Nanoszerkezetek és biomarkerek a gyógyítás szolgálatában

Dékány Imre
MTA r.tagja

MTA SZTE Szupramolekuláris és Nanoszerkezetű Anyagok

Kutatócsoport

SZTE AOK Orvosi Vegytani Intézet

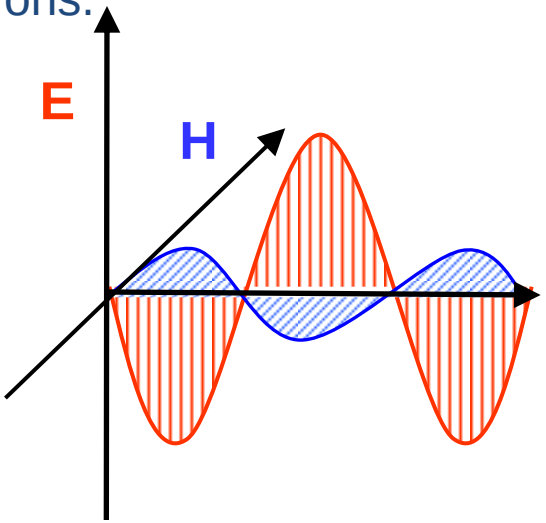
Szeged, Dóm tér 8.

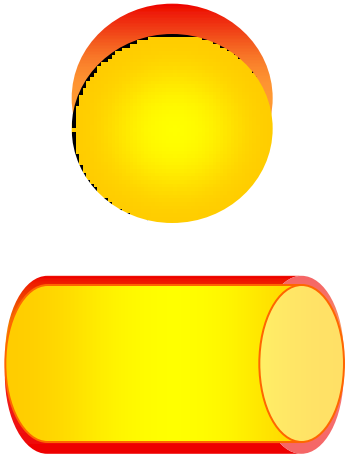
Tartalmi összefoglaló

- SPR 2D technológia alkalmazása az adszorpció kvantitativ mérésére: aminosavak és proteinek
- Hatóanyagok és toxikius molekulák megkötése funkcioanlizált Au felületeken
- Au nanorészecskék lipid membránokban
- Au plazmonikus csatolás biomolekulákkal
- Hatóanyagok nanokapszulázása

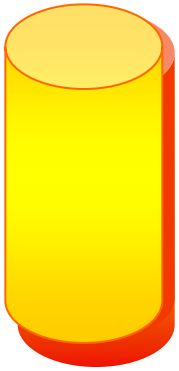
Au és Ag nanorészecskék optikai tulajdonságai

Surface plasmon resonance (SPR) ⇨ These phenomena occur when electromagnetic field interacts with conduction band electrons and induces the coherent oscillation of electrons.

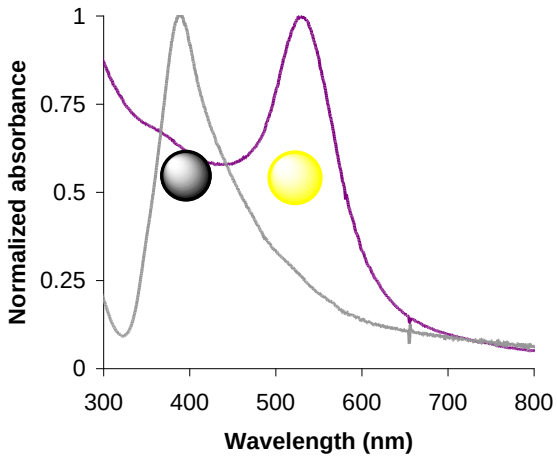




transverse oscillation (**TSP**)



longitudinal oscillation (**LSP**)

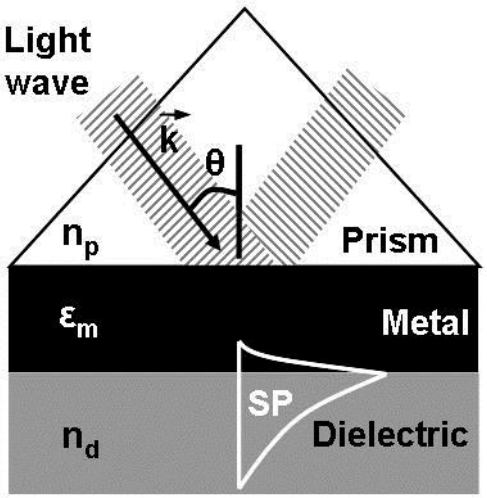


Plasmon bands of spherical gold and silver nanoparticles

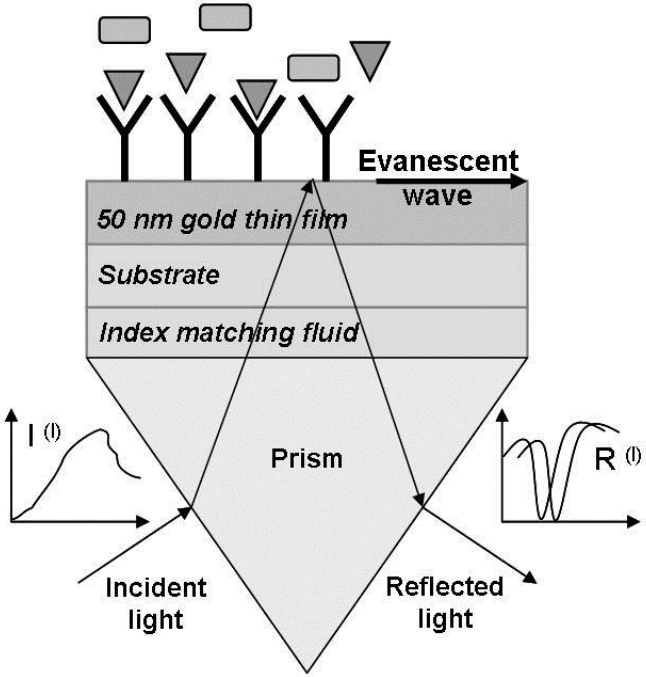
Ag TSP : λ_{max1} = 385 – 420 nm ~ 1/r

Au TSP : λ_{max1} = 500 – 550 nm ~ 1/r

Az SPR mérés kísérleti elrendezése

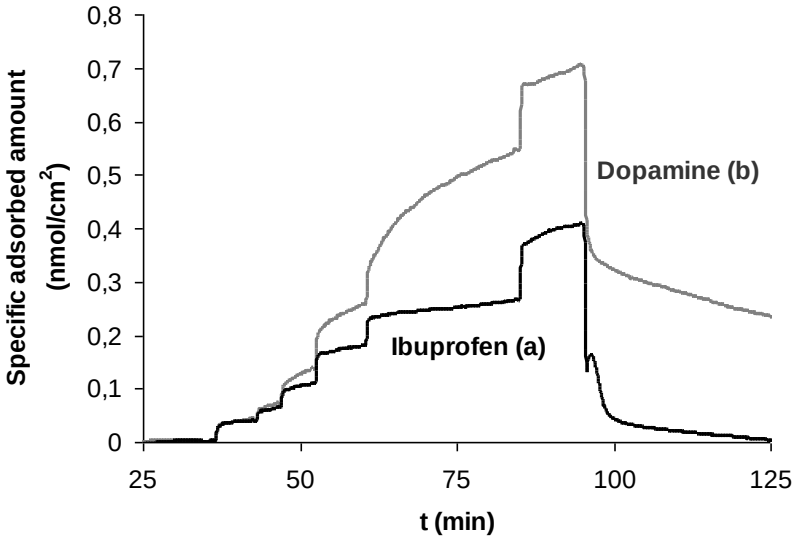


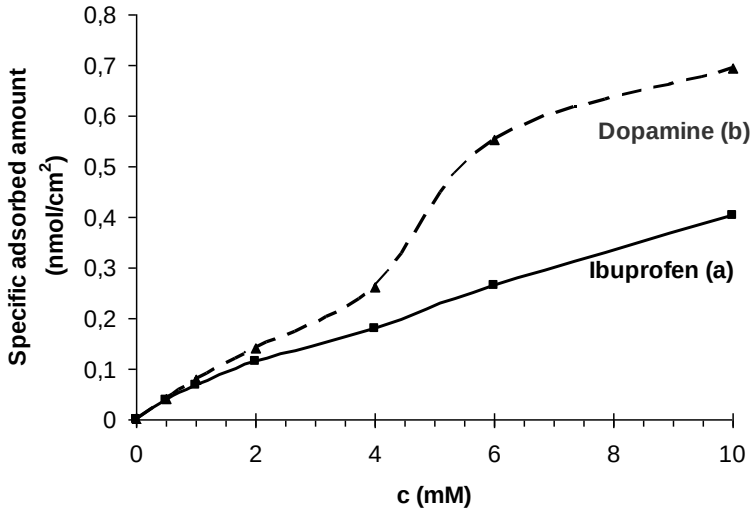
The prism coupling (Kretschmann-configuration) in SPR technique



Schematic representation of Kretschmann-configuration

SPR mérések és adszorpciós izotermák

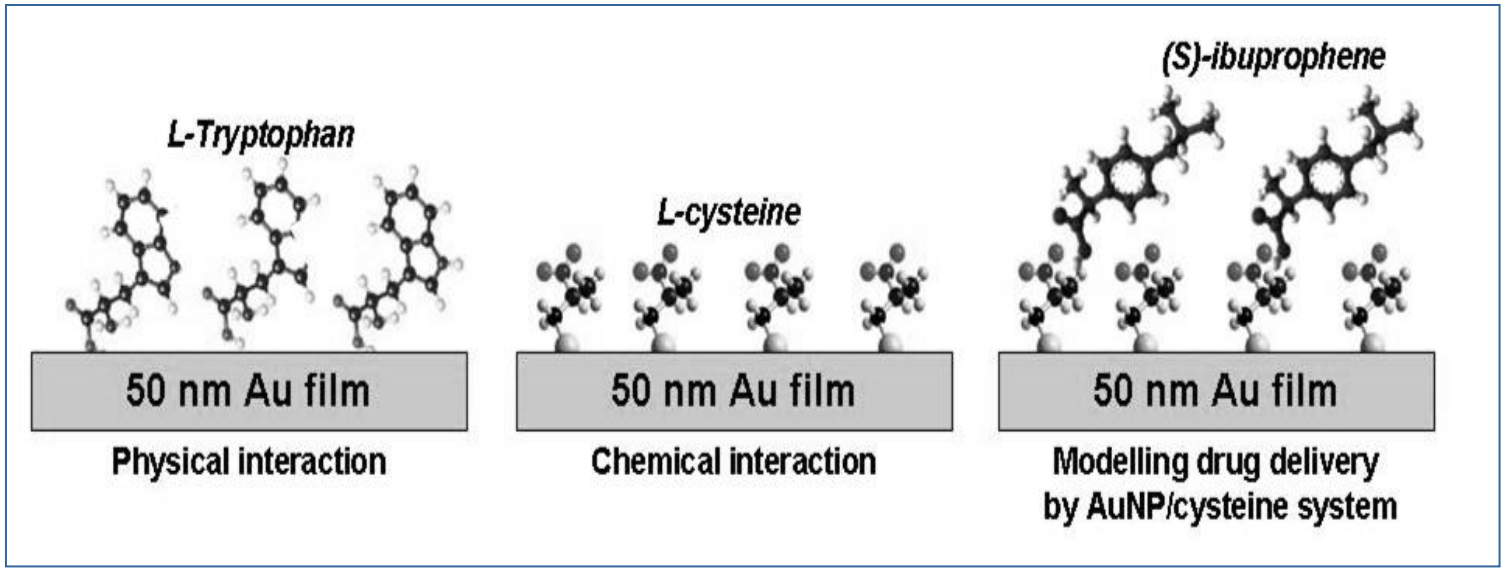




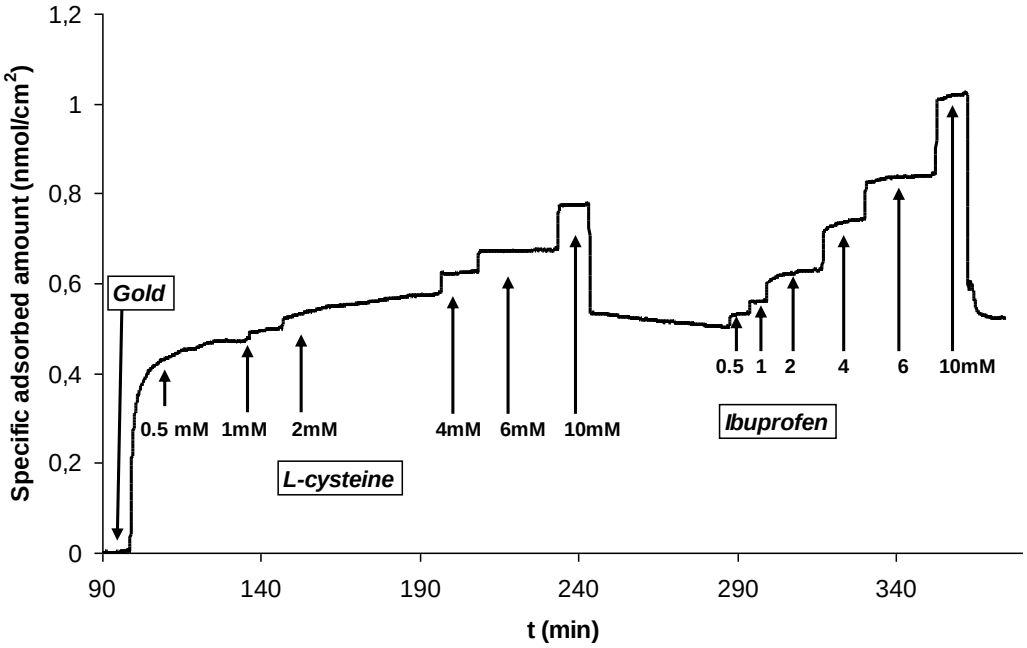
The plasmonic curves of adsorption of (a) ibuprofen and (b) dopamine on gold surface at different concentrations (0.5, 1, 2, 4, 6, 10 mmol/dm^3 aqueous solutions)

The adsorption isotherm of (a) ibuprofen and (b) dopamine from aqueous solution on gold surface

Adszorpció és felületi orientáció



SPR mérések: Au-cisztein- ibuprofen rendszer



The plasmonic curves of adsorption of ibuprofen on *L*-cysteine functionalized gold surface from aqueous solutions (at 0.5, 1, 2, 4, 6, 10 mmol/dm^3 concentrations)

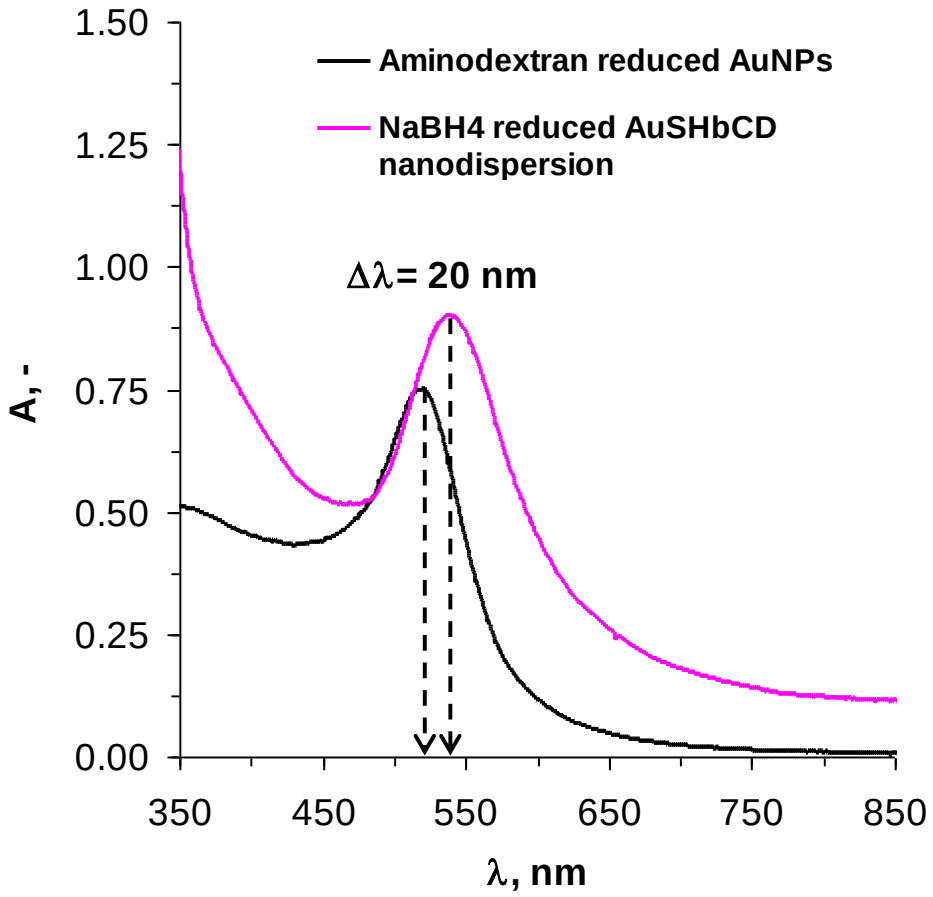
Table 1. The monolayer adsorption capacities (G_{mono}) and molecular cross section areas (a) on gold surface for different bioconjugated systems as obtained from adsorption isotherms

Molecules on gold surface	Monolayer capacity, $\Gamma_{m.}$/nmol cm^{-2} Eq. (5)	Cross sectional area, $a_{m.}$/nm^2 Eq. (6)	$a_m/a_{m,calc}$	Calculated cross sectional area * $a_{m,calc}$/nm^2	Surface orientation
L-Cysteine	0.325	0.513	1.425	0.360	parallel
L-Glutathion	0.135	1.234	1.505	0.820	parallel
Ibuprofen	0.330	0.505	0.789	0.640	parallel
Dopamine	0.860	0.194	0.359	0.540	perpendicular

Molecules on functionalised gold surface	Adsorptioncapacity, $\Gamma_{m.}$/nmol cm^{-2} Eq. (5)	Cross sectional area, $a_{m.}$/nm^2 Eq. (6)	$a_m/a_{m,calc}$	Calculated cross section area, *$a_{m,calc}$/nm^2	Surface orientation
L-Cyst-Ibuprofen	0.325	0.513	0.801	0.640	parallel
L-Glut-Ibuprofen	0.180	0.926	1.447	0.640	parallel
L-Cyst-Dopamine	0.640	0.260	0.481	0.540	perpendicular
L-Glut-Dopamine	0.580	0.287	0.531	0.540	perpendicular

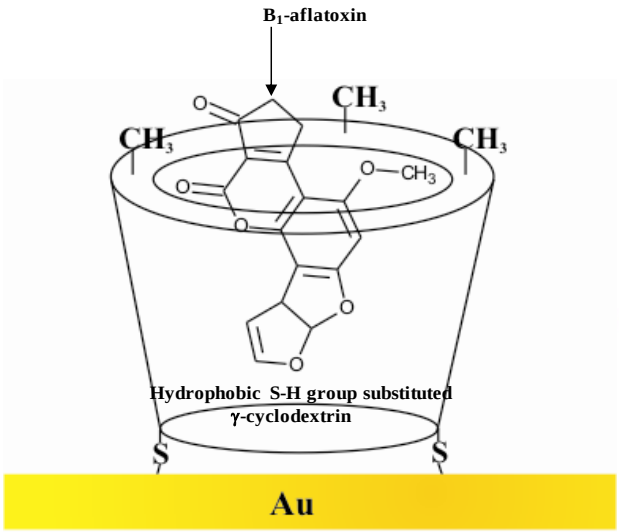
Arany nanoszenzorok aflatoxinok kimutatására

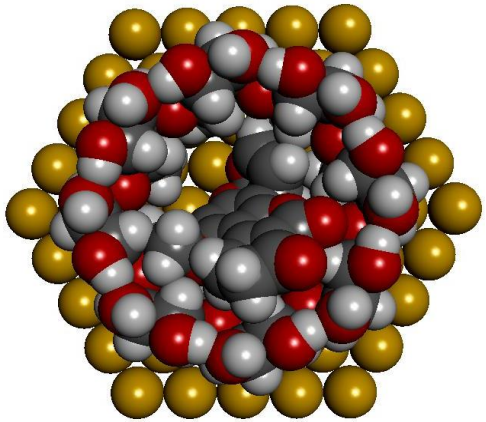
UV-Vis plazmonikus spektrumok

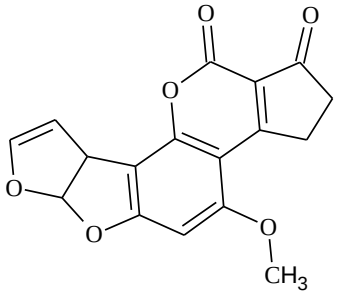


The changes of plasmon bands of aminoldextran reduced gold nanodispersion and AuSHβCD gold nanodispersions(164 mg/ml; 0.5 mM Au) registered by UV-Vis spectroscopy.

