

Přístrojové metody molekulární biofyziky

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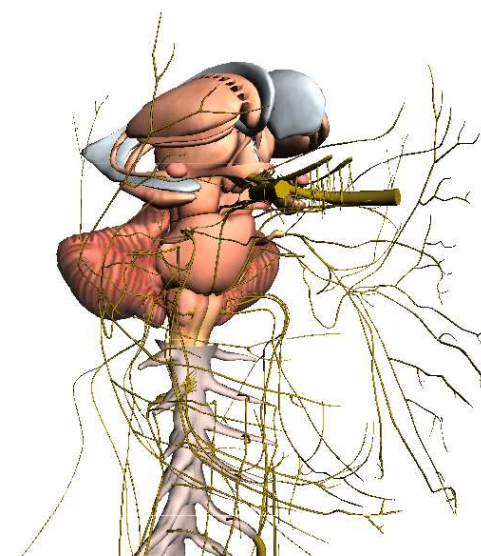
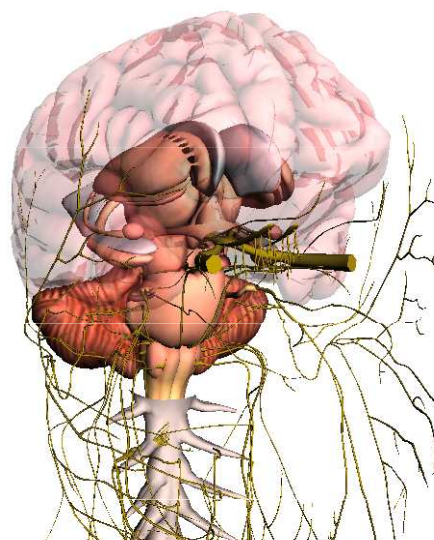
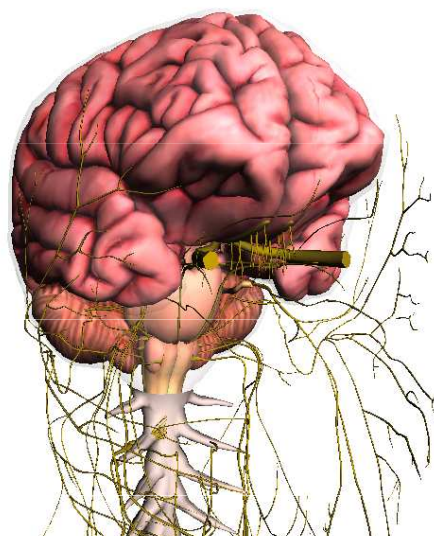
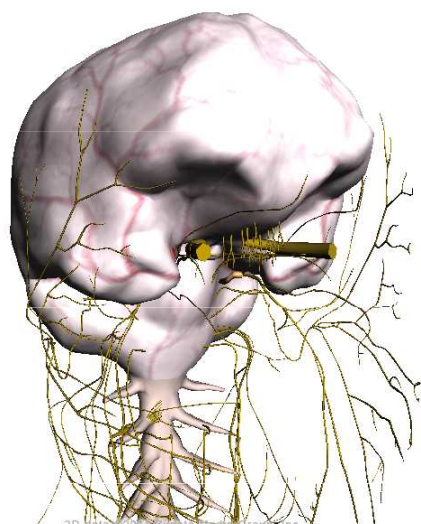
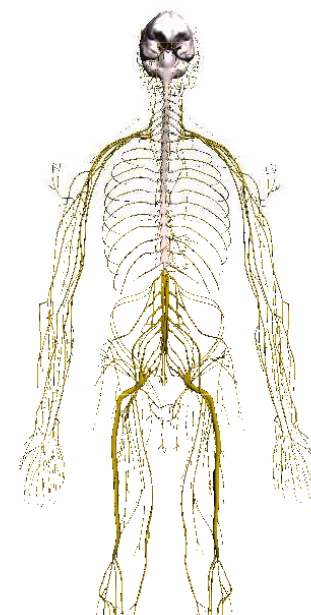
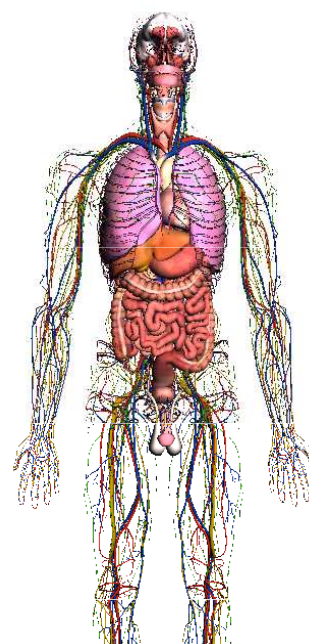
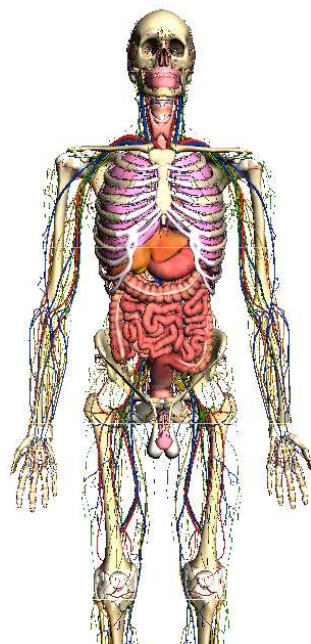
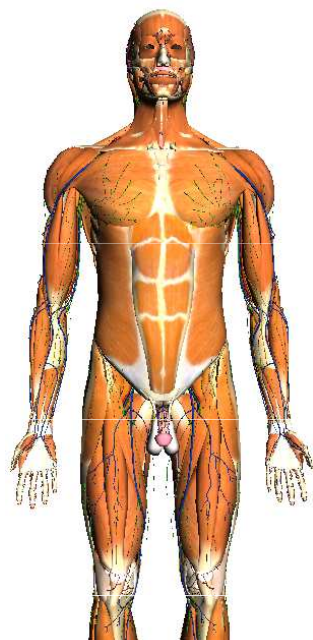
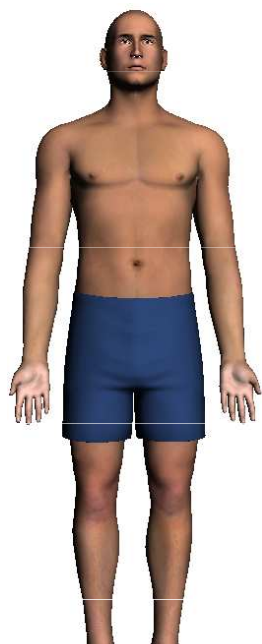
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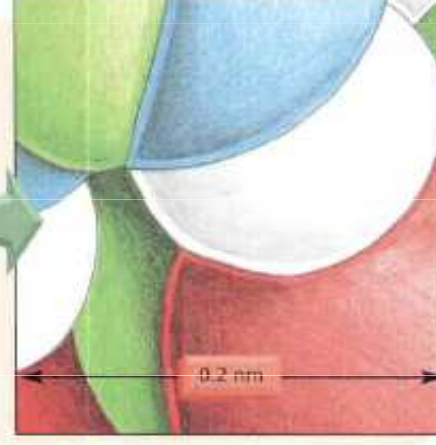
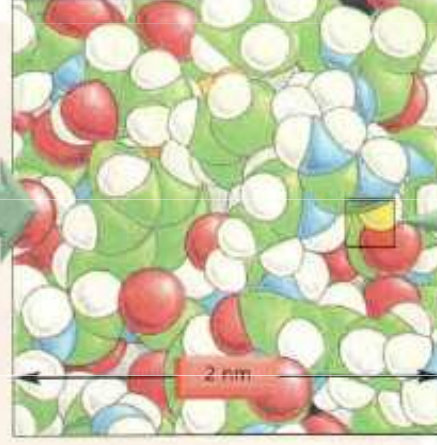
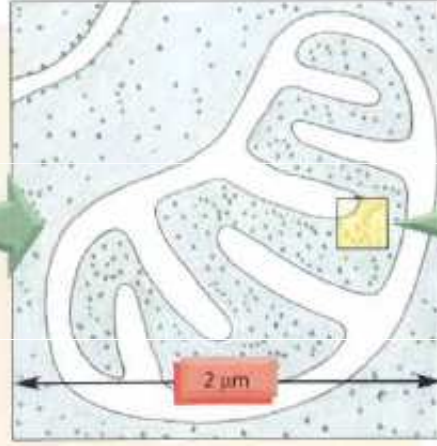
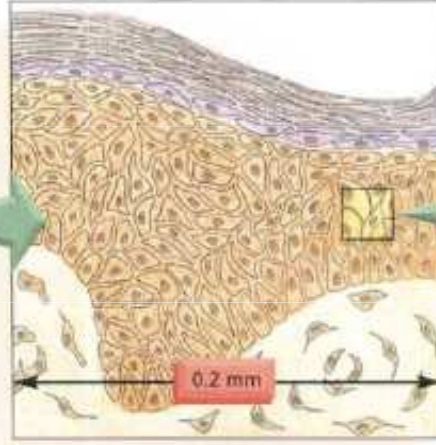
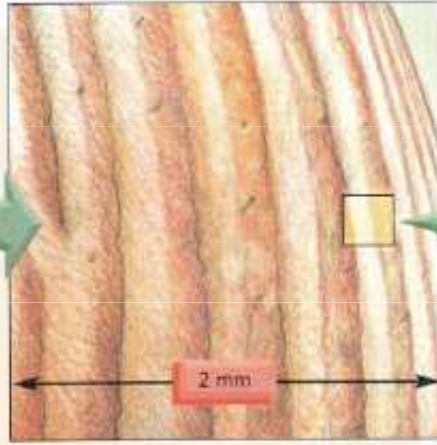
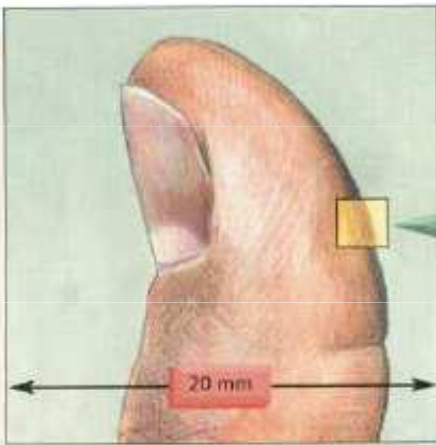
- **Biomolekulární vědy mají klíčový význam pro molekulární medicínu.**
 - Budeme se zabývat zařízeními pro studium struktury, měření koncentrace (*in-vitro* i *in-vivo*), a pro studium vlastností membrán
- Nejběžnější zařízení založená na interakci elektromagnetického záření s makromolekulami
 - VIS, UV a IR spektrofotometry
 - Ramanovy spektrometry
 - Zařízení pro měření cirkulárního dichroismu
 - Zařízení pro rentgenstrukturní analýzu
 - Nukleární magnetickou rezonancí
 - Hmotnostní spektrometrie
 - Fluorescenční techniky
- Zařízení založená na jiných vlastnostech biomolekul (např. mechanických a elektrických)
 - Elektroforéza
 - Langmuir-Blodgettové technika
- Zařízení pro měření membránových potenciálů a koncentrace iontů v buňkách

Biofyzika a biomolekulární výzkum

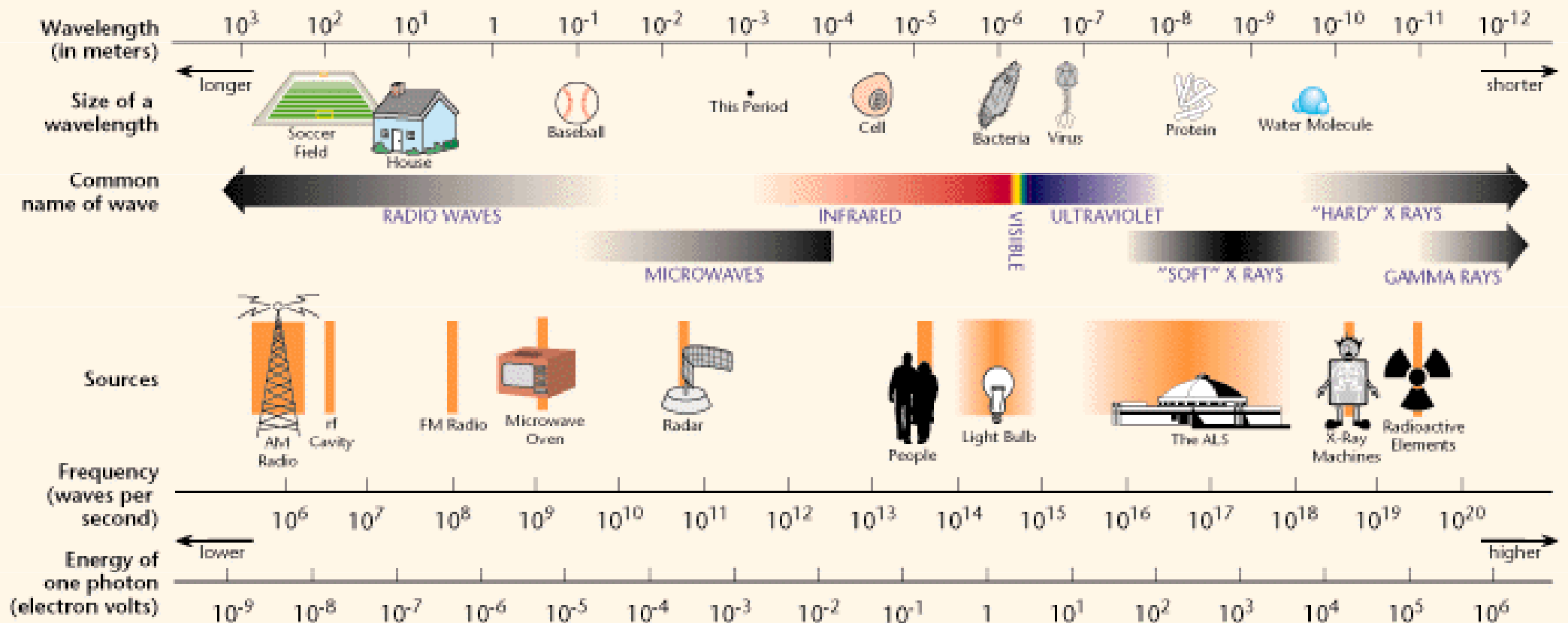
Tento výzkum je orientován zejména na strukturální studie, které umožňují porozumět např.:

- Specifičnosti enzymatických a imunologických reakcí
- Účinkům některých léků (např. cytostatik) na molekulární úrovni.
- Mechanismům pasivního i aktivního transportu
- Buněčnému pohybu
-





THE ELECTROMAGNETIC SPECTRUM



Composition of the Earth's Crust, Seawater, and the Human Body*

Earth's Crust		Seawater		Human Body [†]	
Element	%	Compound	mM	Element	%
O	47	Cl ⁻	548	H	63
Si	28	Na ⁺	470	O	25.5
Al	7.9	Mg ²⁺	54	C	9.5
Fe	4.5	SO ₄ ²⁻	28	N	1.4
Ca	3.5	Ca ²⁺	10	Ca	0.31
Na	2.5	K ⁺	10	P	0.22
K	2.5	HCO ₃ ⁻	2.3	Cl	0.08
Mg	2.2	NO ₃ ⁻	0.01	K	0.06
Ti	0.46	HPO ₄ ²⁻	<0.001	S	0.05
H	0.22			Na	0.03
C	0.19			Mg	0.01

*Figures for the earth's crust and the human body are presented as percentages of the total number of atoms; seawater data are millimoles per liter. Figures for the earth's crust do *not* include water, whereas figures for the human body do.

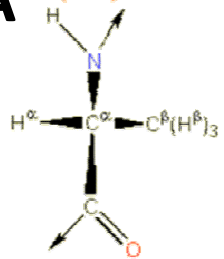
[†]Trace elements found in the human body serving essential biological functions include Mn, Fe, Co, Cu, Zn, Mo, I, Ni, and Se.

Chemické složení lidského těla

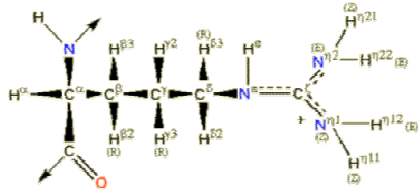
- a. (65 %) Voda
- b. (20 %) Proteiny
- c. (12 %) Lipidy (tuky)
- d. (1.1 %) Nukleové kyseliny
- e. Ionty (Na^+ , K^+ , Cl^- , PO_4^{3-} ...)
- f. Plyny (O_2 , CO_2 , ...), karbohydráty (glukóza), hydroxyapatit (forma vápníku a fosfátu – zuby, kosti), volné radikály, etc.

Proteiny

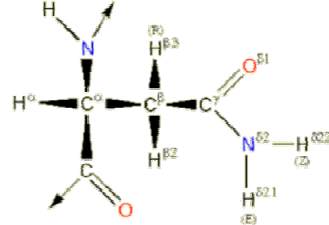
A L-Alanine (Ala)



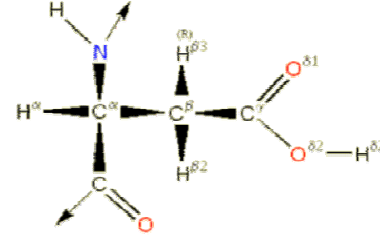
R L-Arginine (Arg)



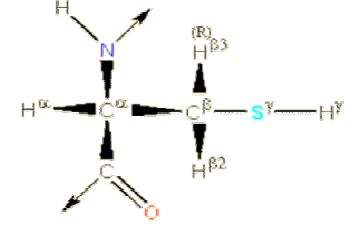
N L-Asparagine (Asn)



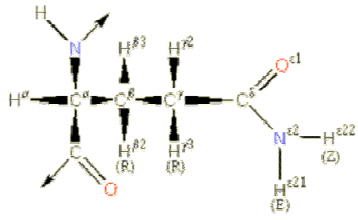
D L-Aspartic Acid (Asp)



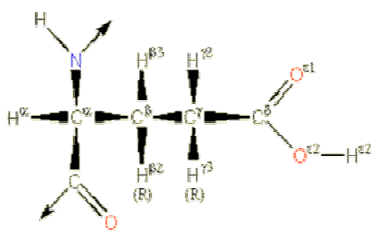
C L-Cysteine (Cys)



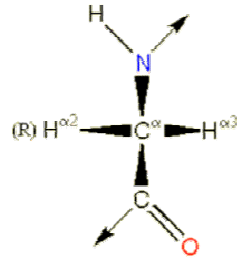
Q L-Glutamine (Gln)



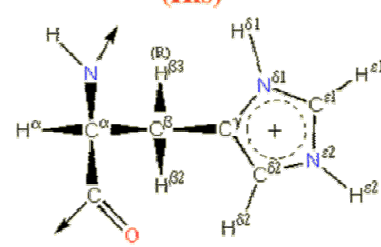
E L-Glutamic Acid (Glu)



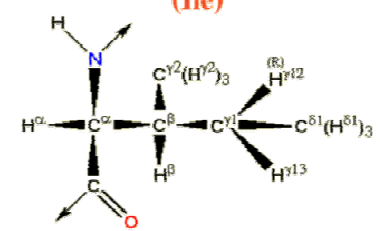
G Glycine (Gly)



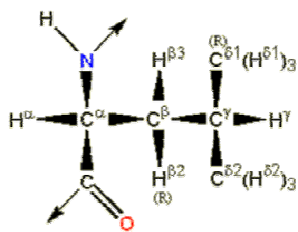
H L-Histidine (His)



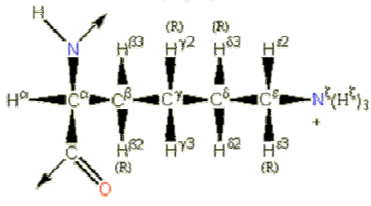
I L-Isoleucine (Ile)



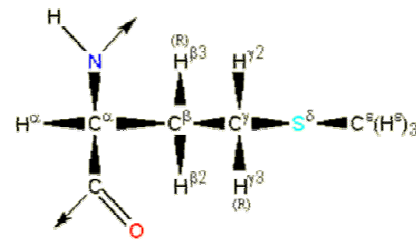
L L-Leucine (Leu)



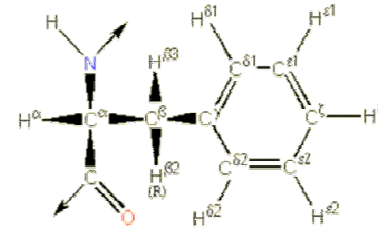
K L-Lysine (Lys)



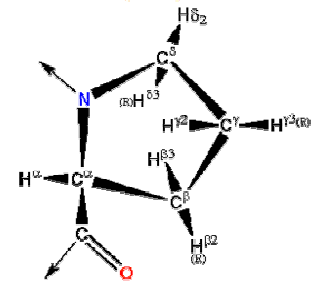
M Methionine (Met)



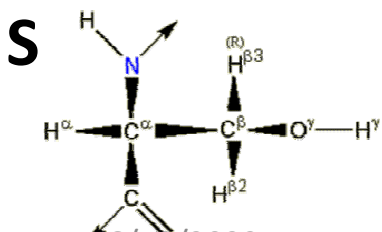
F L-Phenylalanine (Phe)



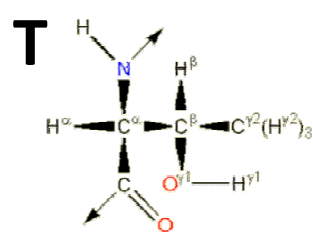
P L-Proline (Pro)



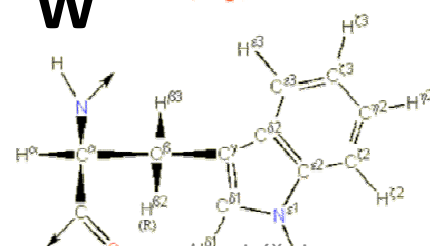
L-Serine (Ser)



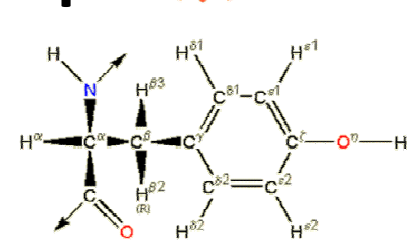
L-Threonine (Thr)



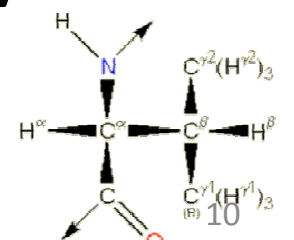
L-Tryptophan (Trp)



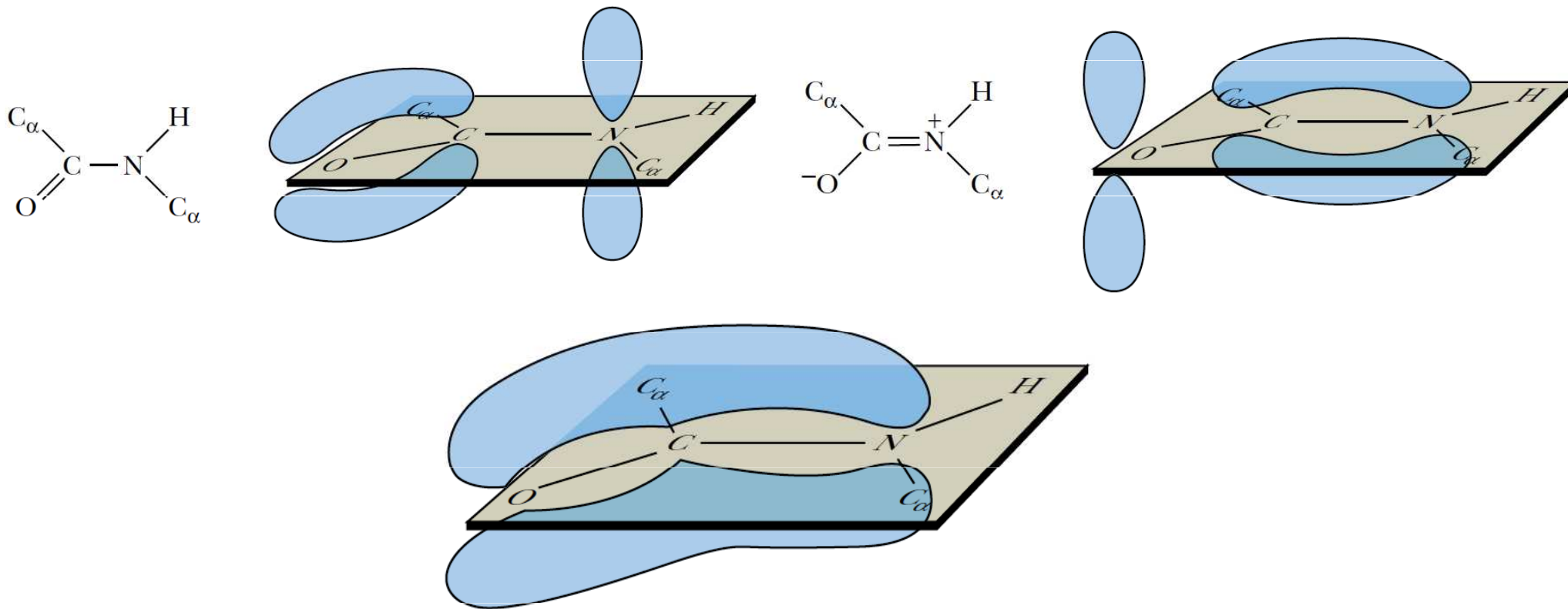
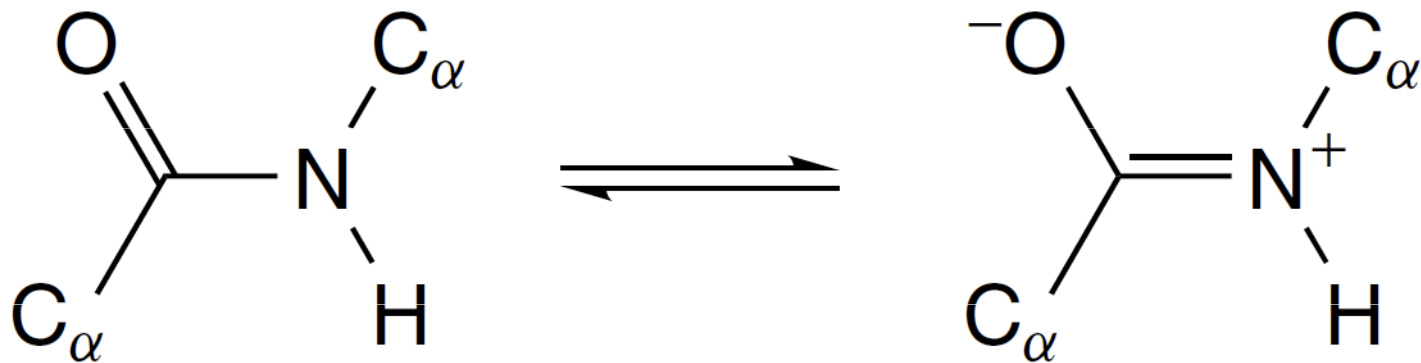
L-Tyrosine (Tyr)



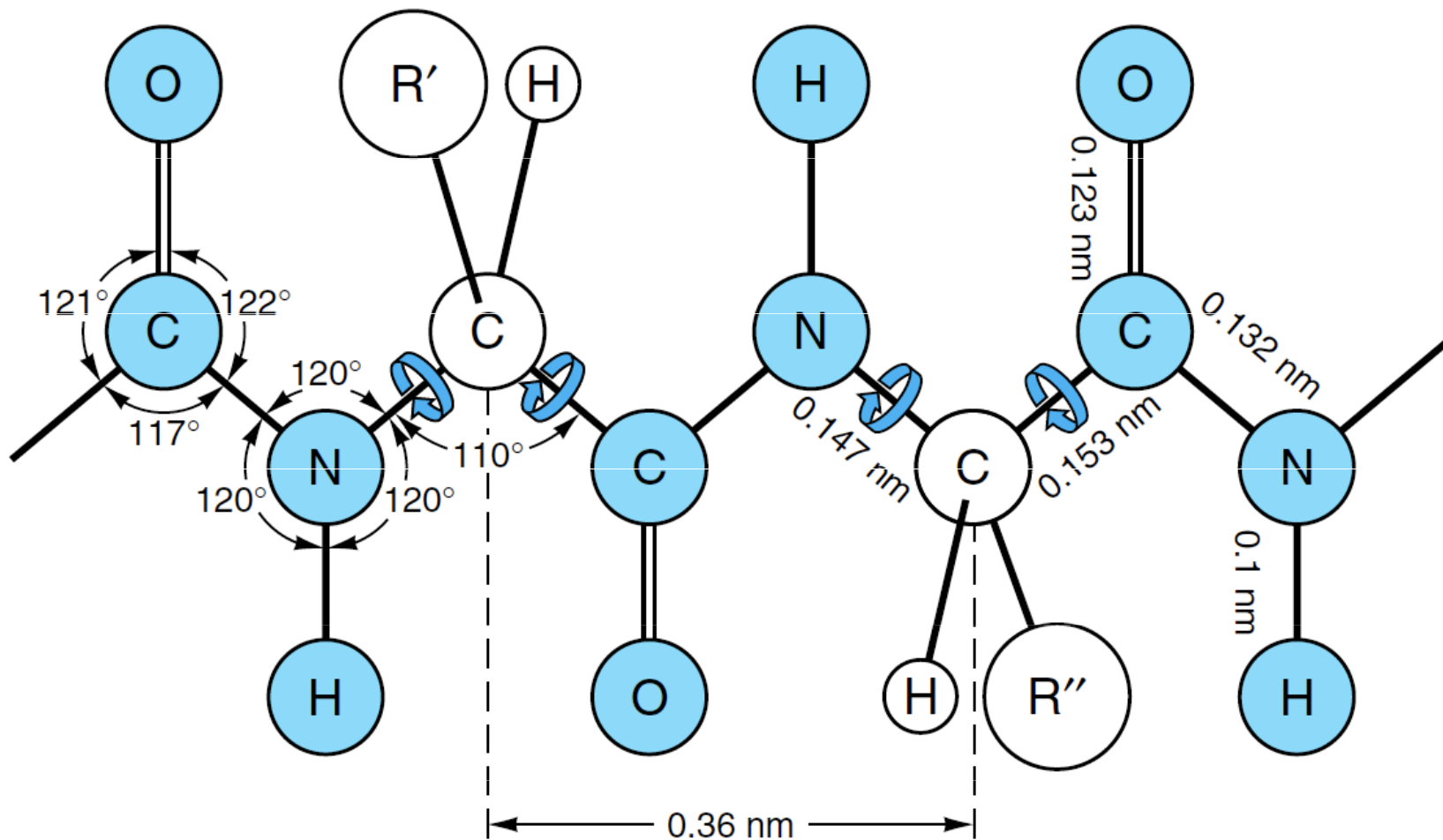
L-Valine (Val)

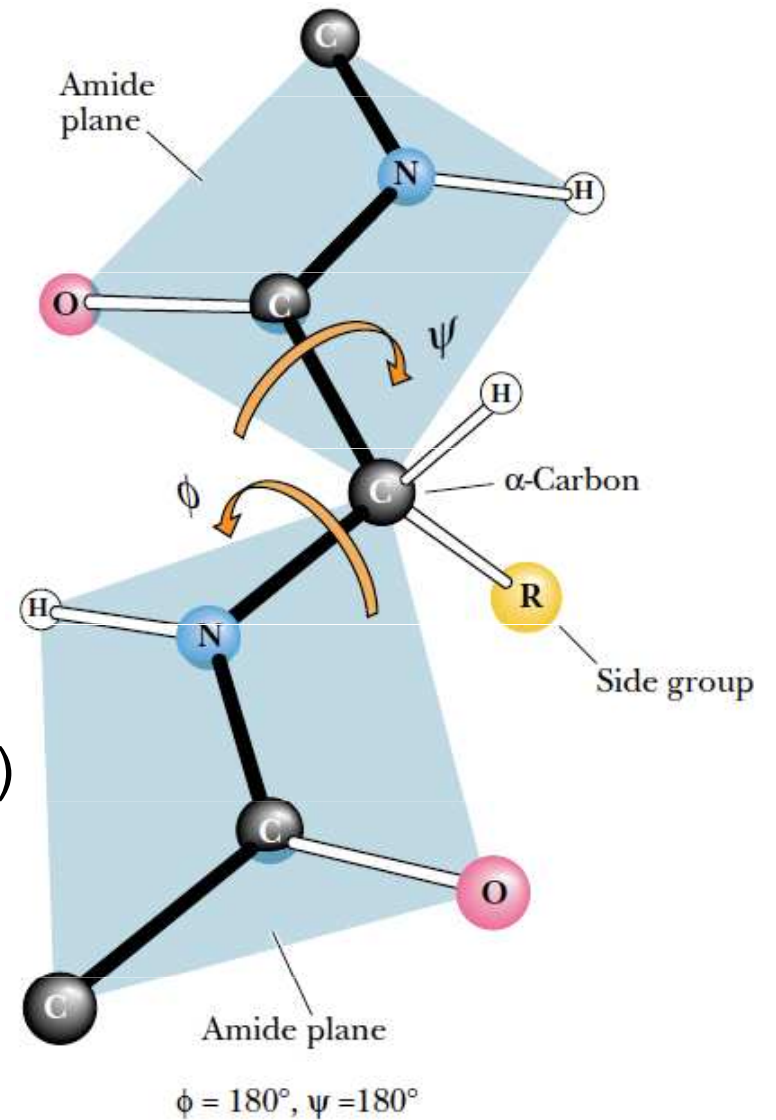
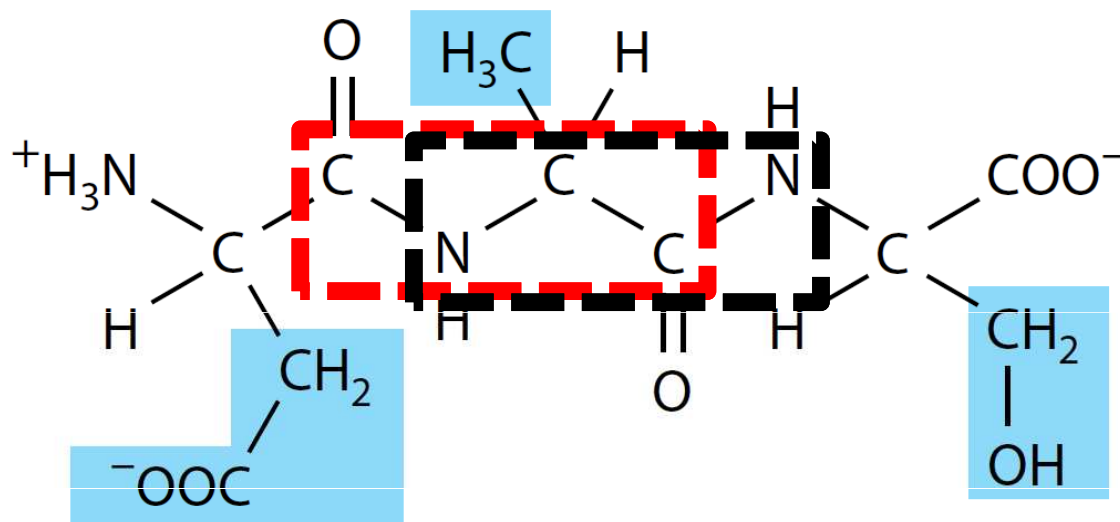
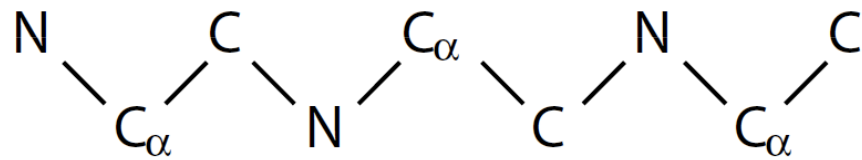


IV] Peptidová vazba – pseudo dvojitá vazba => amidová rovina



Proteinová páteř, primární struktura, číslování od **N**-konce (terminu) směrem k **C**-konci





ϕ – **CO, N, C_α, CO** (CO někdy značeno C')

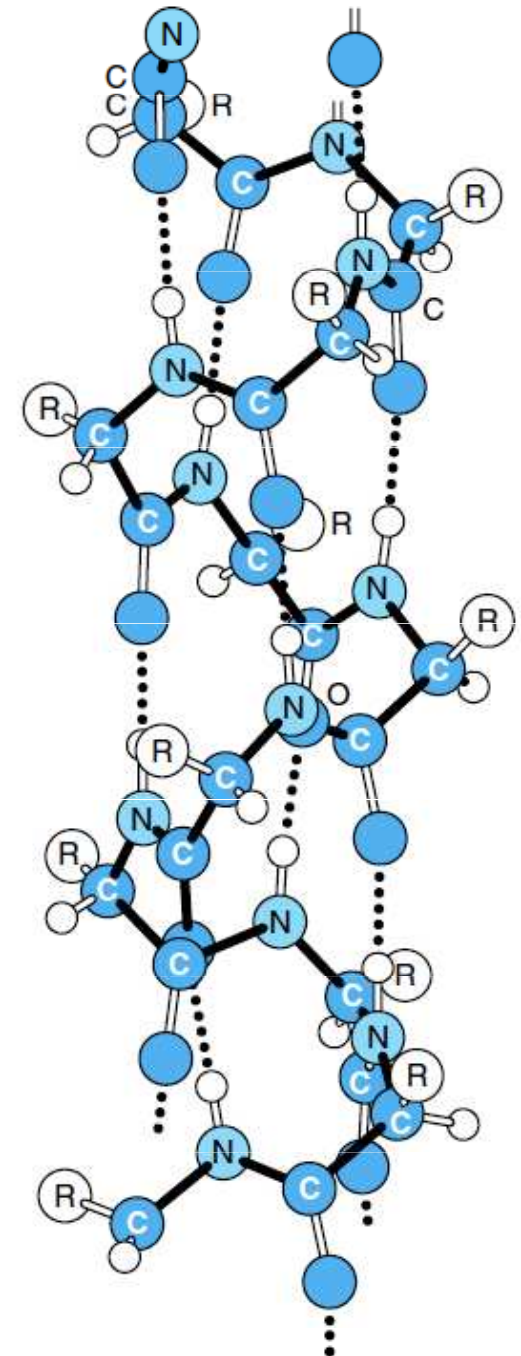
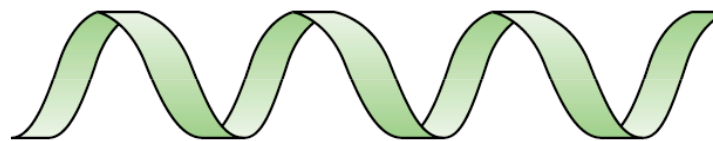
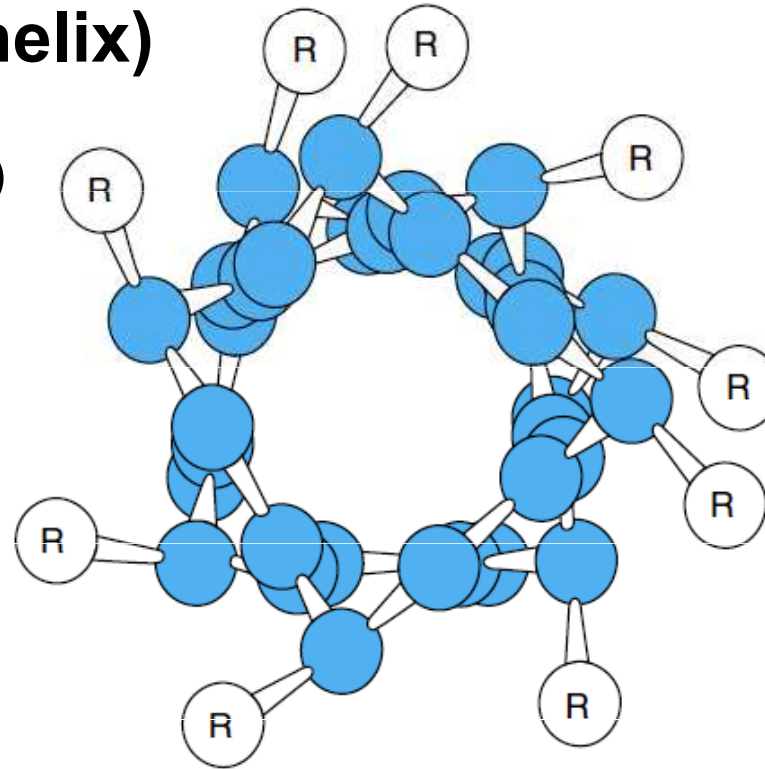
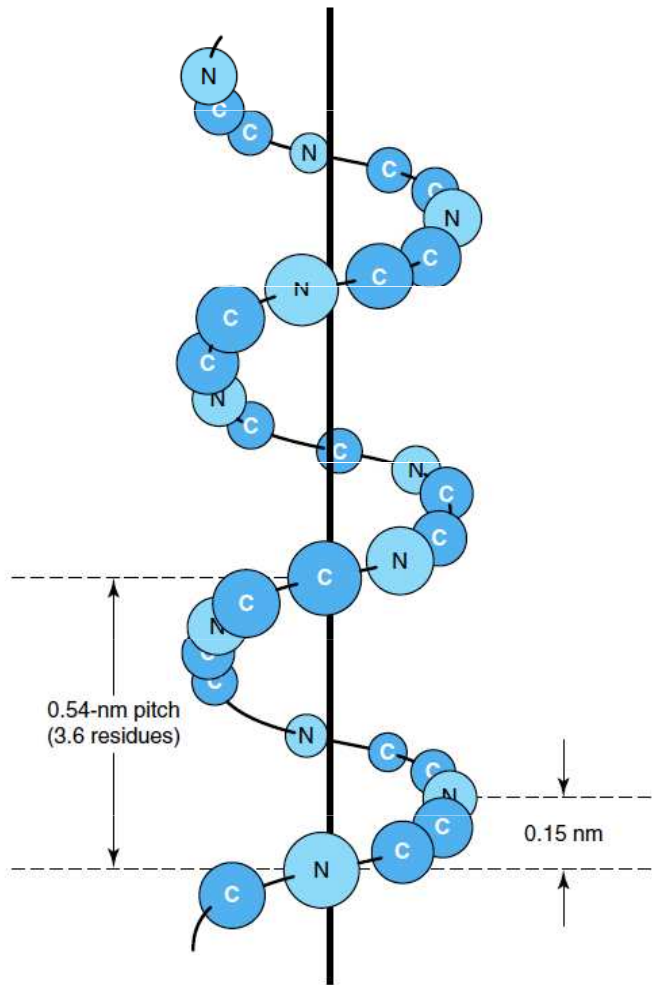
ψ – **N, C_α, CO, N**

VII] Sekundární struktura

1) α -šroubovice (α -helix)

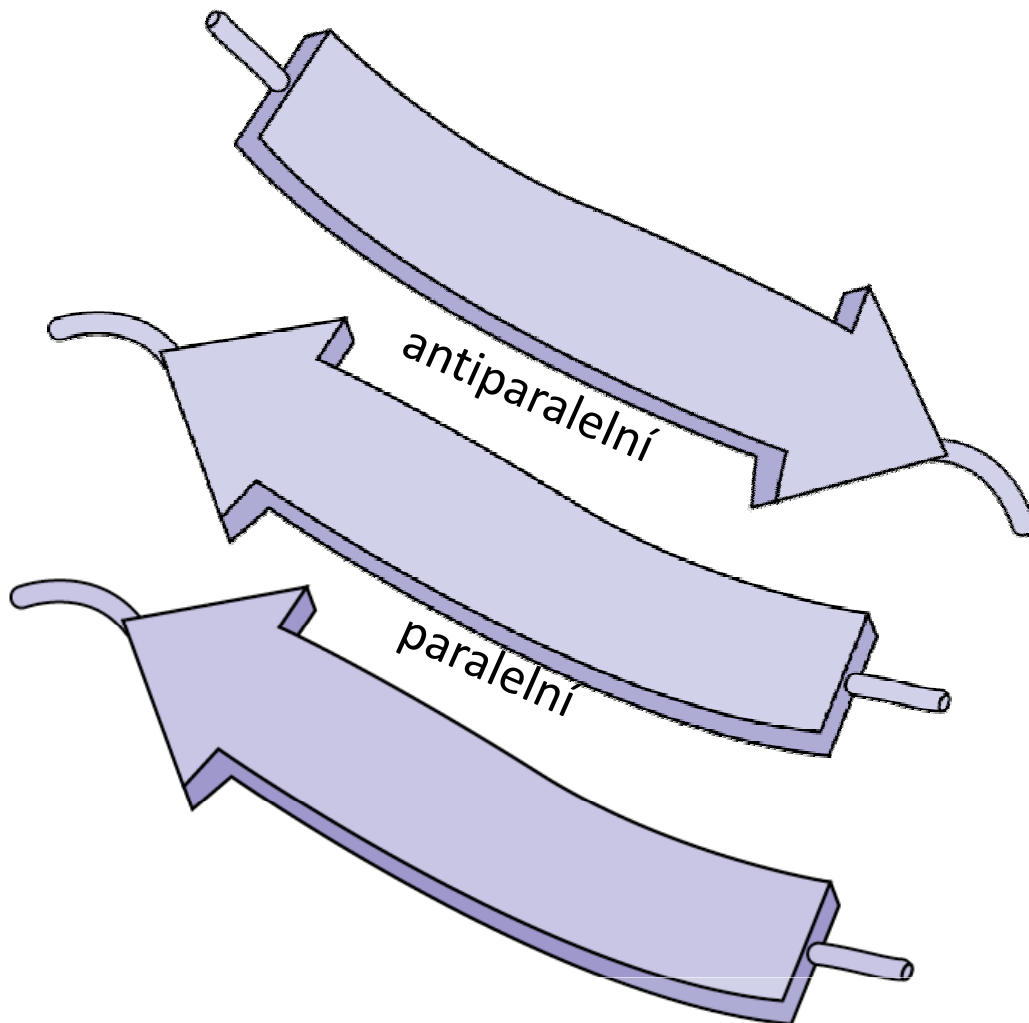
2) β -skládání list (beta-sheet)

3) Ohyb, smyčka (loop/turn)

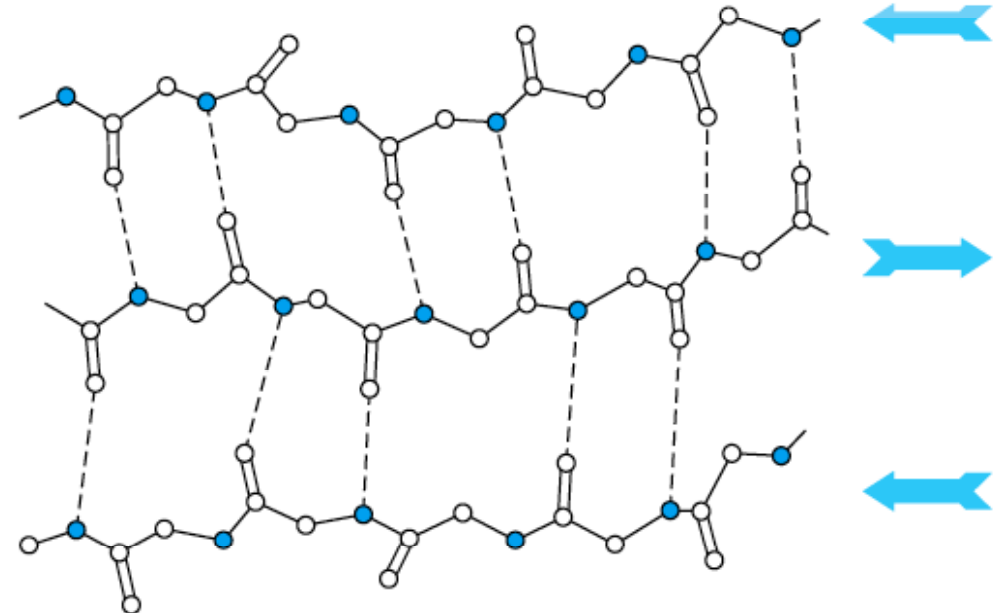


VII] Sekundární struktura

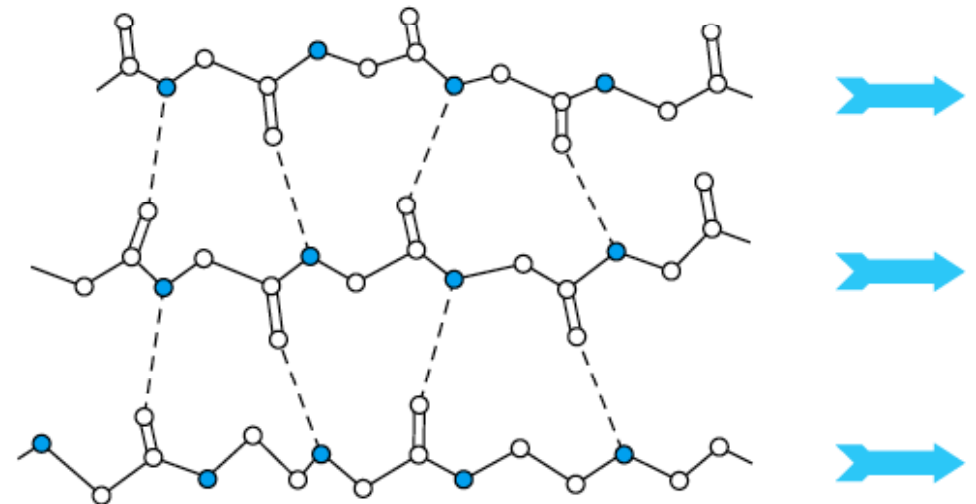
- 1) α -šroubovice (α -helix)
- 2) β -skládání list (β -sheet)
- 3) Ohyb, smyčka (loop/turn)



antiparalelní uspořádání



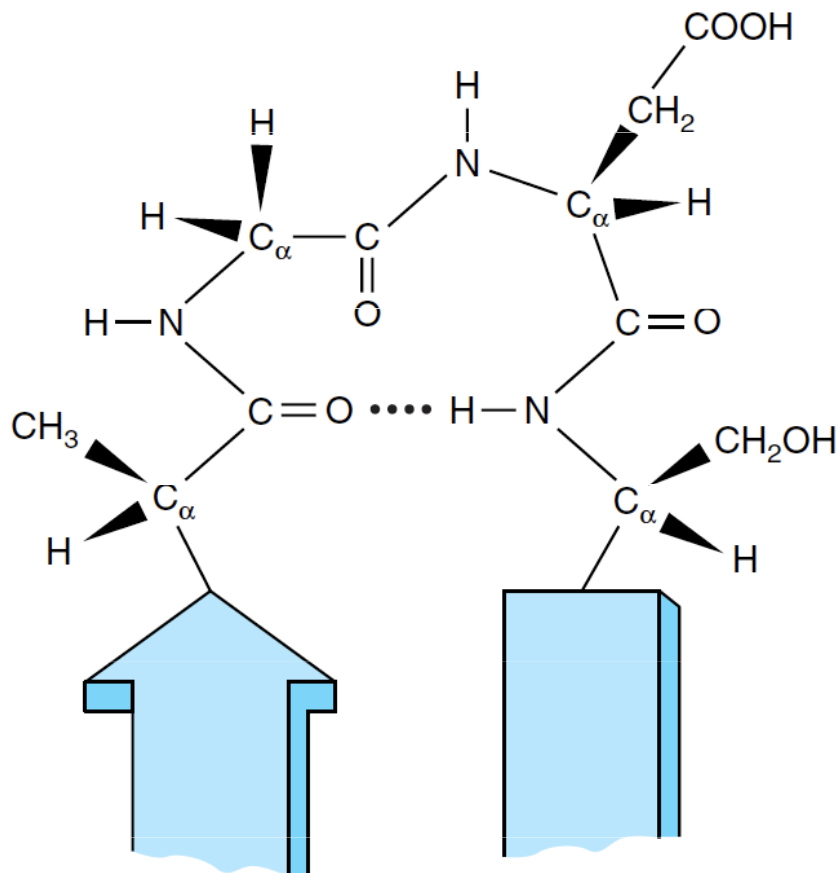
paralelní uspořádání



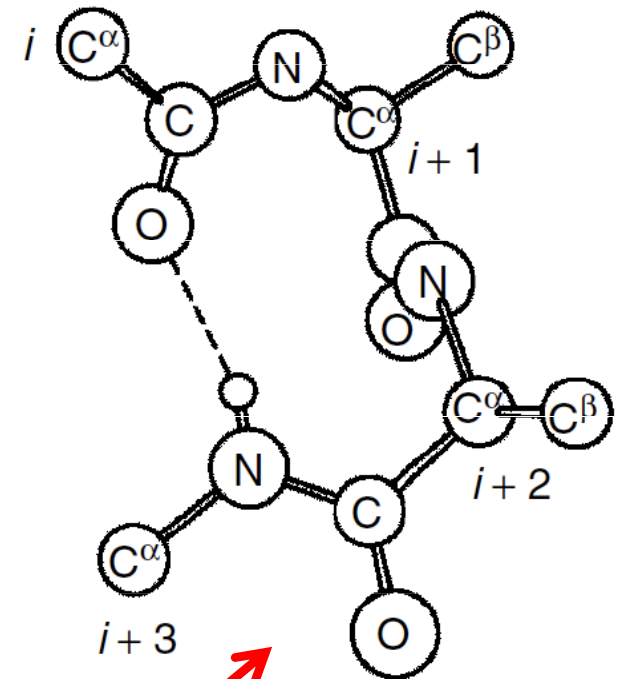
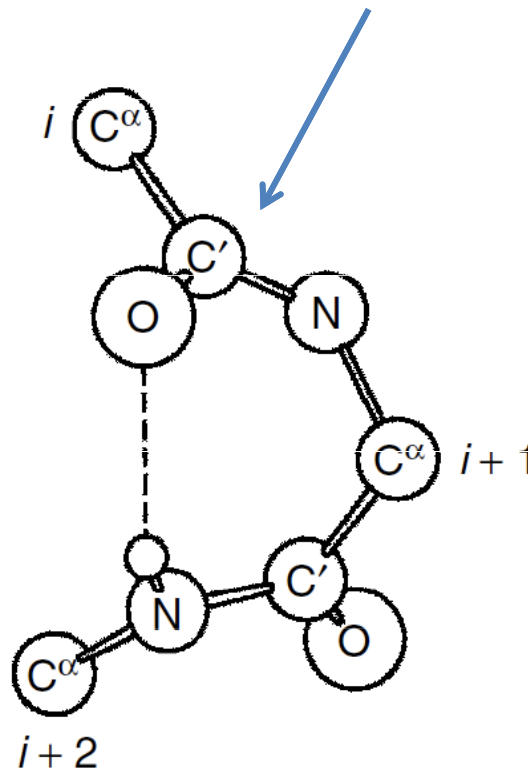
VII] Sekundární struktura

- 1) α -šroubovice (α -helix)
- 2) β -skládání list (β -sheet)

3) Ohyb, smyčka (loop/turn)

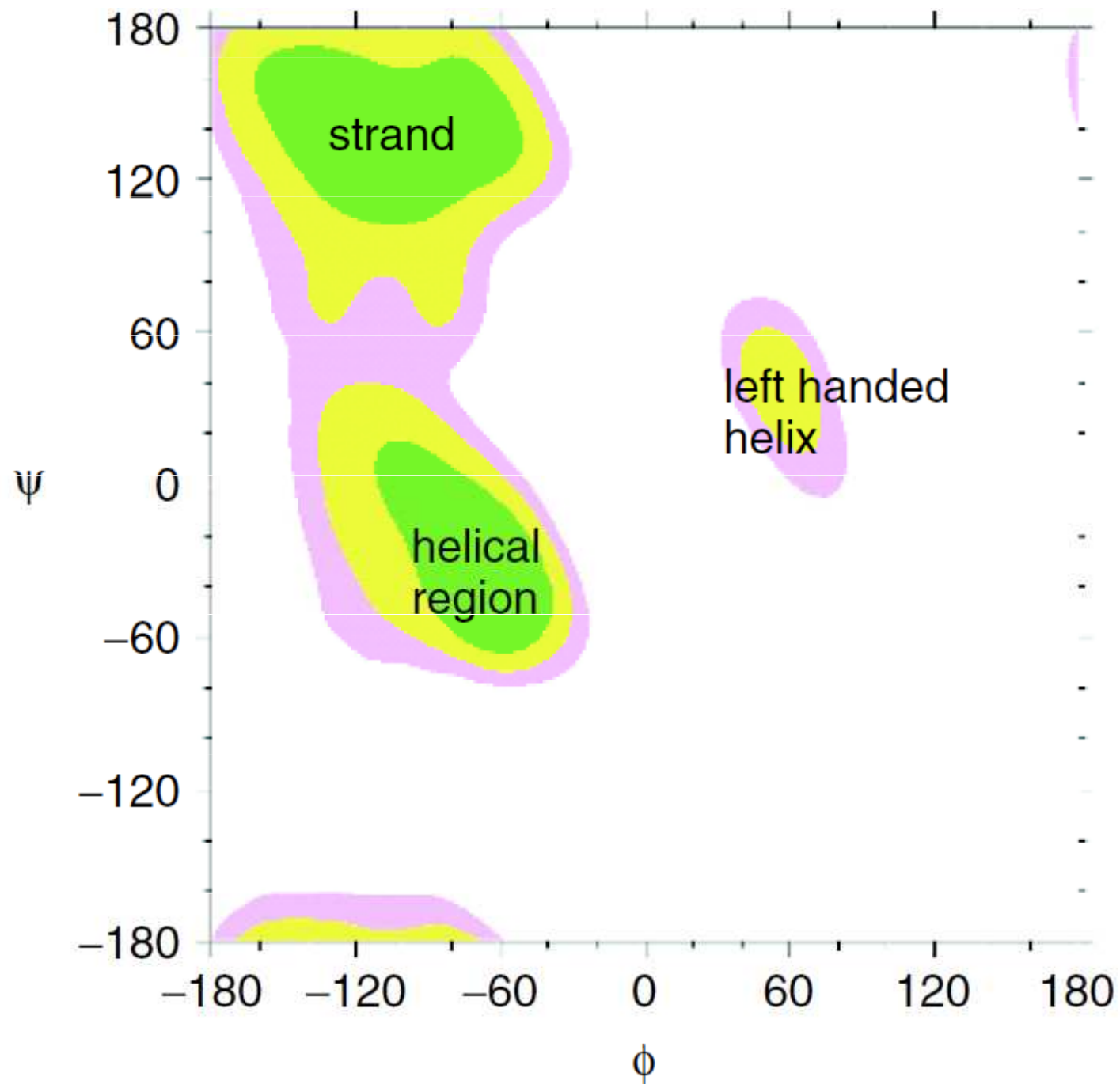


γ -smyčka/ohyb (3 residua)



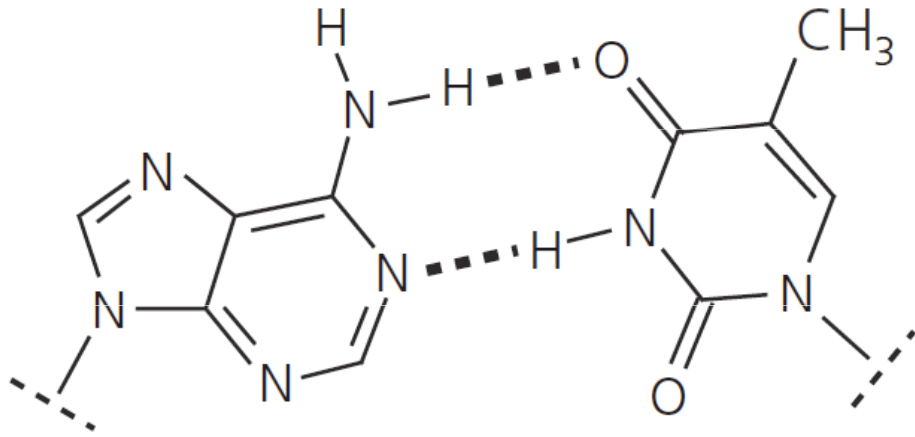
β -smyčka/ohyb (4 residua)

