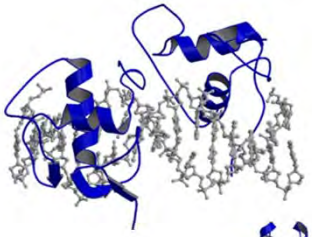

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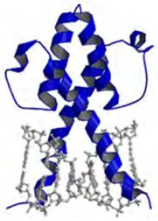

Is a motif enriched?

How do you find a motif from unaligned seqs?

If I had found these sites using ChIP-Seq, how would I describe the specificity?

TGACTCC
TGACTCA
TGACAAA
TGACTCA
TTACACA
TGACTAA
TGACTAA
TGACTCA
TGACTCA
TGACTCA

A

If I had found these sites using ChIP-Seq, how would I describe the specificity?

```
TGACTCC
TGACTCA
TGACAAA
TGACTCA
TTACACA
TGACTAA
TGACTAA
TGACTCA
TGACTCA
TGACTCA
```

Position Frequency Matrix (PFM)

A:	0	0	10	0	2	3	9
C:	0	0	0	10	0	7	1
G:	0	9	0	0	0	0	0
T:	10	1	0	0	8	0	0

If I had found these sites using ChIP-Seq, how would I describe the specificity?

```
TGACTCC
TGACTCA
TGACAAA
TGACTCA
TTACACA
TGACTAA
TGACTAA
TGACTCA
TGACTCA
TGACTCA
```

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G:	0	9	0	0	0	0	0
T:	10	1	0	0	8	0	0

Position Probability Matrix (PPM)

A:	0.000	0.000	1.000	0.000	0.200	0.300	0.900
C:	0.000	0.000	0.000	1.000	0.000	0.700	0.100
G:	0.000	0.900	0.000	0.000	0.000	0.000	0.000
T:	1.000	0.100	0.000	0.000	0.800	0.000	0.000

Define motif model → Define background model → Compare the models

Is a region a valid binding site?

- Steps:
  1. Define a mathematical model for matching sequences

$$Model\_prob = \prod_{i=1}^{w} p_{\text{model}}(b, i)$$

Position Probability Matrix (PPM)

A:	0.000	0.000	1.000	0.000	0.200	0.300	0.900
C:	0.000	0.000	0.000	1.000	0.000	0.700	0.100
G:	0.000	0.900	0.000	0.000	0.000	0.000	0.000
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G:	0.000	0.900	0.000	0.000	0.000	0.000	0.000
T:	1.000	0.100	0.000	0.000	0.800	0.000	0.000

  2. Define a model for sequences that don't match: $P_{background} = 0.25$

Is the sequence more probably a motif or a random genomic region?

- Steps:
  3. Quantitatively compare the two hypotheses

$$Model\_prob = \prod_{i=1}^{w} p_{\text{model}}(b,i)$$

$$Background\_prob = \prod_{i=1}^{w} p_{\text{background}}(b)$$

Odds ratio

$$\frac{Model\_prob}{Background\_prob} = \prod_{i=1}^{w} \frac{p_{\text{model}}(b,i)}{p_{\text{background}}(b)} = \prod_{i=1}^{w} odds(b,i)$$

B

Define motif model | Define background model | Compare the models

$$\frac{Model\_prob}{Background\_prob} = \prod_{i=1}^{w} \frac{p_{model}(b,i)}{p_{background}(b)} = \prod_{i=1}^{w} odds(b,i)$$

Position Probability Matrix (PPM) = *Model_prob*

A:	0.000	0.000	1.000	0.000	0.200	0.300	0.900
C:	0.000	0.000	0.000	1.000	0.000	0.700	0.100
G:	0.000	0.900	0.000	0.000	0.000	0.000	0.000
T:	1.000	0.100	0.000	0.000	0.800	0.000	0.000

Assume that all bases are equally likely in the rest of the genome

$P_{background} = 0.25$

Odds Ratio

A:	0	0	4.000	0	0.800	1.200	3.600
C:	0	0	0	4.000	0	2.800	0.400
G:	0	3.600	0	0	0	0	0
T:	4.000	0.400	0	0	3.200	0	0

Define motif model → Define background model → Compare the models

Likelihood = Prob(sequence | model)

What is the likelihood of TGACTCA?
What is the likelihood of AGACTCA?

