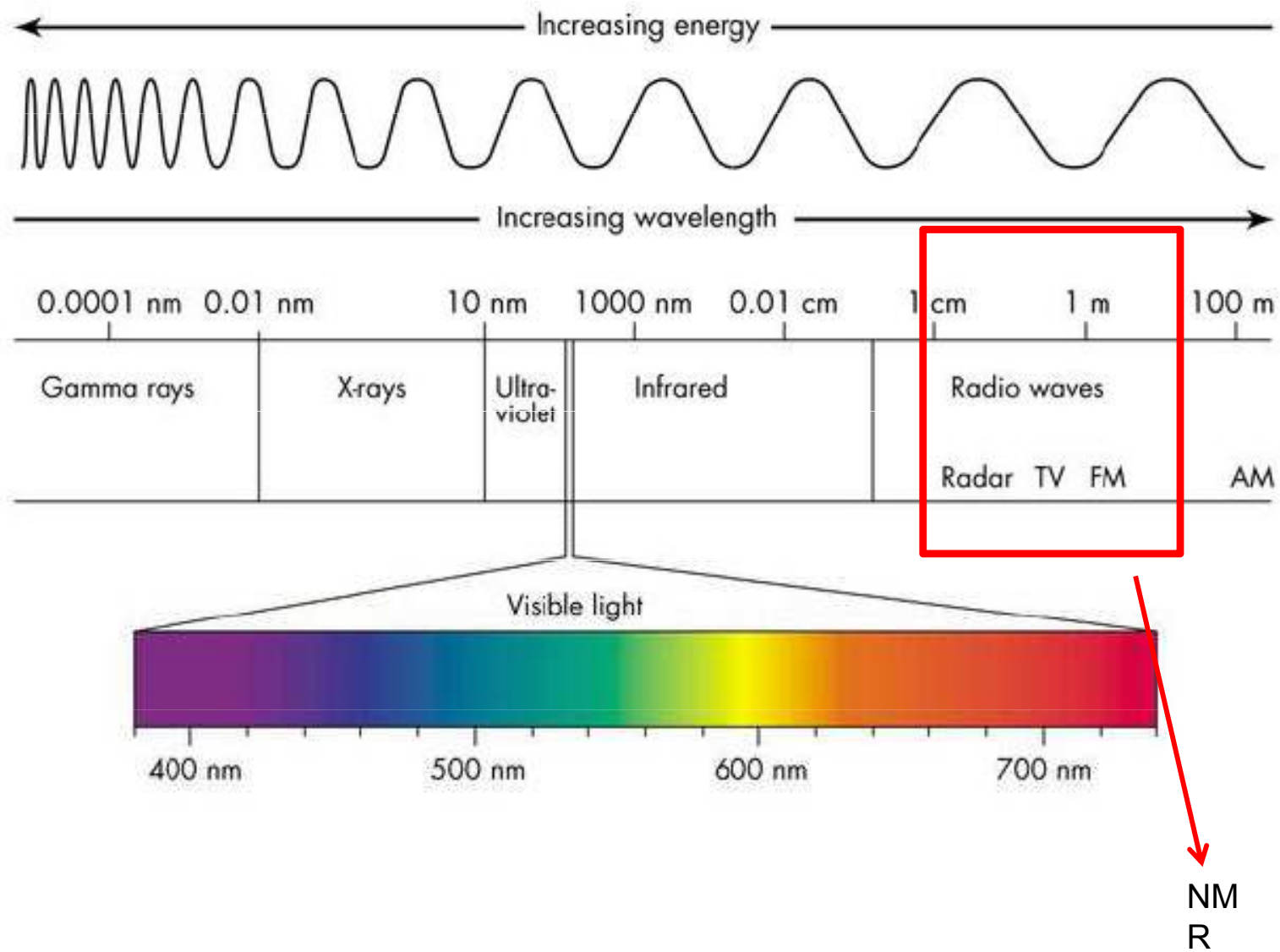


**doc. Mgr. Karel Kubíček, Ph.D.,**

**Ústav fyziky kondenzovaných látek,  
CEITEC MU,**

**e: [karelk77@gmail.com](mailto:karelk77@gmail.com)  
[20563@mail.muni.cz](mailto:20563@mail.muni.cz)**

**t: +420 549 49 3253**



# NMR

1) Jaderný spin  $\neq 0$  ( $^1\text{H}$ ,  $^{13}\text{C}$ ,  $^{15}\text{N}$ ,  $^{31}\text{P}$ )

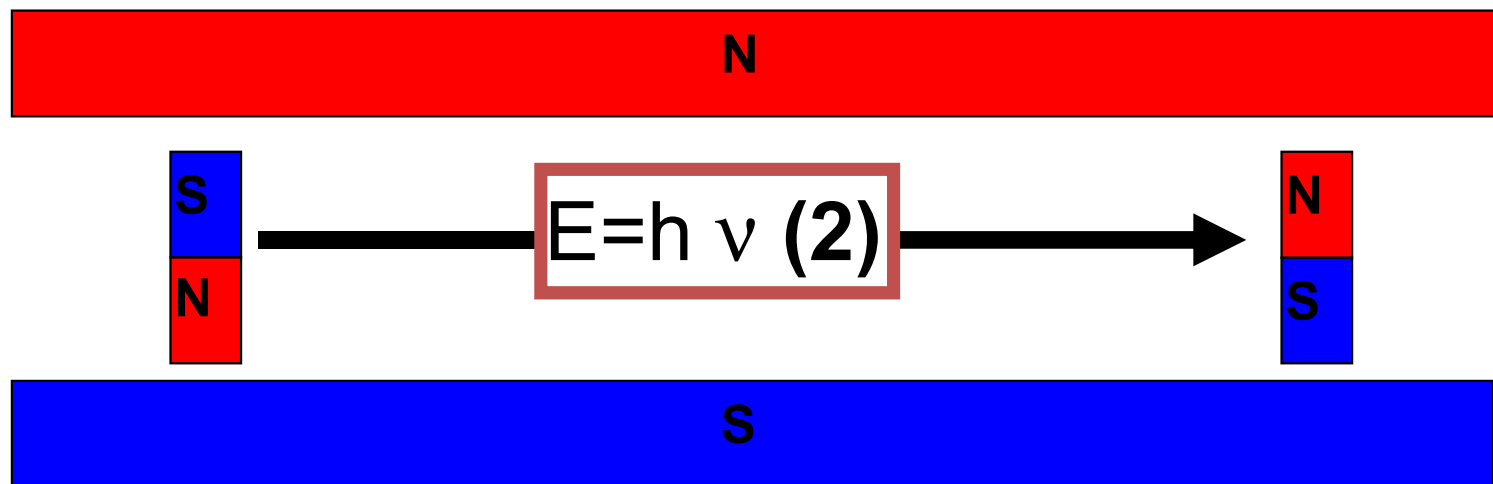
- počet neutronů **a** počet protonů jsou sudá čísla ( $^{12}\text{C}=6\text{p}+6\text{n}$ )  $\Rightarrow$  **nulový spin**

- počet neutronů **plus** počet protonů je liché číslo ( $^1\text{H}=\text{p}$ ,  $^{13}\text{C}=6\text{p}+7\text{n}$ )  $\Rightarrow$  **neceločíselný spin** (i.e.  $\frac{1}{2}$ ,  $\frac{3}{2}$ ,  $\frac{5}{2}$ )

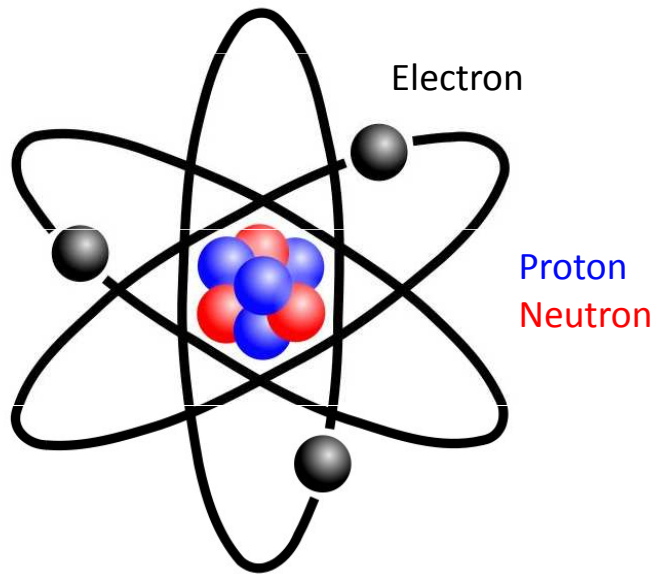
- počet neutronů **a** počet protonů jsou lichá čísla ( $^2\text{H}=\text{p}+\text{n}$ )  $\Rightarrow$  **celočíselný spin** (i.e. 1, 2, 3)

1)  $\nu = \gamma \cdot B$  (1) – pokud vložíme do magnetického pole intezity **B**, jádro mající nenulový spin může absorbovat foton frekvence  $\nu$ . Frekvence  $\nu$  závisí na gyromagnetickém poměru  $\gamma$  jader

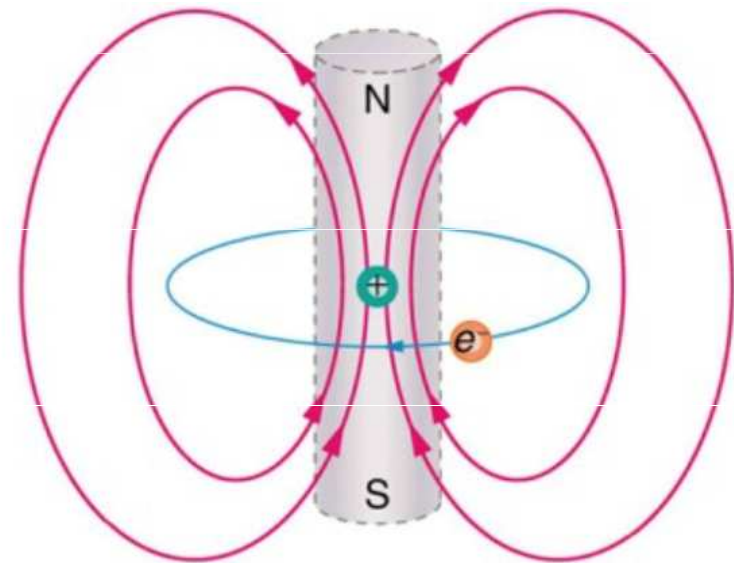
2) Z kvantové mechaniky víme, že spin  $I$  může nabývat  $2I + 1$  orientací  $\Rightarrow$  **jádro se spinem  $\frac{1}{2}$  může mít dvě orientace v externím magnetickém poli – nižší / vyšší energie**



# Atom



=



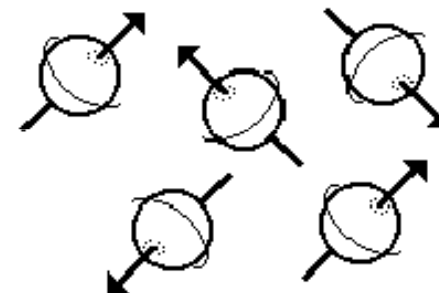
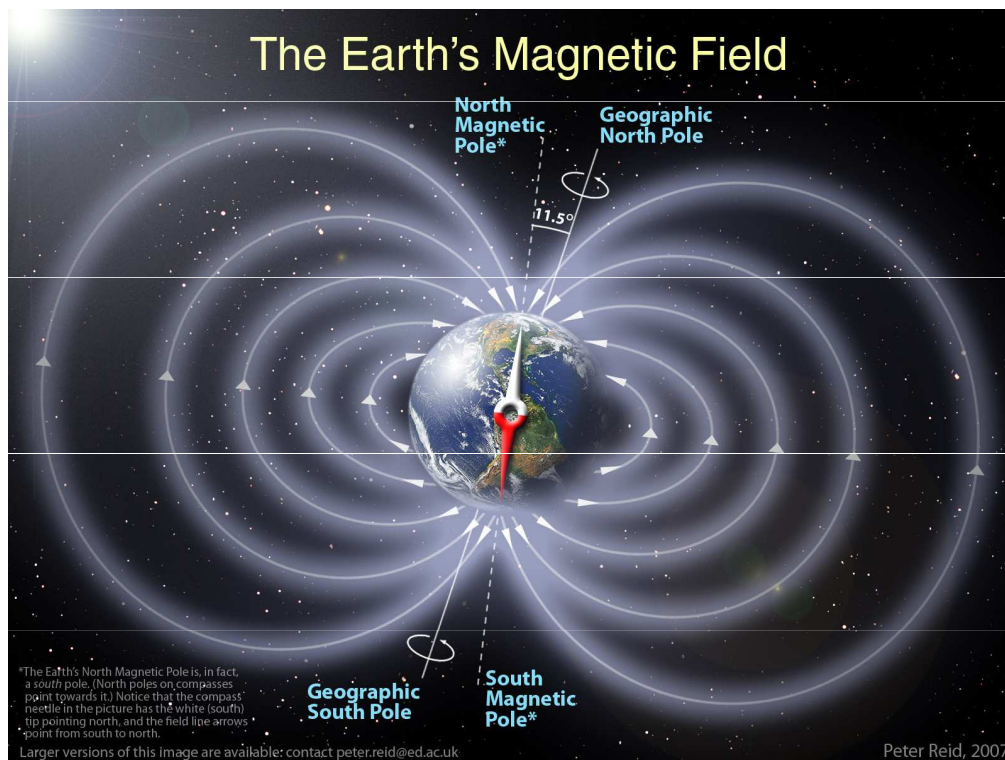
In the planetary model of the atom, an **electron orbits a nucleus**, forming a closed-current loop and **producing a magnetic field** with a north pole and a south pole.

Molecule is hence a group of small magnetic fields and each atom within the molecule experiences different local magnetic field.



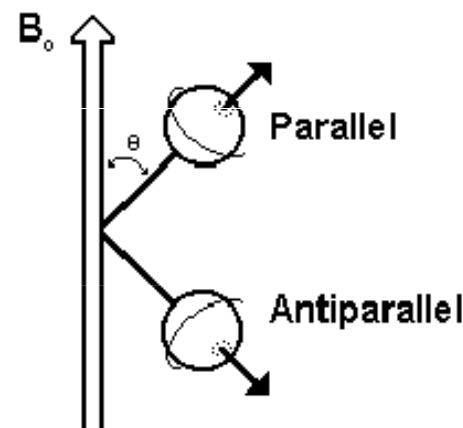
For NMR, nuclear spin is needed!!!

