Elżbieta Pędziwiatr – Werbicka
University of Lodz, Faculty of Biology and Enviromental Protection
Deparment of General Biophysics
PROF. MARIA BRYSZEWSKA

KBO Katedra Biofizyki Ogólnej
WYDZIAŁ BIOLOGII i OCHRONY ŚRODOWISKA
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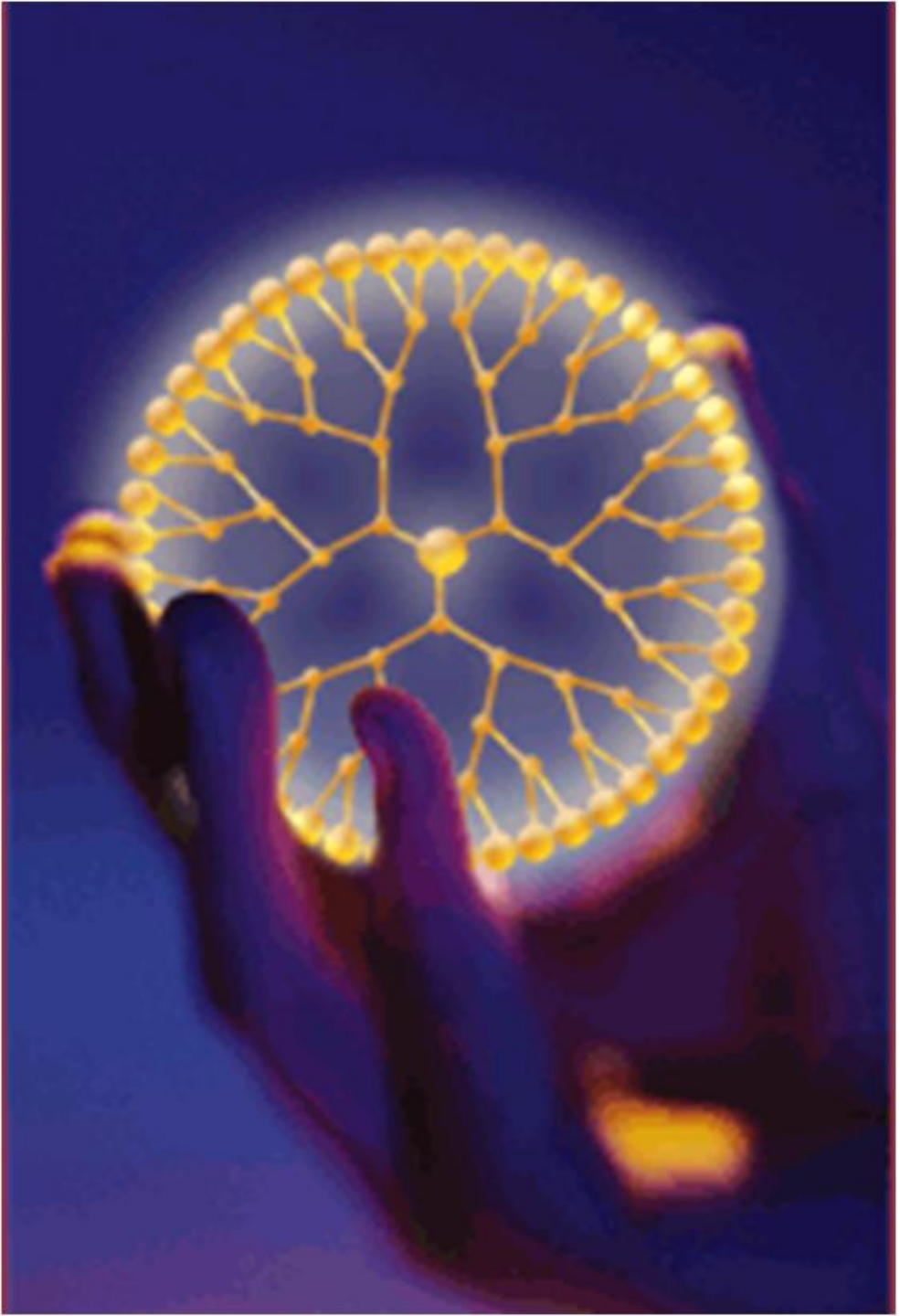
NANOPARTICLES AS THE BASIS FOR TREATMENT OF HIV/AIDS AND CANCER

ROLE OF DENDRIMERS IN THE TREATMENT OF HIV-1 INFECTIONS



PLAN OF PRESENTATION

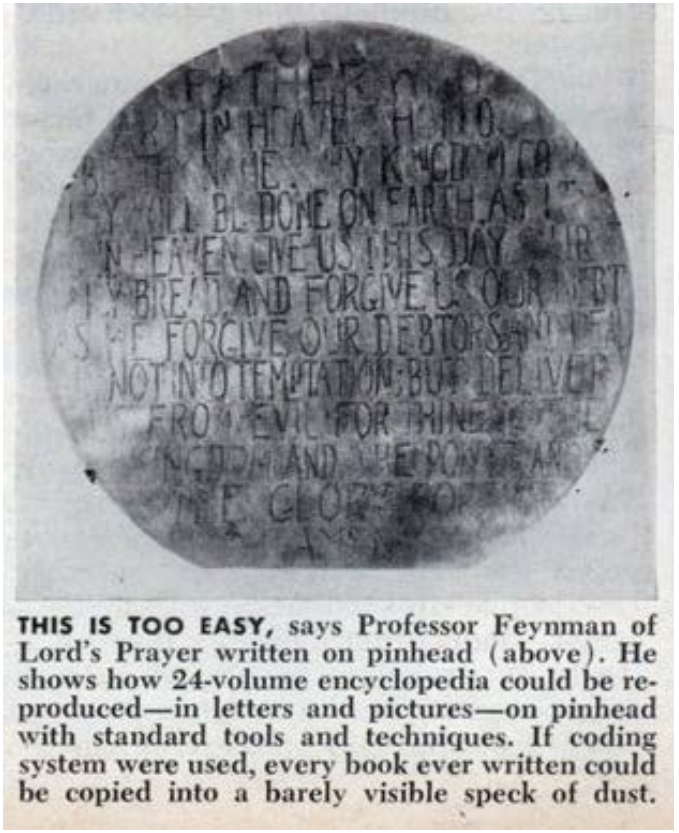
1. NANOworld
2. DEPARTMENT OF GENERAL BIOPHYSICS
3. DENDRIMER CHARACTERIZATION (structure, unique properties, possible applications)
4. APPLICATION OF DENDRIMERS AS anti-HIV THERAPY: AS CARRIERS FOR anti-HIV SHORT NUCLEIC ACIDS (ODNs, siRNAs)

Lecture in1959

There's Plenty of Room at the Bottom

the initiations for an independent development of nanoscience and nanotechnology

"What I want to talk about is the problem of manipulating and controlling things on a small scale"

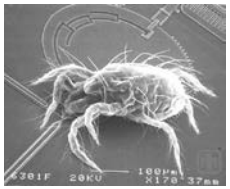


THIS IS TOO EASY, says Professor Feynman of Lord's Prayer written on pinhead (above). He shows how 24-volume encyclopedia could be reproduced—in letters and pictures—on pinhead with standard tools and techniques. If coding system were used, every book ever written could be copied into a barely visible speck of dust.

Richard P. Feynman (1918-1988)

Nobel Prize (1965 r.)

Popular Science, Nov. 1960

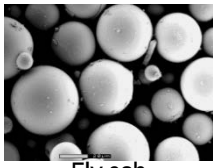


Dust mite

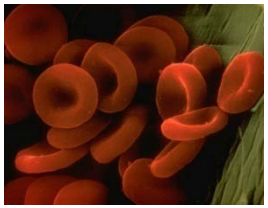
200 μm

Ant
~ 5 mm

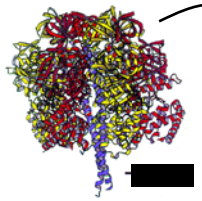
Human hair
~ 60-120 μm wide



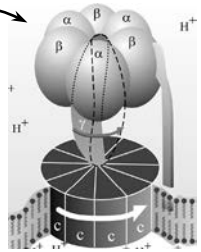
Fly ash
~ 10-20 μm



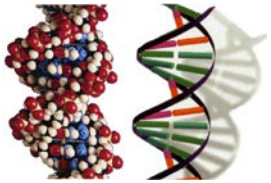
Red blood cells
(~7-8 μm)



~10 nm diameter



ATP synthase

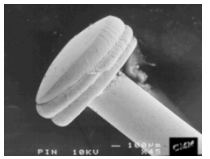


DNA
~2-1/2 nm diameter

Atoms of silicon
spacing 0.078 nm

1 cm
10 mm
10^{-2} m

10^{-3} m
1 000 000 nanometers = 1 millimeter (mm)



Head of a pin
1-2 mm

0.1 mm
100 μm
10^{-4} m

Micro-technology
"The Micro World"

0.01 mm
10 μm
10^{-5} m

10^{-6} m
1 000 nanometers = 1 micrometer (μm)

0.1 μm
100 nm
10^{-7} m

0.01 μm
10 nm
10^{-8} m

Nanotechnology
"The Nano World"

nano – gr. dwarf

10^{-9} m
1 nanometer (nm)

0.1 nm
10^{-10} m

NANOSTRUCTURES

particles, with a size <100 nm in at least one dimension with novel properties and functions because of their small size

low-dimensional structures

- quantum dots
- quantum wires
- quantum wells

carbon and metal structures

- fullerens
- carbon nanotubes
- carbon nanocrystallites
- carbon nanocapsules
- graphene
- nanosilver
- nanogold
- nanotitanium

molecular structures and nanomaterials

- dendrimers **2001**
- clusters
- nanopowders
- nanoporous materials (mezoporous silica)

DEPARTMENT OF GENERAL BIOPHYSICS

prof. dr hab. Maria Bryszewska email marbrys@biol.uni.lodz.pl
dr Elzbieta Pedziwiatr-Werbicka email epedz@biol.uni.lodz.pl

to investigate biological properties of nanoparticles and developing new biomedical applications

1. basic studies of nanomaterials impact on biomolecules

   (interactions between nanoparticles and proteins, nanoparticles - lipids, nanoparticles - nucleic acids)
2. toxicity studies of nanoparticles carried out on cells (cytotoxicity)
3. application phase in three emerging medical fields:

   ✓ using dendrimers as carriers of anticancer drugs to enhance effectiveness of chemotherapy and reducing side-effects,

   ✓ exploring possibility to develop a therapy based on dendrimers for amyloid disorders such as Alzheimer's and prion diseases,

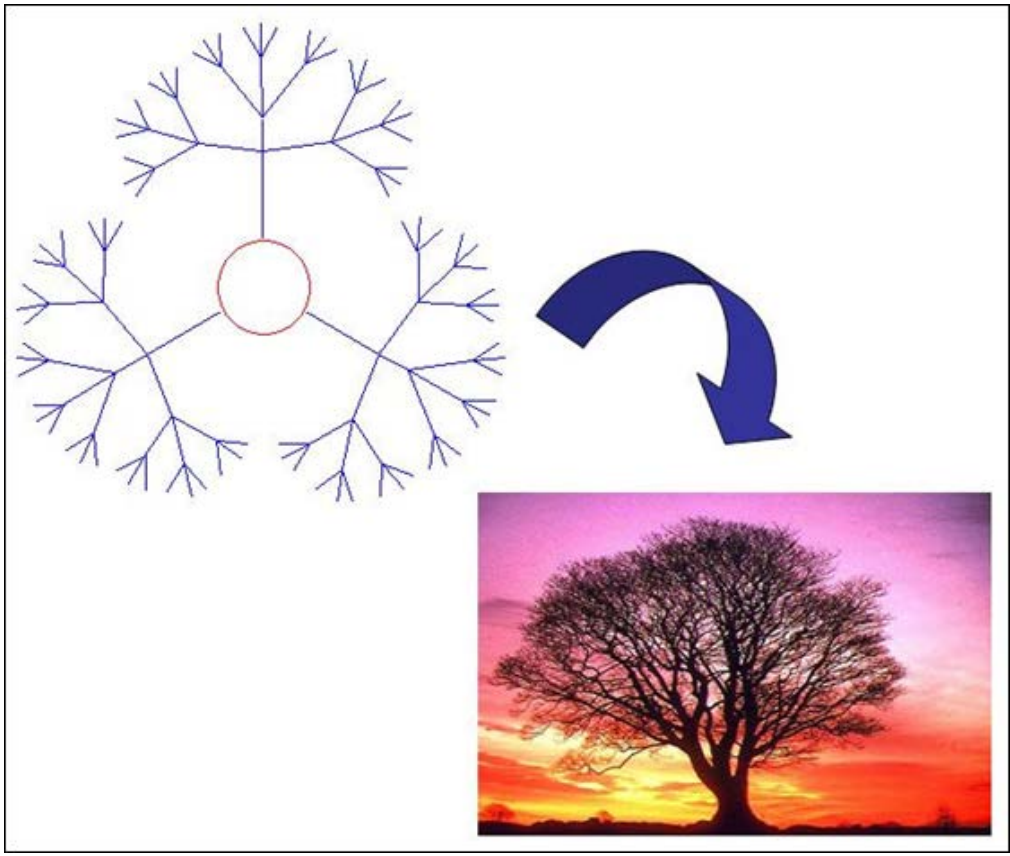
   ✓ employing nanoparticles as carriers of genetic material in gene therapy of AIDS and cancer/of HIV peptides for the development of nano-HIV vaccines

Vögtle's laboratory,
Newkome's laboratory

ARBOROLS from the Latin
***arbor* - tree**

Donald Tomalia – 1985

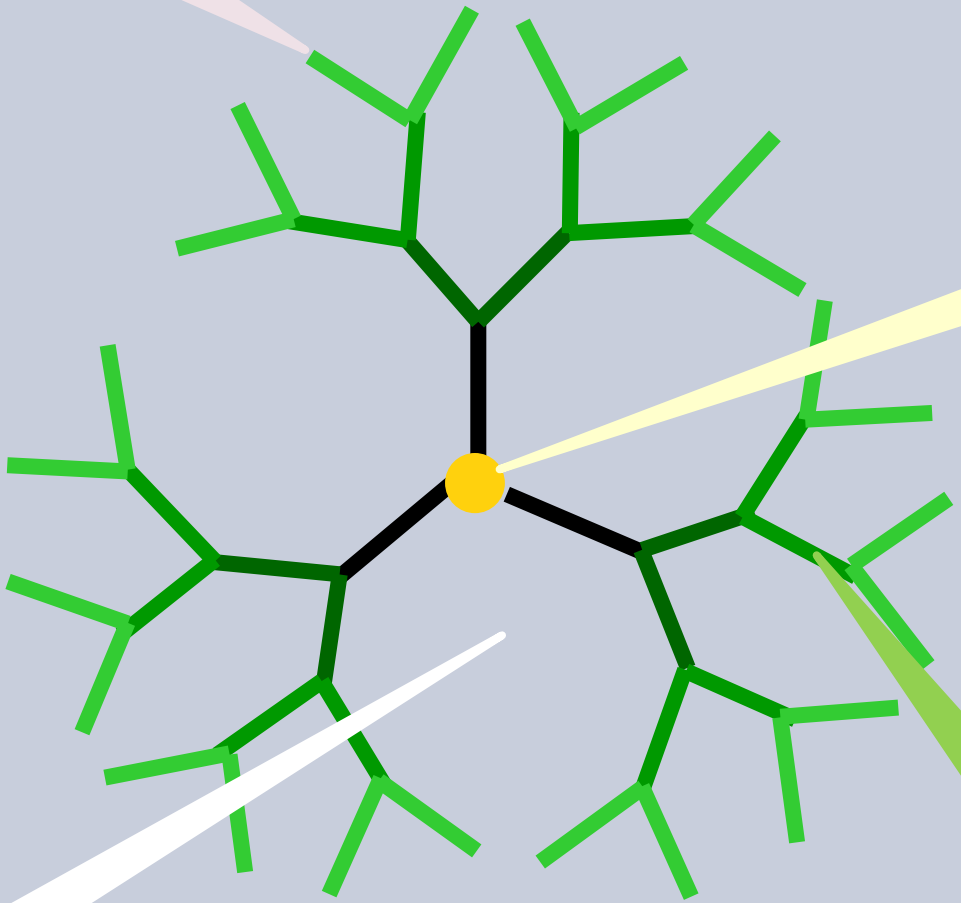
the synthesis of PAMAM dendrimers



DENDRIMER
from Greek *dendron* – *tree*
meros* - *part

consists of a central core molecule which acts as the root from which a number of highly branched, tree-like arms originate in an ordered and symmetric fashion

