SUPPORTING INFORMATION

Multivalent Dendrimer Vectors with DNA Intercalation Motifs for Gene Delivery

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Materials

Unless noted otherwise, all reagents and solvents were purchased from Sigma-Aldrich including folic acid (≥97%), 3,8-diamino-6-phenanthridine (98%), folate binding protein (FBP, bovine milk), riboflavin binding protein (RBP; apo form, chicken egg white) and DNA sodium salt (calf thymus). (−)-Riboflavin (98%) was purchased from Acros Organics. Biosensor chips (CM5) and SPR buffer solutions were purchased from Pharmacia Biosensor AB. pGL4.50[*luc2*/CMV/Hygro] vector encoding the luciferase reporter gene *luc2* (*Photinus pyralis*) was purchased from Promega. Generation 5 (G5) poly(amidoamine) (PAMAM) dendrimer was purchased as a methanol solution (17.5% (w/w); Dendritech, Inc). Prior to use, the dendrimer solution was evaporated *in vacuo* and purified to remove lower generation impurities by dialysis with membrane tubing (MWCO 10 kDa).[1-2] The number (n) of primary amine branches per dendrimer molecule was determined on a mean basis (n = 114) by potentiometric titration[3] using a Mettler Toledo MP230 pH meter equipped with an InLab® Micro electrode.

Analytical Methods

NMR experiments were performed at 499.9 MHz for ^{1}H nucleus using a Varian NMR spectrometer. Chemical shifts (δ) in ^{1}H NMR spectrum were recorded in standard units (ppm) with an internal reference (2,2-dimethyl-2-silapentane-5-sulfonate sodium salt (DSS); $\delta = 0.00$ ppm). All NMR experiments were performed at 297.3 K (±0.2) using standard pulse sequences as noted in the NMR spectra (Supporting Information). UV–vis analysis was performed with a Perkin Elmer Lambda 20 spectrophotometer.

Purity of the dendrimer conjugate was assessed by ultra-performance liquid chromatography (UPLC) performed on a Waters Acquity Peptide Mapping System equipped with a Waters photodiode array detector.[4] Each sample (0.1–1.0 mg mL^{-1} in water) was analyzed by running on a C4 BEH column (150 × 2.1 mm, 300 Å) at a flow rate of 1 mL min^{-1} with a linear gradient method starting with 98:2 (v/v) water/acetonitrile with TFA (0.14 w/v%).

Molecular weights (M_r) of G5 PAMAM dendrimer and its conjugates were measured by matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) with a Waters TOfsPec-2E spectrometer. Gel permeation chromatography (GPC) was performed in an Alliance Waters 2695 separation module equipped with a Wyatt HELEOS Multi Angle Laser Light Scattering detector and an Optilab rEX differential refractometer (Wyatt Technology Corporation). Each GPC sample was prepared at 3–5 mg/mL in 0.1 M citric acid (pH 2.7) for GPC analysis. Its data were analyzed with Astra 5.3.14 software (Wyatt Technology Corporation) to extract the values of weight-average molecular weight (M_w), number-average molecular weight (M_n) and polydispersity index (PDI = M_w/M_n). GPC analysis of unmodified G5 PAMAM dendrimer determined its molecular weights of M_w = 26,550 g mol^{-1} and M_n = 26,270 g mol^{-1} with a PDI value of 1.01.

Synthesis of G5 PAMAM dendrimer conjugates 3–7 (Scheme 1)

(a) Activation of FA: Folic acid (21.6 mg, 49 μmol) was dissolved in DMSO (1.0 mL) containing *N*,*N*-diisopropylethylamine (DIPEA; 26 μL, 151 μmol). To this solution was added 1-hydroxybenzotriazole (HOBt; 9.2 mg, 60 μmol) and (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBop; 31.4 mg, 60 μmol). The mixture was stirred at room temp for 2 h, and used for next step immediately (labeled as “FA-HOBt ester”).

(b) Activation of 1 RF-CO_2H: RF-CO_2H[5] (24 mg, 49 μmol) was dissolved in DMSO (1.0 mL) containing DIPEA (26 μL, 151 μmol), followed by the addition of HOBt (9.2 mg, 60 μmol) and PyBop (31.4 mg, 60 μmol). After stirring at room temp for 2 h, the activated solution was used for next step immediately (labeled as “RF-HOBt ester”).

(c) Activation of 2 DAPP-CO_2H: DAPP-CO_2H[6] (**2**) was synthesized by N_5-alkylation of *N*,*N’*-di-Boc-3,8-di-amino-6-phenylphenanthridine with methyl 4-(bromomethyl)benzoate (MeOH, 80°C), and subsequent hydrolysis of its resulting methyl ester (for details, see Supporting Information). DAPP-CO_2H (32.3 mg, 56.5 μmol) was dissolved in DMSO (1.5 mL) containing DIPEA (30 μL, 170 μmol) prior to addition of HOBt (10.4 mg, 67.8 μmol) and PyBop (44.1 mg, 84.8 μmol). The mixture was stirred at room temp for 2 h, and the activated DAPP-CO_2H solution was divided into three equal parts (0.5 mL each, labeled as “DAPP-HOBt ester”).

(d) Conjugate G5(FA)$_n$ 3. A solution of the "FA-HOBT ester" (1.0 mL) was added to G5 PAMAM dendrimer (104 mg, M_r = 27,600) dissolved in MeOH (10 mL). This mixture was stirred overnight, and divided into two equal parts (5.5 ml each, labeled as "FA-conjugated G5-NH_2"). One part of this reaction mixture was used for the synthesis of G5(FA)$_n$ **3** and the other part was saved for synthesis of G5(FA)$_n$(DAPP)$_m$ **5** to be described below. For completion of G5(FA)$_n$ synthesis, the solution ("FA-conjugated G5-NH_2") was treated with triethylamine (Et_3N, 32 μL, 226 μmol) and acetic anhydride (Ac_2O, 11 μL, 113 μmol) for partial neutralization (~60%) of remaining dendritic amines. After stirring the final mixture for 6 h, it was concentrated *in vacuo*, and the residue was dissolved in 10 mL of phosphate buffered saline (PBS, pH 7.4). This solution was loaded in a membrane dialysis tubing (MWCO 10 kDa), and dialyzed against PBS (2 × 2L) and deionized water (3 × 2L) until unconjugated FA was undetectable as determined by analytical UPLC. Lyophilization of the dialyzed solution afforded G5(FA)$_n$ **3** as pale yellow solid (58 mg). Valency (n = 8.6 ± 0.6) of the folate ligand was determined on a mean basis by a UV–vis method using a folate (FA) calibration curve ([FA] = slope × $A_{280\text{ nm}}$; slope = 4.47×10^{-5}). UV–vis ([**3**] = 3.3–9.9 μM in PBS, pH 7.4): λ_{max} = 350 nm (ε = 62,600 $M^{-1}cm^{-1}$), 287 nm (ε = 203,000 $M^{-1}cm^{-1}$). UPLC: t_r = 7.32 min (cf., t_r = 6.84 min for FA); purity >95%. MALDI-TOF MS (*m/z*, g mol^{-1}): 30,400. ^1H NMR (500 MHz, D_2O): δ 8.11-8.09 (m), 7.81 (br s), 7.75 (br s), 7.40 (br s), 3.53–3.51 (t), 3.35 (br s), 3.31 (s), 3.17–3.15 (t), 2.92 (br), 2.73 (br), 2.48 (br), 1.99 (s) ppm (abbreviation: br = broad; s = singlet; m = multiplet, t = triplet).

