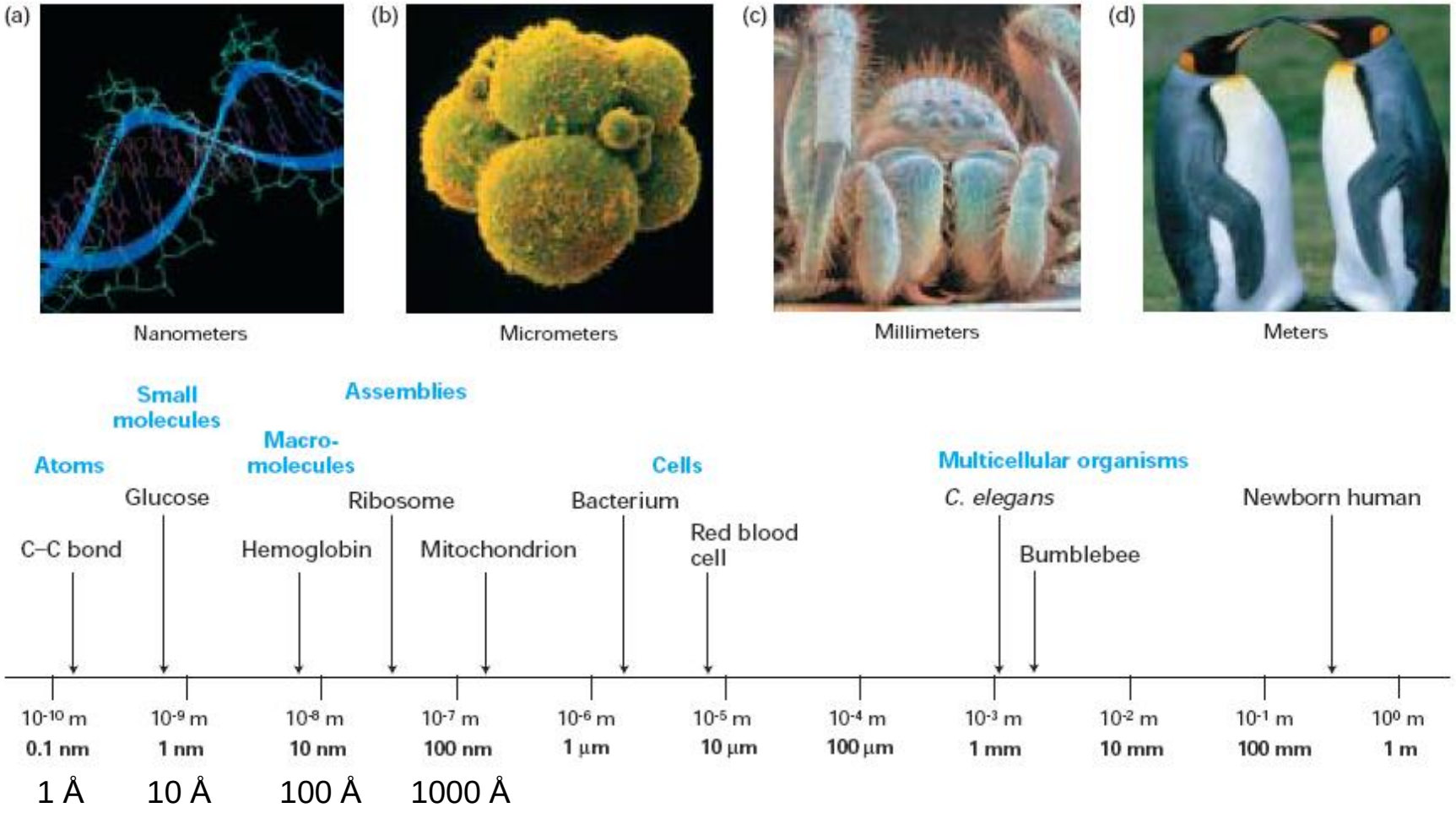
Structural biology

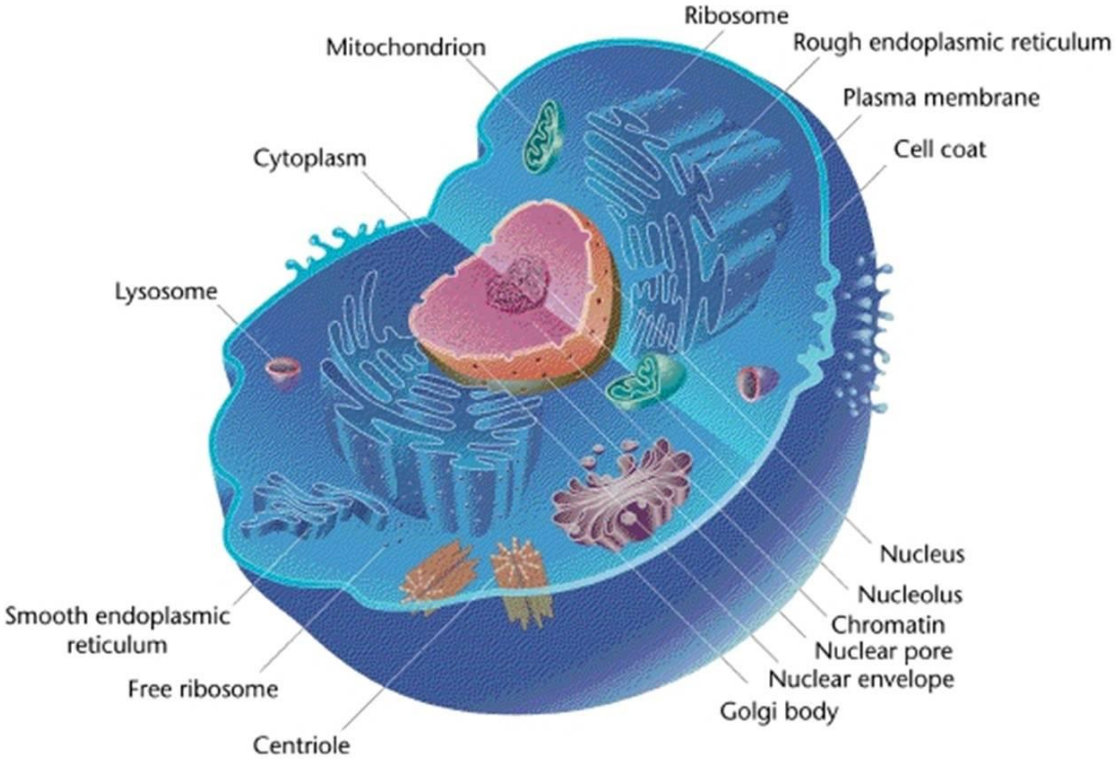
Studies of the shape and architecture of biological macromolecules, including in particular proteins and nucleic acids

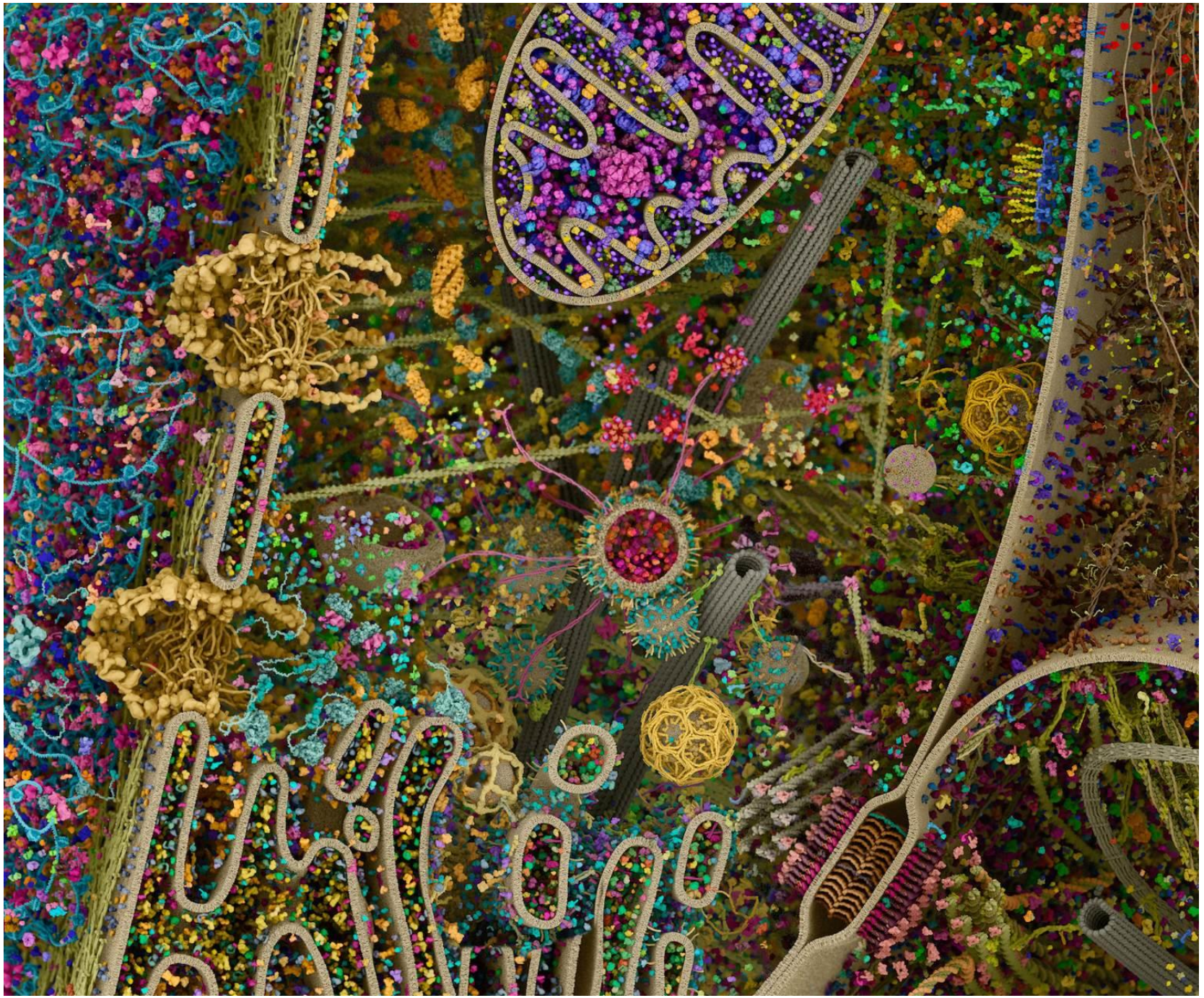
Proper functioning of macromolecules (RNA) requires that they adopt the correct spatial structure

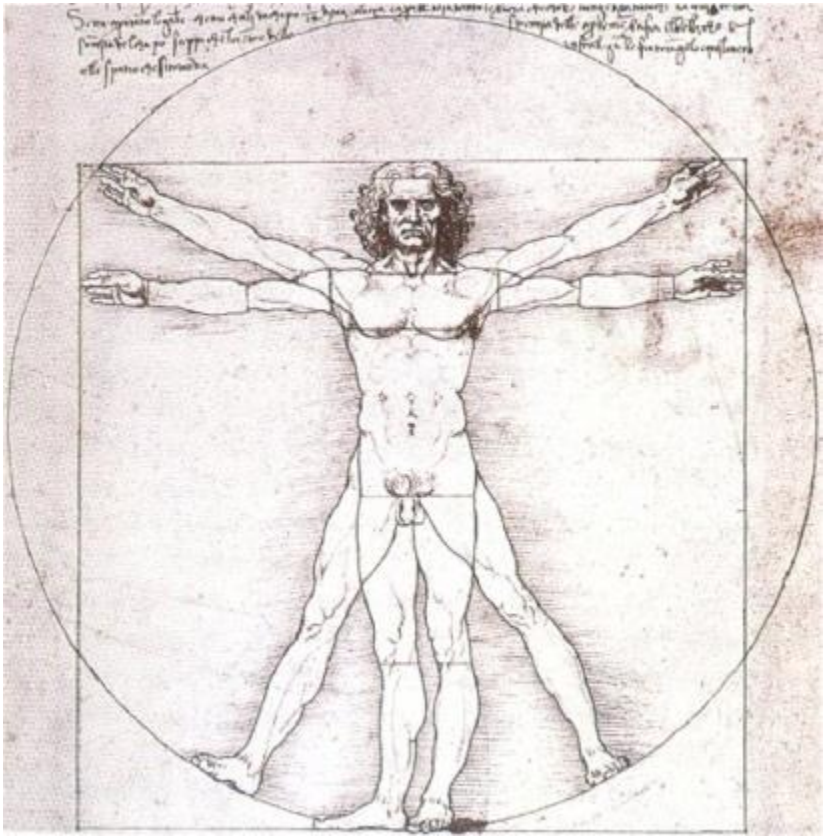
Macromolecules are able to perform their functions due to the precise arrangement of chemical groups in their structure



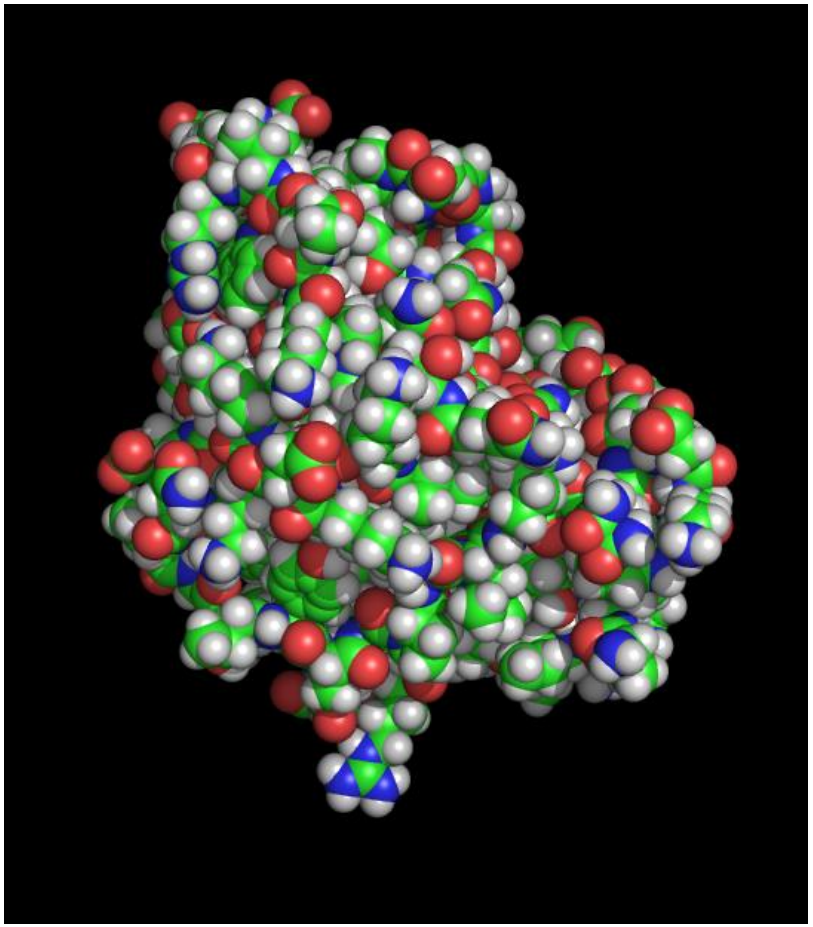
1 Å 10 Å 100 Å 1000 Å


Ribosome
Mitochondrion
Rough endoplasmic reticulum
Plasma membrane
Cytoplasm
Cell coat
Lysosome
Nucleus
Nucleolus
Chromatin
Nuclear pore
Nuclear envelope
Golgi body
Smooth endoplasmic reticulum
Free ribosome
Centriole

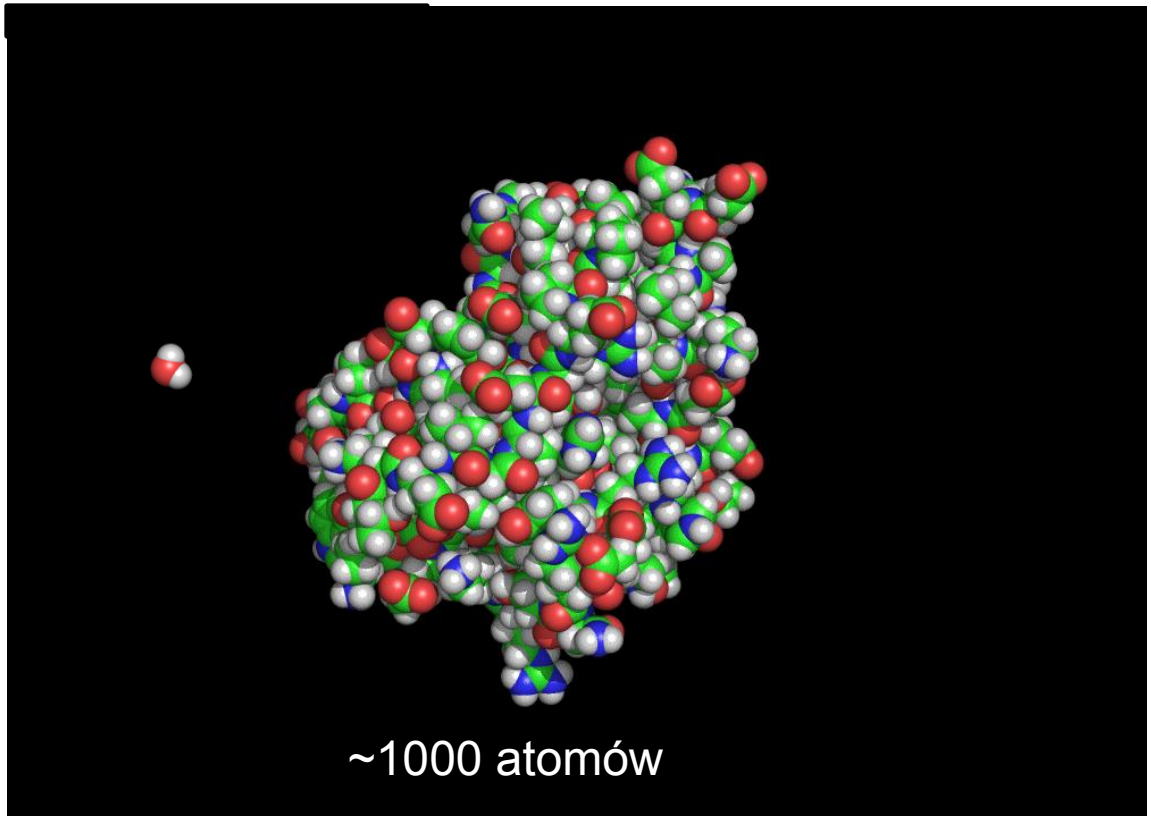


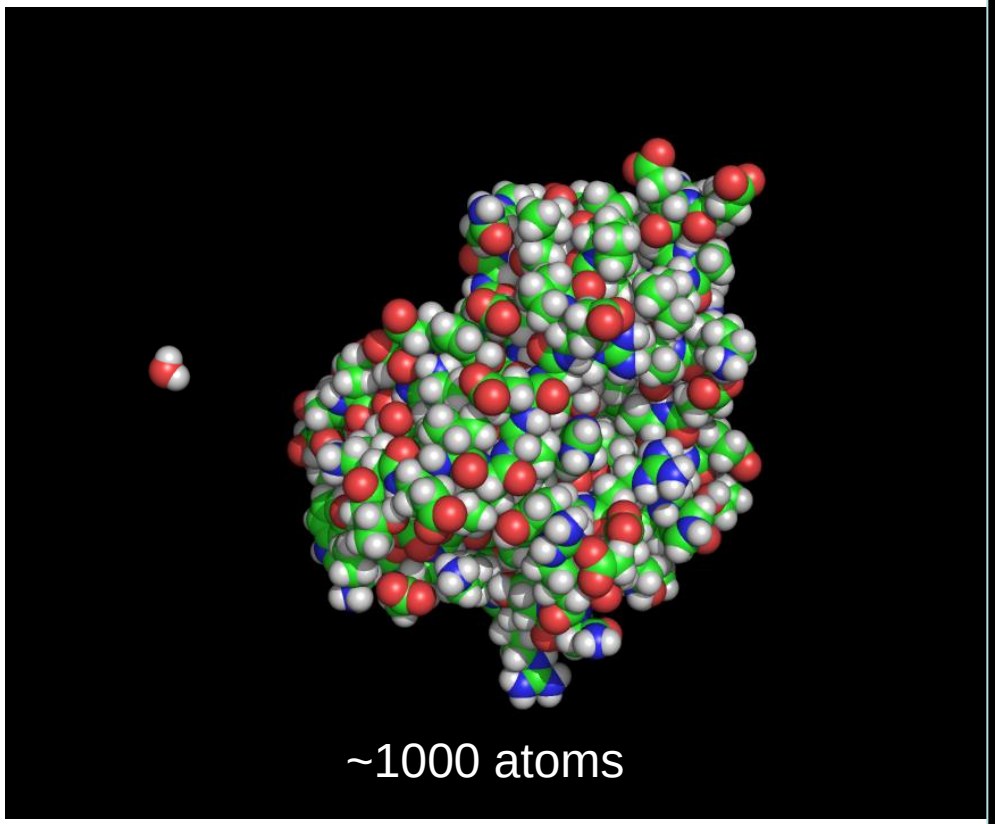


20 000 km

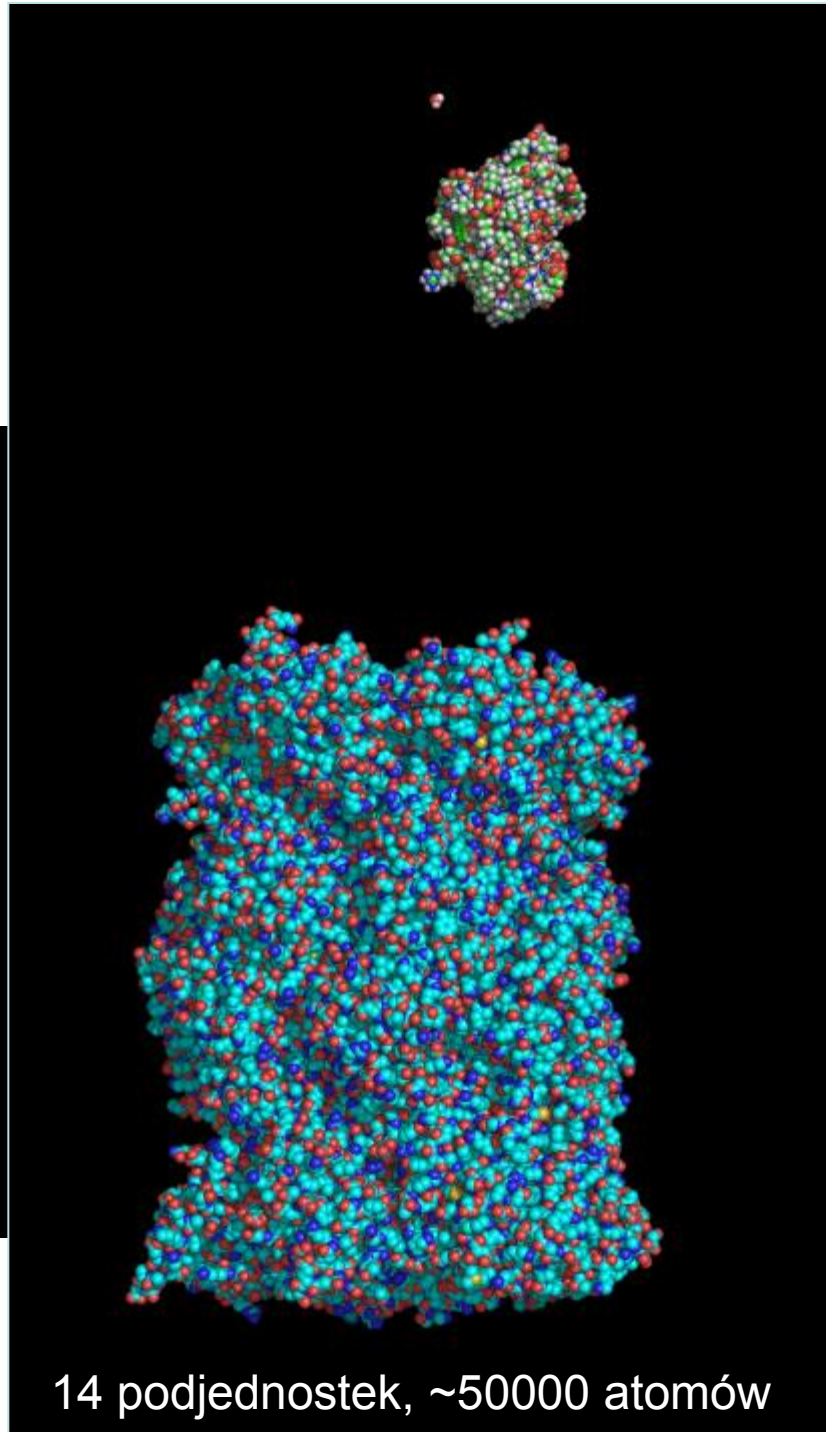


5 cm

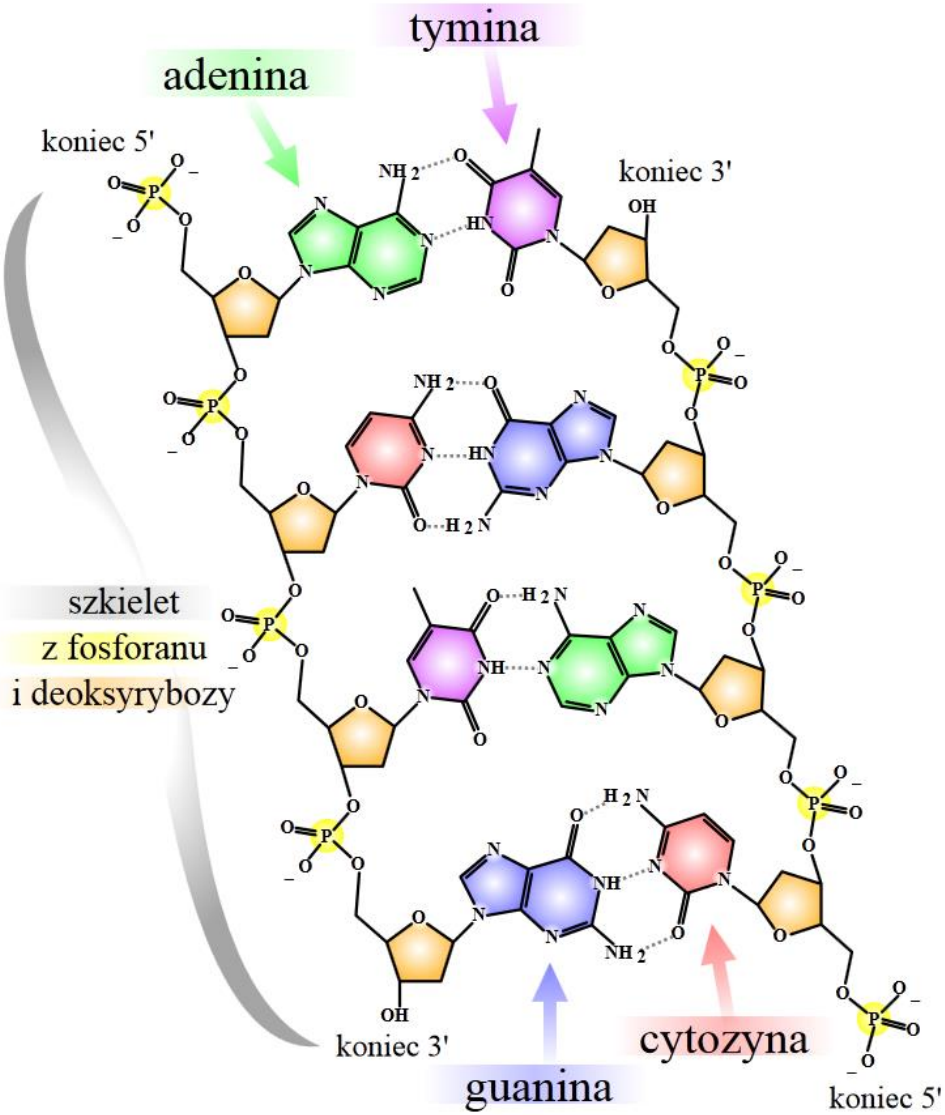

~1000 atomów



~1000 atoms

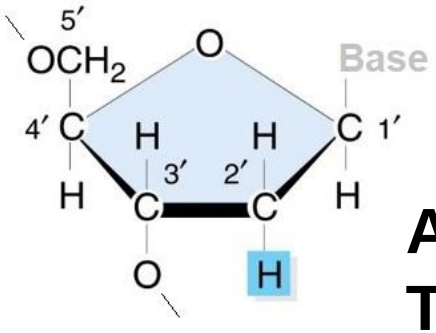


14 podjednostek, ~50000 atomów

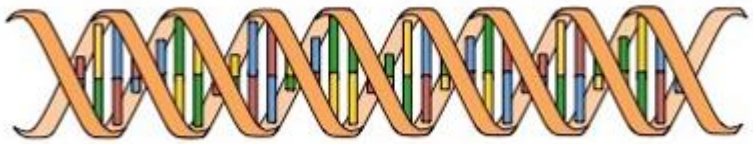

tymina
adenina
koniec 5'
koniec 3'
szkielet
z fosforanu
i deoksyrybozy
koniec 3'
guanina
cytozyna
koniec 5'

chemical structures of DNA and RNA: similar or different?

DNA:

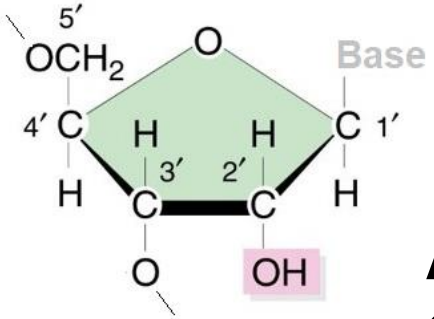


deoxyribose

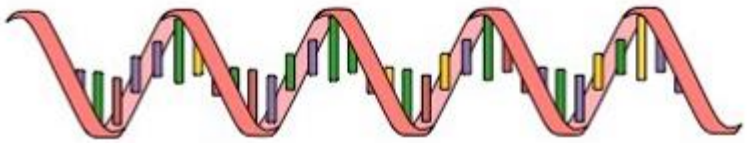


A, G, C, T
Two complementary strands forming an antiparallel double helix

RNA:

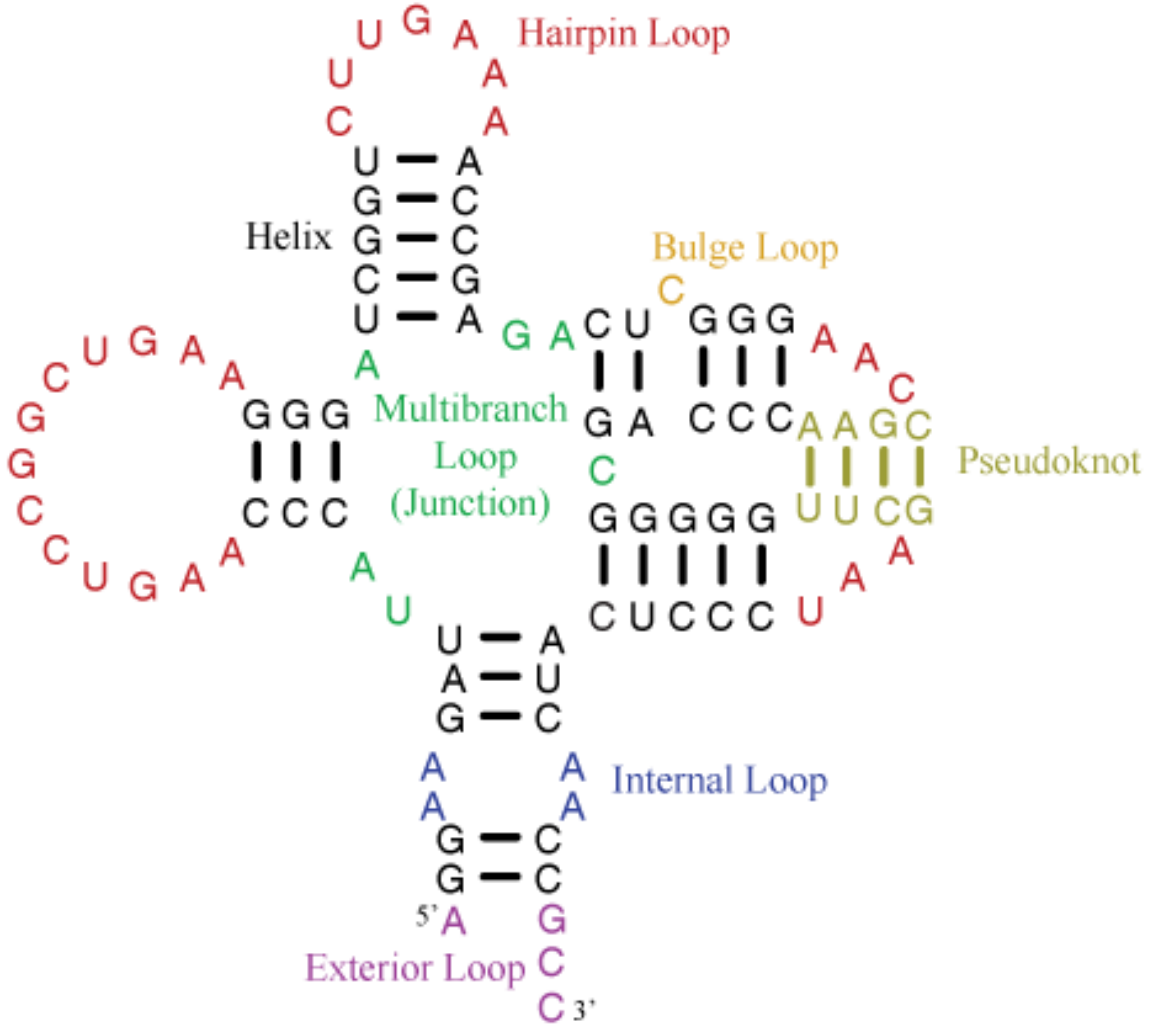


ribose

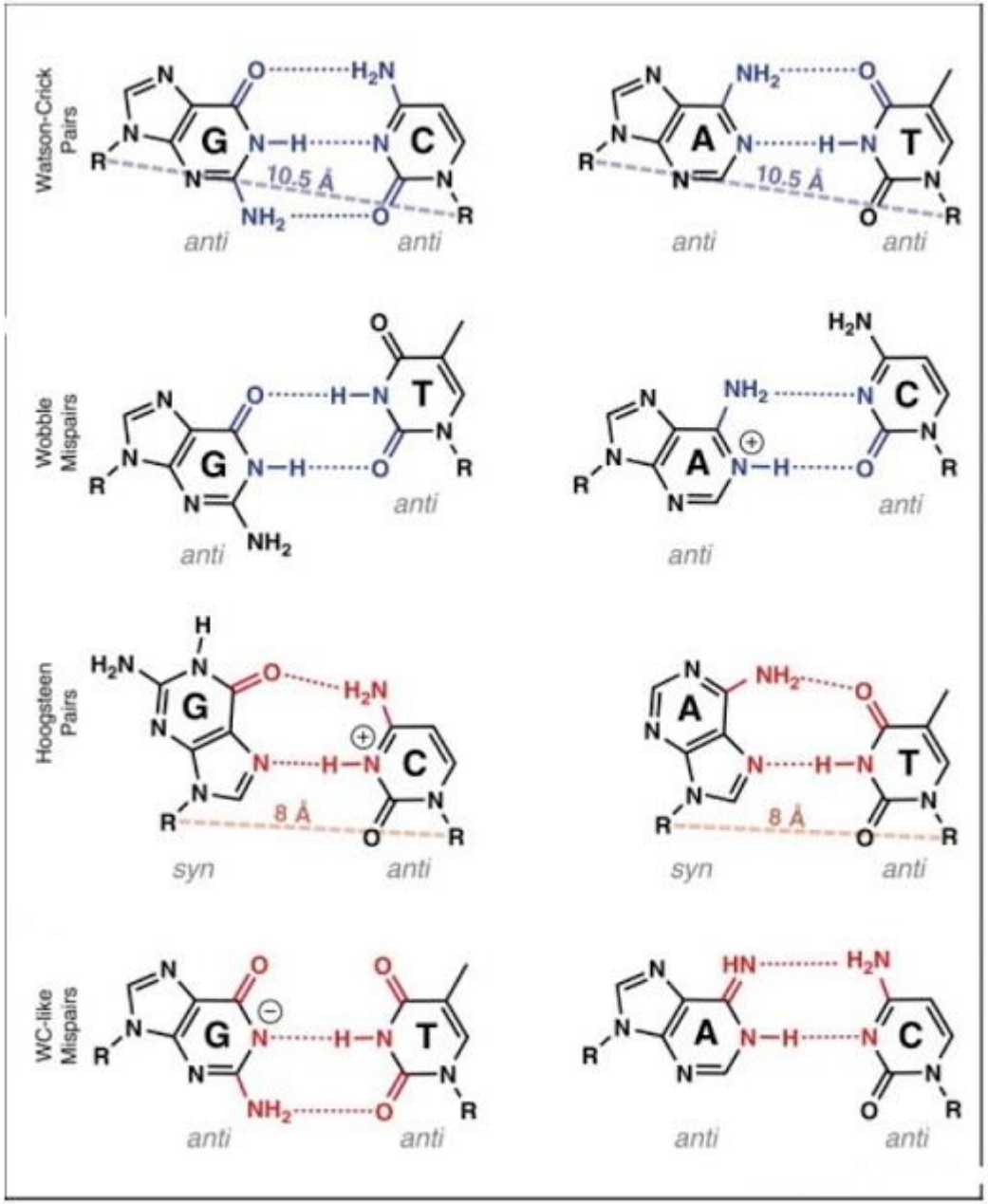


A, G, C, U
One strand
forming… what exactly?

