

## Supporting Information

### **Acid-degradable Dextran as an Image Guided siRNA Carrier for COX-2 Downregulation**

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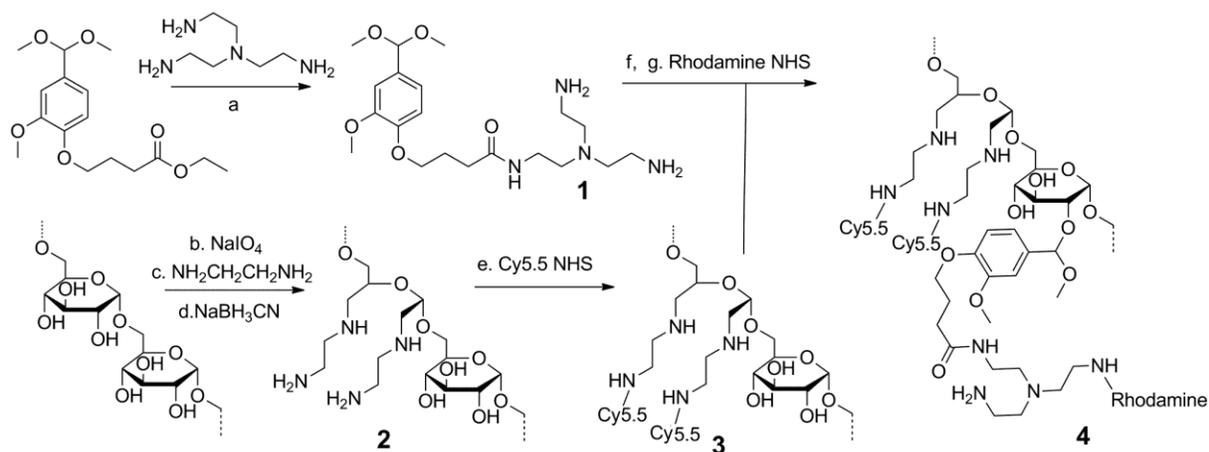
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#### **Materials and General Experimental Methods:**

All organic chemicals and solvents were of analytical grade were from Aldrich (St. Louis, MO), Sigma (Milwaukee, WI) and Alfa Aesar (Ward Hill, MA) unless otherwise specified. Fetal bovine serum, penicillin, streptomycin were from Invitrogen (Carlsbad, CA). Amicon ultra-15 centrifugal filter tubes (10,000 MW cutoff) were from Millipore (Bedford, MA). The MDA-MB-231 human breast cancer cell line was obtained from American Type Culture Collection (Rockville, MD). COX-2 siRNA (sense: 5'- CAUCCCCUCCUUCGAAAUTT -3' and antisense: 5'- AUUUCGAAGGAAGGGAAUGTT-3') was synthesized by Dharmacon (Lafayette, CO). <sup>1</sup>H, <sup>13</sup>C NMR spectra were recorded on a Bruker Avance III 500 MHz NMR spectrometer (Bruker BioSpin Corporation, Billerica, MA), and chemical shifts were reported in ppm relative to tetramethylsilane.

#### **Synthesis Procedure**



**Figure S1:** Synthesis procedure

**Compound 1:** Ethyl 4-(4-formyl-3-methoxy)phenyl butyrate dimethyl acetal <sup>[1]</sup> 400 mg was mixed with tris(2-aminoethyl)amine 1.5 ml. This mixture was stirred at 100 °C under nitrogen atmosphere for 24 h. After reaction, tris(2-aminoethyl)amine was removed by distilling at 100 °C under high vacuum. Yellow oil raw product was further purified by basic aluminum oxide column chromatography (elution: CH<sub>2</sub>Cl<sub>2</sub>/ N (CH<sub>3</sub>CH<sub>2</sub>)<sub>3</sub> = 95/5). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 6.99–6.95 (m, 2H, Ar), 6.86 (d, 1H, J = 8.3 Hz, ArH), 5.31 (s, 1H, CH(OCH<sub>3</sub>)<sub>2</sub>), 4.05 (t, 2H, J = 7.6 Hz, OCH<sub>2</sub>), 3.87 (s, 3H, ArOCH<sub>3</sub>), 3.32 (s, 6H, CH(OCH<sub>3</sub>)<sub>2</sub>), 3.28 (t, 2H, J = 7.4 Hz, CONHCH<sub>2</sub>CH<sub>2</sub>), 2.72 (t, 4H, J = 5.9 Hz, N(CH<sub>2</sub>CH<sub>2</sub>NH<sub>2</sub>)<sub>2</sub>), 2.55 (t, 2H, J = 6.0 Hz, CONHCH<sub>2</sub>CH<sub>2</sub>), 2.52 (t, 4H, J = 6.1 Hz, N(CH<sub>2</sub>CH<sub>2</sub>NH<sub>2</sub>)<sub>2</sub>), 2.41 (t, 2H, J = 7.2 Hz, CH<sub>2</sub>CO), 2.14 (dddd, 2H, J = 7.2, 7.2, 6.4, 6.4 Hz, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>). <sup>13</sup>C NMR (500 MHz, CDCl<sub>3</sub>): δ 172.56, 149.17, 148.39, 131.00, 119.39, 112.42, 109.92, 103.25, 67.94, 57.70, 57.26, 55.92, 53.19, 52.83, 39.89, 38.25, 32.85, 25.44. MALDI HRMS calculated for C<sub>20</sub>H<sub>36</sub>N<sub>4</sub>NaO<sub>5</sub> (M + Na): 435.2578, found: 435.25861

