Nanoparticles as drug delivery systems. The interaction with biomembrane

Z. Garaiová[1], S. Melikishvili[1], D. Wrobel[2], K. Ciepluchl[2], M. Ionov[2], S.Michlewska[2], E. Pedziwiatr-Werbicka[2],I.Waczuliková[1], M. Bryszewska[2], T. Hianik[1],
M.A. Muñoz-Fernandez[3], R. Gomez-Ramirez[4], J.F. de la Mata[4]

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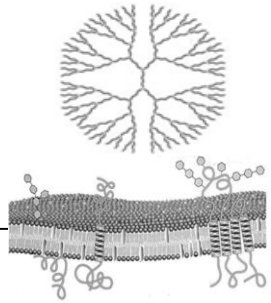
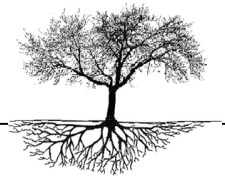
[2] *Department of General Biophysics, Faculty of Biology and Environmental Protection, University of Lodz, Poland*
[3] *Immunomolecular Biology Laboratory, Hospital Gregorio Marañon, Madrid, Spain*
[4] *Inorganic Chemistry Department, University Alcala de Henares, Spain*

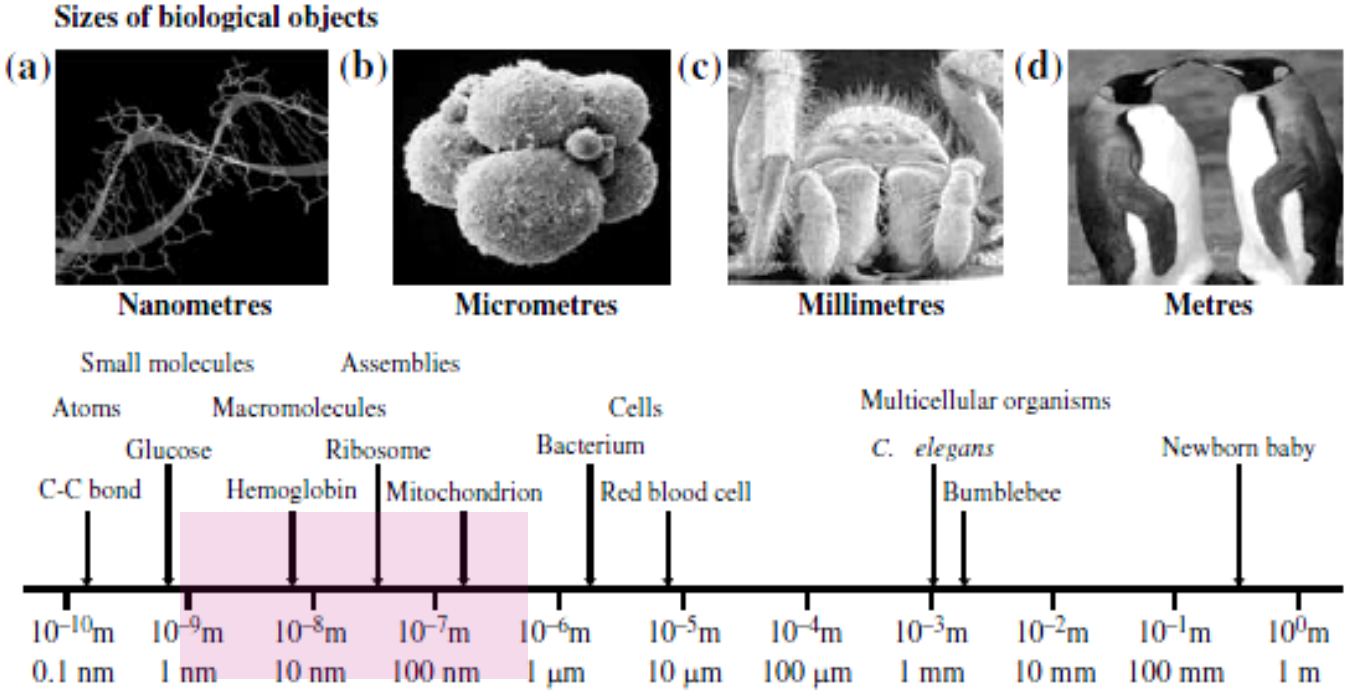


“There is Plenty of Room at the Bottom.
An invitation to Enter a New Field of Physics“

first person to define the uniqueness and enormous potential of studies at nanometric scale

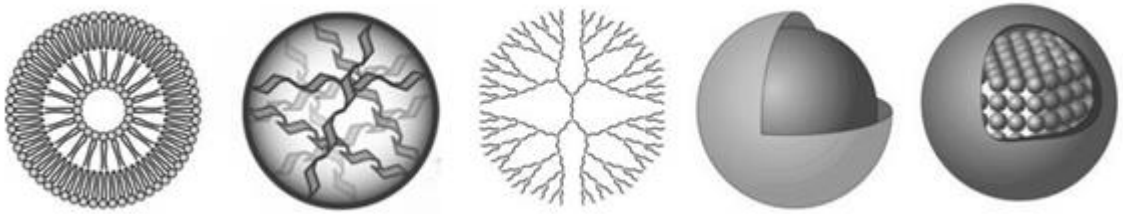


Nanoscale



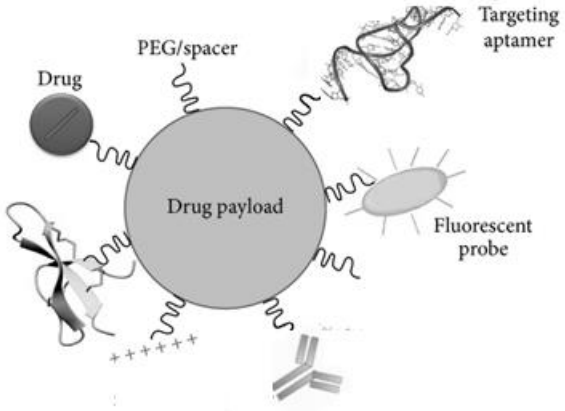
unique properties and size-compatibility
with proteins and nucleic acids

Nanoparticles as drug delivery systems.



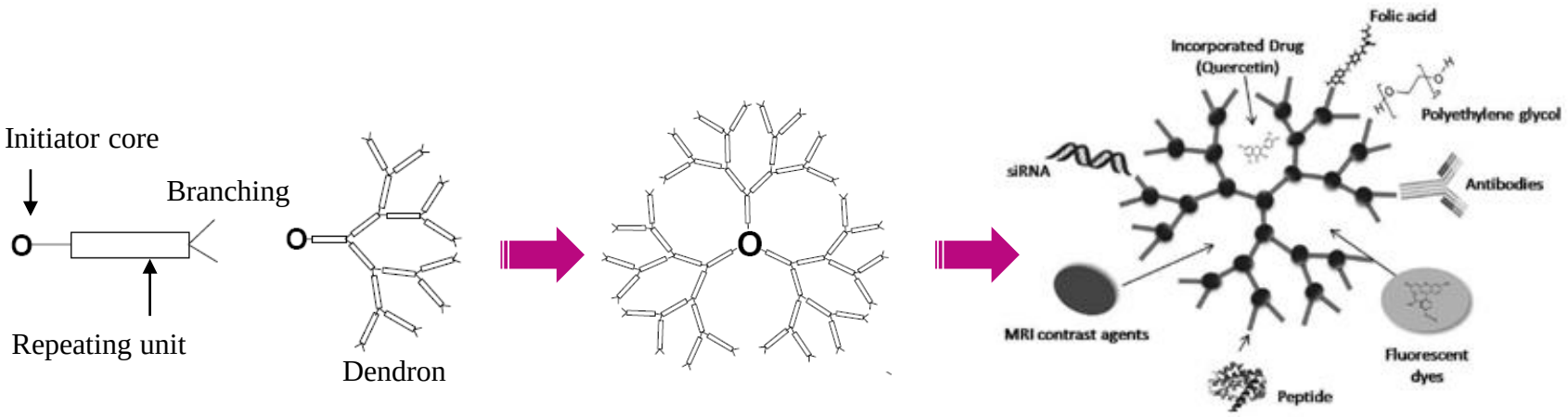
Liposome **Polymer** **Dendrimer** **Core-shell inorganic NPs**

- better control of biological processes, improvements in disease detection, therapy, prevention
- reduce the cost of drug discovery, design and development
- offering solutions to fundamental problems in drug industry ranging from poor solubility to a lack of target specificity



DENDRIMERS as drug delivery systems

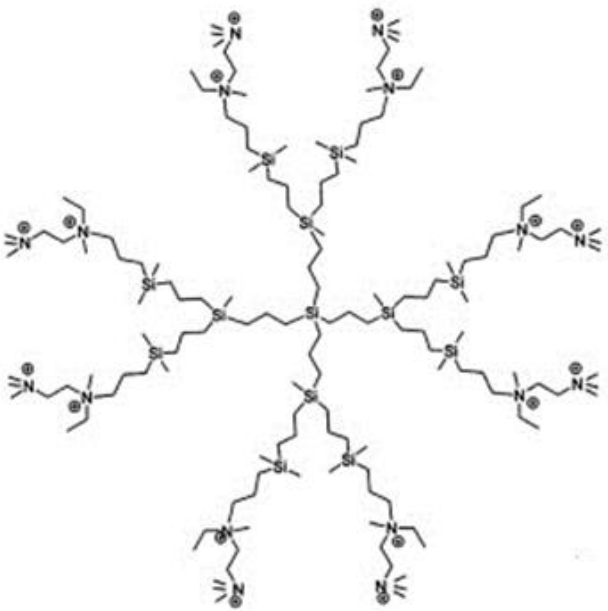
highly branched 3D structure with high degree of surface functionality and versatility



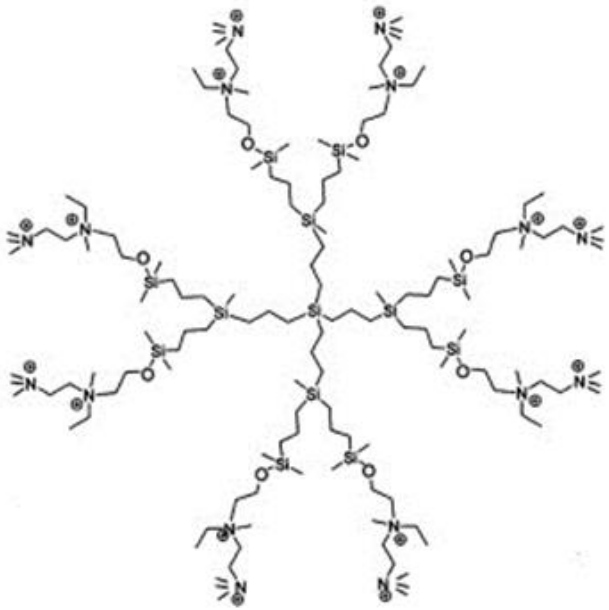
iterative sequence of reaction steps, in which each reaction results in a new so called generation.

DENDRIMERS as drug delivery systems.

CARBOSILABE DENDRIMERS



CBD-CS



CBD-OS

H I V

Human immnodeficiency virus

Acquried immunodeficiency syndrome

A I D S

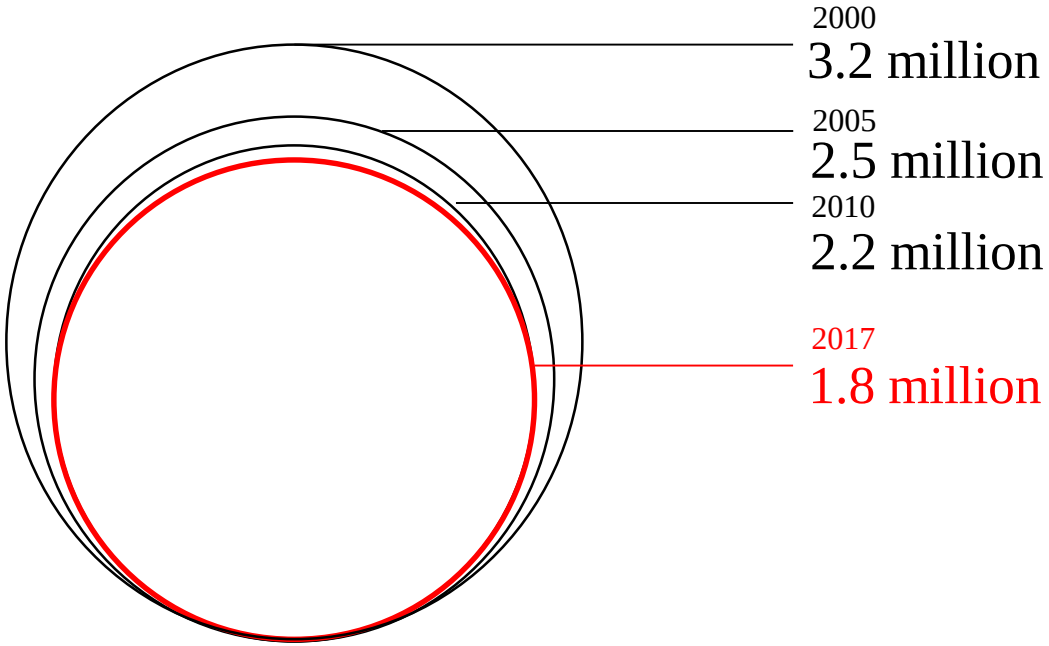
dead immune cells

36.7 million

people worldwide are currently living with HIV/AIDS.