FUNCTIONAL GROUPS

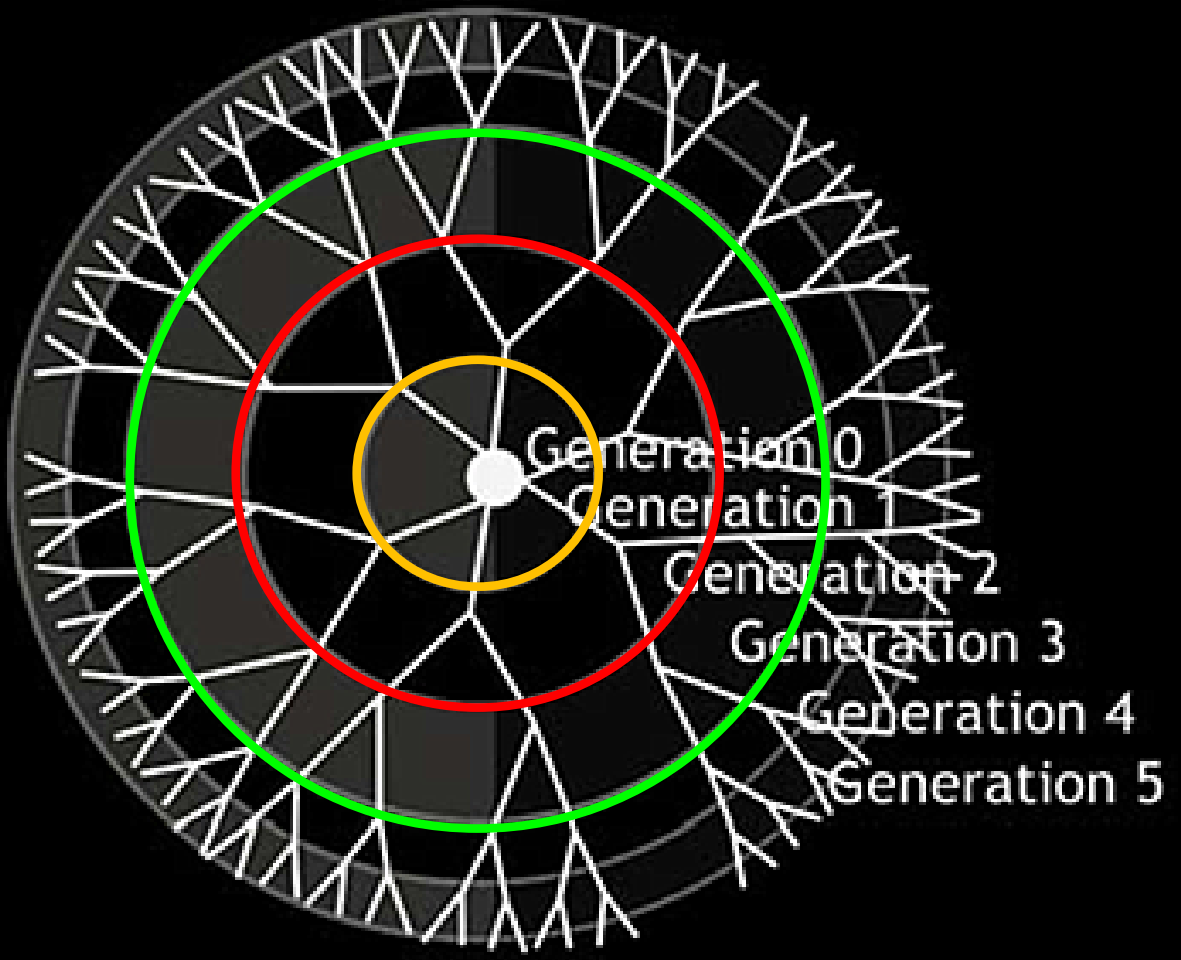


CORE

CAVITIES

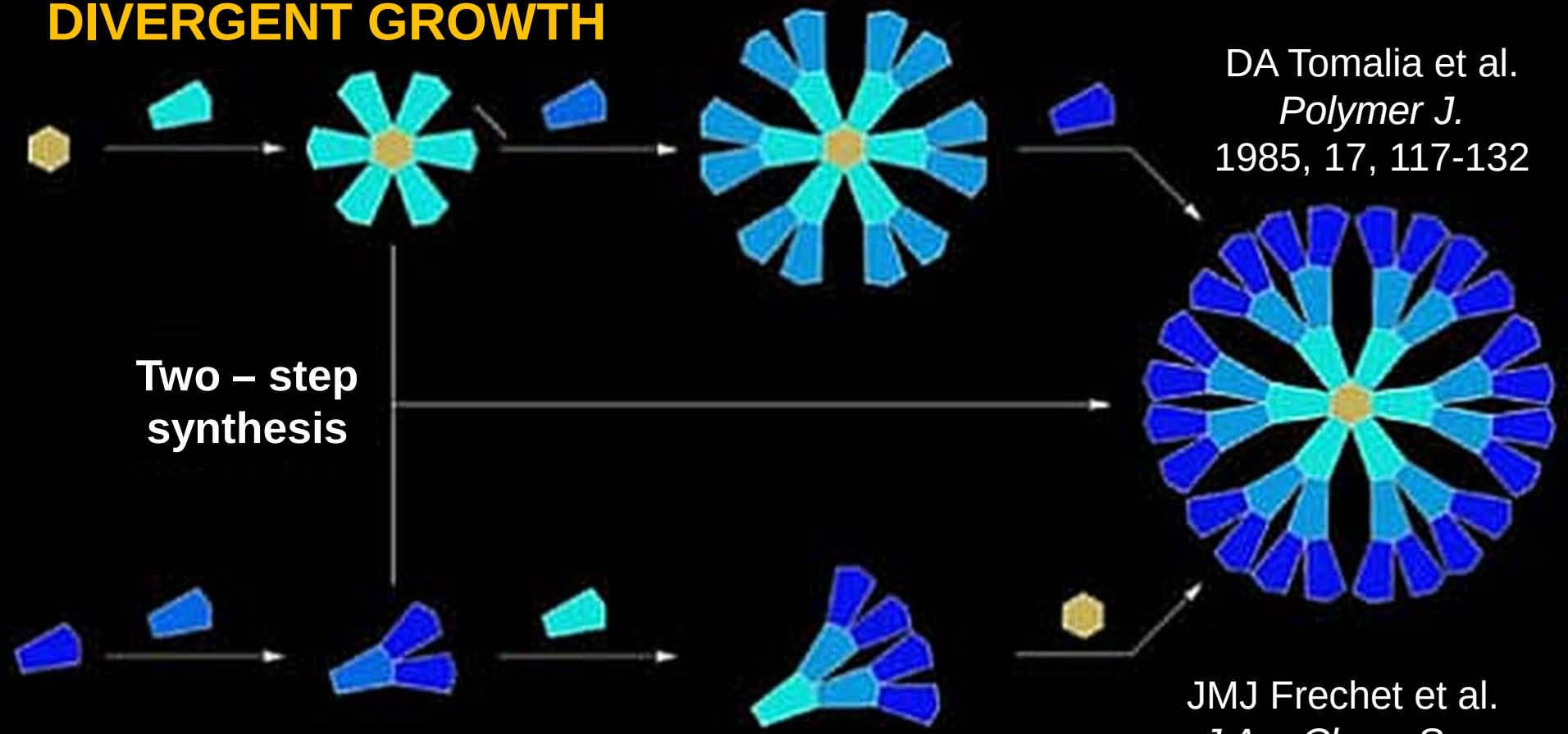
BRANCHES-DENDRONS

DENDRIMER



Dendrimers are built in a regular manner – layer by layer using divergent or convergent synthetic strategy. The more layers of branched units are added the higher generation of dendrimer is obtained.

construct from the root to the leaves



construct from the leaves to the root

DENDRIMERS:

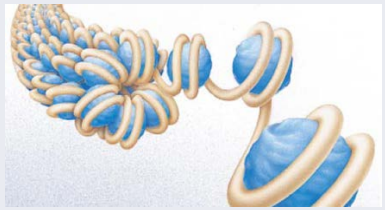
- PAMAM poly(amido amine)
- PPI poly(propyleneimine)
- PLy polylysine
- P phosphorus-containing
- PBzE poly(benzyl ether)
- PHEN polyphenylene
- PMMH thiophosphoryl phenoxymethyl (methylhydrazono)
- carbosilane
- viologen
- with regard to modifications over 100

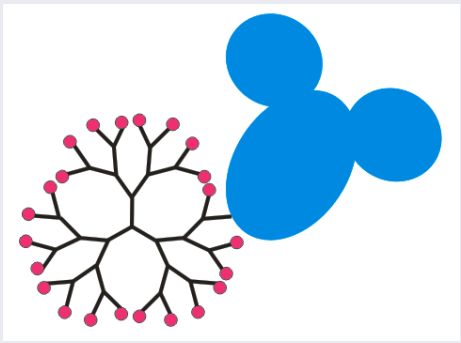
THE UNIQUE PROPERTIES OF DENDRIMERS :

- ✓ uniform and controlled size
- ✓ monodispersity
- ✓ reactivity
- ✓ solubility
- ✓ modifiable surface group functionality

make dendrimers suitable for a wide range of biomedical applications

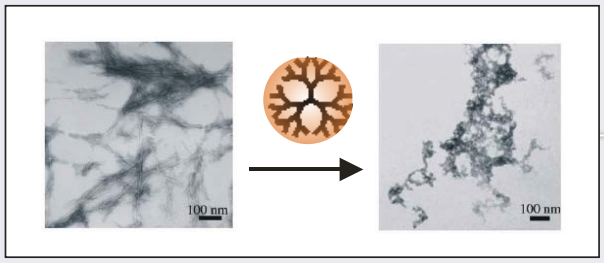
RNA, DNA, oligonucleotide targeting



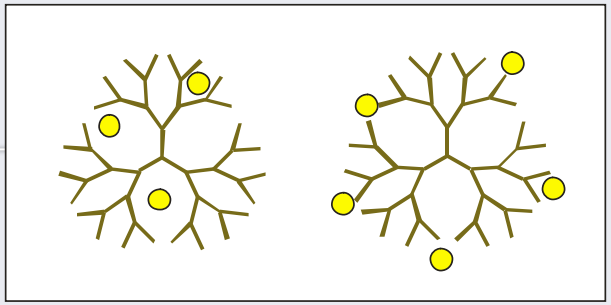


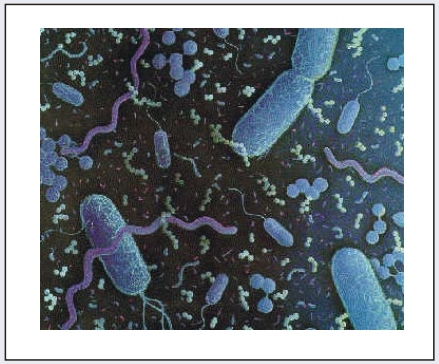
antiviral agents

drug delivery

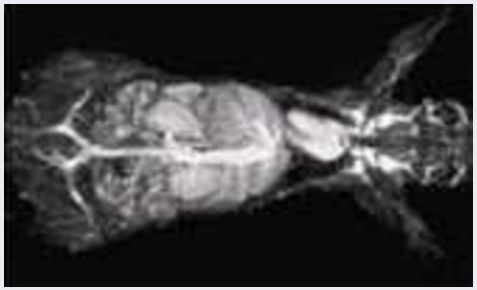


activity against prion diseases



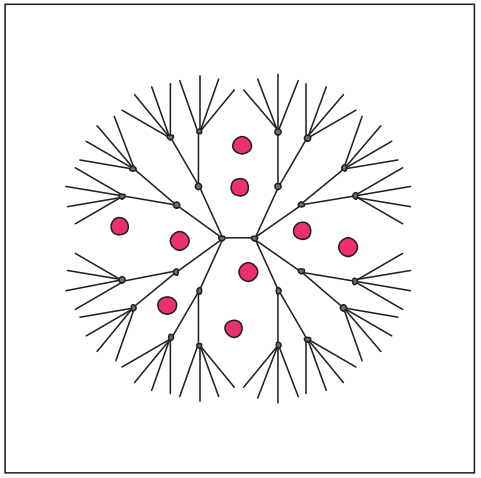


antibacterial, antifungial agents



as diagnostic imaging contrast agent

Drugs can be encapsulated into dendrimer's cavities or electrostatic and covalent complexed to dendrimer's surface

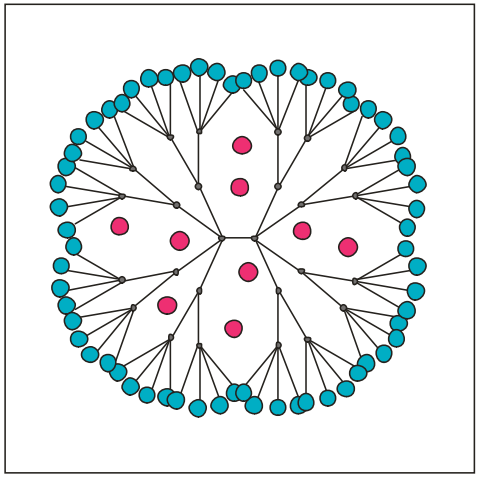


Drug inside dendrimer

np. adriamycin, metotreksat, indometacin

slow release of drug

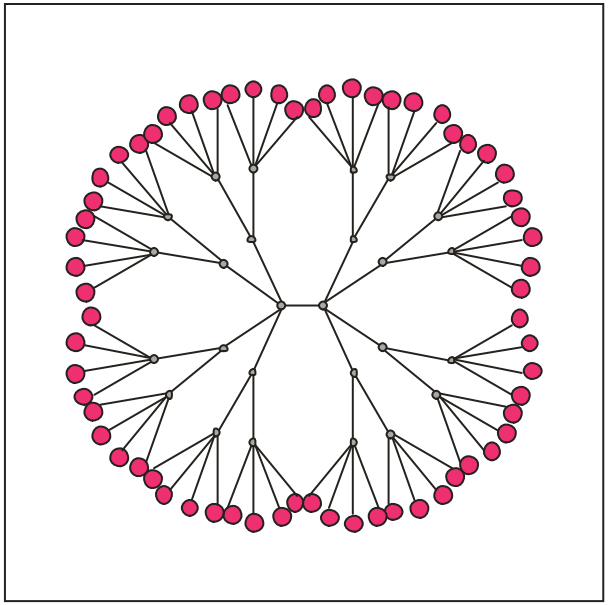
Drug inside dendrimer



np. adriamycin, metotreksat,
indometacin

slow release of drug
(folic acid on the surface)
slow release of drug close to cancer cells