**Compound 2:** An aqueous solution of 1.0 g dextran (70 kDa; 12.5% (w/v); pH 5.5) was oxidized with 2 mL of sodium periodate solution (20 mg/mL) at room temperature for 4 h to yield theoretical

oxidations of 3%. Here the oxidation degree (OD) is defined as the number of oxidized residues per 100 glucose residues). The oxidized dextran was purified through centrifugal filter tubes (Amicon ultra-15, 10,000 MW cutoff) for 3 times (10 minutes per time). The solution was lyophilized to produce the white solid oxidized dextran. Due to the very low OD, the oxidized residues were difficult to identify by NMR. The actual oxidation degree determined by TNBS colorimetric titration assay was  $3.1 \pm 0.2\%$ .<sup>[2]</sup> 100 mg oxidized dextran was dissolved in 2.0 ml PBS buffer (pH 7.4), then 30  $\mu$ l ethylenediamine was added into this solution. The mixture was stirred for 4 h to form the Schiff base. Then the Schiff base of dextran was reduced to the stable amine-carbon bond with adding of 40mg sodium cyanoborohydride. After quenching the reaction with 50  $\mu$ l 10% HCl, dextran was purified through centrifugal filter tubes (Amicon ultra-15, 10,000 MW cutoff) for 2 times in PBS and 2 times in water, and 87.8 mg white solid was obtained after lyophilization. <sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O)  $\delta$ 4.92 (m, 1H, glucose-*H*<sub>1</sub>), 4.00–3.42 (m, 6H, glucose-*H*<sub>2–6</sub>)

**Compound 3:** 40.0 mg compound 2 was dissolved in 2 ml HEPES buffer (pH 8.4), then 1 mg Cy5.5 NHS ester in 100  $\mu$ L DMSO was added into the dextran solution. The mixture was stirred at room temperature for 2 h. After 2 h stirring, the pH value was adjusted to 7.0 by adding 10% HCl. Then 10  $\mu$ l acetic acid was added into this solution, keeping the pH between 6.0-7.0. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) 40 mg was added to form the amide bonds between the acetic acid and the unreacted amines of dextran. This reaction prevented the unreacted amines to be involved in further modifications of the dextran carriers. The Cy 5.5 labeled dextran was purified by centrifugal filter tubes (Amicon ultra-15, 10,000 MW cutoff) for 3 times. After lyophilization, 38.7 mg cyan solid was harvested. Colorimetric assay of Cy5.5 at 670 nm indicated

that there were 1.1 Cy5.5 molecules in one dextran molecule. NMR could not characterize Cy5.5 on dextran due to the very low concentration of Cy5.5.

