Structural Biology: Why you should care

Andrew J Howard

Biology and Physics, IIT

Wheaton North High School Science Club

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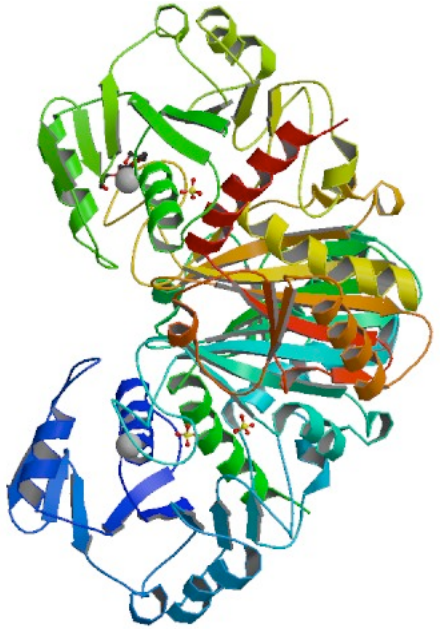
Structure tells us about function

- Proteins and nucleic acids are responsible for how organisms operate.
- Knowing what they look like can enable us to understand how they work.



What we'll discuss

- Why structure matters
- How to study structure
  - Microscopy
  - NMR
  - Other methods
- Crystallography
  - Growing crystals
  - Measuring diffraction intensities
  - Solving structures
- Careers in science

Please interrupt whenever you like!

Today: 8 February

- 8 February 1672: Isaac Newton presents his first optics paper before the Royal Society in London
- Relevant because many of the techniques we'll describe are optical techniques (especially when you realize that X-rays are short-wavelength light!)

Why structure matters

- We can't learn *everything* about biology by studying large molecules (proteins, DNA, RNA), but we can learn a lot
- How these macromolecules operate in the cell (and outside it) determines most of what happens in organisms
- If we know what they look like, we can tell how they function

Aren't macromolecules flexible?

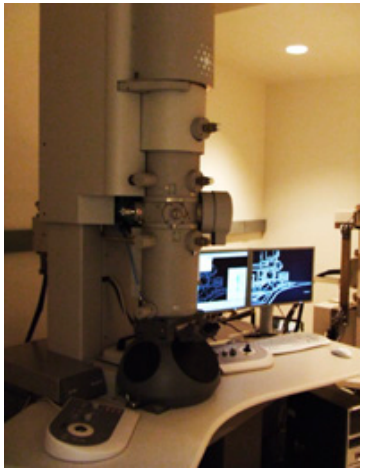
- You betcha.
- But proteins and many RNA molecules have enough structural rigidity that we can say intelligent things about their structures, and draw functional conclusions therefrom.
- We can even draw important conclusions about DNA from its structure.

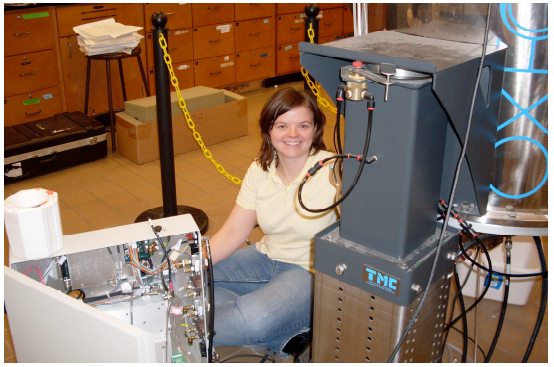
How to study structures

- We can divide structural probes into two categories:
  - Methods that don't require any prior knowledge of the structure
  - Methods that provide specific structural information about regions whose overall fold is already known

Starting from scratch: I

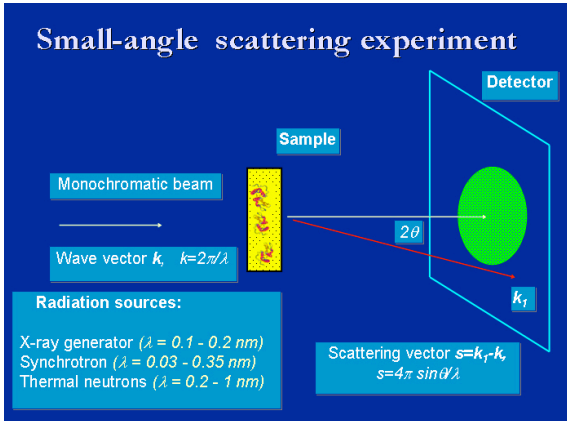
- Electron microscopy
- Nuclear Magnetic Resonance Spectroscopy
- Circular Dichroism

