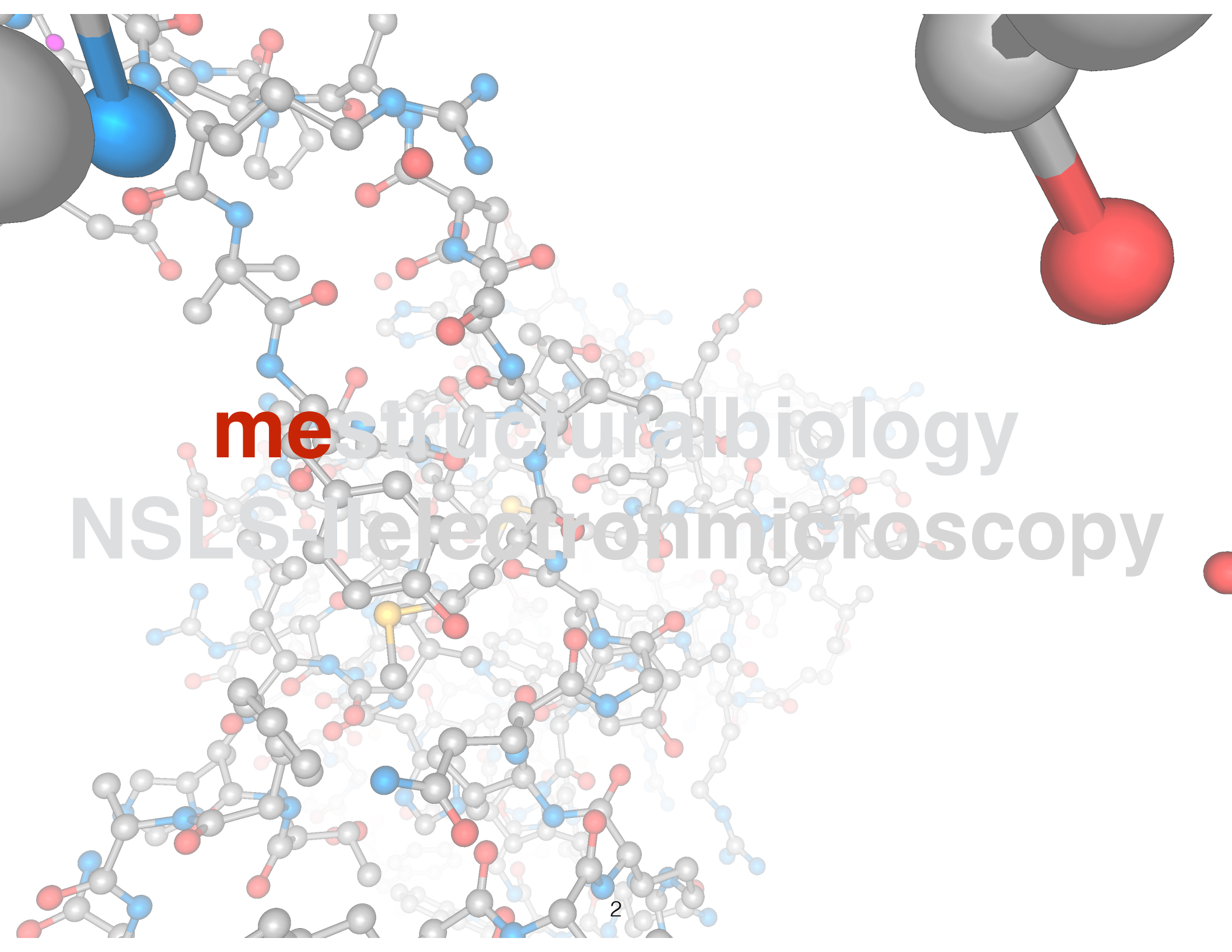
A complex molecular structure is shown in the background, composed of numerous atoms represented by spheres in shades of gray, blue, and red, connected by bonds. The structure is dense and intricate, filling most of the frame. In the top right corner, there is a larger, more detailed view of a specific part of the molecule, showing a gray sphere connected to a red sphere by a thick red rod, with another gray sphere attached to the red one.

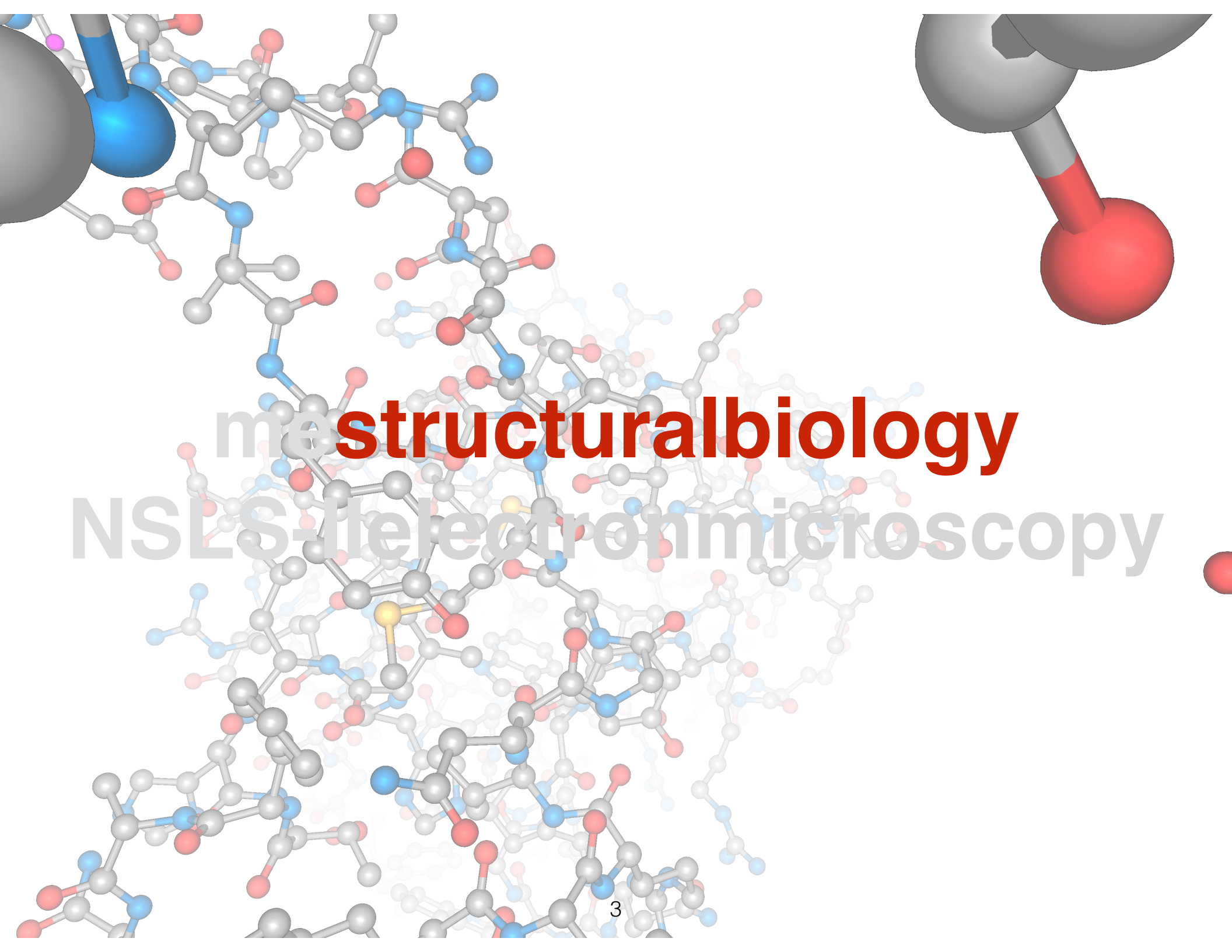
“...everything that living things do  
can be understood in terms of the  
**jiggling and wiggling** of atoms.”

R. Feynman  
Lectures on Physics Vol 1, Chap 3.

Seán McSweeney  
NSLS-II  
[smcsweeney@bnl.gov](mailto:smcsweeney@bnl.gov)