**Compound 4:** 30 mg of compound 3 was dissolved in 2 ml anhydrous DMSO containing 2 g 4Å active molecular sieves. Then p-toluenesulfonic acid monohydrate (270 mg, 4 eq of compound 1) was added into this mixture. This mixture was stirred at room temperature under nitrogen atmosphere for 4 h to remove water in the solution. Meanwhile 150 mg compound 1 (2 eq of glucose unit) was dissolved in anhydrous DMSO (1 mL) with 1 g 4Å active molecular sieves, and the solution was stirred at room temperature under nitrogen atmosphere for 4 h. Then compound 1 solution was transferred into compound 3 solution containing 4Å active molecular sieves, and this mixture was stirred at 65 °C under nitrogen atmosphere for 16 h. This reaction was stopped by adding 100 µl triethylamine, and this DMSO solution was dropped into ethyl acetate to precipitate the cyan product. The suspension was centrifuged to obtain the cyan solid precipitate, which was purified by three times re-precipitation in ethyl acetate. After drying, 55.3 mg product was obtained. This product was dissolved in 2.0 ml HEPES buffer (pH 8.2), then 100 µl DMSO containing 0.5 mg rhodamine 6G NHS ester was added to this HEPES buffer. After stirring at room temperature for 2 h, this dextran product was purified by centrifugal filter tubes (Amicon ultra-15, 10,000 MW cutoff). After repeating purification 2 more times and lyophilization, 49.1 mg purple solid was harvested. Colorimetric assay of rhodamine 6G at 530 nm indicated that there were 1.2 rhodamine molecules in one dextran molecule. <sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O) δ 7.15–6.9 (m, 0.5 × 3H, Ar), 5.32 (m, 0.51 × 1H, acetal-H), 4.92 (m, 1H, glucose-H<sub>1</sub>), 4.37–3.32 (m, 6H, glucose-H<sub>2-6</sub>; 0.5 × 2H, ArOCH<sub>2</sub>; 0.5 × 3H, ArOCH<sub>3</sub>; 0.5 × 2H, CH<sub>2</sub>NHCO; 0.5 × 3H, acetal-OCH<sub>3</sub>), 2.41–2.83 (br. app. s, 0.5 × 4H, NHCH<sub>2</sub>CH<sub>2</sub>NH<sub>2</sub>; 0.5 × 4H, NHCH<sub>2</sub>CH<sub>2</sub>NH<sub>2</sub>; 0.5 × 2H, CONHCH<sub>2</sub>CH<sub>2</sub>NH<sub>2</sub>), 2.35 (br. app. s, 0.5 × 2H, CH<sub>2</sub>CO), 2.05 (br. app. s, 0.5 × 2H, ArOCH<sub>2</sub>CH<sub>2</sub>).

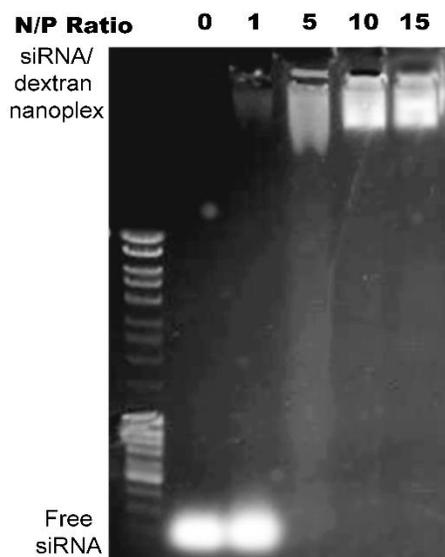
## **Determination of size distribution and zeta potential of compound 4 and siRNA/compound 4 nanoplex**

The hydrodynamic radius and size distribution of compound 4 were determined by dynamic light scattering (DLS, 10 mW He-Ne laser, 633 nm wavelength). Dextran-siRNA nanoplex for size distribution measurement was prepared at N/P ratios of 15 by adding compound 4 in 600  $\mu$ l PBS pH=7.4 buffer to a solution of siRNA (400  $\mu$ l, in DI waters, 50  $\mu$ g/ml), followed by vortexing for 10 s and incubating for 20 min at room temperature. The DLS measurements were performed in triplicate. Dextran-siRNA nanoplex (N/P = 15) for measurement of zeta potential was prepared by adding compound 4 in 600  $\mu$ l DI water to a solution of siRNA (400  $\mu$ L, in DI waters, 50  $\mu$ g/ml), followed by vortexing for 10 s and incubating for 10 min at room temperature. The average zeta potential of natural dextran alone, compound 4 and dextran-siRNA nanoplex (N/P = 15) in DI water solution were measured with a Zetasizer Nano ZS instrument (Malvern) equipped with a clear standard zeta capillary electrophoresis cell cuvette from 20 acquisitions with a concentration of approximately 0.5 mg/ml. The zeta potential measurement of dextran-siRNA nanoplex was performed immediately after the complex was produced. The measurements were performed in triplicate.

## **N/P value dependent stability of siRNA/compound 4 nanoplex**

The N/P ratio is crucial for determining the stability and delivery efficacy of the polymer/siRNA nanoplex. Electrophoretic gel mobility shift assay was used to evaluate the N/P ratio between compound 4 and COX-2 siRNA. Compound 4 and COX-2 siRNA were mixed in PBS (pH = 7.4)