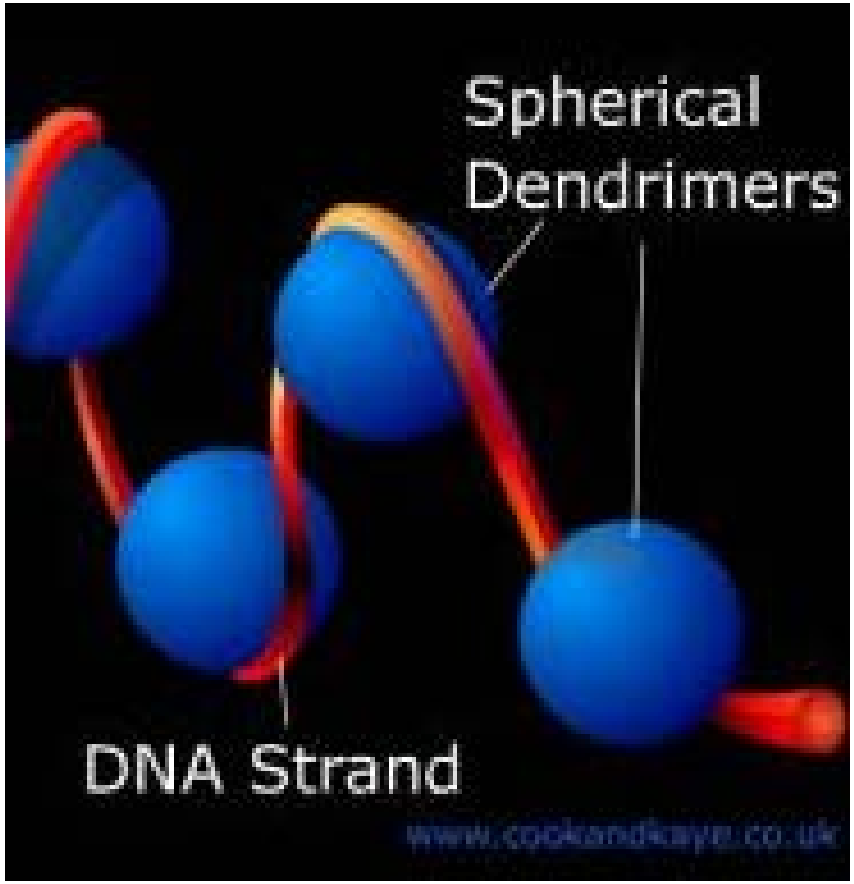
Drug delivery on dendrimer surface



metotreksat, 5-FU

More intensive therapeutic effect

Electrostatic interactions between DNA/RNA and dendrimers



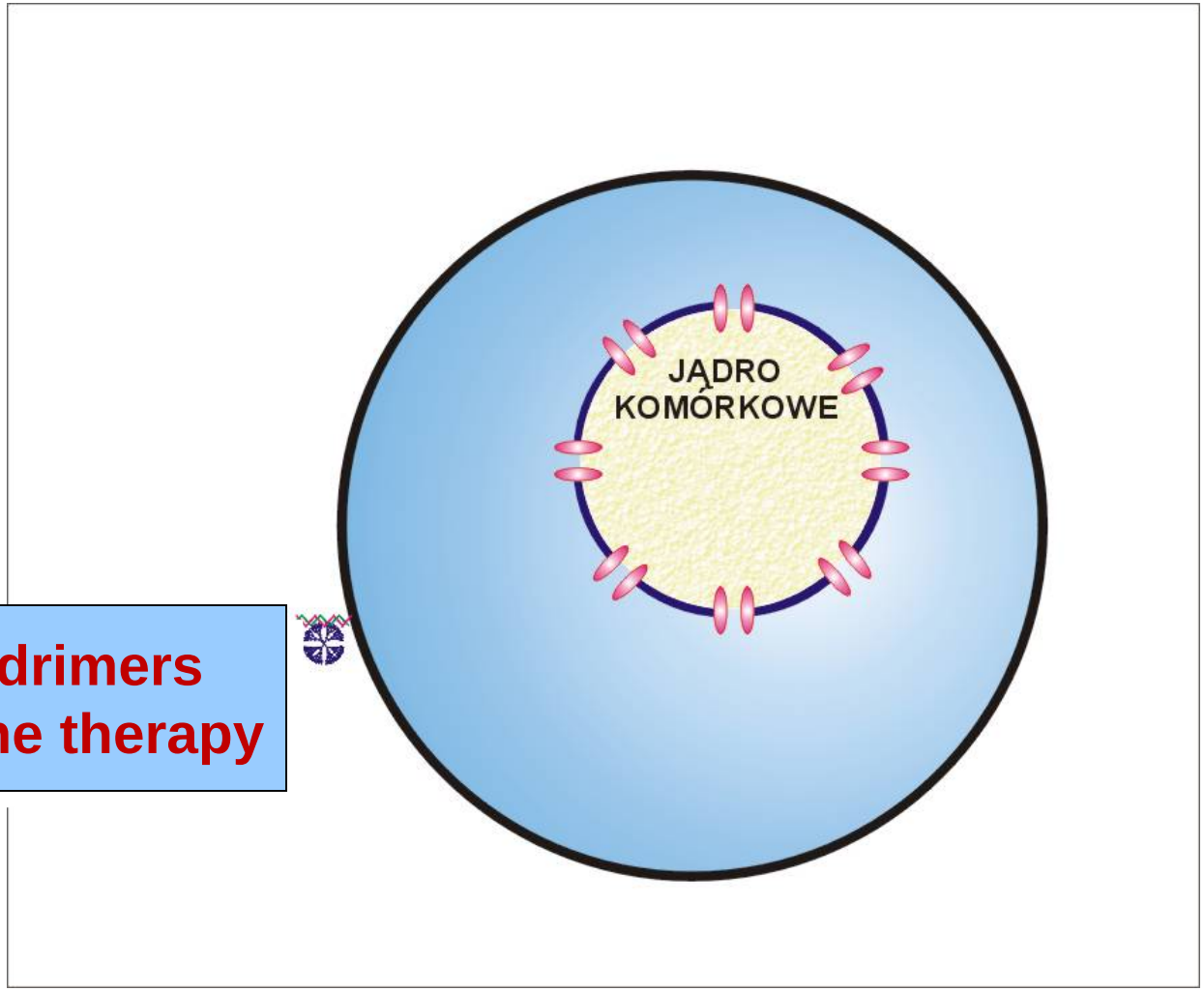
www.polymercentre.org.uk/research

Advantages of dendrimers for gene transfer:

1. dendrimers possess many terminal groups that can be ionized, it means that they can efficiently bind large amount of genetic material

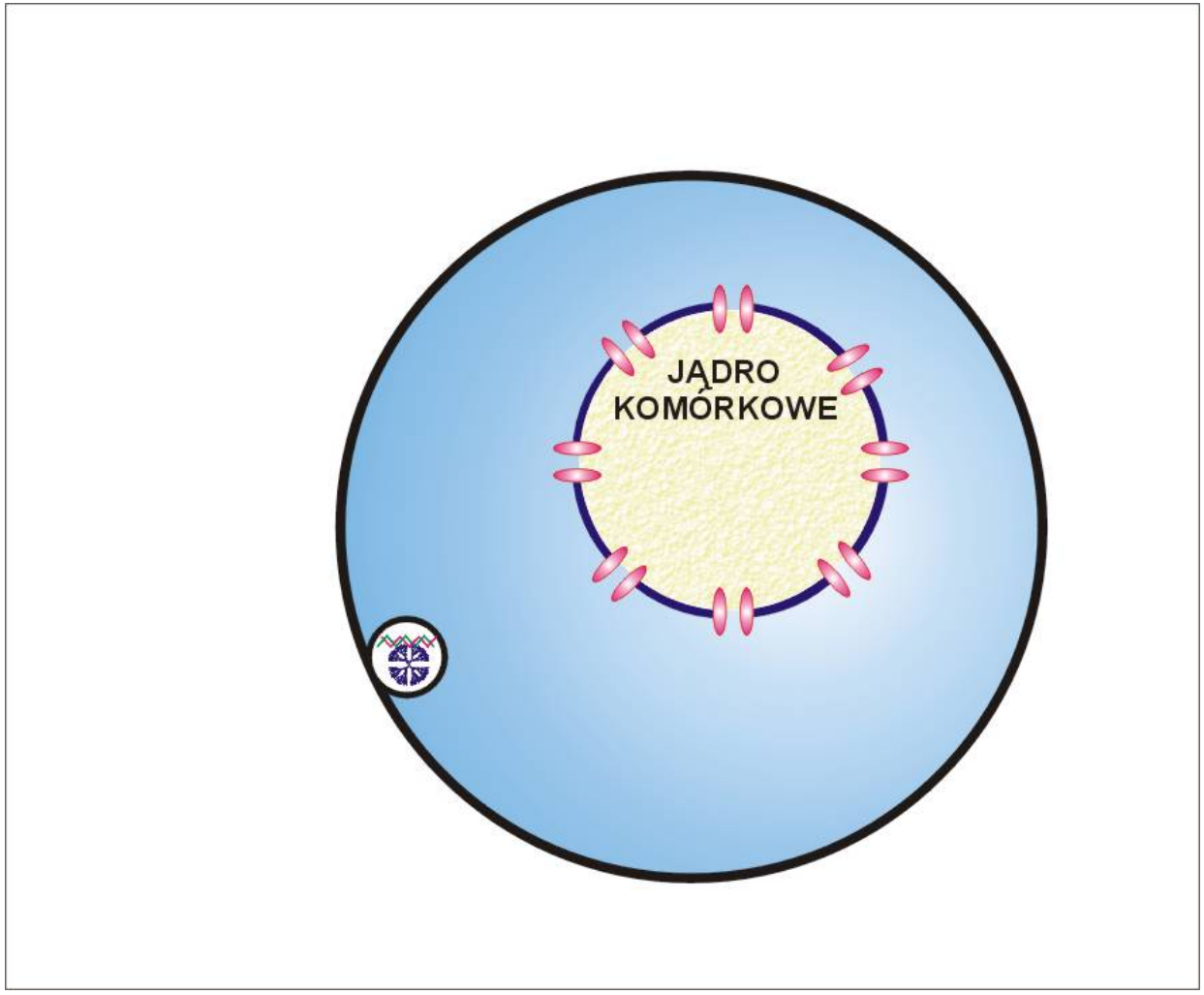
2. dendrimers of large generations can mimic, both in size and shape, histone proteins which condense and store DNA within a eukaryotic cell nuclei

1. Complex of DNA and dendrimer - dendriplex binds to membrane

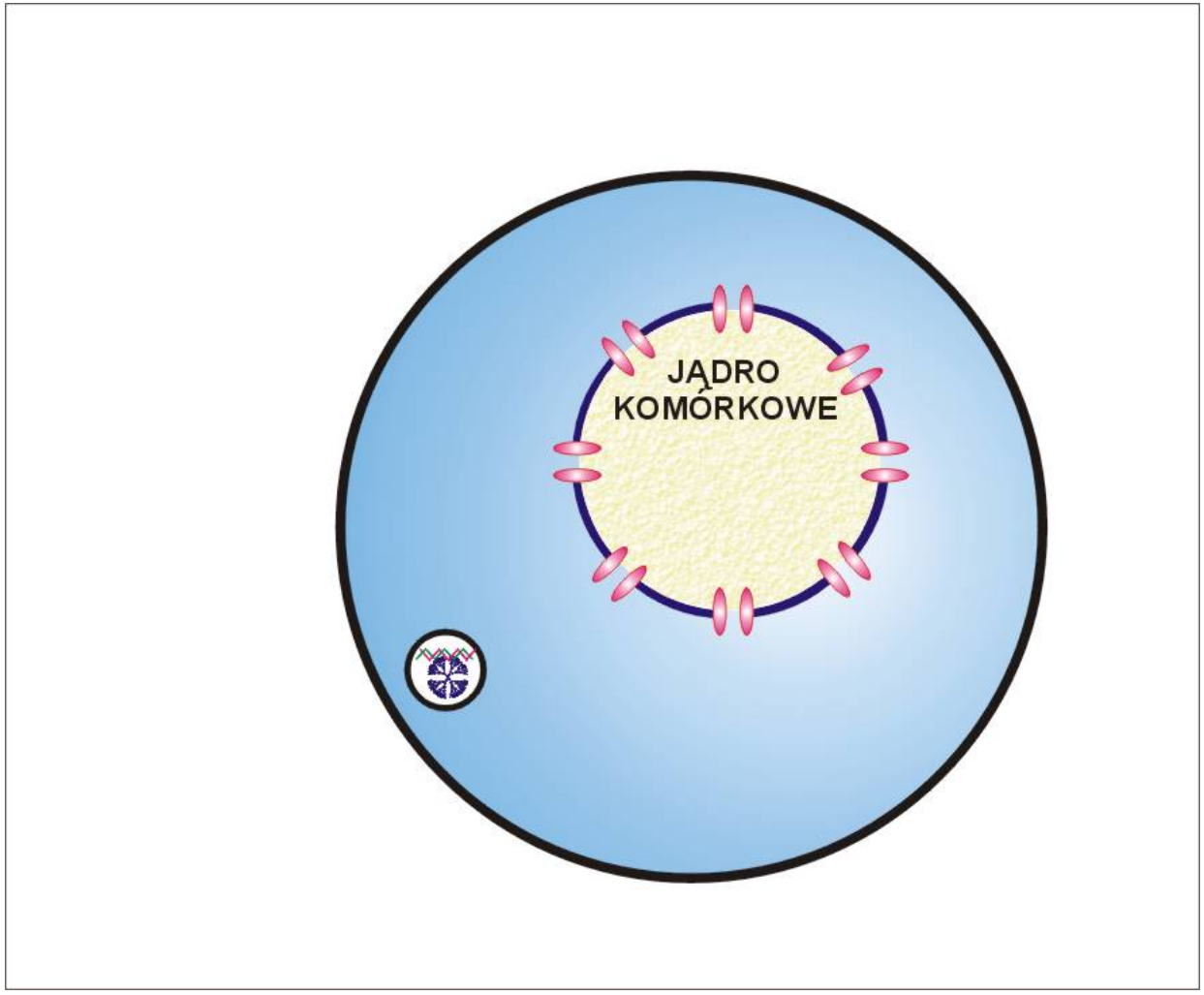


Dendrimers in gene therapy

2. Cellular uptake of dendriplexes by endocytosis



3. Transport DNA to nucleus



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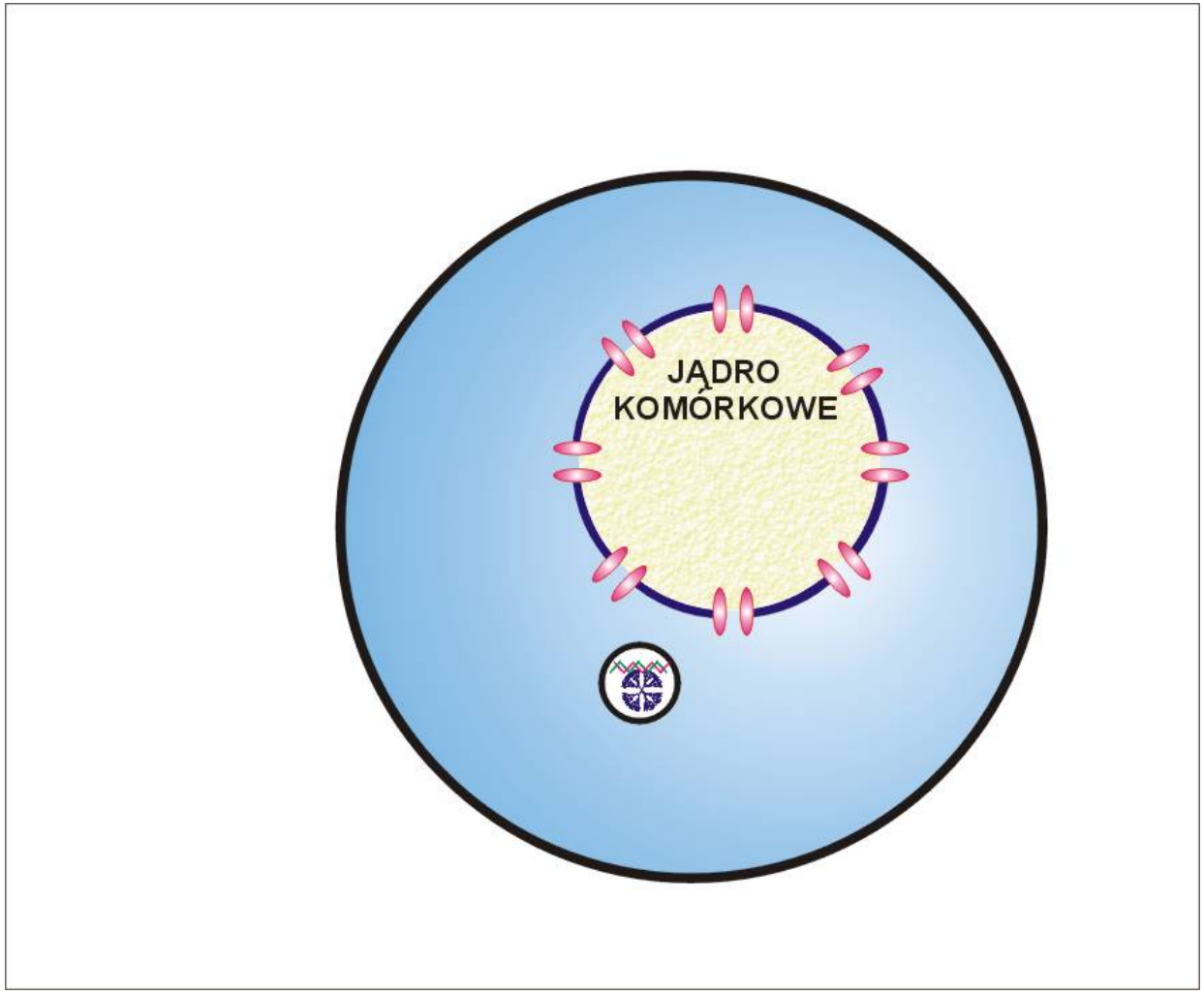
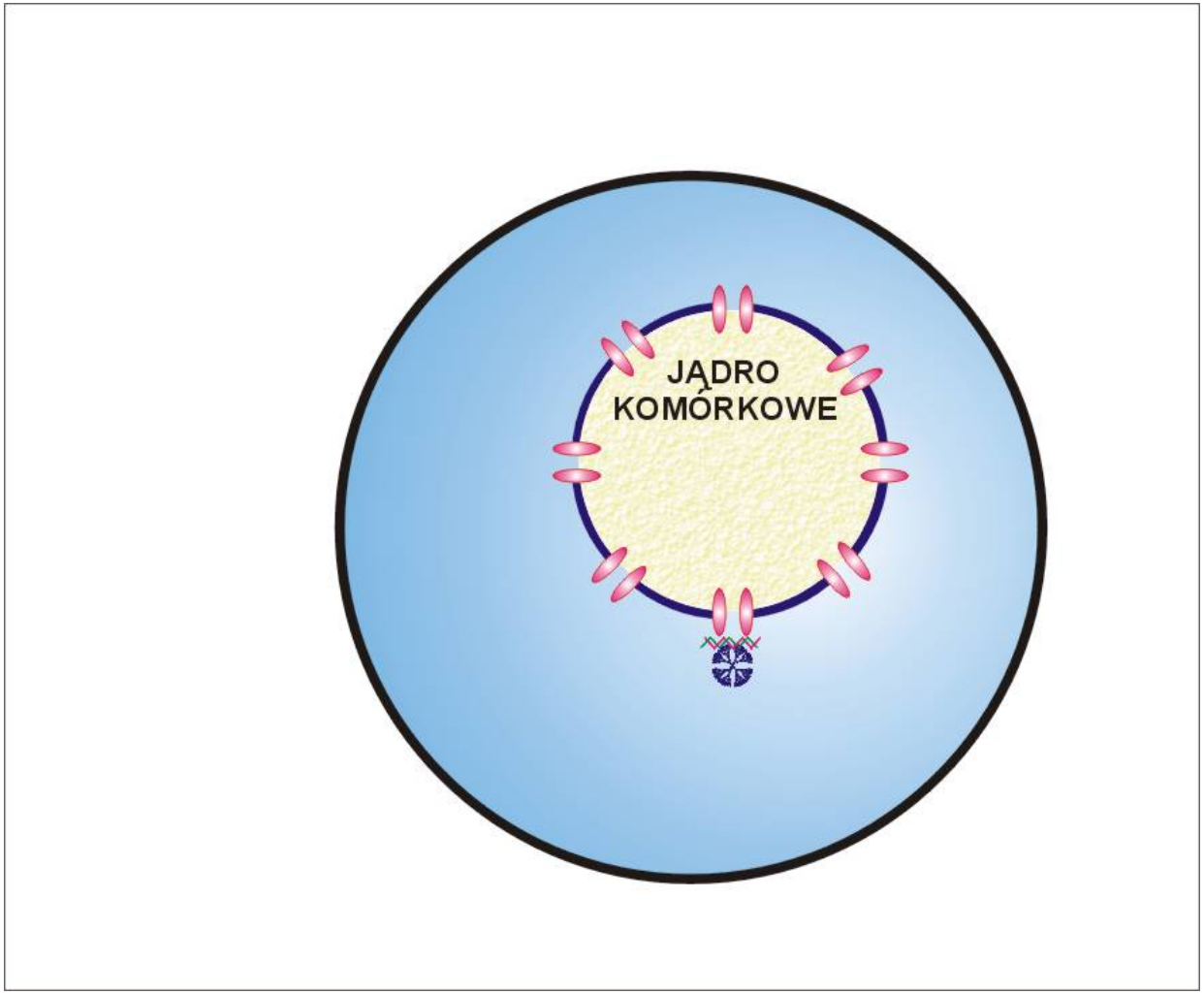
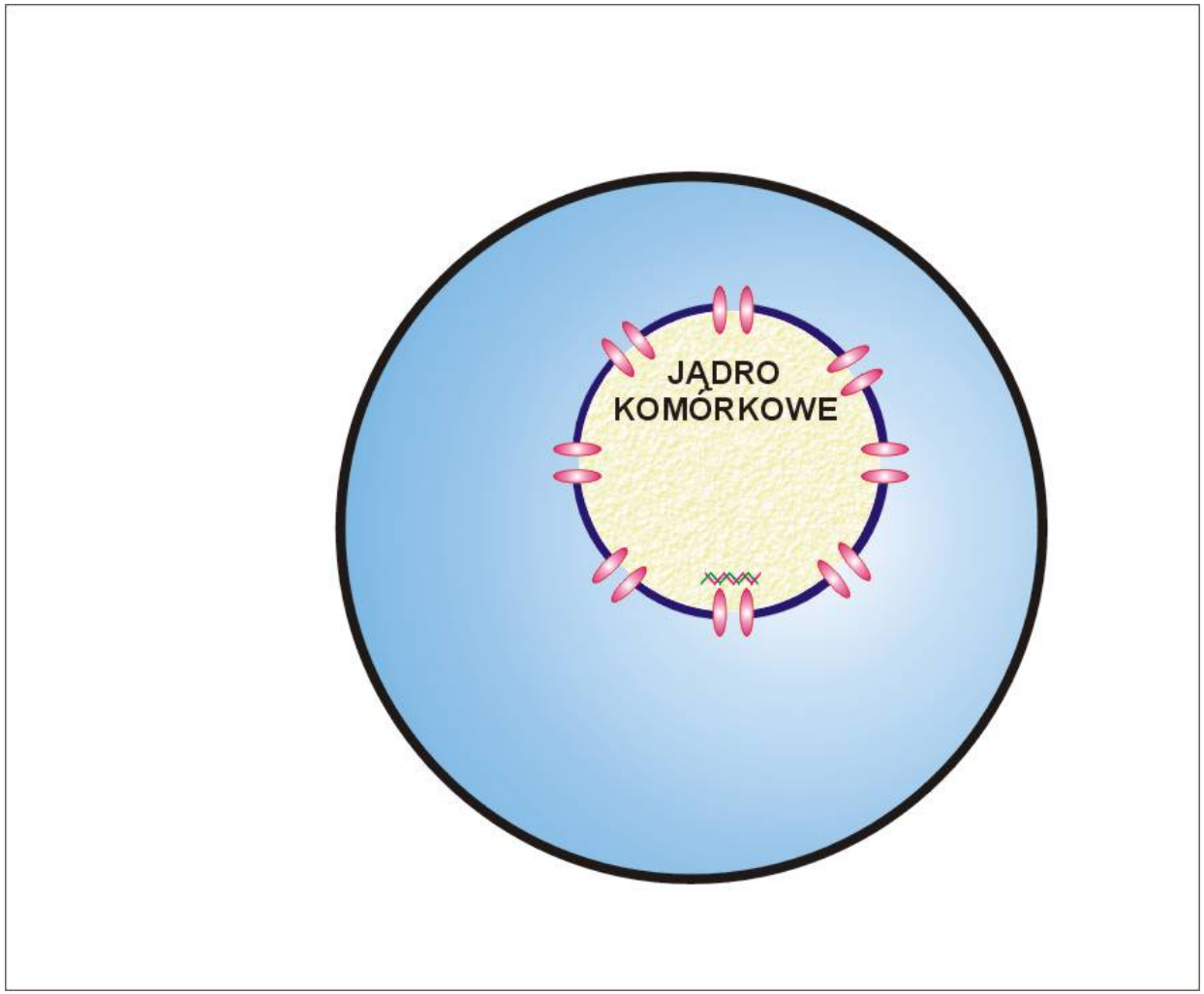


