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Stimuli Responsive Charge-Switchable Lipids: Capture and Release of Nucleic Acids

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Abstract

Stimuli responsive lipids, which enable control over the formation, transformation, and disruption of supramolecular assemblies, are of interest for biosensing, diagnostics, drug delivery, and basic transmembrane protein studies. In particular, spatiotemporal control over a supramolecular structure can be achieved using light activated compounds to induce significant supramolecular rearrangements. As such, a family of cationic lipids are described which undergo a permanent switch in charge upon exposure to 365 nm ultraviolet (UV) light to enable the capture of negatively charged nucleic acids within the self-assembled supramolecular structure of the lipids and subsequent release of these macromolecules upon exposure to UV light and disruption of the assemblies. The lipids are composed of either two different tripeptide head groups, Lysine-Glycine-Glycine (KGG) and Glycine-Glycine-Glycine (GGG) and three different hydrocarbon chain lengths (C₆, C₁₀, or C₁₄) terminated by a UV light responsive 1-(2-nitrophenyl)ethanol (NPE) protected carboxylic acid. The photolysis of the NPE protected lipid is measured as a function of time, and the resulting changes in net molecular charge are observed using zeta potential analysis for each head group and chain length combination. A proof of concept study for the capture and release of both linear DNA (calf thymus) and siRNA is presented using an ethidium bromide quenching assay where a balance between binding affinity and supramolecular stability are found to be the key to optimal nucleic acid capture and release.

Graphical Abstract

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Keywords

Lipid; ultraviolet light; stimuli responsive; cationic

1. INTRODUCTION

Lipids self-assemble into a wide range of supramolecular structures including uni-lamellar and multi-lamellar micelles, vesicles, and membranes.^{1,2} In this role, lipids serve as the interface or boundary between aqueous environments, a means to compartmentalize different biologics, as well as a relatively hydrophobic medium for anchoring of surface proteins. The specific supramolecular assemblies formed are dictated by the size and charge of the hydrophilic head group(s), as well as the length of the hydrophobic chain of the lipid.^{3,5} Examples of lipid compositions synthesized include those bearing phosphate, saccharide, nucleoside, quaternary amine, or polypeptide head groups and varying chain configurations (e.g., unsaturated vs. saturated or branched vs. linear).^{6,9}

Stimuli-responsive lipids represent a unique class of functional lipids. These lipids or amphiphiles are employed to induce transitions between supramolecular states or dissolution of the assembly via the application of an external or internal stimulus. For example, temperature,^{10,16} pH,^{17,22} enzymatic,¹⁵ chemical,^{23,30} mechanical (ultrasound),^{31,32} and light^{33,35} responsive systems are described. From an application perspective, these stimuli responsive amphiphiles are used as triggers of supramolecular assembly into biosensing membranes^{36,38}, tunable hydrogels^{39,42}, carriers for drug delivery,^{43,46} and as membranes for the study of transmembrane proteins^{7,47}. For example, Liu et al. developed a phospholipase D responsive biosensor by creating a phospholipid-graphene nanoassembly that mimicked a cellular membrane and provided enhanced fluorescence due to the improved efficiency of fluorescence energy transfer between fluorophores and the nonoxidized graphene. By incorporating a fluorescently labeled phospholipid into this nanoassembly, the activity of phospholipase D was quantified over a wide dynamic range and down to a very low detection limit (0.01 U/L) in real time as the enzyme cleaved the fluorescent phospholipids from the nanoassembled construct.

Among the many possible stimuli, light-activated systems offer a high degree of spatiotemporal control. In fact, photo-caging of small molecules has facilitated the study of enzymes and metabolites. In this strategy, a light activated protecting group is conjugated to the biomolecule effectively “caging” it from an active state allowing for the controlled addition of these molecules to a biologic system upon exposure to the appropriate

wavelength of light.^{48,49} The first use of a photo-caging system was reported by Kaplan et al. to study the activity of a Na, K-ATPase in red blood cells by caging ATP into an inactive form using a 1-(2-nitro)phenylethyl phosphate and providing active ATP under light activated control (340 nm).⁴⁸ While these caging events occur on the individual molecule level, strategies employing similar chemistries to alter or disrupt a supramolecular assembly can be used to release a large number of biomolecules simultaneously or induce a change in conformation within a biosensing membrane.^{33,35, 47} As such, there is a need for the development of stimuli responsive amphiphiles which respond to a specific external stimuli.

Herein, we describe the synthesis of a family of ultraviolet light-activated amphiphiles which capture and release anionic biopolymers such as DNA and siRNA. The approach relies on electrostatic interactions between a cationic amphiphile and the negatively charged biopolymer to capture it, and then release it upon switching the amphiphile charge from cationic to anionic using UV light (Figure 1). Specifically we report: 1) a family of four new lipids with two different cationic tripeptide head groups (KGG or GGG) and three different hydrocarbon chain lengths (C₆, C₁₀, or C₁₄) terminated by a UV light responsive nitrobenzyl protected carboxylic acid; 2) the quantification of the deprotection rate via UV light; and 3) the efficiency of the capture and release of DNA and siRNA.

2. EXPERIMENTAL SECTION

2.1 General Materials and Procedure for Synthesis

All chemicals were purchased from Aldrich. Solvents used during synthesis were dried and distilled prior to use. All reactions were done in dry conditions using nitrogen. NMR spectral analysis was performed using a Varian Mercury spectrometer operating at 300 MHz, unless otherwise noted.

2.2 Synthesis of *O,O'*-(3-{2-[2-(2,6-diaminohexanamido)acetamido]acetoxyl}propane-1,2-diyl) bis[1-(2-nitrophenyl)ethyl] diadipate (KGG-C₆-NPE).6-[1-(2-nitrophenyl)ethoxy]-6-oxohexanoic acid (1a)

Adipic acid (6 g, 41.1 mmol), was dissolved in minimal DMF, DCM was added (1:1 vol), and the solution was cooled to 0 °C. 1-(2-nitrophenyl)ethanol (2.3 g, 13.7 mmol) and cat. DMAP were added. Last, EDCI (3.9 g, 20.5 mmol) was added to the mixture and the reaction was stirred overnight warming up to RT. The solution was taken up into EtOAc (200 mL) and washed with water, 1 N HCl, and sat. NH₄Cl solution. The organic layer was dried over Na₂SO₄. The volatiles were evaporated and the residue was purified on silica gel chromatography (gradient EtOAc:hexanes, 1:2 to 1:1), affording product **2a** as a white thick oil in 66% yield. ¹H NMR (300 MHz, CDCl₃): δ = 1.58-1.72 (m, 7 H), 2.31-2.40 (m, 4 H), 6.32 (q, 1 H, J = 6.6 Hz), 7.41-7.47 (m, 1 H), 7.62-7.68 (m, 2 H), 7.93 (d, 1 H, J = 8.1 Hz). ¹³C NMR (75 MHz, CDCl₃): δ = 21.9, 23.9, 24.1, 33.6, 33.8, 68.0, 124.4, 127.2, 128.4, 133.6, 137.8, 147.7, 172.2, 179.5. HRMS: m/z calcd for C₁₄H₁₇NO₂ [M+Na]⁺: 318.0954; found: 318.0966.

