JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

COMMUNICATION

pubs.acs.org/JACS

Why Is Less Cationic Lipid Required To Prepare Lipoplexes from Plasmid DNA than Linear DNA in Gene Therapy?

Mónica Muñoz-Úbeda,[†,⊥] Santosh K. Misra,[§,⊥] Ana L. Barrán-Berdón,[†] Clara Aicart-Ramos,[‡] María B. Sierra,[†] Joydeep Biswas,[§] Paturu Kondaiah,[∥] Elena Junquera,[†] Santanu Bhattacharya,[*,§] and Emilio Aicart[*,†]

[†]Grupo Química Coloidal y Supramolecular, Departamento de Química Física I, and [‡]Departamento Bioquímica y Biología Molecular I, Facultad de Ciencias Químicas, Universidad Complutense de Madrid, 28040 Madrid, Spain

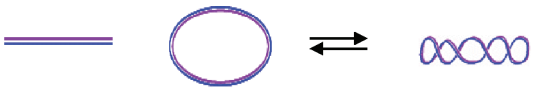
Departments of [§]Organic Chemistry and [∥]Molecular Reproduction Development and Genetics, Indian Institute of Science, Bangalore 560012, India

Supporting Information

ABSTRACT: The most important objective of the present study was to explain why cationic lipid (CL)-mediated delivery of plasmid DNA (pDNA) is better than that of linear DNA in gene therapy, a question that, until now, has remained unanswered. Herein for the first time we experimentally show that for different types of CLs, pDNA, in contrast to linear DNA, is compacted with a large amount of its counterions, yielding a lower effective negative charge. This feature has been confirmed through a number of physicochemical and biochemical investigations. This is significant for both *in vitro* and *in vivo* transfection studies. For an effective DNA transfection, the lower the amount of the CL, the lower is the cytotoxicity. The study also points out that it is absolutely necessary to consider both effective charge ratios between CL and pDNA and effective pDNA charges, which can be determined from physicochemical experiments.

Gene therapy is a novel branch of medicine with the objective of curing a wide variety of diseases, either by supplementation of damaged cellular DNA or by inserting functional genes in living cells.[1−8] Due to the multianionic character of DNA, its permeation across the negative cellular membranes is limited, and vectors for transporting it are needed. One way to achieve DNA delivery is via the use of viral vectors, which show efficient transfection but cause undesirable immune response. Another possibility is to use cationic lipids or polymers that compact DNA of any length to form lipoplexes[6,8−11] or polyplexes,[12−16] respectively, leading to gene transfection without triggering any immune response. Lipoplexes used in gene therapy are constituted by DNA and a mixture of lipids. The mixed lipids consist of a cationic lipid (CL), which interacts electrostatically with DNA and the cellular membrane, and a helper zwitterionic phospholipid (often DOPE), which decreases the toxicity of CL, increases the bilayer fluidity, and makes membrane fusion easier.[11,17−19] Biophysical and biochemical studies of DNA compaction by CLs shed light on both the lipoplex formation and the transfection mechanisms.[6,8−11] The goal is to select lipid mixtures that form stable lipoplexes with high transfection efficiency and minimum toxicity, together with facile DNA release into the cells. For this, the amount of CL must be kept at the lowest that permits effective DNA transfection.

Chart 1. Linear and Plasmid DNA



DNA compaction by CL to form lipoplexes cannot be due only to the Coulombic attractions, because charged macromolecules in solution are surrounded by a sheath of opposite counterions, according to the nonlinear Poisson−Boltzmann theory, which predicts a counterion condensation in the vicinity of its surface (Manning condensation).[20] However, when CL-DNA lipoplexes are formed by positive CL and negative DNA, a percentage of their counterions are released to the solution, resulting in an entropy gain.[12] In addition, CL-DNA lipoplexes must be net cationic to bind to cell surfaces, which suggests that CL charges must be higher than those of DNA in the lipoplex. On the other hand, plasmid DNA (pDNA) is a circular form of DNA that at physiological pH may adopt a supercoiled conformation depending on the ionic strength (Chart 1), while chromosomal DNA fragments, i.e., from calf thymus (ctDNA) or salmon sperm, remain in a linear form. The supercoiling effect renders a less effective negative charge of the biopolymer than its actual charge. Because of this feature and its conformation, it is important to determine the exact amount of CL that is needed to yield a positive lipoplex, which should be adequate for transfection. Indeed, biological studies have shown that pDNA is delivered to cells more efficiently than linear DNA.[21−23] However, why this happens has not been experimentally verified. Also, it must be noted that in biological studies, i.e., transfection or cytotoxicity assays, less CL vector (and, accordingly, less cytotoxicity) could be used. Physicochemical experiments can determine the reasons. However, these are usually done with the cheaper and commercially available linear DNA,[6,9,10,12,24] since large amounts of DNA are needed in such studies. Thus, physicochemical and biological investigations have so far been carried out differently. It is therefore essential that these studies are performed under identical conditions. Herein we report the