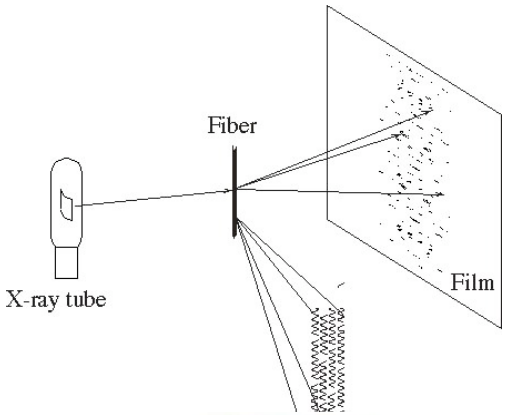


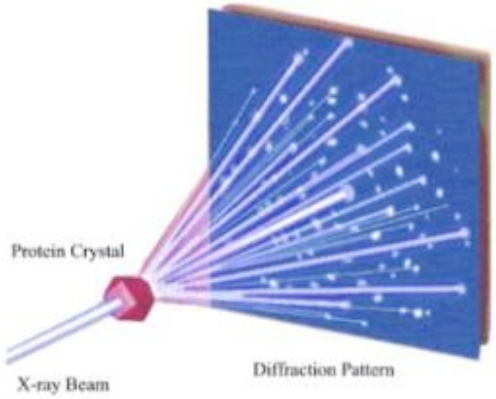


Starting from scratch: II

- Solution scattering
- Fiber diffraction
- Single-crystal X-ray diffraction

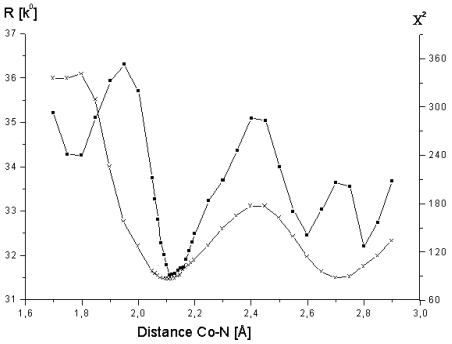


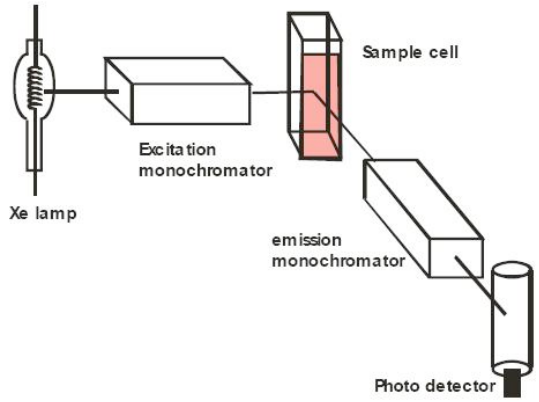




Answering specific questions

- X-ray spectroscopy
- EPR spectroscopy
- Fluorescence techniques
- …



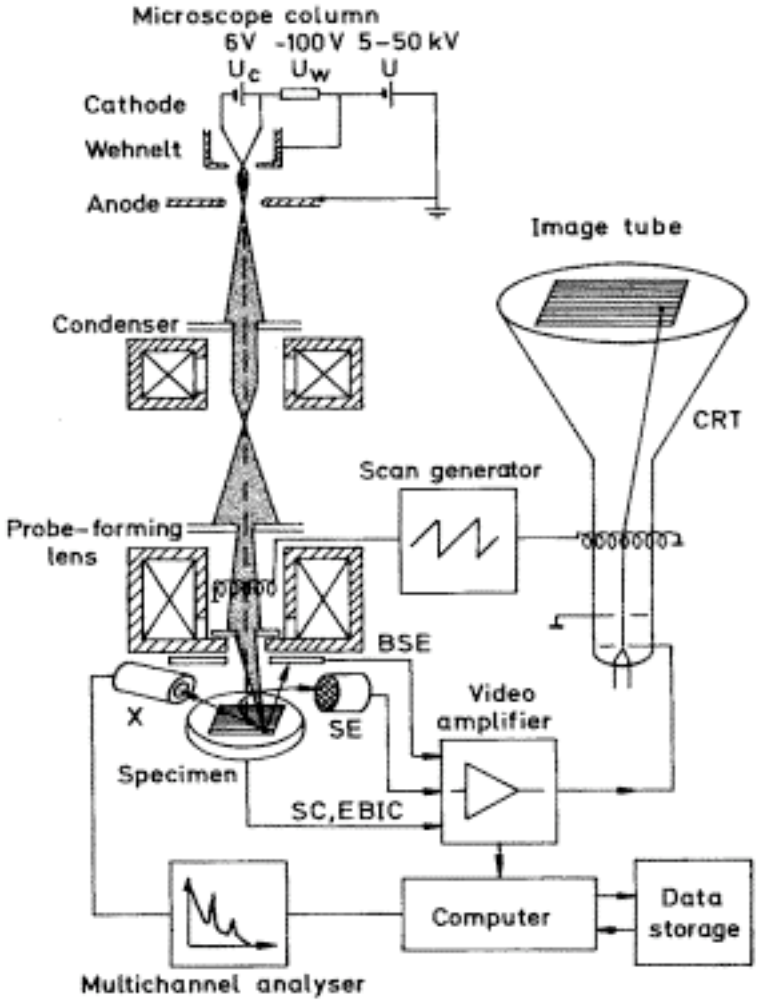


Why can't we just look at the structures under a microscope?