2.2.1, *O,O'*-(3-[bis(4-methoxyphenyl) (phenyl)methoxy]propane-1,2-diyl) bis[1-(2-nitrophenyl)ethyl] diadipate (2a)—3-[Bis(4-methoxyphenyl)(phenyl)methoxy]

propane-1,2-diol (200 mg, 0.51 mmol), compound **1a** (389 mg, 1.32 mmol), and cat. DMAP were dissolved in DCM (10 mL) and the solution was cooled to 0 °C. EDCI (340 mg, 1.77 mmol) was added to the mixture and the reaction was stirred overnight warming up to RT. The solution was washed with sat. NaHCO₃ and sat. NH₄Cl solutions. The organic layer was dried over Na₂SO₄. The volatiles were evaporated and the residue was purified by silica gel chromatography (gradient hexanes:EtOAc, 4:1 to 1:1 with 1% TEA). The product **2a** was obtained as white thick oil in 35% yield. ¹H NMR (300 MHz, CDCl₃): δ = 1.49-1.55 (m, 14 H), 2.15-2.25 (m, 8 H), 3.13 (d, 2 H, J = 3.9 Hz), 3.68 (s, 6 H), 4.09-4.28 (m, 2 H), 5.16-5.18 (m, 1 H), 6.23 (q, 2 H, J = 6.0 Hz), 6.73 (d, 4 H, J = 8.4 Hz), 7.11-7.33 (m, 11 H), 7.47-7.53 (m, 4 H), 7.81 (d, 2 H, J = 7.8 Hz). ¹³C NMR (75 MHz, CDCl₃): δ = 21.9, 24.1, 24.2, 33.5, 33.8, 55.2, 61.9, 63.0, 68.0, 70.6, 86.1, 113.1, 124.4, 126.9, 127.2, 127.8, 128.0, 128.4, 130.0, 133.6, 135.6, 137.9, 144.5, 147.7, 158.5, 172.0, 172.5, 172.8. HRMS: m/z calcd for C₅₂H₅₆N₂O₁₅ [M+Na]⁺: 971.3578; found: 971.3577.

2.2.3 O,O'-(3-hydroxypropane-1,2-diyl) bis[1-(2-nitrophenyl)ethyl] diadipate (**3a**)

—Compound **2a** (100 mg, 0.11 mmol) was dissolved in DCM (3.5 mL) and the solution was cooled to 0 °C. To the solution was added TFA (175 μL), and the reaction was left to stir at 0 °C for 15 min. Cold sat. NaHCO₃ (3.5 mL) was added to the solution and the layers were separated. The organic layer was dried over Na₂SO₄. The volatiles were evaporated without heat and the residue was purified by silica gel chromatography (gradient hexanes:EtOAc, 2:1 to 0:1). The volatiles were evaporated without heat and the product **3a** was obtained as white oil in 72% yield. ¹H NMR (300 MHz, CDCl₃): δ = 1.49-1.70 (m, 14 H), 2.15-2.45 (m, 8 H), 3.71 (s, 2 H), 4.09-4.23 (m, 2 H), 4.28-4.33 (m, 1 H), 5.06-5.08 (m, 1 H), 6.32 (q, 2 H, J = 6.3 Hz), 7.41-7.47 (m, 2 H), 7.63-7.65 (m, 4 H), 7.93 (d, 2 H, J = 8.1 Hz). ¹³C NMR (75 MHz, CDCl₃): δ = 21.9, 24.0, 24.1, 24.2, 33.6, 33.7, 33.8, 61.3, 62.1, 68.0, 68.1, 72.2, 124.4, 128.4, 133.6, 137.7, 137.9, 147.7, 172.1, 172.3, 172.8, 173.1. HRMS: m/z calcd for C₃₁H₃₈N₂O₁₃ [M+Na]⁺: 669.2272; found: 669.2296.

2.2.4 O,O'-{10-[(*tert*-butoxycarbonyl)amino]-2,2-dimethyl-4,11,14,17-tetraoxo-3,18-dioxo-5,12,15-triazahenicosane-20,21-diyl} bis[1-(2-nitrophenyl)ethyl] diadipate (**4a**)

—Boc-Lys(Boc)-Gly-Gly-OH tripeptide (purchased from GenScript; 34.9 mg, 0.076 mmol) and compound **3a** (49 mg, 0.076 mmol) were dissolved in ice cold DCM (5 mL). EDCI (14.5 mg, 0.076 mmol) and a catalytic amount of DMAP was added to the solution. The reaction was allowed to stir for 18 hours while warming up to RT. The solution was taken up in EtOAc, and washed with sat. NaHCO₃ and sat. NH₄Cl solutions. The organic layer was dried over Na₂SO₄. The volatiles were evaporated and the residue was purified by silica gel chromatography (gradient EtOAc:hexanes 2:1 to 1:0). The volatiles were evaporated and the product **4a** was obtained as a yellow oil in 40% yield. ¹H NMR (300 MHz, CDCl₃): δ = 1.22-1.27 (m, 4), 1.31 - 1.49 (m, 18 H), 1.54 - 1.68 (m, 16 H), 2.26 - 2.36 (m, 8 H), 3.02-3.16 (m, 2 H), 3.86 - 4.20 (m, 9 H), 4.24-4.33 (m, 2 H), 4.75 (br s, 1 H), 5.20 - 5.23 (m, 1 H), 5.49 (br s., 1 H), 6.29 (q, 2 H, J = 6.6 Hz), 7.02 (br s, 1 H), 7.30 (br s, 1 H), 7.43 (dd, 2 H, J¹ = 8.4, J² = 4.0 Hz), 7.59-7.65 (m, 4 H), 7.91 (d, 2 H, J = 7.95 Hz). ¹³C NMR (75 MHz, CDCl₃): δ = 21.1, 21.9, 24.0, 24.1, 28.3, 28.4, 33.5, 33.8, 39.5, 41.1, 42.8, 55.4, 60.4, 62.0, 62.8, 64.4, 68.0, 68.7, 79.2, 80.4,

124.4, 127.2, 128.4, 133.6, 137.8, 139.0, 147.7, 156.4, 169.1, 172.2, 172.4, 172.9. HRMS: m/z calcd for $C_{51}H_{72}N_6O_{20}$ $[M+H]^+$: 1089.48; found: 1089.4973.

2.2.5 *O,O'*-(3-{2-[2-(2,6-diaminohexanamido) acetamido]acetox}propane-1,2-diyl) bis[1-(2-nitrophenyl)ethyl] diadipate TFA salt (**5a**; KGG-C₆-NPE)—

Compound **4a** (164 mg, 0.15 mmol) was dissolved in 1:1 TFA:DCM solution (5 mL), and the reaction was stirred at RT for 1 hour. The volatiles were evaporated under high vacuum, affording **5a** as a yellow oil in quantitative yield, without the need for further purification. 1H NMR (300 MHz, $CDCl_3$): δ = 1.22 - 1.34 (m, 2 H), 1.48 - 1.69 (m, 16 H), 1.97-2.00 (m, 2 H), 2.24-2.40 (m, 10 H), 2.80-3.22 (m, 2 H), 3.78 - 4.26 (m, 9 H), 5.20 (br s, 1 H), 6.21-6.34 (m, 2 H), 7.39 - 7.49 (m, 2H), 7.58-7.69 (m, 4 H), 7.84 - 7.94 (m, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 21.7, 23.9, 29.7, 31.6, 33.5, 33.7, 40.3, 68.1, 84.1, 87.3, 95.1, 124.3, 127.2, 128.6, 133.8, 137.6, 147.7, 153.9, 166.9, 172.5, 192.0. HRMS: m/z calcd for $C_{41}H_{56}N_6O_{16}$ $[M+Na]^+$: 911.3650; found: 911.3669. UV/Vis (CH_3OH): λ_{max} (ϵ) = 204 (21000), 255 nm (6000).