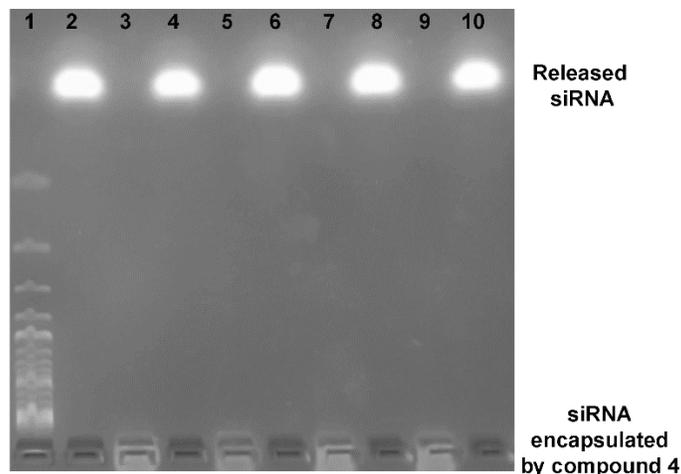
buffer with N/P values of 0, 1, 5, 10, and 15. After 30 min incubation at room temperature, the resulting nanoplexes were loaded on a 5% agarose gel (1.0  $\mu\text{g}$  COX-2 siRNA per well). After electrophoresis, the nucleotide was stained by ethidium bromide and UV light images were obtained.



**Figure S2:** Electrophoretic gel mobility shift assay of stability of dextran-siRNA nanoplex.

### **The Stability of Encapsulated siRNA in Fresh Mouse and Human Serum**

A nuclease stability assay was conducted to determine the stability of siRNA encapsulated in siRNA/compound 4 nanoplex. The nanoplex was formulated in PBS, pH 7.4 with an N/P ratio of 15 for 20 min. The nanoplex was incubated for 4 and 8 h in 70% fresh serum at 37 °C. At the end of incubation, all encapsulated siRNA was displaced from the nanoplex by adding 2% SDS and loaded to the wells (1  $\mu\text{g}$ /well siRNA) containing 4% agarose gel.



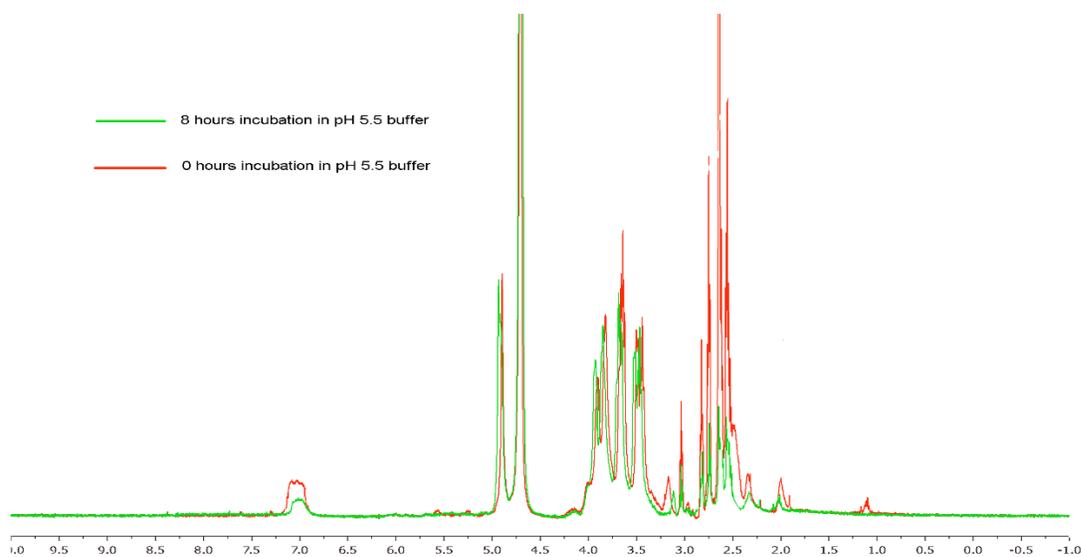
**Figure S3:** Stability of siRNA encapsulated in siRNA/compound 4 nanoplex in fresh mouse serum (Sigma-Aldrich, St. Louis, MO) or human serum (Sigma-Aldrich, St. Louis, MO).

(Lane 1: Ladder; Lane 2: COX-2 siRNA control; Lane 3: siRNA /compound 4 nanoplex in fresh mouse serum for 24 h; Lane 4: siRNA /compound 4 nanoplex in fresh mouse serum for 24 h with SDS; Lane 5: siRNA /compound 4 nanoplex in fresh mouse serum for 72 h; Lane 6: siRNA /compound 4 nanoplex in fresh mouse serum for 72 h with SDS; Lane 7: siRNA /compound 4 nanoplex in fresh human serum for 24 h; Lane 8: siRNA /compound 4 nanoplex in fresh human serum for 24 h with SDS; Lane 9: siRNA /compound 4 nanoplex in fresh human serum for 72 h; Lane 10: siRNA /compound 4 nanoplex in fresh human serum for 72 h with SDS.