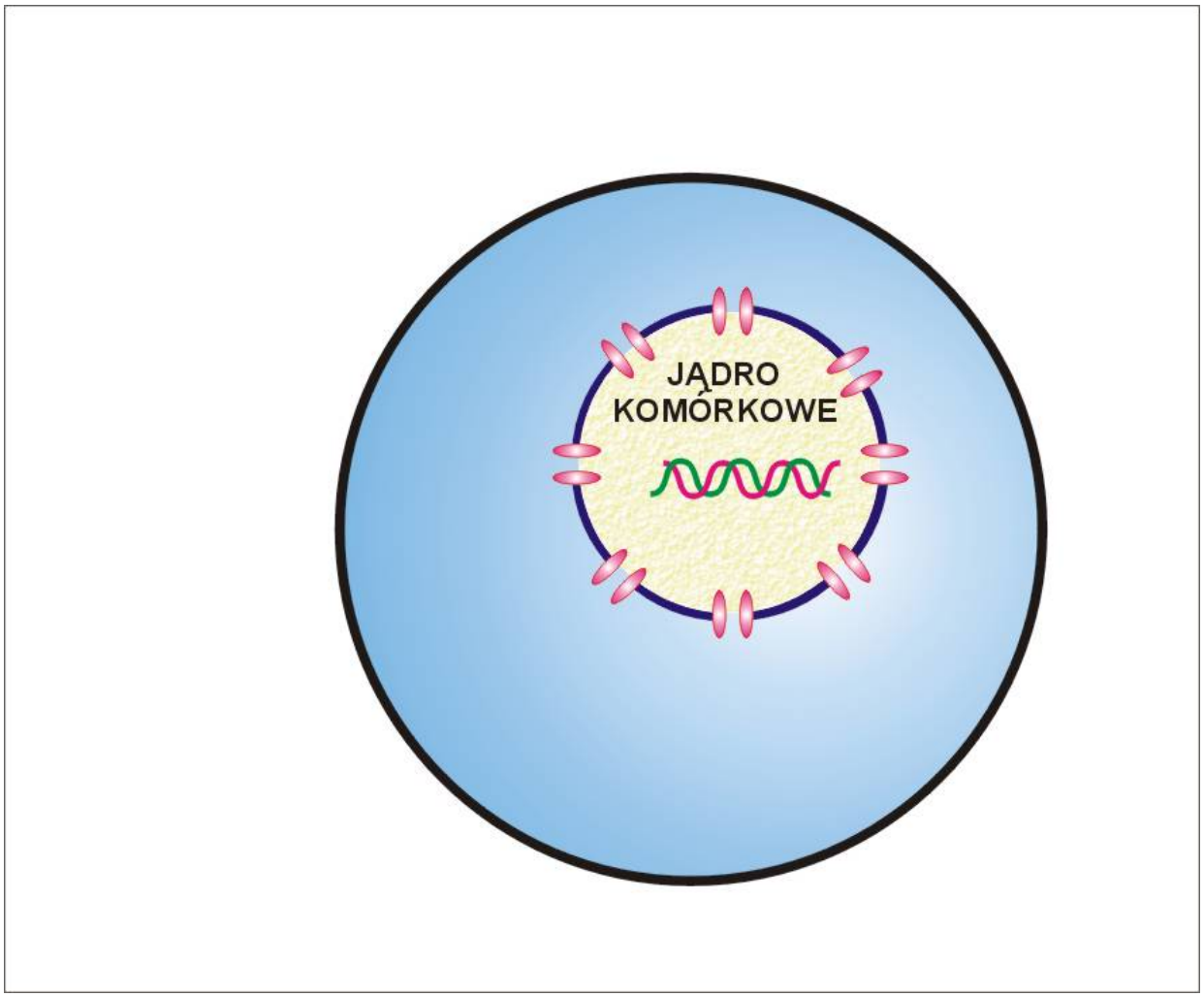
4. Transport of unload DNA to nucleus



4. Transport of unload DNA to nucleus



5. Transcription and tranlation



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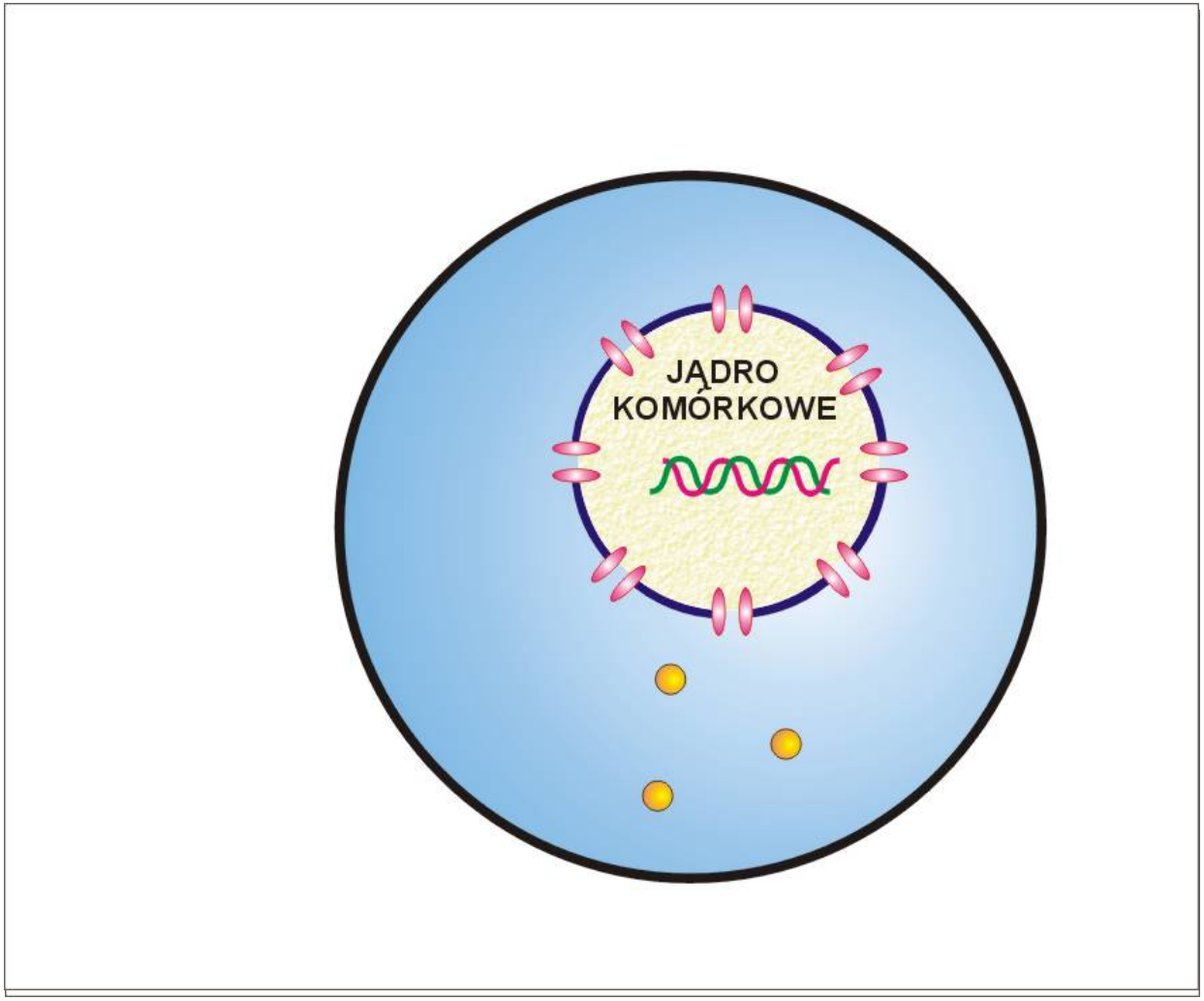
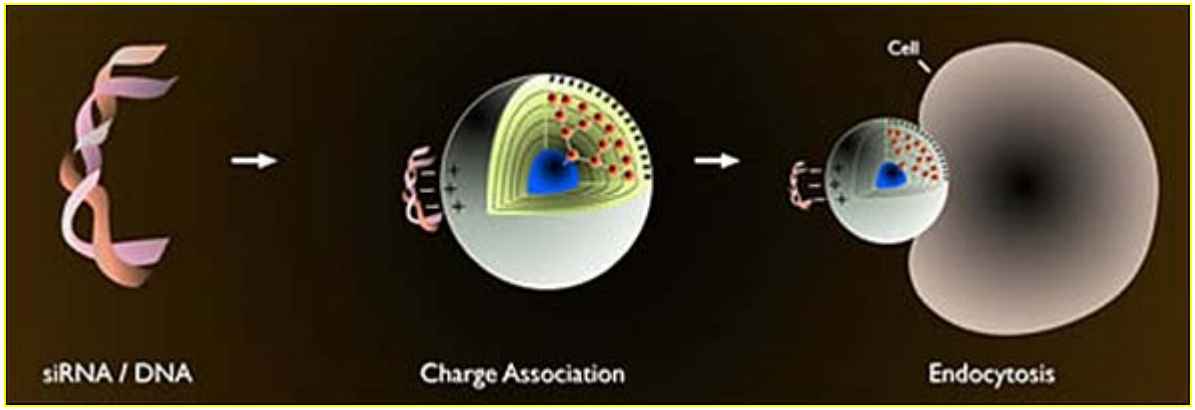


Gene therapy offers not only a possibility to insert DNA into a cell to express a protein, but also allows disrupting the expression of disease-related genes in antisense therapy using ODNs and siRNAs. In this case nucleic acids don't reach the nucleus, but they work within cytoplasm.