(e) Conjugate G5(DAPP)$_m$ 4. A solution of the "DAPP-HOBt ester" (0.5 mL) was added to the G5 PAMAM dendrimer (52 mg) dissolved in MeOH (5 mL). This mixture was stirred overnight, and the remaining amines were partially (~60%) neutralized by reacting with Et_3N (32 μL, 226 μmol) and Ac_2O (11 μL, 113 μmol) for 6 h. The reaction mixture was concentrated *in vacuo*, and the residue was purified by membrane dialysis (MWCO 10 kDa) against PBS (2 × 2L) and deionized water (3 × 2L) and monitoring its purity (>95%) by analytical UPLC. Lyophilization of the dialyzed solution afforded **4** G5(DAPP)$_m$ as a pale purple solid (65 mg). Valency (m = 5.4 ± 0.6) of **2** DAPP ligand was determined on a mean basis by a UV–vis method using an ethidium (Et) calibration curve ([Et] = slope × $A_{290\text{ nm}}$; slope = 9.63×10^{-5}). UV–vis ([**4**] = 3.3–9.9 μM in PBS, pH 7.4): λ_{max} = 350 nm (ε = 99,980 $M^{-1}cm^{-1}$), 290 nm (ε = 206,400

$M^{-1}cm^{-1}$). UPLC: t_r = 7.14 min (cf., t_r = 6.84 and 9.23 min for FA and **2**, respectively); purity >95%. GPC: M_w = 30,310, PDI (M_w/M_n) = 1.501. MALDI-TOF MS (*m/z*, g mol^{-1}): 30,200. ^1H NMR (500 MHz, D_2O): δ 8.25 (br), 8.11–8.09 (m), 7.78 (br d), 7.69 (br d), 7.39–7.37 (m), 3.55–3.52 (t), 3.37 (br s), 3.32 (s), 3.19–3.17 (t), 2.94 (br s), 2.75 (br), 2.49 (br), 2.00 (s) ppm (abbreviation: d = doublet).

(f) Conjugate $G5(FA)_n(DAPP)_m$ 5. To the remaining solution (5.5 mL) of "FA-conjugated $G5-NH_2$" was added the solution of "DAPP-HOBT ester" (0.5 mL). This mixture was stirred overnight at room temp, and the remaining amines of the dendrimer were partially (~60%) neutralized by reacting with Et_3N (32 μL, 226 μmol) and Ac_2O (11 μL, 113 μmol) for 6 h. The mixture was concentrated *in vacuo*, and the residue was purified by membrane dialysis (MWCO 10 kDa) against PBS (2 × 2L) and deionized water (3 × 2L). Lyophilization of the dialyzed solution afforded **5** $G5(FA)_n(DAPP)_m$ as a pale purple solid (65 mg). UV–vis ([**5**] = 3.3–9.8 μM in PBS, pH 7.4): λ_{max} = 350 nm (ε = 85,800 $M^{-1}cm^{-1}$), 285 nm (ε = 203,900 $M^{-1}cm^{-1}$). UPLC: t_r = 7.32 min (cf., t_r = 9.23 min for **2**); purity >95%. MALDI-TOF MS (*m/z*, g mol^{-1}): 30,500. ^1H NMR (500 MHz, D_2O): δ 8.25 (m), 8.10 (m), 7.79 (br), 7.40 (br m), 3.54 (br), 3.44 (br), 3.31 (s), 3.19 (t), 3.7–2.7 (br), 2.57 (br), 1.99 (s) ppm. GPC: M_w = 23,400, PDI (M_w/M_n) = 1.066.

(g) Conjugate $G5(RF)_{n'}$ 6. A solution of the "RF-HOBT ester" (1.0 mL) was added to G5 PAMAM dendrimer (104 mg, M_r = 27,600) dissolved in MeOH (10 mL). This mixture was stirred overnight, and divided into two equal parts (5.5 ml each, labeled as "RF-conjugated G5-NH_2"). One part of this reaction mixture was used for the synthesis of $G5(RF)_{n'}$ **6** and the other part was saved for synthesis of $G5(RF)_{n'}(DAPP)_{m'}$ **7** to be described below. For completion of $G5(RF)_{n'}$ synthesis, the "RF-conjugated $G5-NH_2$" solution (5.5 mL) was treated with triethylamine (Et_3N, 32 μL, 226 μmol) and acetic anhydride (Ac_2O, 11 μL, 113 μmol) for partial neutralization (~60%) of remaining dendritic amines. After stirring the final mixture for 6 h, the mixture was concentrated *in vacuo*, and the residue was purified by membrane dialysis (MWCO 10 kDa) against PBS (2 × 2L) and deionized water (3 × 2L). Lyophilization of the dialyzed solution afforded **6** $G5(RF)_{n'}$ as a pale yellow solid (74 mg). Valency (*n'* = 4.9) of RF ligand was determined on a mean basis by a UV–vis method using a RF calibration curve ([RF] = slope × $A_{448\ nm}$; slope = 9.70 × 10^{-4}). UV–vis ([**6**] = 3.3–10 μM in PBS, pH 7.4): λ_{max} = 448 nm (ε = 5,900 $M^{-1}cm^{-1}$), 350 nm (ε = 3,800 $M^{-1}cm^{-1}$). UPLC: t_r = 7.31 min (cf., t_r = 7.01 min for RF);

purity >95%. GPC: M_w = 29,500, PDI (M_w/M_n) = 1.198. MALDI-TOF MS (*m/z*, g mol^{-1}): 30,100. ^{1}H NMR (500 MHz, D_2O): δ 8.22 (br), 8.11–8.03 (m), 7.79–7.78 (d), 7.68-7.67 (d), 7.39 (m), 3.52 (t), 3.35 (s), 3.17 (t), 2.92 (br), 2.72 (br), 2.48 (br), 1.99 (s) ppm.

(h) Conjugate G5(RF)$_{n'}$(DAPP)$_{m'}$ 7. To a remaining solution (5.5 mL) of "RF-conjugated G5-NH$_2$" was added the "DAPP-HOBT ester" solution (0.5 mL), each prepared above. This mixture was stirred overnight at room temp, and the remaining amines of the dendrimer were partially (~60%) neutralized by reacting with Et_3N (32 μL, 226 μmol) and Ac_2O (11 μL, 113 μmol) for 6 h. The mixture was concentrated *in vacuo*, and the residue was purified by membrane dialysis (MWCO 10 kDa) against PBS (2 × 2L) and deionized water (3 × 2L). G5(RF)$_{n'}$(DAPP)$_{m'}$ **7** was obtained as a pale purple solid (67 mg). Valency (*m'* = 6.9 ± 2.1) of **2** DAPP ligand was determined on a mean basis by a UV–vis method using an ethidium (Et) calibration curve ([Et] = slope × $A_{290\ nm}$; slope = 9.63 × 10^{-5}). UV–vis ([**7**] = 3.3–10 μM in PBS, pH 7.4): λ_{max} = 448 nm (ε = 48,100 M^{-1}cm^{-1}), 350 nm (ε = 163,000 M^{-1}cm^{-1}), 285 nm (ε = 302,000 M^{-1}cm^{-1}). UPLC: t_r = 7.25 min (cf., t_r = 7.01 and 9.23 min for RF and **2**, respectively); purity >95%. GPC: M_w = 32,300, PDI (M_w/M_n) = 1.413. MALDI-TOF MS (*m/z*, g mol^{-1}): 30,000. ^{1}H NMR (500 MHz, D_2O): δ 8.22 (br), 8.10 (m), 7.77–7.54 (br d), 7.68–7.67 (br d), 7.37 (br), 3.55–3.53 (t), 3.44 (br), 3.31 (s), 3.19–3.17 (t), 3.10 (br), 3.03–2.70 (br), 2.58 (br), 2.00 (s) ppm.

The conjugation efficiency for each ligand which is defined as $[\text{ligand}]_{\text{attached}} \div [\text{ligand}]_{\text{added}}$) × 100% was in the range of 38% (RF), 54–69% (DAPP) to 66% (FA).

Surface Plasmon Resonance (SPR) Spectroscopy

SPR experiments were performed on a Biacore® X instrument (Pharmacia Biosensor, AB) using a CM5 sensor chip immobilized with G5(DAPP) dendrimer or FBP. Each of the chips was prepared by dendrimer or protein immobilization following an EDC/NHS (1:1 mixture of 0.4 M EDC and 0.1 M NHS, each in water; 70 uL) coupling protocol in which the dendrimer or protein was covalently attached to the chip surface coated with carboxymethylated dextran.[7-9] Each chip was prepared by injecting 35 μL of **4** G5(DAPP)$_{5.4}$ (2.5 mg/mL) or FBP (1.67 mg/mL), during the immobilization process which remains otherwise identical.[10] A sensor chip with G5(DAPP)$_{5.4}$ was prepared with an increase in response units (RU) of 13,060, a value equivalent to dendrimer immobilization at a surface density of ~13 ng G5(DAPP)/mm^2. A FBP sensor chip was prepared with an increase in RU of 13,090 (density = ~13 ng/mm^2). SPR experiments for the kinetic study were carried out by injection (50 μL) of serially-diluted analyte solutions, each prepared in HBS–EP buffer, at a flow rate of 30 μL/min unless noted otherwise in each figure legend. At the end of each dissociation phase ($t > 600$ s), the chip surface was treated with 10 μL of 10 mM glycine–HCl (pH 2.5) for surface regeneration.