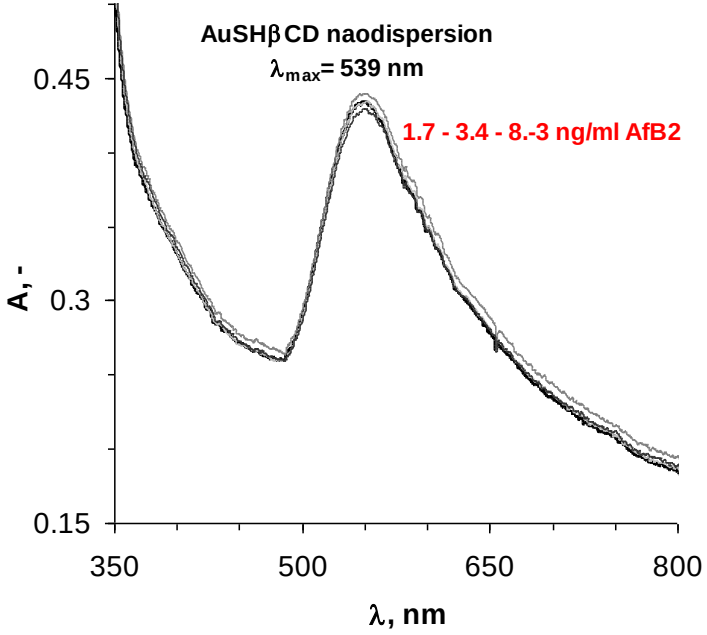
UV-Vis mérések



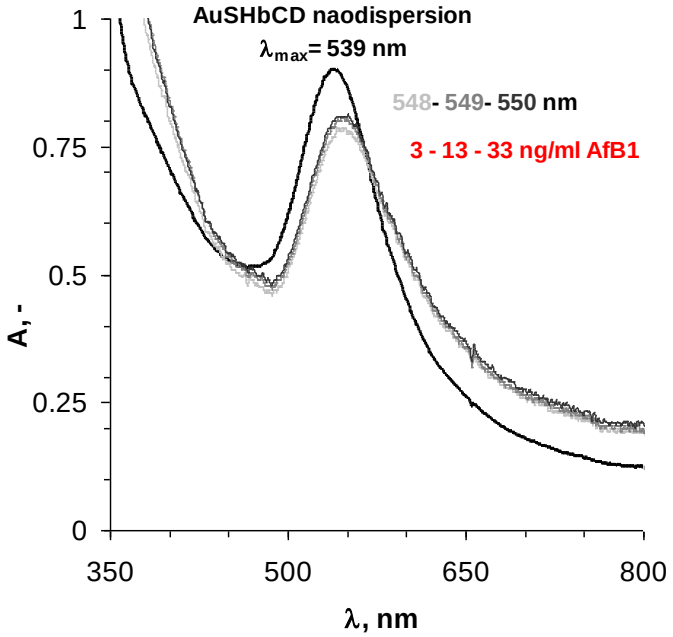




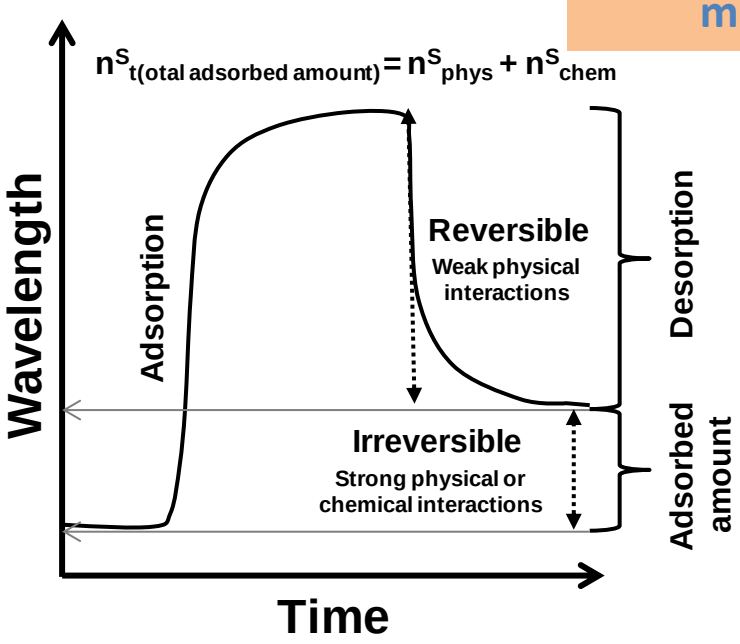
Attachment of Aflatoxin B1 molecules to cyclodextrin modified gold surface and β-cyclodextrin on gold crystal surface (111).



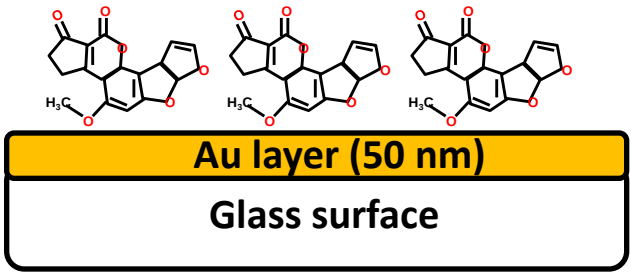
The changes of plasmon bands of AuSHβCD gold nanodispersions (164 mg/ml; 0.5 mM Au) added AfB1 (in concentration range 3-33 ng/ml AfB1 and 1.7-8.3 ng/ml AfB2) to the solution are also registered by UV-Vis spectroscopy in dilute acetonitril solution.



SPR mérések AfB1 molekulával



1) **Aflatoxin B1 (AfB1) molecules**



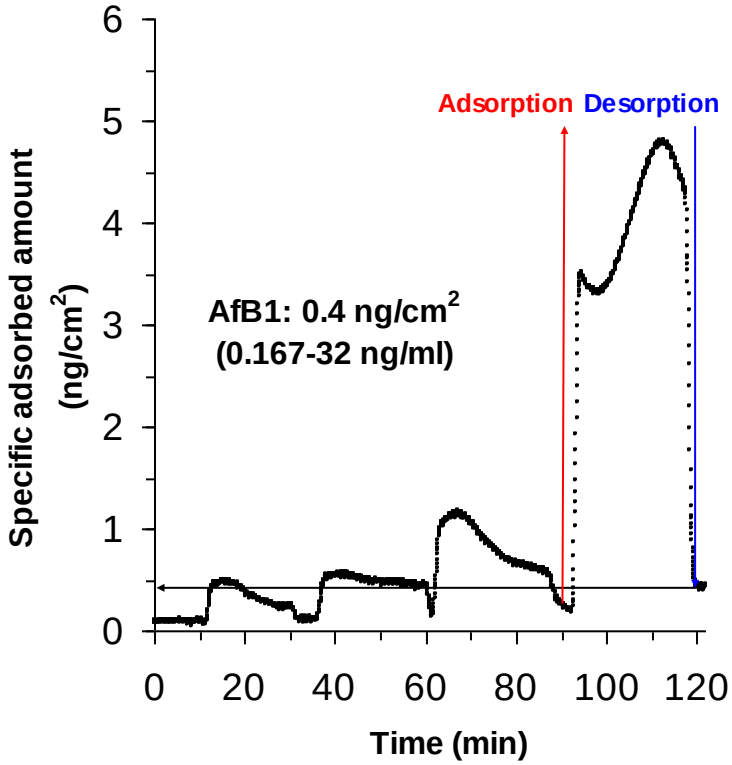
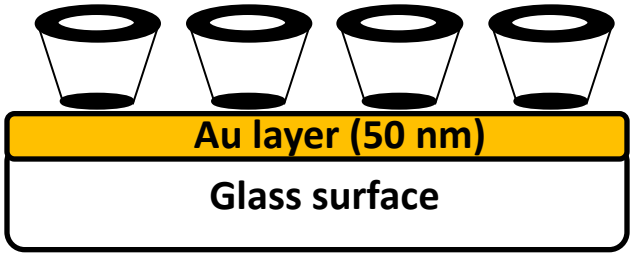
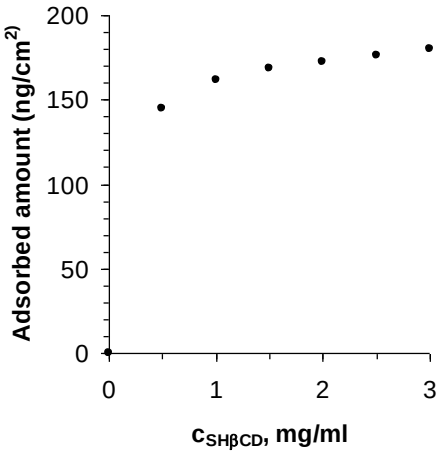


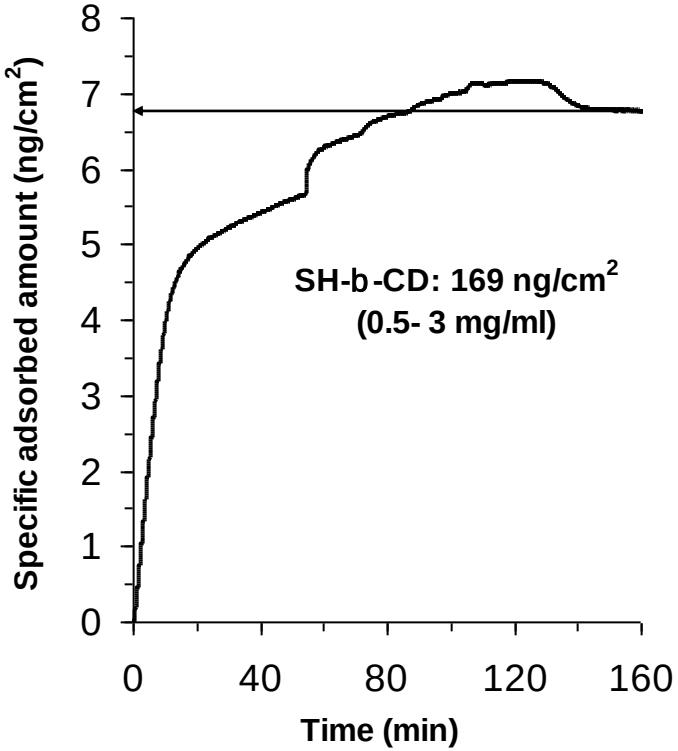
Fig. 7. Results of SPR measurements: AfB1 solution (0.167-32ng/ml) to gold surface.

2)

SH modified βCD (βCD-SH) molecules







3)

AfB1 to βCD-SH covered surface

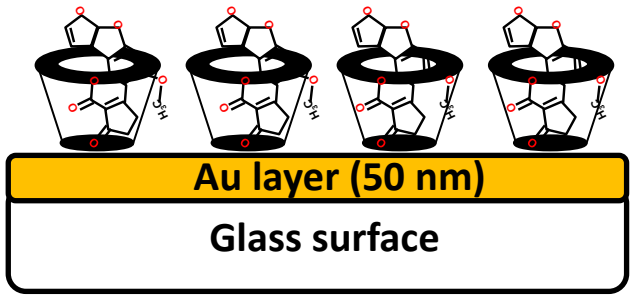
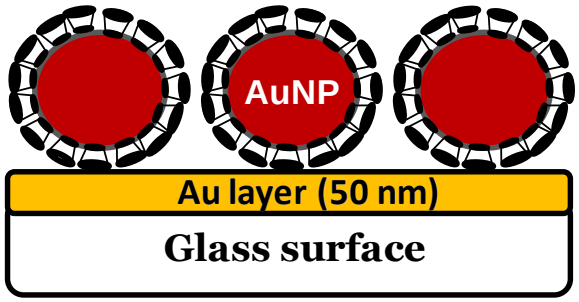
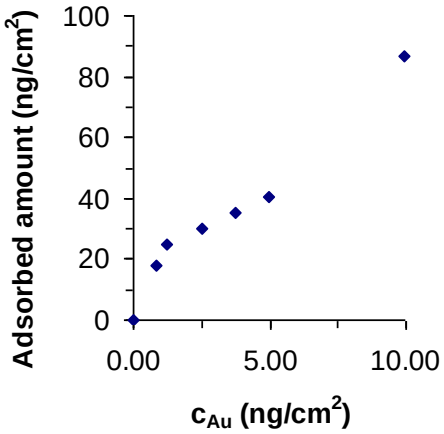


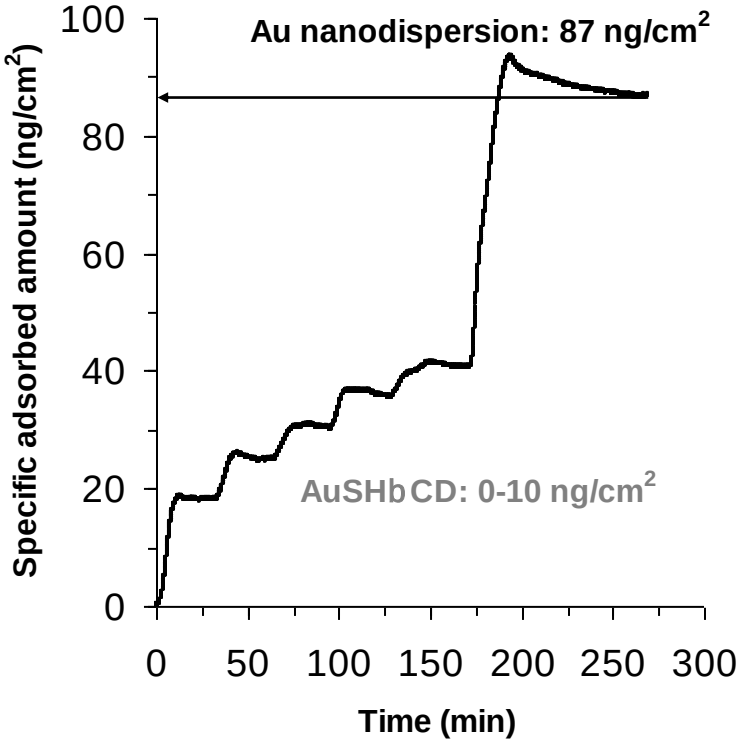
Fig. 8. Results of SPR measurements: βCD-SH (0.5-3 mg/ml) to gold surface.

4)

AuNPs with βCD-SH (βCDAuNPs)







Results of SPR measurements: AuSHβCD gold nanodispersions (0-10 ng/ml Au).