Received: May 23, 2011
Published: October 10, 2011

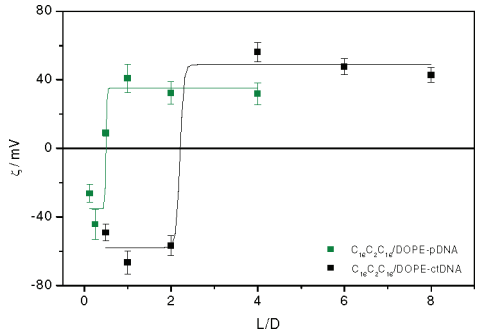


Figure 1. Plot of ζ potential against lipoplex composition (L/D) of $C_{16}C_2C_{16}$/DOPE-ctDNA and $C_{16}C_2C_{16}$/DOPE-pDNA at α = 0.5 in HEPES buffer at 298.15 K, pH 7.4. Solid line: sigmoidal fit of experimental values. Errors are within ±5%, [DNA] = 0.1 mg/mL and [L] was varied, depending on CL composition, to cover a wide L/D range.

results of our investigations where for the first time both physicochemical and biological experiments have been performed using pDNA.

Physicochemical characterization of the DNA compaction process requires the knowledge of the mass ratio (L/D) between lipid and DNA and the charge ratio (ρ) between positive CL and negative DNA phosphate groups. These quantities are related to the CL composition (α) of the lipid mixture as given by

$$\alpha = (L^+/M_{L^+})/[(L^+/M_{L^+}) + (L^0/M_{L^0})] \quad (1)$$

$$\rho = [n^+/n^-] = (q^+_{L^+}L^+/M_{L^+})/(q^-_{DNA}D/\bar{M}_{bp}) \quad (2)$$

where L^+ and L^0 are the masses of the cationic and neutral helper lipids (thus, $L = L^+ + L^0$, is the total mass of lipid); M_{L^+} and M_{L^0} are the molar masses of cationic and helper lipids; D is the DNA mass; n^+ and n^- are the number of moles of positive and negative charges, coming from CL and DNA; $q^+_{L^+}$ and q^-_{DNA} are the charges of the CL and DNA base pairs; and $\bar{M}_{bp}$ is the average molar mass per DNA base pair. In particular, it is important to know the electroneutrality ratio ($(L/D)_\phi$) where the positive and negative charges balance ($\rho = 1$), because it marks the lower limit from which the net charge of the lipoplex is positive, thus becoming a potentially adequate cell transfecting agent.[6,10] This relation must be determined experimentally but can be also estimated (see the Supporting Information (SI)) by combining eqs 1 and 2 with the definition of L/D ratio, as given by

$$(L/D)_\phi = q^-_{DNA}[\alpha M_{L^+} + (1-\alpha)M_{L^0}]/(q^+_{L^+}\alpha\bar{M}_{bp}) \quad (3)$$

To analyze the effect of both DNA conformation and the type of CL on CL-DNA interaction and on the extent of the counterion release, we have employed in this work several lipoplexes formed by (i) a gemini CL, such as 1,2-bis(hexadecyl dimethylammonium)ethane ($C_{16}C_2C_{16}$), 1,2-dioleoyl-*sn*-glycero-3-ethylphosphocholine (DOEPC), or 3β-[*N*-(*N*′,*N*′-dimethylaminoethane)carbamoyl]cholesterol (DC-Chol); (ii) the helper lipid 1,2-dioleoyl-*sn*-glycero-3-phosphatidylethanolamine (DOPE); and (iii) either of the two types of DNA, pEGFP-C3 pDNA or linear ctDNA. It must be mentioned that ctDNA is commercial, whereas the pDNA has been amplified and purified by us. The study has been performed using ζ potential, GelRed fluorescence intercalation assay, small-angle X-ray scattering (SAXS), gel electrophoresis, gene transfection, confocal microscopy, and cell viability (cytotoxicity) techniques (see SI).

