Angewandte Chemie
Eine Zeitschrift der Gesellschaft Deutscher Chemiker

Supporting Information

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Light-Responsive Helical Polypeptides Capable of Reducing Toxicity and Unpacking DNA: Toward Nonviral Gene Delivery**

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Supporting Information

Materials and cell lines:

All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used as received unless otherwise specified. γ-(4-Vinylbenzyl)-$_L$-glutamate NCA (VB-$_L$-Glu-NCA) and $_L$-glutamic acid copper (II) complex were synthesized according to reported procedures[1]. Dry nitrobenzene (NB) was prepared by treating regular nitrobenzene with CaH_2 followed by distillation under reduced pressure. Anhydrous tetrahydrofuran (THF) and hexane were dried by a column packed with alumina. Anhydrous dimethylformamide (DMF) was dried by a column packed with 4Å molecular sieves and stored in a glove box. Plasmid DNA encoding luciferase (pCMV-Luc) and plasmid DNA encoding enhanced green fluorescent protein (pEGFP) was obtained from Elim Biopharm (Hayward, CA, USA). Bright-Glo luciferase assay system and BCA protein assay kit were purchased from Promega (Madison, WI, USA). Lipofectamine 2000, YOYO-1, and 3-(4,5)-dimethylthiahiazo (-z-y1)-3,5-di-phenyltetrazo liumromide (MTT) were purchased from Invitrogen (Carlsbad, CA, USA).

The HeLa cell line (human cervix adenocarcinoma) and COS-7 cell line (African Green Monkey SV40-transf'd kidney fibroblast) were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA) and cultured in Dulbecco's Modified Eagles Medium (DMEM, Grand Island, NY, USA) supplemented with 10% (v/v) fetal bovine serum.

Instrumentation.

^1H and ^{13}C NMR spectra were recorded on a Varian UI400 MHz, a UI500NB MHz or a VXR-500 MHz spectrometer. Chemical shifts are reported in ppm and referenced to the solvent protio impurities and solvent ^{13}C resonances. Gel permeation chromatography (GPC) experiments were performed on a system equipped with an isocratic pump (Model 1100, Agilent Technology, Santa Clara, CA, USA), a DAWN HELEOS multi-angle laser light scattering detector (MALLS) detector (Wyatt Technology, Santa Barbara, CA, USA), and an Optilab rEX refractive index detector (Wyatt Technology, Santa Barbara, CA, USA). The detection wavelength of HELEOS was set at 658 nm. Separations were performed using serially connected size exclusion columns (100 Å, 500 Å, 10^3Å and 10^4 Å Phenogel columns, 5 μm, 300 × 7.8 mm, Phenomenex, Torrance, CA, USA) at 60 °C using DMF containing 0.1 M LiBr as the mobile phase. The MALLS detector was calibrated using pure toluene with no need for calibration using polymer standards and can be used for the determination of the absolute molecular weights (MWs). The MWs of polymers were determined based on the d*n*/d*c* value of each polymer sample calculated offline by using the internal calibration system processed by the ASTRA V software (version 5.1.7.3, Wyatt Technology, Santa Barbara, CA, USA). Infrared spectra were recorded on a Perkin Elmer 100 serial FTIR spectrophotometer calibrated with polystyrene film. Circular dichroism (CD) measurements were carried out on a JASCO J-700 CD spectrometer. The polymer samples were prepared at concentrations of 0.02-0.25 mg/mL in general unless otherwise specified. The solution was placed in a quartz cell with a path length of 0.5 cm. The mean residue molar ellipticity of each polymer was calculated based on the measured apparent

ellipticity according reported formulas: Ellipticity ([θ] in deg·cm^2·dmol^{-1}) = (millidegrees × mean residue weight)/(path length in millimeters × concentration of polypeptide in mg ml^{-1}). The helicity of the polypeptides was calculated by the following equation: helicity = (−[θ_{222}] + 3,000)/39,000. [2] Ozone was produced by an OZV-8S ozone generator manufactured by Ozone Solutions Inc (Hull, IA, USA). Lyophilization was performed on a FreeZone lyophilizer (Labconco, Kansas City, MO, USA). UV light (365 nm) was generated from an OmiCure S1000 UV lamp (EXFO, Mississauga, Canada). NIR light pulses (70 fs) was generated from a Ti:Sapphire laser system (Spectra-physics Tsunami®, Mai Tai® DeepSee™) that has a maximum output power of 2.4 W, working at continuous wave mode at 750 nm and repetition rate of 80 MHz. Polypeptide solution was placed in a quartz curvette while cells were grown on 384-well plates, and was irradiated with NIR light (750 nm, laser power 2 W, and beam spot size of 1 mm in diameter) at 3.2 μJ/cm^2/pulse.

Methods:

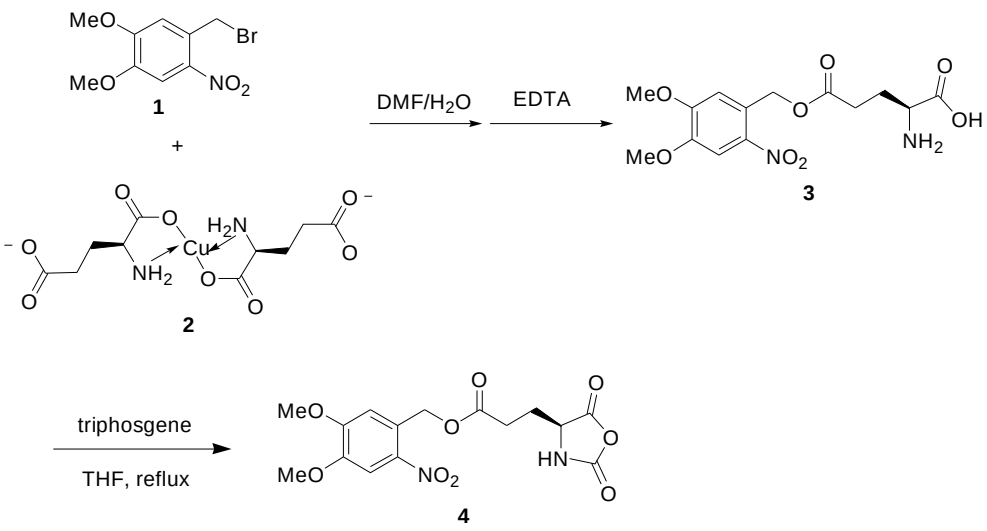
Synthesis of γ-4,5-dimethoxy-2-nitrobenzyl-$_L$-glutamate (DMNB-$_L$-Glu, 3)

In a 300-mL round bottom flask, *N*,*N*,*N'*,*N'*-tetramethylguanidine (1.1 mL, 87 mmol) was added slowly to a stirred mixture of $_L$-glutamic acid (0.64 g, 4.3 mmol) and $_L$-glutamic acid copper (II) complex (1.05 g, 2.14 mmol) in DMF (4 mL) and distilled water (0.6 mL). The mixture turned to dark blue. After all solids were dissolved (~ 2h), additional DMF (3.1 mL) was added. Then, 4,5-dimethoxy-2-nitro-1-bromomethylbenzene (DMNB-Br, 2.5 g, 9 mmol) was added to the above solution in one portion. The reaction solution became darker and was kept at 40 ^{o}C for 38 h. After that, acetone (100 mL) was added to the mixture and stirred until a fine precipitate was obtained (~1 h). The violet solid was collected by filtration, followed by addition of freshly prepared EDTA (2 g)/sodium bicarbonate (1 g) aqueous solution (15 mL) and further stirring for 24 h. The product was collected by filtration and washed with DI water. Further purification was performed by recrystallizing from H_2O/isopropanol. Lyophilization yielded the purified product as a yellow crystalline solid. Obtained 1.51 g (yield: 49%). ^{1}H NMR [D_2O/DCl (1wt%), δ, ppm]: 7.52 (s, 1H, ArH), 6.90 (s, 1H, ArH), 5.21 (s, 2H, ArC*H*$_2$-), 3.95 (t, 1H, -C*H*NH$_2$), 3.74 (s, 3H, CH_3O-), 3.70 (s, 3H, CH_3O-), 2.55 (t, 2H, -COC*H*$_2$CH$_2$-), 2.08 (m, 2H, -COCH$_2$C*H*$_2$-).

Synthesis of γ-4,5-dimethoxy-2-nitrobenzyl-$_L$-glutamic acid-based *N*-carboxylanhydride (DMNB-$_L$-Glu-NCA, 4)

DMNB-$_L$-Glu (1.51 g, 4.4 mmol) and triphosgene (0.66 g, 0.22 mmol) were dispersed in anhydrous THF (30 mL) in a round-bottomed flask. The reaction mixture was heated up to reflux temperature and then removed from heat when the mixture became clear. THF was distilled at room temperature under vacuum. The yellow solid residue was purified by recrystallization from THF/hexane (v/v=1/3). The product was obtained as a yellowish needle-like crystalline solid (obtained 1.0 g, yield: 62%). ^{1}H NMR ($CDCl_3$, δ, ppm): 7.69 (s, 1H, ArH), 6.97 (s, 1H, ArH), 6.42 (s, 1H, NH), 5.49 (m, 2H, ArC*H*$_2$-), 4.46 (t, 1H, -C*H*NH), 4.00 (s, 3H, CH_3O-), 3.96 (s, 3H, CH_3O-), 2.65 (t, 2H, -COC*H*$_2$CH$_2$-), 2.10-2.35 (m, 2H, -COCH$_2$C*H*$_2$-). ^{13}C NMR ($CDCl_3$, δ,

ppm): 172.13, 169.51, 153.71, 151.72, 148.88, 140.52, 125.81, 111.62, 108.57, 64.43, 56.98, 56.77, 56.67, 29.80, 27.07.