Spin analogy to a compass needle



magnetic field =  
0

Randomly oriented  
nuclear magnetic moments



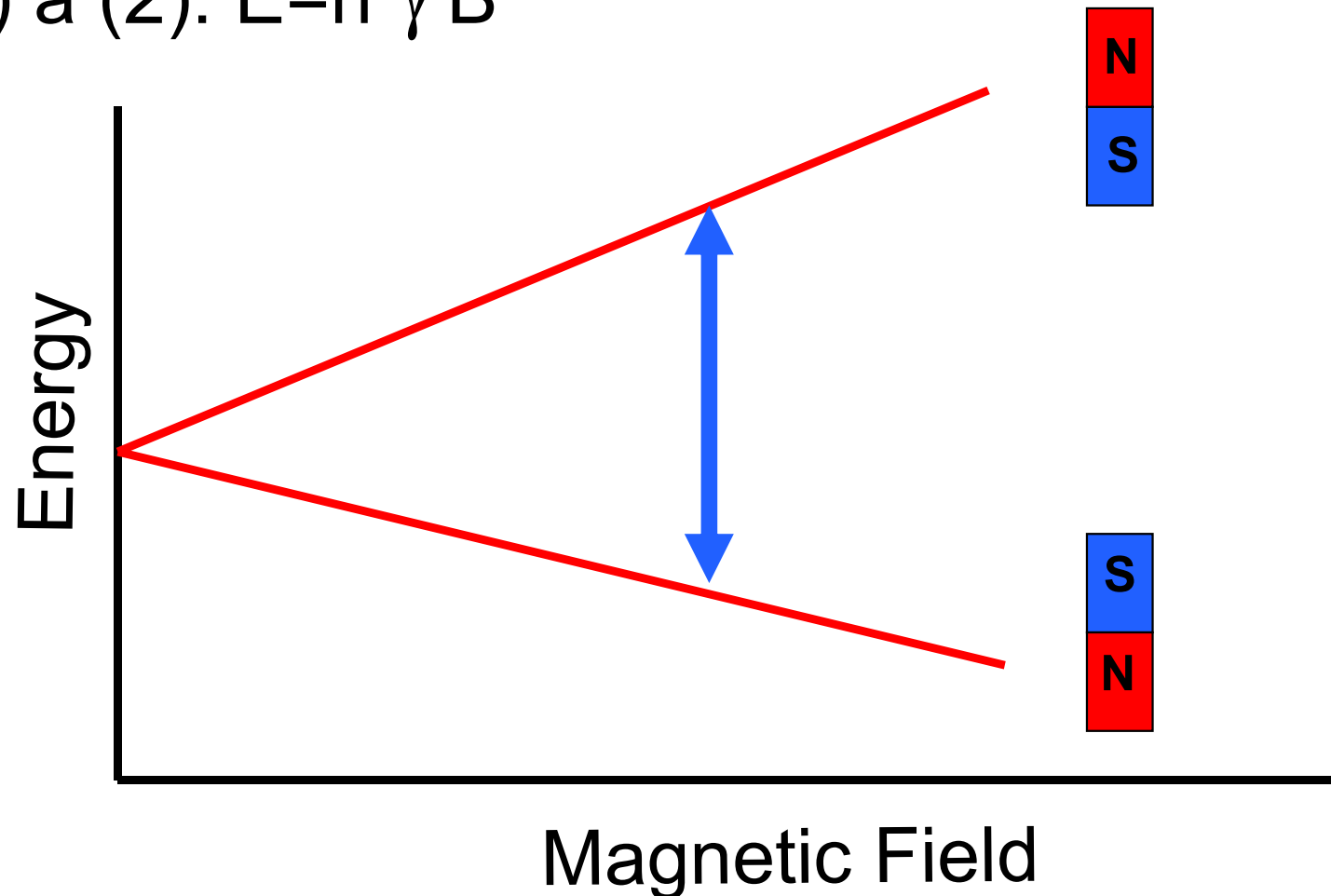
magnetic field >  
0

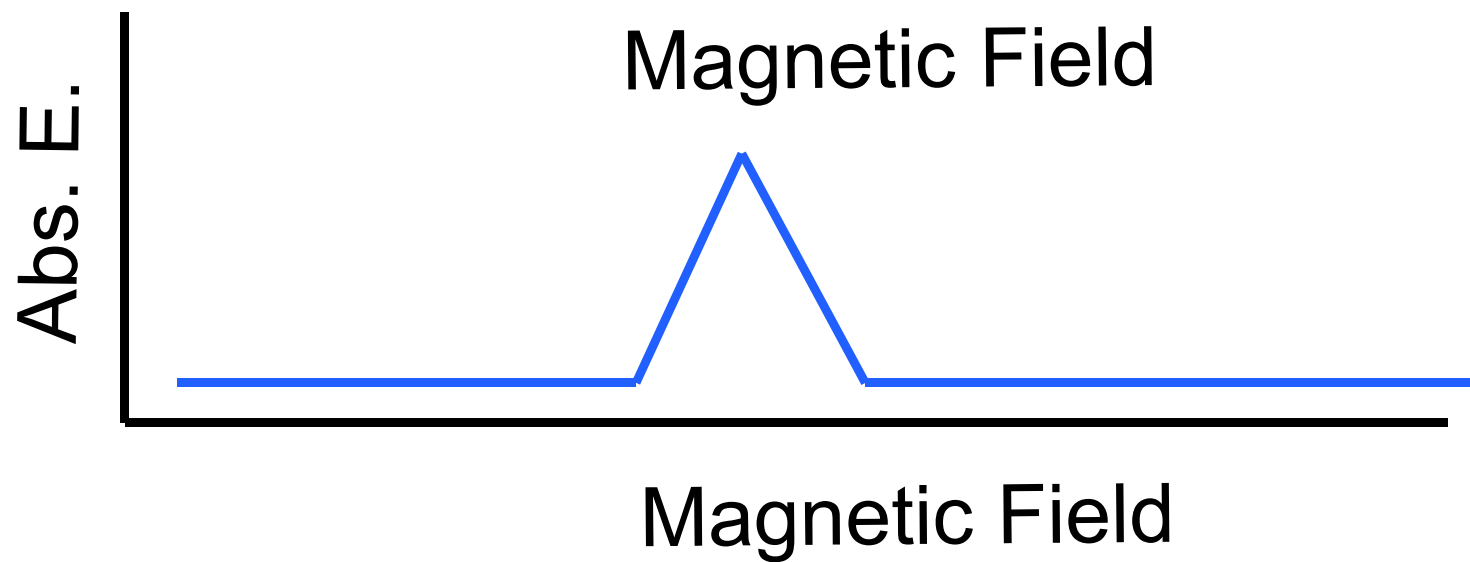
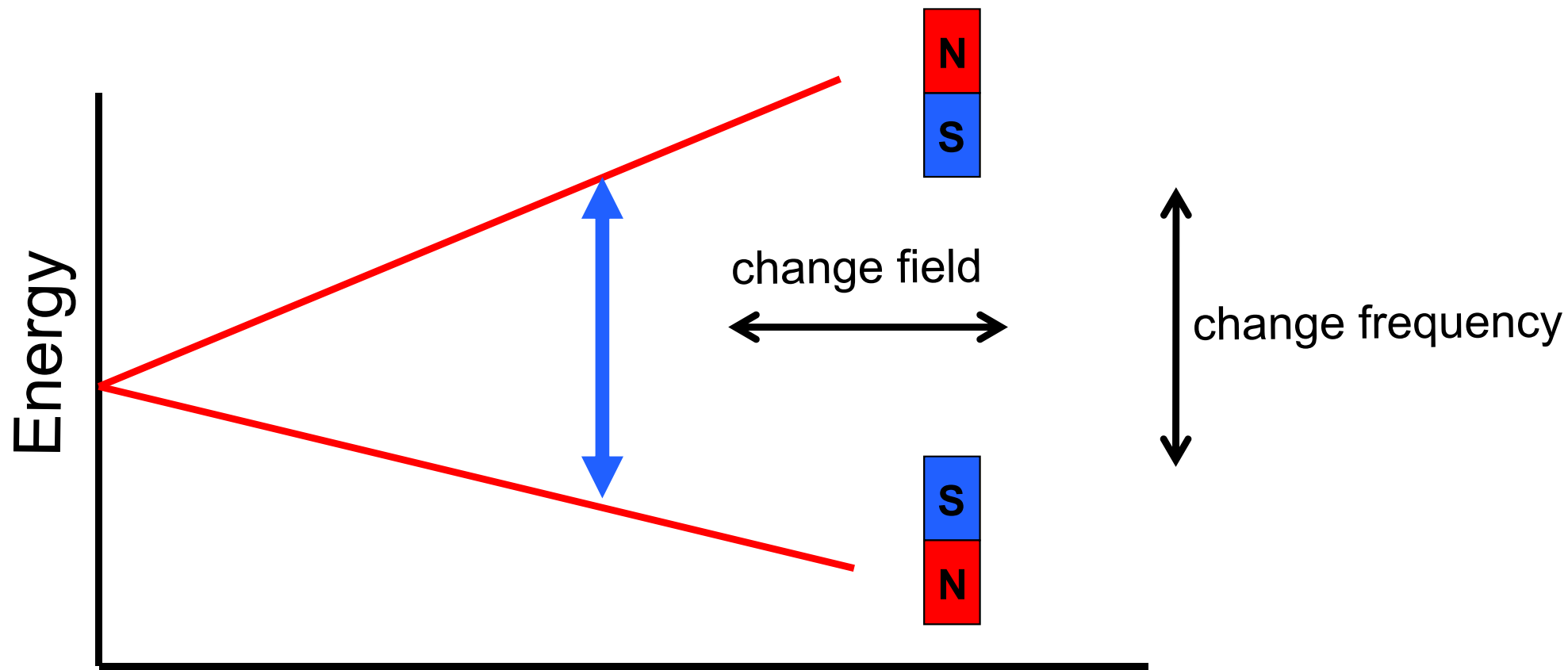
Nuclear magnetic moments  
in the presence of an external field

# Nuclear Magnetic Resonance

## Stručně

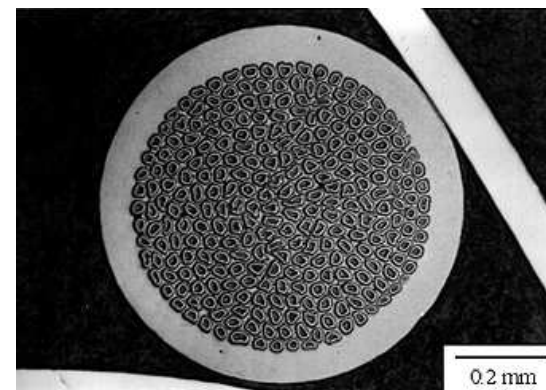
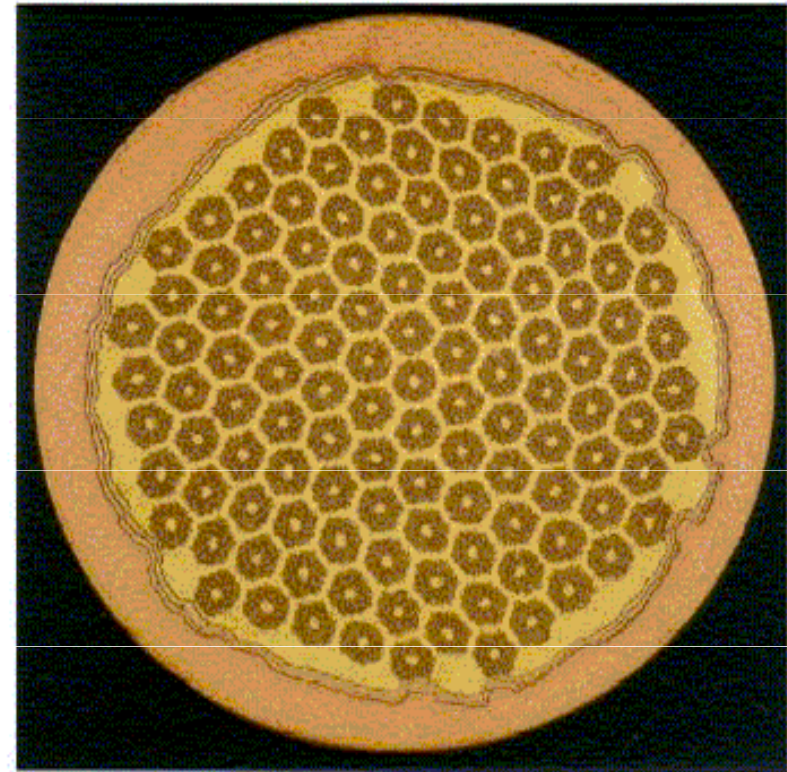
Z (1) a (2):  $E = h \gamma B$





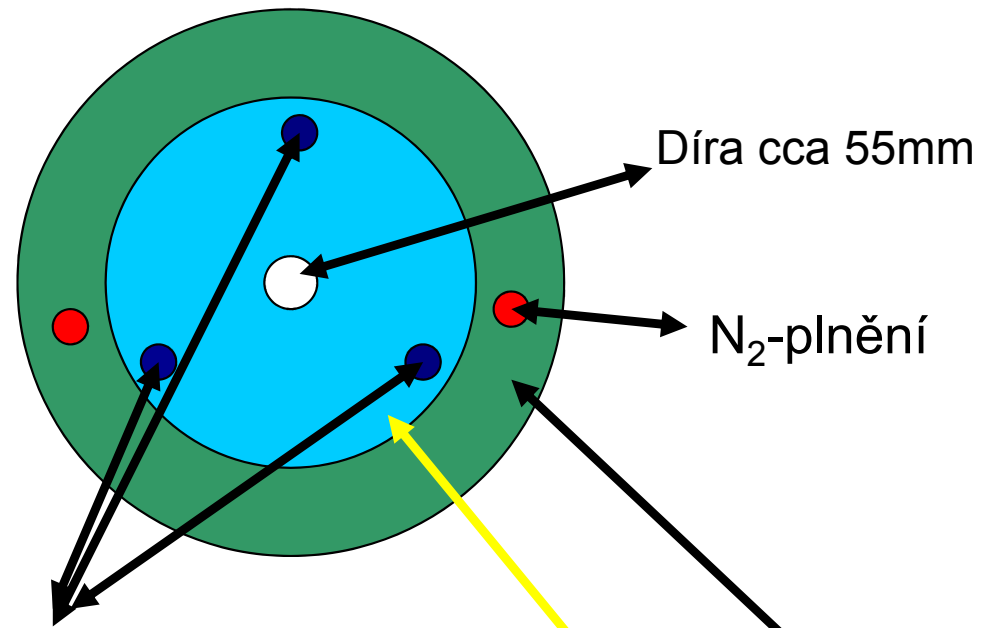
## Magnet

- supravodivé solenoidy na bázi
- Nb a Sn ponořené do heliové a dusíkové lázně
- He-lázeň **~4 K** dále snížena J-T
- pumpou na **~2.1 K**
- v současnosti až 22 Tesla
- magnetické pole země  $\sim 50\mu\text{T}$

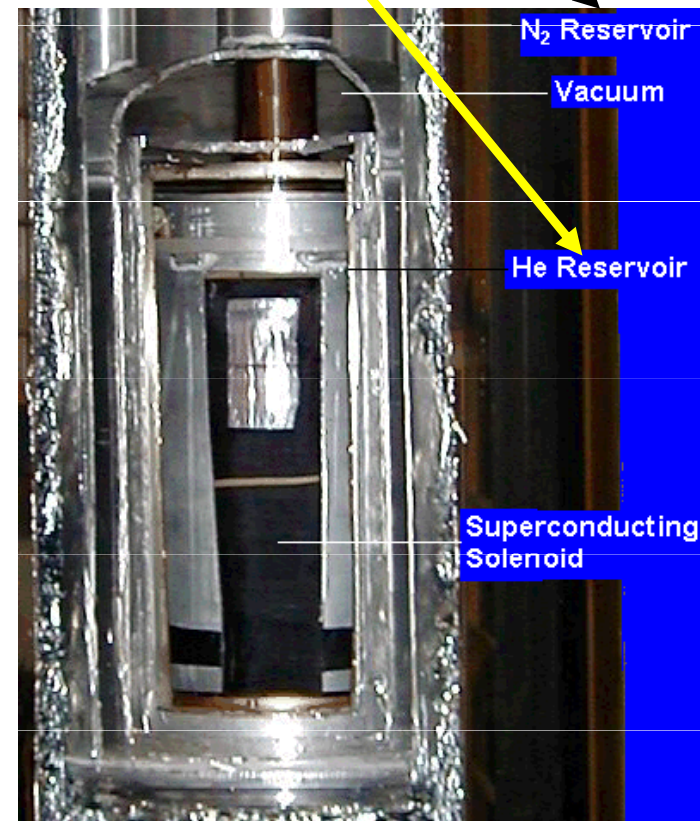


(Nb, Ta)<sub>3</sub>Sn supravodič o šířce 0.81 mm s 271 vlákny vnořenými do OFHC měděné matrice





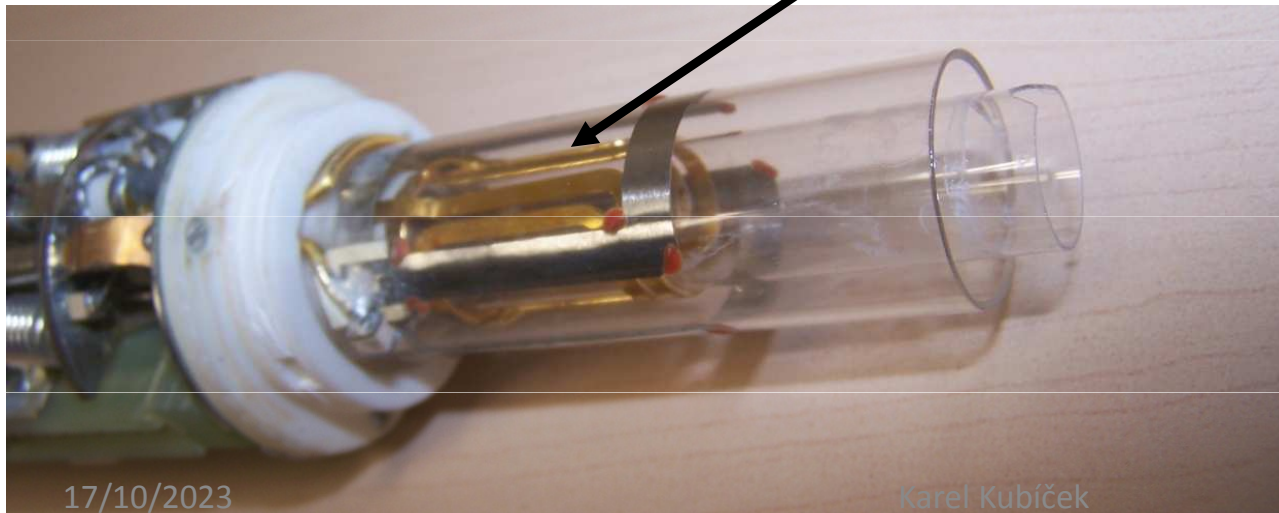
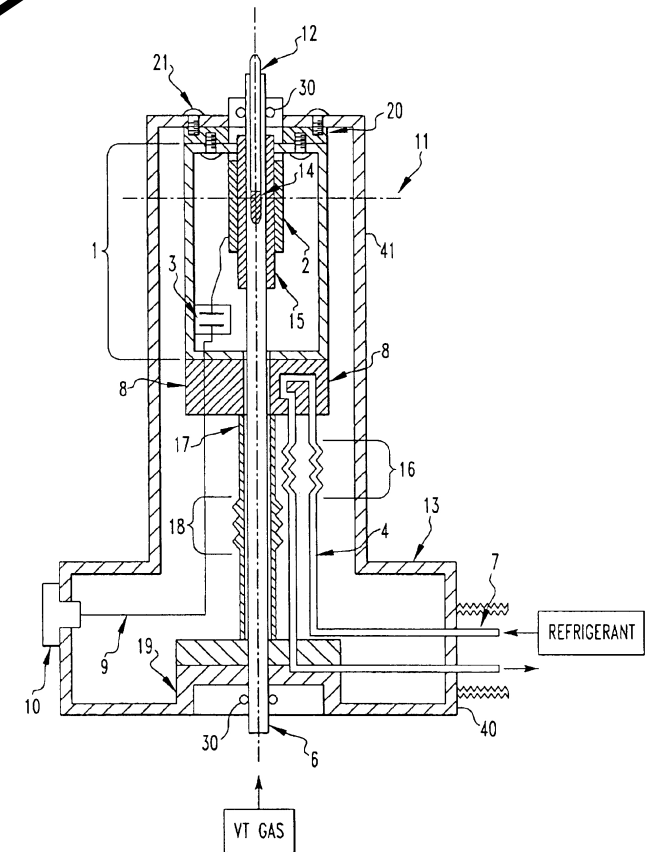
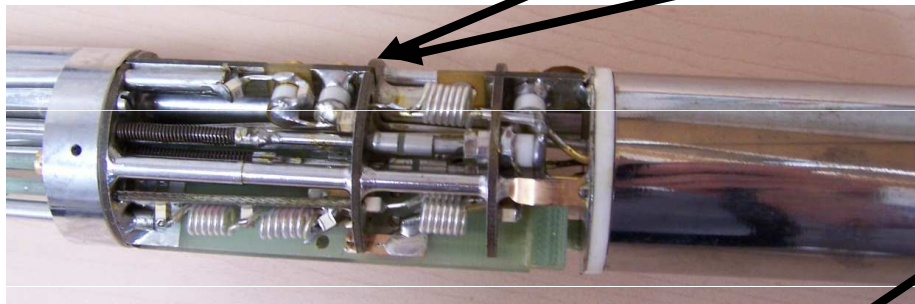
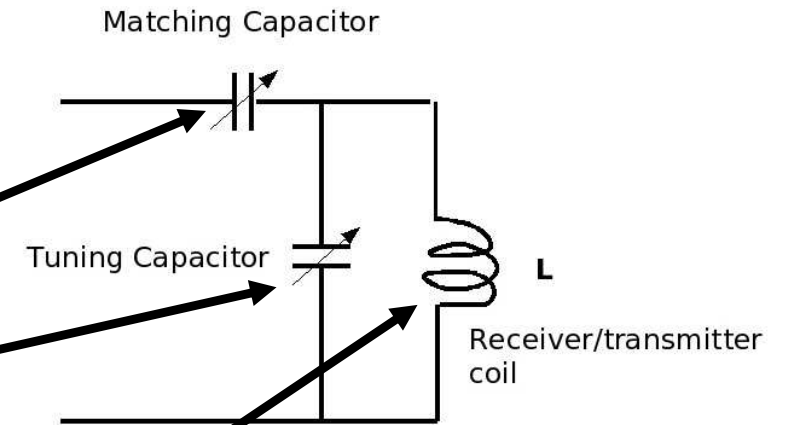
He-plnění







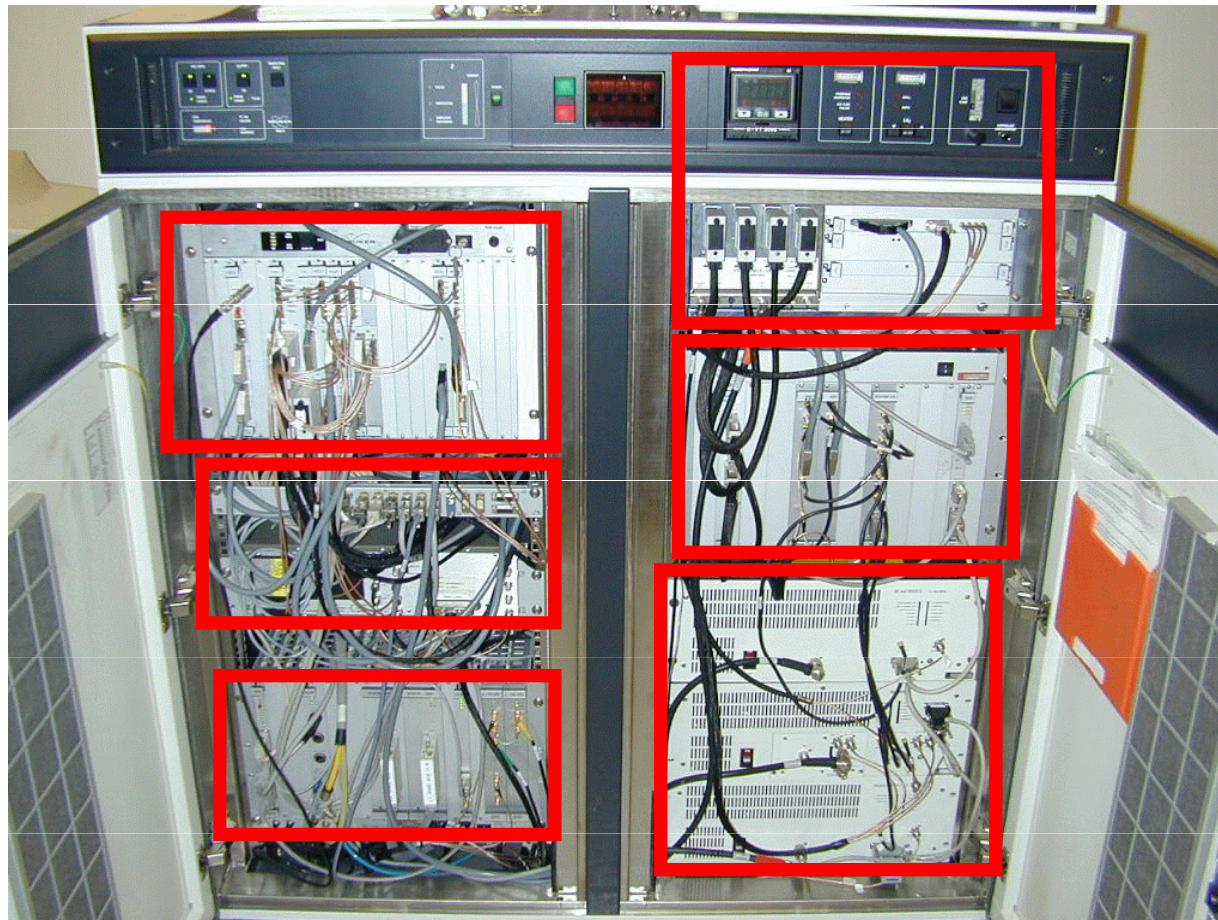
# NMR měřicí sonda





# Spektrometr

**CBU**  
Control board  
unit  
**FGU**  
Frequency  
gen. u.  
**Shimms**



**Temperature  
Unit**  
**Acquisition Con  
troller**  
**Transmitter**

### Size

### Relaxation



slow (i.e. long  $t_2$  time)