Source: UNAIDS/WHO

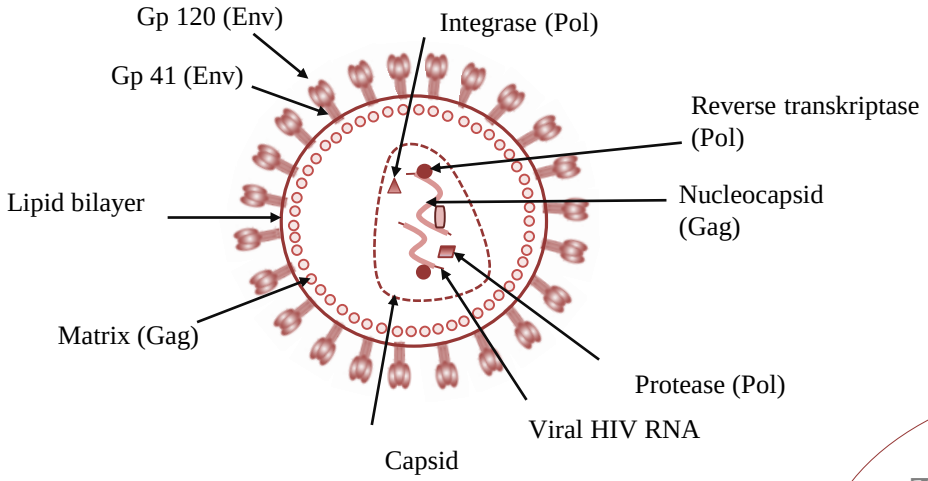
Number of newly infected people with HIV



Source: UNAIDS/WHO

~120 nm

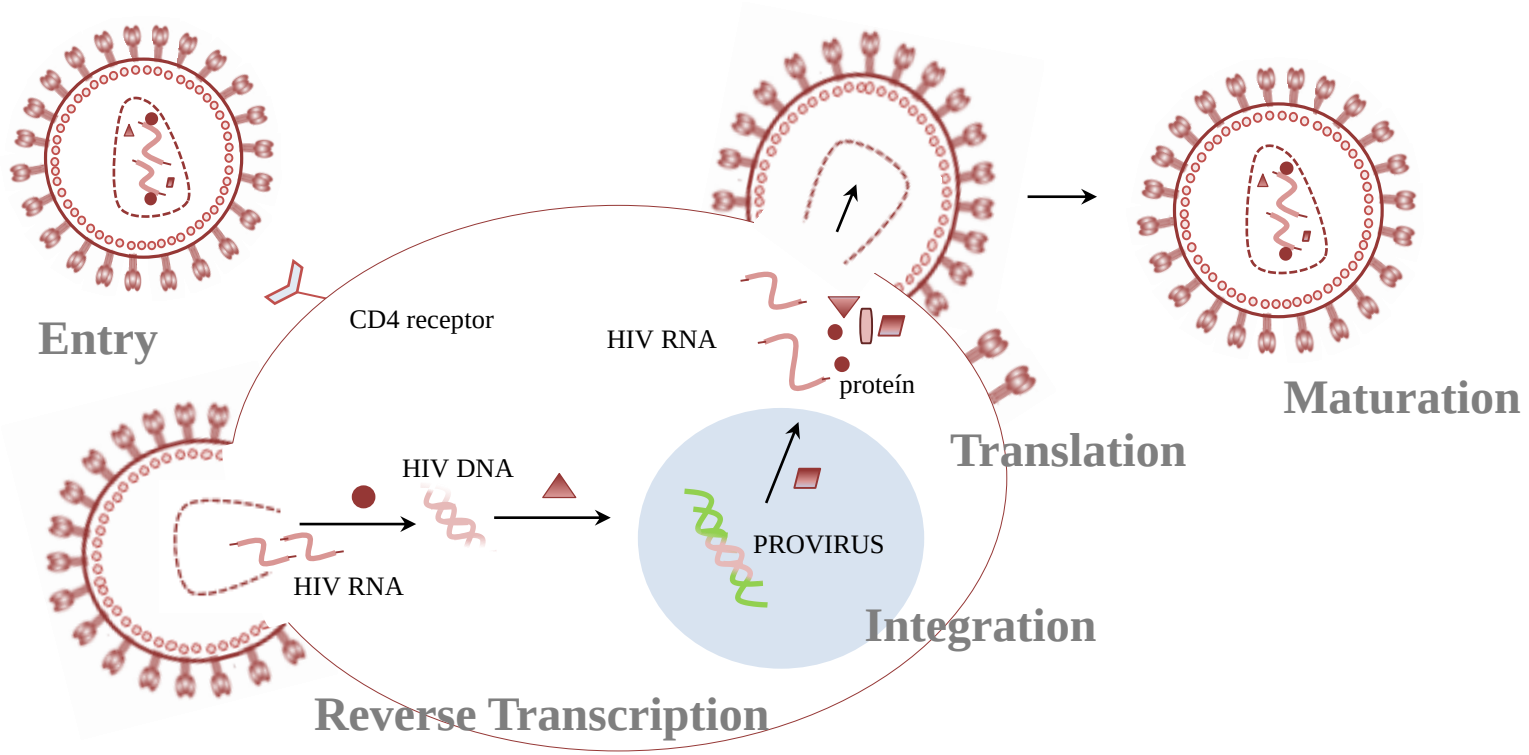
HIV virus

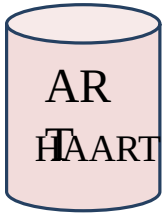


TARGET

T-cells (CD4+ Tcells)
Macrophage
Dendritic cells

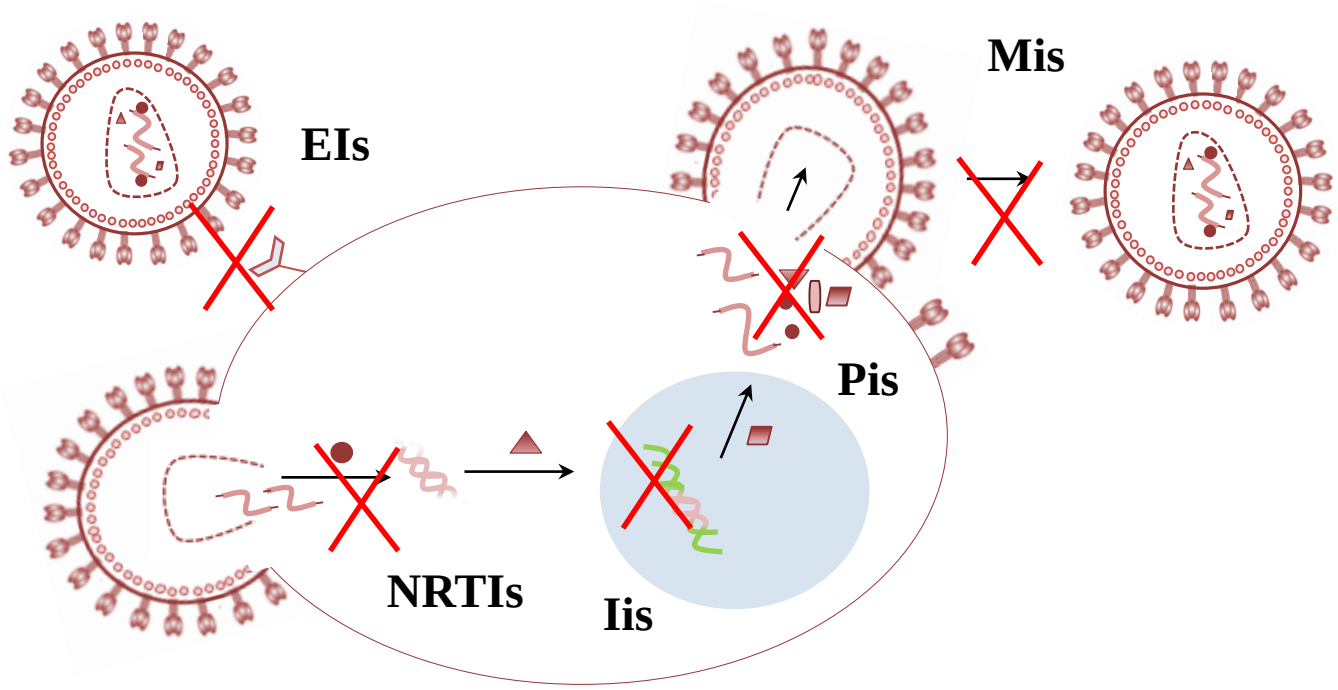
Viral cycle of HIV





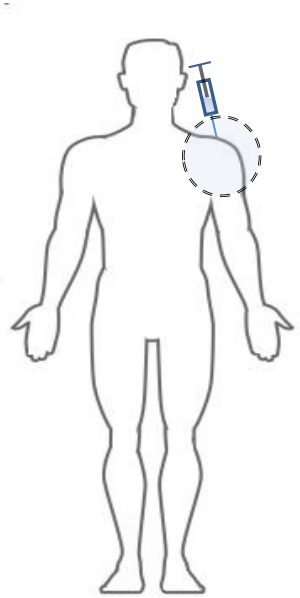
Antiretroviral agents

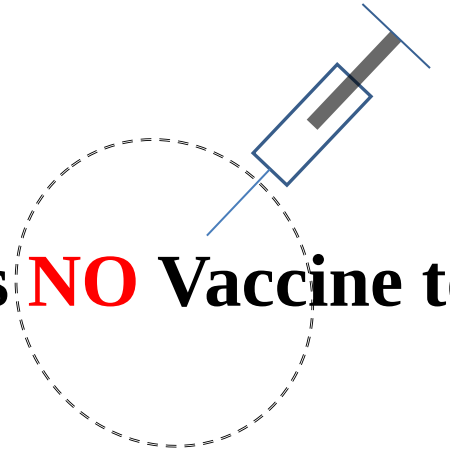
that act on different stages of the HIV life-cycle



can **decrease viral load** but **with limitations**

HIV VACCINE ?

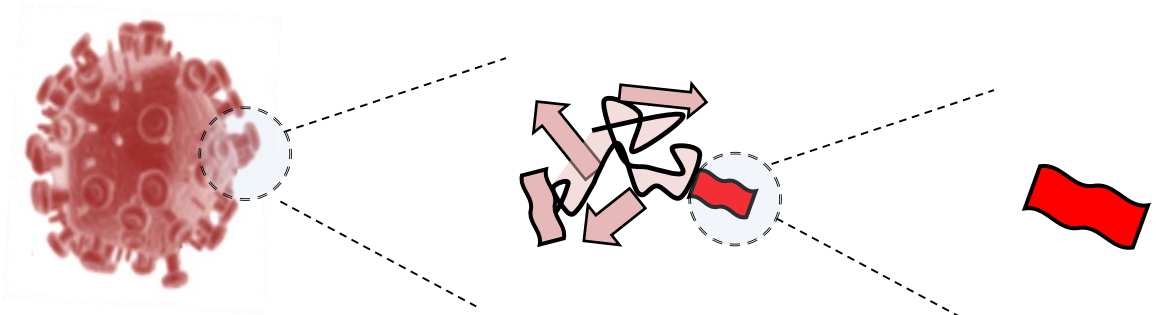




Currently there is NO Vaccine to prevent HIV

Vaccination strategies

To create immune memory



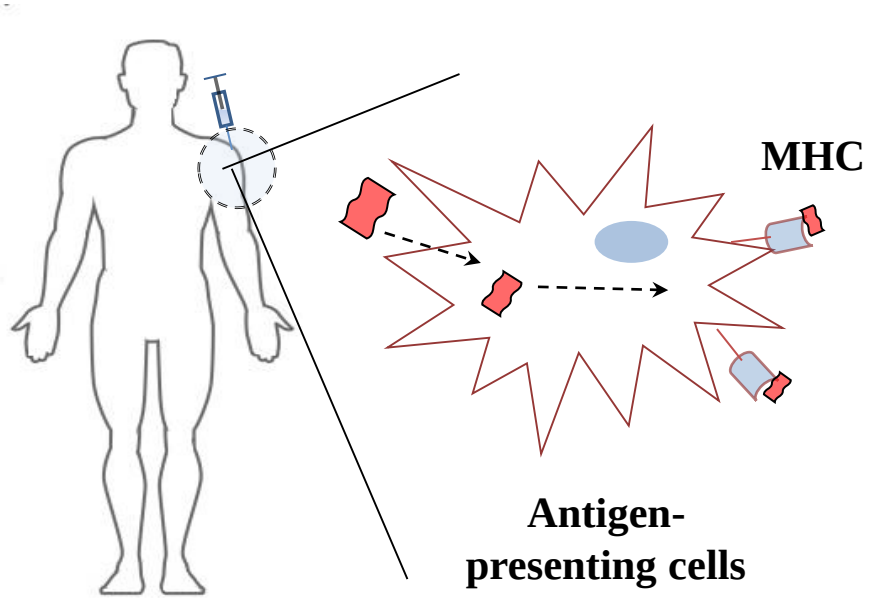
Live attenuated
Inactivated
microorganism

protein

Peptid
(synthetic)