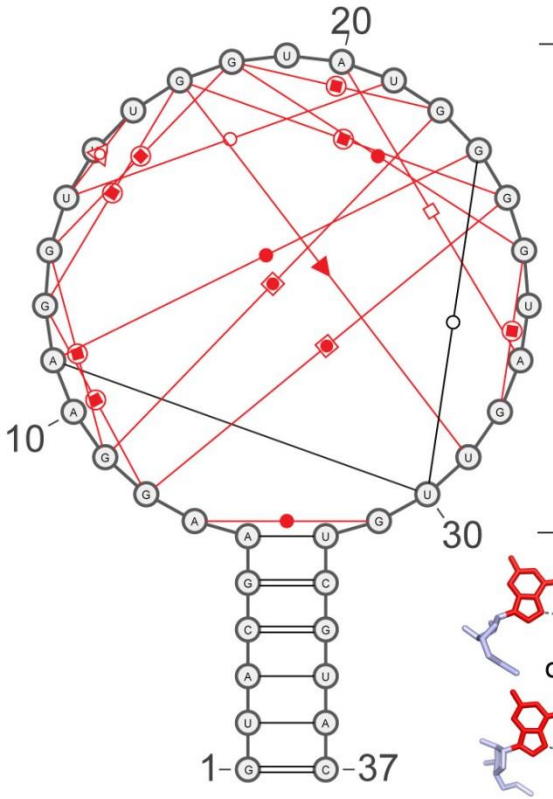
RNA can form canonical A-U & C-G pairs and fold into helical regions divided by loops



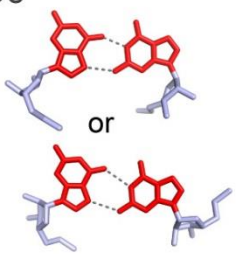
https://x3dna.org/articles/exterior-loop-in-rna-secondary-structure

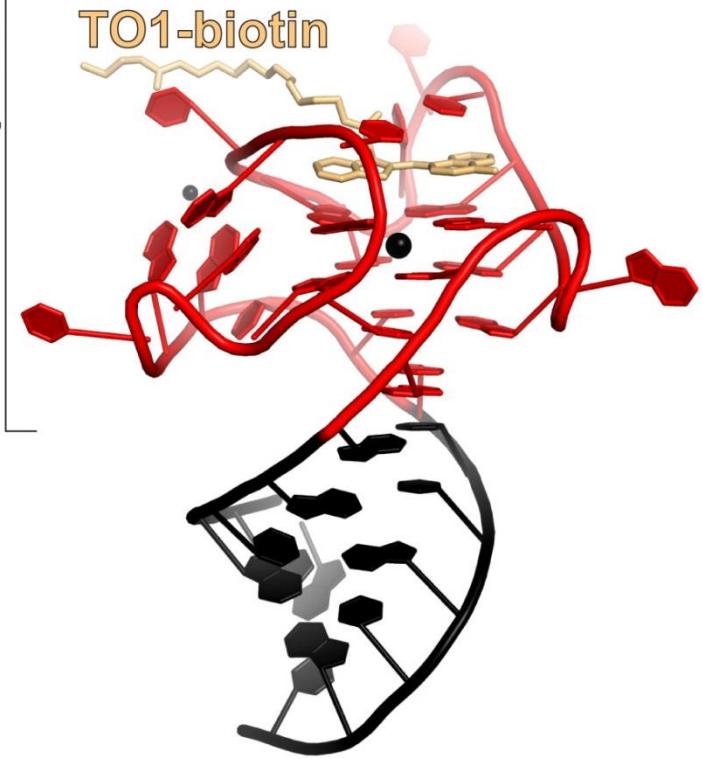


RNA can form non-canonical A-U & C-G pairs



This region *is not* 'unstructured' (as the 'empty loop' within the secondary structure model shown in black might suggest)

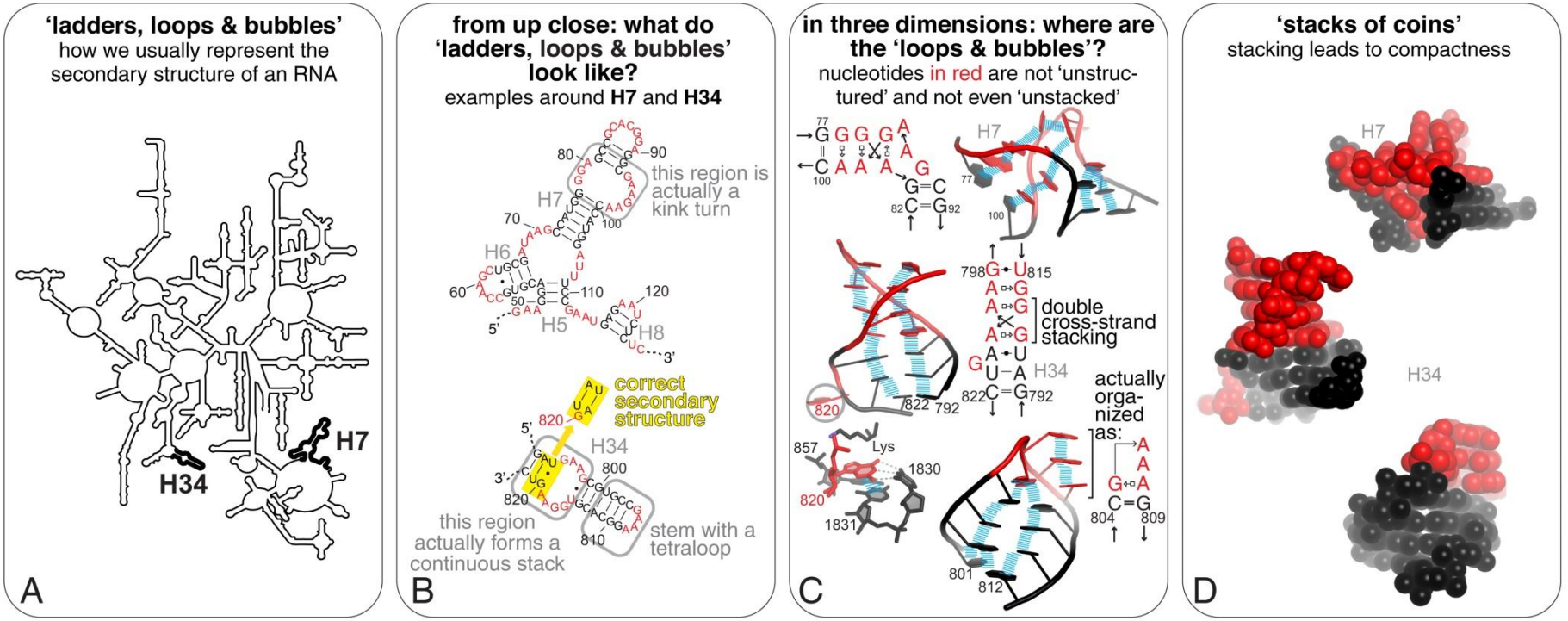




iMango-III fluorescent aptamer

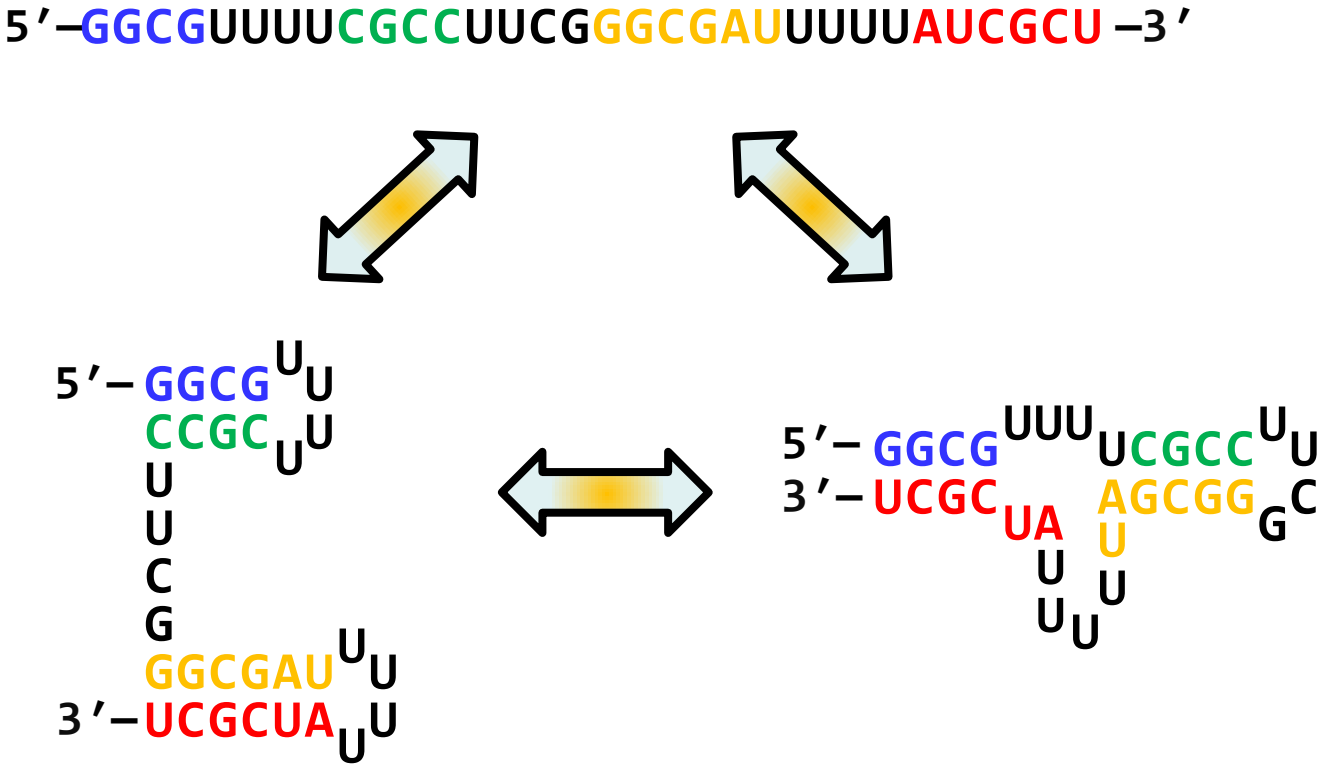
PDB ID 6PQ7

Canonical and non-canonical pairs and other interactions lead to complex 3D motifs

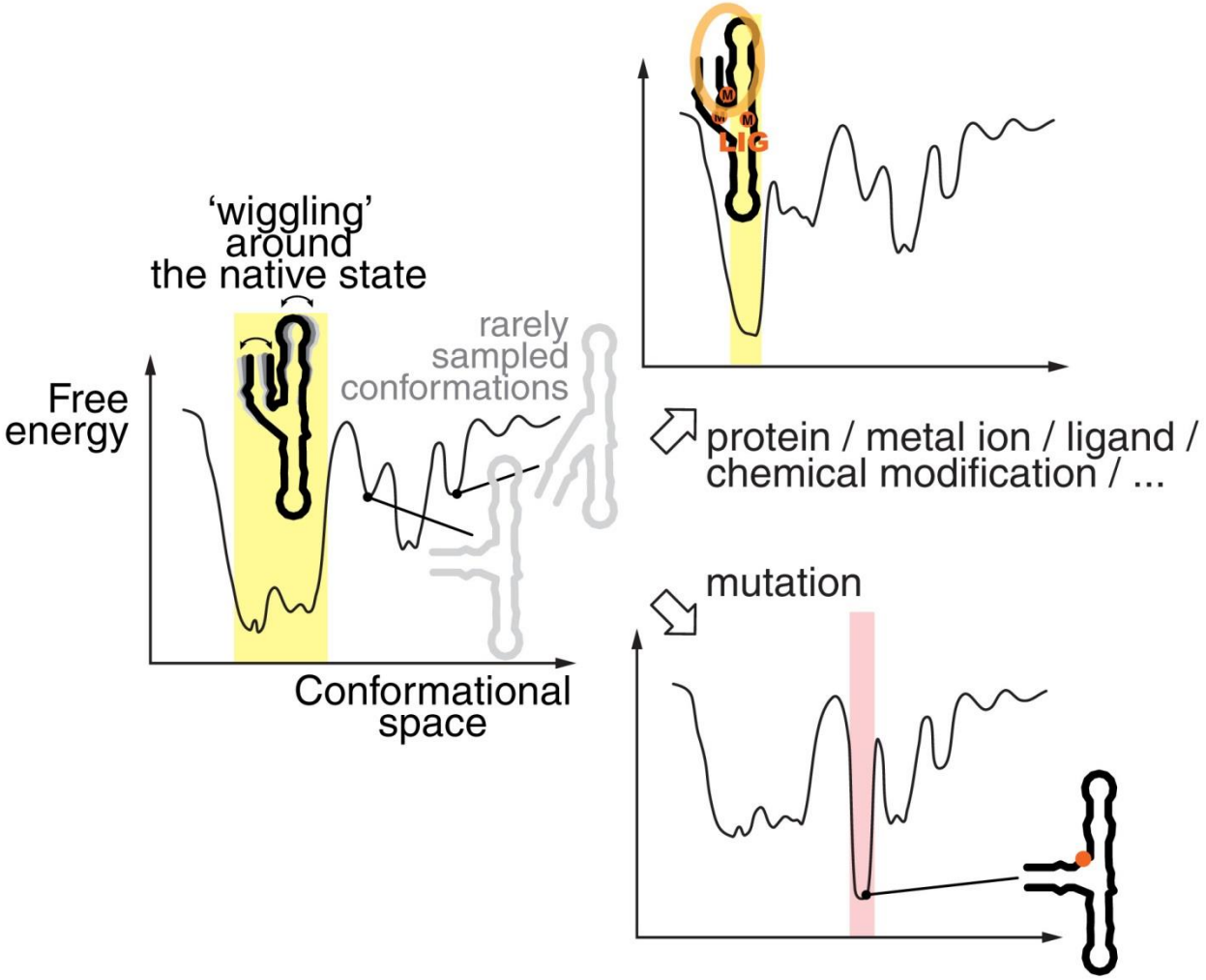


Thoughts on how to think (and talk) about RNA structure
Quentin Vicens, Jeffrey S Kieft, Proc Natl Acad Sci U S A, 2022 Apr 26;119(17):e2112677119.

RNA molecules
can form alternative, different structures

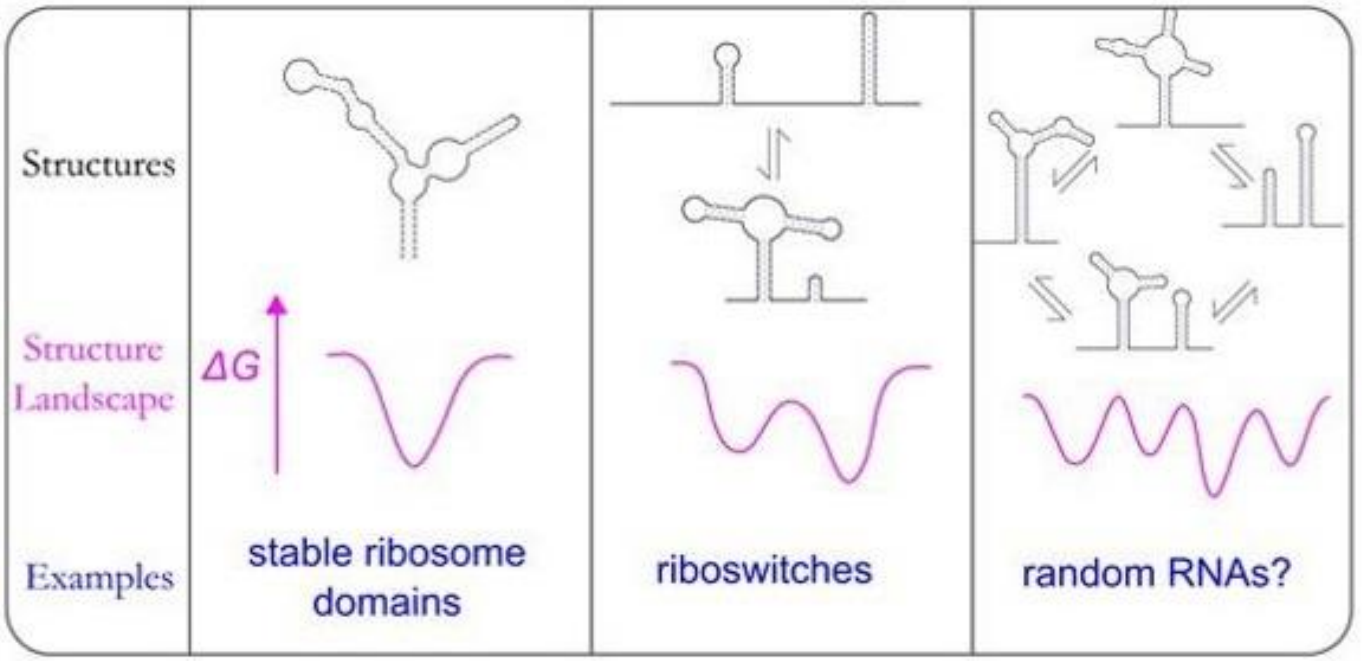


RNA molecules
can form alternative, different structures



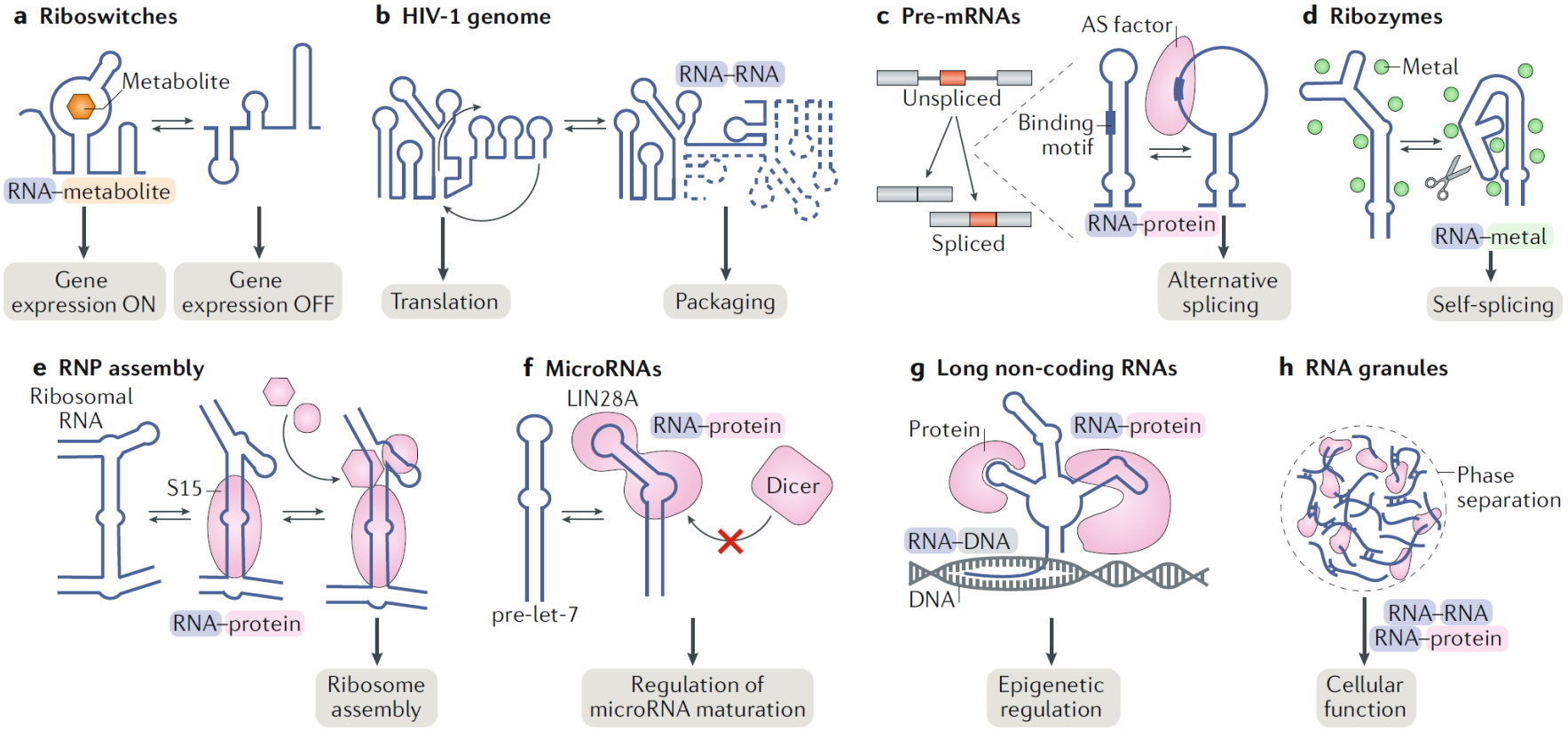
Thoughts on how to think (and talk) about RNA structure
Quentin Vicens, Jeffrey S Kieft, Proc Natl Acad Sci U S A, 2022 Apr 26;119(17):e2112677119.

Functional RNAs
have undergone evolutionary selection to form a restricted number of relatively stable structures



Cordero P, Das R
Rich RNA structure landscapes revealed by mutate-and-map analysis
PLoS Comput Biol 11(11): e1004473.

Dynamic RNA structures play important roles in biological processes



Ganser LR, Kelly ML, Herschlag D, Al-Hashimi HM
The roles of structural dynamics in the cellular functions of RNAs
Nature Rev Mol Cell Biol 2019, 20, 474–489

RNA structure is stabilized mainly by stacking interactions

Structured RNA is not static

RNA is often compact

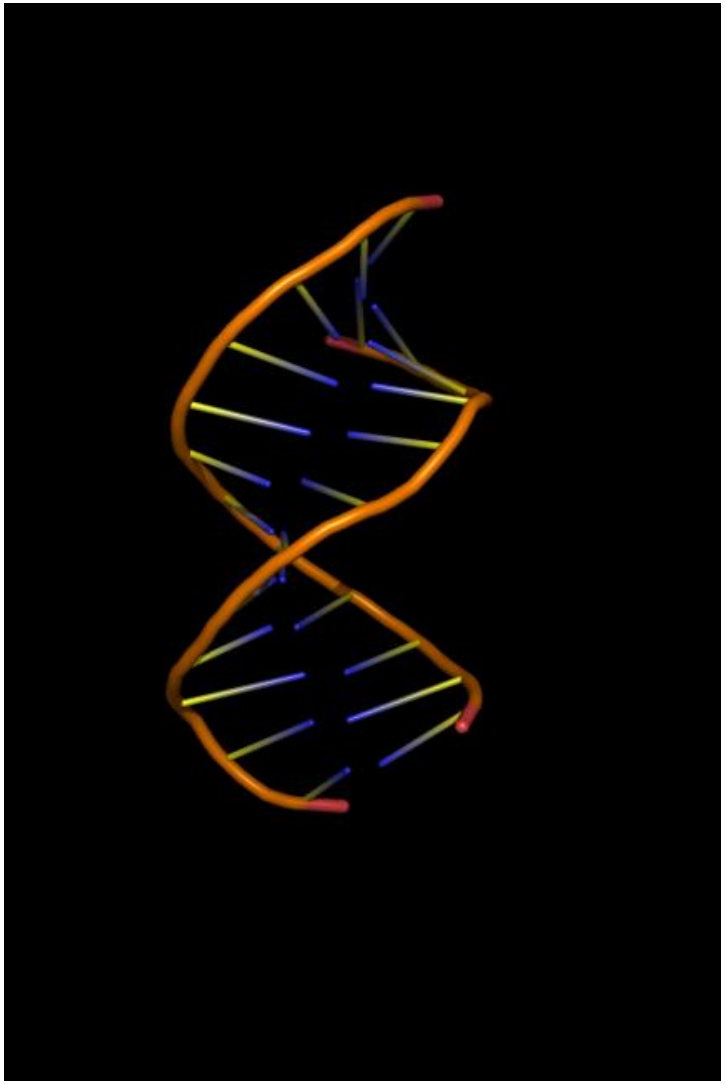
Watson-Crick pairing is important, but not the only one

Non-canonical base pairs play a key role

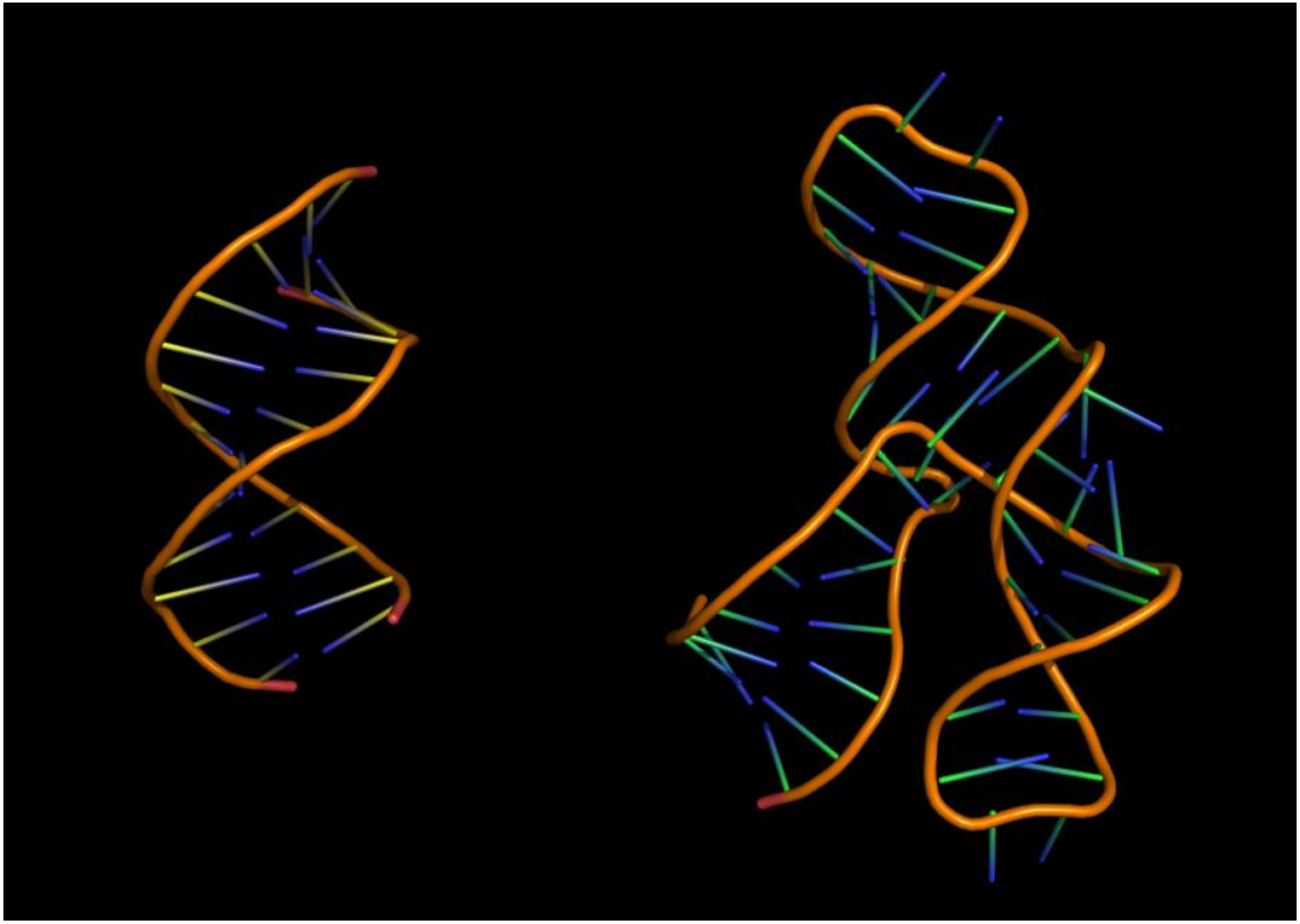
Simple diagrams based on W-C pairs - treat with care

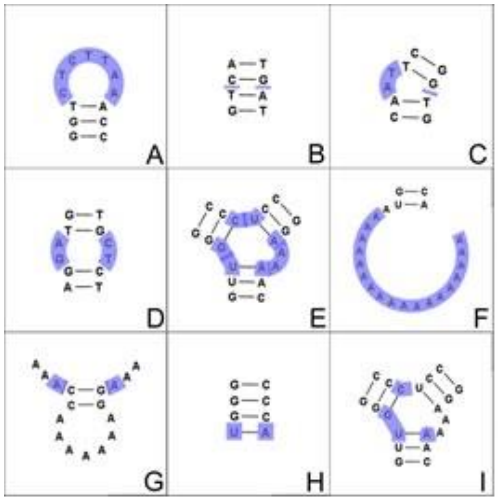
Thoughts on how to think (and talk) about RNA structure
Quentin Vicens, Jeffrey S Kieft, Proc Natl Acad Sci U S A, 2022 Apr 26;119(17):e2112677119.