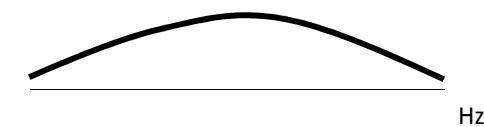
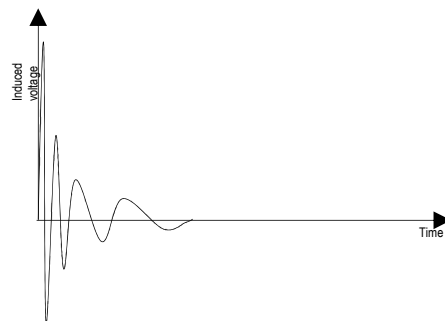
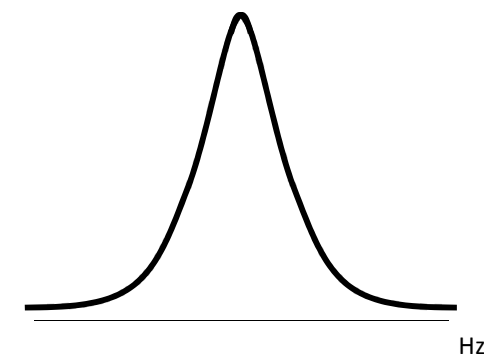
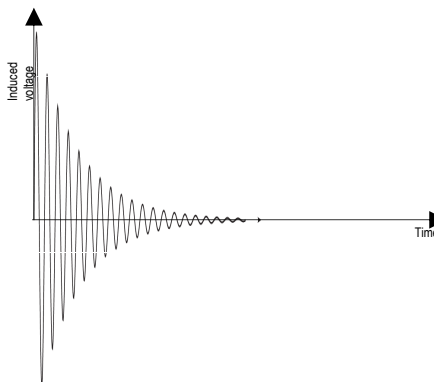
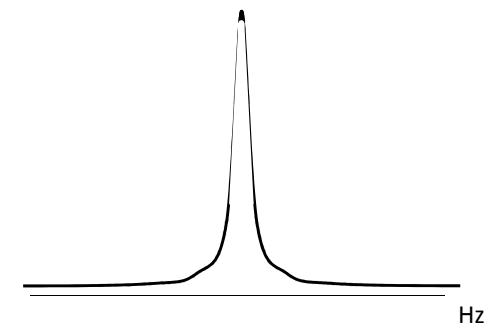
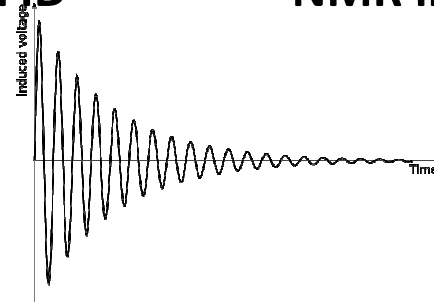
medium



fast

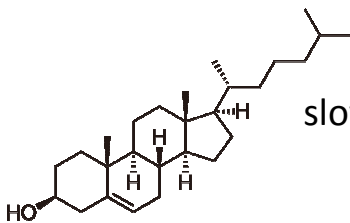
### FID

### NMR line(width) after FT



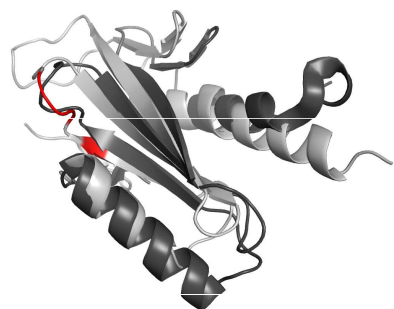
### Size

### Relaxation



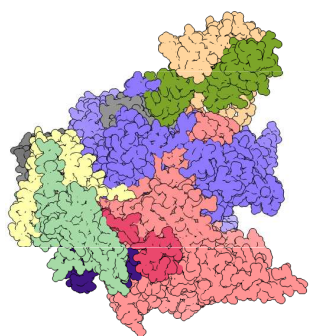
e.g. Cholesterol

slow (i.e. long  $t_2$  time)



medium

Biomolecules 5-30 kDa

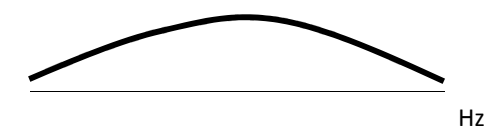
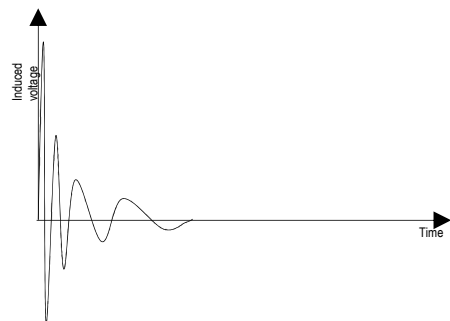
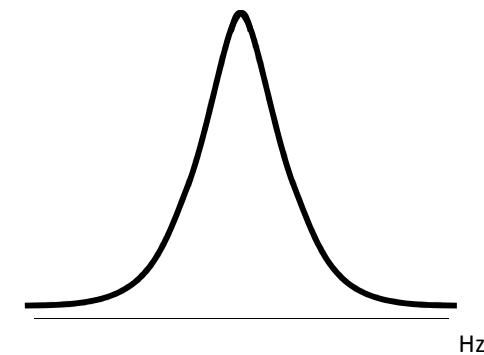
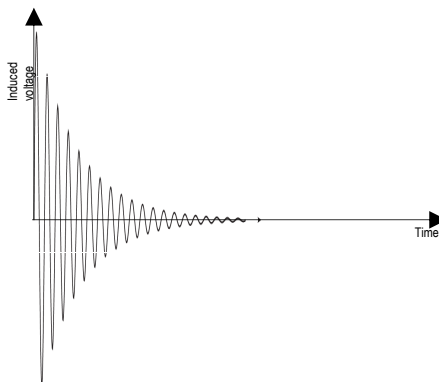
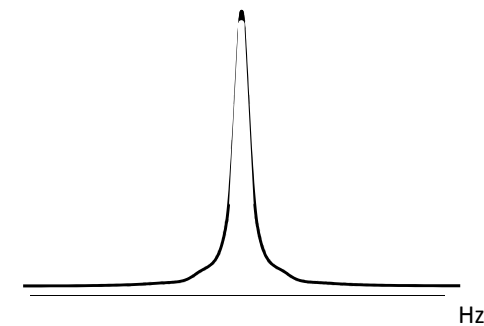
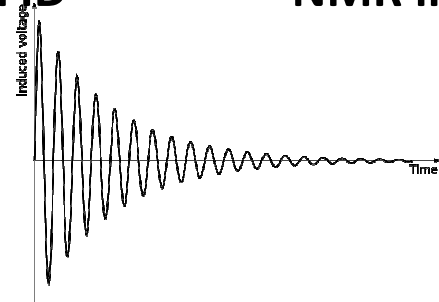


fast

Large molecules 50+ kDa

### FID

### NMR line(width) after FT



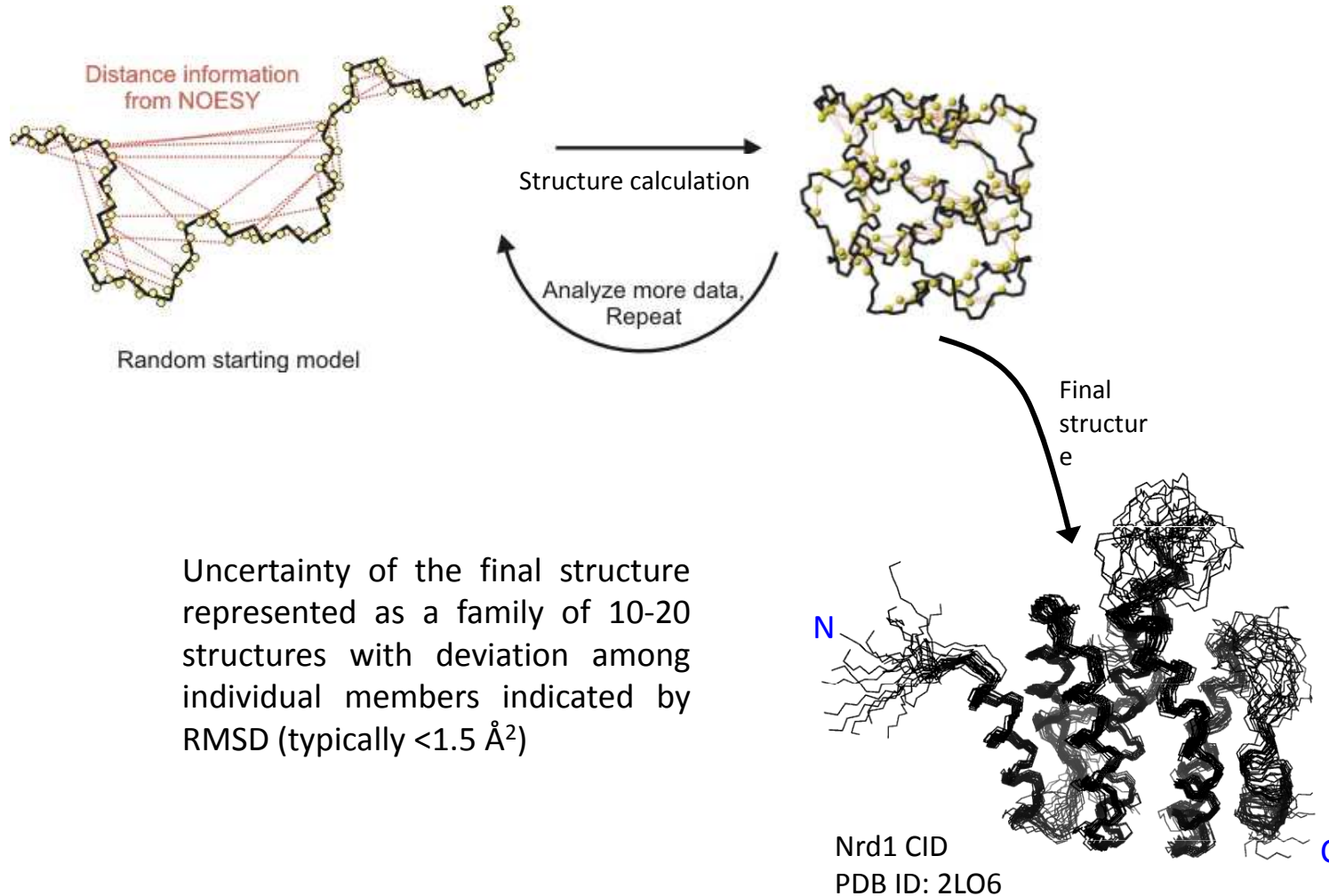
NMR as a tool for study **structure**, **dynamics** and **interactions** of biomolecules

- 1) Structure determination of NAs and proteins
- 2) **Protein – metal interaction**
- 3) **Protein – ligand interaction**

For most of the modern applications, enrichment by  $^{13}\text{C}$ ,  $^{15}\text{N}$  and often  $^2\text{H}$  needed!

Isotope	Ground state spin	Natural abundance [%]	Rel. Sensitivity
$^1\text{H}$	$\frac{1}{2}$	~100	$1.00 \times 10^0$
$^{13}\text{C}$	$\frac{1}{2}$	1.10	$1.59 \times 10^{-2}$
$^{15}\text{N}$	$\frac{1}{2}$	0.37	$1.04 \times 10^{-3}$
$^{19}\text{F}$	$\frac{1}{2}$	100	$8.30 \times 10^{-1}$
$^{31}\text{P}$	$\frac{1}{2}$	~100	$6.63 \times 10^{-2}$
$^{12}\text{C}$	0	98.90	-
$^{16}\text{O}$	0	~100	-

## Iterative procedure of structure determination by NMR



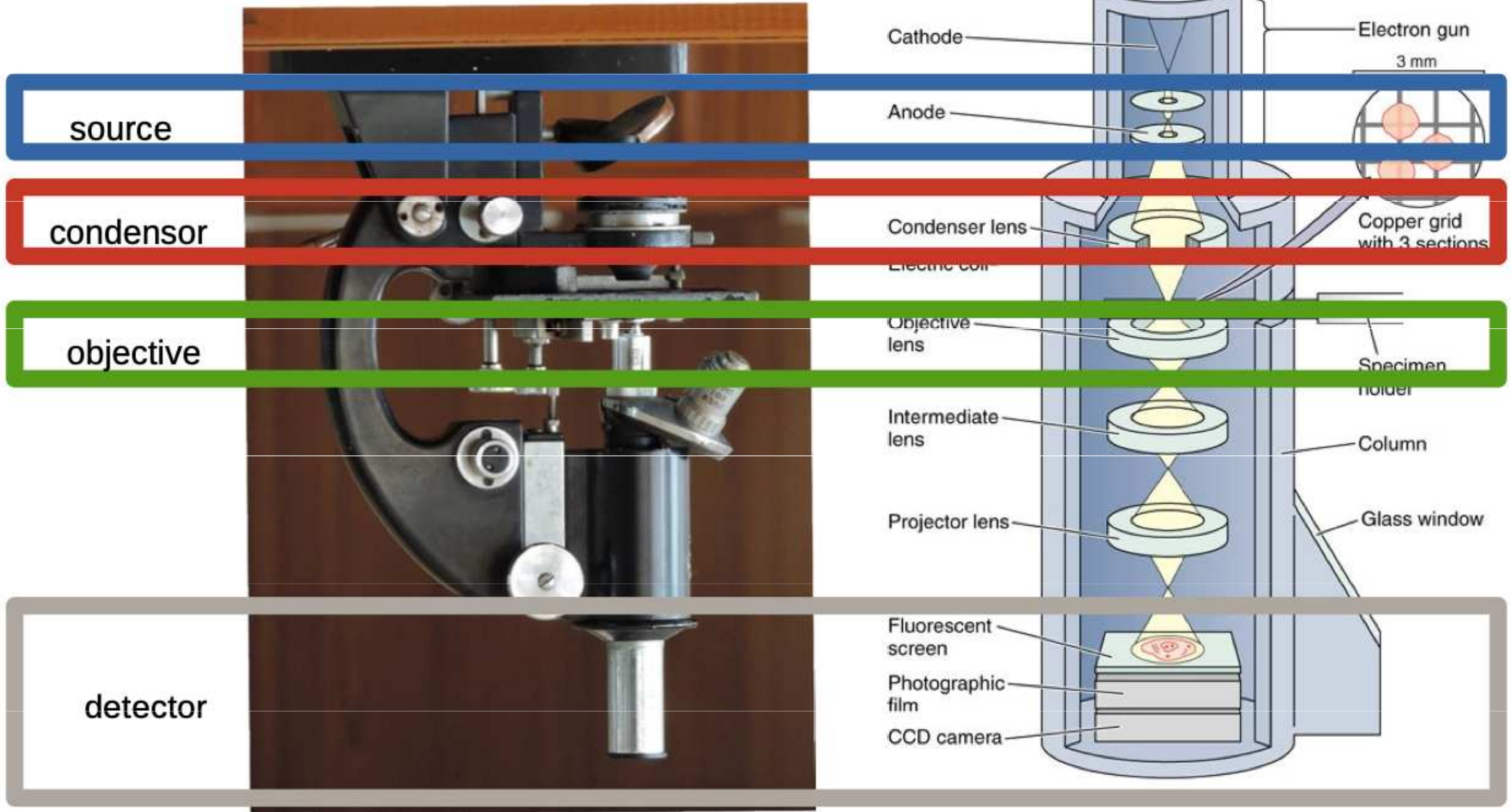
Uncertainty of the final structure represented as a family of 10-20 structures with deviation among individual members indicated by RMSD (typically  $<1.5 \text{ \AA}^2$ )

[http://www.fbreagents.com/basics\\_nmr/9proteins.htm](http://www.fbreagents.com/basics_nmr/9proteins.htm)

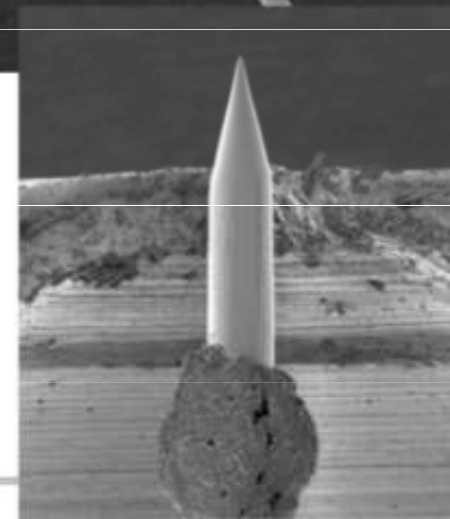
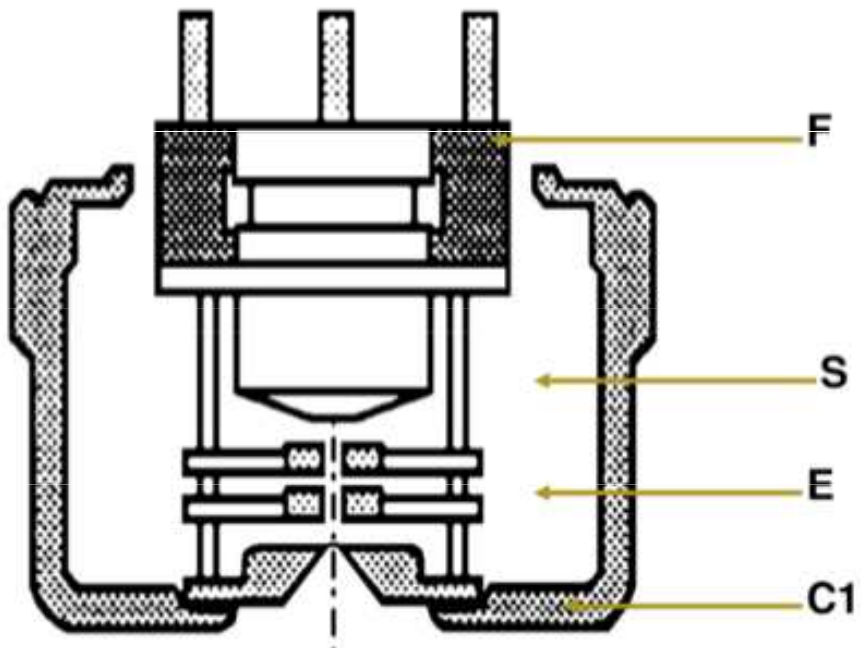
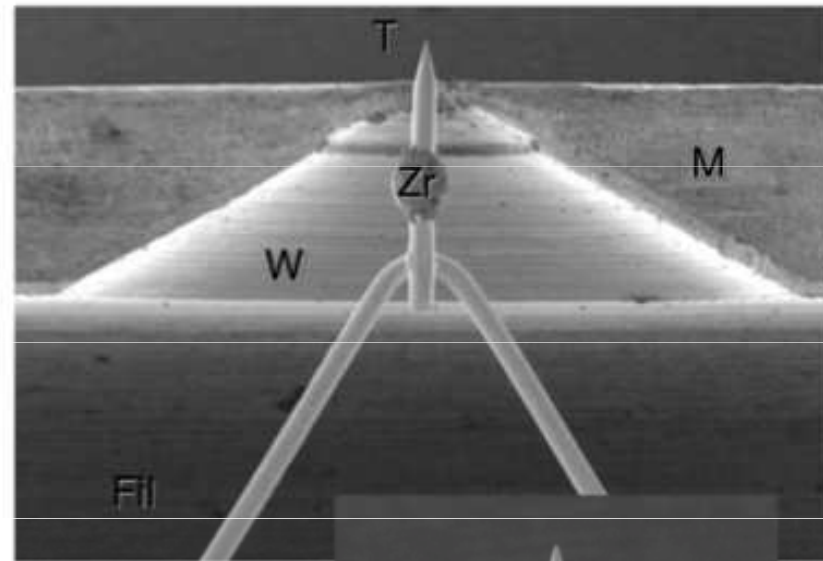
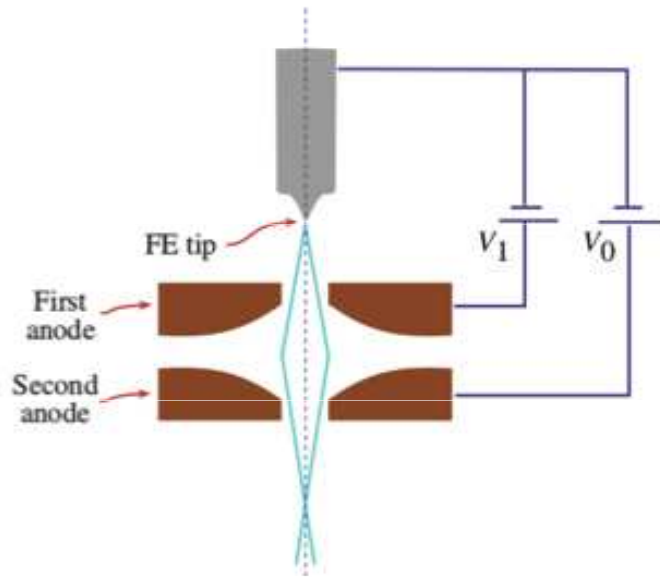