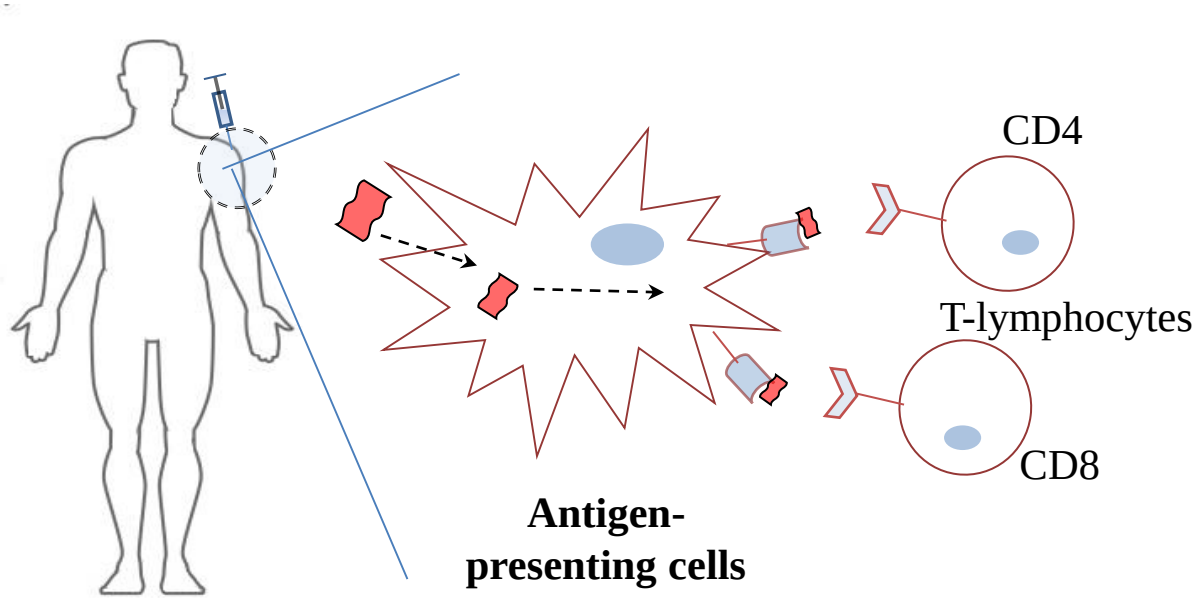
antigens

Peptide-based subunite vaccination strategy

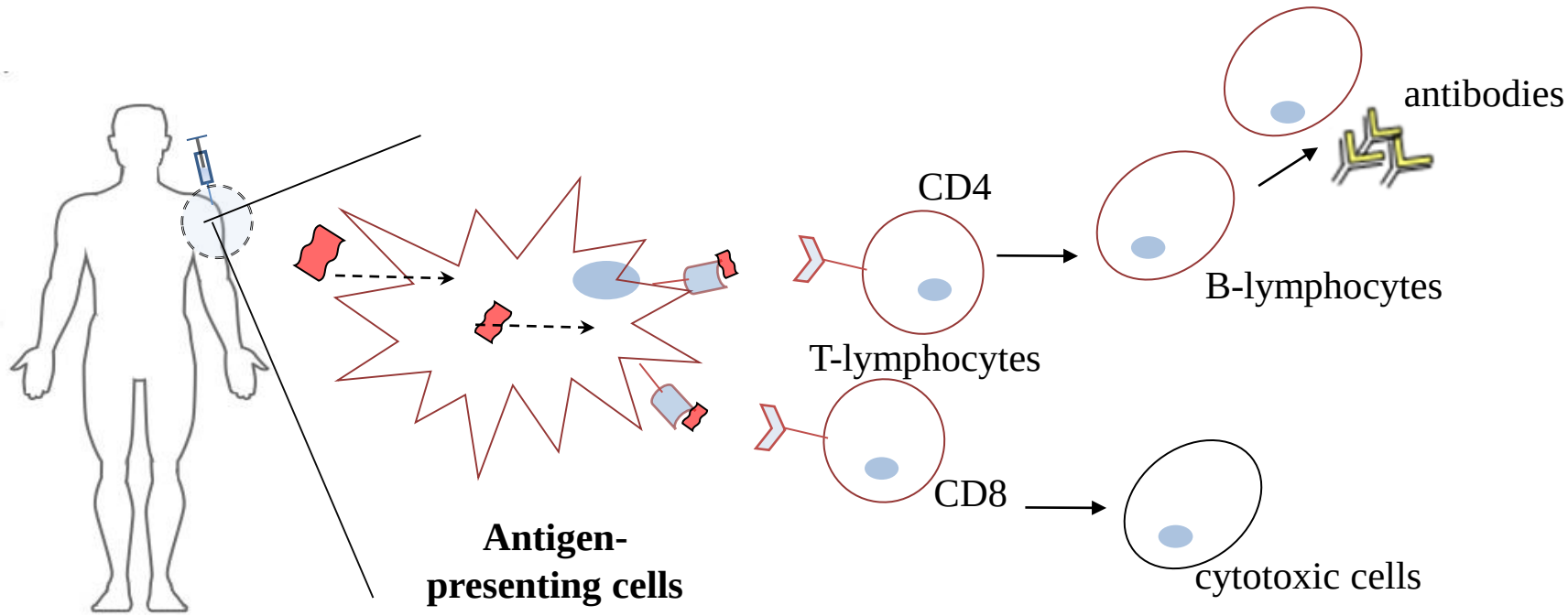


Dendritic cells

Peptide-based subunite vaccination strategy



Peptide-based subunite vaccination strategy



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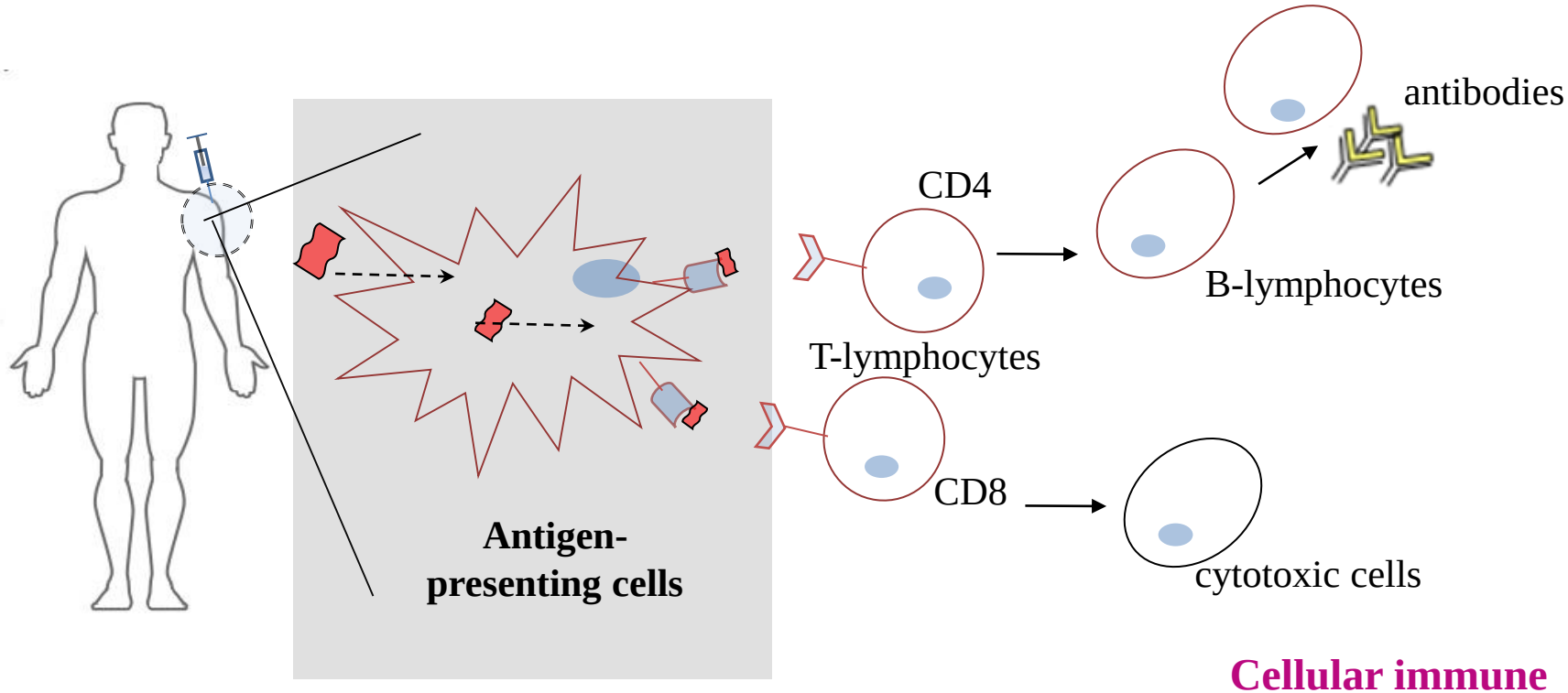
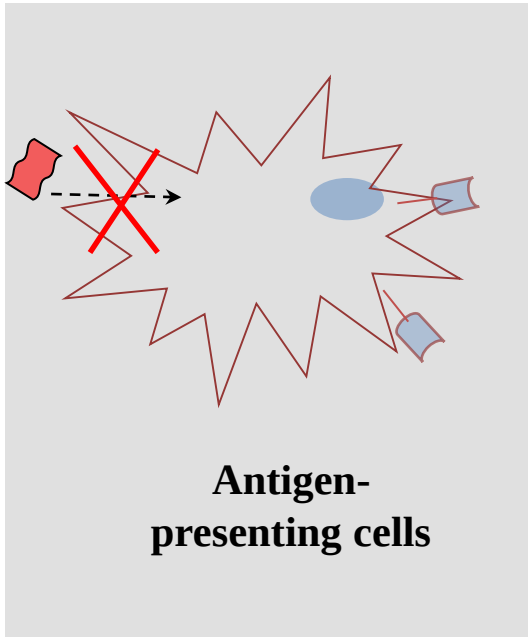


Peptide-based subunite vaccination strategy

generally safe, able to induce very specific but weak immunity response

Require use of immunostimulants (adjuvants)



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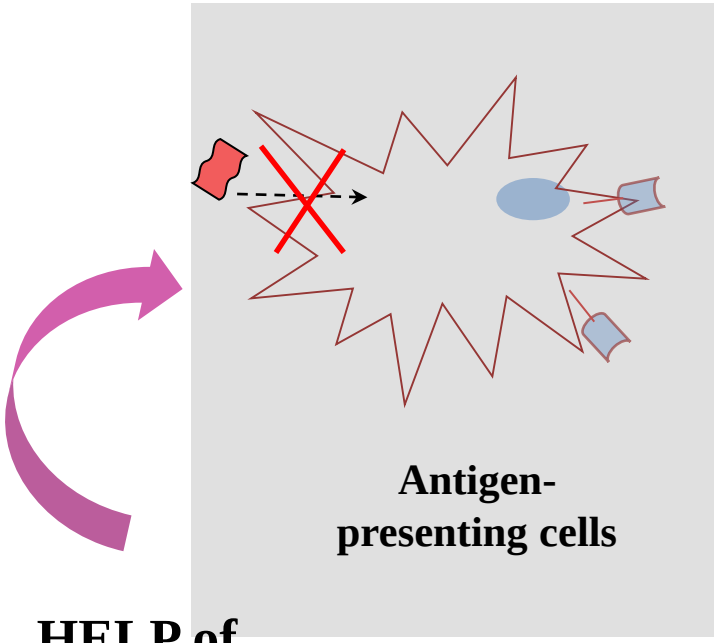