Odds Ratio

A:	0	0	4.000	0	0.800	1.200	3.600
C:	0	0	0	4.000	0	2.800	0.400
G:	0	3.600	0	0	0	0	0
T:	4.000	0.400	0	0	3.200	0	0

Laplace's rule takes into account rare events

$$F(base = b) = \frac{C(base = b)}{Total\_counts}$$

$$F(base = b) = \frac{C(base = b) + 1}{Total\_counts + 4}$$

Pseudocounts:

add a small frequency ρ to all bases

$$F(base = b) = \frac{C(base = b)}{Total\_counts}$$

$$F(base = b) = \frac{C(base = b) + 1}{Total\_counts + 4}$$

$$F(base = b) = \frac{C(base = b) + p}{Total\_counts + 4p}$$

C

In next slides p=0.25% of Total_Counts

Position Probability Matrix (PPM)

A:	0.000	0.000	1.000	0.000	0.200	0.300	0.900
C:	0.000	0.000	0.000	1.000	0.000	0.700	0.100
G:	0.000	0.900	0.000	0.000	0.000	0.000	0.000
T:	1.000	0.100	0.000	0.000	0.800	0.000	0.000

Odds Ratio

A:	0	0	4.000	0	0.800	1.200	3.600
C:	0	0	0	4.000	0	2.800	0.400
G:	0	3.600	0	0	0	0	0
T:	4.000	0.400	0	0	3.200	0	0

With Pseudocounts

D

Position Probability Matrix (PPM)

A:	0.0025	0.0025	0.9926	0.0025	0.2005	0.2995	0.8936
C:	0.0025	0.0025	0.0025	0.9926	0.0025	0.6955	0.1015
G:	0.0025	0.8936	0.0025	0.0025	0.0025	0.0025	0.0025
T:	0.9926	0.1015	0.0025	0.0025	0.7946	0.0025	0.0025

Odds Ratio

A:	0.0099	0.0099	3.9703	0.0099	0.8020	1.1980	3.5743
C:	0.0099	0.0099	0.0099	3.9703	0.0099	2.7822	0.4059
G:	0.0099	3.5743	0.0099	0.0099	0.0099	0.0099	0.0099
T:	3.9703	0.4059	0.0099	0.0099	3.1782	0.0099	0.0099

Log-Odds

$$\frac{Model\_prob}{Background\_prob} = \prod_{i=1}^{w} \frac{p_{model}(b,i)}{p_{background}(b)} = \prod_{i=1}^{w} odds(b,i)$$

$$\log\left[\frac{Model\_prob}{Background\_prob}\right] = \log\left[\prod_{i=1}^{w} odds(b,i)\right] = \sum_{i=1}^{w} \log\left(odds(b,i)\right)$$

E

Define motif model | Define background model | Compare the models

Log-odds matrix is often called PWM position weight matrix or PSSM position-specific scoring matrix

$$log\left[\frac{P_{model}}{P_{background}}\right] = log[P_{model}] - log[P_{background}]$$

Log Odds

```
A: -6.658 | -6.658 |  1.989 | -6.658 | -0.318 |  0.261 |  1.838 |
C: -6.658 | -6.658 | -6.658 |  1.989 | -6.658 |  1.476 | -1.301 |
G: -6.658 |  1.838 | -6.658 | -6.658 | -6.658 | -6.658 | -6.658 |
T:  1.989 | -1.301 | -6.658 | -6.658 |  1.668 | -6.658 | -6.658
```

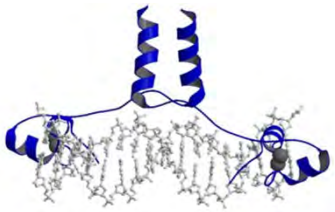
Define motif model → Define background model → Compare the models

Is a region a valid binding site?

- Steps:
  1. Define a mathematical model for matching sequences
  2. Define a model for sequences that don't match
  3. Quantitatively compare the two hypotheses

ACGTAGATCGATCCCCTGATCAAAATCGTGTTGAGCGCGCGTAATATCGCTAGCTAGCAAATTCCGATA

Match?