- The details are too fine!
- Microscopy has an inherent limit called the diffraction limit:
  - No details smaller than $\lambda/2$ can be visualized
  - Visible light has $\lambda \sim 500$ nm; too coarse for molecular details, where the characteristic length scale is ~0.1 nm
  - Size of the macromolecule itself is ~ 10 nm, so it won't even appear as a featureless dot

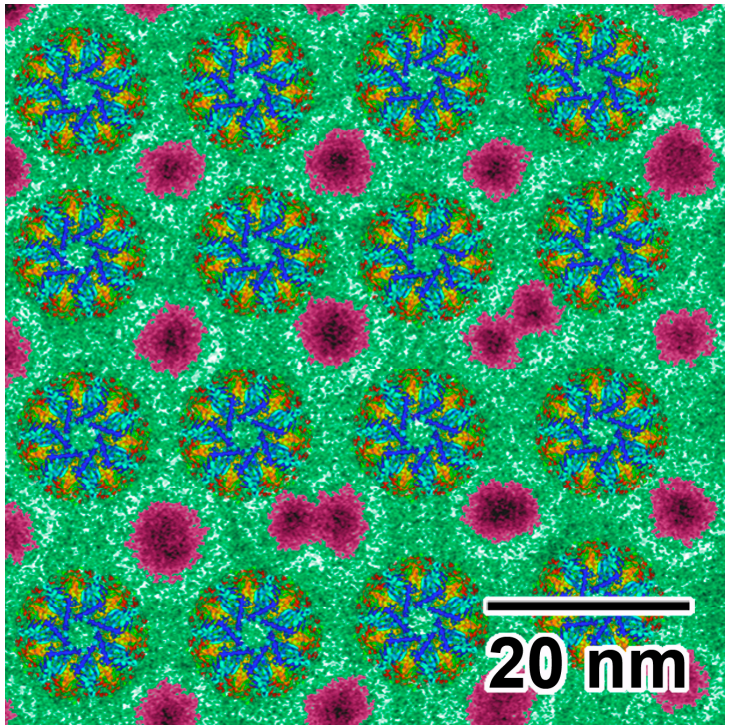
Electron microscopy

- Depositing molecules onto a surface and allowing electrons to scatter from them
- Measure the scattering allows us to infer the structures that are doing the scattering



Can we do better?

- Yes: an ordered 2D array will give us details that one protein molecule at a time can't
- Computer averaging of the images of the individual molecules lets us look at finer distinctions
- Clever tags attached to molecules make them more visible

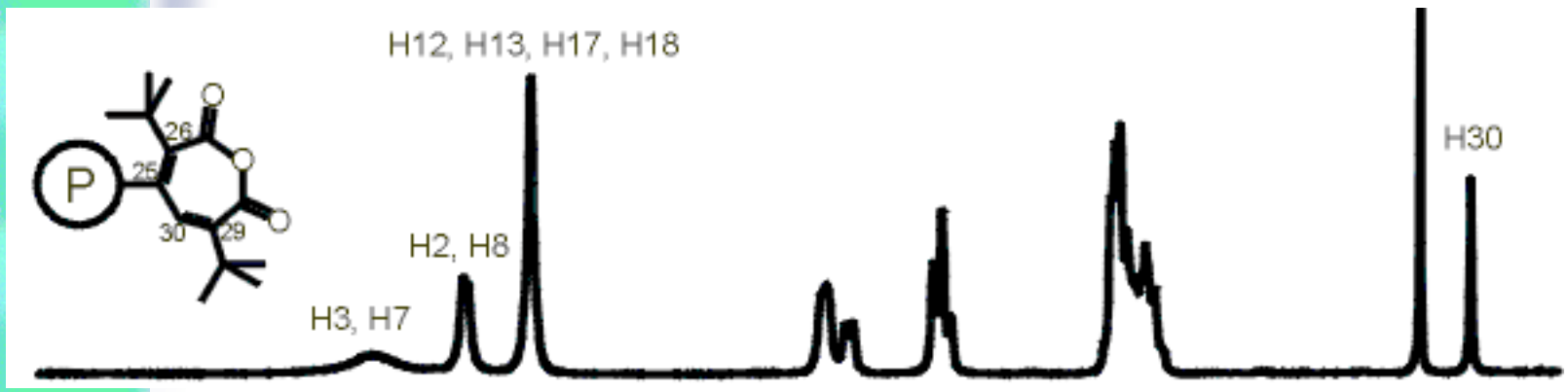


What's the problem, then?

