Nanoparticles as drug delivery systems. The interaction with biomembrane


1 Department of Nuclear Physics and Biophysics, FMFI UK Bratislava, Slovakia
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
unique properties and size-compatibility with proteins and nucleic acids
Nanoparticles as drug delivery systems.

- 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
HIV Human immunodeficiency virus

Acquired immunodeficiency syndrome

dead immune cells

AIDS
36.7 million people worldwide are currently living with HIV/AIDS.

Source: UNAIDS/WHO
Number of newly infected people with HIV

- 2000: 3.2 million
- 2005: 2.5 million
- 2010: 2.2 million
- 2017: 1.8 million

Source: UNAIDS/WHO
HIV virus

- Gp 120 (Env)
- Gp 41 (Env)
- Reverse transcriptase (Pol)
- Integrase (Pol)
- Protease (Pol)
- Nucleocapsid (Gag)
- Capsid
- Viral HIV RNA
- Lipid bilayer
- Matrix (Gag)

~120 nm

TARGET

- T-cells (CD4+ Tcells)
- Macrophage
- Dendritic cells
Viral cycle of HIV

Entry

CD4 receptor

HIV RNA

HIV DNA

Reverse Transcription

Translation

Integration

PROVIRUS

Maturation

RBC 2018
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 subunit vaccination strategy

Dendritic cells

Antigen-presenting cells

MHC
Peptide-based subunite vaccination strategy

Antigen-presenting cells

CD4

T-lymphocytes

CD8
Peptide-based subunite vaccination strategy

Antigen-presenting cells

CD4

T-lymphocytes

CD8

B-lymphocytes

cytotoxic cells

humoral memory cells

antibodies

Cellular immune response
Peptide-based subunite vaccination strategy

Antigen-presenting cells

CD4

T-lymphocytes

CD8

B-lymphocytes

Cytotoxic cells

Humoral memory cells

Antigen-presenting cells

Cellular immune response
Peptide-based subunite vaccination strategy

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

Require use of immunostimulants (adjuvants)
**Peptide-based subunite vaccination strategy**

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

Require use of immunostimulants (adjuvants)

HELP of NANOPARTICLES To deliver and support biological activity of transported material
Peptide-based subunite vaccination strategy generally safe, able to induce very specific but weak immunity response

Require use of immunostimulants (adjuvants)
**Peptide-based subunite vaccination strategy**

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

Require use of immunostimulants (adjuvants)

1. COMPLEXATION OF HIV-peptides with CBDs dendrimers
Complexation of HIV derived peptides with carbosilane dendrimers

Maksim Ionov\textsuperscript{a,\ast}, Karol Ciepluch\textsuperscript{a}, Barbara Klajnert\textsuperscript{a}, Sława Glińska\textsuperscript{b}, Rafael Gomez-Ramirez\textsuperscript{c}, Francisco Javier de la Mata\textsuperscript{c}, María Angeles Munoz-Fernandez\textsuperscript{d}, Maria Bryszewska\textsuperscript{a}

\textsuperscript{a} Department of General Biophysics, University of Lodz, Poland
\textsuperscript{b} Laboratory of Electron Microscopy, Faculty of Biology and Environmental Protection, University of Lodz, Poland
\textsuperscript{c} Departamento Química Inorgánica, Universidad de Alcalá de Henares, CIBER-BBN Alcalá de Henares, Spain
\textsuperscript{d} Laboratorio Inmunobiología Molecular, General Hospital Universitario Gregorio Marañón, CIBER-BBN, Madrid, Spain

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figures}
\caption{Graphs showing zeta potential and average size of dendrimers with peptide ratios.}
\end{figure}
Peptide-based subunite vaccination strategy

Require use of immunostimulants (adjuvants)

Antigen-presenting cells

PEPTIDES

2. INTERACTION OF DENDRIMERS WITH BIOMEMBRANES DENDRIPLEXES
Peptide-based subunite vaccination strategy

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

PEPTIDES

2. INTERACTION OF DENDRIMERS WITH BIOMEMBRANES

DENDRIPLEXES
Peptide-based subunite vaccination strategy

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

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 \((C_{128}H_{316}I_{16}N_{16}O_{8}Si_{13}^{+16})\)

**HIV derived peptides**

<table>
<thead>
<tr>
<th>Peptide</th>
<th>HIV-HXB2</th>
<th>Length (aa)</th>
<th>Charge</th>
</tr>
</thead>
<tbody>
<tr>
<td>P24</td>
<td>NH-DTINEEAAEW-COOH</td>
<td>10</td>
<td>-4</td>
</tr>
<tr>
<td>Gp160</td>
<td>NH-EIDNYTNIYTHEE-COOH</td>
<td>15</td>
<td>-4</td>
</tr>
<tr>
<td>Nef</td>
<td>NH-EIDNYTNIYTHEE-COOH</td>
<td>2.0</td>
<td>-3</td>
</tr>
</tbody>
</table>