5) **AfB1 molecules attach to βCDs holes of βCDAuNPs**

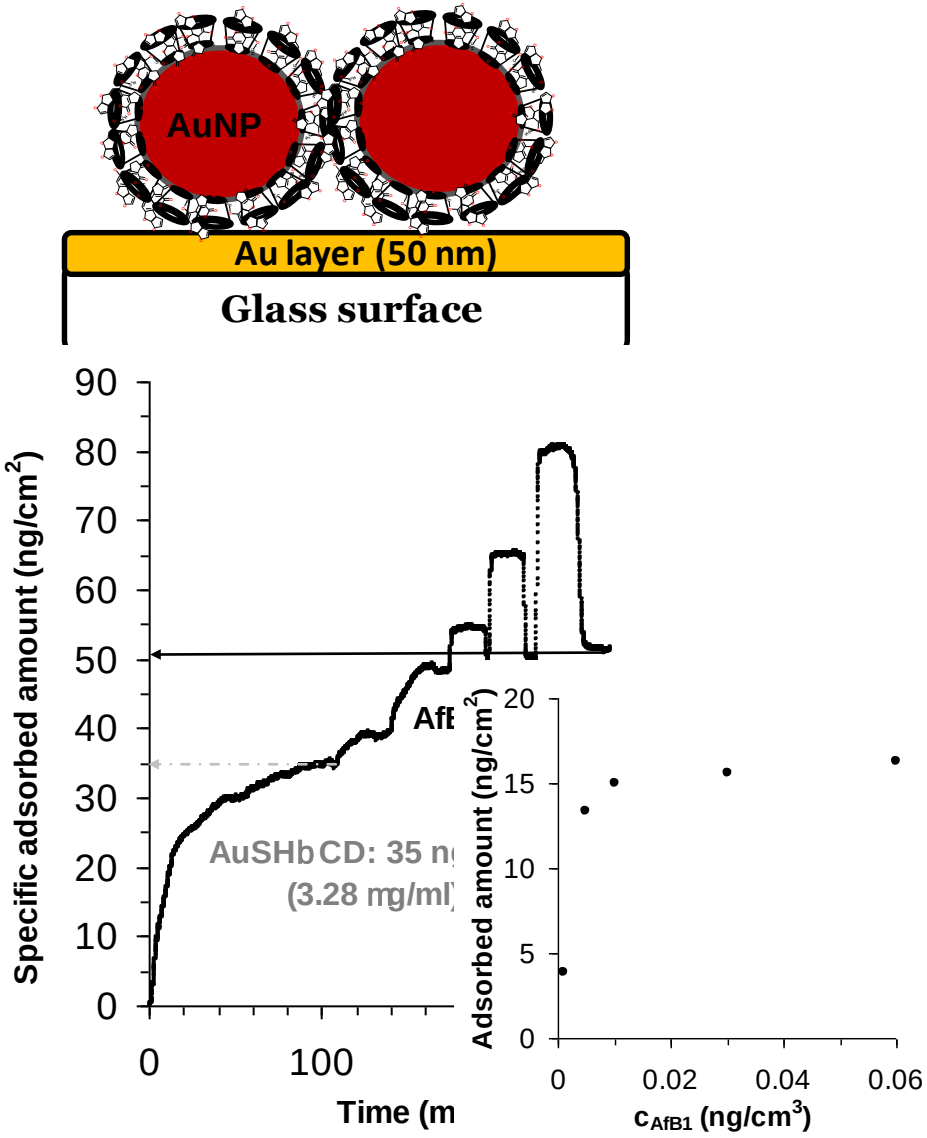
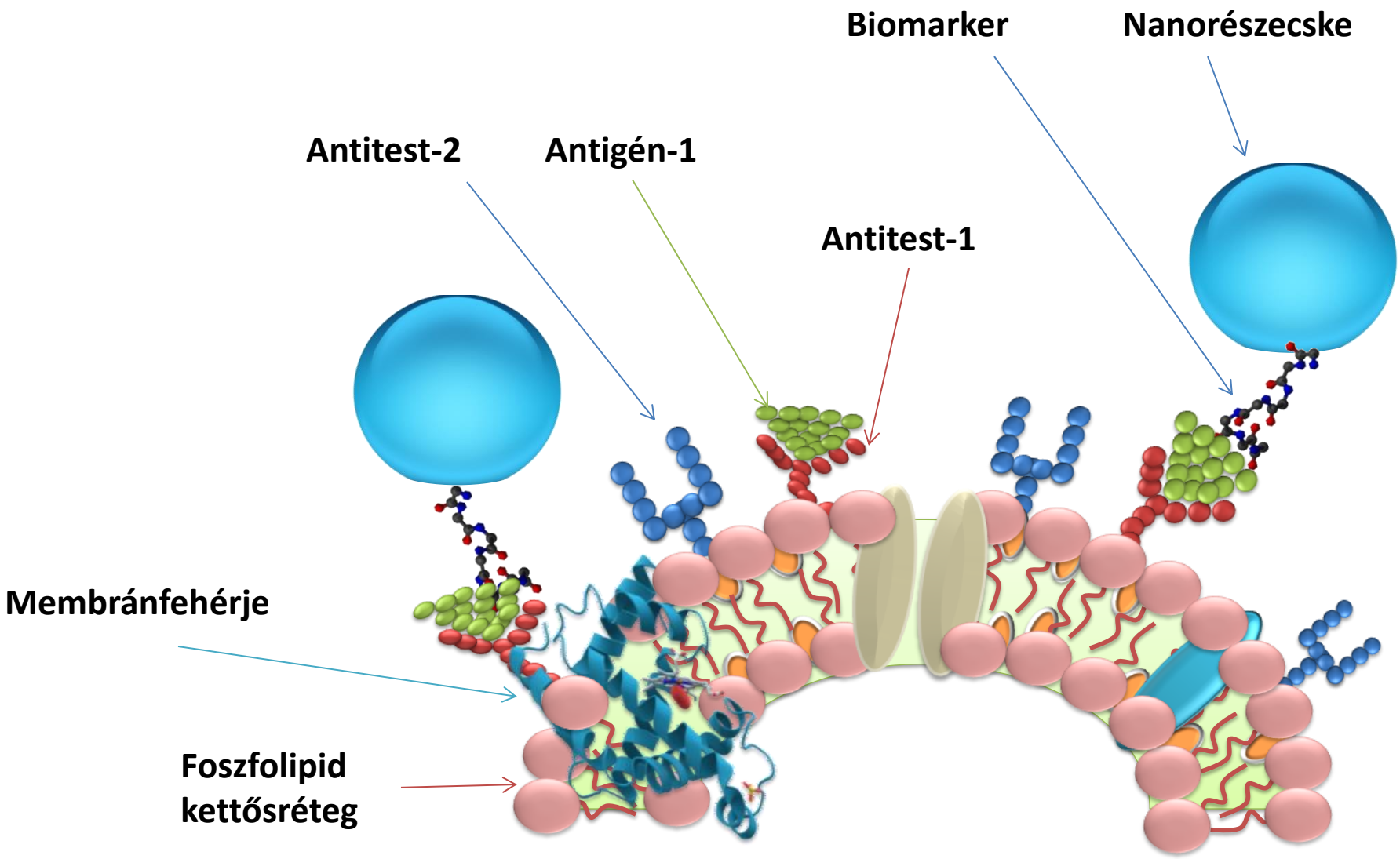
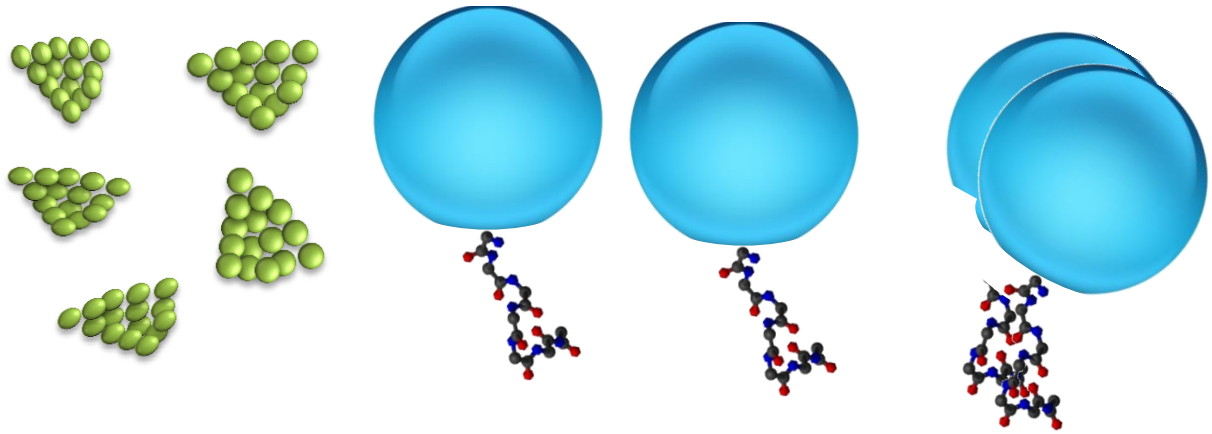
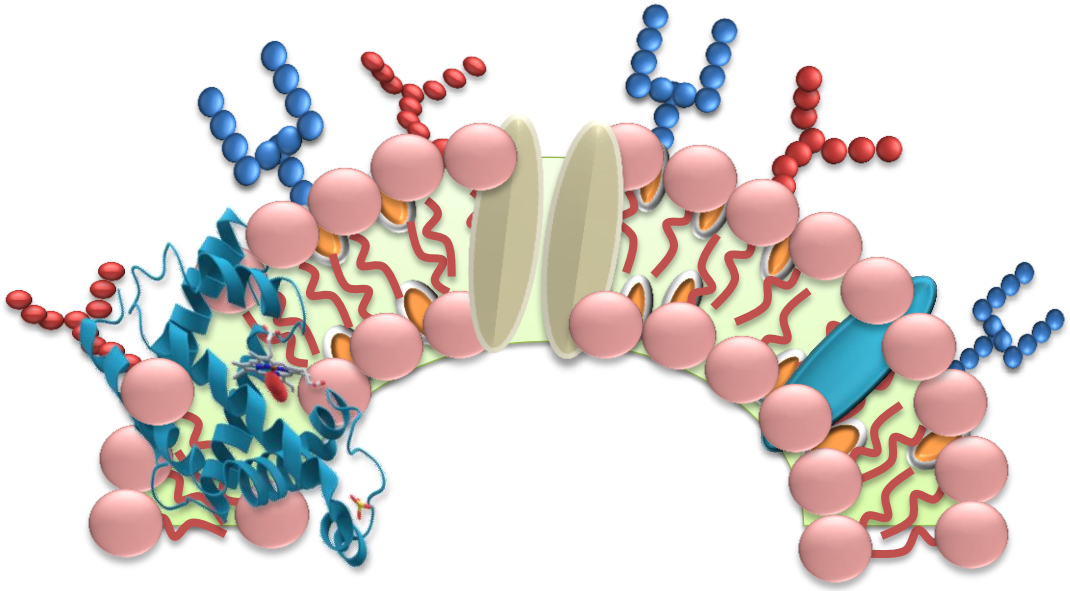


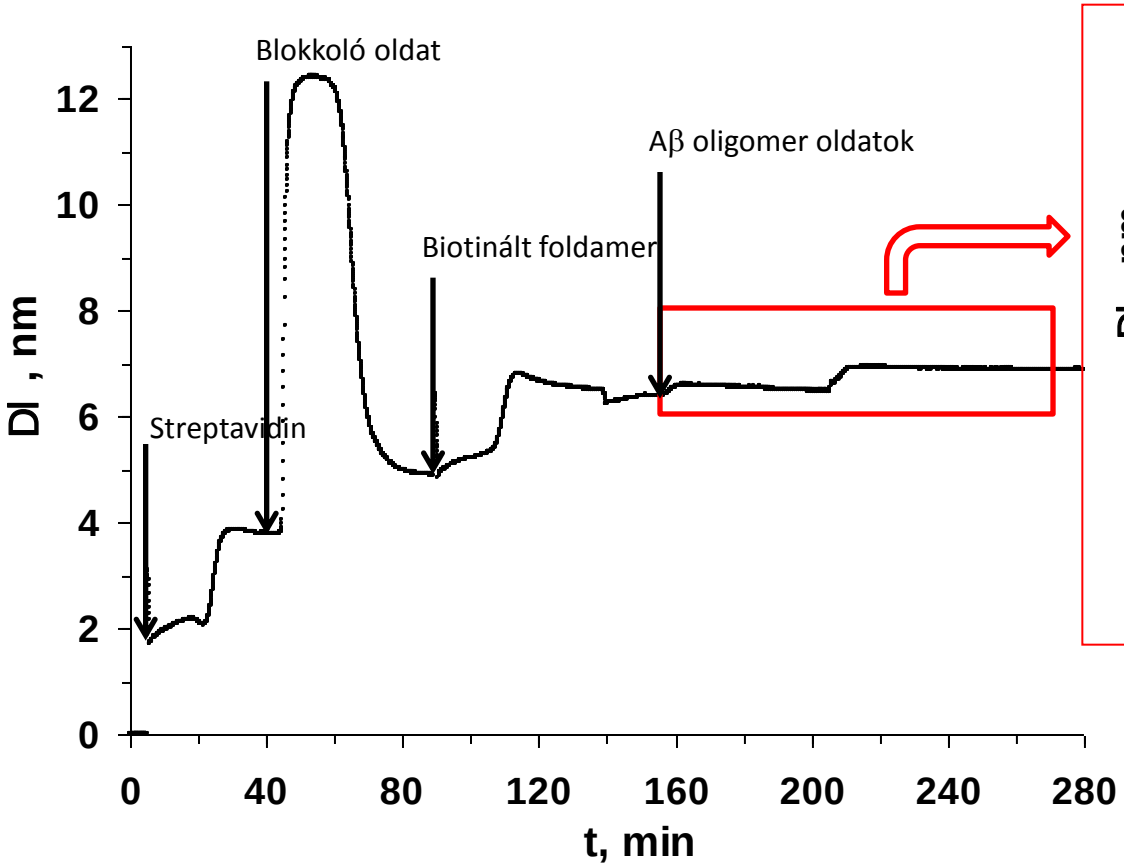
Fig. 11. Results of SPR measurements: in first step Au nanodisperson and after added AfB1 (in concentration range 0-32 ng/ml AfB1) in dilute acetonitril solution.

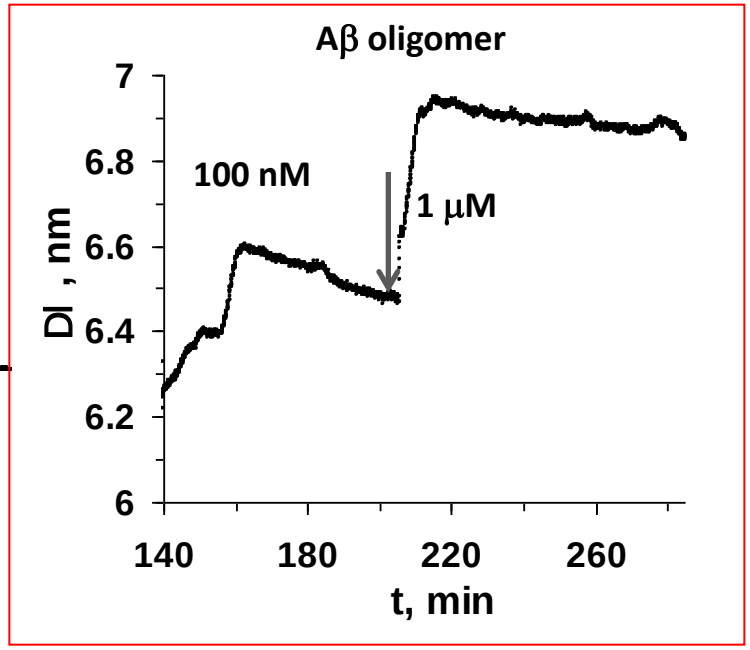

Biomarker
Nanorészecske
Antitest-2
Antigén-1
Antitest-1
Membránfehérje
Foszfolipid
kettősréteg



Az animáció kattintásra indul.



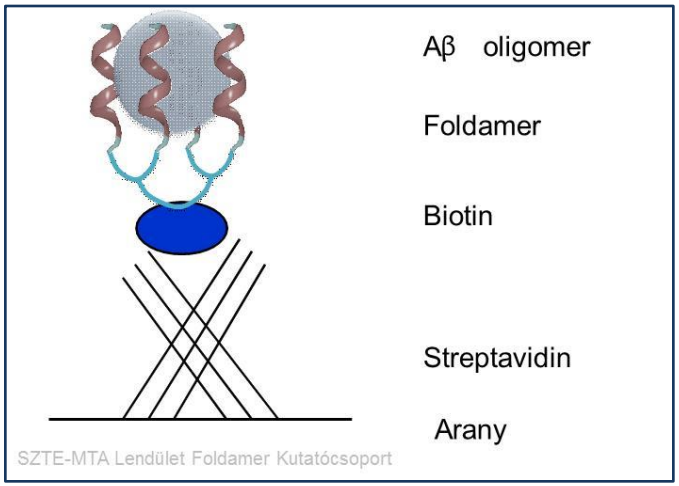




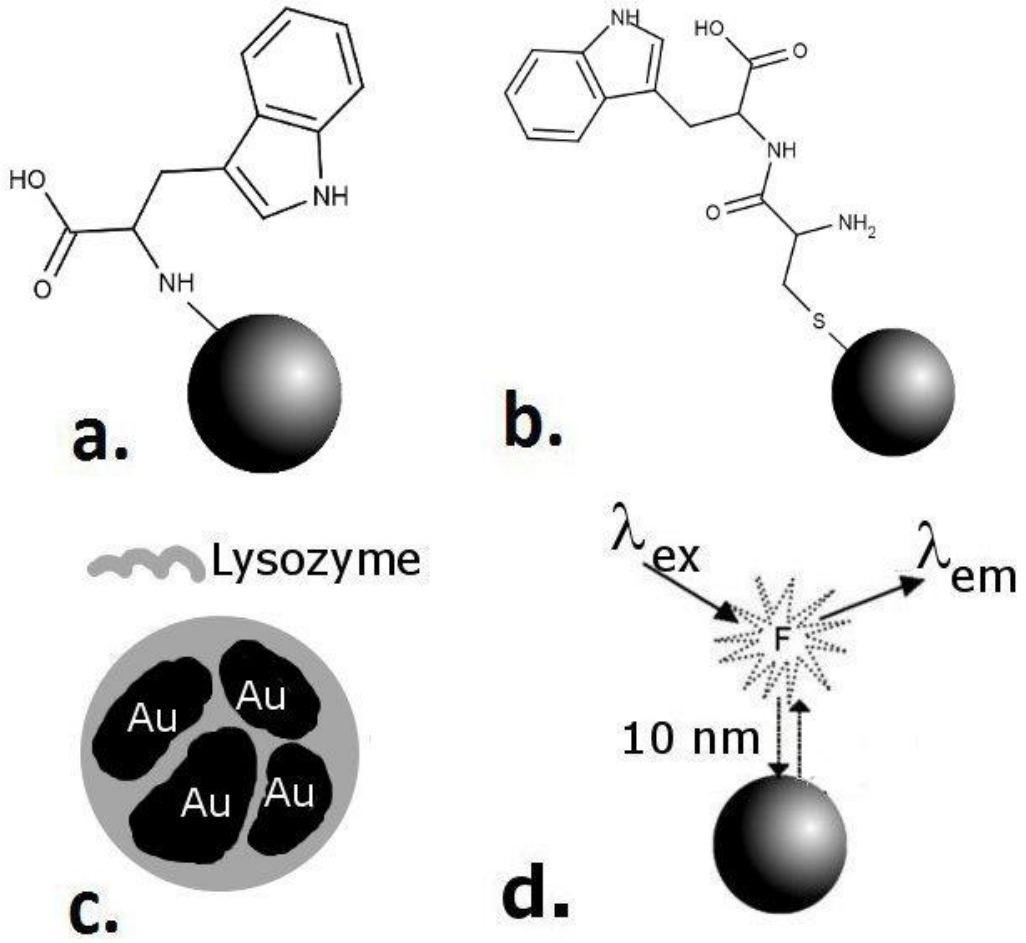
Közeg: PBS puffer oldat
Áramlási sebesség: 20 μL/perc

500 μL folyadéktérfogatok kerültek beadagolásra

1. Lépés: Streptavidin 10 μM
2. Lépés: BSA 1% oldata
3. Lépés: Biotinált foldamer 20 μM oldata
4. Lépés: Aβ oligomer 100 nM
5. Lépés: Aβ oligomer 1 μM