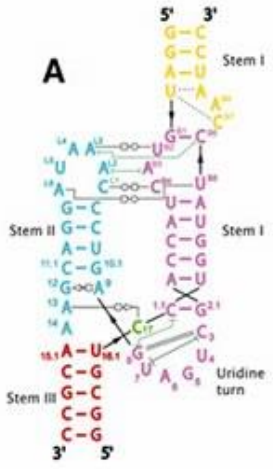
Rybozym

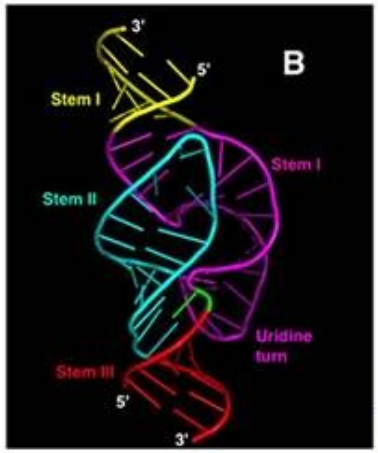


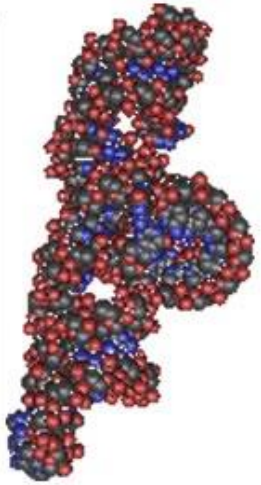
Rybozym



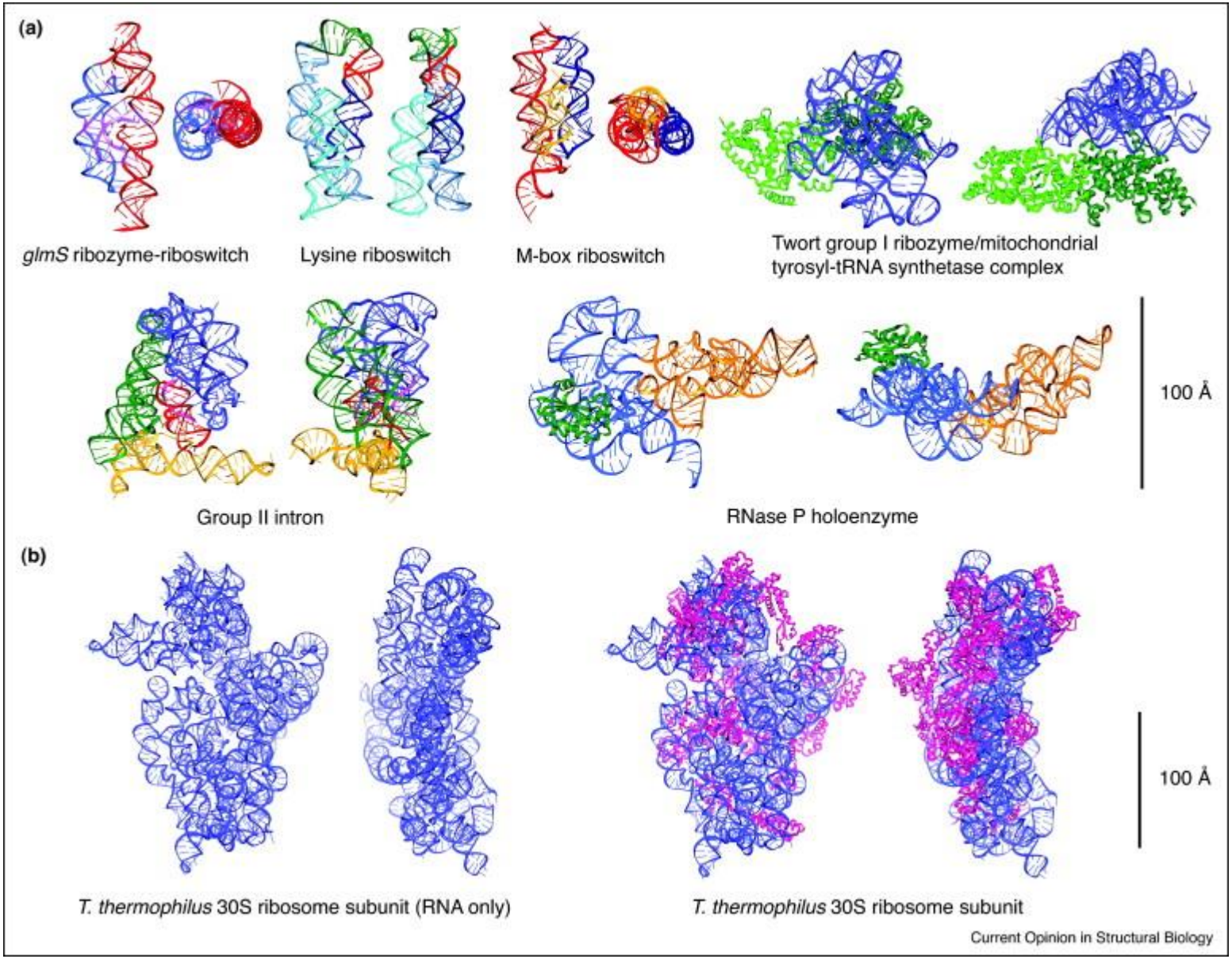

A
B
C
D
E
F
G
H
I


A
5'
3'
Stem I
Stem II
Stem I
Stem III
Uridine turn
3'
5'

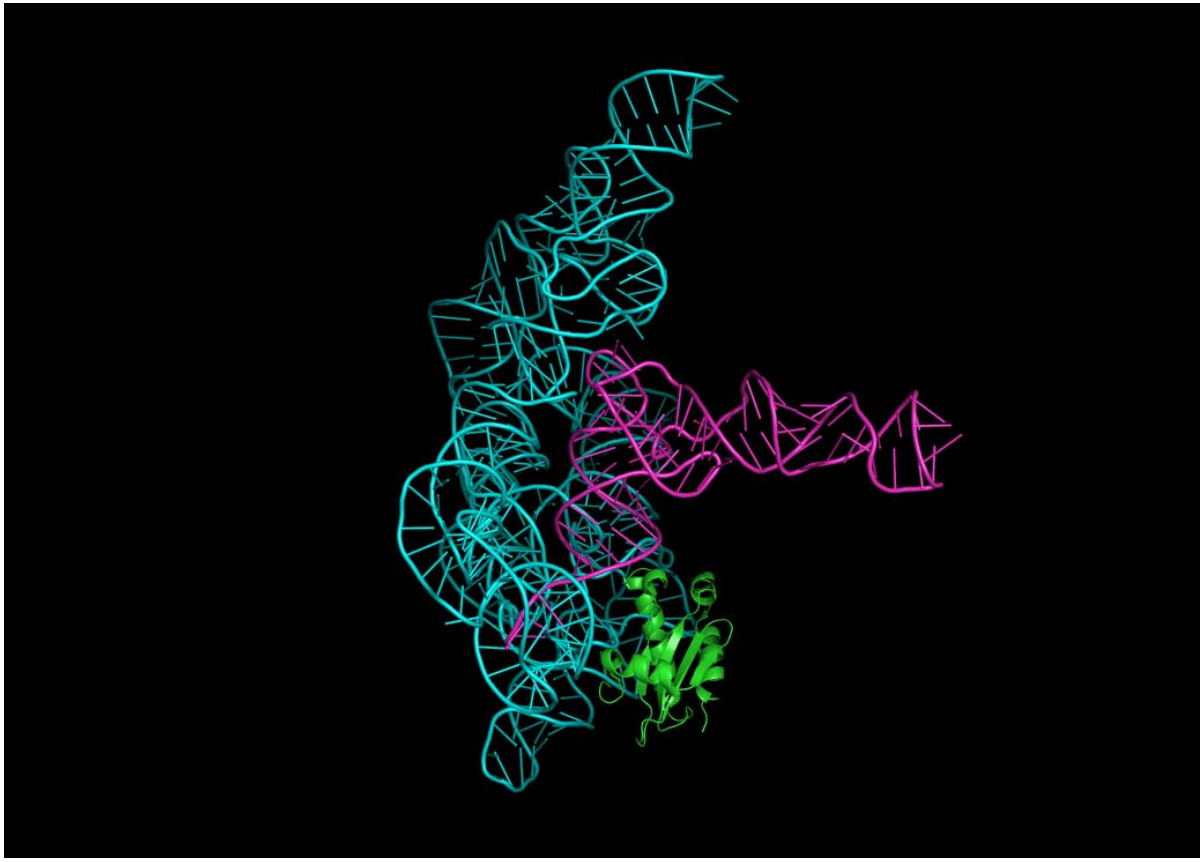

B
3'
5'
Stem I
Stem II
Stem I
Uridine turn
Stem III
5'
3'



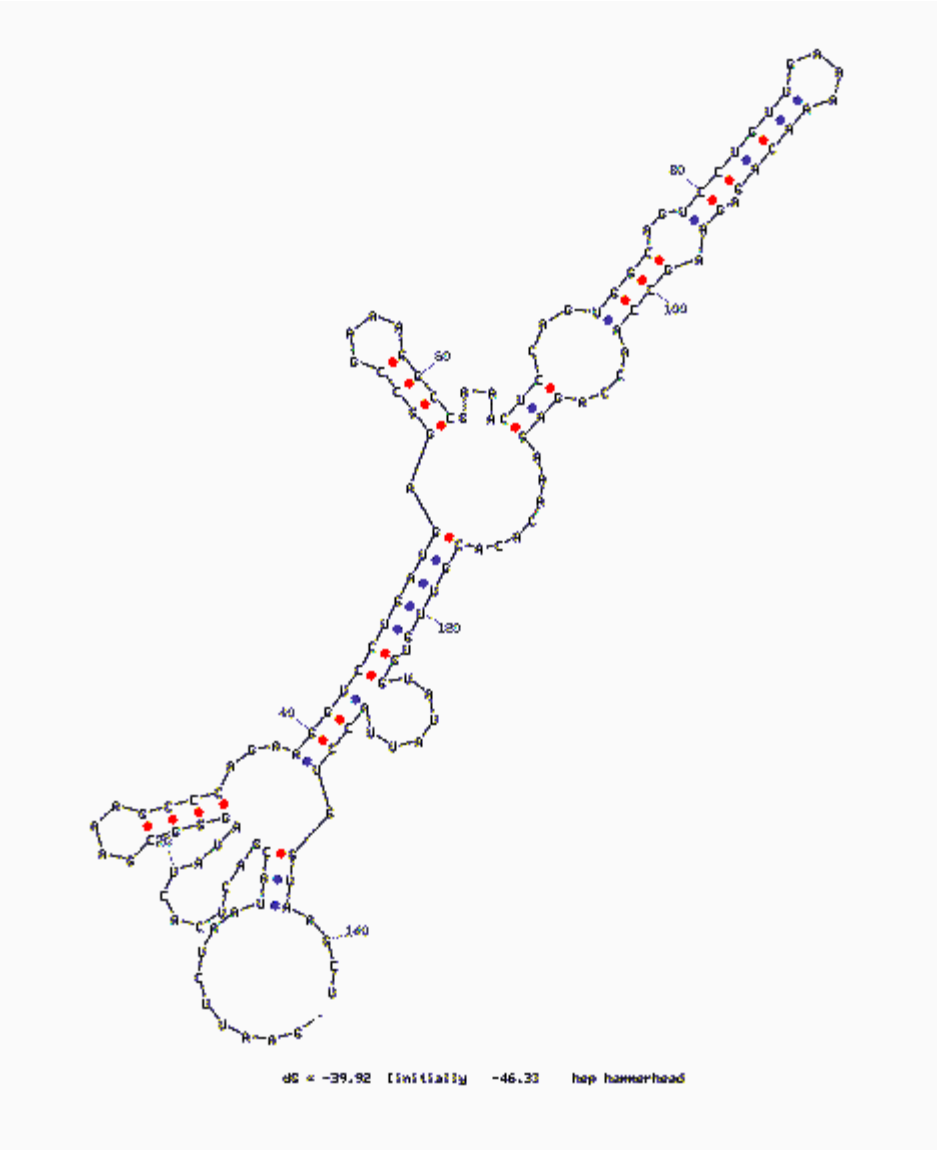
Podstawy struktury RNA



RNase P



RNA secondary structure can be predicted relatively accurately by sequence comparison or thermodynamic methods



Chemical methods

Small angle X-ray scattering (SAXS)

Electron microscopy (EM)

Nuclear Magnetic Resonance (NMR).

Crystallography

Computational methods

Specialized methods (e.g., FRET)

RNA production:
Synthesis (up to a few ten nucleotides)
In vitro transcription
Natural sources

Purification under denaturing conditions:
HPLC
Sequential gels

Purification under native conditions:
Chromatographic methods (gel filtration)

Chemical methods for RNA testing (chemical probing)

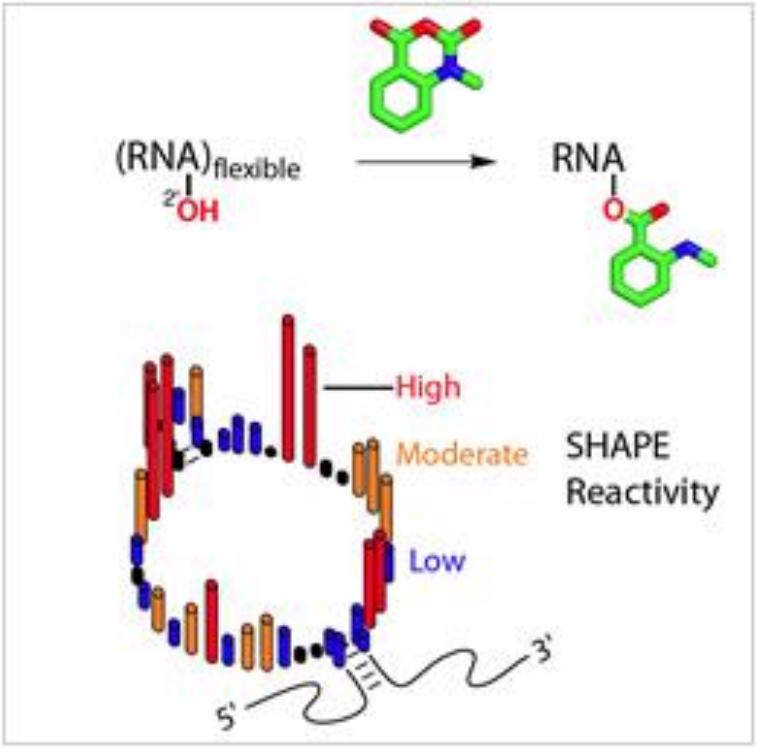
Enzymes

Nuclease S1 available nucleotides in single-stranded regions
Ribonuclease V1 nucleotides in forming stacking or paired interactions
T1 ribonuclease available (unpaired) guanines, sequencing for guanines (under denaturing conditions)
U2 ribonuclease Available (unpaired) adenines, sequencing for adenines (under denturating conditions)

Chemical reagents

Imidazole Single-stranded regions available
Lead Single-stranded regions available
Ethylnitrosourea ENU Ethylates available phosphates
Hydroxyl radicals (reaction of Fe(II)-EDTA with NaOH or synchortron radiation) - cut the main chain of RNA where C1' or C4' ribose is available
Dimethylsulfate DMS Methylates available N1 adenine, N3 cytosine, N7 guanine
CMCT Modifies available N3 uridine, N1 guanine
DEPC Available N7 adenine
Kethoxal Available N1 and N2 guanines

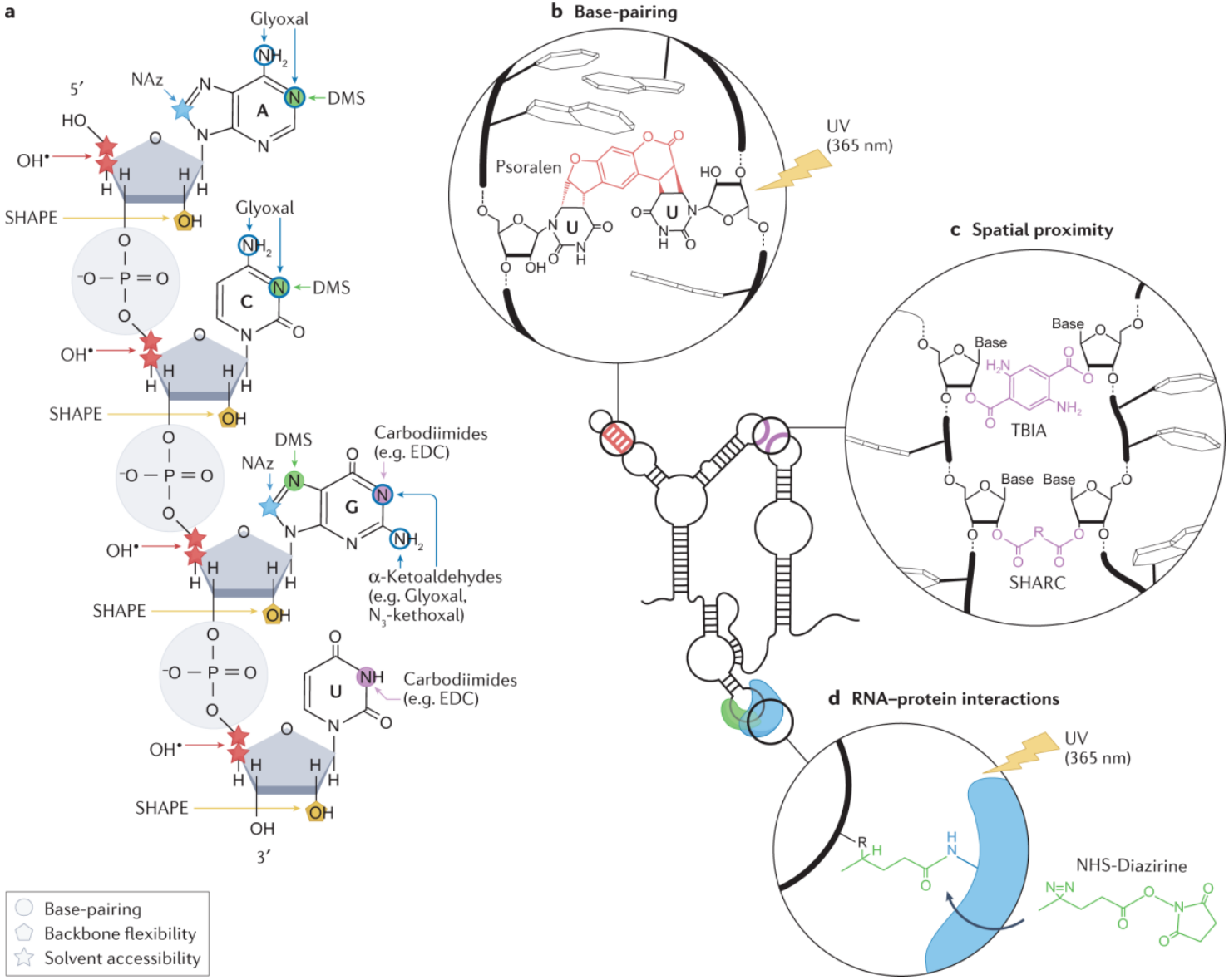
SHAPE



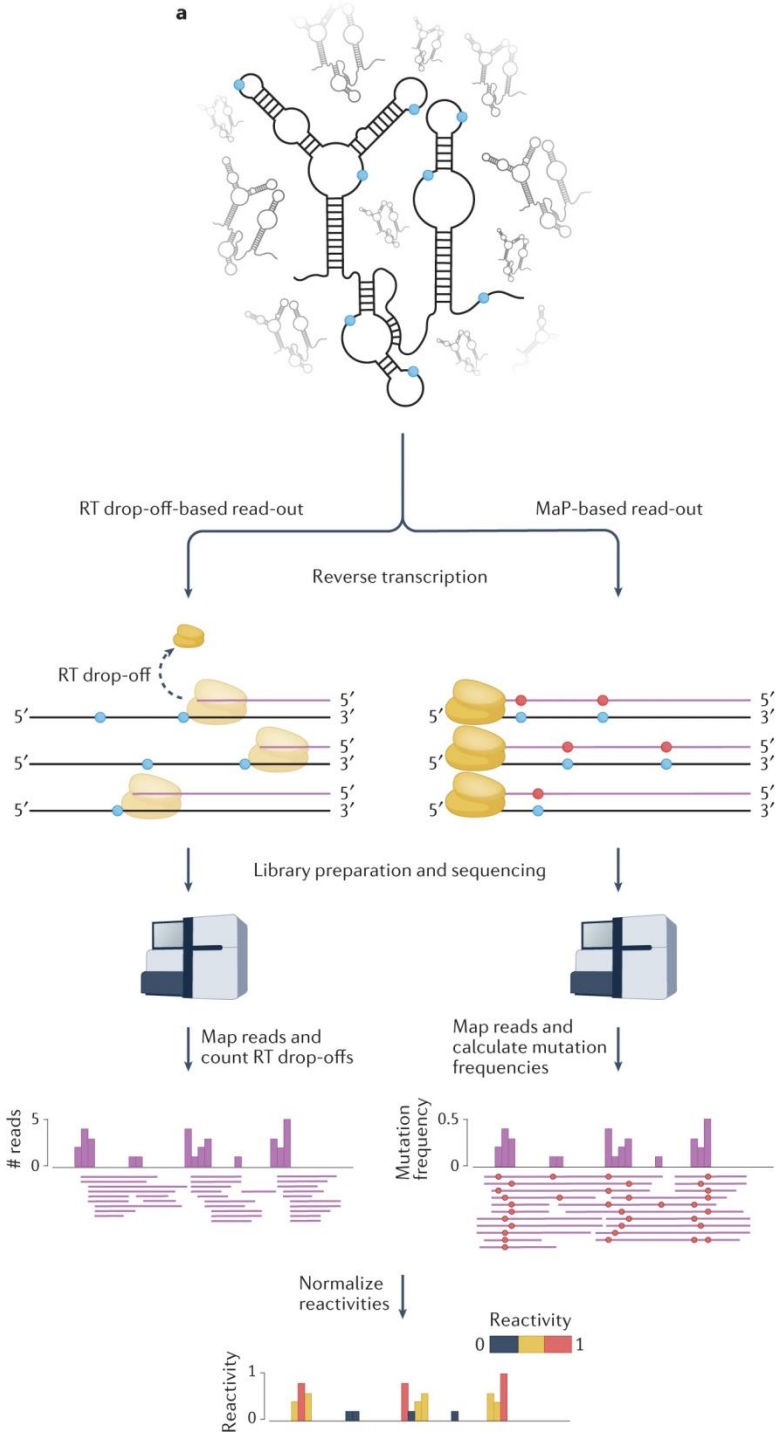
1-methyl-7-nitroisatoic anhydride

Reactivity independent of the type of base, but strongly dependent on the mobility/availability of the nucleotide.

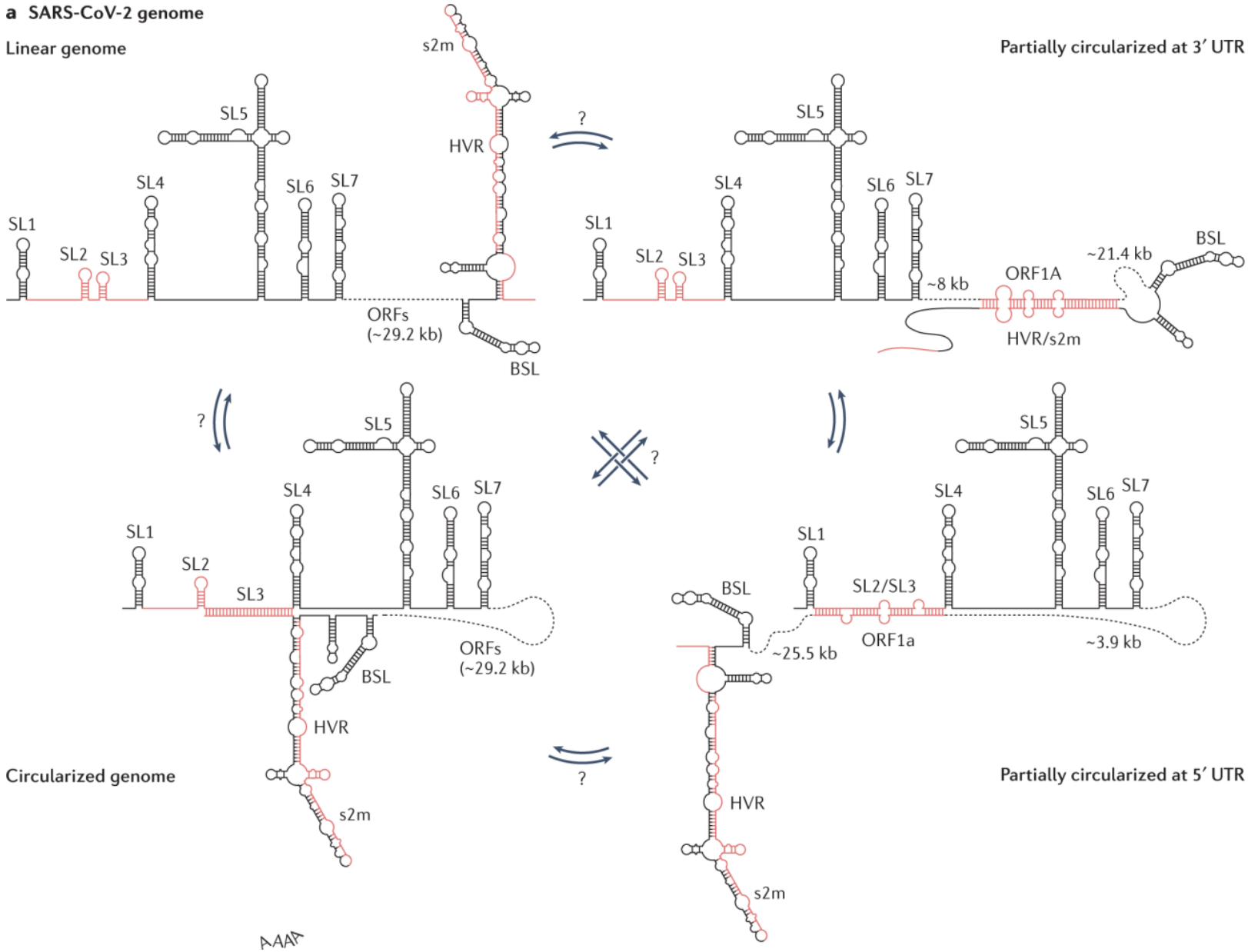
The formation of the reaction product is detected by stopping the DNA primer elongation reaction using reverse transcriptase and comparing with the unmodified control



https://www.nature.com/articles/s41576-022-00546-w



https://www.nature.com/articles/s41576-022-00546-w


a SARS-CoV-2 genome
Linear genome
Partially circularized at 3′ UTR
Circularized genome
Partially circularized at 5′ UTR
s2m
HVR
SL1
SL2
SL3
SL4
SL5
SL6
SL7
ORFs
(~29.2 kb)
BSL
?
~8 kb
ORF1A
~21.4 kb
HVR/s2m
SL2/SL3
ORF1a
~25.5 kb
~3.9 kb
AAA

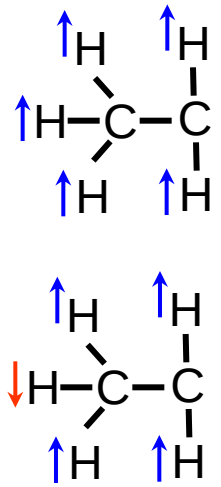
Chemical methods are used to confirm the correctness of crystallographic structures

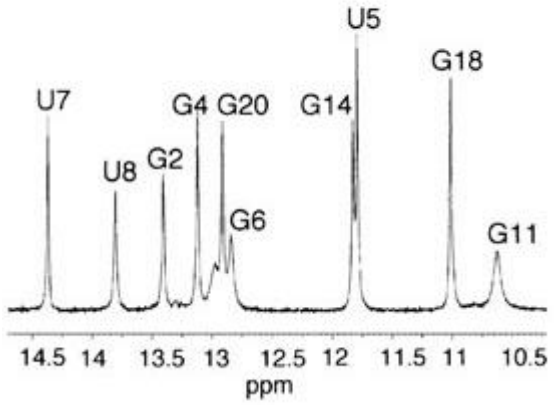
They have the advantage of being used in solution under physiological conditions

These methods have been applied to whole cells (mapping the structure of 16S rRNA and RNase P in bacteria (Adilkshmi, NAR, 2006)

NMR

Nuclear magnetic resonance



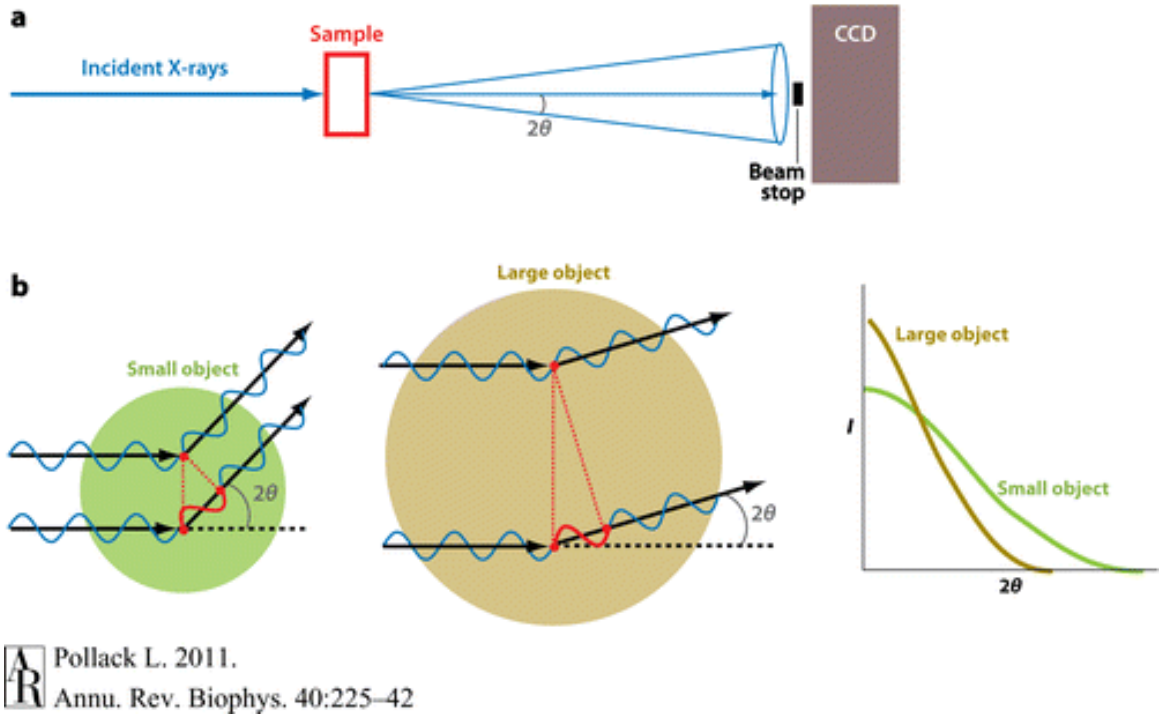


SAXS

SAXS

Provides low-resolution structural information

Advantages: in solution, simple measurement, measurements possibly from a dozen kDa to a few Mda.



Pollack L. 2011.
Annu. Rev. Biophys. 40:225–42

<u>Possible types of analysis:</u>

Comparison of the theoretical SAXS curve with the experimental curve

Calculating the shape of a molecule

Docking the RNA model into shape

Determination of shape ab initio

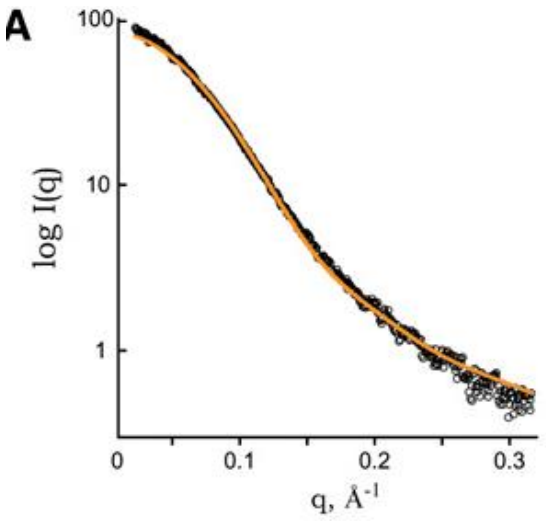
A given volume is filled with spheres.

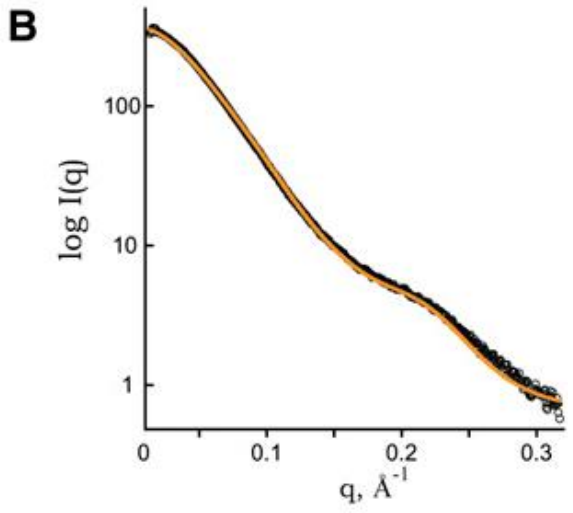
Each sphere can be assigned to a molecule or solution.

The initial assignments are random.

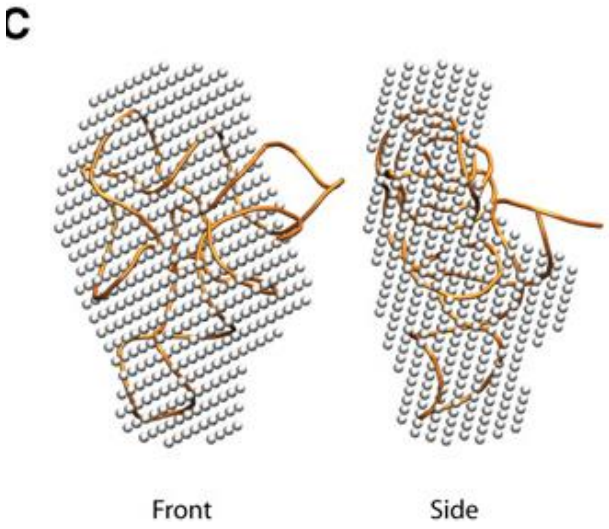
The assignments are randomly shifted until a shape corresponding to the experimental curve is obtained.