SPR data processing and analysis. To correct for the contribution of non-specific adsorption, the reference sensorgram from reference flow cell 2 (RU_2) was subtracted from that recorded from receptor-immobilized flow cell 1 (RU_1) for each dendrimer: ΔRU (corrected) = $RU_1 - RU_2$. For determination of the binding avidity, two kinetic parameters—association (on) rate constant (k_a) and dissociation (off) rate constant (k_d)—were extracted by fitting each corrected sensorgram to the Langmuir adsorption isotherm and using global curve fitting models with BIAevaluation software (version 3).[7-8, 11-12] The equilibrium dissociation constant ($K_D = k_d/k_a$) was calculated from each pair of the two associated rate constants, and a mean value was obtained from four or more independent measurements ($n \geq 4$).

In vitro luciferase transfection

Method Development and Controls: Transfection of pLuc was evaluated for polyplexes of two selected G5 PAMAM dendrimers: the negatively charged G5(GA)[2] (GA = glutaric acid), and the positively-charged unmodified G5-NH_2. G5-NH_2 significantly enhanced the transfection efficiency relative to that of DNA alone, which is consistent with what has been previously reported.[13] Transfection with G5-NH_2 preincubated with 1 μg of pLuc at D/P ratios of either 2:1 or 1:1 showed an enhancement in luciferase expression of 215- and 43-fold relative to pDNA alone (Figure S7B, Supporting Information). In contrast, negatively-charged G5(GA) was ineffective as a transfection agent, and showed lower luciferase activity than cells incubated with pDNA alone. This demonstrates the importance of having cationic charge on the polyplex for effective transfection. For method development, we examined the effects of fetal bovine serum (FBS) on transfection efficiency by performing the 4 h transfection in the presence or absence of FBS with polyplexes made with dendrimer constructs **3**–**7**. The presence of FBS during transfection had minor effects on the magnitude of transfection efficiency, but did not appear to alter the relative order of activity of the dendrimer constructs (data not shown). Thus, the remaining studies were performed in the absence of FBS, as commercial sources of FBS carry trace amounts of FA and RF which could subsequently alter the density of available targeted receptors on the cell surface.

Figure S1. HPLC (A), GPC (B) traces and MALDI-TOF MS data (C) of **3**–**7** PAMAM G5 dendrimer conjugates. FA = folic acid; RF = riboflavin; DAPP = 3,8-diamino-6-phenylphenanthridine; **2** = DAPP-CO_2H.

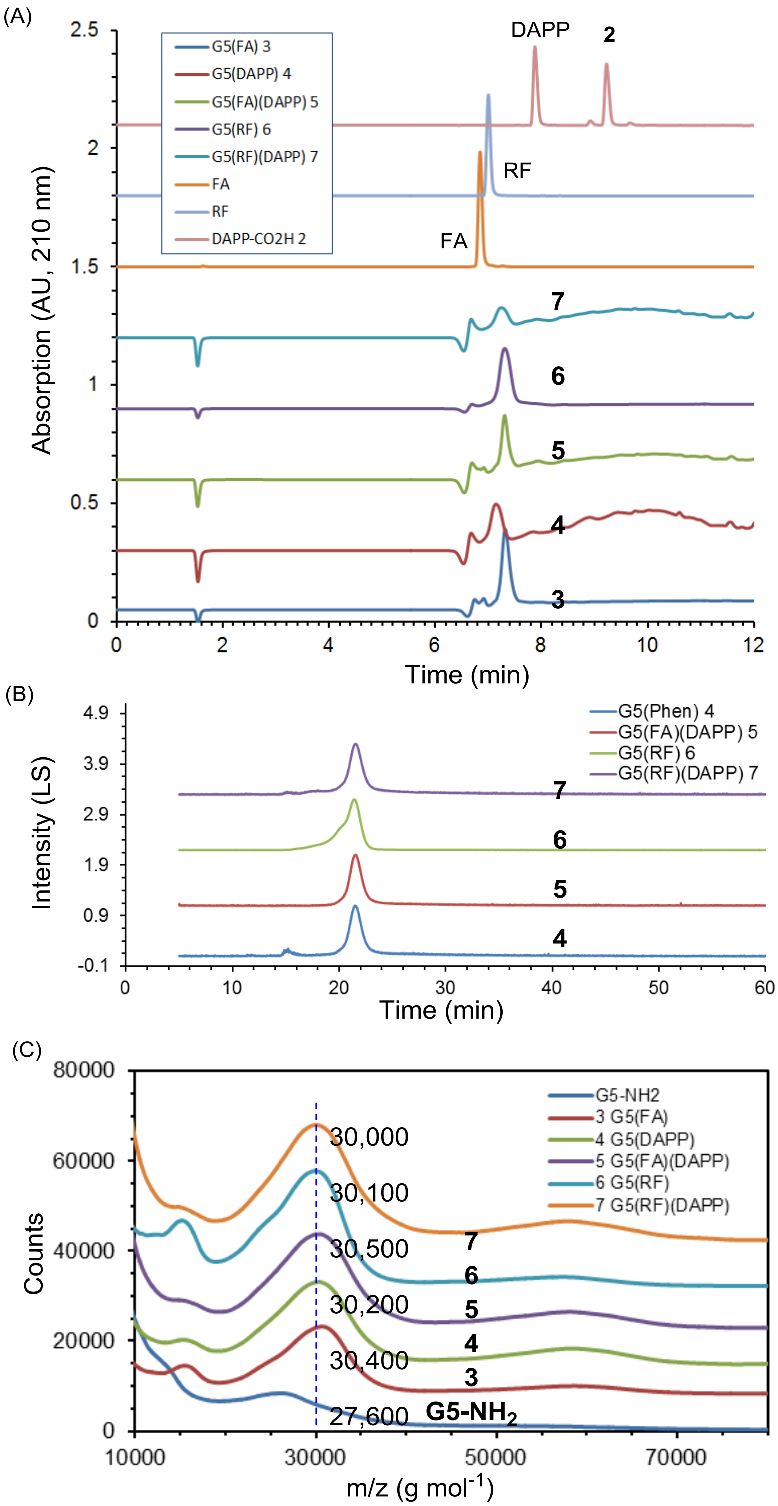
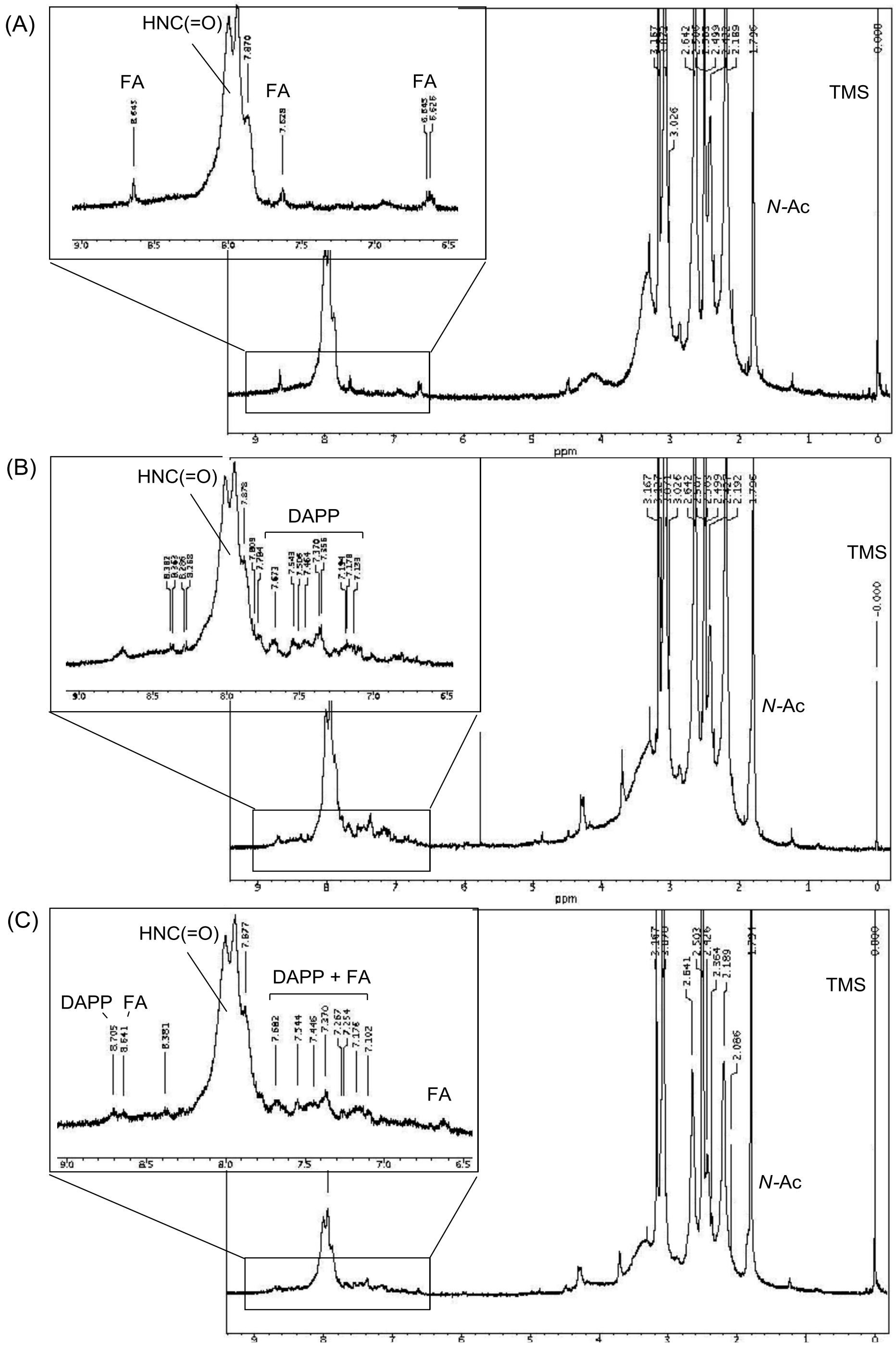
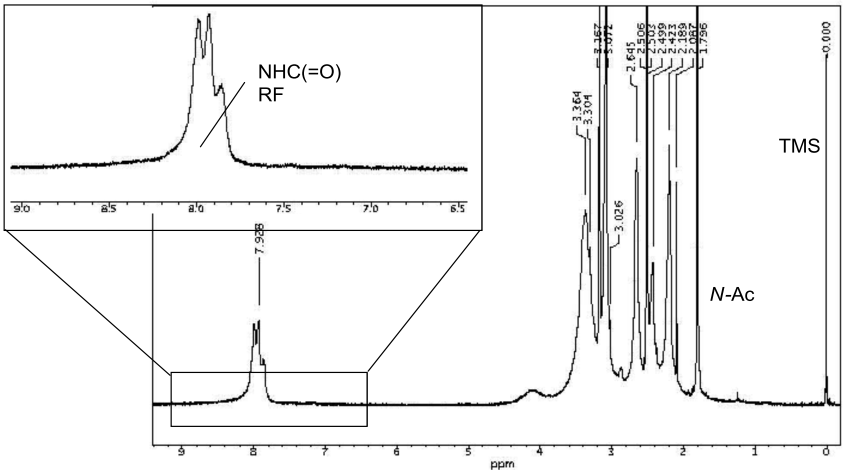