HELP of NANOPARTICLES

To deliver and support biological activity of transported material

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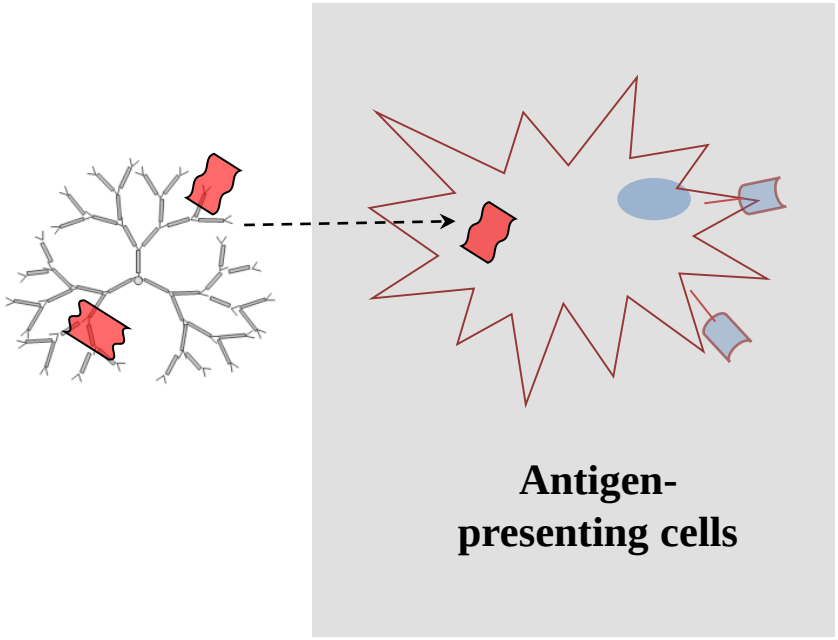

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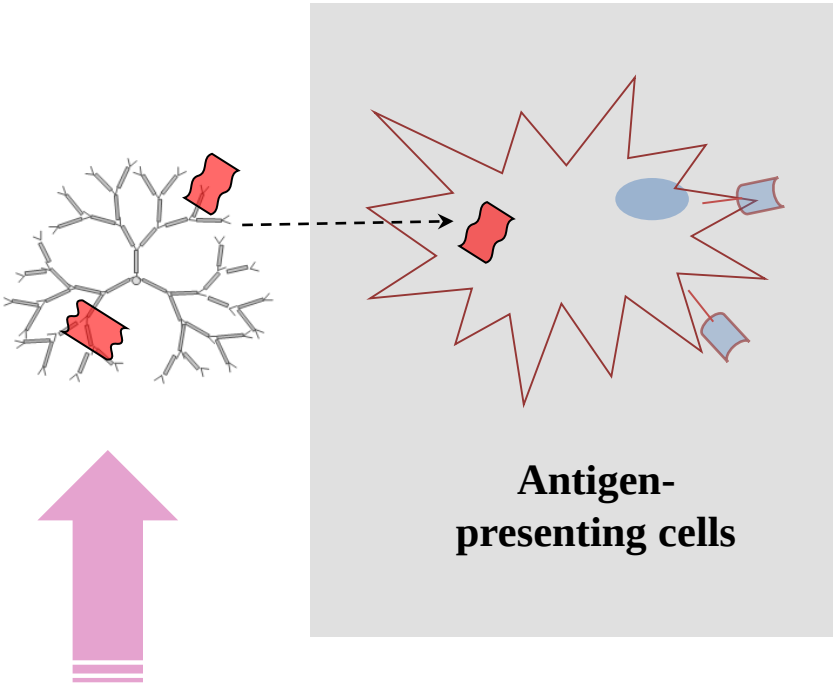


1. COMPLEXATION OF HIV-peptides with CBDs dendrimers

RBC 2018

Contents lists available at SciVerse ScienceDirect

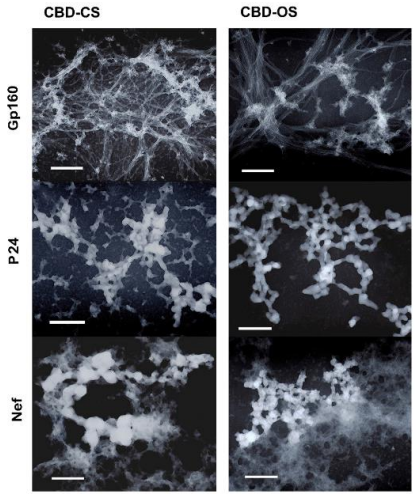
Colloids and Surfaces B: Biointerfaces

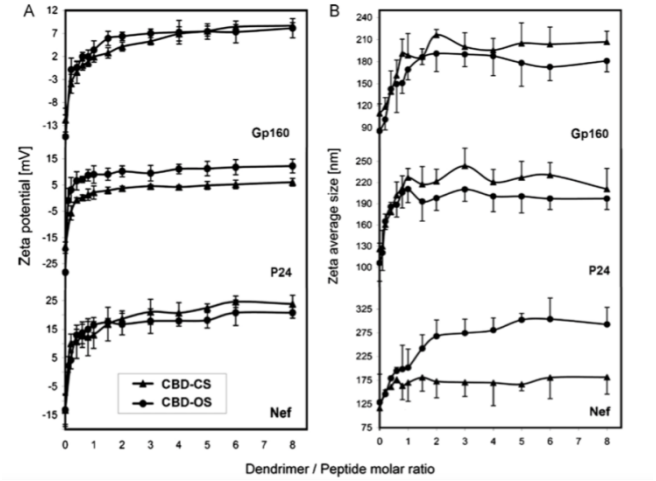
journal homepage: www.elsevier.com/locate/colsurfb

Complexation of HIV derived peptides with carbosilane dendrimers

Maksim Ionov[a,*], Karol Ciepluch[a], Barbara Klajnert[a], Sława Glińska[b], Rafael Gomez-Ramirez[c], Francisco Javier de la Mata[c], Maria Angeles Munoz-Fernandez[d], Maria Bryszewska[a]

[a] Department of General Biophysics, University of Lodz, Poland
[b] Laboratory of Electron Microscopy, Faculty of Biology and Environmental Protection, University of Lodz, Poland
[c] Departamento Química Inorgánica, Universidad de Alcalá de Henares, CIBER-BBN Alcalá de Henares, Spain
[d] Laboratorio InmunoBiologia Molecular, General Hospital Universitario Gregorio Marañon, CIBER-BBN, Madrid, Spain





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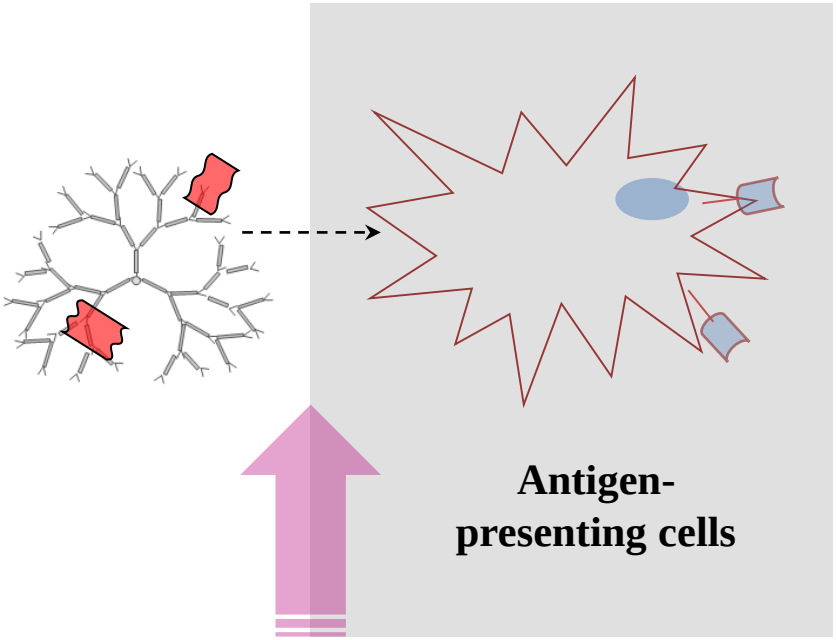


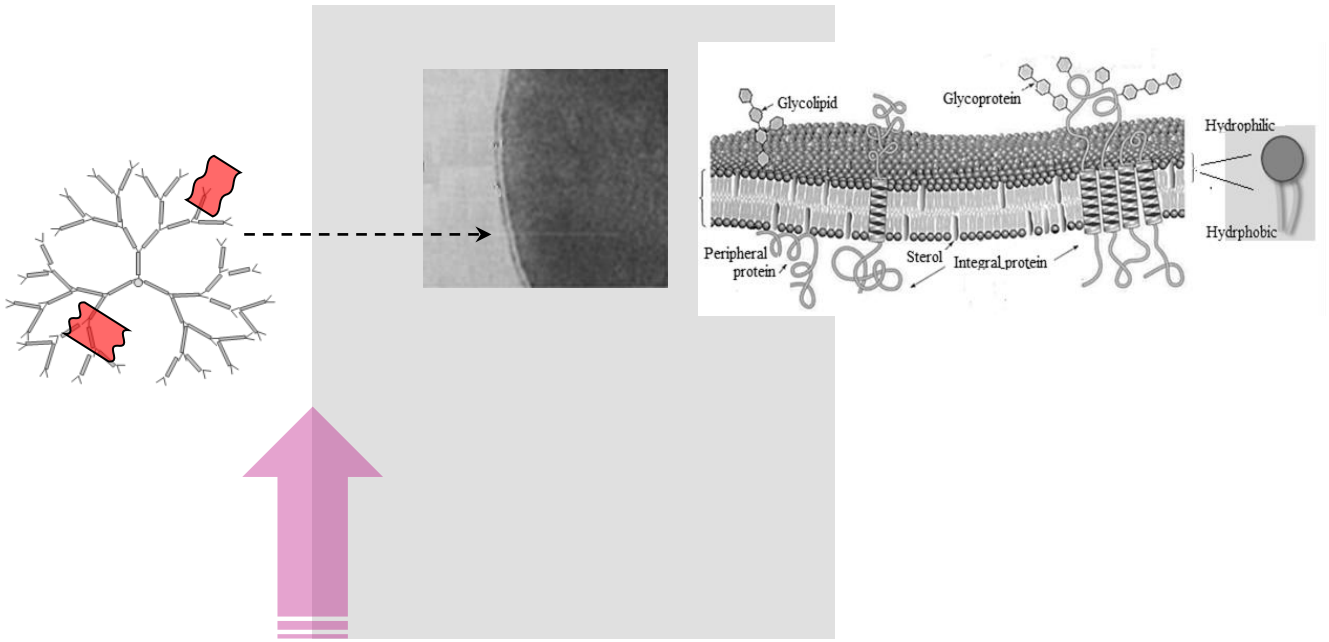
PEPTIDES

2. INTERACTION OF DENDRIMERS WITH BIOMEMBRANES

DENDRIPLEXES

Peptide-based subunite vaccination strategy

BIOLOGICAL MEMBRANES plays an important role in cell life, complex system



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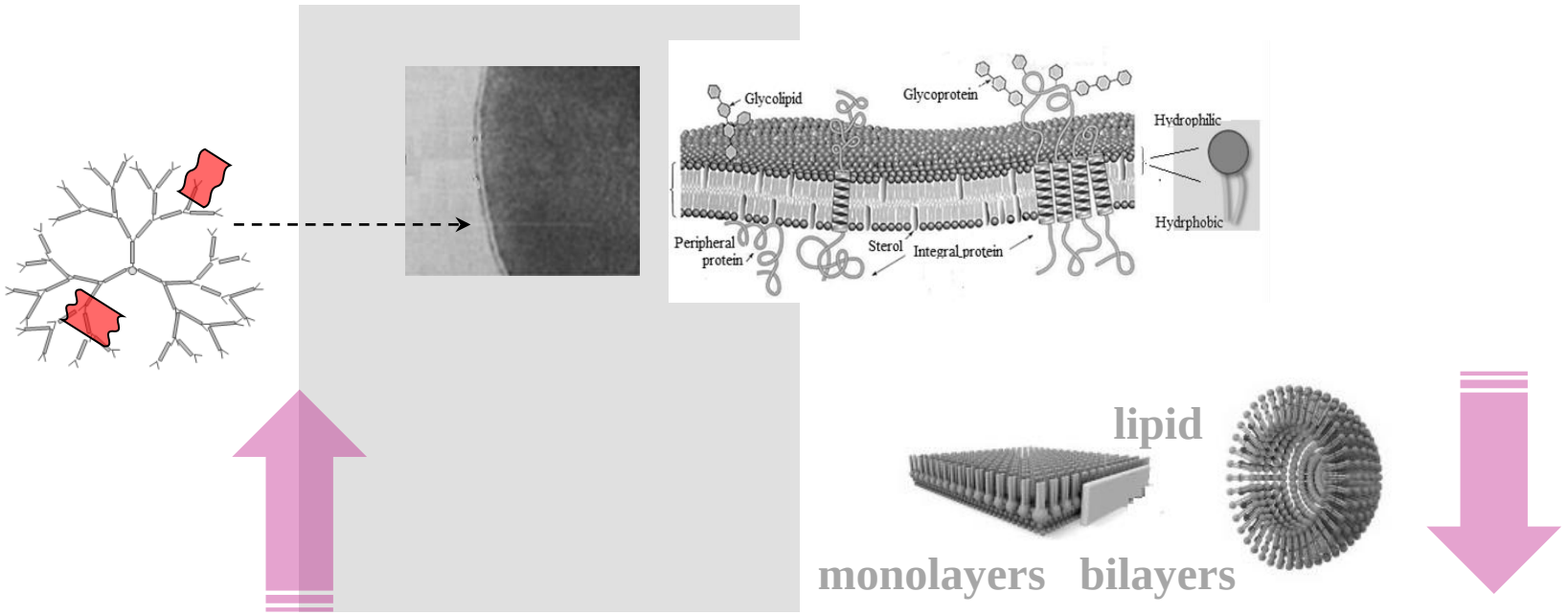