2.3 Synthesis of 1',1-3-{2-[2-(2,6-Diaminohexanamido) acetamido] acetox}propane-1,2-diyl 10-bis[1-(2-nitrophenyl)ethyl] bis(decanedioate) hydrochloric acid salt (KGG-C₁₀-NPE)

2.3.1 10-[1-(2-Nitrophenyl)ethoxy]-10-oxodecanoic acid (1b**)**—Decanedioic acid (1 g, 4.9 mmol), cat. DMAP, and 1-(2-nitrophenyl)ethanol (613 mg, 3.7 mmol) were dissolved in DMF (10 mL), and the solution was cooled to 0 °C. To the mixture was added EDCI (1.2 g, 6.4 mmol) and the reaction was stirred overnight warming up to RT. The solution was taken up into EtOAc (150 mL) and washed with water, 1N HCl, and sat. NH_4Cl solution. The organic layer was dried over Na_2SO_4 . The volatiles were evaporated and the residue was purified on silica gel chromatography (gradient EtOAc:hexanes, 1:3 to 1:2), affording product **1b** as a thick oil in 62% yield. 1H NMR (300 MHz, $CDCl_3$): δ = 1.22-1.29 (m, 8 H), 1.55-1.63 (m, 7 H), 2.30 (dt, 4 H, $J^1 = 5.7$ Hz, $J^2 = 1.8$ Hz), 6.31 (q, 1 H, $J = 6.6$ Hz), 7.38-7.44 (m, 2 H), 7.60 (d, 1 H, $J = 5.1$ Hz), 7.91 (d, 1 H, $J = 8.1$ Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 22.2, 24.8, 25.0, 29.1, 29.2, 34.2, 34.5, 68.1, 124.6, 127.4, 128.6, 133.7, 138.3, 147.9, 172.9, 180.3. HRMS: m/z calcd for $C_{18}H_{25}NO_6$ $[M+H]^+$: 352.1760; found: 352.1749.

2.3.2 1',1-3-[Bis(4-methoxyphenyl)(phenyl) methoxy]propane-1,2-diyl 10-bis[1-(2-nitrophenyl)ethyl] bis(decanedioate) (**2b**)

3-[Bis(4-methoxyphenyl)(phenyl) methoxy] propane-1,2-diol (500 mg, 1.27 mmol), compound **1b** (1.3 g, 3.8 mmol), and cat. DMAP were dissolved in DCM (10 mL) and the solution was cooled to 0 °C. To the mixture was added EDCI (730 mg, 3.8 mmol) and the reaction was stirred overnight while warming up to RT. The solution was taken up in EtOAc and washed with water, sat. $NaHCO_3$, and sat. NH_4Cl solutions. The organic layer was dried over Na_2SO_4 . The volatiles were evaporated and the residue was purified by silica gel chromatography (gradient hexanes:EtOAc, 3:1 to 1:1 with 1% TEA). The product **2b** was obtained as white foam in 60% yield. 1H NMR (300 MHz, $CDCl_3$): δ = 1.23-1.29 (m, 16 H), 1.51-1.65 (m, 14 H), 2.26 (t, 2 H, $J = 7.8$ Hz), 2.20-2.35 (m, 6 H), 3.21 (d, 2 H, $J = 4.2$ Hz), 3.78 (s, 6 H), 4.22-4.38 (m, 2 H), 5.22-5.29 (m, 1 H), 6.32 (q, 2 H, $J = 6.6$ Hz), 6.81 (d, 4 H, $J = 7.2$ Hz), 7.22-7.32 (m, 6 H), 7.38-7.45 (m, 5 H), 7.63 (br s, 4 H), 7.92 (d, 2 H, $J = 8.4$ Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 21.0, 24.7, 24.8, 28.9, 29.0, 29.1, 34.0, 34.2, 34.3, 55.1, 60.3, 61.9, 62.9, 67.8,

70.5, 86.0, 113.0, 124.3, 126.8, 127.1, 127.8, 128.0, 128.3, 129.9, 133.4, 125.7, 128.0, 144.5, 147.7, 158.4, 172.5, 172.9, 173.3. HRMS: m/z calcd for $C_{60}H_{72}N_2O_{15}$ $[M+Na]^+$: 1083.4825; found: 1083.4799.

2.3.3 1',1-3-hydroxypropane-1,2-diyl 10-bis[1-(2-nitrophenyl)ethyl]

bis(decanedioate) (3b)—Compound **2b** (500 mg, 0.47 mmol) was dissolved in DCM (5 mL) and the solution was cooled to 0 °C. TFA (250 μ L) was to the solution, and the reaction was left to stir at 0 °C for 15 min. Next, cold sat. $NaHCO_3$ (5 mL) was added to the solution and the layers were separated. The organic layer was dried over Na_2SO_4 . The volatiles were evaporated without heat and the residue was purified by silica gel chromatography (gradient hexanes:EtOAc, 2:1 to 0:1). The volatiles were evaporated without heat and the product **3b** was obtained as white foam in 88% yield. 1H NMR (300 MHz, $CDCl_3$): δ = 1.27 (s, 16 H), 1.58-1.65 (m, 14 H), 2.32 (dt, 8 H, J = 7.5 Hz), 3.37 (d, 2 H, J = 4.8 Hz), 4.27 (dq, 2 H, J^1 = 4.5 Hz, J^2 = 18.0 Hz), 5.08 (dt, 1 H, J = 1.5 Hz), 6.31 (q, 2 H, J = 6.6 Hz), 7.40-7.46 (m, 2 H), 7.63 (d, 4 H, J = 4.5 Hz), 7.93 (d, 2 H, J = 7.8 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 21.9, 24.7, 28.9, 34.0, 34.2, 61.4, 62.0, 67.8, 72.0, 124.4, 127.1, 128.3, 133.5, 138.0, 147.7, 172.6, 173.3, 173.7. HRMS: m/z calcd for $C_{39}H_{54}N_2O_{13}$ $[M+Na]^+$: 781.3524; found: 781.3522.

2.3.4 1',1-10-(*tert*-Butoxycarbonylamino)-2,2-dimethyl-4,11,14,17-tetraoxo-3,18-dioxa-5,12,15-triazahenicosane-20,21-diyl 10-bis[1-(2-nitrophenyl)ethyl]

bis(decanedioate) (4b)—Boc-Lys(Boc)-Gly-Gly-OH tripeptide (purchased from GenScript; 300 mg, 0.65 mmol) and compound **3b** (740 mg, 0.98 mmol) were dissolved in ice cold DCM (5 mL). EDCI (190 mg, 0.98 mmol) was added to the solution as well as a catalytic amount of DMAP. The reaction was allowed to stir overnight warming up to RT. The solution was taken up in EtOAc, and washed with water, sat. $NaHCO_3$, and sat. NH_4Cl solutions. The organic layer was dried over Na_2SO_4 . The volatiles were evaporated and the residue was purified by silica gel chromatography (gradient hexanes:EtOAc, 1:2 to 0:1). The volatiles were evaporated and the product **4b** was obtained as white foam in 40% yield. 1H NMR (400 MHz, $CDCl_3$): δ = 1.28 (s, 16 H), 1.42 (s, 9 H), 1.44-1.54 (m, 13 H), 1.58-1.65 (m, 14 H), 1.80-1.83 (m, 2 H), 2.30-2.34 (m, 8 H), 3.10 (q, 2 H, J = 5.2 Hz), 3.90-4.25 (m, 7 H), 4.30-4.34 (m, 2 H), 4.80-4.83 (m, 1 H), 5.25-5.31 (m, 1 H), 5.56 (br s, 1 H), 6.32 (q, 2 H, J = 6.8 Hz), 7.14 (br s, 1 H), 7.35 (br s, 1 H), 7.41-7.46 (m, 2 H), 7.63-7.65 (m, 4 H), 7.93 (d, 2 H, J = 8.5 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.9, 22.4, 24.7, 28.2, 28.4, 28.9, 29.7, 31.1, 33.9, 34.0, 34.2, 39.5, 41.0, 42.8, 55.3, 61.9, 63.5, 79.1, 80.3, 124.3, 127.1, 128.1, 133.4, 137.9, 147.7, 156.3, 169.1, 169.5, 172.5, 172.8, 173.0, 173.2. HRMS: m/z calcd for $C_{59}H_{88}N_6O_{20}$ $[M+Na]^+$: 1223.5951; found: 1223.5948.