Ramachandranův diagram

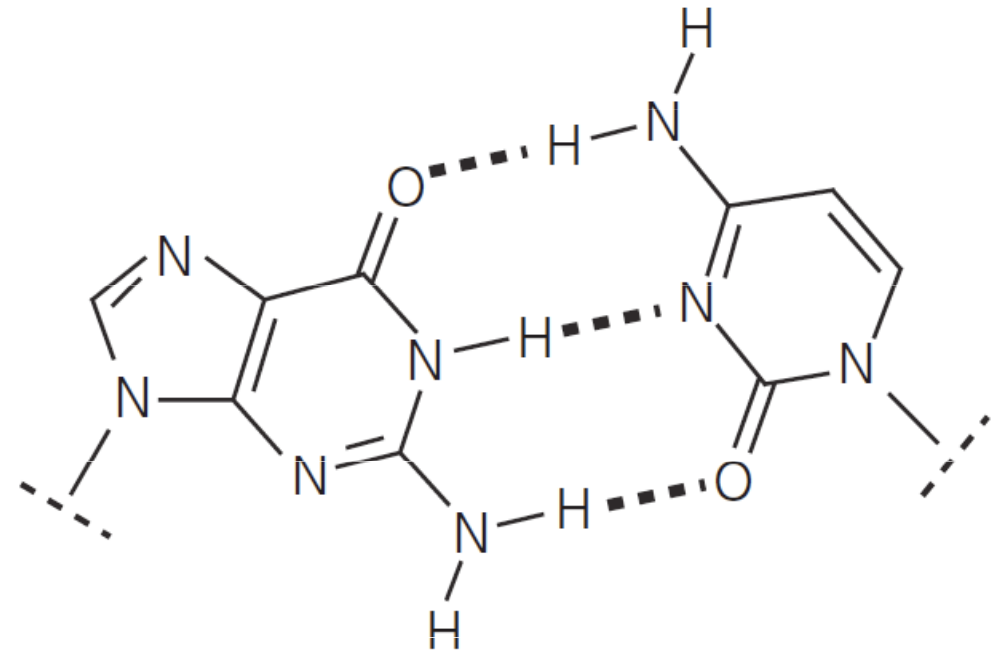


Nukleové kyseliny

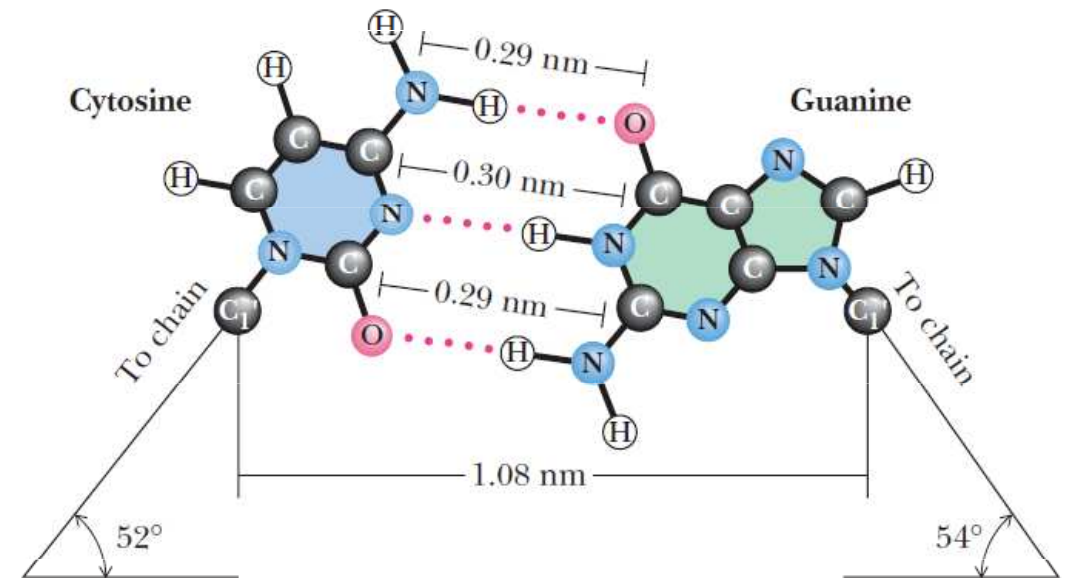
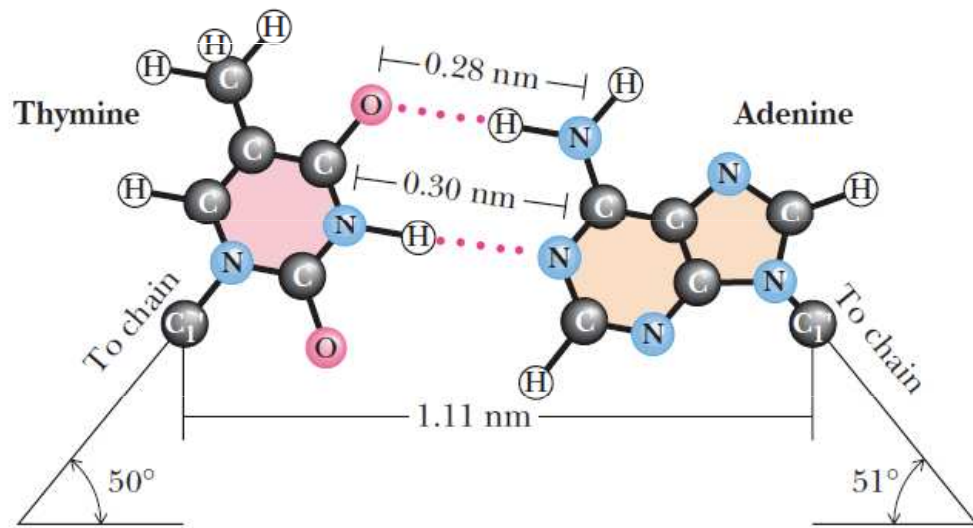
Watson-Crickové párování bází



adenine : thymine



guanaine : cytosine



Watson-Crickovské párování bazí



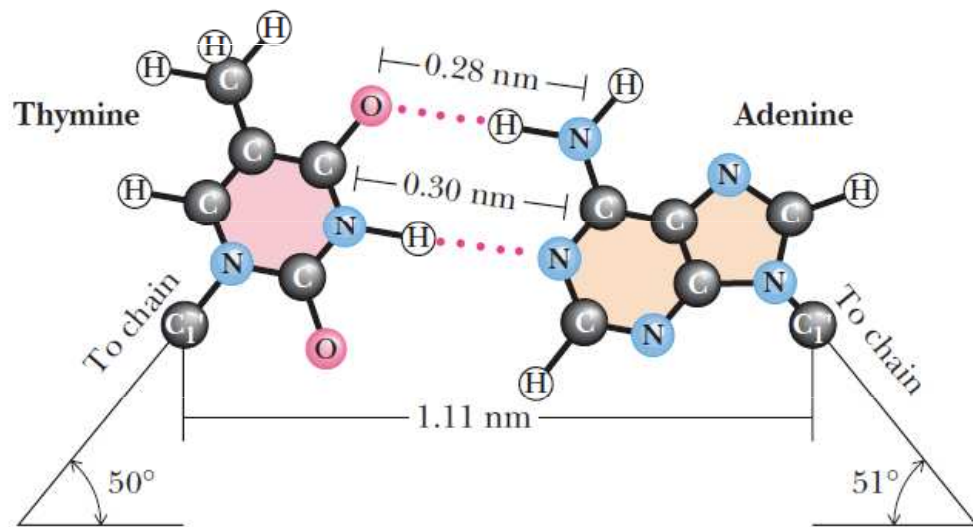
2 vodíkové vazby!!!

adenine : thymine



3 vodíkové vazby!!!

guanaine : cytosine

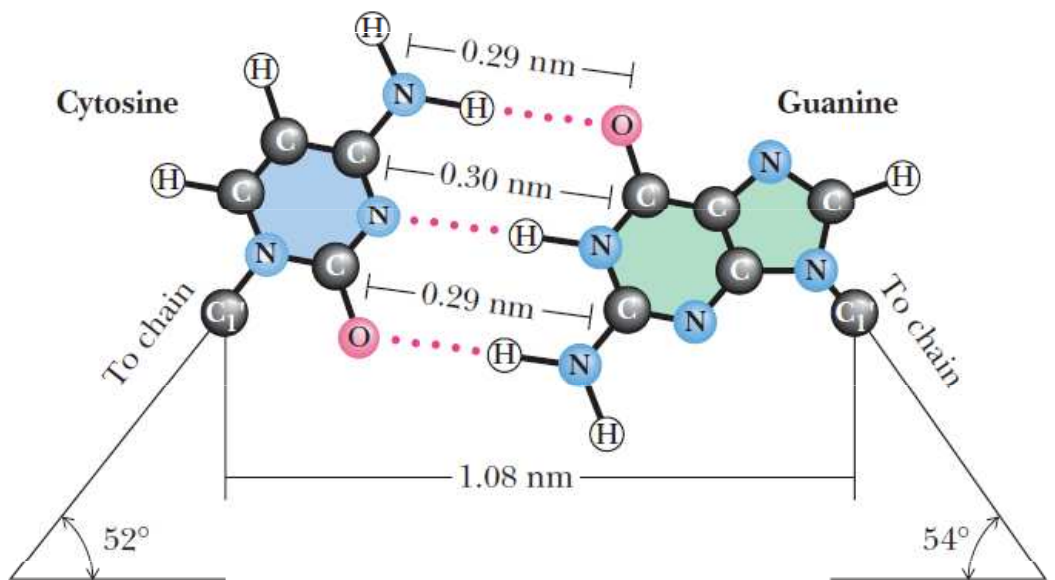


Thymine

Adenine

50°

51°

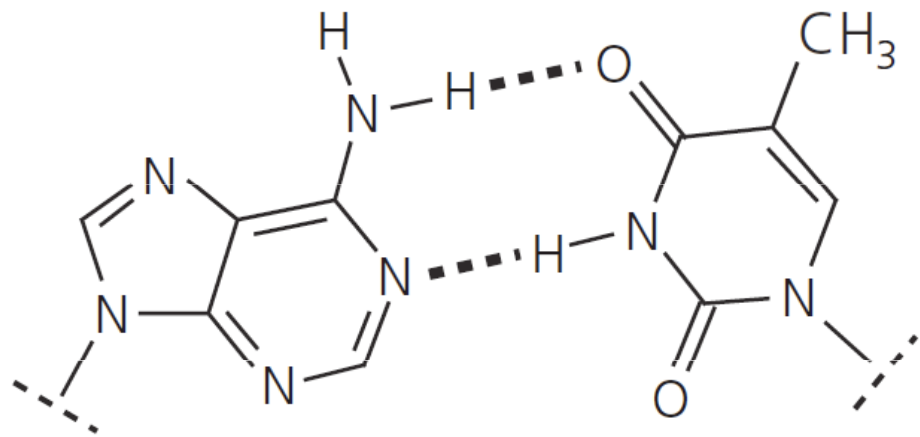


Cytosine

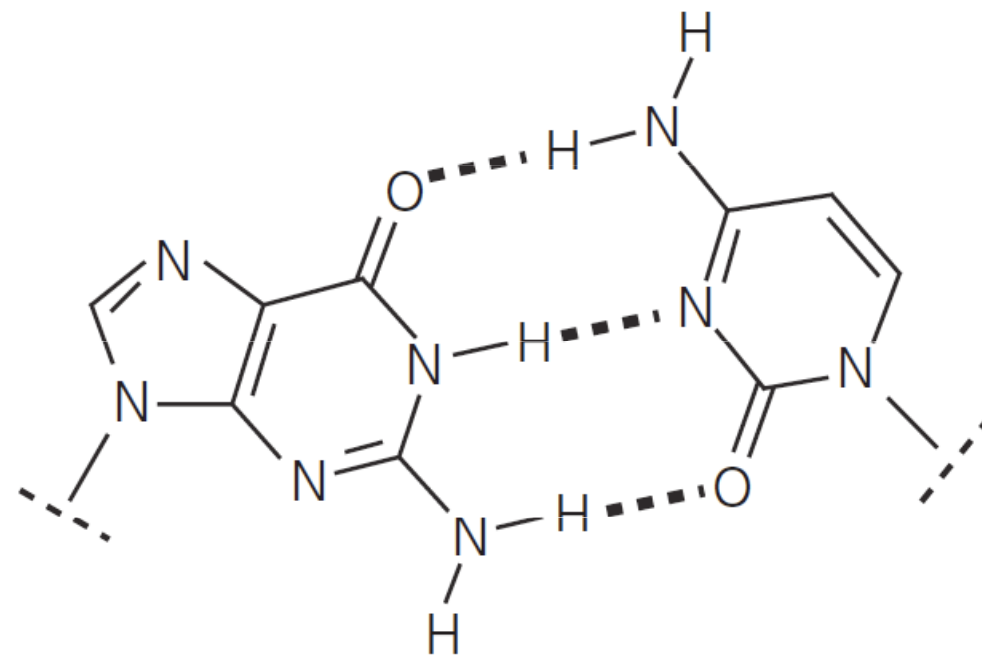
Guanine

52°

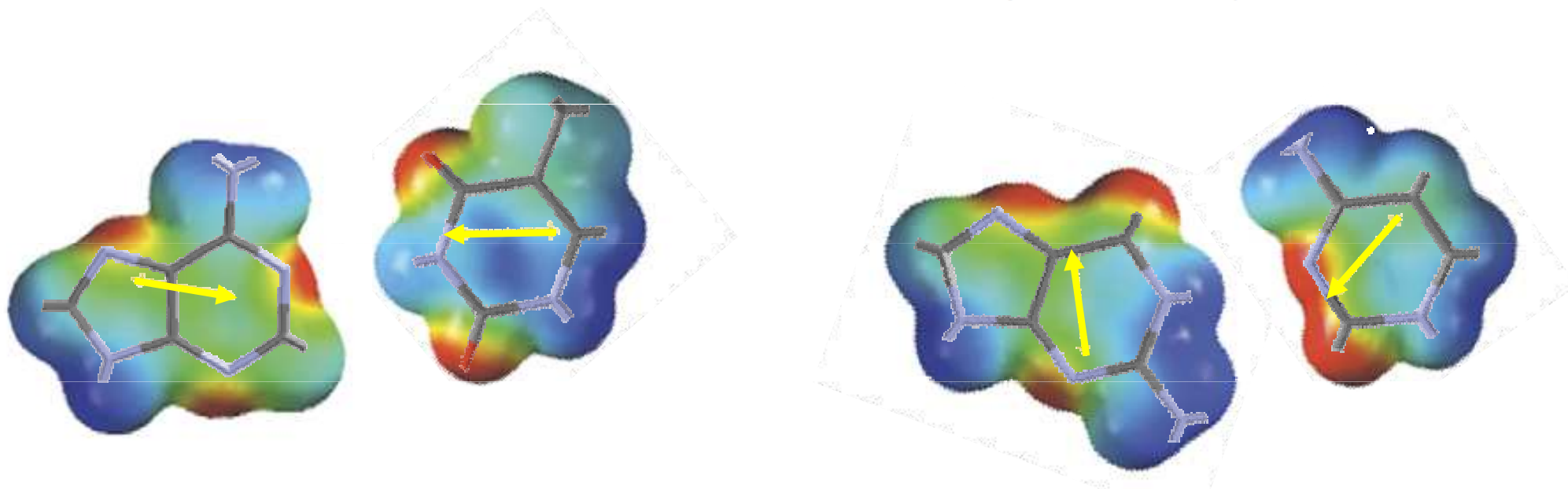
54°



adenine : thymine

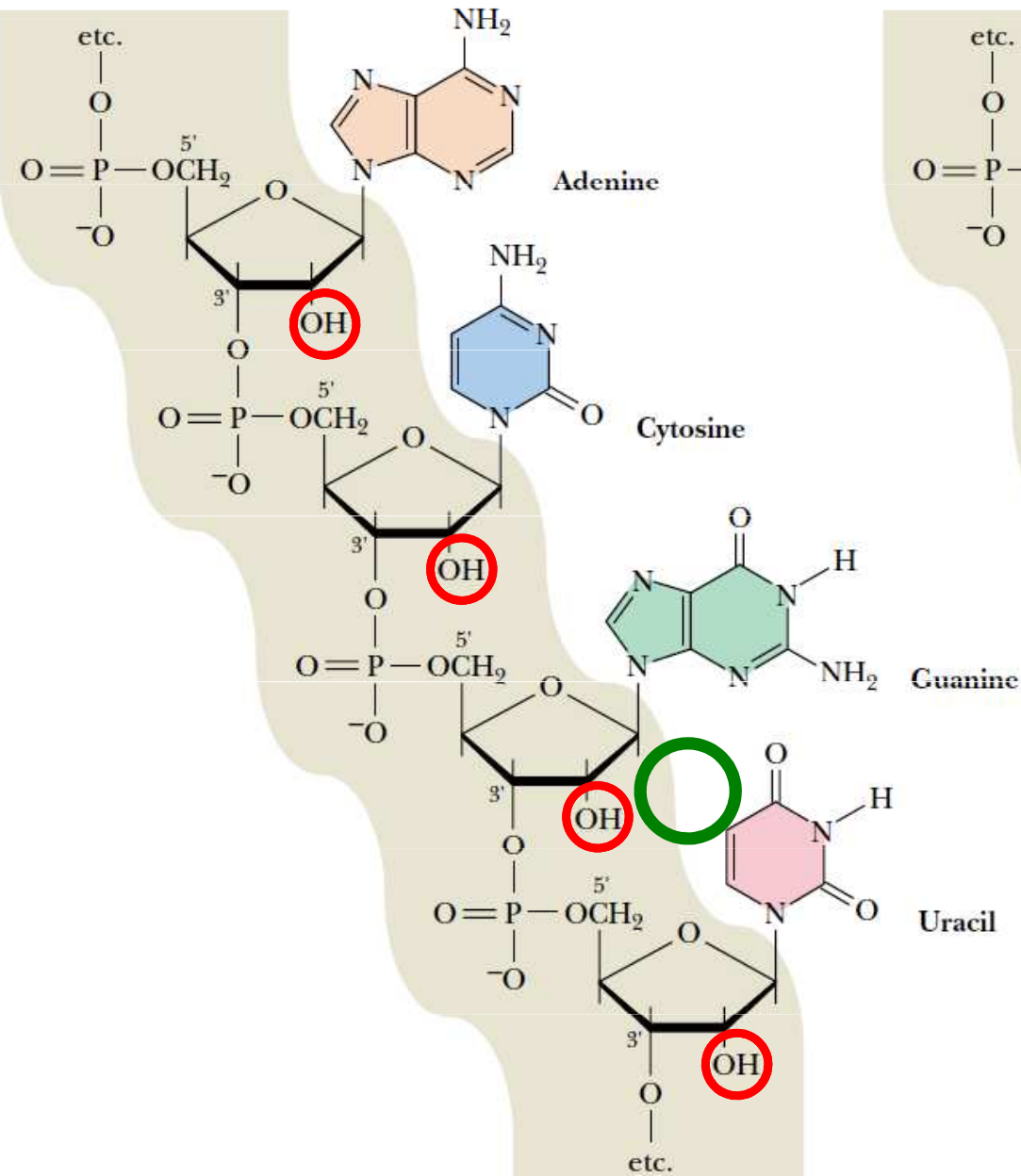


guanine : cytosine

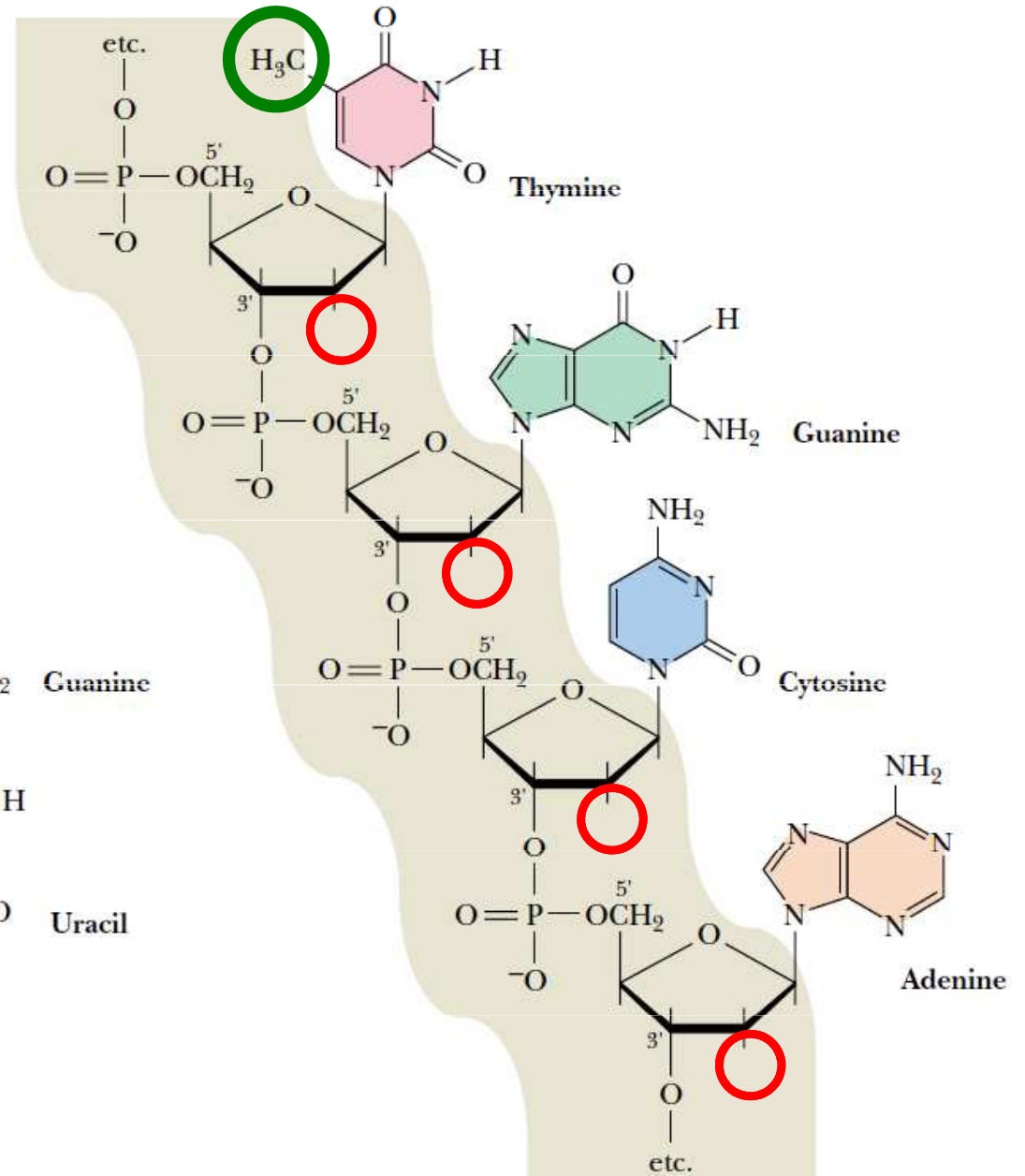


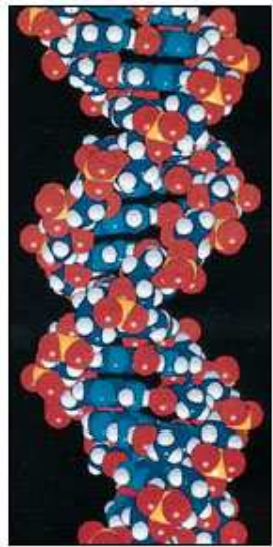
rozložení náboje v nukleobázích + 0 -, šipky označují dipólový moment

RNA



DNA



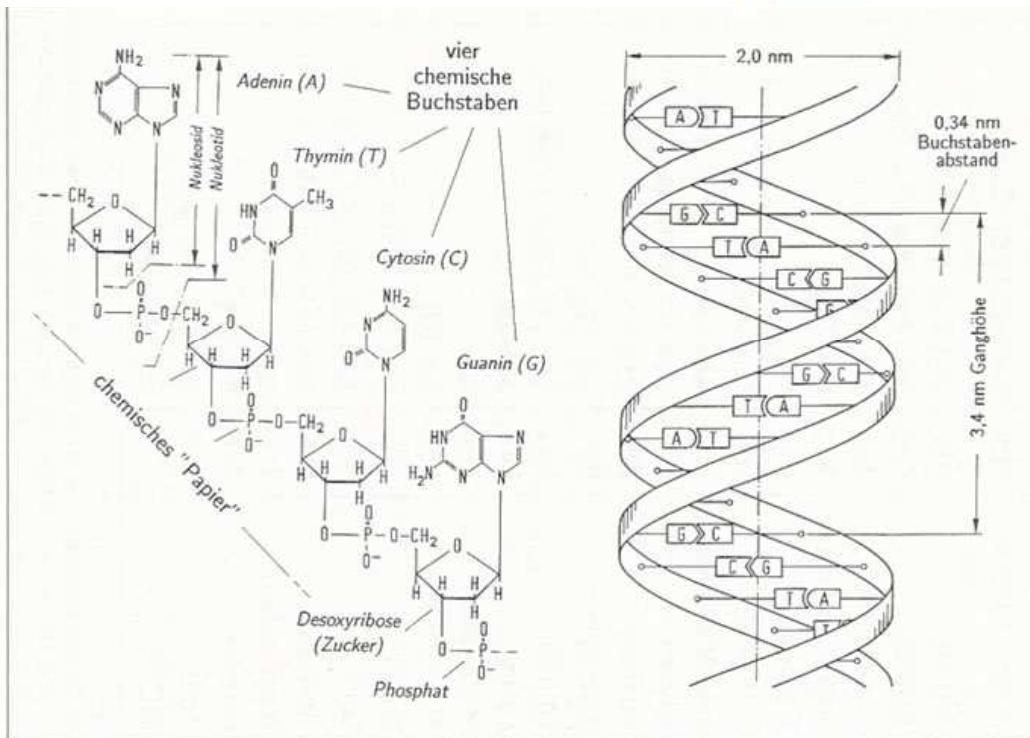
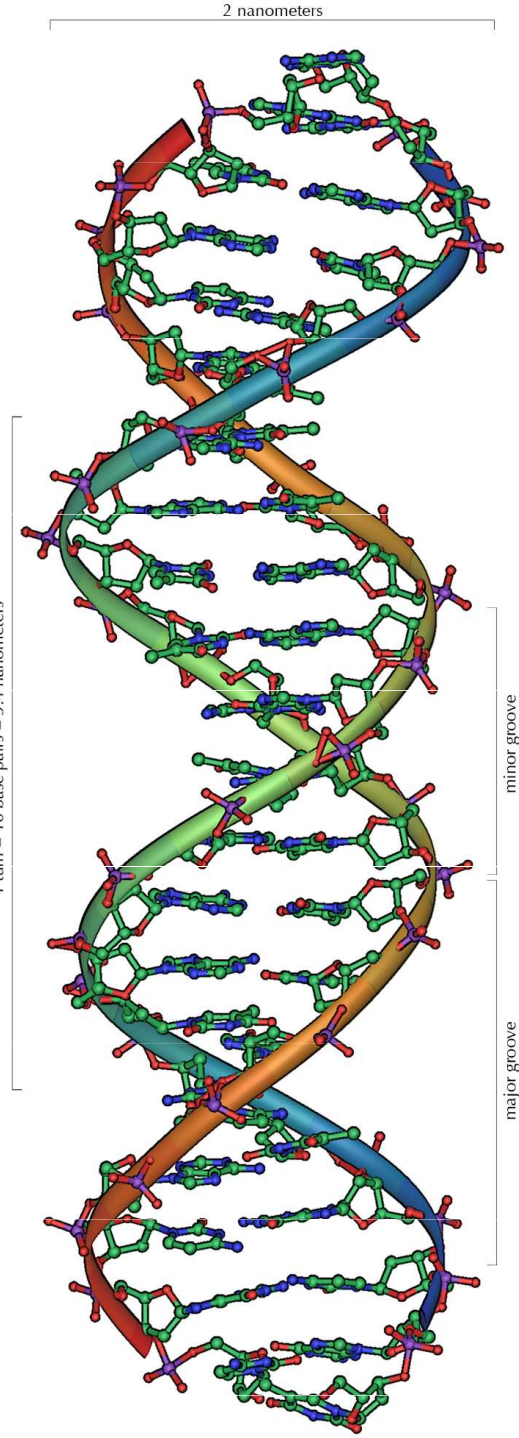
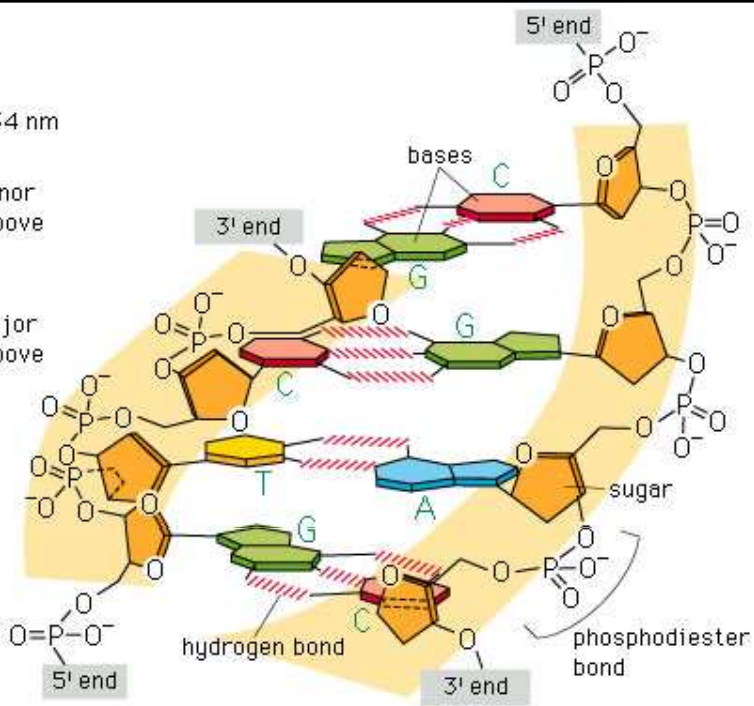


2 nm

0.34 nm

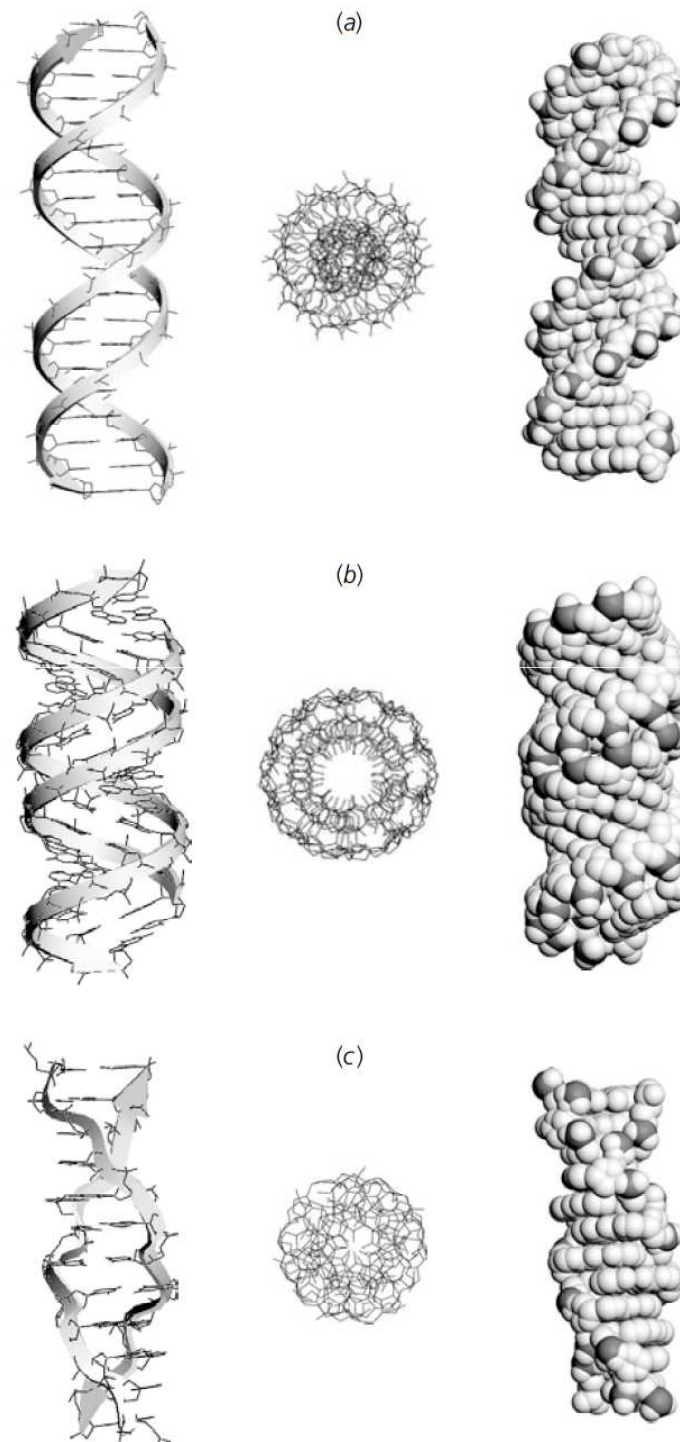
minor groove

major groove



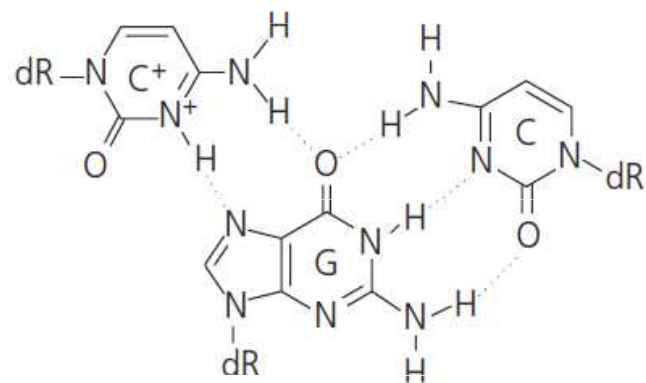
Nejběžnější typy DNA: B-DNA (a), A-DNA (b), Z-DNA (c)

DNA konformace	B	A	Z
Směr vinutí	pravotočivá	pravotočivá	levotočivá
Počet parů bazí na otáčku	10.5	11.0	12.0
Průměr šroubovice	~2.0 nm	~2.6 nm	~1.8 nm
Konformace cukru	C2'-endo	C3'-endo	C2'-endo (pyr) C3'-endo (pur)
Velký žlábek <i>Major groove</i>	široký, hluboký	úzký, hluboký	plochý
Malý žlábek <i>Minor groove</i>	úzký, hluboký	široký, mělký	úzký, hluboký

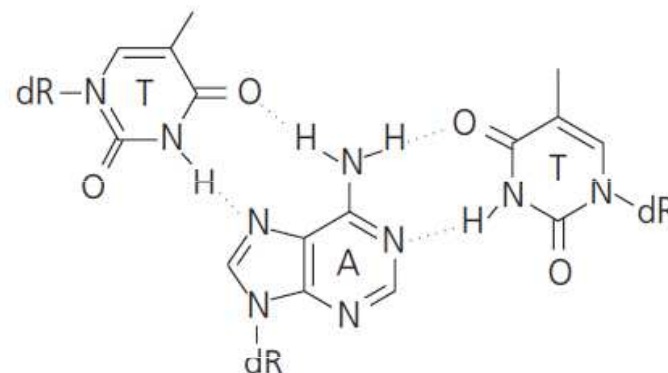


Non-Watson-Crickové (Hoogsteenovo – Karsten Hoogsteen) párování bazí

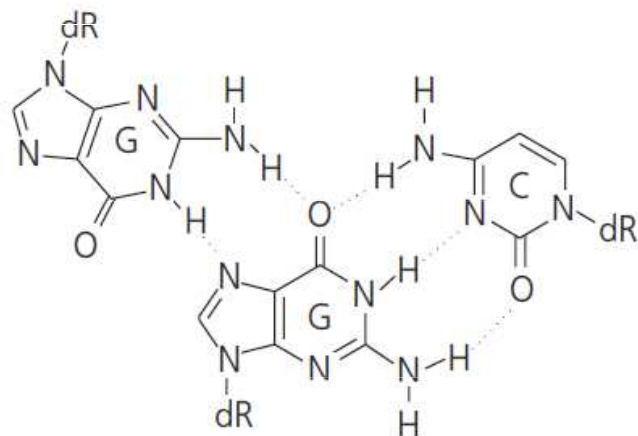
Triplexové struktury



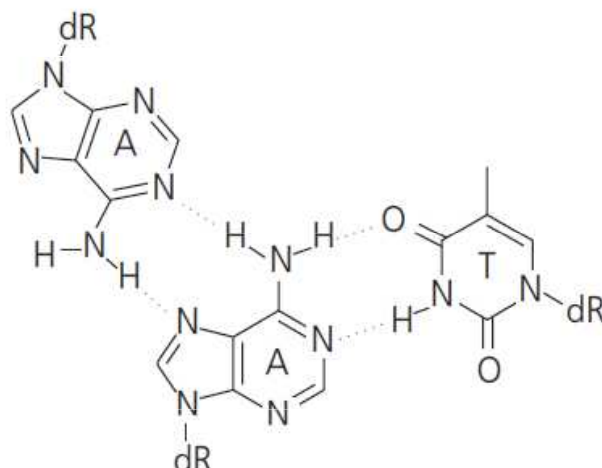
C⁺·GC



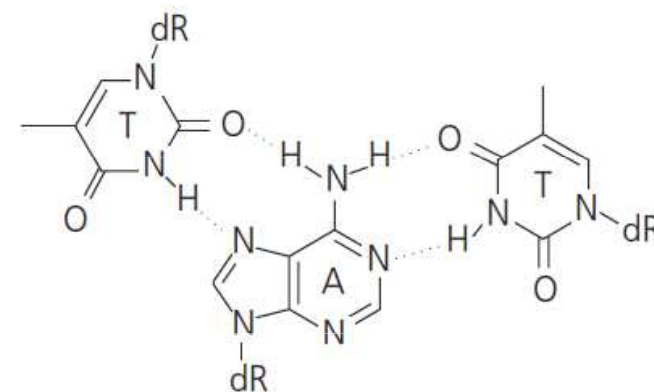
T·AT



G·GC

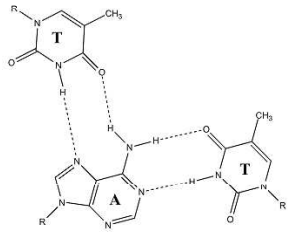


A·AT

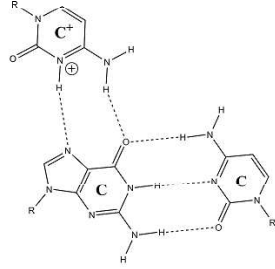


T·AT

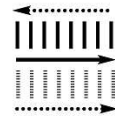
Triplexové struktury



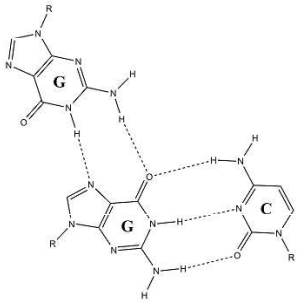
TA*T



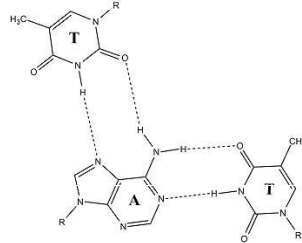
CG*C⁺



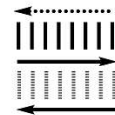
YR*Y



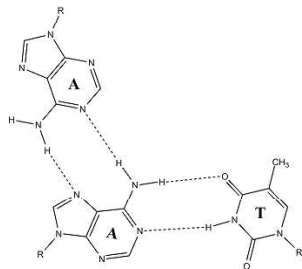
CG*G



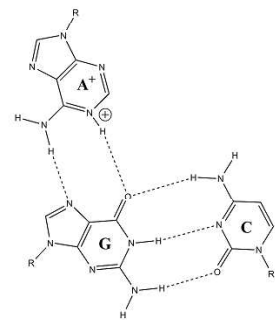
TA*A



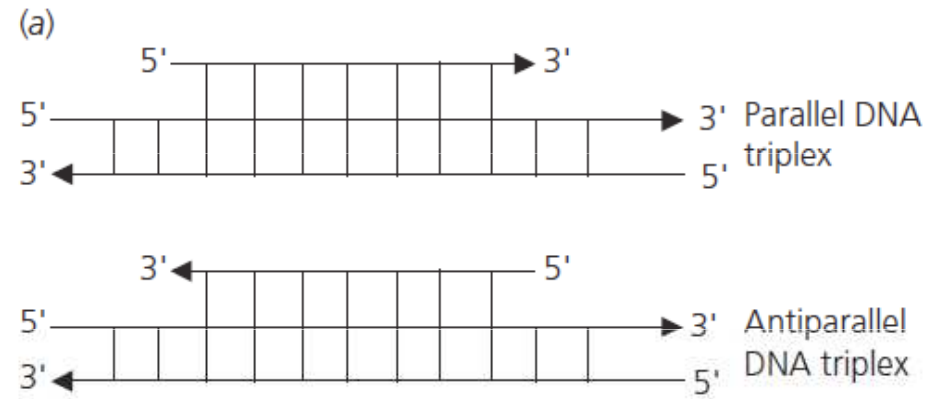
YR*R



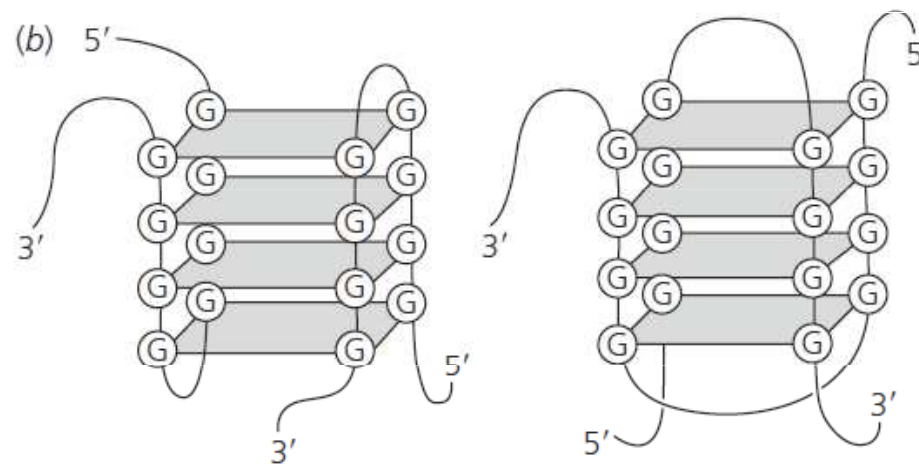
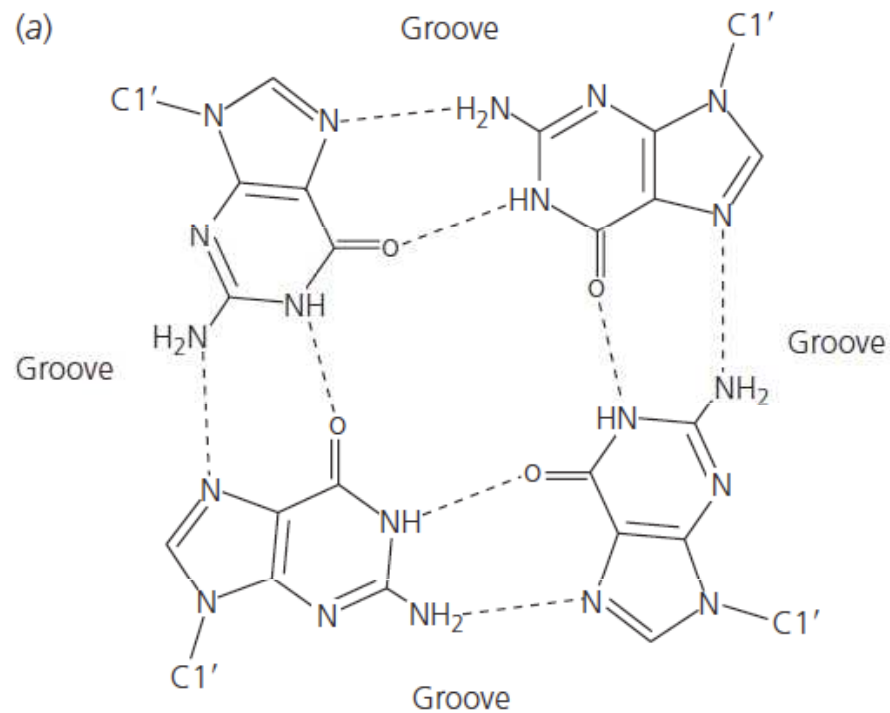
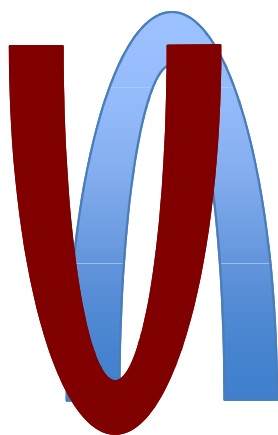
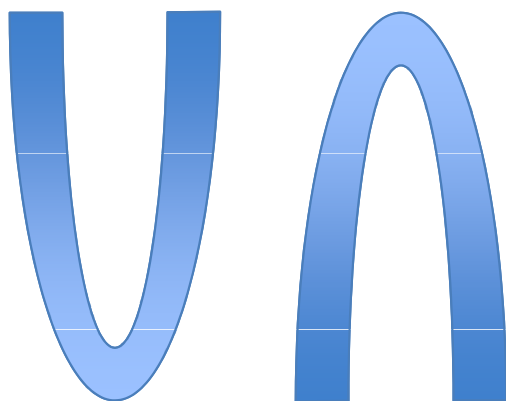
TA*T



CG*A⁺



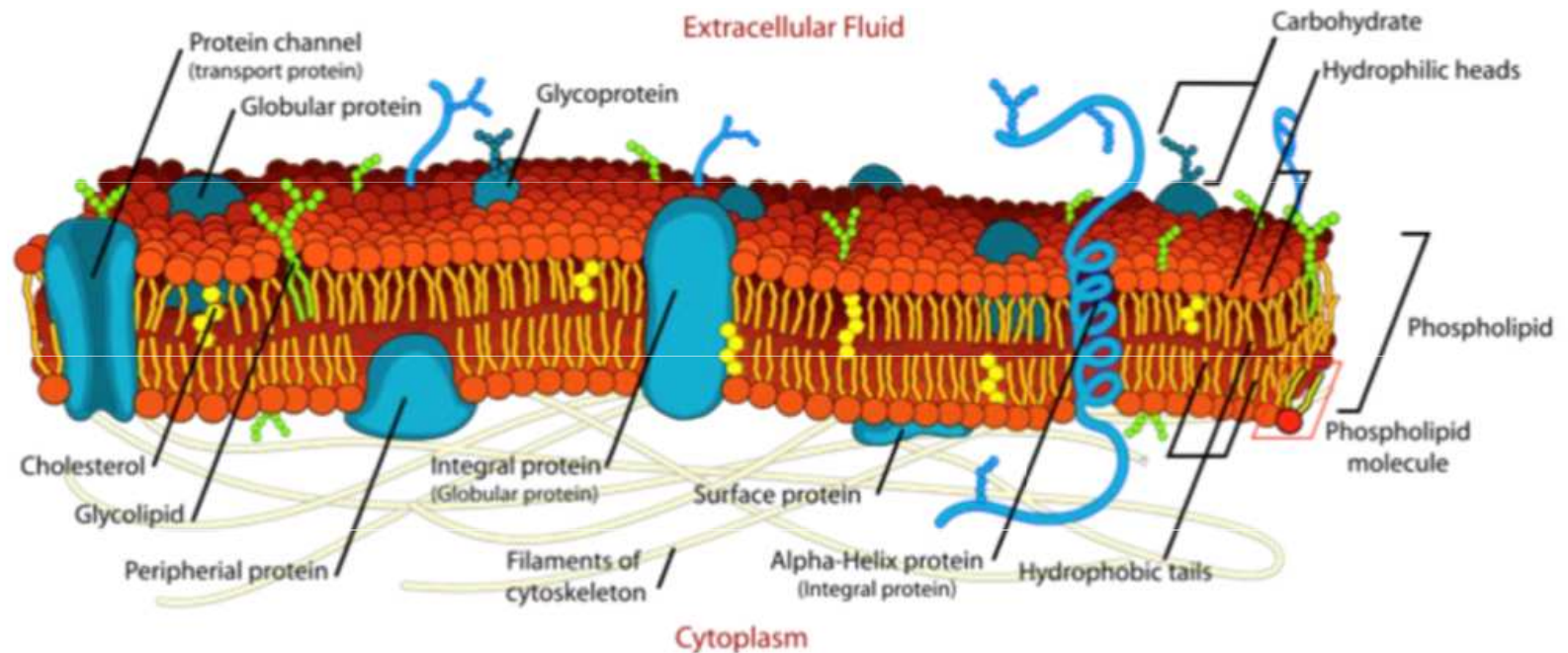
Quadruplexové struktury



Lipidy

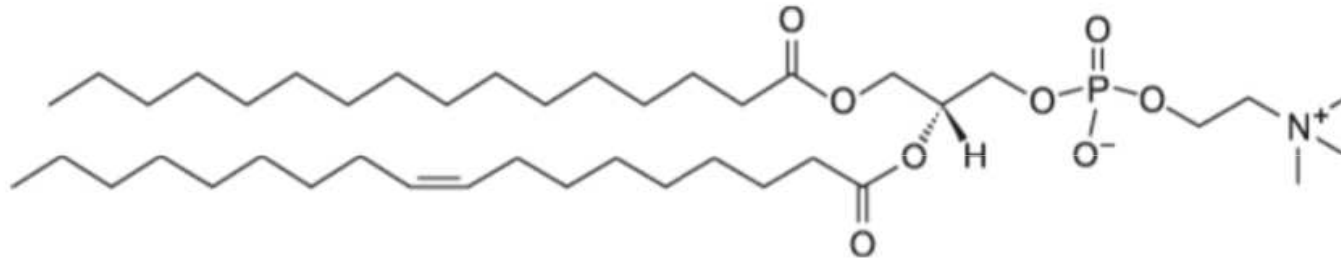
Složení

- lipidy
- cukry
- proteiny

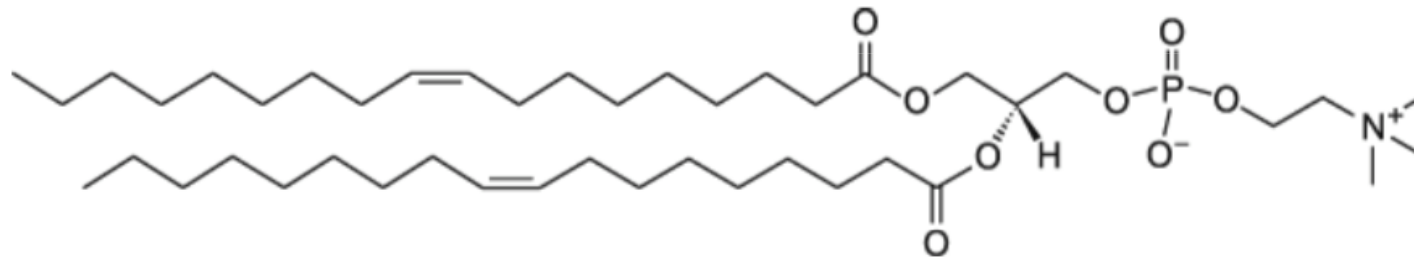


- 1) Lipoprotein: lipid + protein, který je většinou rozpustný v H_2O
- 2) Proteolipid: protein + lipid, -----"----- v organice (e.g. 2:1 = $CHCl_3$: CH_3OH)
- 3) Glycolipid: lipid + carbohydrate. Cukry glycolipidů jsou na vnějším povrchu a velmi pravděpodobně se účastní mezibuněčných komunikací
- 4) Glycoprotein: carbohydrate + protein. Podobně jako glycolipidy, cukerné zbytky glycoproteinů jsou připojeny na ne-cytoplasmické straně membrány.