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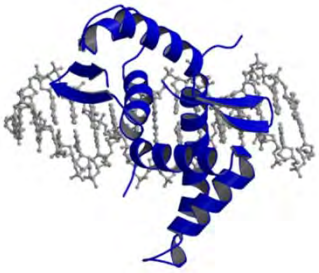

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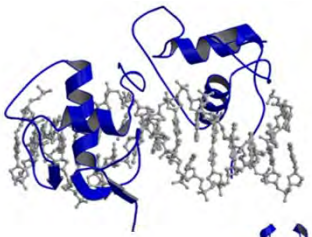

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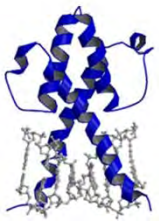
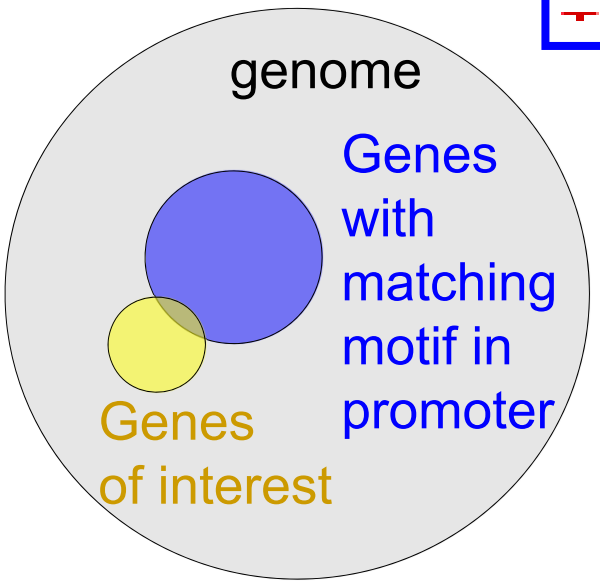
How do I compute a motif from bound sites?

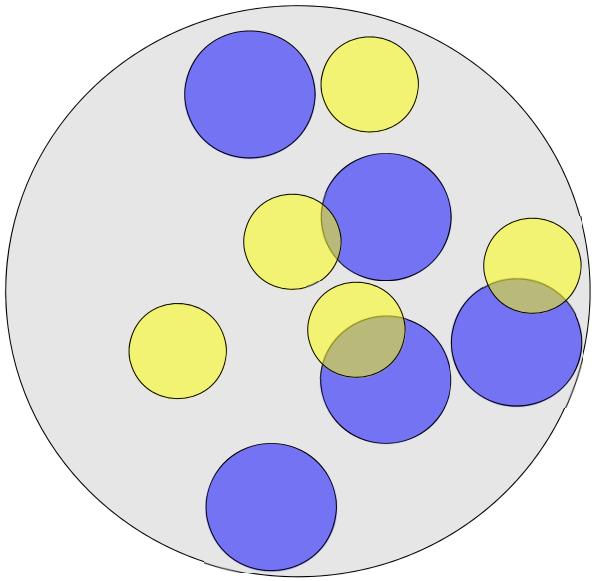


Is a motif enriched?

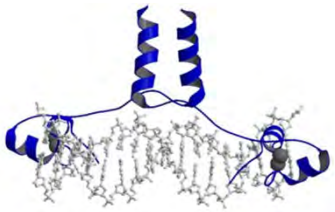
How do you find a motif from unaligned seqs?

Compute Significance With Hypergeometric Distribution





- **Rule-of-thumb**: *60% of the maximum-possible* LLR score is a reasonable threshold for determining a match to a *PWM motif*



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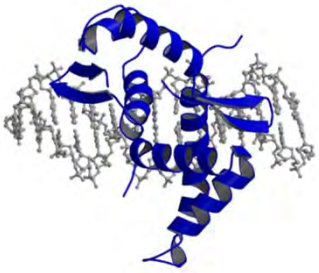

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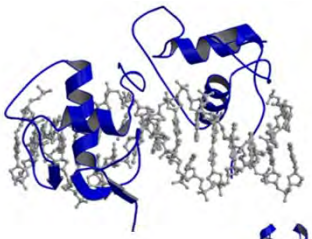

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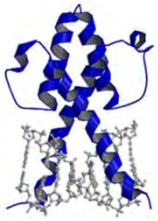

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How do you find a motif from unaligned seqs?