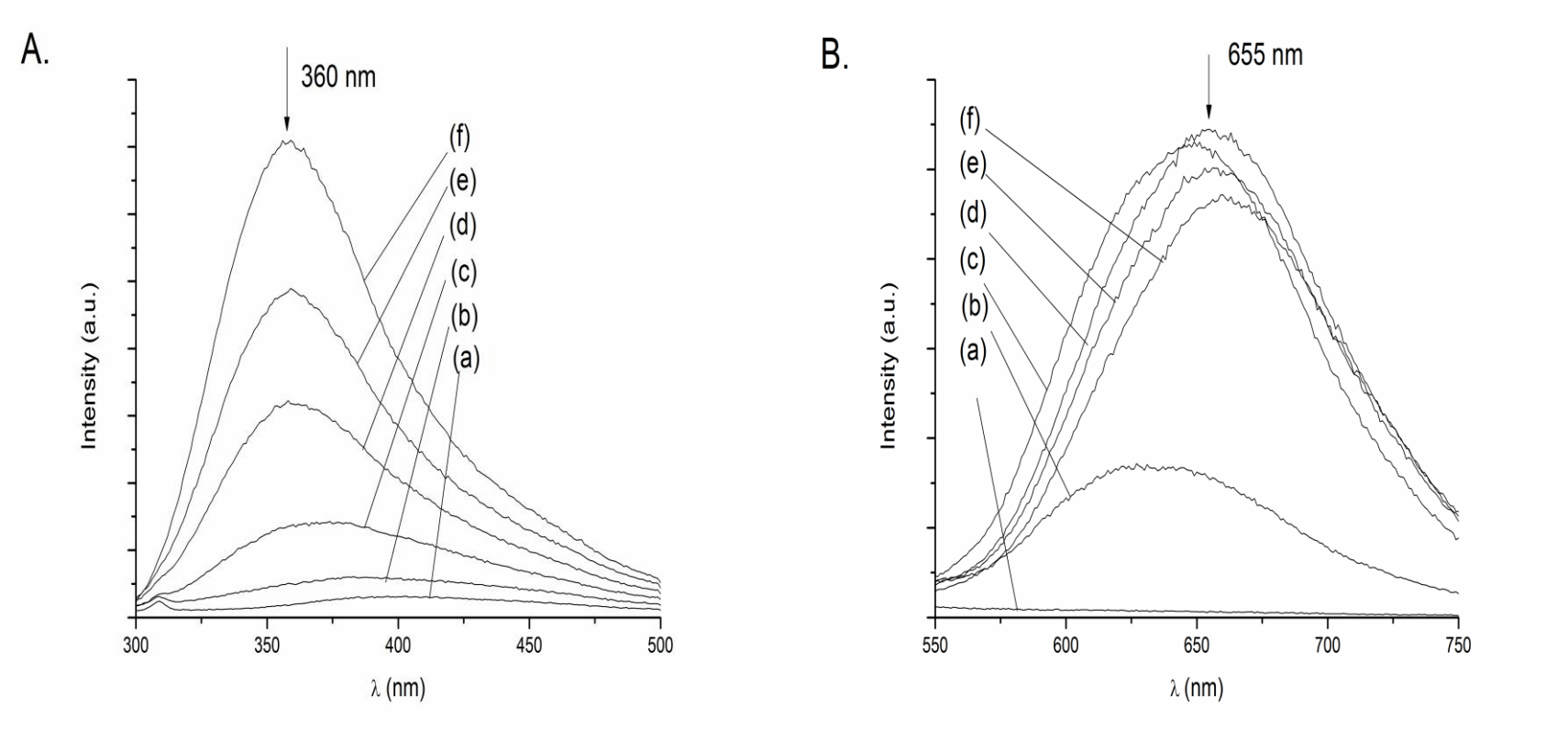


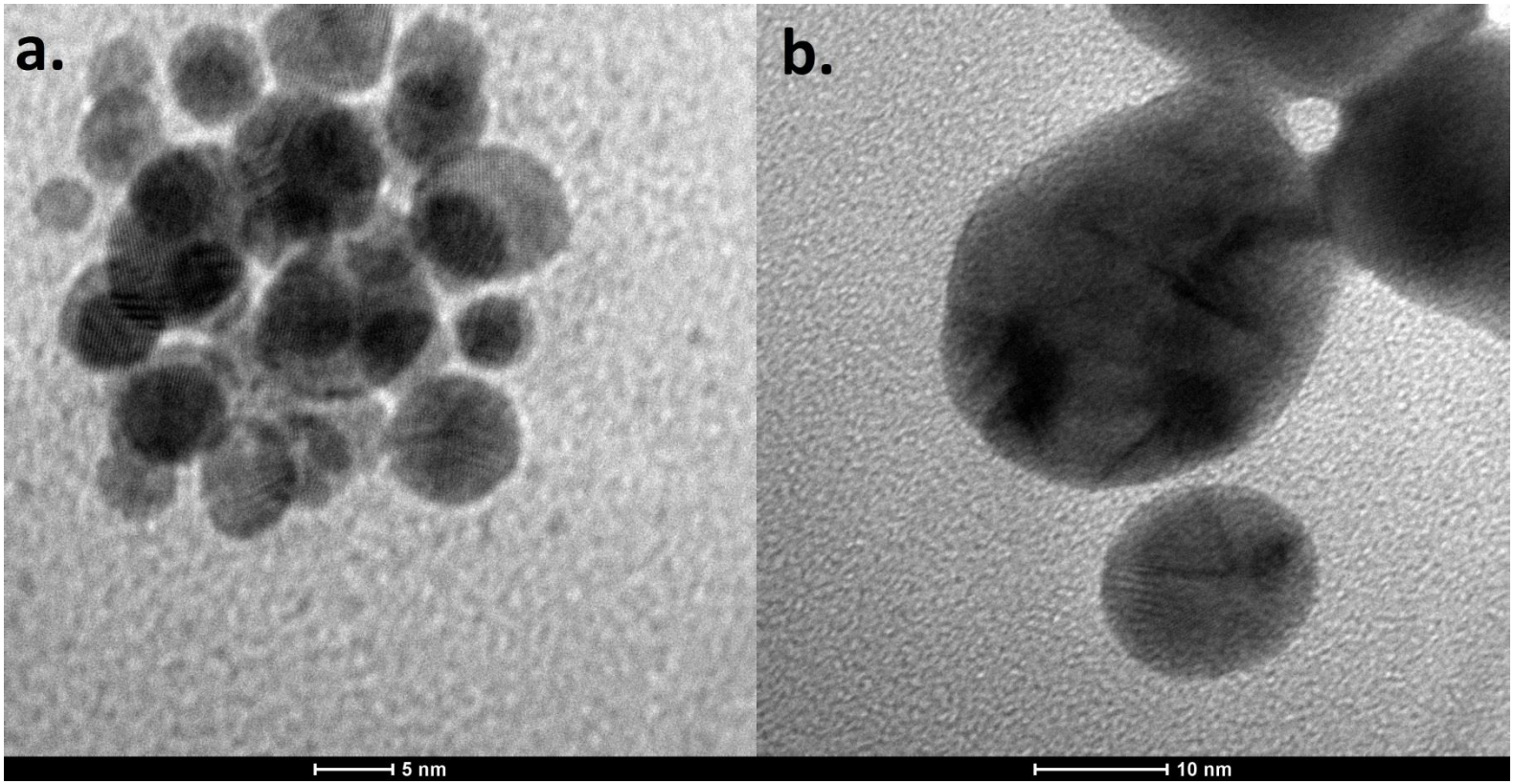
A plazmonikus csatolás lehetőségei



Schematic figures of the prepared AuNP samples (a) AuNP(Trp), (b) AuNP(CysTrp), (c) AuNP(Lys) and (d) the plasmonic coupling effect

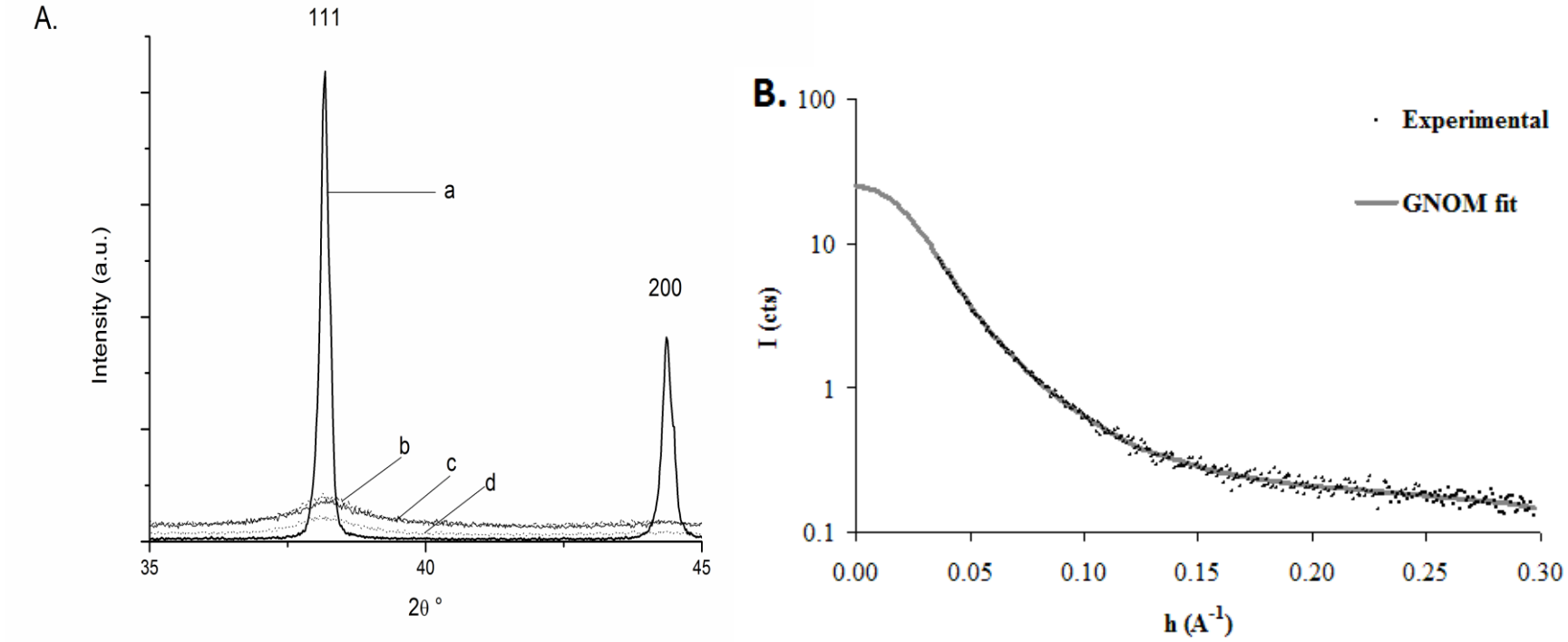
Fluoreszcencia spekrtumok Au-lizozim rendszereken





HRTEM images of the (a) AuNP(CysTrp) and (b) AuNP(Lys) samples with the ratio of $m_{Lys}/m_{Au} = 5$

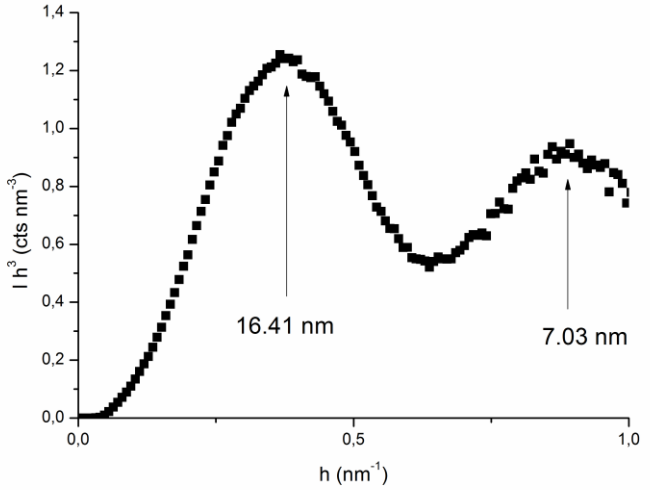
XRD mérések



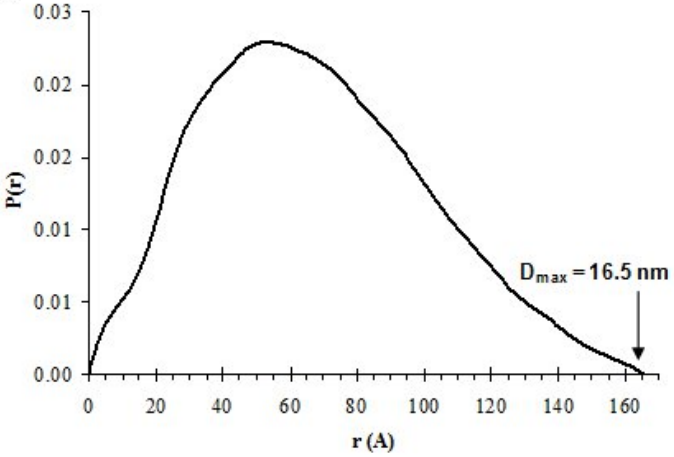
(A) XRD pattern of (a) bulk gold, (b) AuNP(cit), (c) AuNP(Lys) and (d) AuNP(Cys) samples (B) The SAXS curve of (a) AuNP(Lys) sample (c_{Lys}=1 mg/ml) and the calculated SAXS curve (GNOM fit)

SAXS mérések

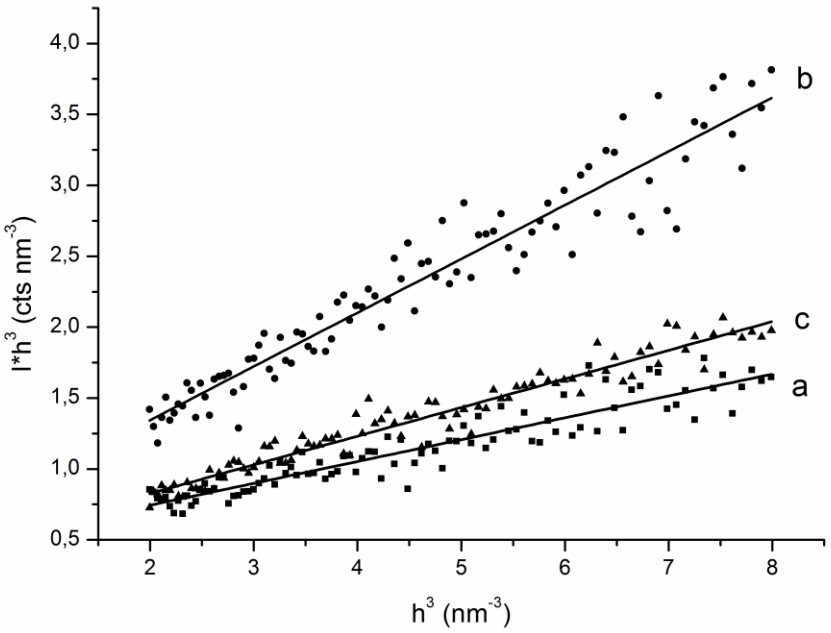
A.



B.

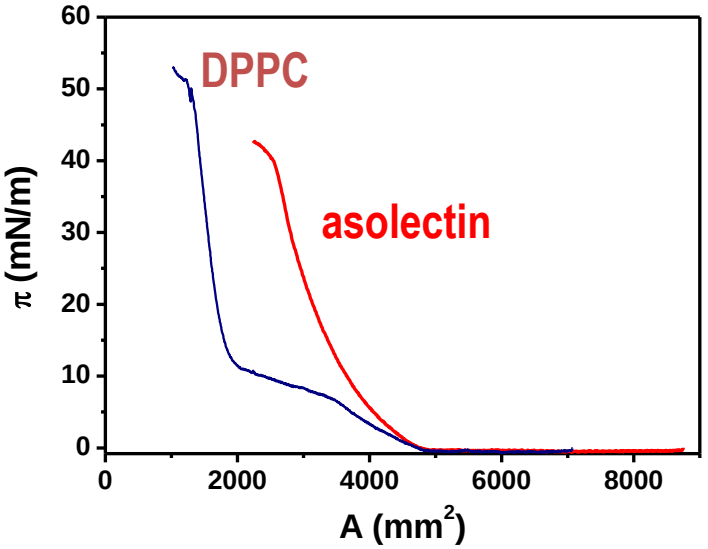


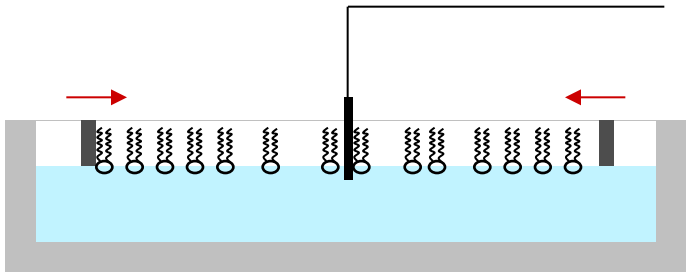
C.



(A) The SAXS curve of the AuNP(Lys) nanoparticles in Kratky representation (B) calculated pair distance distribution function of the AuNP(Lys) samples m_{Lys}/m_{Au} = 5 (C) the SAXS curves of Porod representation m_{Au}/m_{Lys} = 1:5 (a), 1:15 (b), 1:20 (c)

Langmuir monoréteg vizsgálatok





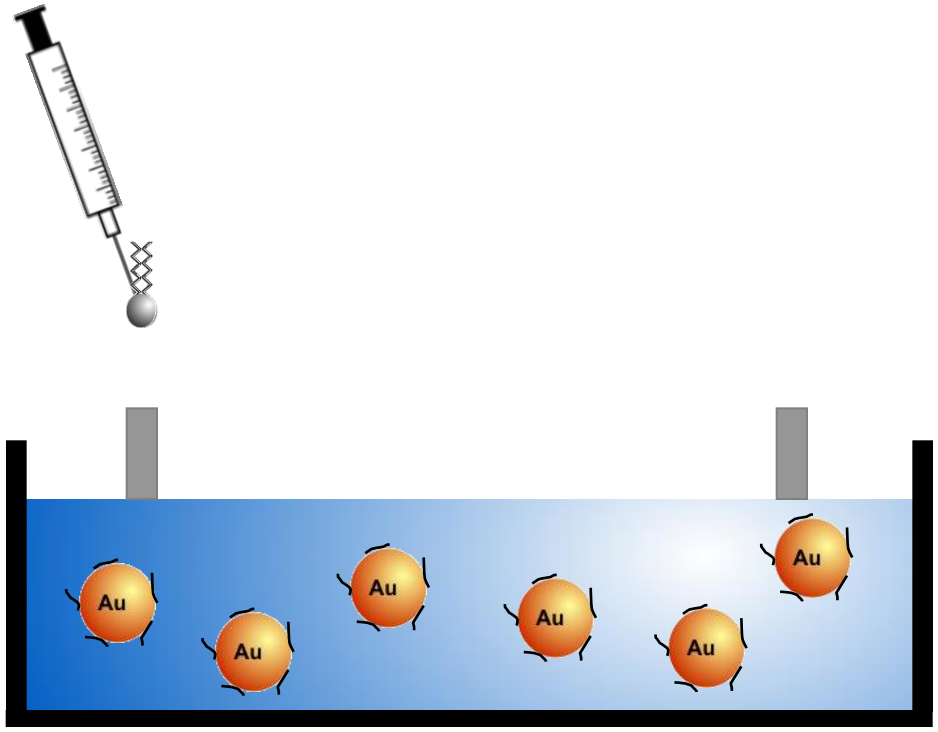
Surface pressure – area isotherms of the model membrane materials: DPPC and asolectin

DPPC: dipalmytoil-phosphatidylcholine
pure phospholipid

asolectin: mixture of phospholipids and fatty acids

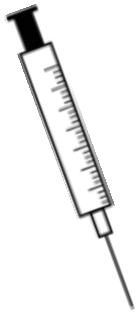
Au nanorészecskék beépülése a lipid membránba

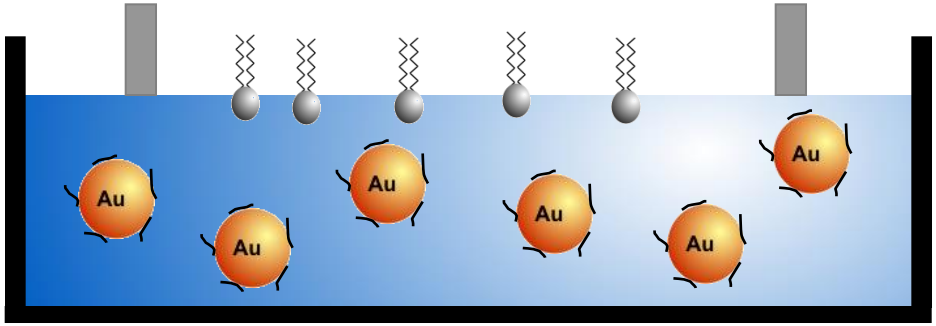
1. **Preparation of phospholipid monolayer at liquid/air interface**