Zkratky



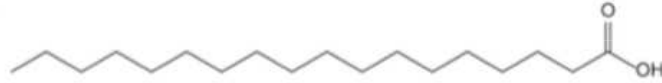
POPC 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine



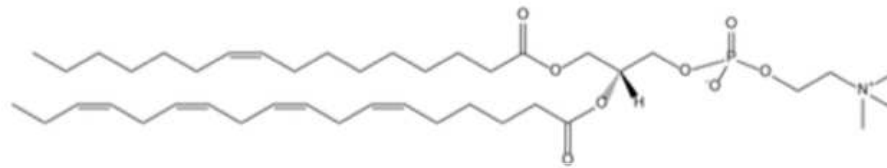
DOPC 1,2-dioleoyl-sn-glycero-3-phosphocholine

Lipidy

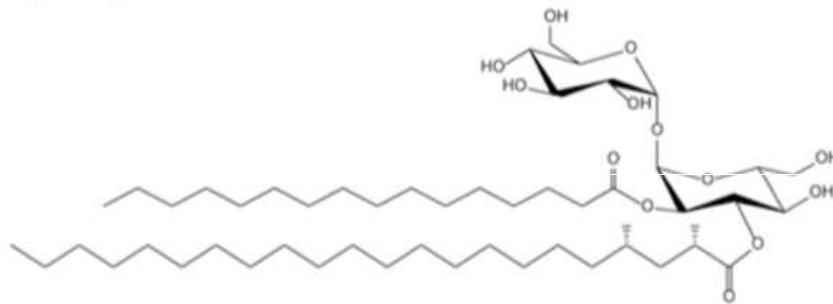
A



(a) Fatty acids: octadecanoic acid

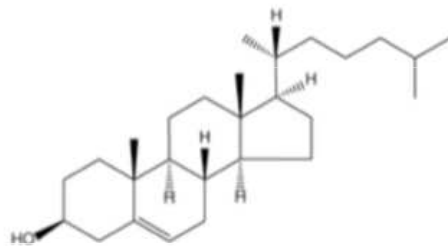


(c) Glycerophospholipids: 1-(9Z-hexadecenoyl)-2-(6Z,9Z,12Z,15Z-octadecatetraenoyl)-sn-glycero-3-phosphocholine



(e) Saccharolipids: 2-O-hexadecanoyl-3-O-(2S,4S-dimethyl-docosanoyl)-α,α-trehalose

B



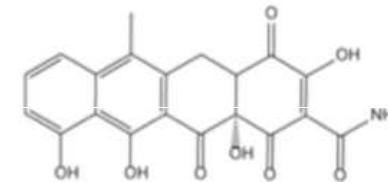
(g) Sterol lipids: cholest-5-en-3β-ol



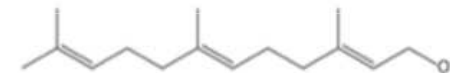
(b) Glycerolipids: 1-hexadecanoyl-2-(9Z,12Z,15Z-octadecatrienoyl)-sn-glycerol



(d) Sphingolipids: N-(hexadecanoyl)-sphing-4-enine

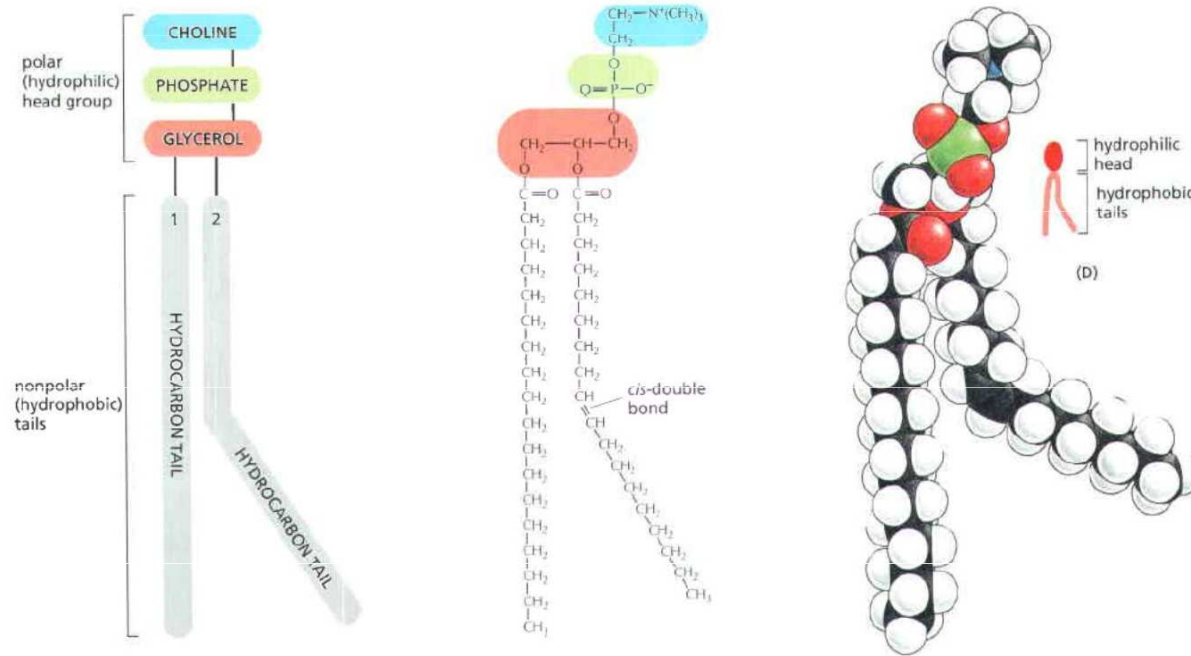


(f) Polyketides: 4-Dedimethylamino-4-oxo-anhydrotetracycline

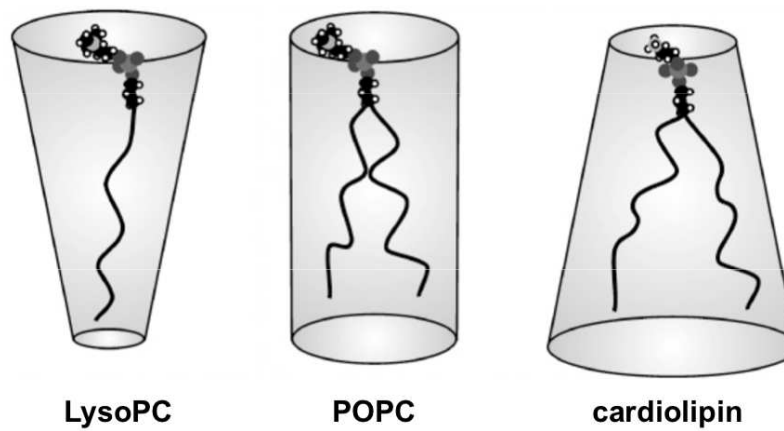


(h) Prenol lipids: 2E,6E-farnesol

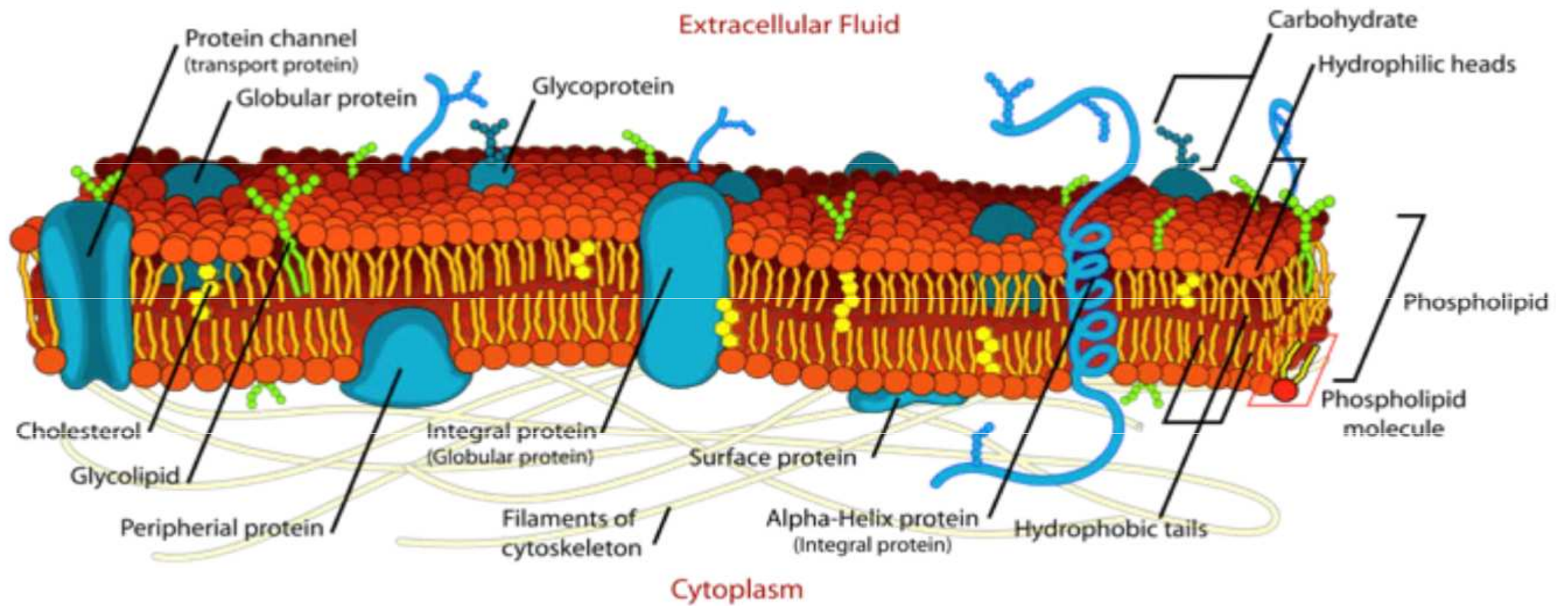
Fosfolipidy



Tvar lipidů



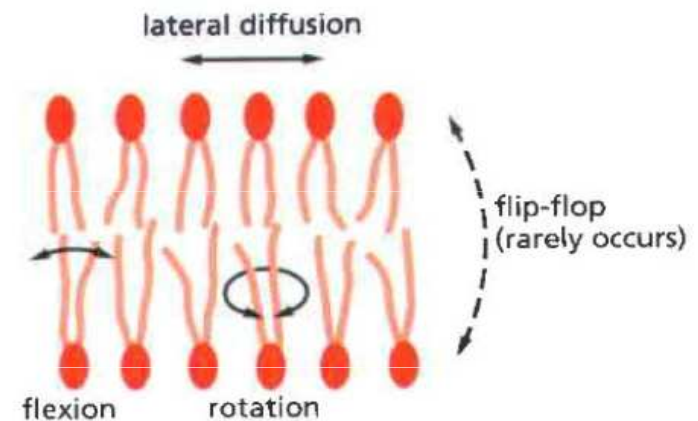
Současný model membrány



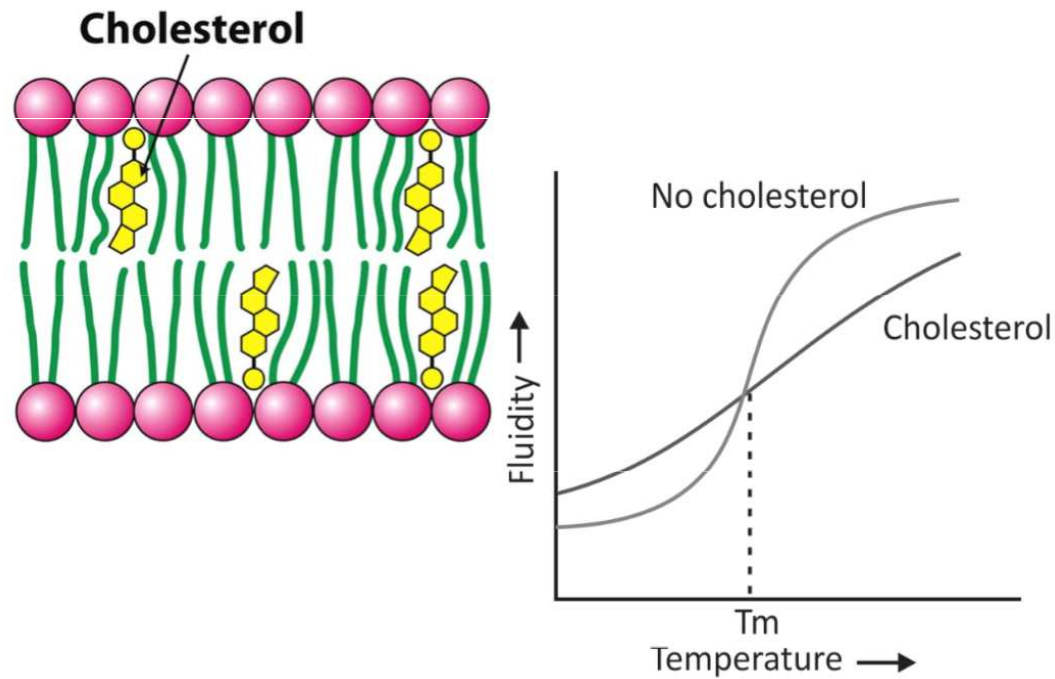
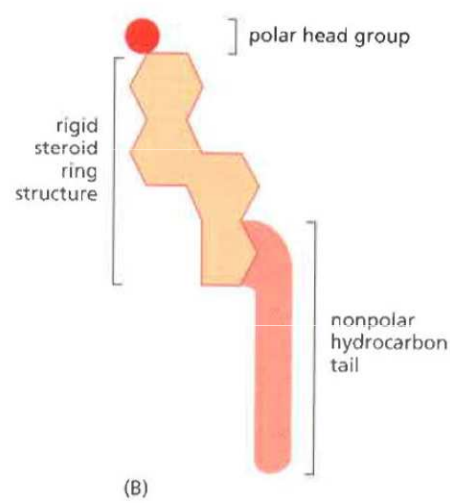
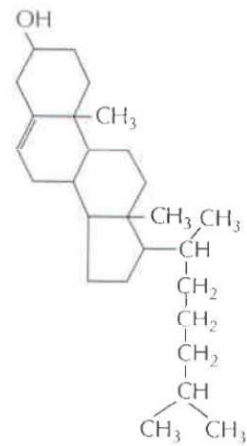
fluid mosaic model – 2d tekutá vrstva



3-5 nm



Cholesterol



Zařízení založená na interakci elektromagnetického záření s makromolekulami

Druhy spektrofotometrů

- **Spektrofotometry** jsou laboratorní přístroje používané pro měření koncentrace látek absorbujících nebo emitujících infračervené, viditelné nebo ultrafialové světlo. Mohou být též použity pro studium jejich chemické struktury.
- **Absorpční spektrofotometry:** založeny na spektrální závislosti absorpce světla.
- **Emisní spektrofotometry:** Zdrojem světla je sama analyzovaná látka, jež je injektována nebo rozprašována do bezbarvého plamene. Emitované světlo prochází optickým hranolem nebo mřížkou, takže můžeme získat celé emisní spektrum. Frekvence přítomné ve spektru umožňují identifikovat např. přítomné ionty.
- **Spektrofluorimetry:** emise světla je vyvolána světlem o vlnové délce kratší než je vlnová délka světla emitovaného.

Absorpční spektrofotometry: Lambertův-Beerův zákon

Absorpční spektrofotometrie je založena na absorpci světla při průchodu vrstvou roztoku. Jeho koncentrace může být zjištěna pomocí **Lambertova-Beerova zákona**:

$$I = I_0 \cdot 10^{-\epsilon c x}$$

c je koncentrace rozpuštěné látky, x tloušťka vrstvy, I_0 původní intenzita světla, I je intenzita světla po průchodu vrstvou. Konstanta ϵ (epsilon, absorpční nebo extinkční koeficient) **závisí na vlnové délce světla, na rozpuštěné látce a rozpouštědle**. Její hodnoty pro běžné chemické sloučeniny lze nalézt v tabulkách. Tyto hodnoty jsou vždy udávány pro určitou vlnovou délku (obvykle absorpční maximum). Číselné hodnoty tohoto koeficientu závisejí na tom, jak je vyjadřována koncentrace rozpuštěné látky. Když použijeme mol.l^{-1} , hovoříme o **molárním absorpčním koeficientu**.

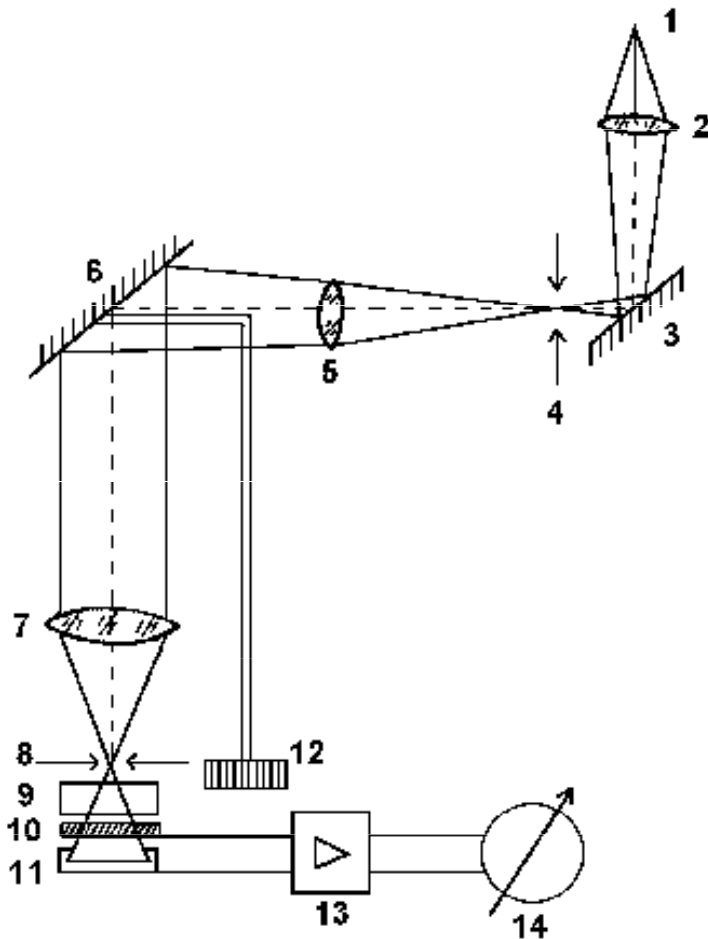
Poměr intenzit světla prošlého a dopadajícího se nazývá **transmittance** (dříve transparence). Dekadický logaritmus převrácené hodnoty transmittance se nazývá **absorbance** A . S ohledem na L.-B. zákon je tedy absorbance přímo úměrná koncentraci rozpuštěné látky a tloušťce absorbující vrstvy roztoku.

$$A = \varepsilon \cdot c \cdot X$$

Druhy absorpčních spektrofotometrů

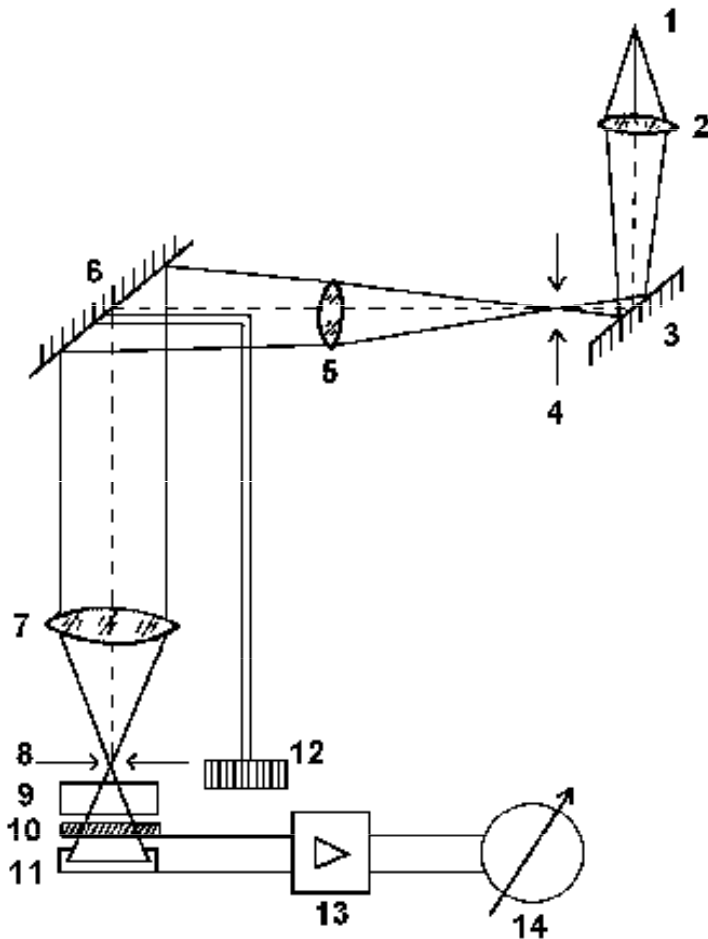
- Podle konstrukce rozdělujeme spektrofotometry na jednopaprskové a dvoupaprskové.
- U **jednopaprskových spektrofotometrů** jeden svazek světla prochází nejdříve srovnávacím a pak měřeným vzorkem (kyvety obsahující roztoky musí být pohyblivé). U **dvoupaprskových spektrofotometrů** jeden svazek světla prochází měřeným vzorkem a druhý srovnávacím vzorkem (blankem). Dvoupaprskové přístroje umožňují podstatně rychlejší měření, avšak jsou dražší. U jednoduchých přístrojů je nastavování vlnové délky světla ruční. U pokročilejších přístrojů se toto nastavování děje automaticky, což umožňuje přímo získávat **absorpční křivky**, tj. grafy závislosti absorbance na vlnové délce světla.

Jednopaprskový spektrofotometr



Zdrojem světla (1) je žárovka s wolframovým vláknem. Její polychromatické světlo prochází kondenzorem (2) a odráží se od zrcadla (3) na vstupní štěrbinu (4) **monochromátoru** (části 4 až 8, plus 12). Světlo je soustřeďováno čočkou (5) na **odrazovou optickou mřížku (6)**, která tvoří barevné spektrum. Téměř monochromatické světlo je promítáno objektivem (7) na **výstupní štěrbinu (8)** monochromátoru.

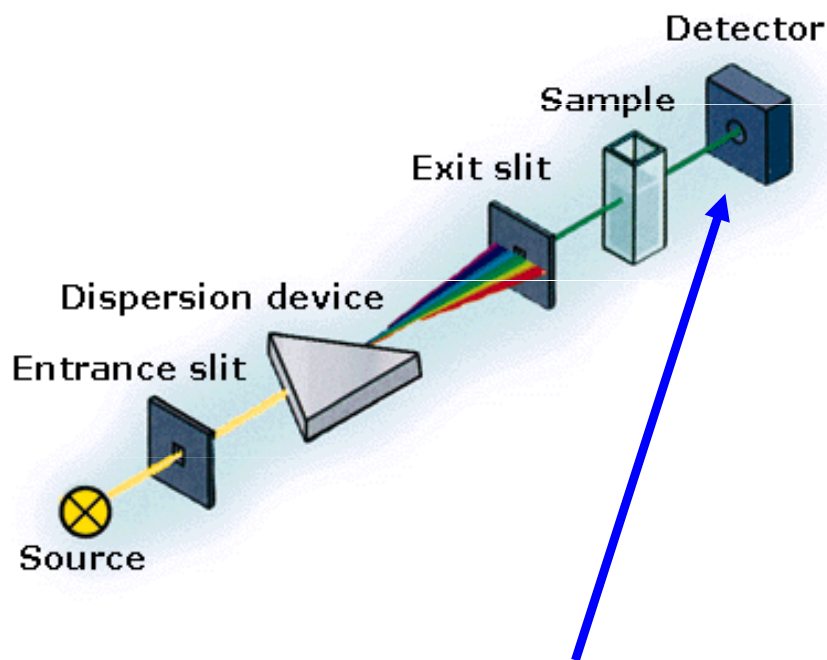
Jednopaprskový spektrofotometr



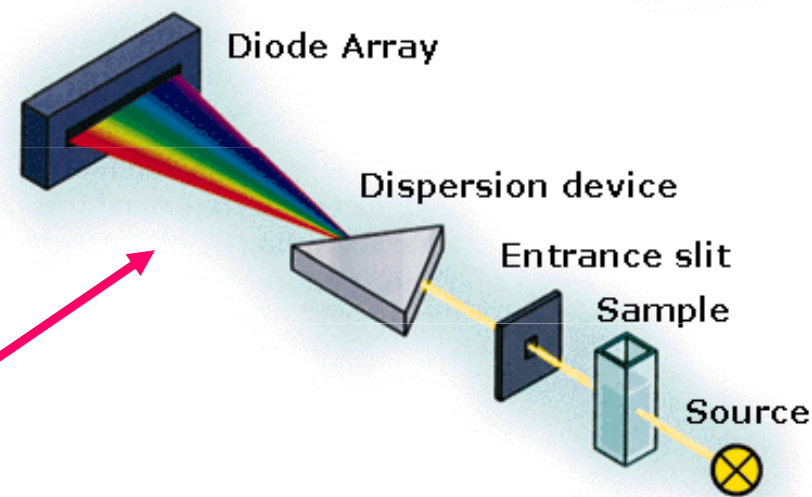
S mřížkou lze otáčet pomocí **ovladače vlnových délek** (12), čímž se zaměřuje světlo o určité vlnové délce na výstupní štěrbinu. Svazek světla pak prochází **kyvetou** (9) se vzorkem. Intenzita prošlého světla je měřena fotodetektorem (10, 11). Jeho signál je zesilován **zesilovačem** (13). Hodnota absorbance je zobrazena na displeji (14). Intenzita světla prošlého srovnávacím roztokem je vždy srovnávána s intenzitou téhož svazku světla prošlého měřeným vzorkem.

Moderní UV/VIS/NIR spektrofotometr

NIR = near infrared = blízká infračervená oblast



Světlo **jedné vybrané vlnové délky** nebo **celé prošlé spektrum** může být měřeno



Absorpční UV spektrofotometrie

- Ultrafialové (**UV**) světlo **je absorbováno** různými sloučeninami, zejména těmi, které mají **konjugované dvojně vazby**. Jak bílkoviny, tak nukleové kyseliny silně absorbují UV světlo, což lze využít pro jejich zkoumání.
 - Aminokyseliny **tryptofan** a **tyrosin** mají absorpční maxima při přibližně **280 nm**. **Fenylalanin** při **255 nm**.
 - **Nukleotidy** (dusíkaté báze) mají absorpční maxima v oblasti **260 - 270 nm**.
 - **Chromofory** – jejich absorpční vlastnosti se mění podle chemického složení prostředí.

UV/Vis spektroskopie proteinů

Absorpční spektrum volného fenylalaninu, tyrosinu a tryptofanu v UV oblast

Podle:<http://www.fst.rdg.ac.uk/courses/fs460/lecture6/lecture6.htm>

Bathochromní efekt (červený posun)



Hypsochromní efekt (modrý posun)



Hypochromní efekt – snížení intenzity



Hyperchromní efekt – zvýšení intenzity

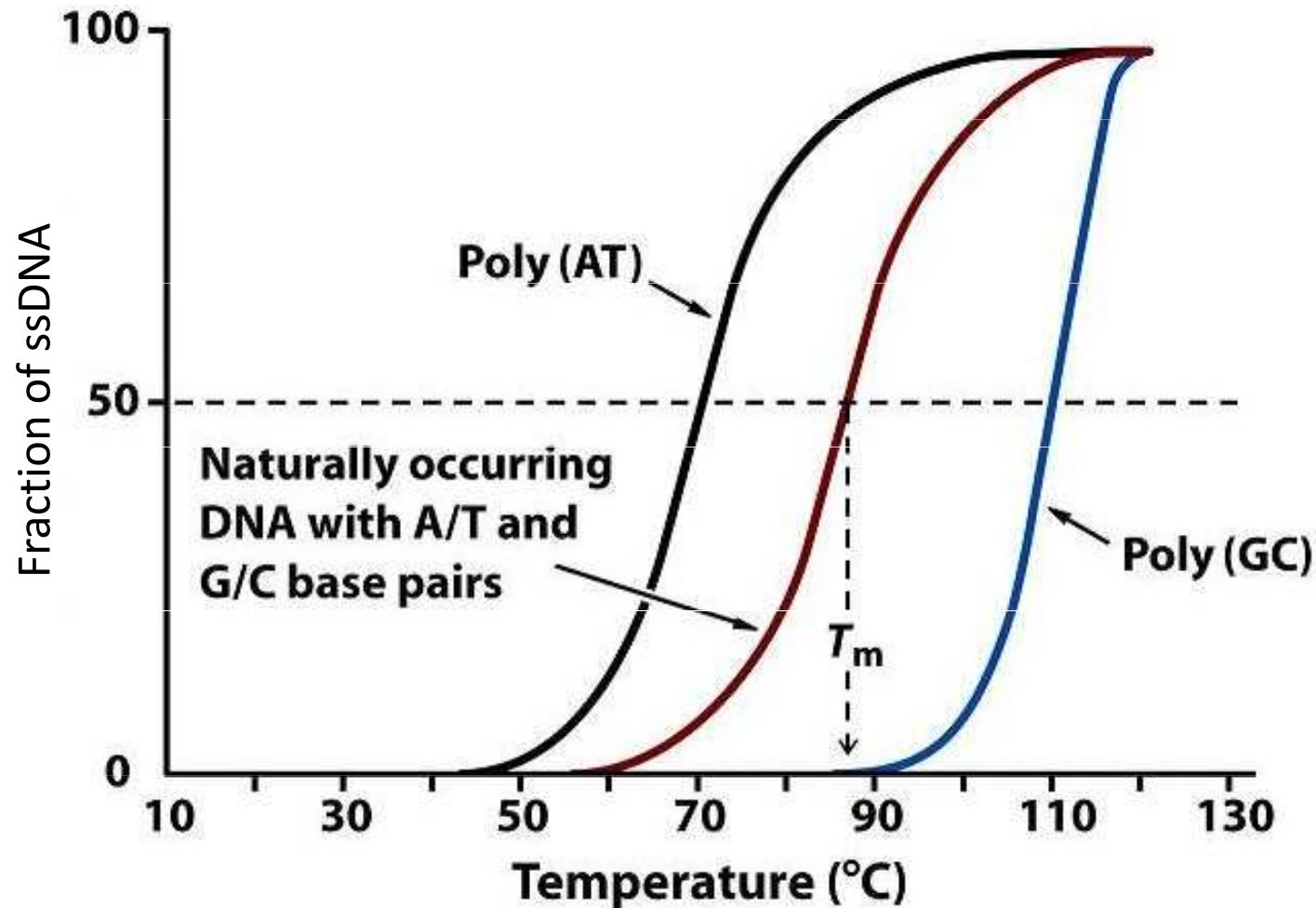


Hypochromní efekt (HE)

- Absorpce světla je ovlivňována dipólovými momenty chemických vazeb, které interagují s fotony. Stochasticky (náhodně) orientované dipólové momenty (denaturovaná bílkovina) absorbují světlo lépe než ve stavu uspořádaném (šroubovice). U **bílkovin** je HE způsoben peptidovými vazbami, které mají UV absorpční maximum kolem 190 nm.
- Dvoušroubovice **DNA** absorbuje UV světlo hůře vlivem patrových a vodíkových interakcí než jednořetězcová (denaturovaná/neuspořádaná).
 $A_{\text{DNA}260\text{nm}}$ v horké vodě > ve studené vodě
- **Helicita** – relativní zastoupení uspořádaných částí makromolekuly

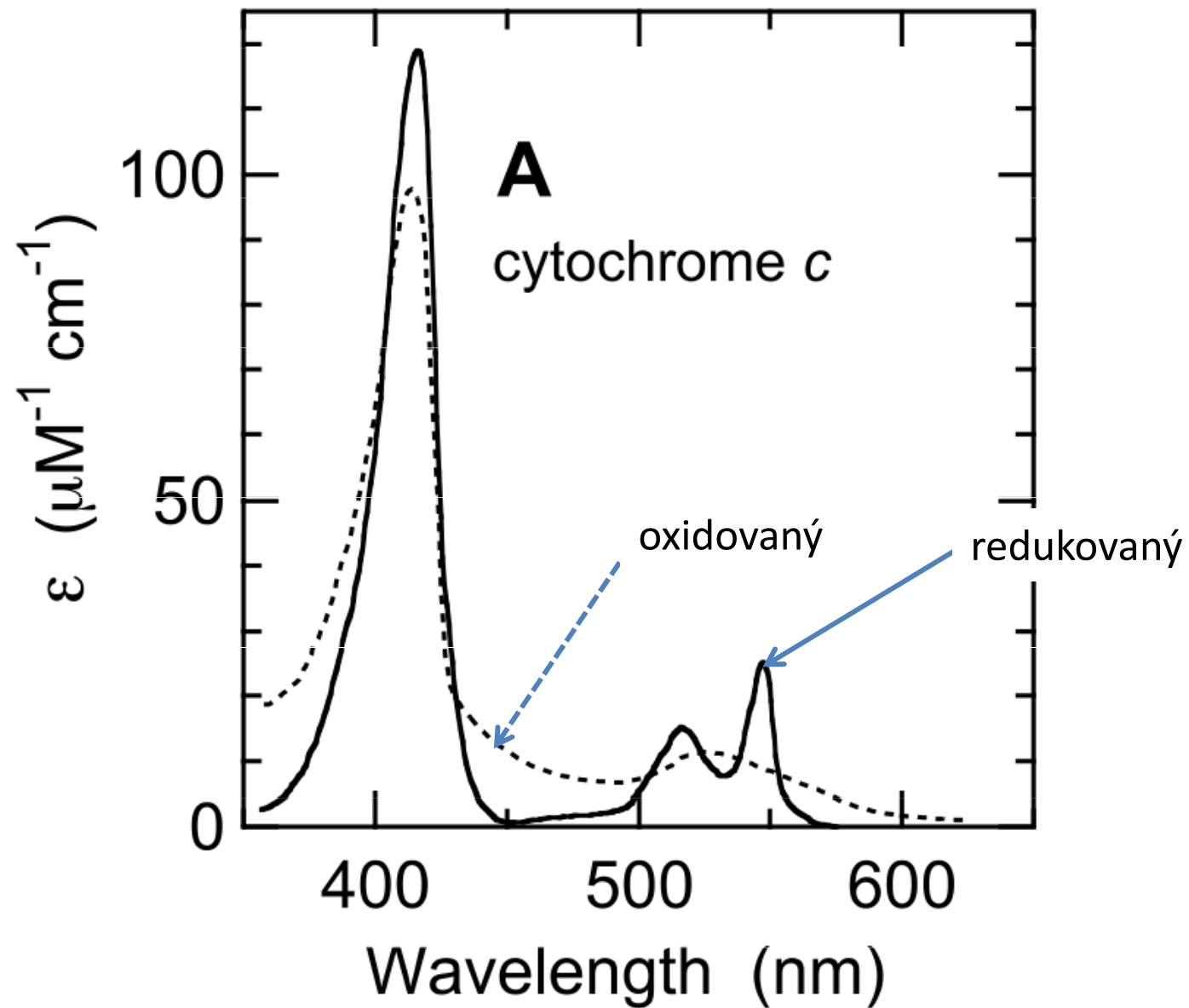
Hypochromní efekt u kys. polyglutamové. Při pH 7 tento polypeptid tvoří stochastické (neuspořádané) klubko (1), při pH 4 získává šroubovicovou strukturu (2). Absorpční maximum peptidových vazeb je sníženo vlivem jejich prostorového uspořádání. ϵ je molární absorpční koeficient a λ je vlnová délka UV světla. [dle: Kalous a Pavlíček, 1980]

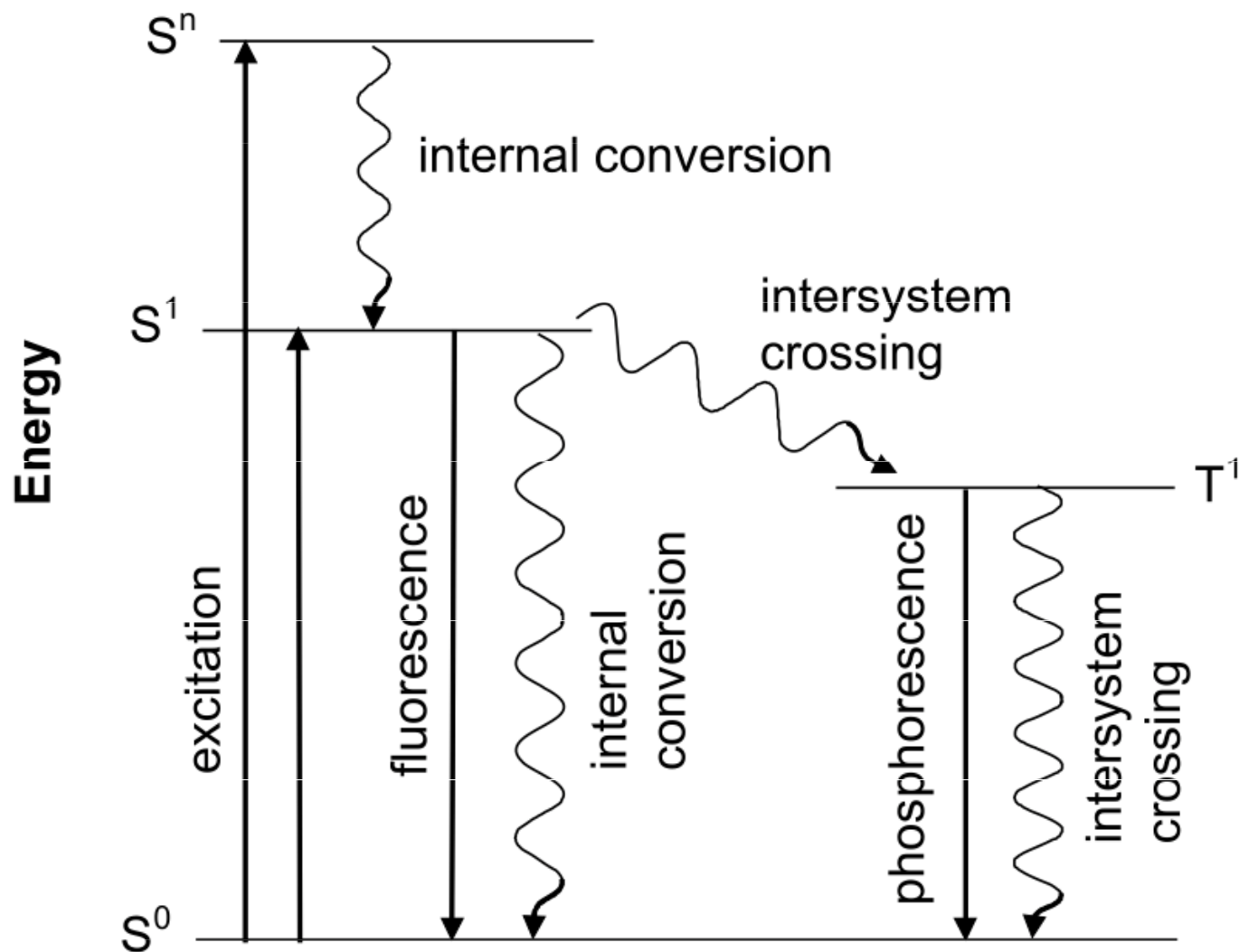
Teplota tání duplexů DNA



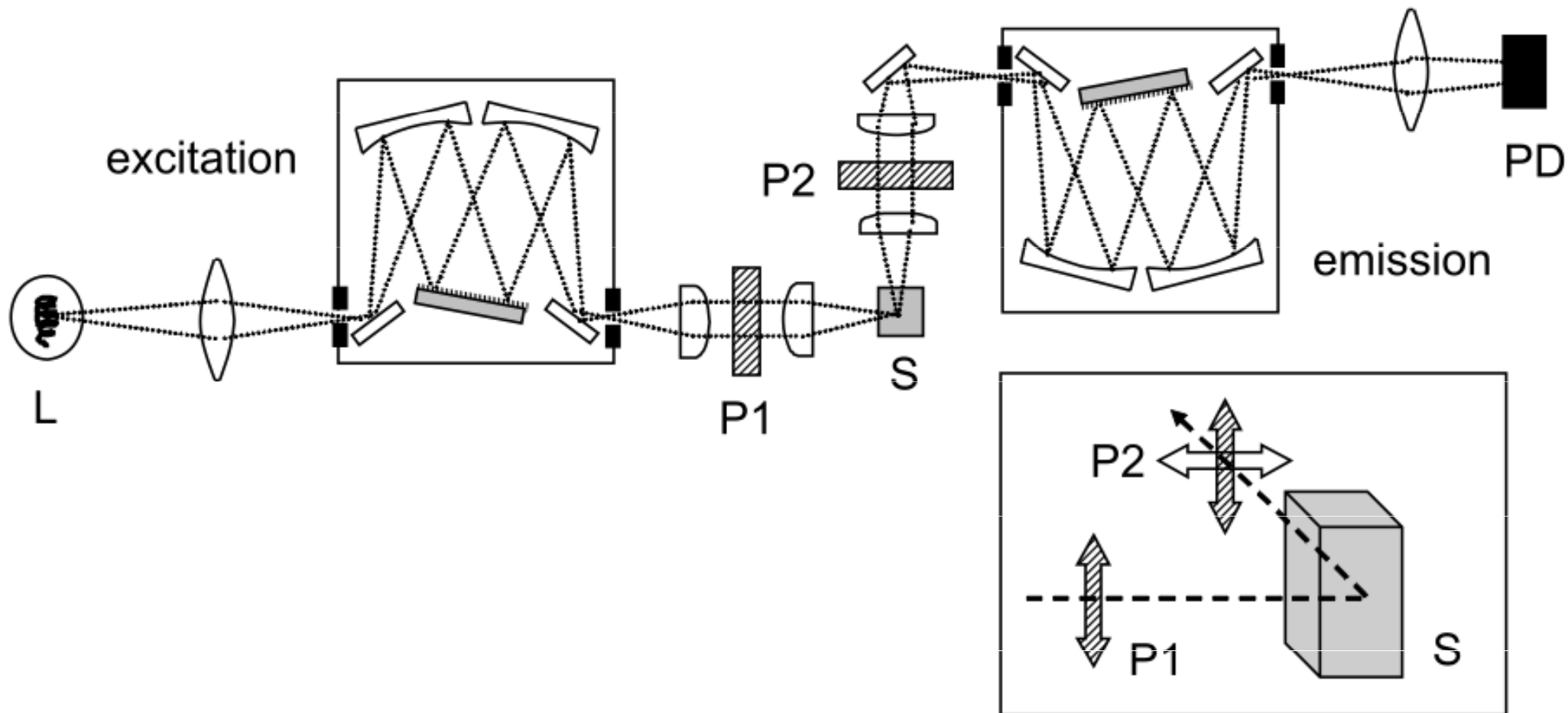
<https://sandwalk.blogspot.com/2007/12/dna-denaturation-and-renaturation-and.html>

<https://www.khanacademy.org/test-prep/mcat/physical-sciences-practice/physical-sciences-practice-tut/e/melting-point-and-thermodynamics-of-double-stranded-dna-1>



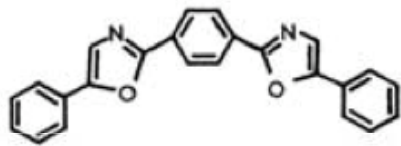


Zářivé procesy (absorpce, fluorescence, fosforescence) jsou indikovány rovnými šipkami. Nezářivé procesy jsou naznačeny zvlněnými šipkami. Diagramy tohoto typu byly zavedeny A. Jablonskim v jeho práci z r. 1935 na téma mechanismu fosforescence. Horizontální osa nemá fyzikální význam.

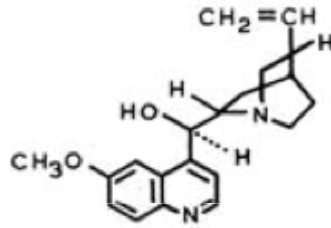


Zařízení pro měření **fluorescenční anisotropie**. V hlavním schématu polarizační filtr nebo hranol (P1) polarizuje dopadající světlo. Intenzita fluorescence je měřena druhým polarizátorem (P2), který může být kolmý nebo paralelní k P1. L – lampa, S – vzorek, PD – fotodetektor.

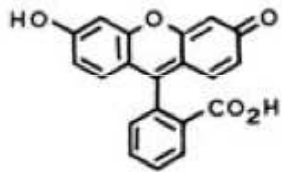
Fluorescenční (Försterův) přenos energie



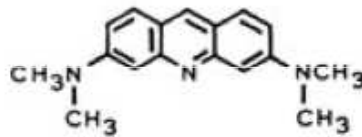
POPOP



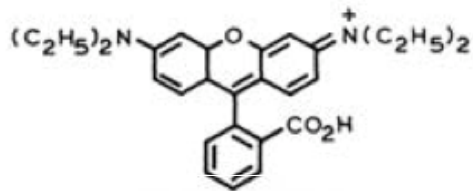
Quinine



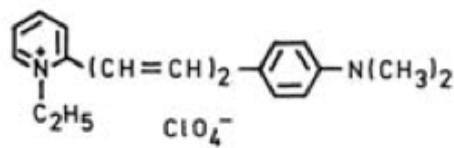
Fluorescein



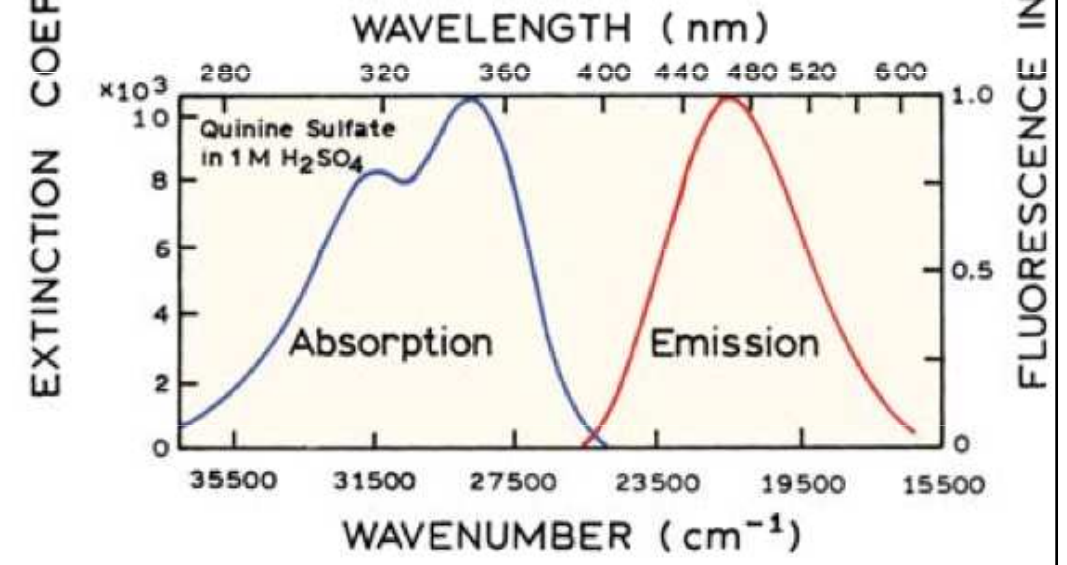
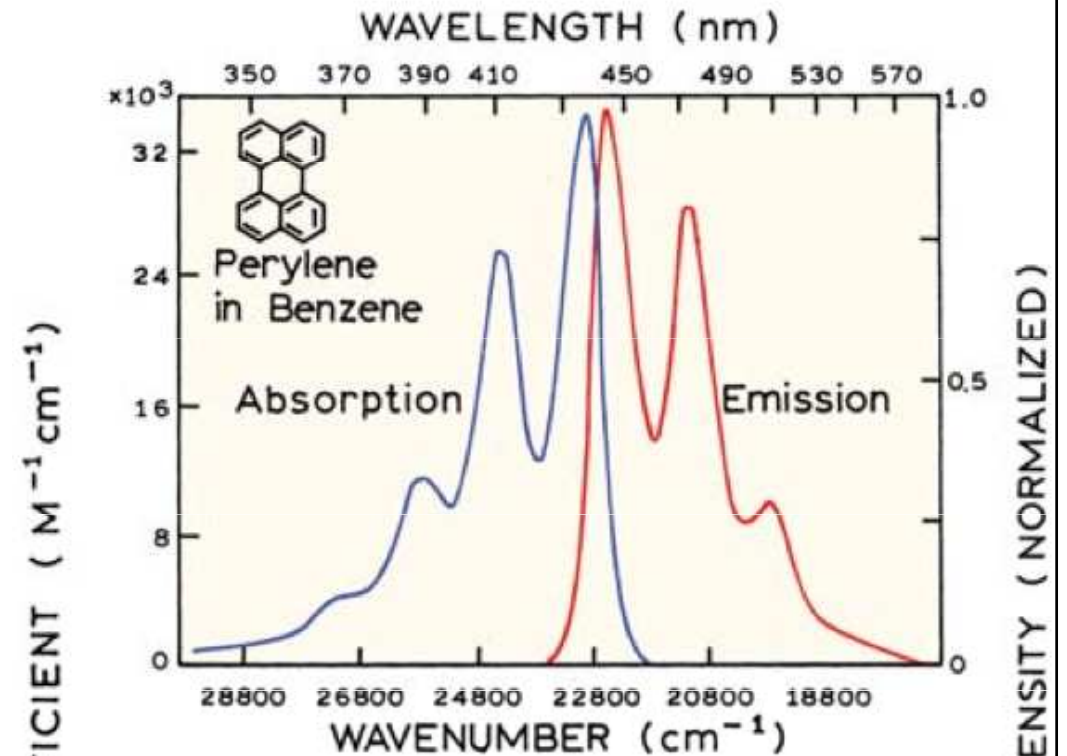
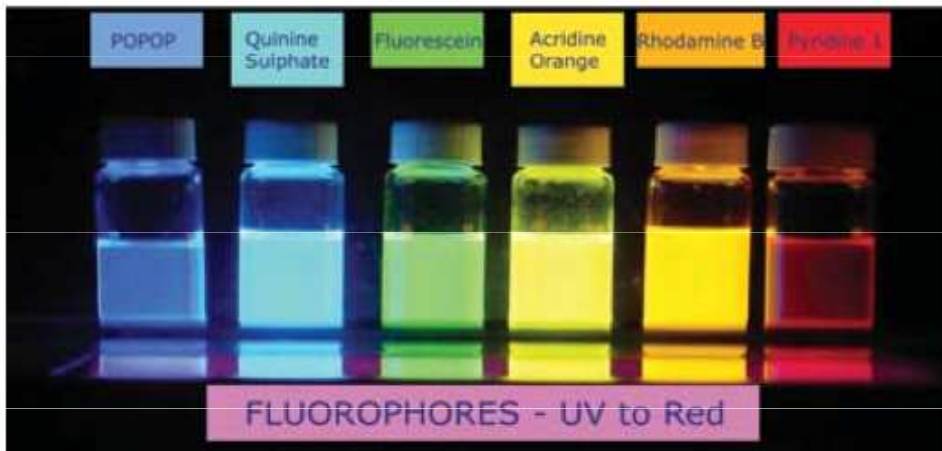
Acridine Orange



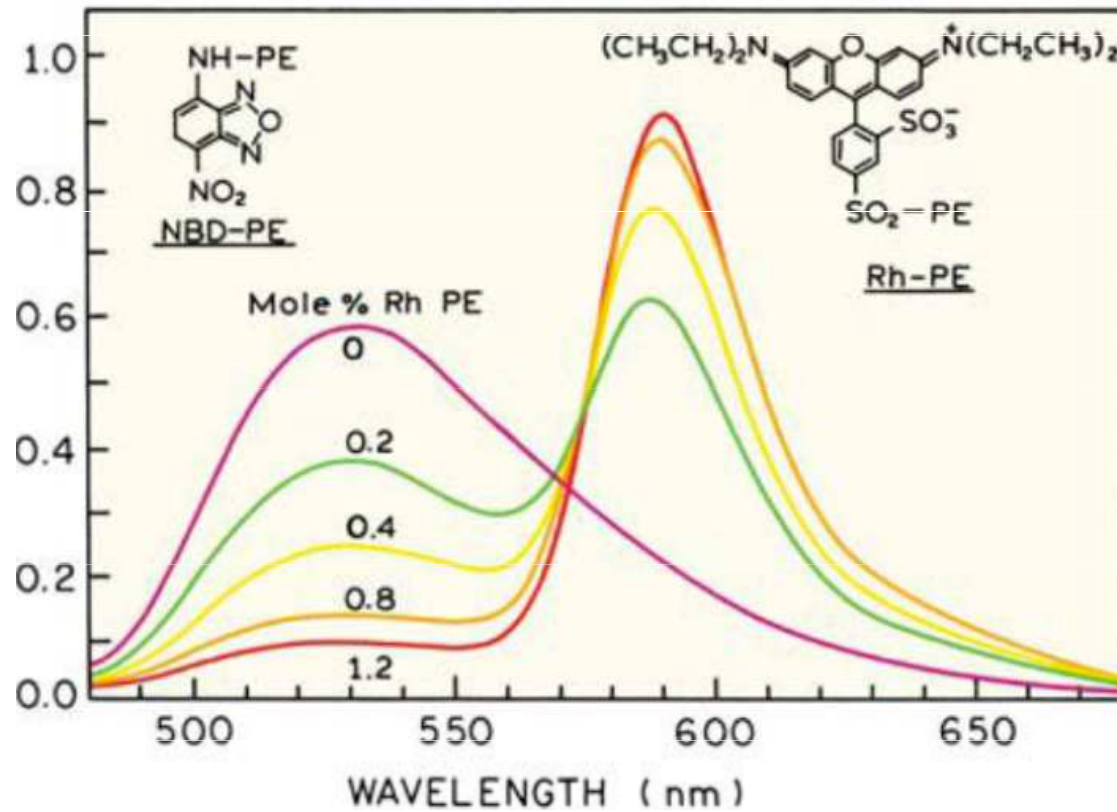
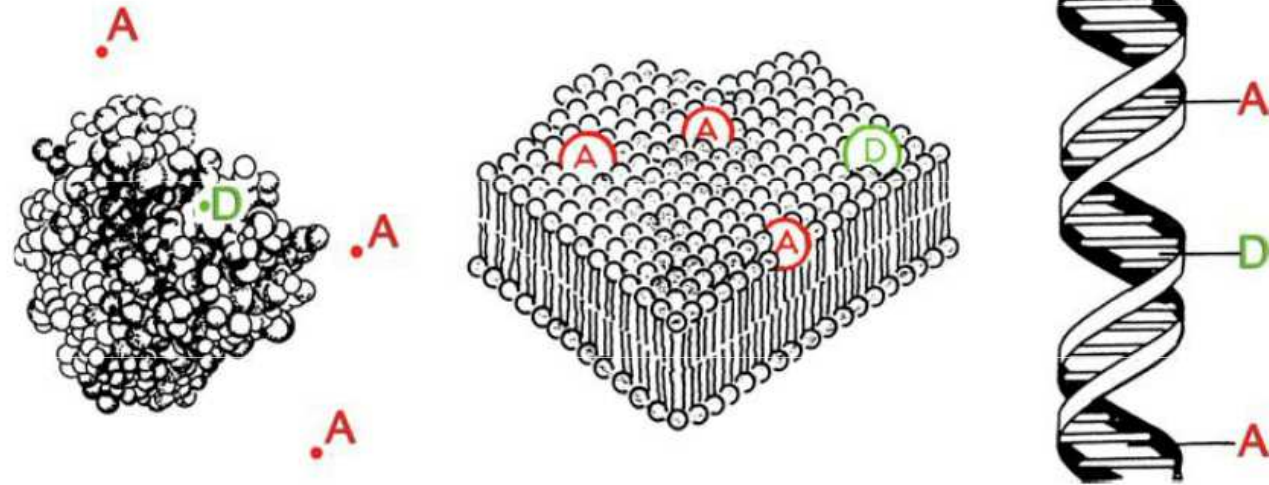
Rhodamine B

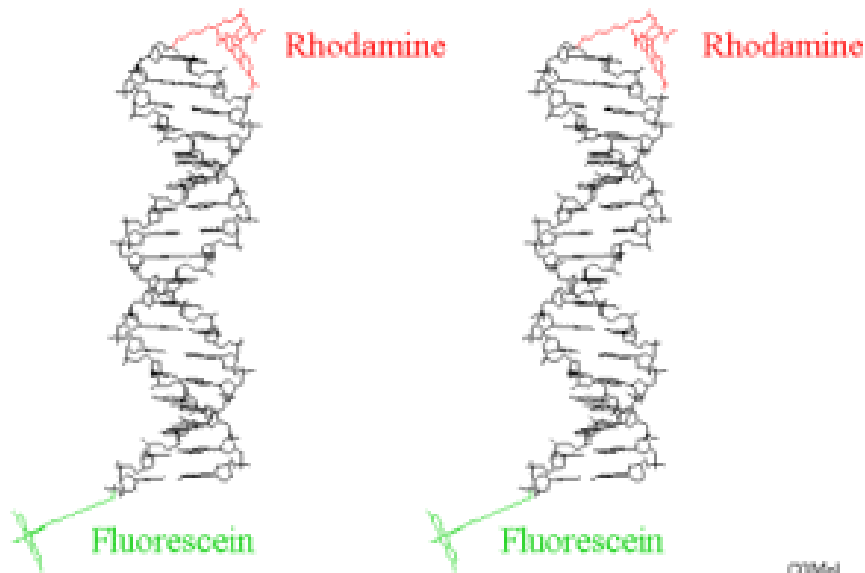


Pyridine 1

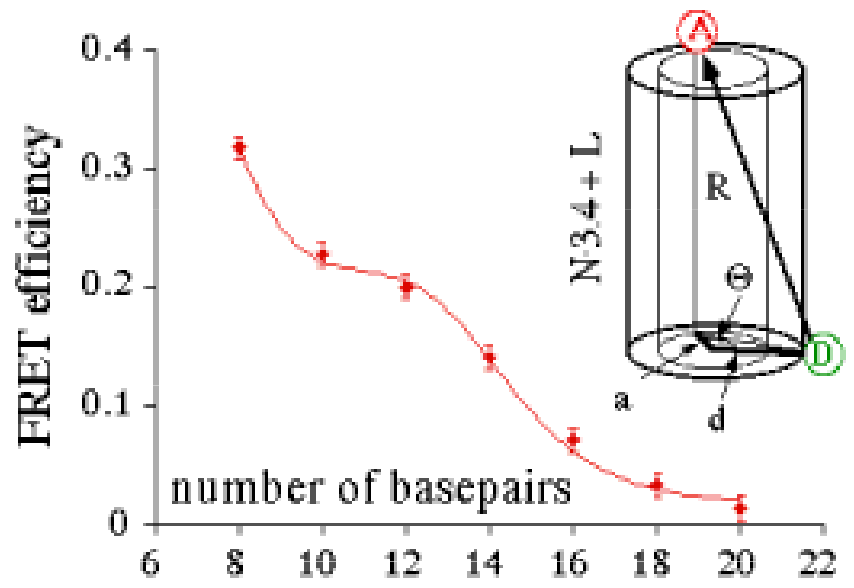
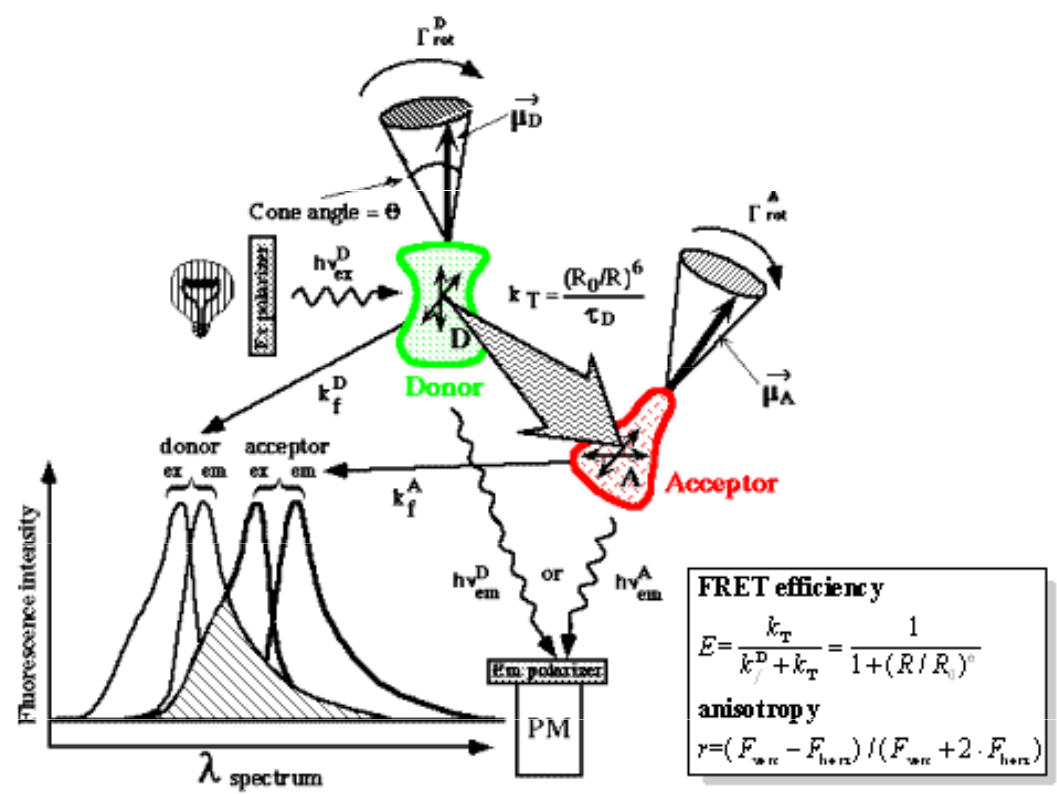


Fluorescenční (Försterův) přenos energie



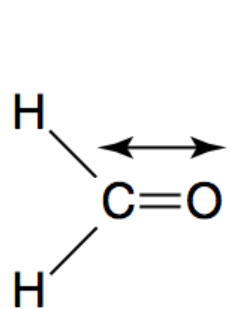


©MMel

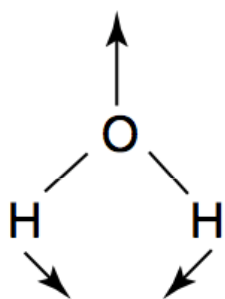


IR spektrofotometrie

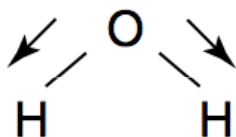
- Infračervené záření (IR) působí na rotační a vibrační stavy molekul. Složité molekuly mohou vibrovat nebo rotovat mnoha různými způsoby (módy). Různé chemické skupiny (-CH₃, -OH, -COOH, -NH₂ atd.) mají specifické vibrační a rotační frekvence, a proto absorbují IR světlo o specifických vlnových délkách.
- Z tohoto důvodu mají infračervená absorpční spektra mnoho maxim. Změna chemické struktury se projevuje jako změna polohy těchto maxim ve spektru.