# Optical microscope

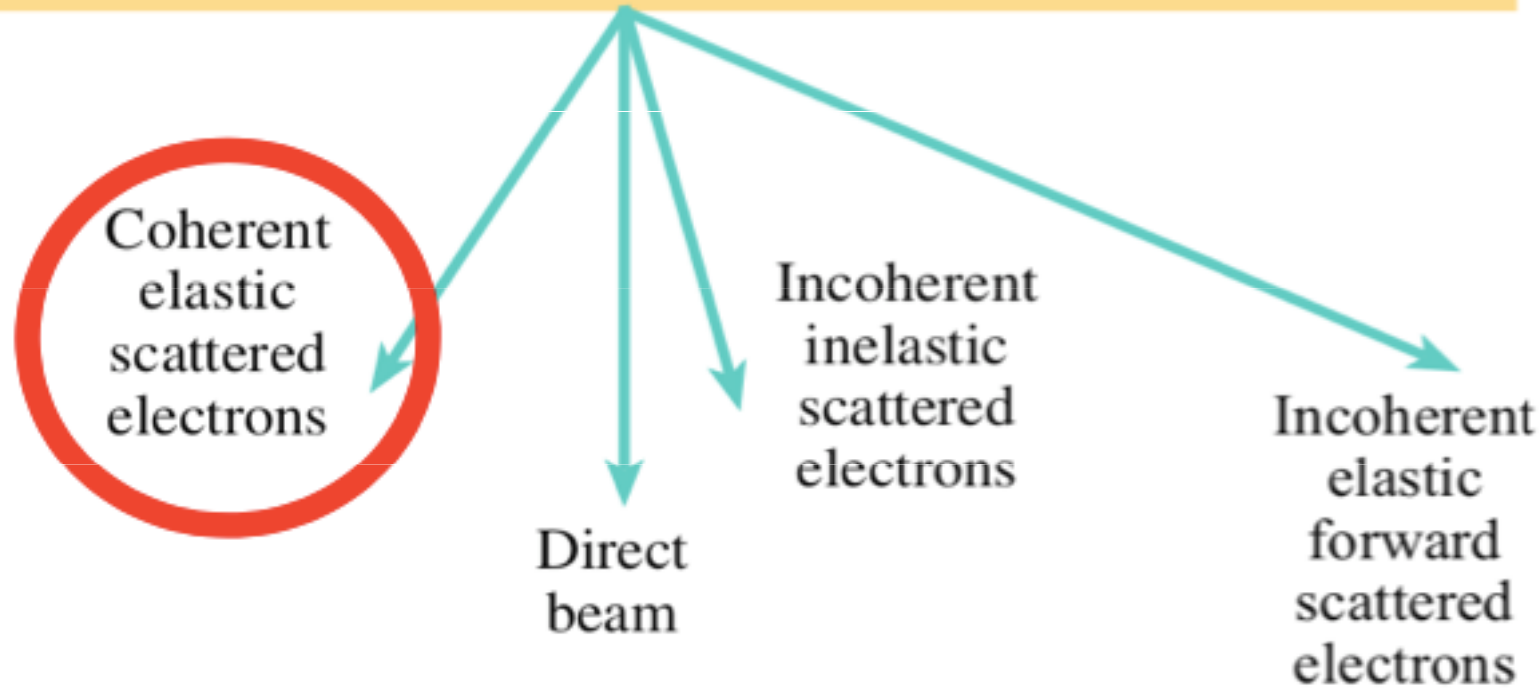
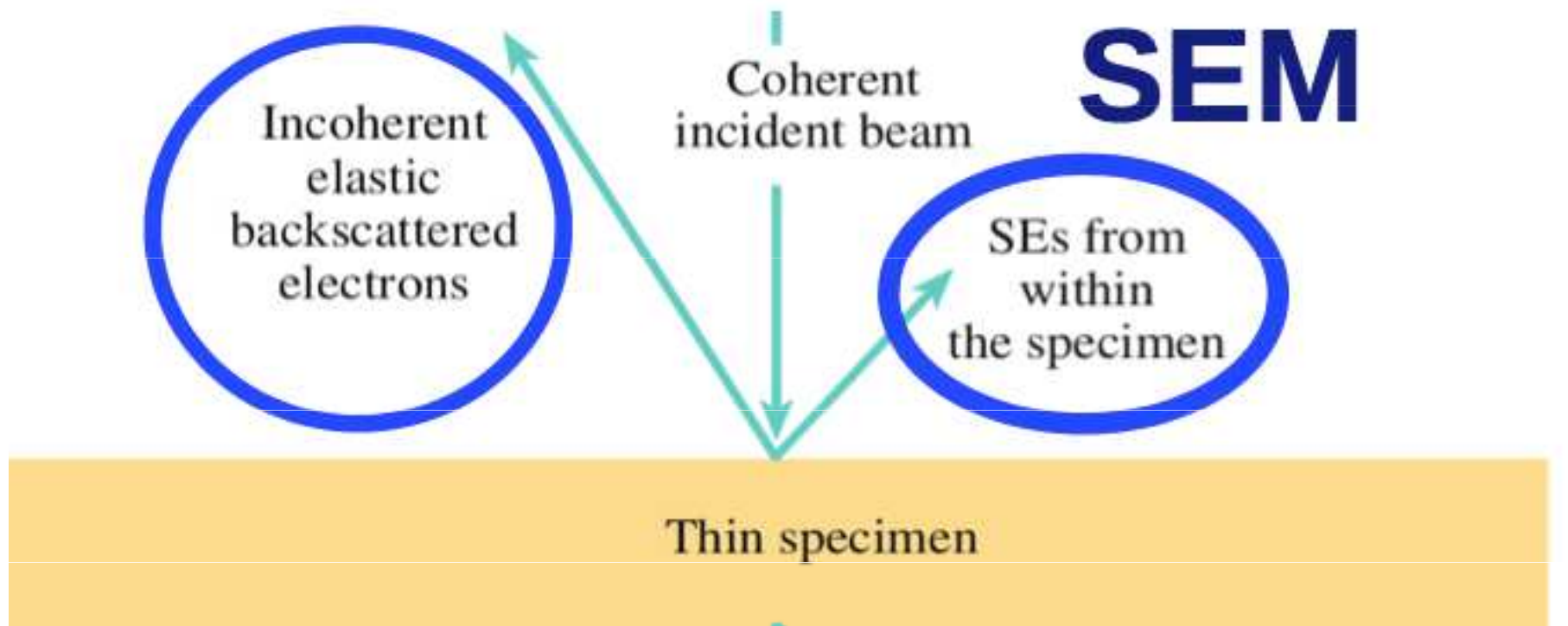
# Electron microscope



# Electron source - FEG



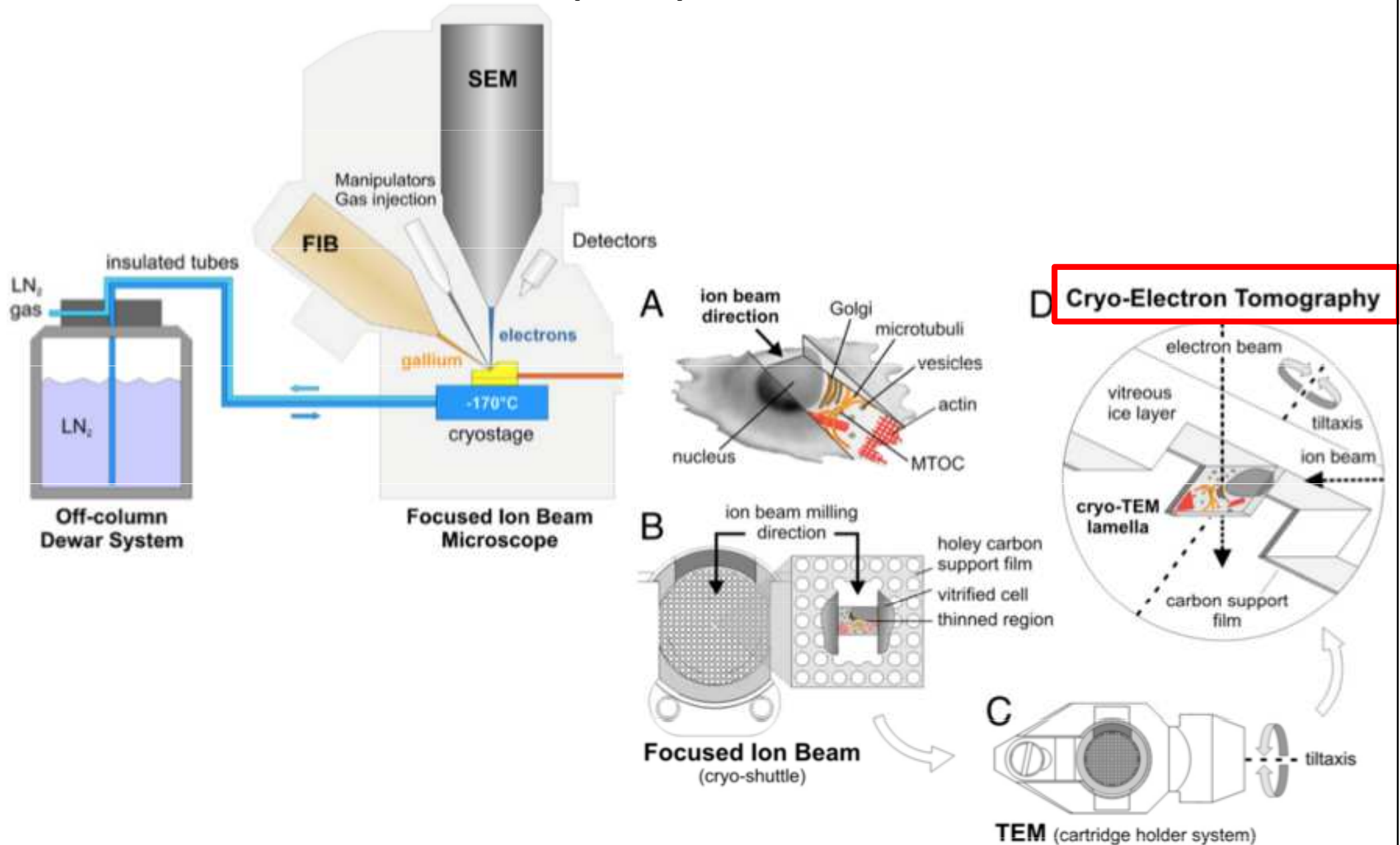
FEG - Field Emission Gun

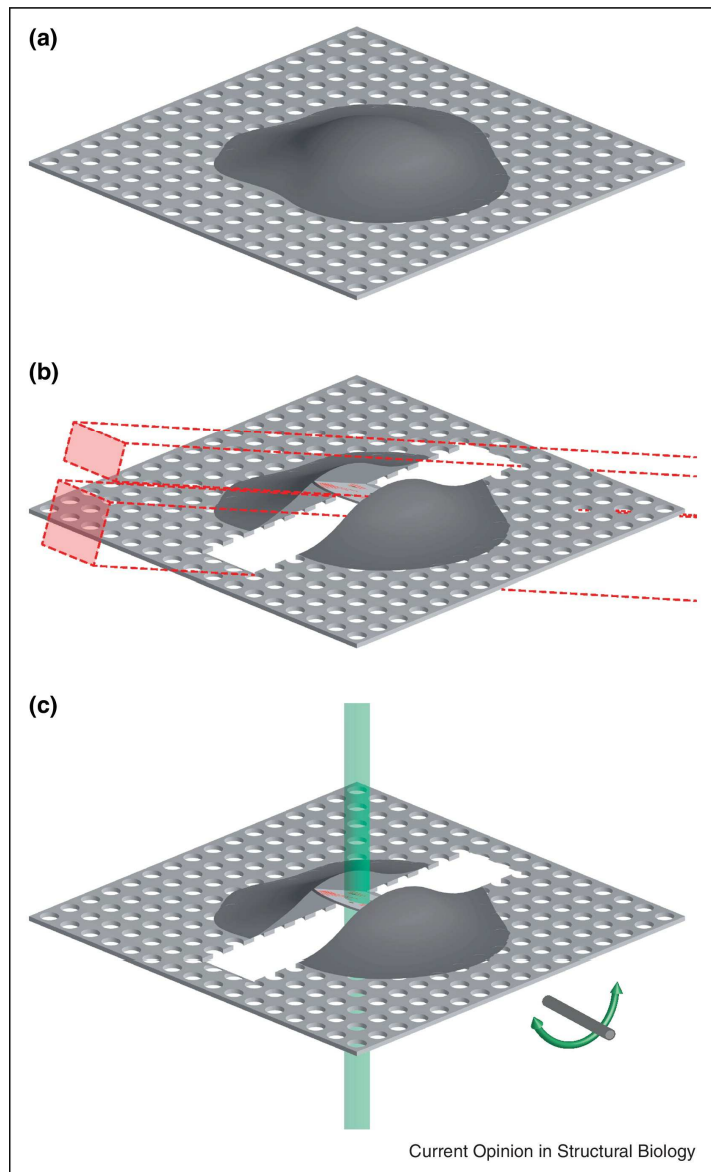


**TEM**

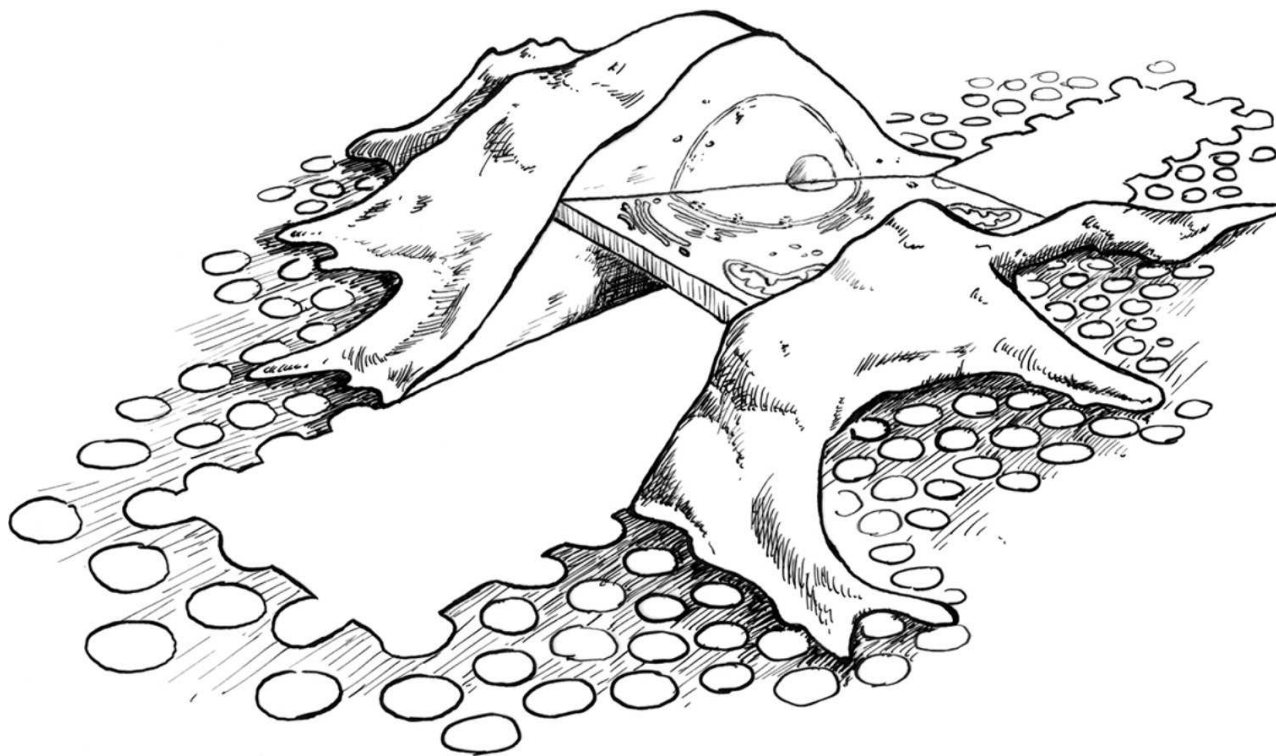
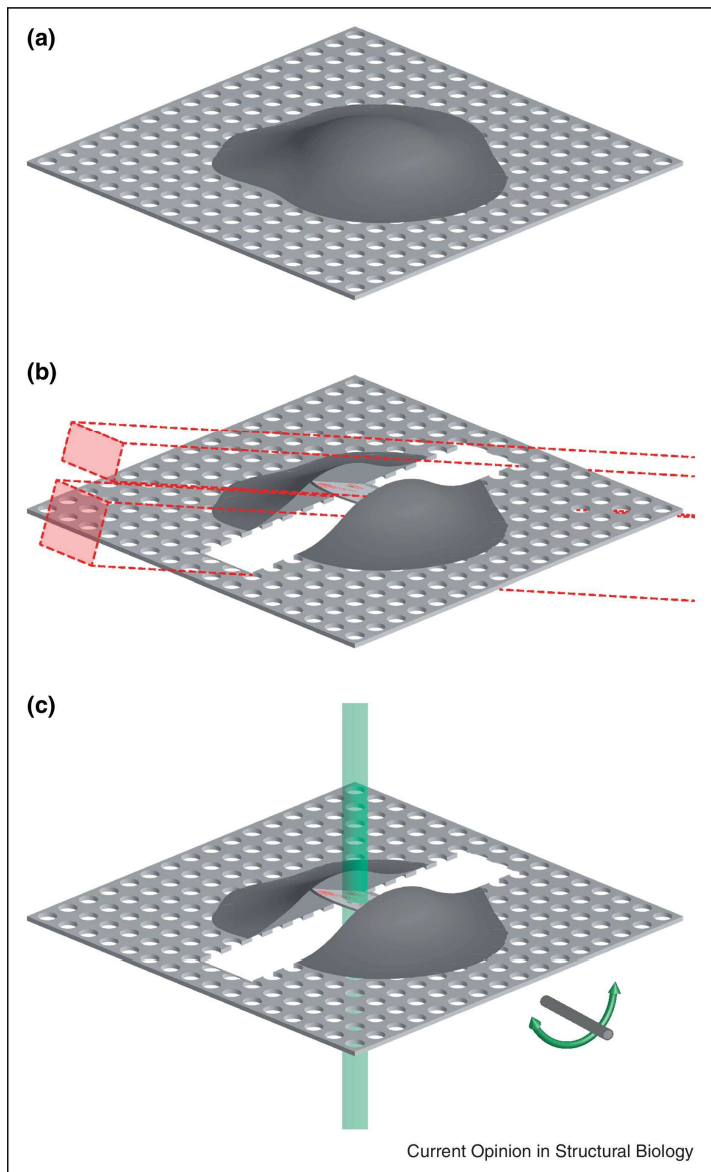