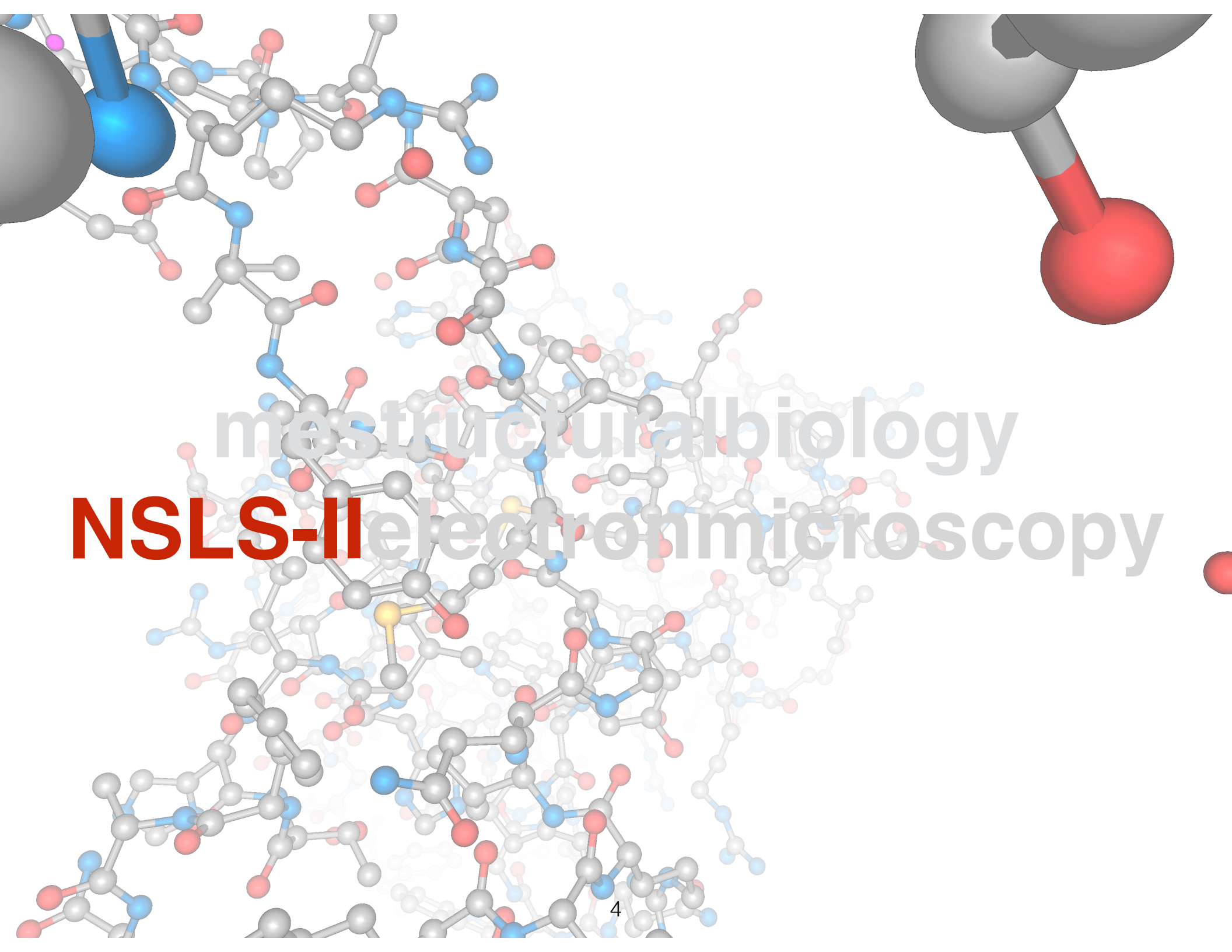


**me** structural biology  
NSLS-II electron microscopy



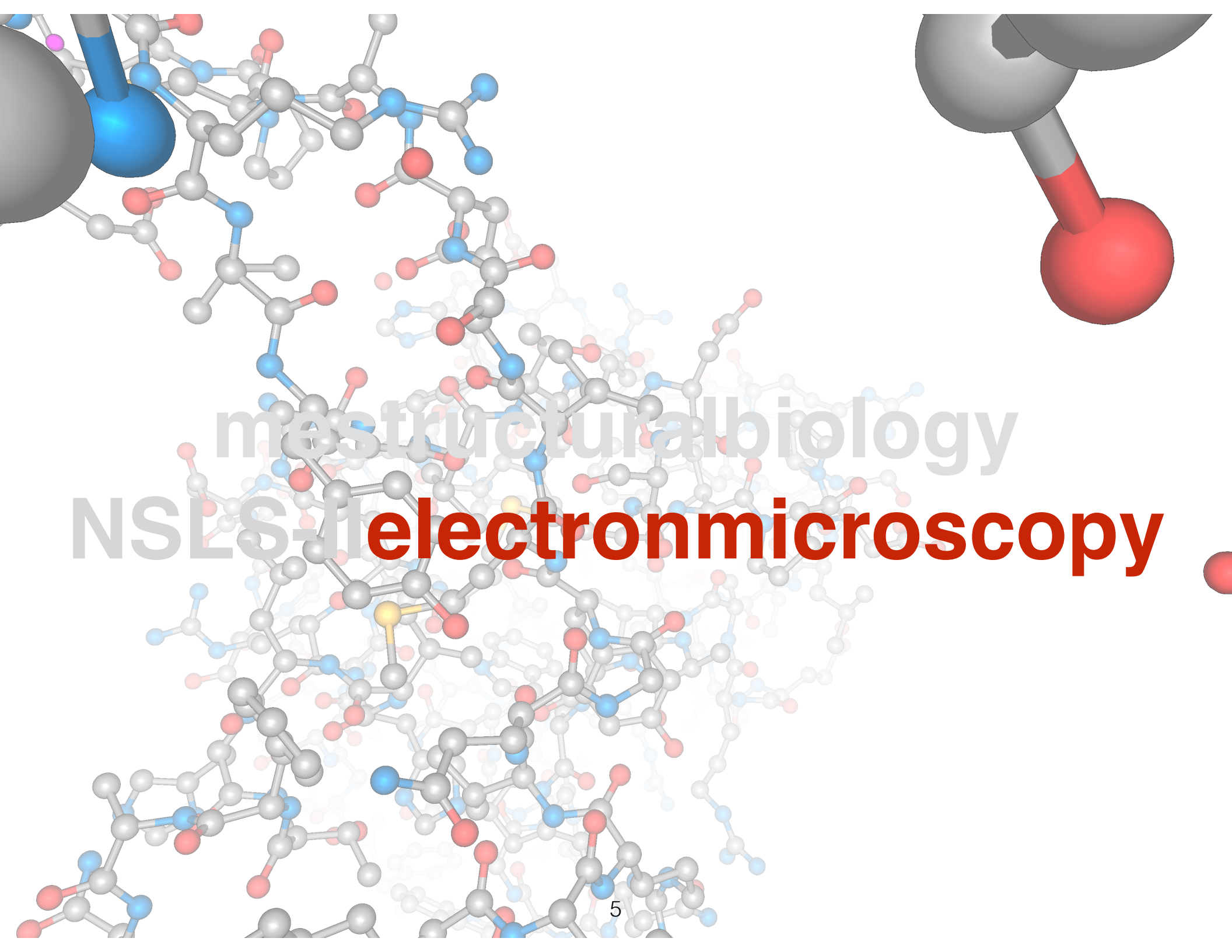
me **structural biology**

NSLS-II electron microscopy



structural biology

**NSLS-II** electron microscopy

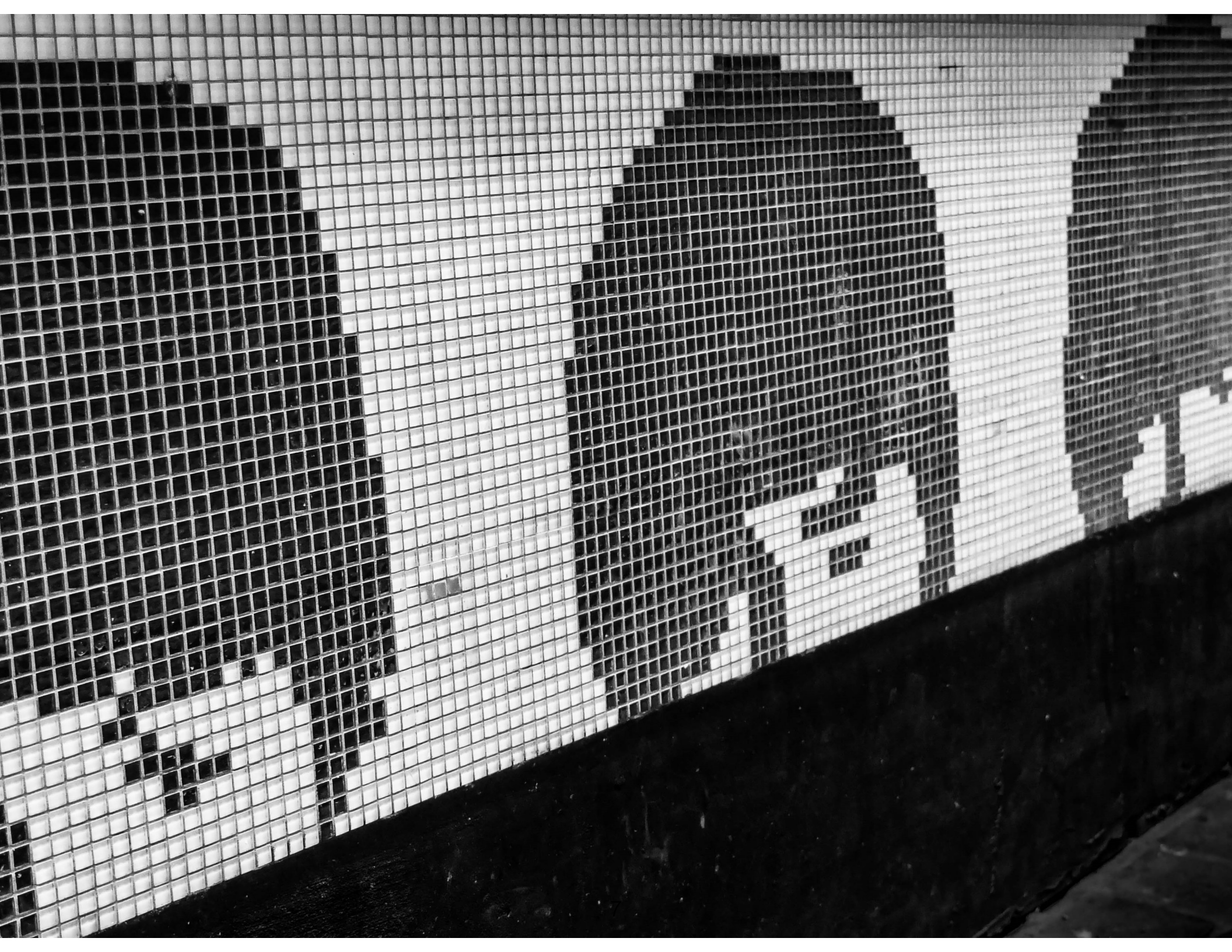



structural biology

NSLS-II **electron microscopy**

I am originally from a small town in the North West of England.



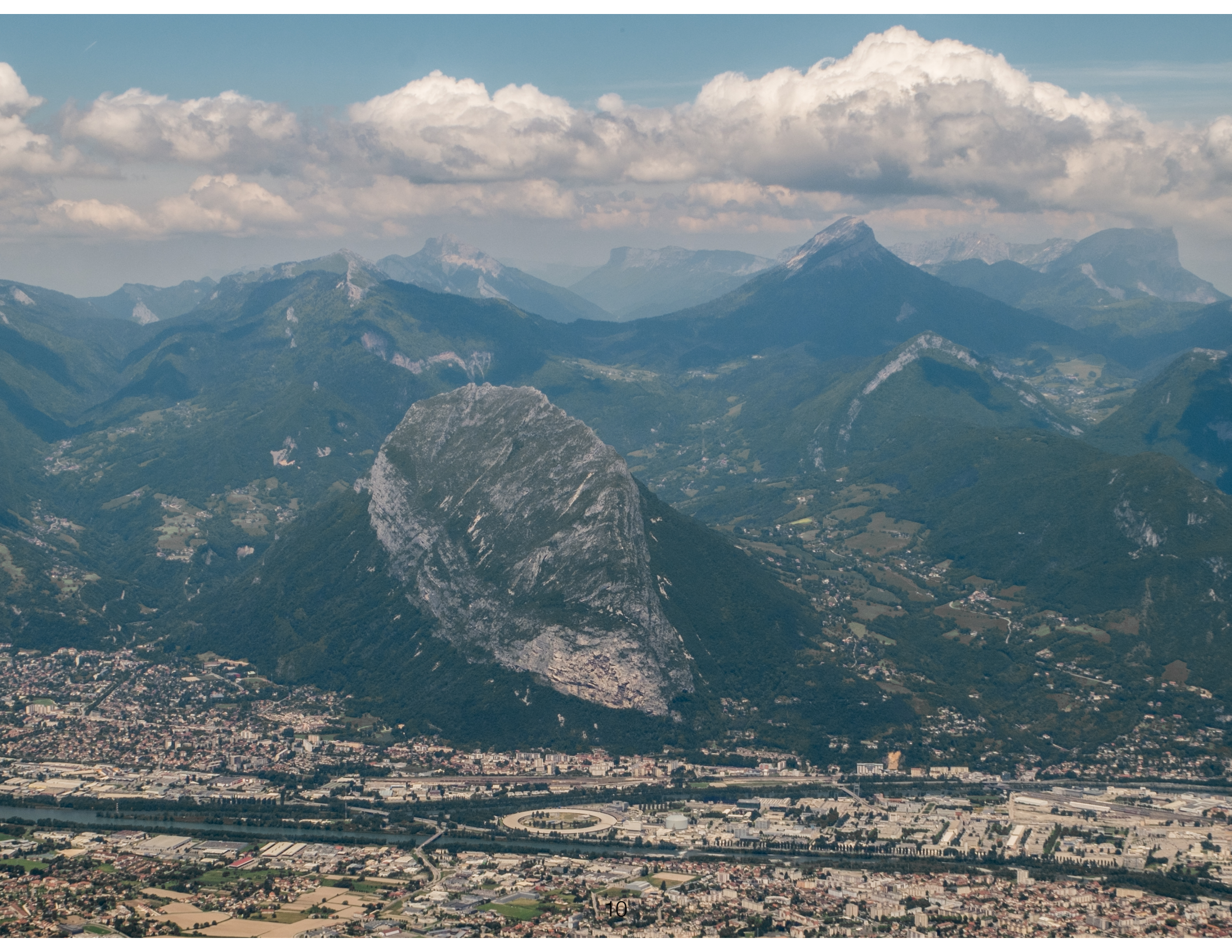




MERSEYSIDE



For close to twenty years I worked at the European synchrotron, the ESRF, in Grenoble France. There I built **structural biology research teams** with varying degrees of success.





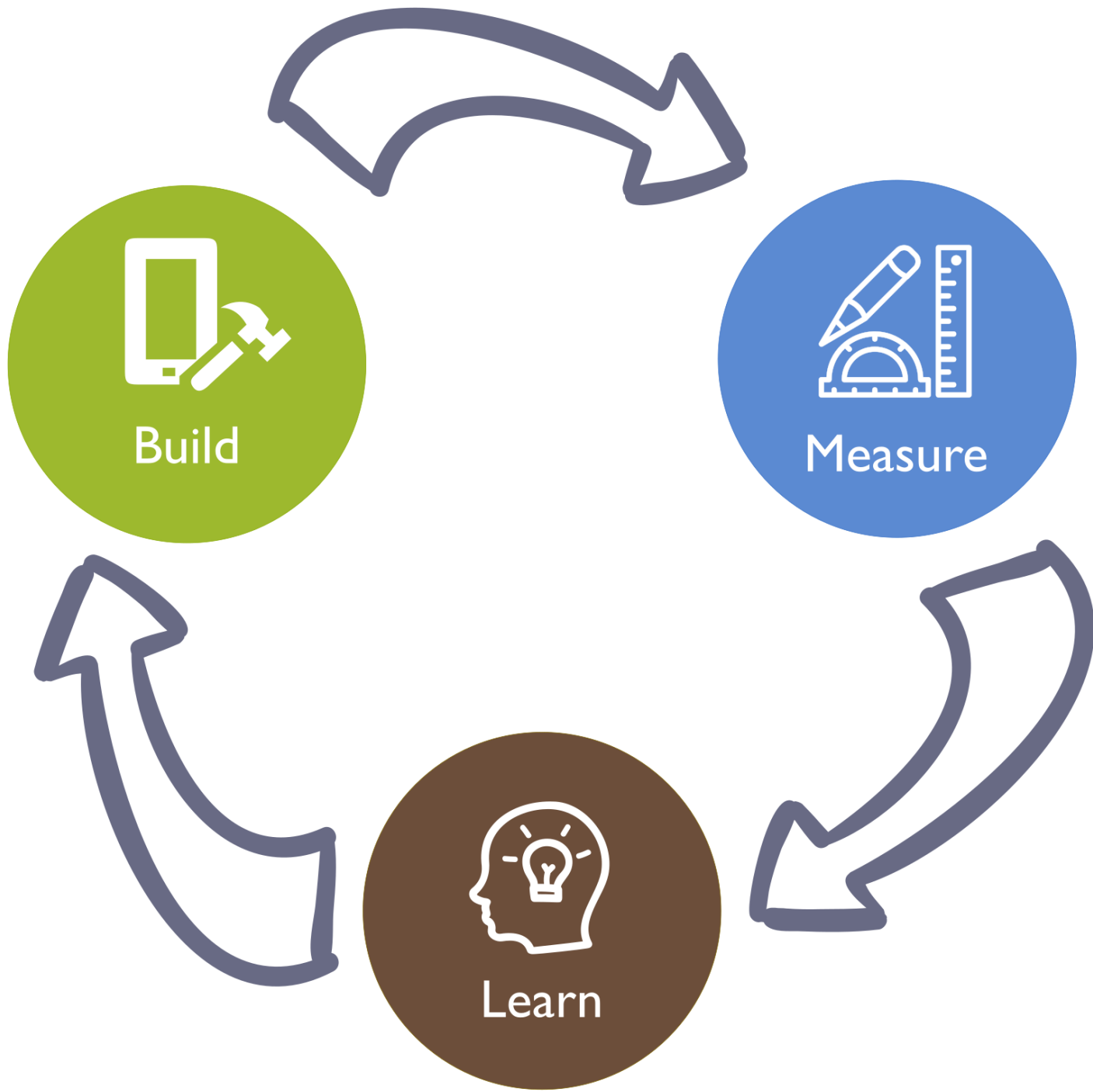
DO YOU

ATTENTION

VOTRE

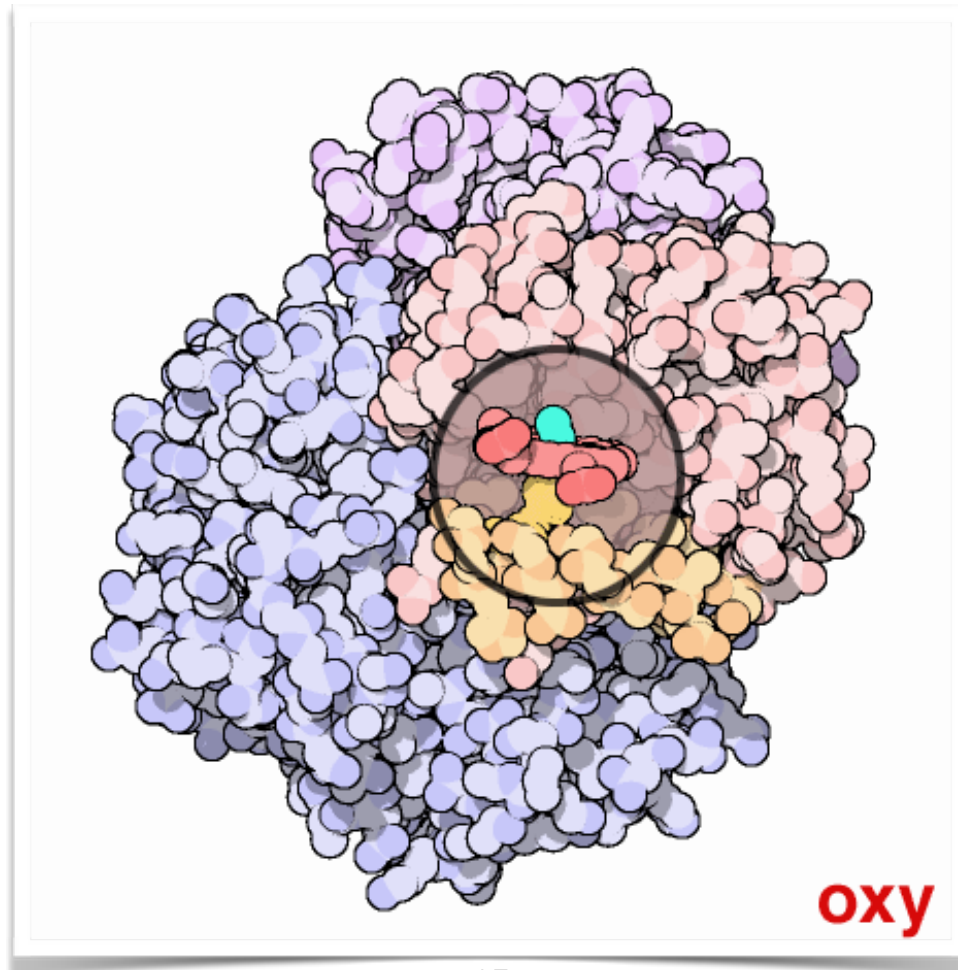
TÊTE

BITTE



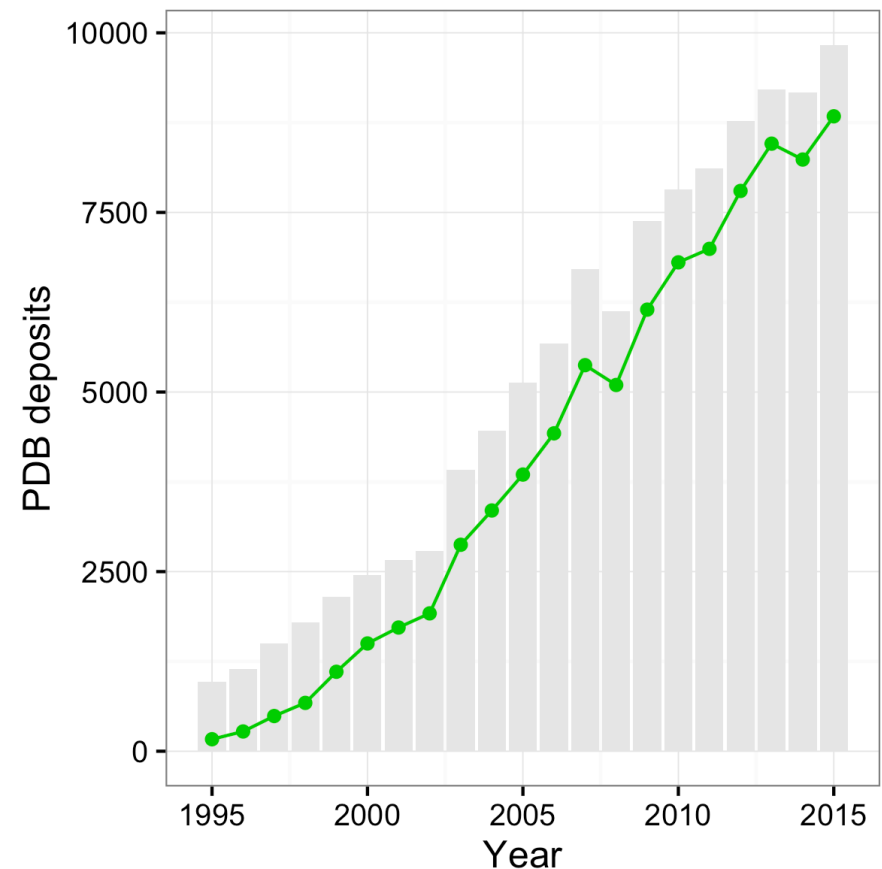
**Structural biology** is the way to understand the role of biological molecules with an atomistic and a cellular view.

