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Supporting Information

Synthesis and Anticancer Properties of Fucoidan-Mimetic Glycopolymer Coated Gold Nanoparticles

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General Methods. All chemicals used were purchased from Sigma-Aldrich unless stated otherwise. ^{1}H NMR spectra (300 MHz) were recorded on a Varian 300 spectrometer in D_2O at 25°C. The resonance of the deuterated solvent was used as internal standard for all compounds (^{1}H NMR, $\delta = 4.79$)[1]. NMR spectra were processed in MestReNova v8.1.2-11880. Dialysis was performed using Spectra/Por® Dialysis Membrane 6. Prior to all dialyses filtering was done using Acrodisc® LC PVDF 0.45 μm filters. Gel permeation chromatography (GPC) was done at Polymer Standards Service GmbH, Mainz, Germany using a PG13 instrument equipped with a PSS SECcurity 1100 HPLC pump; a PSS MCX, 10 μm, Guard, ID 8.0 mm x 50 mm precolumn; a PSS MCX, 10 μm, 1000 Å, ID 8.0 mm x 300 mm column; a PSS MCX, 10 μm, 100000 Å, ID 8.0 mm x 300 mm column and a PSS SECcurity refractive index detector with 0.05 M KH_2PO_4 /

0.05 M K_2HPO_4 in deionized water as eluent at a flow rate of 1.0 mL/min at 23.0°C. Calibration was done with dextran/pullulan standards. Chromatograms were processed using the PSS – WinGPC UniChrom Version 8.2 software. Elemental analysis was performed at Eurofins Mikro Kemi AB, Uppsala, Sweden. The particle size determination was performed in Zetasizer Nano ZS from Malvern at 25 °C and UV-Vis spectroscopy was performed in Lambda 35 UV-Vis spectrophotometer from PerkinElmer. SEM images of Glycopolymer coated gold nanoparticles were recorded using SEM, MERLIN Zeiss.

Synthesis of Glycopolymer 2. 2-Methacrylamidoethyl 2,3,4-tri-*O*-Acetyl-α-L-fucopyranoside (**1**)[2] (2.025 g, 5.045 mmol, 1 equiv.), 2-(trimethylsilyl)ethanethiol (0.0325 mL, 0.203 mmol, 0.04 equiv.) and AIBN (0.0082 g, 0.050 mmol, 0.01 equiv.) were dissolved in 1,4-dioxane (4.465 mL). The solution was degassed through bubbling with N_2(g) for 20 minutes and then stirred at 70°C under an N_2(g) stream for 6 hours until the solution turned viscous. Another 5 mL 1,4-dioxane was added and the temperature was raised to 80°C to lower the viscosity. The per-acetylated glycopolymer was precipitated by adding the solution drop-wise to 900 mL diethyl ether and then stirred at room temperature overnight. Filtration, washing with diethyl ether and drying under vacuum for 48 hours gave the per-acetylated glycopolymer (1.186 g) as a white powder.

The per-acetylated glycopolymer (1.178 g) was suspended in methanol (40 mL) and sodium methoxide (0.160 g, 2.962 mmol) added. The suspension was stirred at room temperature for 2 days, during which the suspension turned into a homogenous solution. The solution was neutralized by addition of Dowex Marathon C, filtered and the beads washed with methanol. The solvent was evaporated and the crude glycopolymer re-dissolved in deionized water. Dialysis against deionized water (MWCO 1000) followed by lyophilisation afforded glycopolymer **2**

(0.719 g, 53% monomer conversion over two steps) as a white powder. GPC analysis gave M_n= 90600 Da, M_w = 119000 and PDI = 1.32.

Synthesis of FM-Glycopolymer 3. Glycopolymer **2** (0.062 g) was dissolved in DMF (22 mL) upon which sulfur trioxide pyridine complex (0.538 g, 3.380 mmol, 5 equiv. per hydroxyl group) was added and a precipitate formed almost instantly. The precipitate was scraped off the walls of the flask, crushed with a spatula and stirred at room temperature for 2 days. Further precipitation was allowed by cooling in a freezer for 3 hours and the solution then decanted off. The precipitate was dissolved in a solution of $NaHCO_3$ (0.752 g, 8.951 mmol) in deionized water (25 mL) and stirred overnight. The solution was dialyzed against deionized water (MWCO 1000) and lyophilized which gave the partially *O*-sulfated FM-glycopolymer **3** (0.108 g) as a white powder. Elemental analysis showed contents to be carbon 24.9 wt. % and sulfur 14.4 wt. % corresponding to an 87% conversion of hydroxyl groups to sulfate esters.