# Focused Ion Beam (FIB) + SEM



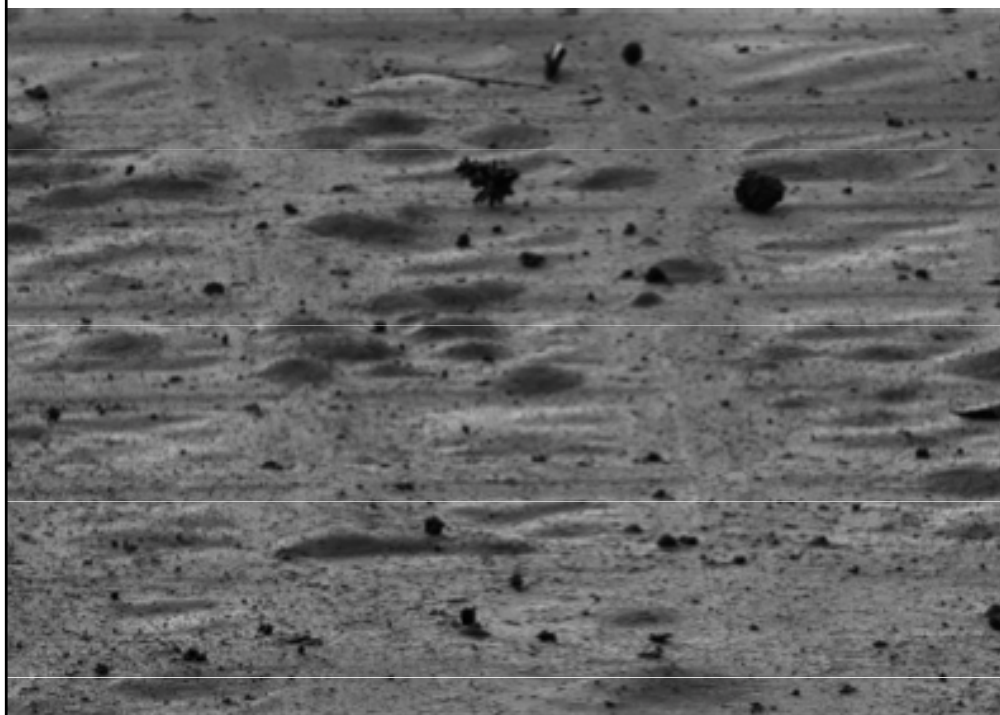


Villa et al. Current Opinion in Structural Biology, 23(5), 2013, 771-7

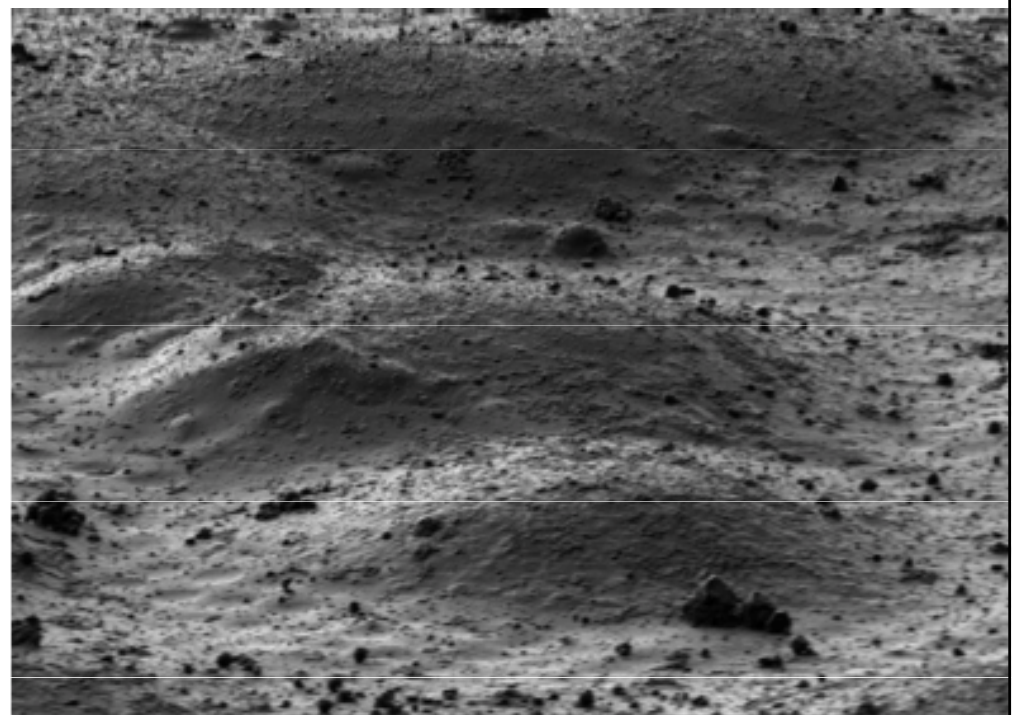


Villa et al. Current Opinion in Structural Biology, 23(5), 2013, 771-7

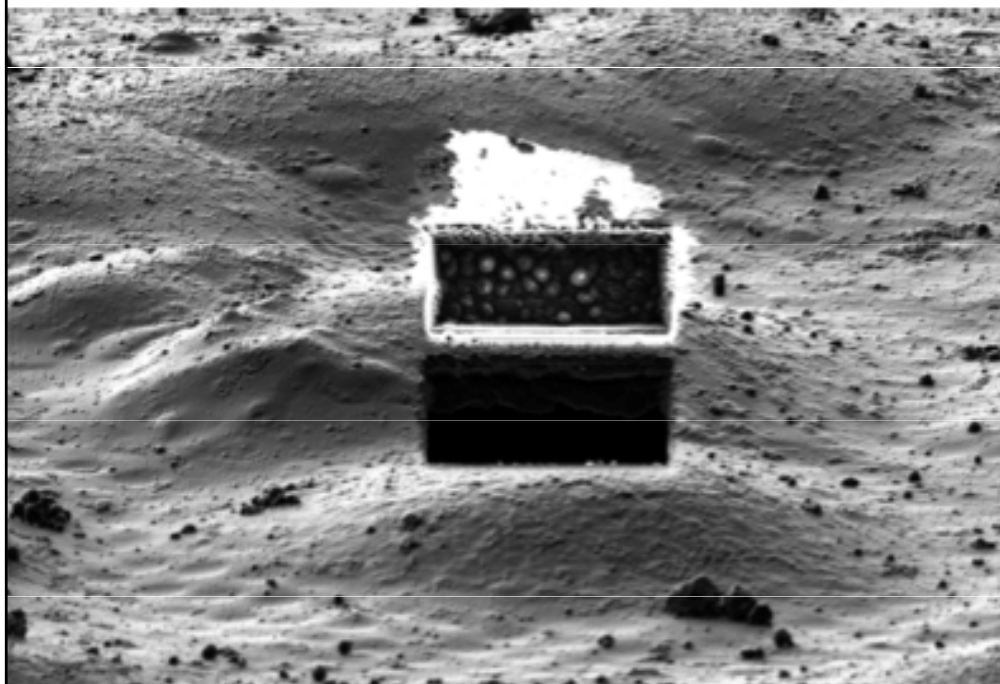




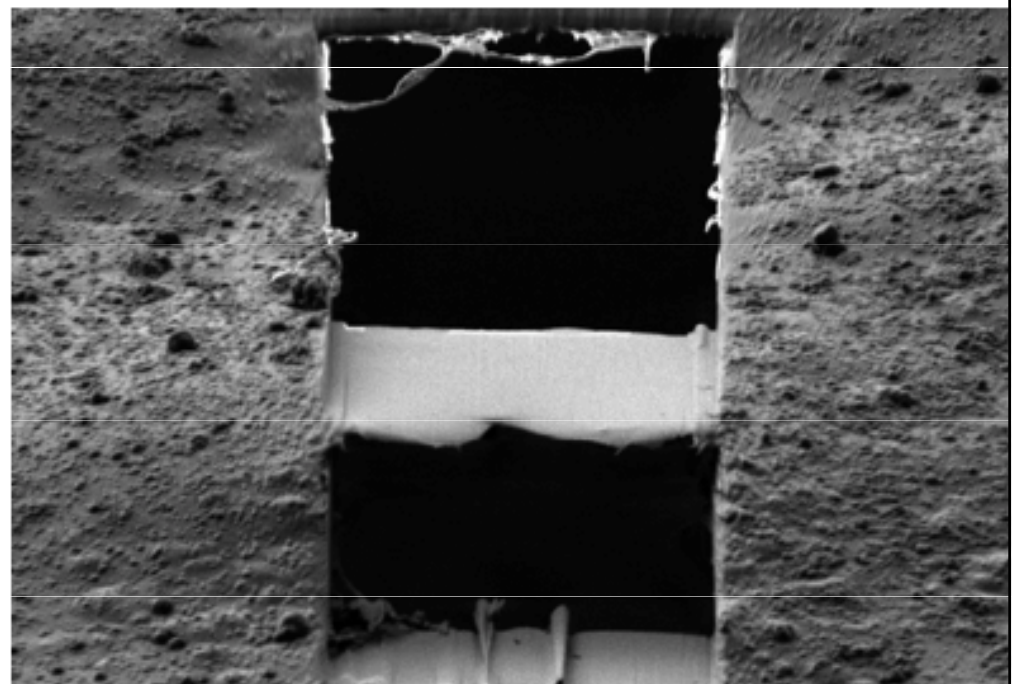
HV	curr	HPW	mag	pressure	det	mode	WD	tilt	50 μm	
30.00 kV	10 pA	319 μm	650 x	8.10e-5 Pa	ETD	SE	19.0 mm	10 °	Versa 3D	



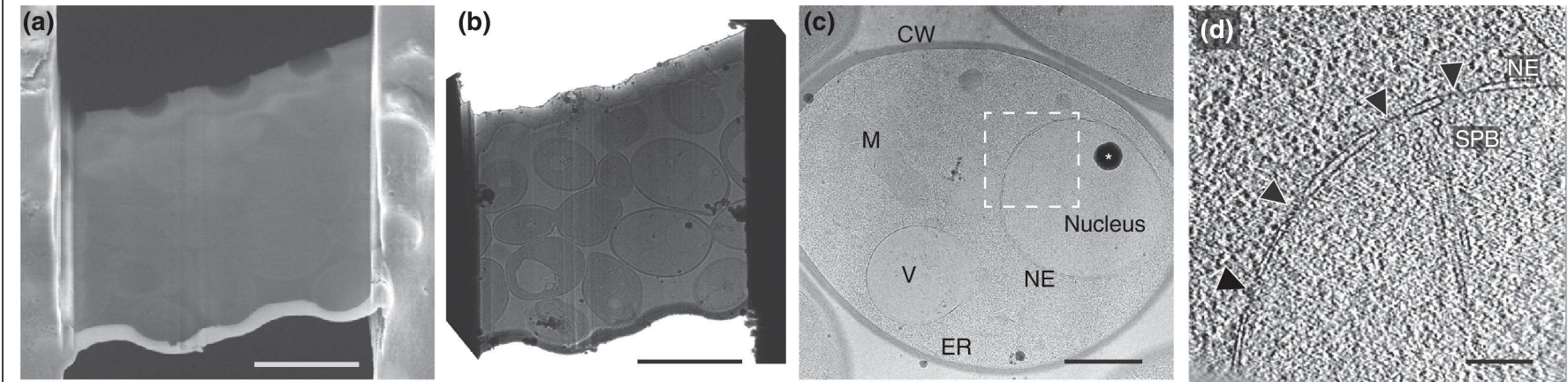
HV	curr	HPW	mag	pressure	det	mode	WD	tilt	5 μm	
30.00 kV	10 pA	41.4 μm	5 000 x	7.57e-5 Pa	ETD	SE	19.4 mm	10 °	Versa 3D	



HV	curr	HPW	mag	pressure	det	mode	WD	tilt	5 μm	
30.00 kV	10 pA	41.4 μm	5 000 x	7.75e-5 Pa	ETD	SE	19.4 mm	10 °	Versa 3D	



HV	curr	HPW	mag	pressure	det	mode	WD	tilt	5 μm	
30.00 kV	1.5 pA	25.9 μm	8 000 x	7.57e-5 Pa	ETD	SE	19.5 mm	20 °	Versa 3D	

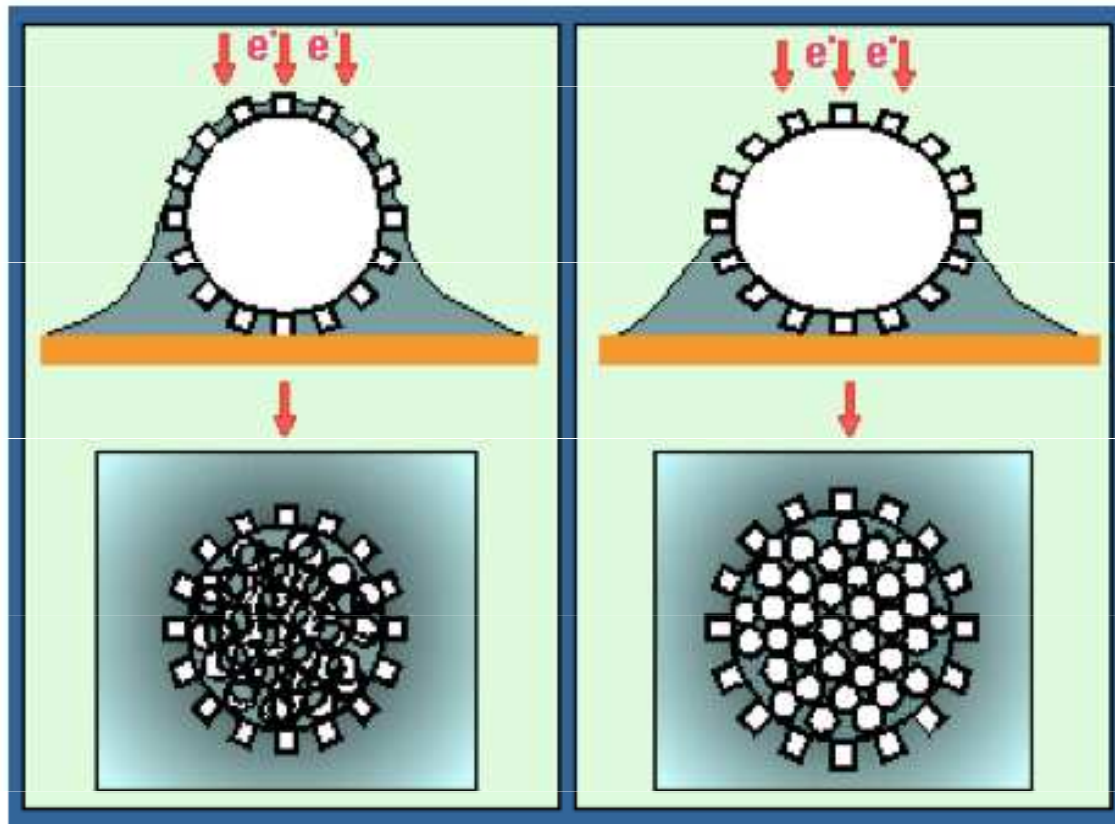


Current Opinion in Structural Biology



# TEM – Sample preparation

## Negative staining



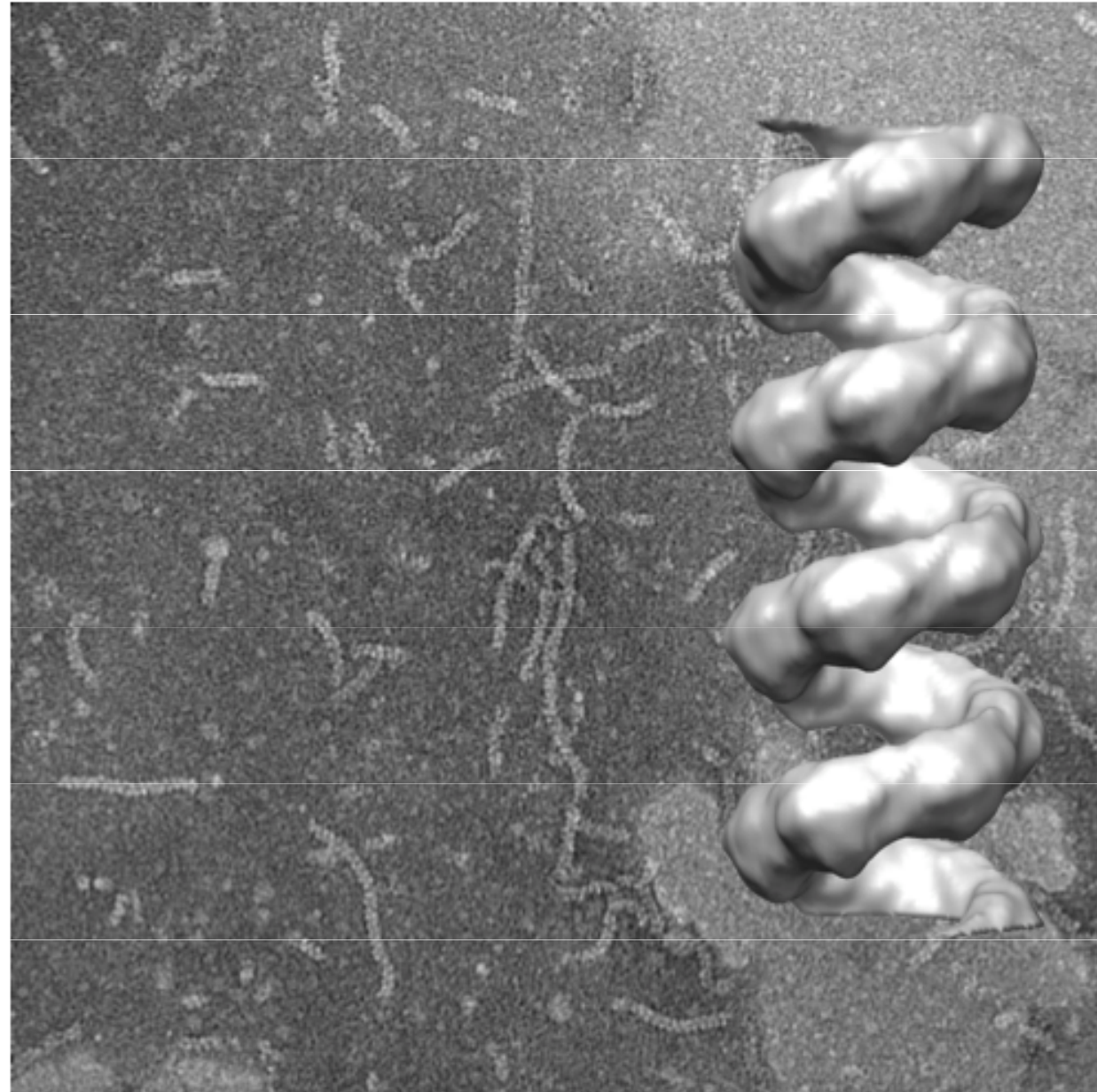
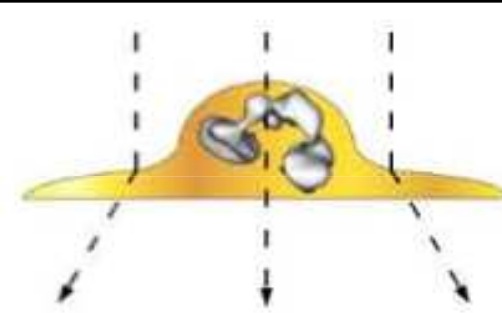
Pros: quick sample screening  
high amplitude contrast  
less prone to beam damage