APPLICATION OF DENDRIMERS AS anti-HIV THERAPY

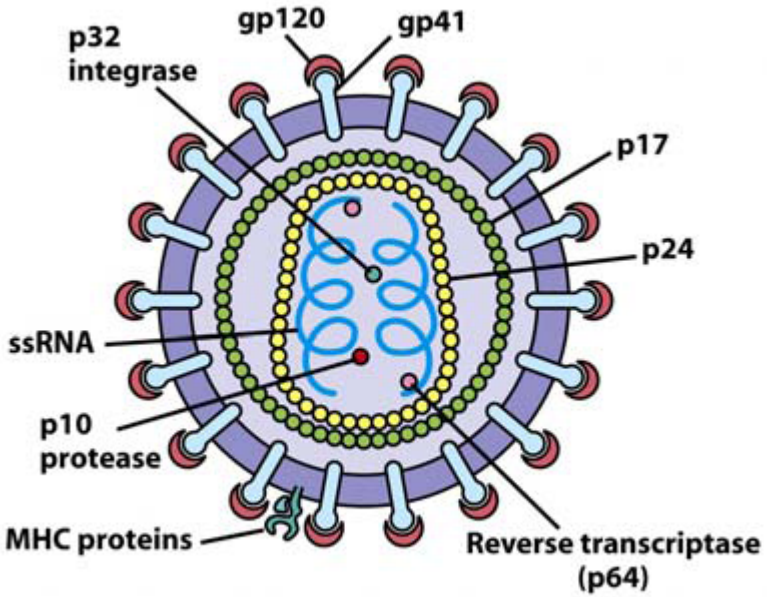
1) using dendrimers as anti-HIV drug and gene carriers

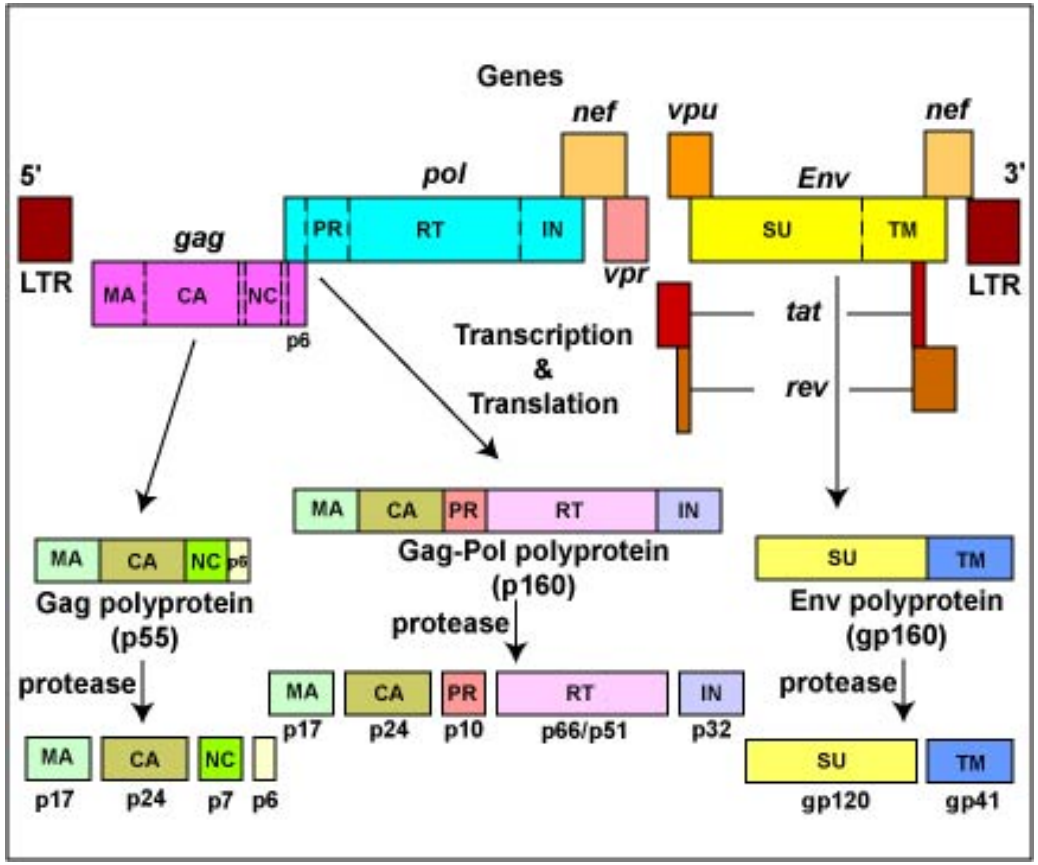


V. Dzmitruk, D. Shcharbin, E. Pedziwiatr-Werbicka, M. Bryszewska: **Dendrimers in anti-HIV therapy.** In: ADVANCES IN NANOCOMPOSITE TECHNOLOGY, pp. 361-374, InTech, Ed. Abbas Hashim, 2011, ISBN 978-953-307-347-7

1. EU MNT ERANET **"Anti-HIV short nucleic acids transported by nanovehicles based on dendrimers as novel therapeutical approach for HIV-1 infections"**
2. project operated within the Foundation for Polish Science Team Programme co-financed by the EU European Regional Development Fund Foundation of Polish Science **"Biological Properties and Medical Applications of Dendrimers"**
3. bilateral cooperation between Poland and Slovakia **"Dendrimers as carriers for siRNA targeted against HIV-1 virus – interactions with membranes"**
4. Project Iuventus Plus (Ministry of Science and Higher Education) **"Dendrimers as carriers of drugs used in anti-HIV gene therapy"**

HIV
STRUCTURE AND GENOME





HIV genome consist of nine genes. Three of them *gag, pol* and *env* code structural proteins of HIV. There are six regulatory genes :*tat, rev, nef, vif, vpr, vpu* (*vpx* in HIV-2). The HIV genome also has a "Long Terminal Repeat" (LTR) at each end of its genome - which serves some structural and regulatory purposes.

DENDRIMERS as anti-HIV NUCLEIC ACIDS CARRIERS

- PPI unmodified: PPIG2, PPIG3, PPIG4
- PPI maltose and maltotriose modified: PPIG4 (100%, 50%, 25%)
- PG3, PG4

*Pedziwiatr-Werbicka E. et al. **(2011)** Colloids Surf B Biointerfaces 83: 360-366*
Shcharbin D. et a. (2011) Pharmaceutics , 3, 458-473

Carbosilane dendrimers CBS:

- ✓ water soluble
- ✓ ammonium terminated
- ✓ second generation
- ✓ divided into 2 groups: NN and IMe

Carbosilane dendrimers BDEF:

- ✓ water soluble
- ✓ ammonium terminated
- ✓ first generation BDEF007
- ✓ second generation BDEF008
- ✓ third generation BDEF009

*Pedziwiatr-Werbicka et al. **(2013)** Colloids Surf Biointerfaces 109 :183–189*

- *Weber N. et al. **(2008)** J Control Release 132: 55–64*
- *Gras R. et al. **(2009)** PharmRes 26: 577 - 586*
- *Gonzalo T. et al. **(2010)** ChemMedChem 5: 921-929*
- *Ortega P. et al. **(2010)**Tetrahedron 66:3326–3331*
- *Shcharbin D. et al. **(2011)** Colloids Surf B Biointerfaces 83: 388-391*

OLIGODEOXYNUCLEOTIDES ODNs:

- SREV
- AT
- GEM91

siRNAs:

- siP24

PHOSPHOROTHIOATE OLIGONUCLEOTIDES
PS-ODNs

ANTI –TAR (MW 5192 g/mol)
16 bases 5'-GCTCCCGGGCTCGACC-3'
targeted against *tat* gene and the TAR element

Tat - transcriptional transactivator , binds to the transactivation response element (TAR)
The binding of Tat to TAR activates transcription

GEM91 (MW 8112,5 g/mol)
25 bases 5'-CTCTCGCACCCATCTCTCTCCTTCT-3'
targetet against *gag* gene

Gag – major structural protein

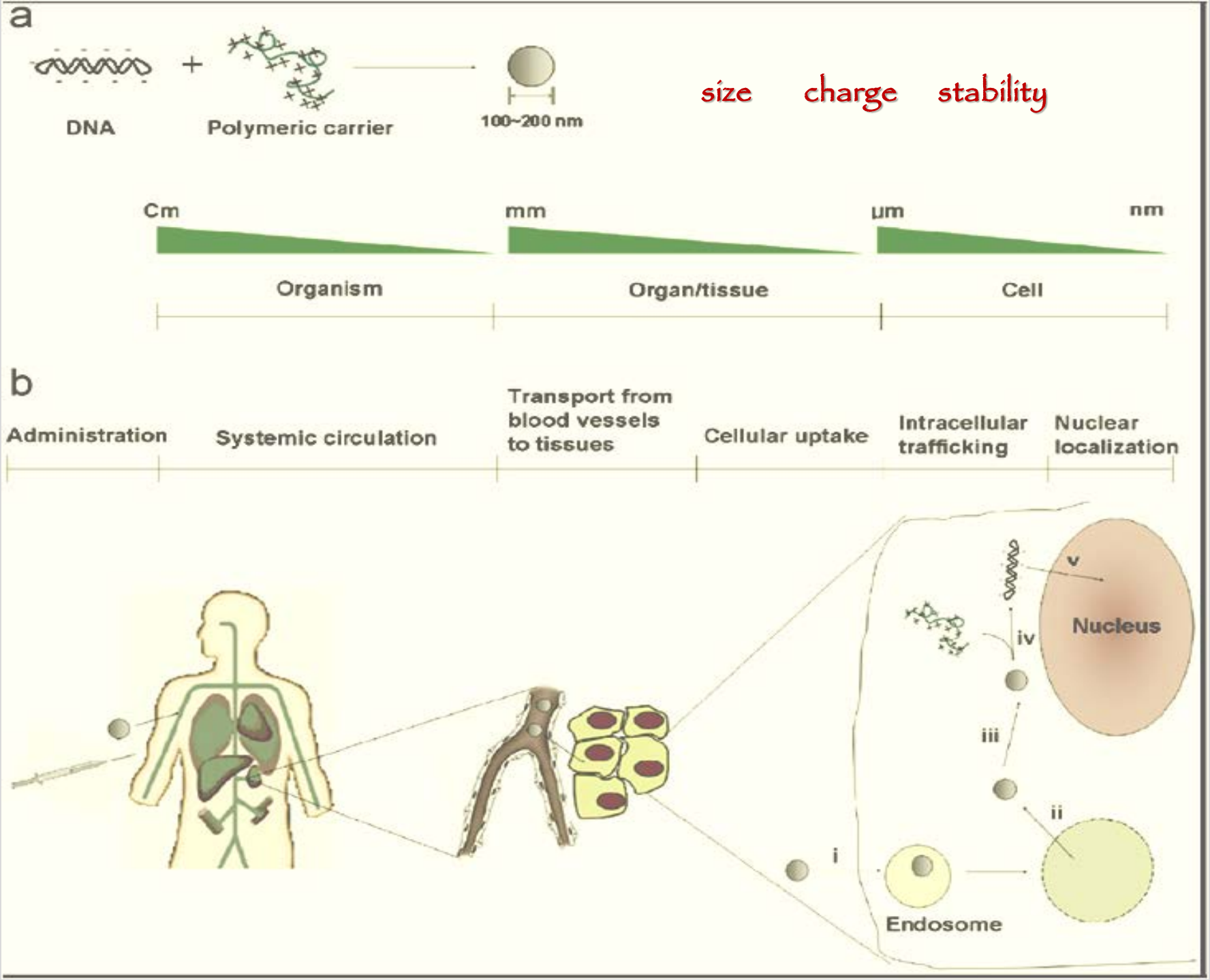
SREV (MW 9086 g/mol)
28 bases 5'-TCGTCGCTGTCTCCGCTTCTTCCTGCCA-3'
targeted against *rev* gene

Rev – regulatory protein

siP24

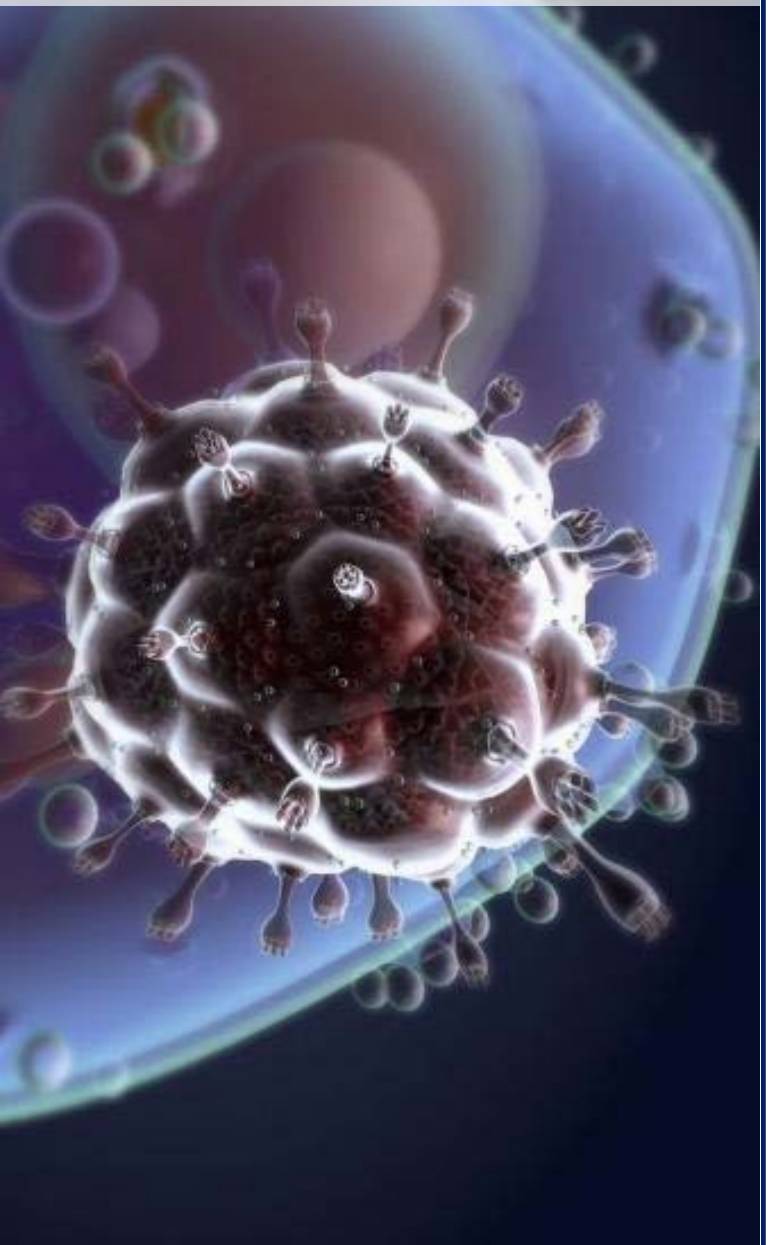
42 bases

Sense sequence:
GAUUGUACUGAGAGACAGGCU
Antisense sequence:
CCUGUCUCUCAGUACAAUCUU


a
DNA
Polymeric carrier
100~200 nm
size
charge
stability
Cm
mm
μm
nm
Organism
Organ/tissue
Cell
b
Administration
Systemic circulation
Transport from blood vessels to tissues
Cellular uptake
Intracellular trafficking
Nuclear localization
i
ii
iii
iv
v
Nucleus
Endosome

Many regions of the HIV genome have been targeted with antisense, including the *rev, tat, gag, pol,* and *env* genes, and the 5'-untranslated region and psi sequences

Lavigne et al. (2001) *AAPS PharmSci*. 3,E7



GEM91 (Trecovirsen) effectively reduces HIV replication *in vitro*

Lisziewicz et al. (1994) Proc. Natl. Acad. Sci. U.S.A. 91, 7942– 7946
Yamaguchi et al. (1997) *AIDS Res. Hum. Retroviruses* 13, 545–554

It reached to phase III clinical trial, but it was interrupted due to secondary effects

Wagner and Flanagan (1997) *Mol Med Today* 3(1), 31-38

At clinically relevant doses in mice, rats, monkeys, and humans, more than 96% of the PS-ODNs is bound to plasma proteins

Geary et al. (2002) *Clin. Pharmacokinet*. 41,255– 260

To achieve the desired therapeutic effects higher doses of ODNs are necessary . It can induce toxic effects.

DO NANOPARTICLES PROTECT ODNs FROM BINDING TO SERUM ALBUMIN?

- **interactions between ODNs and HSA**
- **interactions between HSA and dendrimers**
- **interactions between ODNs, dendrimers and HSA**
  - ***dendriplexes (ODN/dendrimer complex) and HSA***
  - ***addition of dendrimers to ODN+HSA complexes***

AIM OF STUDY

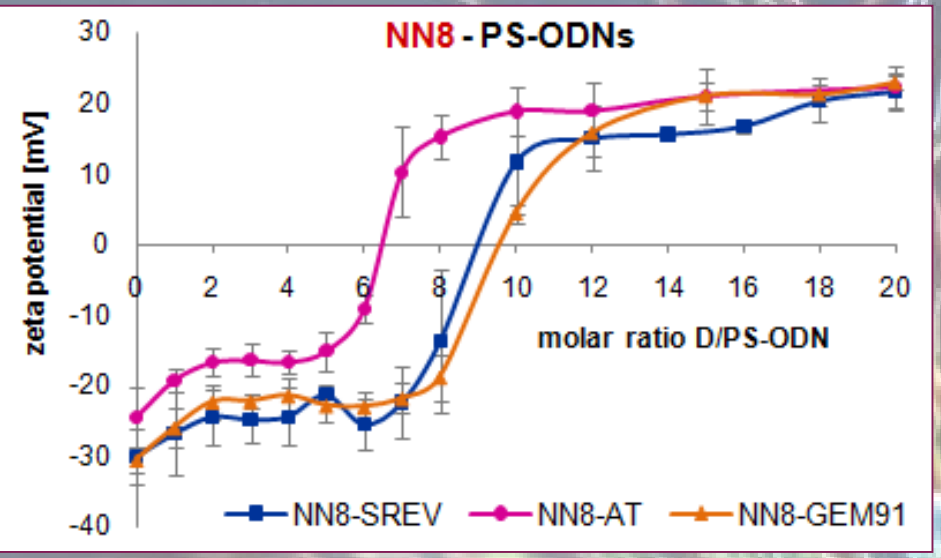
to check the toxicity and antigenicity of dendrimers (nanoparticles)
-MTT toxicity assay
- visual examination under a phase-contrast light microscope
- flow cytometry,
-Trypan Blue (TB) uptake
- DAPI staining
- time-lapse video microscopy
- hemolysis