Scheme S1. The synthetic route of γ-4,5-dimethoxy-2-nitrobenzyl-$_L$-glutamic acid-based *N*-carboxylanhydride (**4**).

Synthesis of poly(γ-4,5-dimethoxy-2-nitrobenzyl-$_L$-glutamate)-*r*-poly(γ-vinylbenzyl-$_L$-glutamate) (PDMNBLG-*r*-PVBLG) (6)

A representative copolymerization of VB-$_L$-Glu-NCA and DMNB-$_L$-Glu-NCA is as follows: Inside a glove-box, VB-$_L$-Glu-NCA (116 mg, 0.4 mmol) and DMNB-$_L$-Glu-NCA (37 mg, 0.1 mmol) were dissolved in the mixture of DMF (2.0 mL) and nitrobenzene (100 μL), followed by addition of HMDS solution in DMF (25.0 μL, 0.1 M, M/I = 200). FTIR was used to monitor the polymerization until the conversion was above 99% (~48 h). The resulting polymer was precipitated from cold methanol and collected by centrifuge. Obtained 106 mg (yield: 80%). ^{1}H NMR ($CDCl_3$/TFA-*d*, *v* : *v* = 80 : 20, δ, ppm): 7.31 (s, 2H, ArH), 7.19 (d, 2.7H, ArH), 6.96 (m, 0.7H, ArH), 6.61 (t, 1H, CH_2=C*H*-), 5.69 (d, 1H, CH_2=CH-), 5.38 (d, 1.4H, ArCH_2-), 5.21 (d, 1H, CH_2=CH-), 5.05 (s, 2H, ArCH_2-), 4.63 (s, 1.7H, -C*H*NH), 3.94 (s, 2.1H, CH_3O-), 3.92 (s, 2.1H, CH_3O-), 2.49 (s, 3.4H, -COCH_2CH_2-), 1.80-2.30 (d, 3.4H, -$COCH_2CH_2$-).

Synthesis of poly(γ-4,5-dimethoxy-2-nitrobenzyl-$_L$-glutamate)-*r*-poly(γ-adehydobenzyl-L-glutamate) (PDMNBLG-*r*-PABLG) (7) and PDMNBLG-*r*-PVBLG-8 (8)

A representative side-chain modification of PDMNBLG-*r*-PVBLG was as follows: The copolymer (68 mg, 0.17 mmol of vinyl groups) was reacted with benzyl chloroformate (>100 equiv.) in chloroform (60 mL) at room temperature for two hours to protect the end groups. The solution was then cooled to -78 ^{o}C. O_2 was bubbled into the mixture for 1 min followed by bubbling of O_3 until the solution became blue, indicating the reaction was completed. O_3 was then replaced by O_2 and bubbled into the solution for another 2 min until the solution became colorless. The solution was then degassed and back filled with nitrogen. Ph_3P (262 mg, 1.0 mmol)

was then added to the mixture and stirred at room temperature for 2-3 h, after which the solvent was removed under vacuum. The resulting polymer (**7**) was purified by stirring in methanol (3 × 20 mL) to remove unreacted Ph_3P and other impurities. Obtained 62 mg (yield: 90%). ^{1}H NMR ($CDCl_3$/TFA-*d*, *v* : *v* = 80 : 20, δ, ppm): 9.82 (s, 1H, -CHO), 7.87 (d, 2H, ArH), 7.66 (m, 0.7H, ArH), 7.43 (d, 2H, ArH), 6.94 (m, 0.7H, ArH), 5.38 (d, 1.4H, ArCH_2-), 5.16 (s, 2H, ArCH_2-), 4.63 (s, 1.7H, -C*H*NH), 3.92 (s, 2.1H, CH_3O-), 3.90 (s, 2.1H, CH_3O-), 2.56 (s, 3.4H, -COCH_2CH_2-), 1.80-2.30 (d, 3.4H, -$COCH_2CH_2$-). The resulting polymer (62 mg, 0.15 mmol of aldehyde groups) was then reacted with 1-(2-aminoethyl)piperidine (0.20 g, 1.5 mmol, 10 eqv.) in DMF (4 mL) at 50 ^{o}C for 24 h. Borane pyridine (0.2 mL, 1.5 mmol 10 eqv.) was then added and the solution was stirred at 50 ^{o}C for another 24 h. A 3 M HCl solution was added to the above DMF solution and then dialyzed against DI water (MWCO=8 kDa). Lyophilization yielded the solid products. Obtained 30 mg (yield: 45%). ^{1}H NMR (D_2O, δ, ppm): 6.80-7.45 (br d, 5.4H, ArH), 4.84 (br s, 3.4H, $ArCH_2O$-), 4.32 (br s, 1.7H, -C*H*NH), 4.13 (br s, 2H, ArCH2NH-), 3.43 (s, 2.1H, CH_3O-), 3.38 (s, 2.1H, CH_3O-), 3.08 (s, 2H, -HNCH_2CH_2N-), 2.77 (s, 2H, -$HNCH_2CH_2$N-), 1.00-2.50 (br m, 14H, -$COCH_2CH_2$- and –$NCH_2CH_2CH_2$-).

Synthesis of rhodamine-labeled PDMNBLG-*r*-PVBLG-8

PDMNBLG-*r*-PABLG (20.6 mg, 0.05 mmol) was first reacted with *N*-(6-aminohexyl)rhodamine 6G-amide bis(trifluoroacetate) (3.9 mg, 5 μmol, 10 mol% equiv) in DMF (2 mL) at 50 °C for 24 h in dark. 1-(2-Aminoethyl)piperidine (0.07 g, 0.5 mmol, 10 equiv) was then added and allowed to react at 50 °C for another 24 h in dark. The product was then reduced by borane pyridine, acidified by HCl, and dialyzed against DI water as mentioned above. The conjugation efficiency of rhodamine was determined to be 2~4 mol% for all polymers (P10, P20, P30, and P40).

Photo-responsiveness of polypeptides

Polypeptides were dissolved in water (1 mg/mL) and transferred to a quartz cuvette before irradiation with UV light (365 nm, 20 mW/cm^2) for specific periods of time. The UV-Vis spectra were recorded after each irradiation to demonstrate the photo-triggered side chain cleavage. CD spectra of non-irradiated and UV-irradiated polypeptides were also recorded to evaluate the alteration in secondary structure.

Preparation and characterization of polypeptide/DNA complexes

Polypeptides and pCMV-Luc were dissolved in deionized water at 1 mg/mL. Complexes were allowed to form by addition of the polypeptide solution to pCMV-Luc at different N/P ratios followed by vortex for 30 s and incubation at 37 °C for 30 min. The suspension was subject to assessment of particle size and Zeta potential on a Malvern Zetasizer. To evaluate the light-responsiveness, complexes were UV-irradiated for 10 min (20 mW/cm^2) before assessment of particle size and Zeta potential.

Ethidium bromide (EB) exclusion assay

Complexes were formed and UV-irradiated as described above. EB solution was added to the complex suspension at DNA/EB ratio of 10:1 (w/w), and the mixture was incubated at RT for 30 min before quantification of fluorescence intensity on a microplate reader (λ_{ex}=510 nm, λ_{em}=590 nm). A pure EB solution and the DNA/EB solution without any polypeptide were used as negative and positive controls, respectively. The DNA condensation efficiency (%) was defined as:

$$\text{DNA condensation efficiency (\%)} = (1 - \frac{F - F_{EB}}{F_0 - F_{EB}}) \times 100$$

Where F_{EB} and F_0 denote the fluorescence intensity of pure EB solution and the DNA/EB solution without any polypeptide, respectively.

Heparin replacement assay

Complexes were formed and UV-irradiated as described above, and they were incubated with heparin (0.5 mg/mL) at 37 °C for 1, 4, and 12 h, respectively. EB solution was then added to the solution at the DNA/EB ratio of 10:1 (w/w), and the fluorescence intensity was measured by spectrofluorimetry (λ_{ex}=510 nm, λ_{em}=590 nm). The DNA release rate (%) was calculated as mentioned above.

Membrane disruption/destabilization towards light irradiation

The membrane disruption capacity of the polypeptides was evaluated in terms of the cell uptake of membrane-impermeable dye, fluorescein isothiocyanate (FITC) in its non-reactive form. FITC was first dissolved in DMSO and diluted with Tris buffer (0.2 M, pH 7.4) to make the stock solution, which was kept in dark at room temperature overnight. In such case, the FITC (ESI: calcd $C_{21}H_{12}NO_5S$ $[M+H]^+$ *m*/*z* 390.39, found 390.1) would have been converted to fluorescein-tris(hydroxymethyl)methanethiourea evidenced by the MS analysis (ESI: calcd $C_{25}H_{23}N_2O_8S$ $[M+H]^+$ *m*/*z* 511.11, found 511.12) (abbreviated as FITC-Tris), which serves a biomarker for the study of membrane pore formation.[3] HeLa cells and COS-7 cells were seeded in 24-well plate at 5×10^4 cells/well and incubated for 24 h. The medium was changed to serum-free DMEM, into which non-irradiated P20 or UV-irradiated P20 (20 mw/cm^2, 10 min) was added at 5 μg/well and FITC was added at 1 μg/well. After incubation for 2 h, cells were washed with cold PBS for three times and then lysed with the RIPA lysis buffer. FITC content in the cell lysate was quantified by spectrofluorimetry, and protein content was determined using the BCA kit. Cells incubated with free FITC in the absence of polypeptide served as the control. The uptake level was expressed as ng FITC associated with 1 mg of cellular protein.