Structure and function are intimately related.



# light sources have revolutionized structural biology.

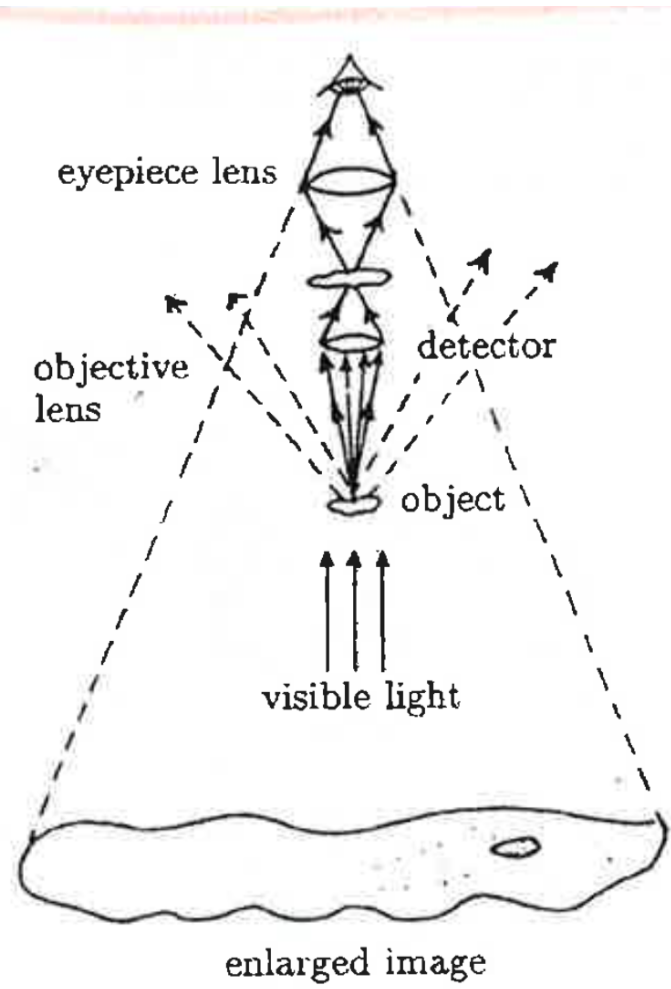
- structural biology has become the most important source of publications and user “foot fall” at most modern light sources.
- now electron microscopes are making big changes for us.



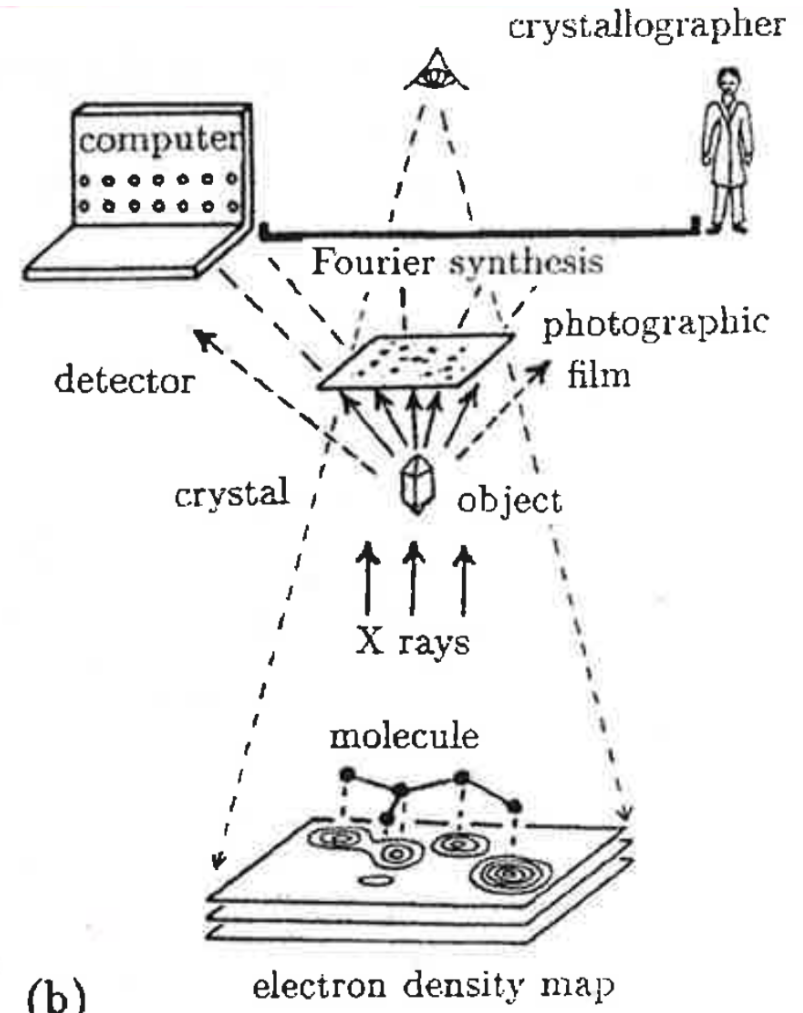
**Grey:** protein structures made publicly available each year. **Green;** the structures owing to data from a light source



structural biology in a  
few slides.



(a)



(b)

the principle method  
for determining  
structures is  
**x-ray crystallography.**

# An early example of “interesting”

- The antibacterial property of hen egg white, due to the lysozyme it contains, was first observed by Laschtschenko in 1909, although it was not until 1922 that the name 'lysozyme' was coined, by **Alexander Fleming** (1881–1955), the discoverer of **penicillin**.
- Large amounts of lysozyme can be found in egg white.



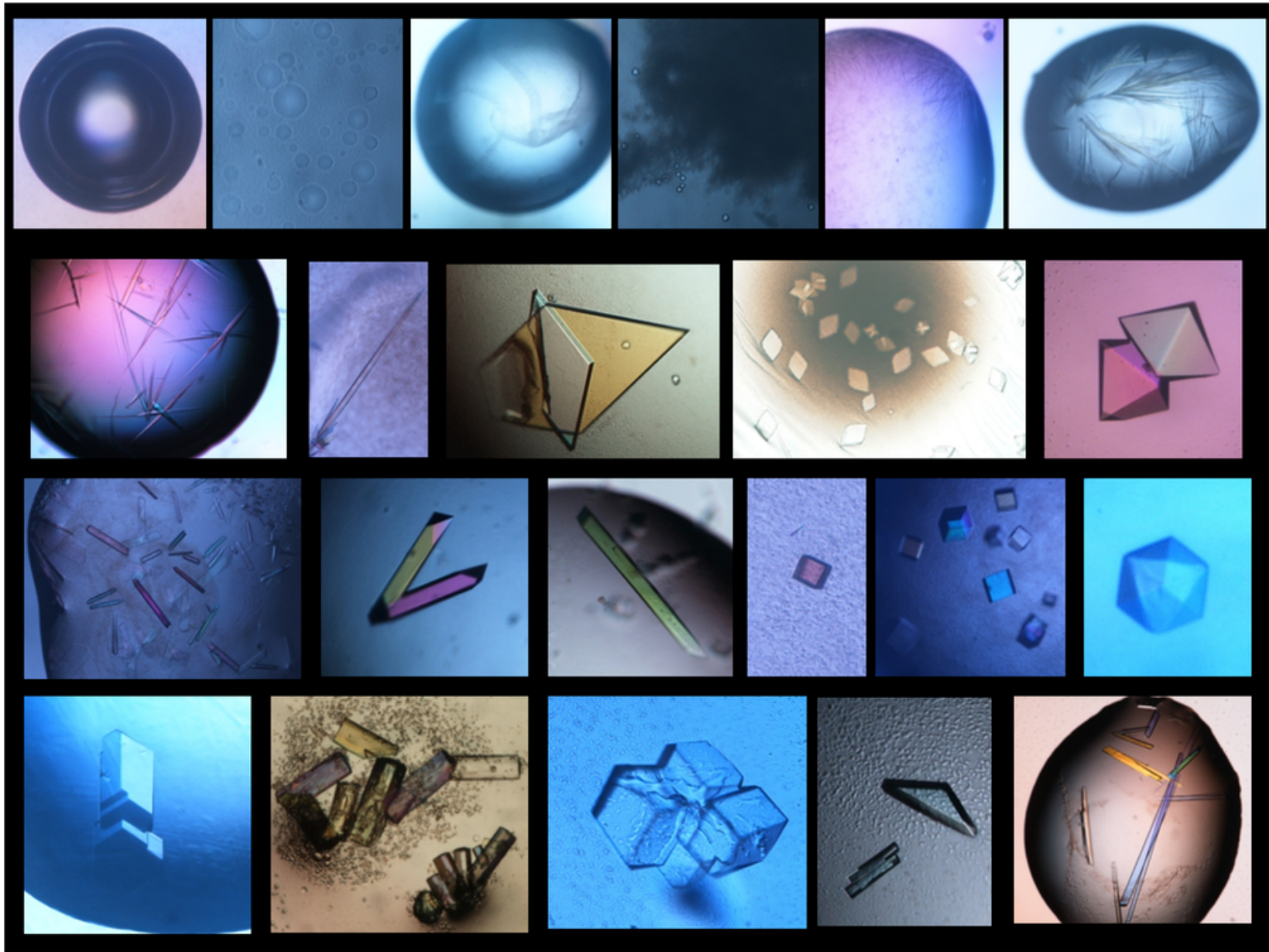
**get lots of sources for the  
protein.**



**OR** induce your protein producer to generate a lot of material.



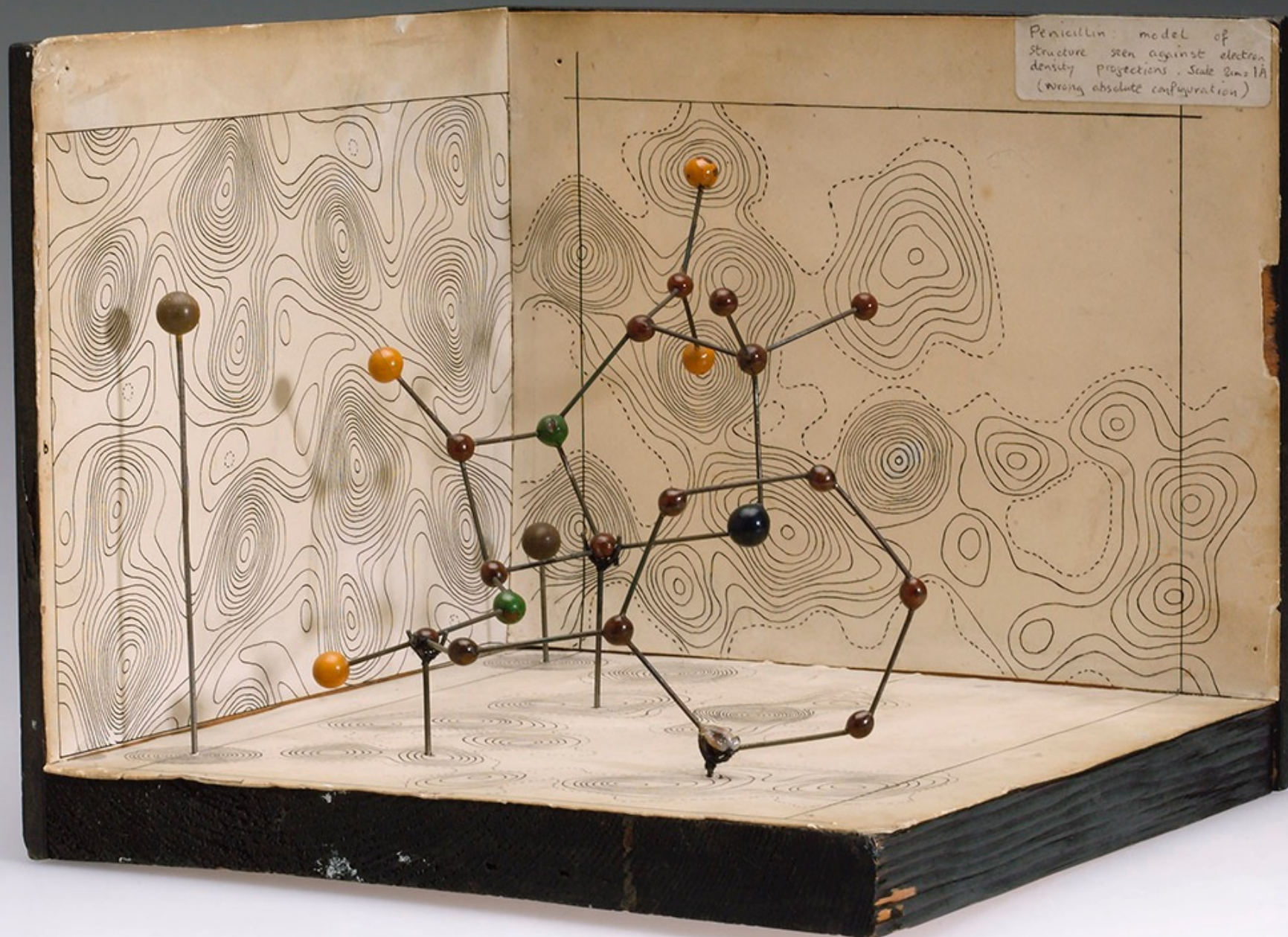
or **better** get lots of producers to produce lots of material.