Figure S2. ^{1}H NMR (500 MHz, DMSO-d_6) spectra of G5 dendrimer conjugates **3**–**7**: (A) **3** G5(FA)$_{8.6}$; (B) **4** G5(DAPP)$_{5.4}$; (C) **5** G5(FA)$_{8.6}$(DAPP)$_{5.4}$; (D) **6** G5(RF)$_{4.9}$; (E) **7** G5(RF)$_{4.9}$(DAPP)$_{6.9}$. Signal peaks for the two aromatic protons of a free RF molecule appear at 7.90 and 7.92 ppm, each as a singlet (DMSO-d_6). Thus, those pertinent RF signals in **6** (D) and **7** (E) are overlaid in broad amide peaks centered around 8 ppm.

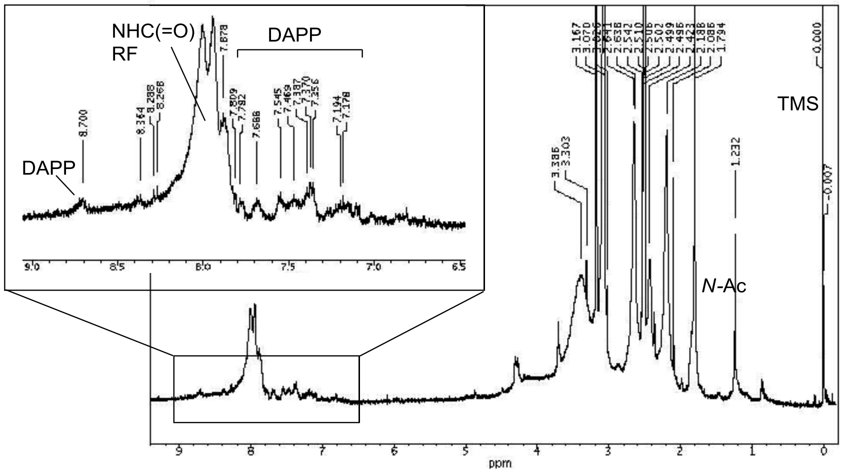


(D)



NHC(=O)
RF
9.0
8.5
8.0
7.5
7.0
6.5
7.928
3.364
3.304
3.167
3.072
3.026
2.645
2.506
2.503
2.499
2.423
2.189
2.087
1.796
0.000
TMS
N-Ac
9
8
7
6
5
4
3
2
1
0
ppm

(E)



NHC(=O)
RF
DAPP
DAPP
8.700
8.354
8.288
8.268
7.878
7.809
7.782
7.688
7.545
7.469
7.387
7.370
7.356
7.194
7.178
9.0
8.5
8.0
7.5
7.0
6.5
3.385
3.303
3.167
3.070
3.026
2.641
2.638
2.542
2.510
2.506
2.502
2.499
2.496
2.423
2.188
2.086
1.794
1.232
0.000
-0.007
TMS
N-Ac
9
8
7
6
5
4
3
2
1
0
ppm

Figure S3. UV–vis absorption spectra of **3**–**7** PAMAM conjugates, each measured in PBS (pH 7.4). (A) **3** G5(FA)$_{8.6}$; (B) **4** G5(DAPP)$_{5.4}$; (C) **5** G5(FA)$_{8.6}$(DAPP)$_{5.4}$; (D) **6** G5(RF)$_{4.9}$; (E) **7** G5(RF)$_{4.9}$(DAPP)$_{6.9}$.

