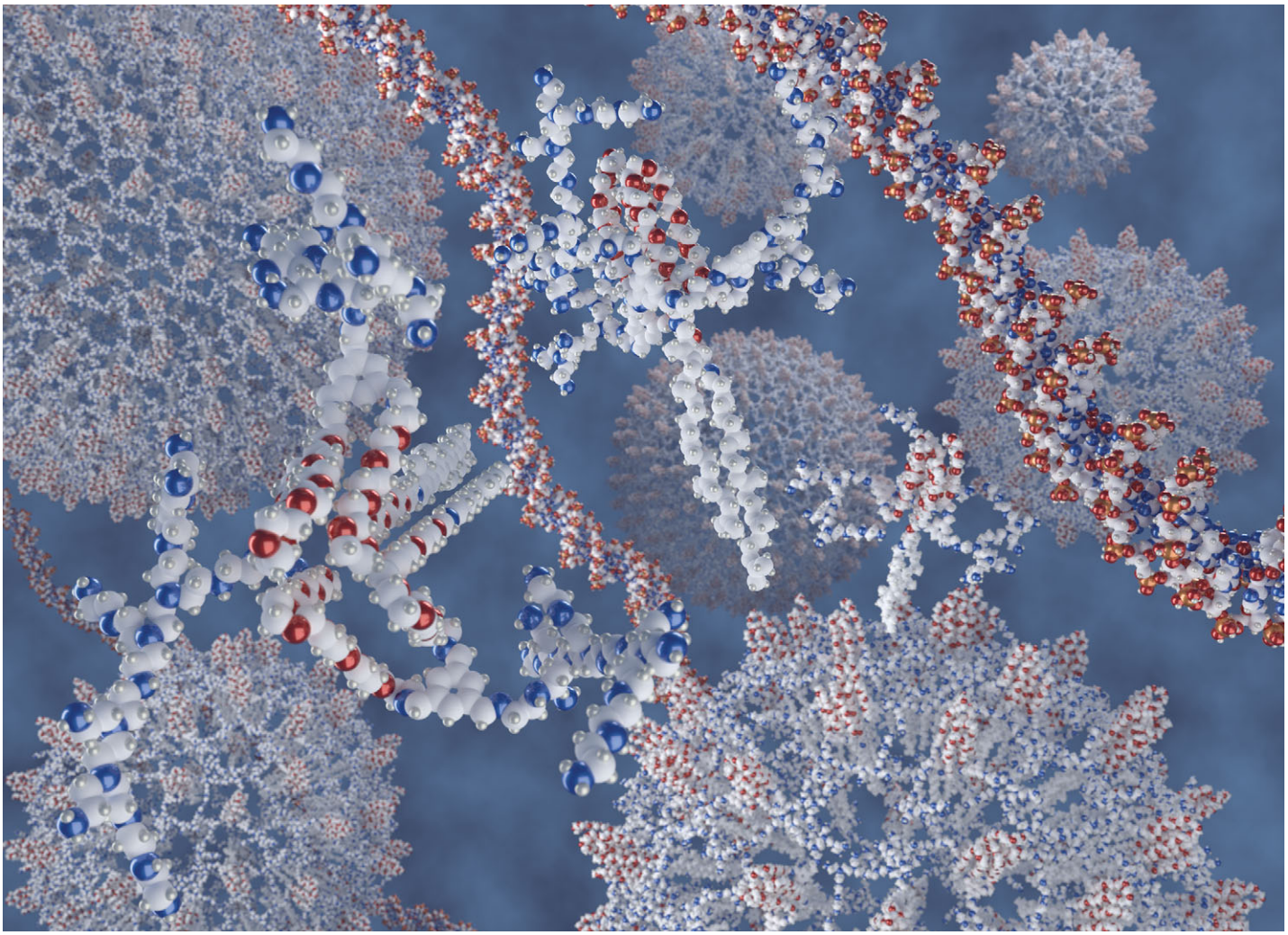

Showcasing research from Mihail Barboiu's laboratory, Institut Européen des Membranes, Montpellier, France and from Mariana Pinteala's laboratory, INTELCENTRU, Iasi, Romania.

DyNAvectors: dynamic constitutional vectors for adaptive DNA transfection

Dynamic Constitutional Frameworks are prepared and tested as modular DyNAvectors for DNA transfection. Depending on their tunable structure, they constitutionally self-adapt to the DNA targets, allowing a rapid identification of most effective vectors with high complexation ability, good transfection efficiency, and well tolerated by mammalian cells.