2.3.5 1',1-3-{2-[2-(2,6-Diaminohexanamido) acetamido]acetoxyp}propane-1,2-diyl 10-bis[1-(2-nitrophenyl)ethyl] bis(decanedioate) hydrochloric acid salt (5b); KGG-C₁₀-NPE

—Compound **4b** (300 mg, 0.25 mmol) was dissolved in 4 N HCl in dioxane solution (5 mL), and the reaction was stirred at RT for 12 h. The volatiles were evaporated under high vacuum, affording **5b** as a white solid in quantitative yield, without the need for further purification. 1H NMR (300 MHz, $DMSO-d_6$): δ = 1.16 (s, 16 H), 1.33-1.53 (m, 12 H), 1.53 (s, 3 H), 1.55 (s, 3 H), 1.70-1.75 (m, 2 H), 2.22-2.27 (m, 8 H),

2.73 (t, 2 H, $J = 7.5$ Hz), 3.76-3.88 (m, 5 H), 4.10-4.29 (m, 4 H), 5.14-5.20 (m, 1 H), 6.04 (q, 2 H, $J = 6.6$ Hz), 7.54 (dt, 2 H, $J^1 = 6.9$ Hz, $J^2 = 1.5$ Hz), 7.65-7.76 (m, 4 H), 7.93 (d, 2 H, $J = 8.4$ Hz). ^{13}C NMR (100 MHz, CDCl_3): $\delta = 21.9, 24.7, 25.4, 28.3, 29.0, 29.6, 34.0, 34.2, 64.1, 67.8, 124.4, 127.2, 128.4, 133.6, 137.9, 147.7, 170.2, 172.5, 173.4$. HRMS: m/z calcd for $\text{C}_{49}\text{H}_{72}\text{N}_6\text{O}_{16}$ $[\text{M}+\text{H}]^+$: 1001.5083; found: 1001.5064. UV/Vis (CH_3OH): λ_{max} (ϵ) = 205 (26000), 255 nm (8000).

2.4 Synthesis of 25-(2-nitrophenyl)-11-({10-[1-(2-nitrophenyl)ethoxy]-10-oxodecanoyl}oxy)-2,5,8,14,23-pentaoxo-9,13,24-trioxa-3,6-diazahexacosan-1-aminium (GGG-C₁₀-NPE)

2.4.1 O¹,O¹-(2,2-dimethyl-4,7,10,13-tetraoxo-3,14-dioxa-5,8,11-triazaheptadecane-16,17-diyl) 10-bis[1-(2-nitrophenyl)ethyl] bis(decanedioate) (4c)—Boc-Gly-Gly-Gly-OH tripeptide (purchased from GenScript; 57.2 mg, 0.198 mmol) and compound **3b** 150 mg, 0.198 mmol) were dissolved in ice cold DCM (2 mL). EDCI (37.9 mg, 0.198 mmol) was added to the solution as well as a catalytic amount of DMAP. The reaction was allowed to stir overnight warming up to RT. The solution was washed with sat. NH_4Cl solution. The organic layer was dried over Na_2SO_4 . The volatiles were evaporated and the residue was purified by silica gel chromatography (gradient hexanes:EtOAc, 1:2 to 0:1). The volatiles were evaporated and the product **4c** was obtained as yellow oil in 38% yield. ^1H NMR (300 MHz, CDCl_3): $\delta = 1.28$ (s, 16 H), 1.44 (s, 9 H), 1.57-1.65 (m, 14 H), 2.32 (dt, 8 H, $J = 7.5$ Hz), 3.83 (d, 2 H, $J = 5.4$ Hz), 4.02-4.36 (m, 8 H), 5.26-5.29 (m, 1 H), 5.53-5.56 (m, 1 H), 6.32 (q, 2 H, $J = 6.6$ Hz), 7.19-7.23 (m, 2 H), 7.41-7.47 (m, 2 H), 7.64 (d, 4 H, $J = 4.8$ Hz), 7.93 (d, 2 H, $J = 7.8$ Hz). ^{13}C NMR (75 MHz, CDCl_3): $\delta = 12.7, 13.2, 18.1, 20.0, 21.0, 23.7, 27.3, 27.9, 28.0, 29.6, 32.9, 33.1, 33.2, 40.1, 41.8, 43.4, 59.4, 60.9, 62.2, 63.4, 66.8, 67.6, 79.4, 123.4, 126.2, 127.4, 132.5, 137.0, 146.7, 155.5, 168.2, 168.2, 169.4, 170.2, 171.7, 172.0, 172.3$. HRMS: m/z calcd for $\text{C}_{50}\text{H}_{71}\text{N}_5\text{O}_{18}$ $[\text{M}+\text{H}]^+$: 1030.4872; found: 1030.4869.

2.4.2 25-(2-nitrophenyl)-11-({10-[1-(2-nitrophenyl)ethoxy]-10-oxodecanoyl}oxy)-2,5,8,14,23-pentaoxo-9,13,24-trioxa-3,6-diazahexacosan-1-aminium TFA salt (5c; GGG-C₁₀-NPE)—Compound **4c** (77.3 mg, 0.075 mmol) was dissolved in 1:1 TFA/DCM solution (5 mL), and the reaction was stirred at RT for 1 h. The volatiles were evaporated under high vacuum, affording **5c** as a yellow oil in quantitative yield, without the need for further purification. ^1H NMR (300 MHz, CDCl_3): $\delta = 1.26$ (s, 16 H), 1.57-1.65 (m, 14 H), 2.32 (dt, 8 H, $J = 5.4$ Hz), 3.98-4.31 (m, 10 H), 5.25 (s, 1 H), 6.31 (q, 2 H, $J = 6.3$ Hz), 7.14 (s, 4 H), 7.741-7.46 (m, 2 H), 7.64 (d, 4 H, $J = 4.2$ Hz), 7.92 (d, 2 H, $J = 8.1$ Hz), 8.06 (s, 1 H). ^{13}C NMR (75 MHz, CDCl_3): $\delta = 20.7, 21.9, 24.7, 28.9, 33.9, 34.0, 34.2, 67.9, 124.4, 127.2, 128.0, 128.4, 133.6, 137.8, 147.7, 170.0, 173.1, 173.6, 173.9$. HRMS: m/z calcd for $\text{C}_{45}\text{H}_{64}\text{N}_5\text{O}_{16}$ $[\text{M}+\text{H}]^+$: 930.4348; found: 930.4341. UV/Vis (CH_3OH): λ_{max} (ϵ) = 205 (27000), 255 nm (9000).