Cons: limited resolution (20Å)  
flattening artefacts  
denaturation of proteins

# Heavy metal staining

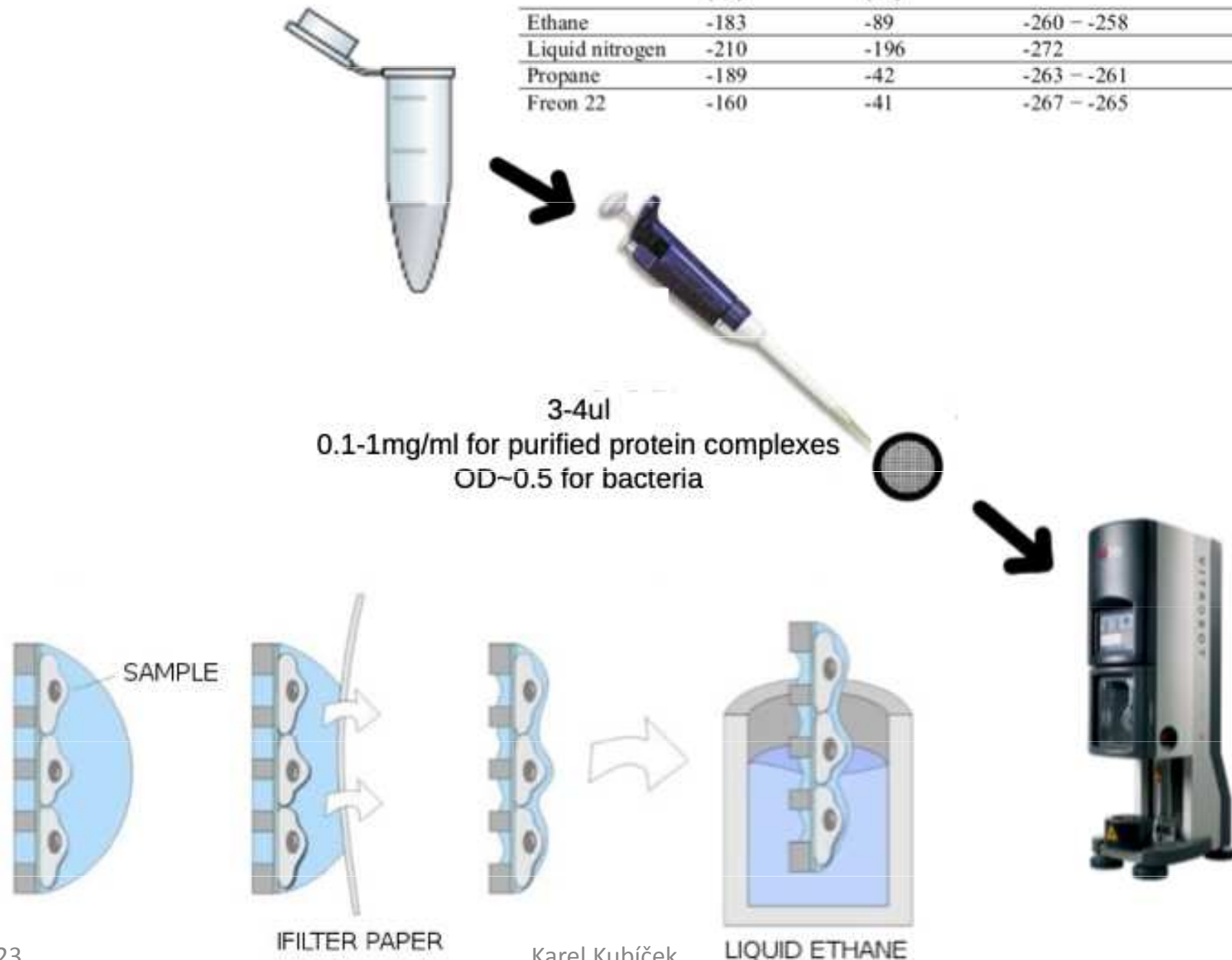
e.g. uranyl acetate

## Negative staining



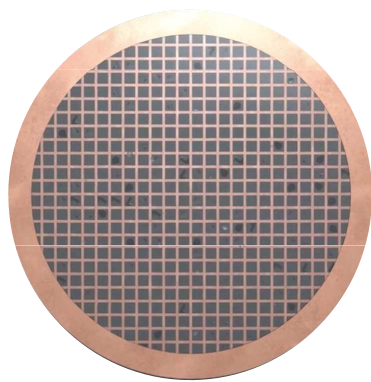
# Sample vitrification

Cryogens	Melting point (°C)	Boiling point (°C)	Cooling rate ( $10^3$ °C/s)	Relative cooling efficiency*
Ethane	-183	-89	-260 - -258	1.3
Liquid nitrogen	-210	-196	-272	0.1
Propane	-189	-42	-263 - -261	1.0
Freon 22	-160	-41	-267 - -265	0.7





Grid



**C-clip**



**AutoGrid**



ThermoFisherScientific



**Grid Box Tool**

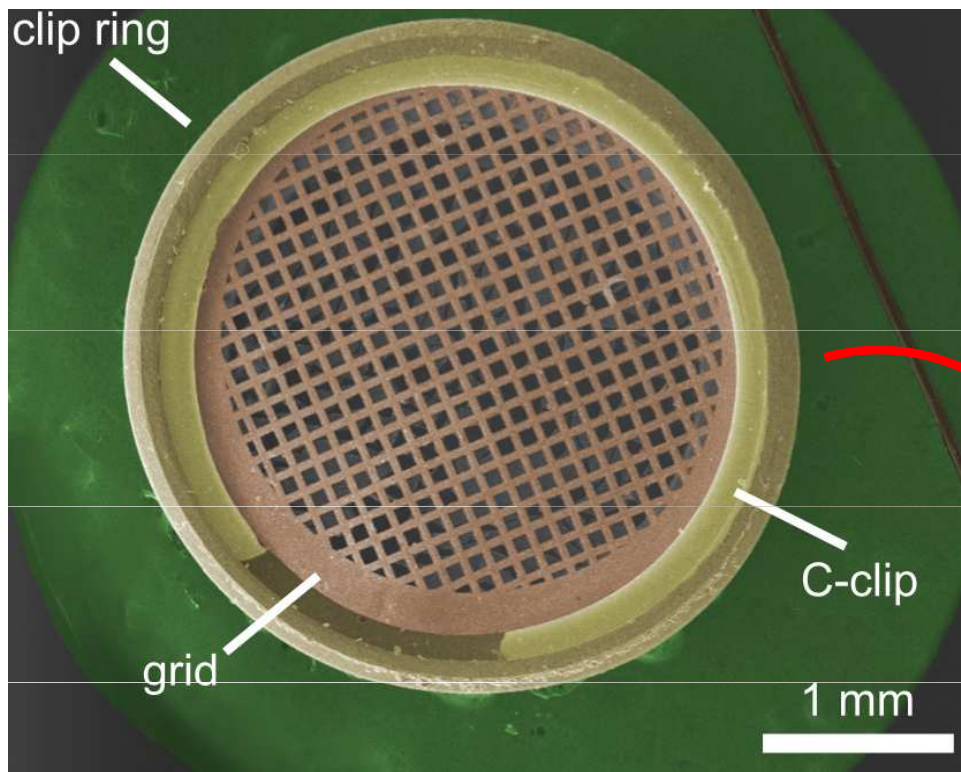


**Clipping Tool**



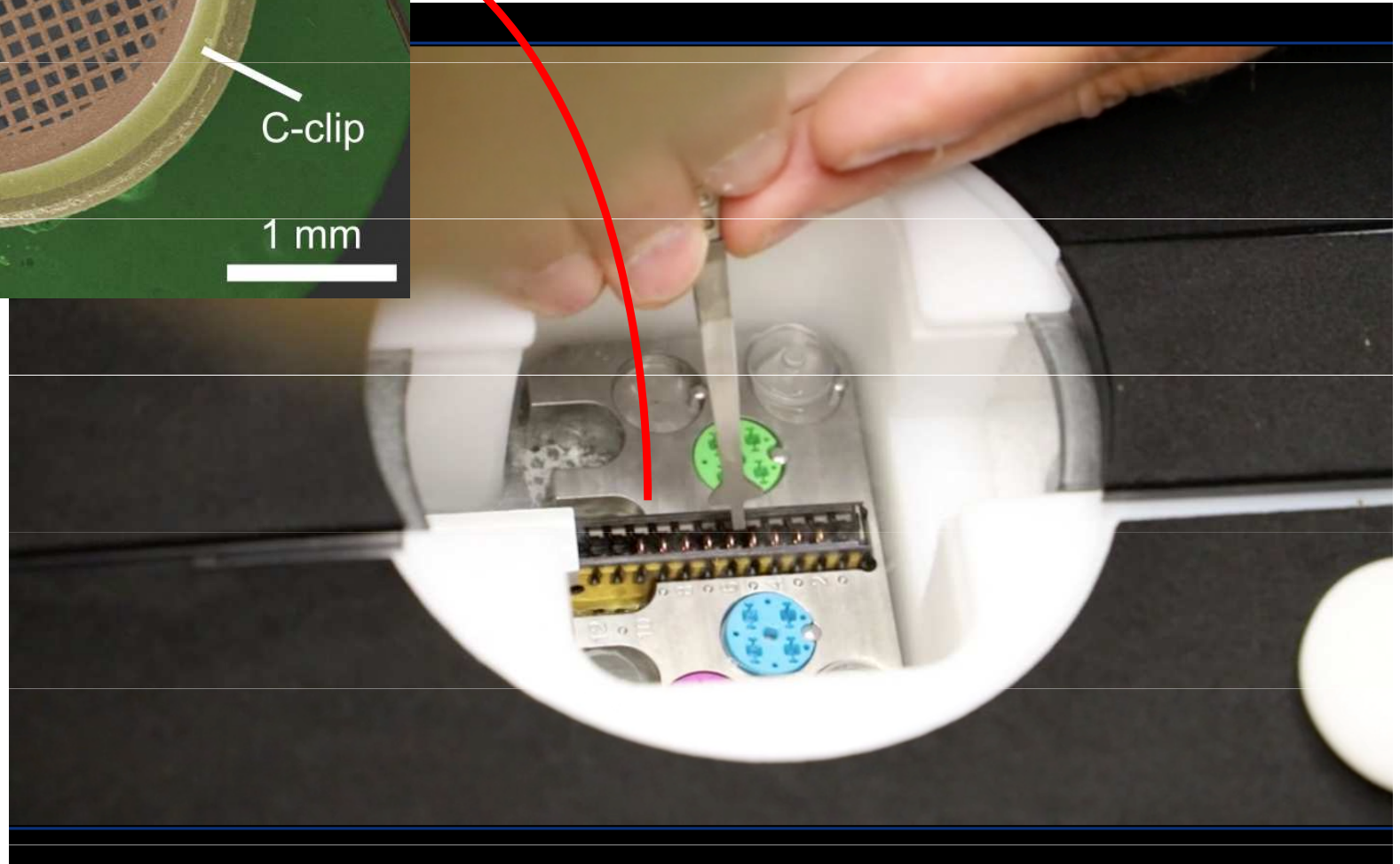
**Autogrid Tweezer**





Autogrid:  
grid + C-shape spring (C-clip) + clip ring

Auto-loader: up to 12 autogrids

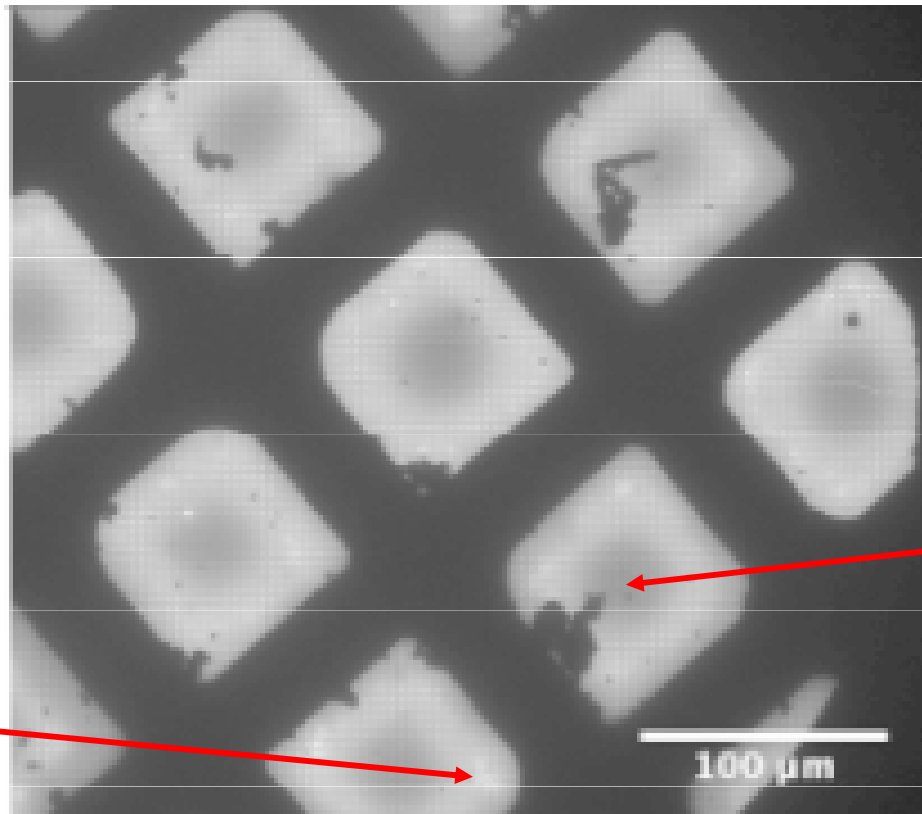
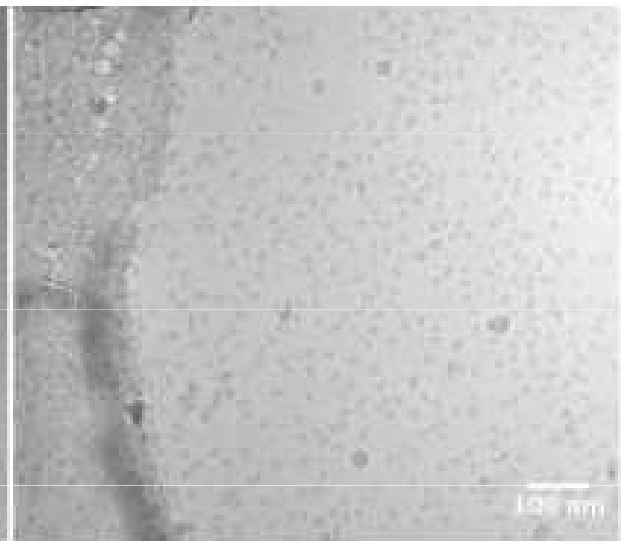
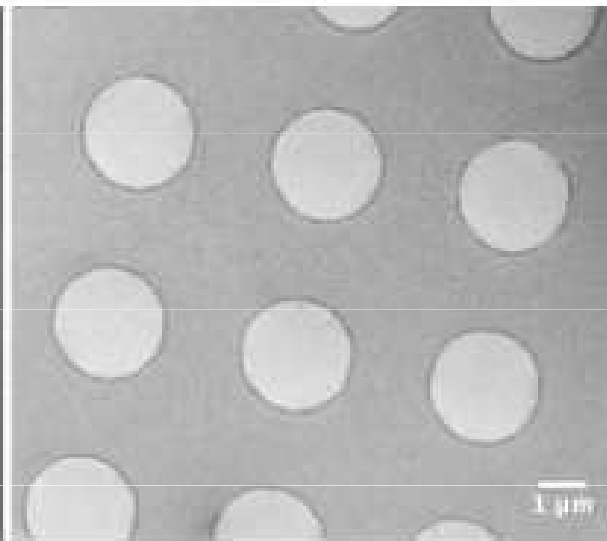
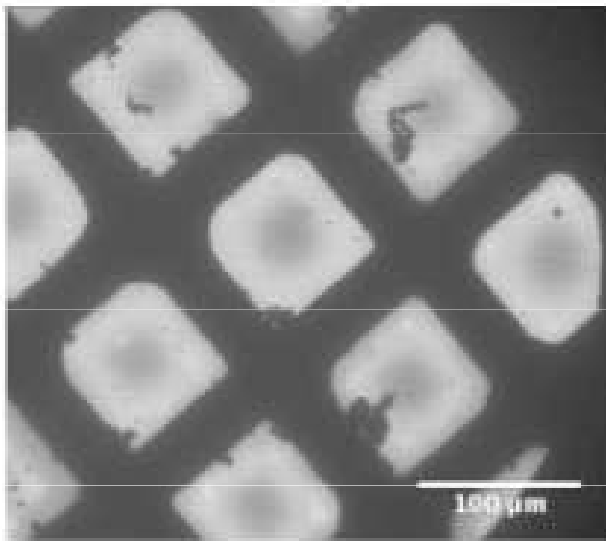