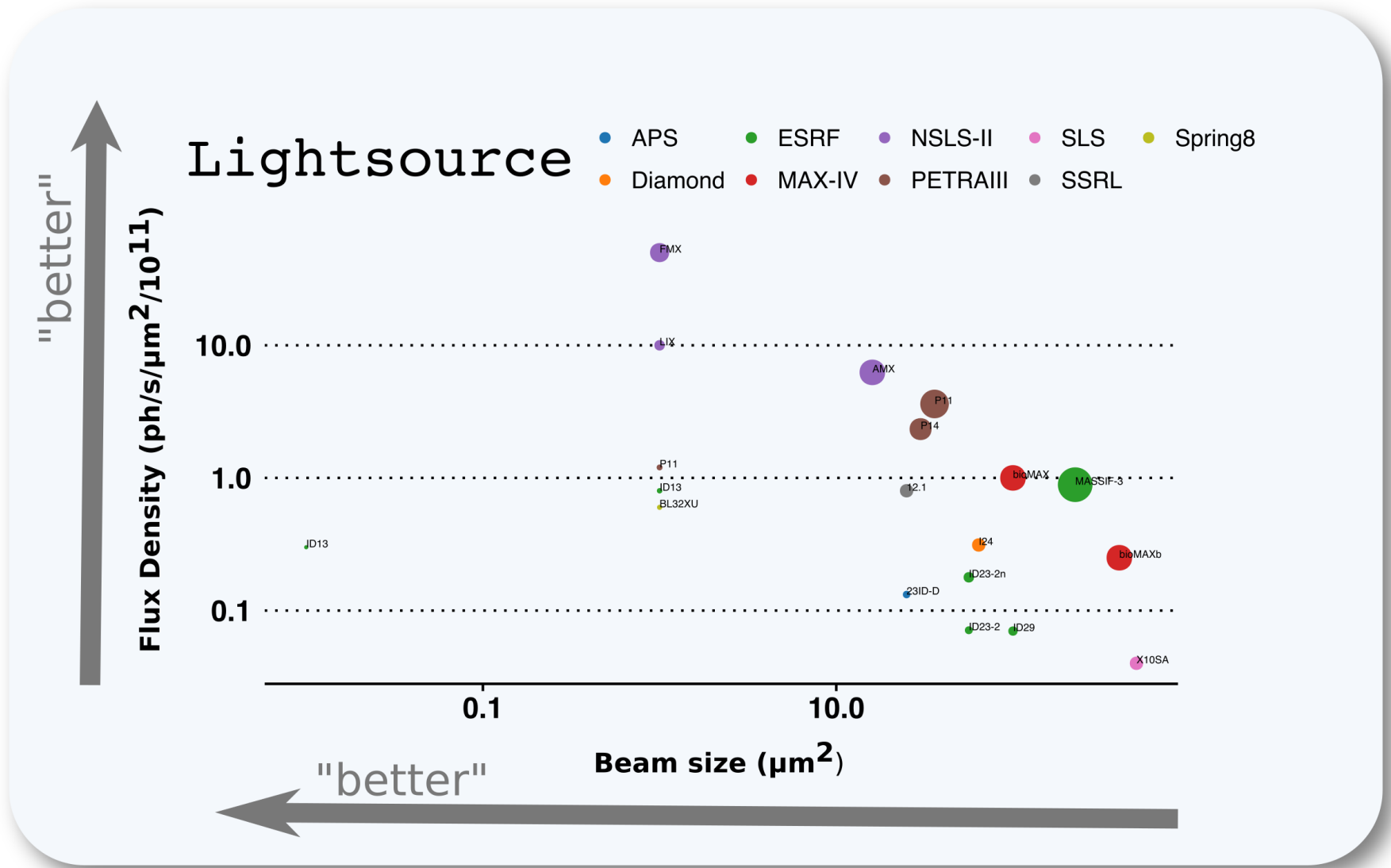


if you are lucky crystals can be created.



if lucky the diffraction will be lovely

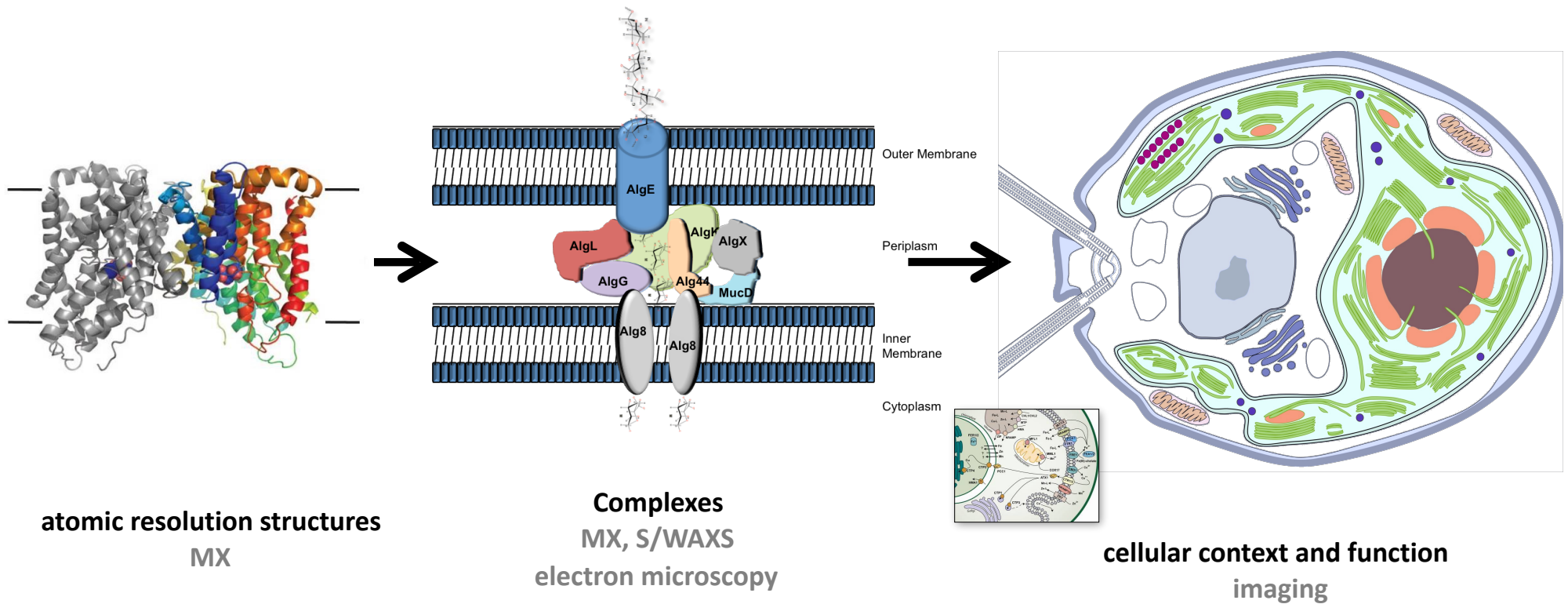




# NSLS-II is leading the pack

So why are we so excited about electron microscopy?

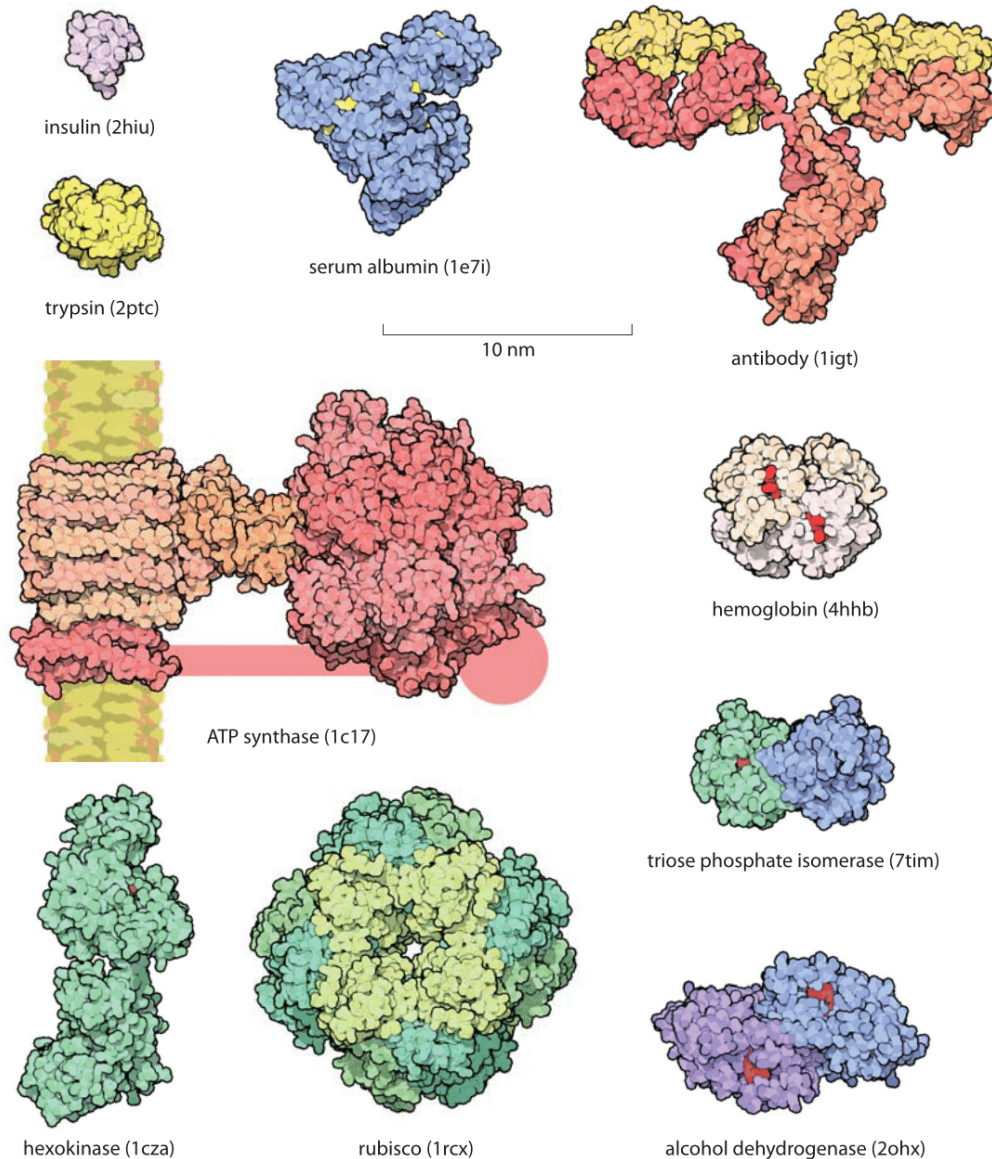
# LIFE: atomic to cellular



# What if you can't get a crystal?

- until recently you were stuck!.
- complex biology runs through complex machines.
- electron microscopy has been through many technical advances in the last few years.