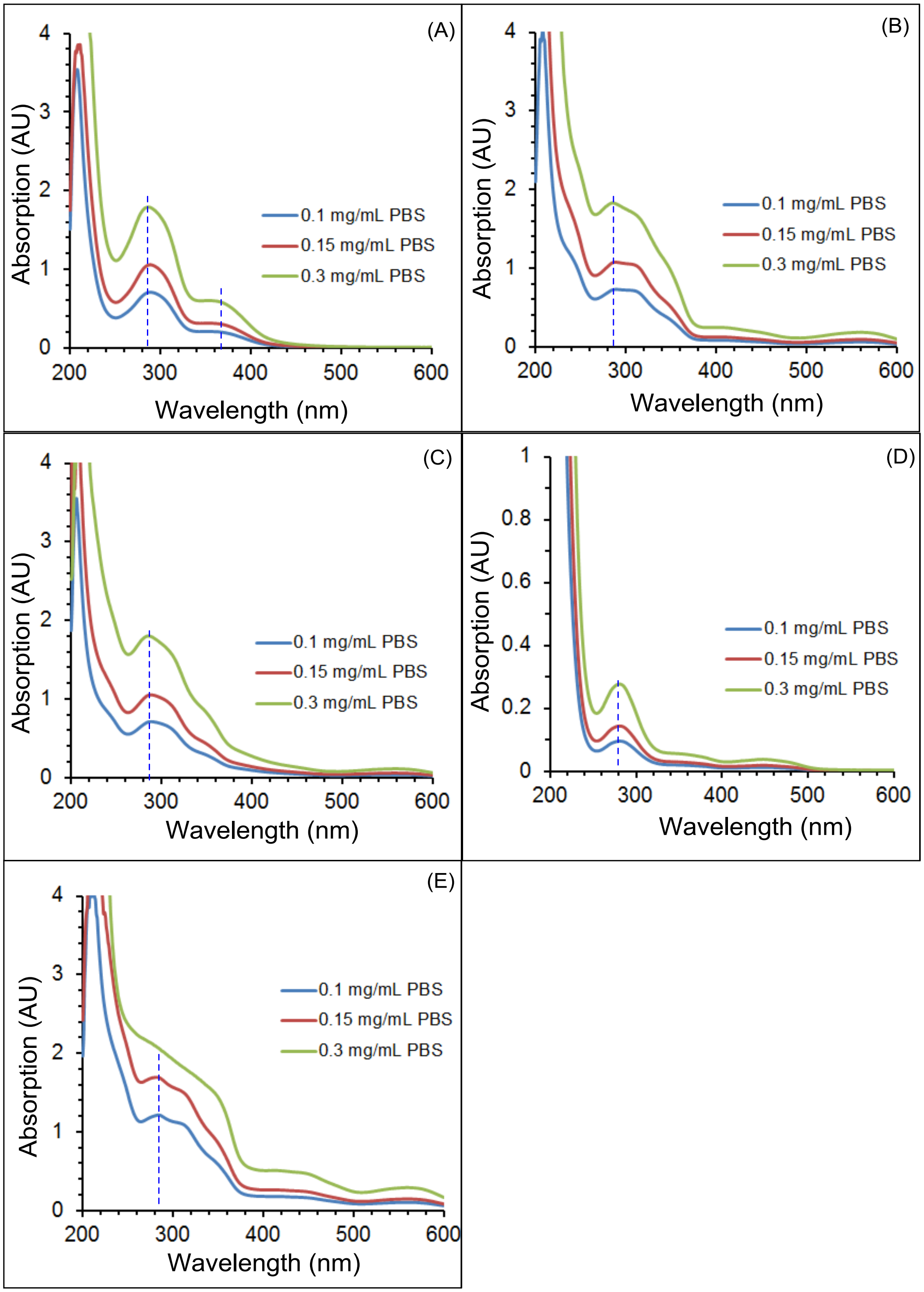


Figure S4. (A, B) Immobilization on the CM5 sensor chip surface. Two chips were prepared, each immobilized with **4** G5(DAPP)$_{5.4}$ (A) or folate binding protein (FBP; B) in flow cell 1. In each chip, the flow cell 2 (reference surface) was treated in the same manner but without injection of the dendrimer or the protein. (C) Dose-RU sensorgrams of folic acid (FA) to the FBP chip. Abbreviations: EDC = *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride, NHS = *N*-hydroxysuccinimide, ETA = 2-ethanolamine, RU = response unit

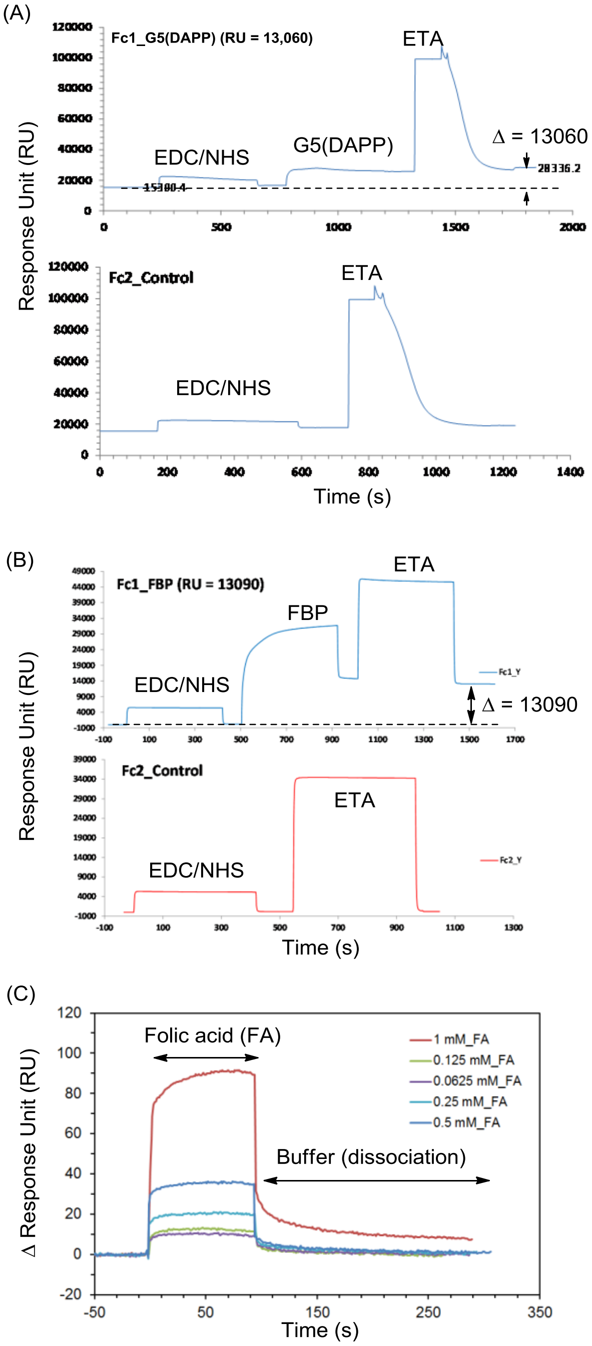
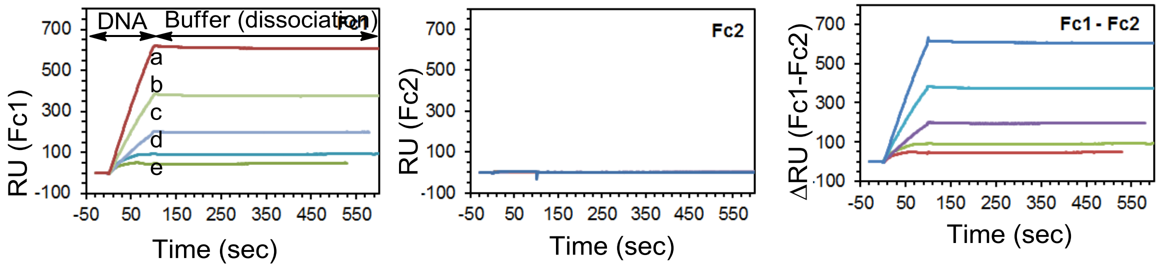
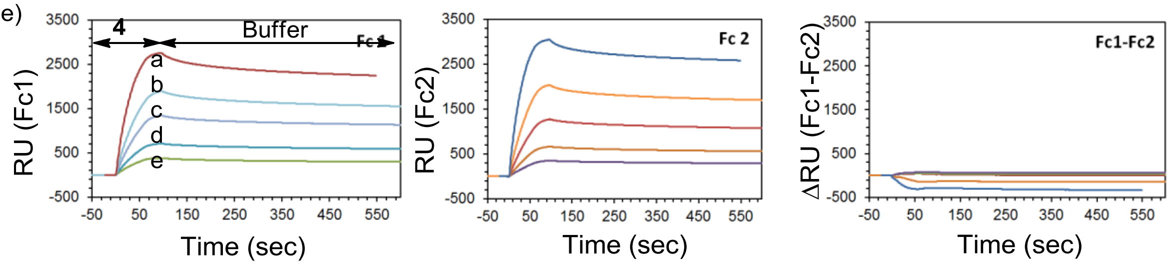


Figure S5. SPR sensorgrams for binding kinetics of dsDNA (calf thymus) to a CM5 sensor chip immobilized with **4** G5(DAPP)$_{5.4}$. Each sensorgram refers to a raw sensorgram acquired from flow cell 1 (Fc1; **4** immobilized), flow cell 2 (Fc2; reference witout **4** immobilized), or to a corrected sensorgram obtained by subtraction of the reference sensorgram: ΔRU (Fc1 – Fc2) = RU (Fc1) – RU (Fc2). (A) DNA alone injected at various concentrations. (B) **4** alone injected at the concentrations indicated. Sensorgrams for various polyplexes, each made of dsDNA with **4** (C), **5** G5(FA)$_{8.6}$(DAPP)$_{5.4}$ (D), **6** G5(RF)$_{4.9}$ (E) or **7** G5(RF)$_{4.9}$(DAPP)$_{6.9}$ (F), were acquired at the concentrations indicated.