To visualize the FITC uptake and intracellular distribution, cells were seeded on coverslips in 6-well plate at 5×10^4 cells/well and incubated for 24 h. The medium was changed to serum-free DMEM, into which P20 (non-irradiated or UV-irradiated) was added at 20 μg/well and FITC was added at 4 μg/well. After incubation for 2 h, cells were washed with cold PBS for three times and fixed with 4% paraformaldehyde for 10 min at RT. Cells were further washed with PBS for three times and the nuclei were stained with DAPI (5 μg/mL) for 10 min. After three more PBS

washes, the coverslips were embedded in Prolong Gold® (Invitrogen) and cells were visualized by CLSM.

Cytotoxicity of polypeptides

HeLa cells and COS-7 cells were seeded in 96-well plate at 1×10^4 cells/well (100 μL media/well) and incubated for 24 h. Polypeptides (non-irradiated or UV-irradiated at 20 mW/cm^2 for 10 min) were added at 10, 5, 2, 0.5, and 0.2 μg/well, respectively. After incubation for 4 h, the medium was replaced by fresh serum-containing DMEM and cells were further incubated for 20 h. Cell viability was then evaluated via the MTT assay. Cells without polypeptide treatment served as the control and results were expressed as percentage viability of control cells.

To simulate the transfection process, cytotoxicity of the polypeptide/DNA complexes (N/P ratio of 20) were also monitored. Briefly, cells in 96-well plates were incubated with complexes at 0.5 μg DNA/well for 4 h in 100 μL media/well. The media were subsequently replaced by fresh serum-containing DMEM, and cells were irradiated with UV light (20 mW/cm^2) for 5 min at room temperature. Both UV-irradiated cells and non-irradiated cells were further cultured for 20 h before viability assessment using the MTT assay.

Cell uptake of complexes

DNA (1 mg/mL) was labeled with YOYO-1 (20 μM) at one dye molecule per 50 bp DNA[4]. The resultant YOYO-1-DNA was then allowed to form complexes with the polypeptide at the N/P ratio of 20:1 as described above. Lipofectamine 2000/DNA complexes (weight ratio of 2.5) were formed according to manufacturer's protocol.

HeLa and COS-7 cells were seeded on 96-well plates at 1×10^4 cells/well, and cultured in serum-containing DMEM for 24 h to reach confluence. The media was then replaced by fresh serum-free DMEM and complexes were added at 0.1 μg YOYO-1-DNA/well. After incubation at 37 °C for 4 h, the medium was aspirated and cells were washed with PBS three times before lysis with 100 μL of RIPA lysis buffer. YOYO-1-DNA content in the lysate was quantified by spectrofluorimetry (λ_{ex}=485 nm, λ_{em}=530 nm) and protein content was measured using the BCA kit. Uptake level was expressed as ng YOYO-1-DNA per μg of protein. To evaluate the time-resolved cell internalization, complexes were incubated with cells for 0.5, 1, 2, 3, 4, 6 and 8 h before analysis of the uptake level of YOYO-1-DNA. To explore the mechanism involved in cell internalization, cells were pre-incubated with endocytosis inhibitors including sodium azide (10 mM)/deoxyglucose (50 mM), chlorpromazine (10 μg/mL), genistein (100 μg/mL), methyl-β-cyclodextrin (mβCD, 5 mM), and wortmannin (10 μg/mL) for 30 min prior to complex addition and throughout the 4-h uptake experiment at 37 °C. Results were expressed as percentage uptake of the control where cells were incubated with complexes at 37 °C for 4 h.

CLSM observation on the intracellular complex unpackaging

To visualize the intracellular unpackaging of polypeptide/DNA complexes, RhB-polypeptide and YOYO-1-DNA were allowed to form complexes at the N/P ratio of 20 and incubated with HeLa cells as described above. Following incubation for 4 h, the medium was refreshed by

serum-containing DMEM and cells were irradiated with UV light (365 nm, 20 mW/cm^2) for 5 min before further incubation for another 2 h. Cells were then washed with cold PBS, fixed with 4% paraformaldehyde, stained with DAPI (1 μg/mL), and subjected to observation using CLSM (LSM700, Zeiss).

Subcellular distribution of plasmid DNA

For further assessment of the nucleic and cytoplasmic distribution of DNA, an image-based quantification method was adopted[5]. Briefly, polypeptide/YOYO-1-DNA complexes (N/P ratio of 20) were incubated with HeLa cells in serum-free DMEM for 4 h, after which the cells were washed and the media was replaced by serum-containing DMEM. Cells were then irradiated with UV light (20 mW/cm^2) for 5 min before further incubation for 2 h. The cells were subsequently visualized by CLSM and the total pixel area for clusters of YOYO-1-DNA in the nucleus or the cytoplasm in each cell was calculated to determine the subcellular fraction of YOYO-1-DNA.

***In vitro* transfection**

HeLa and COS-7 cells were seeded on 96-well plates at 1×10^4 cells/well and incubated for 24 h prior to transfection studies. The media was replaced by fresh DMEM containing 10% FBS (100 μL), into which polypeptide/DNA complexes (N/P ratio of 20:1) were added at 5 μg DNA/mL. After incubation for 4 h, the medium was replaced by fresh medium and cells were irradiated by UV light (365 nm, 20 mW/cm^2) for 5 min. Subsequently, cells were allowed to be cultured for another 20 h before assessment of luciferase expression and protein content. Results were expressed as relative luminescence unit (RLU) associated with 1 mg of protein. Lipofectamine 2000/DNA complexes (weight ratio of 2.5) were formed according to manufacturer's protocol.

pEGFP was used as another DNA to monitor the transfection efficiency. Cells were seeded on 24-well plates at 5×10^4 cells/well and incubated for 24 h prior to transfection studies. The media was replaced by fresh DMEM containing 10% FBS (500 μL), into which polypeptide/DNA complexes (N/P ratio of 20:1) were added at 5 μg DNA/mL. After incubation for 4 h, the medium was replaced by fresh medium and cells were irradiated by UV light (365 nm, 20 mW/cm^2) for 5 min. Subsequently, cells were allowed to be cultured for another 44 h before assessment of GFP expression by flow cytometry.

For the evaluation of NIR-responsive gene transfection, HeLa cells were seeded on 384-well plates at 3×10^3 cells/well (30 μL/well), incubated for 24 h, and treated with polypeptide/DNA complexes (N/P ratio of 20:1) at 5 μg DNA/mL for 4 h. The medium was replaced by fresh medium and cells were irradiated by NIR light (750 nm, 3.2 μJ/cm^2/pulse) for 1.5 h. The gene transfection efficiency was measured 20 h later as described above.

Statistical analysis

Statistical analysis was performed using Student's t-test and differences were judged to be significant at $*p<0.05$ and highly significant at $**p<0.01$.

Supplementary Figures

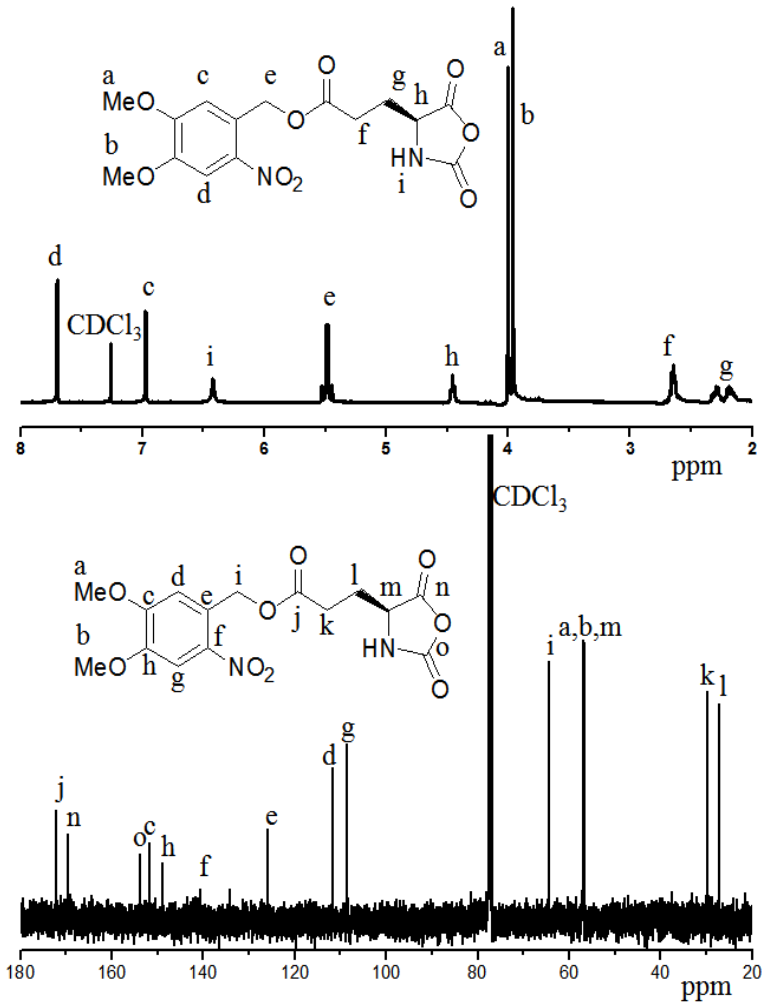


Figure S1. ^{1}H NMR (upper) and ^{13}C NMR (lower) spectra of DMNB-$_{L}$-Glu-NCA (**4**) in $CDCl_3$.