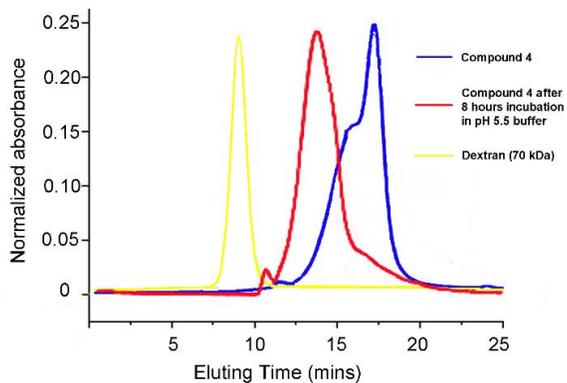
#### Degradation of compound 4

Compound 4 was incubated in pH 5.5 buffer for 8 h, after which the pH value was changed to pH 7.4 by adding  $\text{NH}_3 \cdot \text{H}_2\text{O}$ . After dialysis in pH 7.4  $\text{NH}_3 \cdot \text{H}_2\text{O}$  solution, partially degraded

compound 4 solid was collected by lyophilization. This solid was dissolved in D<sub>2</sub>O for <sup>1</sup>H NMR spectroscopy, or dissolved in PBS pH7.4 buffer for gel permeation chromatography (GPC).



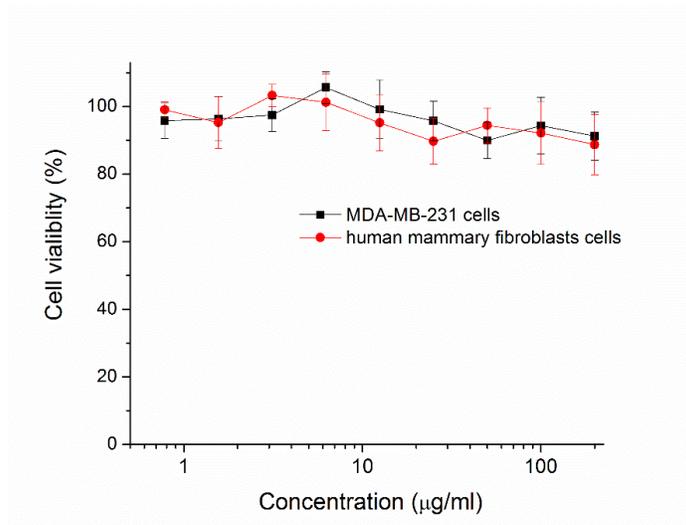
**Figure S4:** <sup>1</sup>H NMR spectra of compound 4 before and after incubation in pH 5.5 buffer. The intensity of peaks was normalized to the peak of dextran at 4.9 ppm.



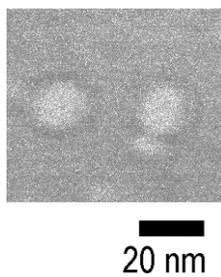
**Figure S5:** The effluent peak of dextran and compound 4 before and after incubation in pH 5.5 buffer.

### Determination of Cytotoxicity of Compound 4 *in vitro*

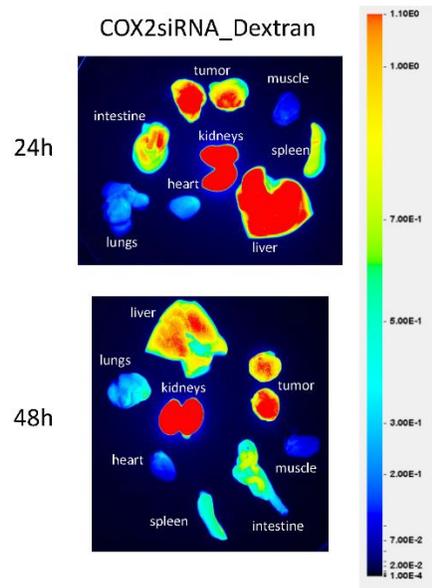
The concentration dependent cytotoxicities of compound 4 in MDA-MB-231 human breast cancer cells and human mammary fibroblasts after 3 days incubation were measured using an MTT assay. As demonstrated in Figure S6, compound 4 did not show toxicity up to 3 days incubation with concentrations up to 100  $\mu\text{g/ml}$ .



