Supporting Information Available

Highlighting the Role of Polymer Length, Carbohydrate size, and Nucleic Acid Type in Potency of Glycopolycation Agents for pDNA and siRNA Delivery

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Gel Binding Assay

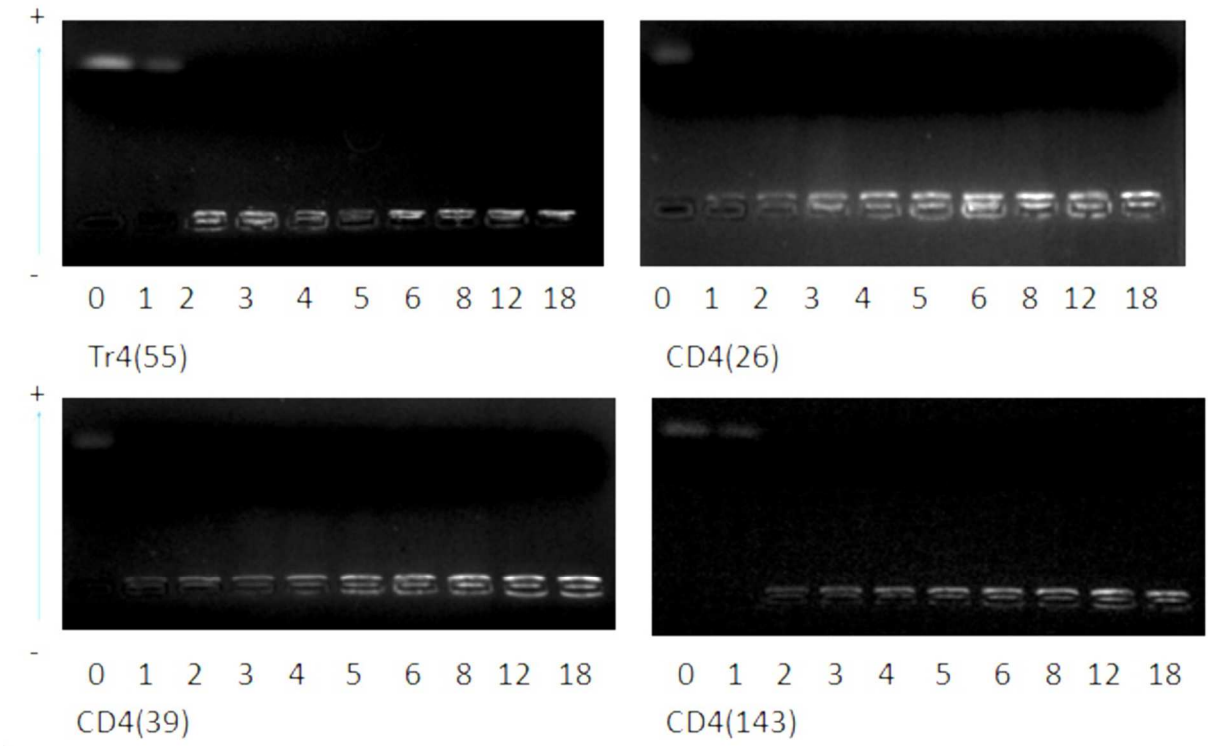

Figure S1: The binding efficiency of polymers with siRNA examined by agarose gel electrophoresis. Photographs of gels for T4(55), CD4(26), CD4(39) and CD4(143) at N/P ratios: 1, 2, 3, 4, 5, 6, 8, 12 and 18.

NMR Measurements

^{1}H NMR measurements were performed with a temperature-controlled Varian 400-MR spectrometer operating at a frequency of 399.7 MHz. Samples were prepared in D_2O (HOD internal standard).