γ-4,5-Dimethoxy-2-nitrobenzyl-$_{L}$-glutamic acid based *N*-carboxylanhydride (DMNB-$_{L}$-Glu-NCA) was synthesized by a multi-step synthetic route, including the mono-esterification between $_{L}$-glutamic acid copper (II) complex and 4,5-dimethoxy-2-nitro-1-bromomethylbenzene, and cyclization of γ-4,5-dimethoxy-2-nitrobenzyl-$_{L}$-glutamic acid in the presence of triphosgene (Scheme S1). DMNB-$_{L}$-Glu-NCA was purified by recrystallization from anhydrous THF/hexane for three times to yield a light yellow crystalline product with high purity. The molecular structure of DMNB-$_{L}$-Glu-NCA was verified by ^{1}H NMR and ^{13}C NMR (Figure S1).

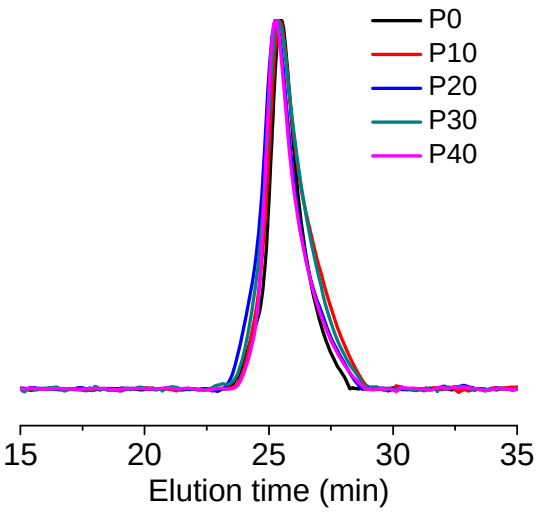


Figure S2. GPC curves of PVBLG-8 (P0) and PDMNBLG-*r*-PVBLG with different PDMNBLG contents.

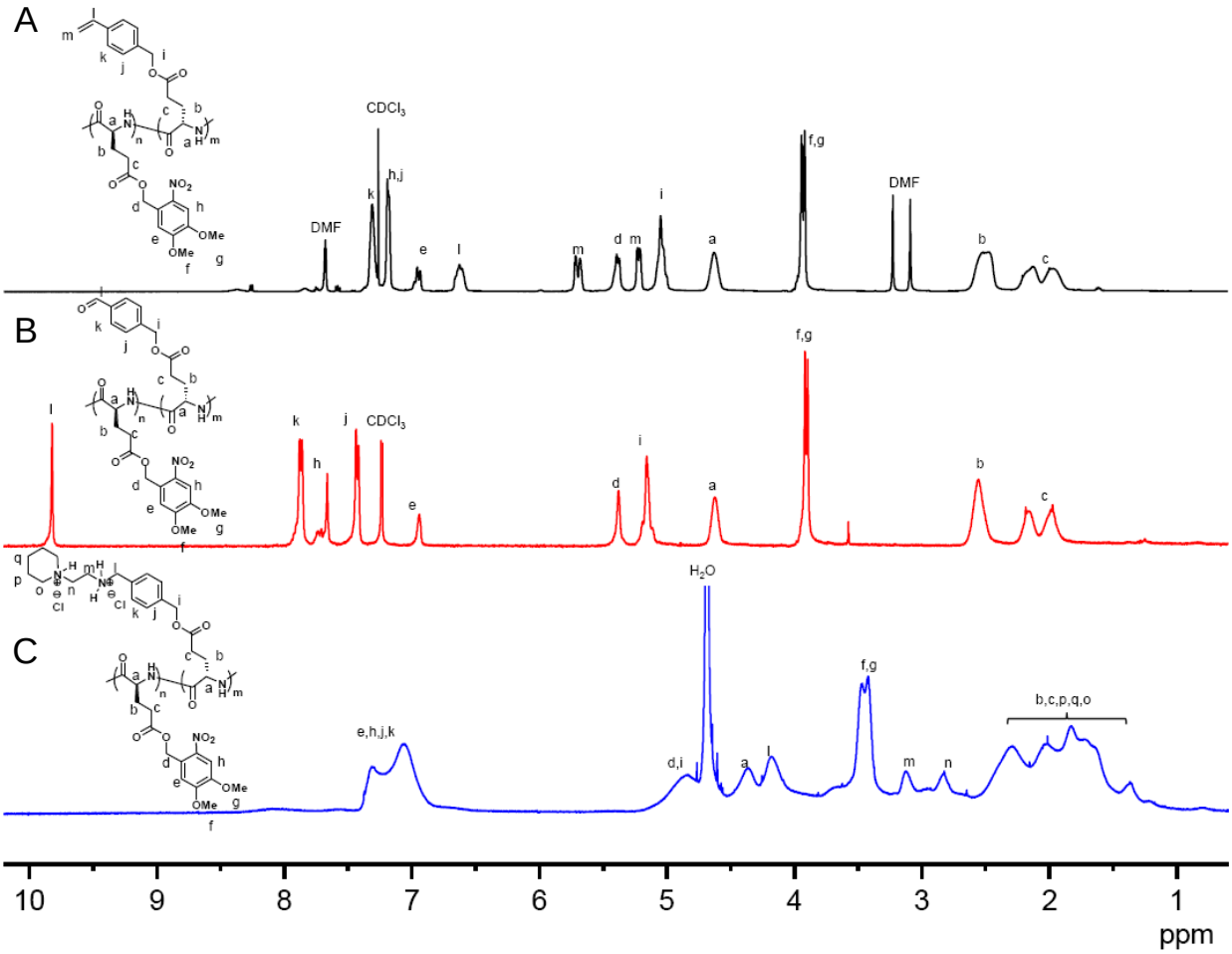


Figure S3. ^{1}H NMR spectra of PDMNBLG-*r*-PVBLG (A), PDMNBLG-*r*-PABLG in $CDCl_3$/TFA-*d* (v/v=80/20) (B), and PDMNBLG-*r*-PVBLG-8 in D_2O (C).

PDMNBLG-*r*-PVBLG was further derivatized into poly(γ-4,5-dimethoxy-2-nitrobenzyl -$_L$-glutamate)-*r*-poly(γ-adehydobenzyl-$_L$-glutamate) (PDMNBLG-*r*-PABLG) by oxidation with ozone and reduction in the presence of Ph_3P in chloroform at -78 ^{o}C. ^{1}H NMR analysis of the resulting PDMNBLG-*r*-PABLG revealed a quantitative conversion, as evidenced by the disappearance of the vinyl peak at 6.61 ppm and the appearance of the aldehydo peak at 9.82 ppm (Figure S3B). Quantitative aldehydo substitution was observed for the copolymers.

The aldehydo groups on the PDMNBLG-*r*-PABLG side-chains were orthogonally derivatized via the amine-aldehyde reaction, followed by reaction with borane-pyridine complex to yield PDMNBLG-*r*-PVBLG-8 with cationic charges on the side-chains (Scheme 1B). In the ^{1}H NMR spectrum of PDMNBLG-*r*-PVBLG-8 (Figure S3C), the disappearance of the aldehydo groups indicated a high grafting efficiency.

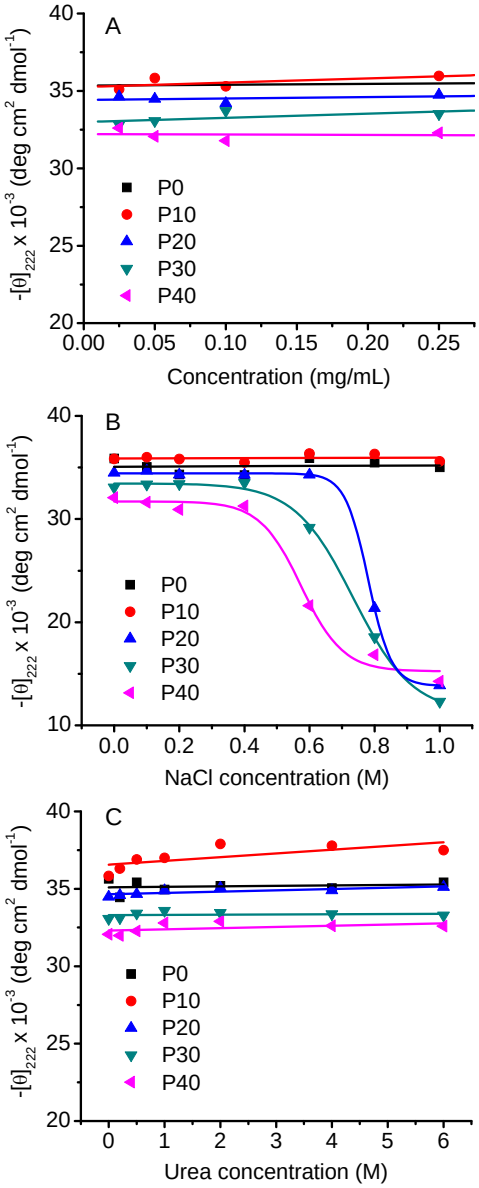


Figure S4. Effects of polypeptide concentration (A), urea concentration (B), and salt concentration (C) on the residue molar ellipticity at 222 nm for PDMNBLG-*r*-PVBLG-8.