Table 1. Representative $(L/D)_\phi$ Values Obtained from ζ Potential and GelRed Experiments, and Estimated from Eq 3

	$(L/D)_{\phi,exp}$		
α	pDNA	ctDNA	$(L/D)_{\phi,est}$
	$C_{16}C_2C_{16}$/DOPE		
0.15	1.6	7.1	7.6
0.50	0.5; 0.5[a]	2.2	2.3
0.80	0.4		1.4
	DOEPC/DOPE		
0.25	1.3	9.2; 9.0[a]	9.5
0.50	0.5	5.1; 5.6[a]	4.9
0.75	0.4	4.0; 4.2[a]	3.5
	DC-Chol/DOPE		
0.25	0.8	8.3	8.5
0.50	0.8	4.2; 4.4[a]	3.9
0.75	1.0	3.4	2.4

[a] GelRed experiments.

Three methods have been used to determine the electroneutrality ratio: (i) ζ potential, since it shows a sign inversion at this particular L/D value;[10,24] (ii) GelRed fluorescence intercalation assay, since the maximum fluorescence intensity of the probe decreases as soon as it is displaced from the hydrophobic interior of DNA double-helix to the bulk when the lipoplex is formed, reaching a constant value at $(L/D)_\phi$; and (iii) estimated using eq 3, if preassumed values of $q^+_{L^+}$ and q^-_{DNA} are used.

Figure 1 shows the variation of ζ potential against L/D for the lipoplexes formed by $C_{16}C_2C_{16}$/DOPE at α = 0.5 with pDNA or ctDNA (for other lipoplexes and CL compositions, see SI Figures S-1 to S-6). Two sigmoidal curves are observed, with an inversion of sign taking place at the electroneutrality ratio (ρ = 1) for a certain $(L/D)_\phi$ value. Table 1 reports $(L/D)_\phi$ determined from the ζ potential measurements and from the GelRed fluorescence assays (see SI Figures S-7 and S-8) for the investigated systems at several CL compositions together with $(L/D)_\phi$ values estimated using eq 3 assuming total release of the counterions when lipoplexes are formed, i.e., $q^+_{L^+}$ = 2 for $C_{16}C_2C_{16}$, $q^+_{L^+}$ = 1 for DOEPC or DC-Chol, and q^-_{DNA} = −2. For each CL/DOPE-ctDNA lipoplex, an excellent agreement is observed between $(L/D)_\phi$ values obtained from the ζ potential studies or GelRed fluorescence assays and the ones estimated using eq 3. This means that, in all the cases, CL/DOPE is able to release all the Na^+ counterions from ctDNA when lipoplexes are formed. However, in the case of lipoplexes containing pDNA, $(L/D)_\phi$ values experimentally determined using both ζ potential measurements and GelRed fluorescence are considerably lower than those estimated from eq 3. This indicates that a significant percentage of counterions remains bound with the pDNA when the lipoplex is formed. Thus the effective pDNA charge becomes less negative than 2. It is plausible that as a result of a more compact conformation, it may be more difficult for the CLs, due to geometric constraints, to fully match with the charge of the pDNA and displace all the bound counterions of pDNA. Therefore more counterions are still present in the vicinity of the pDNA even after the CL binding. In any case, although a significant percentage of counterions remains associated with the phosphate groups, those that are expelled to the bulk drive the lipoplex formation contributing to a clear entropy gain.[12] Rearranging eq 3

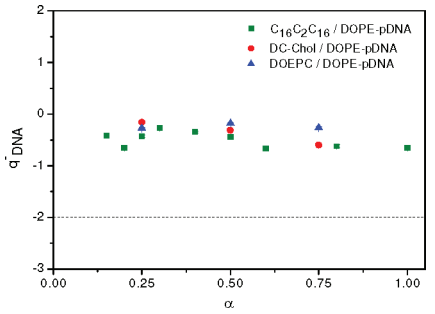


Figure 2. Effective pDNA charge for $C_{16}C_2C_{16}$/DOPE, DC-Chol/DOPE and DOEPC/DOPE mixed lipids against CL composition (α). Dashed line corresponds to a preassumed value of $q^-_{pDNA} = -2$.