2.5 Synthesis of 2-(2-nitrophenyl)-20-({14-[1-(2-nitrophenyl)ethoxy]-14-oxotetradecanoyl}oxy)-4,17,23,26,29-pentaoxo-3,18,22-trioxa-25,28-diazatetratetriacontane-30,34-diaminium (KGG-C₁₄-NPE)

2.5.1 N-(2-{1-[(13-carboxytridecanoyl)oxy]ethyl}phenyl)-N-oxohydroxylammonium (1c)—Tetradecanedioic acid (4.883 g, 18.9 mmol), cat. DMAP,

and 1-(2-nitrophenyl)ethanol (1.055 g, 6.3 mmol) were dissolved in DMF (40 mL), and then added to dry DCM (100 mL). The solution was cooled to 0 °C. EDCI (1.81 g, 9.8 mmol) was added to the mixture and the reaction was stirred for 18 hours and allowed to warm to RT. The solution was taken up into EtOAc (200 mL) and washed with water, 0.1 M HCl, and sat. NH_4Cl solution. The organic layer was dried over Na_2SO_4 . The volatiles were evaporated and the residue was purified on silica gel chromatography (gradient EtOAc:hexanes 1:3 to 1:2), affording product **1c** as a thick oil in 58% yield. ^1H NMR (300 MHz, CDCl_3): δ = 1.15-1.3 (m, 16 H), 1.48-1.62 (m, 7 H), 1.91-1.99 (m, 1 H), 2.18-2.47 (m, 4 H), 6.25 (q, 1 H, 6 Hz), 7.3-7.4 (m, 1 H), 7.55-7.60 (m, 2 H), 7.83 (d, 1 H, J = 9 Hz). ^{13}C NMR (75 MHz, CDCl_3): δ = 20.9, 21.83, 24.73, 29.3, 34.2, 35.1, 60.3, 67.7, 77.2, 124.25, 127.1, 128.26, 133.45, 138.0, 147.7, 169.53, 171.2, 172.6, 179.9. HRMS: m/z calcd for $\text{C}_{22}\text{H}_{34}\text{NO}_6$ $[\text{M}+\text{Na}]^+$: 430.2206; found: 430.2196.

2.5.2 O¹,O¹-{3-[bis(4-methoxyphenyl)(phenyl) methoxy]propane-1,2-diyl} 14-bis[1-(2-nitrophenyl)ethyl] ditetradecanedioate (2c)—3-(bis(4-methoxyphenyl)(phenyl)methoxy)propane-1,2-diol (0.45 g, 1.14 mmol), compound **1c** (1.115 g, 2.73 mmol), and cat. DMAP were dissolved in DCM (10 mL) and the solution was cooled to 0 °C. EDCI (0.765 g, 3.99 mmol) was added to the mixture and the reaction was stirred for 18 hours and allowed to warm to RT. The solution was taken up in EtOAc and washed with sat. NaCO_3 and sat. NH_4Cl solutions. The organic layer was dried over Na_2SO_4 . The volatiles were evaporated and the residue was purified by silica gel chromatography (gradient EtOAc:hexanes, 1:4 to 1:1 with 1% TEA). The product **2c** was obtained as a yellow oil in 77% yield. ^1H NMR (300 MHz, CDCl_3): δ = 1.17-1.37 (m, 32 H), 1.51-1.70 (m, 14 H), 2.2-2.39 (m, 8 H), 3.24 (d, 2 H, J = 4.4 Hz), 3.76 (s, 3 H), 4.05-4.29 (m, 2 H), 4.32-4.41 (m, 1 H), 6.32 (q, 2H, J = 6 Hz), 6.82 (d, 4 H, J = 9 Hz), 7.14-7.46 (m, 9 H), 7.62 (dd, 6 H, J^1 = 10 Hz, J^2 = 10 Hz), 7.90 (d, 2 H, J = 9 Hz). ^{13}C NMR (75 MHz, CDCl_3): δ = 20.9, 21.9, 24.8, 29.4, 34.2, 55.0, 60.3, 62.8, 67.7, 70.4, 77.2, 86.0, 113.0, 124.3, 127.1, 128.3, 130.0, 133.4, 135.6, 138.0, 144.6, 158.5, 170.9, 172.5, 172.9. HRMS: m/z calcd for $\text{C}_{68}\text{H}_{88}\text{N}_2\text{O}_{15}$ $[\text{M}+\text{Na}]^+$: 1195.6082; found: 1195.6127.

2.5.3 O¹,O¹-(3-hydroxypropane-1,2-diyl) 14-bis[1-(2-nitrophenyl)ethyl] ditetradecane-dioate (3c)—Compound **2c** (1.02 g, 0.878 mmol) was dissolved in DCM (5 mL) and the solution was cooled to 0 °C. TFA (250 μL) was added to the solution and the reaction was left to stir at 0 °C for 15 minutes. Cold sat. NaHCO_3 (5 mL) was added to the solution and the layers were separated. The organic layer was dried over Na_2SO_4 . The volatiles were evaporated without heat and the residue was purified by silica gel chromatography (gradient EtOAc:hexanes, 1:2 to 100% EtOAc). The volatiles were evaporated without heat and the product **3c** was obtained as a yellow oil in 64.1% yield. ^1H NMR (300 MHz, CDCl_3): δ = 1.20-1.39 (m, 32 H), 1.50-1.71 (m, 14 H), 2.21-2.42 (m, 8 H), 3.72 (d, 2 H, J = 4.42 Hz) 4.03-4.26 (m, 2 H), 4.30-4.40 (m, 1 H), 6.31 (q, 2 H, J = 6.4 Hz), 7.37-7.49 (m, 2 H), 7.58-7.72 (m, 4 H), 7.92 (d, 2 H, J = 8.2 Hz). ^{13}C NMR (75 MHz, CDCl_3): δ = 20.9, 21.8, 24.7, 29.3, 34.2, 60.3, 61.1, 64.9, 67.7, 72.0, 77.2, 124.3, 127.1, 128.2, 133.4, 138.0, 147.7, 171.0, 172.5, 173.5. HRMS: m/z calcd for $\text{C}_{47}\text{H}_{70}\text{N}_2\text{O}_{13}$ $[\text{M}+\text{Na}]^+$: 893.4776; found: 893.4785.

2.5.4 O¹,O¹- (2,2-dimethyl-4,11,14,17- tetraoxo-10-pivalamido- 3,18-dioxa-5,12,15-triaza- henicosane-20,21-diyl) 14-bis[1-(2-nitrophenyl)ethyl] ditetradecanedioate (4d)—Boc-Lys(Boc)-Gly-Gly-OH tripeptide (purchased from GenScript; 0.259 g, 0.56 mmol) and compound **3c** (0.49 g, 0.56 mmol) were dissolved in ice cold DCM (5 mL). EDCI (0.107 g, 0.56 mmol) and cat. DMAP were added to the mixture. The reaction was allowed to stir for 18 hours as the temperature increased to RT. The solution was taken up in EtOAc, and washed with sat. NH₄Cl solution. The organic layer was dried over Na₂SO₄. The volatiles were evaporated and the residue was purified by silica gel chromatography (gradient EtOAc:hexanes, 2:1 to 100% EtOAc). The volatiles were evaporated and the product **4d** was obtained as a yellow oil in 52% yield. ¹H NMR (300 MHz, CDCl₃): δ = 1.26–1.28 (m, 34 H), 1.37–1.50 (m, 18H), 1.55–1.72 (m, 18 H), 2.32 (t, 8 H, J = 6.9 Hz), 3.03–3.18 (m, 2 H), 3.87–4.47 (m, 10 H), 4.93 (br s, 1 H), 5.28 (br s, 1 H), 5.67 (br s., 1 H), 6.22–6.48 (m, 2 H), 7.24 (br s, 1 H), 7.40–7.55 (m, 2 H), 7.64 (d, 4 H, J = 3.5 Hz), 7.94 (d, 2 H, J = 8.0 Hz). ¹³C NMR (75 MHz, CDCl₃): δ = 20.95, 21.9, 24.7, 25.5, 28.3, 29.0, 29.5, 29.7, 34.0, 34.2, 41.0, 42.8, 60.3, 61.9, 63.0, 67.7, 68.5, 76.7, 77.1, 77.5, 80.2, 124.3, 127.1, 128.3, 133.4, 138.0, 147.7, 169.1, 171.1, 172.6, 172.9, 173.0, 173.3. HRMS: m/z calcd for C₆₇H₁₀₄N₆O₂₀ [M+H]⁺: 1313.7384; found: 1313.7673.