**AutoGrid Container**

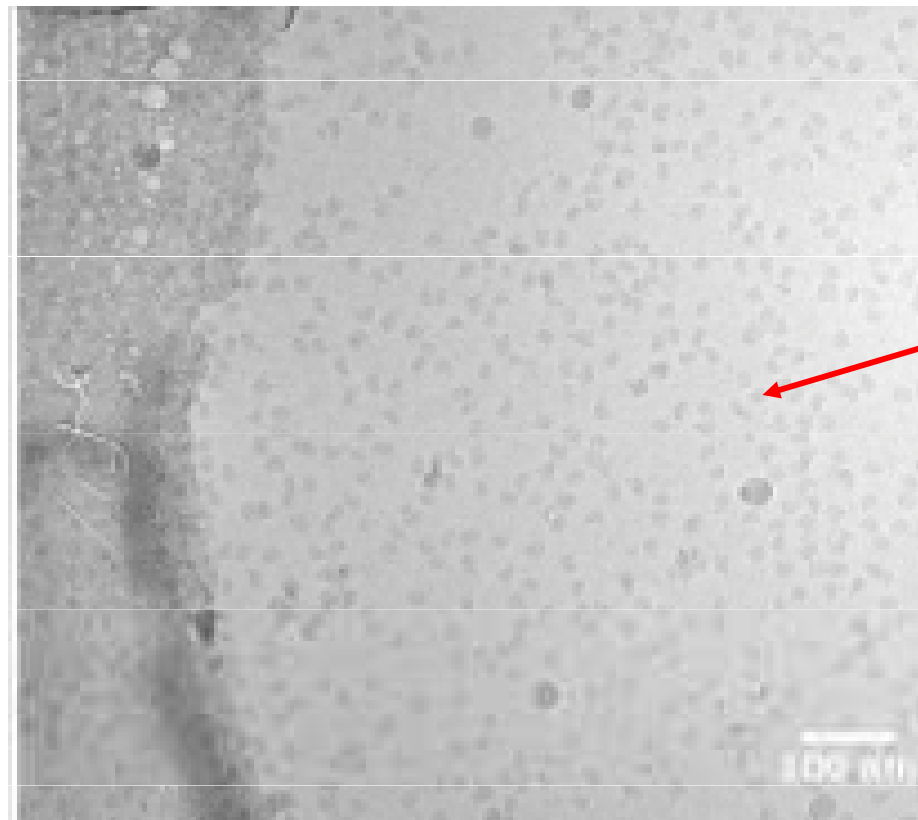
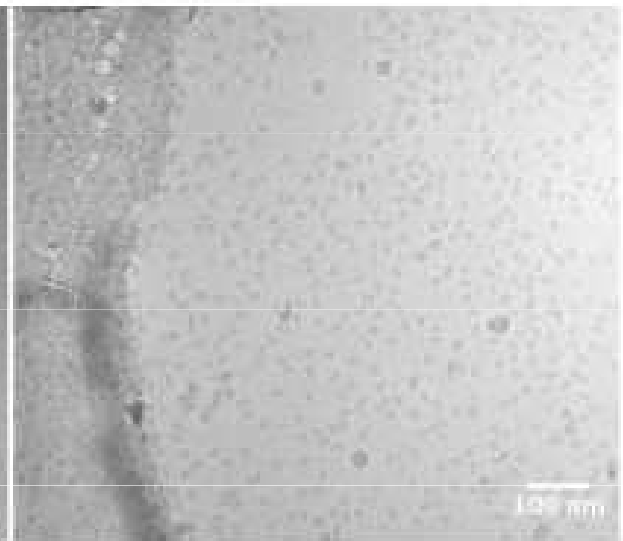
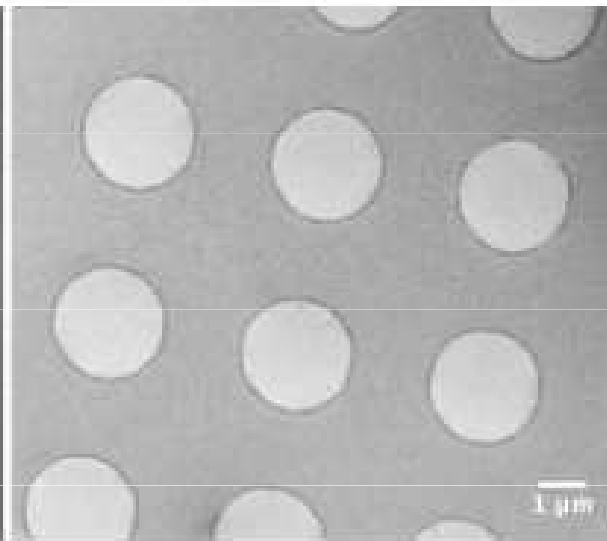
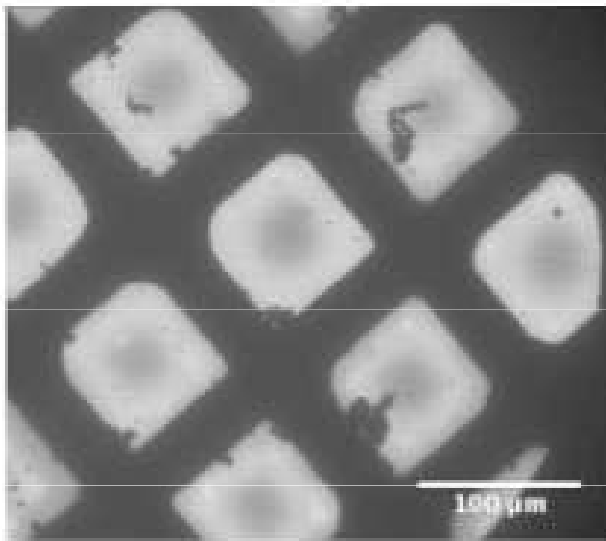


ThermoFisherScientific; MiTeGen



Grids get  
damaged

Note the ice  
thickness



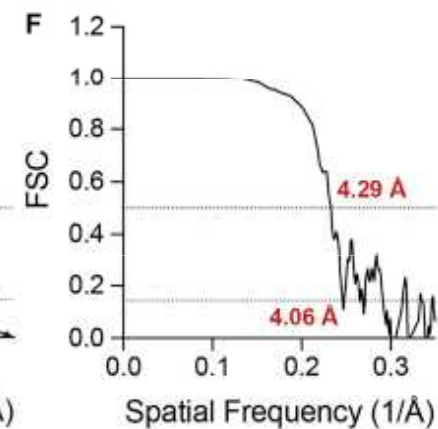
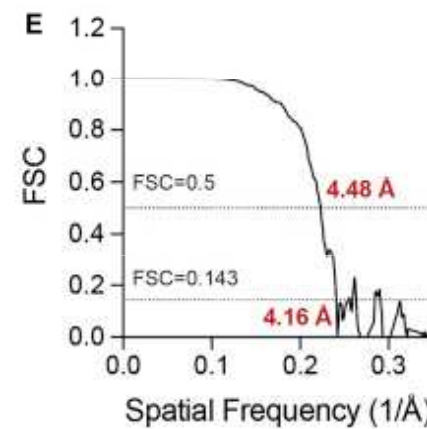
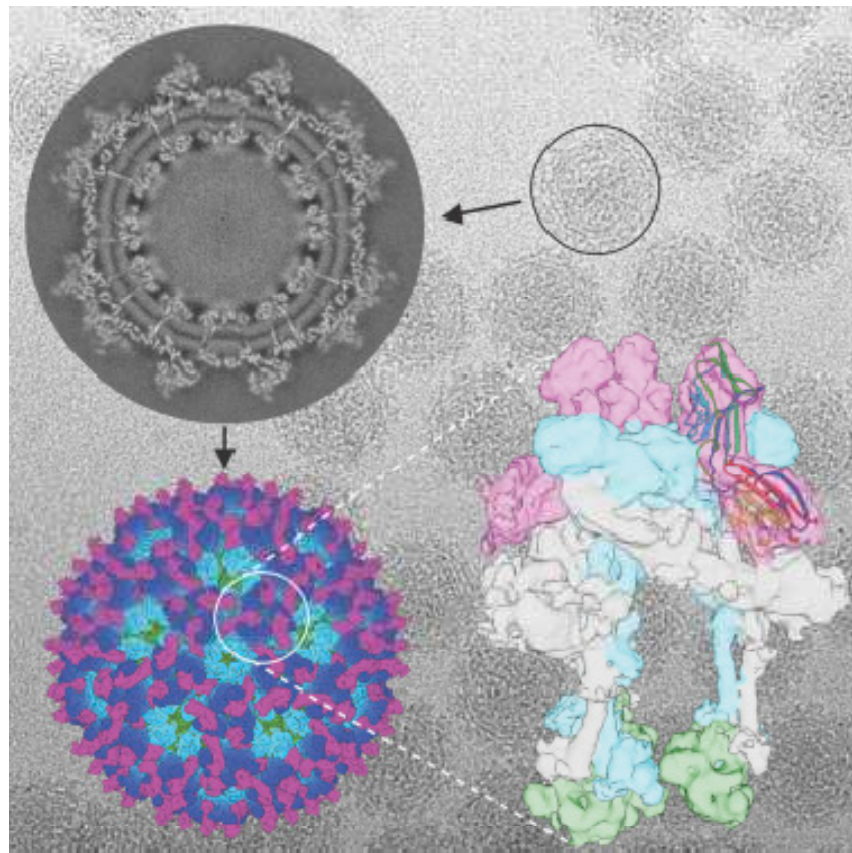
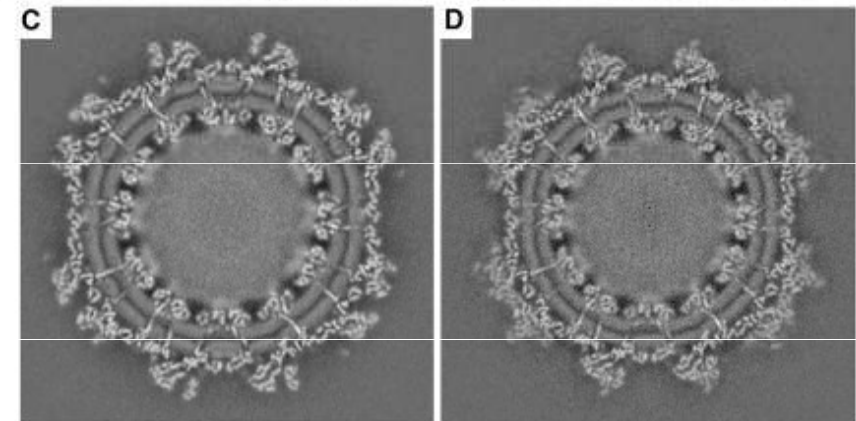
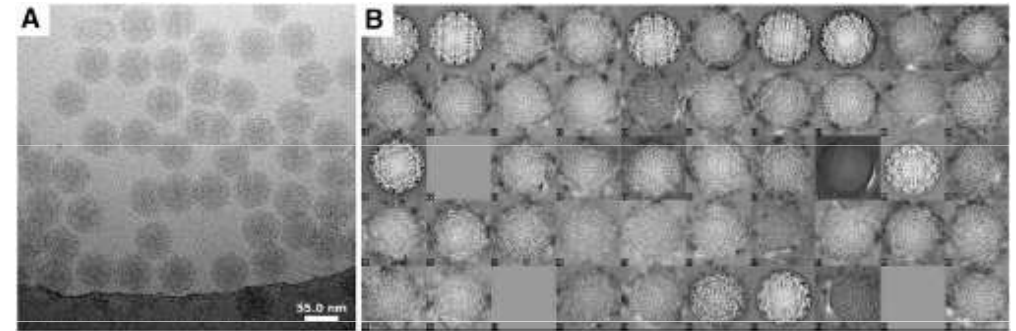
single particles



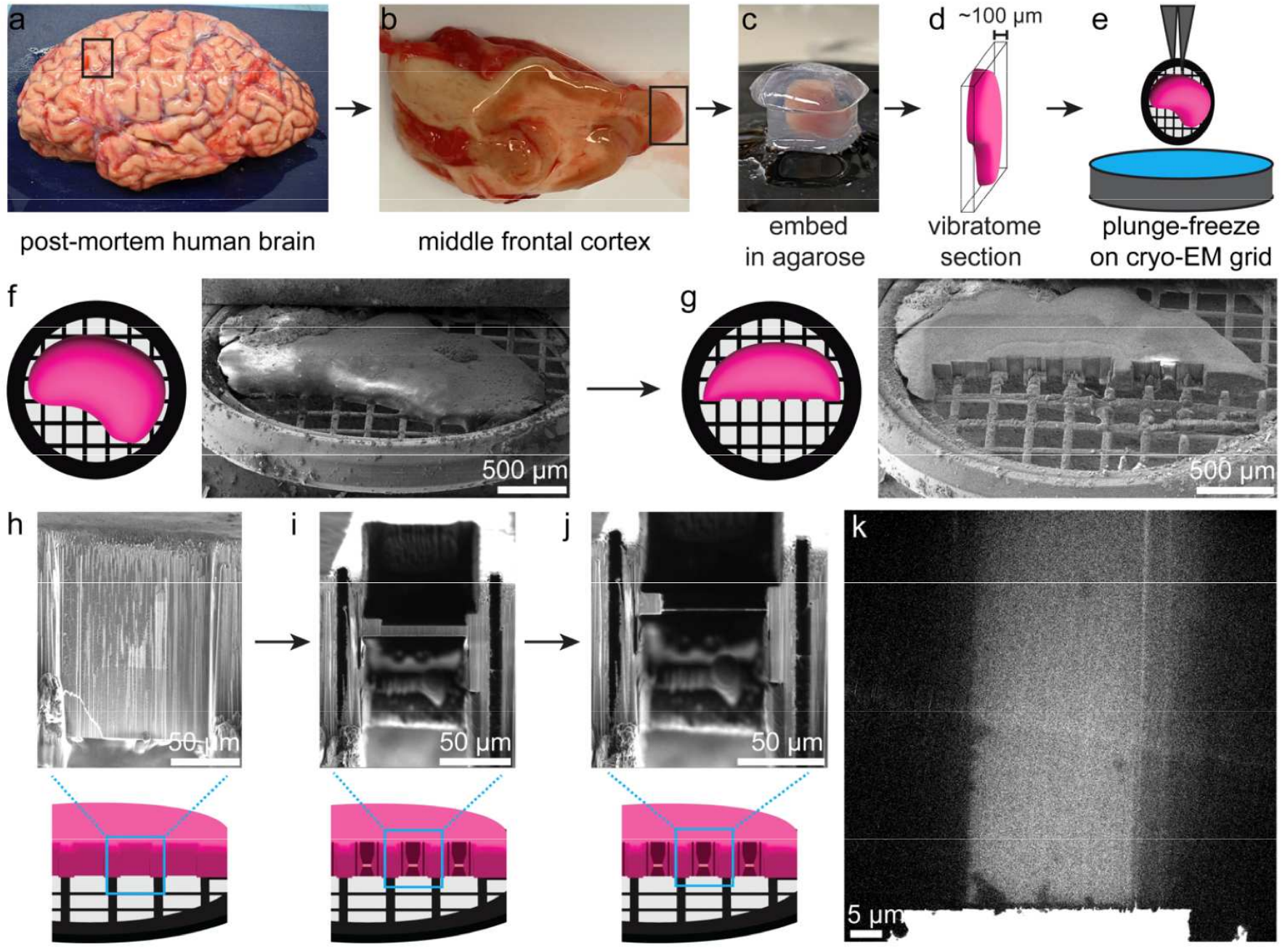


# Structure determination using single particle cryoEM

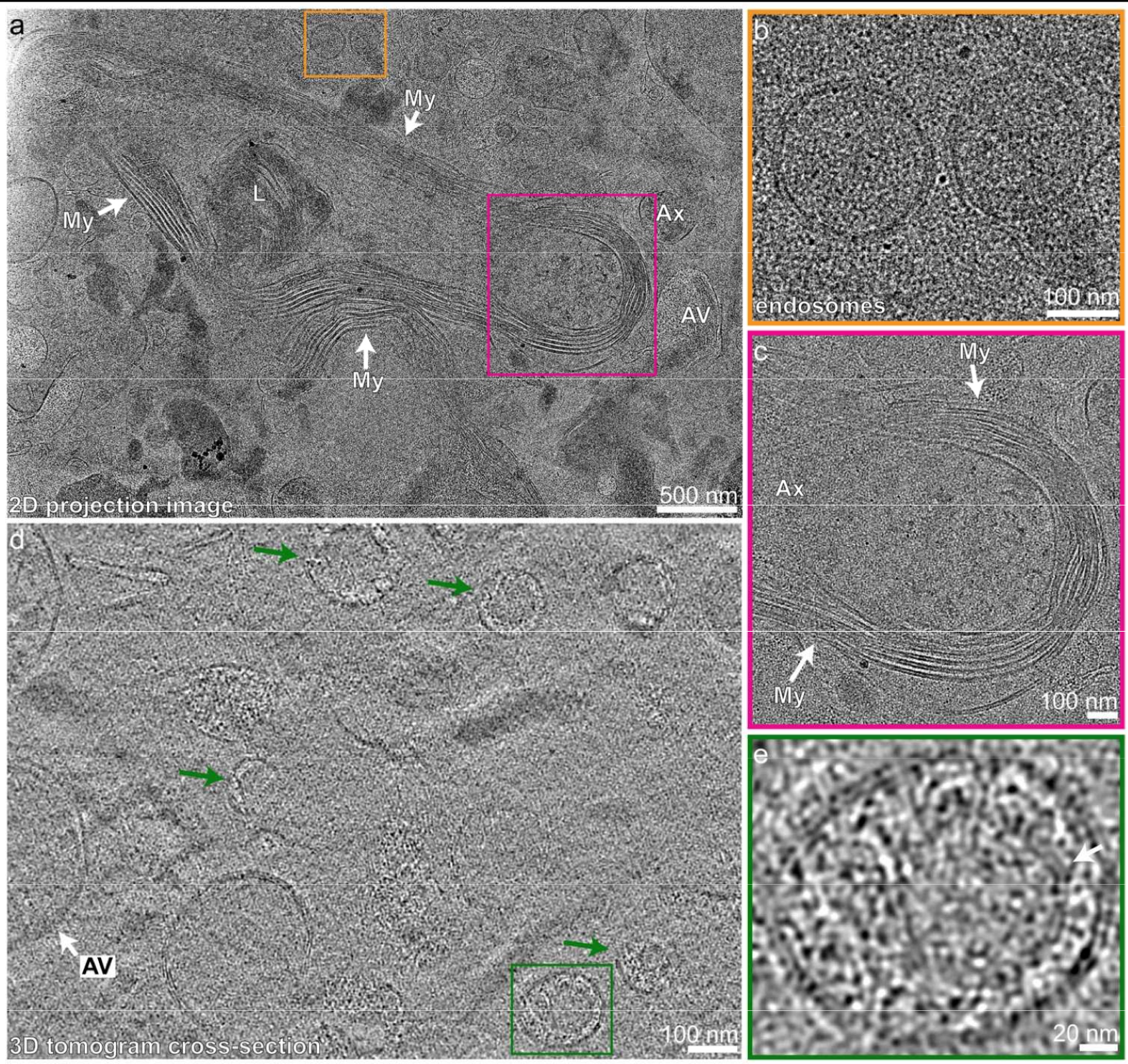
- 1) Sample preparation – 2) vitrification – 3) measurement – 4) particle picking – 5) 2D classification – 6) 3D model reconstruction



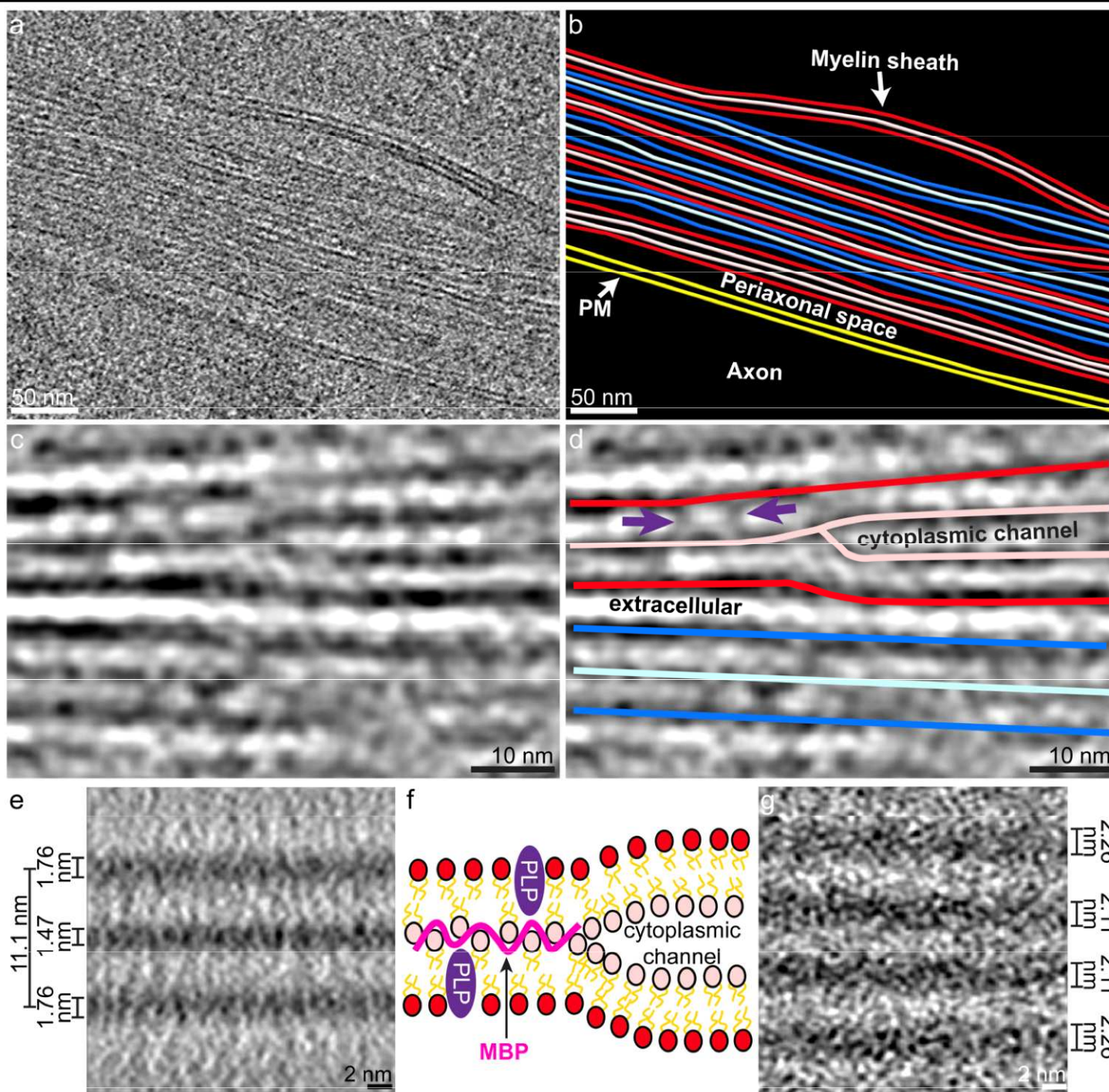
<https://doi.org/10.1016/j.cell.2019.04.006>