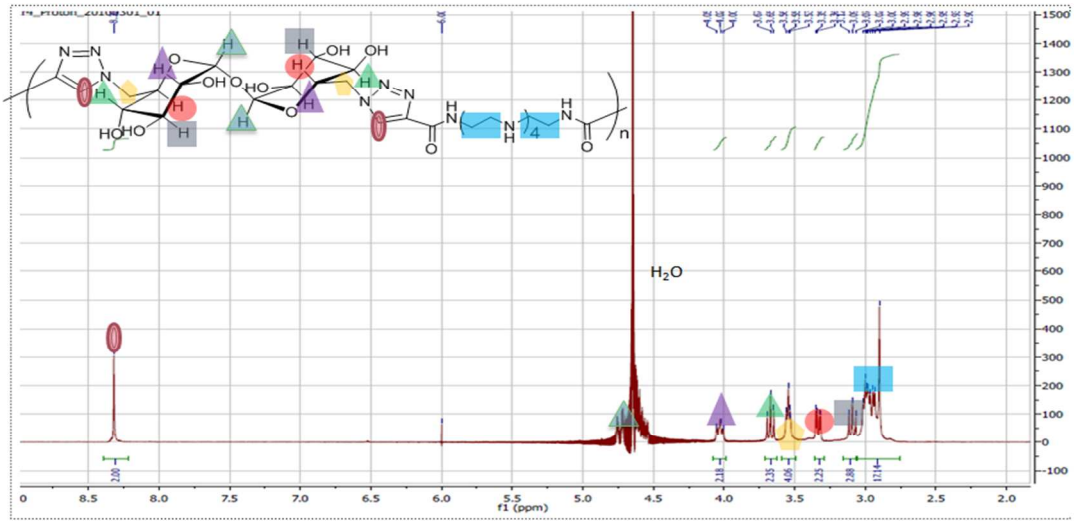

Figure S2: 1H-NMR of Tr4(77). δ 2.90-3.05 m 17H, 3.05-3.12 t 2H, 3.30-3.35 dd 2H, 3.50-3.52 d 4H, 4.00-4.02 t 2H, 8.28 s 2H. The corresponding peaks were assigned according to 1H1H-COSY.

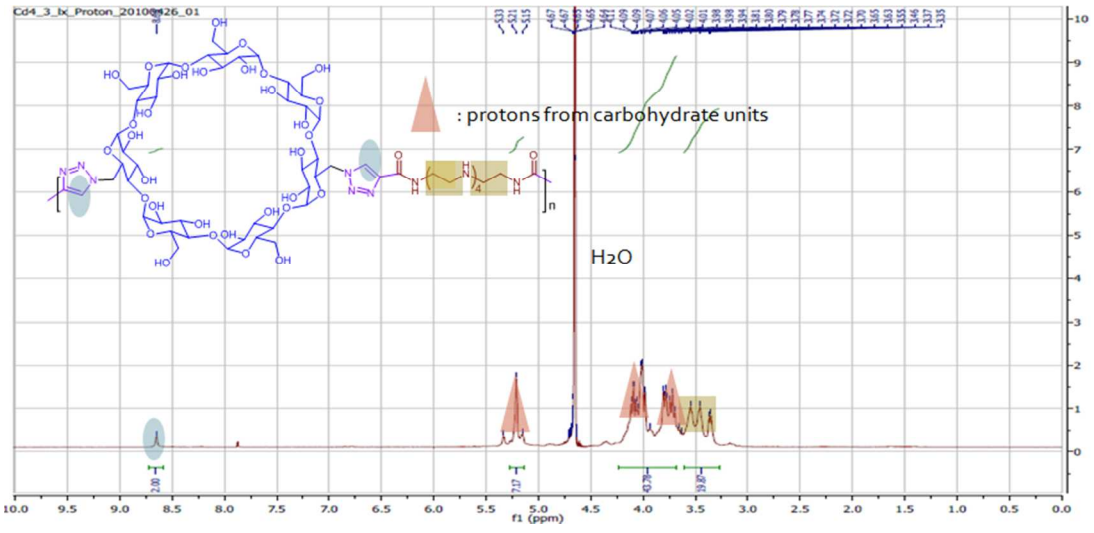

Figure S3: 1H-NMR of CD4(26). δ 3.30-3.60 m 20H, 3.65-4.11 m 42H, 5.15-5.33 d 7H, 8.60 s 2H.

Size Exclusion Chromatography

Size exclusion chromatography (SEC) was applied to determine the number-average molecular weight (Mn) and polydispersity indices (PDIs) for trehalose polymers and cyclodextrin polymers. The mobile phase for trehalose polymer is a solution of 0.5% sodium acetate (pH=5.5 with acetic acid) containing 20% acetonitrile. The mobile phase for cyclodextrin polymers is a solution of water: methanol: acetic acid (70:25:5).

A flow rate of 0.3 mL/min in the column (Eprogen Inc., IL), on a Wyatt HELEOS II (Santa Barbara, CA) light scattering detector (λ = 662 nm), and an Optilab rEX refractometer (λ = 658 nm) were used. The Mn, PDI, and dn/dc of the polymers were determined by Astra V (version 6.0, Wyatt Technologies).

