Today, Lec 4

Introns and exons & RNA splicing (Quickly)

X-ray crystallography
My dog Lucky
Pre-mRNA (or Nuclear RNA)
Copy of DNA with lots of expressed sequences (exons) and introns (non-expressed sequences)

Present in eukaryotes, not in “simpler” bacteria or archea.

http://en.citizendium.org/wiki/Intron
Definition of introns, exons

A segment of a gene situated between exons that does not function in coding for protein synthesis. After transcription of a gene to [pre-]messenger RNA, the transcriptions of **introns** are removed, and the exons are spliced together by enzymes before translation [into an mRNA] and assembly of amino acids into proteins.

Also called **intervening sequence**.

**any portion of an interrupted gene that is represented in the RNA product and is translated into protein.**
Watching the Spliceosome and RNA get edited

https://www.youtube.com/watch?v=aVgwr0QpYNE

Only eukaryotes have spliceosomes and metazoans (Animals) have a second spliceosome, the minor spliceosome.  https://en.wikipedia.org/wiki/Spliceosome
X-ray Diffraction

One of the premier techniques of Structural Biology

Can get Angstrom resolution of nearly every atom in biological macromolecules —now-a-days get up to (beyond?) 2 MD (Ribosomes).

1st one: Whale myoglobin
Watson & Crick 1953

**X pattern**

**X-ray Diffraction**

- **Period**
  - \( P = 3.4 \text{ nm} \)

- **Interbase distance**
  - \( 0.34 \text{ nm} = P/10 \)

- **Minor Groove**
  - \( 1.2 \text{ nm} = 3P/8 \)

- **Major Groove**
  - \( 2.2 \text{ nm} = 5P/8 \)

- **Diameter**
  - \( 2 \text{ nm} \)
From diffraction pattern → actual image

“Photo 51” – Rosalind Franklin 1952

X pattern
Layer Lines
Missing 4th layer
Diamond Pattern
X-rays behave as electromagnetic waves

1. They interfere

- **Constructive Interference** (Bright Spot)
  \[ \Delta L = \lambda / 2 \]

- **Destructive Interference** (Dark Spot)
  \[ \Delta L = \lambda / 2 \]

**“In Phase”**

**“Out of Phase”**

wavelength \( \lambda \)

amplitude
Constructive interference

\[ \Delta L = n\lambda \quad n = 0, 1, 2, \ldots \]
Destructive interference

\[ \Delta L = \frac{(2n' + 1)}{2} \lambda \quad n' = 0, 1, 2, \ldots \]
ConstrucDve Interference (Bright Spot)

DestrucDve Interference (Dark Spot)
Two-wave Interference Pattern

Interference depends on waves traveling different distances

Constructive interference

Destructive interference
X-rays behave as electromagnetic waves

2. They diffract

What if there are two (or more) atoms?
Young’s Double Slit: Diffraction and Interference

Bottom wave travels extra $\lambda$

Both waves travel same distance

Top wave travels extra $\lambda$
Imagine a simple 1-D crystal...

\[ \Delta L = 0 \quad \text{Constructive Bright Spot} \]
\[ \Delta L = \frac{\lambda}{2} \]

**Destructive Dark Spot**

\[ \Delta L = \frac{\lambda}{2} \]

**Destructive Interference (Dark Spots)**

\[
\Delta L = P \sin \theta = \frac{(2n' + 1)}{2} \lambda \\
n' = 0, 1, 2, \ldots
\]

**Constructive Interference (Bright Spots)**

\[
\Delta L = P \sin \theta = n \lambda \\
n = 0, 1, 2, \ldots
\]
Question about Crystal diffraction

In a NaCl crystal, the spacing between atoms is 0.282 nm. Which of the following wavelengths could be used to see a clear diffraction pattern?

\[ \sin \theta_1 = \frac{\lambda}{d} \]

Need very short wavelength light: X-rays!

A. \( \lambda = 0.1 \) nm  
B. \( \lambda = 1 \) nm  
C. \( \lambda = 10 \) nm
Can get P, \( \Theta, \lambda, P \)
**X-ray crystallography**

Given X-ray wavelength $\lambda$, diffraction angles $\theta$ provide information about distance $d$ between atoms in crystal

\[ \sin \theta_m = m \frac{\lambda}{d} \]

As long as $\lambda < d$, small features lead to large $\theta$. **BUT** need regular ordering of atoms – i.e. a crystal!

“Photo 51” Rosalind Franklin

Crystalline fiber of DNA
2-D crystals...

Four simple arrays...

...and their diffraction patterns

Notice the *inverse* relationship between spacing in the array and spacing in the diffraction pattern.

The array in (x,y,z) real space and the diffraction space (h,l,m) are Fourier Transform of each other. (Will learn about this.)

Rows of spots are perpendicular to original lines

\[ \theta = \sin^{-1} \frac{n\lambda}{P} \]
The first three mask patterns

Basic pattern

A  } d  D  } d  C  } d
A helix (flattened into 2 dimensions) is very similar to a zig zag.

X-shape = Helix!
You discover a new structure of DNA in which the diffraction pattern is the same as the “normal” DNA in every respect EXCEPT that the cross makes a more acute angle $\alpha$.

Which statement regarding the new DNA structure must be true?

A. It cannot be a helix

B. The helix repeat distance $P$ must be different

C. It must be a wider molecule
**Question:** What does increasing the diameter of the helix do to slope of decreases the slope?  

Decreases.
X pattern: Helix

Layer Lines: Helix period P

Angle $\alpha$: Helix radius

10 layers lines/diamond: Interbase spacing $P/10$

Missing 4$^{th}$ Layer line?
\[ \Delta L = \frac{\lambda}{2} \]

Destructive Dark Spot

\[ \Delta L = P \sin \theta \]

Destructive Interference (Dark Spots)

\[ \Delta L = P \sin \theta = \frac{(2n' + 1)}{2} \lambda \quad n' = 0, 1, 2, \ldots \]

Constructive Interference (Bright Spots)

\[ \Delta L = P \sin \theta = n \lambda \quad n = 0, 1, 2, \ldots \]
Missing lines $\rightarrow$ Additional Interference

Even if Blue is in phase with Blue,

$$\sin \theta = \frac{n\lambda}{P}$$

Blue may be out of phase with Red

$$\sin \theta = \frac{(2n' + 1)\lambda}{2q}$$

$$\Rightarrow q = \frac{(2n' + 1)p}{2n}$$

For 4th layer line, $n = 4$

This is mathematically the correct answer. However, $P/8$ would make the atoms collide: 10.4bp/8 = 1.3 bases apart. For 5th layer lines you get 5P/8, but this is the same as $3P/8$

$$q = \frac{(2n' + 1)p}{8}$$

$$q = \frac{1}{8}P \text{ or } \frac{3}{8}P$$

Notice that both the red and blue dots are spaced 1P apart, but because they’re offset from each other by an amount $q$. Two helices identical except the starting point, their phase.
Notice where blue and red dots totally interfere: only at Layer line $4\lambda/P$. Therefore no signal there.
Class evaluation

1. What was the most interesting thing you learned in class today?

2. What are you confused about?

3. Related to today’s subject, what would you like to know more about?

4. Any helpful comments.

Answer, and turn in at the end of class.