Motif Discovery

- **Given**: a set of sequences
- **Find**: the PWM for an over-represented motif

```
ACGTGTCTGCTACAAAATGCAAATACGATGATAAATGCAGCAATTGT
ACGTAAATGCAATTACGATGATAAATGCAGCAACCGTTATCGACTTG
ATCTTACTAGCATGGCCATCATCAACATGCAAAGCAGGTTGTGCCCT
ATAAATGCCCAATTGATTTGTCTCCACTACATAATGCAAATACCATG
```

Motif Discovery

- **Given**: a set of sequences
- **Find**: the PWM for an over-represented motif

ACGTGTCTGCTACA**AAATGCAAA**TACGATGATAAATGCAGCAATTGT

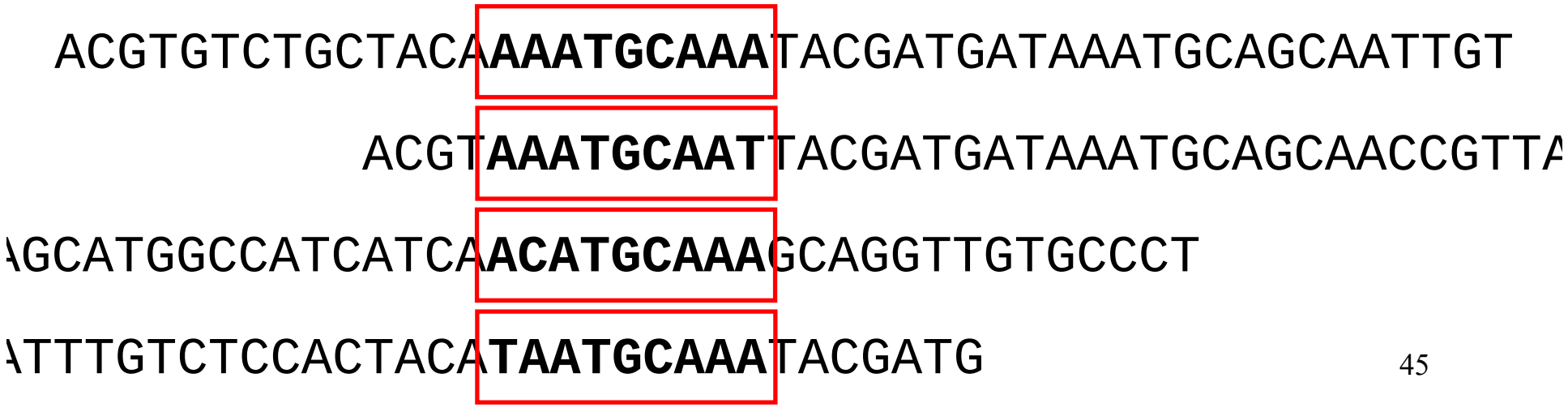
ACGT**AAATGCAAT**TACGATGATAAATGCAGCAACCGTTATCGACTTG

ATCTTACTAGCATGGCCATCATCA**ACATGCAAA**GCAGGTTGTGCCCT

ATAAATGCCCAATTGATTTGTCTCCACTACA**TAATGCAAA**TACGATG

Motif Discovery

- **Note 1:**
  - If you know the PWM, you can easily align the sequences
- **Note 2:**
  - If the sequences are aligned, you can easily find the PWM



The Expectation Maximization (EM) Algorithm

- When we begin
  - we don't know the PWM
  - we don't know the location of the binding sites
- We iteratively:
  - assume we know the motif and look for the most likely binding site
  - assume we know the binding site and compute the best motif

Expectation Maximization

- E step – calculate expected motif locations given the current motif

Given our current best guess about the motif,
Where do we think the protein is binding?