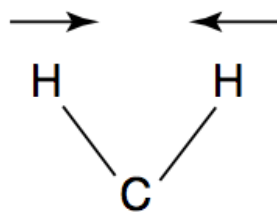
Stretching



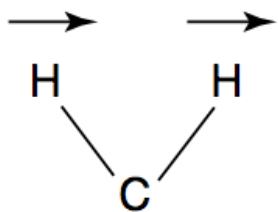
Bending



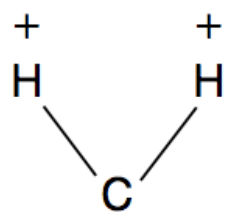
Stretching



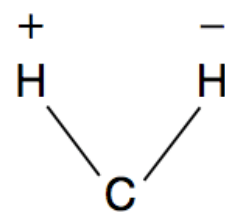
Deformation



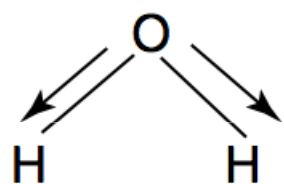
Rocking



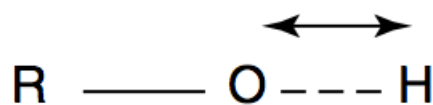
Wagging



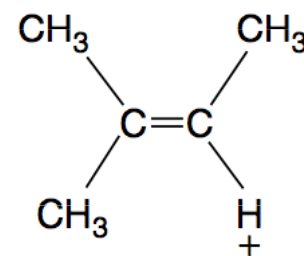
Twisting



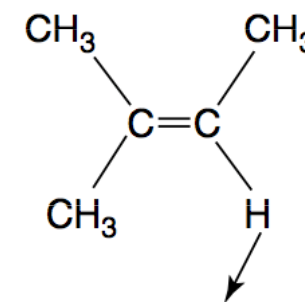
Symmetric stretching



Asymmetric stretching



Out-of-plane bending



In-plane bending

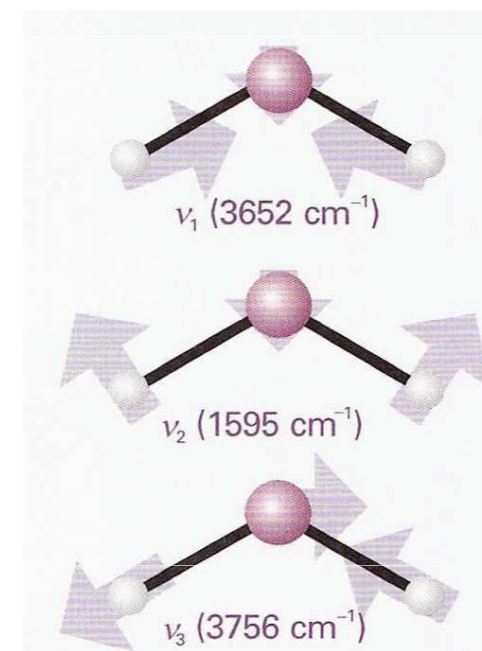
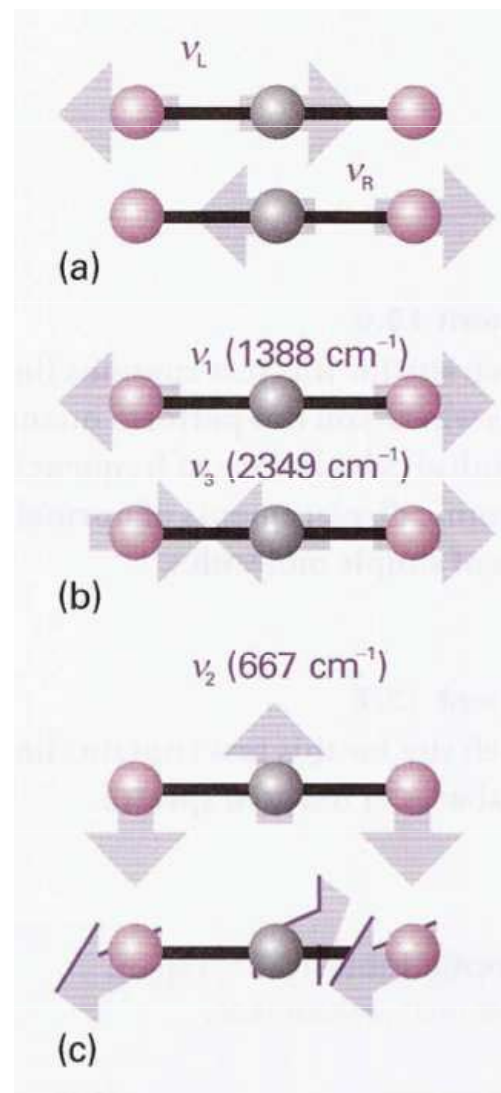
Vibrace

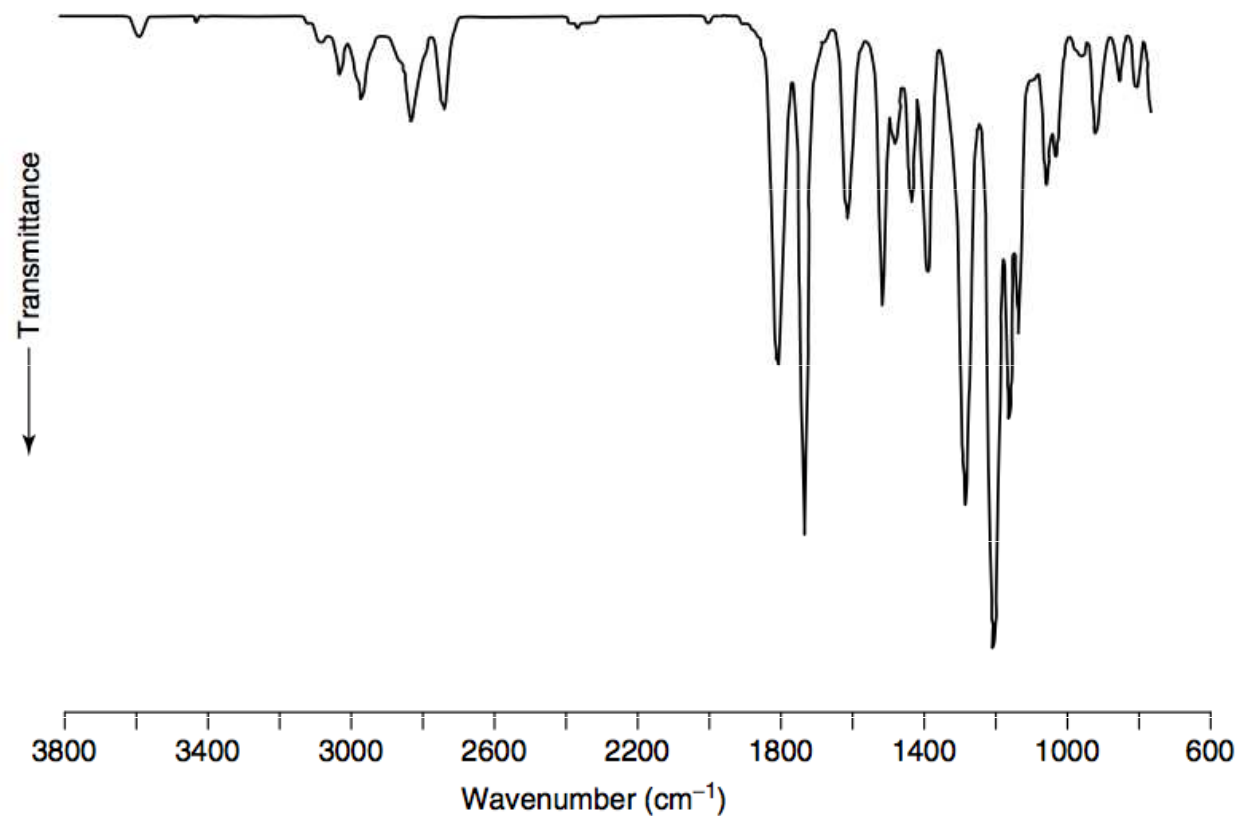
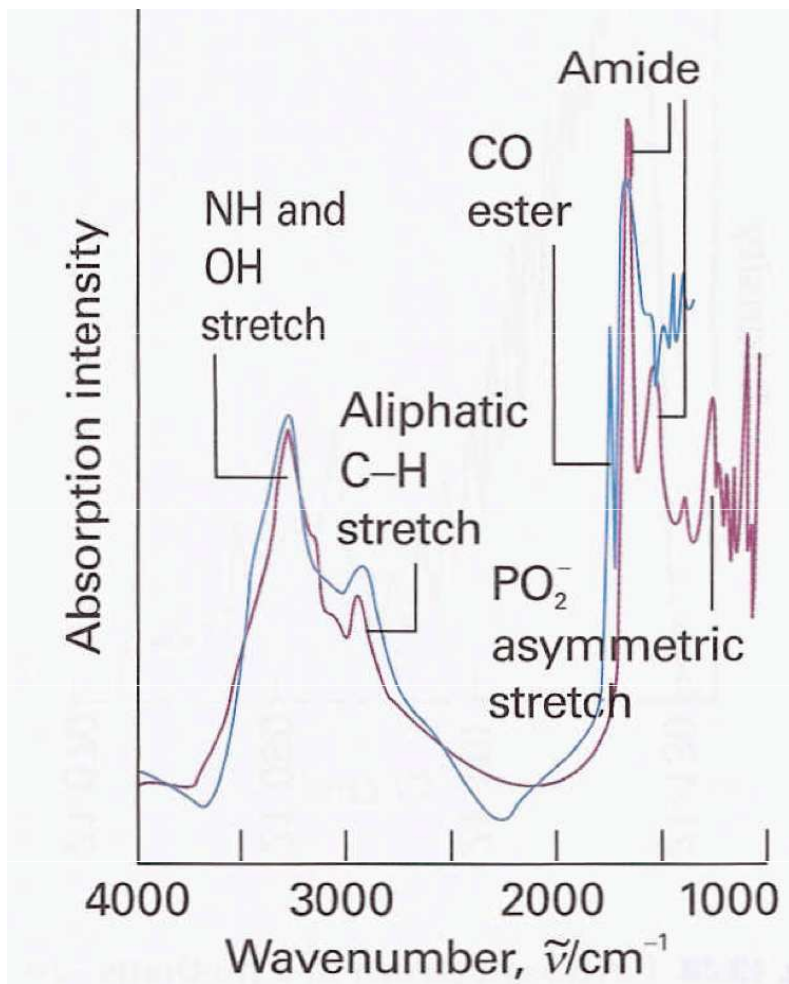
ν/cm^{-1}

C—H stretch	2850–2960
C—H bend	1340–1465
C—C stretch, bend	700–1250
C=C stretch	1620–1680
C≡C stretch	2100–2260
O—H stretch	3590–3650
H-bonds	3200–3570
C=O stretch	1640–1780
C≡N stretch	2215–2275
N—H stretch	3200–3500
C—F stretch	1000–1400
C—Cl stretch	600–800
C—Br stretch	500–600
C—I stretch	500
CO_3^{2-}	1410–1450
NO_3^-	1350–1420
NO_2^-	1230–1250
SO_4^{2-}	1080–1130
Silicates	900–1100

CO_2

H_2O



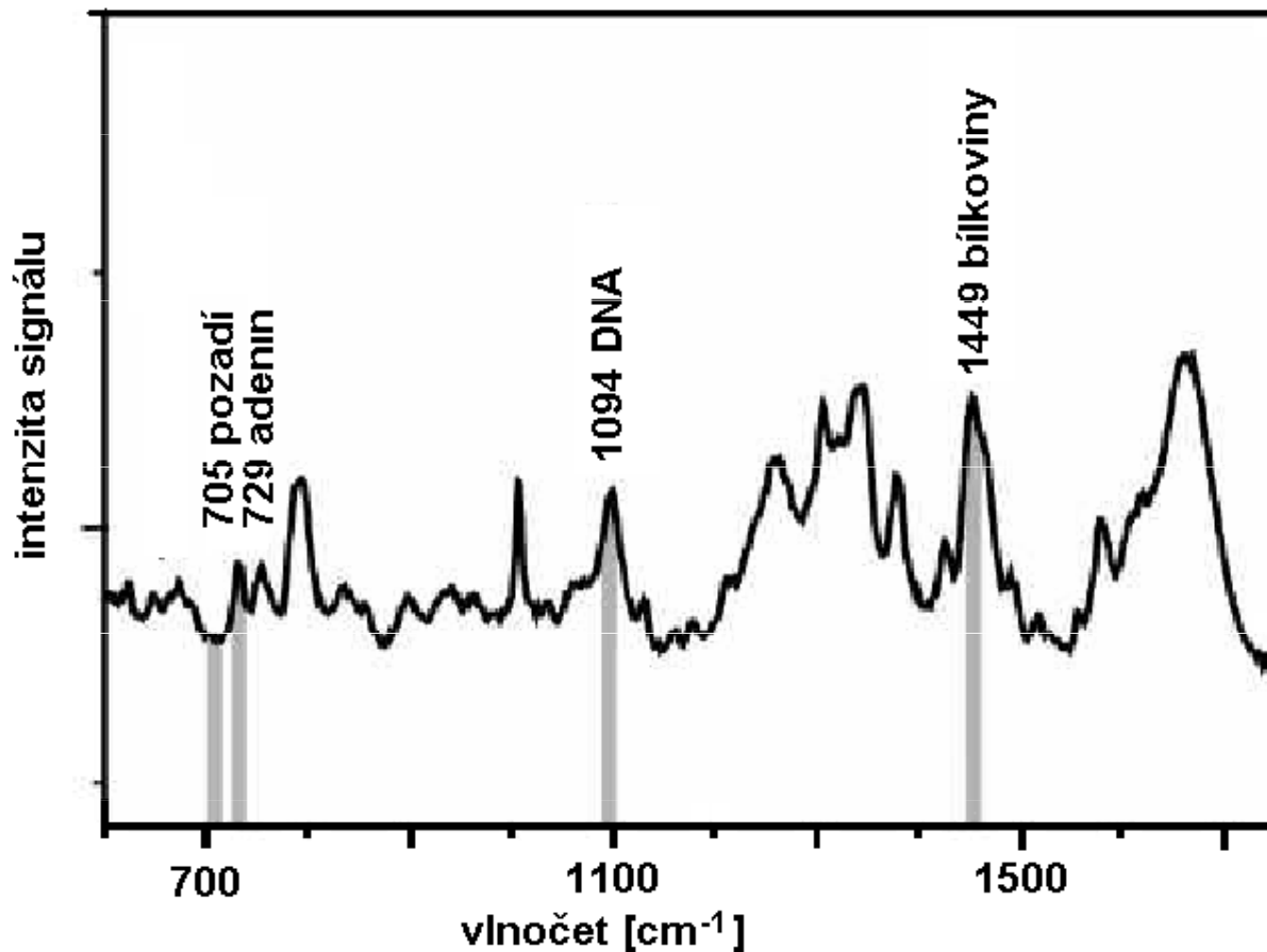


Ukázka IR spekter. Vlevo absorpční spektrum živé (fialově) a umírající (modře) buňky. Vpravo spektrum vanilinu.

Ramanova spektroskopie

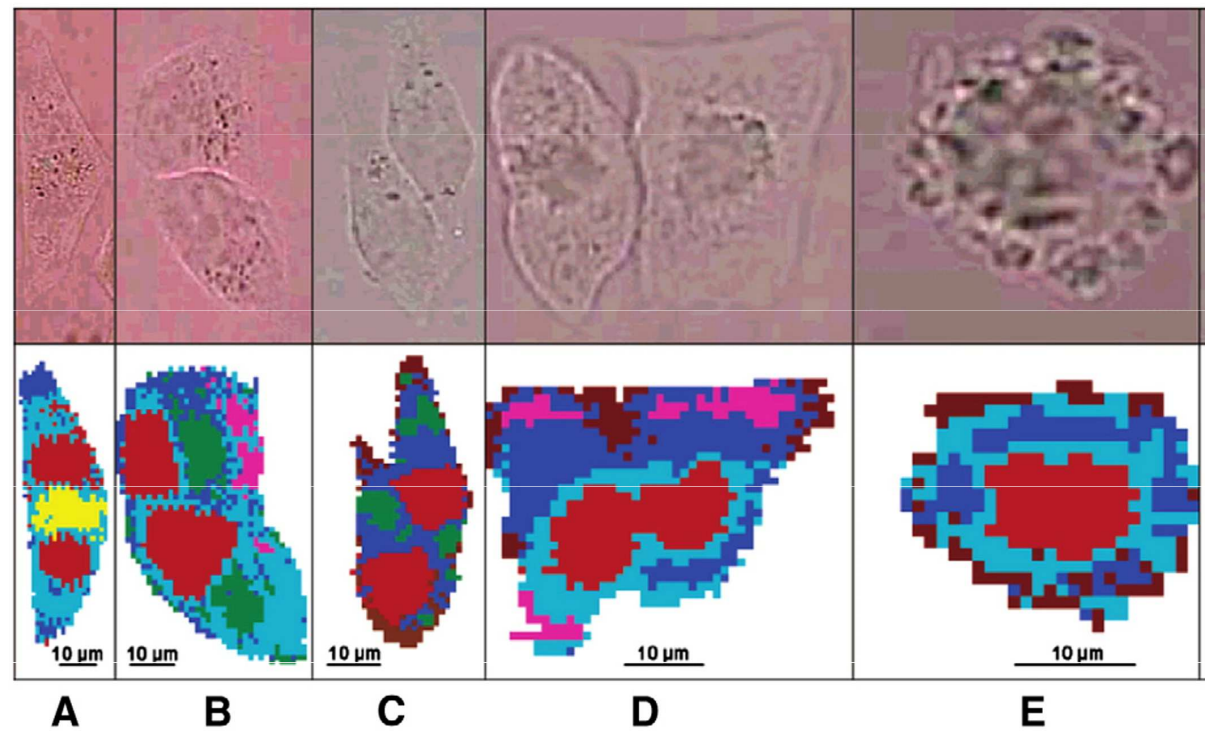
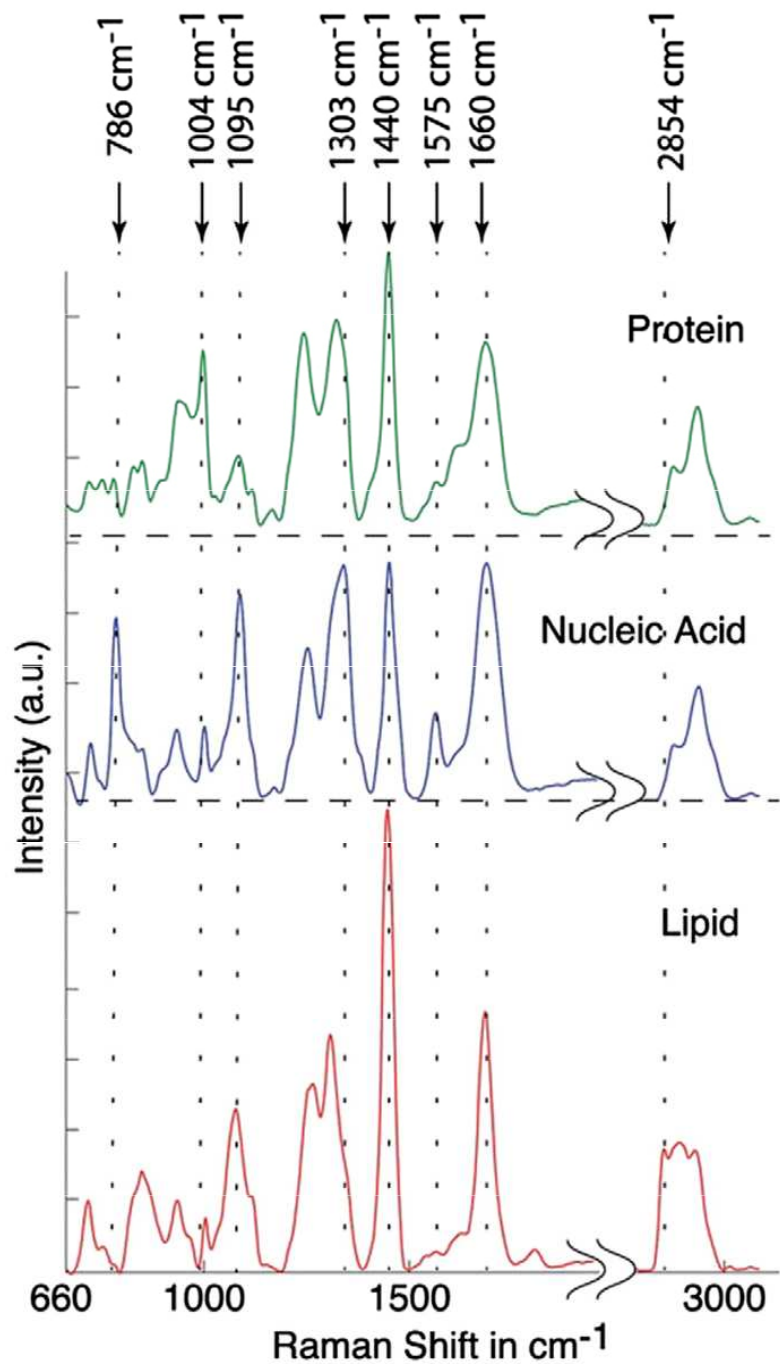
- **Sir Chandrasekhara Venkata Raman** – NC 1930 za fyziku „za jeho práci o rozptylu světla a objevu efektu pojmenovanám po něm“
- **Rayleighův rozptyl světla.** Nastává interakce fotonů s molekulami, jež se projevuje jen velmi malou nebo žádnou změnou vlnové délky. Intenzita rozptýleného světla závisí na molekulové hmotnosti a také na úhlu rozptylu, což lze využít pro odhad tvaru makromolekul.
- **Ramanova spektrometrie.** Při rozptylu fotonů nastává malá změna (posun) vlnové délky, způsobená malým poklesem nebo zvýšením energie rozptýlených fotonů během přechodu z původního do změněného vibračního nebo rotačního stavu interagující molekuly. Tyto stavy se mohou měnit v důsledku strukturálních změn molekul.
- Proto změny v Ramanových spektrech (intenzita signálu v závislosti na posunu vlnové délky) odrážejí konformační změny molekul.

Ramanova spektroskopie



Ramanovo spektrum polytenního chromosomu pakomára Chironomus.

Při zvolených vlnočtech lze uskutečnit ramanovskou mikroskopií. Vybuzeo laserovým světlem o vlnové délce 647.1 nm.



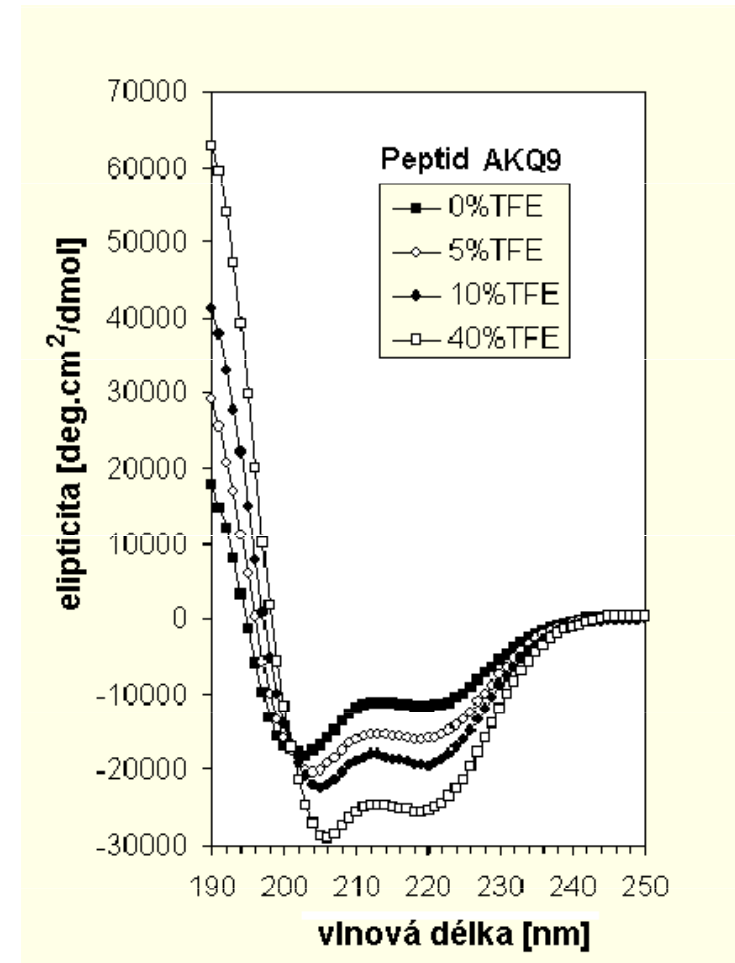
Colors assigned to different cluster memberships of the Raman spectra of:

- 1) nucleus (red),
- 2) cytoplasm (blue, cyan),
- 3) microtubule (yellow),
- 4) vesicles (green),
- 5) peripheral membrane (brown),
- 6) inclusion bodies (magenta).

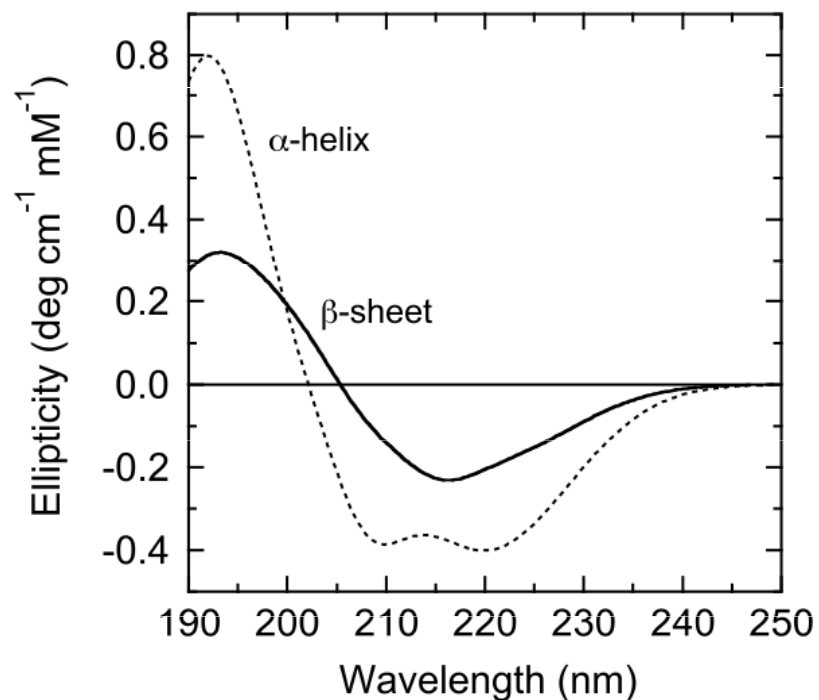
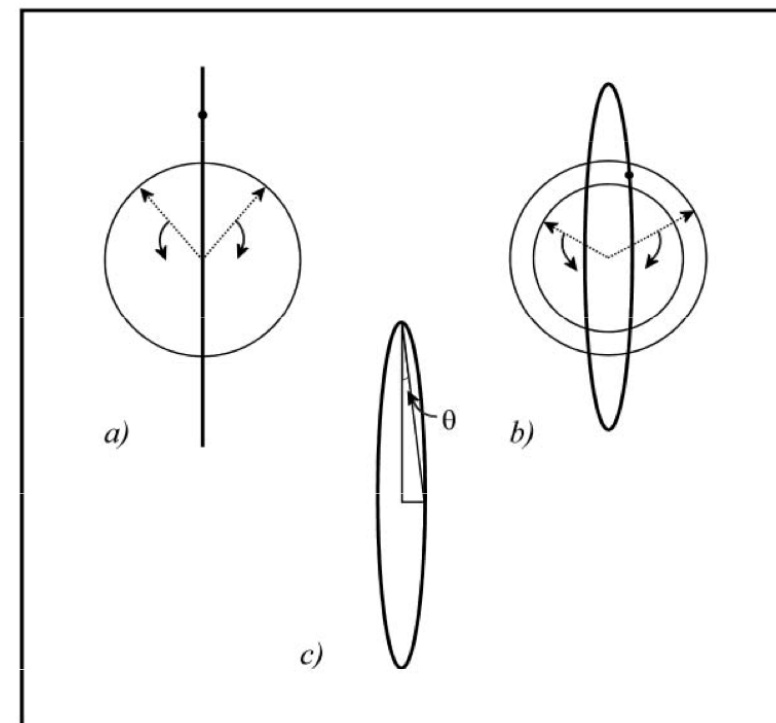
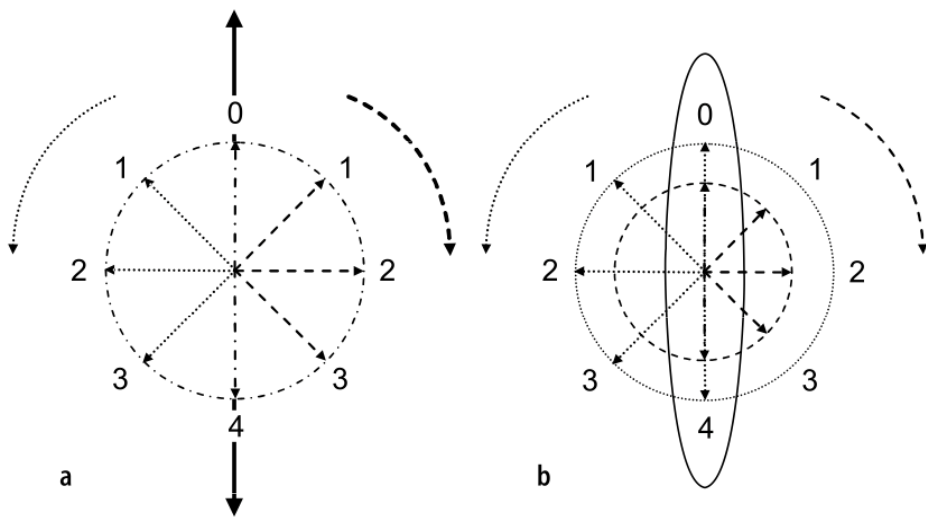
Cirkulární dichroismus (CD)

- Měření **optické aktivity** (schopnosti stáčet rovinu polarizovaného světla). Konformační změny molekul mohou být sledovány jako změny optické aktivity při použití speciálního polarimetru.
- U metody CD srovnáváme absorbance levotočivě a pravotočivě cirkulárně polarizovaného světla, jehož vlnová délka je blízká absorpčnímu maximu bílkoviny.
- CD lze využít též pro studium struktury nukleových kyselin.

Obrázek ukazuje změny **elipticity** syntetického polypeptidu, obsahujícího dlouhé sekvence poly-glu, po přidavku trifluoroethanolu (TFE), který zvyšuje podíl α -šroubovice. <http://www-structure.llnl.gov/cd/polyq.htm>



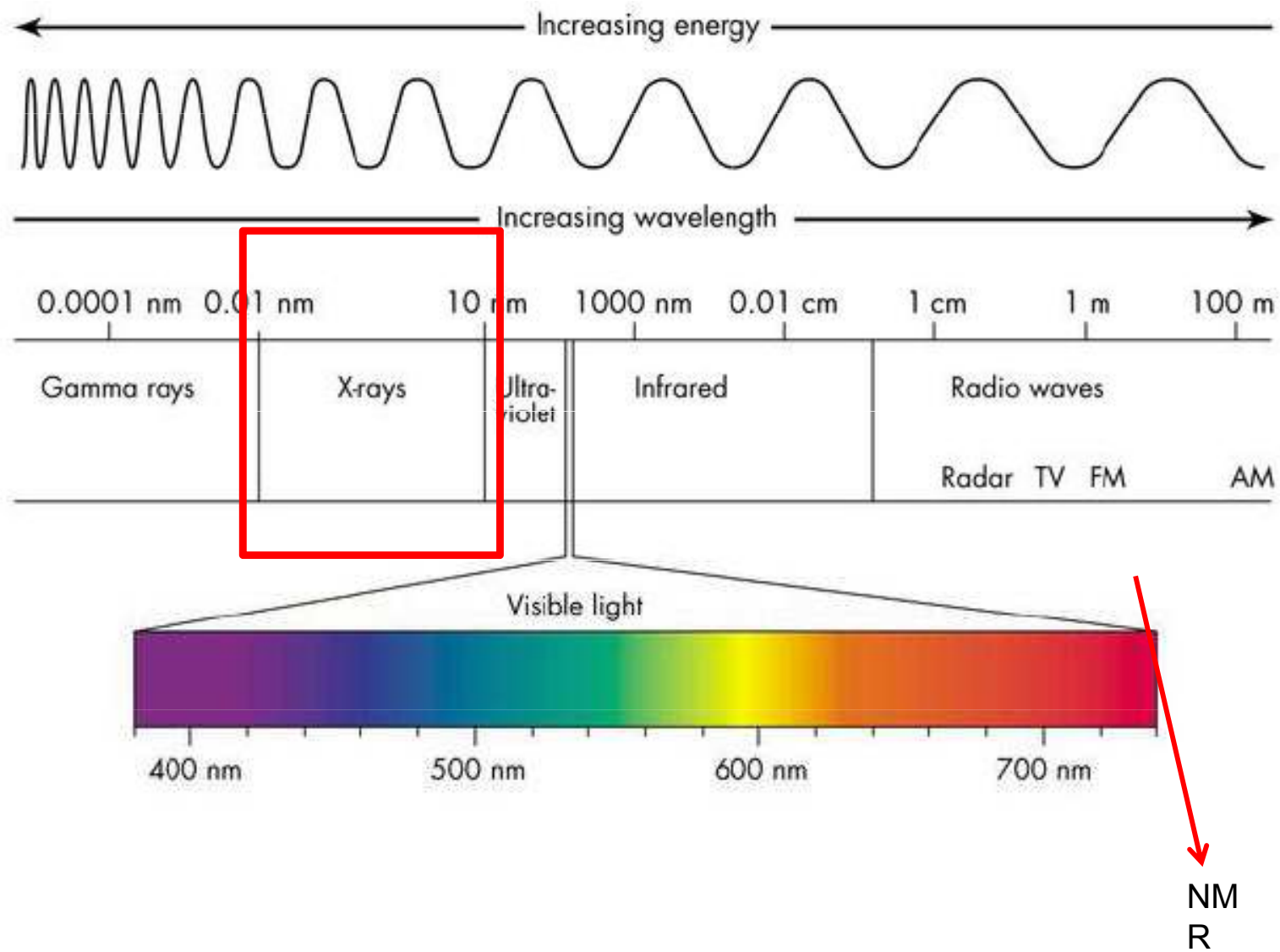
$$\theta_r = \frac{2.303}{4} \cdot (A_L - A_R) \cdot [\text{rad}]$$



Nejčastěji se CD vyjadřuje jako závislost na Delta Epsilon.

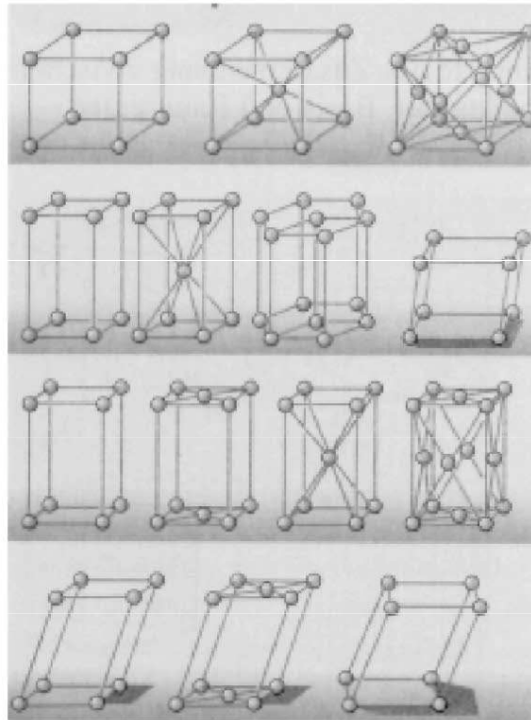
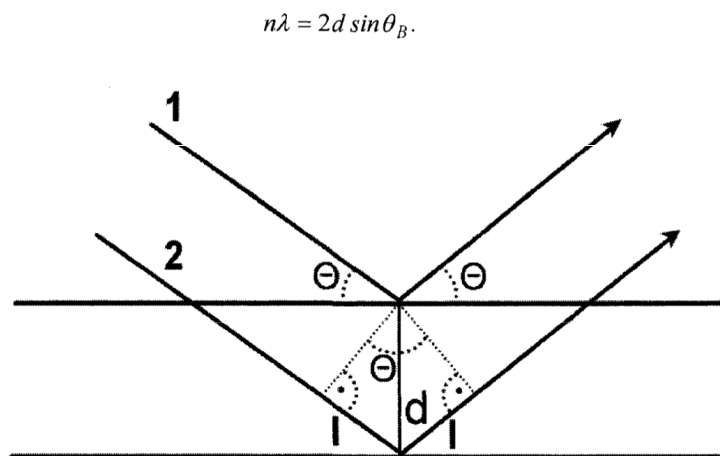
$$\Delta \epsilon = \epsilon_L - \epsilon_R = (A_L - A_R) / (dl)$$

ϵ_L a ϵ_R jsou definované levo-a pravo-točivé extinkční koeficienty, l je délka dráhy a d je molární koncentrace vzorku.



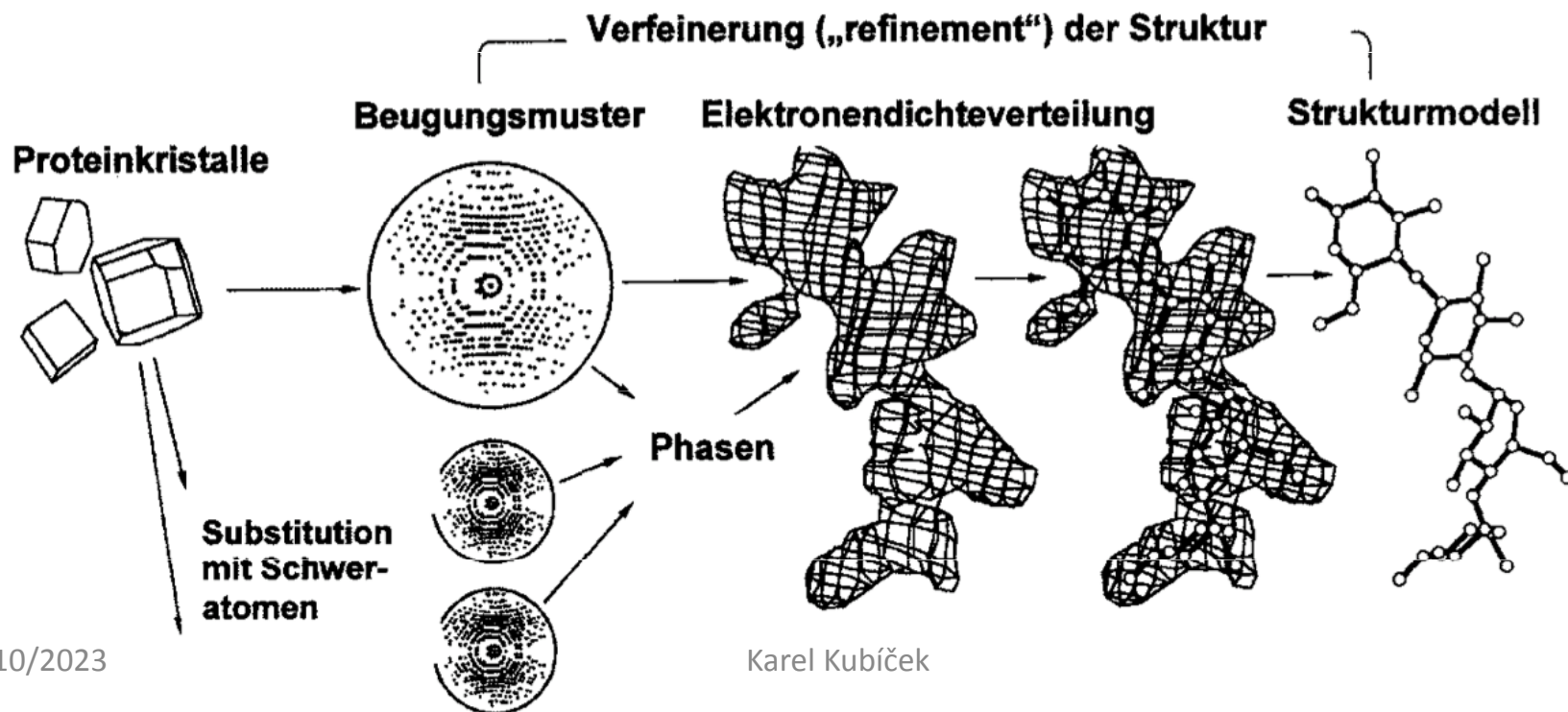
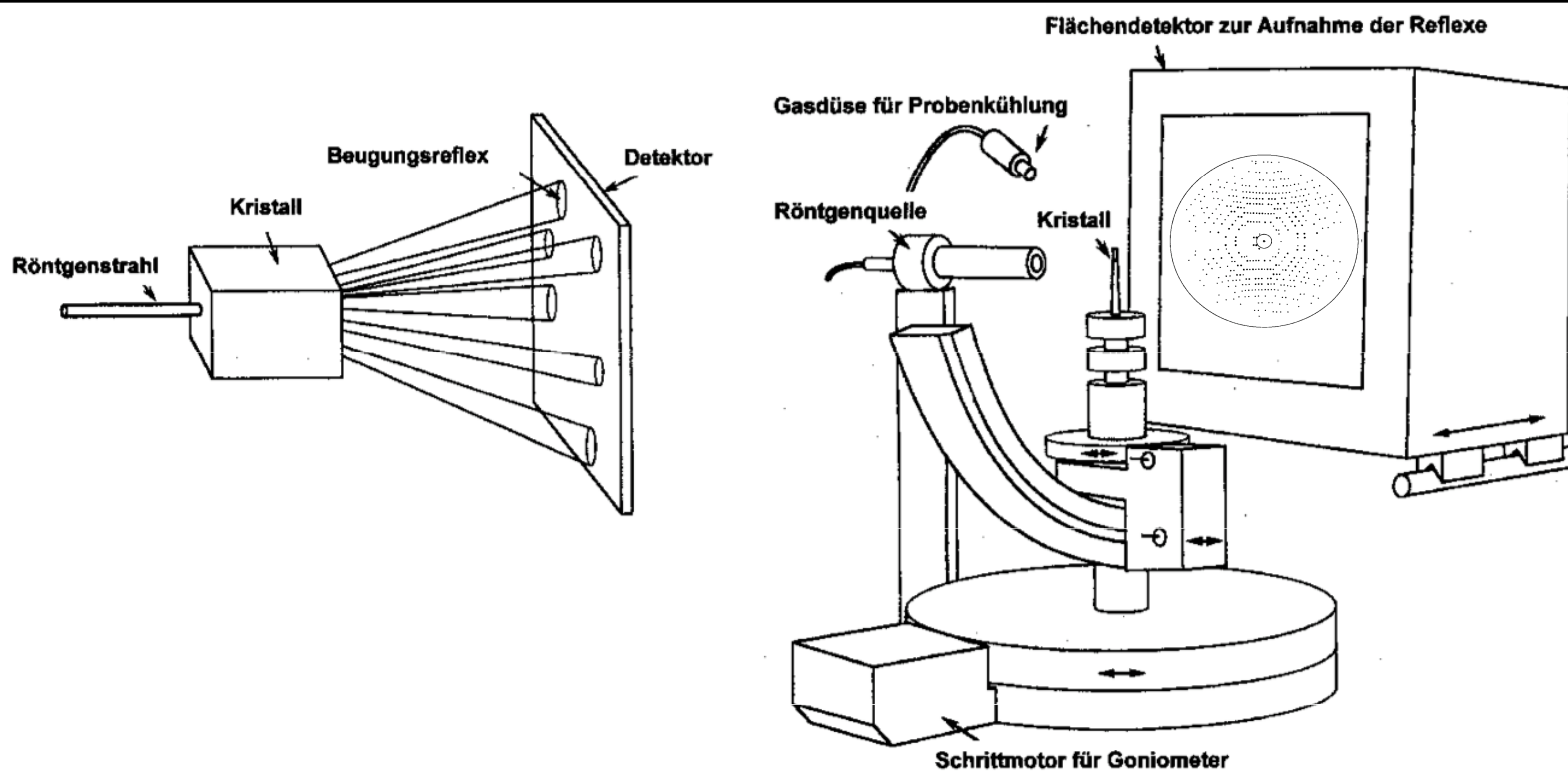
Rentgenstrukturní analýza

Krystalová mřížka působí na rentgenové záření jako optická mřížka na viditelné světlo. Nastávají ohybové jevy a na stínítku se objevuje difrakční obrazec. Tyto obrazce mohou být matematicky analyzovány, aby se získala informace o rozložení elektronů v molekulách tvořících krystal.

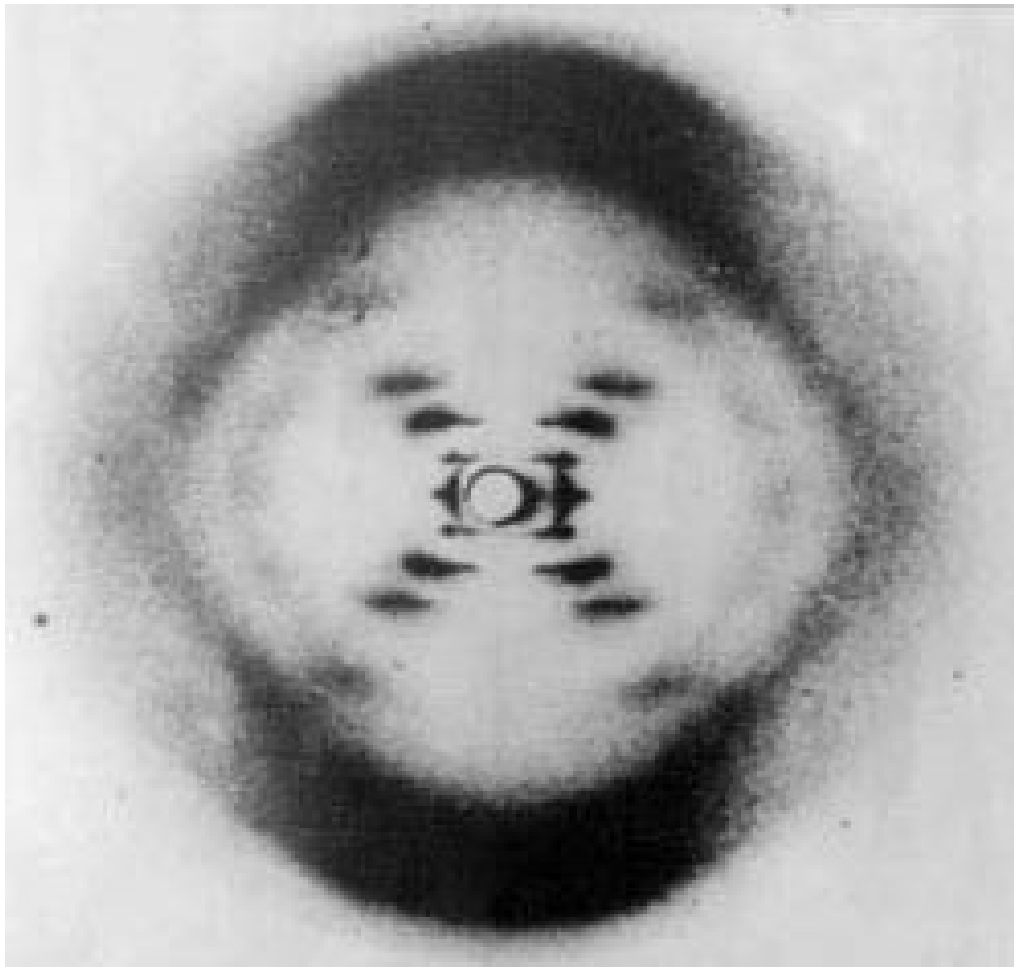


Raumgitter

$\alpha_1, \alpha_2, \alpha_3 \neq 90^\circ$:	triklin
$\alpha_1 = \alpha_2 = 90^\circ$, $\alpha_3 \neq 90^\circ$:	monoklin
$\alpha_1 = \alpha_2 = \alpha_3 = 90^\circ$, $a_0 \neq b_0 \neq c_0$:	orthorhombisch
$\alpha_2 = \alpha_3 = 90^\circ$, $\alpha_1 = 60^\circ$, $b_0 = c_0$:	hexagonal
$\alpha_1 = \alpha_2 = \alpha_3 \neq 90^\circ$, $a_0 = b_0 = c_0$:	trigonal (rhomboedrisch)
$\alpha_1 = \alpha_2 = \alpha_3 = 90^\circ$, $a_0 = b_0 \neq c_0$:	tetragonal
$\alpha_1 = \alpha_2 = \alpha_3 = 90^\circ$, $a_0 = b_0 = c_0$:	kubisch



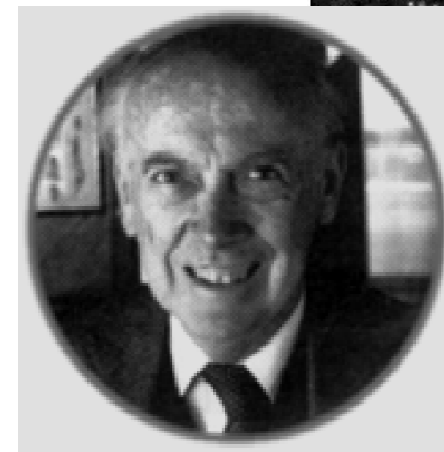
Krystalogram **B-DNA** získaný v r. **1952** Rosalindou E. **Franklinovou**, na jehož základě **Watson** a **Crick** navrhli dvoušroubovicový model struktury DNA. **C. & W.** dostali v r.**1962** společně s Mauricem Hugh Frederick Wilkinsem NC za fyziologii a medicínu „za jejich objevy týkající se molekulární struktury nukleových kyselin a jejich významu při přenosu informací v živých organizmech“



F



C



W

Confidence in structural features of proteins determined by X-ray crystallography

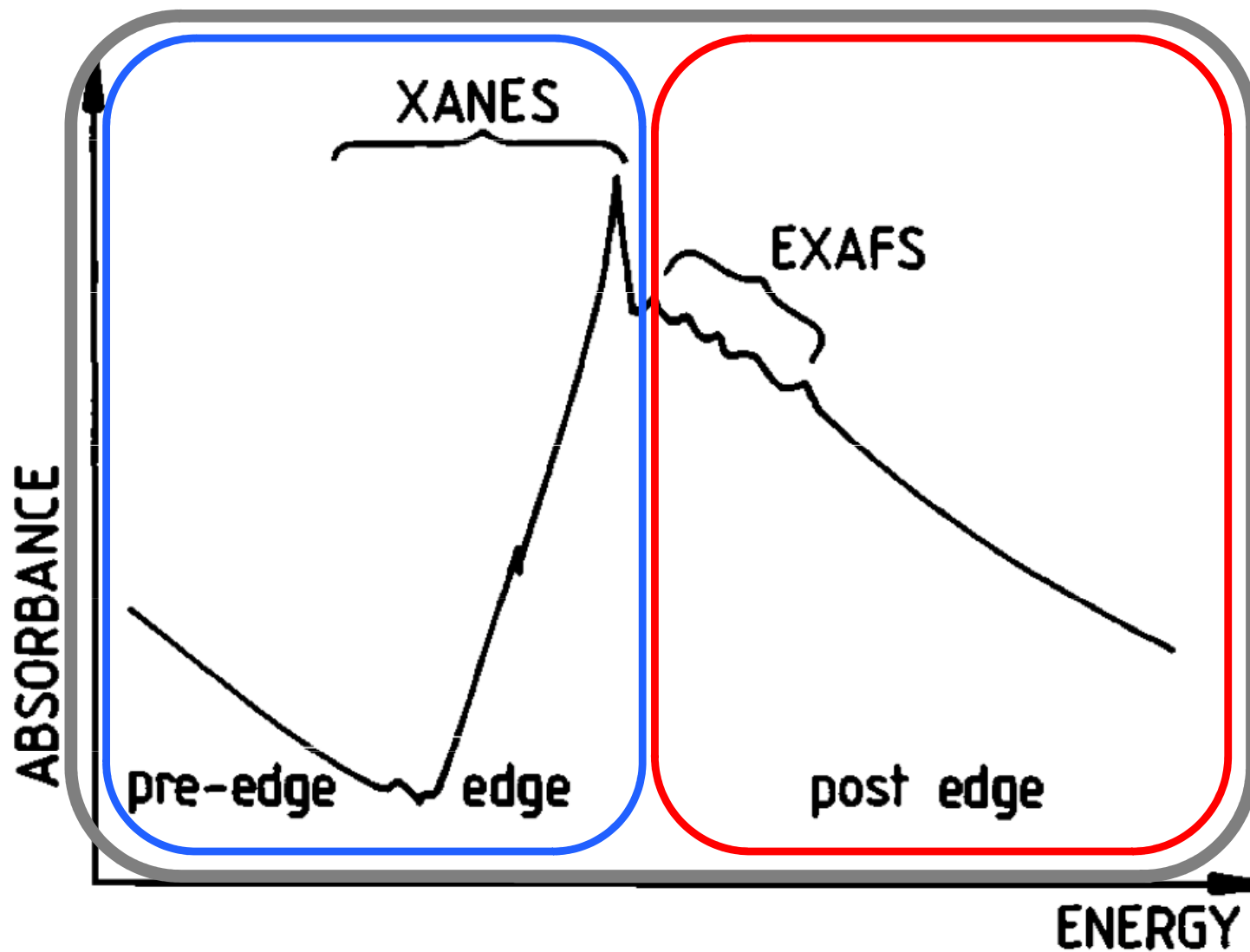
(estimates are very rough and strongly depend on the quality of the data)

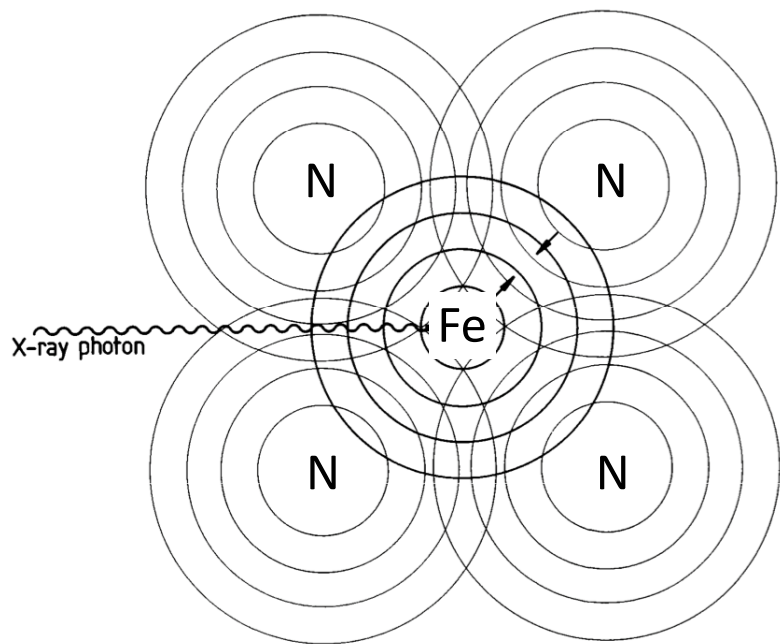
Structural feature			Resolution		
	5 Å	3 Å	2.5 Å	2 Å	1.5 Å
Chain tracing	-	Fair	Good	Good	Good
Secondary structure	Helices fair	Fair	Good	Good	Good
Sidechain conformations	-	-	Fair	Good	Good
Orientation of peptide planes	-	-	Fair	Good	Good
Protein hydrogen atoms visible	-	-	-	-	Good

XAS = X-ray Absorption Spectroscopy

XANES = X-ray Absorption Near Edge Structure

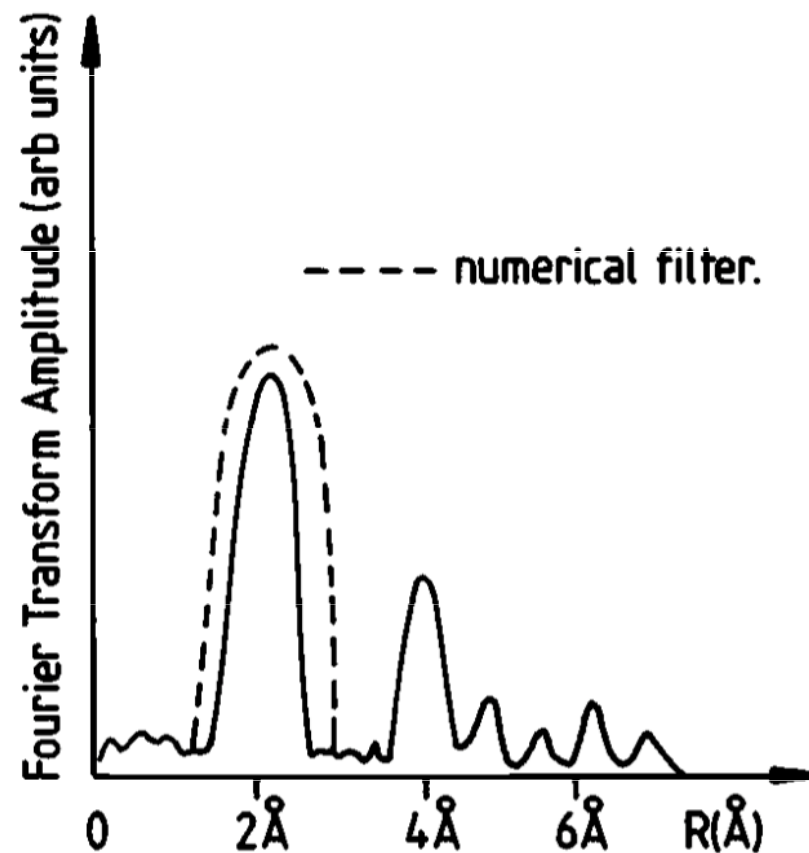
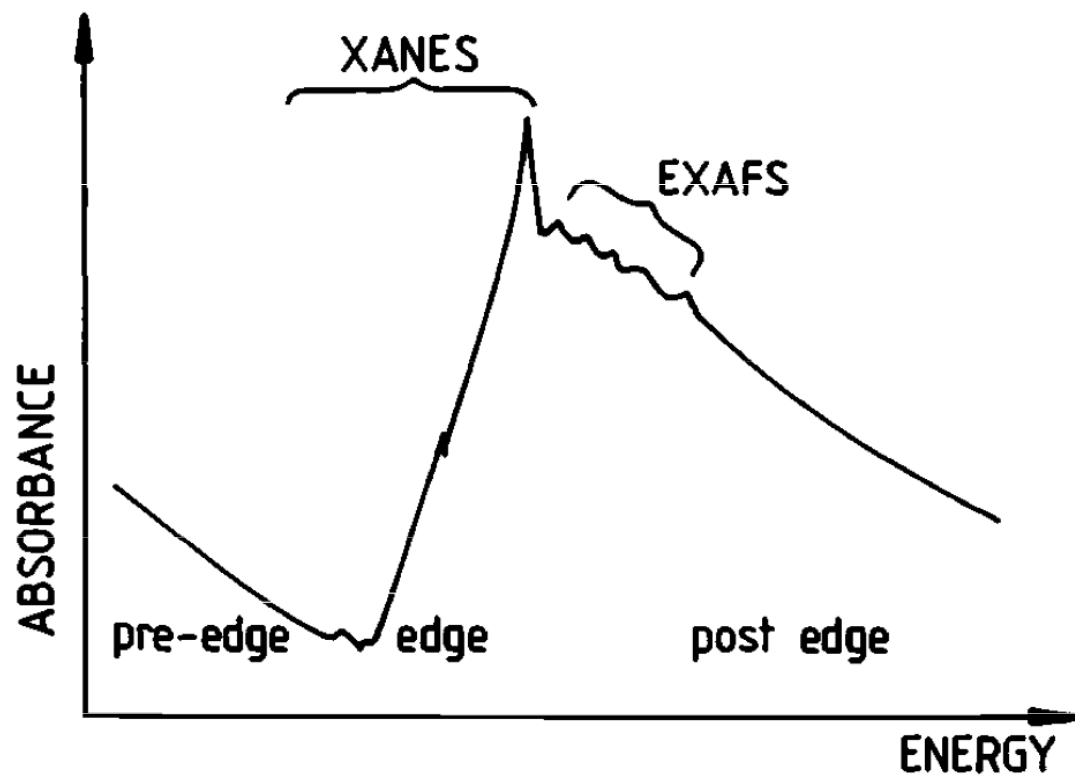
EXAFS = Extended X-Ray Absorption Fine Structure



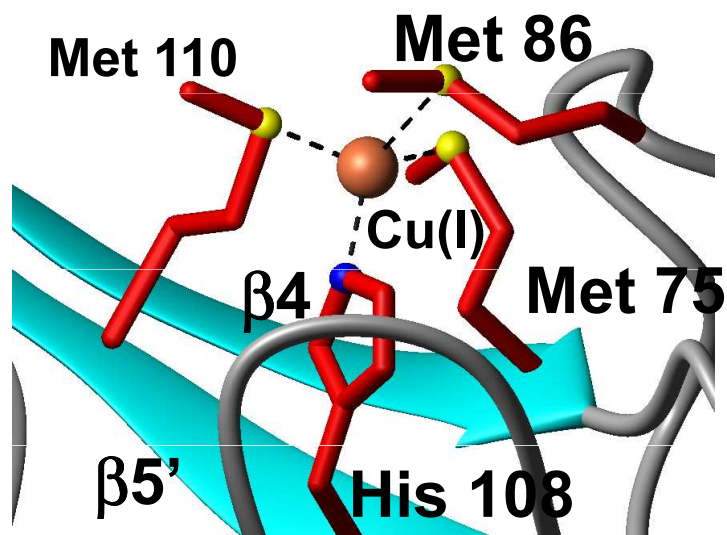


$$I = I_0 \exp(-\mu x)$$

EXAFS (Extended X-ray Absorption Fine Spectroscopy) poskytuje informace o nejbližších slupkách atomů sousedících s absorbujícím atomem