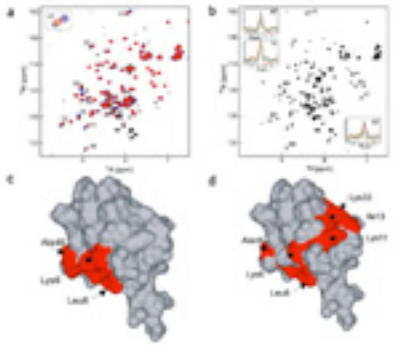
- Even under ideal conditions, it's difficult to do better than 0.4nm resolution
- Electrons destroy the samples quickly, preventing full characterization
- You can partially compensate for this by cooling: thus cryoEM
- Best way to study some viruses, protein complexes
- Immobilizing the molecules on the EM grid can alter the structure

Nuclear Magnetic Resonance

- Protons and certain other nuclei have unpaired spins
- These interact with magnetic fields in ways that tell us how far apart the spins are from one another
- Careful analysis of these distances enables us to determine the positions of those nuclei

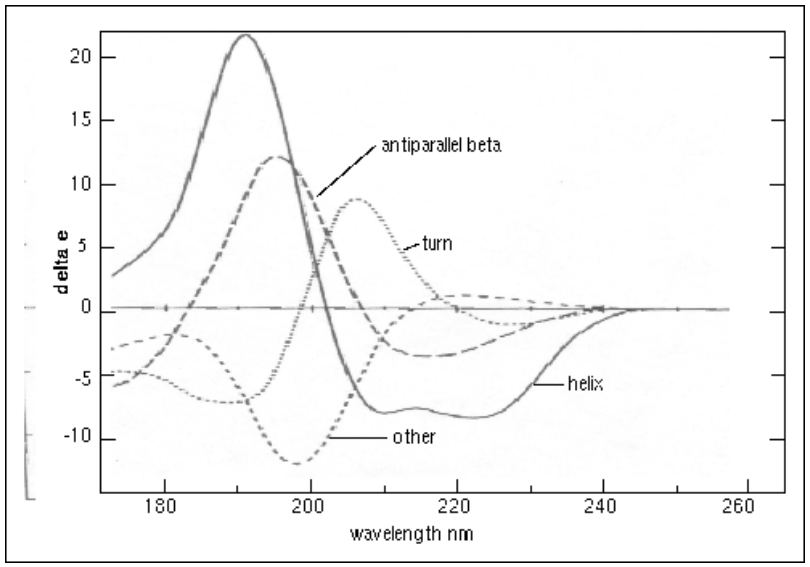


NMR for proteins



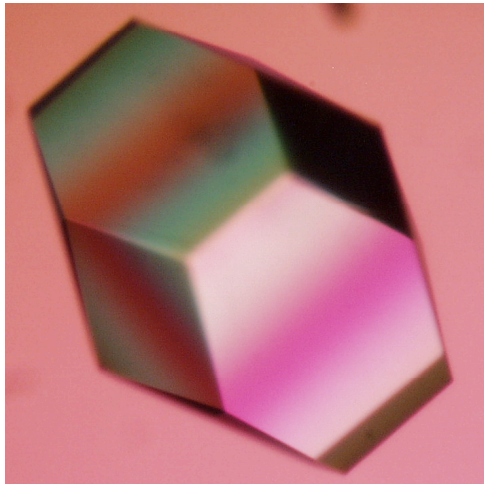
- Lots of resonances
- A simple 1-dimensional excitation produces so many that they overlap and you can't use them
- Subtle techniques involving multiple dimensions allow this to work
- Need very strong magnets (leave your wristwatch outside!) and many weeks or months of computer analysis

Circular dichroism

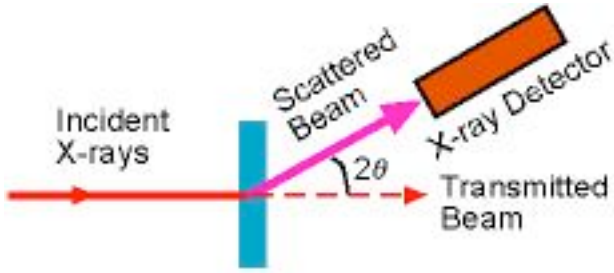


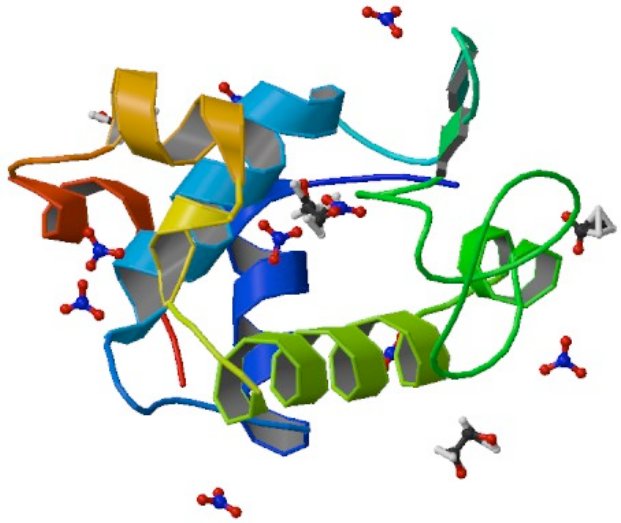
- Many molecules rotate polarized light
- Amount of rotation depends on the wavelength of the incoming light
- Certain structural elements produce characteristic spectra
- This is a quick and non-destructive way to look at proteins
- Most useful if proteins have a lot of helical content

X-ray Crystallography



- The rest of this talk will focus on this approach
- Why?
  - It's the most widely used technique
  - It's what I do.