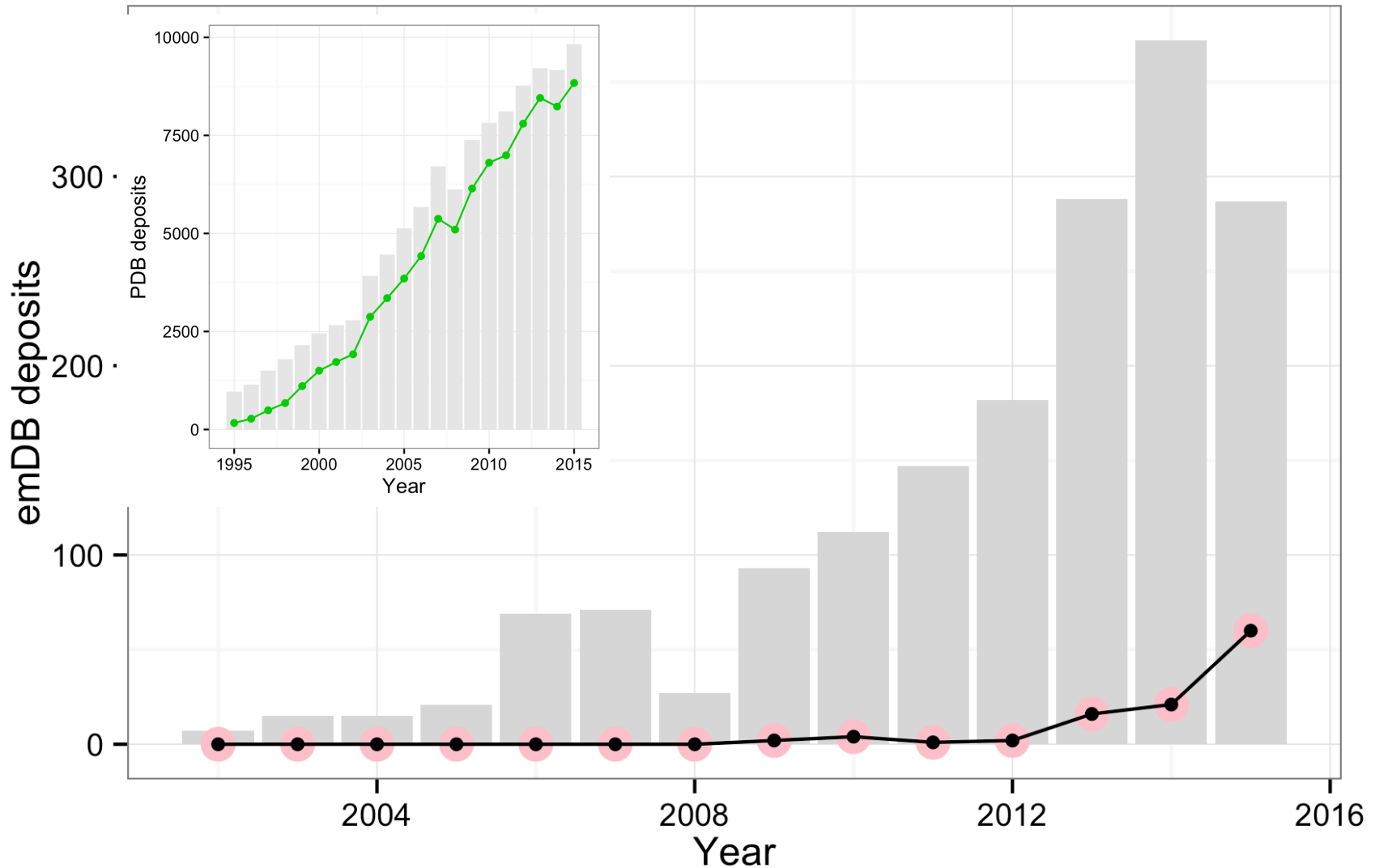
# Introduction



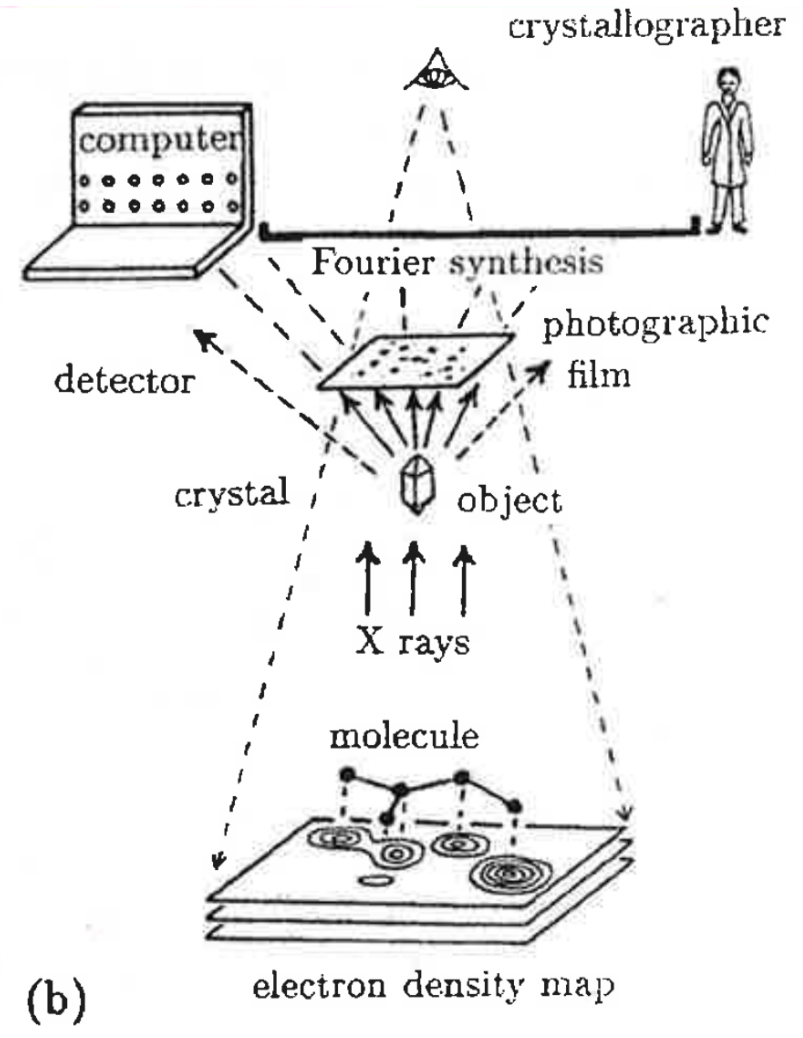
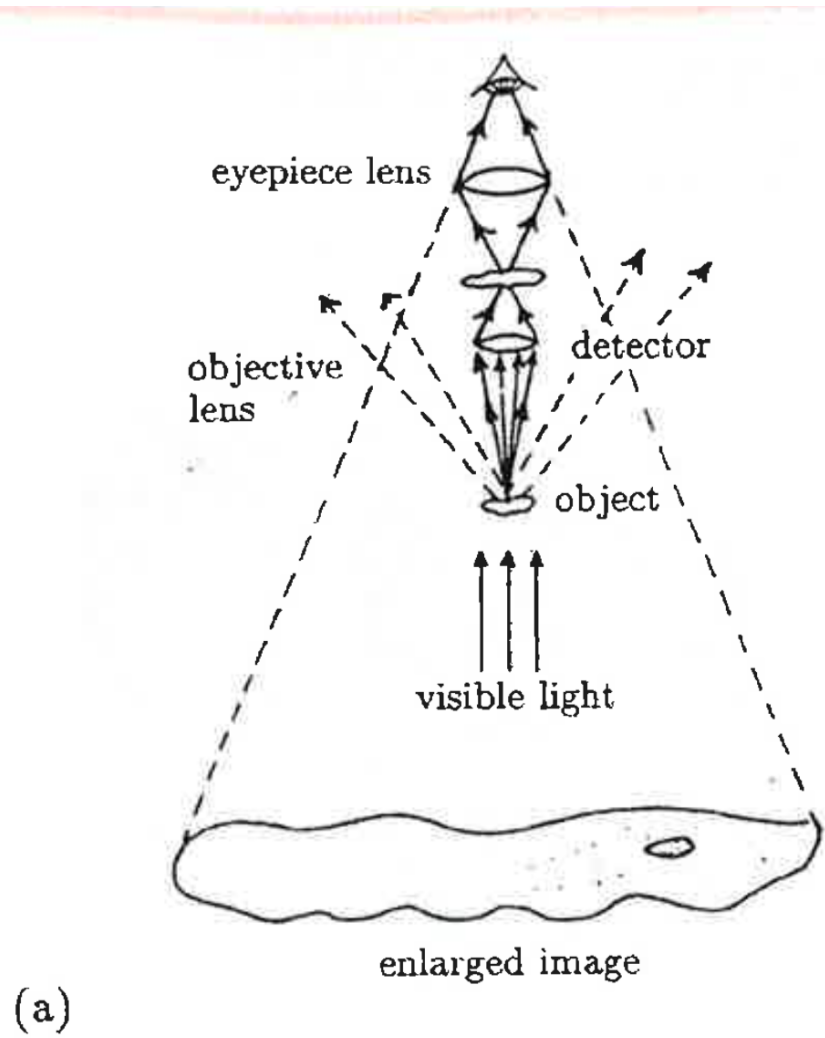
Proteins and their complexes make up the machinery of life. Until recently the only way to resolve these structures at atomic resolution was **X-ray crystallography**.

However recent advances in **cryo-EM** have changed this perspective dramatically.

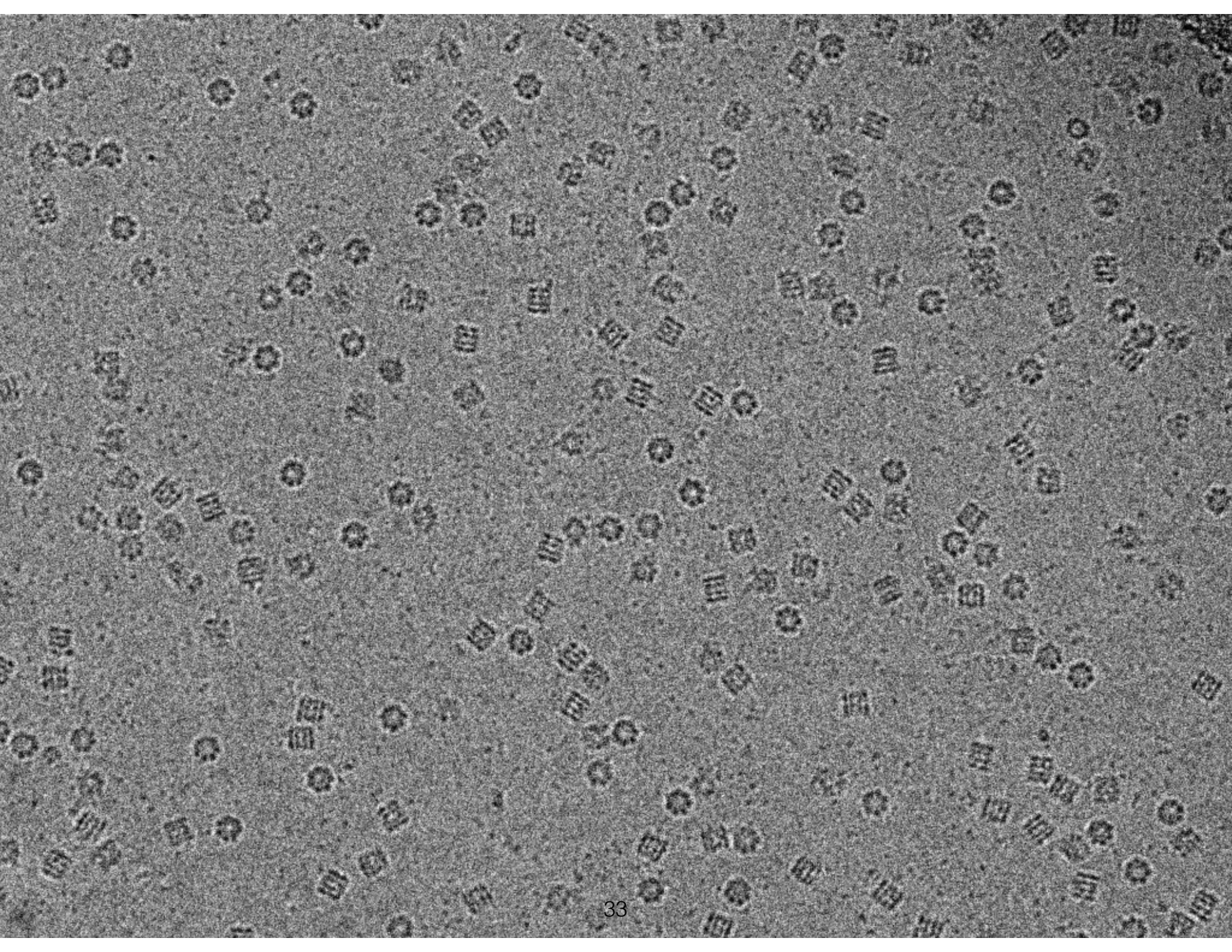
# This method is now 20% of all EM structures.



Publically available structures due to EM methods. gray bars: total enveloped deposited, black line : those structures due to single-particle cryoEM methods at a resolution sufficient for polypeptide chain tracing. Single-particle cryoEM now accounts for 21% of all deposits from 1% in 2012

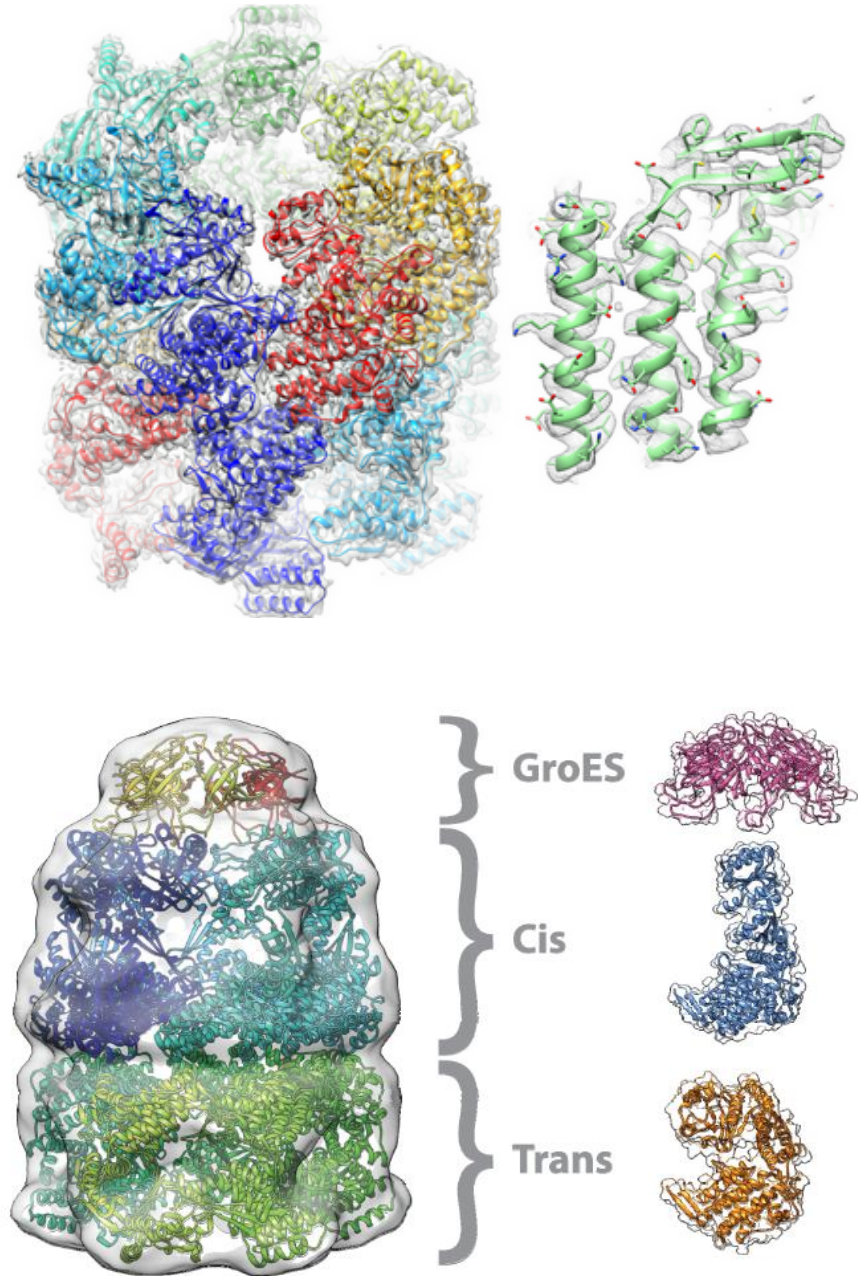






## GROEL

- **GroEL** belongs to the **chaperonin** family of **molecular chaperones**, and is found in a large number of bacteria.<sup>[3]</sup>
- It is required for the proper **folding** of many proteins.
- To function properly, GroEL requires the lid-like cochaperonin protein complex **GroES**.
- In **eukaryotes** the proteins **Hsp60** and **Hsp10** are structurally and functionally nearly identical to GroEL and GroES, respectively.

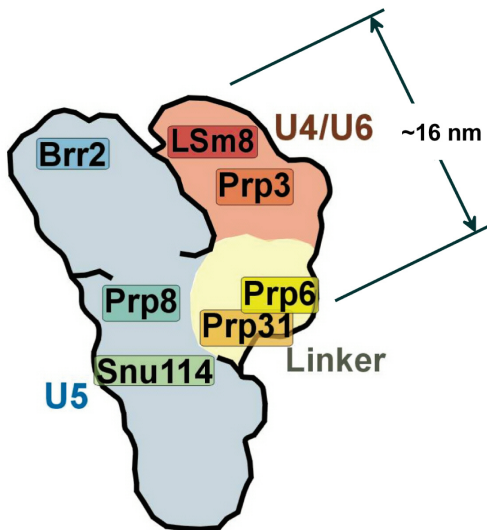


# The EM Revolution

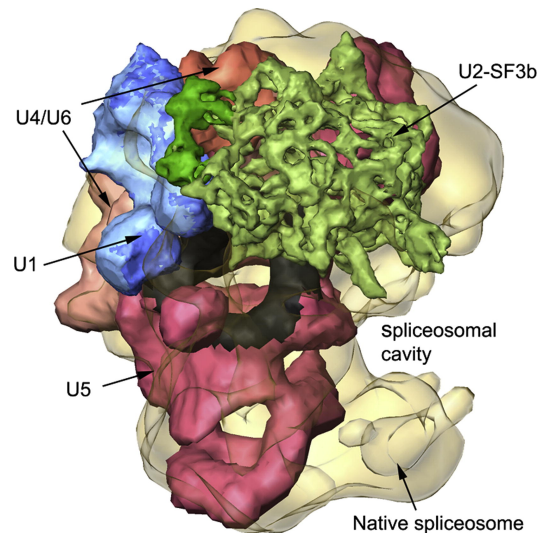
In the past three years, it has become feasible to obtain atomic resolution with cryo-EM for the first time. This is sparking a revolution in structural biology.