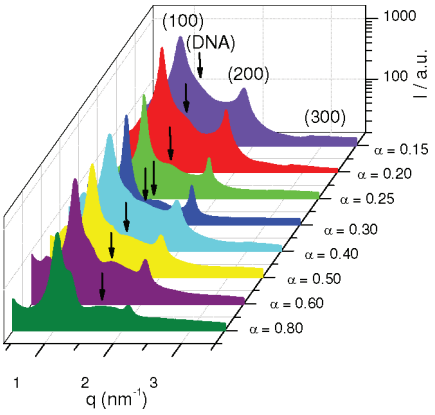


Figure 3. SAXS diffractograms of $C_{16}C_2C_{16}$/DOPE-pDNA lipoplexes at several CL compositions (α) and an effective charge ratio $\rho_{eff} = 2$.

and using experimentally determined $(L/D)_\phi$, the effective pDNA net charge (q^-_{pDNA}) in the lipoplexes may be obtained from

$$q^-_{pDNA} = (L/D)_\phi \left(q^+_{L^+}\alpha \bar{M}_{bp}/[\alpha M_{L^+} + (1-\alpha)M_{L^0}]\right) \quad (4)$$

Results of q^-_{pDNA} for CL/DOPE-pDNA lipoplexes, when plotted against α in Figure 2, clearly show that pDNA is less negatively charged than expected, being $q^-_{pDNA} = (-0.4 \pm 0.1)$ per base pair on average, irrespective of the CL composition. This feature implies that a smaller amount of CL is needed to form electrically neutral complexes using pDNA because pDNA is less negatively charged than the linear one, and hence less CL is necessary to obtain a positive lipoplex with pDNA. It is remarkable that this is the first experimental determination of the effective charge on pDNA. More importantly, it opens up the correct way of preparing lipoplexes with the effective charge ratio (ρ_{eff}), which unifies both the physicochemical and biological transfection studies. Since q^-_{pDNA} is around −0.4 on average, ρ_{eff} is higher than ρ estimated (ρ_{est}) with $q^-_{pDNA} = -2$. These results have been further confirmed by SAXS of $C_{16}C_2C_{16}$/DOPE-pDNA lipoplex at different CL compositions and $\rho_{eff} = 2$, since the structure of the lipoplexes is of crucial importance and this is currently of core interest to groups investigating DNA complexation by simulation/theoretical analysis.[25,26] Note that the peaks on diffractograms shown in Figure 3 for the whole range of composition index well with a lamellar structure, L_α. Values of periodic distance (not shown) of the lamellar structure (d) versus α, at $\rho_{eff} = 2$, decrease slightly (7 to 6 nm). This may be attributed to (i) a thinner bilayer (d_m) as α increases, since the length of

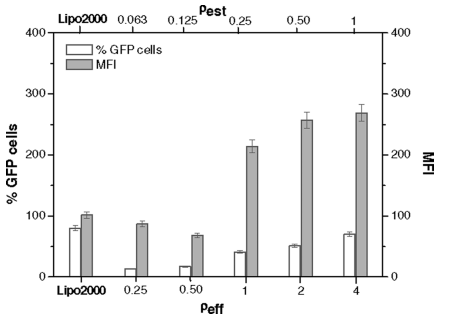


Figure 4. Transfection (% GFP cells and MFI) of pEGFP-C3 pDNA using $C_{16}C_2C_{16}$/DOPE in HEK293T cells at $\alpha = 0.5$ against ρ_{eff} (bottom) and ρ_{est} (up). Also shown is the transfection by Lipofectamine2000, as a control.