PDMNBLG-*r*-PVBLG-8 showed concentration-independent helicity (Figure S4A), suggesting that it remained monomeric in aqueous solution. Additionally, the copolymers exhibited remarkable stability against strong denaturing reagent (urea) (Figure S4C), and they showed stable helical structure when the NaCl concentration was increased from 0 to 0.4 M (Figure S4B). With these observations, it was assumed that PDMNBLG-*r*-PVBLG-8 would ideally maintain its stable helical structure under physiological conditions.

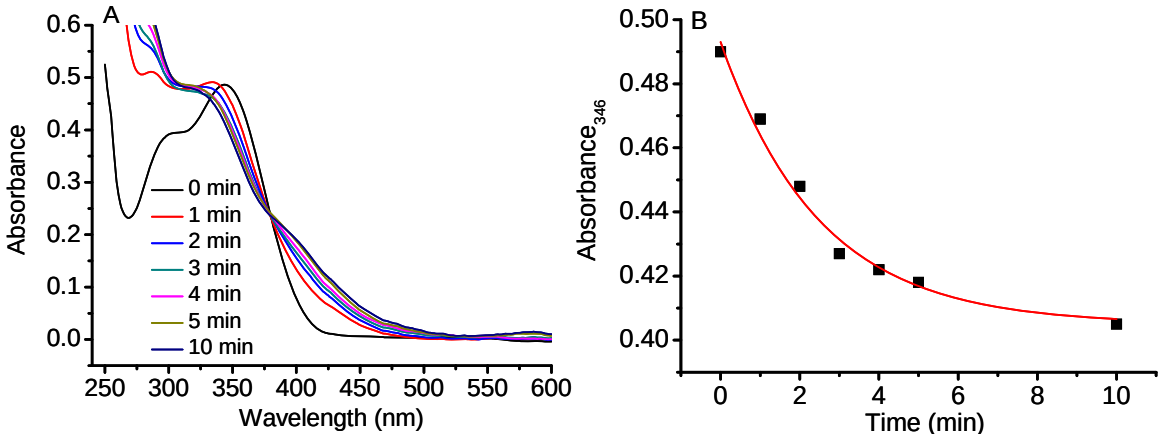


Figure S5. UV/Vis spectra of P40 (1 mg/mL) upon irradiation with UV light (λ=365 nm, 20 mW/cm^2) (A). OD_{346} of P40 following UV irradiation (B).

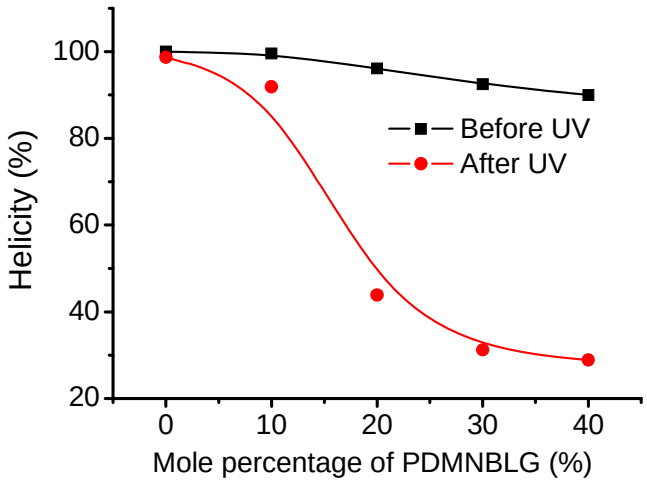


Figure S6. The helicity of PDMNBLG-*r*-PVBLG-8 with various PDMNBLG contents (10-40 mol%) before and after UV irradiation (20 mW/cm^2, 10 min).

The helicity of the PDMNBLG-*r*-PVBLG-8 decreased after UV-irradiation for 10 min while the control homopolypeptide (P0) without PDMNBLG showed no change after the same treatment (Figure S6). The helicity reduction was slight for P10 while was remarkable for P20, P30, and P40 that contained adequate amount of the responsive PDMNBLG moiety.

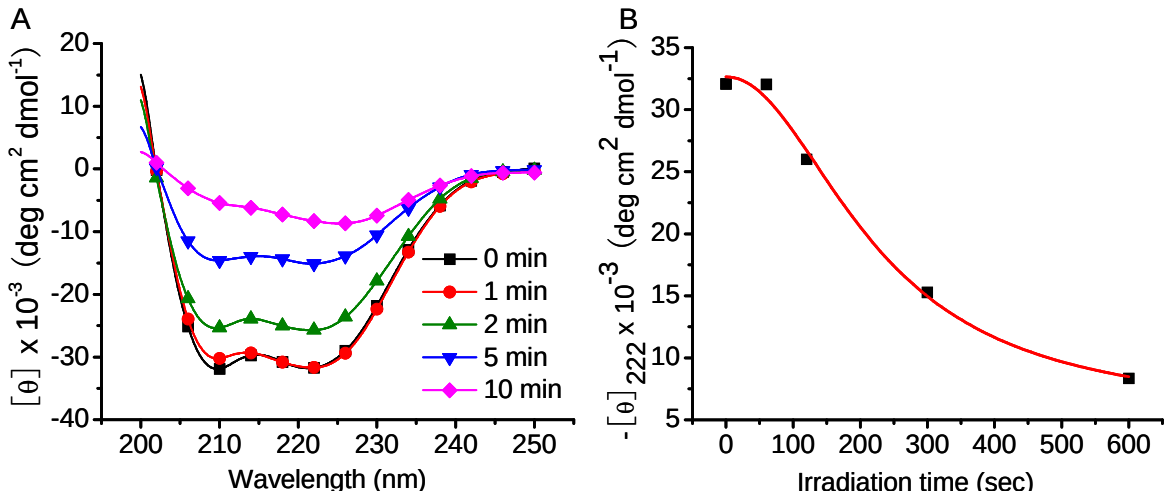


Figure S7. CD spectra (A) and residual molar ellipticity at 222 nm (B) of P40 following UV irradiation (20 mW/cm^2) for different time.

Kinetic studies on the UV-triggered helix alteration were performed with P40 as the representative. An increase in the irradiation time led to significant reduction in the residue molar ellipticity, and following 10-min irradiation, the polypeptide lost majority of its helicity (Figure S7). Further prolonging the irradiation time did not induce further decrease in the polypeptide helicity (data not shown).

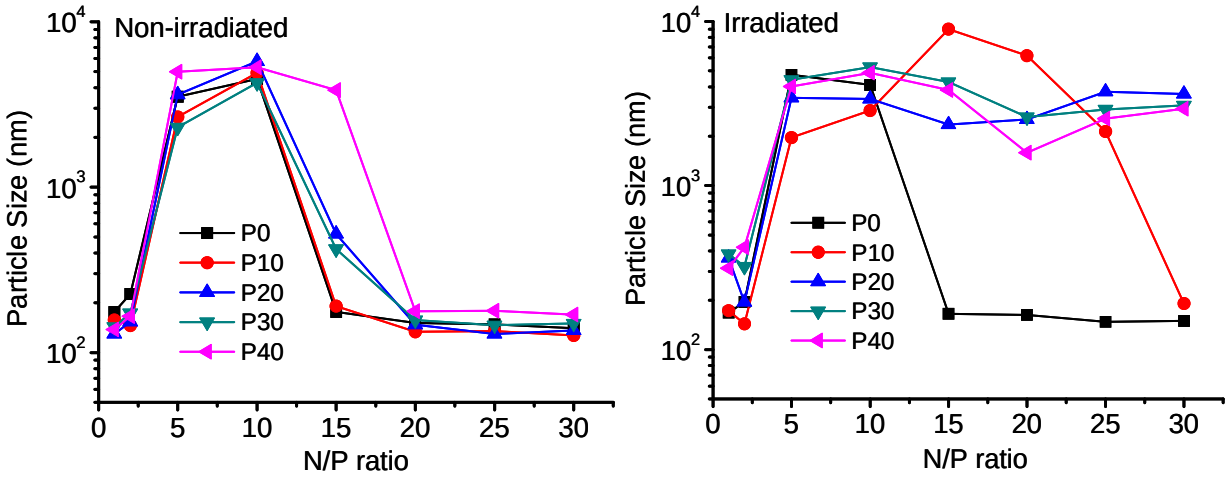


Figure S8. Particle size of polypeptide/DNA complexes in DI water before and after UV-irradiation (20 mW/cm^2, 10 min).