Ex: Spliceosome

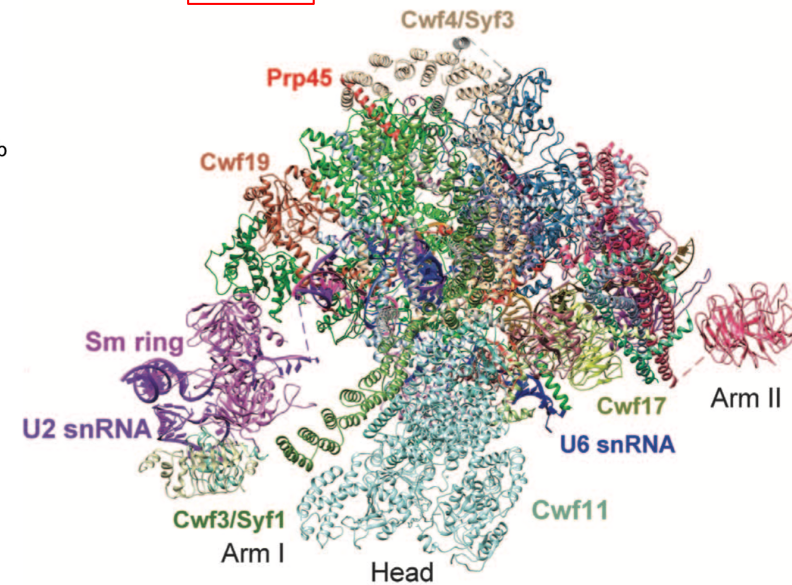
2008



2012



2015

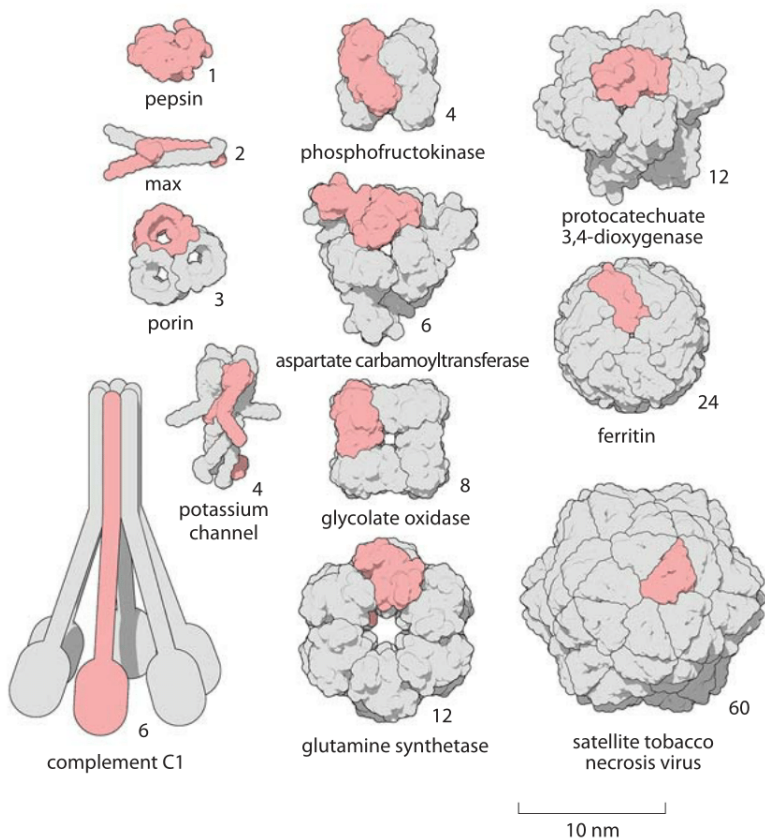


Hacker *et-al*, *Nature Structural & Molecular Biology*  
15, 1206 - 1212 (2008)

Frankenstein *et-al* *Structure*  
(2012), 6, 1097-1106

Yan *et-al* *Science* (2015), 349,  
1182-1191

# CryoEM and X-ray Crystallography are Complementary.



- X-ray crystallography will continue to be the method of choice for very high resolution measurements of small, crystallizable proteins.
- X-ray sources and techniques have not yet reached their limit and will continue to improve (NSLS-II)
- x-ray structures will be essential to provide the components within higher order complexes.
- The co-location of cryoEM with NSLS-II and CFN will provide the opportunity to measure the structure of systems and the components of the systems at one integrated facility with simple, transparent, access procedures.

# NY gives \$15M for cryo-electron microscope at Brookhaven lab

Updated May 18, 2017 8:12 PM

By Delthia Ricks [delthia.ricks@newsday.com](mailto:delthia.ricks@newsday.com)

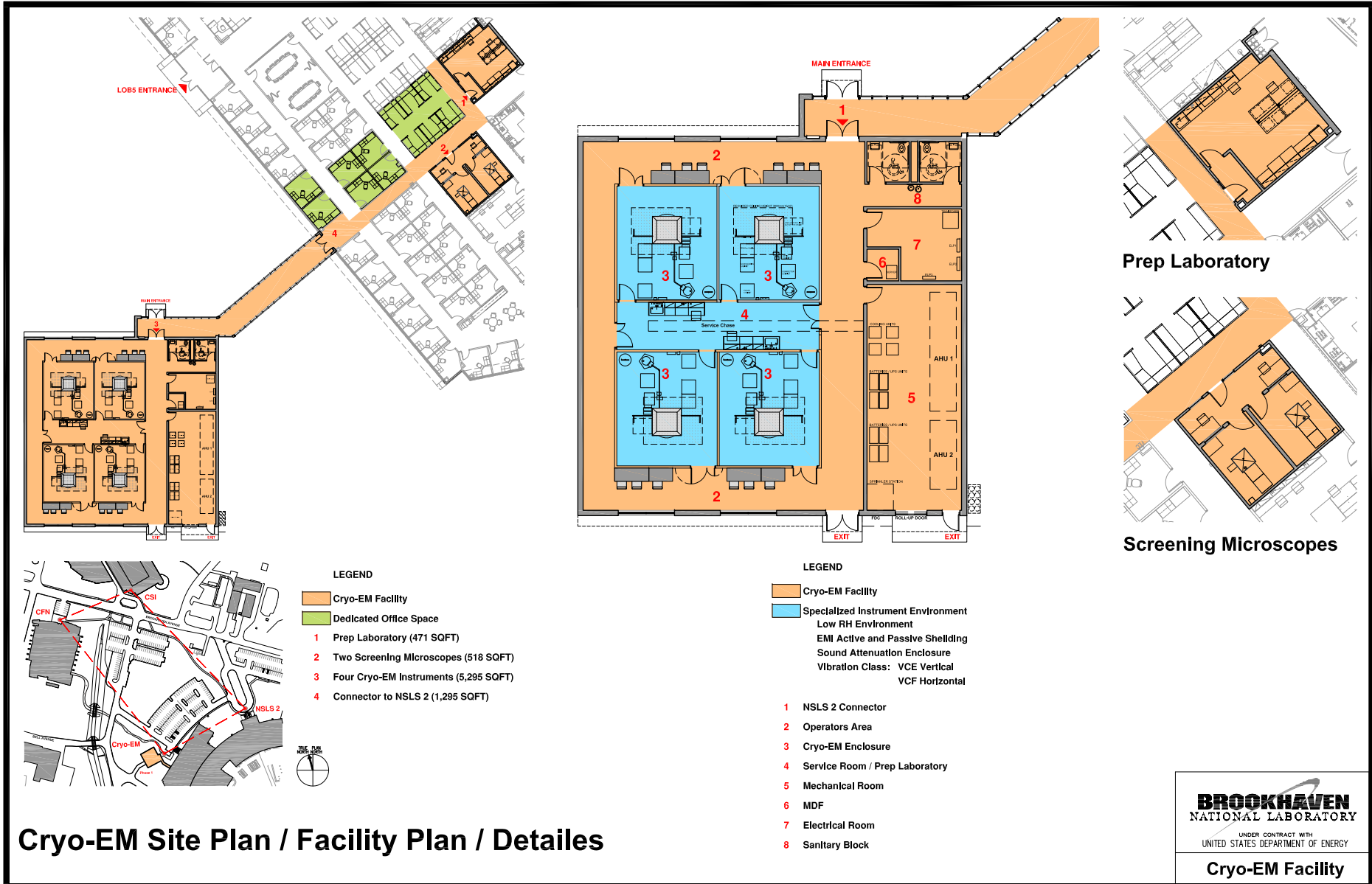
New York is with us  
and we are building our  
plans and team.



Brookhaven National Laboratory on Thursday, May 18, 2017, announced that the state will allocate \$15 million for a state-of-the-art cryo-electron microscope at the lab. The advanced imaging technology is expected to increase scientists' understanding of disease, with the microscope helping to discover new treatments, and boost Long Island's biotechnology and pharmaceutical industries, officials said. (Credit: Newsday)

New York State is allocating \$15 million for a cryo-electron microscope at Brookhaven National Laboratory as part of a collaborative effort involving three Long Island scientific powerhouses, officials at the lab announced Thursday.

The state-of-the-art imaging technology is at the heart of a new center, the Long Island Facility for Electron Microscopy, which is to be established on the Brookhaven...



**Cryo-EM Site Plan / Facility Plan / Details**

- LEGEND**
- Cryo-EM Facility
  - Dedicated Office Space
  - 1 Prep Laboratory (471 SQFT)
  - 2 Two Screening Microscopes (518 SQFT)
  - 3 Four Cryo-EM Instruments (5,295 SQFT)
  - 4 Connector to NSLS 2 (1,295 SQFT)

- LEGEND**
- Cryo-EM Facility
  - Specialized Instrument Environment
    - Low RH Environment
    - EMI Active and Passive Shielding
    - Sound Attenuation Enclosure
    - Vibration Class: VCE Vertical
    - VCF Horizontal
  - 1 NSLS 2 Connector
  - 2 Operators Area
  - 3 Cryo-EM Enclosure
  - 4 Service Room / Prep Laboratory
  - 5 Mechanical Room
  - 6 MDF
  - 7 Electrical Room
  - 8 Sanitary Block

**Prep Laboratory**

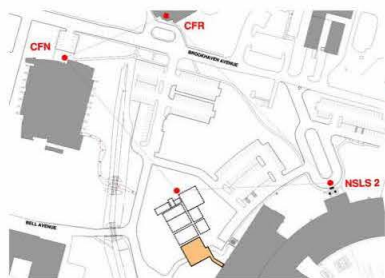
**Screening Microscopes**

**BROOKHAVEN**  
NATIONAL LABORATORY

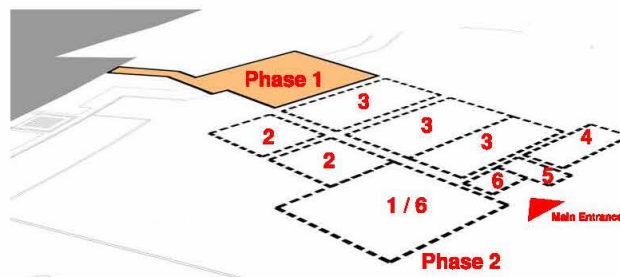
UNDER CONTRACT WITH  
UNITED STATES DEPARTMENT OF ENERGY

**Cryo-EM Facility**

# We are planning and building for the future.



- LEGEND**
- Cryo-EM Facility
  - 1 Auditorium
  - 2 Laboratories
  - 3 Twelve Cryo-EM Instruments
  - 4 Offices
  - 5 Sanitary Block / Stair Tower
  - 6 Lobby

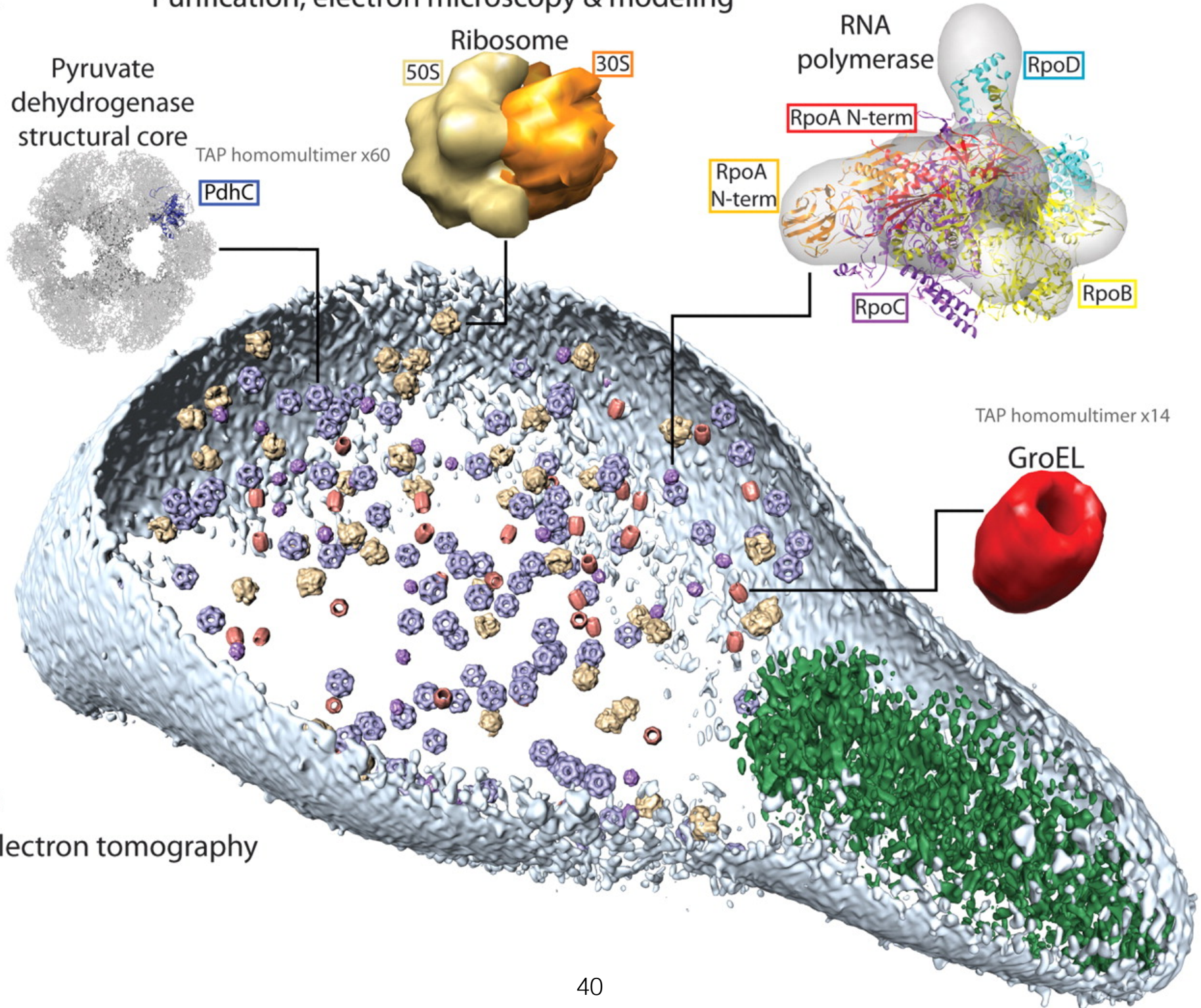


**BROOKHAVEN**  
NATIONAL LABORATORY

UNDER CONTRACT WITH  
UNITED STATES DEPARTMENT OF ENERGY

PROJECT  
CRYO-EM FACILITY

# A Purification, electron microscopy & modeling



# B Electron tomography



FINIS.