What the experiment entails

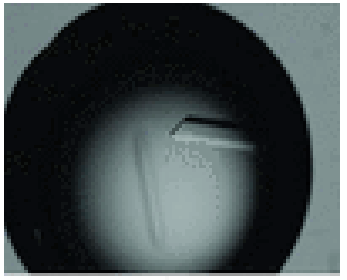
- Induce a macromolecular solution to form a three-dimensionally ordered array of molecules, i.e., a crystal
- Shoot X-rays at the crystal
  - Record a diffraction pattern on an electronic detector
  - Measure the intensities of the spots
- Rotate the sample to expose a different pattern and repeat
- Analyze the pattern to determine the structure

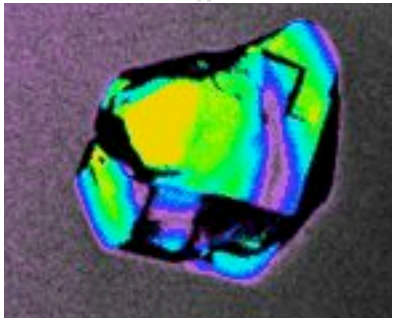
Growing protein or nucleic acid crystals

- Purify the macromolecule
- Get it into solution
- Gradually move it into conditions under which it is insoluble
- If you're successful, it will fall out of solution as an ordered 3-D array
- If you fail, you get an amorphous precipitate :-(

Typical protein crystals

- Barely visible: typical dimensions ~ 0.05 mm
- Rotate polarized light
- Usually display flat faces parallel to the axes of the repeating unit (the unit cell)
- Often, but not always, beautiful



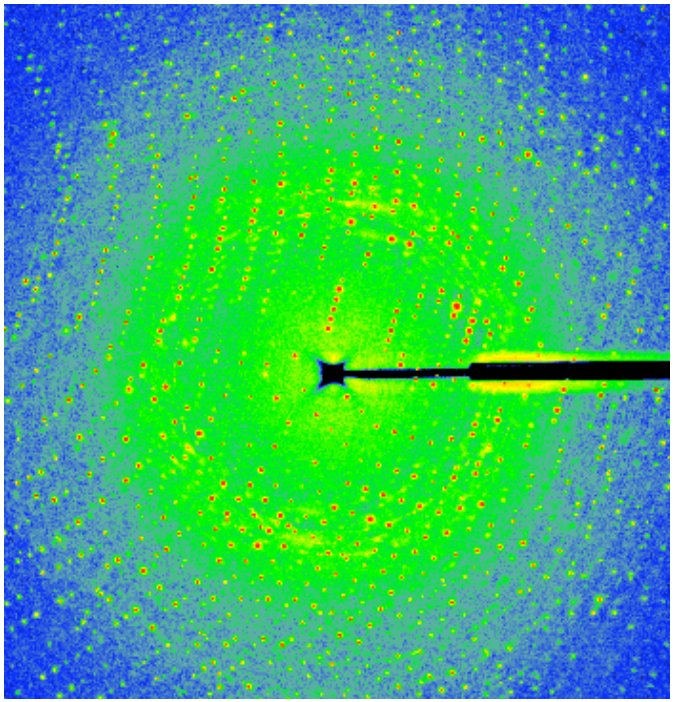


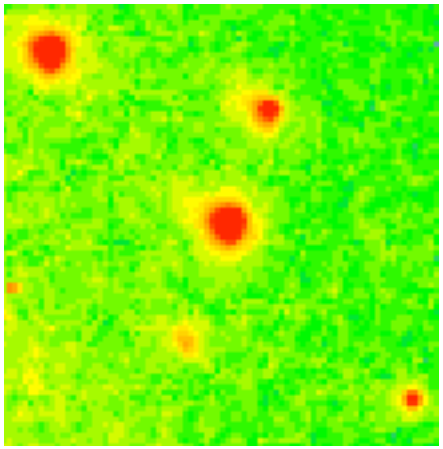
Measuring intensities

- We're shooting electromagnetic waves at an ordered 3-D array of molecules
- The waves scatter, and we get *destructive* interference in most directions
- In a few specified directions we get *constructive* interference
- 3-D array means each of those directions where constructive interference occurs can be characterized by 3 integer values called Miller indices *hkl*

How do we do the measurements?

- Stick an X-ray sensitive detector into the path of the outcoming diffraction directions
- We get intensity=0 except where those constructive interference conditions apply; there we get nonzero intensity
- Identify which (*hkl*) indices apply for each spot and measure how bright it is: get I(*hkl*)





Is that all the information I need?