X-Ray Absorption Spectroscopy

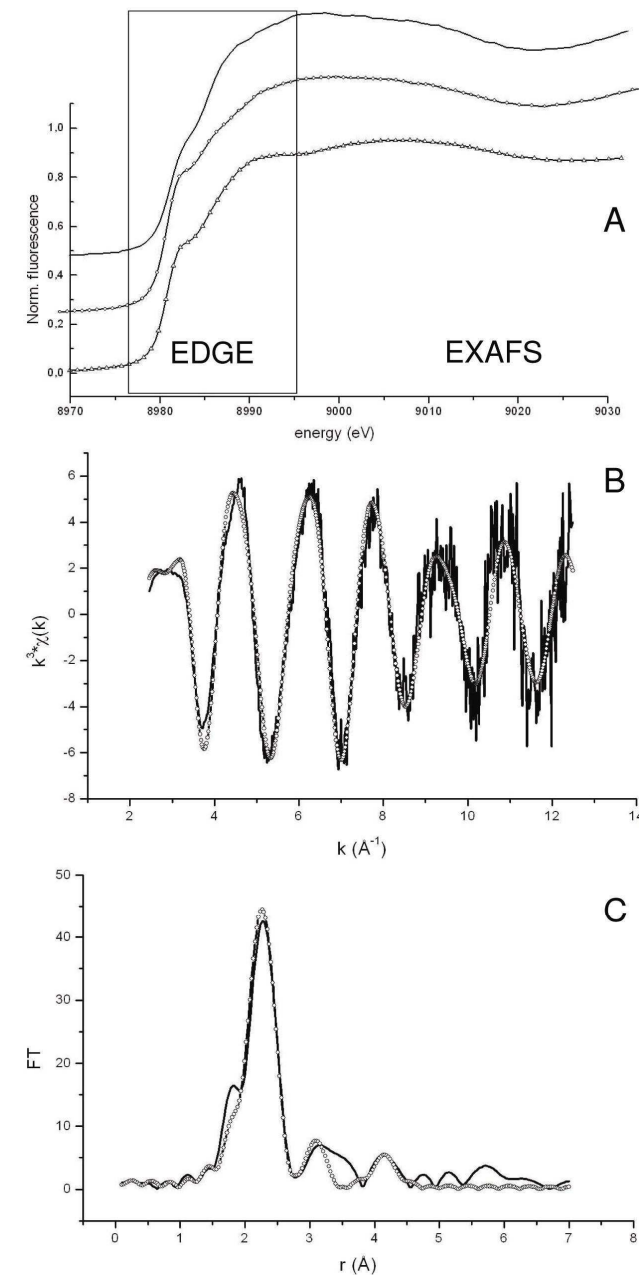


Cu(I)DR1885 $\Delta E = -10.3$ eV

	Ligand	r(Å)	$2\sigma^2 \cdot 10^3(\text{Å}^2)$	R-exafs	$\epsilon(\text{fit index})$
Fit1 (1shell)	2S	2.299	4(1)	0.446	0.49
Fit2 (1shell)	3S	2.301	9(1)	0.403	0.41
Fit3 (2shells)	3S	2.300	8(1)	0.334	0.29
	1N [§]	1.982	4(1)		
Fit4 (2shells)	3S	2.303	8(1)	0.305	0.27
	1N*	1.999	7(2)		

§ no MS

*His, MS



SAXS – Small Angle X-ray Scattering (RTG rozptyl pod malým úhlem)

Terminology

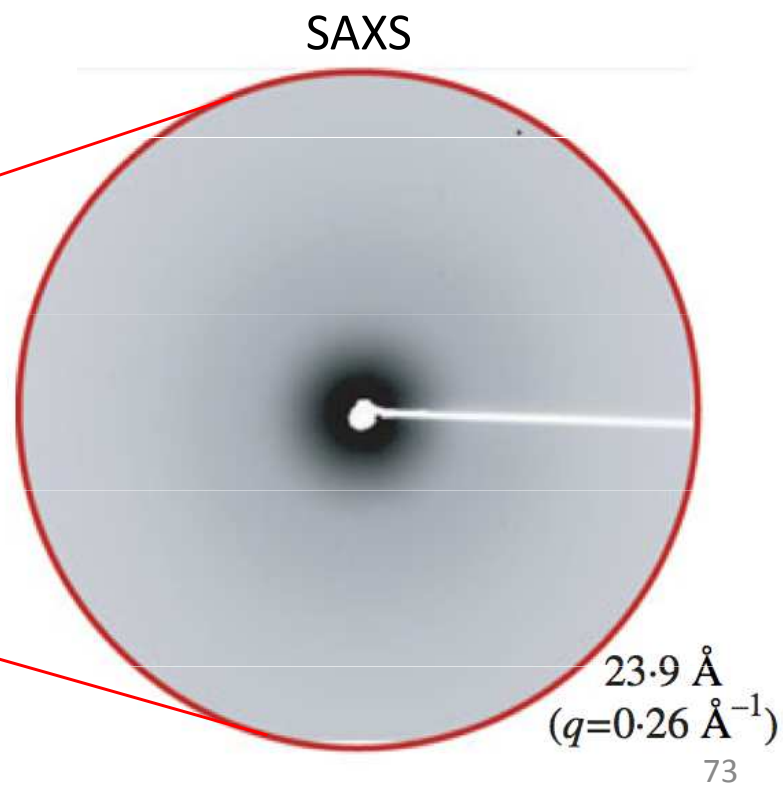
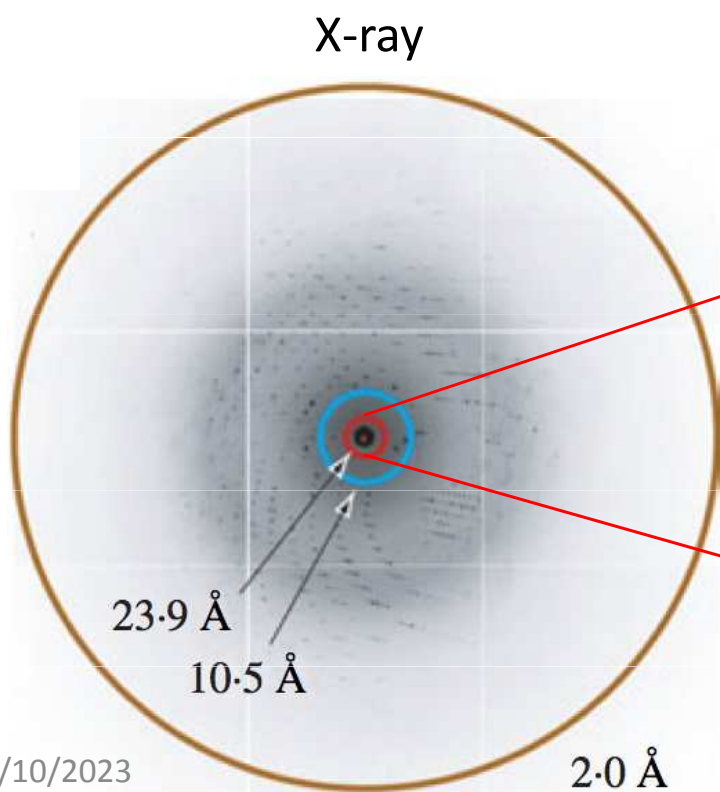
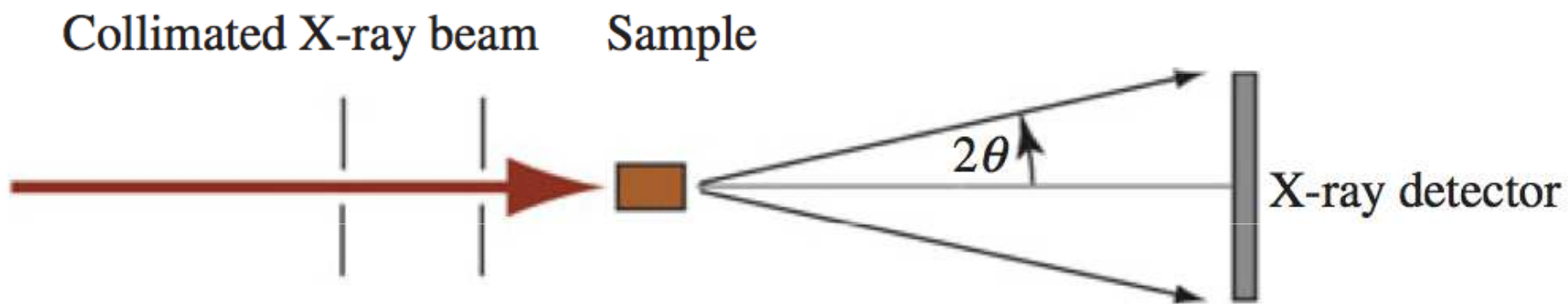
- 1) X-ray – diffraction / scattering
- 2) XS - X-ray scattering
- 3) SAXS/WAXS - Small/Wide Angle X-ray Scattering
- 4) SANS - -----”----- Neutron ---”---

- A) Otto Kratky (1902, Vienna-1995, Graz)
- B) Günter Porod (1919 near Villach, 1984 Graz)
- C) Dmitri I. Svergun

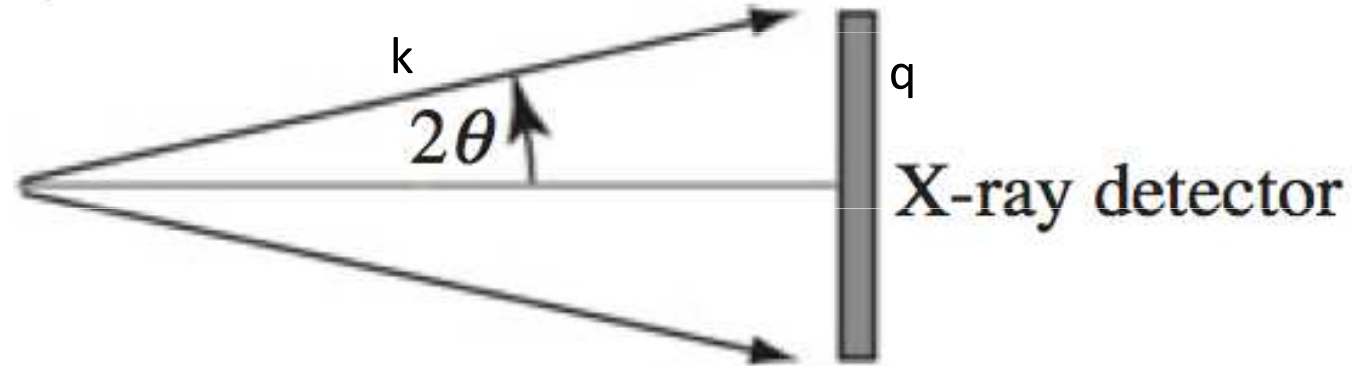
- I) Scattering
- II) Scattering curve
- III) Guinier plot
- IV) PDF (Pair-distribution function)

- a) Bead model
- b) Bead model - / SAXS - envelope

Experimental setup



(very tiny) bit of theory

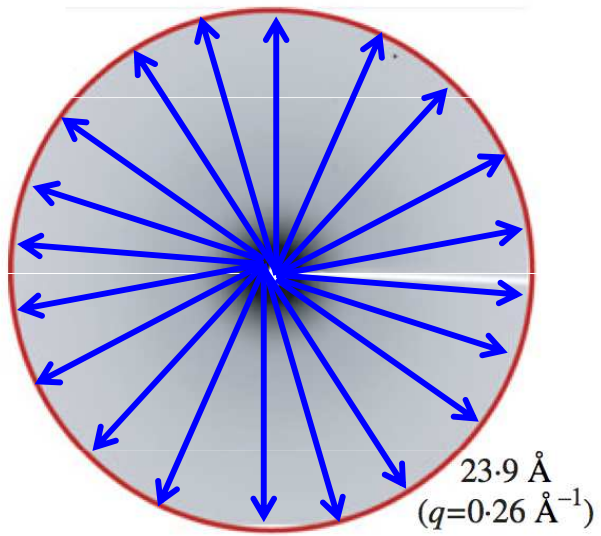


$$q=2k.\sin\theta \quad \Rightarrow \quad q/2/k=\sin\theta,$$

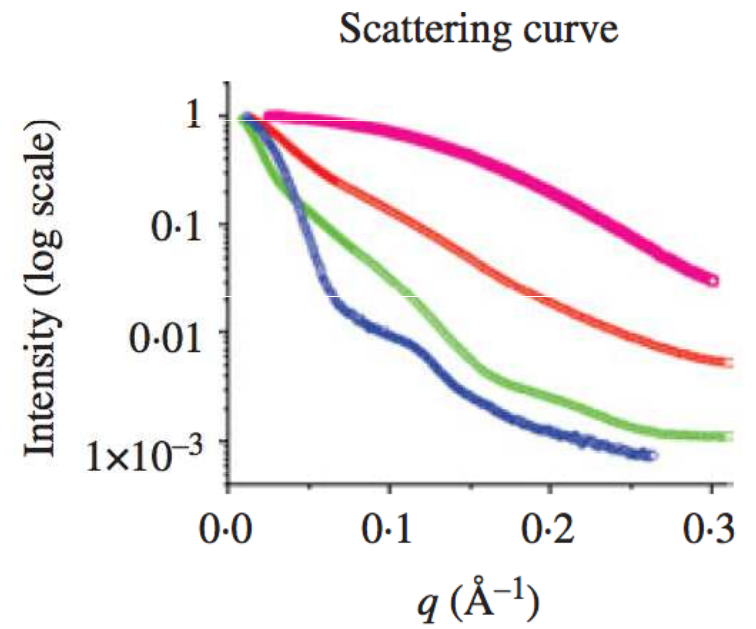
$$k=2\pi/\lambda \quad \Rightarrow \quad \mathbf{q=4\pi/\lambda.\sin\theta}$$

Often q is denoted as s

1st step: scattering to scattering curve

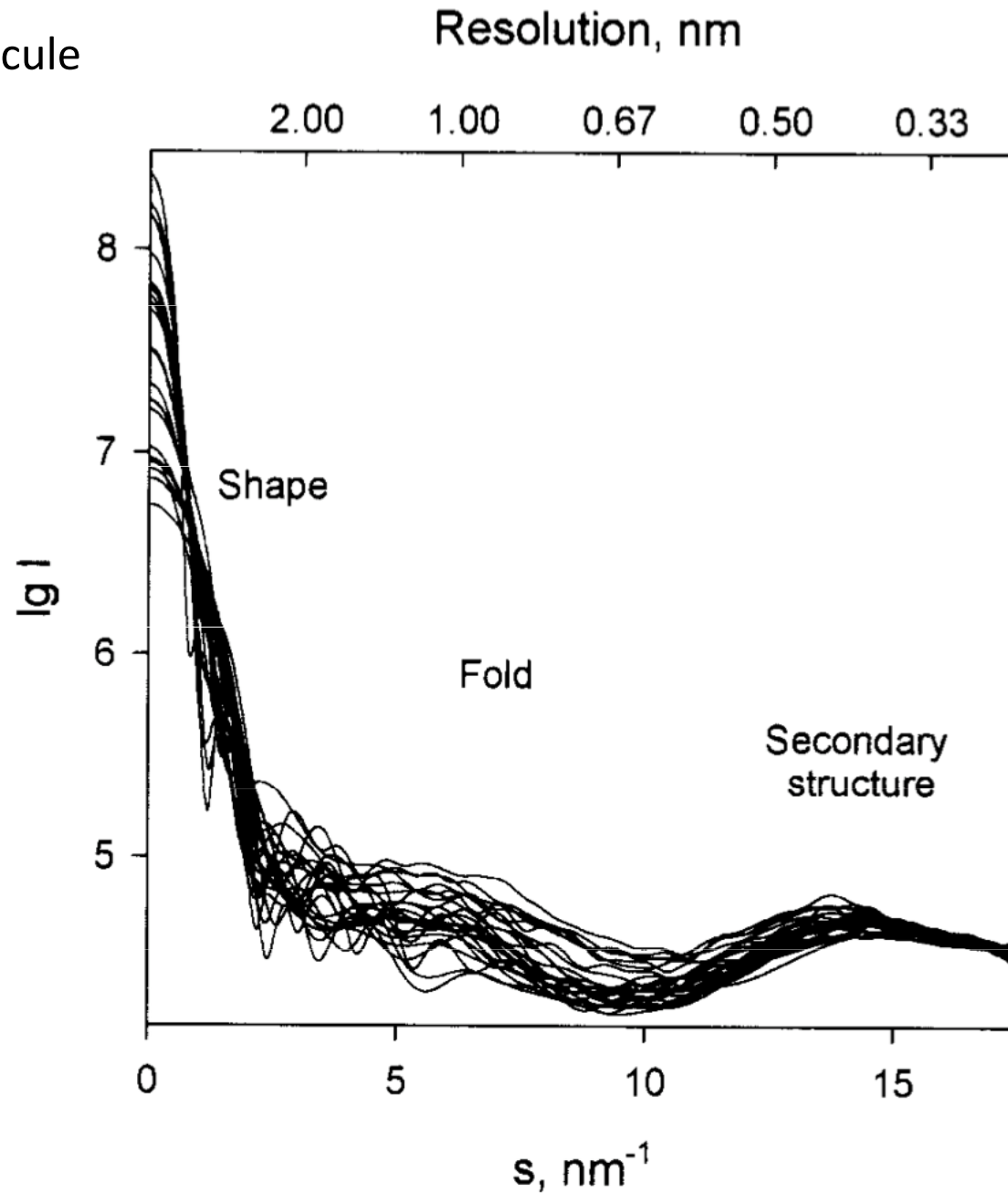


\Rightarrow

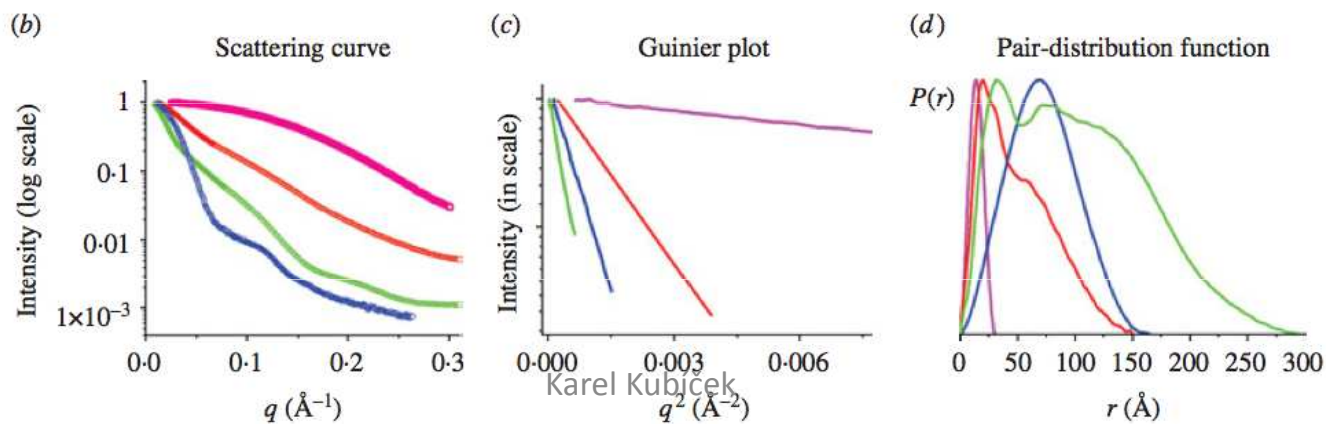
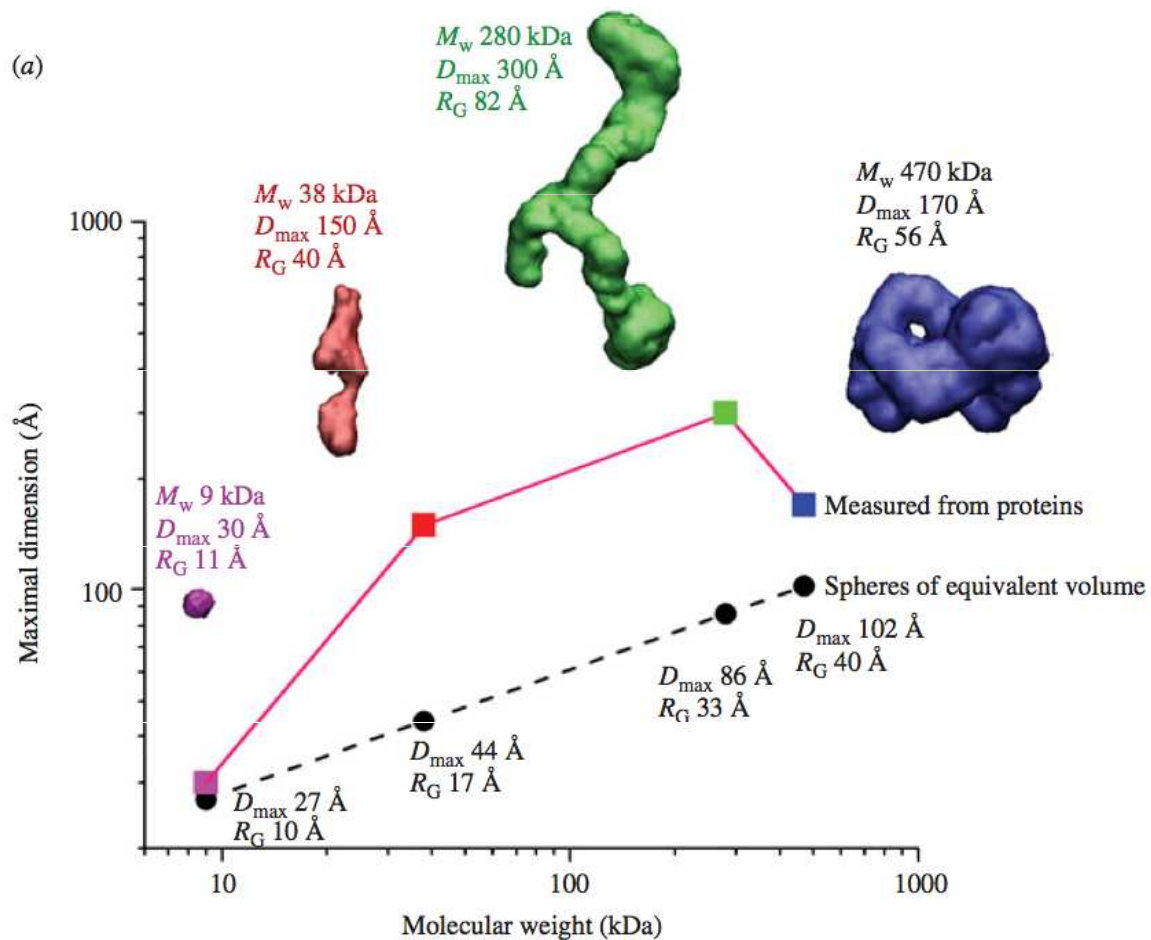


What can we learn from the scattering curve:

- 1) Shape of the studied molecule
- 2) Fold
- 3) Secondary structure

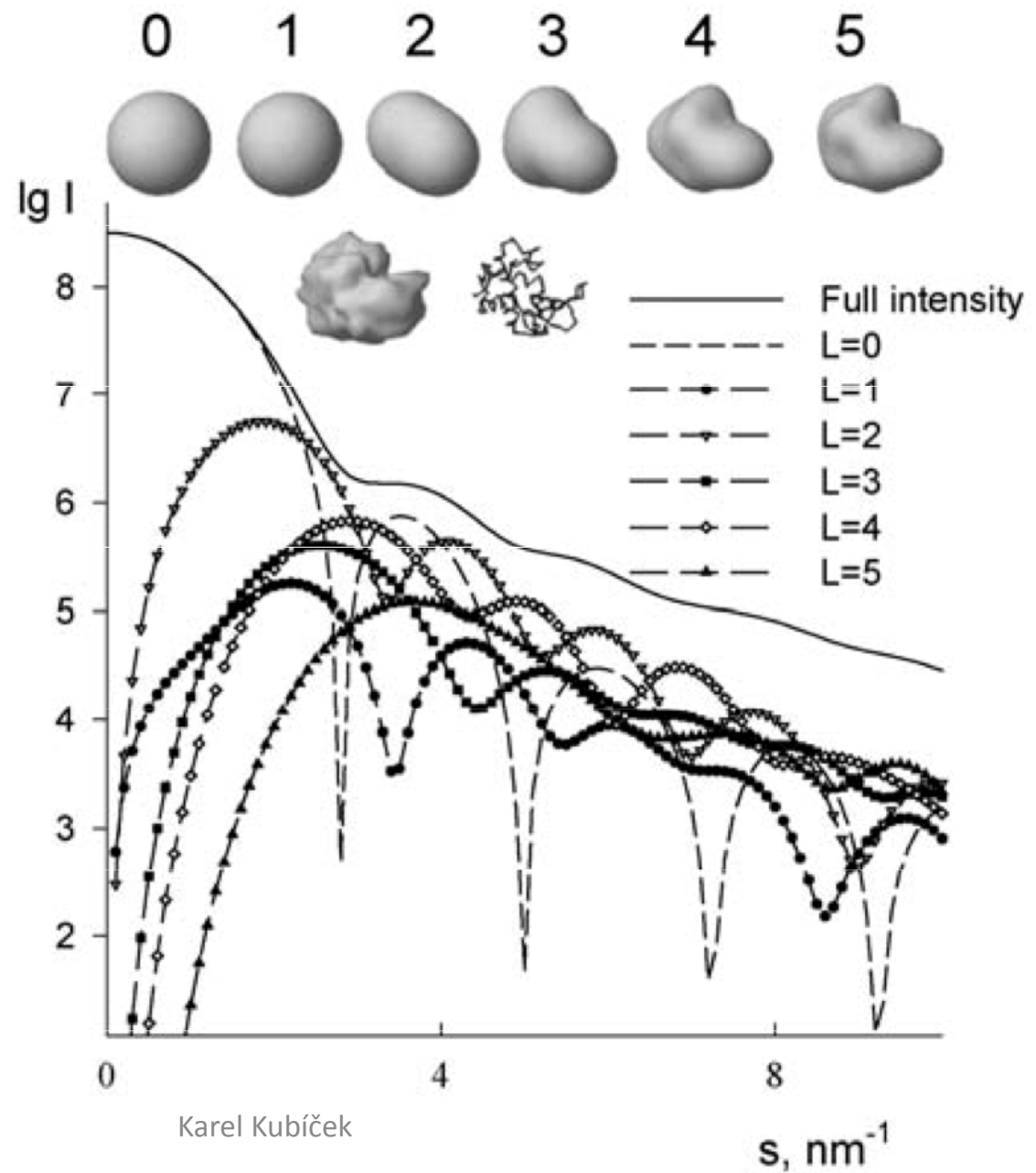


All information from the scattering curve together



Bead model

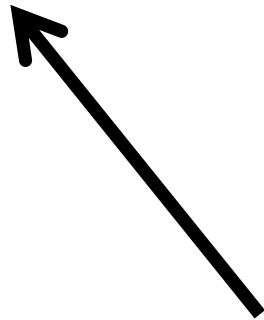
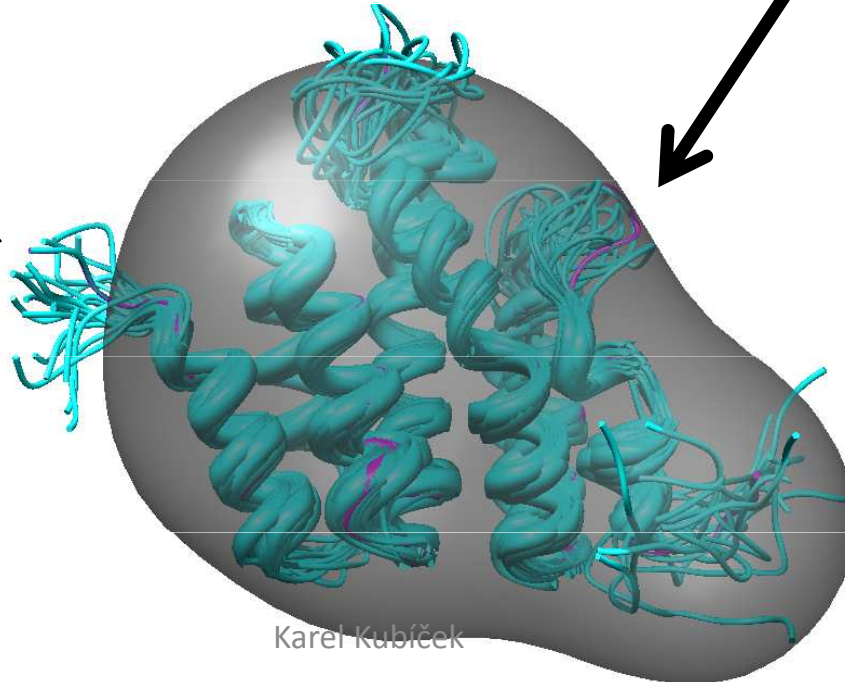
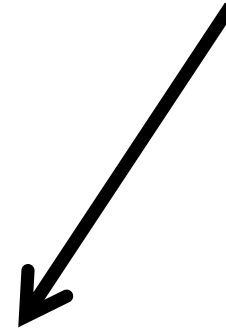
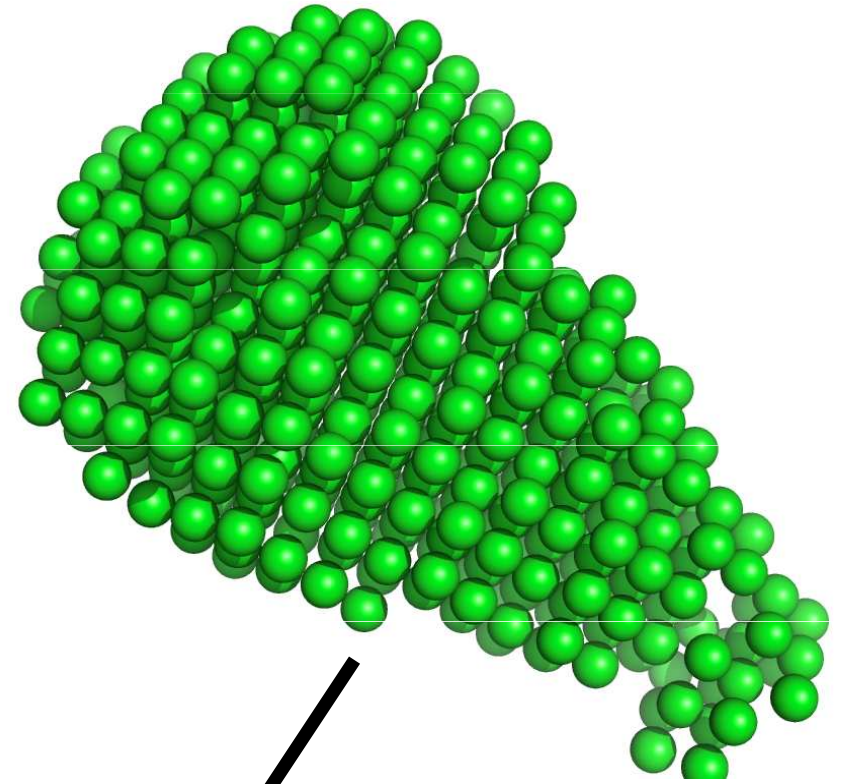
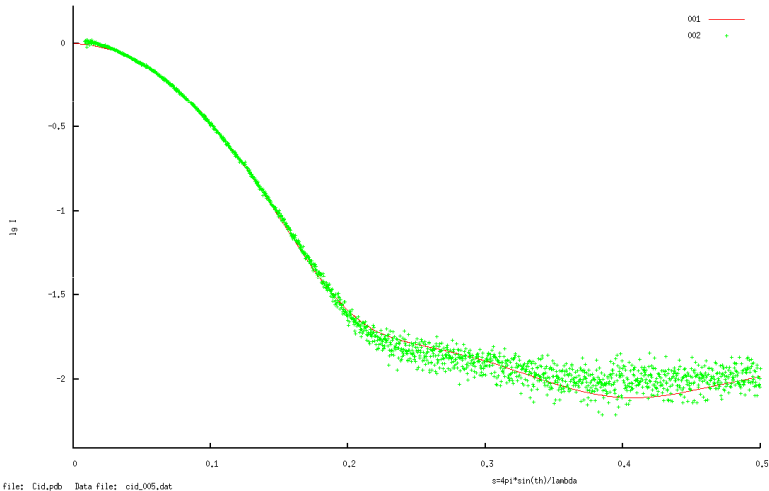
Spherical harmonics problem to be solved

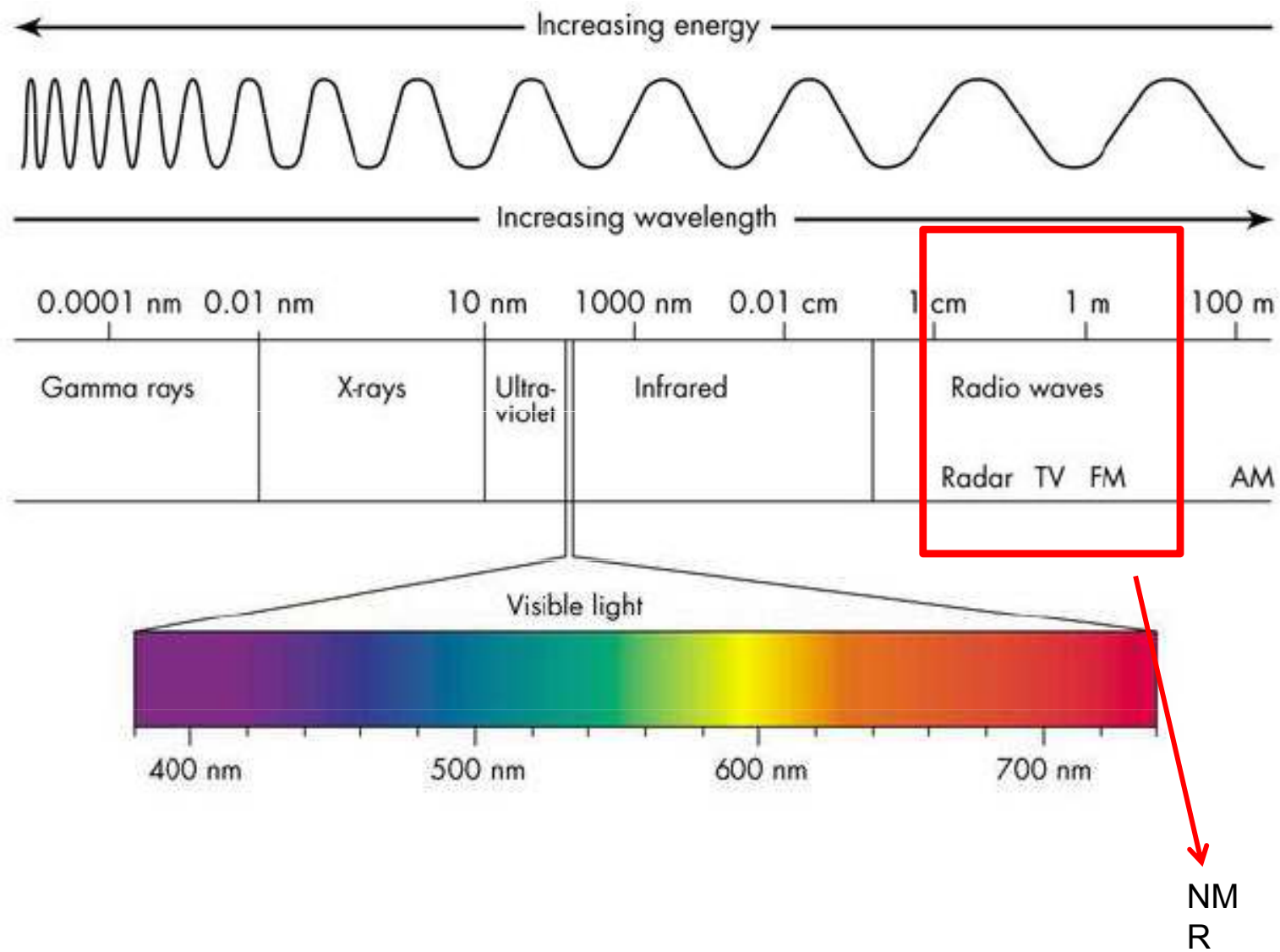


Evaluation of the experimental data

12-Dec-2011 16:16:12

Dro: 0,075 Ra: 1,460 RRE: 18,53 RGT: 18,22 Vol: 486476, Chi: 1,472





NMR

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

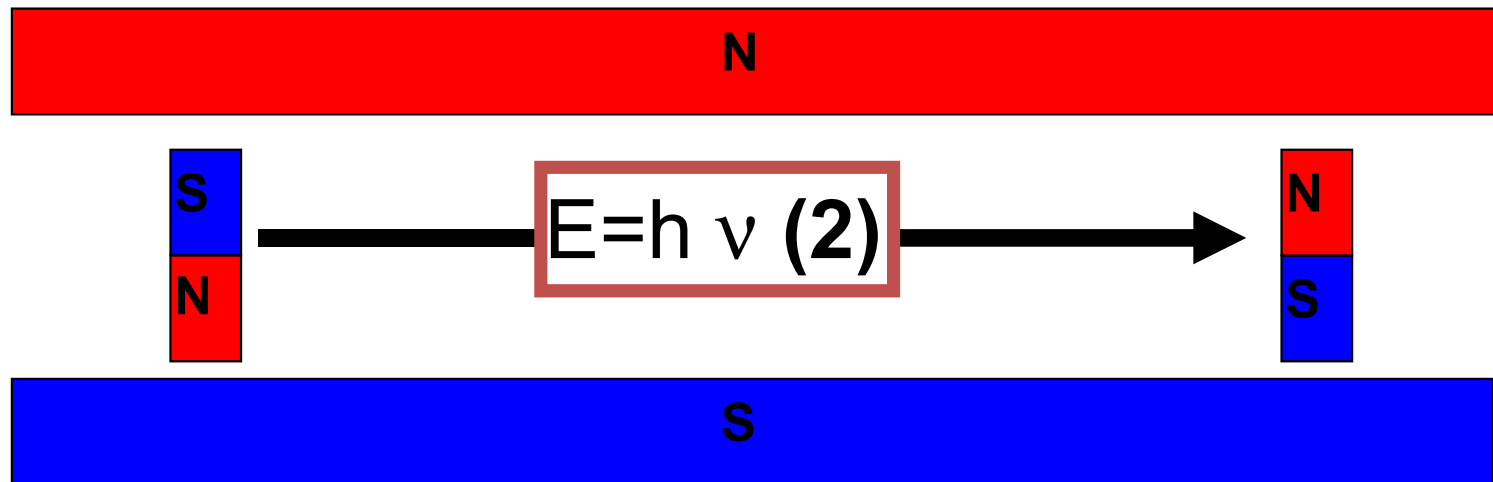
- počet neutronů **a** počet protonů jsou sudá čísla ($^{12}\text{C}=6\text{p}+6\text{n}$) \Rightarrow **nulový spin**

- 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 ν . Frekvence ν závisí na gyromagnetickém poměru γ jader

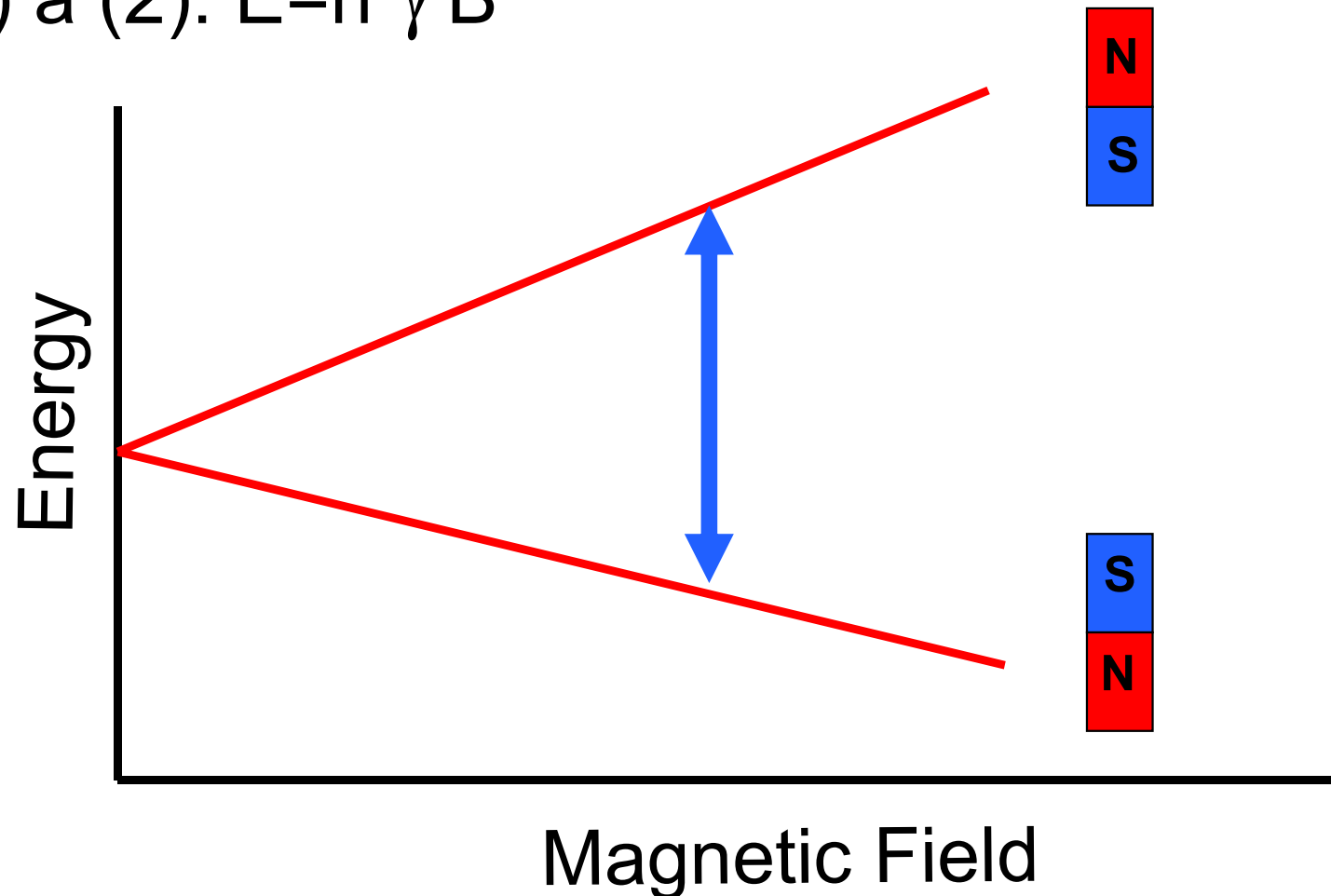
2) Z kvantové mechaniky víme, že spin I může nabývat $2I + 1$ orientací \Rightarrow **jádro se spinem $\frac{1}{2}$ může mít dvě orientace v externím magnetickém poli – nižší / vyšší energie**

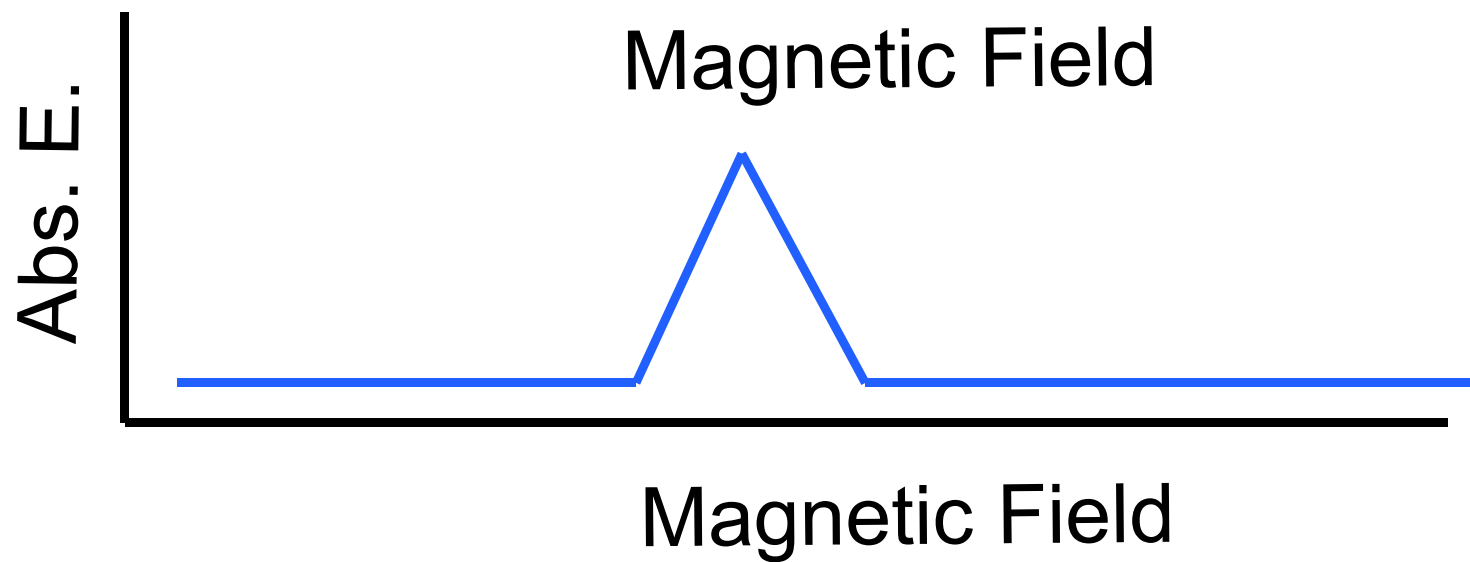
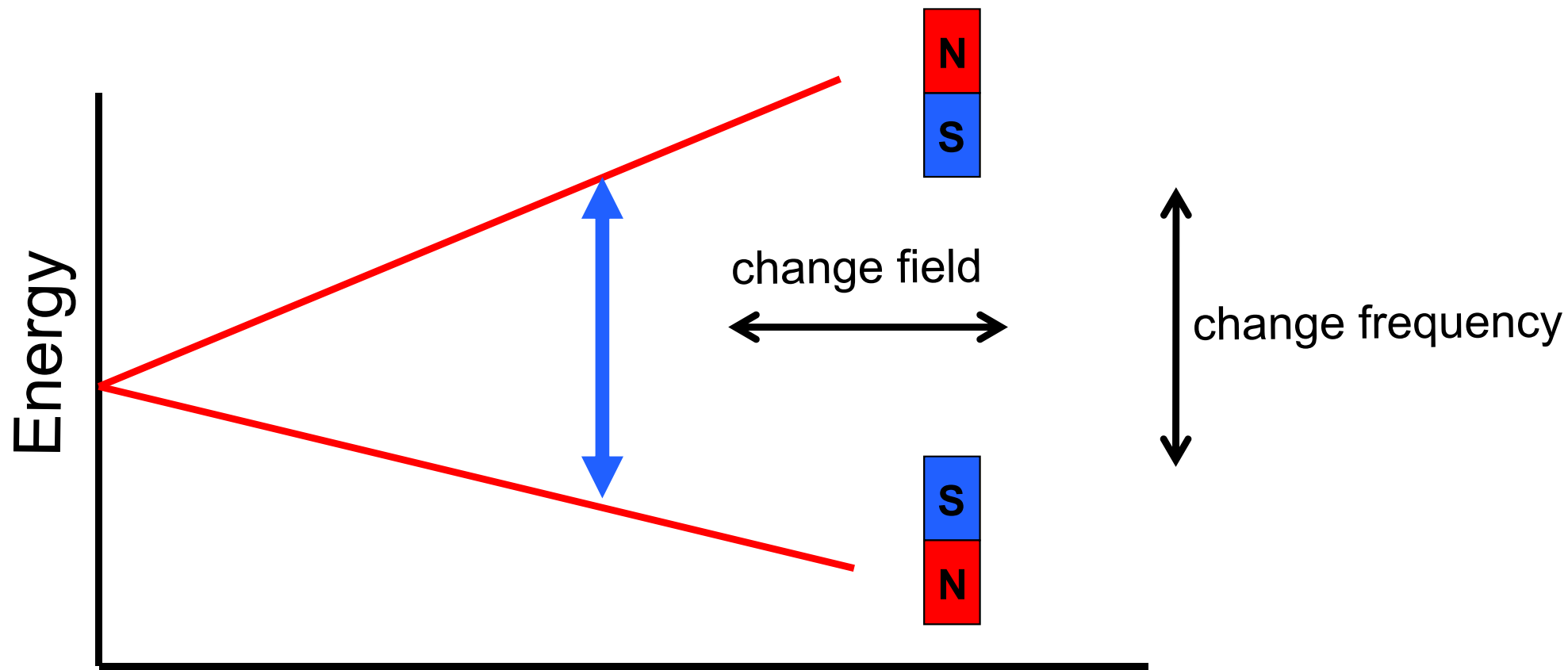


Nuclear Magnetic Resonance

Stručně

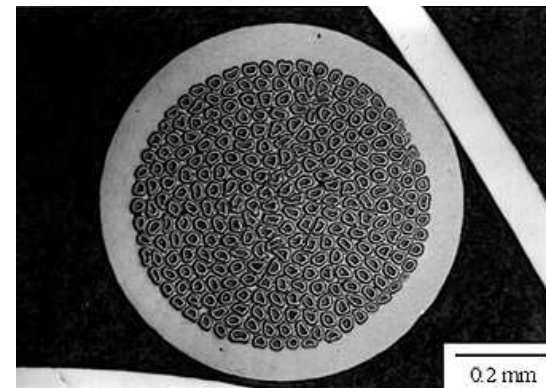
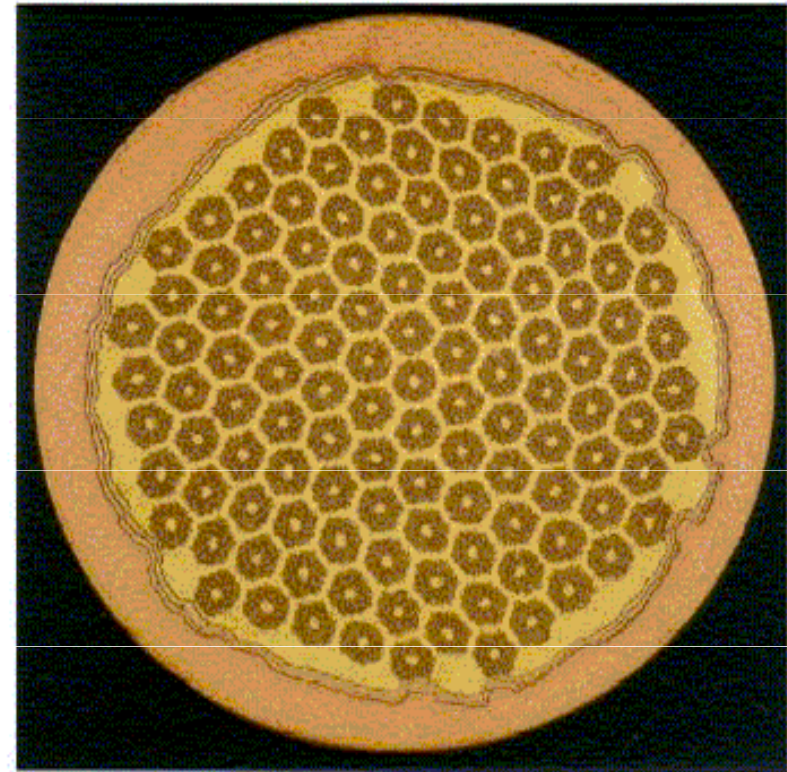
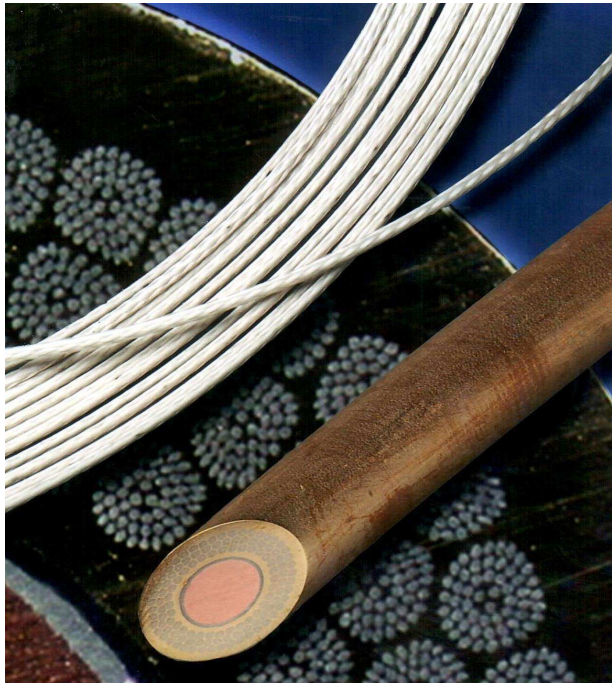
Z (1) a (2): $E = h \gamma B$



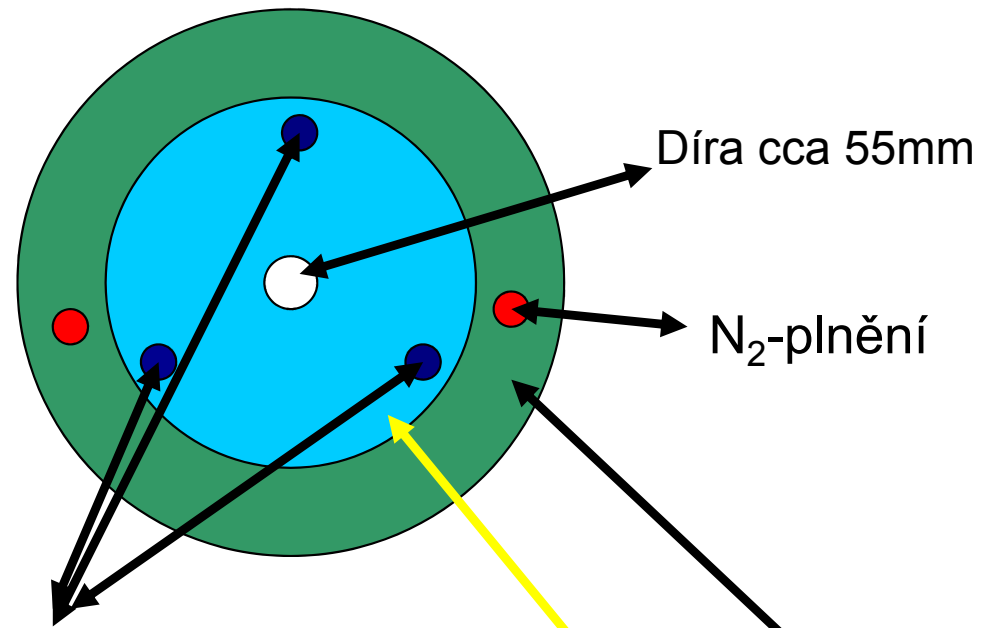


Magnet

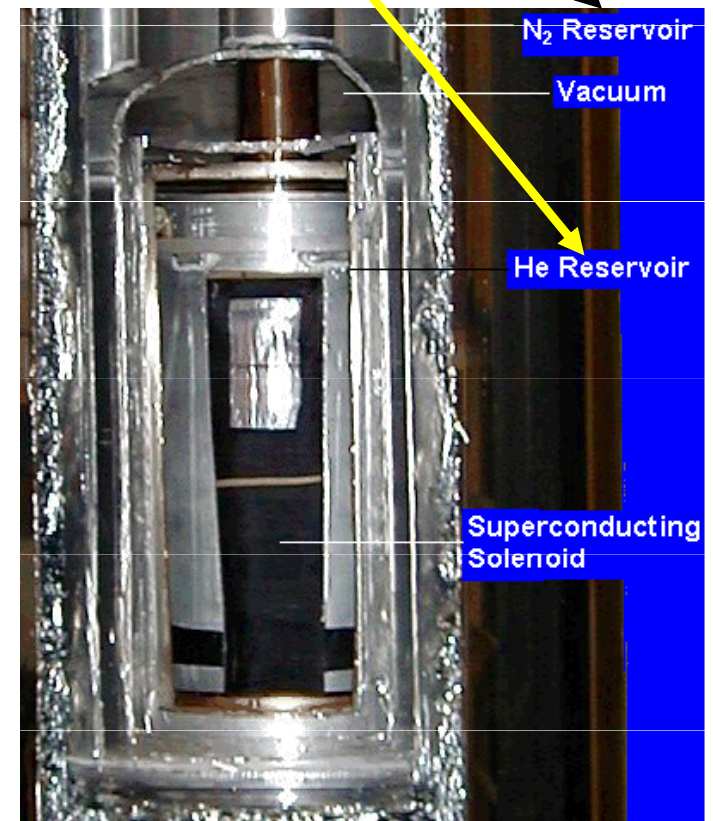
- supravodivé solenoidy na bázi
- Nb a Sn ponořené do heliové a dusíkové lázně
- He-lázeň **~4 K** dále snížena J-T
- pumpou na **~2.1 K**
- v současnosti až 22 Tesla
- magnetické pole země $\sim 50\mu\text{T}$



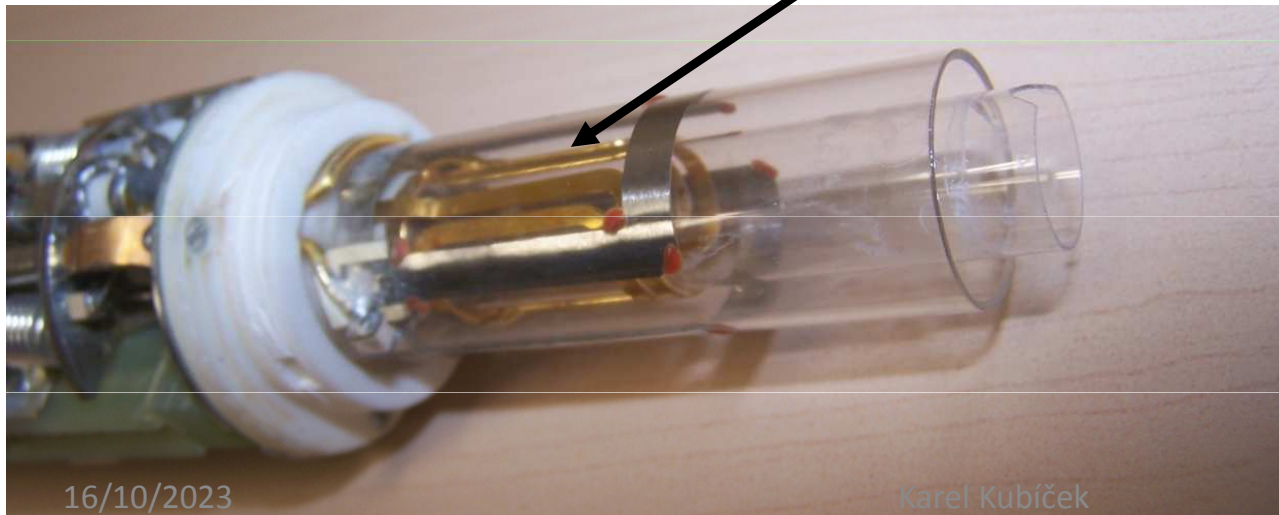
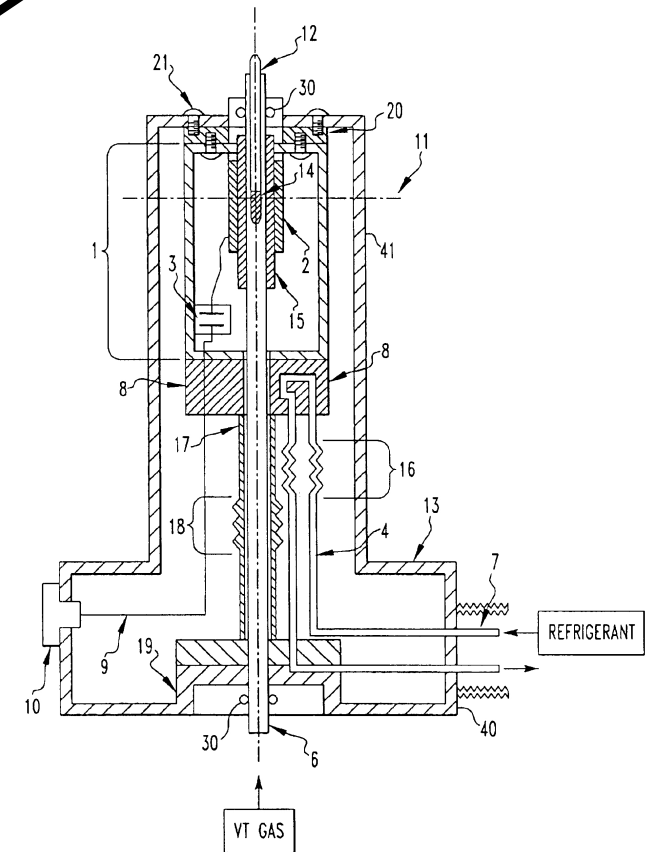
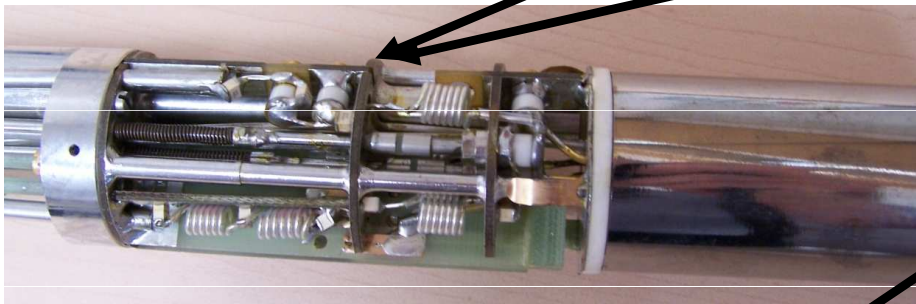
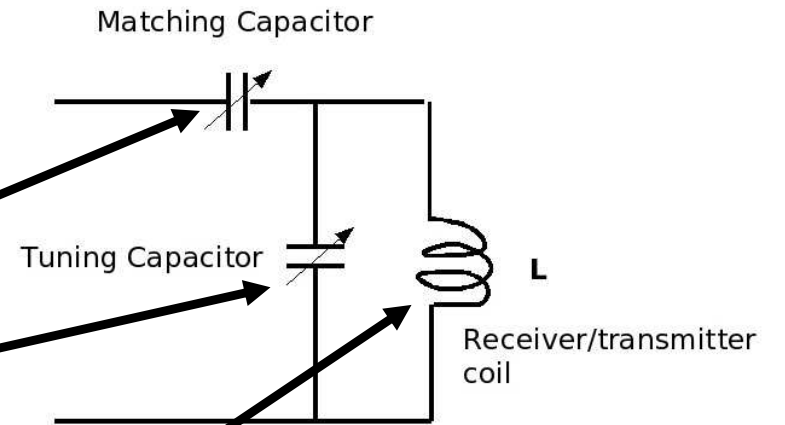
(Nb, Ta)₃Sn supravodič o šířce 0.81 mm s 271 vlákny vnořenými do OFHC měděné matrice



He-plnění

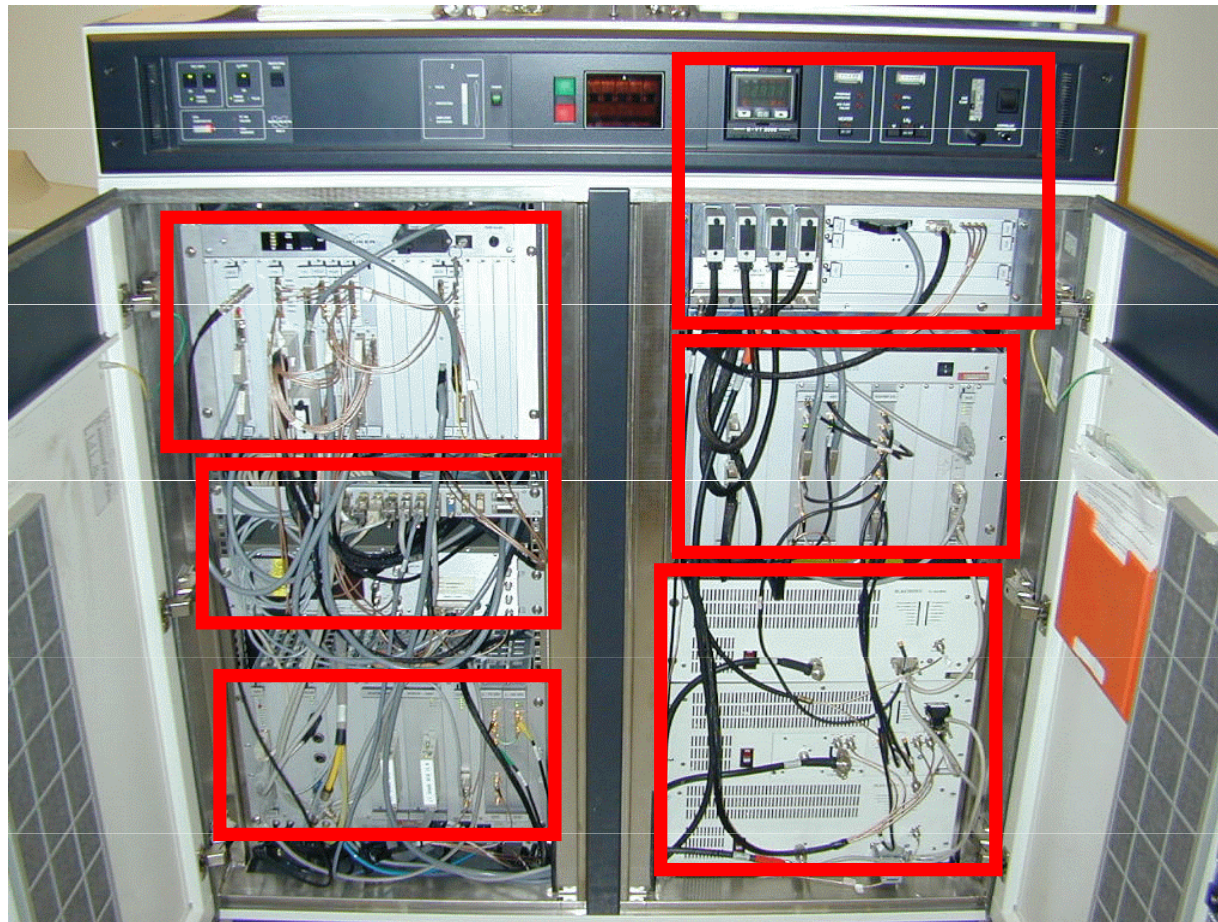


NMR měřicí sonda



Spektrometr

CBU
Control board
unit
FGU
Frequency
gen. u.
Shimms



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

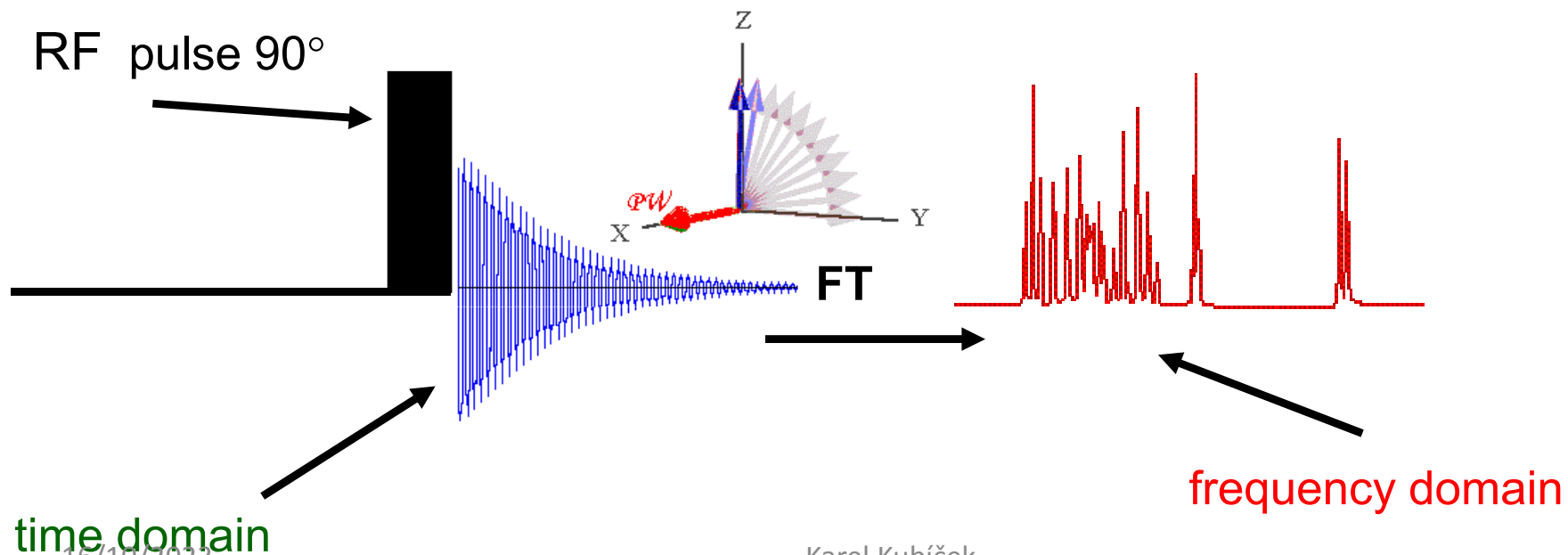
CW vs. Fourier transform NMR

Solution II.