Extra slides.

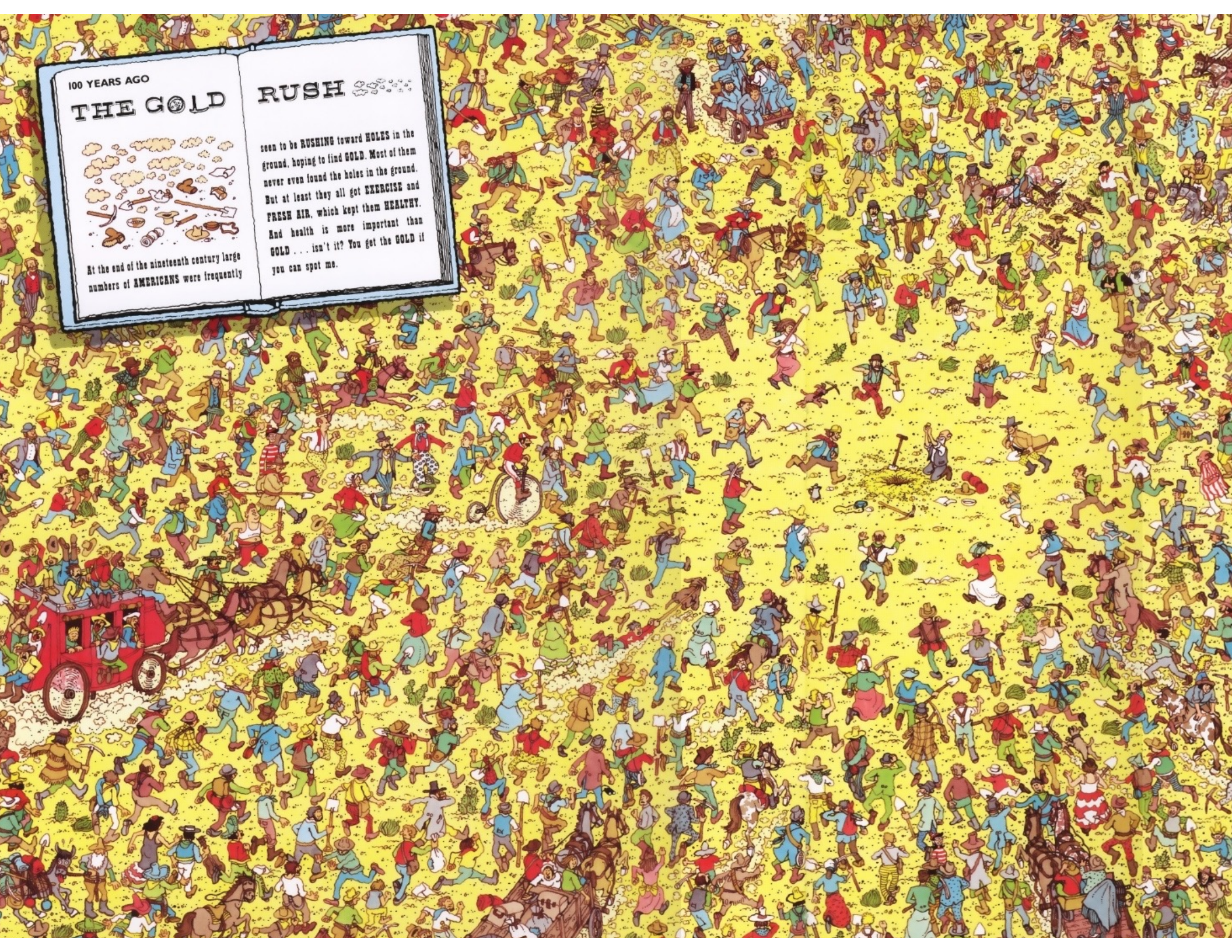
100 YEARS AGO

# THE GOLD RUSH



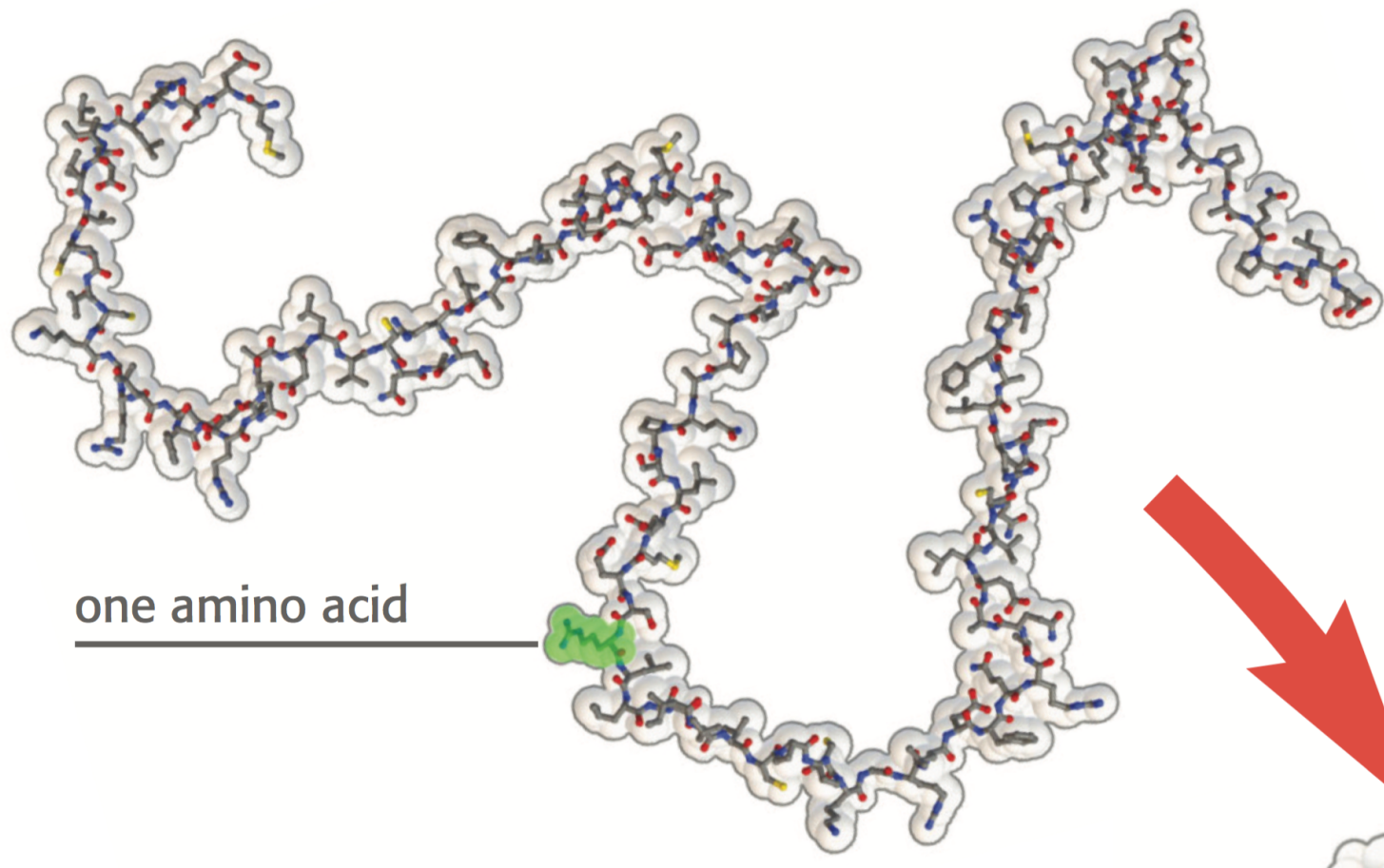
At the end of the nineteenth century large numbers of AMERICANS were frequently

seen to be **RUSHING** toward **HOLES** in the ground, hoping to find **GOLD**. Most of them never even found the holes in the ground. But at least they all got **EXERCISE** and **FRESH AIR**, which kept them **HEALTHY**. And health is more important than **GOLD** . . . isn't it? You get the **GOLD** if you can spot me.