$C_{16}C_2C_{16}$ gemini CL is shorter than that of DOPE and/or (ii) a higher compaction of pDNA, and consequently a decrease in the thickness where the pDNA is sandwiched (d_w) as α increases (see SI Chart S-1). In any case, if a value of $d_m \approx 4-5$ nm is assumed for the L_α structure, the thickness obtained for the pDNA monolayer ($d_w \approx 2$ nm) is consistent with a highly compacted pDNA conformation compared with linear ctDNA ($d_w \approx 2.5$ nm).[1,9,27] Values of d_{pDNA} (not shown), plotted against α, at $\rho_{eff} = 2$, decrease slightly (from 5 to 4 nm). This is expected since, at constant ρ_{eff}, d_{pDNA} should decrease as α in the lipoplex increases, as previously found for most of the lipoplexes reported in literature.[24,28−30] A comparison between the structures formed by both lipoplexes, CL/DOPE-pDNA and CL/DOPE-ctDNA, indicates that (i) they have similar values (within experimental uncertainty) for the lamellar spacing (d) and also for the DNA peak position (d_{pDNA} and d_{ctDNA}) and (ii) the parameters (d, and d_{pDNA} or d_{ctDNA}) decrease slightly versus α in a similar trend.

To confirm the viability of lipoplexes with such a low CL quantity ($\rho_{eff} \geq 1$ using $q^-_{pDNA} = -0.4$, instead of the $\rho_{est} \ll 1$ assuming $q^-_{pDNA} = -2$, as erroneously done in literature), transfection and cytotoxicity assays were also performed under the same conditions using HEK293T cells. Figure 4 shows the transfection efficiency (% green fluorescence protein (GFP) cells, and mean fluorescence intensity (MFI)) for the $C_{16}C_2C_{16}$/DOPE-pDNA lipoplex at $\alpha = 0.5$, covering the range $0.25 < \rho_{eff} < 4$ (with $q^-_{pDNA} = -0.4$) or $0.0625 < \rho_{est} < 1$ (if q^-_{pDNA} was −2). See SI Figure S-9 for results obtained for transfection with other cell lines. Notice that $C_{16}C_2C_{16}$/DOPE is a better transfecting agent than Lipofectamine2000 in the case of HEK293T, H460, and CHO. As pointed out previously, in order for transfection to occur, the lipoplex has to be positively charged, i.e., at $\rho_{eff} \geq 1$, which is exactly what is observed in Figure 4 only if $q^-_{pDNA} = -0.4$, and not with $q^-_{pDNA} = -2$, which would yield a $\rho_{est} \geq 0.25$.

The findings shown in Figure 4 are corroborated further by the pDNA internalization seen using confocal fluorescence microscopy. For the $C_{16}C_2C_{16}$/DOPE-pDNA lipoplex at $\alpha = 0.5$, and $\rho_{eff} = 2$, there is even more effective GFP expression, in the presence of serum,[31] as is evident in Figure 5.

Cytotoxicity of CL-pDNA lipoplexes at several CL compositions and at two charge ratios, $\rho_{eff} = 2$ and 4, with $q^-_{pDNA} = -0.4$, that are equivalent to $\rho_{est} = 0.5$ and 1, with $q^-_{pDNA} = -2$, are shown in SI Figure S-10. Note that the cell viability is close to 100%, pointing to a negligible cytotoxicity due to the low amount of CL used as vector to achieve high transfection efficiency.

Electrophoresis on 1% agarose gel SI indicates that, at $\rho_{eff} = 2$, irrespective of the molar fraction of CL in the bilayer (α), the pDNA is fully protected by CL after the lipoplex formation (see

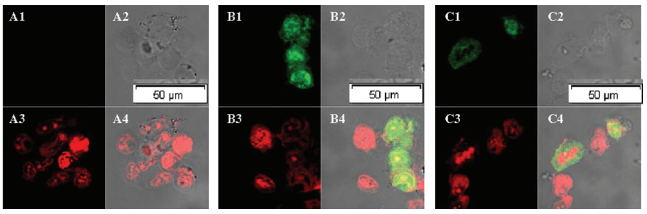


Figure 5. Confocal fluorescence microscopy of HEK293T cells using $C_{16}C_2C_{16}$/DOPE−pDNA at $\alpha = 0.5$ and $\rho_{eff} = 2$. (A1−A4) Negative control (cells only); cells incubated with the same lipoplex at $\alpha = 0.5$ and $\rho_{eff} = 2$ with (B1−B4) and without (C1−C4) serum. A1, B1, and C1 show the GFP in the cell, and A2, B2, and C2 show the phase contrast of the cells. A3, B3, and C3 show propidium iodide stain, and A4, B4, and C4 are overlaps of panels 1 and 3 in each case. Red, propidium iodide; green, GFP.

