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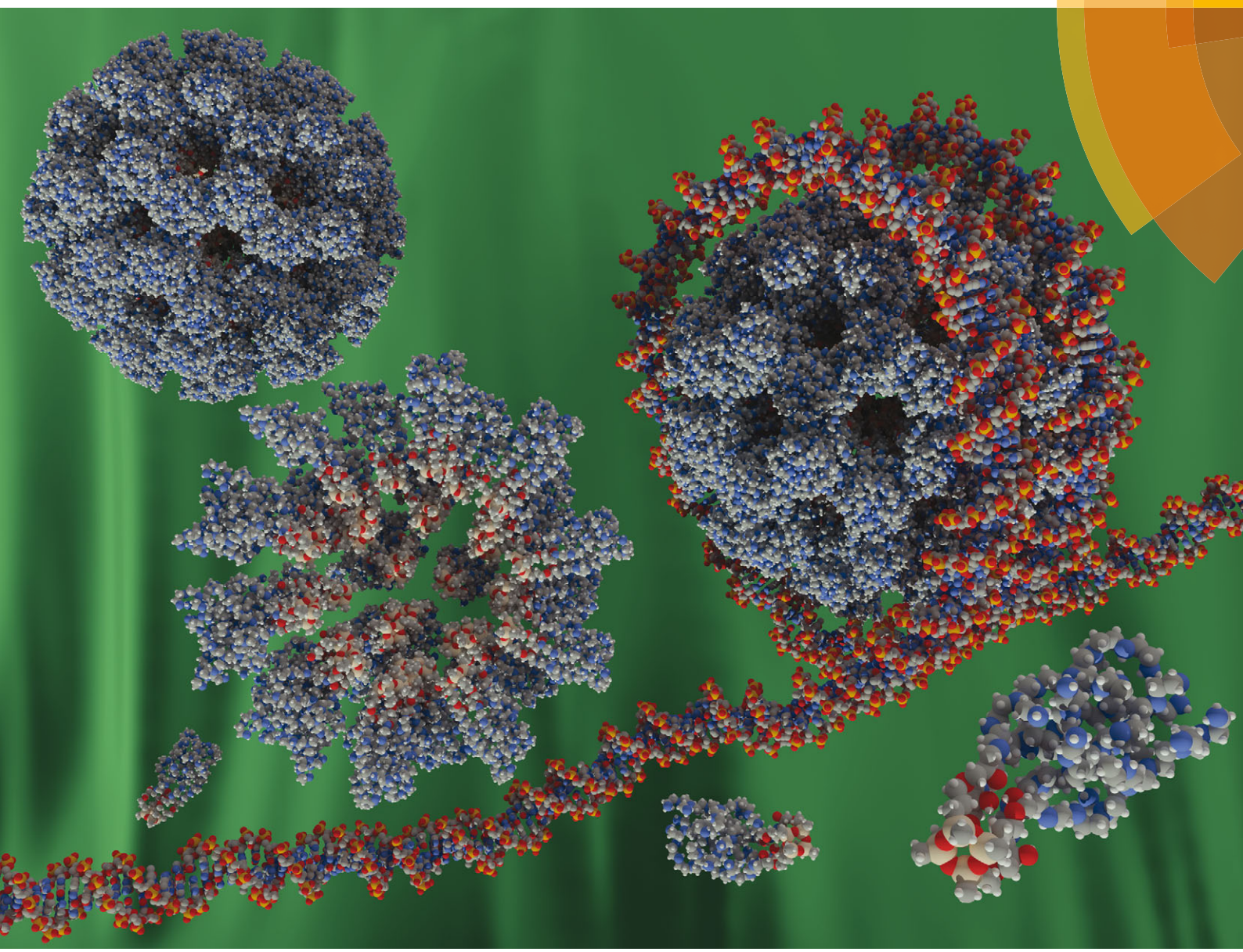

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PAPER

Mariana Pinteala *et al.*

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Flexible cyclic siloxane core enhances the transfection efficiency of polyethylenimine-based non-viral gene vectors†