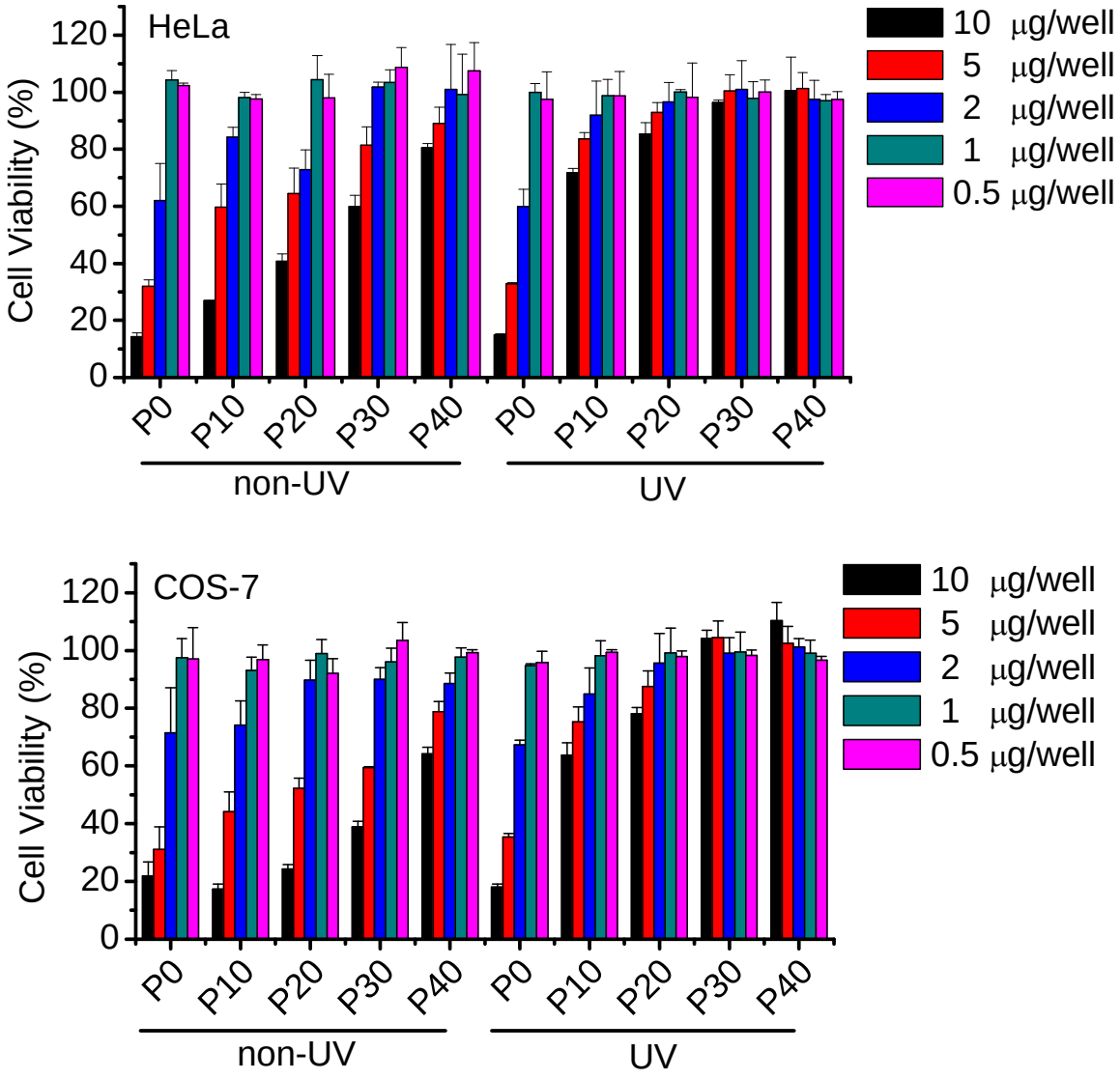


Figure S9. Cytotoxicity of non-irradiated and UV-irradiated polypeptides towards HeLa and COS-7 cells as determined by the MTT assay (n=3). Polypeptides (1 mg/mL) were irradiated for 10 min (365 nm, 20 mW/cm^2) before being incubated with cells.

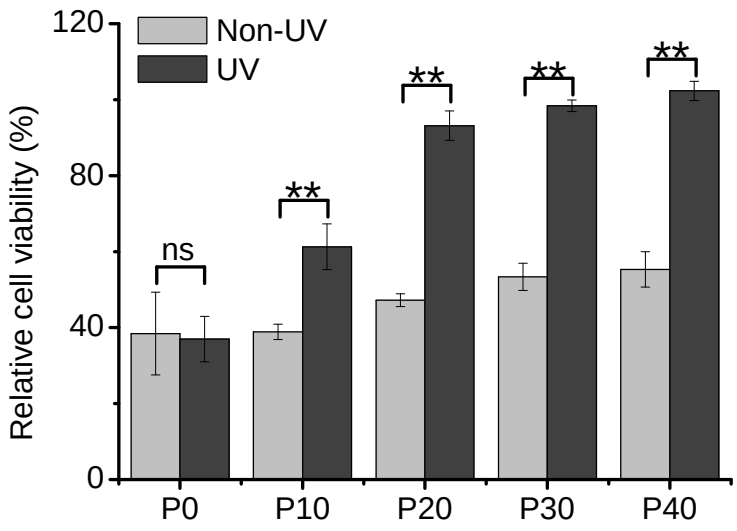


Figure S10. Cytotoxicity of polypeptide/DNA complexes (N/P ratio of 20, 5 μg DNA/mL) in COS-7 cells following treatment for 4 h, UV-irradiation for 5 min (20 mW/cm^2), and further incubation for 20 h. Viability was monitored using the MTT assay (n=3).

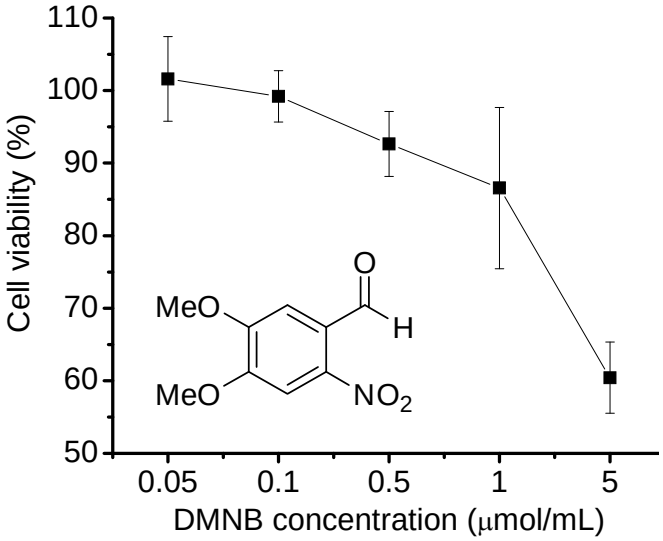


Figure S11. Cytotoxicity of DMNB in HeLa cells following 24-h incubation (n=3).

DMNB-$_L$-Glu (10 mg) was dissolved in water (pH = 6, 5 mL) and was then irradiated with UV light (λ = 365 nm, 20 mW/cm^2, 10 min) to cleave the DMNB ester bond. The cleaved DMNB was extracted using dichloromethane (5 mL × 2). The organic layers were combined, washed using DI water (2×5 mL), and dried over anhydrous Na_2SO_4. The solvent was removed under vacuum to obtain a yellow solid powder. The obtained DMNB was dissolved in DMSO (50 μmol/mL), and was subjected to cytotoxicity measurement by the MTT assay.