FT-NMR \Rightarrow *all frequencies* in a spectrum are *irradiated simultaneously* with a radio frequency pulse.

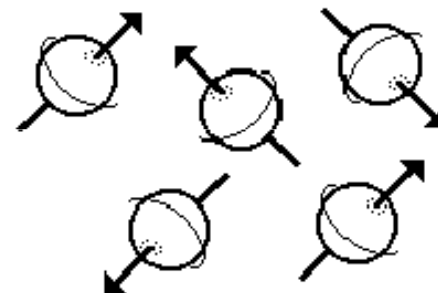
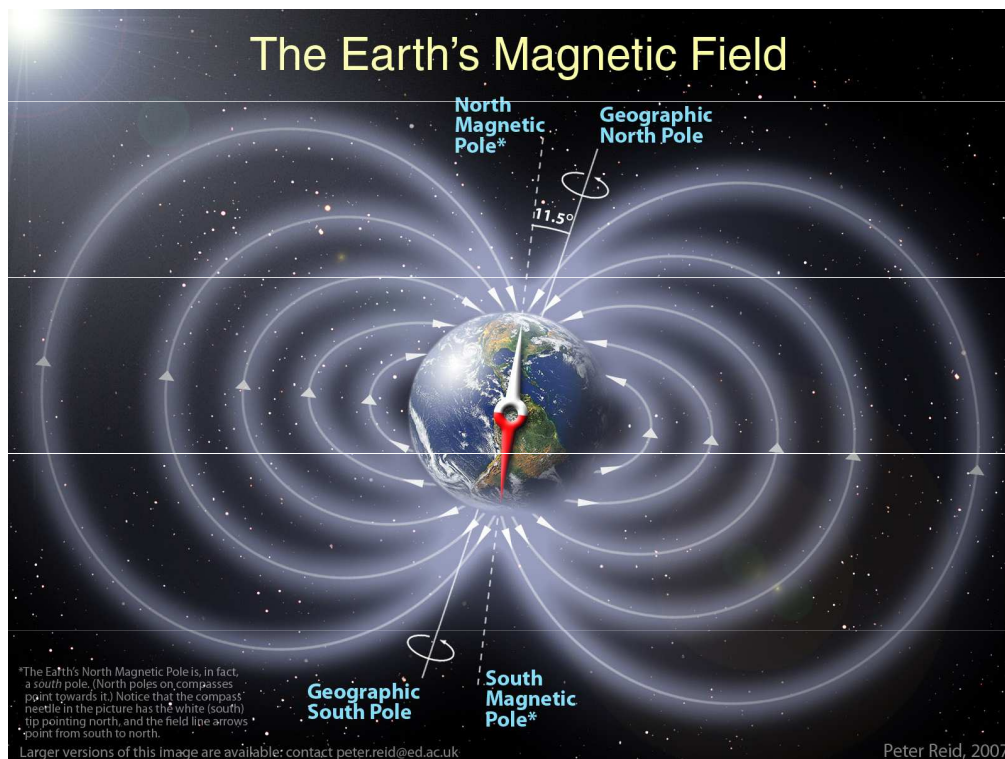
Following the pulse, the nuclei return to thermal equilibrium. A *time domain* emission signal is recorded by the instrument as the nuclei relax.

A *frequency domain* spectrum is *obtained by Fourier transformation*.



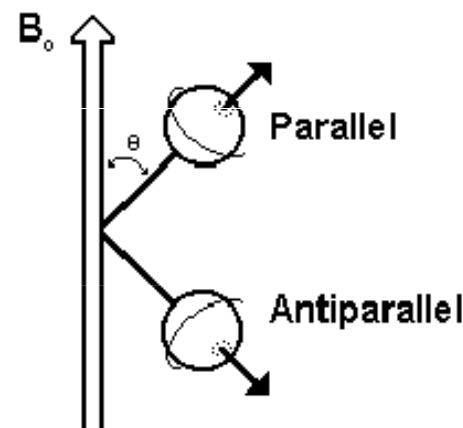
For NMR, nuclear spin is needed!!!

Spin analogy to a compass needle



magnetic field =
0

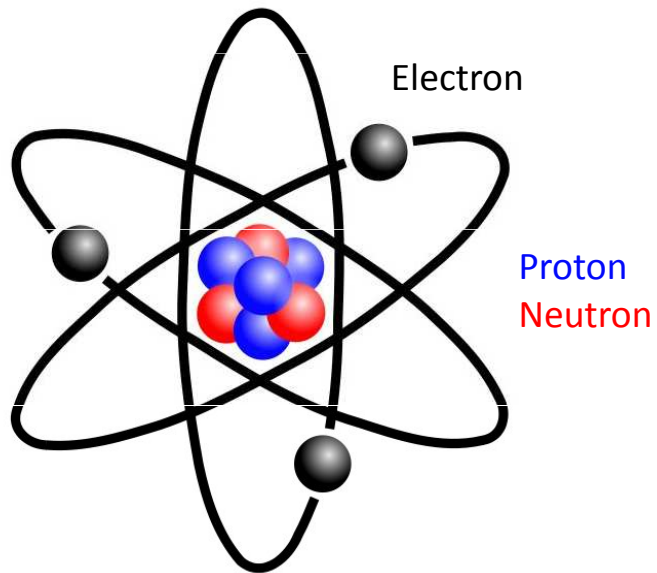
Randomly oriented
nuclear magnetic moments



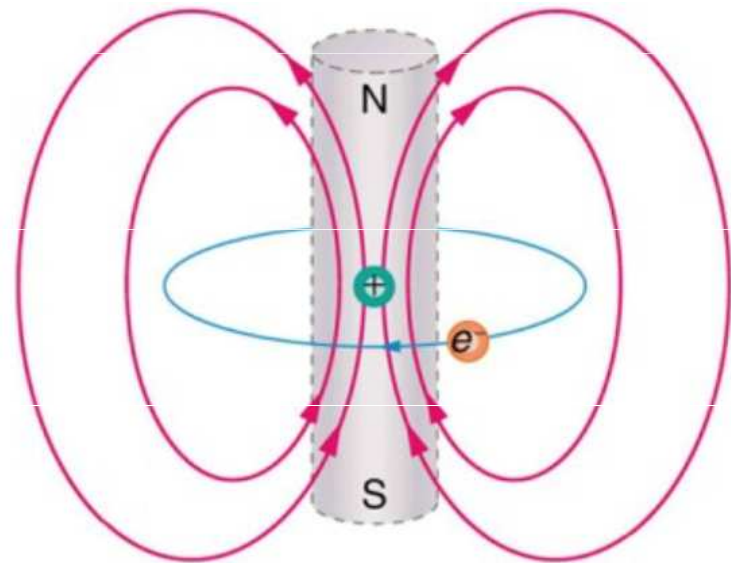
magnetic field >
0

Nuclear magnetic moments
in the presence of an external field

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.

Size

Relaxation



slow (i.e. long t_2 time)



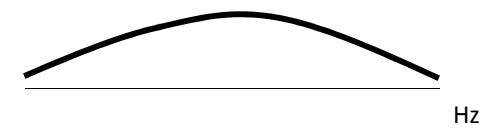
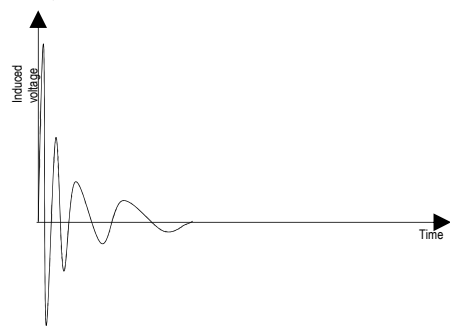
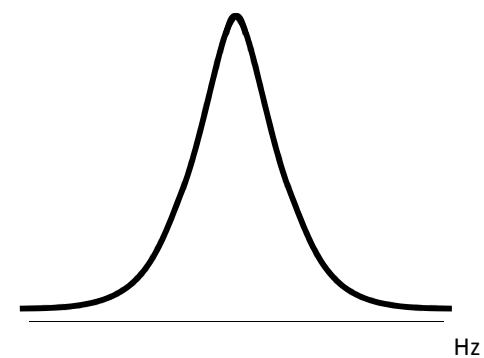
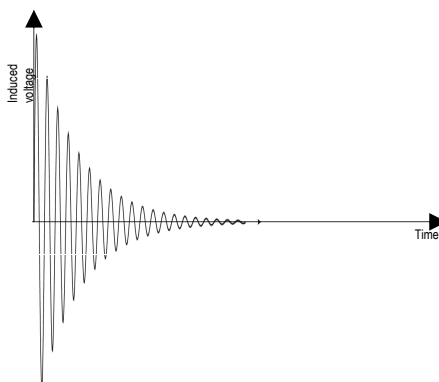
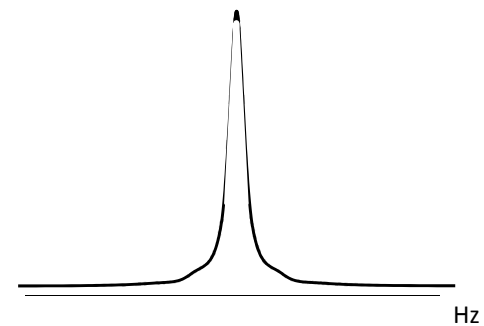
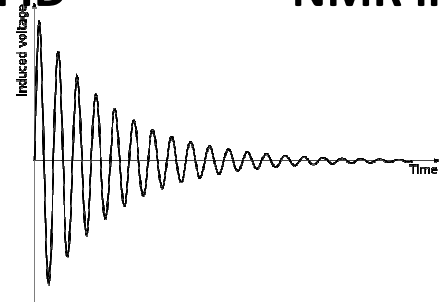
medium



fast

FID

NMR line(width) after FT

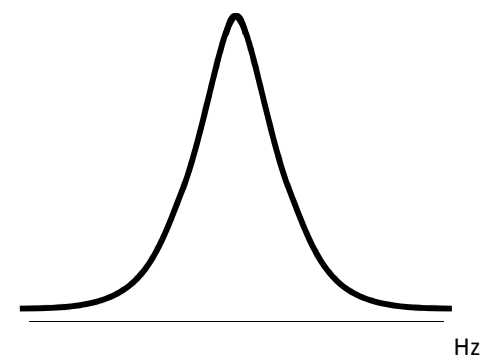
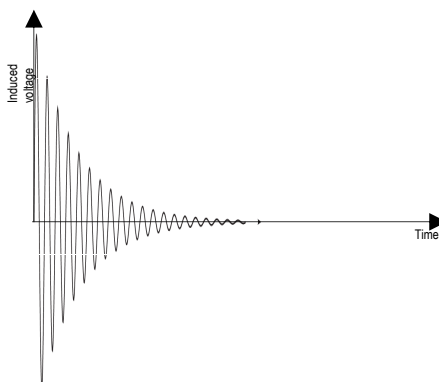
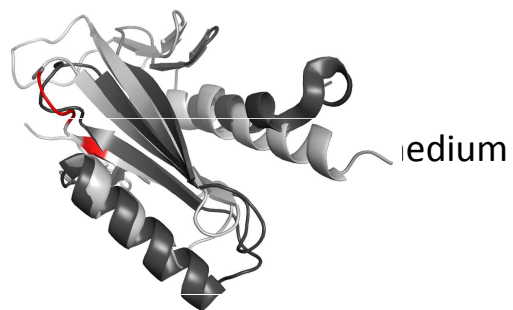
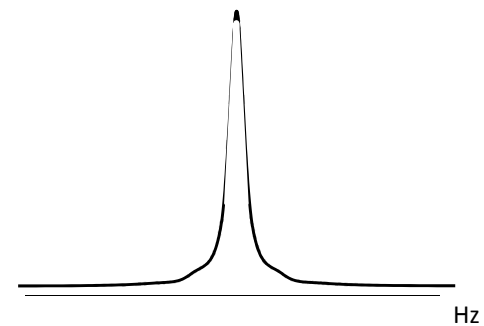
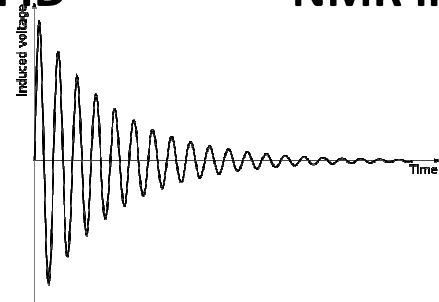
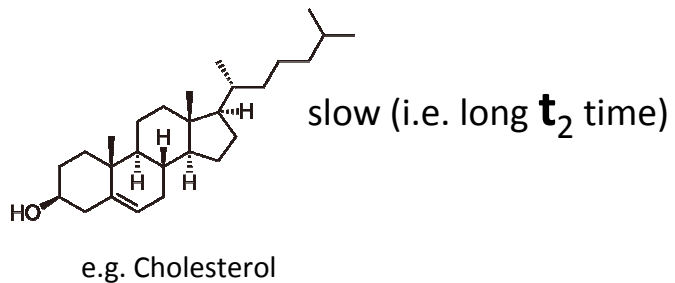


Size

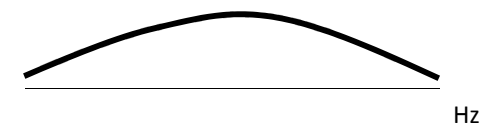
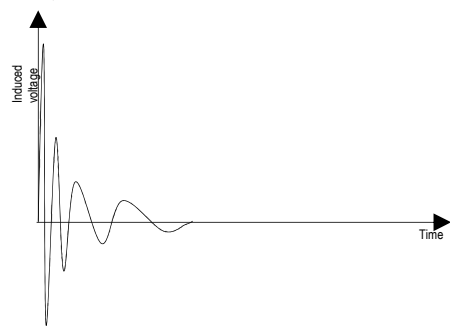
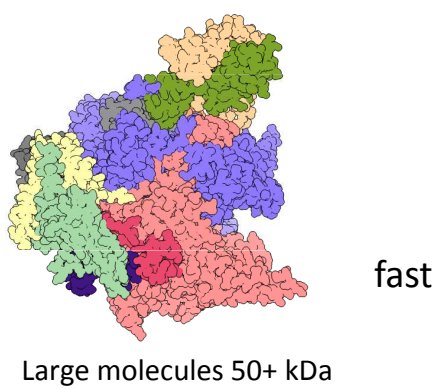
Relaxation

FID

NMR line(width) after FT



Biomolecules 5-30 kDa



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**

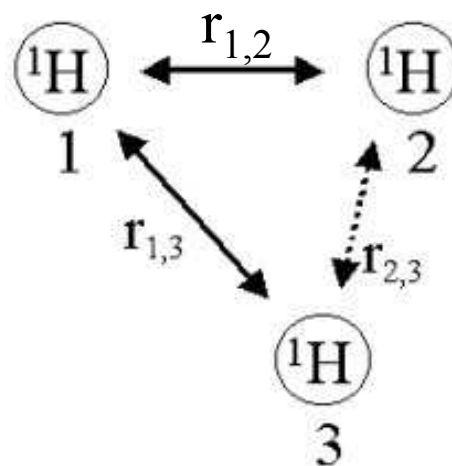
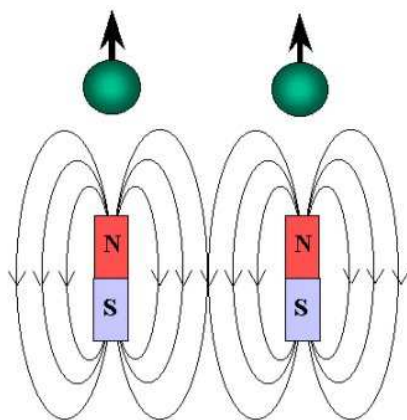
For most of the modern applications, enrichment by ^{13}C , ^{15}N and often ^2H needed!

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

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

- 0) AA/NA sequence, resonance assignment, standard chemical shifts
- 1) Structure determination of proteins/NAs
- 2) NMR can provide detailed information about the structure at the atomic level resolution relying on the spatial proximity of two interacting protons – nuclear Overhauser enhancement (NOE)
- 3) Additional structural information can be obtained (residual dipolar couplings – RDCs, J -couplings, backbone chemical shifts - CSI)

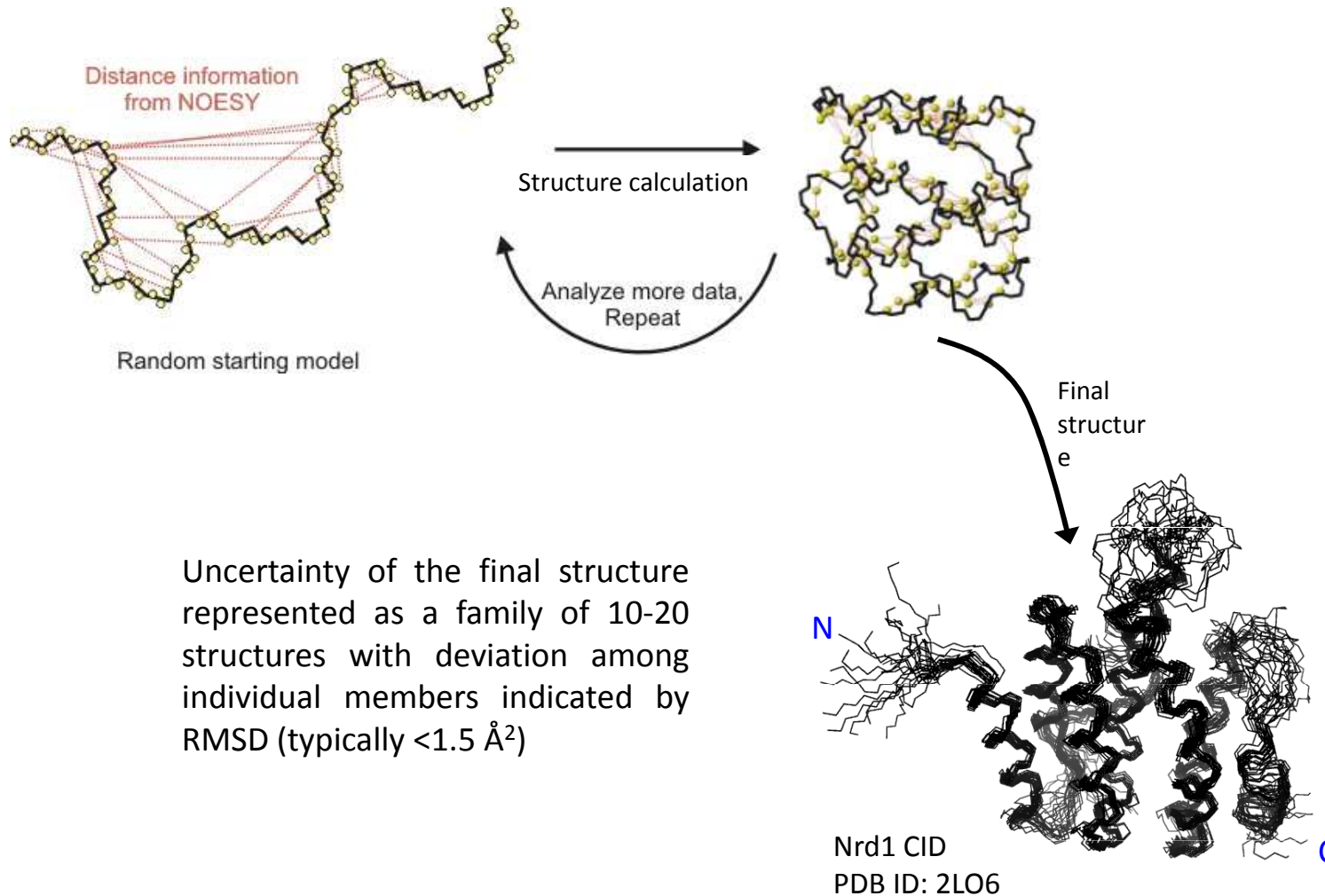
NOE:



$$r_{1,2}; r_{1,3}; r_{2,3} \leq 6 \text{ \AA}$$

$$1 \text{ \AA} = 1 \cdot 10^{-10} \text{ m}$$

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

Studying interactions by NMR titration

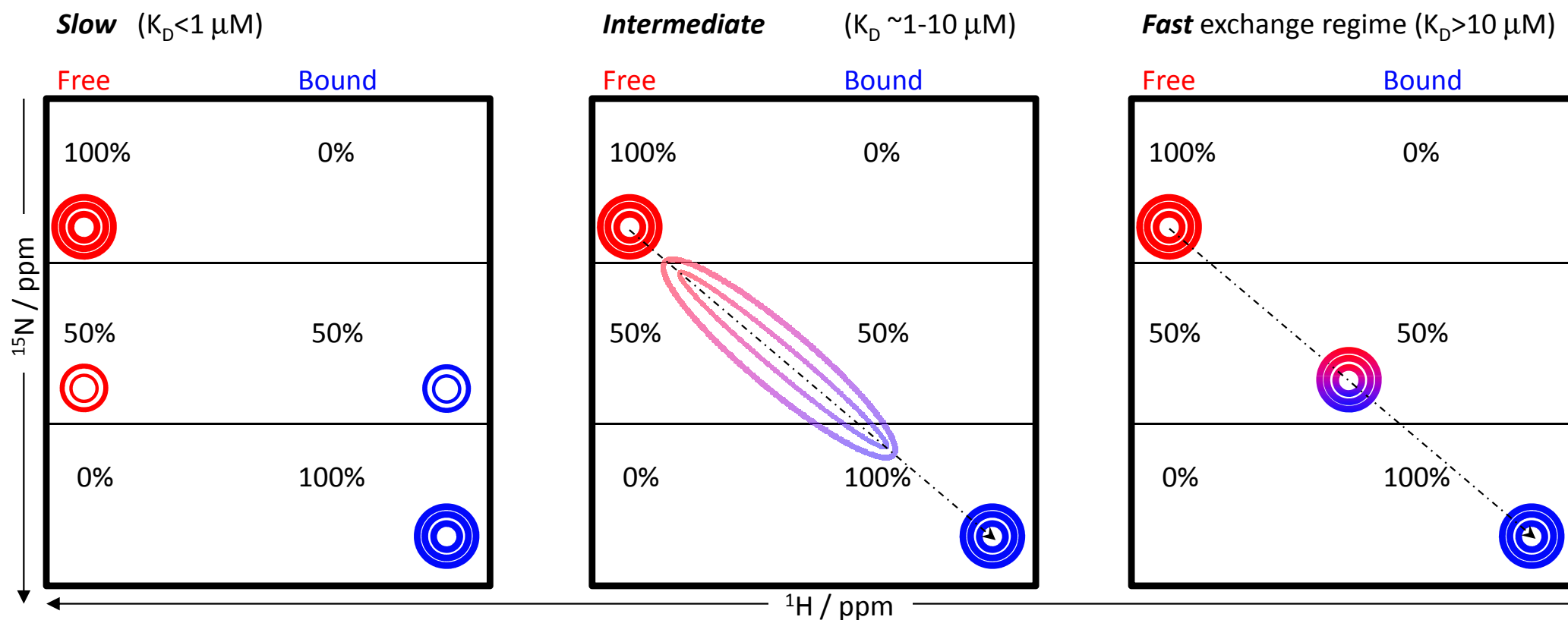
1) **Slow** exch. regime (on the NMR timescale)

– individual peaks for each of the studied states (e.g. free / complexed forms of a protein), peak intensity representing population of a given state

2) **Intermediate** exchange regime

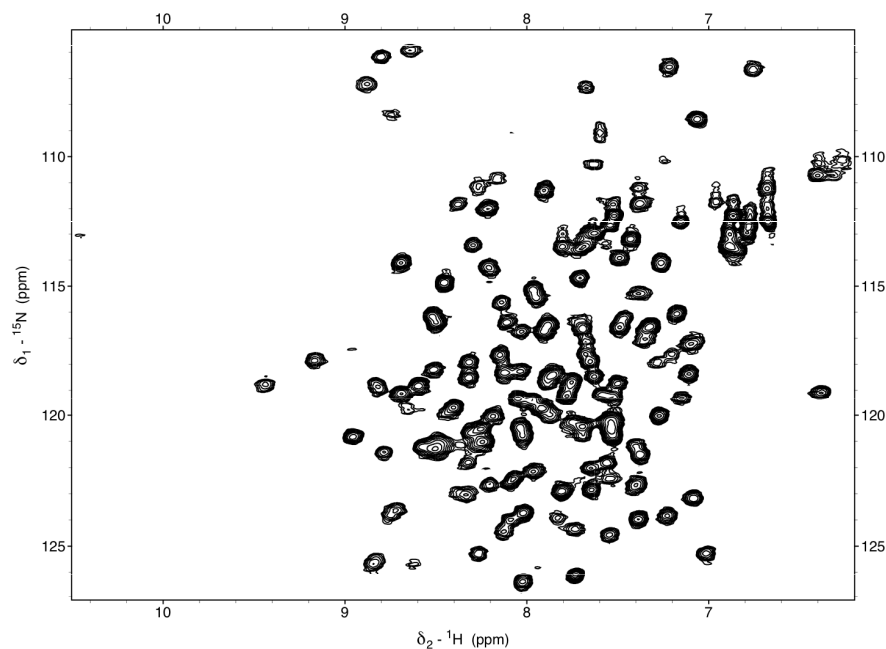
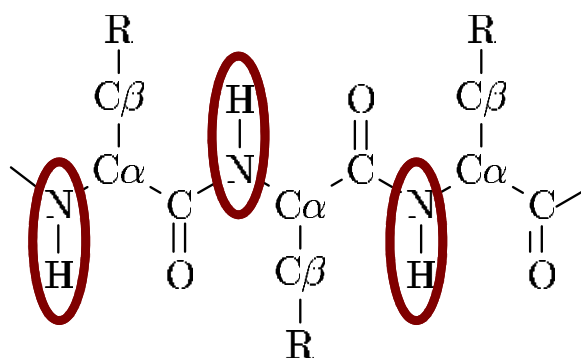
3) **Fast** exchange regime

– single peak whose chemical shift position is given by the molar ratio of the states present in solution

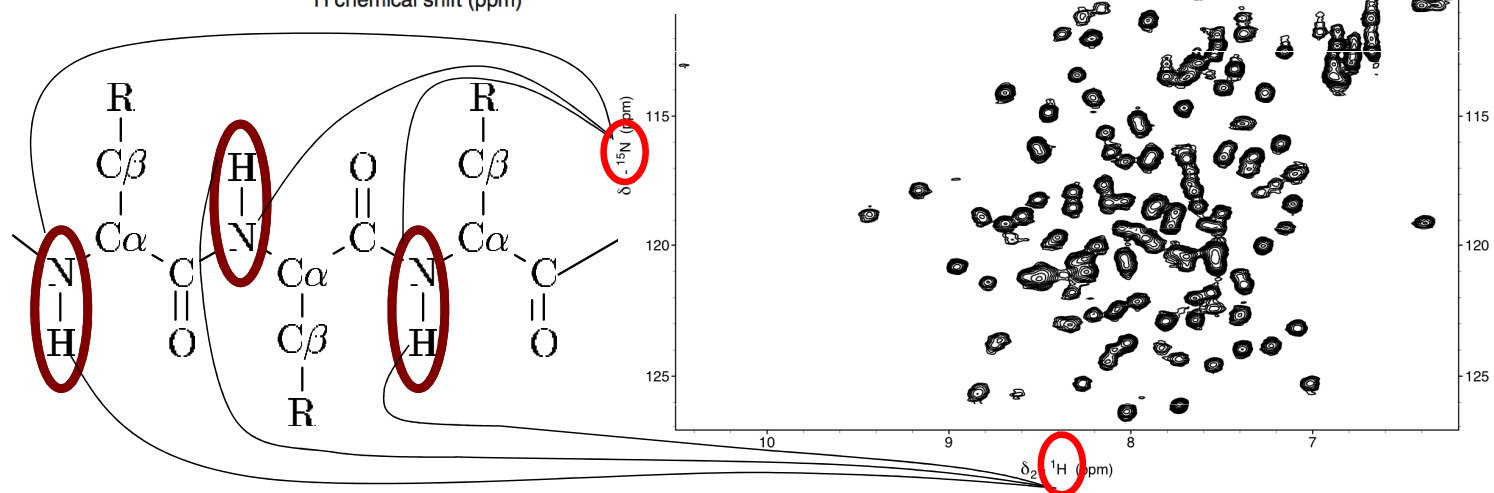
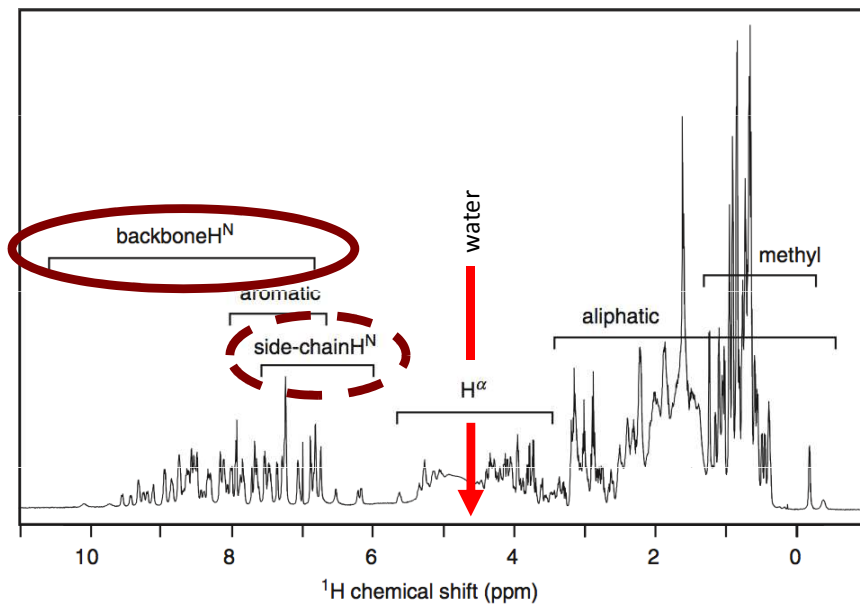


¹⁵N-¹H HSQC – Heteronuclear Single Quantum Coherence

- 1) 1 peak \cong 1 amino acid
- 2) good estimate of the protein folding status
- 3) no information about sequential assignment (which peak is which amino acid)
- 4) for sequential assignment third dimension needed (¹³C)
- 5) once assignment of the peaks known – HSQC is optimal tool for monitoring interactions by NMR through titrations (i.e. stepwise addition of small amounts of ligand to the nearly constant volume solution with the isotopically enriched molecule)



^1H - ^{15}N HSQC, cca 155 aa, well folded, 600MHz, 293K

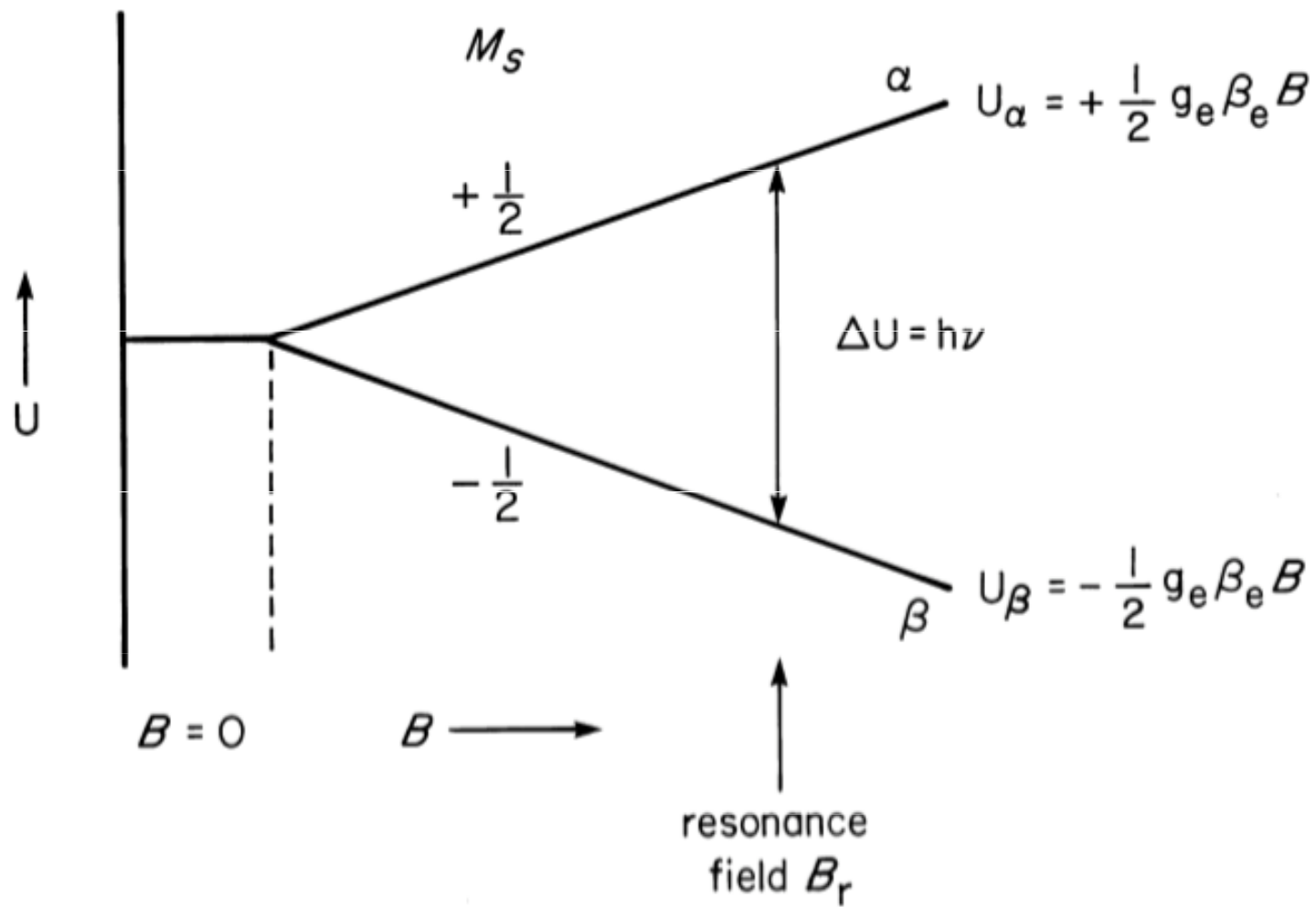


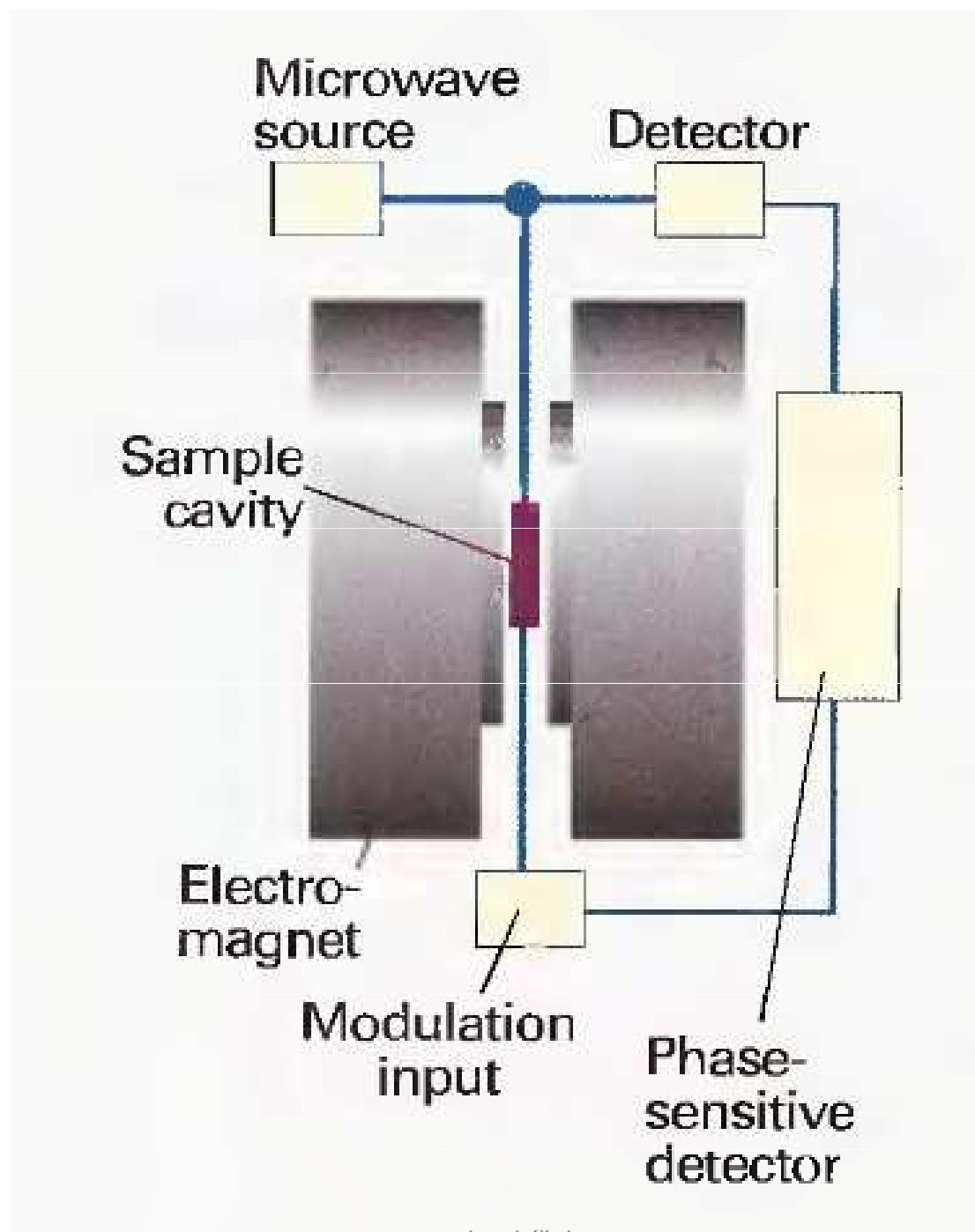
^1H - ^{15}N HSQC, cca 155 aa, well folded, 600MHz, 293K

EPR - Electron Paramagnetic Resonance

When the molecules exhibit **paramagnetism** as a result of **unpaired electron** spins, transitions can be induced between spin states by applying a **magnetic field** and then supplying **electromagnetic energy**, usually in the **microwave range** of frequencies. The **resulting absorption spectra** are described as electron spin resonance (**ESR**) or electron paramagnetic resonance (**EPR**).

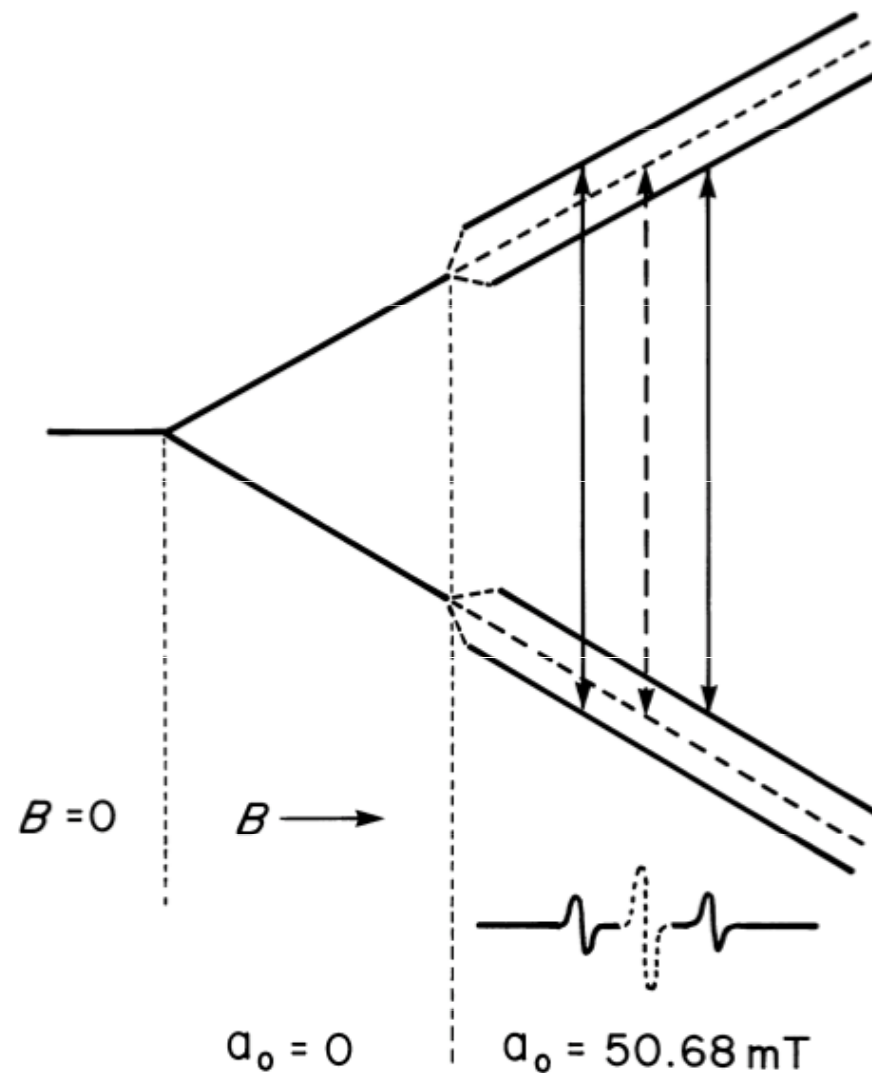
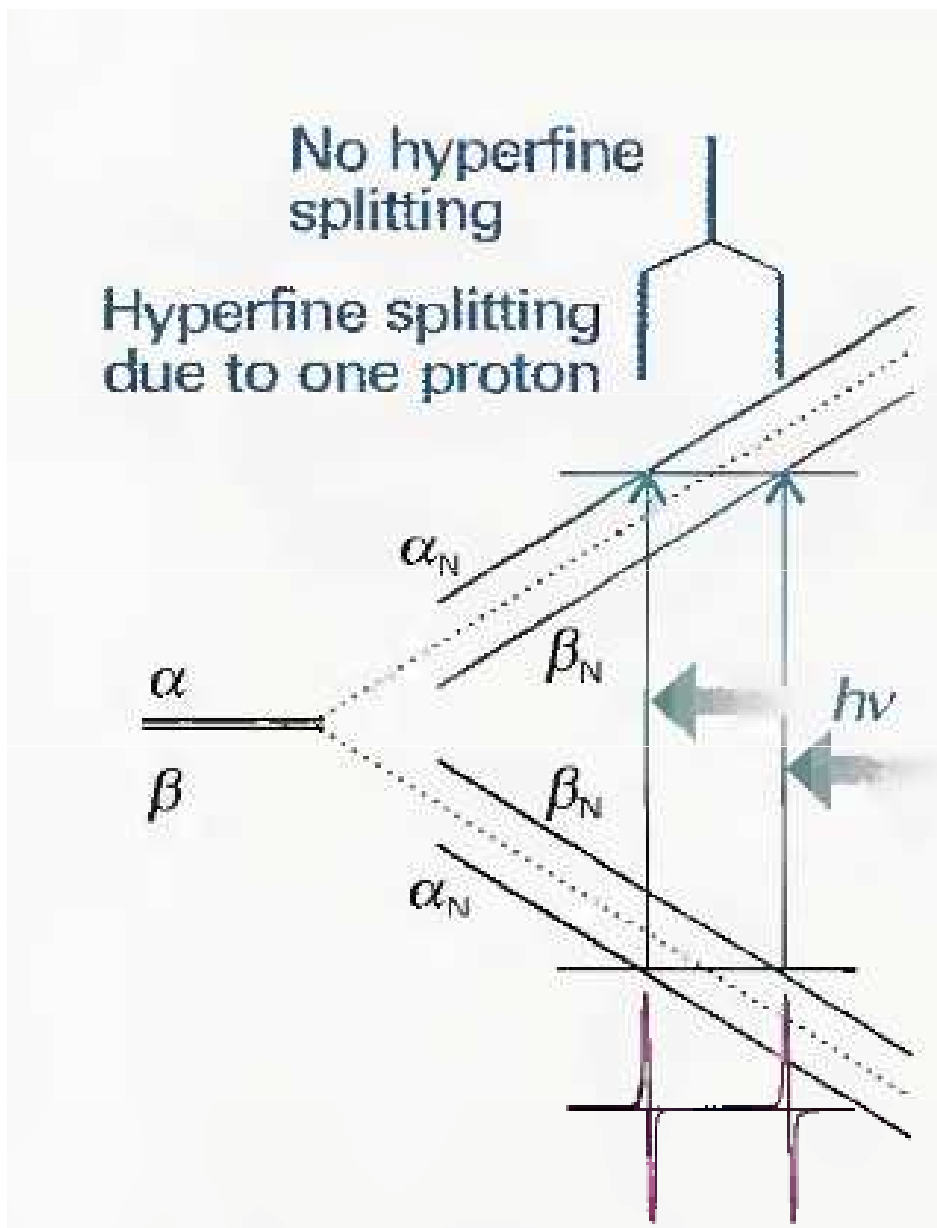
1st EPR experiment in Kazan (Tatarstan, USSR), E.K.Zavoisky on $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, rf source @133 MHz.

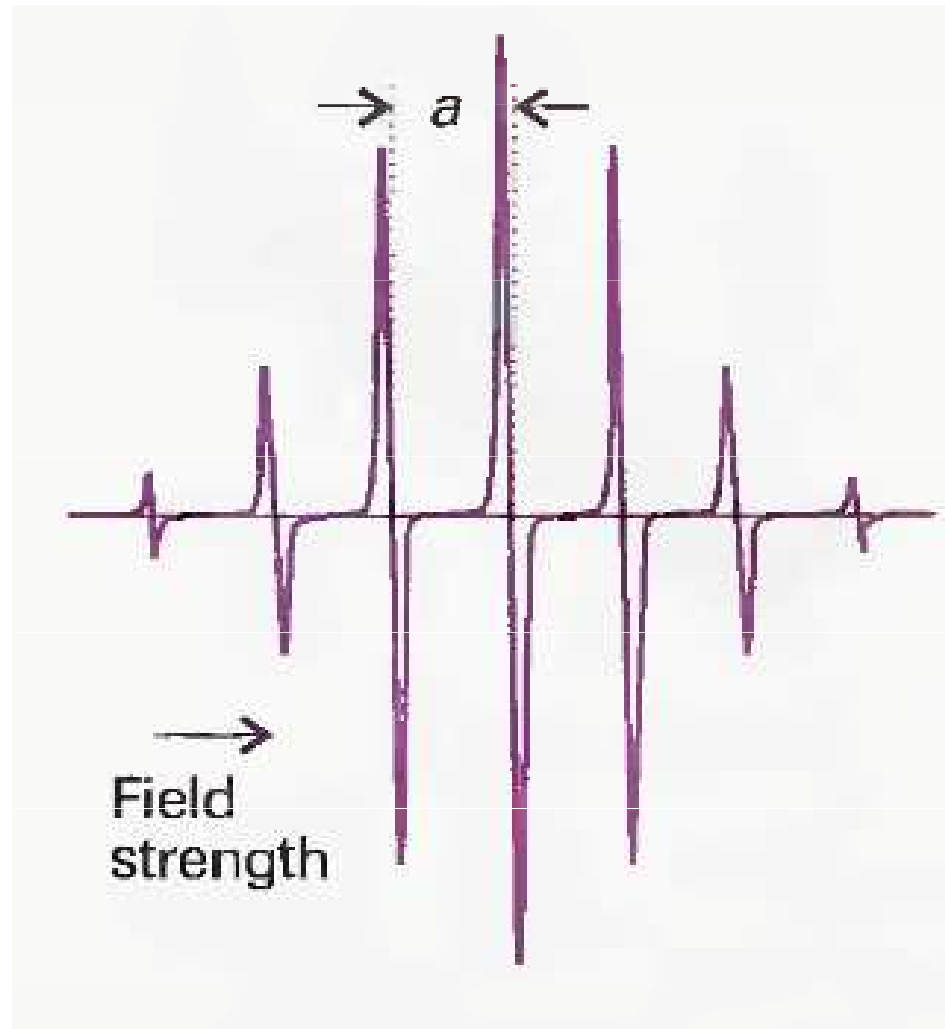


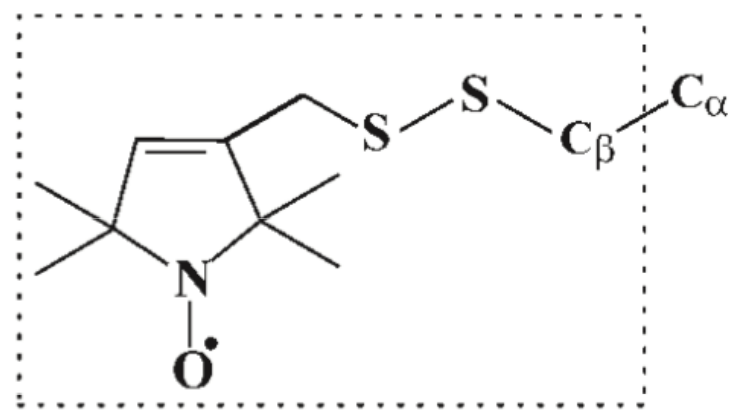
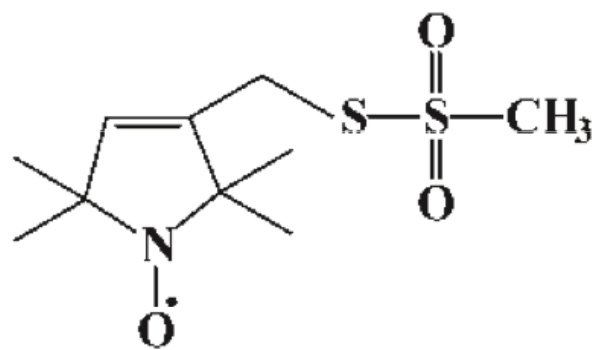


An unpaired electron can move between the two energy levels by either absorbing or emitting a photon of energy $h\nu$ such that the resonance condition, $h\nu = \Delta E$, is obeyed. This leads to the fundamental equation of EPR spectroscopy: $h\nu = g_e \mu_B B_0$.