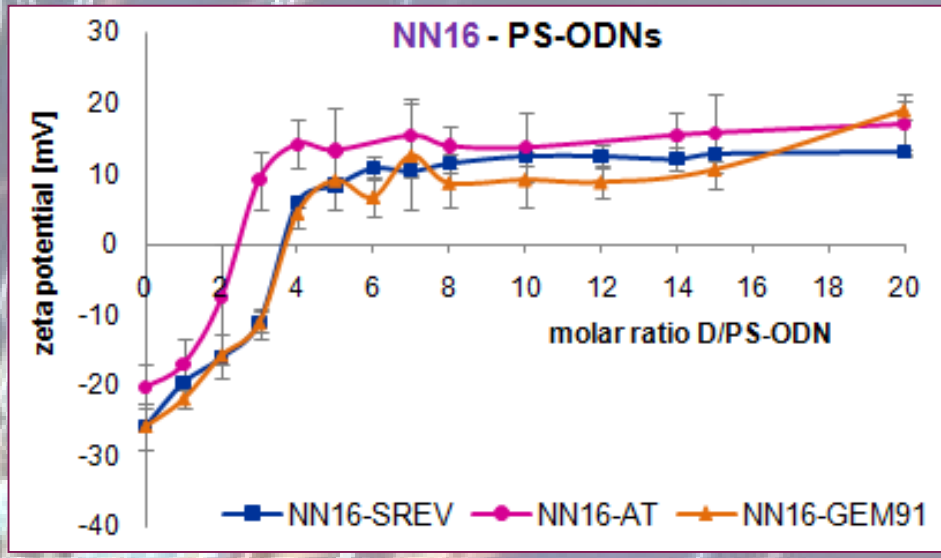
to characterize binding properties of dendrimers (ANS, DNA, proteins)

to answer question: do dendrimers (nanoparticles) protect ODNs from binding to serum albumin by fluorescence and gel electrophoresis

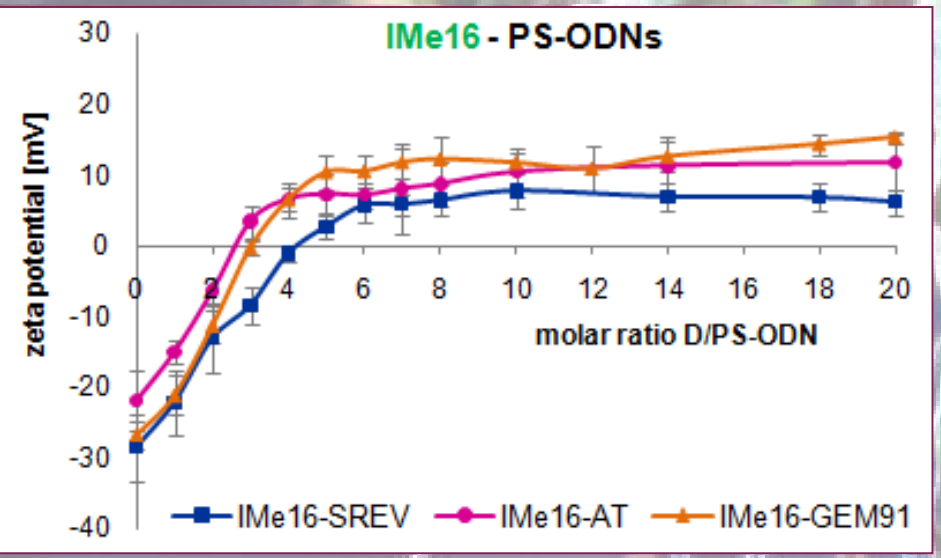
to characterize physicochemical properties of dendrimer/NA complexes formed in different molar/charge ratios: zeta potential (Laser Doppler Electrophoresis), **hydrodynamic diameter** (Dynamic Light Scattering), **morphology** (TEM, AFM), **stability in time, in pH, in the presence of nucleases** (fluorescence, gel electrophoresis), **conformational changes of NA** (circular dichroism)

to study cellular uptake and transfection efficiency (confocal microscopy, flow cytometry)

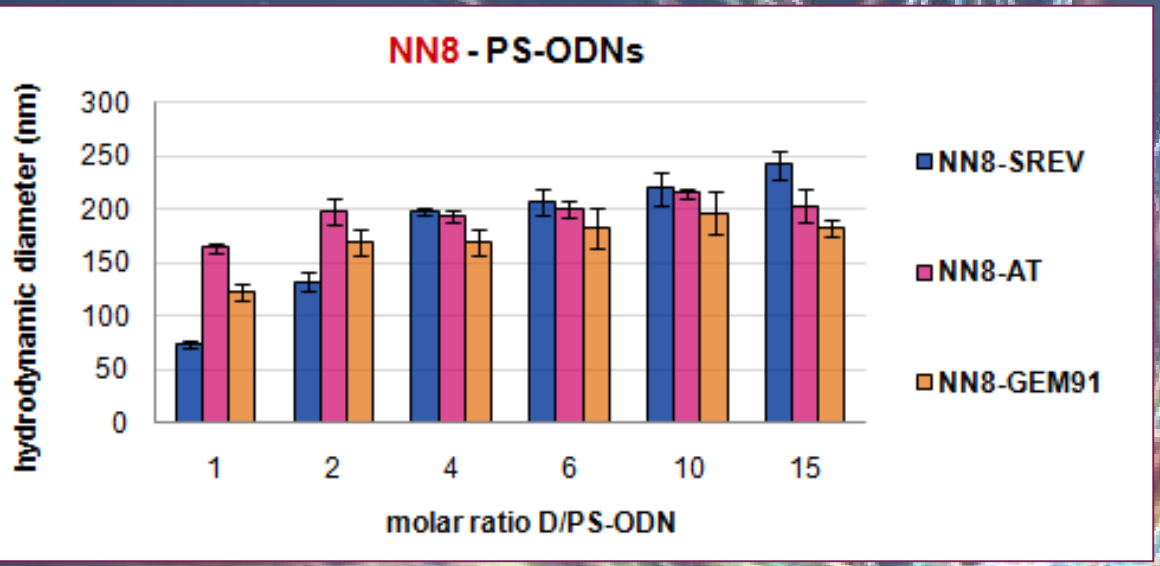




ZETA POTENTIAL of complexes formed in different charge/molar ratio by laser Doppler electrophoresis

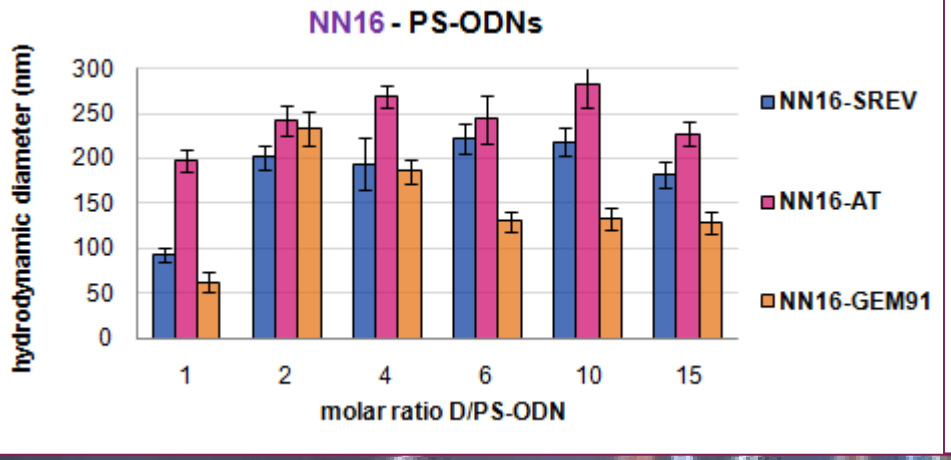


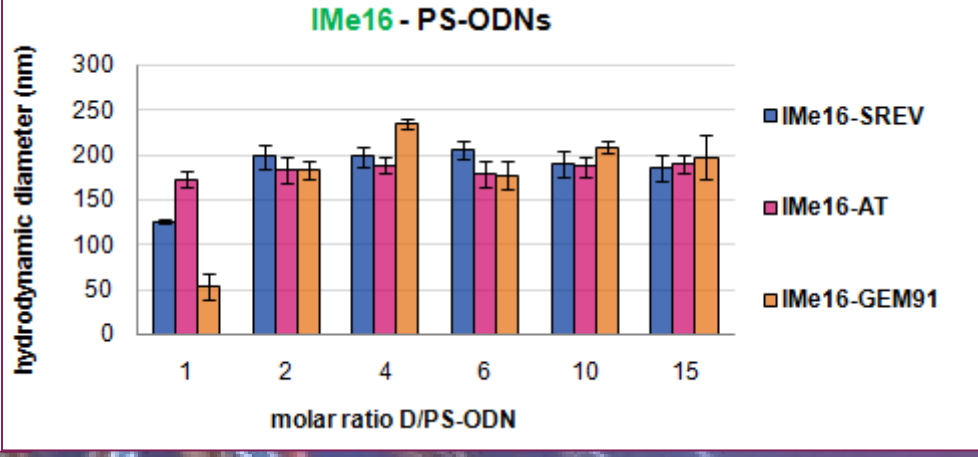
		SREV	AT	GEM91
NN8	molar ratio D - PS-ODN	10-12	8	12-14
	charge ratio +/-	**2,85-3,42**	**4**	**3,84-4,4**
NN16	molar ratio D - PS-ODN	6	4	5
	charge ratio +/-	**3,42**	**4**	**3,2**
IMe16	molar ratio D - PS-ODN	6	4	5
	charge ratio +/-	**3,42**	**4**	**3,2**



- low polydispersity index (PI = 0,1 - 0,2)
- addition of dendrimers increased the hydrodynamic diameter of dendriplexes
- it varied from 50 nm – 75 nm to 150 nm - 200 nm (for SREV and GEM91 complexes)
- in case of AT - dendrimers complexes hydrodynamic diameter didn't change as a function of molar ratio and it amounted 160 nm - 270 nm

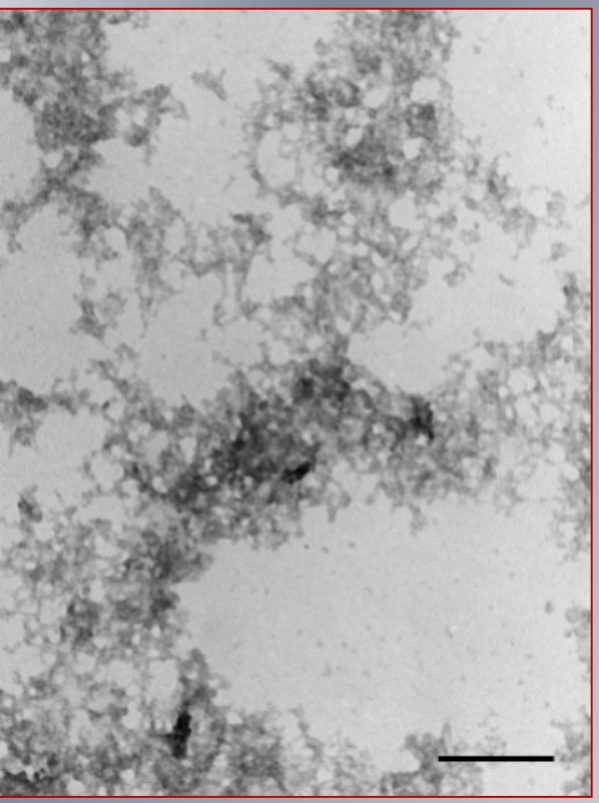
HYDRODYNAMIC DIAMETER of complexes formed in different charge/molar ratio by Dynamic Light Scattering



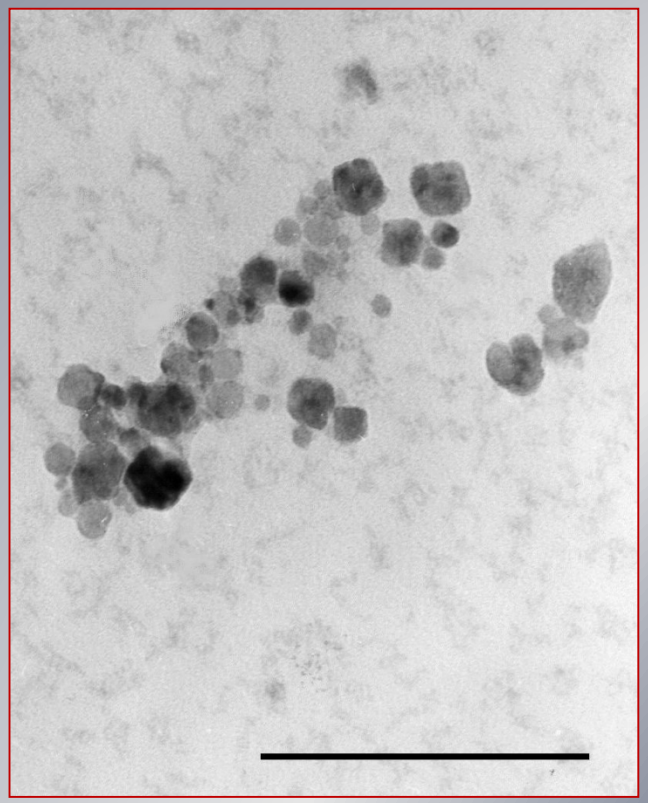


Laboratory of Electron Microscopy
University of Lodz

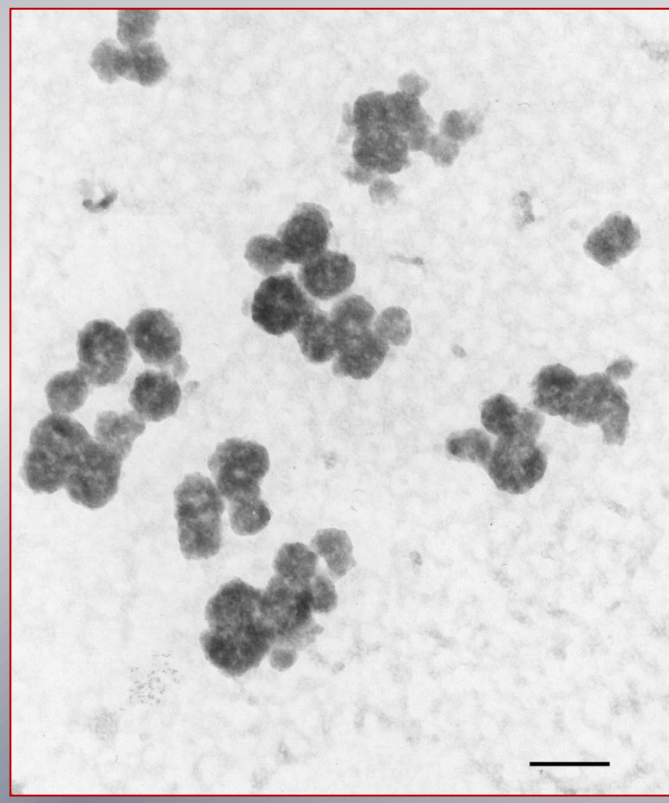
TEM Joel JEM-1010
siatki: Formvar/Carbon 200 mesh, Copper



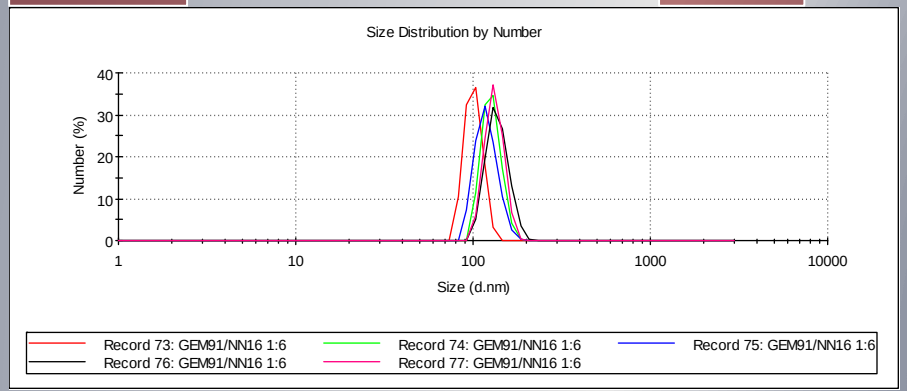
GEM91



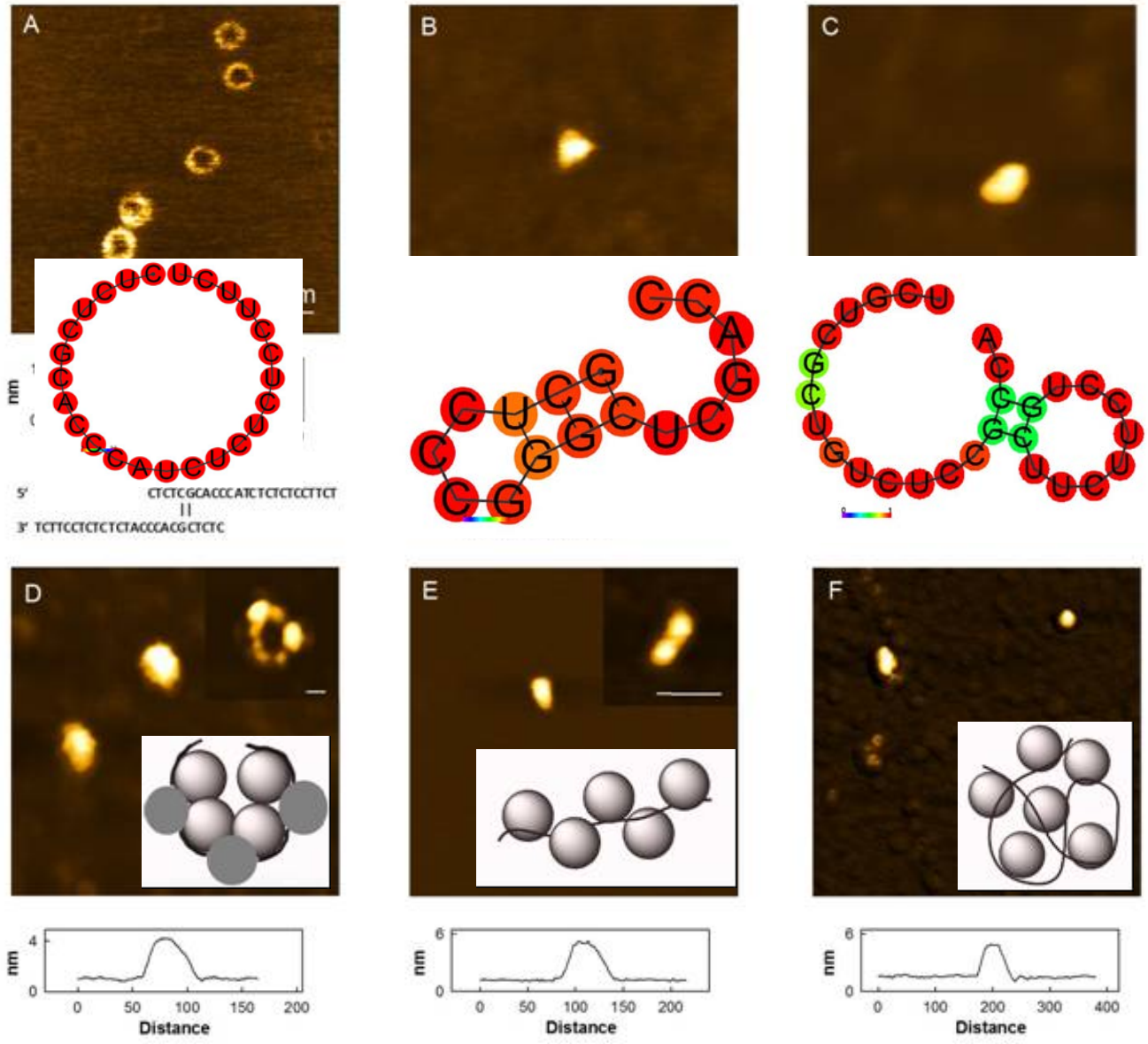
NN16



NN16/GEM91
(charge ratio 3/1, molar. 6,25/1)