Schematic representation of the nanoparticles penetration into lipid membrane

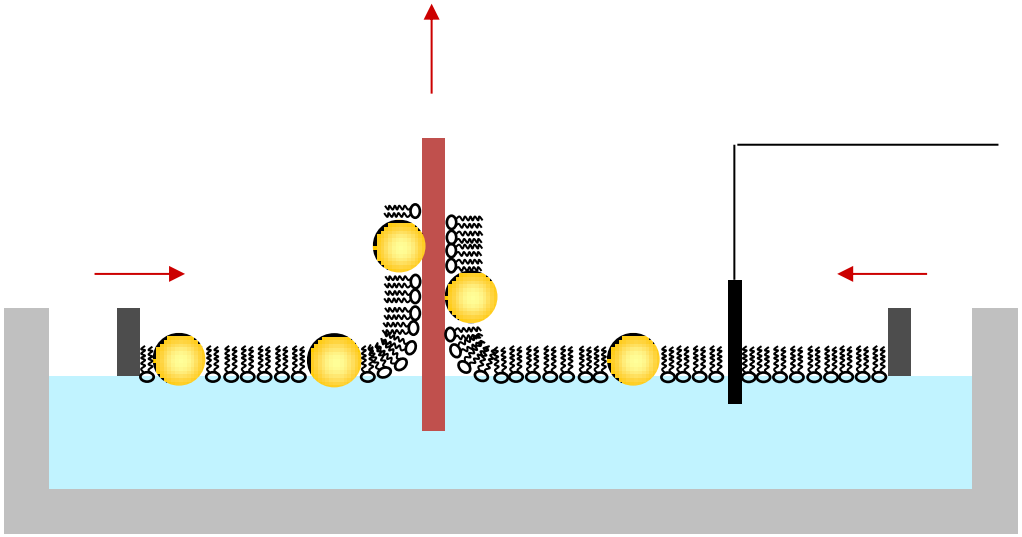
1. Preparation of phospholipid monolayer at liquid/air interface
2. Compression
3. Penetration of functionalized nanoparticles into membrane



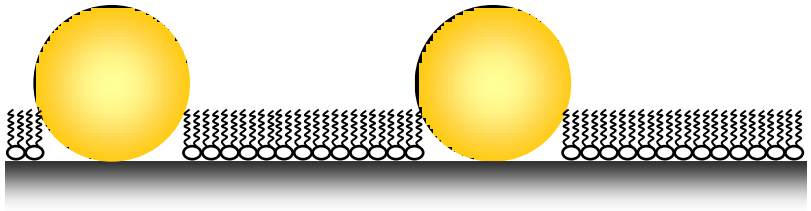


A monomolekulás réteg átvitele szilárd felületre

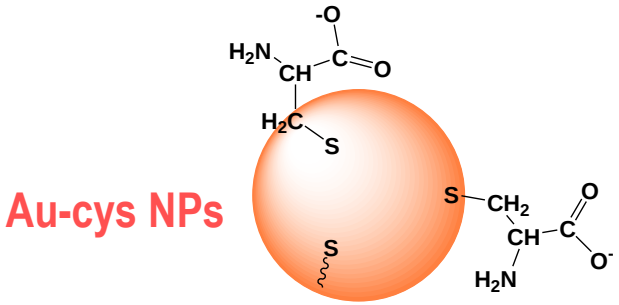
Langmuir-Blodgett method

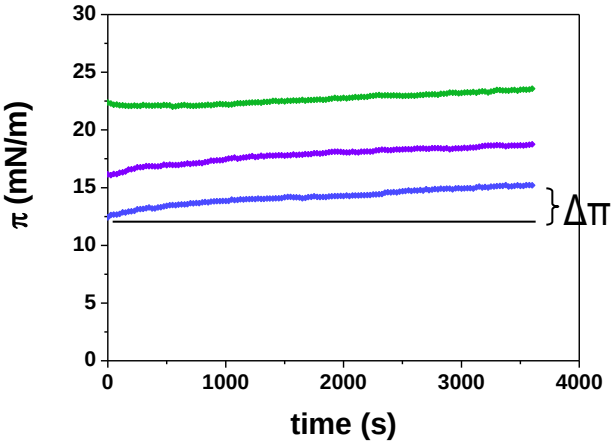


Solid supported monolayer membrane with Au NPs incorporated

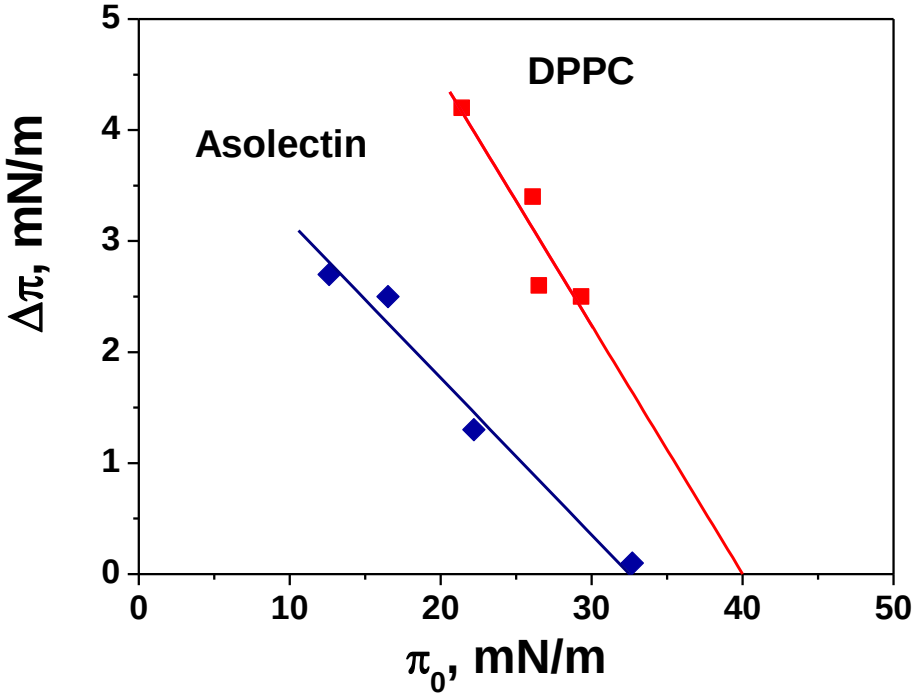


Au- cisztein beépülése monoréteges membránba



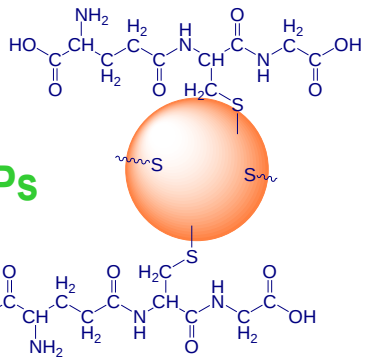


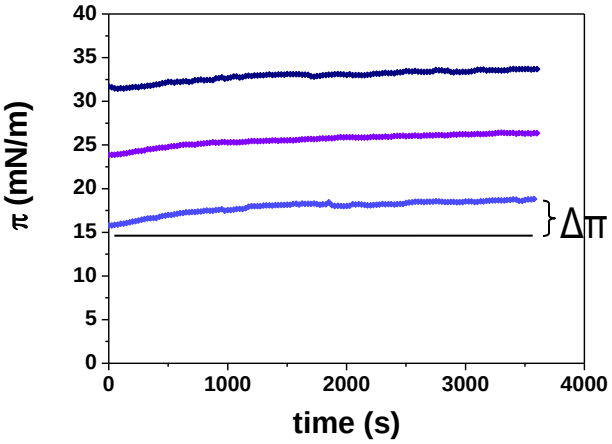
Change in the surface pressure of the monolayer during the incorporation



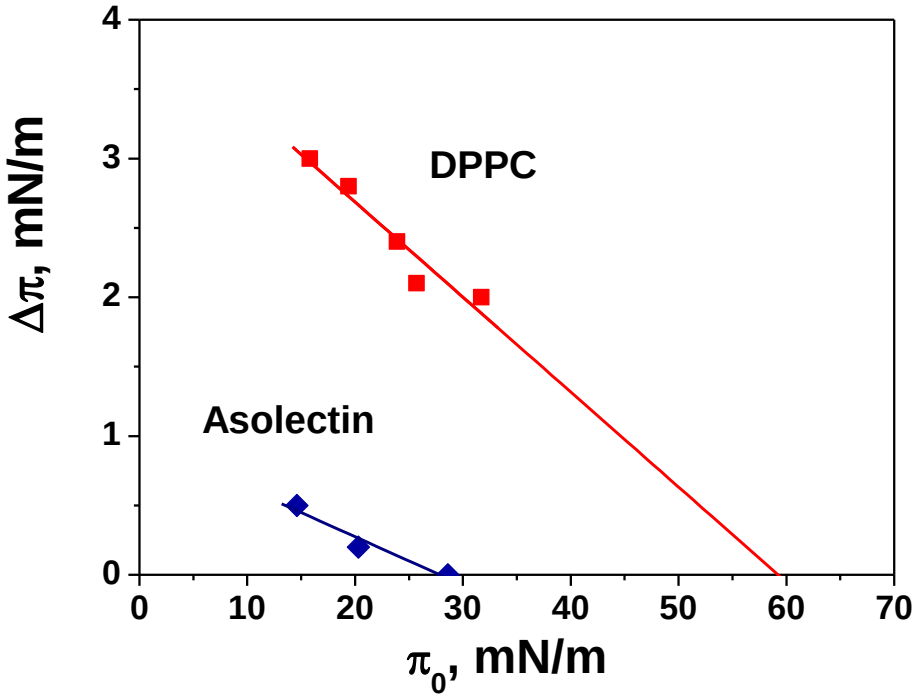
Surface pressure increase (Δπ) as a function of the initial surface pressure (π_0) due to the nanoparticle incorporation

Au - glutation beépülése a monoréteges memránba





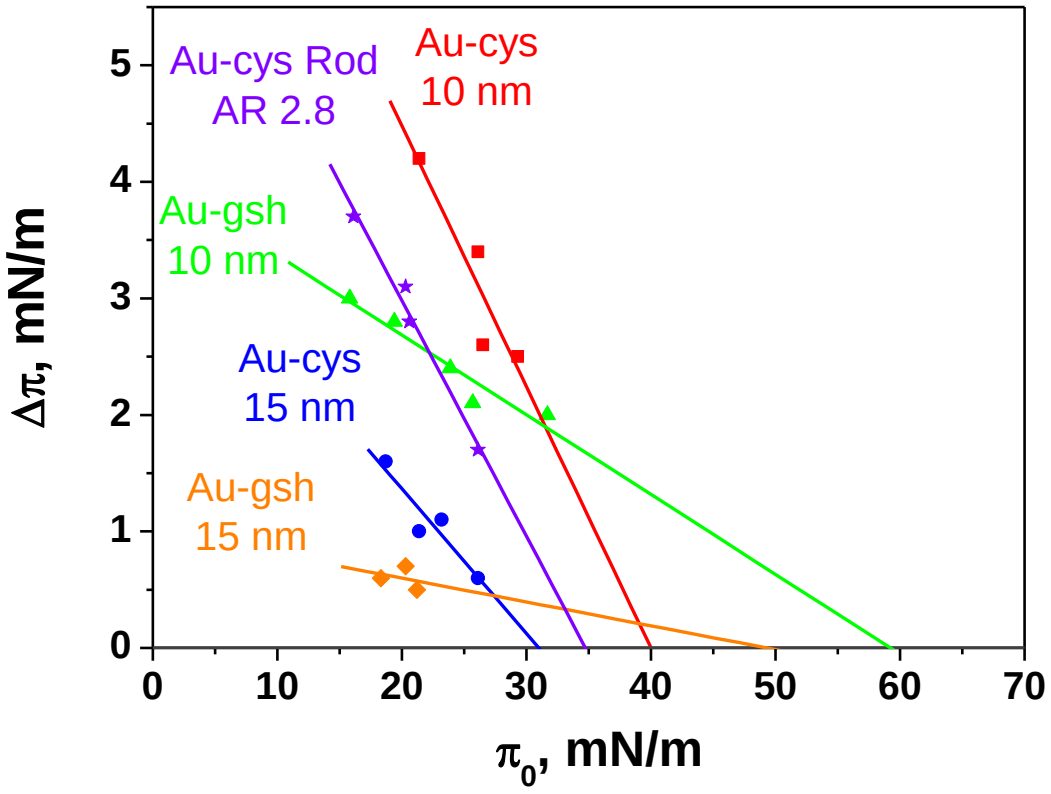
Change in the surface pressure of the monolayer during the incorporation



Surface pressure increase (Δπ) as a function of the initial surface pressure (π_0) due to the nanoparticle incorporation

Biofunkcionalizált Au NR beépülése a DPPC membránba

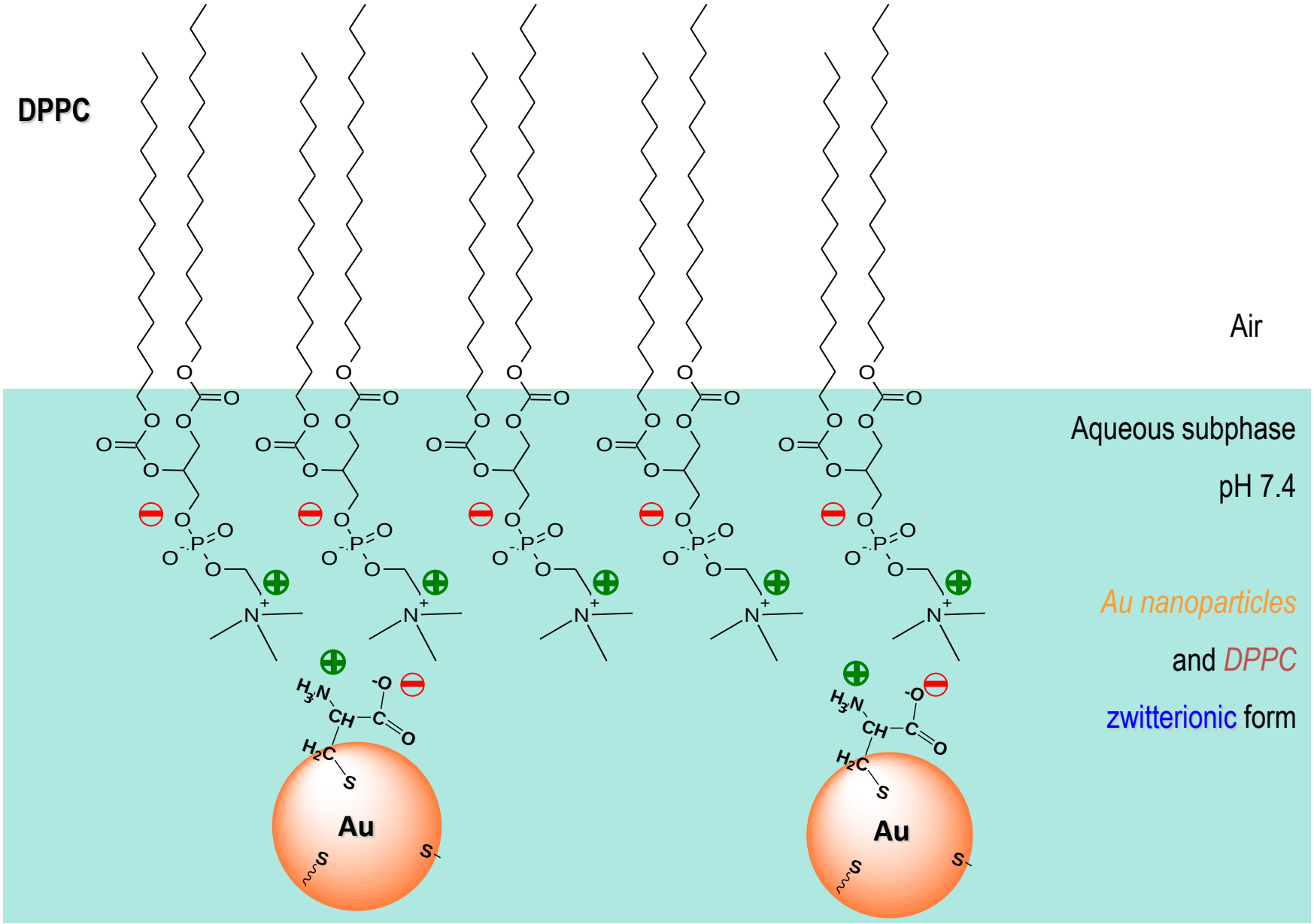
Penetration of the biofunctionalized Au NPs into the different model membranes at different compactnesses (eg. initial surface pressures)

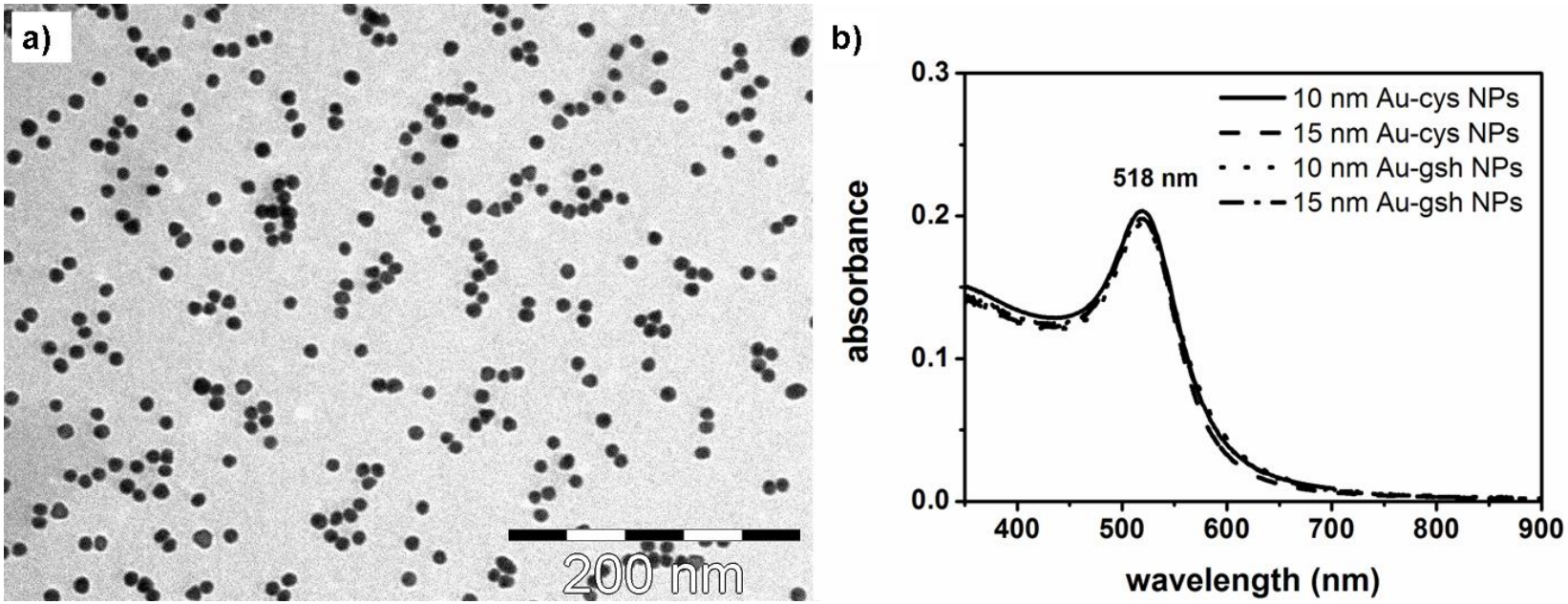


➡ increase in surface pressure means the penetration of the particles into the membrane