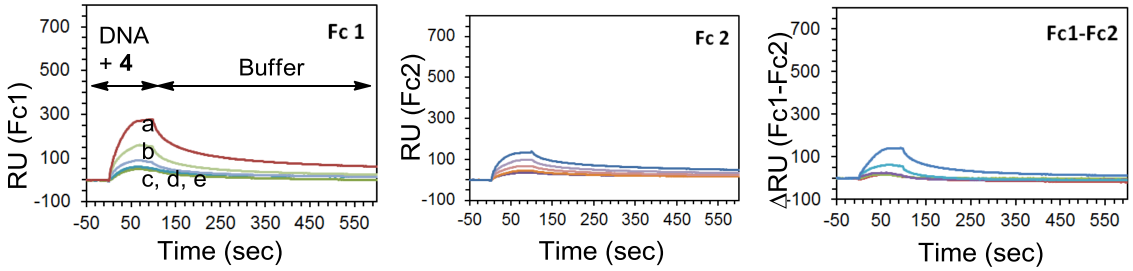
(A) DNA alone: 8 μg/mL (a), 4 (b), 2 (c), 1 (d), 0.5 (e)



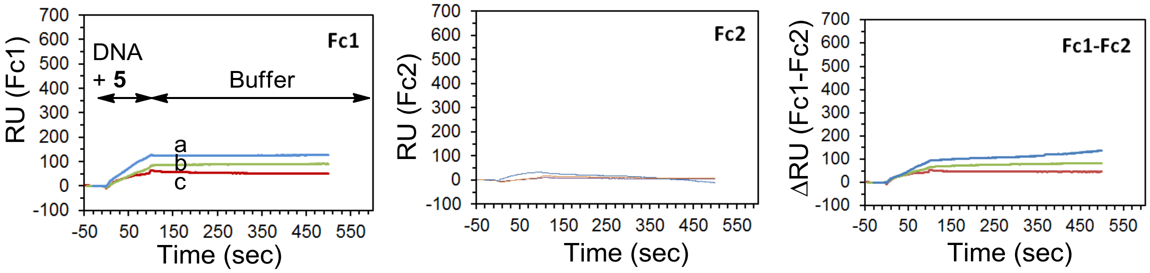
(B) **4** G5(DAPP)$_{5.4}$ alone: : 8 μg/mL (a), 4 (b), 2 (c), 1 (d), 0.5 (e)



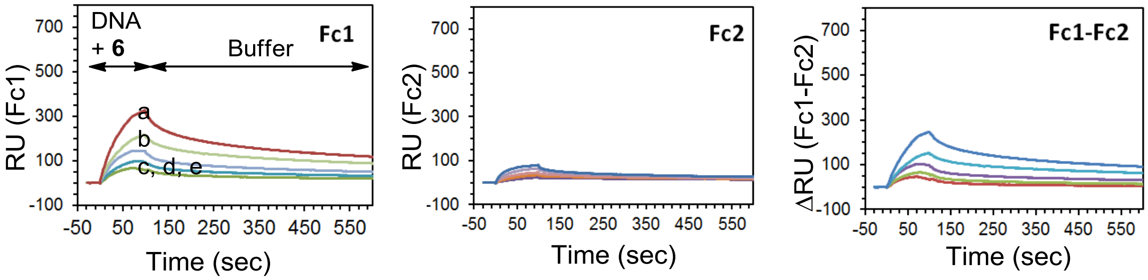
(C) DNA + **4** (1:1 w/w): : 8 μg/mL (a), 4 (b), 2 (c), 1 (d), 0.5 (e)



(D) DNA + **5** G5(FA)$_{8.6}$(DAPP)$_{5.4}$ (1:1 w/w): 2 μg/mL (a), 1.0 (b), 0.5 (c)



(E) DNA + **6** G5(RF)$_{4.9}$ (1:1 w/w): 8 μg/mL (a), 4 (b), 2 (c), 1 (d), 0.5 (e)



(F) DNA + **7** G5(RF)$_{4.9}$(DAPP)$_{6.9}$ (1:1 w/w): 8 μg/mL (a), 4 (b), 2 (c), 1 (d), 0.5 (e)

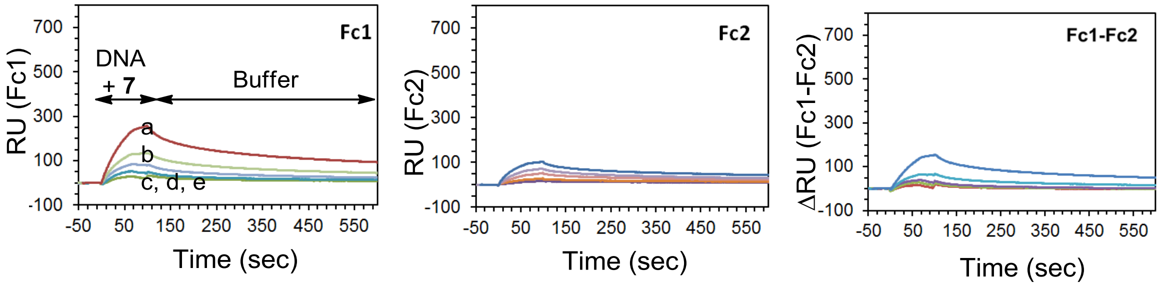
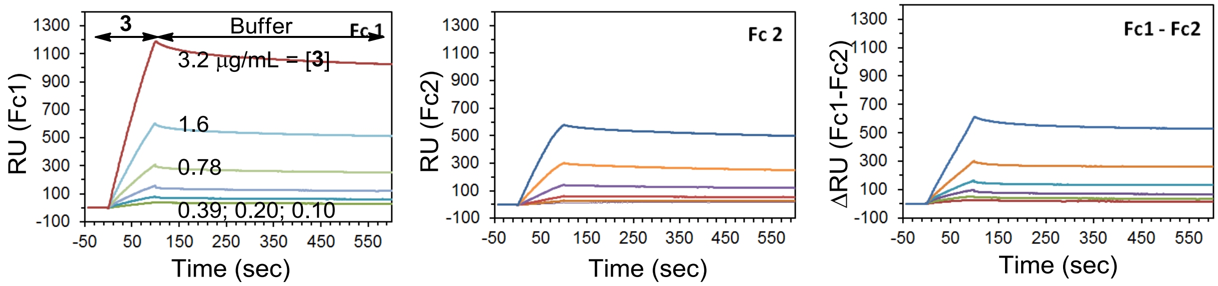
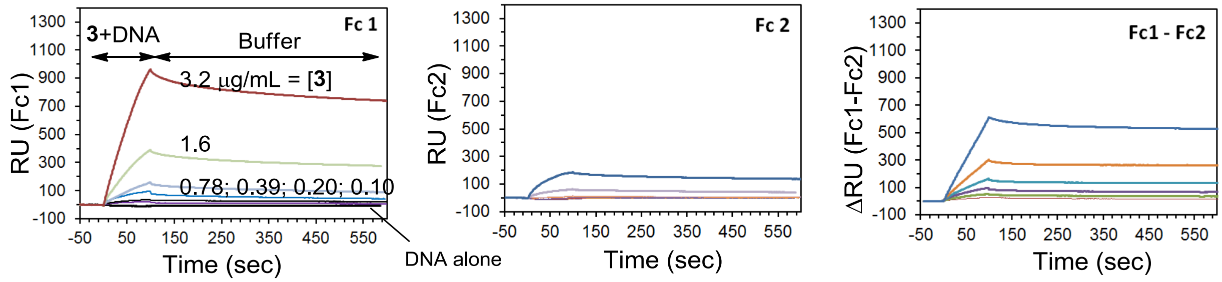


Figure S6. SPR sensorgrams of dendrimer and its polyplex with dsDNA (calf thymus) binding to a CM5 sensor chip immobilized with folate binding protein (FBP). (A) **3** G5(FA)$_{8.6}$ alone; (B) **3** G5(FA)$_{8.6}$ + dsDNA (0.25 μg/mL; fixed); (C) **5** G5(FA)$_{8.6}$(DAPP)$_{5.4}$ alone; (D) **5** G5(FA)$_{8.6}$(DAPP)$_{5.4}$ + dsDNA (0.25 μg/mL; fixed). For definition of RU (Fc1), RU (Fc2) and ΔRU (Fc1 – Fc2), see the legends in Figure S4 and Figure S5.