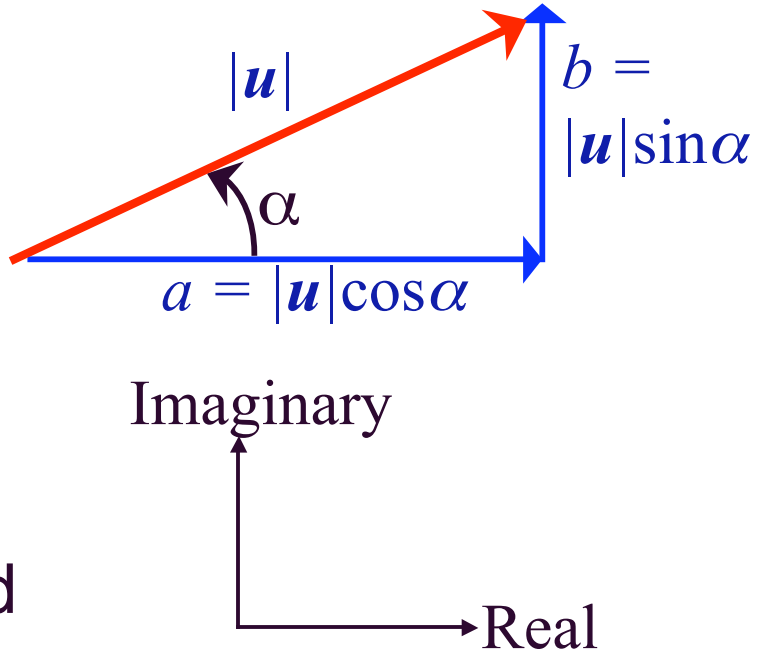
- No, unfortunately.
- The electron density (i.e., information about where the electrons are in the crystal's unit cell) is determined from $\rho(x,y,z) = (1/V) \sum_h \sum_k \sum_l \mathbf{F}_{hkl} e^{-2\pi i(hx+ky+lz)}$
- It turns out $\mathbf{F}_{hkl}$ is a complex number
- You can determine its length from the experiment but not its direction

Wait. What does that mean?

- You've probably already been introduced to complex numbers
- You described them as a real part and an imaginary part:
  $\boldsymbol{u} = a + ib$
  where a is the real part
  and b is the imaginary part
- But think about that geometrically!

Geometry and Euler's formula

- Complex number ***u*** can be expressed as a real and imaginary part (a +ib) or as a length |***u***| and an angle α:
- ***u*** = |***u***|$e^{i\alpha}$ = a + ib = |***u***|(cosα + isinα) with |***u***| = $(a^2+b^2)^{1/2}$
- Note that a = |***u***|cosα, b = |***u***|sinα
- The relationship $e^{i\alpha}$ = cosα + isinα is called *Euler's formula*



Back to the crystallography…

- So it turns out that the intensity of the individual spot I_{hkl} is intimately related to the length of this complex number $\mathbf{F}_{hkl}$:
  $I_{hkl} = Q|\mathbf{F}_{hkl}|^2$
  where Q is a constant characteristic of the experiment
- So we can measure I_{hkl} and determine $|\mathbf{F}_{hkl}|$ from that: $|\mathbf{F}_{hkl}| = (I_{hkl}/Q)^{1/2}$
- But we still don't have the angle α_{hkl}!

We need a way of finding α_{hkl}

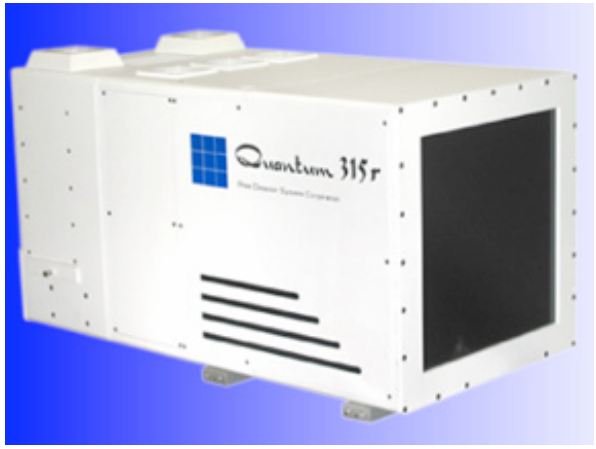
- We express $\mathbf{F}_{hkl} = |\mathbf{F}_{hkl}|e^{i\alpha hkl}$
- We've just shown (or at least asserted) that we can measure $|\mathbf{F}_{hkl}|$
- We still need the phase angles α_{hkl}
- There are various techniques that can be used, including guessing, comparing with other structures, and doing additional related experiments

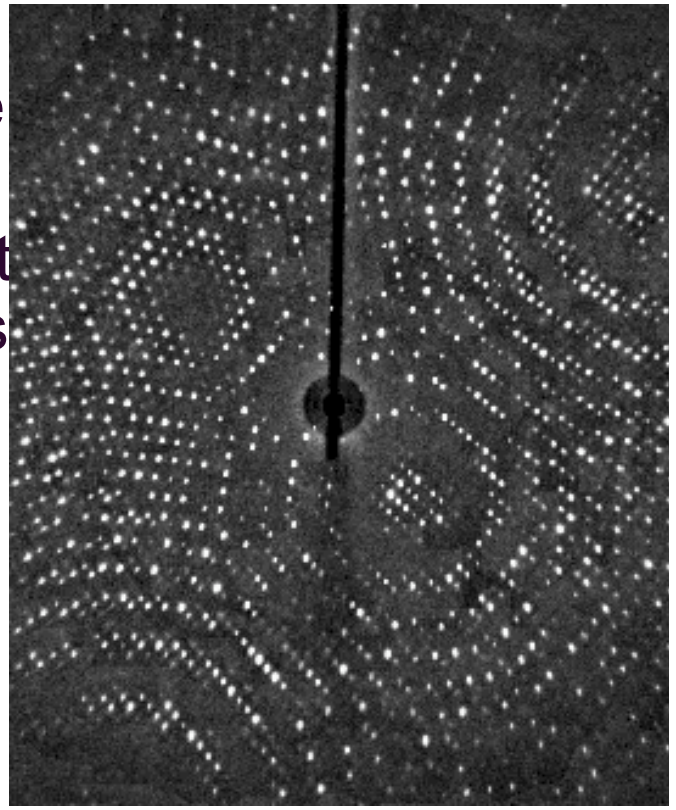
Where do we do this?