Cristina M. Uritu,[a] Manuela Calin,[b] Stelian S. Maier,[ac] Corneliu Cojocaru,[a] Alina Nicolescu,[a] Dragos Peptanariu,[a] Cristina Ana Constantinescu,[b] Daniela Stan,[b] Mihail Barboiu[d] and Mariana Pinteala*[a]

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Transfection of nucleic acid molecules, large enough to interfere with the genetic mechanisms of active cells, can be performed by means of small carriers, able to collectively collaborate in generating cargocomplexes that could be involved in passive mechanisms of cellular uptake. The present work describes the synthesis, characterization, and evaluation of transfection efficacy of a conjugate molecule, which comprises a cyclic siloxane ring (2,4,6,8-tetramethylcyclotetrasiloxane, cD_4^H) as the core, and, on average, 3.76 molecules of 2 kDa polyethyleneimine (PEI) as cationic branches, with an average molecular mass of 7.3 kDa. As demonstrated by *in silico* molecular modeling and dynamic simulation, the conjugate molecule (cD_4^H–AGE–PEI) tends to adopt an asymmetric structure, specific for amphipathic molecules (confirmed by a log*P* value of -1.902 ± 0.06), that favors a rapid interaction with nucleic acids. The conjugate and the polyplexes with the pEYFP plasmid were proved to be non-cytotoxic, and capable of ensuring transfection yields better than 30%, on HEK 293T cell culture, superior to the value obtained using the SuperFect® reagent. We presume that the increased transfection efficacy originates in the ability of the conjugate to locally tightly encompass pDNA molecules by electrostatic interaction mediated by the short PEI branches, and consequently to expose the siloxane hydrophobic moiety, which decreases the interaction energy with the lipid layers.

Introduction

Regardless of their nature and type, gene vectors must be able to pass through physiological barriers, and need to fight against the cells' defensive systems, which are able to detect and silence the "intruder" DNA.[1] In addition, synthetic DNA carriers must be able to pack or wrap genetically significant segments of DNA, of more than 27 kilobases, which is the average dimension of a human active gene that contains introns too.[2]

Carrying and targeted delivery of large segments of nucleic acids is proved to be efficient when viral vectors are used,[3] but still represents a challenge in producing and using non-viral gene vectors. In principle, there are no size limitations in the case of cationic lipid-based vectors,[4] but evident drawbacks have been revealed when liposomes were applied to deliver DNA into cells (poor transfection efficiency, poor entrapment stability in *in vivo* conditions,[5] short lifetime, inactivation by serum proteins, cytotoxicity of cationic lipids in large amounts, especially on phagocytes[6]). If they are non-cytotoxic, nanometric sized particulate polycationic entities,[7,8] possibly endowed with cell penetrating[9,10] and active targeting[11] components, decorated with protectant molecules,[12] and featured with stimuli responsiveness,[13,14] are feasible DNA carriers, both as individual particles, and as collectively encapsulated ones.[15,16]

Spatially extended cargocomplexes generated by the electrostatic self-assembly between numerous individual carrier entities and the DNA segments might represent a practical solution to deliver genes and plasmids of large size. In this respect, different chemical architectures of the individual polymeric cationic carriers were tested, in the pursuit of smaller dimensions accompanied by stable unfolded states of the macromolecular chains.

[a] *Centre of Advanced Research in Bionanoconjugates and Biopolymers, "Petru Poni" Institute of Macromolecular Chemistry, Iasi, 700487, Romania. E-mail: pinteala@icmpp.ro*
[b] *"Nicolae Simionescu" Institute of Cellular Biology and Pathology of the Romanian Academy, Bucharest, 050568, Romania*
[c] *"Gheorghe Asachi" Technical University of Iasi, Iasi, 700050, Romania*
[d] *Adaptative Supramolecular Nanosystems Group, Institut Européen des Membranes, ENSCM/UMII/UMR-CNRS 5635, 34095 Montpellier, Cedex 5, France*

† Electronic supplementary information (ESI) available: Additional experimental data of cD_4^H–AGE–PEI conjugate and its precursors including FTIR spectra, XPS wide scan spectra, a table resuming high resolution spectra C 1s and N 1s assignments, TGA/DTG and DSC curves, a table comprising summary results of molecular dynamics simulation regarding DNA/cD_4^H–AGE–PEI. See DOI: 10.1039/c5tb01342a