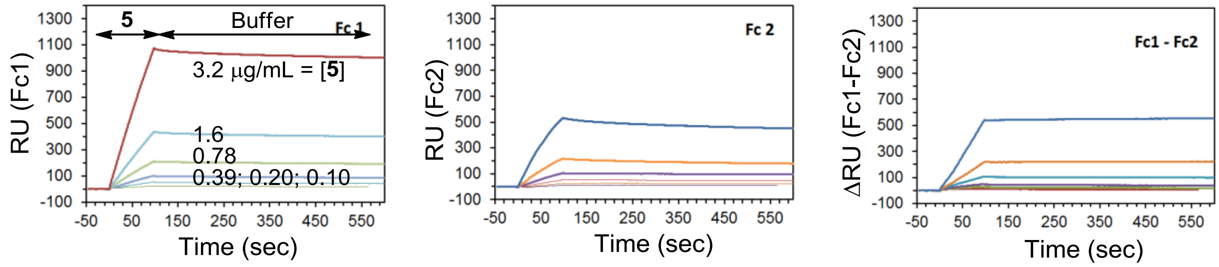
(A) **3** G5(FA)$_{8.6}$



(B) **3** + DNA (fixed at 0.25 µg/mL)



(C) **5** G5(FA)$_{8.6}$(DAPP)$_{5.4}$



(D) **5** + DNA (fixed at 0.25 µg/mL)

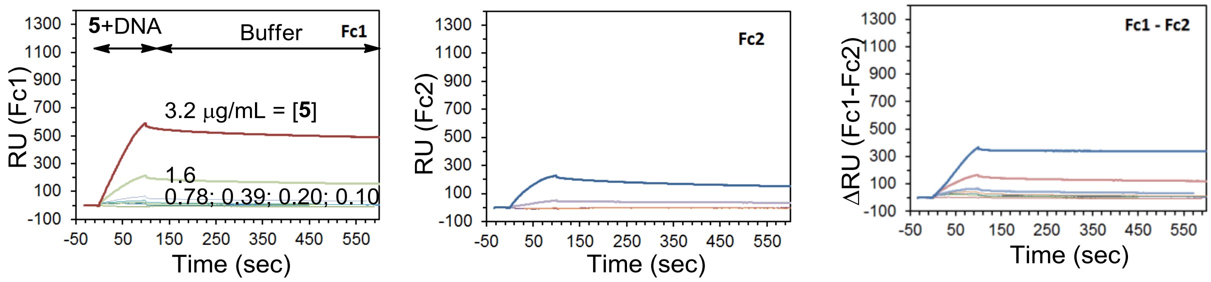


Figure S7. Transfection of luciferase plasmid DNA (pDNA) in KB cancer cells performed in folate- and riboflavin-depleted media. (A) Dose-dependent transfection of luciferase plasmid in complex with TransFast reagent in variable ratio and incubation time (1, 4 h). (B) Transfection of luciferase plasmid in various polyplexes made with dendrimers G5-NH_2, G5(GA) under various dendrimer-to-plasmid DNA (D/P; w/w) ratios and doses. G5(GA) = G5(glutaric acid). RLU = relative light unit.

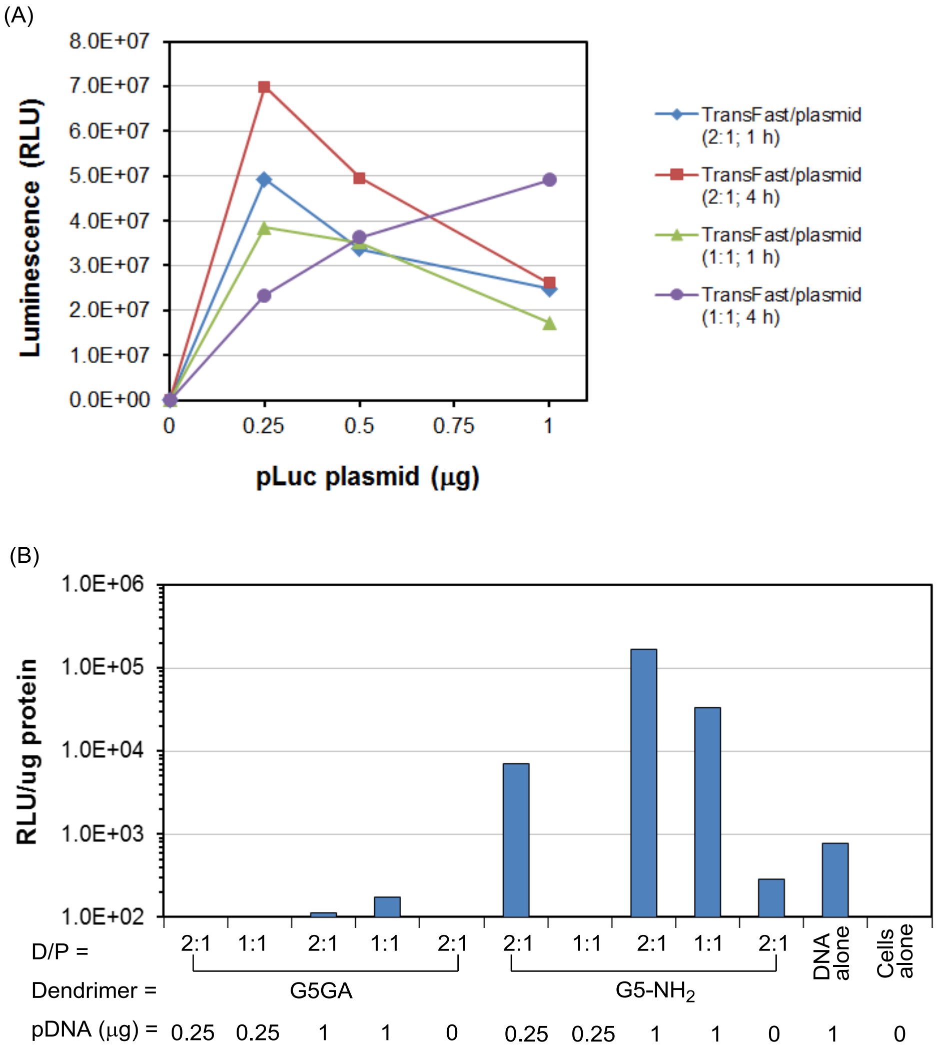


Figure S8. Effect of fetal bovine serum (FBS) on transfection efficiency. Transfection efficiency of luciferase plasmid in various dendrimer polyplexes was measured in the medium without or witih FBS. Inset: expanded portion at lower RLU (relative light unit).

