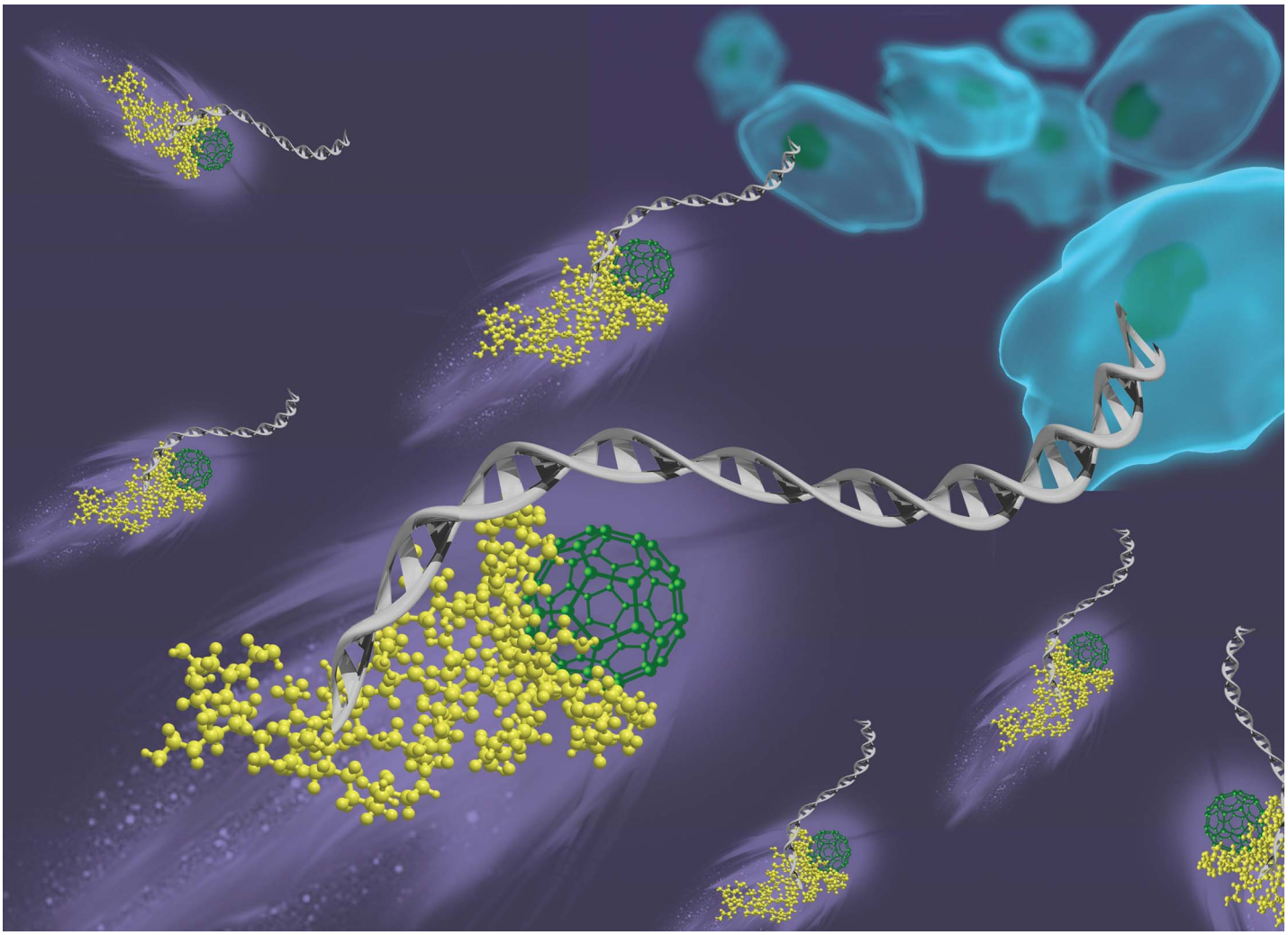

Showcasing collaborative research from Mihail Barboiu's laboratory, Adaptive Supramolecular Nanosystems, Institut European des Membranes, Montpellier, France and Mariana Pinteala's laboratory, INTELCENTRU, "Petru Poni" Institute of Macromolecular Chemistry, Iasi, Romania.

Title: Hybrid fullerene conjugates as vectors for DNA cell-delivery

C60-PEI and C60-PEG-PEI conjugates act as efficient binders of double stranded DNA (dsDNA) polyplexes that exhibit good transfection efficiency and are performant in terms of expression of EYFP reporter gene in cultured cells and exhibited high cytocompatibility, determining cell proliferation up to 200%.