**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

\[ r = \frac{I_{VV} - I_{VH}G}{I_{VV} + 2I_{VH}G} \]
\[ D_H = \frac{kT}{6\pi\eta D} \]
\[ U_e = \frac{2\epsilon\zeta f(ka)}{3\eta} \]
Cationic carbosilane dendrimers–l lipid membrane interactions

Dominika Wrobel\textsuperscript{a,},* Arkadiusz Klys\textsuperscript{b}, Maksim Ionov\textsuperscript{a}, Pavol Vitovic\textsuperscript{c}, Iveta Waculikowa\textsuperscript{c}, Tibor Hianik\textsuperscript{c}, Rafael Gomez-Ramirez\textsuperscript{d}, Javier de la Mata\textsuperscript{d}, Barbara Klajnert\textsuperscript{a}, Maria Bryszewska\textsuperscript{a}

\textsuperscript{a} Department of General Biophysics, University of Lodz, Poland
\textsuperscript{b} Laboratory of Molecular Spectroscopy, Department of Chemistry, University of Lodz, Poland
\textsuperscript{c} Faculty of Mathematics, Physics and Informatics, Comenius University, Bratislava, Slovakia
\textsuperscript{d} Departamento Quimica Inorganica, Universidad de Alcala de Henares, Spain
RBC 2018

**DMPC**

**DMPC/DPPG**

**DPPG**

CBD O-S

CBD C-S

CBD C-S

CBD O-S
TMA - DPH

DMPC

DMPC/DPPG

DPPG

CBD O-S

CBD C-S

RBC 2018

A

B

CBD C-S

CBD O-S

surface pressure changes [%]

surface pressure changes [%]

DMPC

DMPC 30% DPPG

pure DPPG

DMPC

DMPC 30% DPPG

pure DPPG
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

a Department of General Biophysics, Faculty of Biology and Environmental Protection, University of Łódź, Pomorska 141/143, 90-236 Łódź, Poland
b Faculty of Mathematics, Physics and Informatics, Comenius University, Milíčov 7, 842 48 Bratislava, Slovakia
c Laboratory of Electron Microscopy, Faculty of Biology and Environmental Protection, University of Łódź, 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
Peptides only

CBD C-S

CBD O-S

DMPC TMA - DPH
DMPC/DPPG

DMPC     DPH     DMPC/DPPG
Peptides only

CBD C-S

CBD O-S
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, Tomas Vary d, Julius Cirak d, Maria Angeles Munoz-Fernandez 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 Instituto de Bioquimica Molecular, Hospital General Universitario Gregorio Maranon, Spanish HIV BioBank and Instituto de Investigacion Sanitaria Gregorio Maranon, Madrid, Spain
f Networking Research Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Spain
g Departamento Quimica Organica y Quimica Inorganica, Universidad de Alcali Henares, Spain
Summary

- **HIV/AIDS** remains a 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 Garaiová, Sopio Melikishvili, Sylwia Michlewská, Maksim Ionov, Elzbieta Pedziwiatr-Werbicka, Iveta Waczulikova, Tibor Hianik, M. Angeles Muñoz-Fernández, Rafael Gomez-Ramírez, F. Javier de la Mata, Maria Bryszewska

*Department of Nuclear Physics and Biophysics, Faculty of Mathematics, Physics and Informatics, Comenius University, Bratislava, Slovakia; Laboratory of Microscopic Imaging and Specialized Biological Techniques, Faculty of Biology and Environmental Protection, University of Lodz; Department of General Biophysics, Faculty of Biology and Environmental Protection, University of Lodz, Lodz, Poland; Immunomolecular Biology Laboratory, Hospital Gregorio Marañón, Dr. Esquerdo Madrid, Spain; Inorganic Chemistry Department, University Alcalá, Alcalá de Henares, Spain
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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)

\[ \Pi = \text{Force/}l = \gamma_0 - \gamma \]

\( \gamma_0 \) 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

\[
F_0 = \rho_p g Lwt + 2\gamma_0(t + w)\cos\theta_0 - \rho_L g t w h
\]

\[
F_m = \rho_p g Lwt + 2\gamma(t + w)\cos\theta_m - \rho_L g t w h
\]

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

\[
t << w \text{ and the plate is completely wetted } \theta = 0, \cos \theta = 1
\]

Rectangular plate of dimension \(w, L, t\)
Material density \(\rho_p\)
Immersed to a depth \(h\)
In a liquid of density \(\rho_L\)

\[
\Delta F = 2w(\gamma - \gamma_0) = 2w\Delta\gamma
\]

\[
\pi = -\Delta\gamma = -\Delta F/2w
\]
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)

- 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.