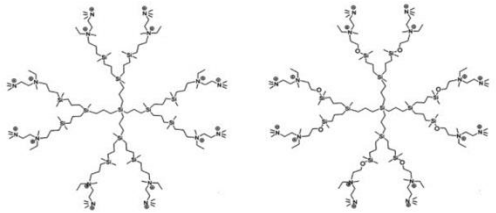
PEPTIDES

2. INTERACTION OF DENDRIMERS WITH MEMBRANE MODELS

DENDRIPLEXES

MATERIAL

Dendrimers : CBD-CS ($C_{144}H_{348}I_{16}N_{16}Si_{13}^{+16}$)
CBD-OS ($C_{128}H_{316}I_{16}N_{16}O_8Si_{13}^{+16}$)

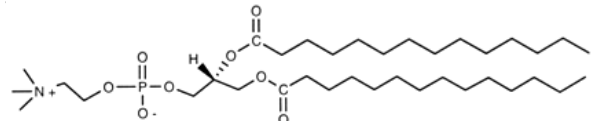


CBD-CS CBD-OS

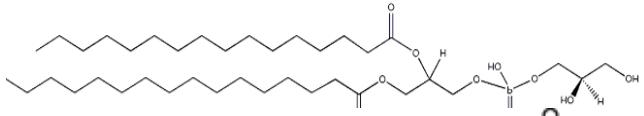
HIV derived peptides

		Length (aa)	charged
P24	HIV-HXB2 (71e80): NH-DTINEEAAEW-COOH	10	-4
Gp160	HIV-HXB2 (634e648): NH-EIDNYTNTIYTLEE-COOH	15	-4
Nef	HIV-HXB2 (172e191): NH-EIDNYTNTIYTLEE-COOH	2.0	-3

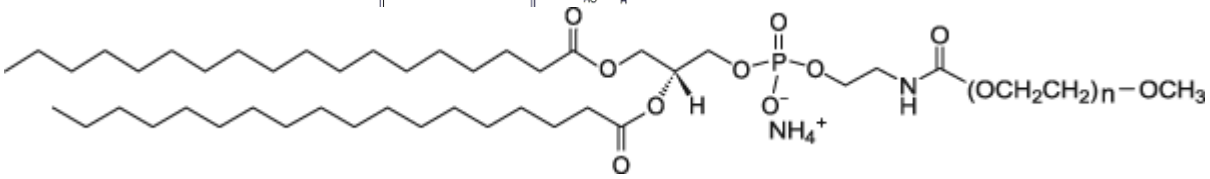
Lipids



DMPC

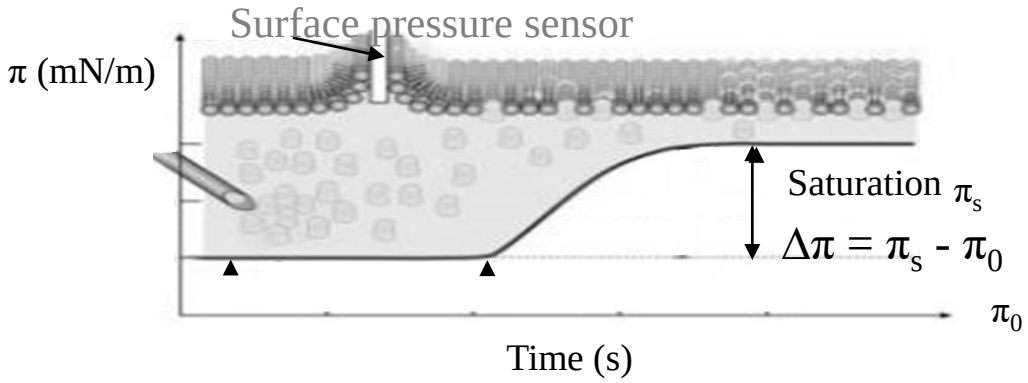


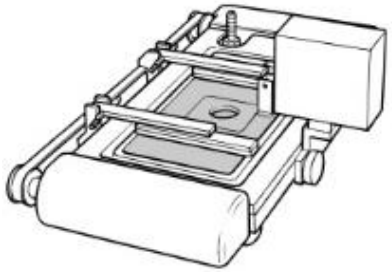
DPPG



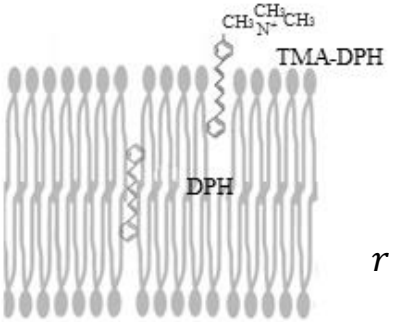
DSPE-PEG

LIPID MONOLAYERS – Monomolecular films of lipids at the air - water interface





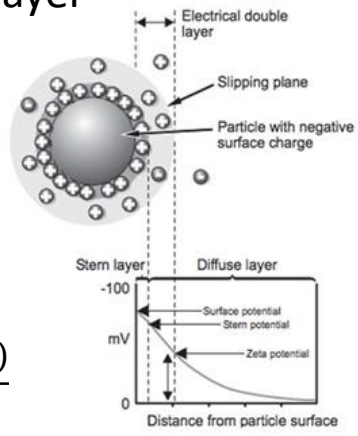
LIPID VESICLES – Spherical vesicles consisting of a phospholipid bilayer



Fluorescence anisotropy measurements

Size and Zeta potential measurements

$$r = \frac{I_{VV} - I_{VH}G}{I_{VV} + 2I_{VH}G} \qquad D_H = \frac{kT}{6\pi\eta D} \qquad U_e = \frac{2\,\epsilon\,\zeta\,f(ka)}{3\eta}$$



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Chemistry and Physics of Lipids

journal homepage: www.elsevier.com/locate/chemphyslip

Cationic carbosilane dendrimers–lipid membrane interactions

Dominika Wrobel[a,*], Arkadiusz Kłys[b], Maksim Ionov[a], Pavol Vitovic[c], Iveta Waczulikowa[c], Tibor Hianik[c], Rafael Gomez-Ramirez[d], Javier de la Mata[d], Barbara Klajnert[a], Maria Bryszewska[a]

[a] Department of General Biophysics, University of Lodz, Poland
[b] Laboratory of Molecular Spectroscopy, Department of Chemistry, University of Lodz, Poland
[c] Faculty of Mathematics, Physics and Informatics, Comenius University, Bratislava, Slovakia
[d] Departamento Quimica Inorganica, Universidad de Alcala de Henares, Spain