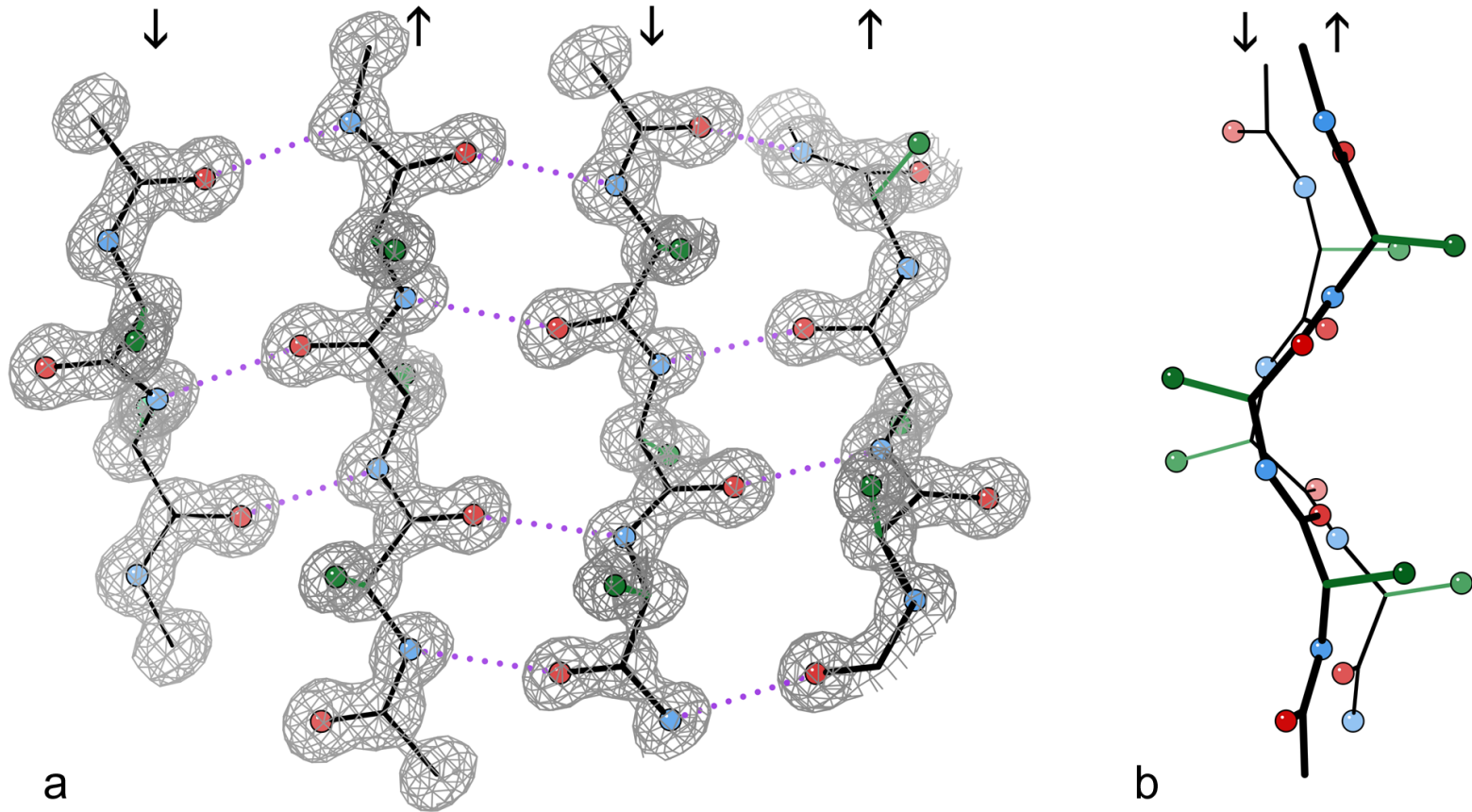


# 1: primary structure.

the linear sequence of amino acids as encoded by the DNA

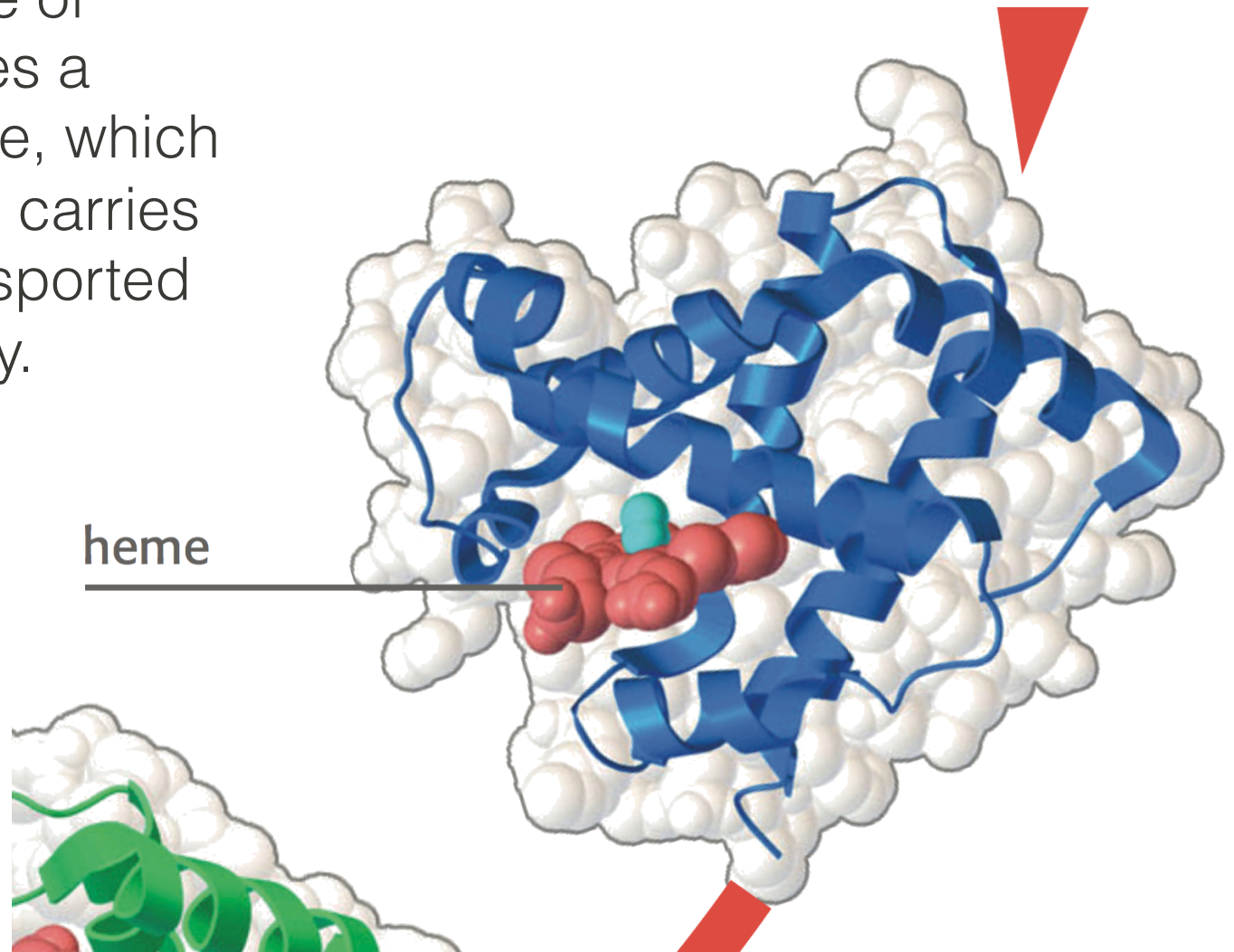


# 2: secondary structure.



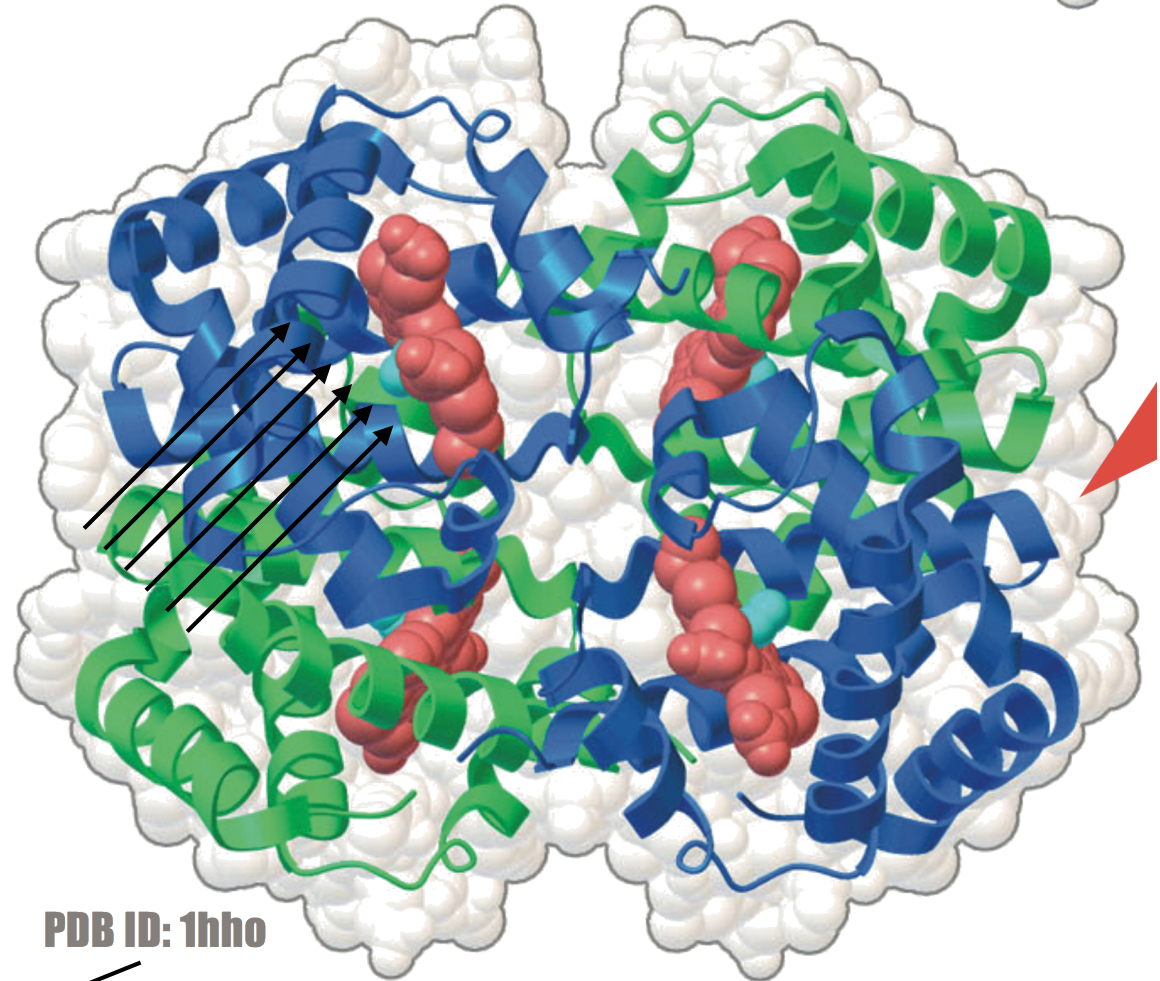
# 3: tertiary structure.

The folded structure of hemoglobin includes a pocket to hold heme, which is the molecule that carries oxygen as it is transported throughout the body.



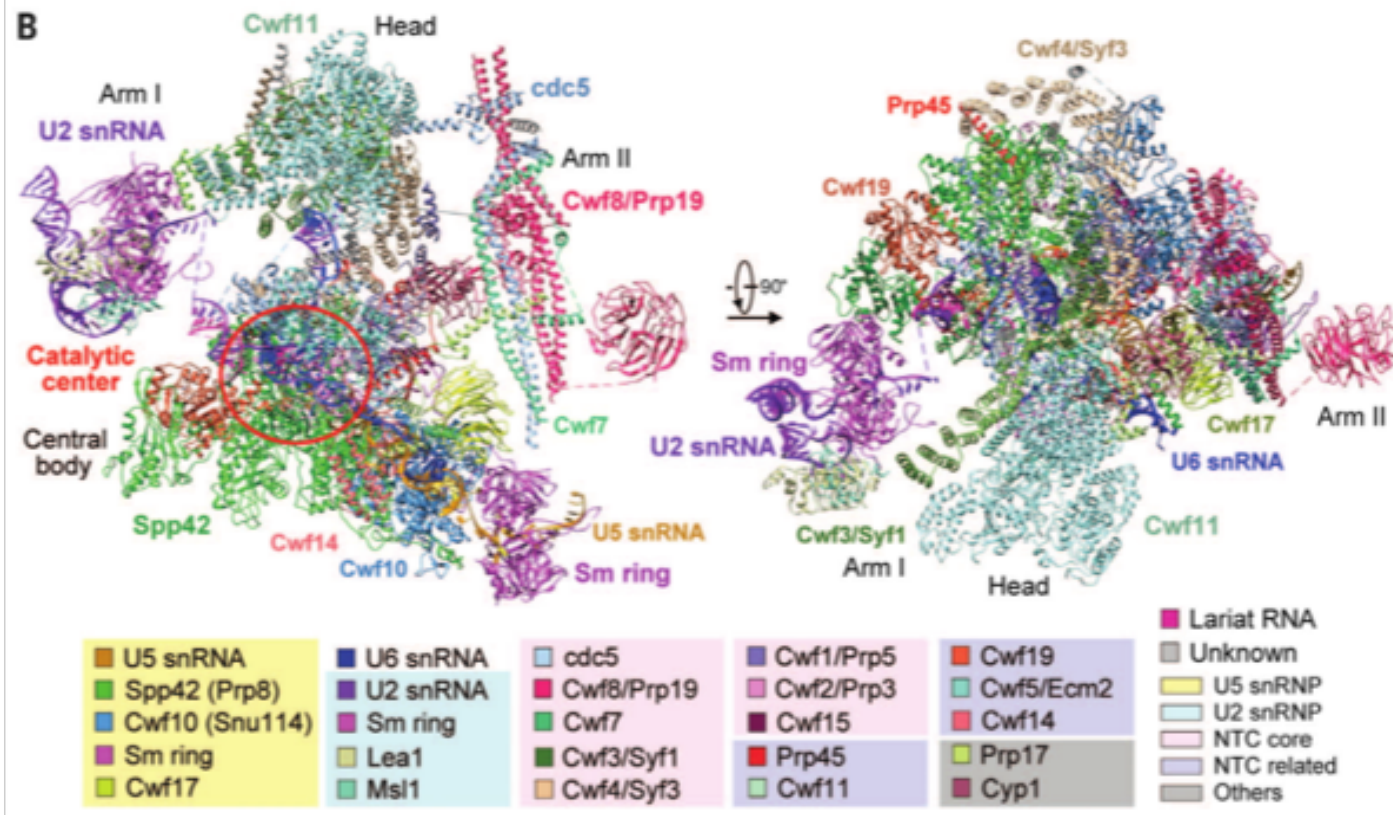
# 4: quaternary structure.

one functional molecule may have several subunits. The four subunits of hemoglobin cooperate so that the complex picks up and delivers more oxygen than is possible with single subunits.



the data is available

# Structure of a yeast spliceosome at 3.6-angstrom resolution

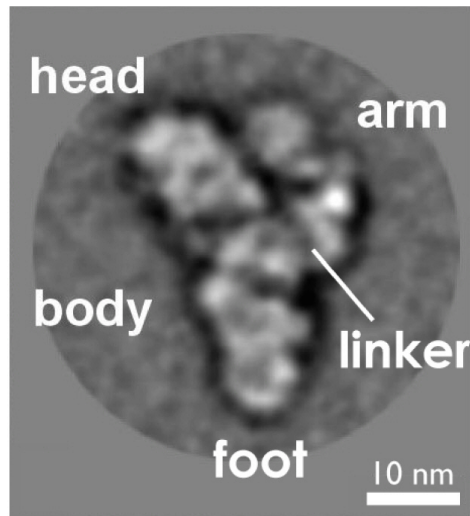


This structure includes 37 proteins, three snRNAs, and one RNA lariat, with a **combined molecular weight of ~1.3 MD**. Among the modeled **10,574 amino acids**, 9312 have been assigned side chains. All 332 RNA nucleotides were tentatively assigned.

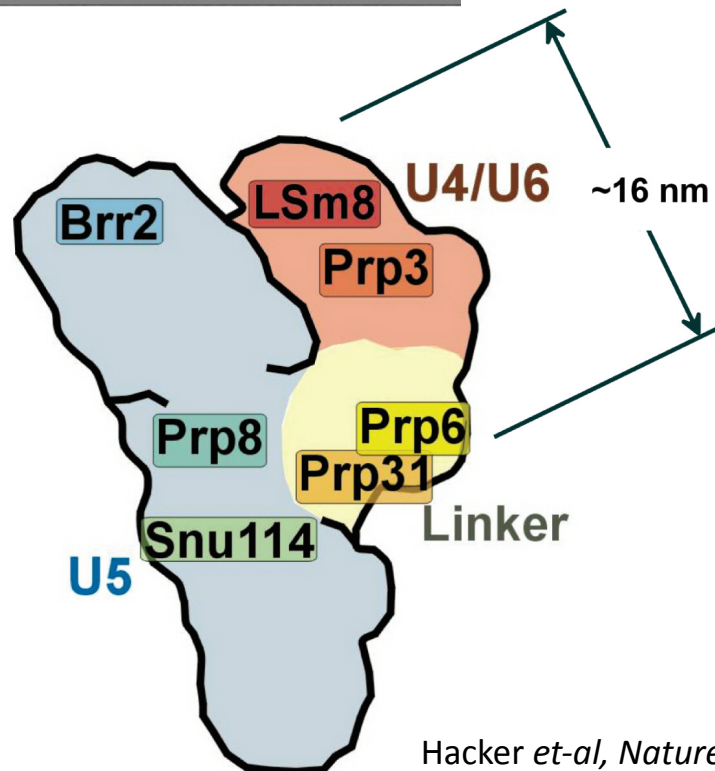
A representative illustration of the yeast spliceosome from two perpendicular views. The protein and RNA components are color-coded.



# Cryo-EM structure of a yeast spliceosome: 2008

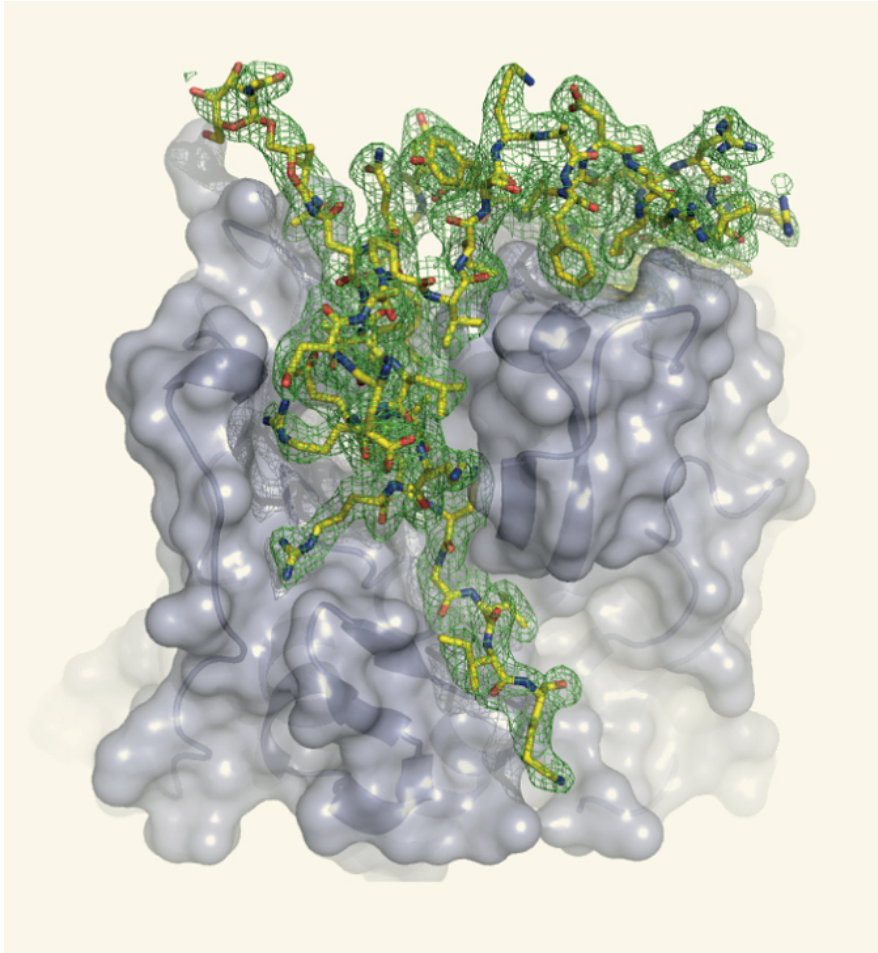


Most eukaryotic genes are expressed as precursor mRNAs that are converted to mRNA by splicing, an essential step of gene expression in which noncoding sequences (introns) are removed and coding sequences (exons) are ligated. The molecular machine responsible for this action is the **spliceosome**, which comprises five small nuclear RNAs and more than 100 associated proteins.



Through x-ray crystallography structural information is available for components of the spliceosome, but the structure of the whole has remained frustratingly elusive.

# The importance of x-ray diffraction



- We are still far from being able to realize the full potential of storage-ring sources.
- In the next decade, scientists will benefit from new innovations.
- For example data-analysis techniques previously developed for FEL experiments enabling structures to be determined from micrometer-scale crystals.