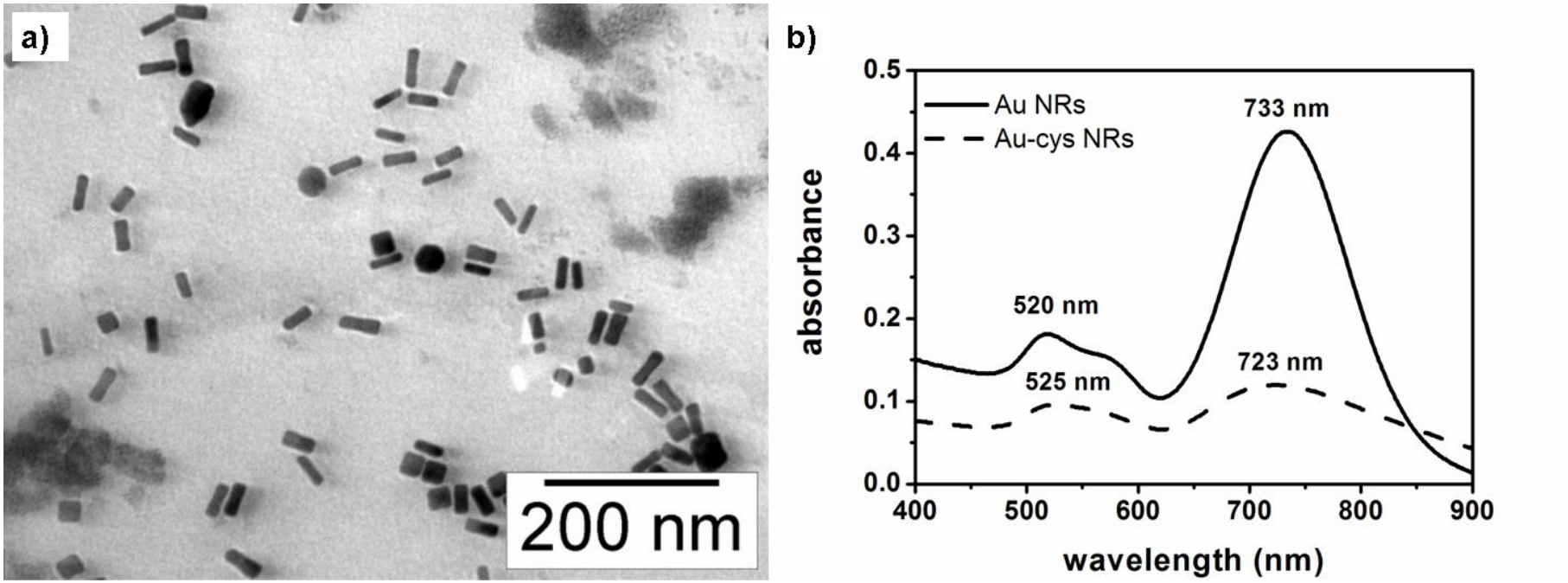
➡ composition and initial surface pressure have marked influence on the penetrated amount

Biofunkcionalizált Au NR és a phospholipid monoréteg

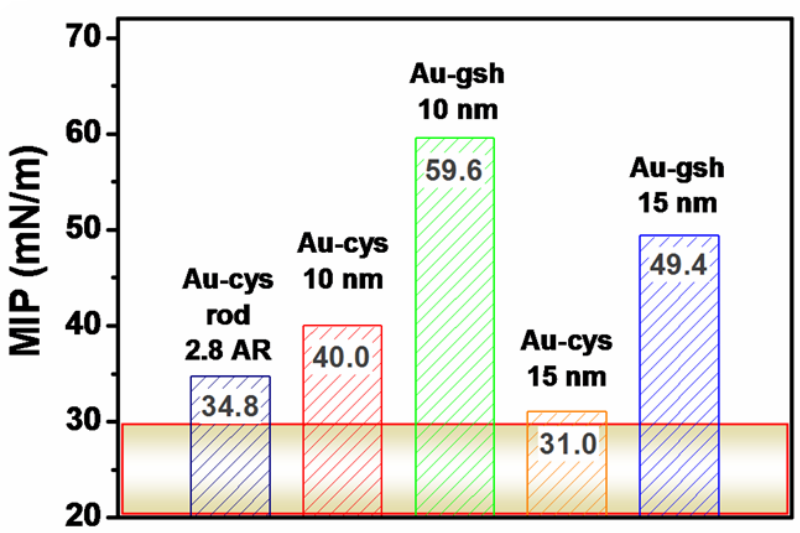


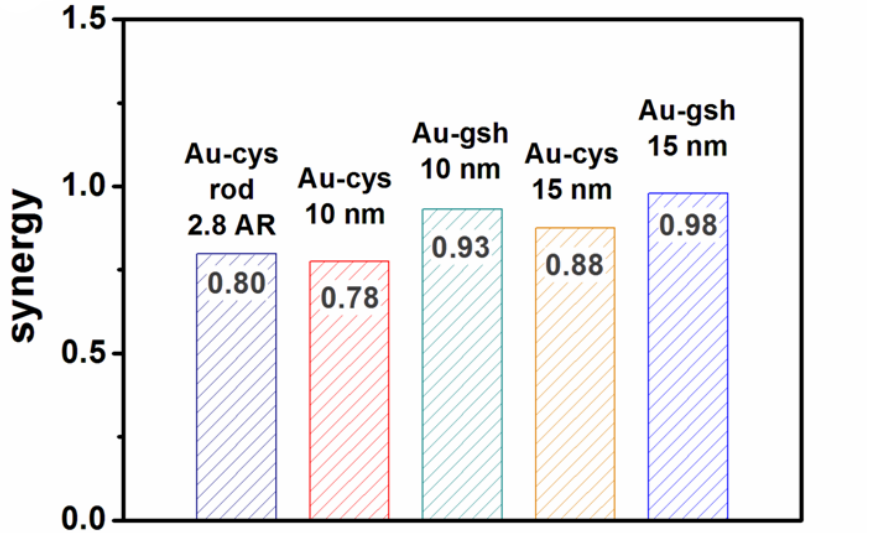


TEM image of the 10 nm spherical Au NPs andUV-Vis absorbance spectra of different sized, bioconjugated Au NPs.



TEM image of the Au nanorods, UV-Vis absorbance spectra of the aqueous dispersions of the Au NRs.

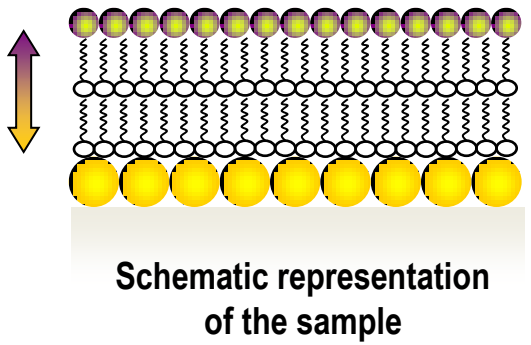




Maximum insertion pressure (MIP) and synergy values obtained for the different sized, shaped, surface functionalized nanoparticles with DPPC membrane.

ZnO-Au plazmonikus csatolás

Au és ZnO nanohibrid filmek



Schematic representation of the sample

ZnO (d=3 nm)
Langmuir-Blodgett layer

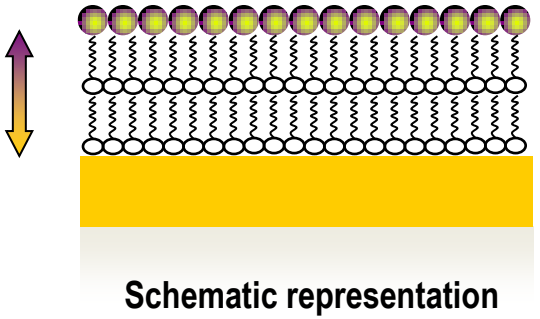
stearic acid (SA)
Langmuir-Blodgett layers

Au nanoparticles – d=10 nm
spray coating technique

quartz

layer numbers:
0, 1, 2

spacer layers between Au and ZnO



Schematic representation of the sample

ZnO (d=3 nm)
Langmuir-Blodgett layer

stearic acid (SA)
Langmuir-Blodgett layers

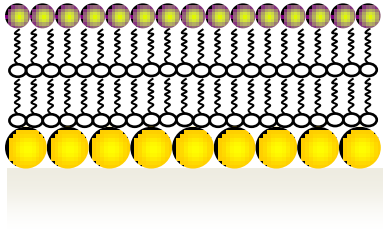
Au film - 47 nm thickness
SPR substrate

glass

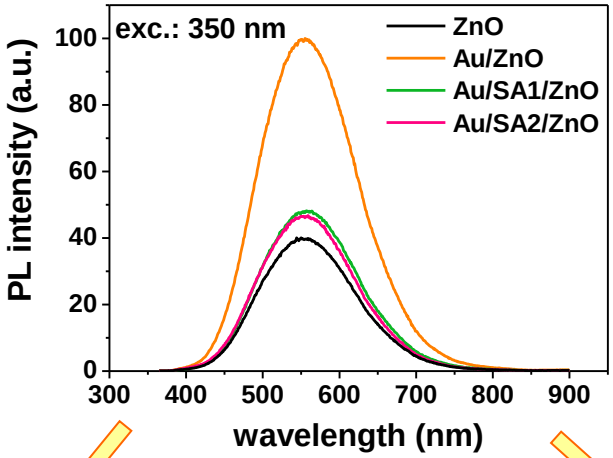
layer numbers:
0, 1, 2, 3, 4, 6

Au-ZnO nanoszerkezetek fotolumineszcenciája

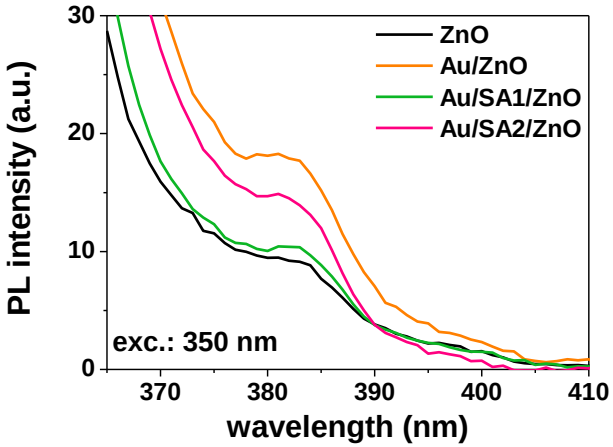
Au NPs based samples



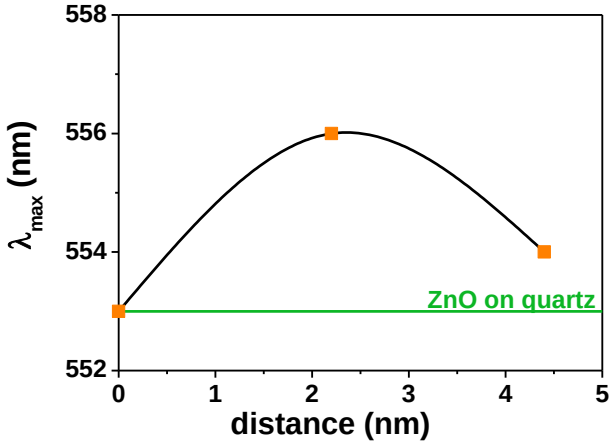
Visible emission



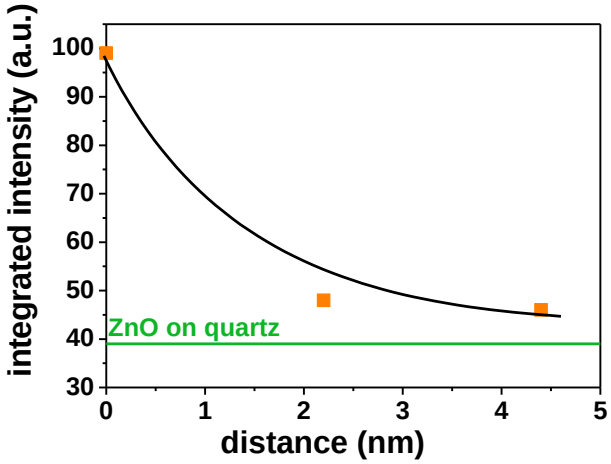
UV emission



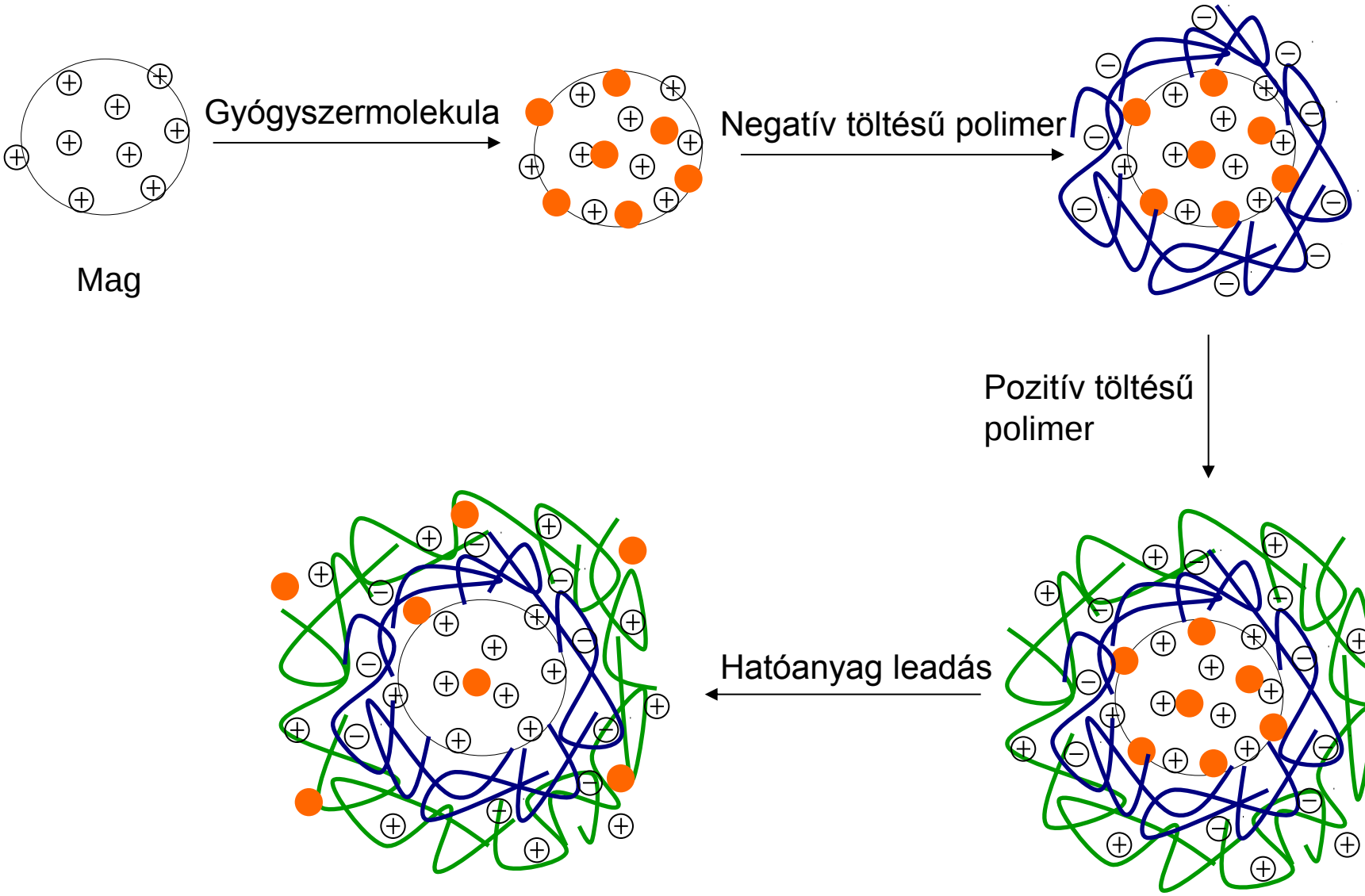
wavelength



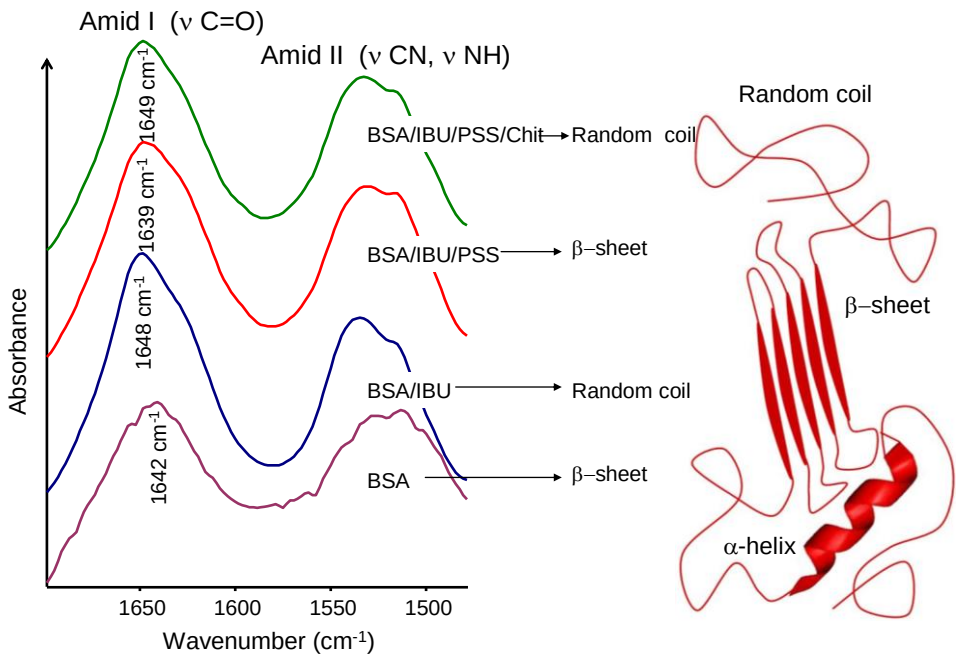
intensity

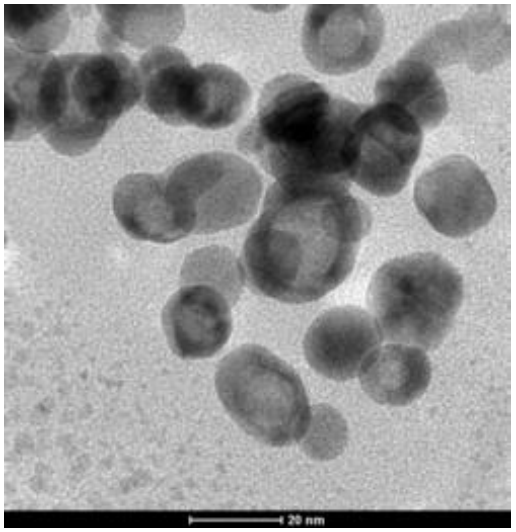


Mag-héj kompozitok



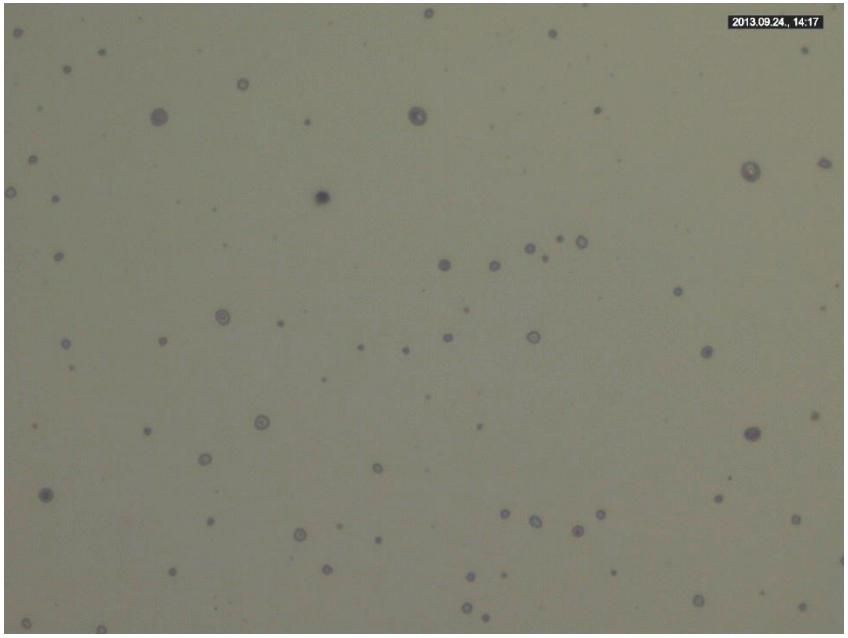
BSA maggal előállított mag-héj kompozitok