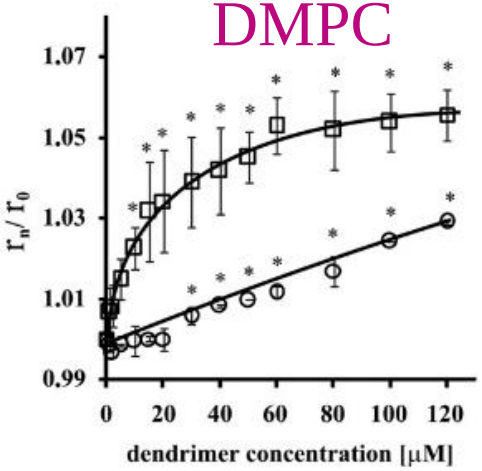
RBC 2018

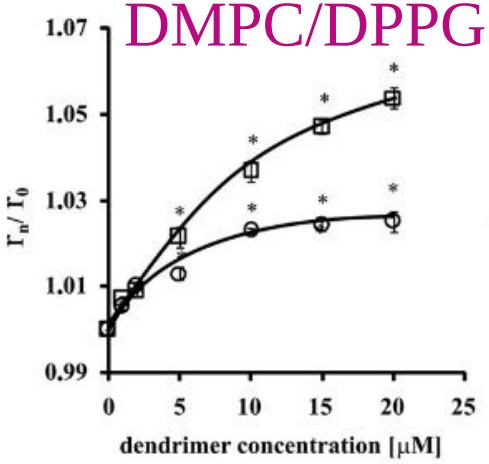
DMPC

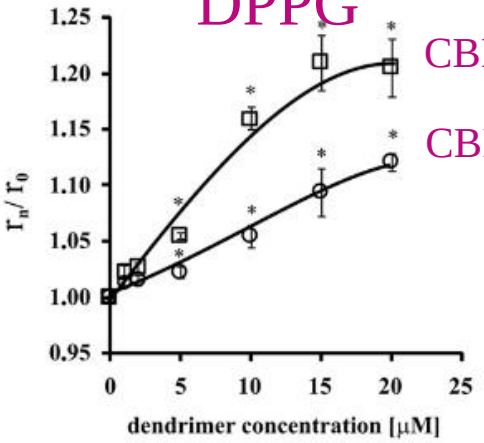
DMPC/DPPG

DPPG

TMA - DPH



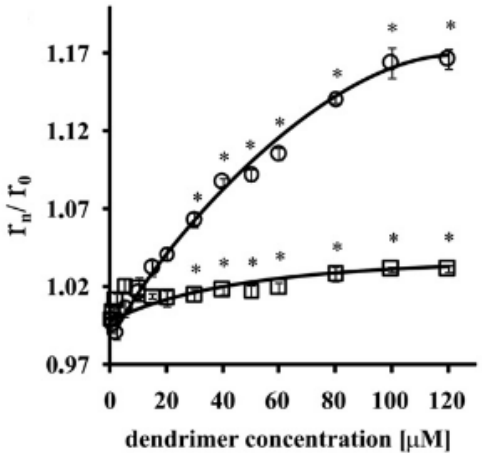


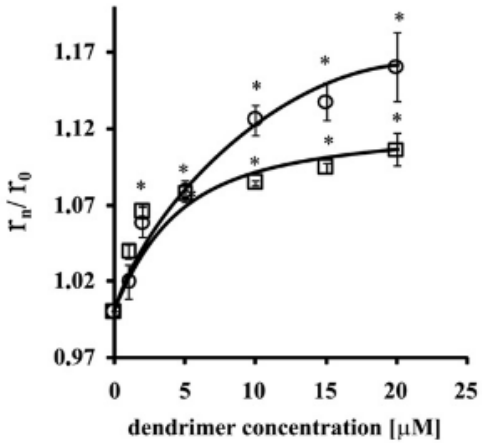


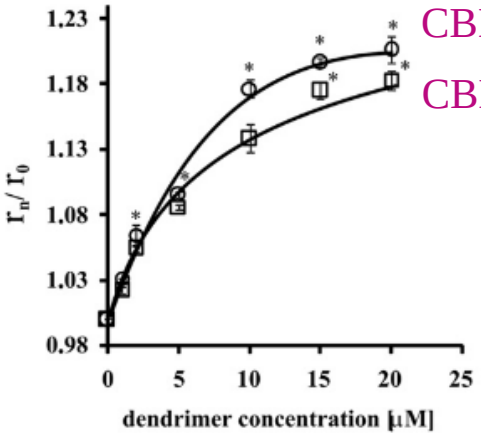
CBD O-S

CBD C-S

DPH







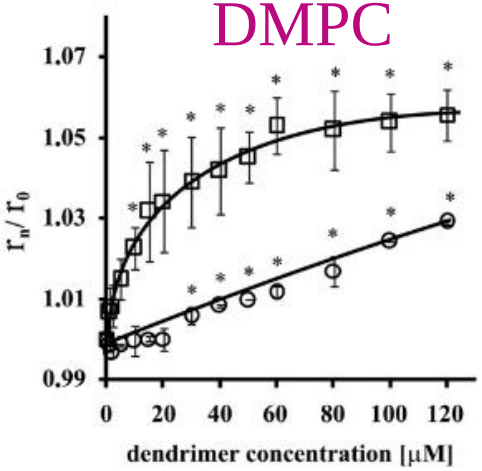
CBD C-S

CBD O-S

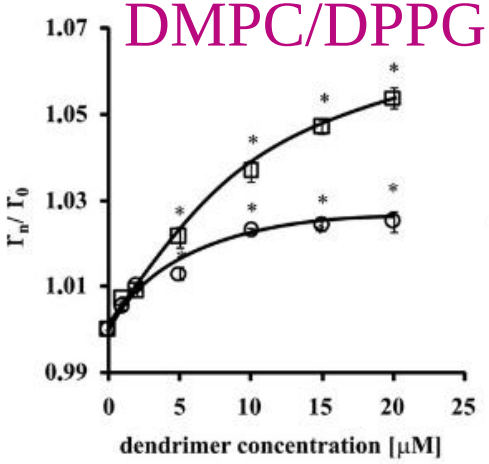
RBC 2018

TMA - DPH

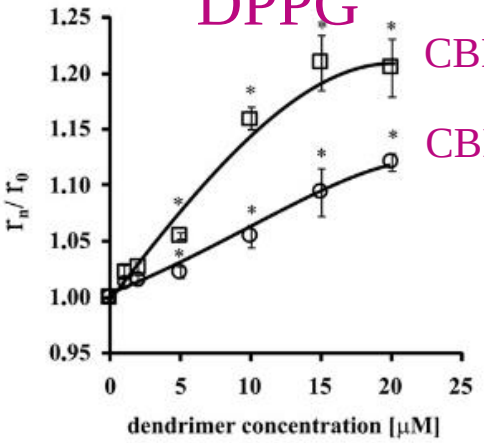
DMPC



DMPC/DPPG



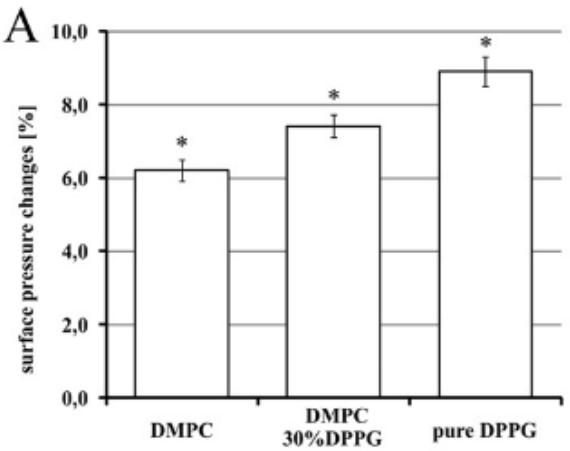
DPPG



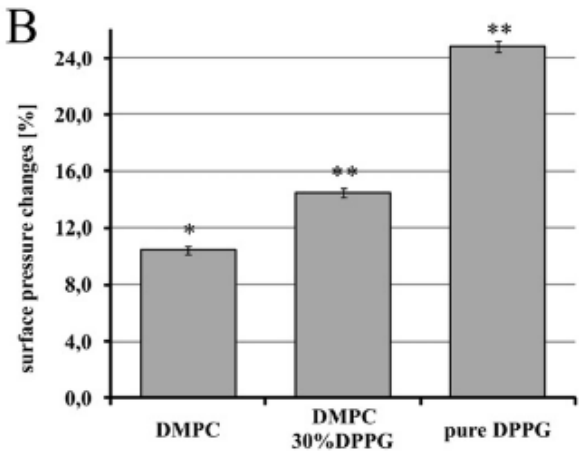
CBD O-S

CBD C-S

CBD C-S



CBD O-S



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Dendrimers complexed with HIV-1 peptides interact with liposomes and lipid monolayers

Maksim Ionov [a,*], Karol Ciepluch [a], Zuzana Garaiova [b], Sophie Melikishvili [b], Sylwia Michlewska [c], Łucja Balcerzak [c], Sława Glińska [c], Katarzyna Miłowska [a], Rafael Gomez-Ramirez [e], Francisco Javier de la Mata [e], Dzmitry Shcharbin [d], Iveta Waczulikova [b], Maria Bryszewska [a], Tibor Hianik [b]

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[b] Faculty of Mathematics, Physics and Informatics, Comenius University, Mlynska dolina, 842 48 Bratislava, Slovakia
[c] Laboratory of Electron Microscopy, Faculty of Biology and Environmental Protection, University of Lodz, Banacha 12/16, 90-237 Łódź, Poland
[d] Institute of Biophysics and Cell Engineering of NASB, Minsk, Belarus
[e] Departamento Química Inorgánica, Universidad de Alcalá de Henares, CIBER-BBN Alcalá de Henares, Spain

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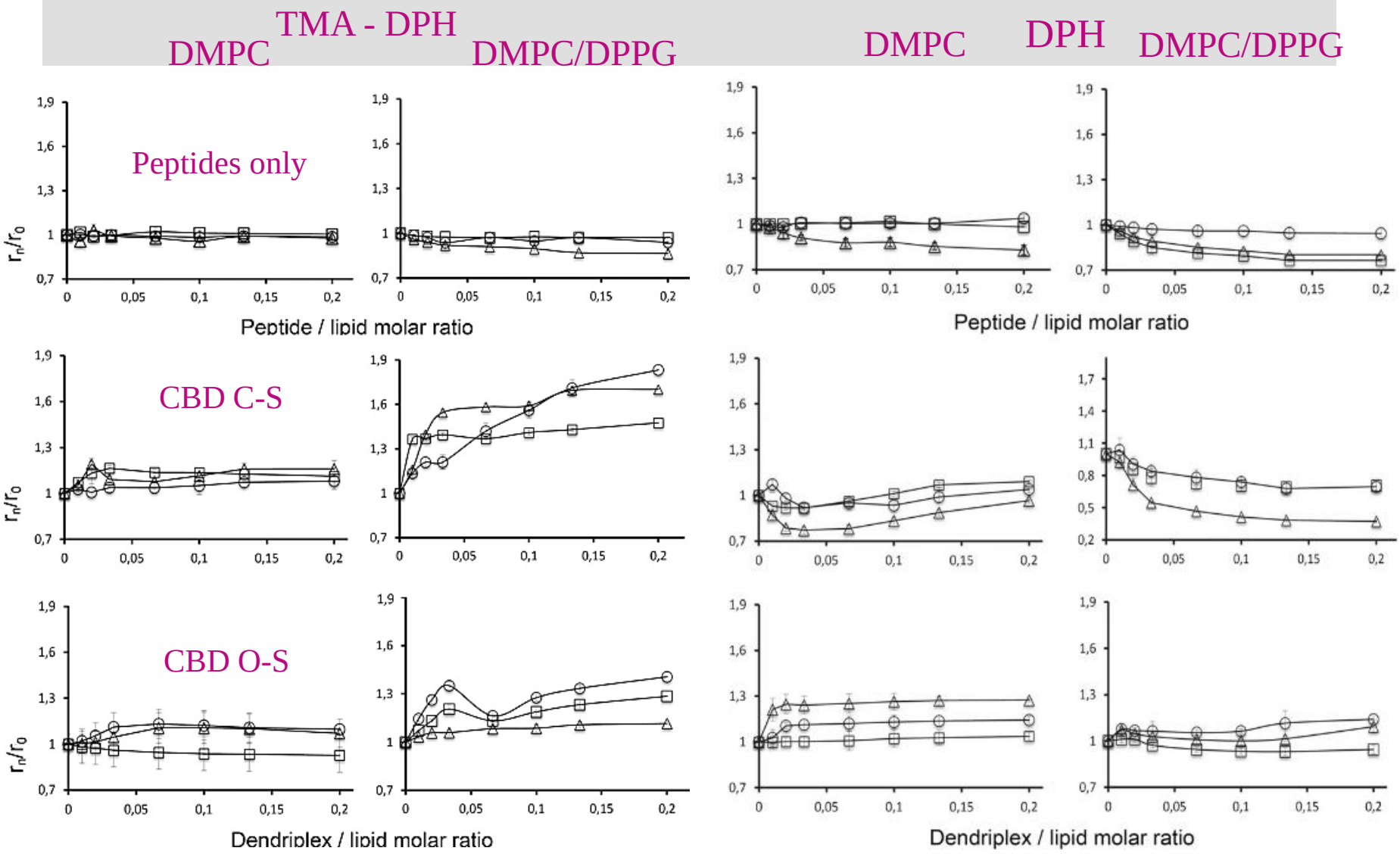
DMPC
TMA - DPH
DMPC/DPPG
DMPC
DPH
DMPC/DPPG
Peptides only
CBD C-S
CBD O-S
r_n/r_0
Peptide / lipid molar ratio
Dendriplex / lipid molar ratio

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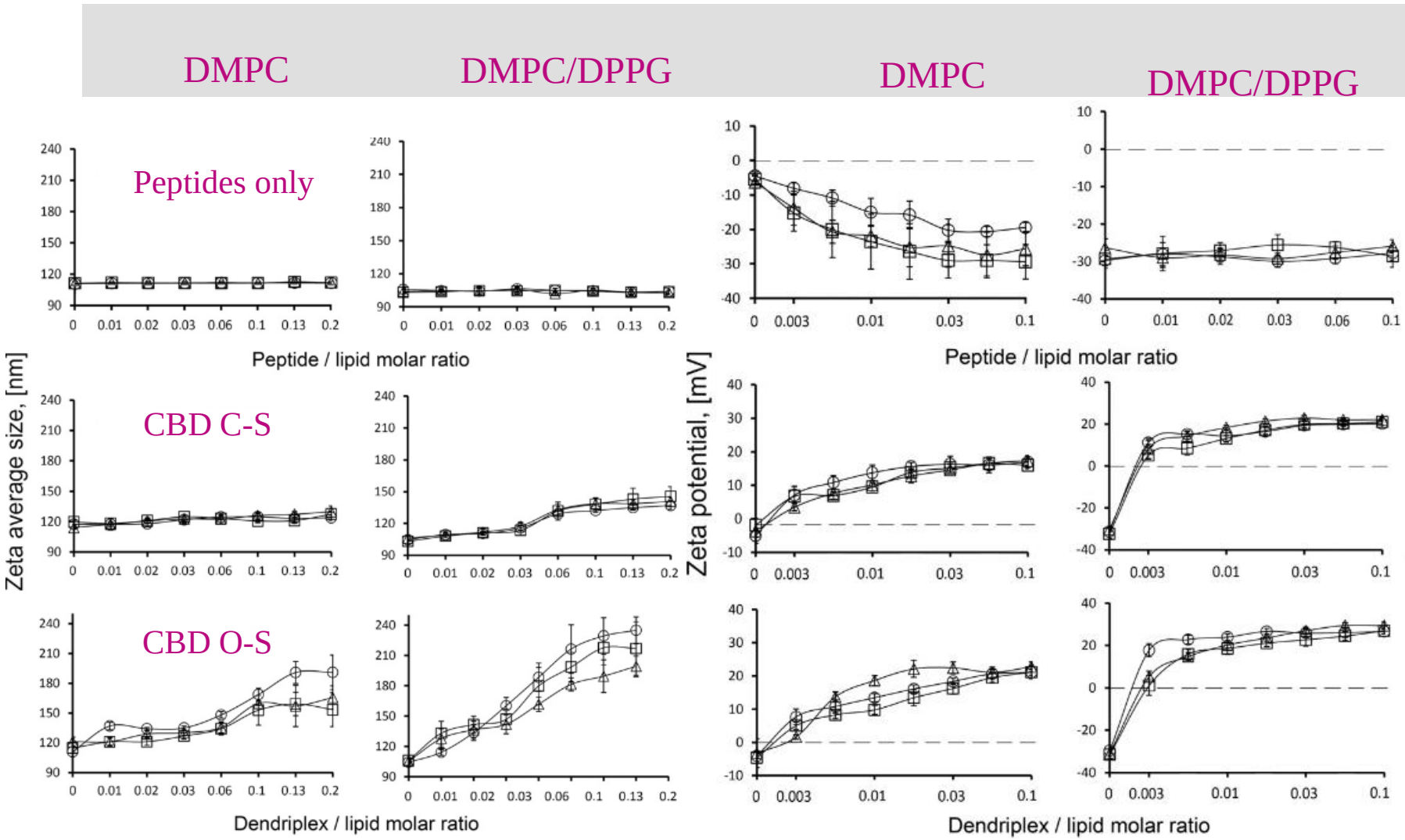
DMPC
DMPC/DPPG
DMPC
DMPC/DPPG
Peptides only
CBD C-S
CBD O-S
Zeta average size, [nm]
Zeta potential, [mV]
Peptide / lipid molar ratio
Dendriplex / lipid molar ratio
Peptide / lipid molar ratio
Dendriplex / lipid molar ratio

RBC 2018

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The effect of polyethylene glycol-modified lipids on the interaction of HIV-1 derived peptide–dendrimer complexes with lipid membranes