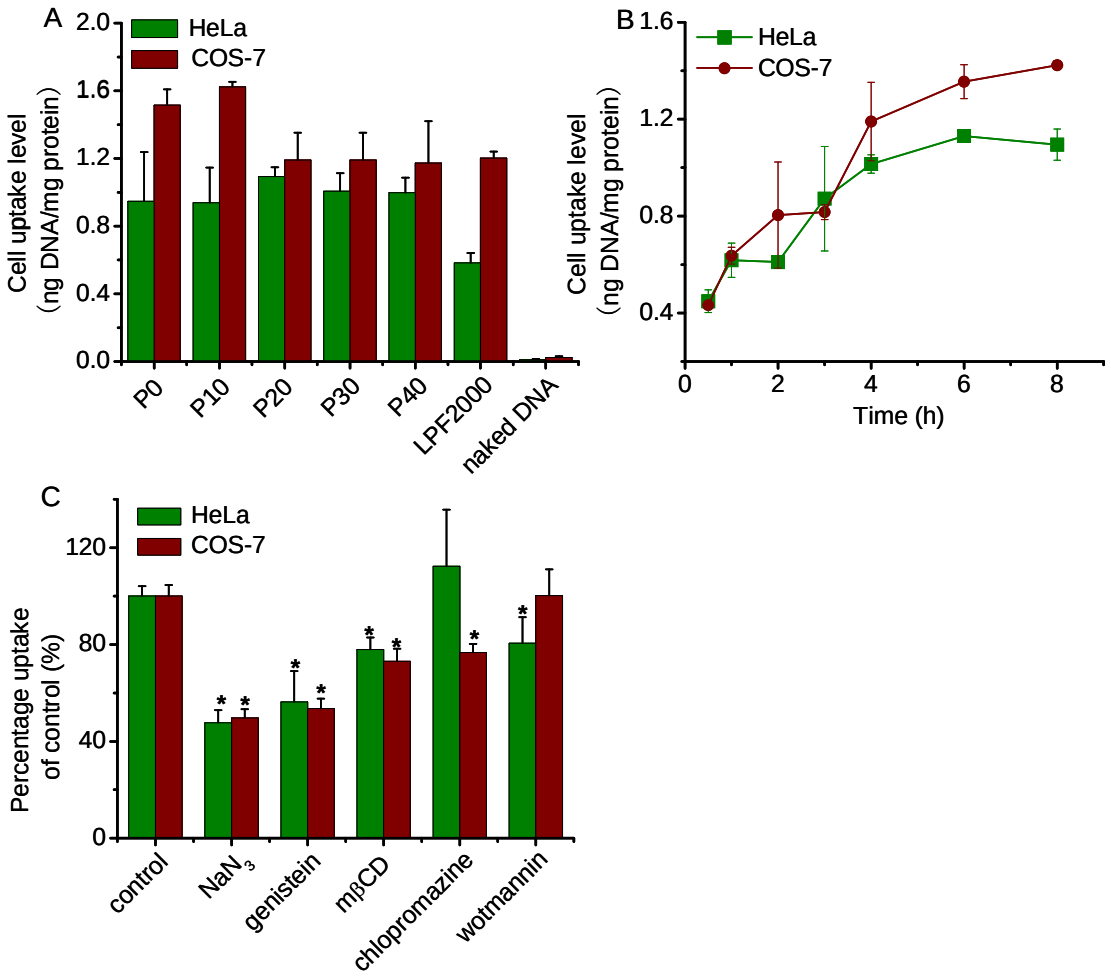


Figure S12. Internalization of polypeptide/YOYO-1-DNA complexes in HeLa and COS-7 cells. (A) Uptake level of polypeptide/DNA complexes after incubation with cells for 4 h at 1 μg YOYO-1-DNA/mL. Results were expressed as ng YOYO-1-DNA associated with 1 μg of cellular protein (n=3). (B) Time-course of the cell uptake of P20/YOYO-1-DNA complexes at 1 μg YOYO-1-DNA/mL (n=3). (C) Elucidation of the mechanism underlying cell uptake of P20/YOYO-1-DNA complexes. The uptake study was performed in the presence of various endocytic inhibitors, and results were expressed as percentage uptake (%) of control cells which were incubated with complexes in the absence of endocytic inhibitors (n=3).

We evaluated the capability of polypeptides in delivering DNA to mammalian cells. DNA was labeled with YOYO-1[4] before forming complexes with polypeptides (N/P ratio of 20), and the uptake level was evaluated in HeLa and COS-7 cells following 4 h incubation. P10~P40 showed comparable or only slightly decreased uptake level to the original PVBLG-8 (Supplementary Figure S12A), suggesting that copolymerization with DMNBLG did not significantly compromise the cell transduction capacity of the polypeptides. A time-course profile revealed that cell uptake level reached a plateau within 4 h (Supplementary Figure S12B). Therefore, in subsequent assessments, cells were treated with complexes for 4 h before light irradiation. The

mechanism of complex internalization was also investigated by using various endocytosis inhibitors. Genistein and methyl-β-cyclodextrin exerted significant inhibitory effect in both cells while chlorpromazine inhibited uptake in COS-7 cells and wortmannin inhibited uptake in HeLa cells (Supplementary Figure S12C), suggesting that the internalization pathway was caveolae- and clathrin-mediated endocytosis in COS-7 cells while caveolae- and macropinocytosis-mediated endocytosis in HeLa cells[6]. Sodium azide treatment to block endocytosis via energy depletion[7] resulted in only ~50% uptake inhibition, indicating that some of the DNA might diffuse through the polypeptide-induced pores on cell membranes in an energy-independent manner.

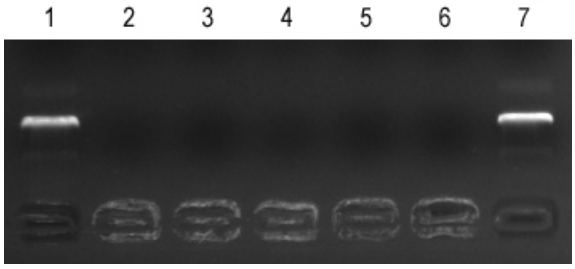


Figure S13. Gel retardation assay showing UV-triggered DNA release in the presence of heparin sulfate. Lane 1: naked DNA; Lane 2: P0/DNA complexes (N/P = 20); Lane 3: P0/DNA complexes (N/P = 20) treated with heparin for 12 h; Lane 4: UV irradiated P0/DNA complexes (N/P = 20, 20 mW/cm^2, 10 min) treated with heparin for 12 h; Lane 5: P20/DNA complexes (N/P = 20); Lane 6: P20/DNA complexes (N/P = 20) treated with heparin for 12 h; Lane 7: UV irradiated P20/DNA complexes (N/P = 20, 20 mW/cm^2, 10 min) treated with heparin for 12 h.

The gel retardation assay revealed that DNA could be completely released from P20/DNA complexes following UV irradiation and heparin replacement in comparison with the minimal DNA release from the non-irradiated complexes, which accorded well with the quantitative EB exclusion measurement (Fig. 4A) and consistently demonstrated that DNA release was due to charge/conformation alteration of the polypeptide. P0 as the non-responsive polymer showed no enhanced DNA release upon UV treatment.

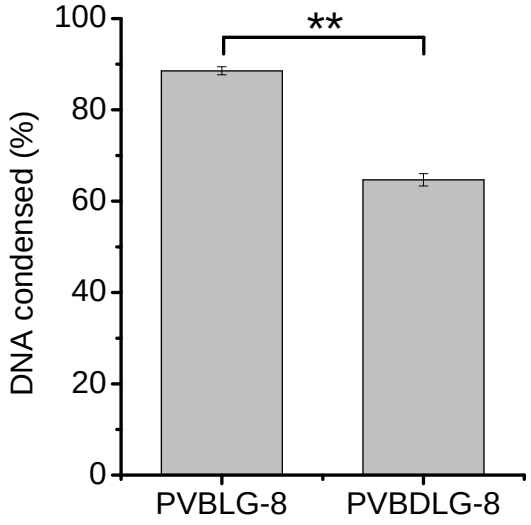


Figure S14. Condensation of DNA by polypeptide (N/P ratio of 20) as demonstrated by the EB exclusion assay (n=3).

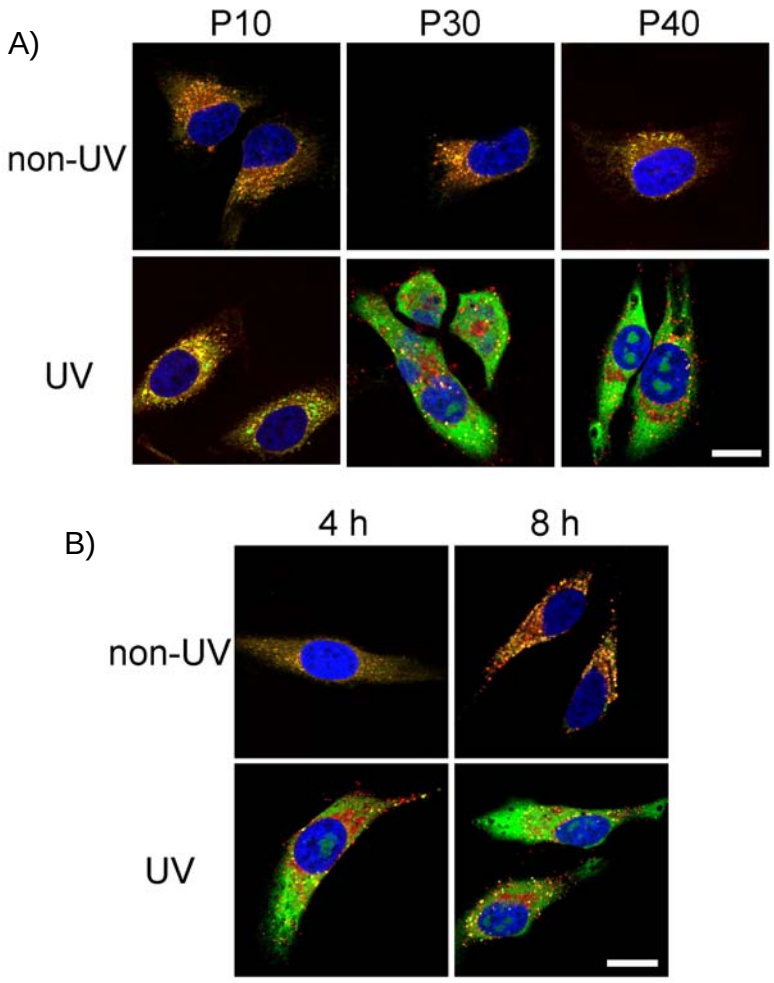


Figure S15. (A) CLSM images of HeLa cells incubated with rhodamine-polypeptide (red)/YOYO-1-DNA (green) complexes with/without UV treatment (bar = 20 μm). The complexes were incubated with cells for 2 h, irradiated by UV light, and further incubated for 2 h before CLSM observation. (B) CLSM images of HeLa cells incubated with RhB-P20 (red)/YOYO-1-DNA (green) complexes with/without UV treatment (bar = 20 μm). Cell were incubated with complexes for 2 h, irradiated by UV light, and further incubated for 4 h and 8 h before CLSM observation.