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DyNAvectors: dynamic constitutional vectors for adaptive DNA transfection†

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Dynamic constitutional frameworks, based on squalene, PEG and PEI components, reversibly connected to core centers, allow the efficient identification of adaptive vectors for good DNA transfection efficiency and are well tolerated by mammalian cells.

Gene therapy is a method used to introduce genetic material into cells to treat disorders. It is well known that viral vectors have superior transfection capacity but their use is limited by their induction of immune responses and virus-pathogenicity.[1,2] Alternatively, the non-viral vectors present lower transfection but their cytotoxicity limits their application to clinical trials.[3] That is why, the rational design of non-viral vectors has been developed.[4] However, the rational design has become limited to a low number of synthetic components fixed in specific positions on the vector backbone and should be completed by Dynamic Constitutional (DC) approaches.[5–7] Extending the DC concepts to materials science led to the emergence of Dynamic Polymers (Dynamers[8]) that are polymers linked through reversible bonds and are able to respond to internal or external factors by exchange of components. We recently proposed the Dynamic Constitutional Frameworks (DCFs), the 3D Dynamers, for DNA recognition.[7] The ability to adaptively implement spatial rearrangements of such reversible materials may induce a high level of correlativity of their 3D architectures and external surfaces in interaction, for example, the DNA and the cell membrane barrier. In other words, this leaves the DNA to self-generate the fittest material, for its own transfection. The DNA target itself is used to self-select an active DyNAvector from a virtual mixture of architectures, resulting in a highly useful simplified screening process. Within this context, the use of dynameric materials for DNA transfection is an emerging field.[7,9] Herein, after the DNA recognition studies we further report an efficient and simple constitutional approach to conceive DCFs as multivalent DyNAvectors for DNA transfection (Fig. 1). They simultaneously exhibit optimal DNA binding, transfection yield to standard agents and preserve high HEK 293T cell viability.

DyNAvector synthesis. The synthesis involves the following components: (a) 1,3,5-benzenetrialdehyde **1** as the core centre, able to cross-link the network components and DNA-binding sites *via* the amino-carbonyl/imine reversible chemistry; (b) PEG-ylated squalene (SQ-PEG) **2** hydrophobic component, known to form stable particles with diameters ~100–200 nm in aqueous solution;[10] (c) poly-(ethylene-glycol)-bis (3-amino-propyl)-terminated (M_n ~ 1500 g mol^{-1}) PEG$(NH_2)_2$ **3** segments, known to favour solubility in water and to reduce the immunogenicity of the systems;[11] and (d) low molecular weight branched polyethyleneimine (bPEI), (M_n ~ 800 g mol^{-1}) **4** as cationic binding sites, able to bind DNA. We know that bPEI2500 (25 kDa) is the most effective vector;[11a] however, they present increased cell toxicity.[12] On contrary, bPEI800 (0.8 kDa) has demonstrated low toxicity and conversely very low transfection activity.[13] We anticipate that the multivalent presentation of bPEI800 on DCF adaptive backbones might increase its transfection efficiency, keeping the toxicity levels low. Treatment of **1** with different equiv. of **2**[14] and **3** (Table S1, ESI†) in CH_3CN (rt, 24 h) resulted in the formation of a mixture of linear and cross-linked DCFs (**5** and **6**), supported by ^{1}H-NMR spectral results. The reactions have been monitored by following the aldehyde chemical shifts corresponding to mono-, M di- D and trialdehyde T-type compounds for which the corresponding imine chemical shifts can be observed in the spectra (Fig. S1, ESI†). By combining **1** and **2** (1 : 1 molar ratio) in the absence of **3**, the M : D : T ratio is found to be 1 : 3 : 1.5. The addition of **3** results in the progression (M : D : T = 1 : 3 : 0.5 at **1** : **2** : **3** molar ratio of 1 : 1 : 0.5) to the complete consumption of T (M : D = 1 : 3 at 1 : 1 : 1 ratio). By decreasing the ratio of component **2** to 1 :0.5 : 1 the ratio M : D remains 1 : 3. Then, the mixture of **5** and **6** was treated with various amounts of bPEI (Table S1, ESI†).

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