2.5.5 2- (2-nitrophenyl)-20- ({14-[1-(2-nitrophenyl)ethoxy]- 14-oxotetradecanoyl}oxy)-4,17,23,26,29-pentaoxo- 3,18,22-trioxa-25,28-diazatetratriacontane-30,34-diaminium TFA salt (5d; KGG-C₁₄-NPE)—Compound **4d** (0.3 g, 0.269 mmol) was dissolved in 1:1 TFA:DCM solution (5 mL), and the reaction was stirred at RT for 1 hour. The volatiles were evaporated under high vacuum, affording **5d** as a yellow oil in 93% yield without need for further purification. ¹H NMR (300 MHz, CDCl₃): δ = 1.13–1.34 (m, 34 H), 1.48–1.71 (m, 18 H), 2.24–2.48 (m, 8 H), 3.12 (br s, 2 H), 3.91–4.42 (m, 10 H), 5.27 (br s, 1 H), 6.33 (q, 2 H, J = 6.2 Hz), 7.09 (br s., 3 H), 7.39–7.49 (m, 2 H), 7.60–7.73 (m, 4 H), 7.93 (d, 2 H, J = 8.0 Hz), 8.19 (br s, 1 H). ¹³C NMR (75 MHz, CDCl₃): δ = 21.0, 21.7, 24.7, 27.5, 28.9, 29.4, 34.5, 40.0, 50.6, 53.8, 62.0, 62.4, 65.3, 68.8, 76.6, 77.0, 77.4, 112.8, 116.6, 120.4, 124.5, 127.2, 128.6, 133.8, 137.3, 147.5, 159.1, 159.7, 160.2, 160.8, 170.0, 171.0, 175.2, 175.7. HRMS: m/z calcd for C₅₇H₉₀N₆O₁₆²⁺ [M+2H]²⁺: 557.8246; found: 557.8269. UV/Vis (CH₃OH): λ_{max} (ε) = 203 (18000), 255 nm (5000).

Liposome and Lipoplex Preparation: The lipid was dissolved in chloroform (1 mg/mL) and dried under vacuum resulting in a thin film deposit of lipid on the flask wall. This lipid film was hydrated with 1x Dulbecco's Phosphate-Buffered Saline solution without calcium and magnesium (1xDPBS, Mediatech, Manassas, VA) at 2 mg/mL, vortexed, and sonicated at RT for 15 min. Lipoplexes were prepared by first diluting the liposomes to the concentration desired for the intended experiment, then adding the DNA or siRNA, and letting the solution incubate for 15 min at RT.⁵⁰

UV Exposure: A 3.5 mW long-wavelength UV lamp (λ = 365 nm, Spectroline, Westbury, NY) was used for UV exposure of the liposome and lipoplex solutions. All irradiation experiments were performed in quartz cuvettes (Starna, Atascadero, CA) with a 1 cm path length to promote transmittance of the light to the sample. The distance of the cuvette from the light source was 5 cm. The model decaging experiments on lipids were performed in

methanol:water (9:1) solution and aliquotes were taken at specified time intervals and analyzed by HPLC/MS.

High Performance Liquid Chromatography-Mass Spectrometry: An Agilent Single-Quad HPLC/MSD VL was used to collect low resolution mass data, while high resolution data were obtained at the Boston University Chemical Instrumentation Center using a Waters Q-ToF. Mass spectrometry analysis was used to characterize synthesized lipids, as well as characterize the cleavage kinetics of the photo-active end group upon UV exposure. During these UV exposure studies, the lipid was dissolved (0.1 mM) in a 9:1 methanol/water solution. The mass was measured for each sample after different durations of exposure to the UV light source (0, 1, 3, 5, 10, 15, and 25 min) and all samples were run in triplicate.

Zeta Potential: The zeta potential of the liposome and lipoplexes was prepared as discussed above with the liposome stock diluted to 73 μM in 1.5 mL deionized water. Measurements were made using the Brookhaven Instruments ZetaPALS Zeta Potential Analyzer (Brookhaven Instruments). Each sample was subjected to 5 runs each consisting of 20 cycles (3 V, 2 Hz). Samples were run in triplicate.

Ethidium Bromide Fluorescence Assay: Using calf thymus DNA or non-sense siRNA, and EtBr purchased from Sigma-Aldrich, EtBr binding and release fluorescence studies were carried out using a fluorimeter and software (Photon Technology International, Birmingham, NJ) according to previously described methods.^{51,52} 3 μL of 1.3 mM EtBr was added to 3000 μL of aqueous buffer (100 mM NaCl, 100 mM Tris, pH 7.4) in a quartz cuvette and baseline fluorescence was recorded ($\lambda_{\text{ex}} = 546 \text{ nm}$, $\lambda_{\text{em}} = 600 \text{ nm}$; 1 cm path length glass cuvette, slit width 6 nm). 6 μL of 1 mg/mL DNA or 40 μL of 10 μM siRNA was added to this solution and allowed to equilibrate for 5 min before measuring the fluorescence. Liposomes were added at the required charge ratios, vortexed, and allowed to equilibrate for 5 min prior to fluorescence measurements. The dissociation constant, K_d , was calculated using the following equation:

$$K_d = \frac{1}{\left(\frac{[\text{EtBr}]}{[\text{IC}_{50}]} \right) \times K_{\text{EtBr}}},$$

where the $[\text{IC}_{50}]$ of the lipid is calculated from the best fit curve using a Gaussian function (minimum $r^2 = 0.99$), $[\text{EtBr}] = 1.0 \times 10^{-6} \text{ M}$, and $K_{\text{EtBr}} = 10^7 \text{ M}^{-1}$.⁵³

3. RESULTS AND DISCUSSION

A family of stimuli responsive charge reversal lipids were synthesized with varied initial charge (+1 vs. +2) and hydrocarbon chain length (C_6 to C_{14}) terminated by a set of carboxylic acids (one per alkyl chain) protected by a 1-(2-nitrophenyl)ethanol (NPE) protecting group to facilitate a permanent charge switching upon exposure to UV light (365 nm) (Figure 2). Two tripeptide head groups, Lysine-Glycine-Glycine (KGG) and Glycine-Glycine-Glycine (GGG) were used to control the initial charge of the amphiphiles, +2 and +1, respectively. Based on previous studies on the interactions between polyanions such as

DNA with cationic amphiphiles, hydrocarbon chain lengths ranging from the more fluid C₆ to the more rigid C₁₄ were chosen for this study, as shown in Figure 2.^{54,55} Each lipid was synthesized by first reacting one equivalent of NPE with either adipic acid (C₆), sebacic acid (C₁₀), or tetradecanedioic acid (C₁₄) through 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) coupling creating mono-protected dicarboxylic acids. A dimethoxytrityl (DMT) protected glycerol was then reacted with two equivalents of each mono-protected hydrocarbon chain using EDCI coupling followed by a trifluoroacetic acid (TFA) deprotection yielding a free secondary alcohol. Next, either a *tert*-butyloxycarbonyl (BOC) protected KGG or GGG tripeptide head group was coupled to the secondary alcohol using EDCI coupling. Acid deprotection of the remaining BOC groups yielded the amphiphiles (Figure 2, Figure S1). The individual reactions proceeded in fair to good yields with milligram amounts produced per variant.