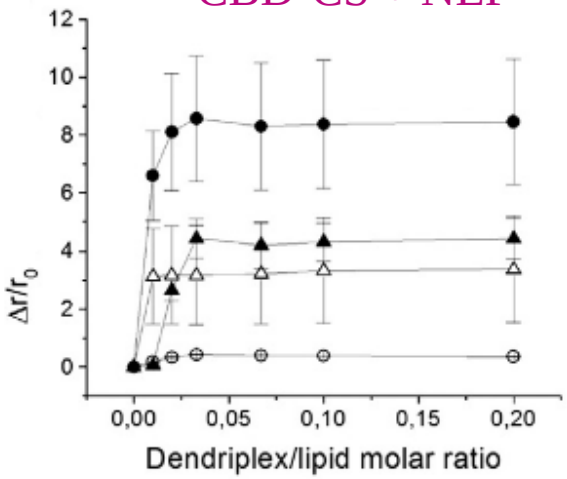
Sophie Melikishvili [a], Alexandra Poturnayova [a,b], Maksim Ionov [c], Maria Bryszewska [c], Tomáš Vary [d], Julius Cirak [d], María Ángeles Muñoz-Fernández [e,f], Rafael Gomez-Ramirez [f,g], Francisco Javier de la Mata [f,g], Tibor Hianik [a,*]

[a] Faculty of Mathematics, Physics and Informatics, Comenius University, Mlynska dolina, 842 48 Bratislava, Slovakia
[b] Institute of Animal Biochemistry and Genetics, Slovak Academy of Sciences, 900 28 Ivanka pri Dunaji, Slovakia
[c] Department of General Biophysics, Faculty of Biology and Environmental Protection, University of Lodz, Pomorska 141/143, 90-236 Lodz, Poland
[d] Institute of Nuclear and Physical Engineering, Slovak University of Technology, Ilkovicova 3, 812 19 Bratislava, Slovakia
[e] Laboratorio InmunoBiología Molecular, Hospital General Universitario Gregorio Marañón, Spanish HIV BioBank and Instituto de Investigación Sanitaria Gregorio Marañón, Madrid, Spain
[f] Networking Research Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Spain
[g] Departamento Química Orgánica y Química Inorgánica, Universidad de Alcalá Henares, Spain

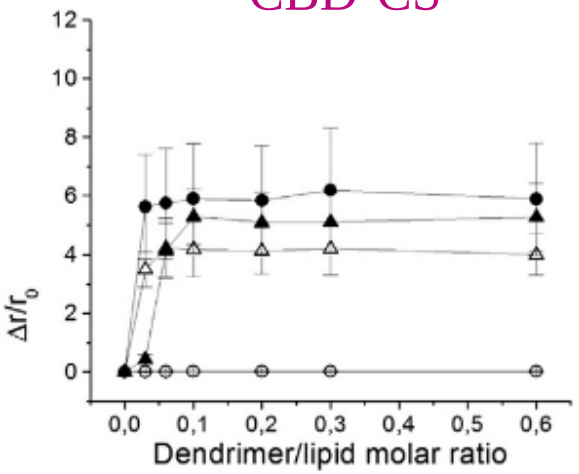
DMPC/DSPE-PEG

TMA - DPH

CBD-CS + NEF



CBD-CS



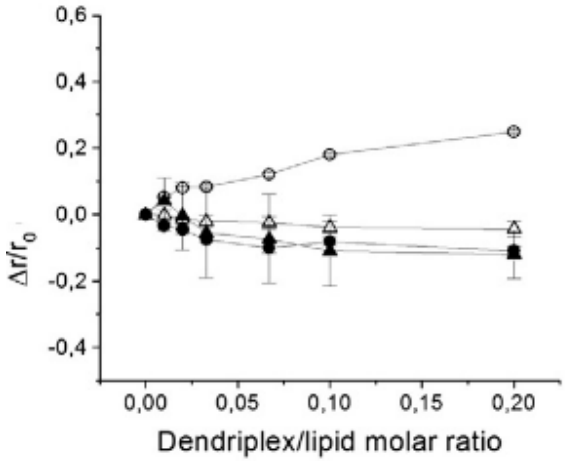
30:1

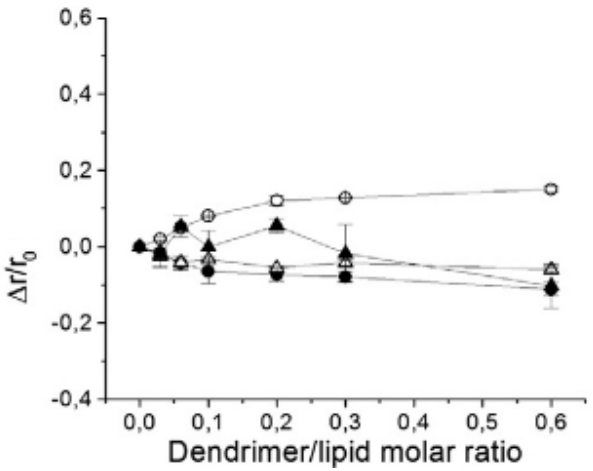
10:1

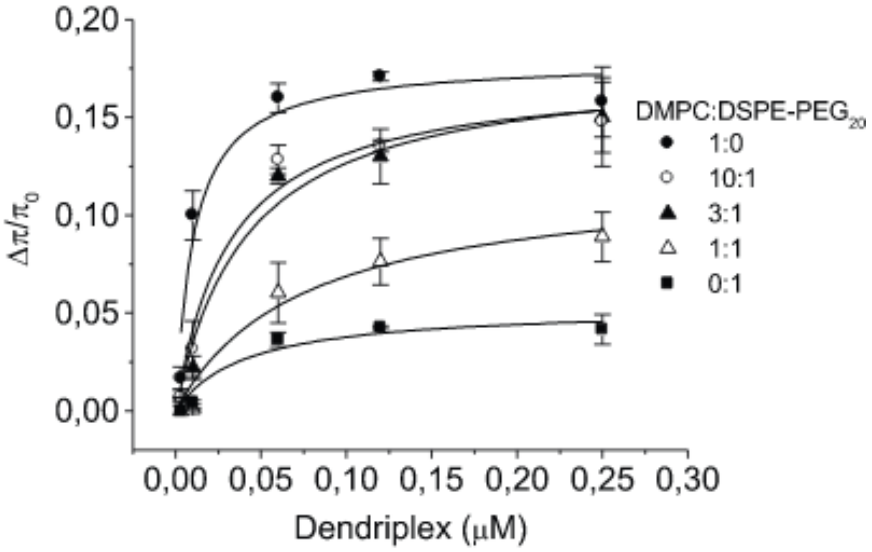
3:1

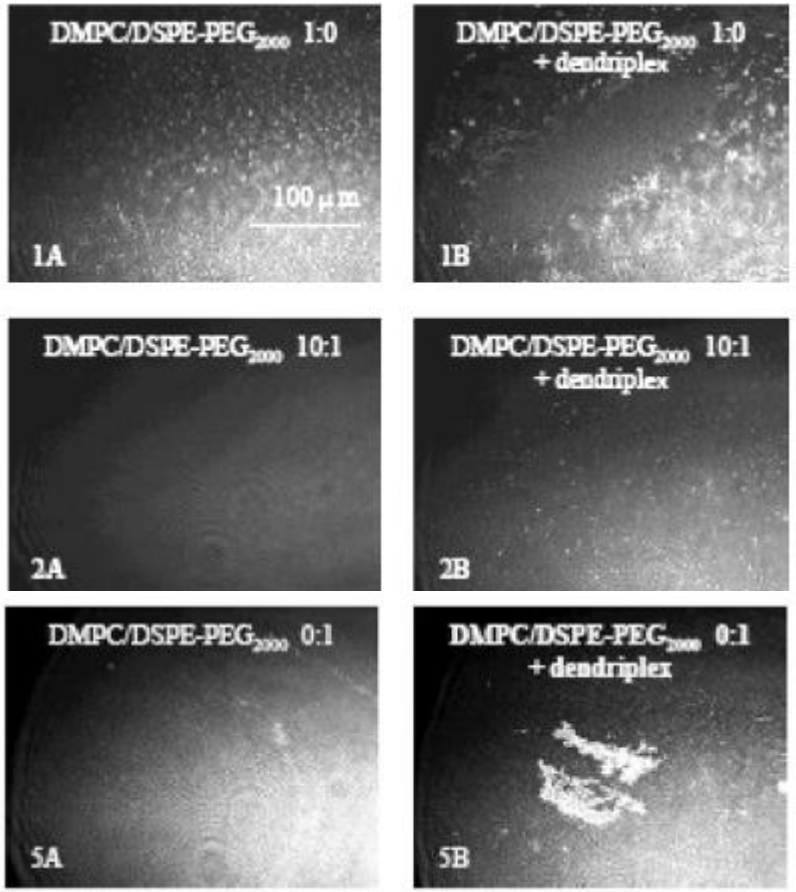
1:0

DPH






0,20
0,15
0,10
0,05
0,00
Δπ/π0
0,00 0,05 0,10 0,15 0,20 0,25 0,30
Dendriplex (μM)
DMPC:DSPE-PEG20
1:0
10:1
3:1
1:1
0:1


DMPC/DSPE-PEG2000 1:0
100 μm
1A
DMPC/DSPE-PEG2000 1:0
+ dendriplex
1B
DMPC/DSPE-PEG2000 10:1
2A
DMPC/DSPE-PEG2000 10:1
+ dendriplex
2B
DMPC/DSPE-PEG2000 0:1
5A
DMPC/DSPE-PEG2000 0:1
+ dendriplex
5B

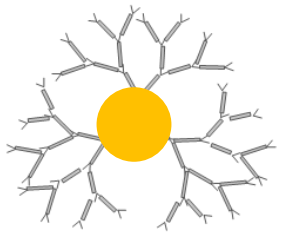
Summary

- **HIV/AIDS** remains serious problem worldwide
- Research in the field of HIV vaccine continues
- **Peptide-based synthetic vaccine** strategy with the help of NANOTECHNOLOGY
- Nanoparticles as carriers

1. **HIV-derived peptides itself** did not induce significant changes in measured parameters – **no/weak interactions** with membrane models
2. **CBDs dendrimers interacted** with model lipid membranes and were able to complex HIV-derived peptides
3. **Dendriplexes interacted** with biomembranes - interaction was **stronger** in the region of lipid headgroups and **for negatively charged membranes**
4. In the case of **pegylated membranes**, dendriplexes **interacted with hydrophilic part**, with ability to adsorb on lipid monolayer with the order of biding strength

DMPC > DMPC/DSPE-PEG 10:1 > DMPC/DSPE-PEG 3:1 DMPC/DSPE-PEG 1:1

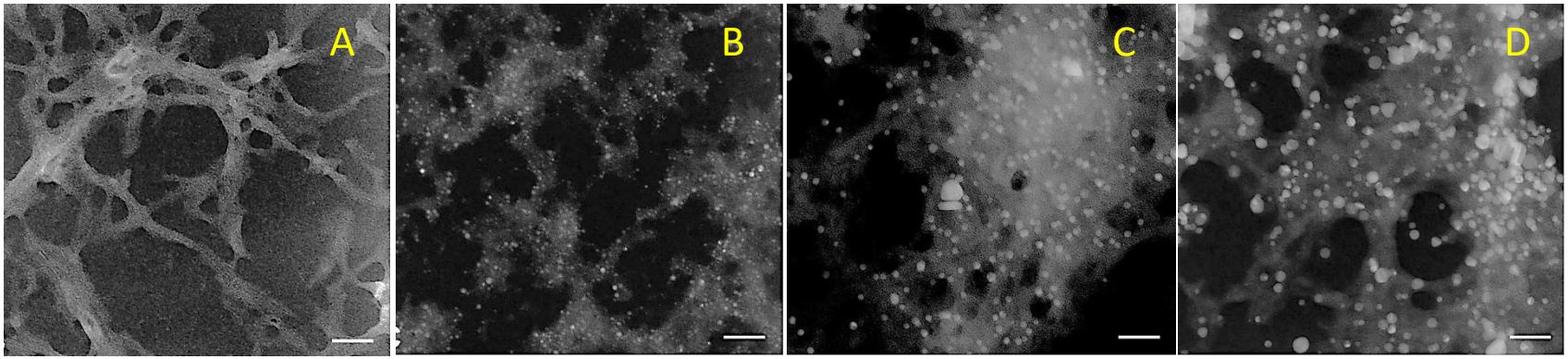
Interactions - due to electrostatic forces, ordering of lipid films
Obtained data – for optimization and potential of CBDs as carriers for HIV peptides



Biophysical study of complex formation between HIV synthetic peptides and dendronized gold nanoparticles

Zuzana Garaiova[a], Sopio Melikishvili[a], Sylwia Michlewska[b,c], Maksim Ionov[c], Elzbieta Pedziwiatr-Werbicka[c], Iveta Waczulikova[a], Tibor Hianik[a], M.Angeles Muñoz-Fernandez[d], Rafael Gomez-Ramirez[e], F. Javier de la Mata[e] Maria Bryszewska[c]

[a]Department of Nuclear Physics and Biophysics, Faculty of Mathematics, Physics and Informatics, Comenius University, Bratislava, Slovakia; [b]Laboratory of Microscopic Imaging and Specialized Biological Techniques, Faculty of Biology and Environmental Protection, University of Lodz; [c]Department of General Biophysics, Faculty of Biology and Environmental Protection, University of Lodz, Lodz, Poland;[d]Immunomolecular Biology Laboratory, Hospital Gregorio Marañon, Dr. Esquerdo Madrid, Spain; [e]Inorganic Chemistry Department, University Alcala, Alcala de Henares, Spain

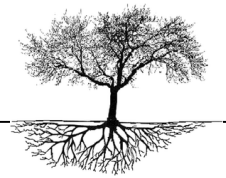


Acknowledgment

This work was supported by Slovak Research and Development Agency, APVV and Polish Ministry of Science and Higher Education, projects No. SK_PL-2015-0021 and APVV-14-0267.