SI Figures S-11 and S-12). Thus, complexes with different membrane charge density and charge ratio but with similar lipid surface area should exhibit comparable DNA binding capacity. This could be due to a size-dependent effect owing to the reduced surface area occupied by the supercoiled DNA with respect to the linear one. As ζ potential measurements show that a much less effective quantity of CLs is required to neutralize the pDNA charges, it may be reasonable to assume that part of the Na^+ counterions remain bound to pDNA. The data reported herein are in agreement with the reported role of surface area of CL membranes in controlling the 2D DNA condensation.[28,32] Thus the spatial dimension available plays a key role in the DNA binding ability of the lipoplexes. A complete DNA protection is absolutely necessary for efficient transfection. However, the use of an excess amount of CL is harmful in terms of toxicity to the cells. Thus our results seem to offer a practical strategy for transfection studies, i.e., employing lipoplex formulations that guarantee full DNA protection with the minimum amount of CL.

To our knowledge, this is the first report that experimentally demonstrates why plasmid DNA, with a lower effective negative charge, is much more efficiently transfected than linear DNA using cationic lipids as vectors in gene therapy. It has been shown that, for different types of CLs irrespective of their differences in molecular architectures, pDNA is compacted, retaining a significant number of counterions in its vicinity. This in turn drives to a lower effective negative charge, and therefore a lower amount of CL is needed. This finding is significant for both *in vitro* and *in vivo* transfection studies, because for an effective DNA transfection, the lower the amount of the CL, the lower is the cytotoxicity. The study also points out why it is necessary to work with both effective charge ratios and effective pDNA charges which can be determined from the physicochemical experiments, as reported herein. An extended study including more CLs in the whole composition and different ρ_{eff} is currently in progress.

■ ASSOCIATED CONTENT

Supporting Information. Materials, methods, and additional results. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

sb@orgchem.iisc.ernet.in; aicart@quim.ucm.es

Author Contributions

⊥These authors contributed equally to this work.

■ ACKNOWLEDGMENT

The authors thank MICINN of Spain (CTQ2009-10002BQU and ACI2009-0867), DST, Govt. of India for JC Bose fellowship to S.B. (DST/INT/SPAIN/P-8/2009), UCM (A05-33-010 and GR58-08), a BM16-CRG/Consorci Laboratori Llum Sincrotró at ESRF, Grenoble, France, F. Fauth for assistance with SAXS studies, and S. Datta and I. Rodriguez-Crespo for synthesis of gemini CL and for pDNA purification, respectively.