ACGTGTCTGCTACA**AAATGCAAA**TACGATGATAAATGCAGCAATTGT

ACGT**TCATGTATT**TACGATGATAAATGCAGCAACCGTTATCGACTTG

ATCTTACTAGCATGGCCATCATCA**ACATGATAA**GCAGGTTGTGCCCT

ATAAATGCCCAATTGATTTGTCTCCACTACA**AAATGCAAT**TACGATG

Expectation Maximization

- M step – re-estimate the motif to maximize likelihood

Given our expectation about where binding occurs,
What is the most likely motif model?

ACGTGTCTGCTACA**AAATGCAAA**TACGATGATAAATGCAGCAATTG

GACATTTTGTACGT**TCATGTATT**TACGATGATAAATGCAGCAACCG

CATGGCCATCATCA**ACATGATAA**GCAGGTTGTGCCCGGTTTACTGA

TTGTCTCCACTACA**AAATGCAAT**TACGATGAGAGGGTGATGGCACT

Expectation Maximization

- M step – re-estimate the motif to maximize likelihood

Old motif

New motif

ACGTGTCTGCTACA**AAATGCAAA**TACGATGATAAATGCAGCAATTG

GACATTTTGTACGT**TCATGTATT**TACGATGATAAATGCAGCAACCG

CATGGCCATCATCA**ACATGATAA**GCAGGTTGTGCCCGGTTTACTGA

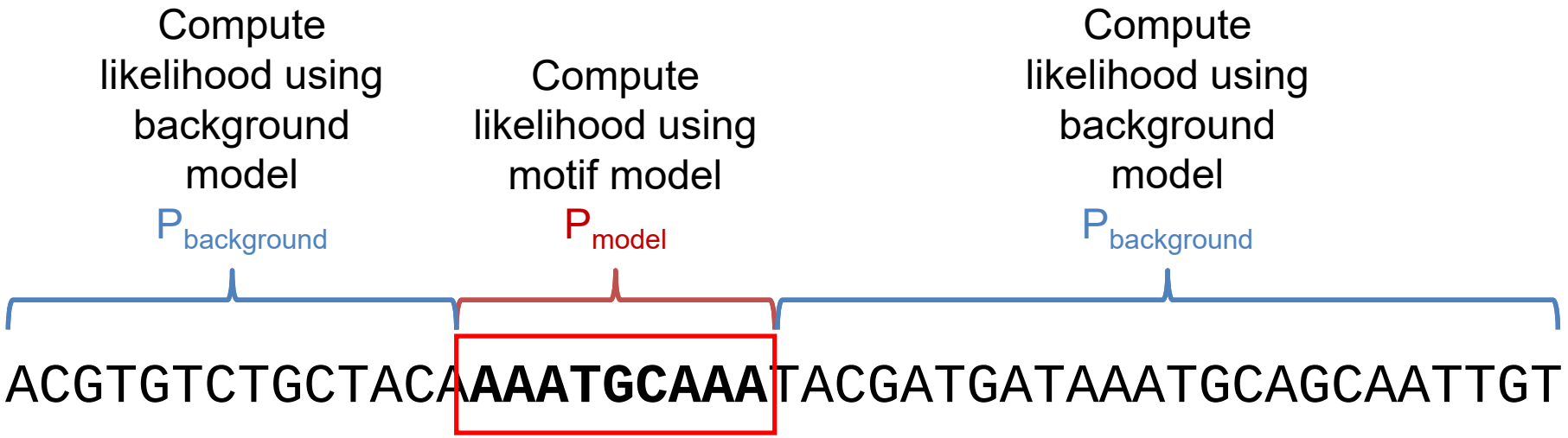
TTGTCTCCACTACA**AAATGCAAT**TACGATGAGAGGGTGATGGCACT

Properties of the EM algorithm

- EM is guaranteed to converge
  - at each step our overall score improves
- EM is not guaranteed to give the right answer
  - had we started with a different initial guess, we might have found a better answer

What do we maximize?

- We maximize the likelihood of the full sequences given our current motif model.



- Remember that each element of the motif is

$$log\left[\frac{P_{model}}{P_{background}}\right] = log[P_{model}] - log[P_{background}]$$