TEM (scale 100 nm)



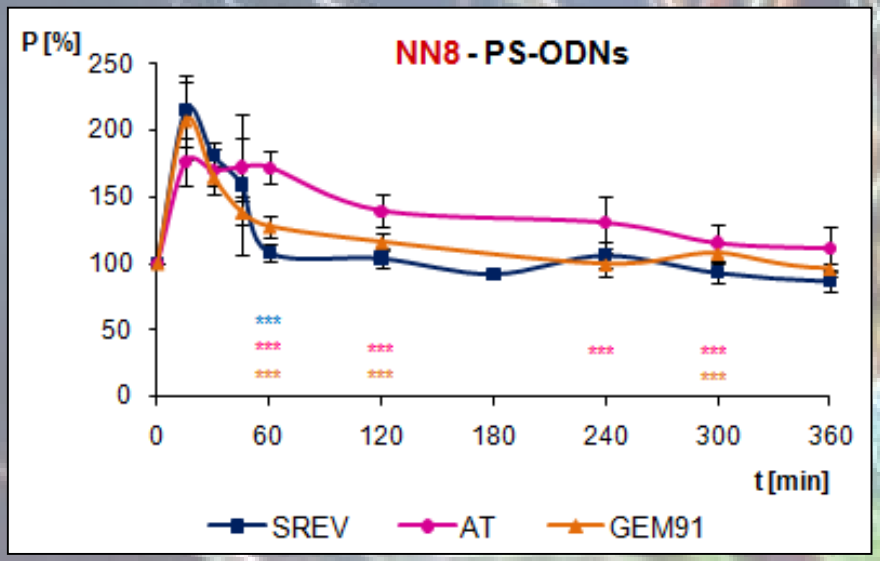
(A) GEM91
(B) AT
(C) SREV
(D) NN16/GEM91 (6/1), inset: NN8/GEM91 (25/1) oligomer
(E) NN16/AT (4/1), inset: dimer
(A) NN8/SREV (10/1)
(A-C) dimer formation (UNAfold simulations)

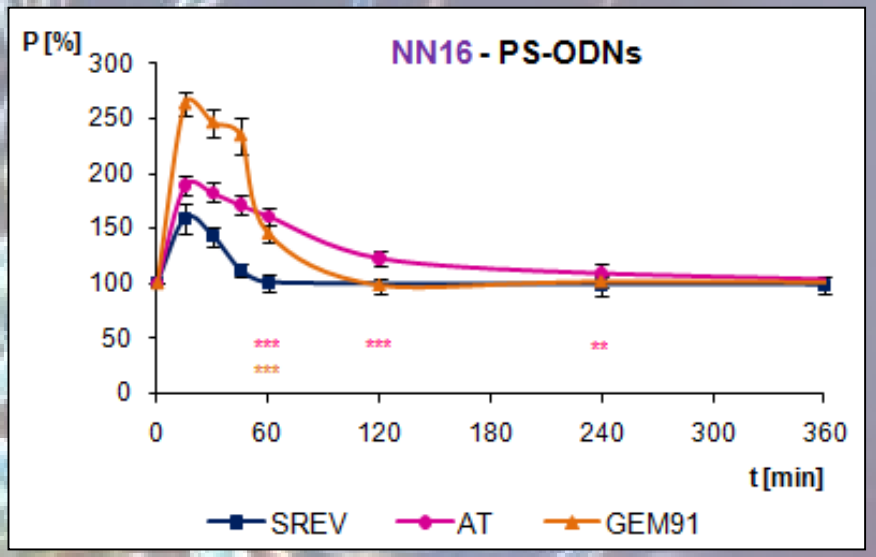
Secondary structures of ODNs (RNAfold web)

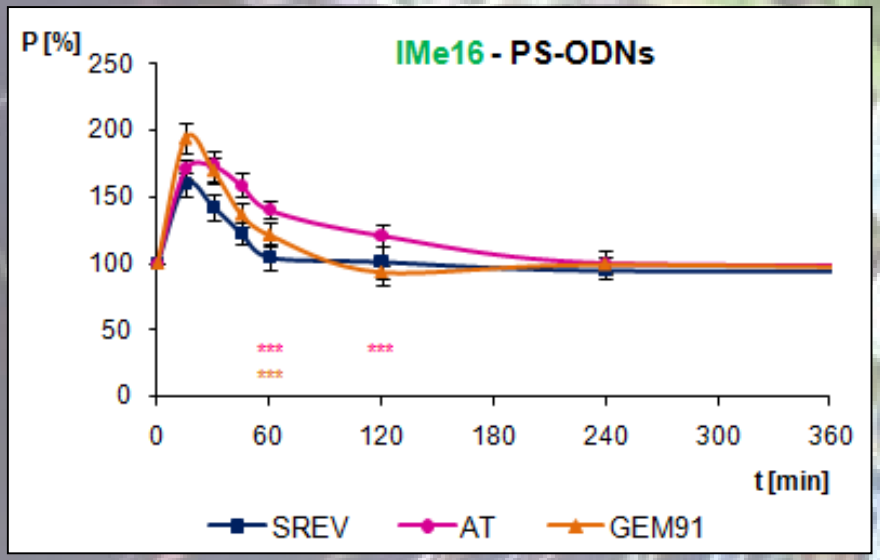
J. E. Purkinje University
Usti nad Labem
Dr Jan Maly

AFM Integra Probe Nanolaboratory (NT-MDT, Russia) – scaner 3×3 µm, semi-contact mode

TIME DEPENDENT STABILITY



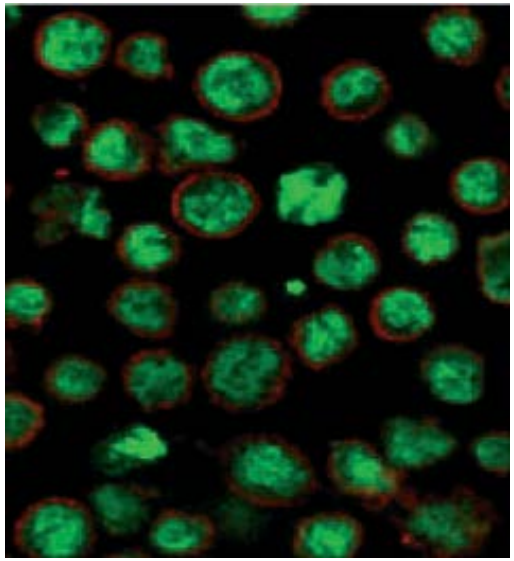




- after 15 minutes of incubation the polarization degree sharply increased indicating the formation of PS-ODN-dendrimer complex
- continuous mixing of PS-ODNs-dendrimer complex led to exponential decrease of relative polarization degree showing the destruction of dendriplex
- stability of complexes ranged between 60 and 300 minutes depending on dendrimer and PS-ODN type
- complexes with AT were the most stable

changes in the fluorescence polarization of fluorescein attached to the ends of the nucleic acids (complexed with dendrimers) in time

The study of the cellular uptake of the dendriplex formed by NN8 and the fluoresceinated PS-ODN into PBMCs by confocal microscopy.



Confocal micrograph of internalization of NN8/ODN dendriplex after 48 h.

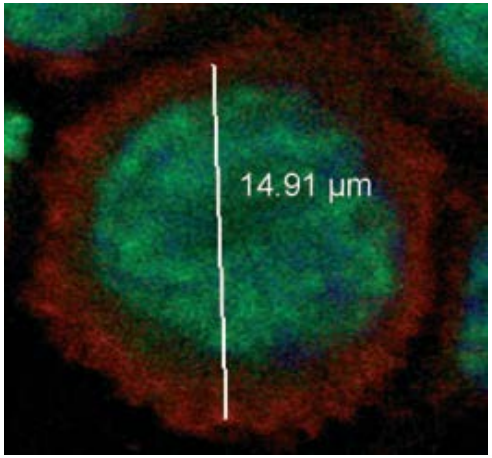
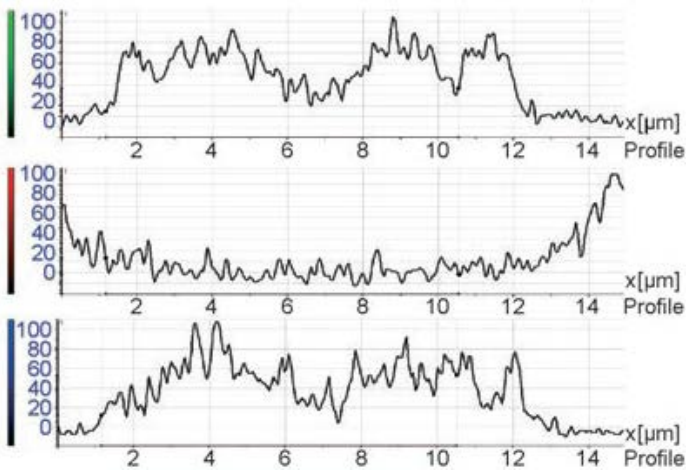
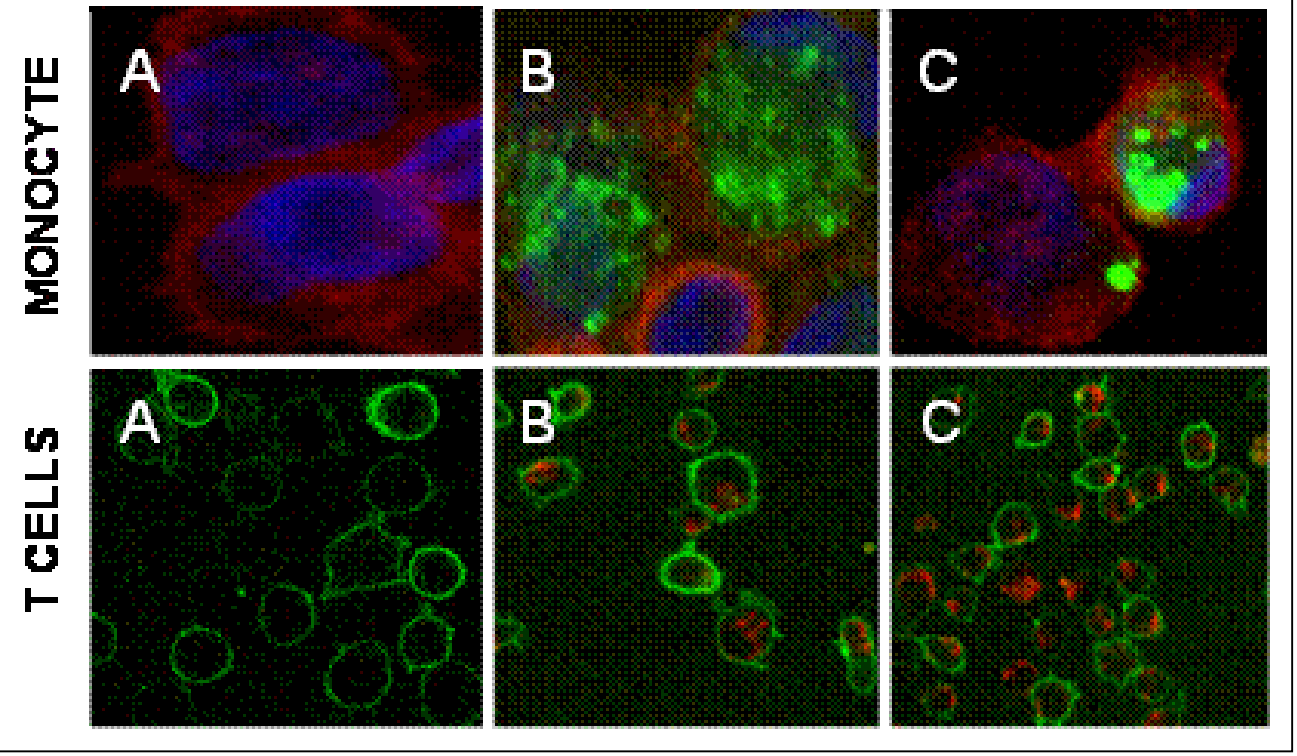


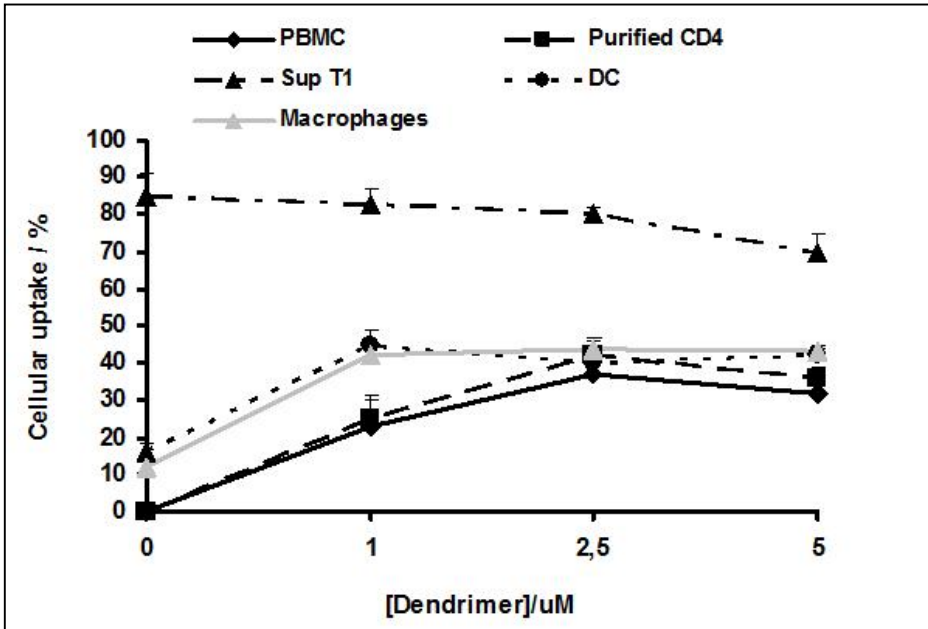
Image of an isolated cell; white line denotes a section through the median plane XY.



Plots of fluorescence emission through the section: green (fluoresceinated ODN), blue (cell nucleus) and red(cell membrane).



Internalization of ODN into monocyte cells or siRNA into T cells. Confocal microscopy images of monocytes after 18 h incubation with mock treated (A) or with FITC-labeled ODN (B) or dendriplex (ODN-FITC/16(C). T cells after 20 h incubation with mock treatment (A) or with Cy3-labeled siRNA (red) alone (B) or complexed with 16 (C). Cell membranes are labeled with αCD14-PE antibodies (red) for monocyte and αCD45-FITC antibodies (green) for T cells.