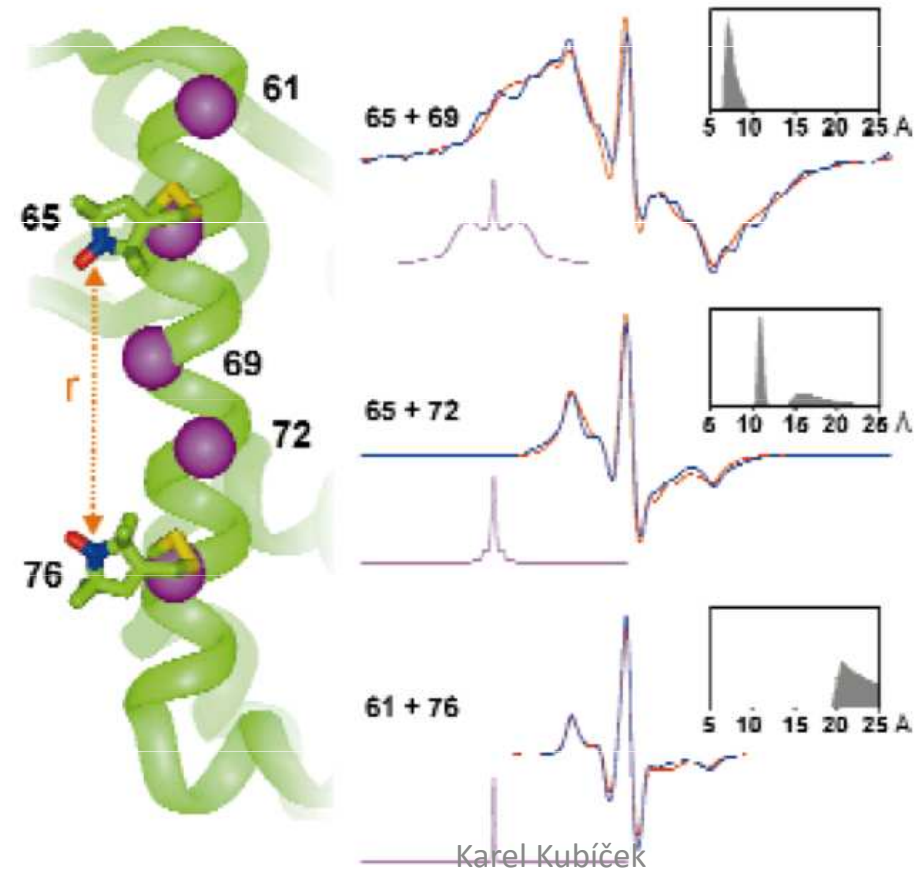
Experimentally, this equation permits a large combination of frequency and magnetic field values, but the great majority of EPR measurements are made with microwaves in the **9000–10000 MHz** (9–10 GHz) region, with fields corresponding to about 3500 G (0.35 T). Furthermore, EPR spectra can be generated by **either varying the photon frequency** incident on a sample **while holding the magnetic field constant or doing the reverse**. In practice, it is **usually the frequency that is kept fixed**.



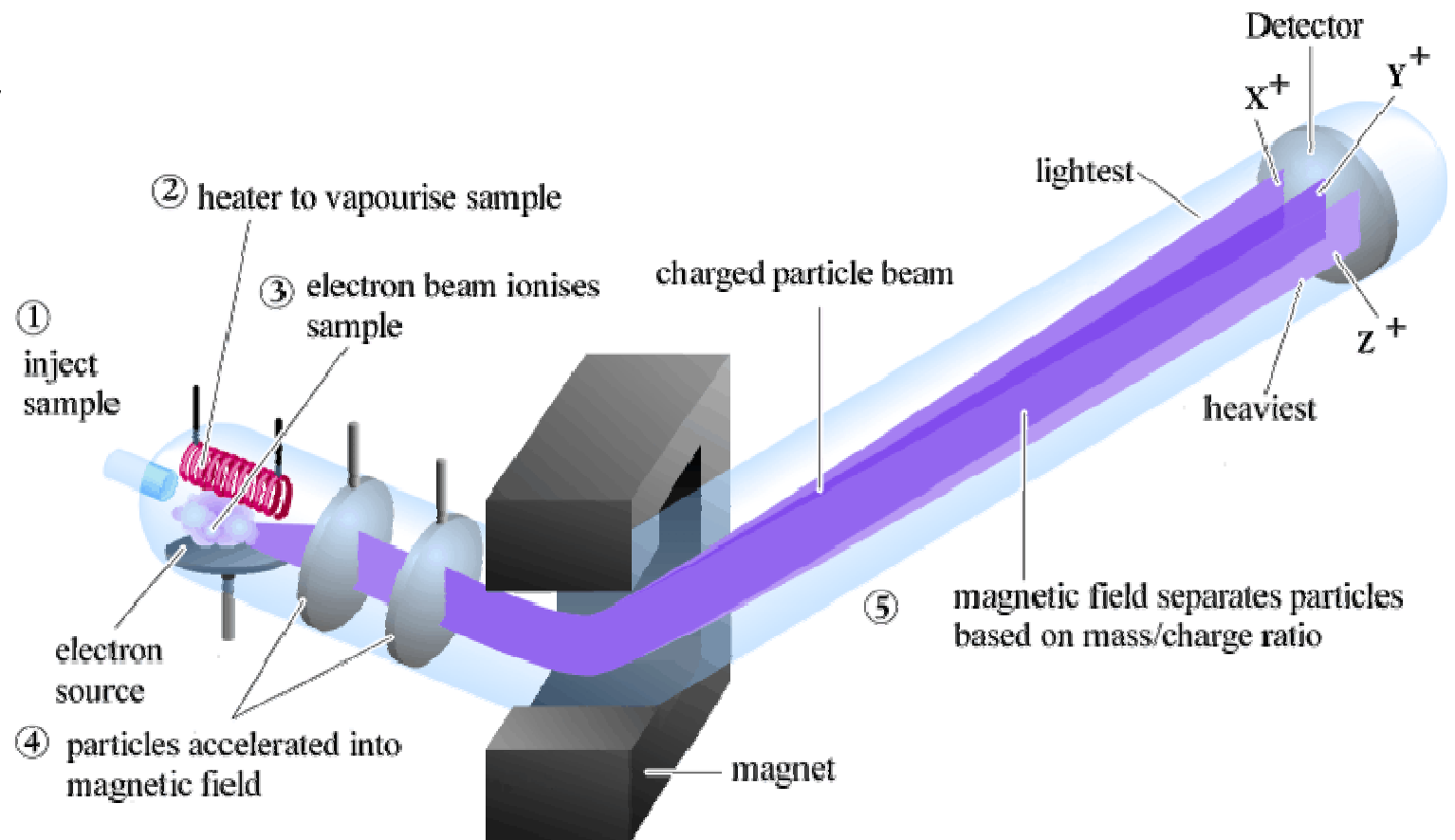




side-chain R1



Mass spectrometry

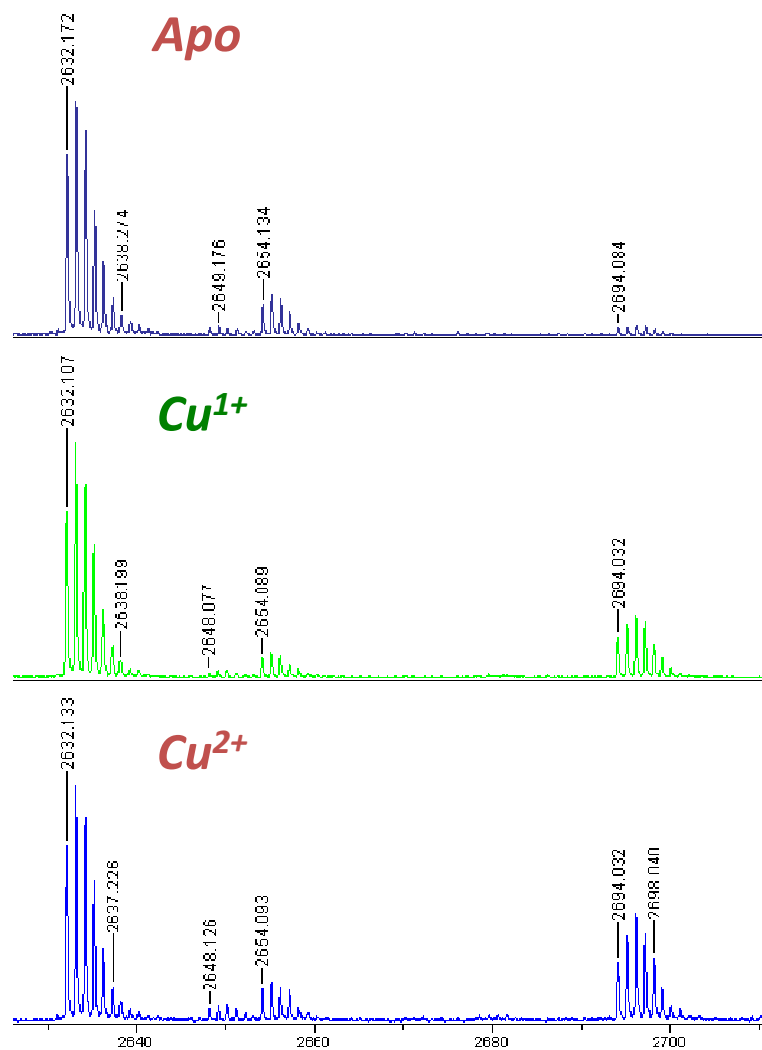


Mass spectrometry is among the most accurate techniques for the determination of molar masses. The procedure consists of ionizing the sample in the gas phase and then measuring the mass-to-charge number ratio (m/z) of all ions. Macromolecules present a challenge because it is difficult to produce gaseous ions of large species without fragmentation. However, two new techniques have emerged that circumvent this problem: **matrix-assisted laser desorption/ionization (MALDI)** and **electrospray ionization**. We shall discuss **MALDI-TOF mass spectrometry**, so called because the MALDI technique is coupled to a time-of-flight (TOF) ion detector.

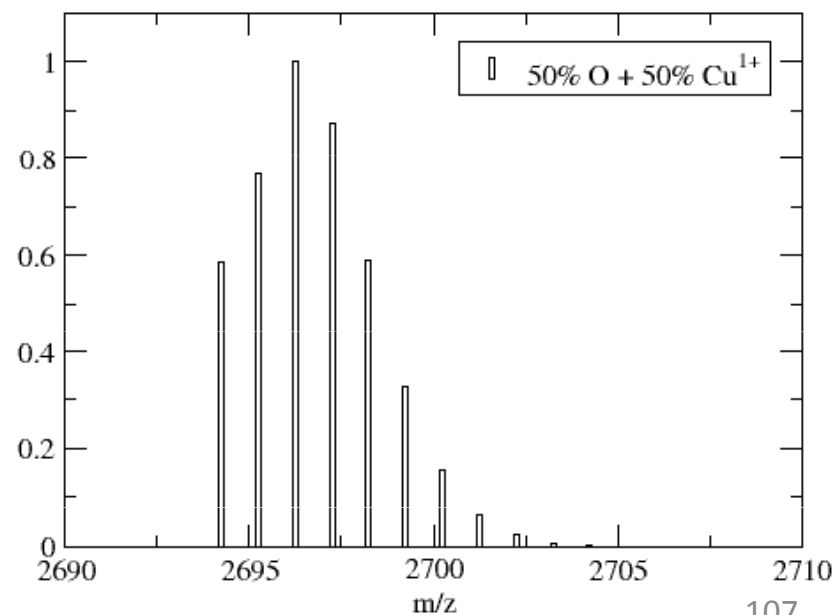
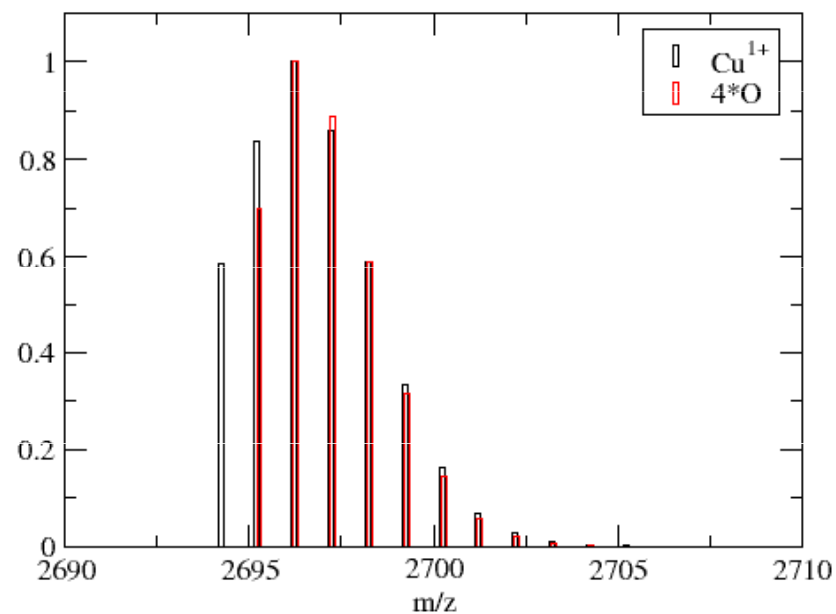
Peptide Mapping with MALDI TOF

Tryptic digestion o/n 1:30-50 (trypsin:protein)

Analysis with PAWS (Genomic Solutions, Inc.)



L61-K87



Elektroforéza

- Elektroforéza – pohyb nabitých molekul v elektrickém poli. Při rovnoměrném přímočarém pohybu **sférické** částice o poloměru r , je elektrostatická síla působící na částici v rovnováze se silou tření, jež je dána viskozitou. Sílu tření lze vypočítat dle **Stokesova vzorce**:

$$F = 6 \cdot \pi \cdot r \cdot \eta \cdot v$$

kde v je rychlost částice a η je dynamická viskozita prostředí.

- Elektrické pole působí na částici silou:

$$F = z \cdot e \cdot E$$

kde z je počet elementárních nábojů nesených částicí, e je elementární náboj ($1,602 \cdot 10^{-19}$ C) a $E [V \cdot m^{-1}]$ je intenzita elektrického pole v daném místě.

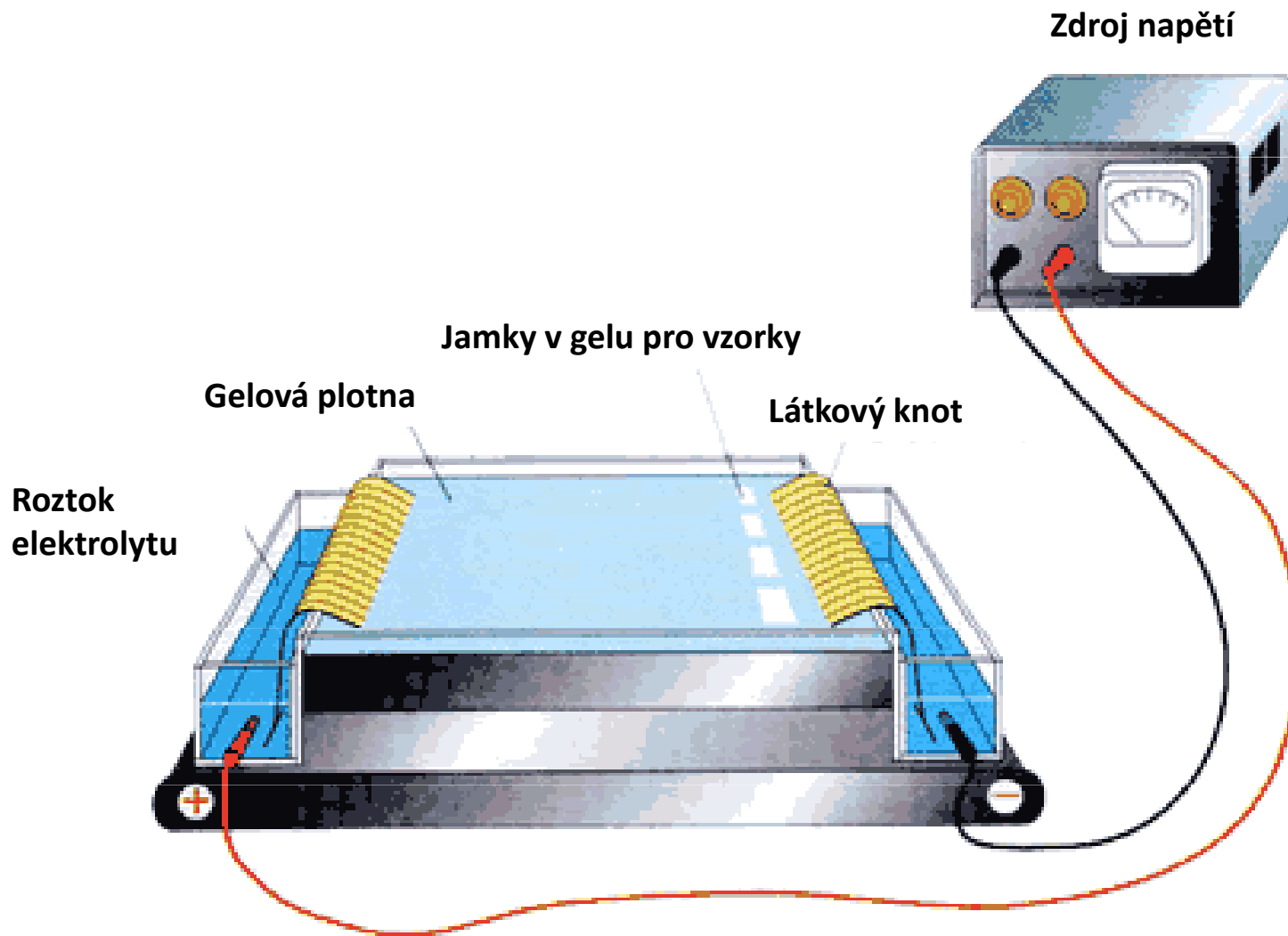
- Rychlost částice je pak v důsledku rovnosti obou sil:

Elektroforetická pohyblivost

- Elektroforetická pohyblivost u nezávisí na intenzitě elektrického pole. Je definována jako podíl rychlosti částice a intenzity elektrického pole. Platí:

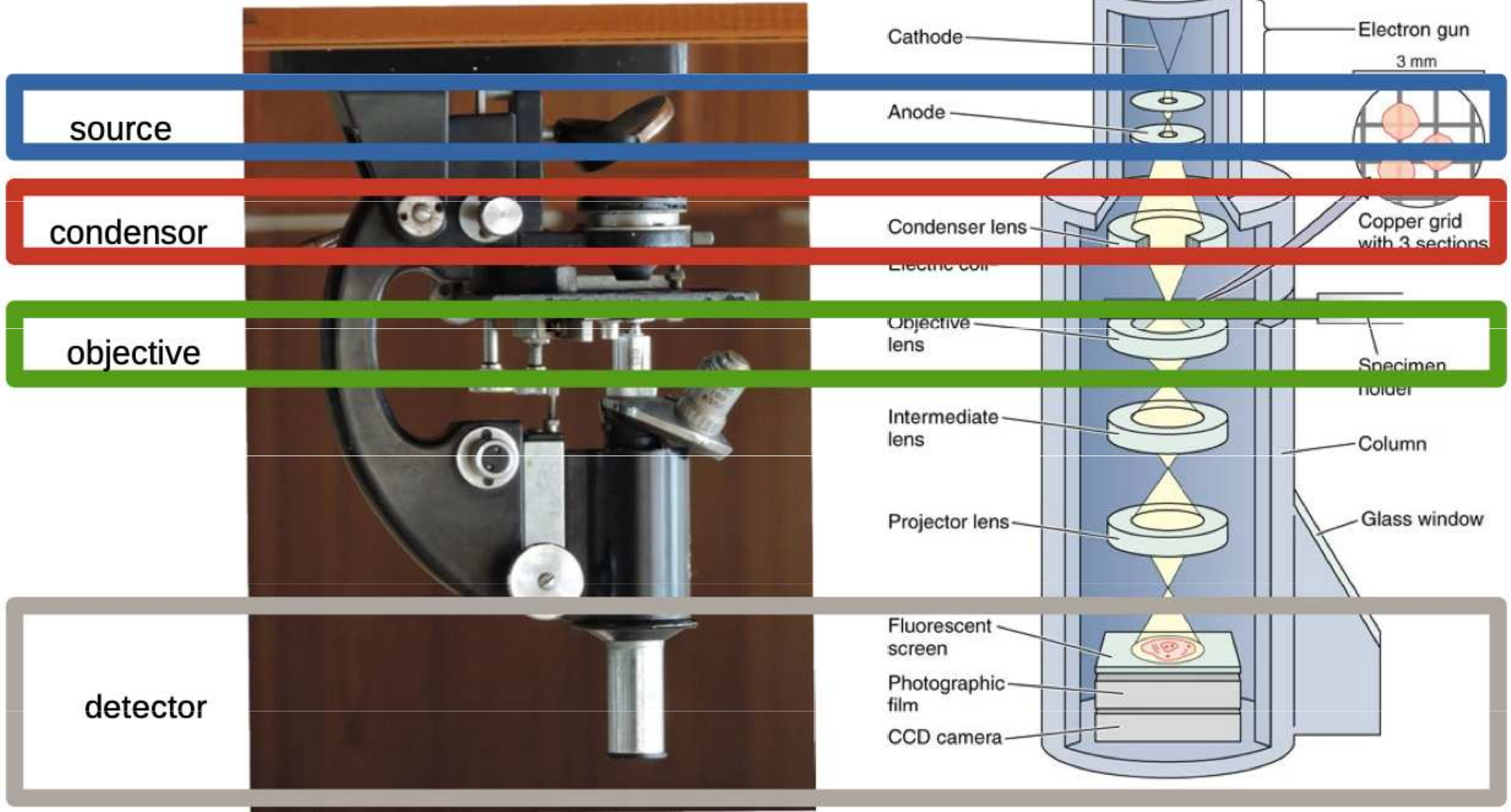
Poznámka. Elektroforéza s dodecylsulfátem sodným. Tato sloučenina, která nese jeden negativní elementární náboj, se váže definovaným způsobem k bílkovinám a eliminuje jejich vlastní elektrický náboj. Molekuly bílkovin se pak pohybují s různou rychlostí jen proto, že mají různou velikost (poloměr).

Zařízení pro elektroforézu

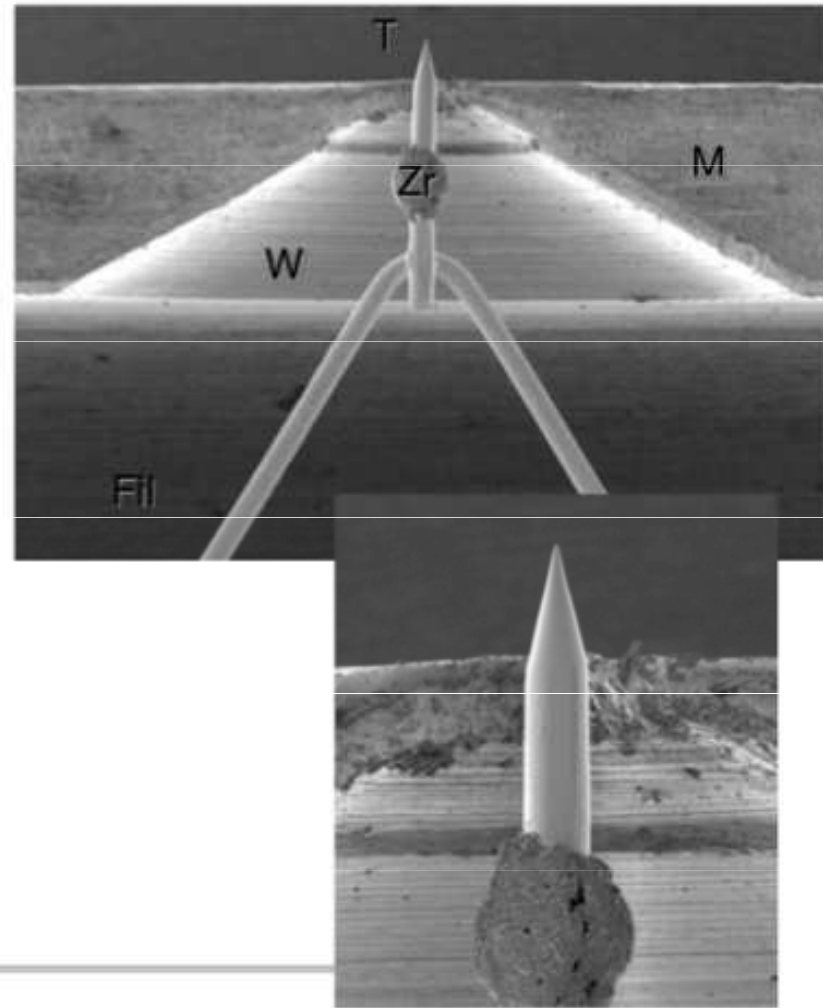
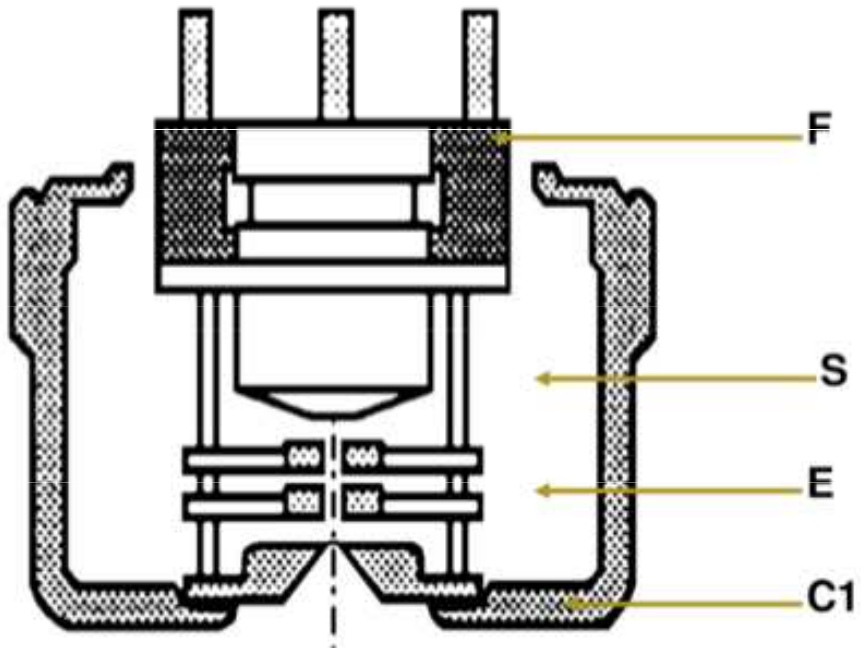
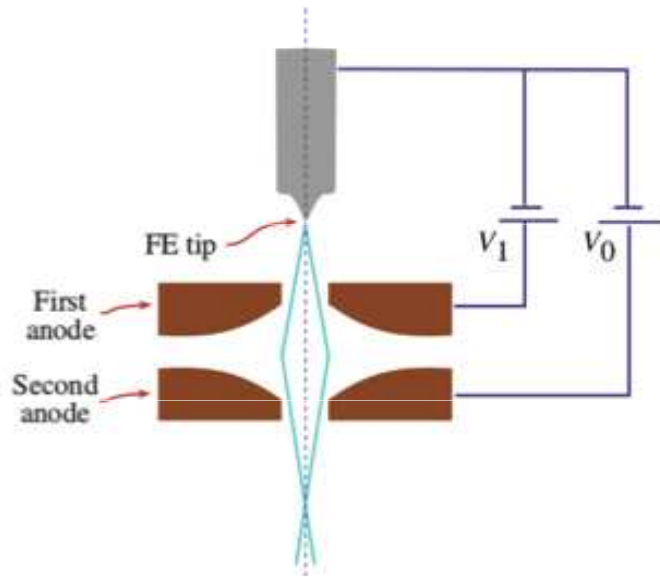


Optical microscope

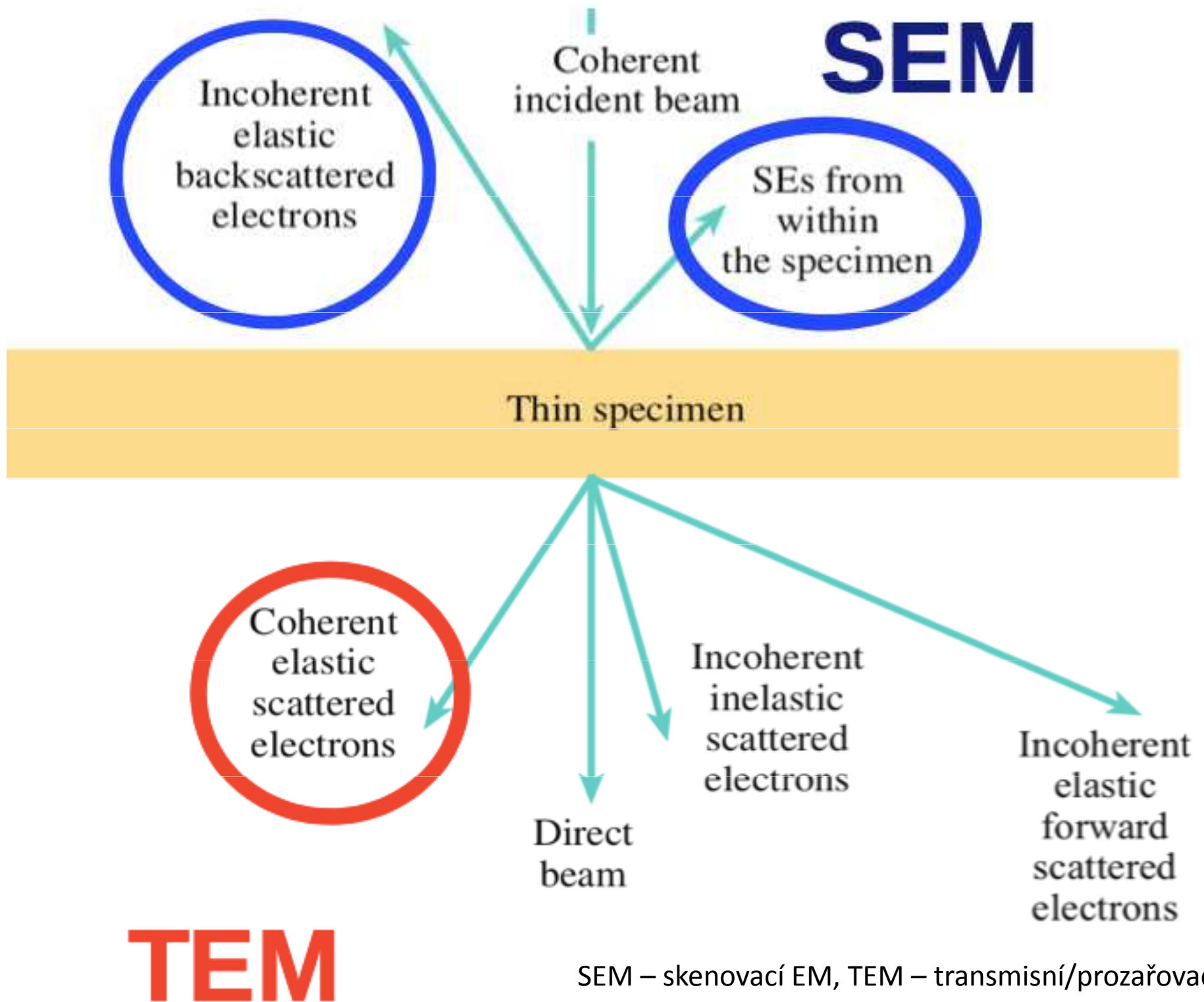
Electron microscope



Electron source - FEG

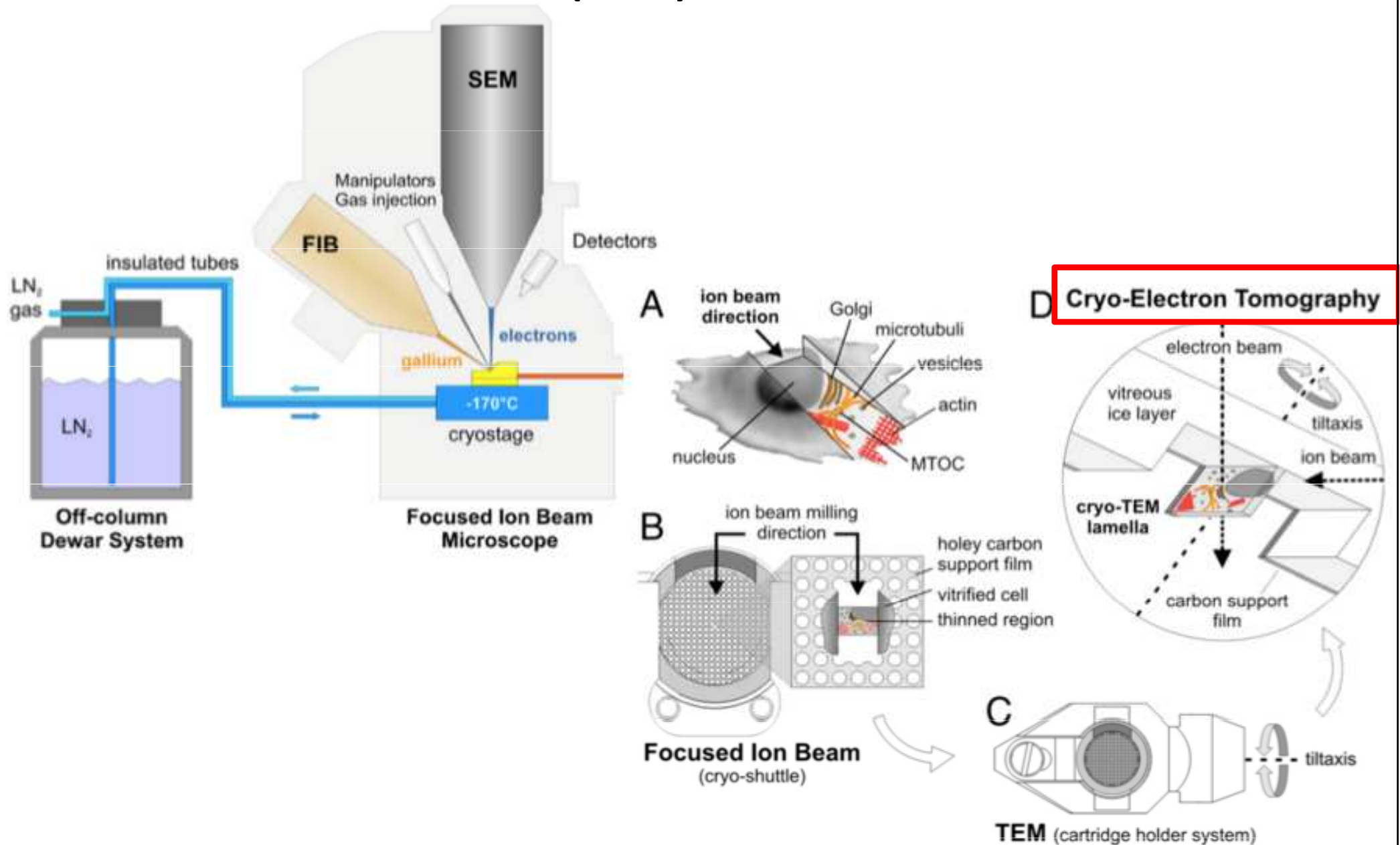


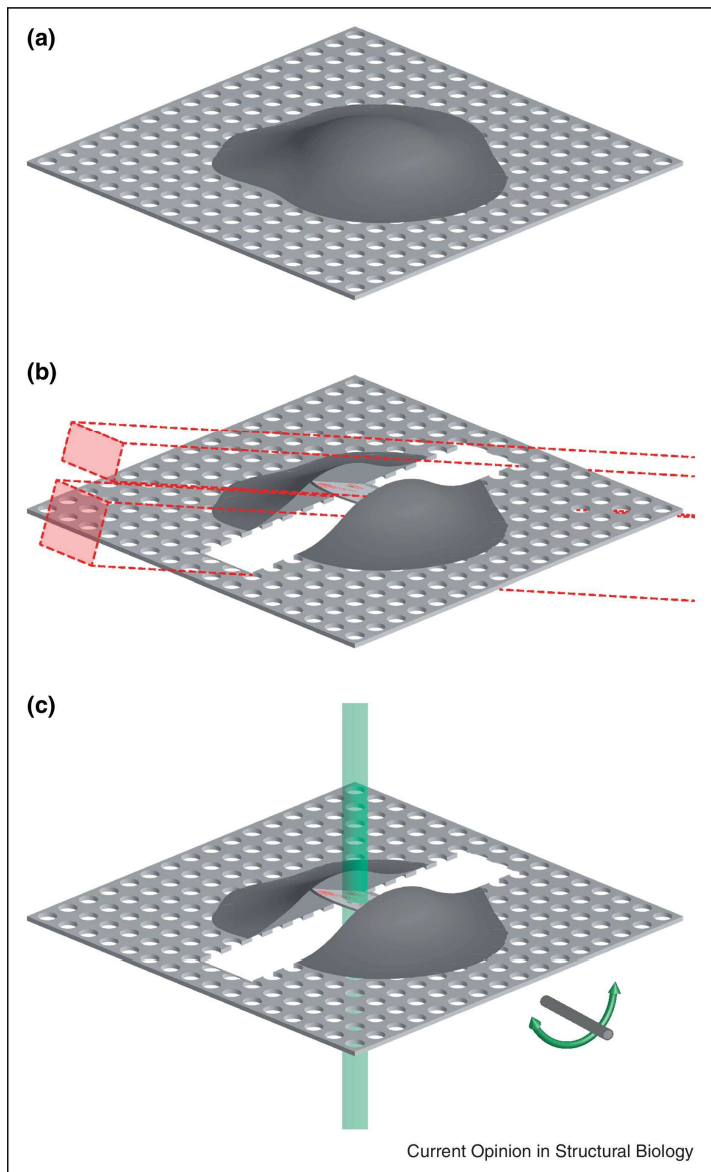
FEG - Field Emission Gun



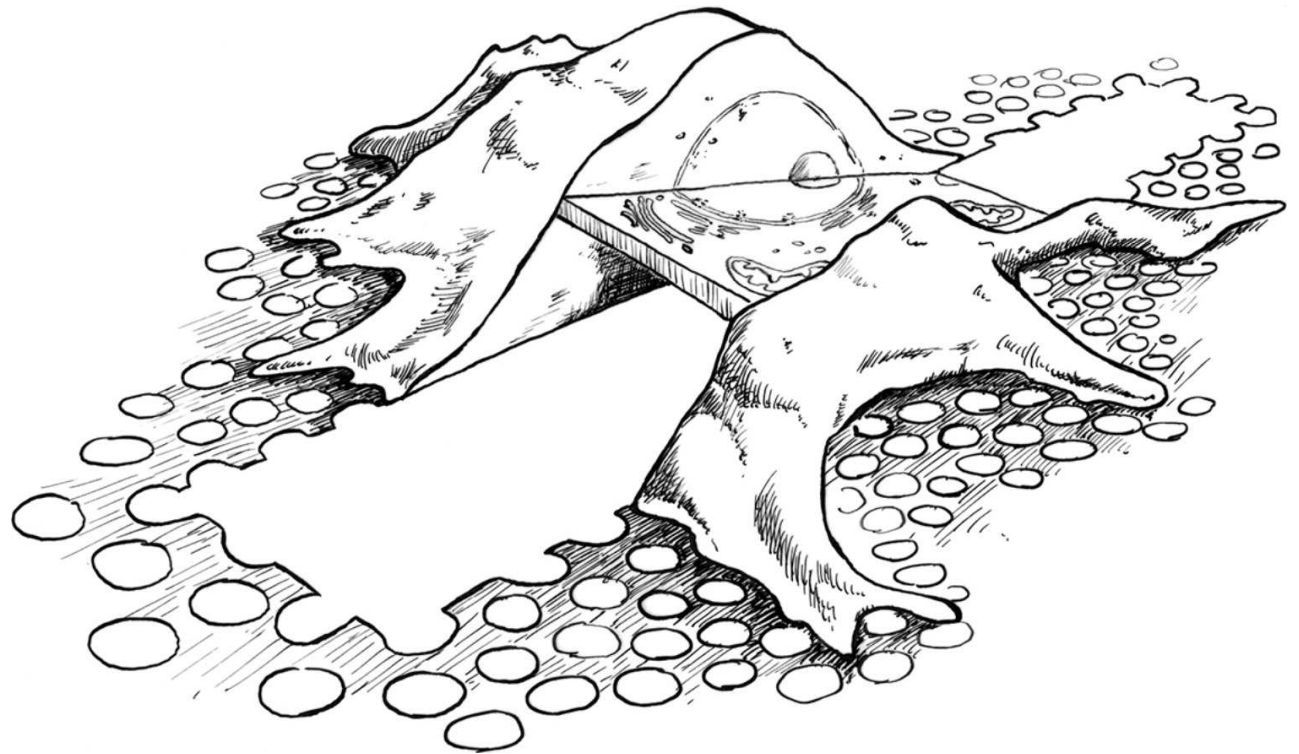
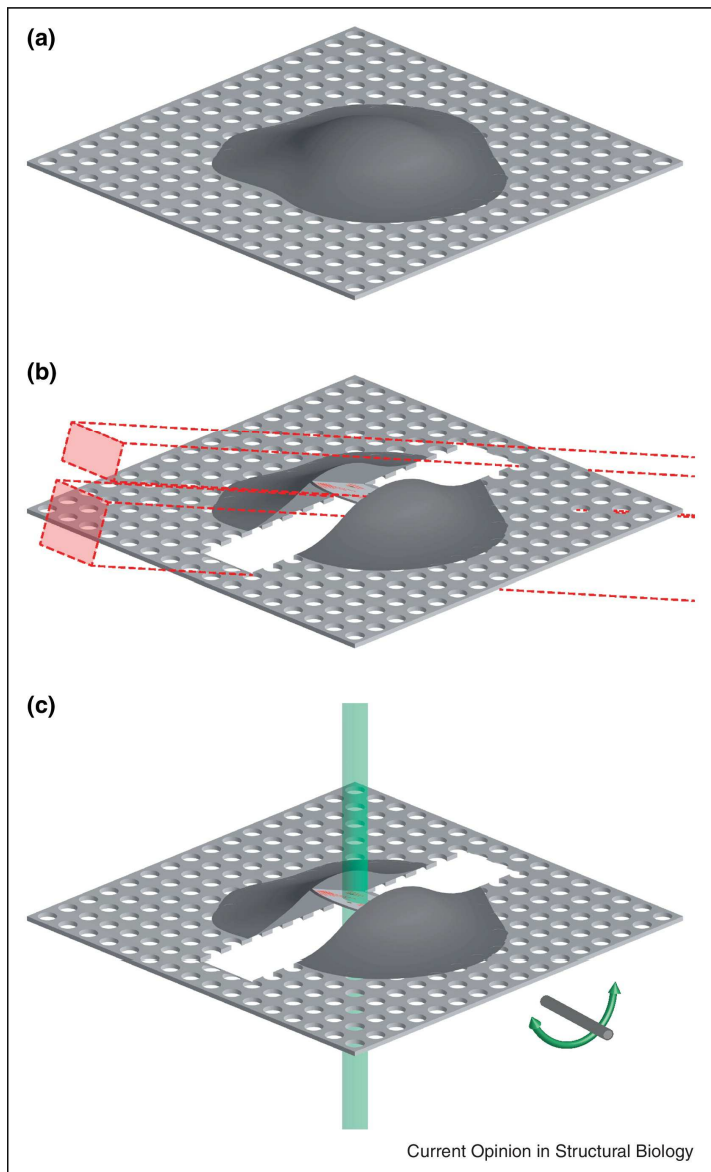
SEM – skenovací EM, TEM – transmisní/prozařovací EM

Focused Ion Beam (FIB) + SEM

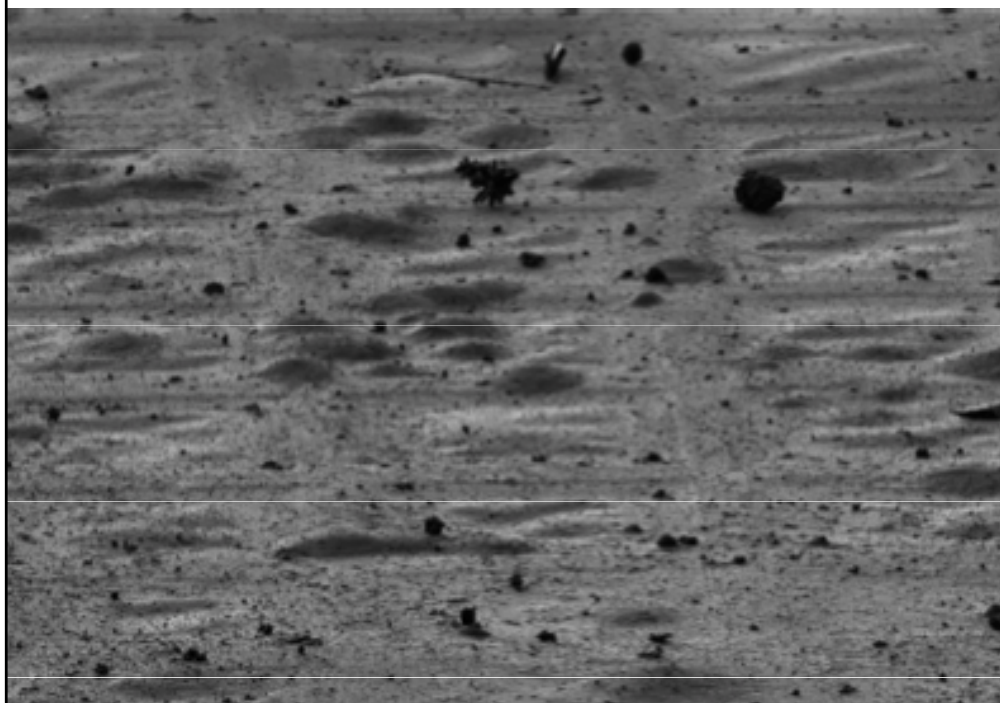




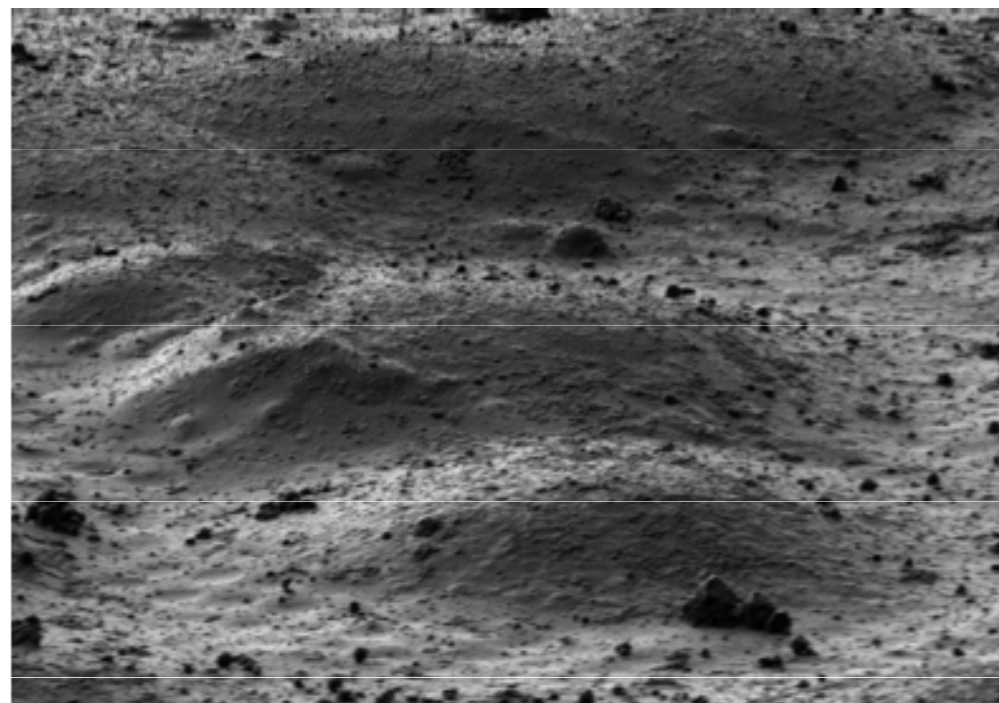
Villa et al. Current Opinion in Structural Biology, 23(5), 2013, 771-7



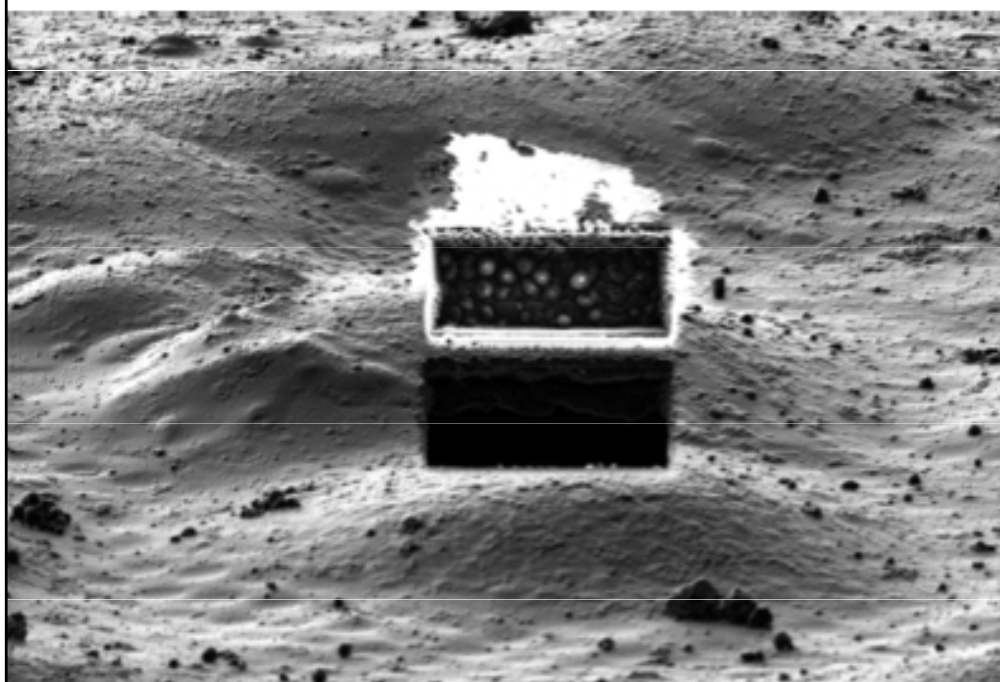
Villa et al. Current Opinion in Structural Biology, 23(5), 2013, 771-7



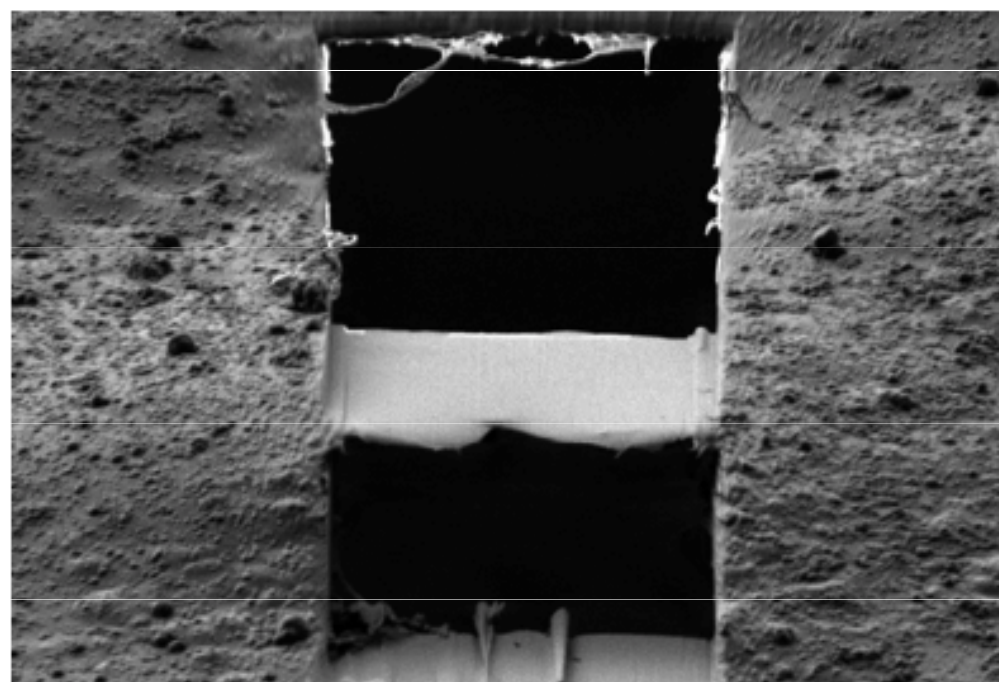
HV	curr	HPW	mag	pressure	det	mode	WD	tilt	50 μm	
30.00 kV	10 pA	319 μm	650 x	8.10e-5 Pa	ETD	SE	19.0 mm	10 °	Versa 3D	



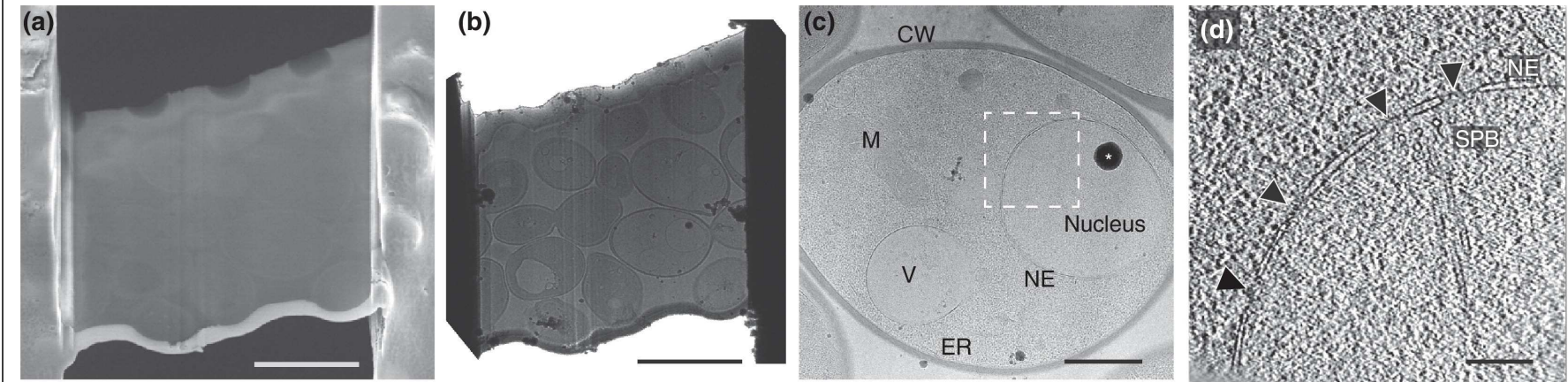
HV	curr	HPW	mag	pressure	det	mode	WD	tilt	5 μm	
30.00 kV	10 pA	41.4 μm	5 000 x	7.57e-5 Pa	ETD	SE	19.4 mm	10 °	Versa 3D	



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	



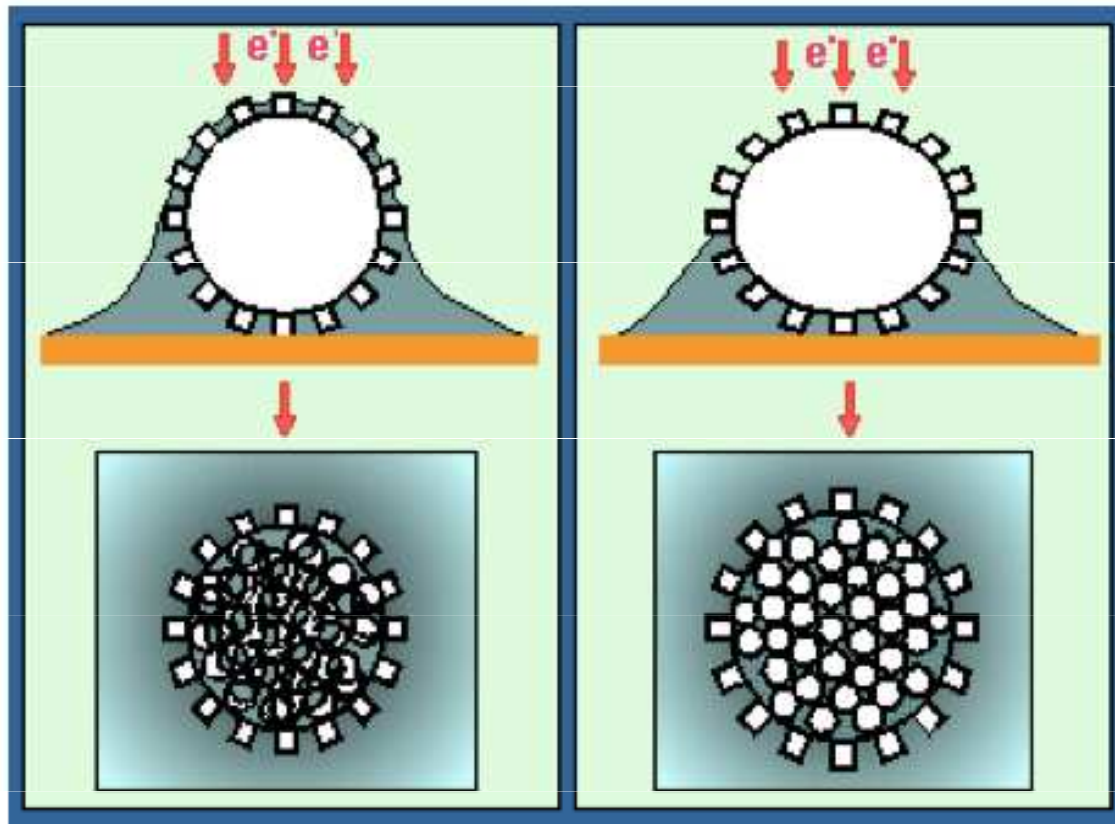
HV	curr	HPW	mag	pressure	det	mode	WD	tilt	5 μm	
30.00 kV	1.5 pA	25.9 μm	8 000 x	7.57e-5 Pa	ETD	SE	19.5 mm	20 °	Versa 3D	