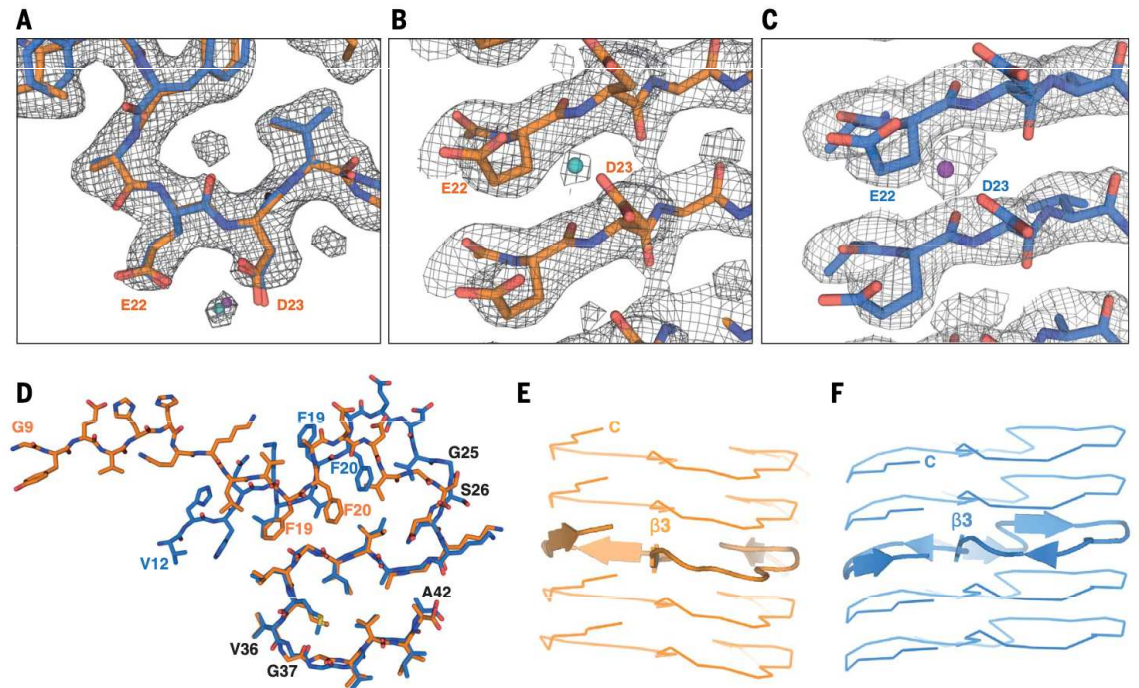
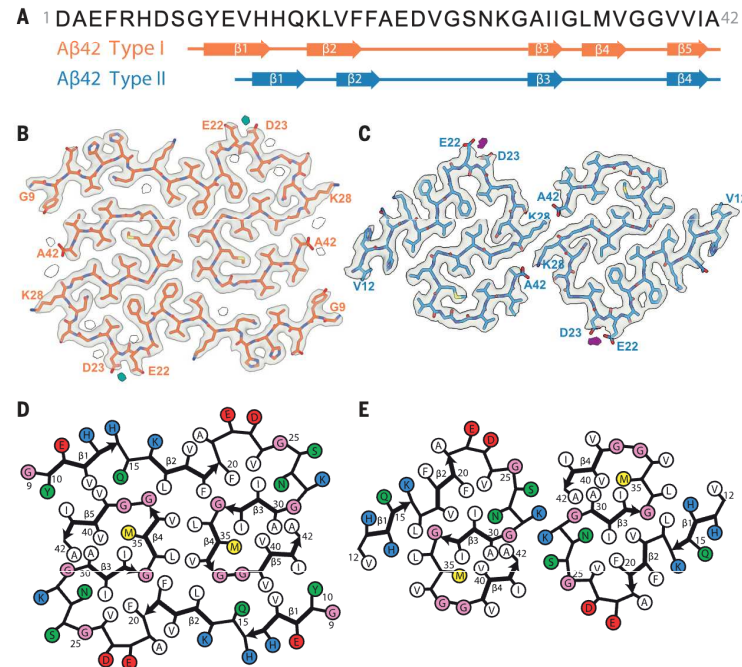
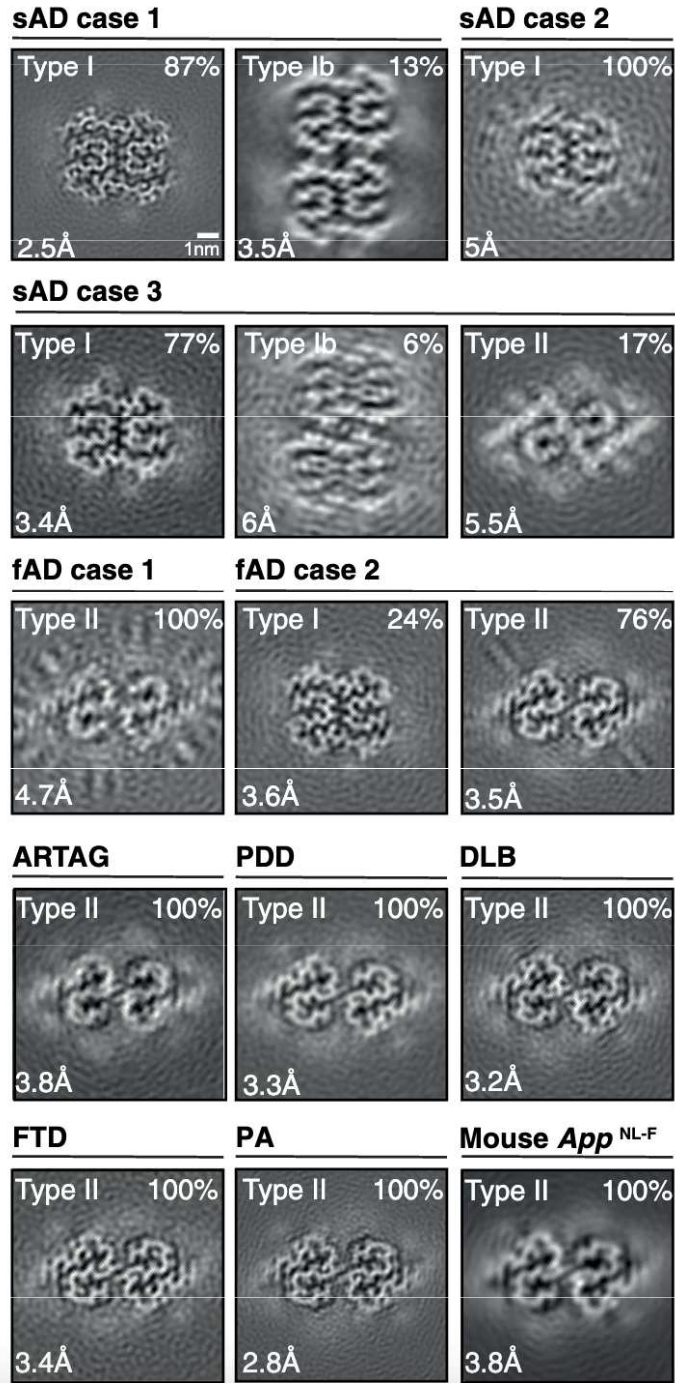




bioRxiv preprint doi: <https://doi.org/10.1101/2023.09.13.557623>



# Cryo-EM structures of amyloid- $\beta$ 42 filaments from human brains



17/10/2023

Karel Kubíček

Yang et al., Science 375, 167–172 (2022)

Mosalaganti, Obarska-Kosinska, Siggel et al

## **Title: Artificial intelligence reveals nuclear pore complexity**

### **Authors:**

Shyamal Mosalaganti<sup>1,2,3†</sup>, Agnieszka Obarska-Kosinska<sup>1,4†</sup>, Marc Siggel<sup>4,5,6†</sup>, Beata Turonova<sup>1,2</sup>, Christian E. Zimmerli<sup>1,2</sup>, Katarzyna Buczak<sup>2‡</sup>, Florian H. Schmidt<sup>2§</sup>, Erica Margiotta<sup>1,2</sup>, Marie-Therese Mackmull<sup>2¶</sup>, Wim Hagen<sup>2</sup>, Gerhard Hummer<sup>5,7\*</sup>, Martin Beck<sup>1,2\*</sup>, Jan Kosinski<sup>2,4,6\*</sup>



# THE BEST INVENTIONS OF 2022

200 innovations changing how we live

How We Chose the List



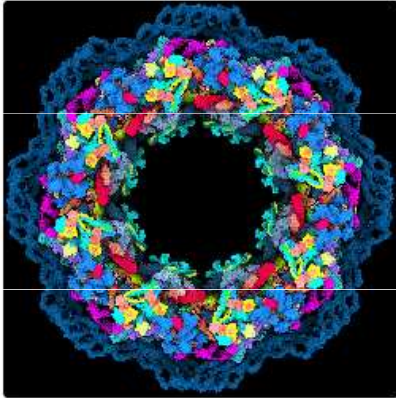
Sergiy Barchuk for TIME

**TIME**  
**S I T E S**

The Best Inventions of 2022 homepage is built on TIME Sites. To learn how businesses use TIME Sites to tell their stories with easy-to-deploy, visually stunning microsites, visit [timesites.com](https://timesites.com).

<https://time.com/collection/best-inventions-2022/6229912/deepmind-alphafold/>

# AI



**MAPPING LIFE'S BUILDING BLOCKS**  
DEEPMIND ALPHAFOLD



**DETECTING DESTRUCTION OF WAR**  
SCALE AI AUTOMATED DAMAGE IDENTIFICATION

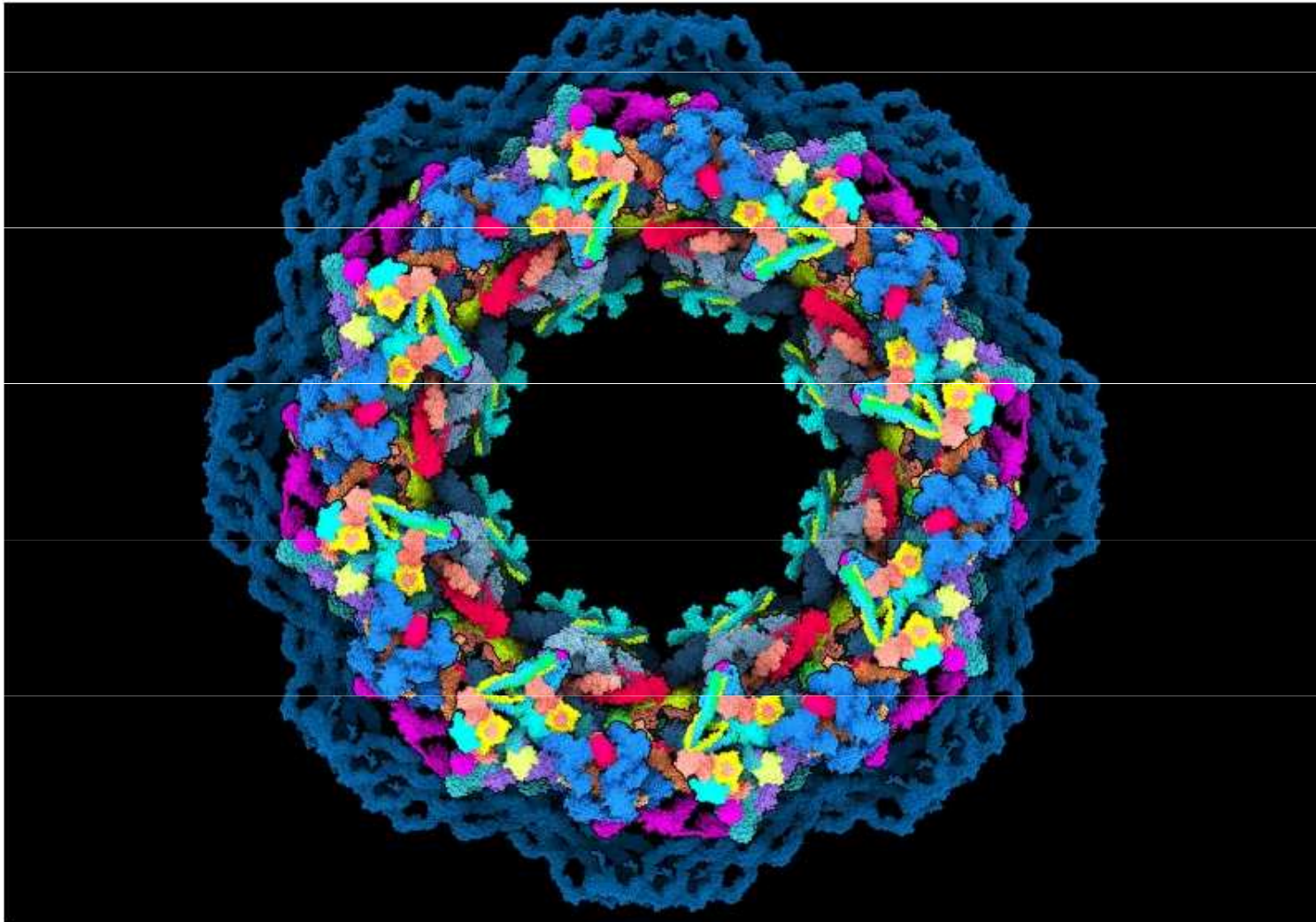


**ARTIFICIAL IMAGINATION**  
OPENAI DALL-E 2



# Mapping Life

DeepMind AlphaFold

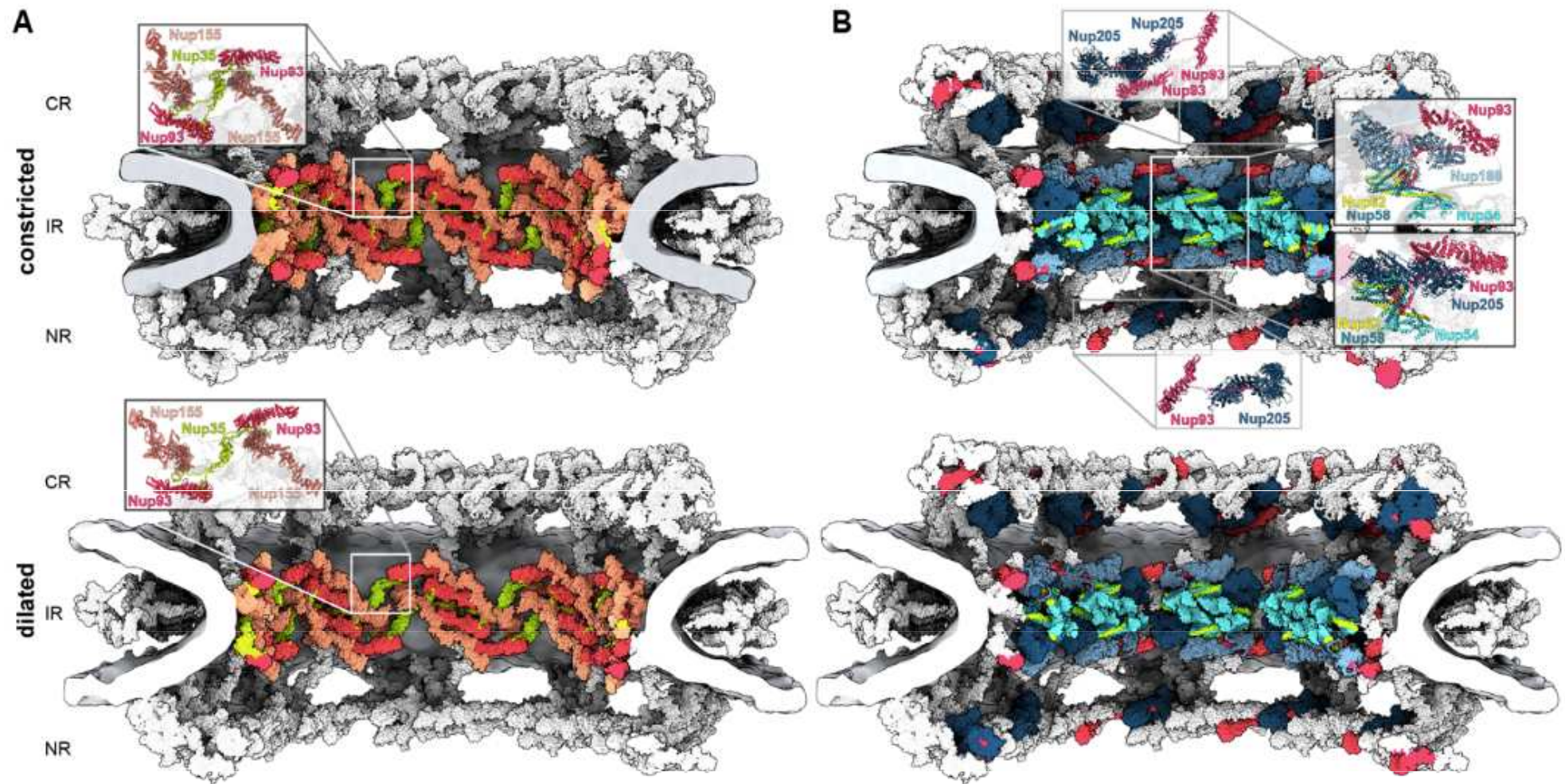


In the recent past, discerning the exact 3D structure of a single protein took around five years. Today the same task is possible in seconds thanks to the machine learning program **AlphaFold**, developed by Alphabet subsidiary DeepMind. In July, the company announced that AlphaFold had predicted the structures of 200 million proteins—nearly all known to humankind. And in what CEO Demis Hassabis described as a “**gift to humanity**,” DeepMind made the structures, along with AlphaFold’s underlying code, freely available to all. That will likely accelerate the work of scientists around the world trying to solve humanity’s toughest problems. The company says AlphaFold is now being used in efforts as diverse as fighting antibiotic resistance and Parkinson’s disease, and tackling plastic pollution.

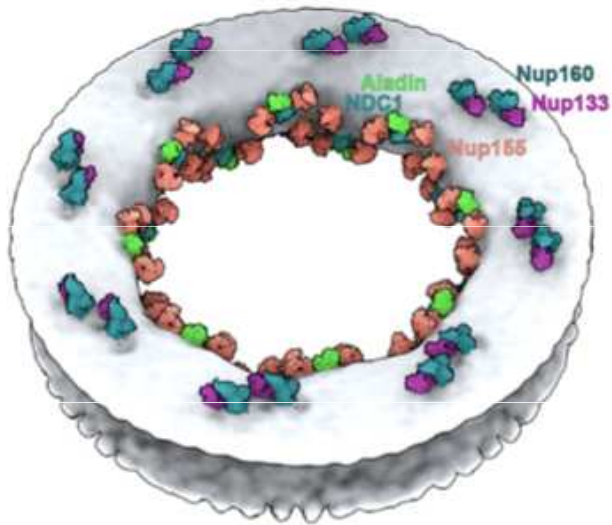








constricted



dilated