Synthesis of FM-Glycopolymer stabilized AuNPs (FMG-AuNP). AuNPs were prepared by chemical reduction of gold salts to yield the corresponding gold nanoparticle by using $NaBH_4$ as the reducing agent. Briefly, $HAuCl_4$ (7.5 mg, 0.0190 mmol, 1 equivalents) was dissolved in 7 mL deionized water and refluxed for 2 hours. This resultant gold solution was cooled to 60 °C. FM-glycopolymer **3** (15.0 mg, 2 equivalents with respect to L-fucoside repeat units) was dissolved separately in 15 mL of deionized water and quickly added to gold solution under vigorous magnetic stirring. $NaBH_4$ (0.35 mg, 0.009 mmol, 0.5 equivalent) was dissolved in 1 mL cold deionized water immediately before use. After stirring for 20 min, the $NaBH_4$ solution was added to the mixture solution with vigorous stirring. The formation of NP was initiated within 10 min,

and the reaction was continued overnight at 60 °C. Formation of AuNP could be observed by the appearance of a deep wine red color. This AuNP solution was subsequently loaded into a dialysis bag (MWCO 3500), dialyzed against deionized water (2 × 2L) for 48 h and lyophilized to obtain 16 mg of FMG-Au-NP as a purple powder material. The λ_{max} for AuNP was found to be 530 nm using UV spectrophotometer.

Synthesis of Chondroitin Sulfate-A coated gold nanoparticles

CS-Au-NP was synthesized following the protocol presented for FMG-Au-NP with slight modification. Briefly, $HAuCl_4$ (11.82 mg, 0.03 mmol, 0.5 equivalents) was dissolved in 10 mL deionized water and refluxed for 2 hours. This resultant gold solution was cooled to 60 °C. Chondroitin sulfate-A (30.0 mg, 1 equivalent with respect to disaccharide units) was dissolved separately in 10 mL of deionized water and added to gold solution under vigorous magnetic stirring. $NaBH_4$ (4.56 mg, 0.12 mmol, 2 equivalents) was added to this solution. The formation of NP was observed immediately with the appearance of violet color and the reaction was progressed for 8 h at 60 °C. This AuNP solution was subsequently loaded into a dialysis bag (MWCO 3500), dialyzed against deionized water (4 × 2L) for 48 h and lyophilized to obtain 28 mg of CS-Au-NP as a purple powder material.

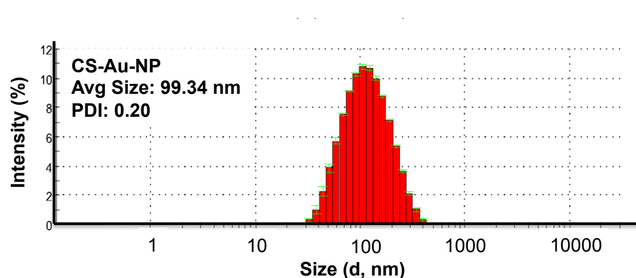


Figure S1. Dynamic light scattering profile of CS-Au-NP in 1 mg/mL concentration measured in deionized water.

Dynamic light scattering study

The particle size distribution of FMG-AuNP before and after lyophilization was carried out using Malvern laser granulometer (Zetasized Nano ZS, Malvern, United Kingdom). Freeze-dried samples were dissolved in deionised water at 1 mg/ml concentration before performing the DLS measurement.

Thermogravimetric Analysis:

Thermogravimetric analysis was performed on a Q500 TGA (TA instruments) in air or N_2 atmosphere (gas flow = 60 ml min^{-1}). Samples (1 mg) were placed on a platinum plate and heated in the TGA at a rate of 20 °C min^{-1} up to 800 or 1000 °C.