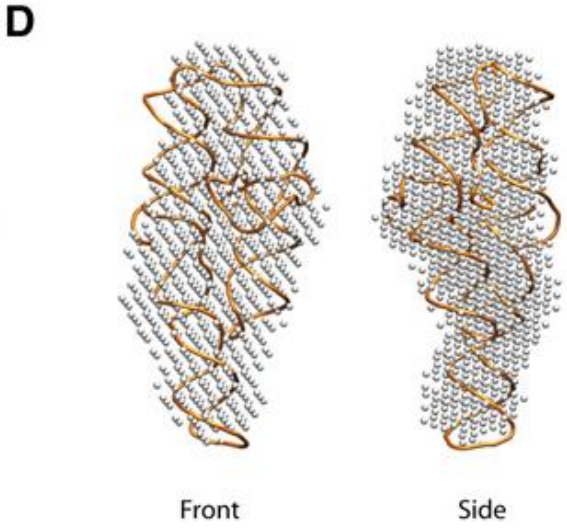
Conditions can be imposed that ensure the compactness of the molecule





$q = 4\pi(\sin \Theta)/\lambda$

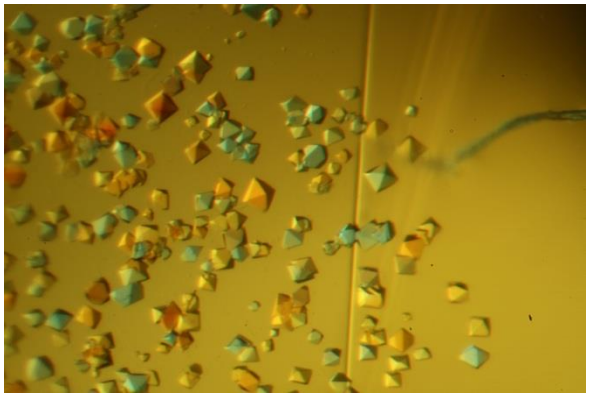


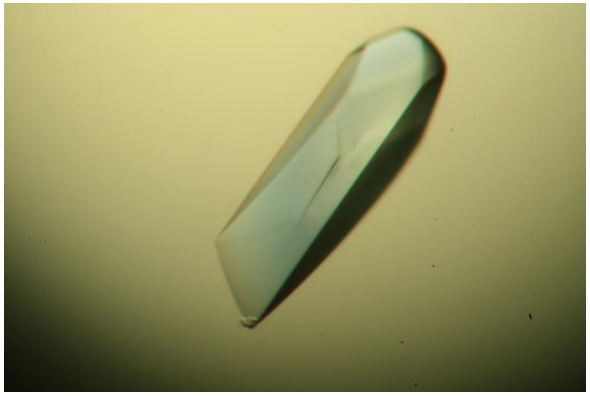


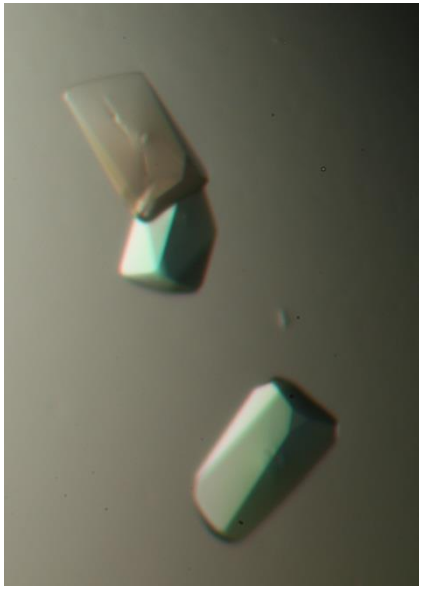
SAM-I riboswitch

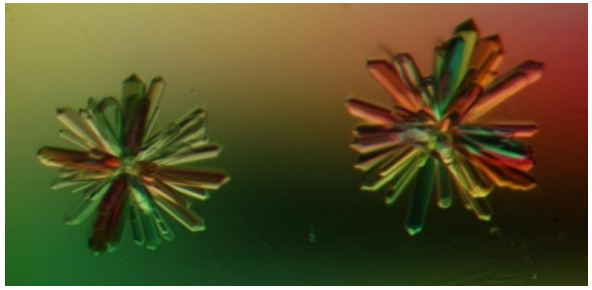
D(C209)P4-P6 domain of the group I intron

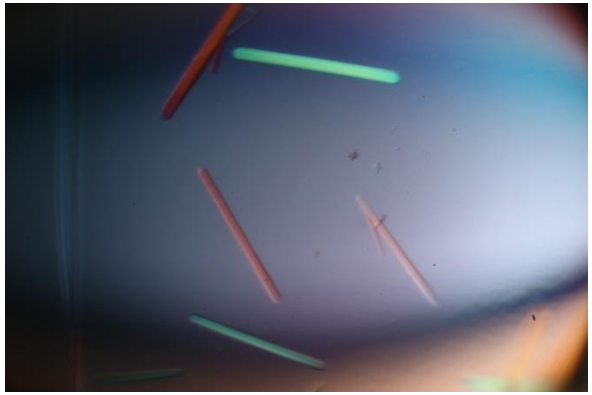
CRYSTALLOGRAPHY

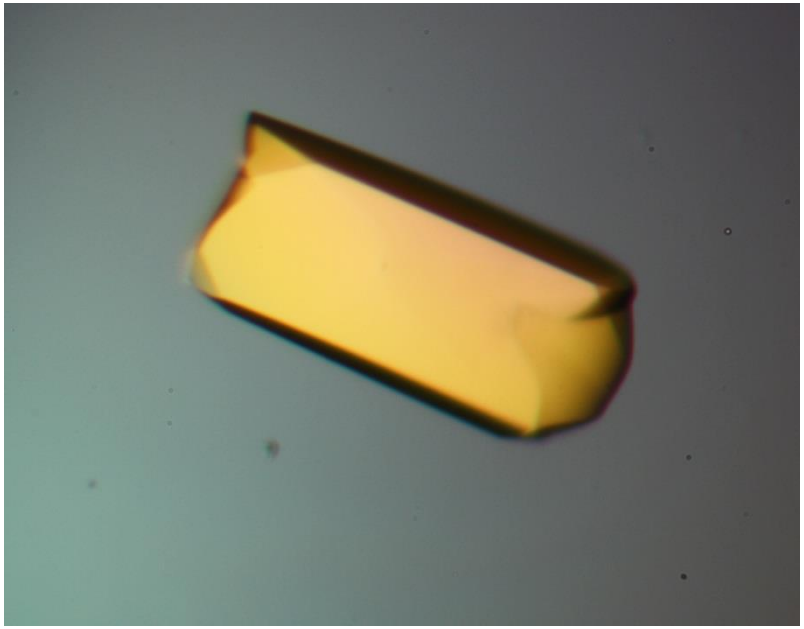


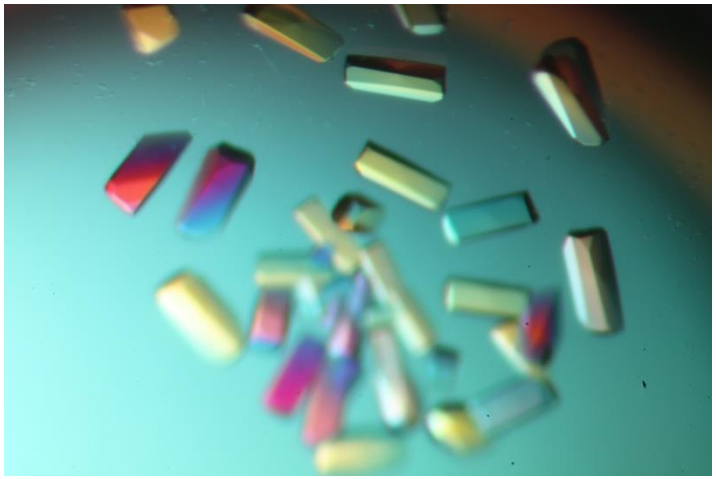


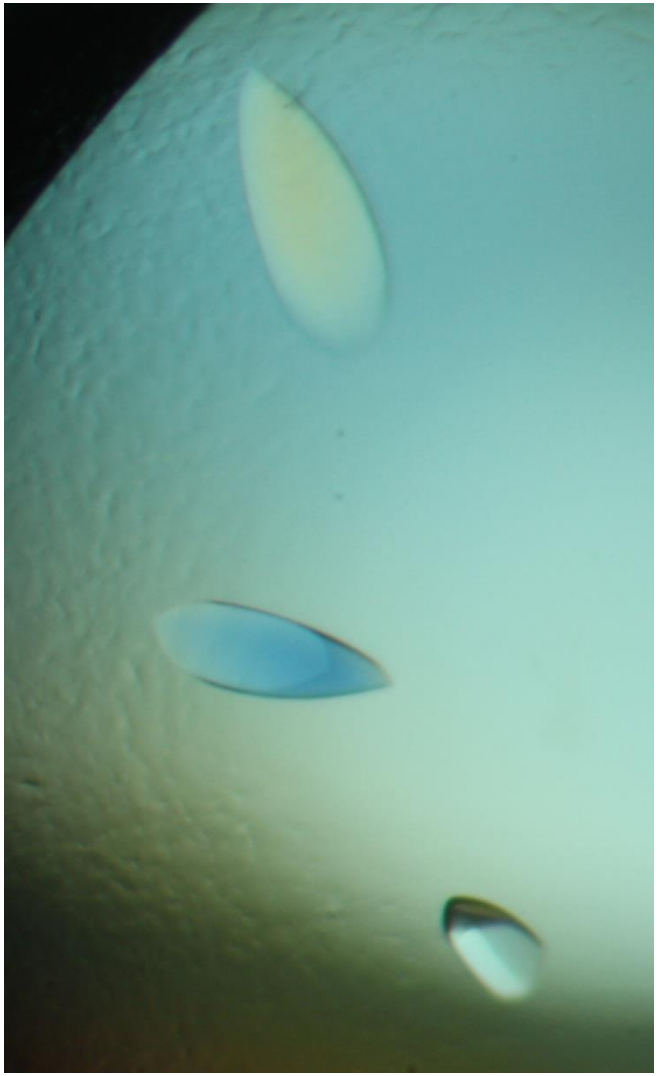


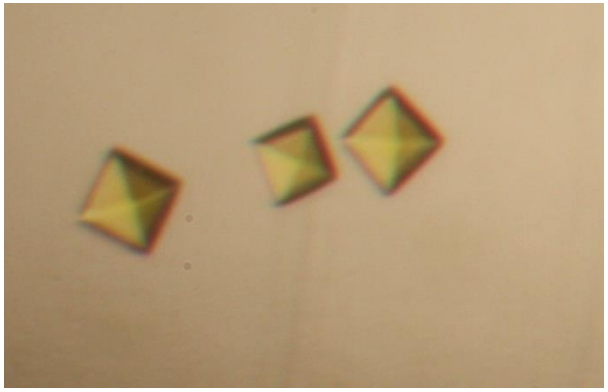


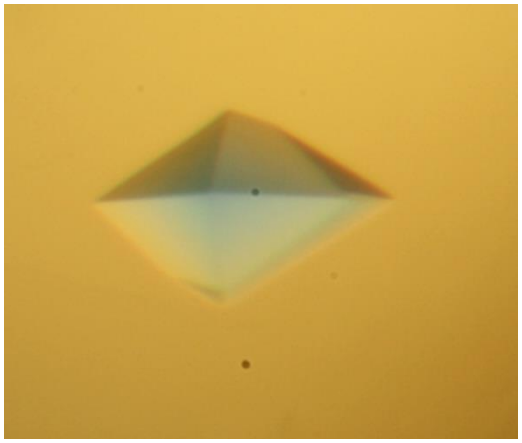


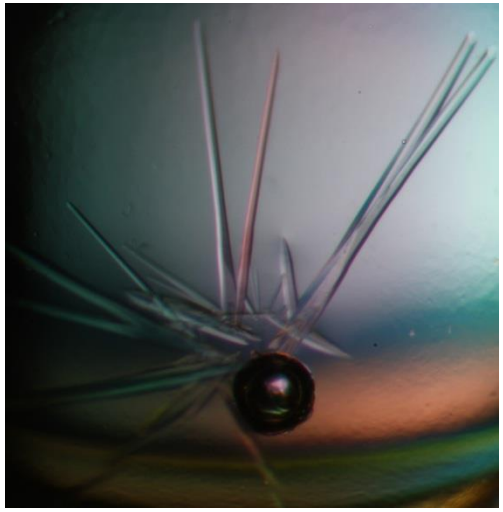


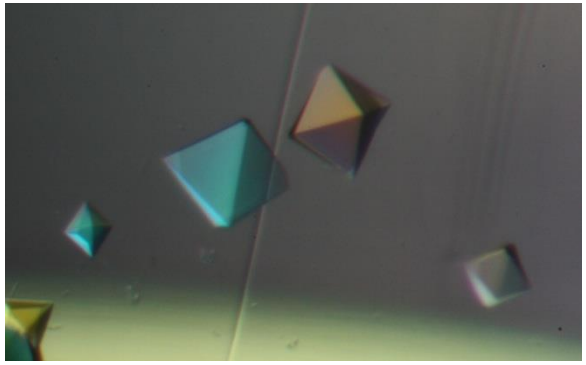






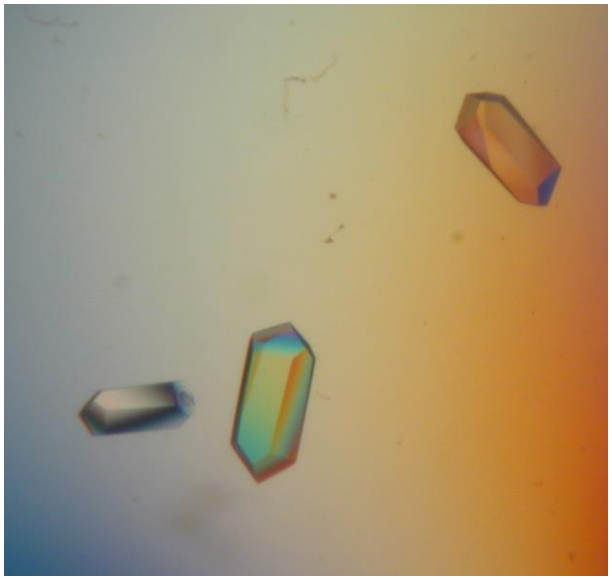


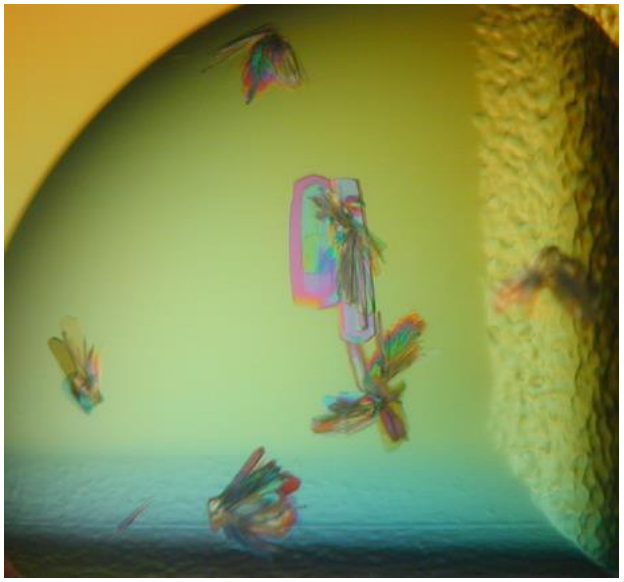


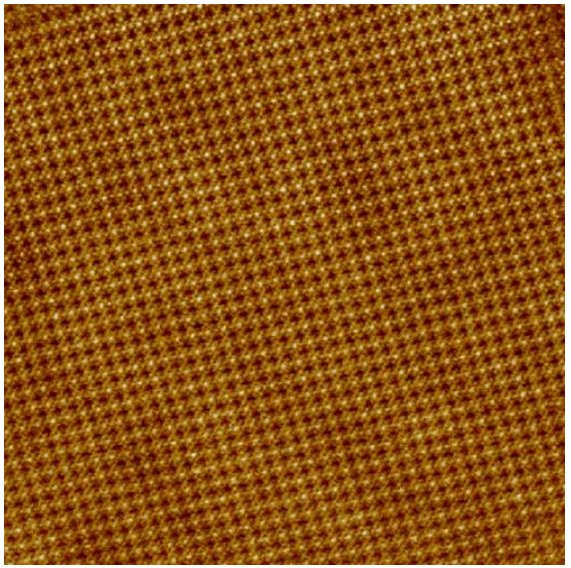


Kryształy dużej podjednostki rybosomu

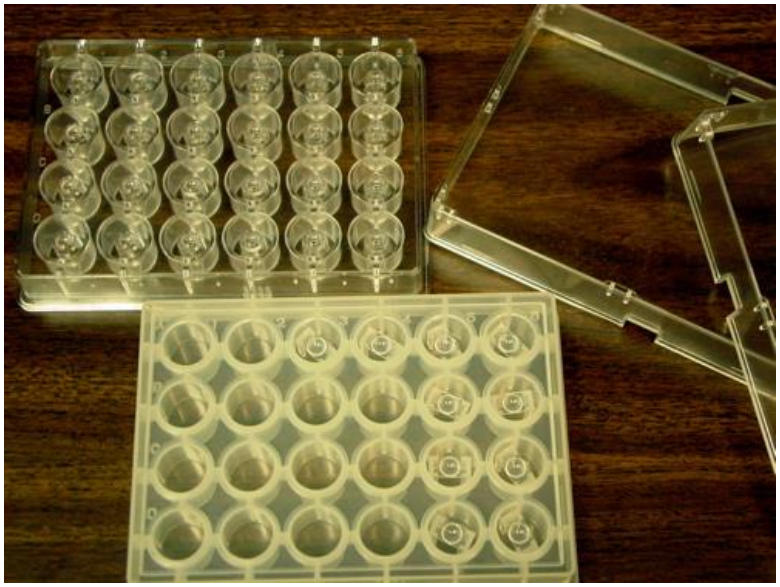
Crystals

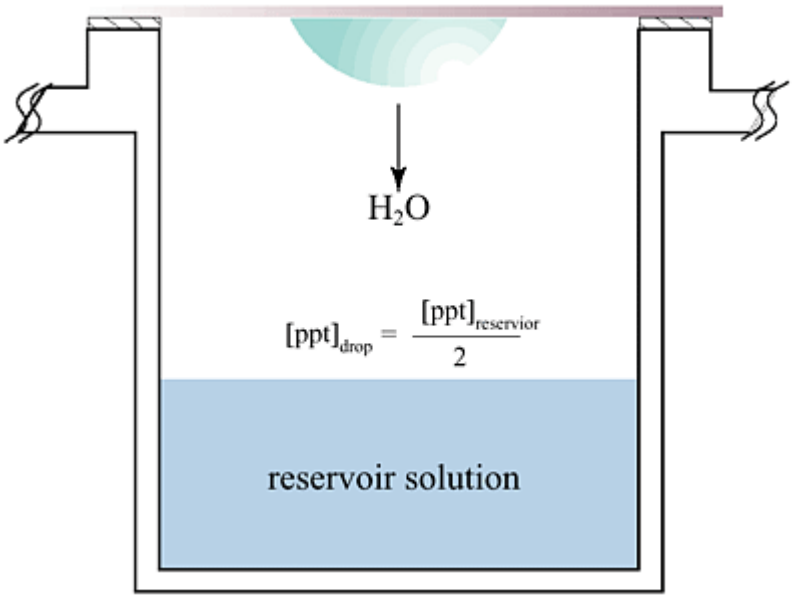




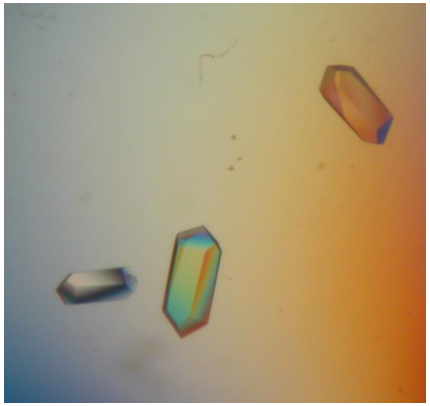


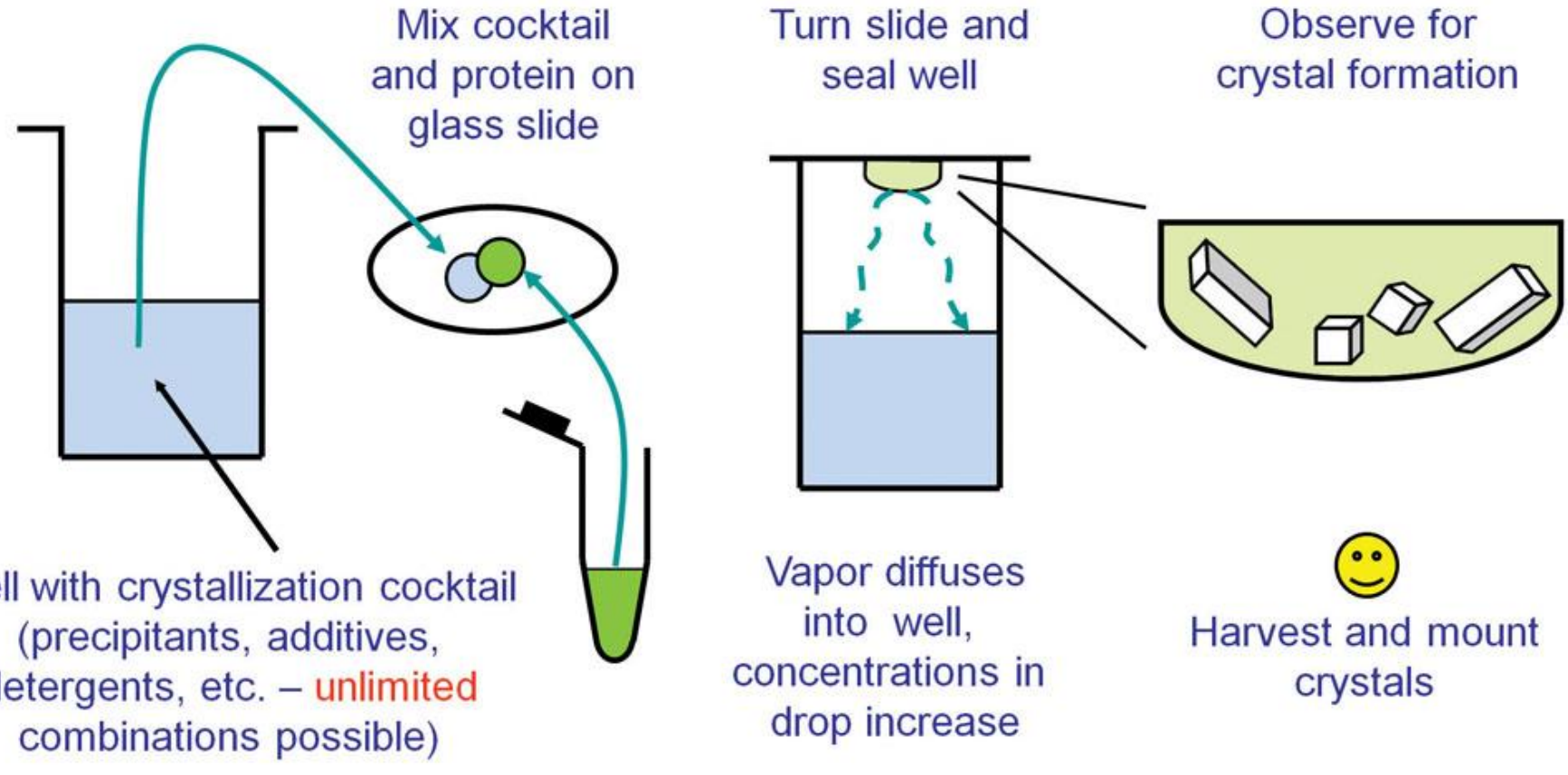
Crystallization

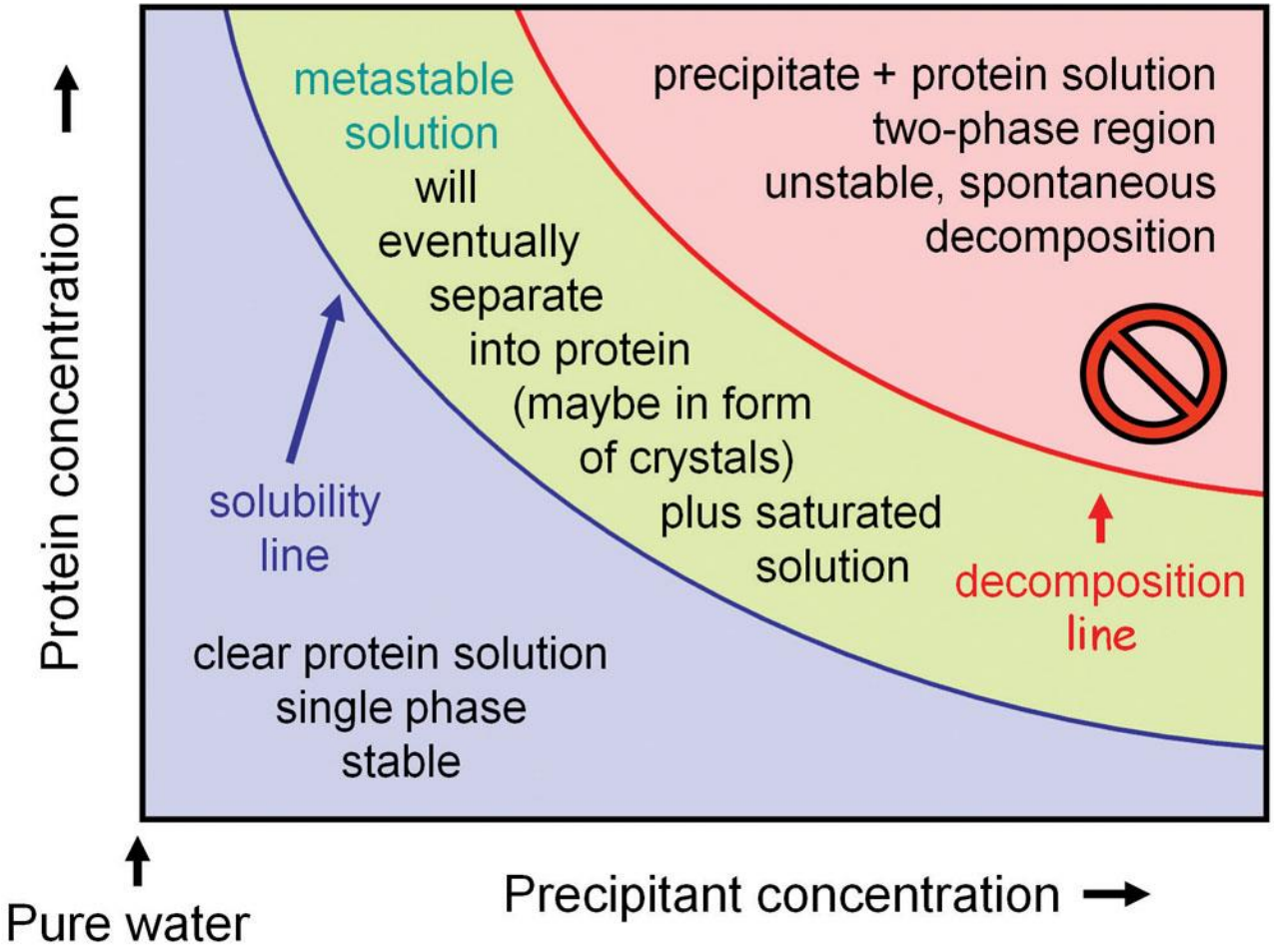


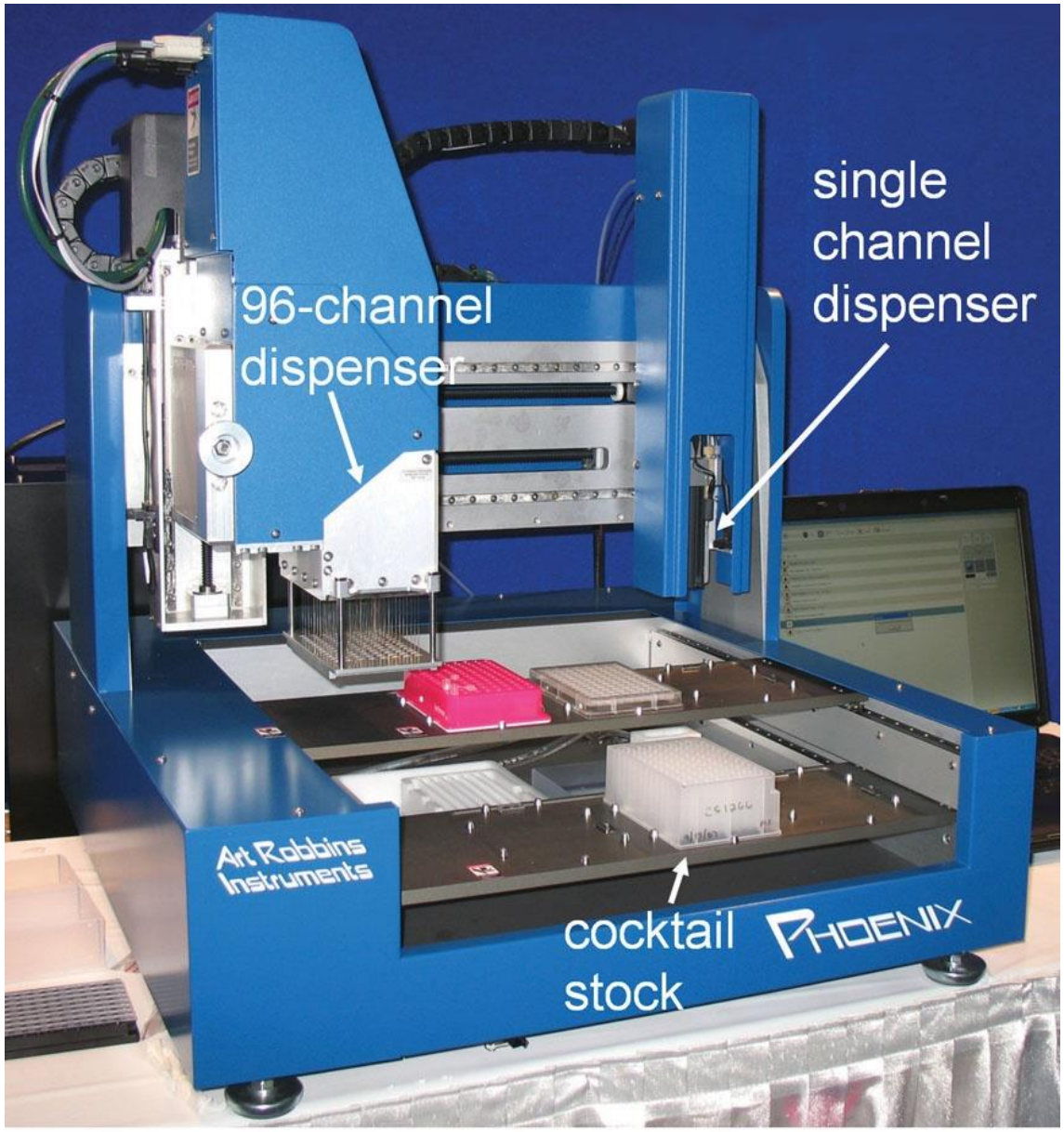


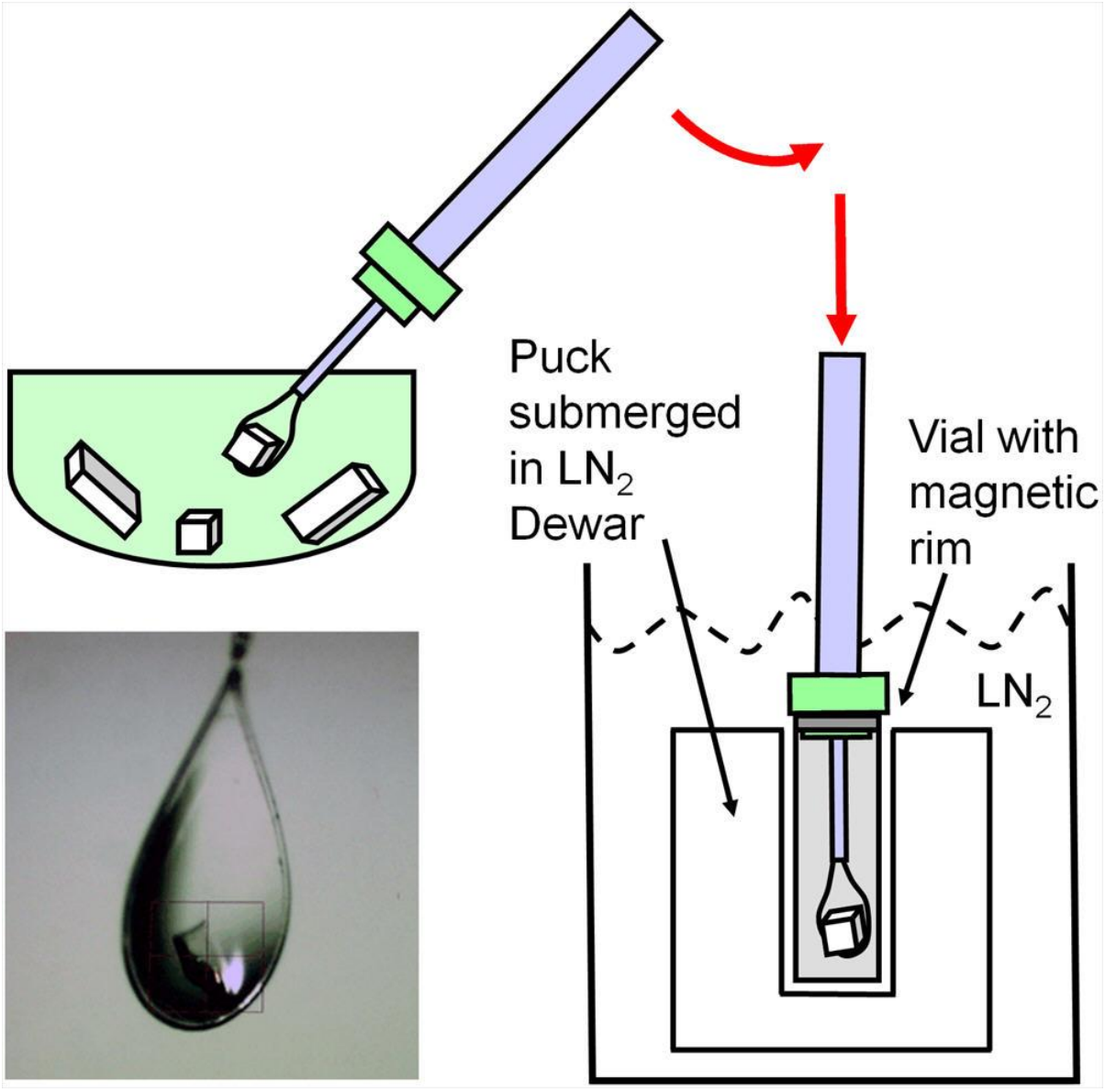
Precipitants:
salts
PEGs
organic solvents