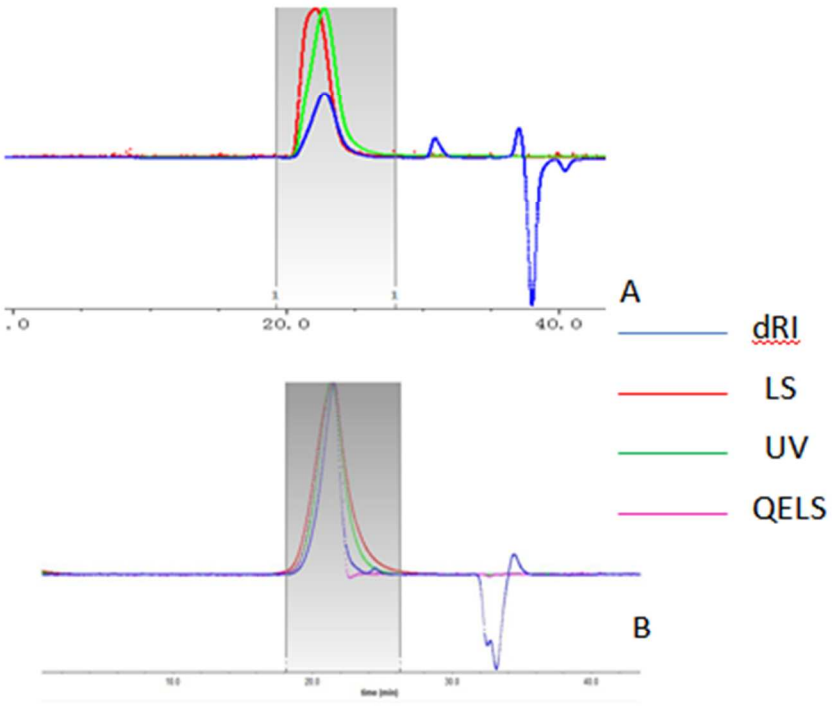

Figure S4: **SEC trace for trehalose polymer Tr4(77) (A) and cyclodextrin polymer CD4(26) (B).**

Dynamic Light Scattering

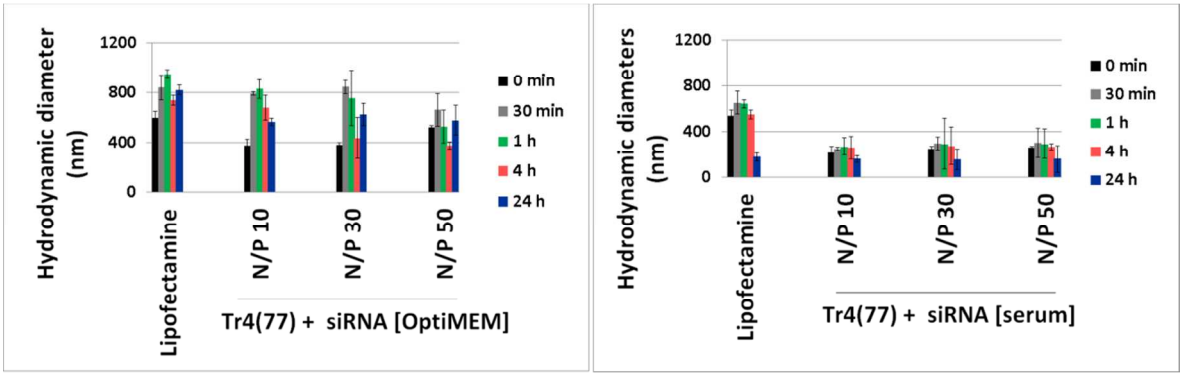

Figure S5: Stability of polyplexes in two kinds of transfection media as measured by dynamic light scattering. Both (A) reduced-serum Opti-MEM and (B) complete DMEM (with 10% FBS) were applied to polyplexes as transfection media, and particle size was measured at indicated time points through 24 h at room temperature. Tr4(77) was the polymer used in this study with three N/P ratios (10, 30, and 50) tested.