The light-activated deprotection of the NPE protecting groups was characterized using the KGG-C₁₀-NPE lipids, prior to supra-molecular assembly, using high performance liquid chromatography / mass spectrometry (HPLC/MS) to detect the percentage of KGG-C₁₀-NPE lipids that were deprotected after various doses of UV light (365 nm) as a model for the other lipids in the family. Using a 9:1 methanol:water solution of the KGG-C₁₀-NPE lipids (concentration: 0.1 mM), samples were collected after UV exposure times ranging from 0 to 25 minutes (i.e., 0 to 4.5 J/cm² UV light dose using a 3 mW UV lamp). Complete deprotection of the lipids was observed within 25 minutes as the KGG-C₁₀-NPE lipids converted into the KGG-C₉-COO⁻ species (Figure 3).

Upon deprotection, the KGG lipid transitions from an overall +2 charge at pH 7.4 to a neutral charge as two carboxylic acids are exposed upon NPE photolysis, while the GGG amphiphiles experience a charge reversal from a +1 to -1 overall charge. Self-assembled supramolecular structures were prepared from each of the lipids using a previously developed thin film method for producing multi-lamellar micelles.⁵⁶ This method involves drying a thin film of each lipid on the walls of Eppendorf tubes from a chloroform solution. These films are then hydrated in PBS solutions, vortexed, and placed in a bath sonicator for 15 minutes to form multi-lamellar structures. The overall charge (i.e. zeta potential) of these “liposomes” was determined. As expected, the GGG lipids (+1 charge) form liposomes with a lower, but still positive, zeta potential than the KGG lipids (+2 charge). Upon the addition of calf thymus DNA at a 12:1 ratio of the positively charged amines in the lipids (N) to negatively charged phosphate backbone of the nucleic acids (P), the formation of “lipoplexes” were observed as the DNA and amphiphiles formed a supramolecular complex based on attractive electrostatic interactions. In the presence of DNA, the KGG-C₆-NPE lipoplexes possessed a near neutral zeta potential (-12.6 ± 5.1 mV) while the GGG-C₁₀-NPE lipoplexes exhibited a significantly negative zeta potential (-36.7 ± 5.7 mV) despite the 12:1 N:P charge ratio of cationic lipids to anionic nucleic acids. Both the KGG-C₁₀-NPE and KGG-C₁₄-NPE lipoplexes maintained a robust positive zeta potential ($+42.1 \pm 4.1$ mV vs. $+39.6 \pm 2.9$ mV, respectively) as expected from the 12:1 N:P ratio (Figure 4).

The release of anionic biopolymers such as nucleic acids from the lipoplex formation was achieved by a disruption of the attractive electrostatic forces via UV photolysis. The

irradiation causes charge switching or reversal, and can be observed as a transition towards more negative zeta potential of the lipoplexes.

The GGG-C₁₀-NPE lipoplexes have a significant negative zeta potential already prior to photolysis and do not seem to exhibit a significant change in zeta potential, compared to the larger transitions observed for the KGG family of lipids upon exposure to UV light. (Figure 4).

We propose that the KGG-C₆-NPE lipoplexes are less stable than the KGG-C₁₀-NPE or KGG-C₁₄-NPE, based on the neutral zeta potential prior to photolysis. The KGG-C₆-NPE exhibited only a slight shift towards a more negative zeta potential after UV irradiation. The KGG-C₁₄-NPE lipoplexes on the other hand, appeared to be extremely stable and resisted the transition from a positive zeta potential to a negative potential, even after 30 minutes of UV exposure (Figure 4). This lipid does possess the longest fatty acid chains which would stabilize the structure. A balance between initial electrostatic attraction and lipoplex stability was reached with the KGG-C₁₀-NPE lipids which experienced a significant transition from positive to negative zeta potential upon UV exposure transitioning from +42.1 mV to -39.1 mV upon exposure to as little as 5 minutes of UV light (Figure 4).

In order to further study the interactions between the highly anionic biopolymers such as DNA (and RNA) and the lipids, an ethidium bromide (EtBr) displacement fluorescence assay was performed to quantify the free vs. condensed nucleic acids before and after lipoplex formation and photolysis. Ethidium bromide intercalates into double stranded nucleic acids resulting in a 20-fold increase in fluorescence intensity (excitation: 526 nm, emission: 605 nm) compared to the fluorescence in aqueous solutions. Figure 5a illustrates the competitive binding assay between EtBr and the cationic lipids with the nucleic acids: 1) the dissolution of 3 μ L of 1.0 mM EtBr into pH 7.4 phosphate buffered saline (PBS); 2) the addition of either 6 μ L of 1 mg/mL calf thymus DNA or 40 μ L of 10 μ M nonsense siRNA (6 μ g of each nucleic acid) resulting in EtBr intercalation and increased fluorescent signal; 3) the addition of UV switchable cationic liposomes which form lipoplexes with the anionic nucleic acids resulting in the loss of EtBr from the nucleic acids and a reduced fluorescent signal; and 4) the application of 365 nm UV light disrupting the lipoplex complex due to a charge switching effect which results in the repulsion of nucleic acids from the lipids and the re-intercalation of EtBr into the nucleic acids affording fluorescence recovery. Based on the zeta potential data, we observed the expected trends in terms of lipoplex stability and UV activated release of the nucleic acids.

The zeta potential data indicated that the monovalent GGG-C₁₀-NPE lipids formed lipoplexes with a net negative charge even at a 12:1 N:P ratio (Figure 4). The EtBr fluorescence assay also indicated a weak association between the liposomes and either the DNA ($K_d = 28.7 \mu$ M) or siRNA ($K_d = 21.9 \mu$ M), as the GGG-C₁₀-NPE liposomes failed to condense either nucleic acid completely even at a 32:1 N:P ratio, (Figure 5). Furthermore, the change in charge from +1 to -1, after exposure to UV light, did not afford substantial release of nucleic acids from the lipoplexes even after 30 minutes of UV exposure (fluorescence recovery after UV exposure: -5.36 ± 2.64 % (DNA), -8.75 ± 0.96 % (siRNA)), (Table 1).

