# Calix[4]arene TMAC4 as efficient non-viral vector in gene therapy

**A.L. Barrán-Berdón**[1]*, Belén Yélamos[2], Luis García-Rio[3], E. Aicart[1] and E. Junquera[1]

[1] Grupo de Química Coloidal y Supramolecular, Dpto. Química Física I, Facultad de Ciencias Químicas, Universidad Complutense de Madrid, Av. Complutense, s/n 28040-Madrid, Spain
[2] Dpto. Bioquímica y Biología Molecular I, Facultad de Ciencias Químicas, Universidad Complutense de Madrid, Av. Complutense, s/n 28040-Madrid, Spain
[3] Centro Singular de Investigación en Química Biológica y Materiales Moleculares. Universidad de Santiago de Compostela, Praza do Obradoiro, s/n 15782-Santiago de Compostela, Spain.

anbarran@pdi.ucm.es

One of the major challenges in the gene therapy process is to find efficiently non-viral gene carriers. Several studies have been done in order to increase the number of compounds able to compact, protect and transport nucleics acids into the cell. The development of several kinds of macrocycles such calixarenes open a new way in the non-viral vector tools in gene therapy. Calixarenes are very promising in gene delivery applications for several reasons: their synthesis is relatively easy, they present low toxicity levels and, possessing two clearly distinct chemical regions, allow an efficient region-selective chemistry.[1-3]

Complexes prepared by mixing the gene vector (formed by calix[4]arene TMAC4 and the zwitterionic lipid 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), at several molar fractions, $\alpha$) with plasmid pEGFP-C3 (pDNA) or linear double-stranded calf thymus DNA (ctDNA). A wide biophysical and biochemical characterization was performed including zeta potential, gel electrophoresis, SAXS, cryo-TEM, fluorescence microscopy and cell viability/cytotoxicity to establish a structure-biological activity relationship. The study was performed at several compositions, $\alpha$, between calixarene and DOPE, and at several effective charge ratios, $\rho_{eff}$, (between the gene vector and the DNA) of the complex.

Electrochemical studies (zeta potential and gel electrophoresis) confirm that pDNA is efficiently compacted by the TMAC4/DOPE system. Structural characterization by SAXS shows that diffractograms correspond to nanoaggregates formed by a lamellar structure at any $\alpha$. Cryo-TEM studies reveal the presence of cluster-type and finger print multilamellar structures. Finally, the biochemical studies *in vitro* show that complexes TMAC4/DOPE-pDNA present moderate transfection efficiency and good cell viability in HEK293T cells lines. Therefore, the reported complexes can be considered as potential DNA vectors for gene therapy *in vivo*.

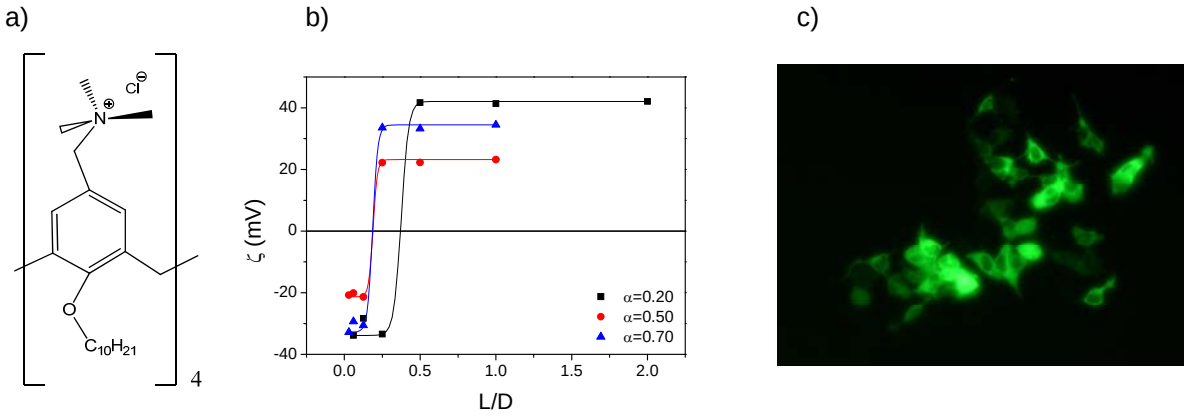


**Figure 1.** a) Molecular structure of the calix[4]arene TMAC4. b) Plot of zeta potential ($\zeta$) against the complex composition (L/D) of TMAC4/DOPE-pDNA at several molar fractions, $\alpha$. c) Fluorescence micrograph of transfected HEK293T cells.

**References**

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