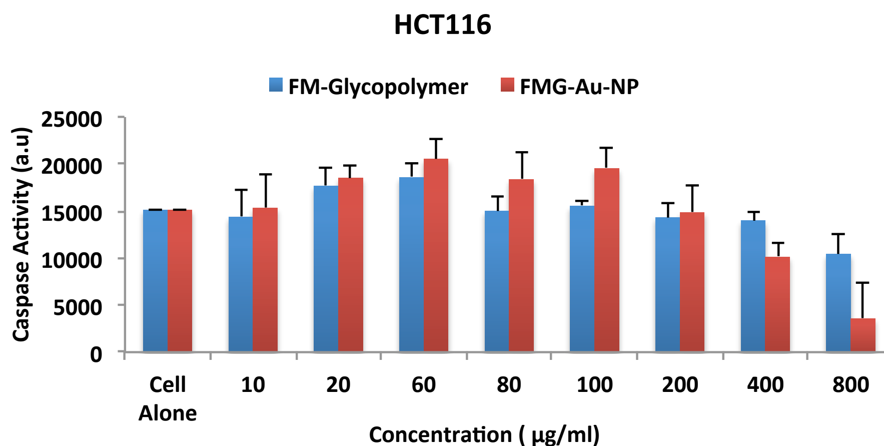
Scanning electron microscopy

FMG-Au-NP was dissolved in deionised water at 1 mg/ml concentration. SEM experiment was performed, by placing a drop of the dissolved sample on silicon wafer chip. Then, these samples were air dried and then dried under vacuum at 5.00 kV voltage. Thereafter, micrographs were taken by using a Zeiss Scanning Microscope MERLIN.

Cytotoxicity Studies and Caspase Activity

Cell viability study and caspase activity was measured using, ApoTox-Glo™ Triplex Assay kit following manufacturer's protocol. Briefly, HCT 116 and NIH3T3 cells were seeded in 384 well BD Facon black plates (1000 cells in 50 µL/well) using automated Biomek FX pipetting workstation and incubated at 37 °C for 24 h for cell attachment. Stock solution of FMG and FMG-AuNP was prepared in cell culture medium (DMEM) respectively. After 24 h, different volume of stock solution was added to each well using non-contact acoustic dispenser (Echo 555), to obtain a gradient concentration ranging from 10 µg/mL to 1000 µg/mL and incubated for additional 48 h at 37 °C. After 48 h, 20 µl of medium was treated with 5µl of cell viability

assay reagent, containing both GF-AFC substrate and bis-AAF-R110 substrate and incubated for 30 min at 37 °C. Fluorescence values were recorded at two wavelength sets: 400Ex/505Em (Viability) and 485Ex/520Em (Cytotoxicity) using Envision Multilabel Plate Reader and the cell viability were obtained as a percentage of the untreated control (100% cell viability). Caspase activity was also measured by adding 25µl of Caspase-Glo® 3/7 Reagent to all wells with incubation for 30 min at room temperature, followed by measuring its luminescence with plate reader (Perkin Elmer). All the cell experiments were measured in triplicate.



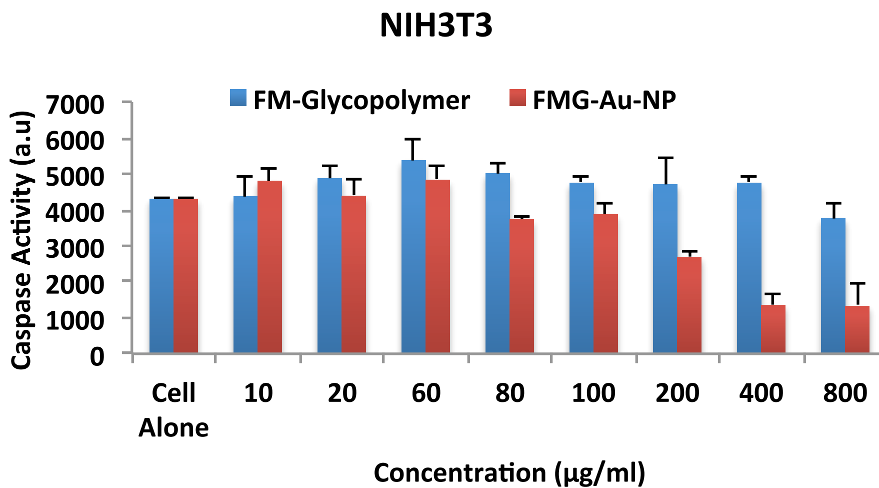


Figure S2. Caspase 3/7 activities of HCT116 and NIH3T3 cell line.

References

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