- Many laboratories have X-ray sources powerful enough to make these measurements
- But stronger and more nimble facilities are available: the Advanced Photon Source at Argonne National Laboratory, not far from you
- Brightest source of X-rays in the western hemisphere!

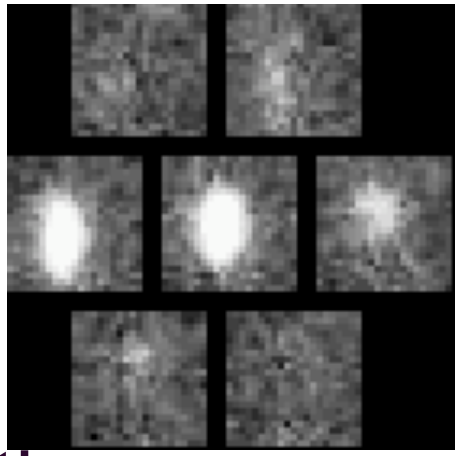
Diffraction Images

- Most crystallographers use two-dimensional position-sensitive detectors to acquire their data
- Raw output of the experiment is a set (20-5000) of disk files that constitute two-dimensional *images*
- Typically analyzed with minimal human examination of the images



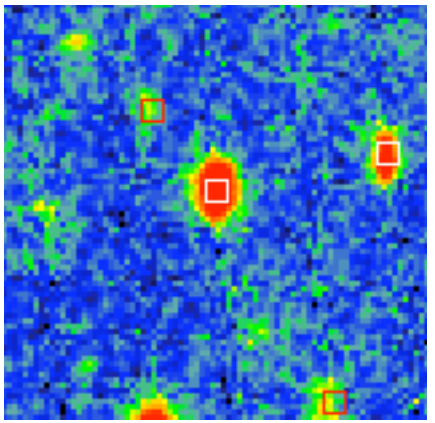


What do we do with these?



- We focus our attention on the diffraction spots!
- $I_{hkl} = Q \Sigma_{XY\omega}\{C(X,Y,\omega) - B(X,Y,\omega)\}$
- $\rho(x,y,z) = (1/V) \Sigma_{hkl} |\mathbf{F}_{hkl}| e^{i\alpha_{hkl}} e^{-2\pi i(hx+ky+lz)}$
- $F_{hkl} \propto \sqrt{I_{hkl}}$; α_{hkl} derivable*
- ** If you're clever*

What's important and what isn't?



- We can predict in advance of the intensity-measurement process which (X,Y,ω) values need to be examined
- So the space between the spots isn't examined except to get background estimates *B*(X,Y,ω)

So what do we do with this?

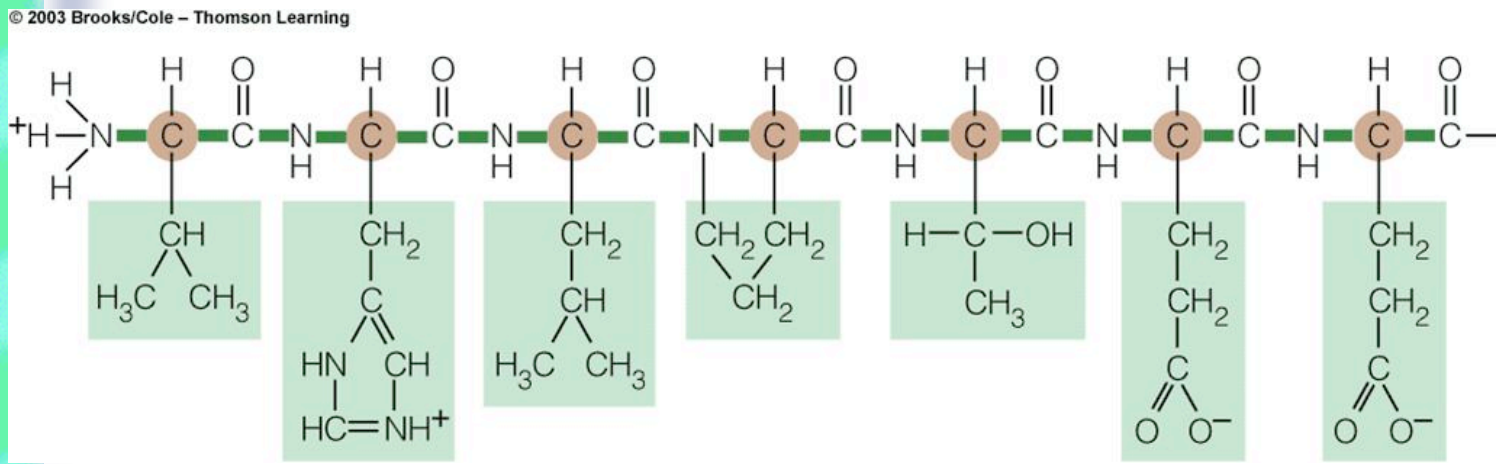
- If we can get the $|F_{hkl}|$ and α_{hkl} measurements, we can get a computer to give us electron density

$$\rho(x,y,z) = (1/V) \Sigma_{hkl} |\mathbf{F}_{hkl}| e^{i\alpha hkl} e^{-2\pi i(hx+ky+lz)}$$