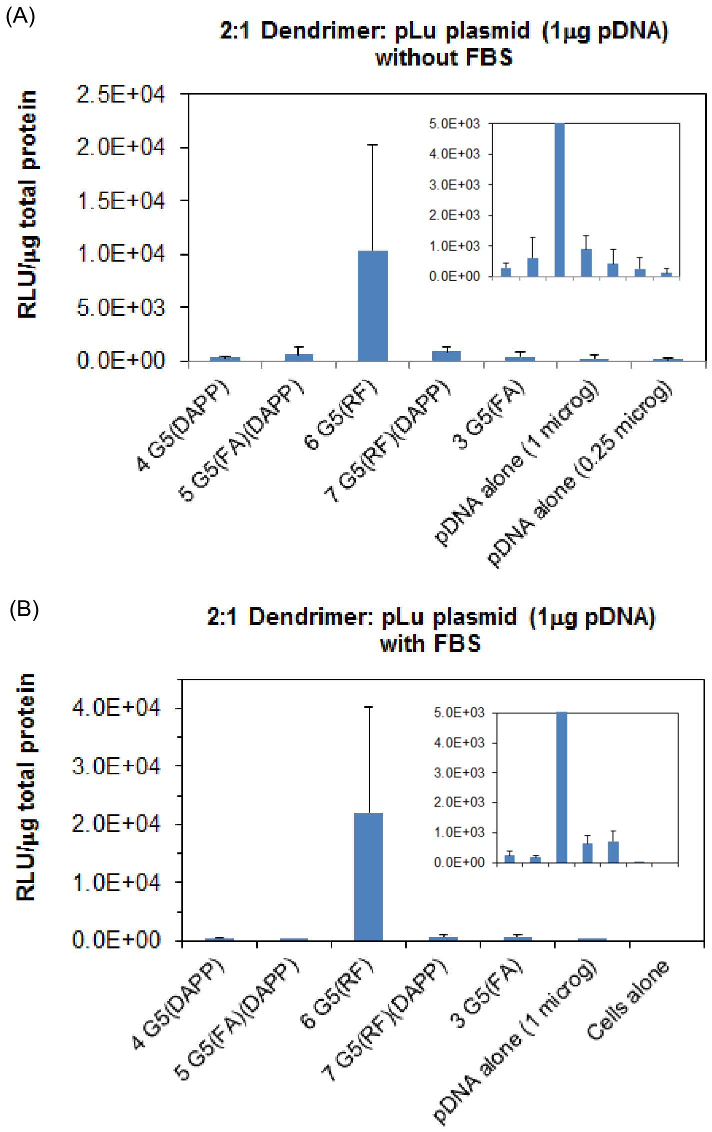


Figure S9. Total cellular protein (μg) measured after transfection of luciferase plasmid DNA (pDNA) and its polyplexes in KB cancer cells. **3** G5(FA)$_{n=8.6}$, **4** G5(DAPP)$_{m=5.4}$, **5** G5(FA)$_{n=8.6}$(DAPP)$_{m=5.4}$, **6** G5(RF)$_{n'=4.9}$, **7** G5(RF)$_{n'=4.9}$(DAPP)$_{m'=6.9}$

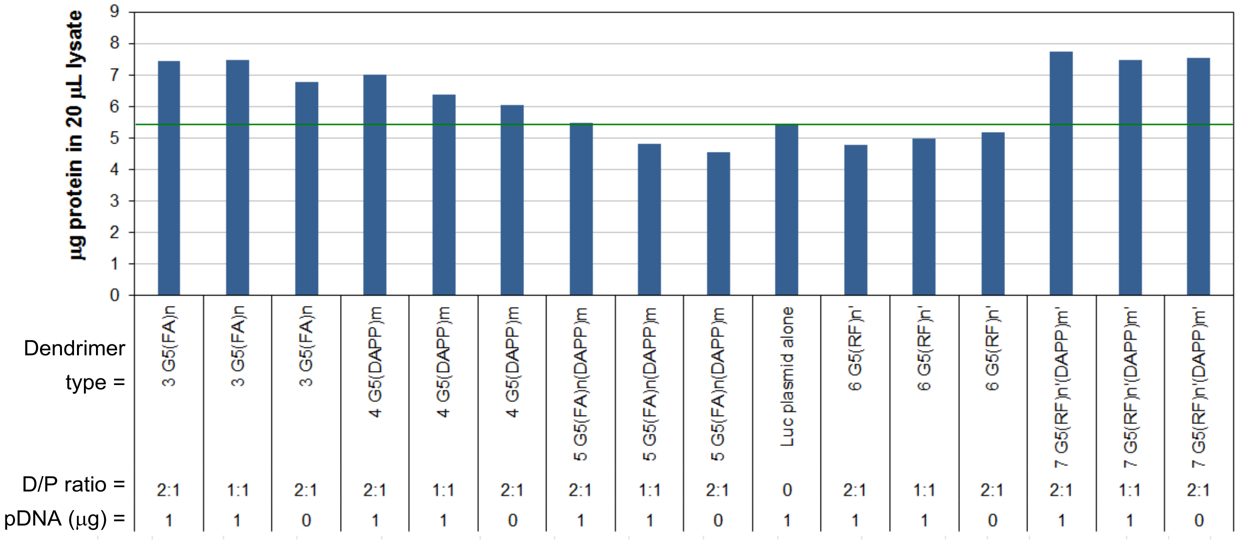


Table S1. Molecular weights and distribution of G5 PAMAM dendrimer conjugates **3**–**7**

Dendrimer Conjugate	M_r (gmol^{-1})[a]	M_w (gmol^{-1})[b]	PDI[c]	Ligand Valency[d]
G5-(NH$_2$)$_{114}$	27,600	26,600	1.01	-
3 G5(FA)$_n$	30,400	nd[e]	nd[e]	n = 8.6
4 G5(DAPP)$_m$	30,200	30,310	1.501	m = 5.4
5 G5(FA)$_n$(DAPP)$_m$	30,500	23,400	1.066	n = 8.6; m = 5.4
6 G5(RF)$_{n'}$	30,100	29,500	1.198	n’ = 4.9
7 G5(RF)$_{n'}$(DAPP)$_{m'}$	30,000	32,300	1.413	n’ = 4.9; m’ = 6.9

[a] measured by matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOFMS), and used for calculating molar concentrations in this study

[b] weight-average molecular weight measured by gel permeation chromatography (GPC)

[c] polydispersity index (PDI) = M_w/M_n (GPC)

[d] mean value determined by UV–vis method

[e] not determined due to insufficient solubility in the GPC eluent (≤4 mg/mL; 0.1 M citric acid, pH 2.7)

Table S2. Hydrodynamic diameter (Z_{ave}, nm) and zeta potential (ZP, mV) of dendrimer conjugates **4**–**7** and the dendrimer polyplexes made with dsDNA (calf thymus) at variable DNA-to-dendrimer ratios (= 0, 0.05, 0.1).

Dendrimer (1 mg/mL)	[DNA], mg/mL	Z_{ave} (d, nm)[a]	PDI[a,b]	ZP (mV)[c]
4 G5(DAPP)$_{5.4}$	0	249.6	0.136	54.6±9.4
	0.05	241.6	0.181	50.7±11.9
	0.1	244.3	0.252	38.4±8.7
5 G5(FA)$_{8.6}$(DAPP)$_{5.4}$	0	118.5	0.191	48.4±8.1
	0.05	130.4	0.292	41.8±6.7
	0.1	133.1	0.302	39.6±6.4
6 G5(RF)$_{4.9}$	0	198.3	0.454	46.7±5.1
	0.05	193.2	0.380	33.1±5.4
	0.1	145.6	0.204	31±5.8
7 G5(RF)$_{4.9}$(DAPP)$_{6.9}$	0	144.1	0.187	52.1±12.7
	0.05	156.4	0.272	45.8±8.6
	0.1	154.8	0.257	40.2±7.3
DNA alone	0.2	1168	1.0	−78.1±3.9

[a] hydrodynamic radius (Z_{ave}) determined by dynamic light scattering (DLS) method

[b] polydispersity index (PDI) determined by DLS method

[c] zeta potential (ZP) measured in HEPES buffer (pH 7.4)

Table S3. Kinetic binding parameters for FA, dendrimers **3** G5(FA)$_{8.6}$, **5** G5(FA)$_{8.6}$(DAPP)$_{5.4}$ and their polyplexes to a CM5 sensor chip surface immobilized with folate binding protein (FBP).

Dendrimer; Polyplex	k_a[a] ($M^{-1}s^{-1}$)	k_d[a] (s^{-1})	K_D[b] (M)	rel avidity[c]	rel mass of adsorption[d]
FA	1.5×10^3	$9.0 (\pm 3.0) \times 10^{-3}$	6×10^{-6}	-	-
3 G5(FA)$_{8.6}$	1.3×10^6	$1.8 (\pm 1.7) \times 10^{-4}$	1.4×10^{-10}	1.0	1.0
3 + dsDNA[e]	5.1×10^6	$6.5 (\pm 3.5) \times 10^{-4}$	1.3×10^{-10}	0.93	0.96 (±0.20)
5 G5(FA)$_{8.6}$(DAPP)$_{5.4}$	1.2×10^6	$2.7 (\pm 2.2) \times 10^{-4}$	2.2×10^{-10}	1.0	1.0
5 + dsDNA[e]	9.5×10^5	$9.3 (\pm 6.9) \times 10^{-4}$	9.8×10^{-10}	0.22	0.86 (±0.27)

[a] mean value (±SD) from serially diluted concentrations ($n \geq 4$)

[b] $K_D = k_d/k_a$ where k_a = association rate constant and k_d = dissociation rate constant

[c] rel avidity = K_D (dendrimer) ÷ K_D (dendrimer + dsDNA)

[d] rel mass of adsorption = ΔRU_{max} (dendrimer + dsDNA) ÷ ΔRU_{max} (dendrimer alone); mean value (±SD) from serially diluted concentrations (n = 6)

[e] calf thymus dsDNA

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