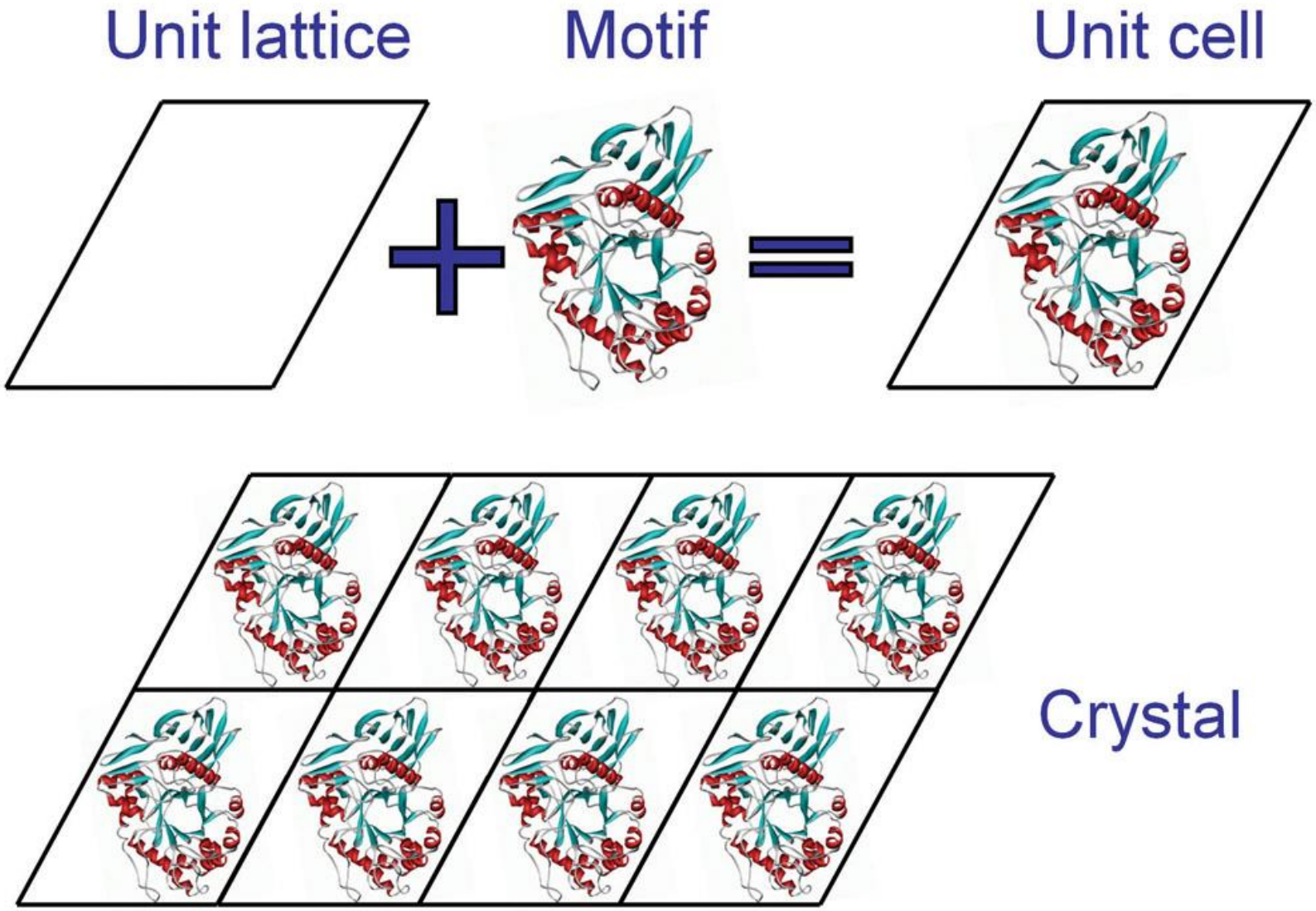


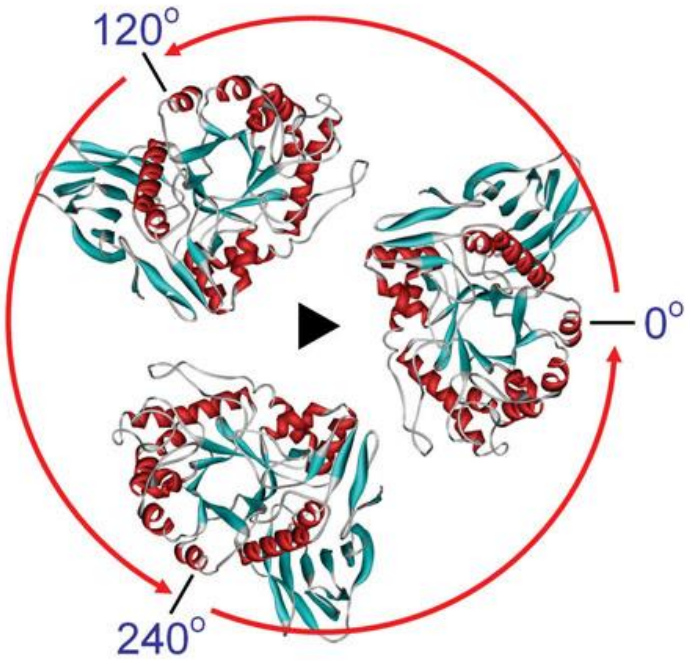


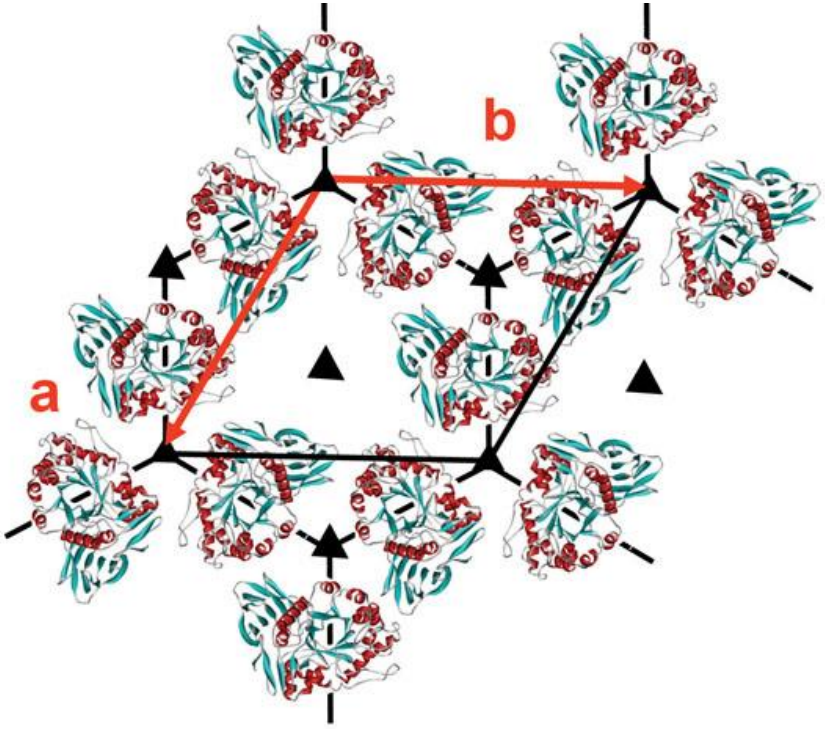


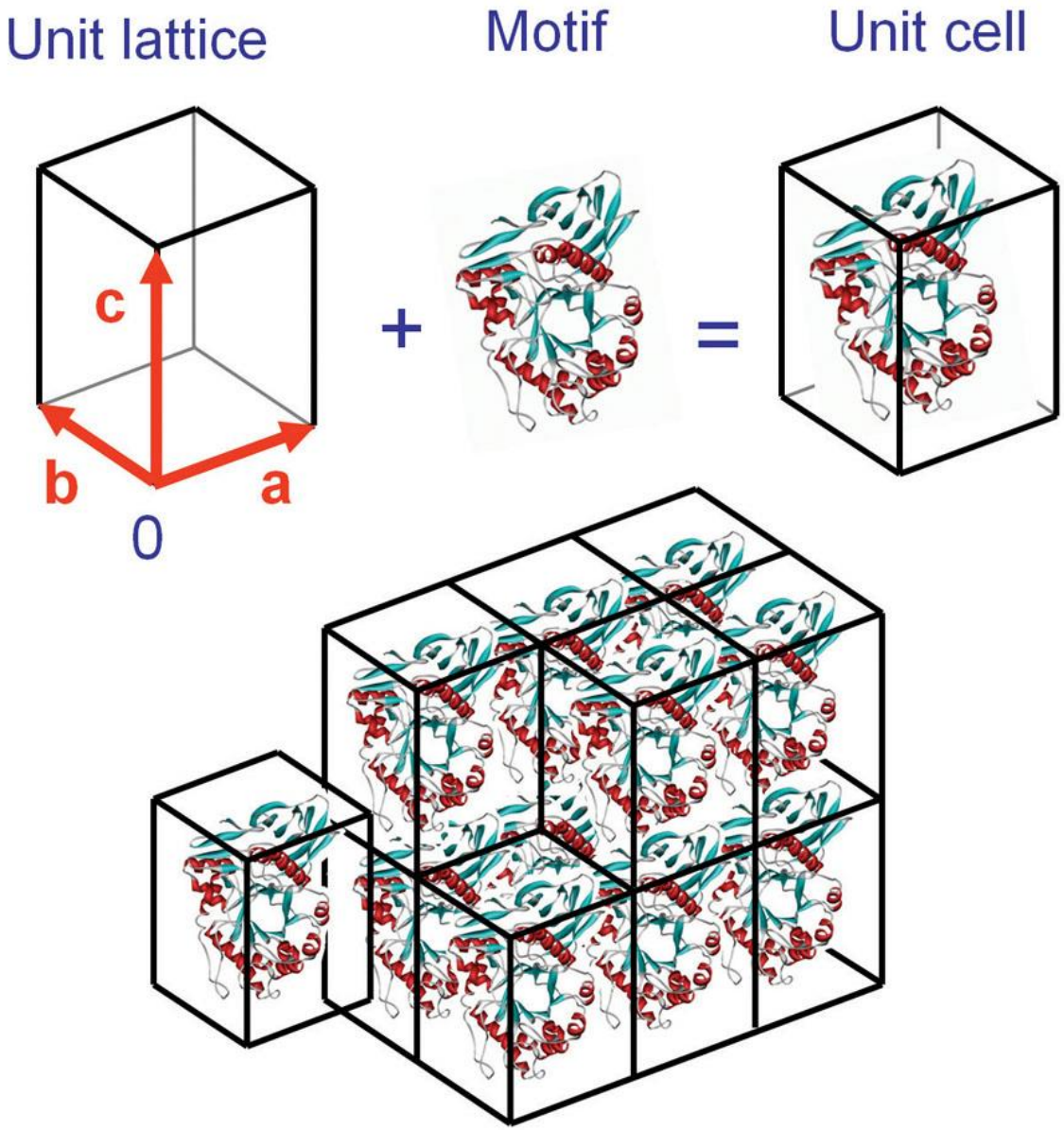


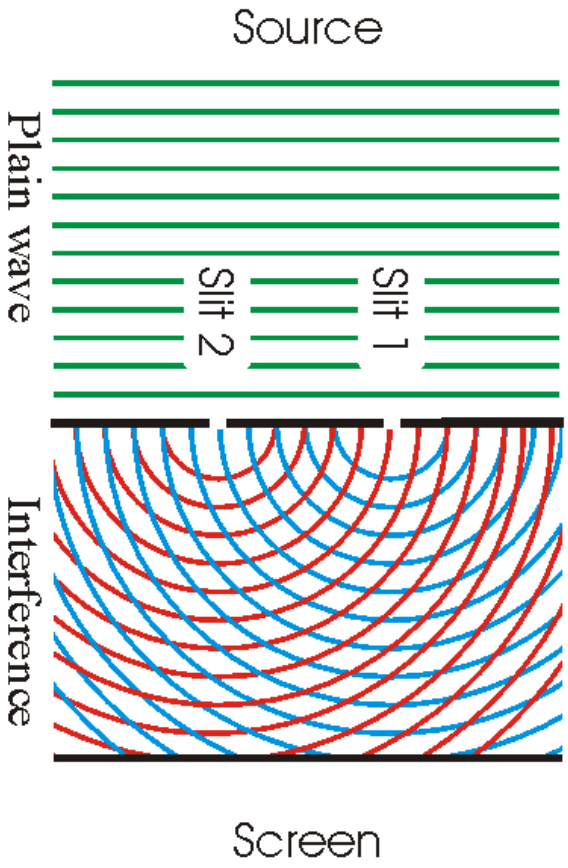

Puck
submerged
in LN_2
Dewar
Vial with
magnetic
rim
LN_2

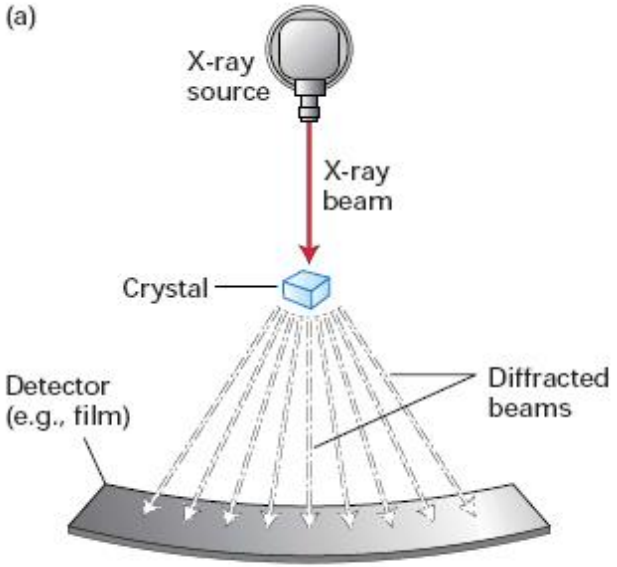


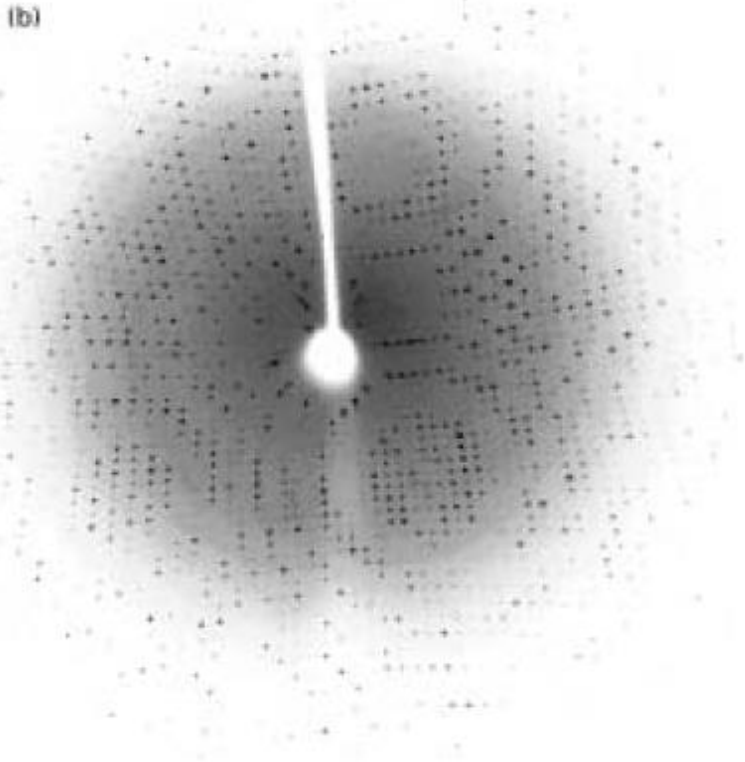




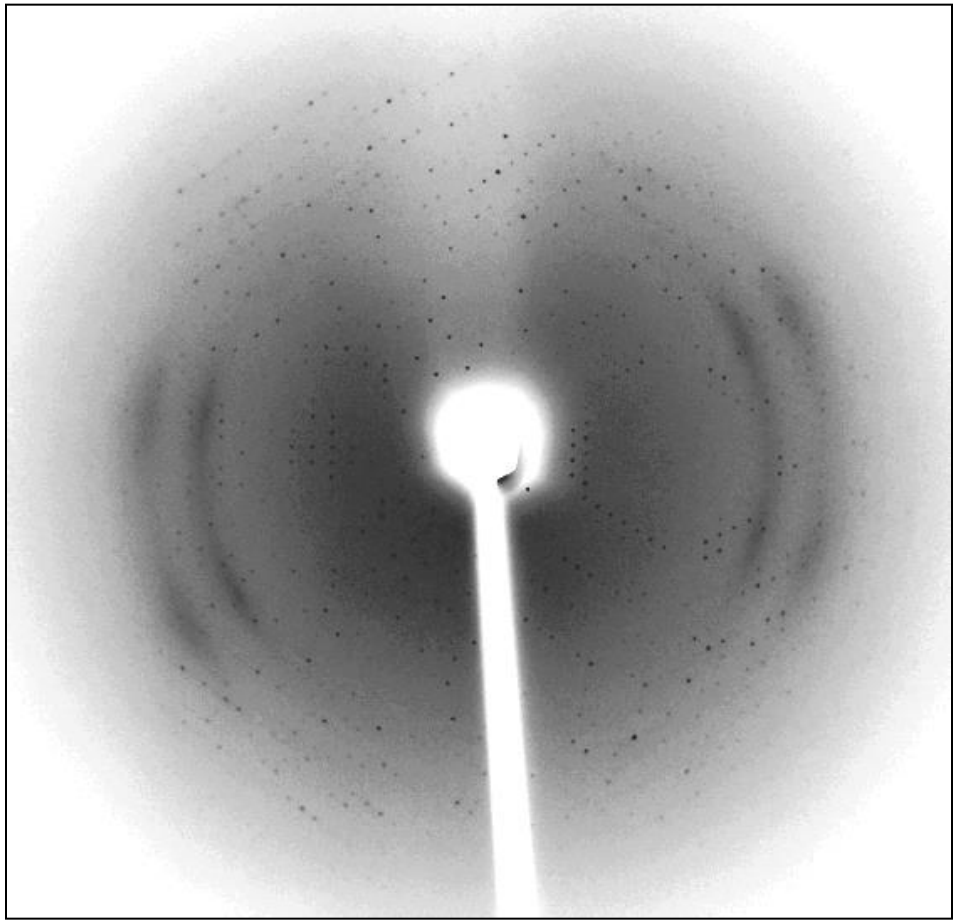


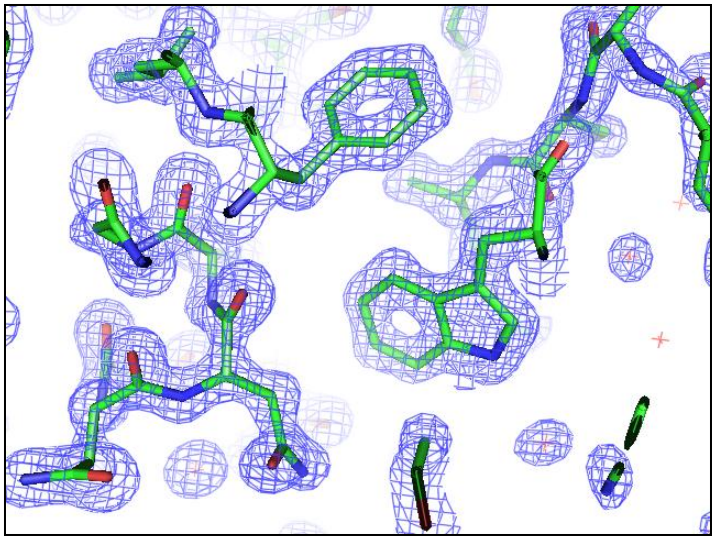

Source
Plain wave
Slit 2
Slit 1
Interference
Screen


(a)
X-ray source
X-ray beam
Crystal
Detector (e.g., film)
Diffracted beams

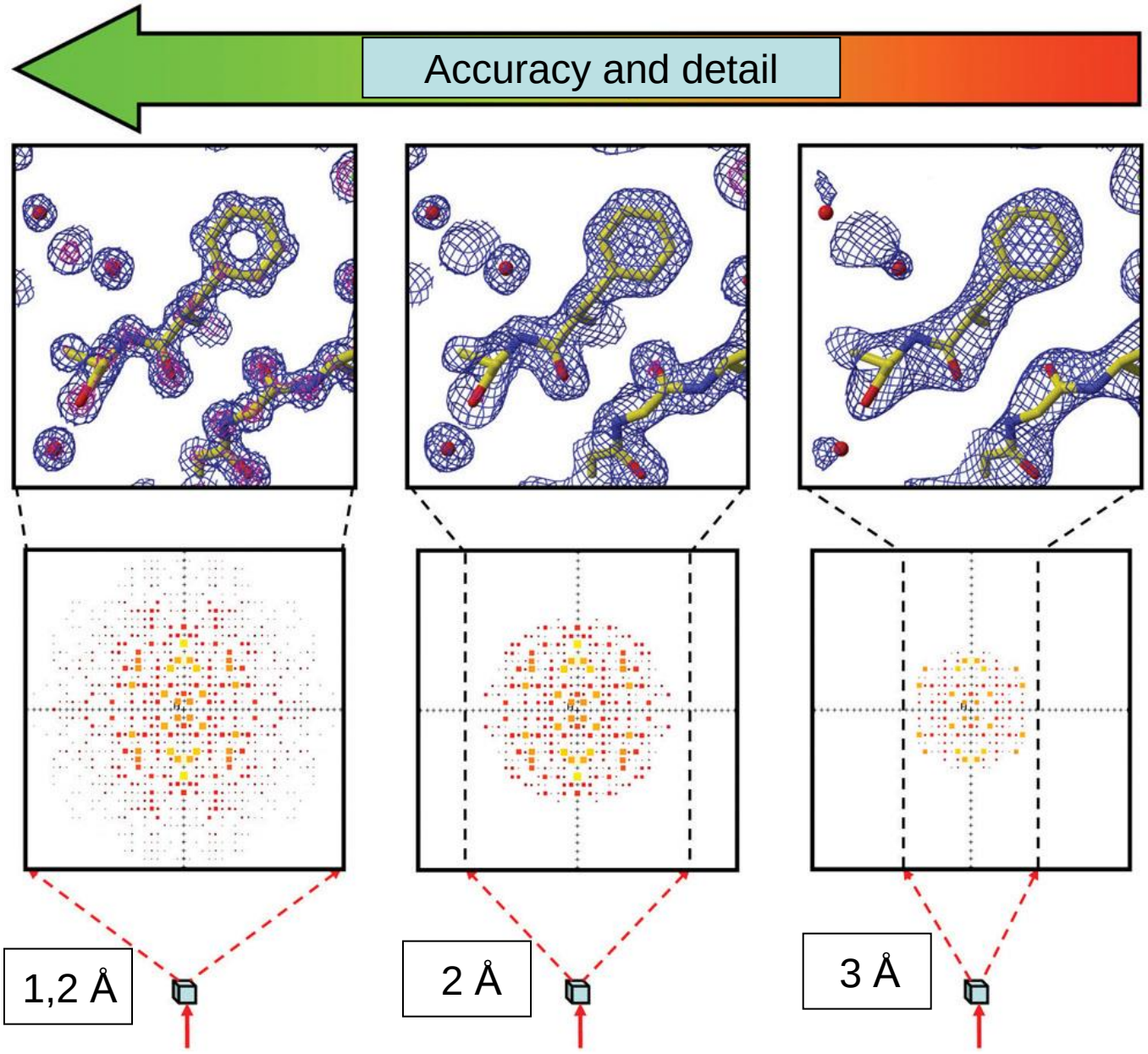

(b)

Crystallography

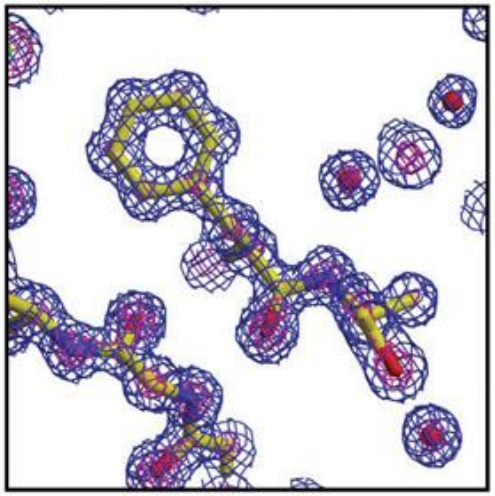


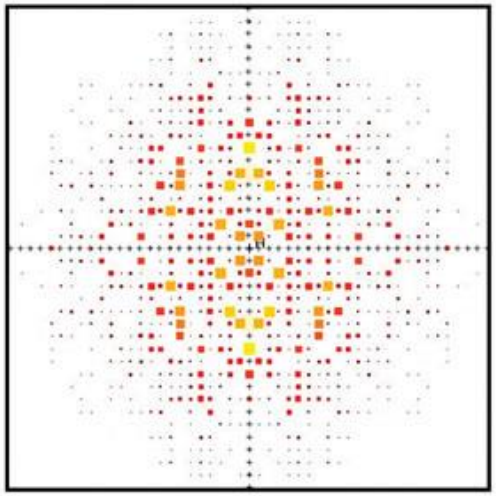


Rozdzielczość: 1,5 Å

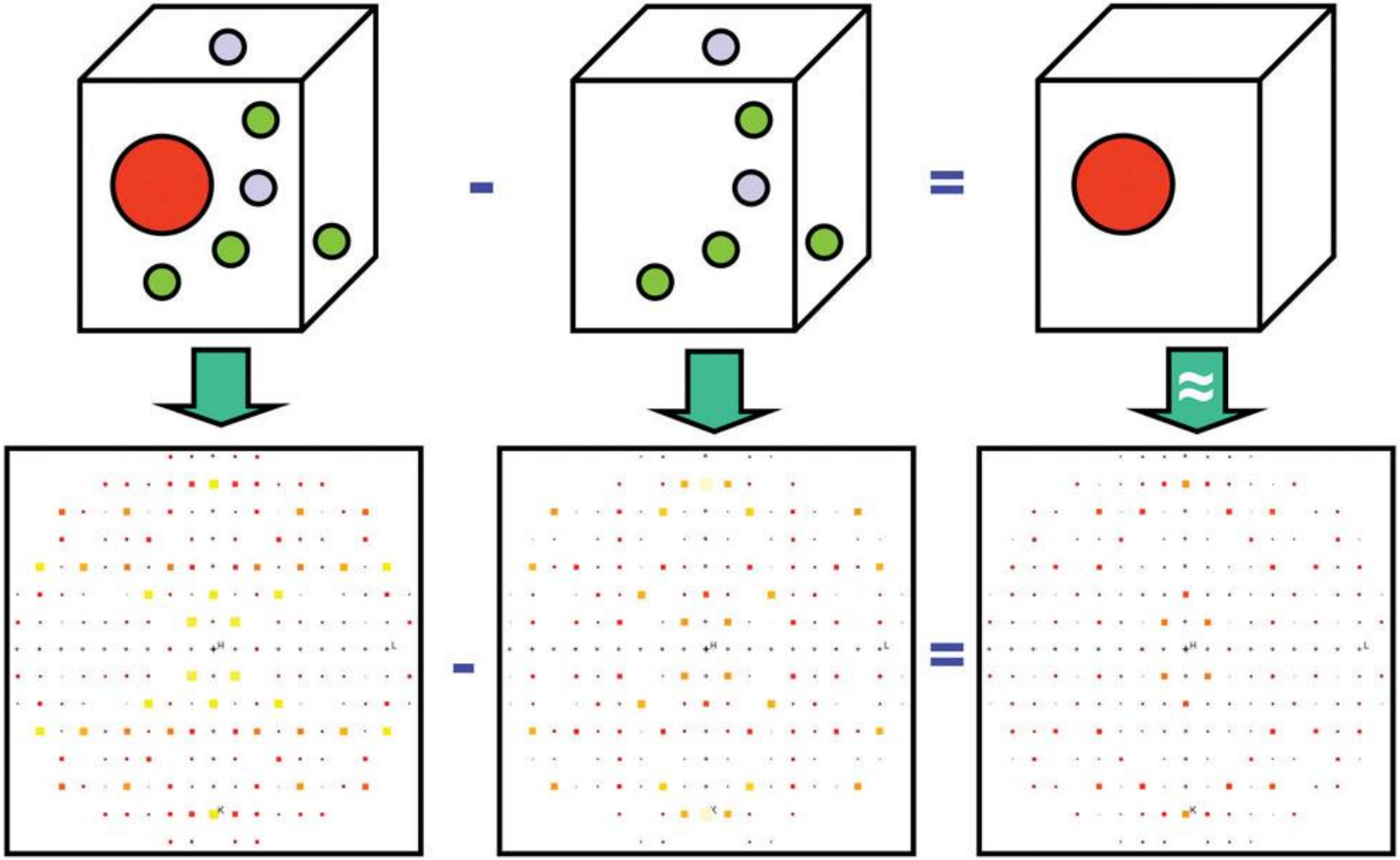


$$\rho(x,y,z) = \frac{1}{V}\sum_{-h}^{h}\sum_{-k}^{k}\sum_{-l}^{l} F_{hkl} \cdot \exp[-2\pi i(hx + ky + lz + \alpha_{hkl})]$$



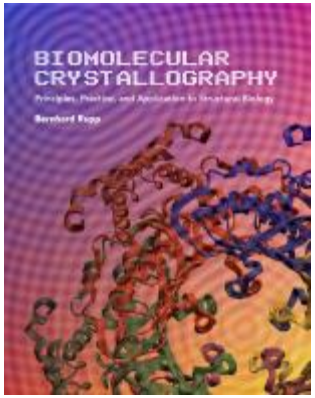


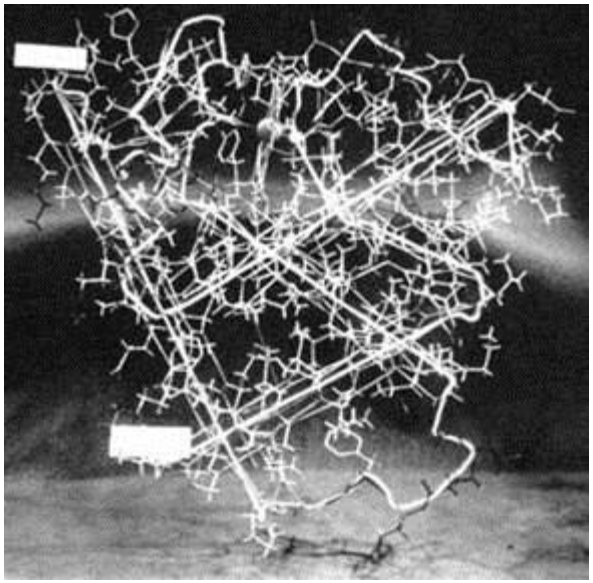
The crystallographic phase problem

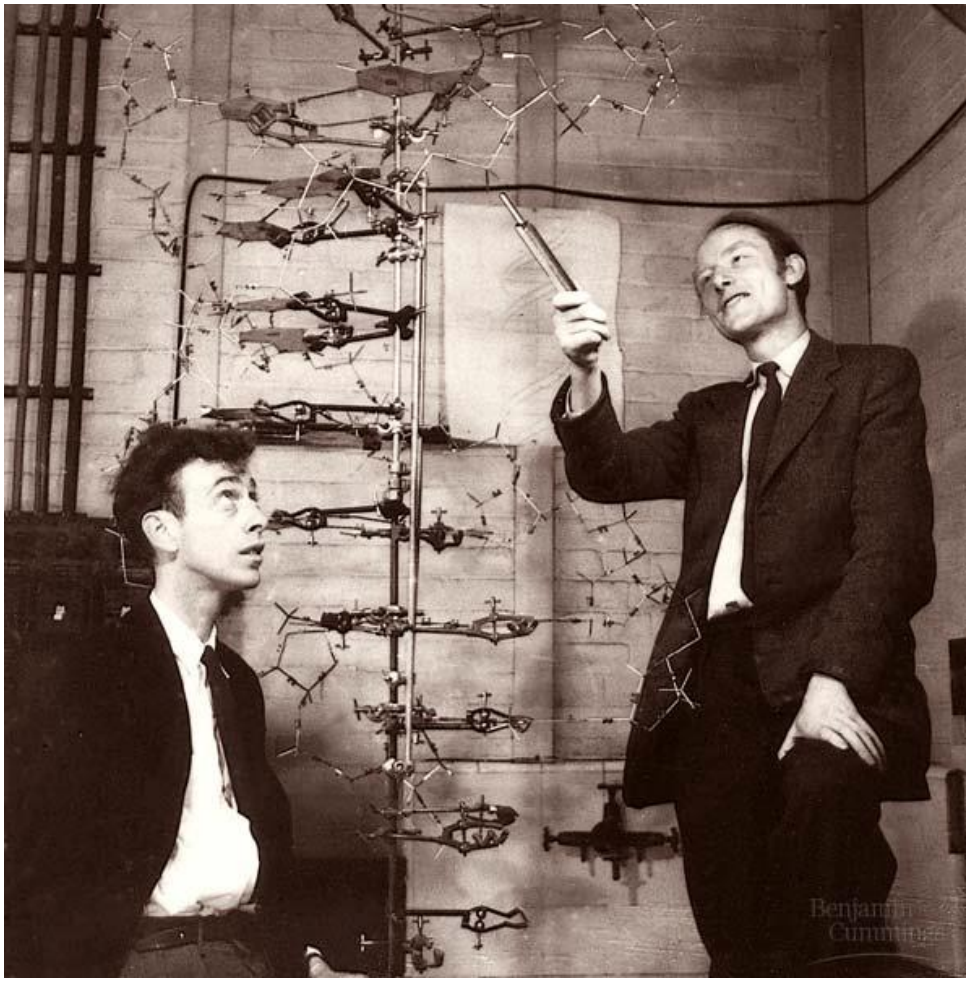


Biomolecular Crystallography: Principles, Practice, and Application to Structural Biology