■ REFERENCES

(1) Rädler, J. O.; Koltover, I.; Salditt, T.; Safinya, C. R. *Science* **1997**, *275*, 810.
(2) Verma, I. M.; Weitzman, M. D. *Annu. Rev. Biochem.* **2005**, *74*, 711.
(3) Koynova, R.; MacDonald, R. C. *Biochim. Biophys. Acta* **2007**, 1768.
(4) Montier, T.; Benvegnu, T.; Jaffres, P. A.; Yaouanc, J. J.; Lehn, P. *Curr. Gene Ther.* **2008**, *8*, 296.
(5) Wasungu, L.; Hoekstra, D. *J. Controlled Release* **2006**, *116*, 255.
(6) Dias, R. S.; Lindman, B. *DNA Interaction with Polymers and Surfactants*; Wiley & Sons: Hoboken, 2008.
(7) Bhattacharya, S.; Bajaj, A. *Chem. Commun.* **2009**, 4632.
(8) Shirazi, R. S.; Ewert, K. K.; Leal, C.; Majzoub, R. N.; Bouxsein, N. F.; Safinya, C. R. *Biochim. Biophys. Acta* **2011**, 1808.
(9) Caracciolo, G.; Marchini, C.; Pozzi, D.; Caminiti, R.; Amenitsch, H.; Montani, M.; Amici, A. *Langmuir* **2007**, *23*, 4498.
(10) Rodriguez-Pulido, A.; Martin-Molina, A.; Rodriguez-Beas, C.; Llorca, O.; Aicart, E.; Junquera, E. *J. Phys. Chem. B* **2009**, *113*, 15648.
(11) Bhattacharya, S.; Mandal, S. S. *Biochemistry* **1998**, *37*, 7764.
(12) Ewert, K.; Slack, N. L.; Ahmad, A.; Evans, H. M.; Lin, A. J.; Samuel, C. E.; Safinya, C. R. *Curr. Med. Chem.* **2004**, *11*, 133.
(13) Tiera, M. J.; Shi, Q.; Winnik, F. M.; Fernandes, J. C. *Curr. Gene Ther.* **2011**, *11*, 288.
(14) Eisele, K.; Gropeanu, R. A.; Zehendner, C. M.; Rouhanipour, A.; Ramanathan, A.; Mihov, G.; Koynov, K.; Kuhlmann, C. R. W.; Vasudevan, S. G.; Luhmann, H. J.; Weil, T. *Biomaterials* **2010**, *31*, 8789.
(15) Mustapa, M. F. M.; Grosse, S. M.; Kudsiova, L.; Elbs, M.; Raiber, E. A.; Wong, J. B.; Brain, A. P. R.; Armer, H. E. J.; Warley, A.; Keppler, M.; Ng, T.; Lawrence, M. J.; Hart, S. L.; Hailes, H. C.; Tabor, A. B. *Bioconjugate Chem.* **2009**, *20*, 51.
(16) Chen, J.; Tian, B.; Yin, X.; Zhang, Y. P.; Hu, D.; Hu, Z.; Liu, M.; Pan, Y.; Zhao, J.; Li, H.; Hou, C.; Wang, J.; Zhang, Y. P. *J. Biotechnol.* **2007**, *130*, 107.
(17) Mahato, R. I.; Kim, S. W. *Pharmaceutical Perspectives of Nucleic Acid-Base Therapeutics*; Taylor & Francis: London, 2002.
(18) Lonez, C.; Vandenbranden, M.; Ruysschaert, J. M. *Prog. Lipid Res.* **2008**, *47*, 340.
(19) Ma, B. C.; Zhang, S. B.; Jiang, H. M.; Zhao, B. D.; Lv, H. T. *J. Controlled Release* **2007**, *123*, 184.
(20) Manning, G. S. *J. Chem. Phys.* **1969**, *51*, 924.
(21) Cherng, J. Y.; Schuurmans-Nieuwenbroek, N. M. E.; Jiskoot, W.; Talsma, H.; Zuidam, N. J.; Hennink, W. E.; Crommelin, D. J. A. *J. Controlled Release* **1999**, *60*, 343.
(22) von Groll, A.; Levin, Y.; Barbosa, M. C.; Ravazzolo, A. P. *Biotechnol. Prog.* **2006**, *22*, 1220.
(23) Chancham, P.; Hughes, J. A. *J. Liposome Res.* **2001**, *11*, 139.
(24) Muñoz-Ubeda, M.; Rodriguez-Pulido, A.; Nogales, A.; Llorca, O.; Quesada-Perez, M.; Martin-Molina, A.; Aicart, E.; Junquera, E. *Soft Matter* **2011**, 5991.
(25) Farago, O.; Gronbech-Jensen, N. *J. Am. Chem. Soc.* **2009**, *131*, 2875.
(26) Farago, O.; Gronbech-Jensen, N. *Soft Matter* **2011**, *7*, 4302.
(27) Muñoz-Ubeda, M.; Rodriguez-Pulido, A.; Nogales, A.; Martin-Molina, A.; Aicart, E.; Junquera, E. *Biomacromolecules* **2010**, *11*, 3332.
(28) Caracciolo, G.; Pozzi, D.; Amici, A.; Amenitsch, H. *J. Phys. Chem. B* **2010**, *114*, 2028.
(29) Farago, O.; Ewert, K.; Ahmad, A.; Evans, H. M.; Gronbech-Jensen, N.; Safinya, C. R. *Biophys. J.* **2008**, *95*, 836.
(30) Zidovska, A.; Evans, H. M.; Ewert, K. K.; Quispe, J.; Carragher, B.; Potter, C. S.; Safinya, C. R. *J. Phys. Chem. B* **2009**, *113*, 3694.
(31) Bajaj, A.; Kondiah, P.; Bhattacharya, S. *J. Med. Chem.* **2007**, *50*, 2432.
(32) Marchini, C.; Montani, M.; Amici, A.; Pozzi, D.; Caminiti, R.; Caracciolo, G. *Appl. Phys. Lett.* **2009**, *94*, 033903.