S. Melikishvili, M. Ionov, S. Michlewska, D. Wrobel, K. Ciepluch, E. Pedziwiatr-Werbicka, I.Waczuliková, M. Bryszewska, T. Hianik, M.A. Muñoz-Fernandez, R. Gomez-Ramirez, J.F. de la Mata

Thank you very much for your attention

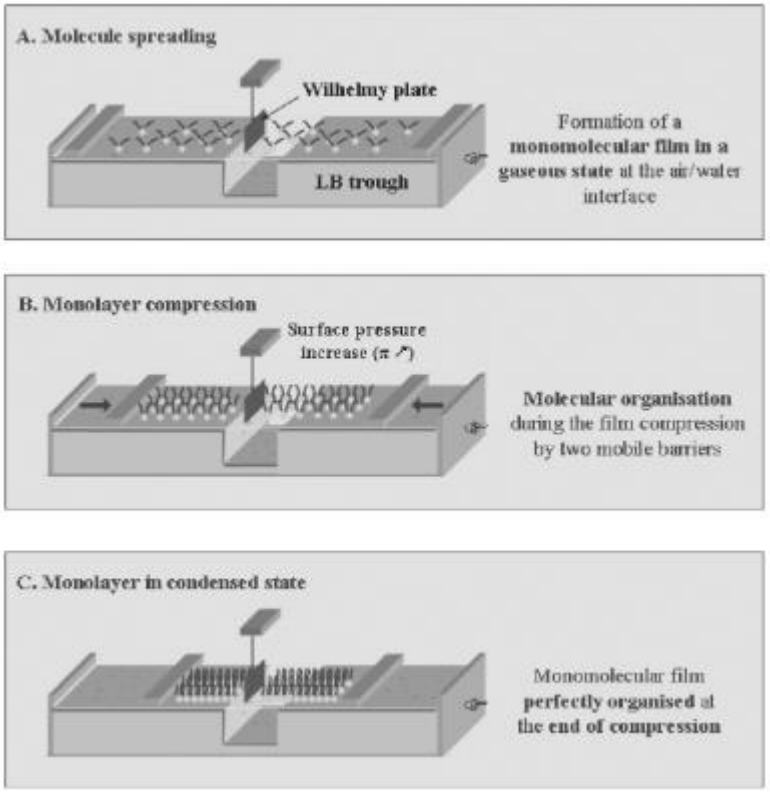


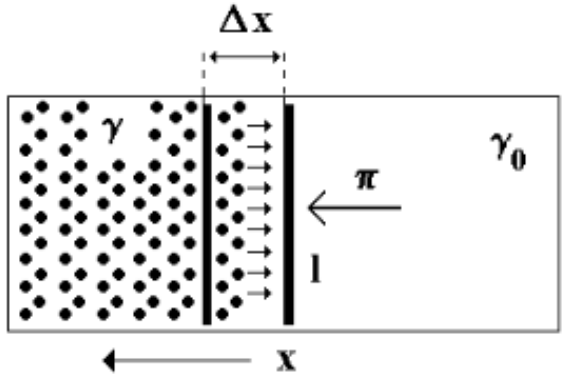
Models of membrane structure

Lipid monolayers

Langmuir monomolecular films
single leaflet of biological membranes

spontaneously formed at an air/water interface (amphiphilic nature of spreading molecules)





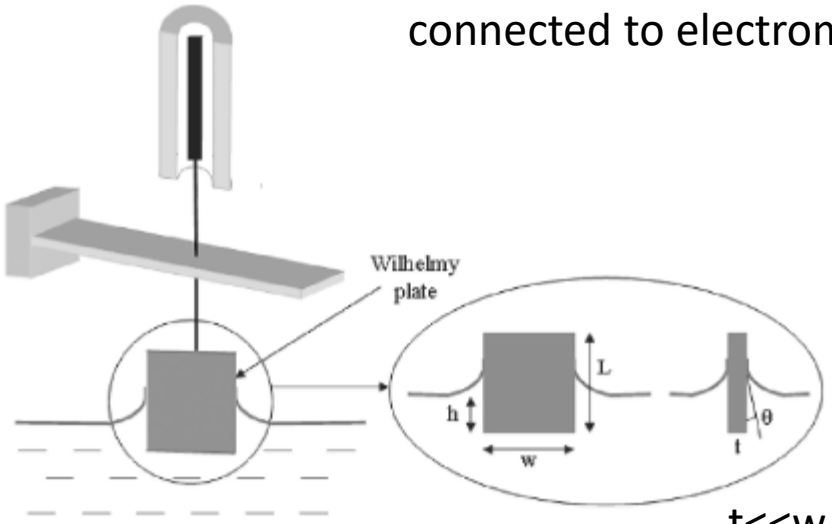
Π = Force/l = $\Upsilon_0 - \Upsilon$

Υ_0 pure water = 72.75 mNm^{-1}

Models of membrane structure

Wilhelm method – the most common method of measurement the surface pressure

_ thin plate of filter paper partially immersed in the liquid phase connected to electromicrobalance



$$F_0 = \rho_p g L w t + 2\gamma_0 (t + w) cos\theta_0 - \rho_L g t w h$$

$$F_m = \rho_p g L w t + 2\gamma (t + w) cos\theta_m - \rho_L g t w h$$

$$\Delta F = F_m - F_0 = 2(t + w)(\gamma cos\theta_m - \gamma_0 cos\theta_0)$$

t<<w and the plate is copletely wetted θ = 0, cos θ = 1

Rectangular plate of dimension w, L,t
Material density ρ_p
Immerssed to a depth h
In a liquid of density ρ_L

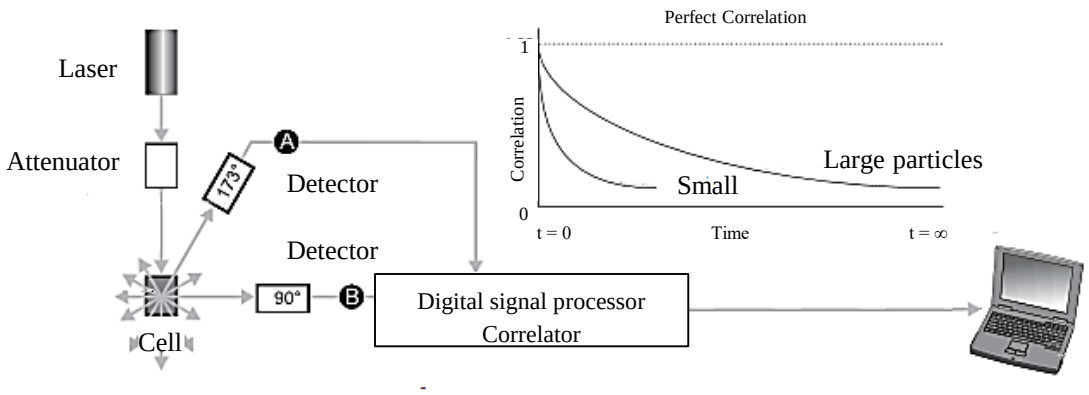
$$\Delta F = 2w(\gamma - \gamma_0) = 2w\Delta\gamma$$

$$\pi = -\Delta\gamma = -\Delta F/2w$$

Models of membrane structure

Size of particles (Zetasizer Nano)

- dynamic light scattering (DLS) (PCS – Photon Correlation Spectroscopy)
- measures Brownian motion and relates this to the size of particles
- illuminating the particles with a beam of monochromatic light
- Brownian motion causes laser light to be scattered at different intensities.
- The time dependent fluctuations in intensity of scattered light is then analyzed and yields the velocity of the Brownian motion called translational diffusion coefficient D.
- hydrodynamic diameter D_H using the Stokes-Einstein relationship



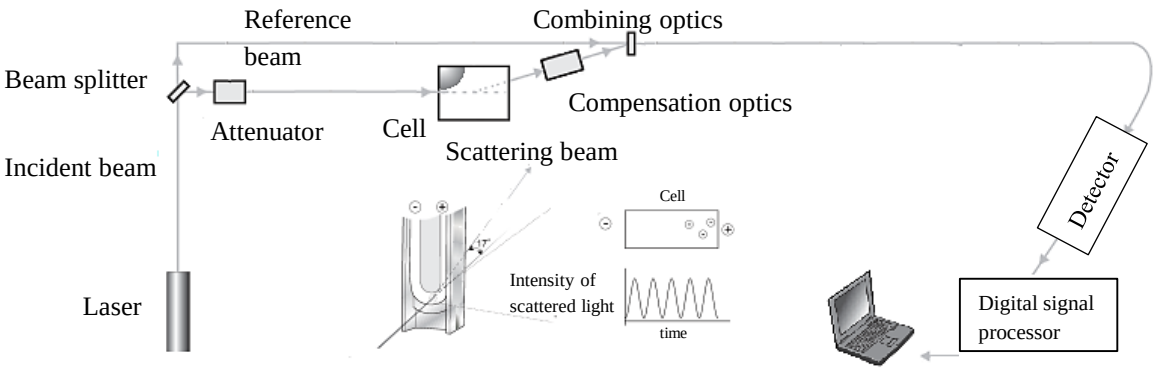
$$D_H = \frac{kT}{6\pi\eta D}$$

Models of membrane structure

Zeta potential (Zetasizer Nano)

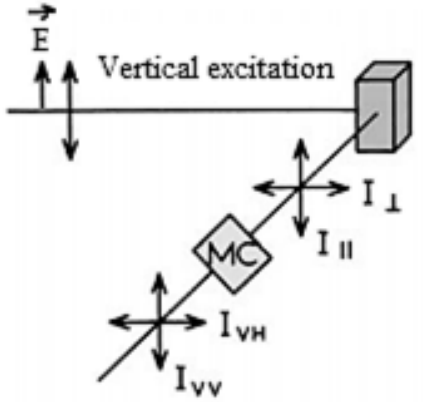
Laser Doppler velocimetry (LDV)

- Light scattered by moving particles experiences a frequency shift measurable
- Incident beam passes through the center of the sample cell and the moving particles scattered the light.
- scattered light + reference beam = modulated beam having the ´beat´ frequency.
- Doppler shift - comparing the beat frequency with that of reference frequency (Fourier transformation, phase analysis light scattering (PALS))
- characteristic frequencies in the scattered light are extracted by a digital signal processor and the mobility of particles is determined



Models of membrane structure

Fluorescence anisotropy (Spectrofluorimeter)



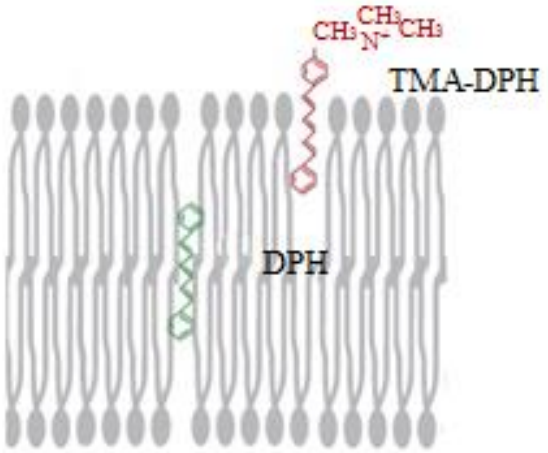
- rotational diffusion of fluorescent object during the interval between the absorption and emission
- exposed to linearly polarized light
- fluorescent target molecules having the absorption transition moments oriented along the electric vector (E) of the incident light will be excited preferentially
- highly polarized fluorescence if molecules do not rotate sample is then said to be anisotropic
- isotropic sample - unpolarized, as the orientation of the emitted light is lost fast rotational diffusion of the excited molecule

$$r = \frac{I_{VV} - I_{VH}G}{I_{VV} + 2I_{VH}G}$$

Models of membrane structure

Fluorescence anisotropy (Spectrofluorimeter)

- fluorescence emission - its degree of depolarization
- fluorescence depolarization - rotational diffusion of the fluorophore during the excited lifetime
- lower the anisotropy value, the faster the rotational diffusion



The changes in ordering of the environment which surrounds the probe are therefore in main interest of fluorescence anisotropy measurement and can be used to provide information about membrane fluidity.

The fluidity of membrane is expressed as reciprocal to lipid order.

Brewster's angle (also known as the **polarization angle**) is an angle of incidence at which light with a particular polarization is perfectly transmitted through a transparent dielectric surface, with *no reflection*. When *unpolarized* light is incident at this angle, the light that is reflected from the surface is therefore perfectly polarized.