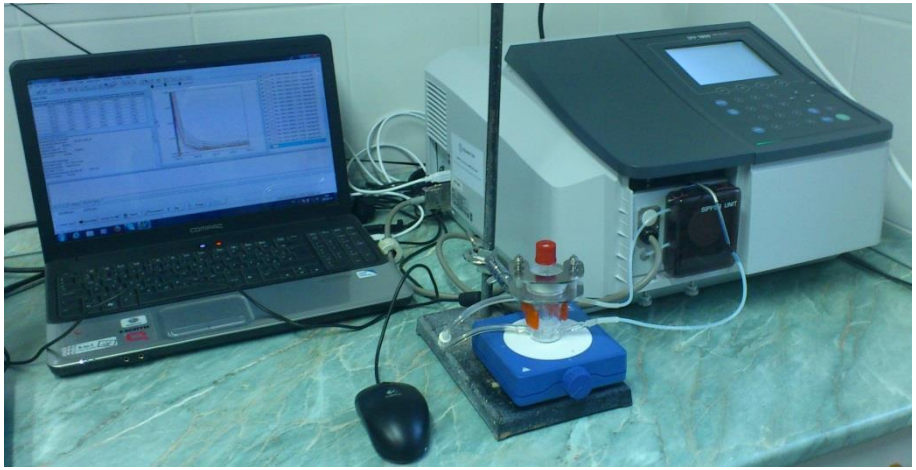
BSA/IBU/PSS/Chit TEM képe

BSA, BSA/IBU, BSA/IBU/PSS valamint a BSA/IBU/PSS/Chit infravörös spektruma, valamint a másodlagos szerkezet változása az amid I sáv eltolódása alapján



BSA/IBU/PSS/Chit fénymikroszkópos képe

Hatóanyag leadás vizsgálata



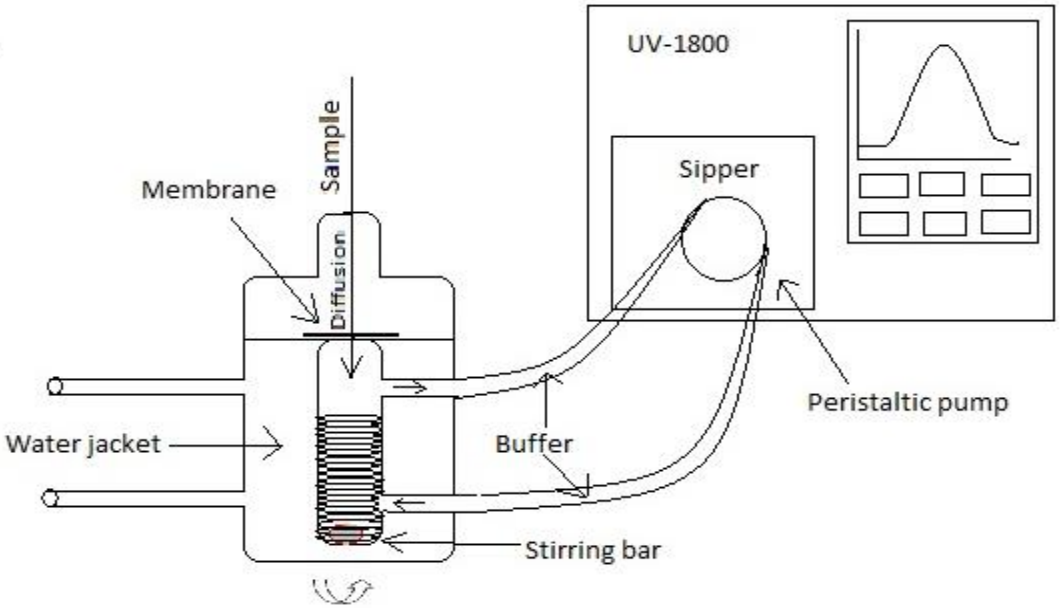
IBUPROFEN meghatározása:

BSA mag jelenlétében: 264 nm, 272 nm

Szilika mag jelenlétében: 222 nm, 264 nm, 272 nm

<u>Mérés:</u>
Foszfát pufferben (PBS, pH=7,4)

HANSON cella-
vertikális diffúzió cella



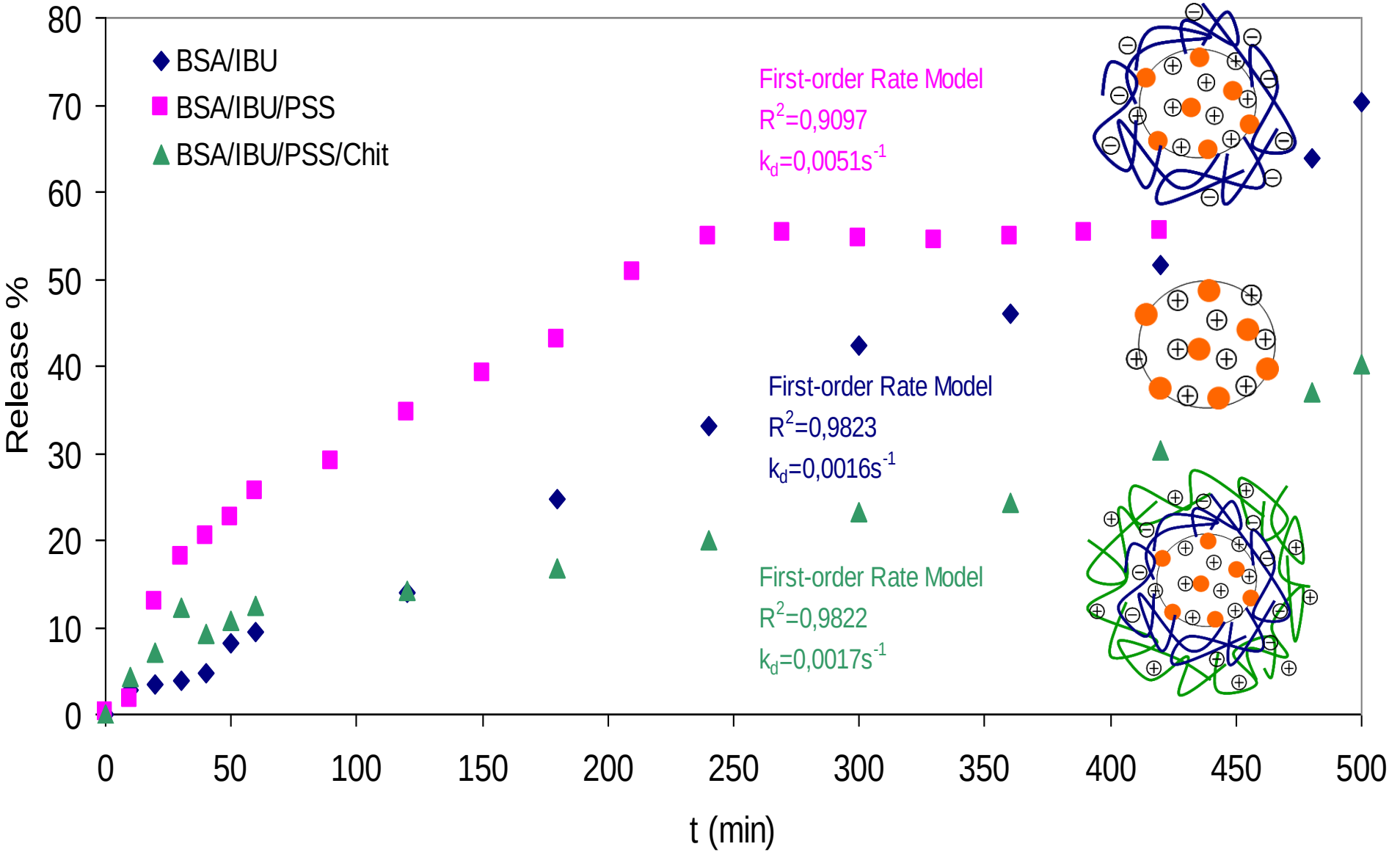
Két kamrából áll: donor and receiving (fogadó) chambers

Membrán :Dialysis tubing cellulose membrane

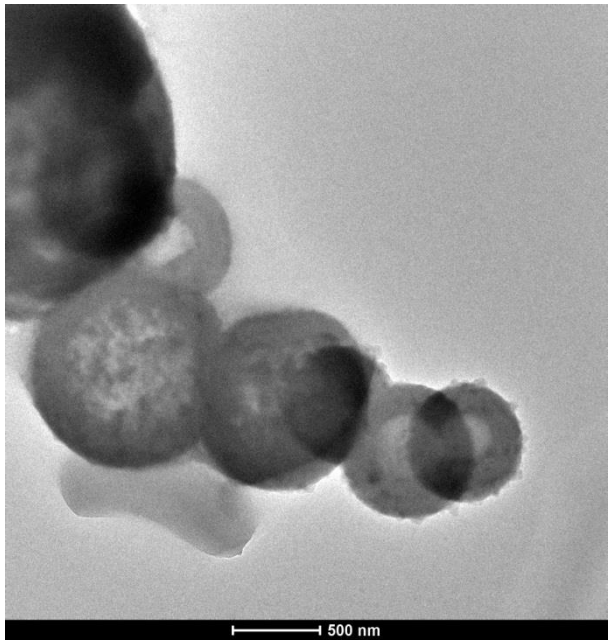
Cella térfogata: 4ml, de a pontos térfogatot meg kell határozni

Keverés sebességet állandó értéken kell tartani a mérés során.

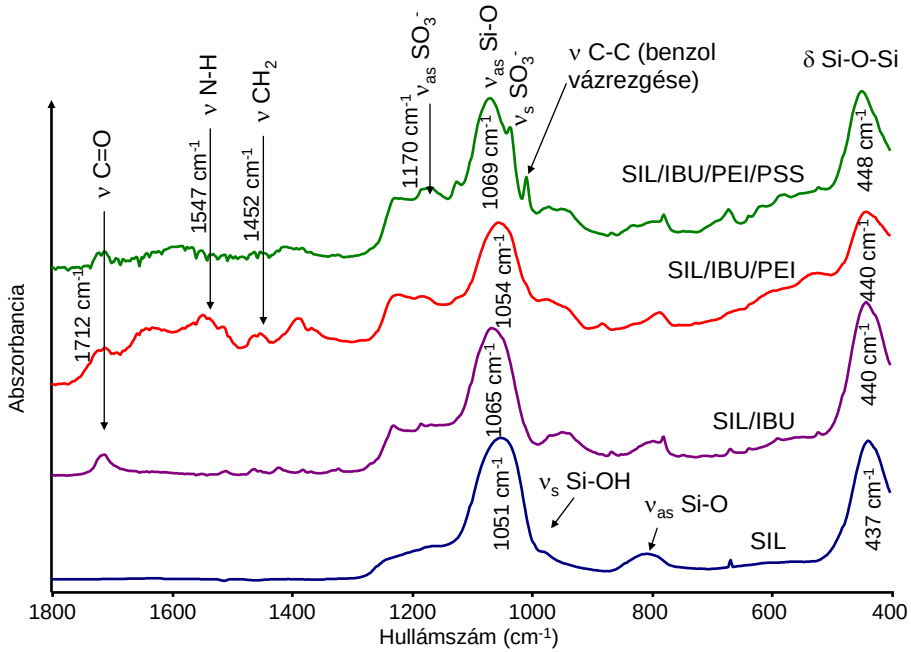
Elsőrendű Sebességi Modell a kioldódás mechanizmusára



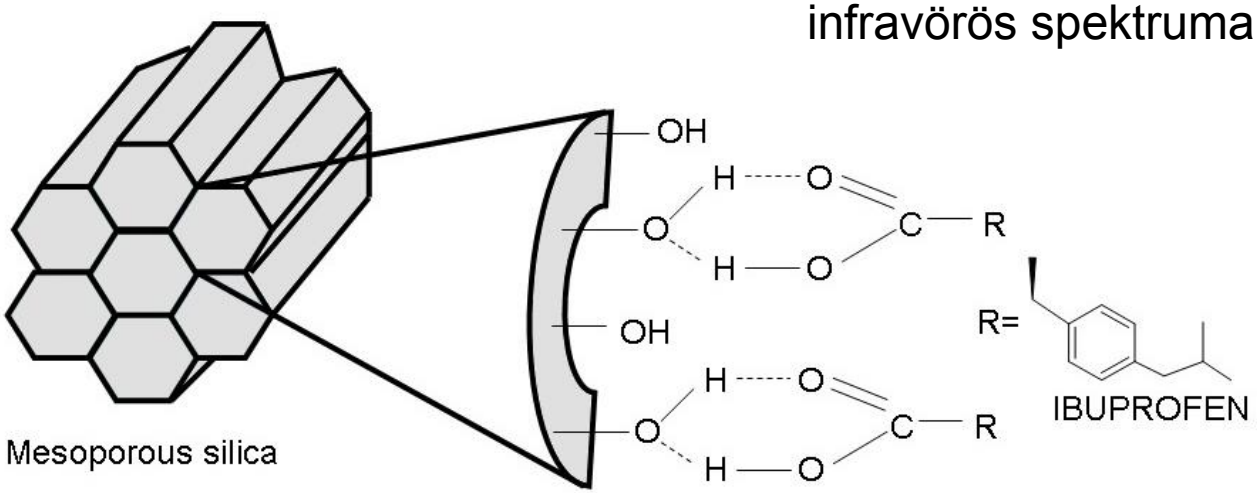
Mezopórusos szilika maggal előállított mag-héj kompozitok



SIL/IBU/PEI TEM képe

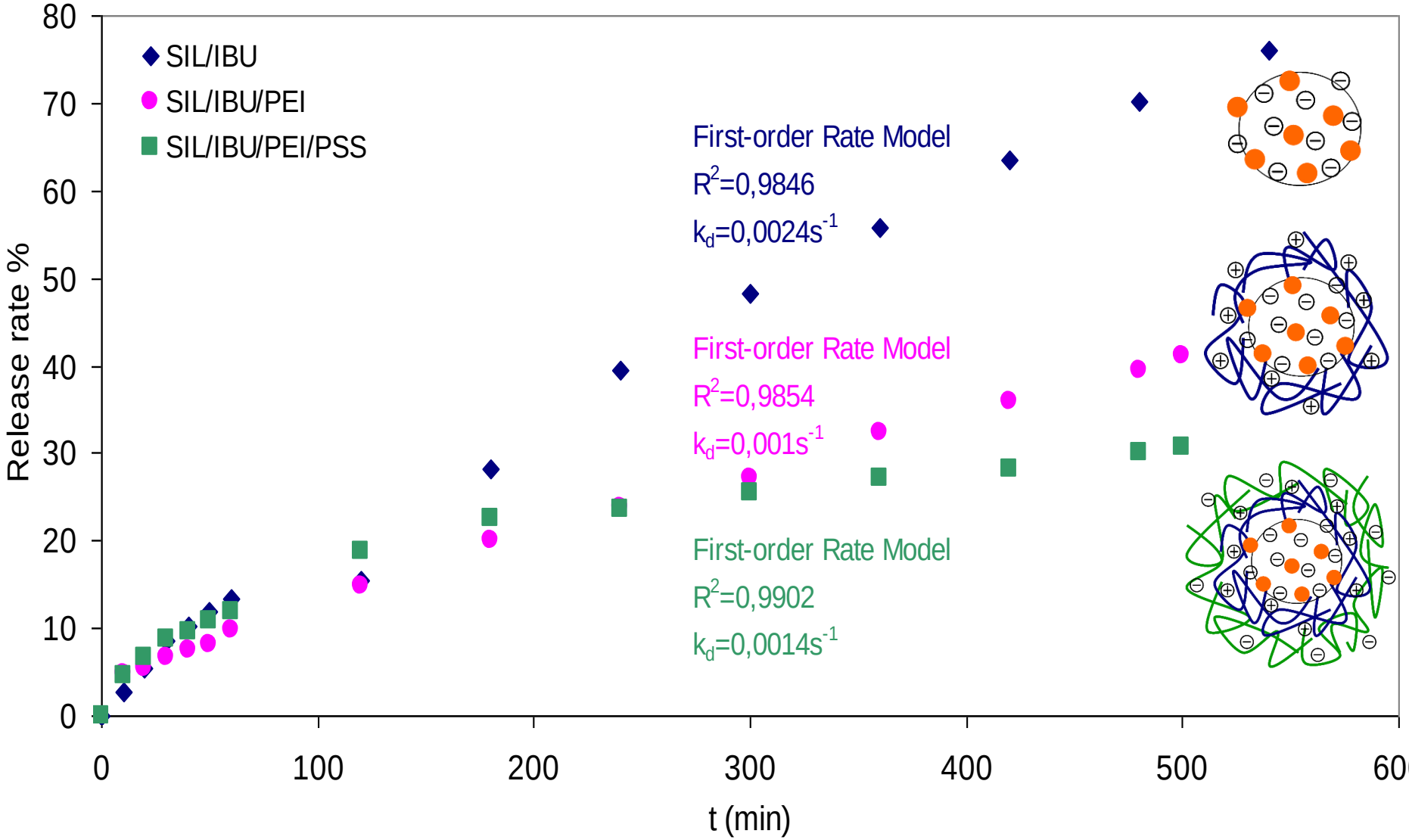


A szilika, valamint a mag-héj kompozitok infravörös spektruma



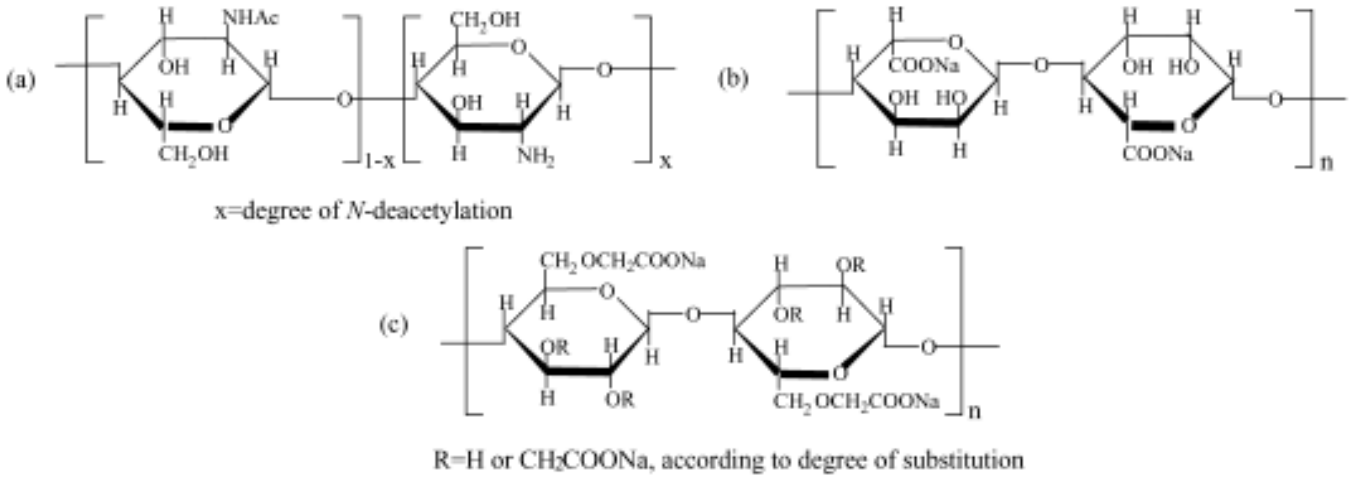
A szilanol csoportok, valamint az ibuprofen karboxil csoportjai közt létrejött H-kötések a szilika pórusaiban