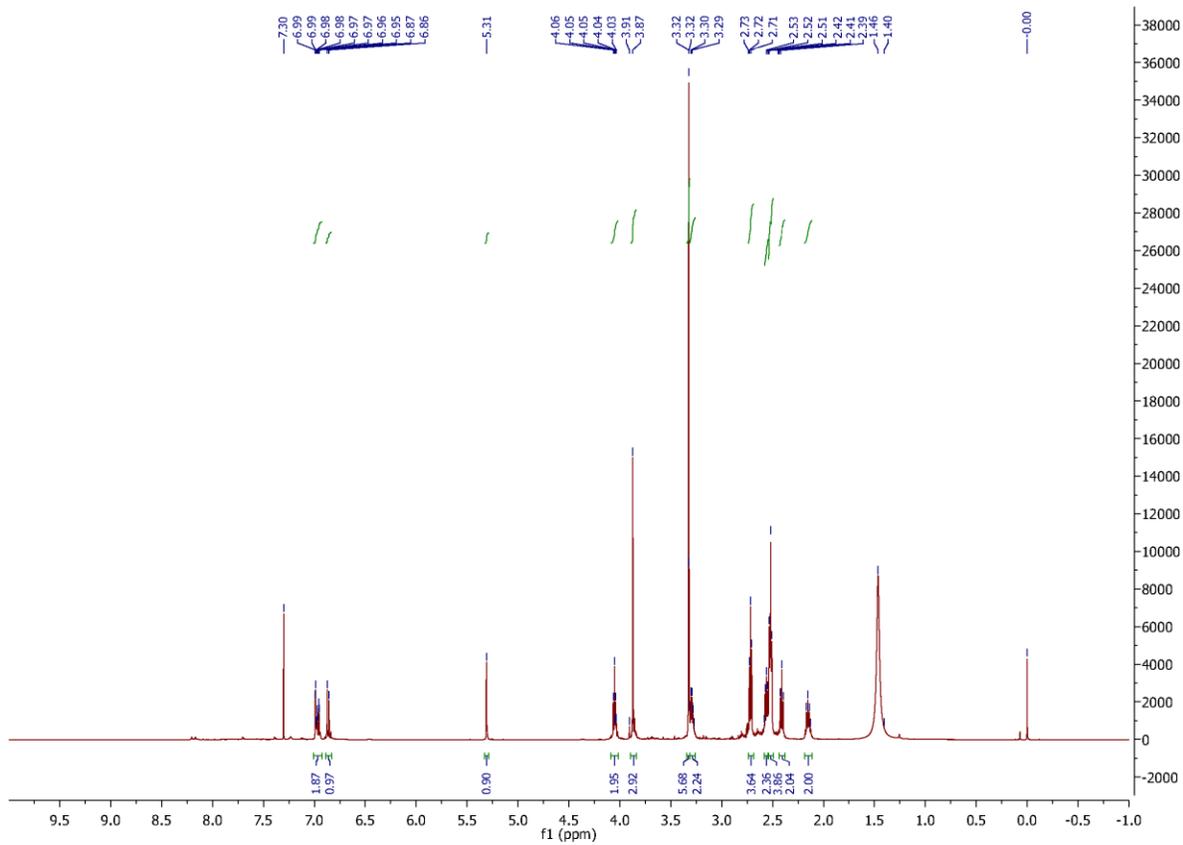
**Figure S6:** Cell viability following treatment with compound 4 in MDA-MB-231 human breast cancer cells and human mammary fibroblasts. (Values represent Mean  $\pm$  SD, n = 3.)