- … For all points in the unit cell of the crystal
- But knowing where the electron density is high tells us where the atoms are.
- The trick is we don't necessarily know which atom goes where!

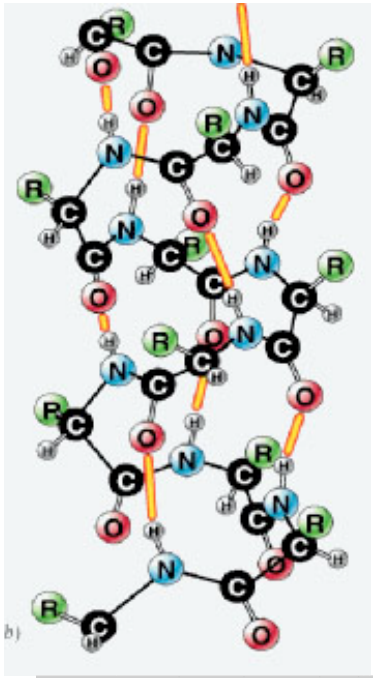
Chemistry helps a lot

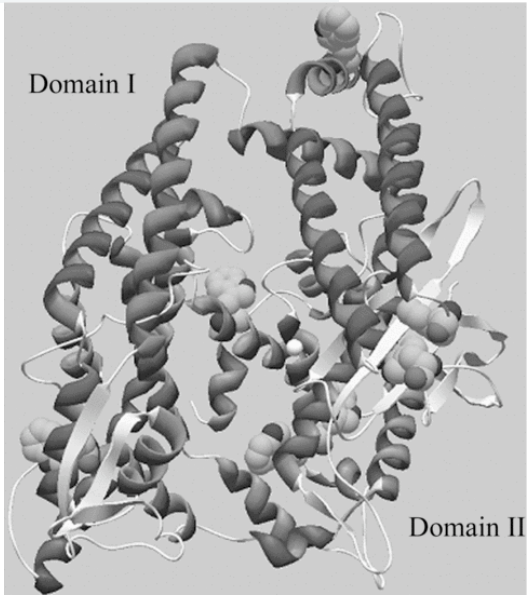
- A protein is a *polymer* made up of amino acid building blocks
- A nucleic acid polymer is similarly made up of nucleotide building blocks
- Biochemist can tell the crystallographer what the sequence of building blocks is for the polymer
- That means we know what connects to what!



Building the structure

- Look for definite features like alpha helices, beta-pleated sheets, and large side-chain motifs like benzene rings
- Start from those well-defined places and try to build outward from them
- Typically we can locate ~98% of the non-hydrogen atoms in our structure





What do we do with this?

- Basic knowledge:
  how do macromolecules work?
- Pharmaceutical Applications:
  - Most (>90%) pharmaceuticals are inhibitors of enzymes, which are proteins that catalyze reactions
  - So finding a better inhibitor or one with fewer side effects is important
- Protein engineering

Careers in research science

- Your high school science teachers have probably told you that a science education prepares you for many professions, including medicine, dentistry, law, and engineering
- That's true!
- But a career in research science is a satisfying option as well

Realities of a science career

- Not a way to make huge pots of money (unless you're J.Craig Venter)
- But unemployment rates are low
- Long hours, long training period, likelihood of disappointments
- But an opportunity to push back the frontiers of knowledge and improve humankind's condition

Where can you do it?

- The obvious answer is in universities
  - Teaching and research go together, although it's often hard to find enough hours in the day
  - Being able to inspire the next generation is satisfying
- Industry: pharamaceutical companies, agribusiness, other biotech
- Government labs (Argonne, others)

My specialty is only one of many

- In the US, there are roughly:
  - 120000 biologists
  - 30000 chemists
  - 20000 physicists
  - ~200000 chemical & biol engineers
  - Perhaps 2000 macromolecular crystallographers
- So my world is small piece of the whole

How to prepare

- Stick it out!
  - Almost all scientific research positions require a PhD
  - Typically 1 or 2 postdoc experiences
- Learn how to communicate and how to sell your ideas to others
- Be ready to live a life of service, patience and dedication

And now, a commercial…

- I teach at Illinois Institute of Technology, a small, independent, technological university on the south side of Chicago
- We have about 3000 undergrads and 7000 students in total
- Strong programs in biology, chemistry, physics
- Significant engineering and architecture programs

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- Small student-faculty ratio
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- Good value
- Strong commitment to X-ray science

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