Bernhard Rupp







RNA crystallography

The first structure - tRNA (1974)

After 2000 - ribosomes, group I introns, P ribonuclease

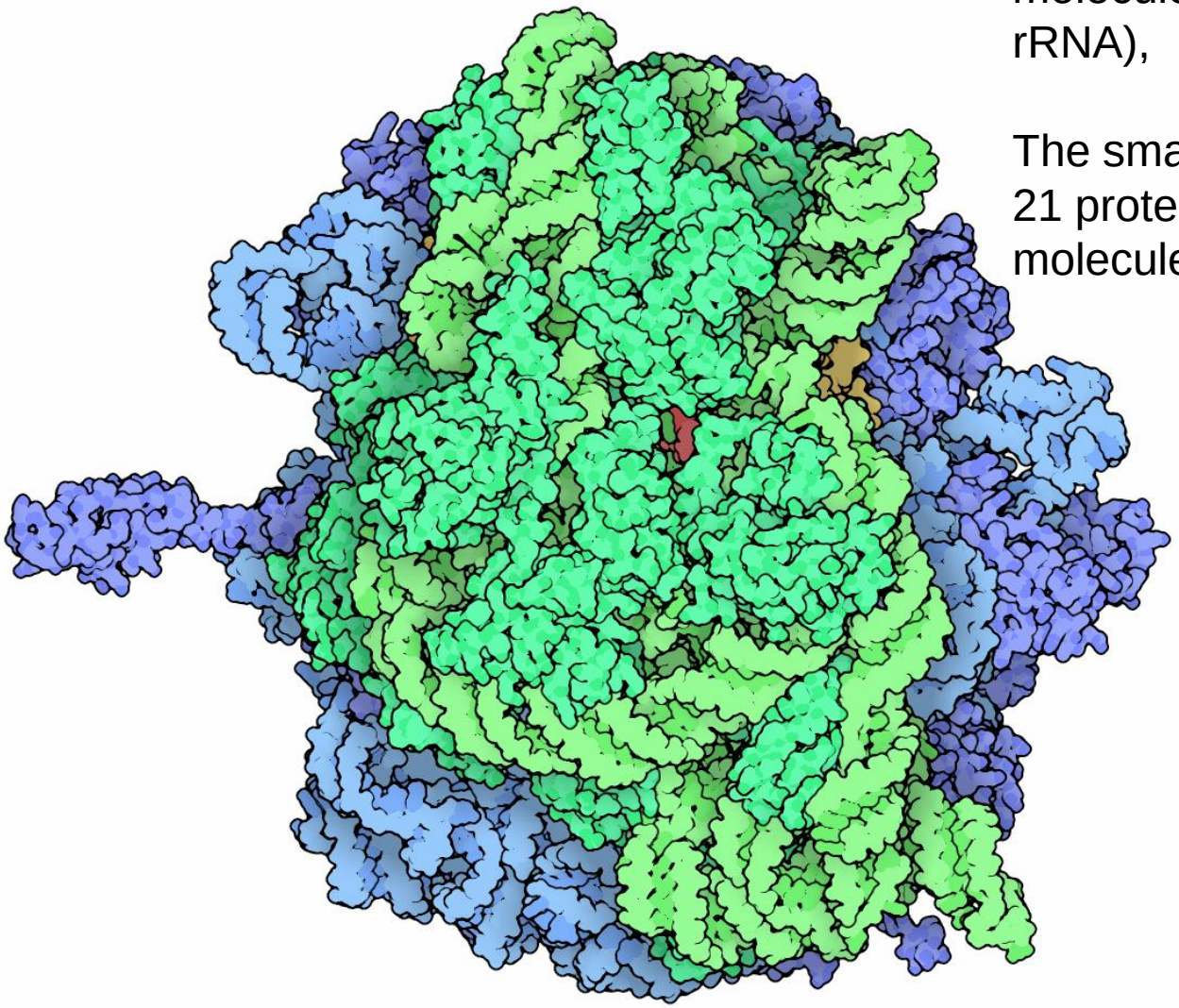
A limitation of crystallography is the heterogeneity of RNA conformations, which often makes it impossible to obtain crystals

RNA crystallization difficult - out of about 80000 structures solved, about 1000 RNA alone and 1000 RNA-protein complexes

Dedicated Nucleic Acid Structure Database (ndbserver.rutgers.edu)
Allows identification of recurring motifs

EXAMPLES

Ribosome



In prokaryotes there are 70S ribosomes

The large subunit (50S) contains 34 proteins and two rRNA molecules (5S rRNA and 23S rRNA),

The small subunit (30S) contains 21 proteins and one rRNA molecule (16S rRNA).

Ribosome

Bacterial ribosome composed of 30S and 50S subunits

A flexible nanomachine that adopts multiple conformations during the peptide bond synthesis cycle

First reconstructions of ribosome structure based on EM in 1970s, first detailed ones around 1995 (Joachim Frank, Holger Stark)

EM has provided a wealth of information about ribosome complexes with tRNA, mRNA elongation factors, and conformational changes of the ribosome during its cycle

EM structures are now available for, among others, bacterial, yeast, and mammalian ribosomes

The first X-ray-scattering bacterial ribosome crystals were obtained in the late 1980s (Ada Yonath)

In 1991, 50S crystals were presented that scattered up to about 3 Å.

In 2000, the first structure of a large subunit from *H. marismortui* was published (T. Steitz)

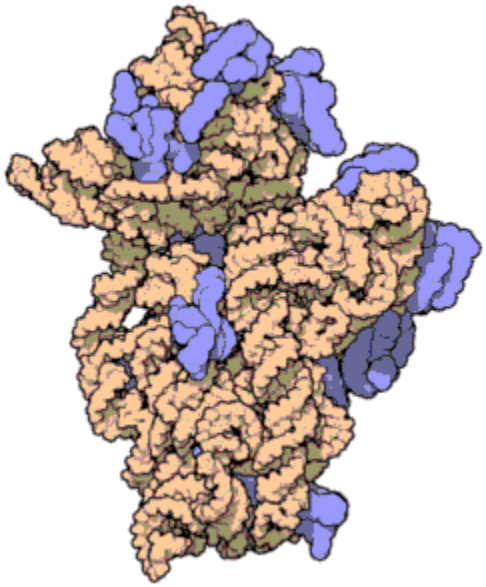
In 2001, the structure of the entire ribosome from *T. thermophilus* (H. Noller)

Currently available multiple complex structures with tRNAs, accessory factors and antibiotics

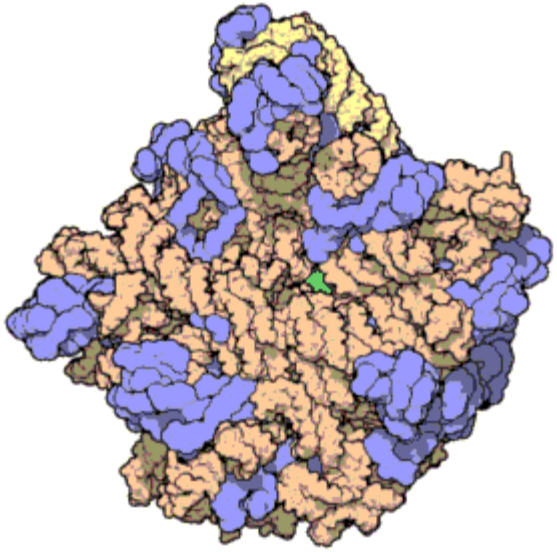
Nobel Prize 2009 - Steitz, Yonath, Ramakrishnan

2010 - Structures of eukaryotic ribosomes

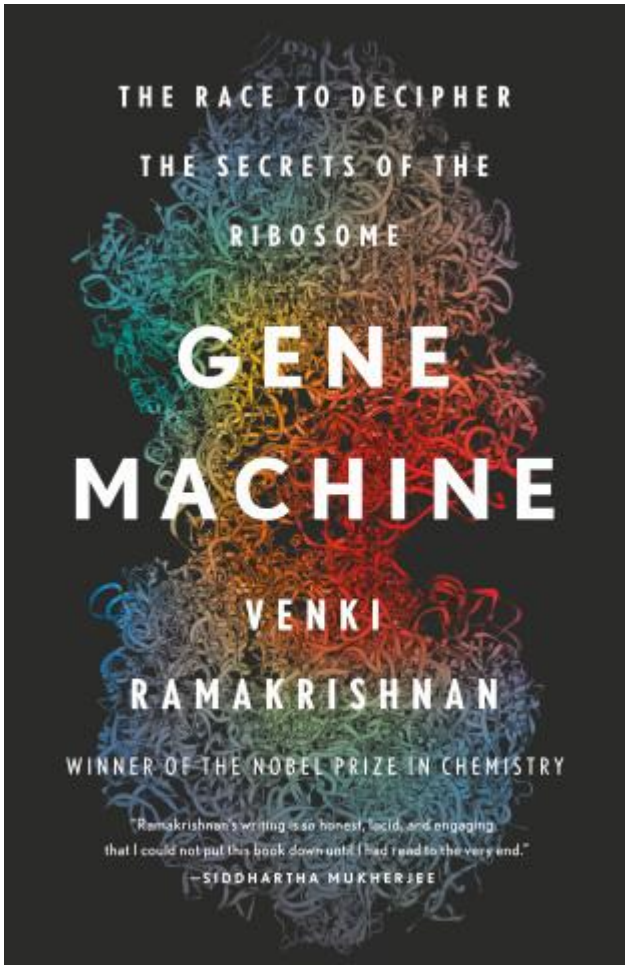
2014 - Structures of mitochondrial ribosomes



Atomic structure of the 30S Subunit from *Thermus thermophilus*. Proteins are shown in blue and the single RNA strand in orange.It is found by MRC Laboratory of Molecular Biology in Cambridge, England.



Atomic structure of the 50S Subunit from *Haloarcula marismortui*. Proteins are shown in blue and the two RNA strands in orange and yellow.[13] The small patch of green in the center of the subunit is the active site.


THE RACE TO DECIPHER
THE SECRETS OF THE
RIBOSOME
GENE
MACHINE
VENKI
RAMAKRISHNAN
WINNER OF THE NOBEL PRIZE IN CHEMISTRY
"Ramakrishnan's writing is so honest, lucid, and engaging
that I could not put this book down until I had read to the very end."
—SIDDHARTHA MUKHERJEE

Electron microscopy

EM's main strength is its ability to visualize large, mobile complexes - there is no upper size limit

Analysis of mobile complexes is possible - classifying different conformations of molecules

For cryo-EM, resolutions of up to 1.0 Å are achieved

EM has proven to be a very useful tool for studying ribosomes, especially their movements in the peptide bond synthesis cycle

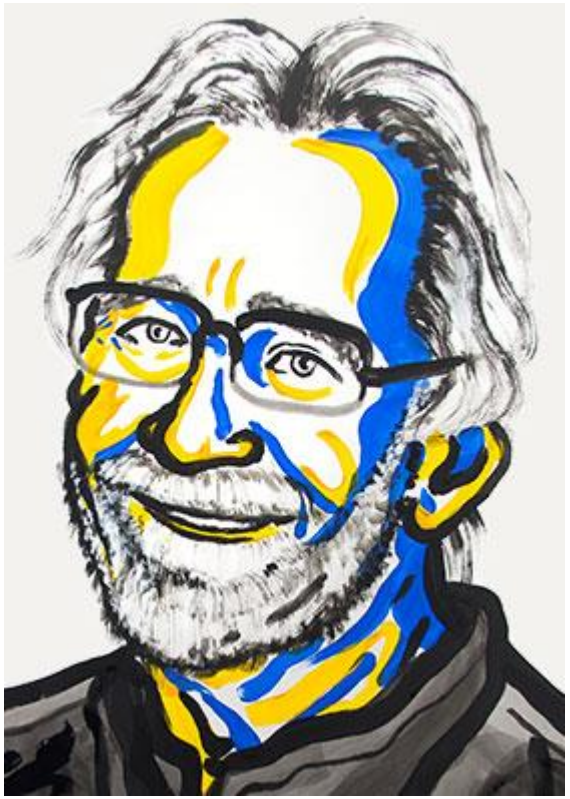
Hybrid methods - a combination of low-resolution EM and high-resolution crystallographic structures - can be particularly useful

EM revolution since 2012

2017 Nobel Prize in Chemistry

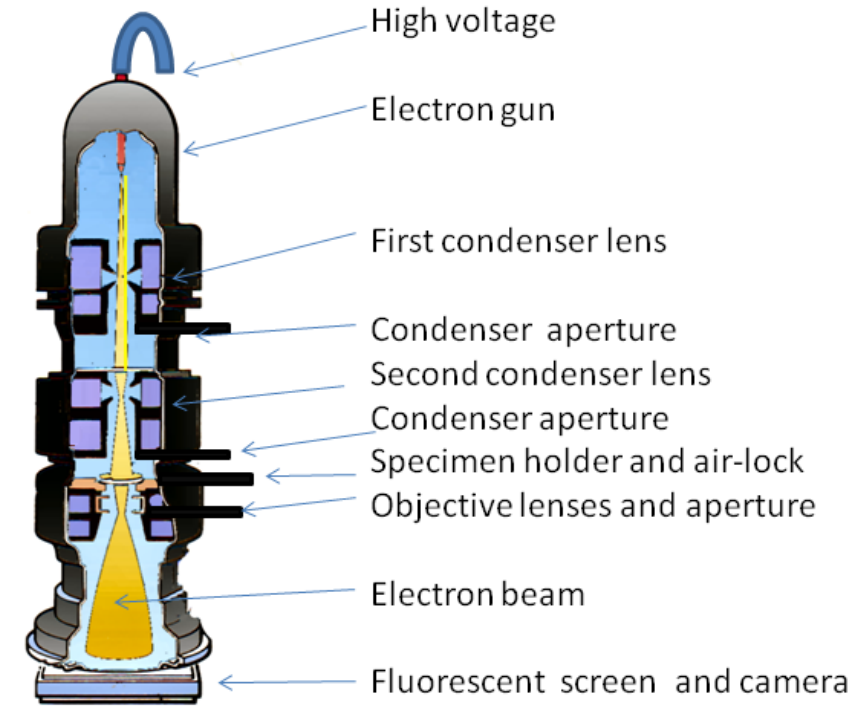
Richard Henderson

Joachim Frank



Jacques Dubochet


TITAN KRIOS

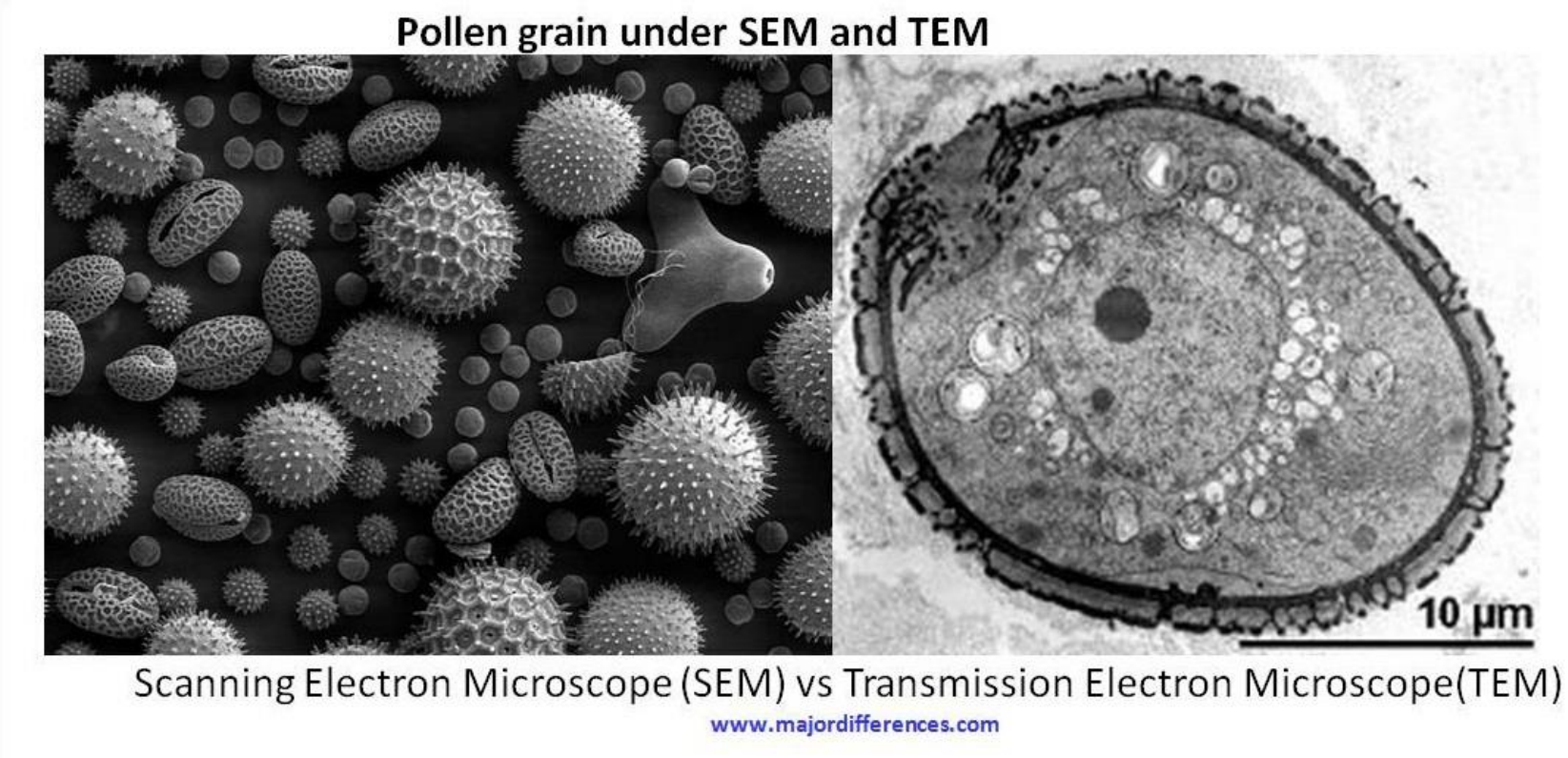


Transmission Electron Microscope

TEM microscope at 300 kV – corresponding wavelength 0.2 Å

EM lenses are poor

TEM vs SEM

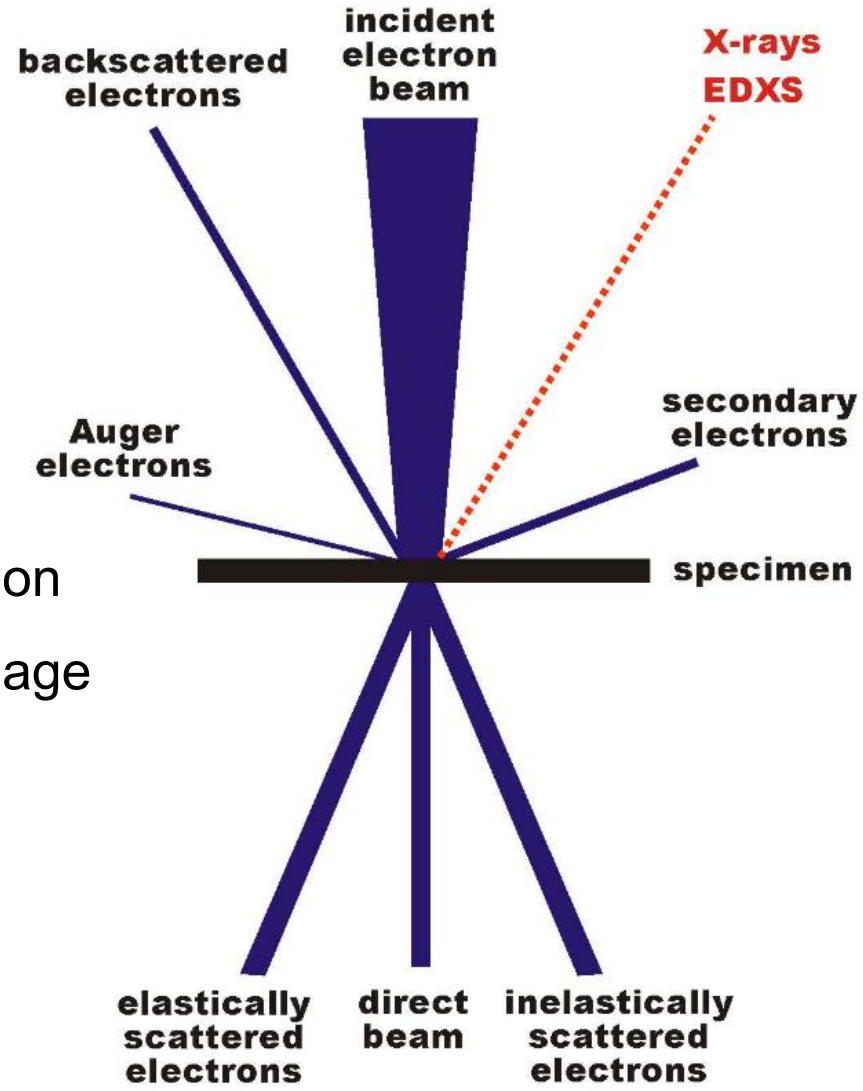


SEM (Scanning Electron Microscope):
- Based on scattered electrons
- surface and composition
- 3D shape
- Lower resolution

TEM (Transmission Electron Microscope):
- Based on transmitted electrons
- Internal structure/composition
- 2D projection
- Higher resolution

Electrons vs matter

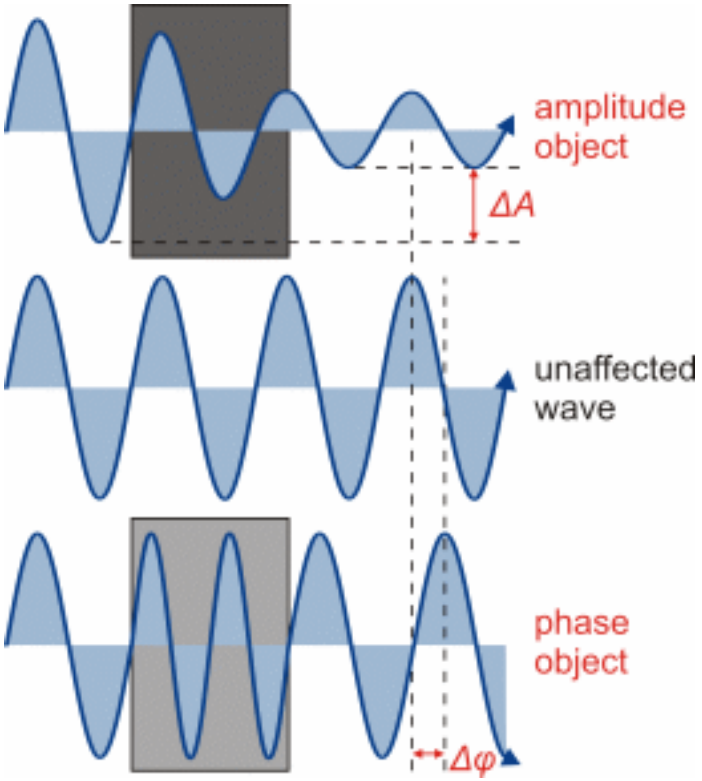
- Coulombic interactions
  - with electrons AND nucleus
- SEM:
  - secondary electrons
- TEM: electrons scattered:
  - elastically → image formation
  - inelastically → radiation damage
- Sources of contrast in TEM:
  - Amplitude contrast
  - Phase contrast



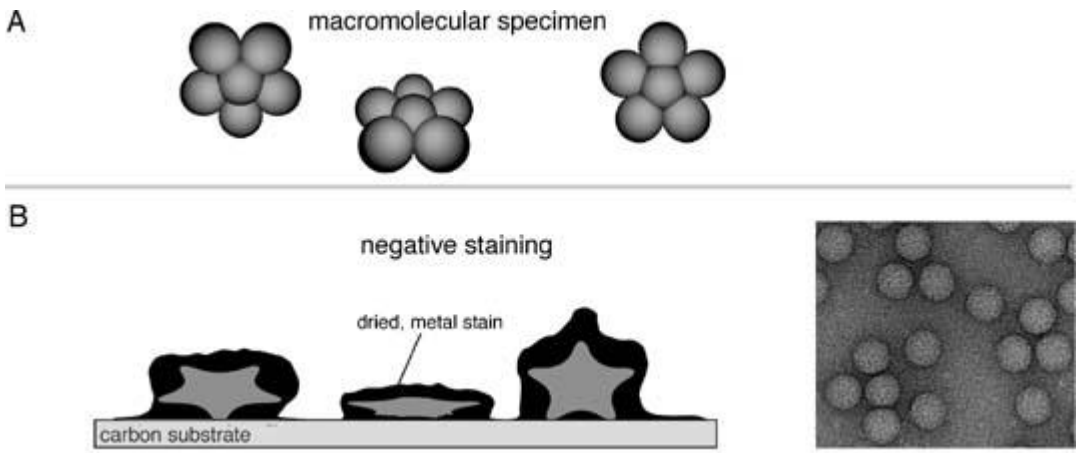
source: Frank Krumeich; Properties of Electrons, their Interactions with Matter and Applications in Electron Microscopy; www.microscopy.ethz.ch

Contrast – summary

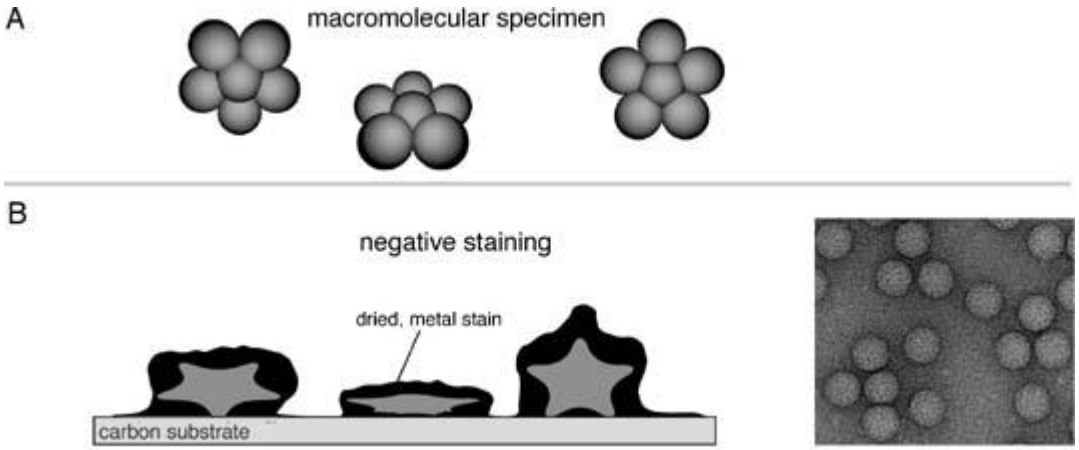
- Amplitude contrast (=mass/scattering contrast)
  - deflected electrons lead to regions of reduced amplitude
  - applicable to strongly scattering and thick samples
- Phase contrast
  - changes in the wavefront phases
- Detectors can only record amplitudes
  - in a in focus and perfect (aberration free) optical system phase objects are invisible to detectors
  - amplitude contrast can be achieved by introducing interferences in the wavefront
  - → defocus!!!



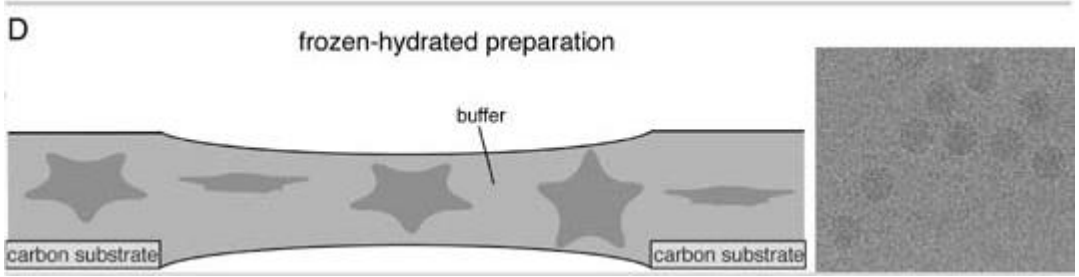
source: http://home.uni-leipzig.de/pwm/web/?section=introduction&page=phasecontrast



Negative stain

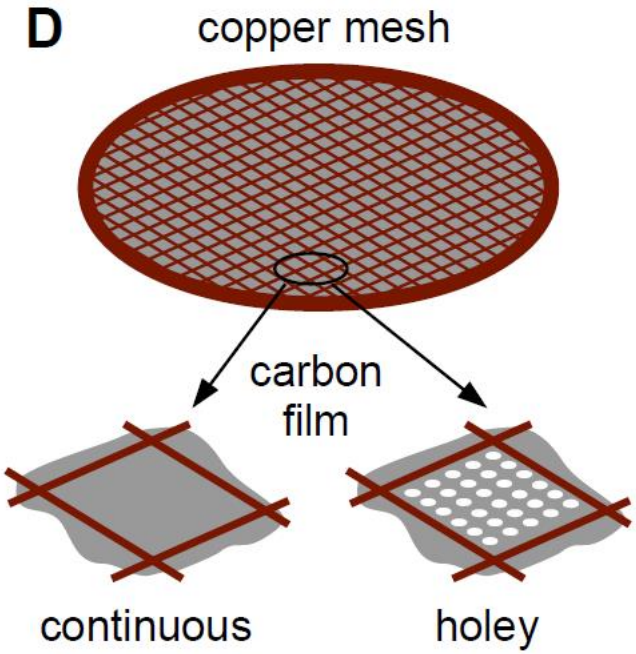


Negative stain

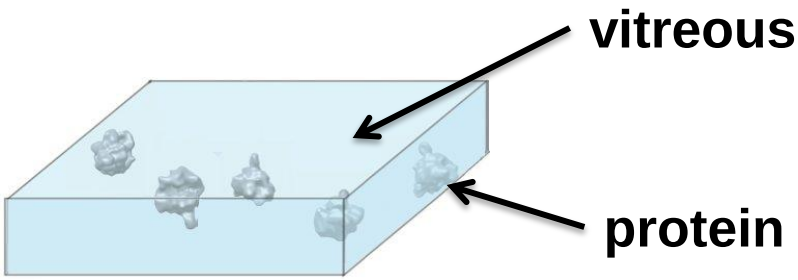


Cryo-EM

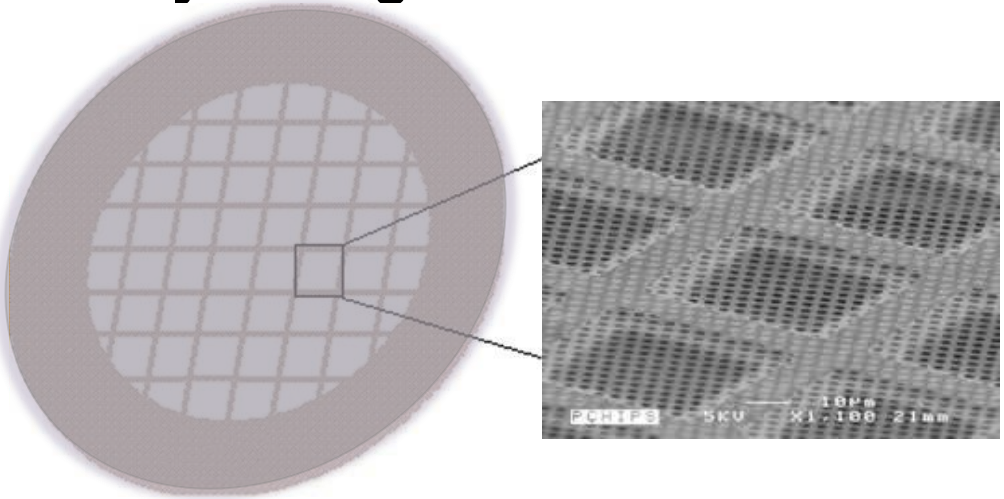

C
1
GROSZ


D
copper mesh
carbon
film
continuous
holey

Preparation of cryo-EM specimen



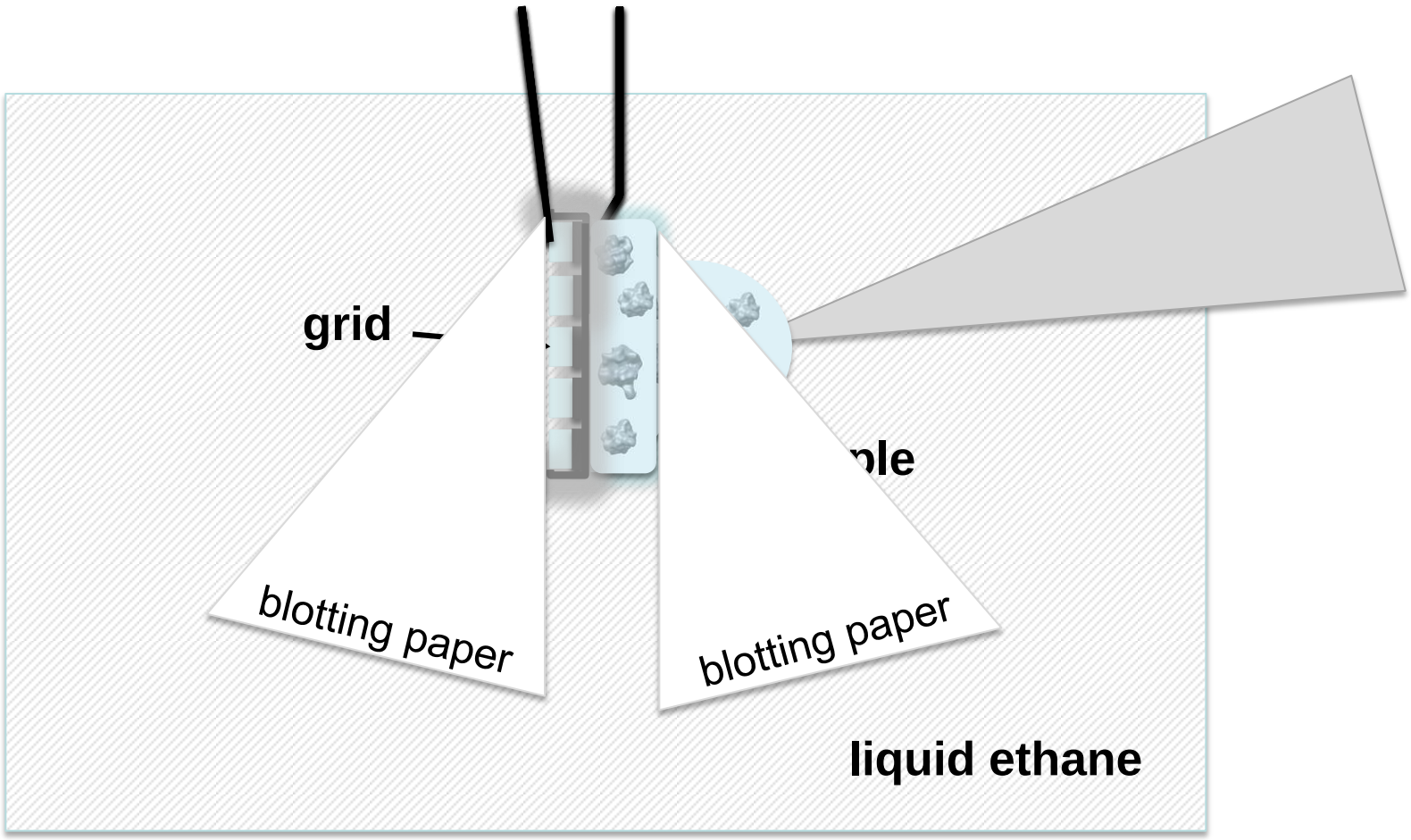
Cryo-EM grid



http://www.emsdiasum.com

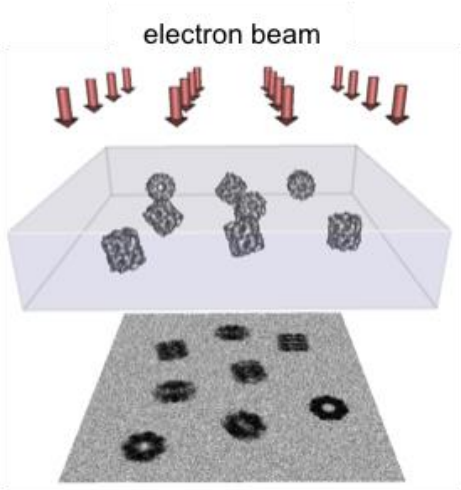
Ania Piasecka

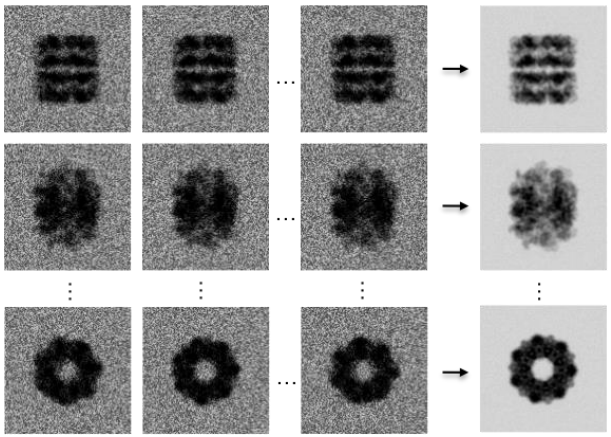
Cryo-EM specimen preparation



SPR – principles

- TEM images: projections of particles
- What kind of projections do we need?
  - many projections of identical particles with different, known orientations