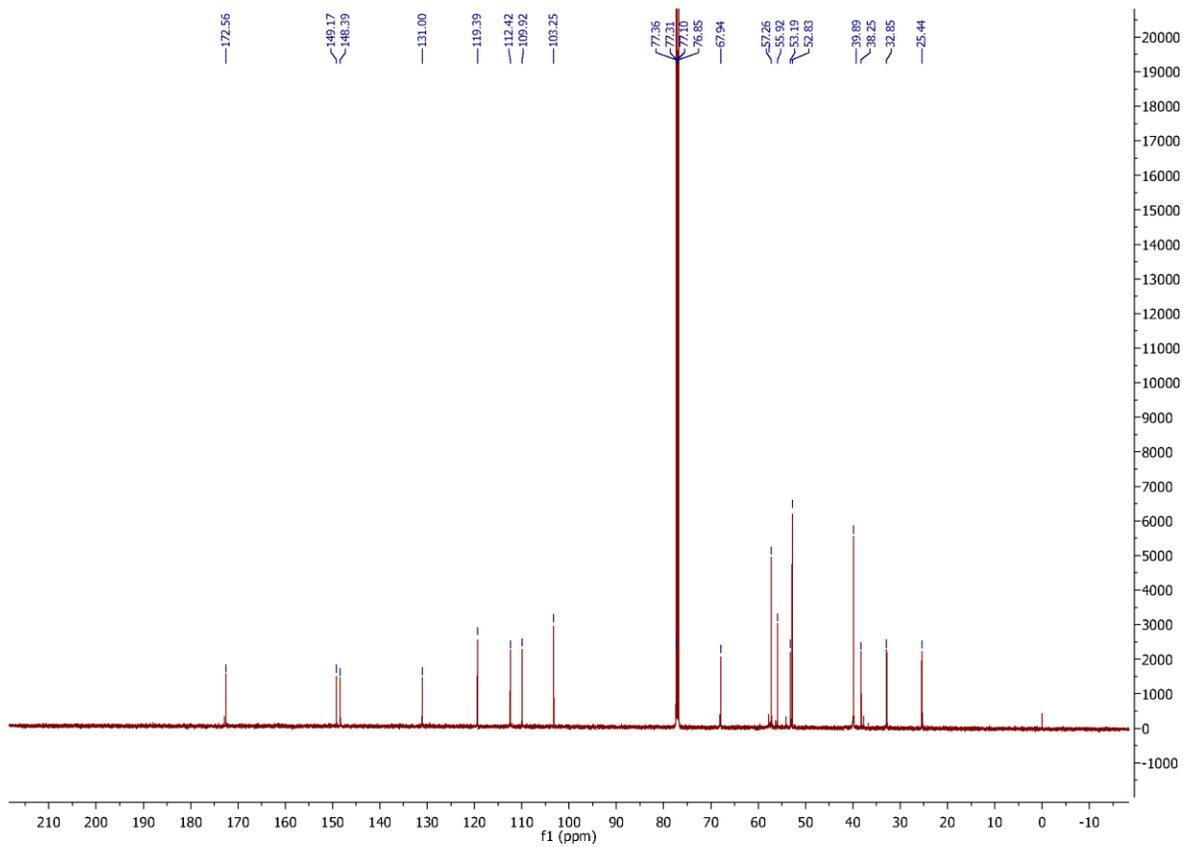
**Figure S7:** TEM imaging of COX-2 siRNA/compound 4 nanoplex.



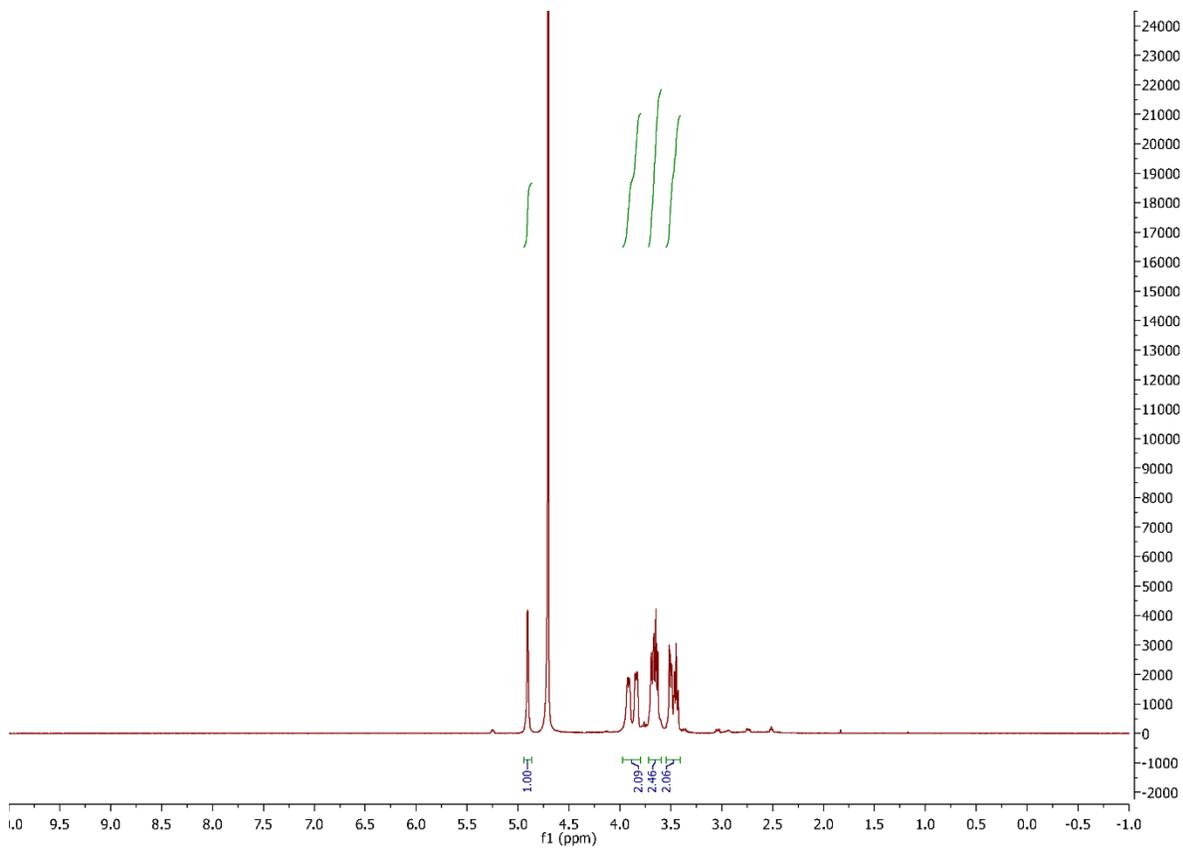
**Figure S8:** *Ex vivo* images of nanoplex in mouse organs.