All the test polymers demonstrated facilitated intracellular DNA release upon UV treatment as evidenced by the separation of rhodamine-polypeptide from YOYO-1-DNA, although DNA release for P10 was not as dramatic as P30 and P40, which could be attributed to the low responsiveness of P10 containing low amount of the DMNB segment. When the incubation time was prolonged to 4 h and 8 h post-transfection, irradiated cells demonstrated significant DNA release from P20 while non-irradiated cells still suffered from poor DNA release as shown by the large co-localization between rhodamine-polypeptide and YOYO-1-DNA.

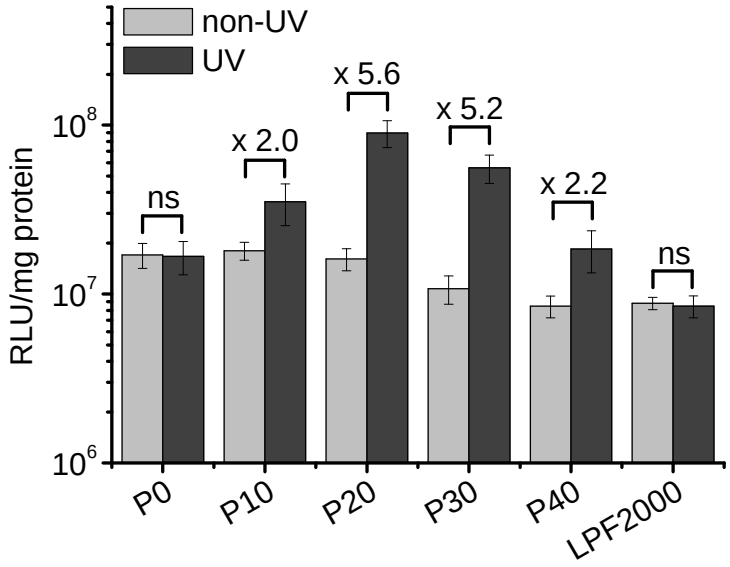


Figure S16. Transfection efficiency of polypeptide/DNA complexes (N/P ratio of 20, 5 μg DNA/mL) in COS-7 cells with/without UV irradiation (20 mW/cm^2, 5 min) (n=3).

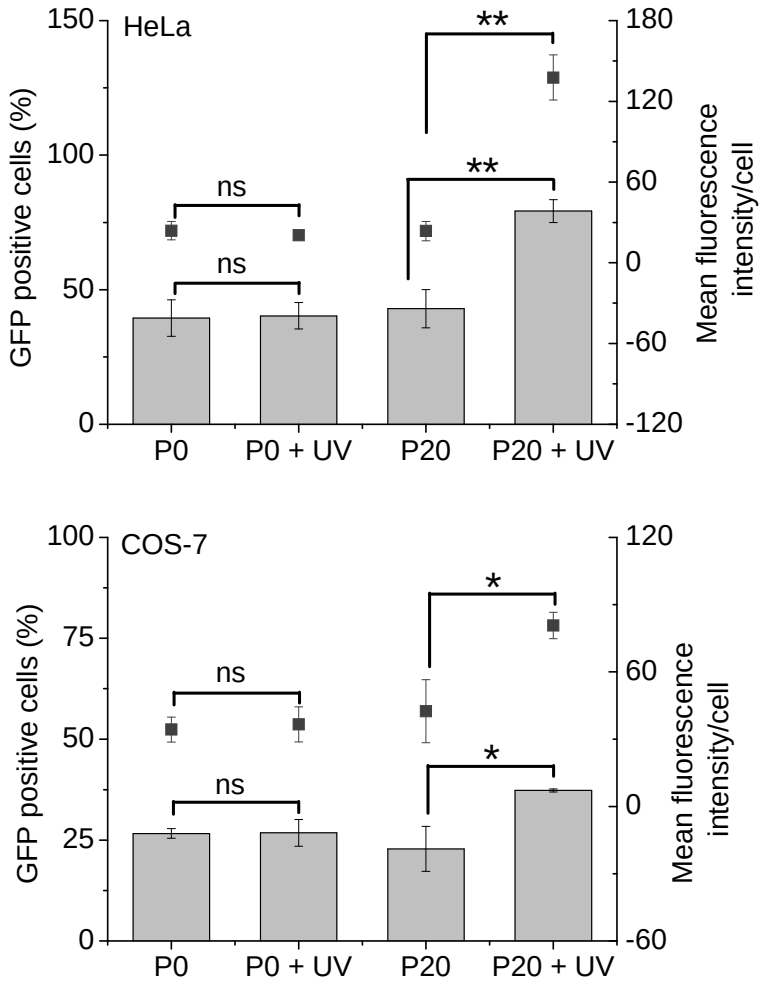


Figure S17. Transfection efficiencies of P20/pEGFP complexes (N/P ratio of 20, 5 μg DNA/mL) in HeLa and COS-7 cells as assessed by flow cytometry in response to UV irradiation (20 mW/cm^2, 5 min). Bar represents GFP positive cells (%) while scatter represents the mean fluorescence intensity/cell.

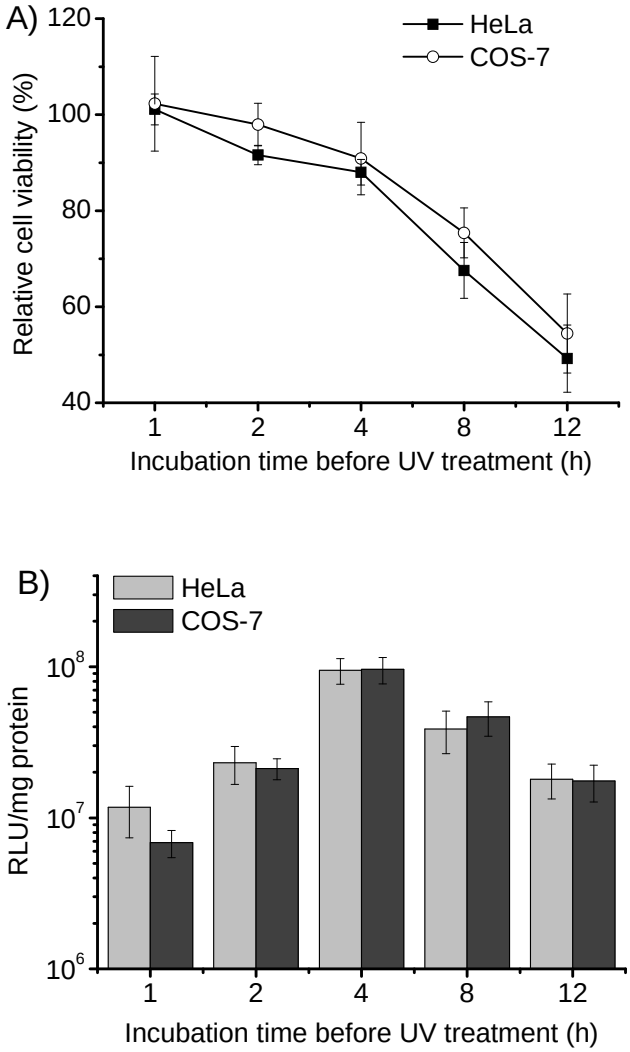


Figure S18. Cytotoxicity (A) and transfection efficiencies (B) of P20/DNA complexes (N/P ratio of 20, 5 μg DNA/mL) in HeLa and COS-7 cells. Cells were incubated with complexes for 1, 2, 4, 8, 12 h, respectively, before UV irradiation (20 mW/cm^2, 5 min).

Earlier UV irradiation resulted in lower cytotoxicity, which was due to the shorter interaction between the cationic polyplexes and cells. However, maximal transfection efficiencies were noted when UV irradiation was performed 4 h after polyplex treatment, according well with the cell uptake level that plateaued 4 h post polyplex treatment. These results collectively indicated that early light irradiation reduced the transfection efficiency due to insufficient cellular internalization and premature DNA release; light irradiation after the cell uptake level peaked could mediate an optimal balance between DNA release and cytotoxicity towards the maximal gene transfection efficiencies.

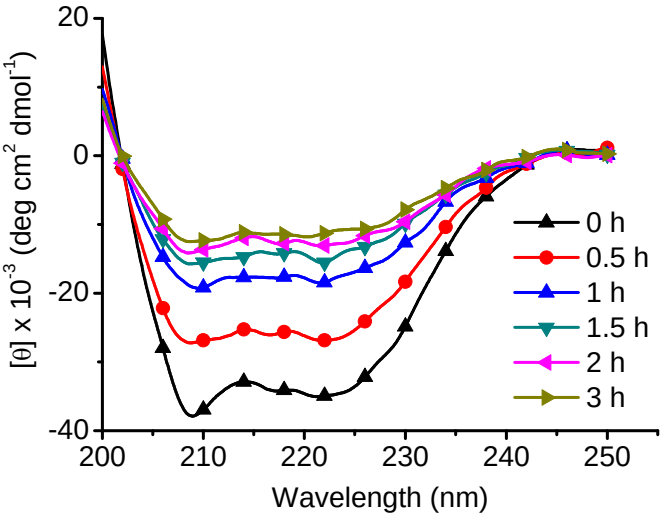


Figure S19. CD spectra of P20 irradiated with NIR light for different time (750 nm, 3.2 μJ/cm^2/pulse).

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