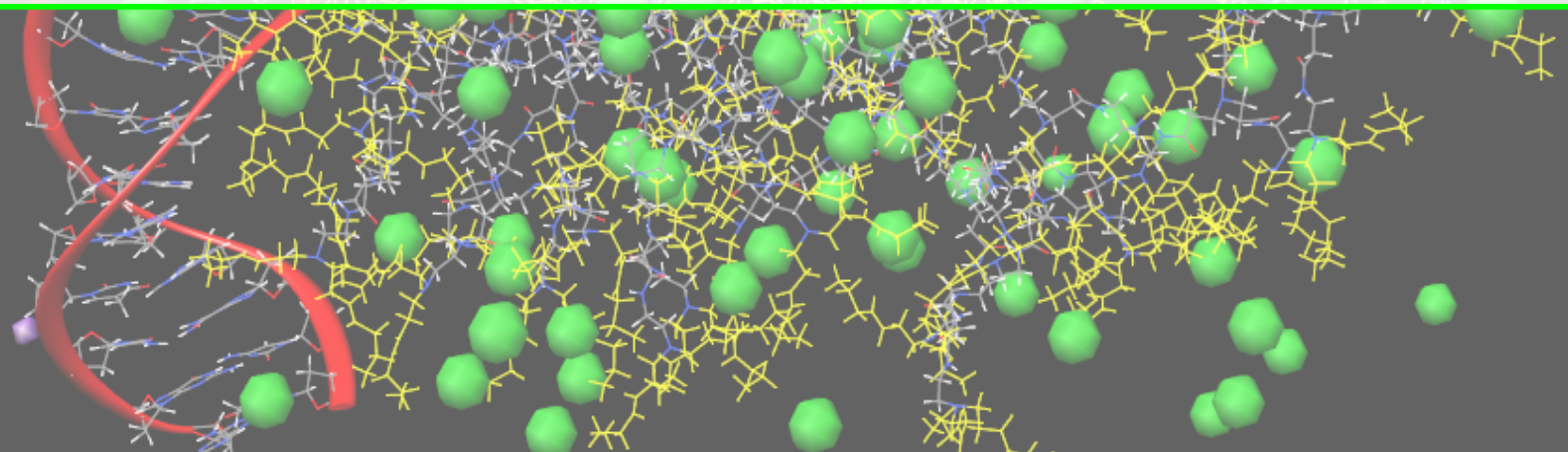


Assays of transfection of the SNA–16 complex. Results are expressed as percentage of cellular uptake measured by flow cytometry relative to NN16 (range 0–5 μM), in different types of cells (PMBCs, purified CD4+ cells, DC, SupT1 lymphocyte cell line and macrophages).

The physicochemical properties of NA-nanoparticles complexes (size, morphology, stability) depend on the type of nanoparticle (structure, number of positive charge) and type of nucleic acid, but independently of dendrimer family, nucleic acid (ODN/siRNA) was fully bound by dendrimer at 1:3-1:4 charge ratio (-/+) (zeta potential measurments).

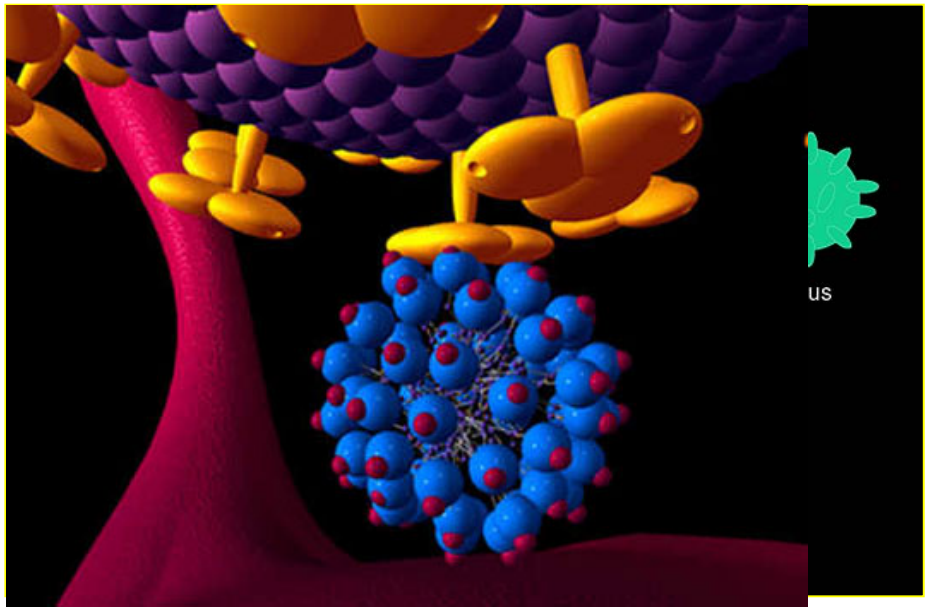
the physicochemical properties of NA-nanoparticles complexes affect cellular uptake and transfection efficiency

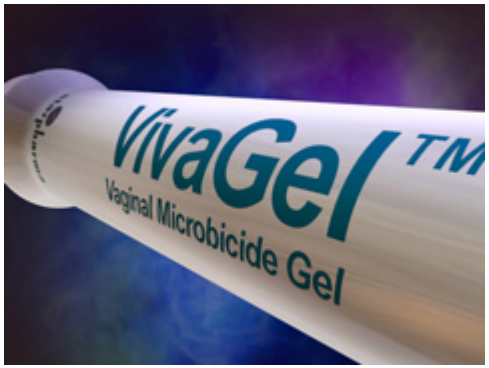
carbosilane dendrimers are the best candidates as carriers for delivery of anti-HIV nucleic acids



APPLICATION OF DENDRIMERS AS anti-HIV THERAPY

2) using dendrimers as anti-HIV therapeutic agents *per se*





VivaGel™, the product of Starpharma.The active ingredient is a dendrimer SPL7013

3) using dendrimers for complexation with HIV peptides

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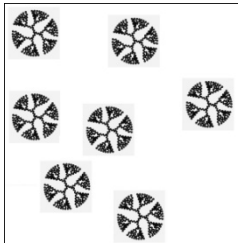
Peptides-associated dendrimers in dendritic cells for the development of new nano-HIV vaccines.

Hospital General Universitario Gregorio Marañón, Laboratorio Inmuno-Biología Molecular, Madrid, Spain

Department of General Biophysics,
Dr Maksim Ionov



Dendrimers

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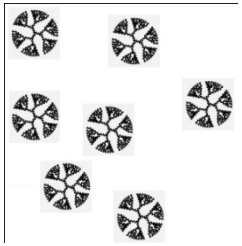

Peptides

Dendrimers

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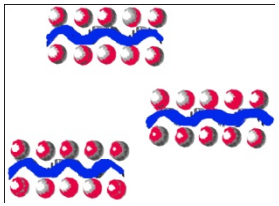

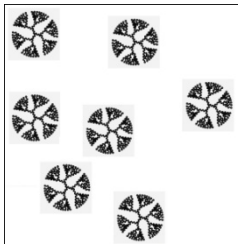

Nanocomplexes

Peptides

Dendrimers

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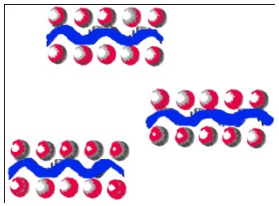
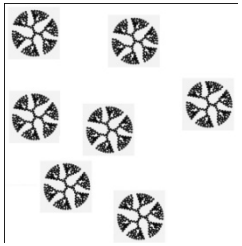


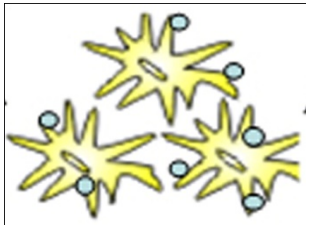
Dendritic cells



Nanocomplexes

Peptides

Dendrimers



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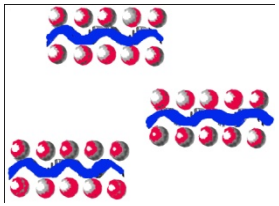
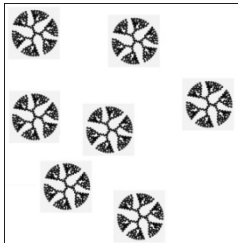
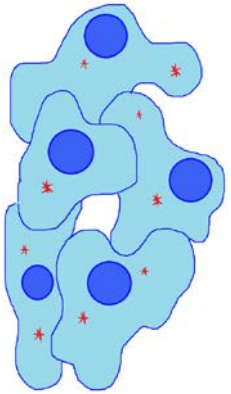


T-limfocytes

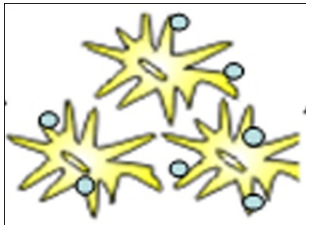
Dendritic cells



Nanocomplexes



Peptides



Dendrimers

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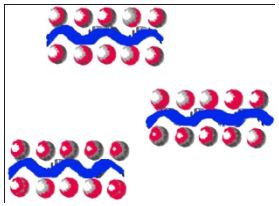
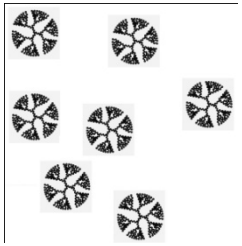
Immunotherapy

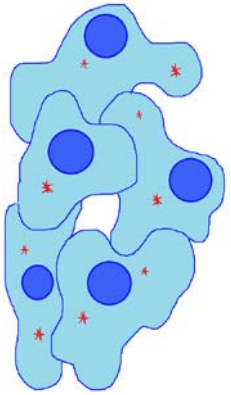


T-limfocytes

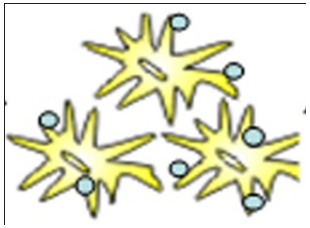
Dendritic cells



Nanocomplexes



Peptides



Dendrimers

POTENTIAL CLINICAL RELEVANCE

Nanomedicine: Nanotechnology, Biology, and Medicine
9 (2013) 972–984

Research Article

nanomedjournal.com

Glycodendrimers as new tools in the search for effective anti-HIV DC-based immunotherapies

E. Vacas Córdoba, MB[a,b,1], M. Pion, PhD[a,b,1], B. Rasines, PhD[c], D. Filippini, PhD[c], H. Komber, PhD[c], M. Ionov, PhD[d], M. Bryszewska, PhD[d], D. Appelhans, PhD[c], M.A. Muñoz-Fernández, PhD, MD[a,b,*]

[a]*Laboratorio InmunoBiología Molecular, Hospital General Universitario Gregorio Marañón and Instituto de Investigación Sanitaria Gregorio Marañón, Madrid, Spain*
[b]*Networking Research Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Spain*
[c]*Leibniz-Institut für Polymerforschung Dresden e.V., Dresden, Germany*
[d]*Department of General Biophysics, University of Lodz, Lodz, Poland*

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Abstract

Dendritic cells (DC), which play a major role in development of cell-mediated immunity, represent opportunities to develop novel anti-HIV vaccines. Dendrimers have been proposed as new carriers to ameliorate DC antigen loading and in this way, we have determined the potential use of maltose decorated neutrally and positively charged G4 glycodendrimers. Thus, immunostimulatory properties of these glycodendrimers on human DC were evaluated in the context of HIV infection. We have demonstrated that DC treated with glycodendrimers were fully functional with respect to viability, maturation and HIV-derived antigens uptake. Nevertheless, iDC and mDC phenotypes as well as mDC functions such as migration ability and cytokines profile production were changed. Our results showed the potential carrier properties of glycodendrimers to activate the immune system by the way of DC stimulation. This is the first study for exploring the use of maltose-functionalized dendrimers-peptides complexes as a potential DC-based vaccine candidate.

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Current Medicinal Chemistry, 2013, *20*, 3935-3943 3935

Biophysical Characterization of Glycodendrimers As Nano-carriers for HIV Peptides

M. Ionov*[,1], K. Ciepluch[1], B.R. Moreno[2], D. Appelhans[2], J. Sánchez-Nieves[3,4], R. Gómez[3,4], F.J. de la Mata[3,4], M.A. Muñoz-Fernández[4,5] and M. Bryszewska[1]

[1]*Department of General Biophysics, Faculty of Biology and Environmental Protection, University of Lodz, Poland;* [2]*Leibniz Institute of Polymer Research, Hohe Straße 6, D-01069 Dresden, Germany;* [3]*Dpto. de Química Orgánica y Química Inorgánica, Universidad de Alcalá, Campus Universitario, E-28871 Alcalá de Henares (Madrid), Spain;* [4]*Networking Research Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN);* [5]*Laboratorio InmunoBiología Molecular, Hospital General Universitario Gregorio Marañón, Madrid, Spain*

Abstract: This paper examines the formation and stability of nano-complexes that could provide a new therapeutic approach against HIV-1 infection. Poly(propylene imine) glycodendrimers decorated with 2nd generation cationic carbosilane dendrons were generated and their use in polyplex formation checked. Owing to their positively-charged terminal amino groups the hybrid glycodendrimers can bind anionic peptides. It was shown that they form nano-complexes with the HIV-derived peptides P24, Gp160 and Nef. Complexes 130-190 nm in size were formed in molar ratios (dendrimer/peptide) of (3-4):1. These were sufficiently stable over time and at different pHs. The results obtained suggest that the hybrid dendrimers studied can be considered as alternative carriers for delivering HIV peptides to dendritic cells.

Keywords: PPI-glycodendrimer, HIV-peptide carrier, complex formation, stability of dendriplexes.

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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 InmunoBiología Molecular, General Hospital Universitario Gregorio Marañon, CIBER-BBN, Madrid, Spain*

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Stability

ABSTRACT

Dendrimers have been proposed as new carriers for selected HIV-1 peptides. This paper reports on the complexation behaviour of the three HIV-derived-peptides: Gp160, NH-EIDNYTNTIYTLLEE-COOH; P24, NH-DTINEEAAEW-COOH and Nef, NHGMDDPEREVLEWRFDSRLAF-COOH with second generation cationic carbosilane dendrimers (CBD) branched with carbon–silicon bonds (CBD-CS) or oxygen–silicon bonds (CBD-OS). Studies on the formation of complexes between HIV peptides and CBDs by fluorescence polarization, zeta-potential, electrophoresis and transmission electron microscopy have shown that both studied dendrimers form complexes with HIV peptides. At a molar ratio of (2.5–3):1 (dendrimer:peptide), the complexes formed were in the size range of 180–275 nm and with significant positive surface charge. The results suggest that interactions between dendrimers and HIV peptides have electrostatic nature due to the negative charge of peptides backbone and positive charge of dendrimer functional groups. Dendriplex stability depended on the type of studied dendrimers. Time of peptides release from the complexes ranged from 1 (CBD-OS) to ~36 (CBD-CS)h. Basing on the obtained results, we propose that the water-soluble cationic carbosilane dendrimers can be considered for delivery of HIV peptides to dendritic cells.

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Interaction of phosphorus dendrimers with HIV peptides—Fluorescence studies of nano-complexes formation

Karol Ciepluch[a,*], Maksim Ionov[a], Jean-Pierre Majoral[b], Maria Angeles Muñoz-Fernández[c], Maria Bryszewska[a]

[a] *Department of General Biophysics, Faculty of Biology and Environmental Protection, University of Lodz, Pomorska Street 141/143, 90-236 Lodz, Poland*
[b] *Laboratoire de Chimie de Coordination du CNRS (LCC), 205 Route de Narbonne, F-31077 Toulouse cedex 4, France*
[c] *Laboratorio InmunoBiología Molecular, Hospital General Universitario Gregorio Marañón, Madrid, Spain*

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ABSTRACT

In this study, dendrimers emerge as an alternative approach for delivery of HIV peptides to dendritic cells. Gp160, NH-EIDNYTNTIYTLLEE-COOH; P24, NH-DTINEEAAEW-COOH and Nef, NHGMDDPEREVLEWRFDSRLAF-COOH peptides were complexed with two types of positively charged phosphorus-containing dendrimers (CPD). Fluorescence polarization, dynamic light scattering, transmission and electron microscopy (TEM) techniques were chosen to evaluate the dendriplexes stability. We were able to show that complexes were stable in time and temperature. This is crucial for using these peptide/dendrimer nano-complexes in a new vaccine against HIV-1 infection.

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- **Prof. Maria Bryszewska -** Department of General Biophysics, University of Lodz, Poland
- **Dr Dzmitry Shcharbin -** Institute of Biophysics and Cell Engineering of NASB, Minsk, Belarus
- **Dr Jan Maly -** Department of Biology, J. E. Purkinje University, Usti nad Labem, Czech Republic
- **Prof. Mª Angeles Muñoz-Fernandez -** Immunomolecular Biology Laboratory, Gregorio Maranón General Hospital, Madrid, Spain
- **Dr Paula Ortega, dr F. Javier de la Mata, dr Rafael Gómez -** Department of Inorganic Chemistry, University of Alcalá de Henares, Spain
- **Dr. Dietmar Appelhans,** Leibniz-Institut für Polymerforschung, Germany
- **Prof. Jean-Pierre Majoral**, CNRS, Toulouse, France

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