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Hybrid fullerene conjugates as vectors for DNA cell-delivery†

Cristina M. Uritu,[a] Cristian D. Varganici,[a] Laura Ursu,[a] Adina Coroaba,[a] Alina Nicolescu,[a] Andrei I. Dascalu,[a] Dragos Peptanariu,[a] Daniela Stan,[b] Cristina A. Constantinescu,[b] Viorel Simion,[b] Manuela Calin,[b] Stelian S. Maier,[ac] Mariana Pinteala[a] and Mihail Barboiu*[d]

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The present study reports fullerene conjugates that act as efficient binders of double stranded DNA (dsDNA) into cytofriendly polyplexes. The conjugates are designed to generate dendrimeric structures, having C60 as the core and bearing linear or branched PEI and polyethyleneglycol (PEG) arms (~2 kDa). Simple and reproducible synthesis pathways provided C60-PEI and C60-PEG-PEI conjugates. They were able to bind linear and plasmidic dsDNA and they form particulate polyplexes of 50 to 200 nm in diameter. The resulted polyplexes toggle between the anionic and cationic state at nitrogen to phosphorous ratios (N/P) of about 5, as revealed by their zeta potential and became colloidally stable at N/P ratios above 10, as determined by atomic force microscopy (AFM). They are electrophoretically unbreakable starting with N/P ratios of 3 and of 5 when salmon sperm DNA and pEYFP-C1 plasmid, respectively are loaded. Both C60-PEI·pEYFP and C60-PEG-PEI·pEYFP polyplexes are non-cytotoxic against HEK 293T cells in culture and exhibit transfection efficiency better than 25% (N/P ratios above 20) and 6% (N/P ratios above 60) respectively, measured by flow cytometry. For comparison, the commercial SuperFect® from Qiagen (positive control) was able to provide an efficiency of 15–20%, under similar conditions. Moreover, the C60-PEG-PEI conjugate is as performant as the positive control in terms of expression of EYFP reporter gene in cultured cells and exhibited high cytocompatibility, determining cell proliferation up to 200%. Our study proved that C60-PEG-PEI is effective vector for DNA delivery being, in addition, easily synthesizable, practically non-cytotoxic and as efficient the commercially available transfection tools.

Introduction

Replacing defective genes with fully functional copies by using gene therapy is one of the most promising techniques in modern medicine. Its basic approach is to selectively transfect DNA into affected cells, by making use of engineered viral or non-viral vectors. Three important features tend to prevail in preferring the use of non-viral vectors against viral ones: (i) the ability to control and to eliminate the occurrence of an immunologic response to the carrying vector, (ii) the simplicity of use in transfection protocols and (iii) the ability to synthetically produce large amounts of vector-entities having highly reproducible structural and functional behaviours. Within this context, sterically exposed polycations are well suited to bind anionic DNAs *via* multiple ionic interactions, in a labile manner enough to act as reversible transporters. Even if cytotoxic *per se*, polyethyleneimines (PEI) having low-to-medium molecular weight (5 to 25 kDa) offer a good compromise in acting as DNA carriers.[1,2] However, below 2 kDa they are unable to transfect cells, while becoming cytofriendly.[3] Highly efficient and essentially nontoxic DNA carriers can be obtained by multivalent linking of short (0.8 kDa) PEI segments through polyacrylates, to build 14–30 kDa conjugates which are cytotoxic.[4a] PEGylated polymers have been used and both toxicity and transfection efficiency are simultaneously reduced.[4b] Concurrently, the design of hybrid nanosystems containing several functions like multivalent DNA binding sites, membrane penetration and anti-opsonisation functions has attracted a great deal of interest.[5] Among them, fullerenes are unique platforms, but their biological applications are drastically limited by their extreme hydrophobicity, which minimizes their solubility in water. The C60 nanometric core is reactionally adaptive to various modifications, dimensionally well adapted to bind DNA

[a]"Petru Poni" Institute of Macromolecular Chemistry of Romanian Academy, 41A, Aleea Gr. GhicaVoda, Iasi, Romania

[b]"Nicolae Simionescu" Institute of Cellular Biology and Pathology, Bucharest, 050568, Romania

[c]"Gheorghe Asachi" Technical University of Iasi, Iasi, 700050, Romania

[d]Institut Européen des Membranes, Adaptive Supramolecular Nanosystems Group – ENSCM-UMII-CNRS UMR-5635, Place Eugène Bataillon, CC 047, F-34095 Montpellier, Cedex 5, France. E-mail: mihail-dumitru.barboiu@univ-montp2.fr

† Electronic supplementary information (ESI) available: Synthetic details, supplementary NMR, FTIR, UV-VIS, XPS spectra, thermal analyses and transfection efficiency details are supplied. See DOI: 10.1039/c4tb02040e