In contrast, each of the divalent KGG lipids successfully condensed both DNA and siRNA by a charge ratio of 12:1; however, the hydrocarbon chain length had an effect on the overall lipoplex stability, which was reflected in the data (Figure 5, Table 1). The binding affinity followed a non-monotonic response as the binding constants for KGG-C₆-NPE and KGG-C₁₄-NPE are lower than for KGG-C₁₀-NPE (Table 1) (K_d : KGG-C₆-NPE < KGG-C₁₄-NPE < KGG-C₁₀-NPE). Upon UV activation, release of nucleic acids occurred readily from the least stable KGG-C₆-NPE lipoplexes (binding affinity: $K_d = 1340$ nM (DNA) and 1930 nM (siRNA); fluorescence recovery after UV exposure: 55.1 ± 0.99 % (DNA) and 56.1 ± 6.94 % (siRNA)). On the other hand, the most stable KGG-C₁₄-NPE lipoplexes with binding affinity of $K_d = 990$ nM (DNA) and 627 nM (siRNA) did not release the nucleic acids efficiently (fluorescence recovery after UV exposure = 7.4 ± 2.28 % (DNA) and 14.6 ± 0.85 % (siRNA)).

Based on both the zeta potential and EtBr fluorescence assay for the KGG-C₁₀-NPE lipid, the most efficient capture and release of both DNA and siRNA molecules from the supramolecular structure was a consequence of the balance between nucleic acid binding affinity ($K_d = 680$ nM (DNA) and 480 nM (siRNA)) and charge switching capability (fluorescence recovery after UV exposure = 62.9 ± 2.70 %, DNA, and 57.2 ± 2.66 %, siRNA). However, from the data obtained in the EtBr assay and presented in Table 1, full fluorescence recovery is not obtained (i.e., 100 %) after irradiation for any of the lipoplexes under study. We hypothesize that some of the nucleic acids remain complexed to the lipid macro-structures even after photolysis and the change in lipid charge.

While the hydrocarbon chain length had an effect on the stability and binding efficiency of the cationic lipids with nucleic acids, the overall utility of these stimuli responsive capture and release lipids was largely dictated by the valency of the hydrophilic tripeptide head group since the liposomes must be able to first capture the nucleic acid cargo prior to release. While a stimuli responsive reversal from a cationic to anionic complex will facilitate a stronger charge repulsion between the lipids and the nucleic acids, the binding constant of the GGG-C₁₀-NPE lipid was too low to observe this phenomenon. Therefore, a sufficient reduction in binding affinity between the lipids and nucleic acids was achieved through a stimuli responsive change in charge from cationic (+2) to neutral by the KGG-C₁₀-NPE lipoplexes.

4. CONCLUSION

A family of stimuli-responsive cationic lipids is reported. The lipids are synthesized via a convergent approach whereby the tripeptide hydrophilic head groups (KGG or GGG) and the light activated hydrophobic chains (C₆, C₁₀, C₁₄) linked to glycerol are first prepared, and then coupled in the last step to afford the lipids. Upon UV exposure, two carboxylates are formed at the ends of each hydrophobic chain. The net effect of the UV exposure is that the lipid switches from an overall charge of +2 to 0 or +1 and -1, depending on the lipid composition. The lipids self-assemble into liposomes and in the presence of either DNA or siRNA, form lipoplexes as the nucleic acids are incorporated into the supramolecular assembly. Both the choice of the head group and the length of the carbohydrate chain play a significant role in activity of these cationic lipids, their ability to form complexes with

nucleic acids, and the subsequent photolytic release of the nucleic acids from the lipoplexes. Lipids with the GGG head group did not form strong interactions with nucleic acids nor form photo-activatable lipid/DNA structures. In contrast, the KGG head group lipids exhibited much higher binding affinity for nucleic acids, as determined from a competitive EtBr fluorescence assay, while the effect of chain length on binding affinity is less pronounced. A non-monotonic relationship between hydrocarbon chain length and nucleic acid binding affinity is observed with the C₁₀ chains resulting in the highest binding affinity. The chain length, on the other hand, does play a much more pronounced role in efficiency of nucleic acid release from the lipoplex macrostructures. The shorter C₆ chain lipoplexes facilitate the most rapid nucleic acid release while the C₁₄ chain lipoplexes, which maintained a positive zeta potential even after UV exposure, display minimal release of nucleic acid upon exposure to UV light. The KGG-C10-NPE lipids exhibited the most ideal balance between the binding affinity, lipoplex stability, and nucleic acid release post irradiation. They form lipoplexes with a strong positive zeta potential, allowing for stability of the macrostructure, while also shifting to strong negative zeta potential upon photolysis. Consequently, they exhibit both high binding affinity for nucleic acids prior to photolysis, and efficient release of the nucleic acids after photolysis.

These results set the stage and provide key data for designing lipids that can capture and release nucleic acids. Of particular note, cationic lipids responsive to near infrared light and/or two photon activation will reduce the likelihood of nucleic acid damage during release and enable applications such as in vivo gene delivery, where light penetration is an important parameter. Continued development of novel stimuli responsive lipids may have broad applicability to a number of challenges in the biotechnology and biomedical engineering fields.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Novel light responsive cationic lipids are synthesized
- Lipids complex DNA and siRNA and form supramolecular structures
- Light triggered dis-assembly of supramolecular lipid-nucleic acid assemblies is demonstrated
- Capture and release of nucleic acids is governed by electrostatic interactions

Figure 2.

Ultraviolet light activated cationic lipid library synthesis. Each lipid has a tripeptide head group, variable alkyl chain length, and a 1-(2-nitrophenyl)ethanol protected terminal carboxylic acid. See the supporting information for more details.

Figure 3. Ultraviolet light (365 nm) deprotection of the NPE protecting group on the KGG-C₁₀-NPE lipid quantified via LC-MS. (Avg $\pm$ StDev, N=3)

Figure 4.

Zeta potential measurements of the cationic lipids as liposomes and lipoplexes with calf thymus DNA at a 12:1 N/P charge ratio, and as lipoplexes after 5 and 30 minutes of UV light exposure. (Avg $\pm$ StDev, N=3)

Figure 5.

a) Schematic diagram (not drawn to scale) of the ethidium bromide competitive binding assay which enables the determination of binding affinities between the nucleic acids and cationic lipids: 1) ethidium bromide (yellow) is added to a phosphate buffered saline solution (blue); 2) nucleic acids (either DNA or siRNA) are added to the mixture causing ethidium bromide intercalation and enhanced fluorescence; 3) cationic amphiphiles are added to the reaction forming lipoplexes with the nucleic acids resulting in the removal of ethidium bromide from the nucleic acids and a reduction in fluorescence; 4) ultraviolet light (365 nm) is applied to the system inducing a charge reversal as the NPE protecting groups are deprotected exposing two carboxylic acid end groups on each lipid leading to the release of the nucleic acids, reintercalation of the ethidium bromide, and an increase in fluorescence intensity. b) Ethidium bromide fluorescence assay using 6 μ g of nucleic acids (top: DNA; bottom: siRNA) and various lipids to DNA charge ratios (N:P) for the KGG-C6-NPE (red), KGG-C10-NPE (blue), GGG-C10-NPE (green), and KGG-C14-NPE (purple). (Avg $\pm$ StDev, N=3)

Table 1

Cationic amphiphiles nucleic acid (DNA and siRNA) binding affinity

	K_d DNA (μM)	K_d siRNA (μM)	Fluorescence Recovery After UV (%) (DNA)	Fluorescence Recovery After UV (%) (siRNA)
KGG-C₆-NPE	1.34	1.93	55.1 ± 0.99	56.1 ± 6.94
KGG-C₁₀-NPE	0.68	0.48	62.9 ± 2.70	57.2 ± 2.66
GGG-C₁₀-NPE	28.7	21.9	−5.36 ± 2.64	−8.75 ± 0.96
KGG-C₁₄-NPE	0.99	0.63	7.44 ± 2.28	14.6 ± 0.85