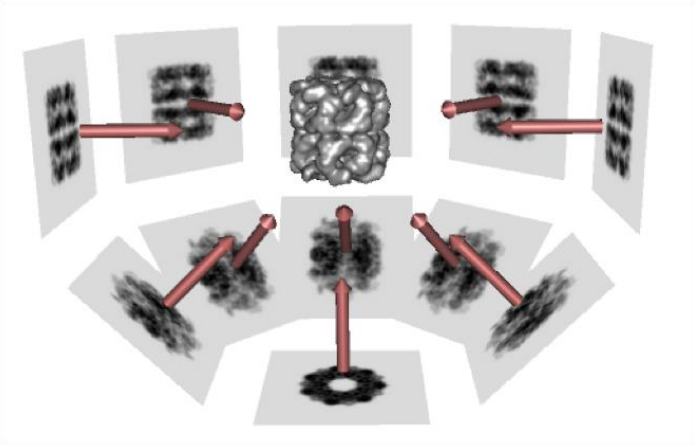
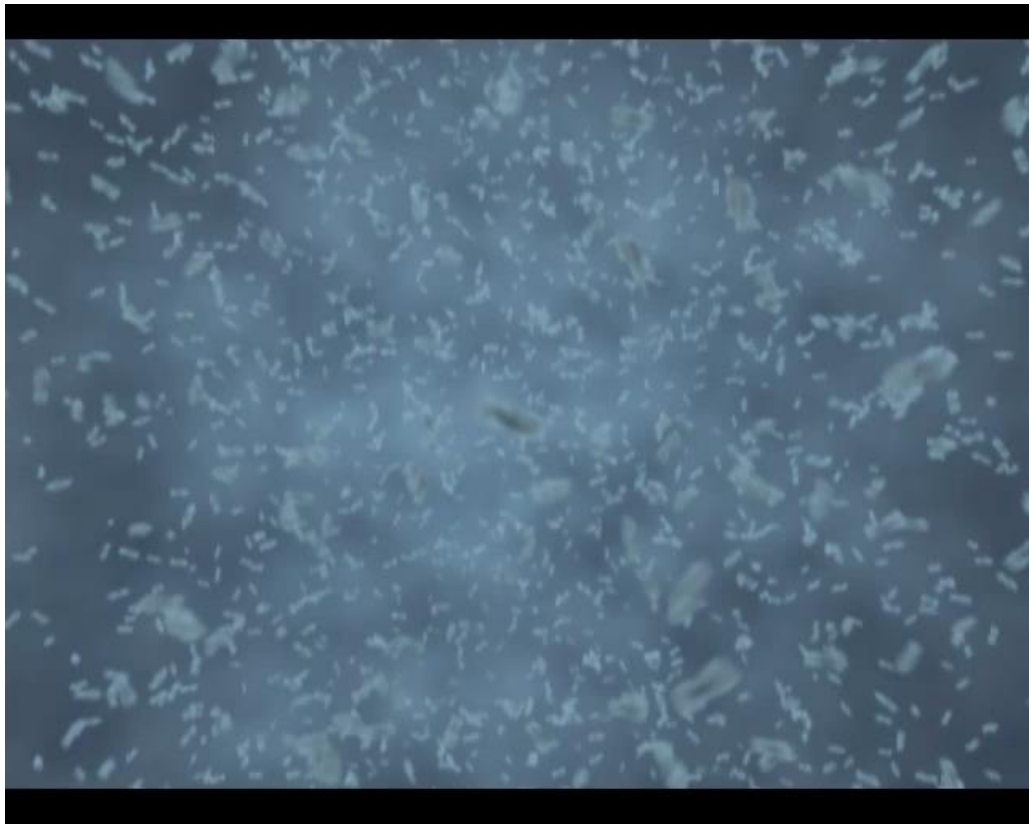
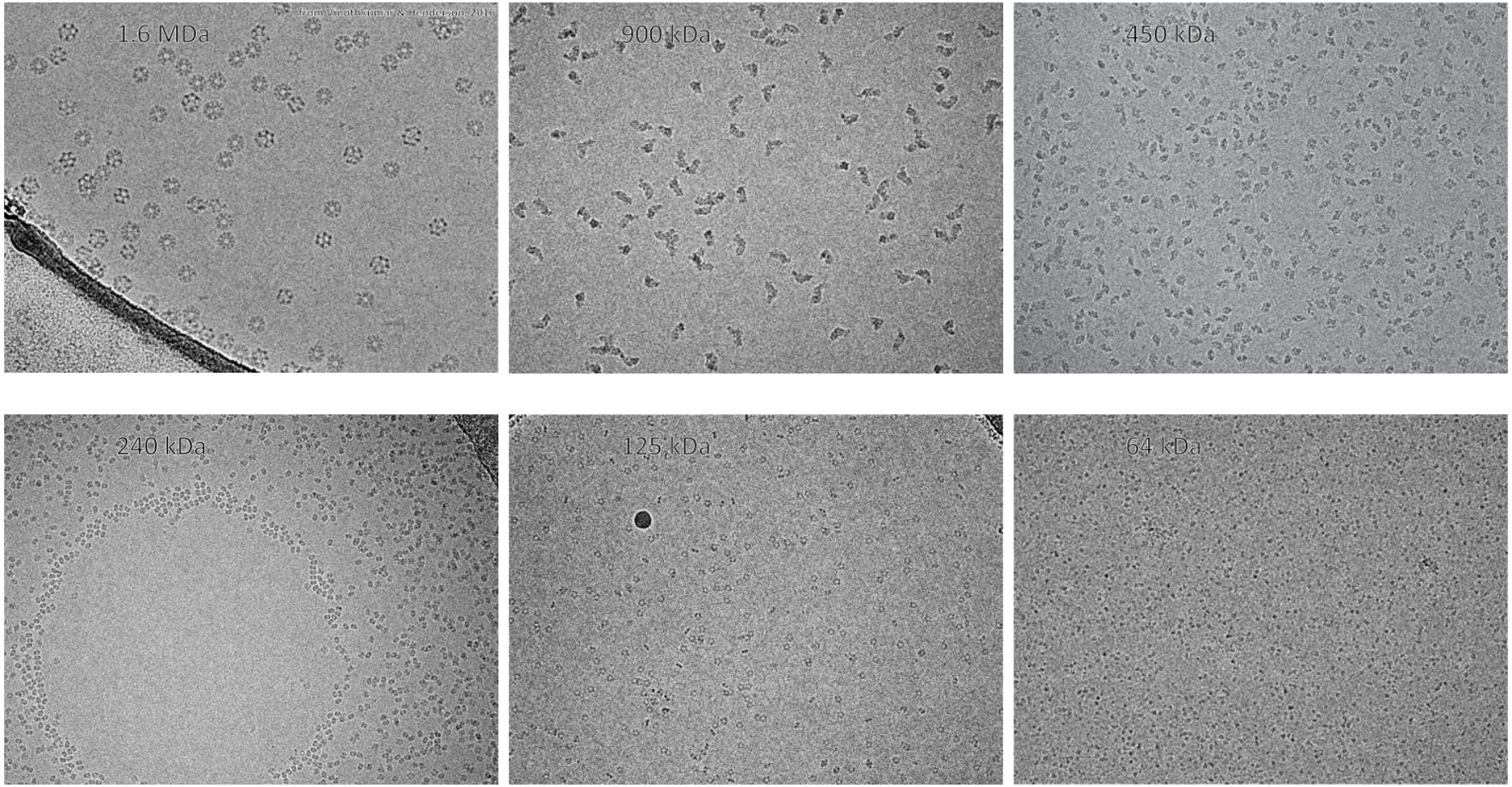


Image source: https://people.csail.mit.edu/gdp/cryoem.html



SPR – size limits

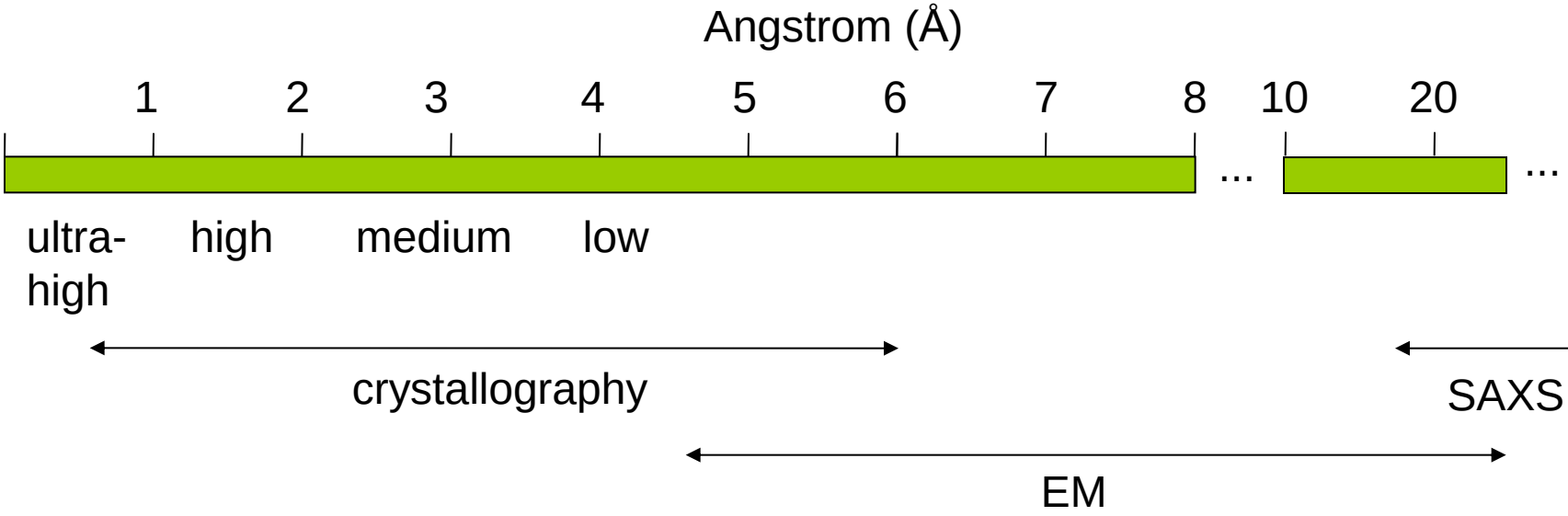


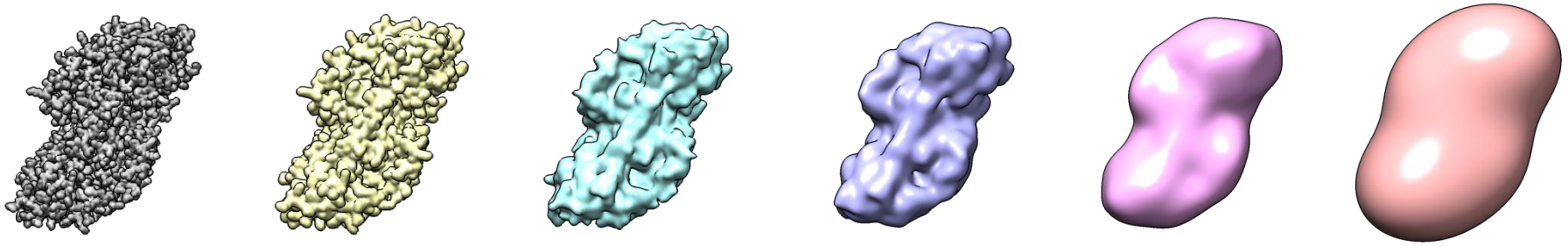
source: Cryo-EM17 Lecture 01 Past Present Future; Richard Henderson, MRC Lab, 2017

PROBLEMS:

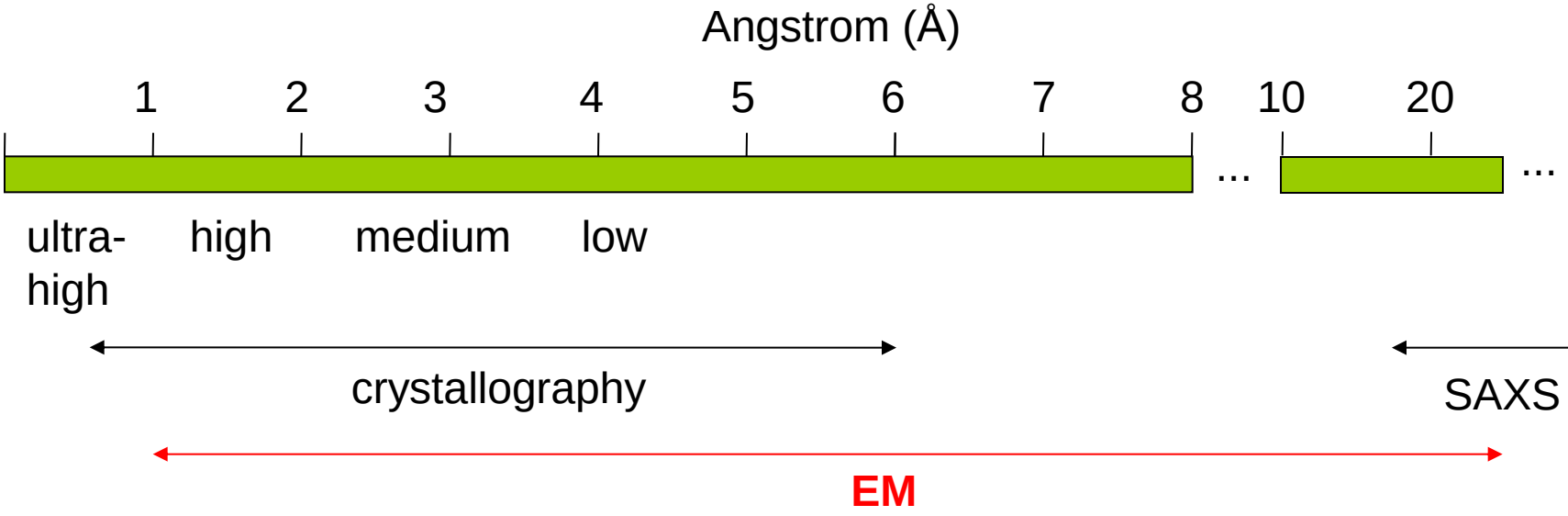
- Low contrast of biological samples
- Radiation damage
- Sample vibrations

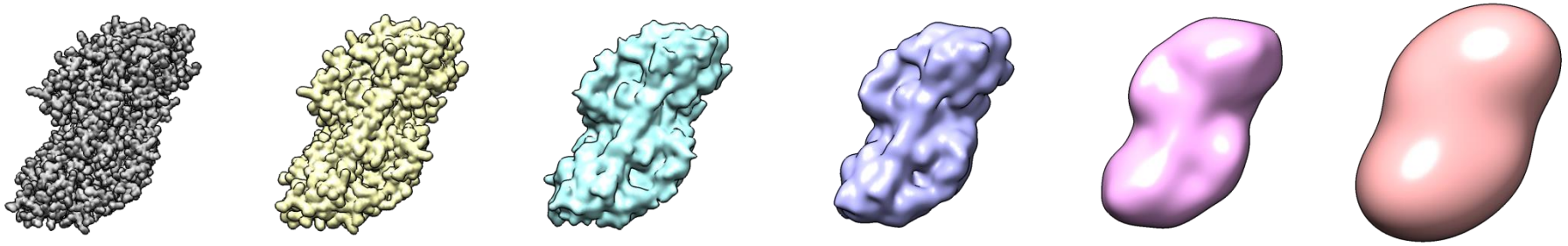
Resolution


Angstrom (Å)
1
2
3
4
5
6
7
8
10
20
...
...
ultra-high
high
medium
low
crystallography
SAXS
EM

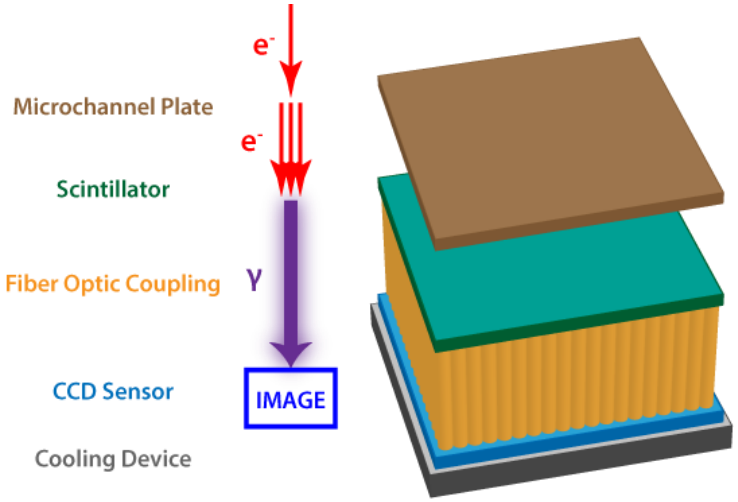

2.5 Å
4 Å
8 Å
12 Å
25 Å
50 Å

Resolution

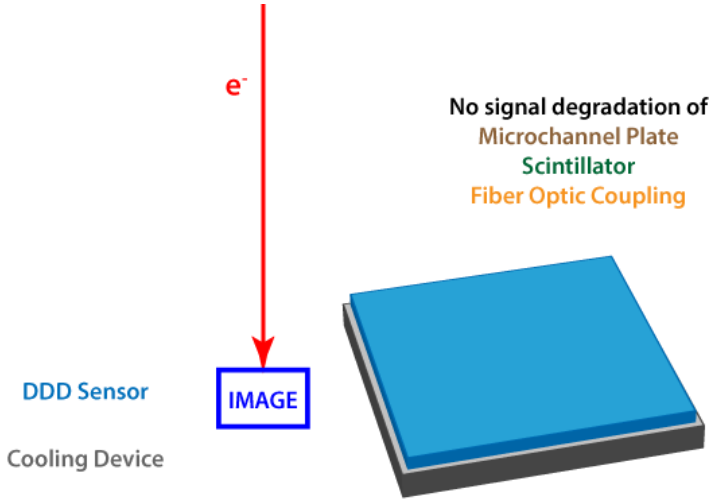

Angstrom (Å)
1
2
3
4
5
6
7
8
10
20
...
...
ultra-high
high
medium
low
crystallography
SAXS
EM


2.5 Å
4 Å
8 Å
12 Å
25 Å
50 Å

Direct detectors



MCP + CCD

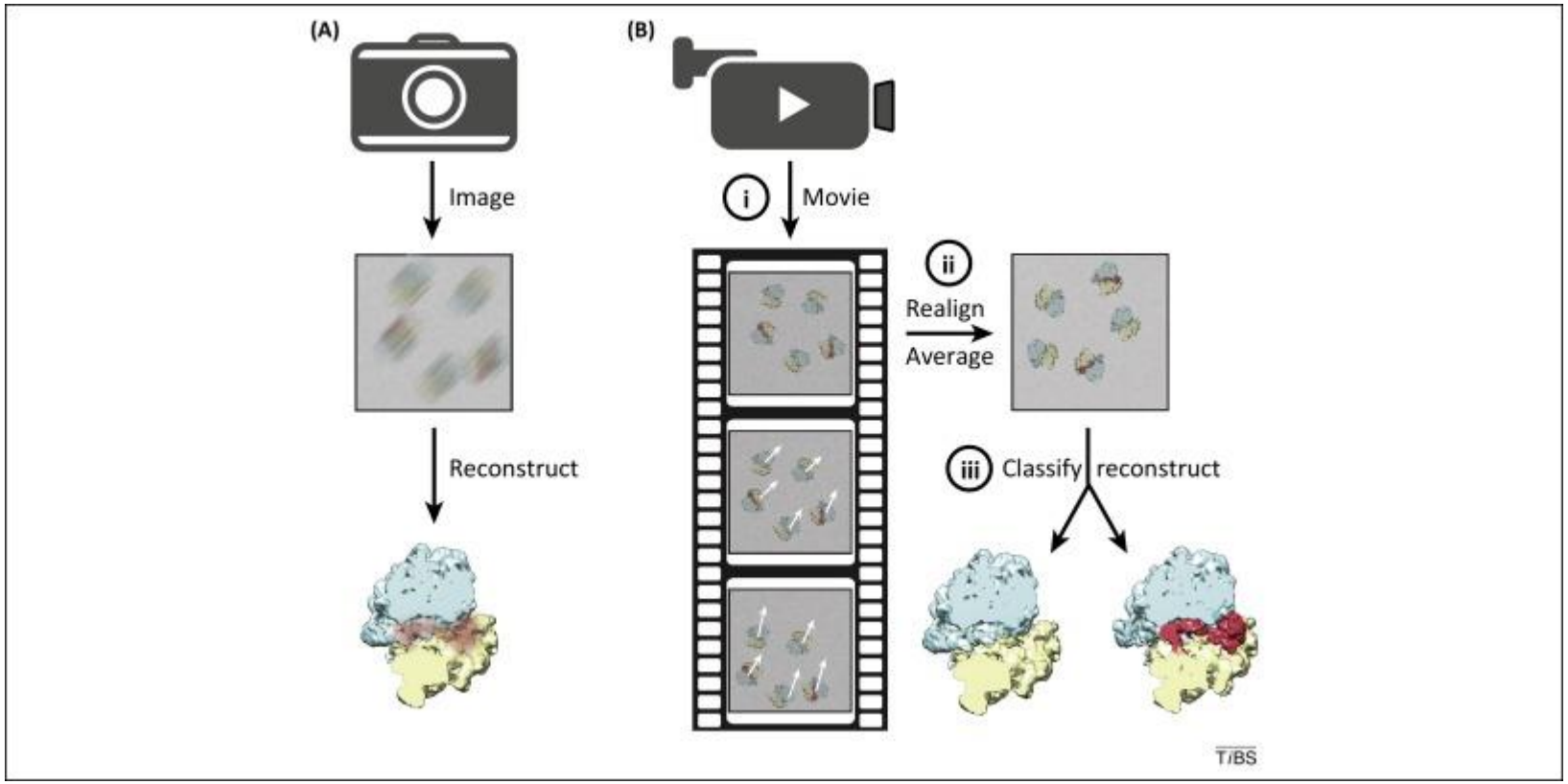


Direct Detection Camera

PROBLEMS:

- Radiation damage
- Sample vibrations

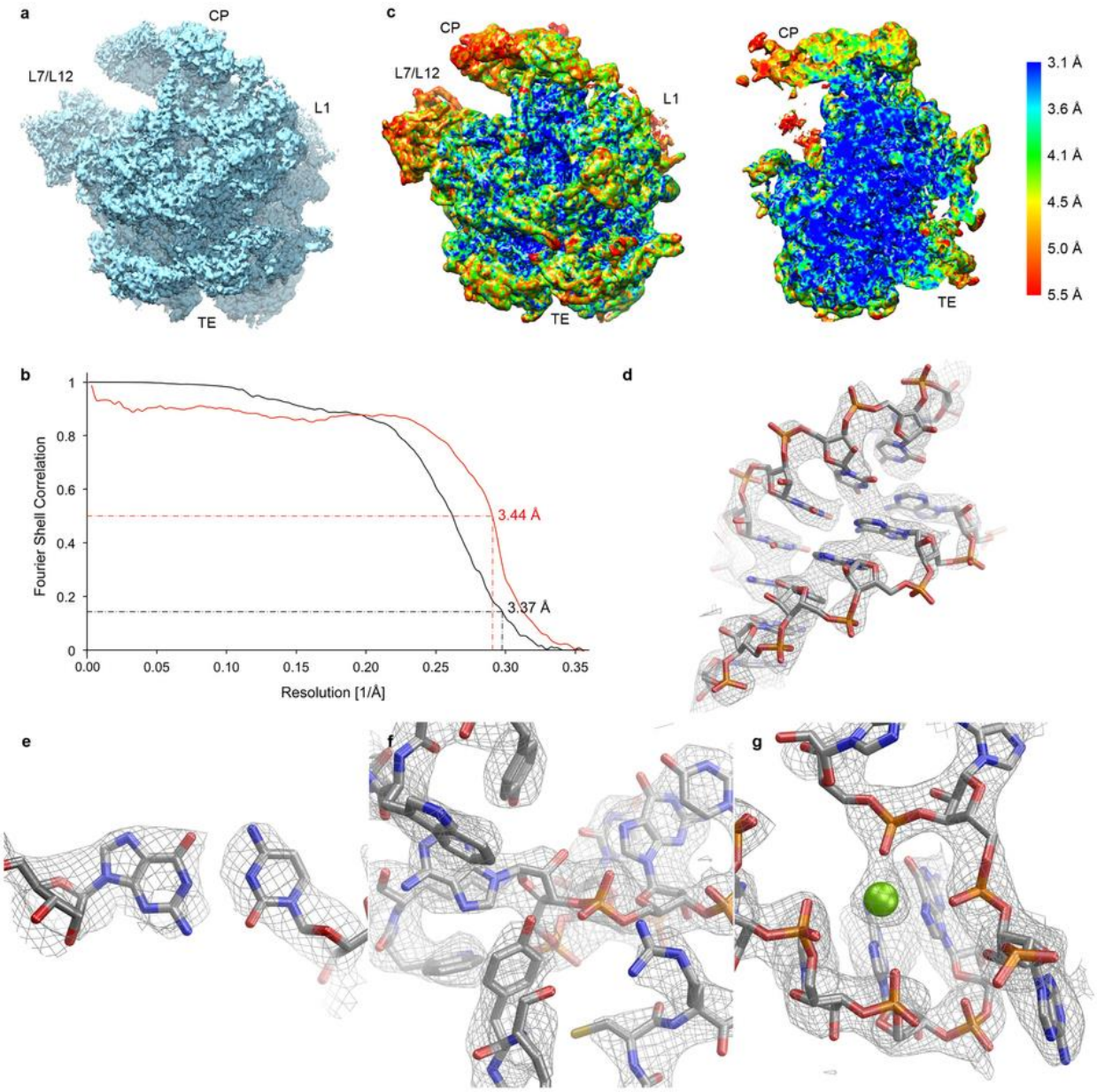
MOVIES



Software packages

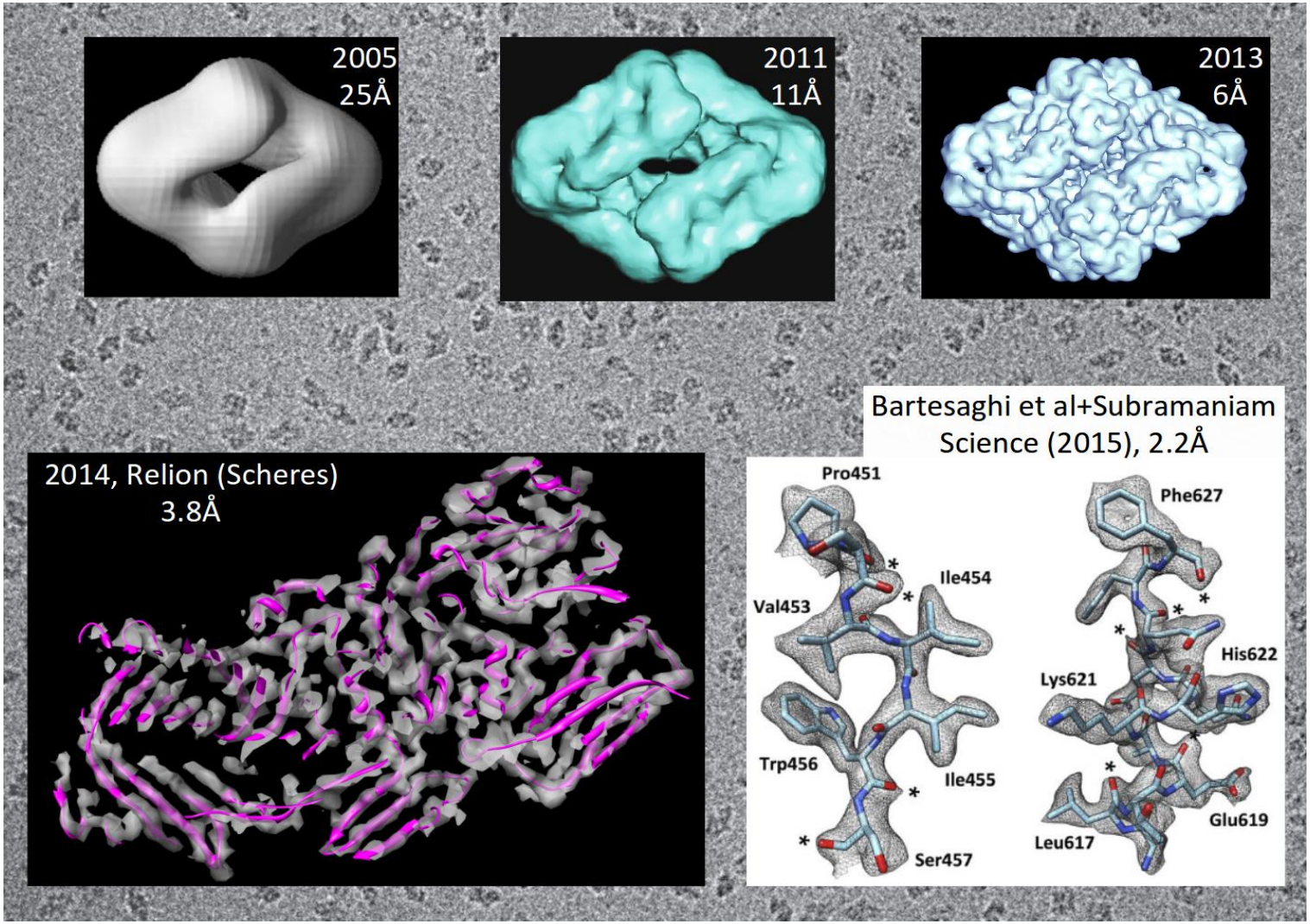
- CryoSPARC (very fast commercial software)
- cisTEM (Computational Imaging System for Transmission Electron Microscopy)
- SIMPLE (Single-particle IMage Processing Linux Engine)
- RELION REgularized LIkelihood OptimizatioN

source: https://www.ebi.ac.uk/pdbe/emdb/statistics_software.html/
more info:
https://en.wikibooks.org/wiki/Software_Tools_For_Molecular_Microscopy
http://www.emdatabank.org/emsoftware.html