Elsőrendű Sebességi Modell a kioldódás mechanizmusára



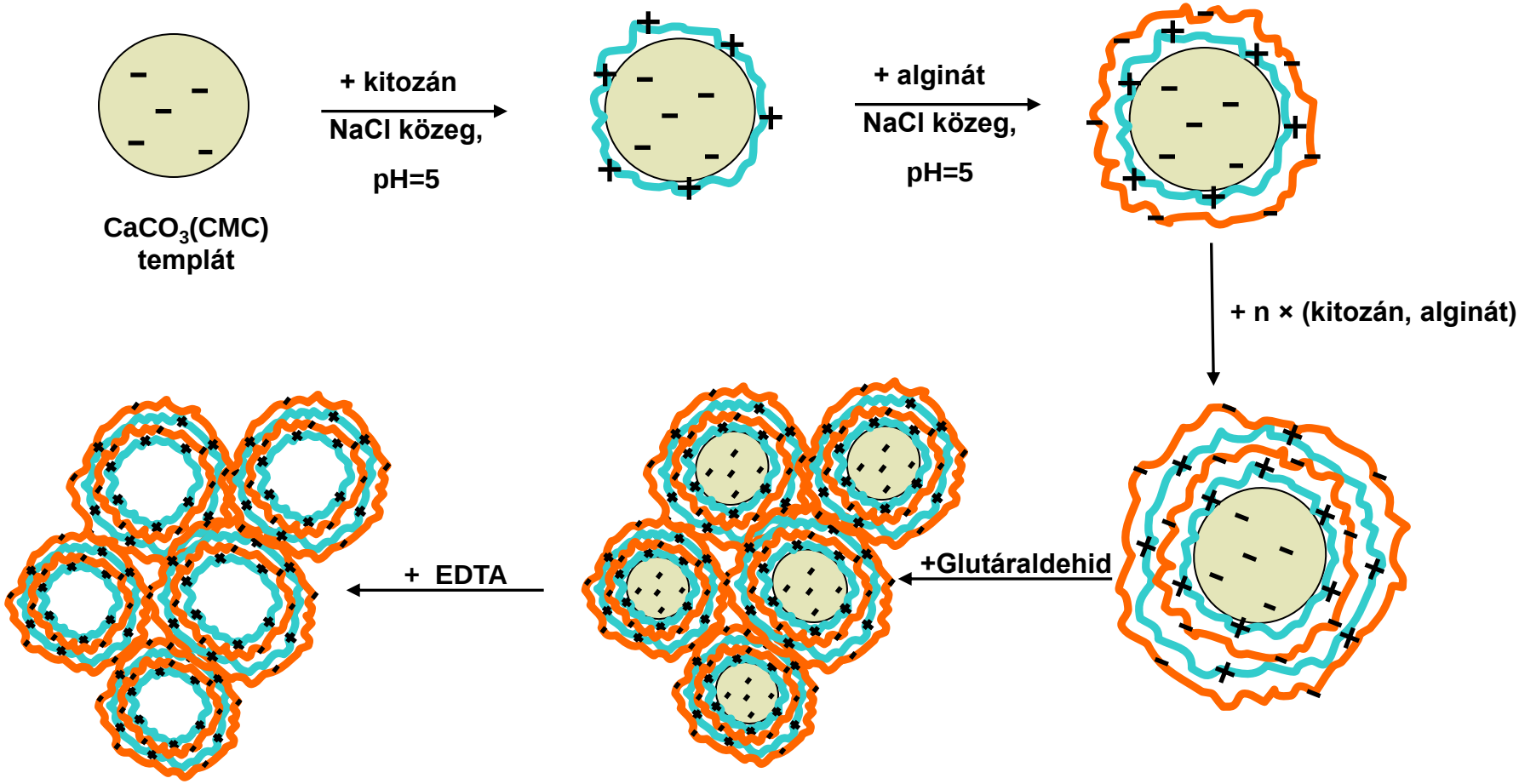
„Hollow spheres” előállítása biokompatibilis poliszacharidokból

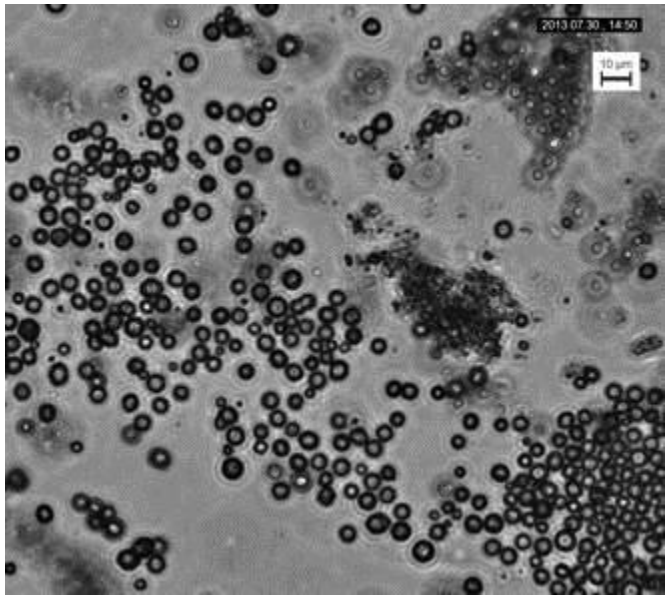
- **kitozán, alginát** - biokompatibilis, biodegradábilis,
  -alkalmazhatóságát széleskörűen vizsgálják hatóanyagok és vakcinák szállítására nyálkahártyán keresztül
- **$CaCO_3$** -nem toxikus,
  -mérete és morfológiája szabályozható,
  -könnyen eltávolítható,
- **CMC** -összekapcsolódik a Ca^+ ionokkal, ezáltal szabályozza a képződő $CaCO_3$ részecskék méretét,
  -úgy viselkedik mint egy „ragasztó”, összefogja a nanorészecskéket egymással és gömb alakú mikrorészecskéket formál.



(a) Kitozán, (b) Na-alginát és (c) karboximetil-cellulóz (CMC) szerkezeti képlete

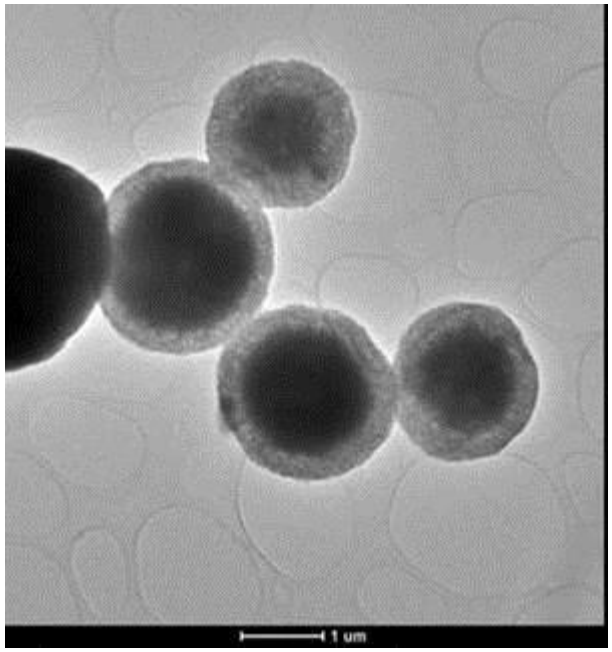
Kitozán alginát „hollow spheres” előállítása



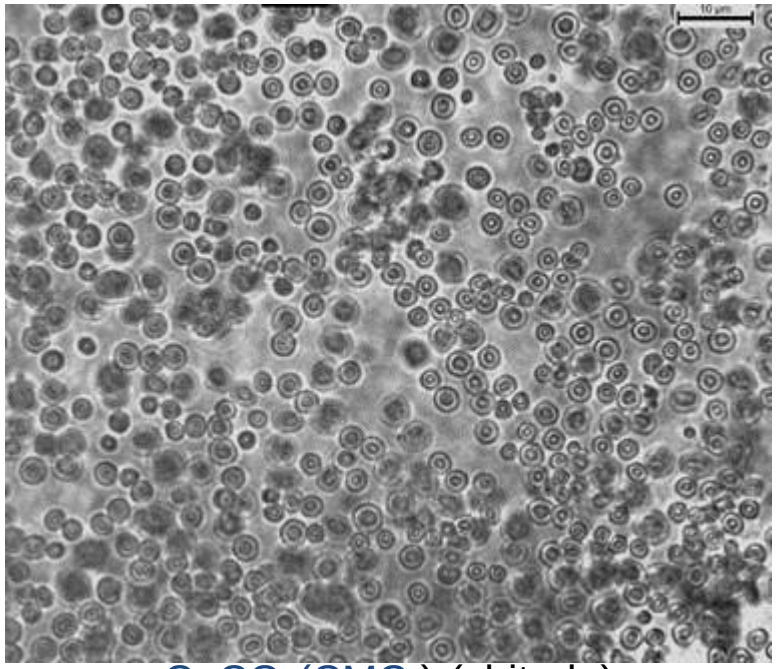


$CaCO_3$(CMC) templát
(Fénymikroszkópos felvétel)

$CaCO_3$(CMC) + n × { 0.05% kitozán (0.5M NaCl, pH=5) ; 0.1% alginát(0.5M NaCl, pH=5) }

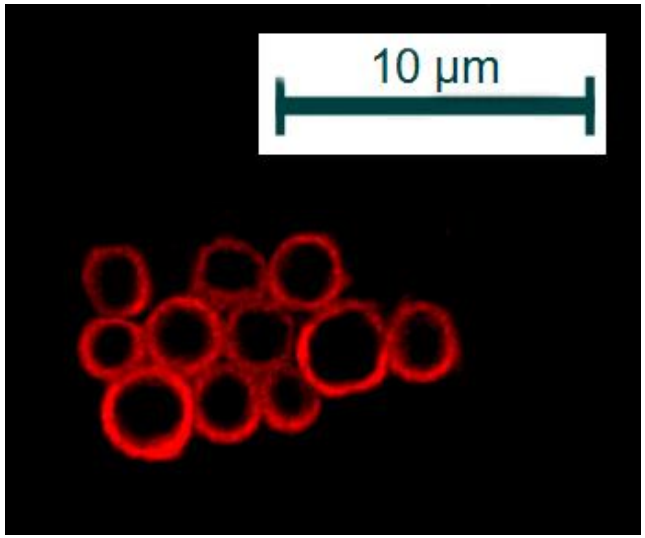


$CaCO_3$(CMC)-$(chit\text{-}alg)_3$ (TEM felvétel)



$CaCO_3$(CMC)-$(chit\text{-}alg)_3$
(Fénymikroszkópos felvétel)

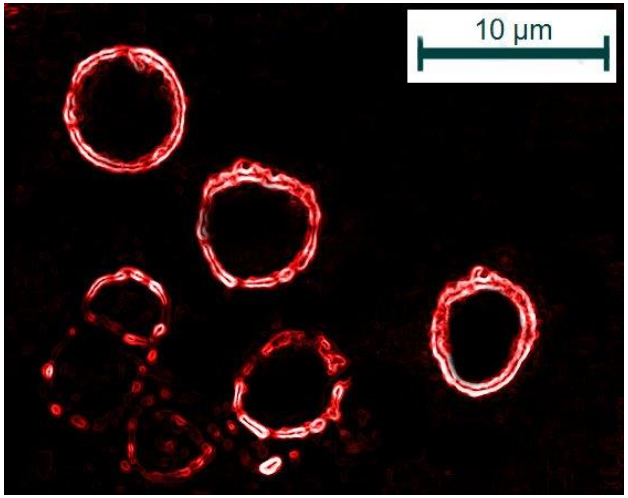
+ Rhodamin B



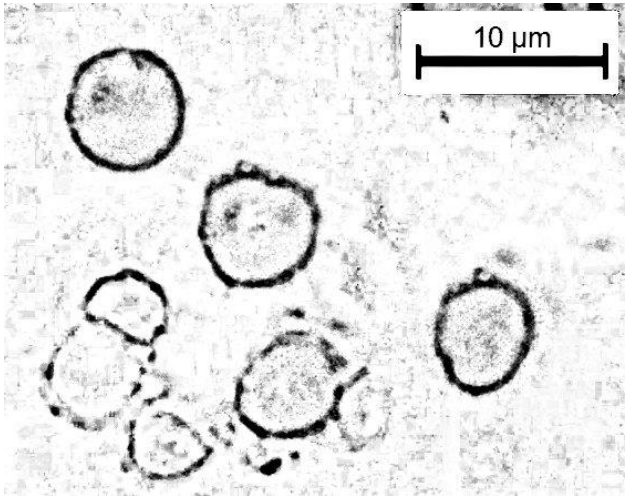
$CaCO_3$(CMC)/$(Chit\text{-}Alg)_3$ – Rhodamin B
fénymikroszkópos felvétele

+ 1% Glutáraldehid

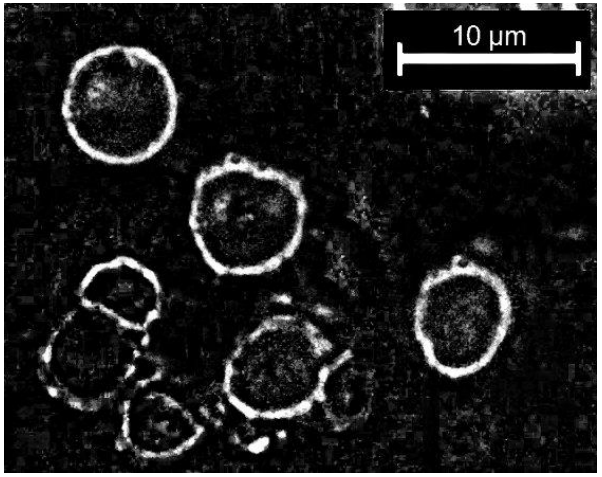
+ 0.2M EDTA

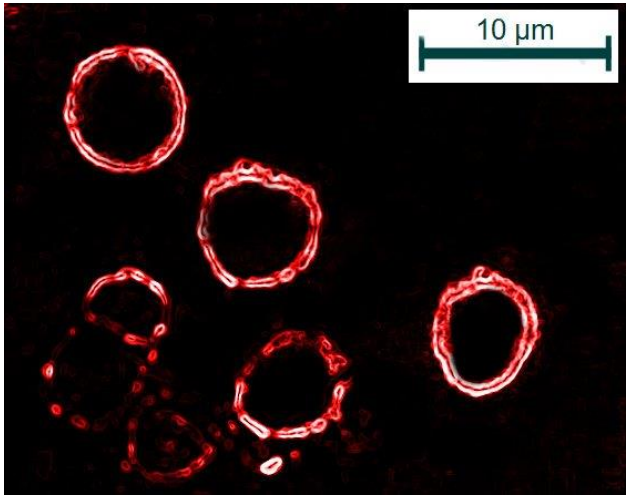


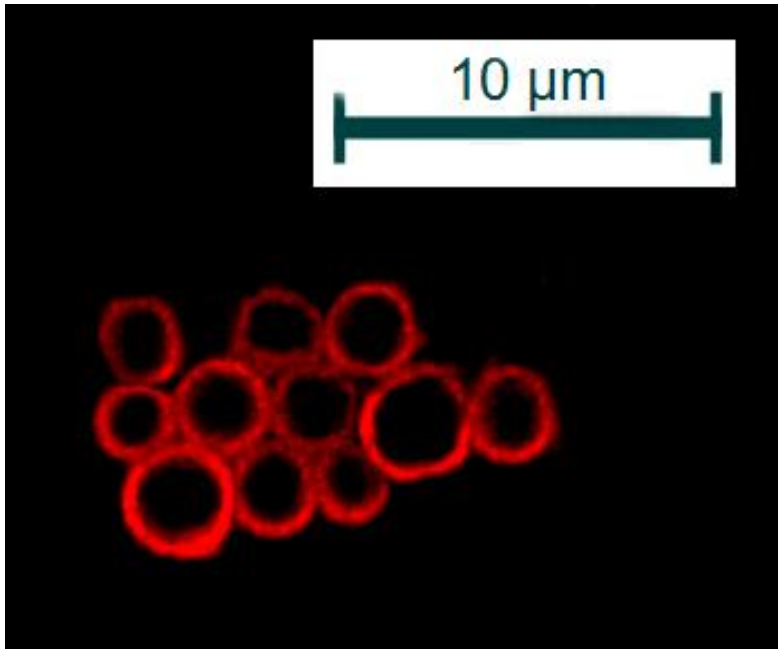
$(chit\text{-}alg)_3$ hollow spheres
fénymikroszkópos felvétele

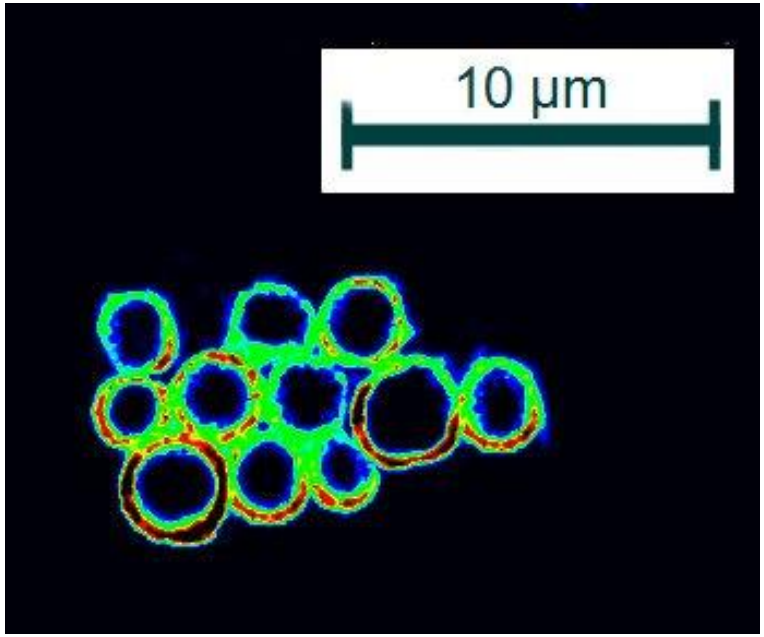

10 µm


10 µm


10 µm


10 µm


10 µm


10 µm

Munkatársak

MTA-SZTE Szupramolekuláris és Nanoszerkezetű Anyagok Kutatócsoport

Juhászné
Dr. Csapó Edit

Dr. Benkő Mária

Dr. Majzik Andrea

Garabné Ábrahám Nóra

Juhász Ádám

Varga Viktória

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Tallóssy Szabolcs

Szalmáné
Ménesi Judit

Veres Ágnes

Dr. Sebők Dániel

Varga Noémi

Köszönöm a megtisztelő figyelemet!