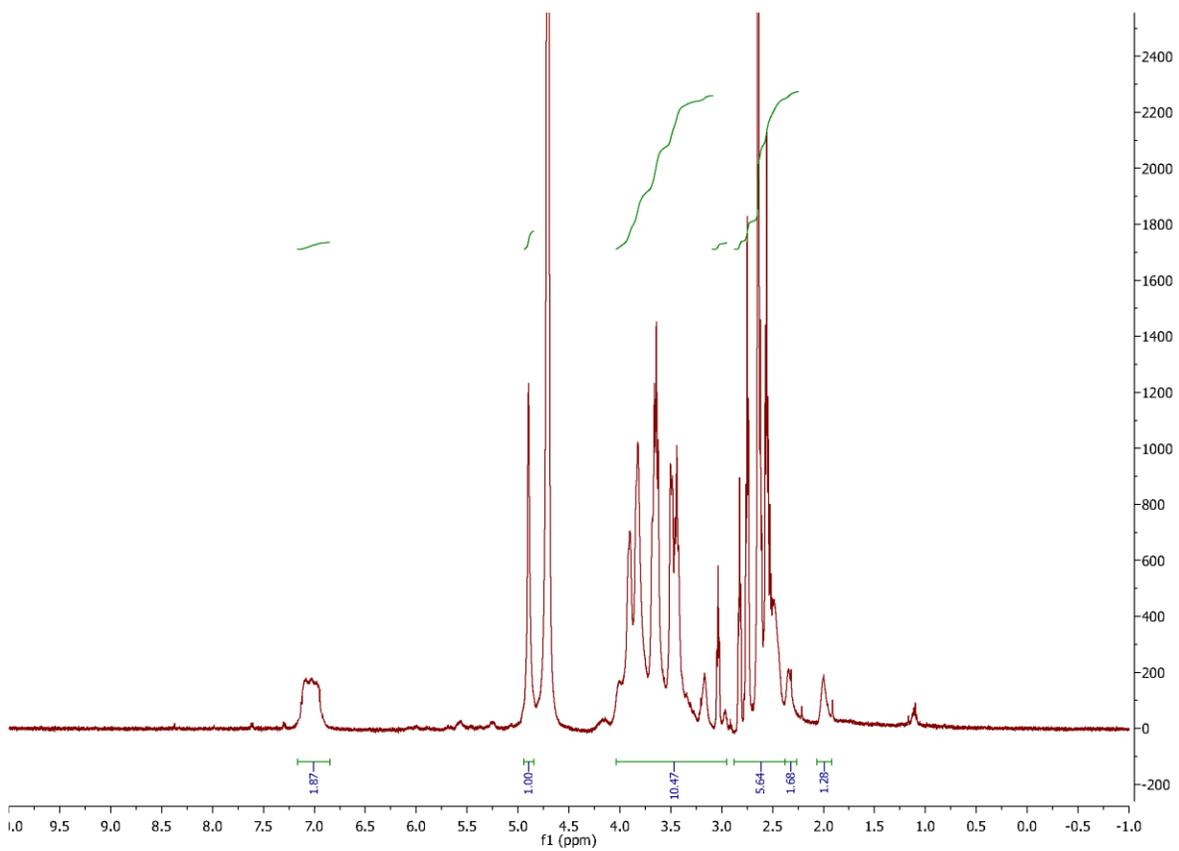
**Figure S9:**  $^1\text{H}$  NMR spectra of compound 1



**Figure S10:**  $^{13}\text{C}$  NMR spectra of compound 1.



**Figure S11:**  $^1\text{H}$  NMR spectra of compound 2



**Figure S12:**  $^1\text{H}$  NMR spectra of compound 4.

References:

- [1]. L. Cui, J. L. Cohen, C. K. Chu, P. R. Wich, P. H. Kierstead, J. M. J. Fréchet, *J. Am. Chem. Soc.*, 2012, 134, 15840–15848.
- [2]. K.H. Bouhadir, D.S. Hausman, D.J. Mooney, *Polymer*, 1999, 40, 3575–3584