Current Opinion in Structural Biology

TEM – Sample preparation

Negative staining



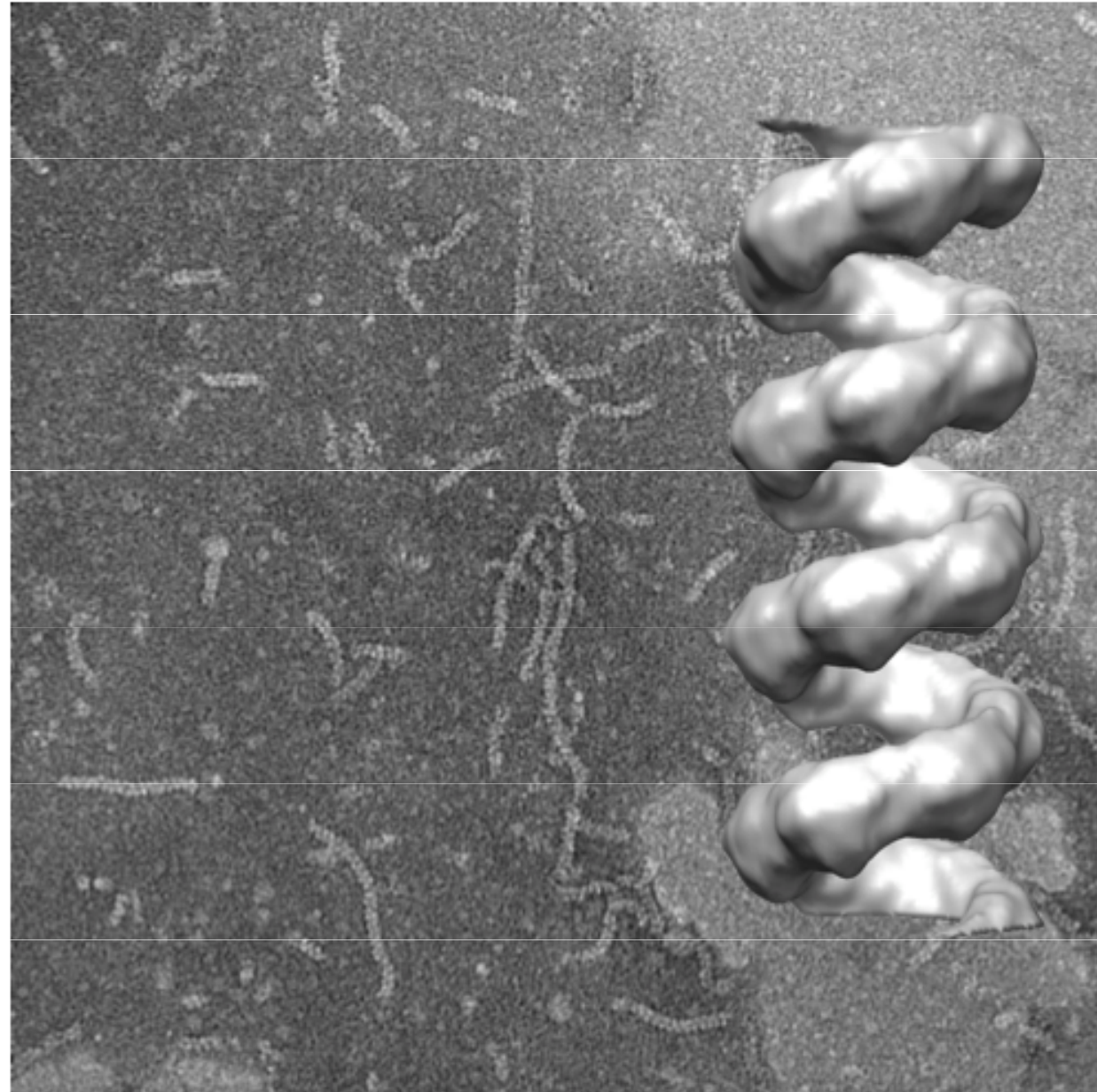
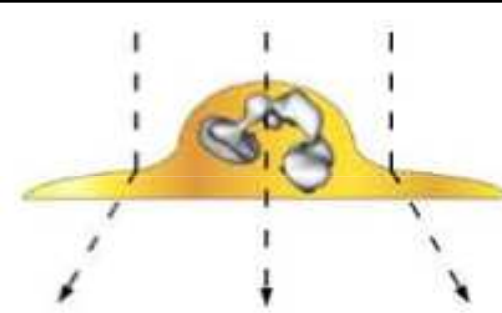
Pros: quick sample screening
high amplitude contrast
less prone to beam damage

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

Heavy metal staining

e.g. uranyl acetate

Negative staining

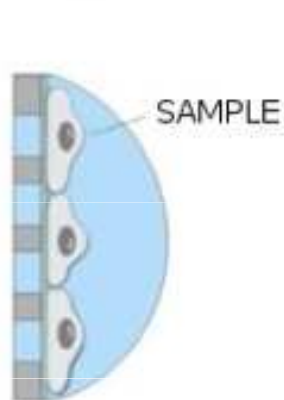


Sample vitrification

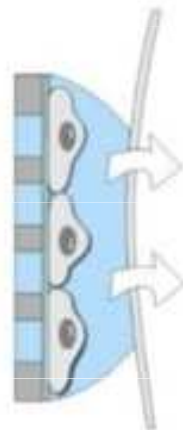
Cryogens	Melting point (°C)	Boiling point (°C)	Cooling rate (10^3 °C/s)	Relative cooling efficiency*
Ethane	-183	-89	-260 - -258	1.3
Liquid nitrogen	-210	-196	-272	0.1
Propane	-189	-42	-263 - -261	1.0
Freon 22	-160	-41	-267 - -265	0.7



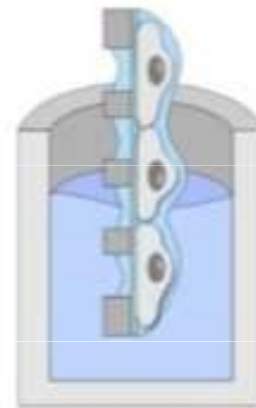
3-4ul
0.1-1mg/ml for purified protein complexes
OD~0.5 for bacteria



SAMPLE



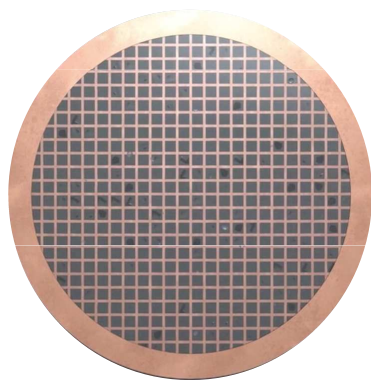
IFILTER PAPER



LIQUID ETHANE



Grid



C-clip



AutoGrid



ThermoFisherScientific



Grid Box Tool

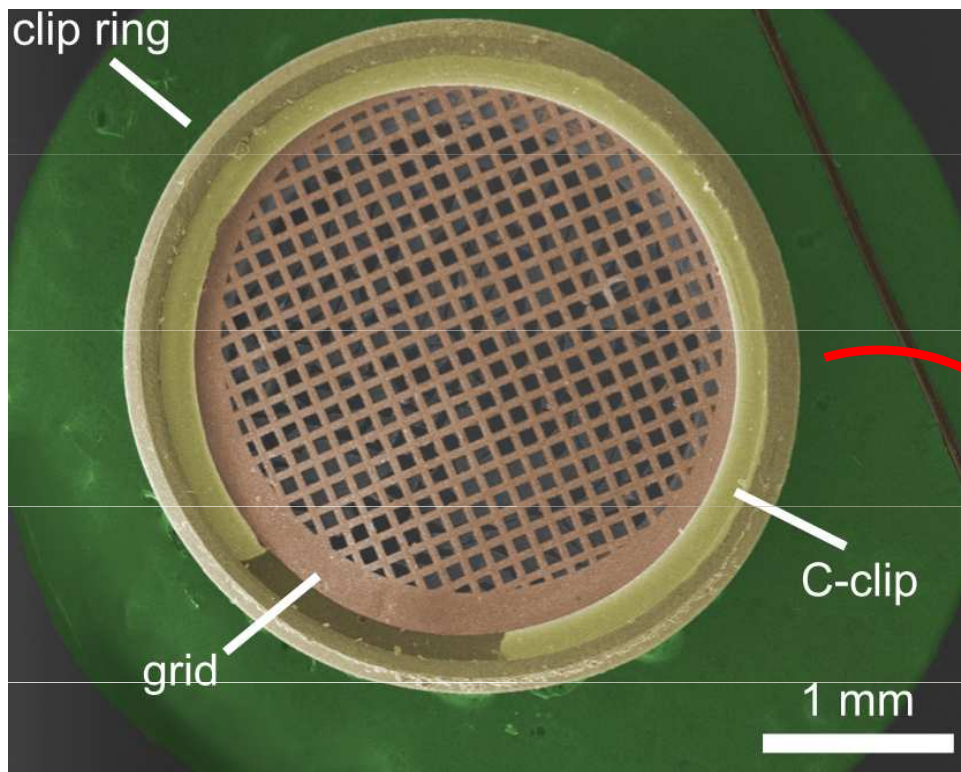


Clipping Tool



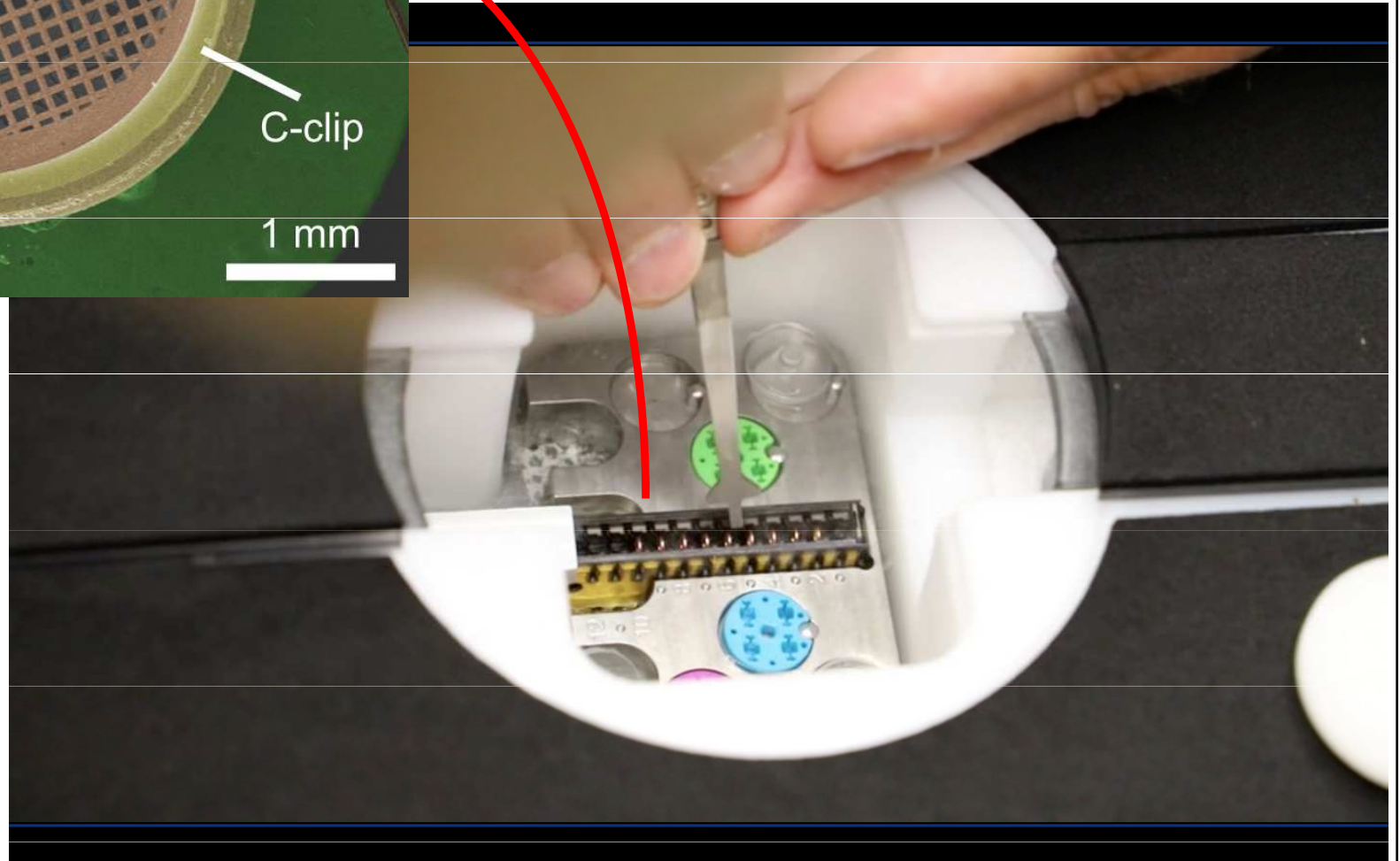
Autogrid Tweezer





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

Auto-loader: up to 12 autogrids

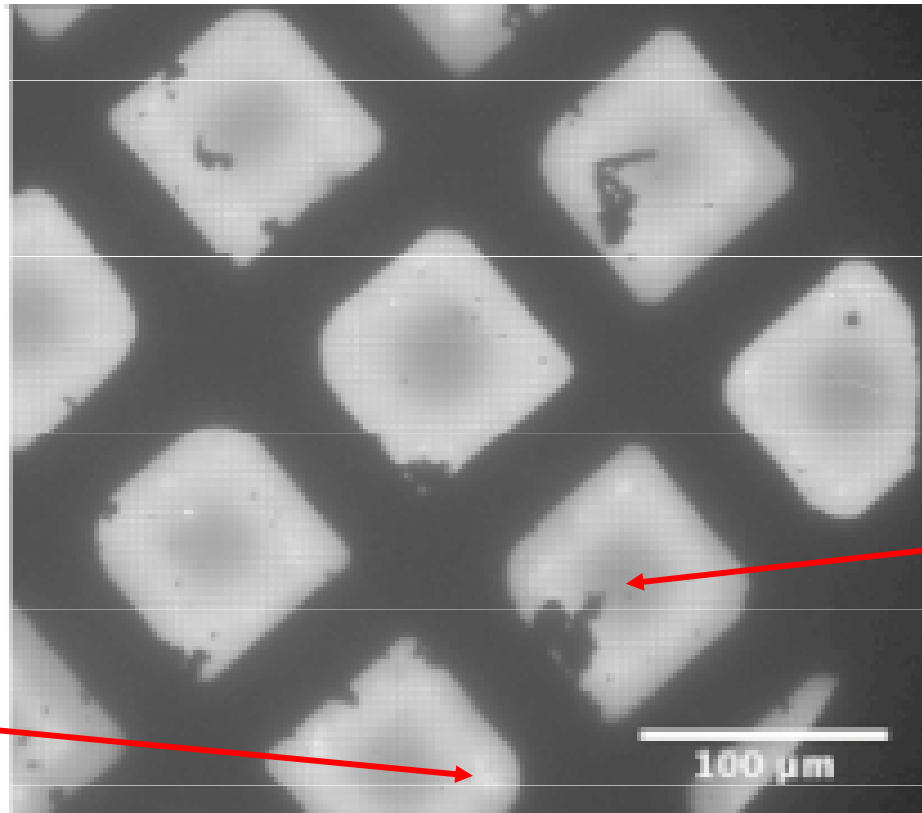
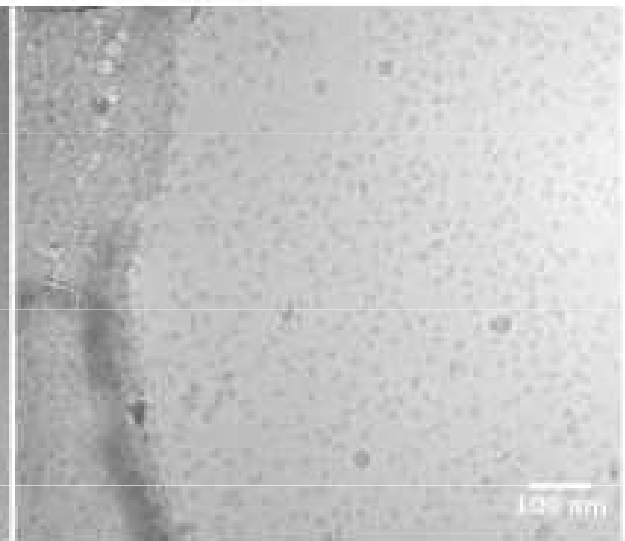
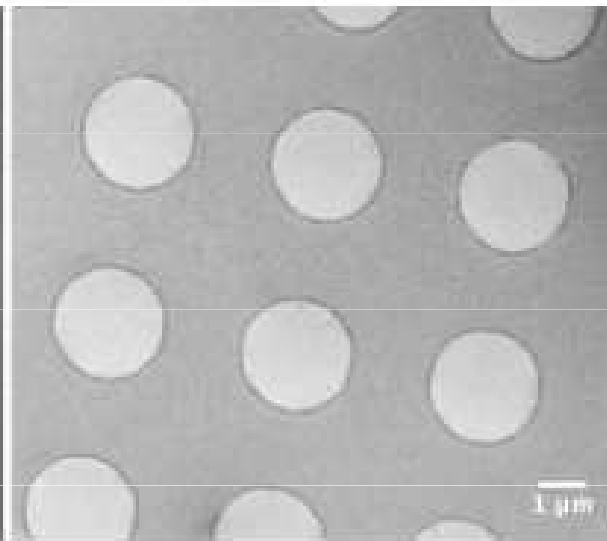
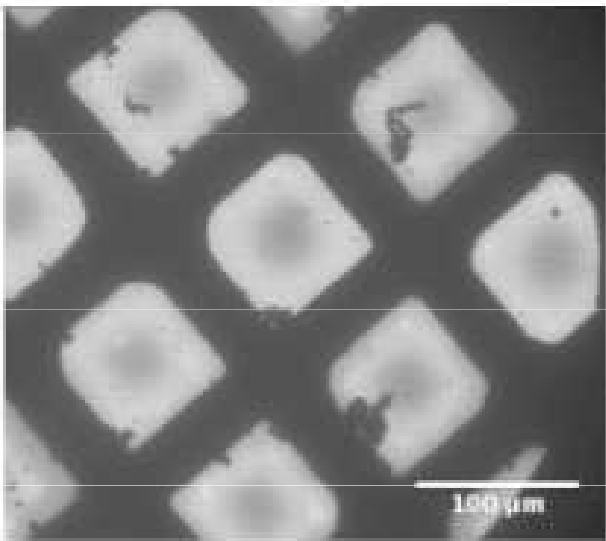




AutoGrid Container

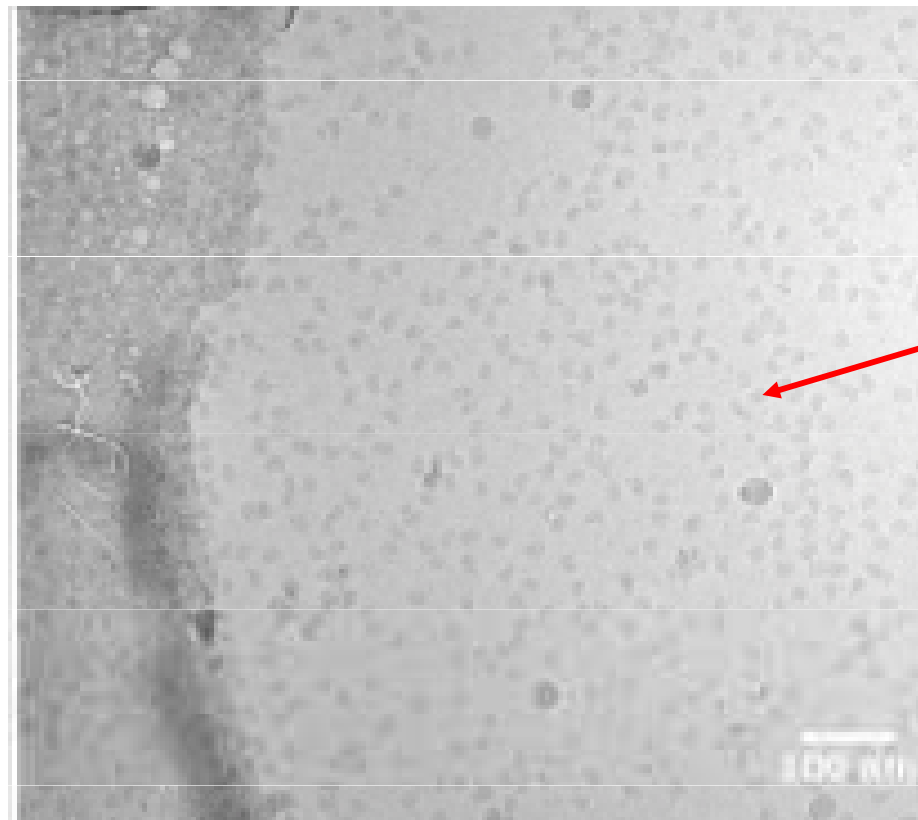
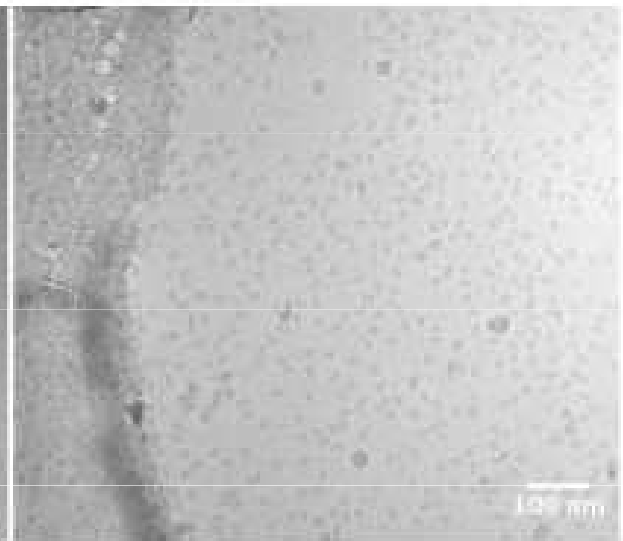
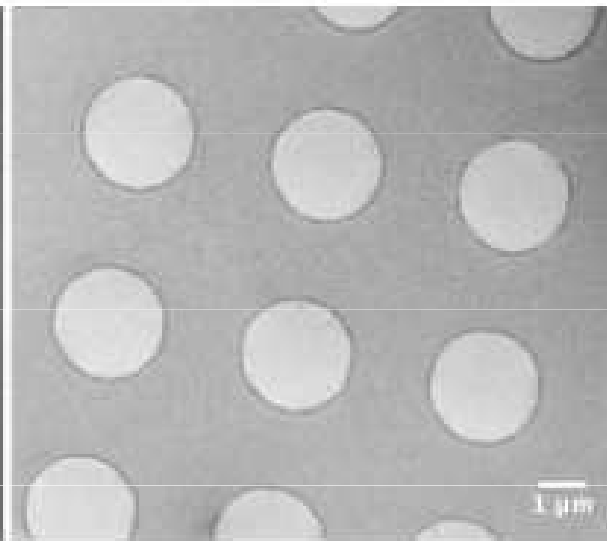
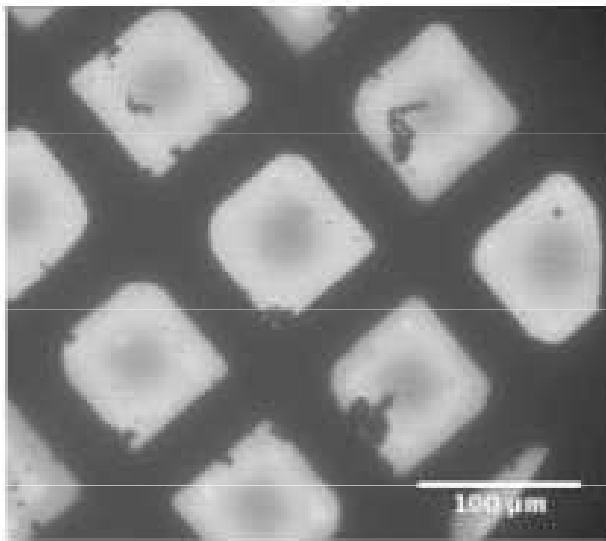


ThermoFisherScientific; MiTeGen



Grids get damaged

Note the ice thickness

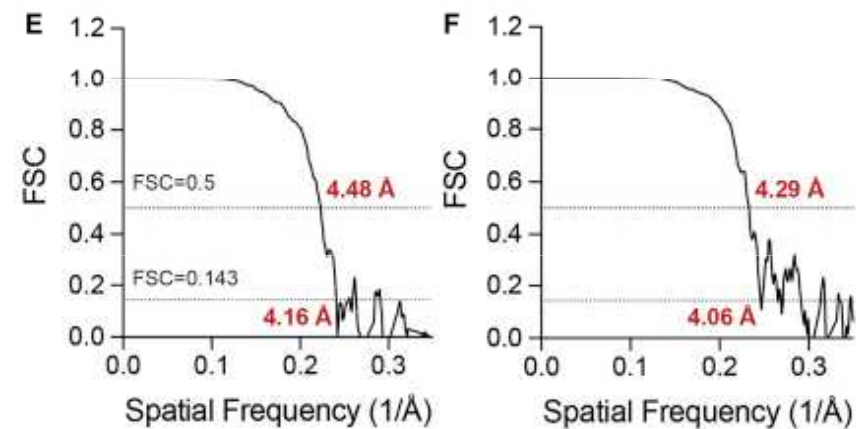
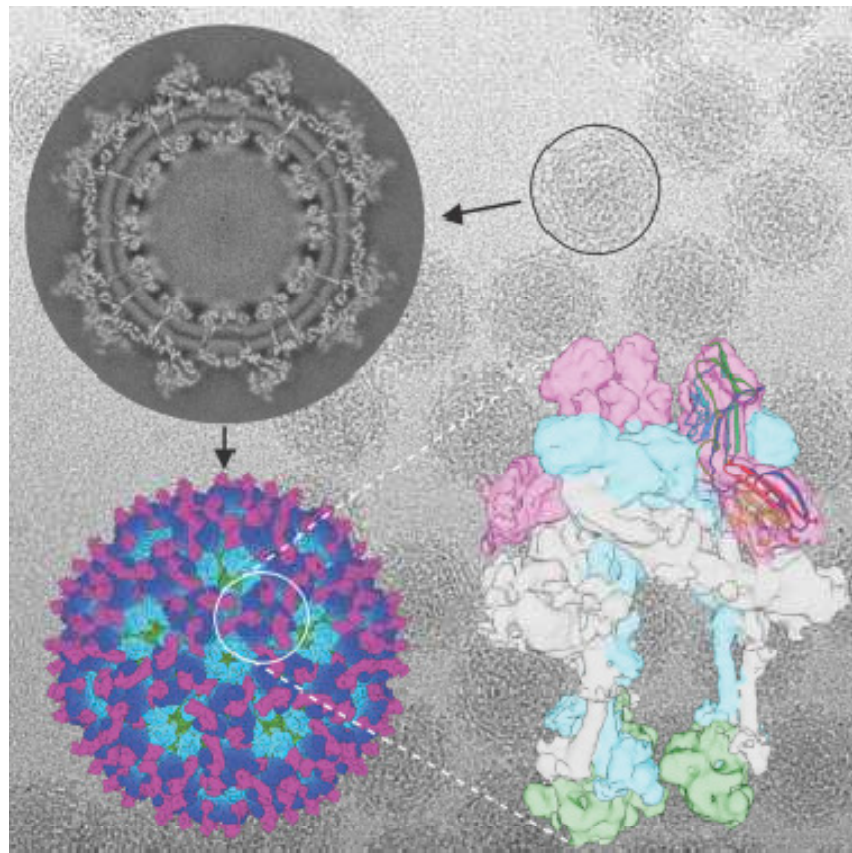
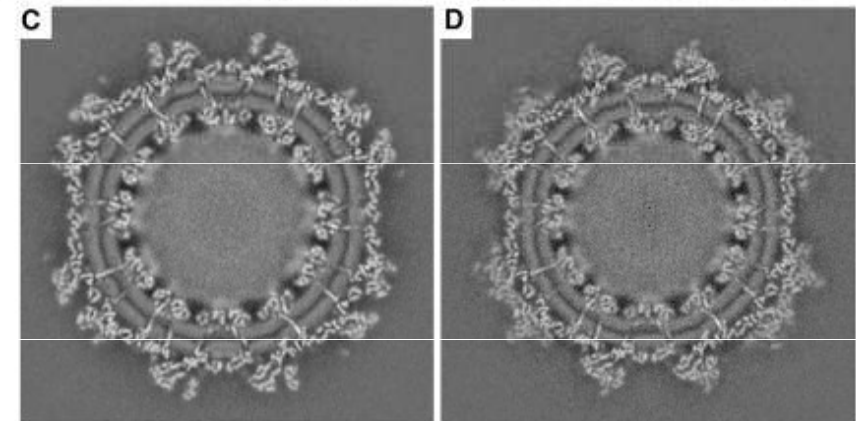
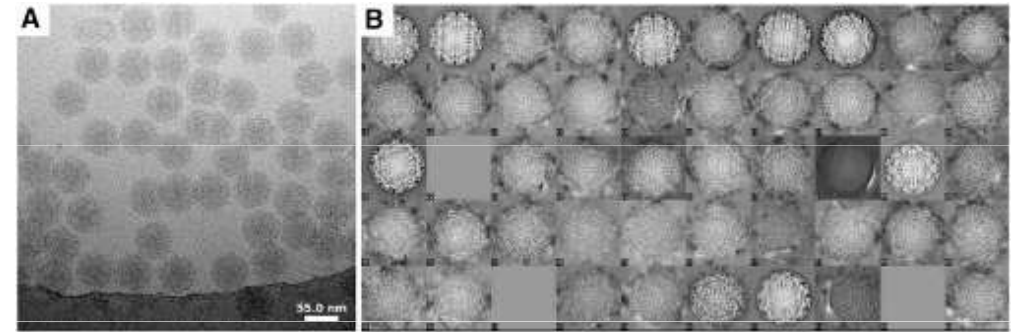


single particles

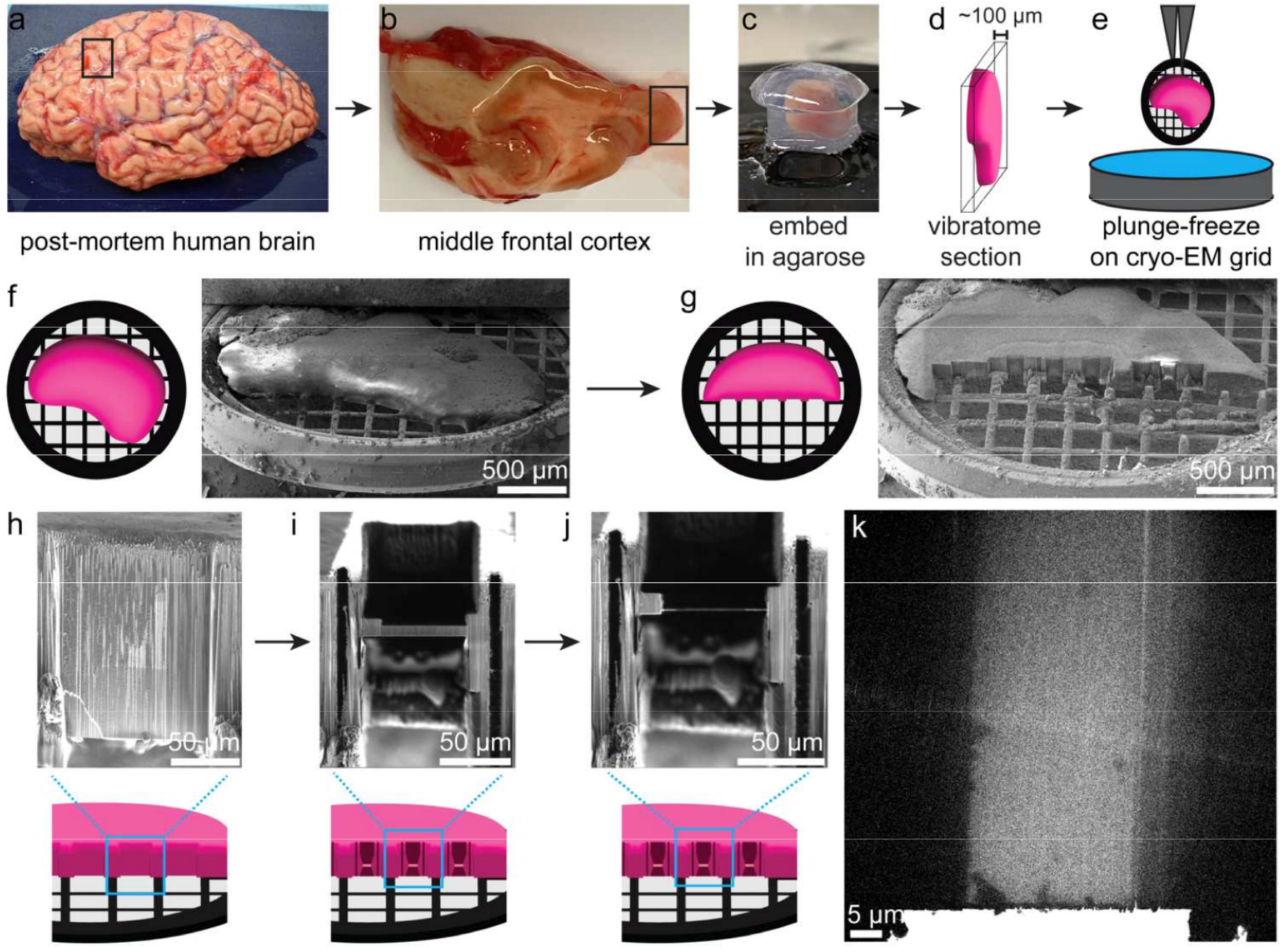


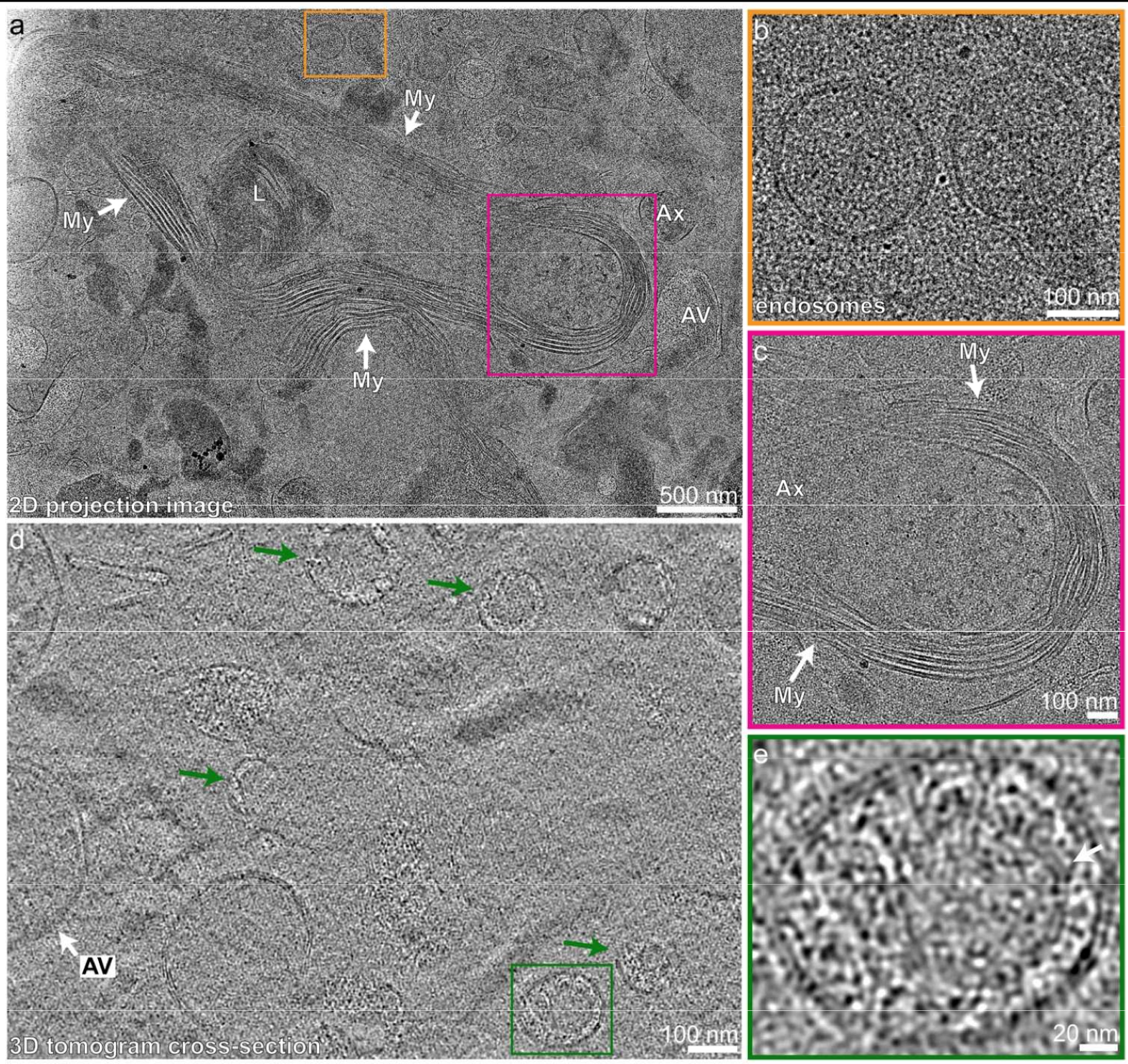
Structure determination using single particle cryoEM

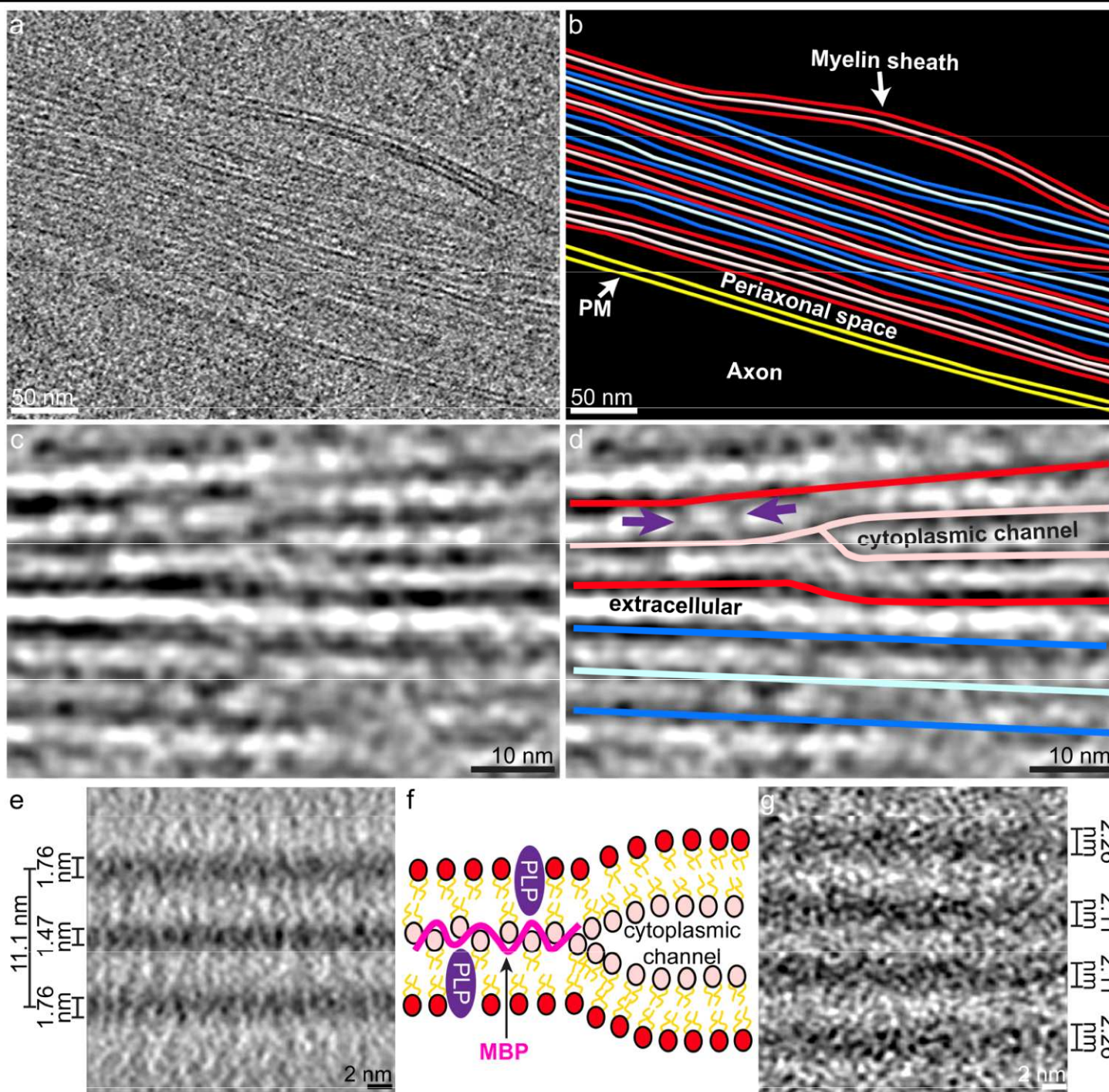
- 1) Sample preparation – 2) vitrification – 3) measurement – 4) particle picking – 5) 2D classification – 6) 3D model reconstruction



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

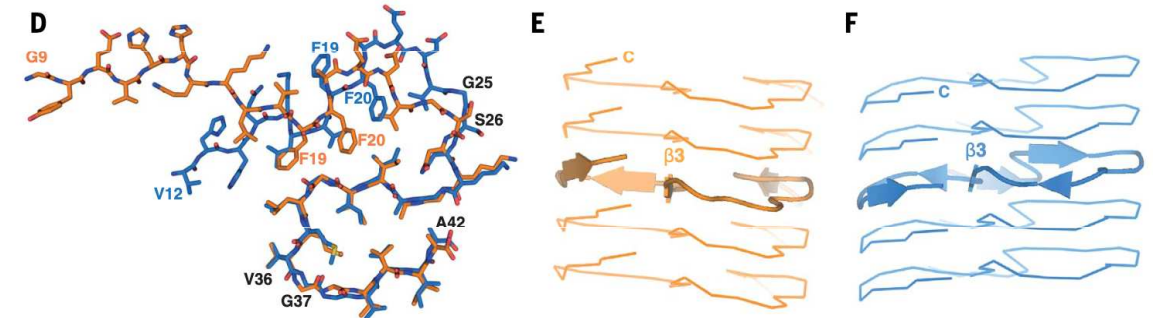
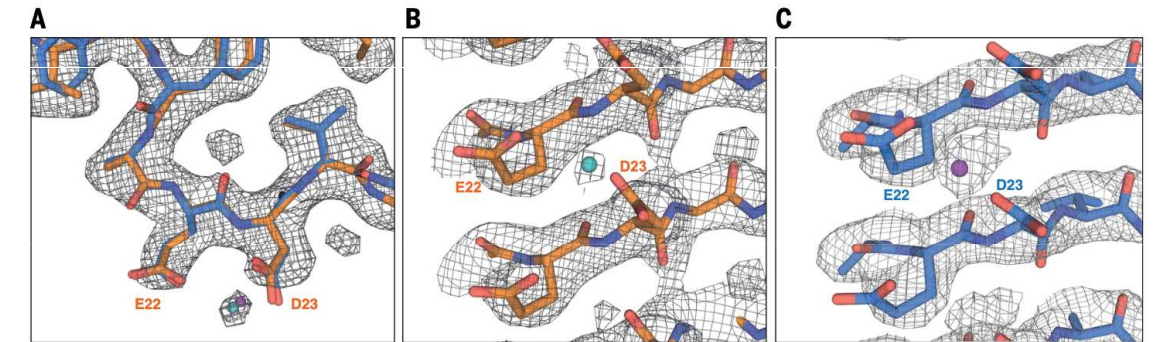
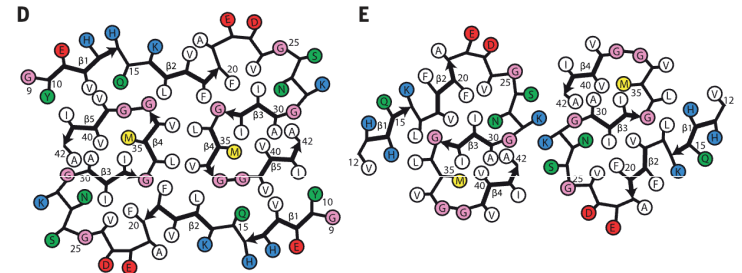
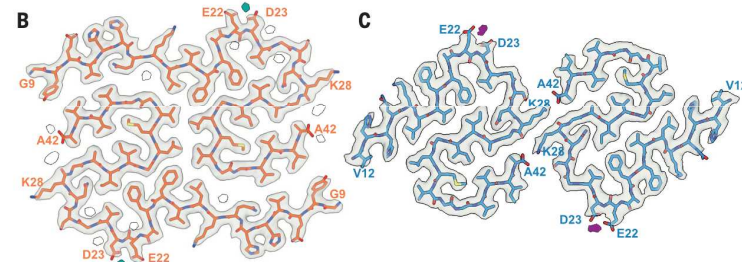
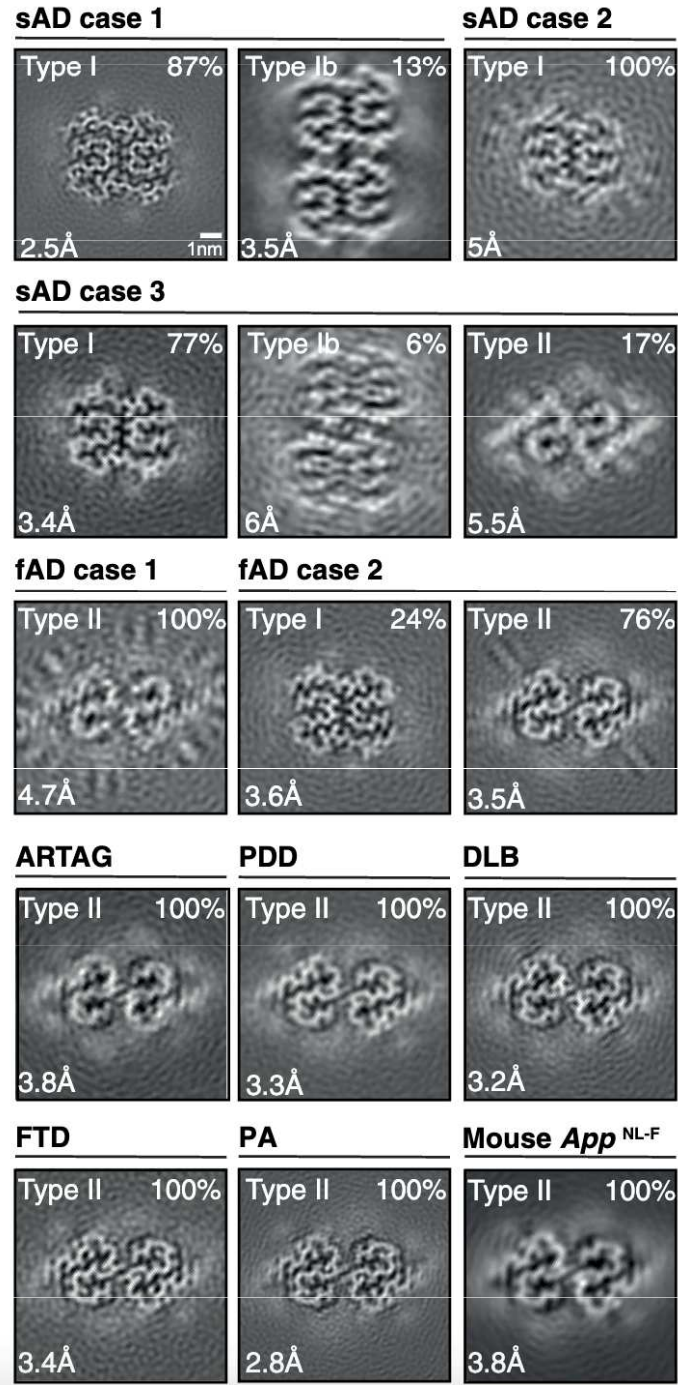






bioRxiv preprint doi: <https://doi.org/10.1101/2023.09.13.557623>

Cryo-EM structures of amyloid- β 42 filaments from human brains



Mosalaganti, Obarska-Kosinska, Siggel et al

Title: Artificial intelligence reveals nuclear pore complexity

Authors:

Shyamal Mosalaganti^{1,2,3†}, Agnieszka Obarska-Kosinska^{1,4†}, Marc Siggel^{4,5,6†}, Beata Turonova^{1,2}, Christian E. Zimmerli^{1,2}, Katarzyna Buczak^{2‡}, Florian H. Schmidt^{2§}, Erica Margiotta^{1,2}, Marie-Therese Mackmull^{2¶}, Wim Hagen², Gerhard Hummer^{5,7*}, Martin Beck^{1,2*}, Jan Kosinski^{2,4,6*}

THE BEST INVENTIONS OF 2022

200 innovations changing how we live

How We Chose the List



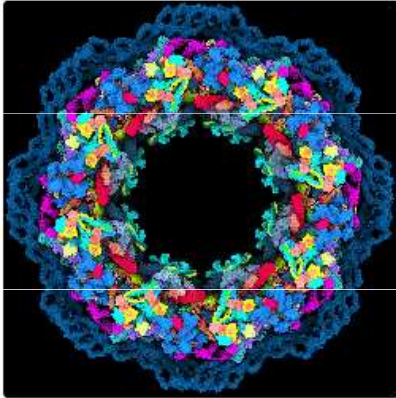
Sergiy Barchuk for TIME

TIME
SITES

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://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

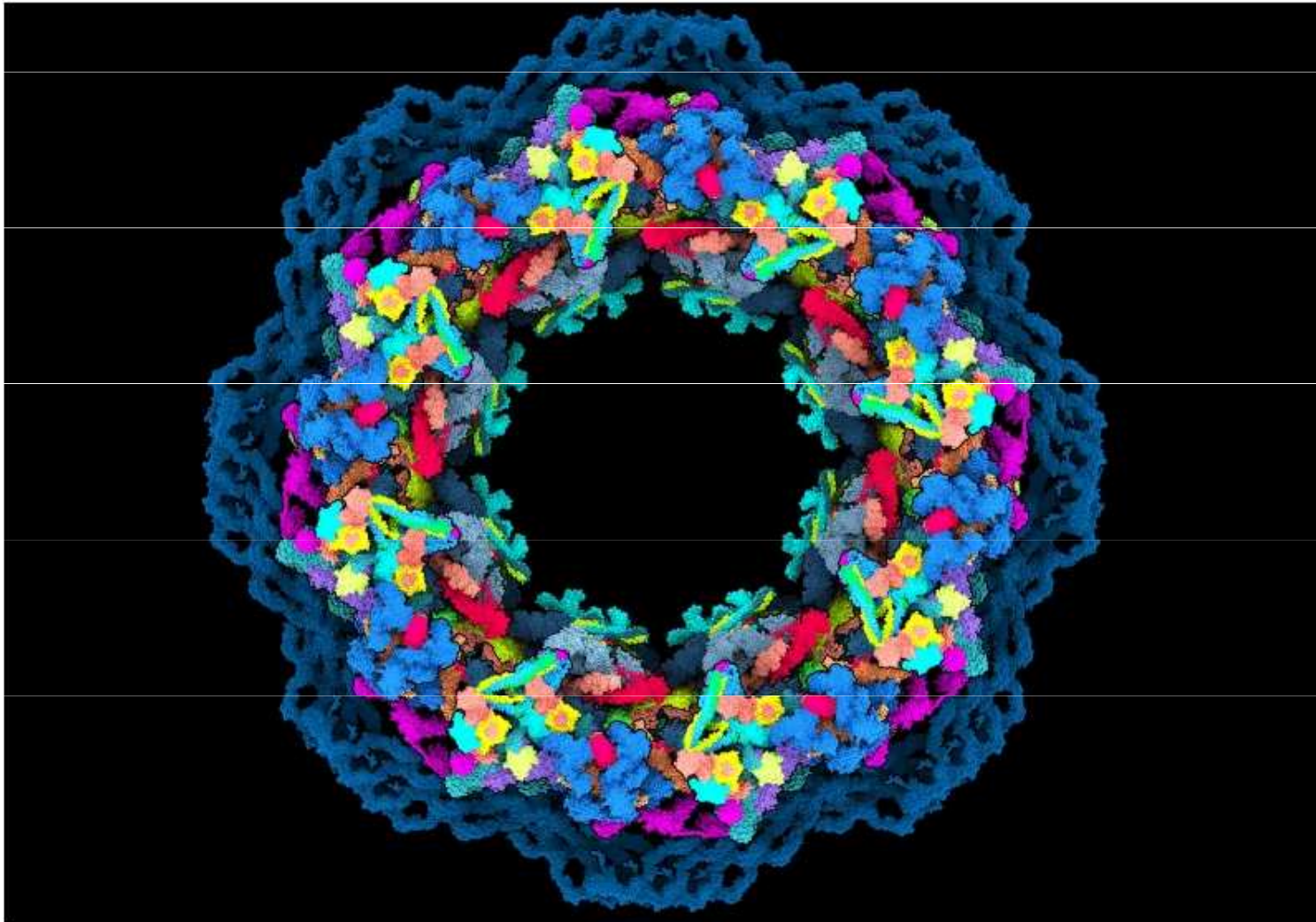


ARTIFICIAL IMAGINATION
OPENAI DALL·E 2

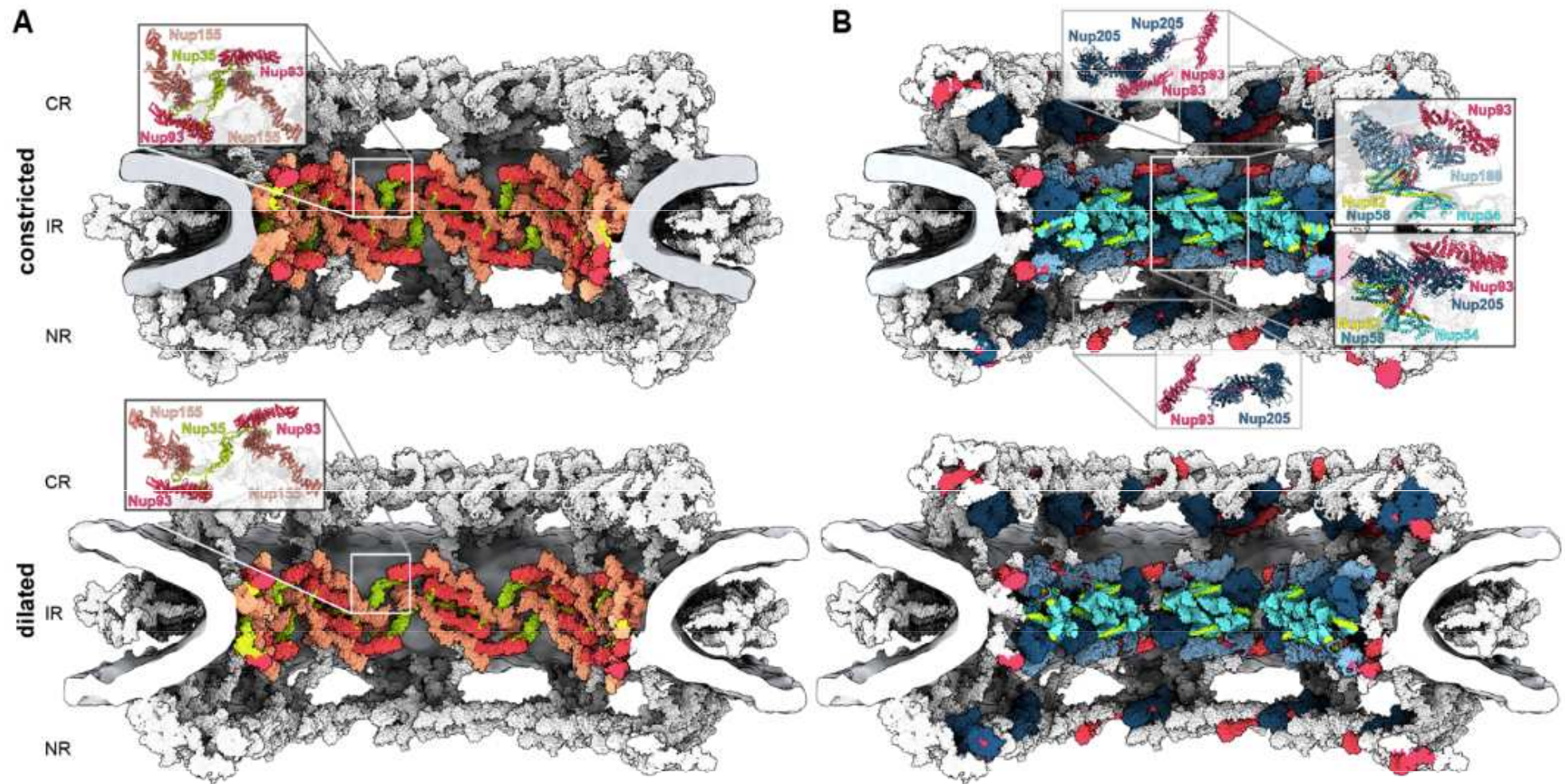


Mapping Life

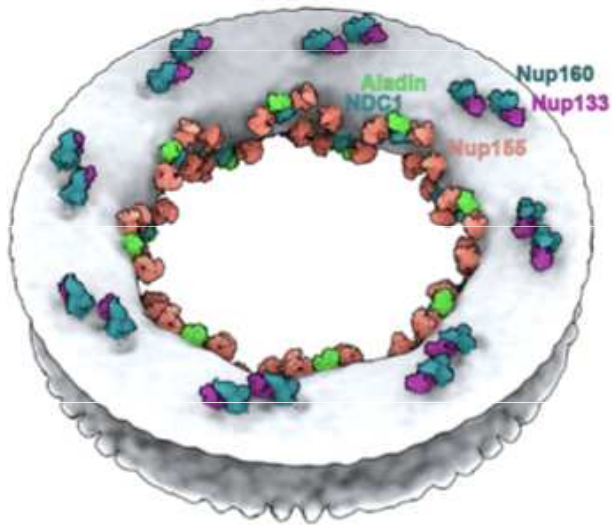
DeepMind AlphaFold



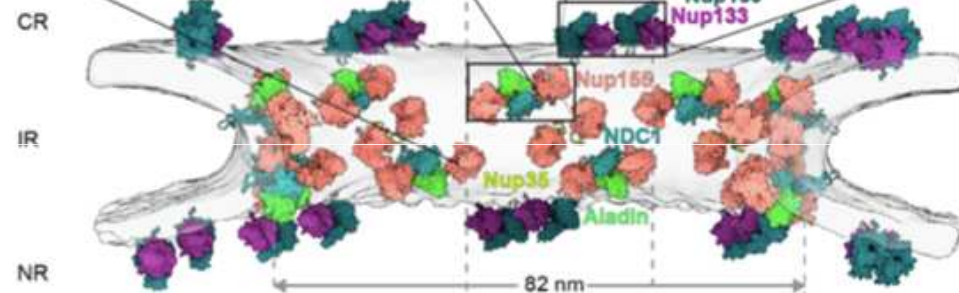
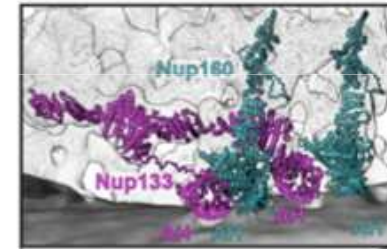
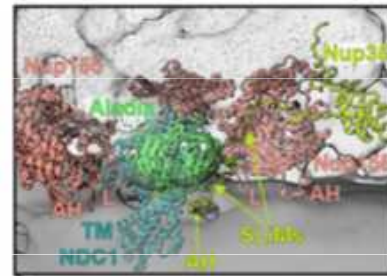
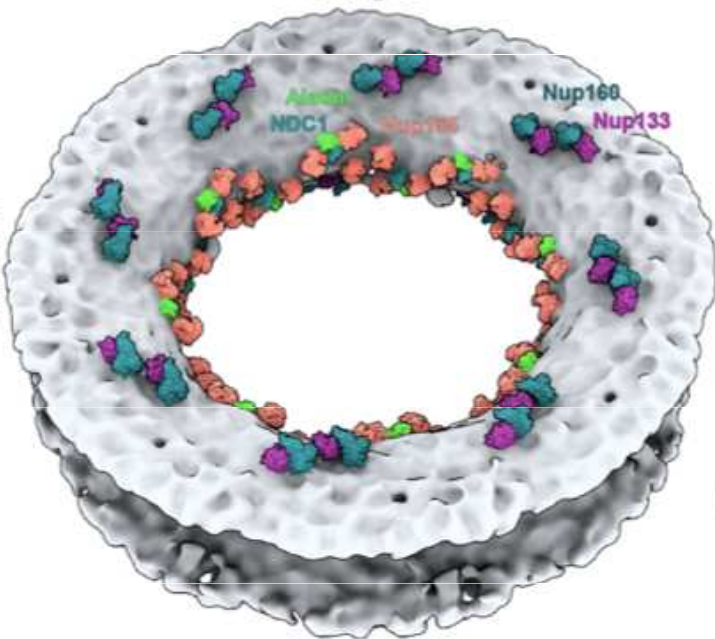
In the recent past, discerning the exact 3D structure of a single protein took around five years. Today the same task is possible in seconds thanks to the machine learning program **AlphaFold**, developed by Alphabet subsidiary DeepMind. In July, the company announced that AlphaFold had predicted the 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



← one spoke →