To study the stability of the polyplexes in reduced-serum and serum-containing transfection media, Tr4(77)-siRNA polyplex sizes were examined in both transfection media at selected timepoints up to 24 h at room temperature (**Error! Reference source not found.**). In Opti-MEM, the polyplexes were relatively large (~400-800 nm) and measurement results fluctuated significantly; however, most measured sizes were larger for all subsequent timepoints than they were for t=0 (this was true for Lipofectamine and for Tr4(77) at N/P=10 and N/P=30; only Tr4(77) at N/P=50 showed no apparent trend in size change over time). In serum-containing DMEM, particles formed with Lipofectamine decreased in size significantly (from ~600 nm to ~200 nm) at 24 h versus

all prior timepoints. It is also should be noted that, in contrast to the particles formed with Lipofectamine, the size of polyplexes formed using Tr4(77) differs significantly between Opti-MEM (~400-800 nm) and complete DMEM (~150-300 nm). In addition, the relative amount of Tr4(77) in the formulation (N/P=10, 30, or 50) did not influence the size of nanoparticles in either transfection medium.

MTT Assay

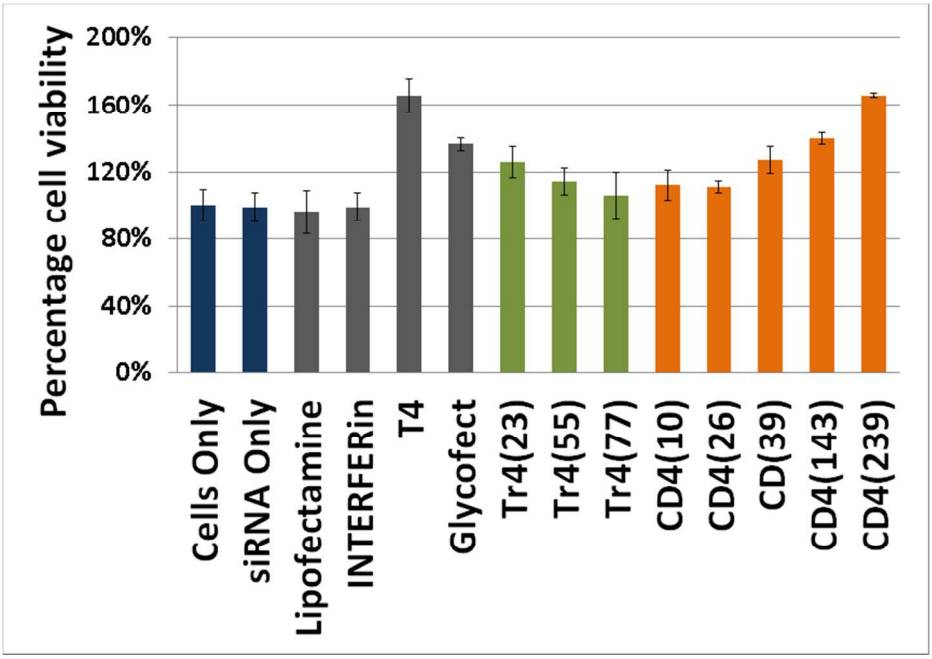

Figure S6: MTT assay to measure the cytotoxicity of siRNA-containing complexes in U87-luc2 glioblastoma cells 24 h after transfection at N/P=50 and a siRNA concentration of 100 nM. All the experiments were performed in triplicate. The y-axis label "Percentage cell viability" stands for the relative activity of mitochondria reductase compared with those without treatment.

Zeta Potential Measurement

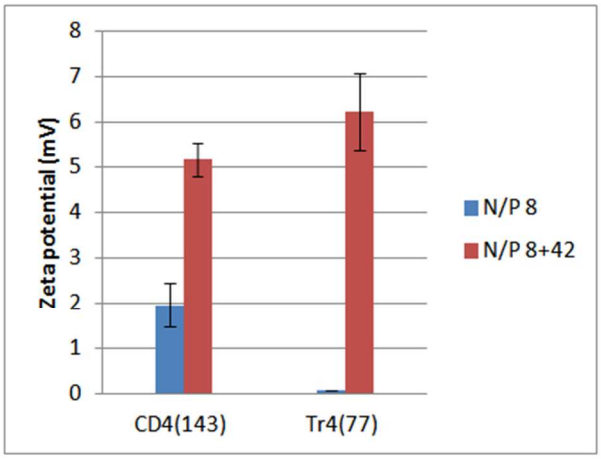

Figure S7: Measurement of zeta potential for polyplexes formed by CD4(143) and Tr4(77) with siRNA before and after addition of free polymers. The polyplexes were formed at N/P=8 in water at the siRNA concentration 1 μM. After 1 hr incubation, the polyplexes then were diluted to 700 μL with OptiMEM to yield the transfection solution. For the treatment of adding free polymers, the solution containing the same amount of N/P=42 polymer was added into the transfection solution for examination. All the experiments were performed in triplicate**.**