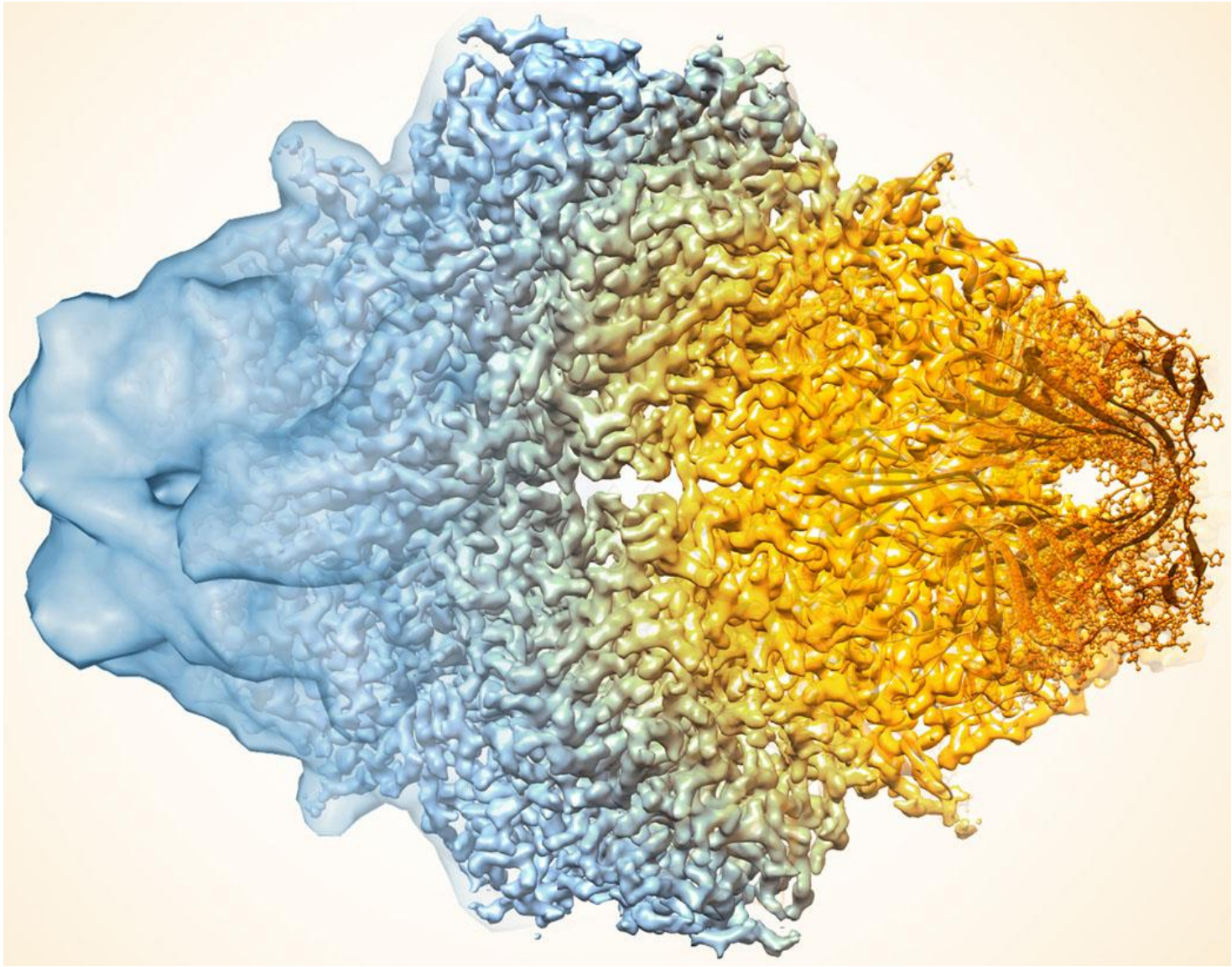


Basil J. Greber, et al. The complete structure of the large subunit of the mammalian mitochondrial ribosome
Nature 515, 283–286 (13 November 2014)

SPR – progress



source: Cryo-EM17 Lecture 01 Past Present Future; Richard Henderson, MRC Lab, 2017

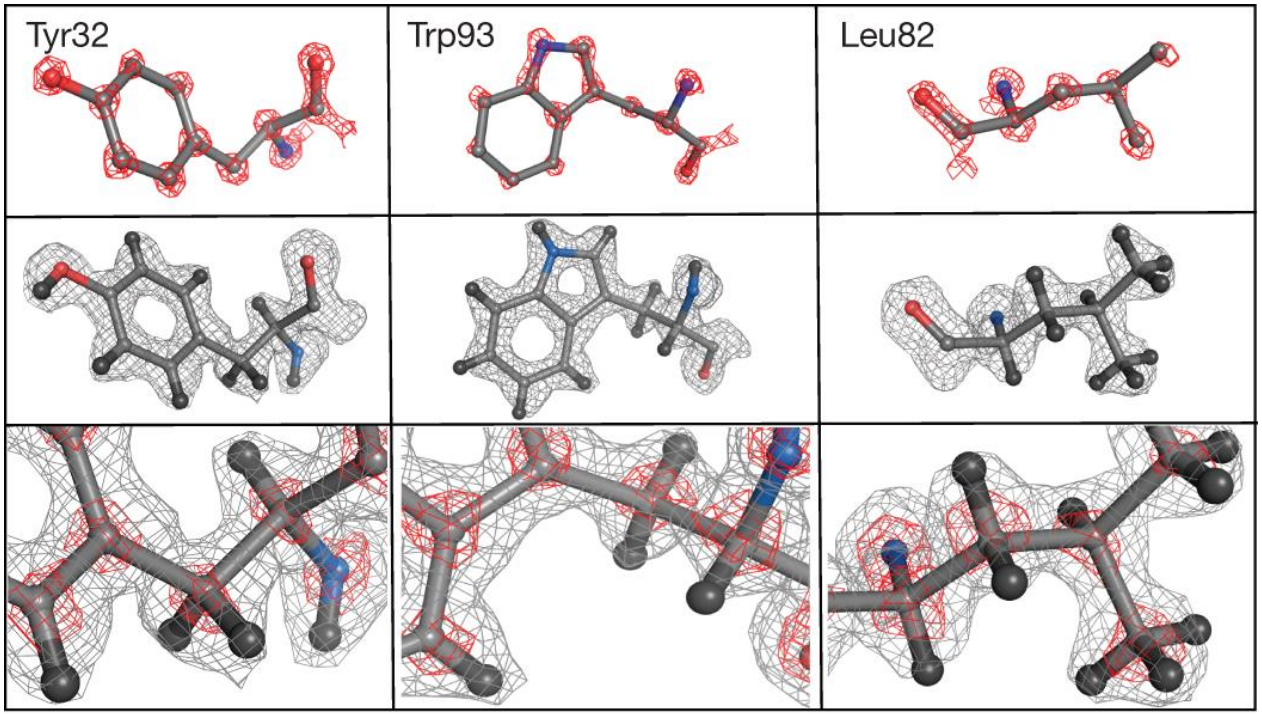


Article | Published: 21 October 2020

Atomic-resolution protein structure determination by cryo-EM

Ka Man Yip, Niels Fischer, Elham Paknia, Ashwin Chari & Holger Stark

Nature **587**, 157–161 (2020) | Cite this article



Title: Translation dynamics in human cells visualized at high-resolution reveal cancer drug action

Authors: Huaipeng Xing[1,2], Reiya Taniguchi[1], Iskander Khusainov[1], Jan Philipp Kreysing[1,3], Sonja Welsch[4], Beata Turoňová[1], Martin Beck[1]*

Affiliations:

[1]Department of Molecular Sociology, Max Planck Institute of Biophysics, 60438 Frankfurt am Main, Germany.

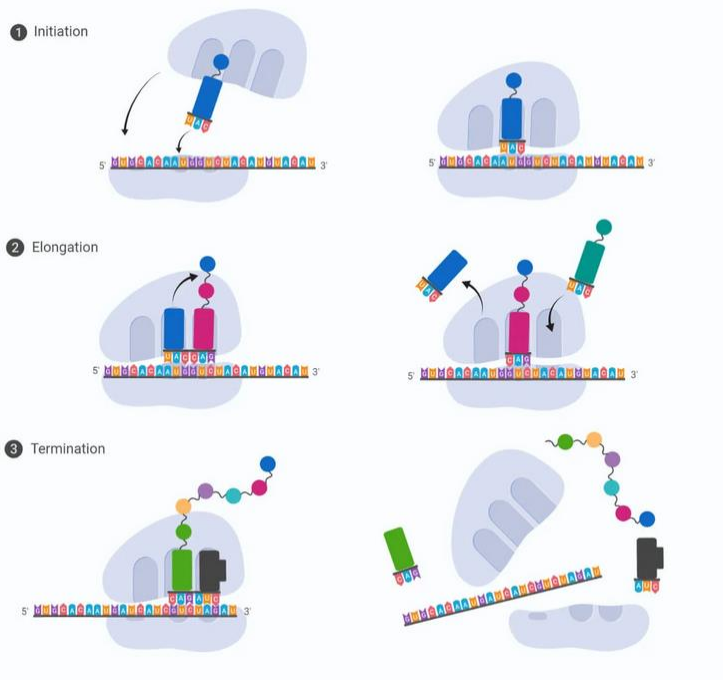
[2]Faculty of Biochemistry, Chemistry and Pharmacy, Goethe University Frankfurt am Main, 60438 Frankfurt am Main, Germany.

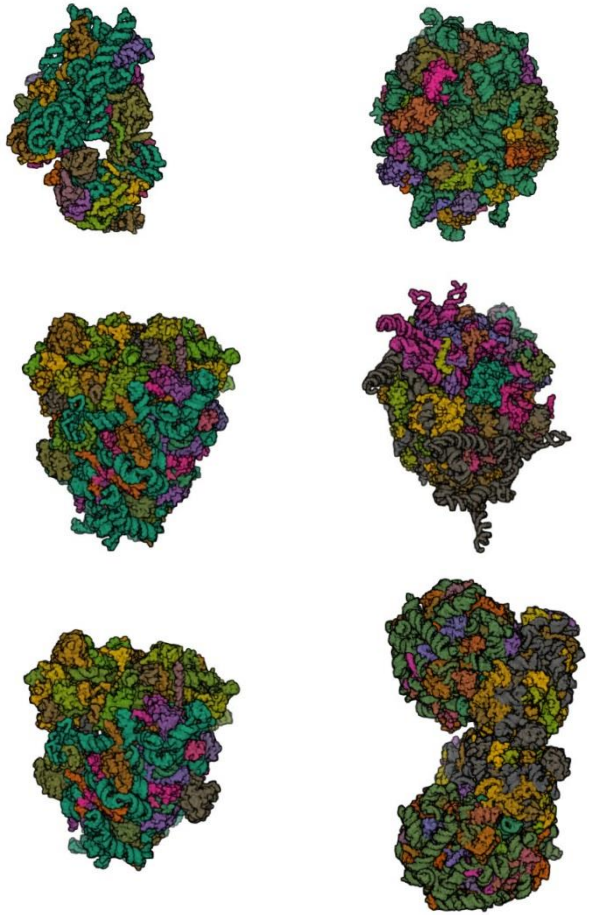
[3]IMPRS on Cellular Biophysics, 60438 Frankfurt am Main, Germany

[4]Central Electron Microscopy Facility, Max Planck Institute of Biophysics, 60438 Frankfurt am Main, Germany.

*Corresponding author. Email: martin.beck@biophys.mpg.de

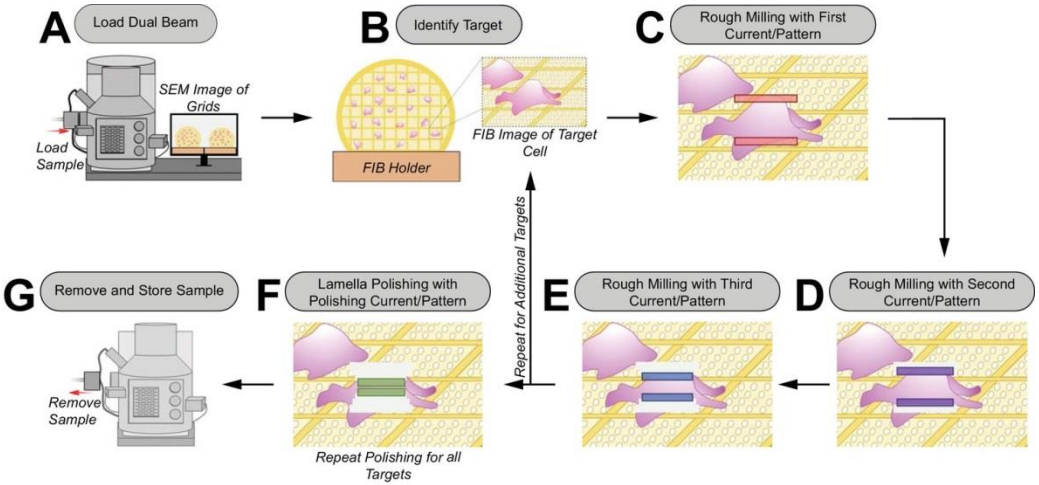
Translation cycle

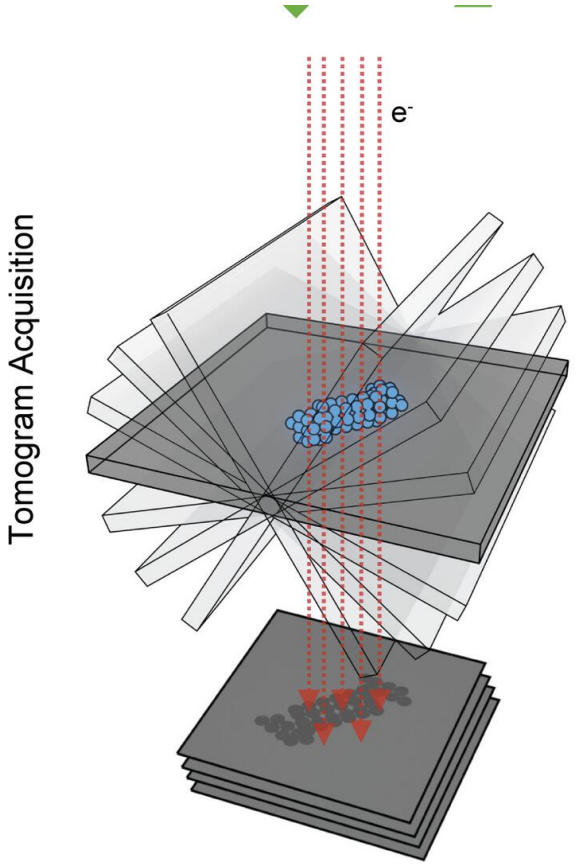




Methodology

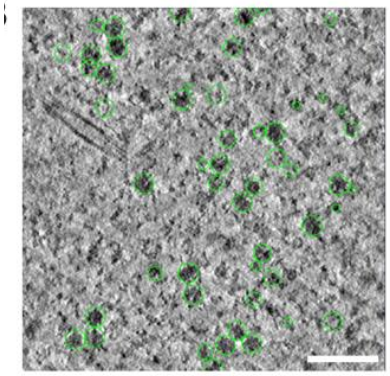
cryo-focused ion beam (cryo-FIB) milling



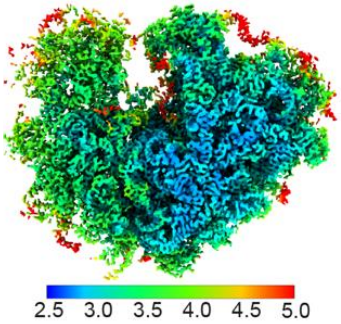


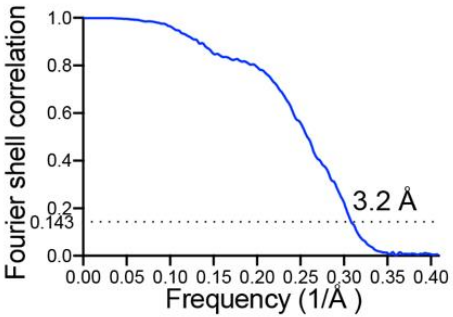
35 cells --- 358 tomograms

Overall ribosome structure

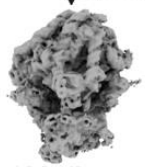


Initial ribosomal candidates (1080 particles)





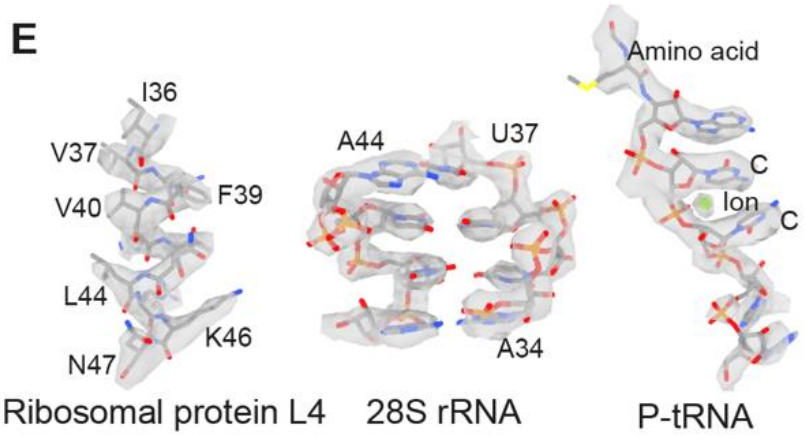
358 tomograms
↓ Template matching
286,400 sub-tomograms
↓ 3D classification and refine in RELION



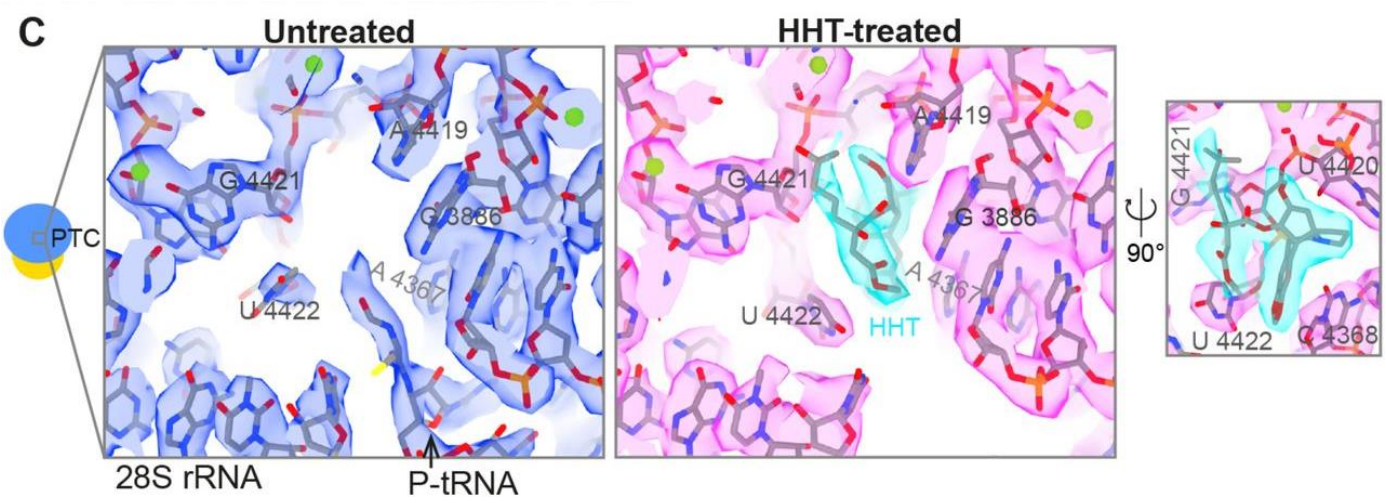
Refine in RELION and M

39.402 ribosomes

3.2 Å

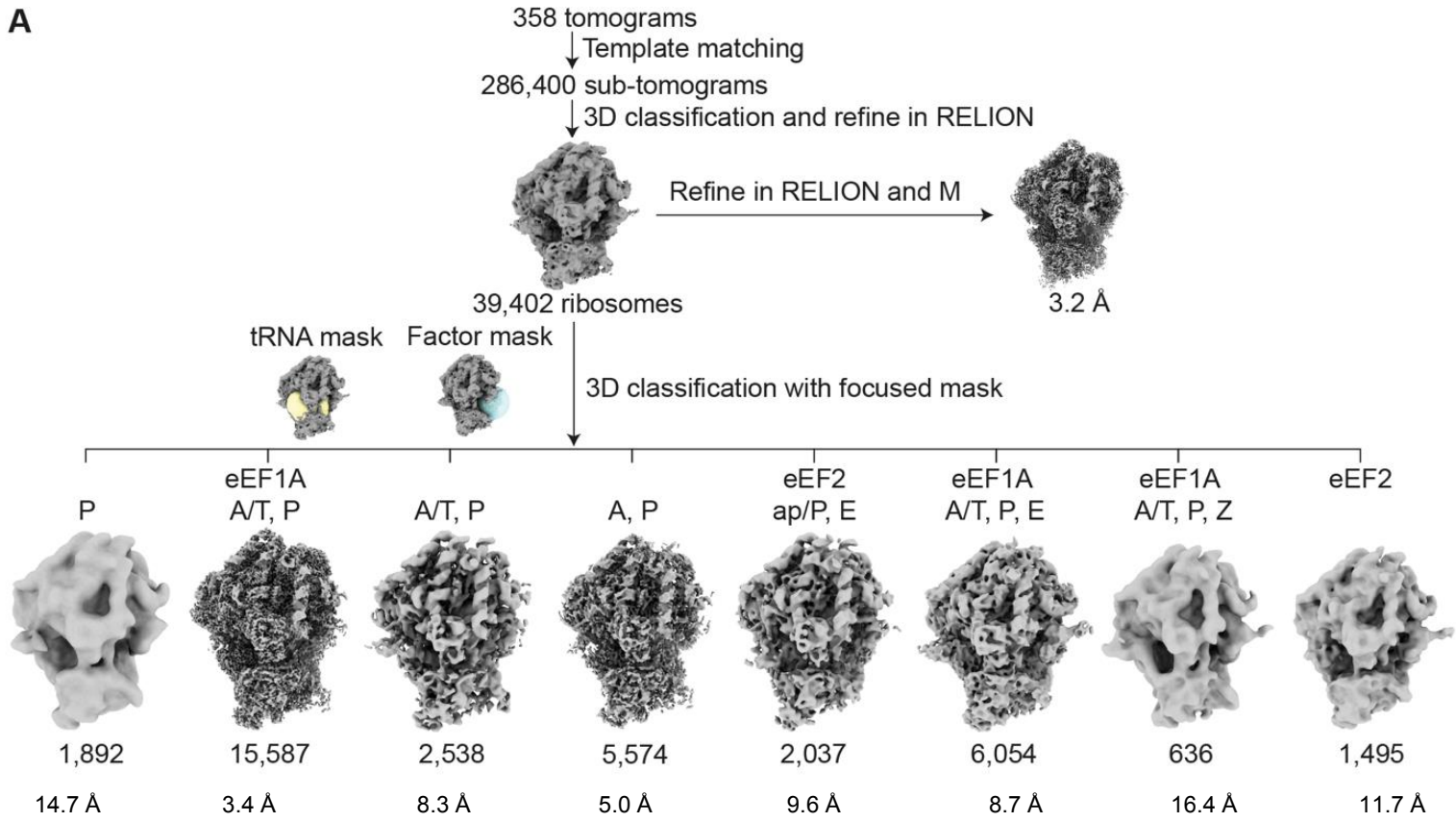


Peptidyl transferase center (PTC)

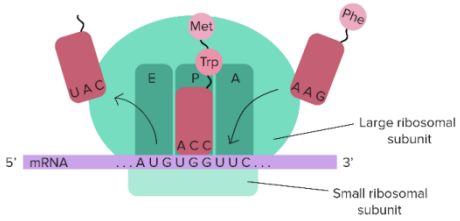


- HHT (homoharringtonine) - natural compound that binds to the ribosome and inhibits protein synthesis
  - drug for chronic myeloid leukemia
  - the exact mechanism of inhibition of protein synthesis not known

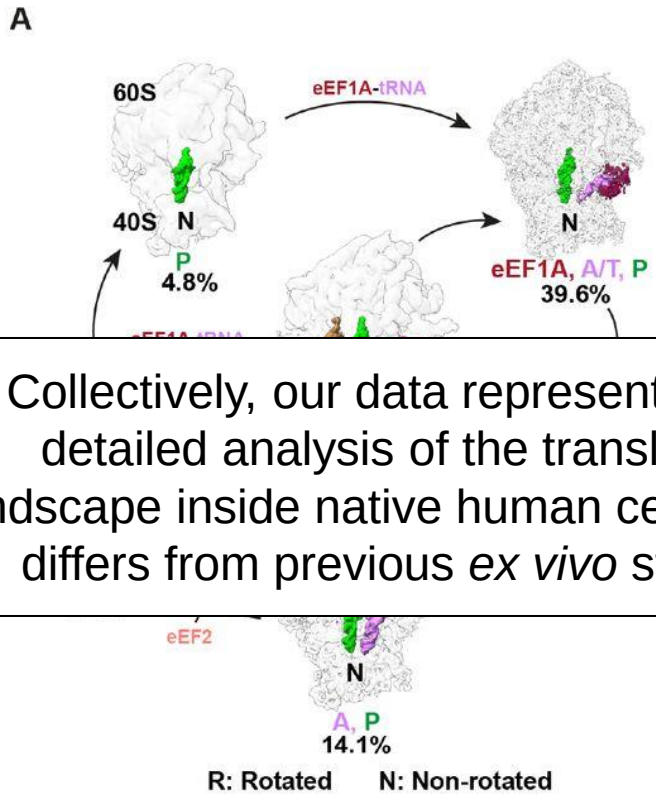
Translation elongation landscape in human cells

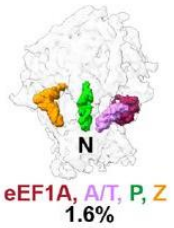


Translation elongation landscape in human cells



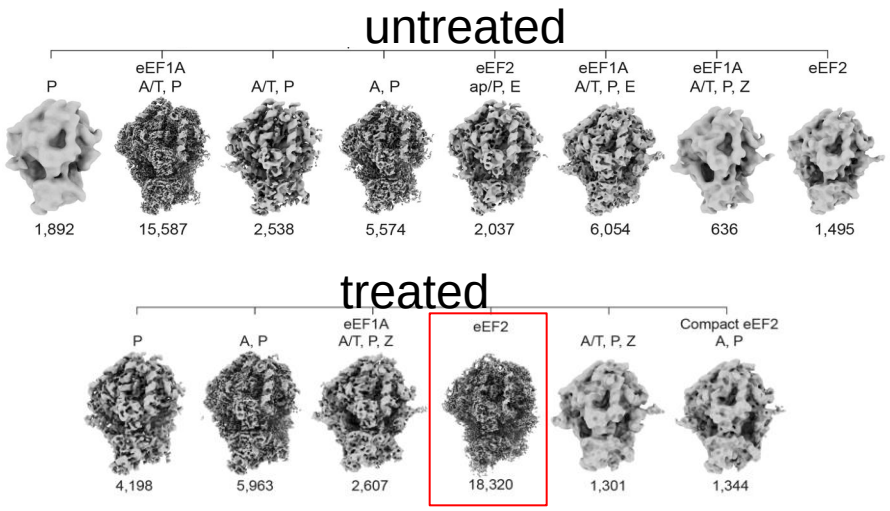
A – amino-acyl site
P – peptidyl site
E – exit site

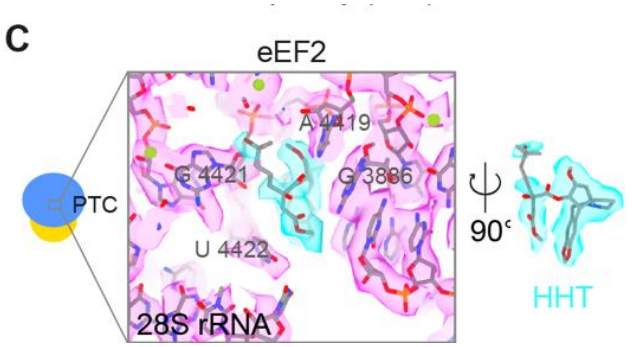


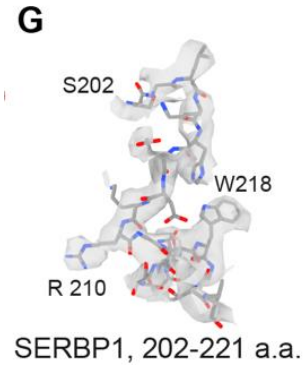


Collectively, our data represented the detailed analysis of the translation landscape inside native human cells, which differs from previous *ex vivo* studies

HHT modifies the translation elongation landscape



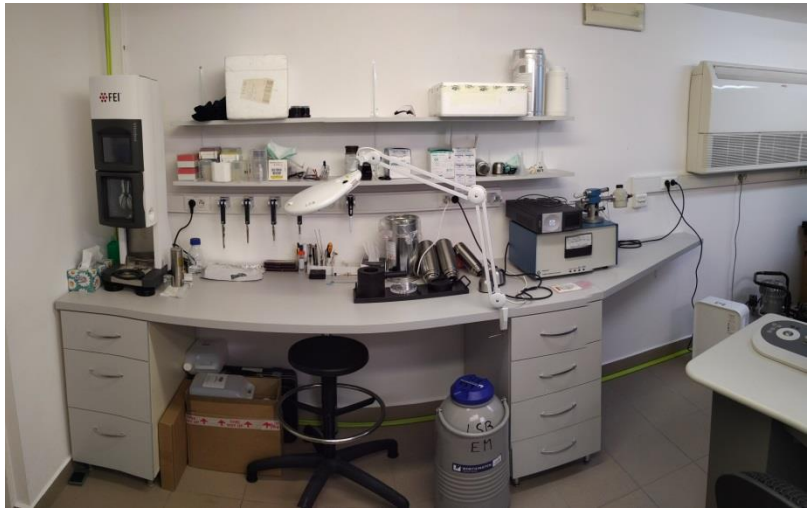


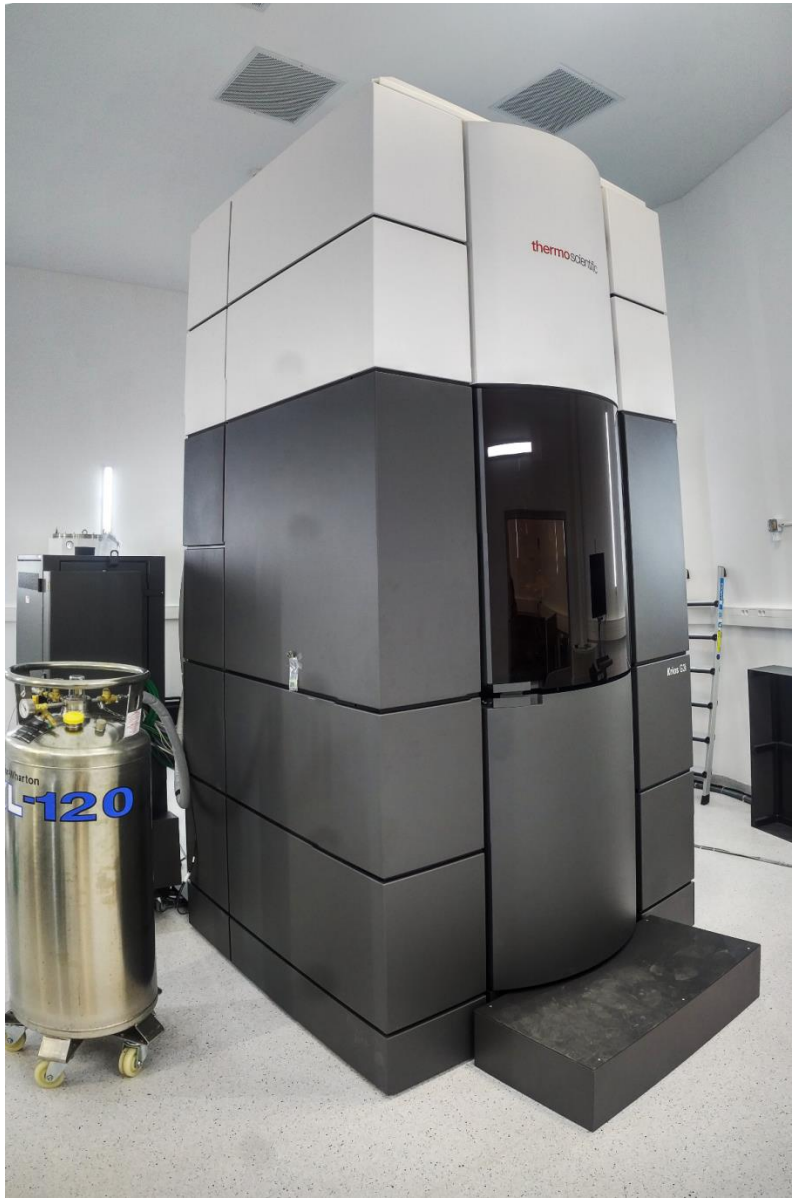


SERBP1(SERPINE1 mRNA-binding protein 1) – marker for hibernating ribosomes

HHT treatment results in the accumulation of ribosome hibernation, which may be representative of the mechanism of the drug action in cancer therapy.

Cryo-EM




thermoscientific
L-120

Protein Data Bank

www.pdb.org

PyMOL

http:// pymol.org

http://www.imb-jena.de/www_bioc/