SUPPLEMENTARY INFORMATION

RNAi-based glyconanoparticles trigger apoptotic pathways for *in vitro* and *in vivo* enhanced cancer-cell killing

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1. Synthesis of Au@Citrate

AuNPs were synthesized by the citrate reduction method described by *Turkevich* and *Frens* and had an average diameter of 14 ± 1 nm. Briefly, in a round-bottom flask, 80 mg of hydrogen tetrachloroaurate(III) hydrate (0.235 mmol, Strem Chemicals) were solved in 200 mL of MiliQ water. It was heated under reflux until the solution started boiling, when 239.3 mg of sodium citrate tribasic dihydrate (0.814 mmol) were added turning the color to intense black and after a few seconds to red. The solution was kept under ebullition and protected from light for 30 minutes and finally cooled down to reach room temperature.

2. Synthesis of N-(20-azido-3,6,9,12,15,18-hexaoxoicosane)-4-mercaptobutanamide (HS-$(CH_2)_3$-CONH-EG(6)-$(CH_2)_2$-N_3) (PEG-N_3)

For the synthesis, 0.6 mmoles of a-Amine-ω-azido hepta(ethylene glycol) were added to 12.5 mL of dimethylsulfoxide under argon atmosphere in a poly(ethylene glycol) bath at 50ºC and under continuous stirring. Subsequently, 0.63 mmol of γ-tiobutirolactone and 5.7 mmol of triethylamine were added. After 5 hr, the mixture was cooled down and lyophilized obtaining a yellow oil. The yield of the reaction was 93%, without any further purification step. The product was characterized by: FT-IR: 3301 cm-1 (NH st), 2870 cm-1 (-CH st), 2530 cm-1 (SH st), 2106 cm-1 (N_3 st), 1663 cm-1 (C=O st); H-RMN 300 MHz (d): 2,00 (m, 2H); 2,25 (t, 2H, *J*=7,30 Hz); 2,65 (t, 2H, *J*=7,00 Hz); 3,01 (s, 1H, SH); 3,32 (t, 4H, *J* = 5,00 Hz); 3,37 (t, 2H, *J* = 5,22 Hz); 3,59 (s, 22H); 6,60 (t, 1H, *J* = 4,76 Hz) and mass spectroscopy [M-H+] 451.1.

3. Functionalization of Au@Citr with thiolated polyethyleneglycol (PEG)

To a 10 nM solution of Au@Citr, it was added SDS 0.028% (w/V), 10.5 μM of HS-$(CH_2)_3$-CONH-EG(6)-$(CH_2)_2$-N_3 and 10.5 μM de HS-EG(8)-$(CH_2)_2$-COOH, followed by NaOH to a final

concentration 25 mM. The mixture was kept stirring at room temperature for 16 hours and washed by centrifugation 3 times at 14000 rpm for 30 minutes at 4˚C.

4. Functionalization with biotin-(PEG)n-NH_2

In first place, EDC (2 µmol) and sulfo-NHS (3.5 µmol) were mixed in buffer MES 0.1M pH 6 and stirred for 10 minutes, followed by the addition of 20 pmol of AuNP@PEG and MiliQ water to a final volume of 1 mL and an incubation of 20 minutes. After the activation, the excess of reactants was eliminated by centrifugation with Amicon-Ultra® of 100KDa, resuspending the AuNPs in 1 mL of MES 50 mM pH 6 for the subsequent addition of the biotin to a final concentration of 2 µM. It was incubated for 2 hours at room temperature before proceeding to centrifuge the samples 3 times at 14000 rpm for 30 minutes at 4ºC in order to remove the excess of biotin.

5. Functionalization with 4-aminophenyl-β-D-glucopyranoside and biotin

The functionalization with 4-aminophenyl-β-D-glucopyranoside was made in the same way as for the biotin. In this case the glucose was added at a final concentration of 20 µM followed by the addition of 2 µM of biotin. The reaction was kept stirring at room temperature for 2 hours and centrifuged at 14000 rpm for 30 minutes at 4ºC. The same process was developed with 4-aminophenyl-β-D-galactopyranoside as control.

6. Functionalization with siRNA

For the functionalization 10 nM AuNP@glucose@biotin were diluted in phosphate buffer 10 mM pH 8 (in DEPC-water) with 0.028% (V/V) sodium dodecylsulfate followed by the addition of thiolated siRNA (Dharmacon, Thermofisher Scientific) at a concentration 5 µM. Next, it started 6 additions of NaCl reaching a final concentration of 0.3M. Each addition was followed by a brief

sonication. The mixture was kept stirring at 4ºC for two days when the excess of siRNA was removed by centrifugation at 14000 rpm for 30 minutes at 4ºC.

Thiolated siRNA (Sense strand: 5'-Thiol-GUGAGGAUAUCUGGAAGAAAUUU-3', Antisense strand: 5'-AUUUCUUCCAGAUAUCCUCACUU-3', from Thermo Scientific Dharmacon) was dissolved in 1 ml of 0.1 M DTT, extracted three times with ethyl acetate, and further purified through a desalting NAP-5 column (Pharmacia Biotech) according to the manufacturer's instructions.

7. Quantification of the HS-EG(8)-$(CH_2)_2$-COOH (PEG-COOH) chains attached

The quantification of HS-EG(8)-$(CH_2)_2$-COOH chains was made by indirect determination by Ellman's assay, based on the colorimetric determination of the reduction product of 5,5'-dithio-bis(2-nitrobenzoic) acid (DTNB) when free thiol groups are present in the medium. The excess of PEG-COOH chains was measured in the supernatants after centrifuging the reaction solution with the AuNPs and the PEG. For the quantification 400 μL of either the stock solution of PEG-COOH or the supernatant were mixed with 100 μL of TRIS buffer 1M pH 8 and 50 μL of DTNB 2 mM in sodium acetate (NaAc) buffer 50 mM. After 10 min of incubation the absorbance at 412 nm was measured. The linear range for the PEG-COOH chain obtained by this method is 1-100 μM (Abs412 = 0.006×[PEG-COOH, μM] + 0.1764)). Subtracting the amount of PEG found in the supernatants from the concentration used for the functionalization, it was possible to calculate the number of PEG chains attached to the nanoparticles.

8. Quantification of the HS-$(CH_2)_3$-CONH-EG(6)-$(CH2)_2$-N_3 (PEG-N_3) chains attached

The quantification of HS-$(CH_2)_3$-CONH-EG(6)-$(CH_2)_2$-N_3 has been carefully described elsewhere [11]. Briefly, after the synthesis, this chain was obtained part as thiol and part as a disulfide complicating the determination by Ellman's method as the DTNB only reacts with free thiol groups. Because of this, the determination of HS-$(CH_2)_3$-CONH-EG(6)-$(CH_2)_2$-N_3 was made by an indirect method varying the amount of this chain used and analyzing its influence in the functionalization

with HS-EG(8)-$(CH_2)_2$-COOH which could be effectively quantified as described before. To this aim, the functionalization was carried out either with different amount of PEG-COOH and a fixed one of PEG-N_3 (**Figure S1B**) or varying the concentration of both chains (**Figure S1C**). In both cases after removing the excess of reactants in this first functionalization, the resultant AuNPs were subsequently incubated with a fixed amount of PEG-COOH and the number of these chains attached was determined by Ellman's method. Results obtained showed a decrease in the amount of PEG-COOH attached in this second addition as the concentration of PEG-N_3 increased, demonstrating the presence of the azide chain on the surface of the AuNPs (**Figure S1**).

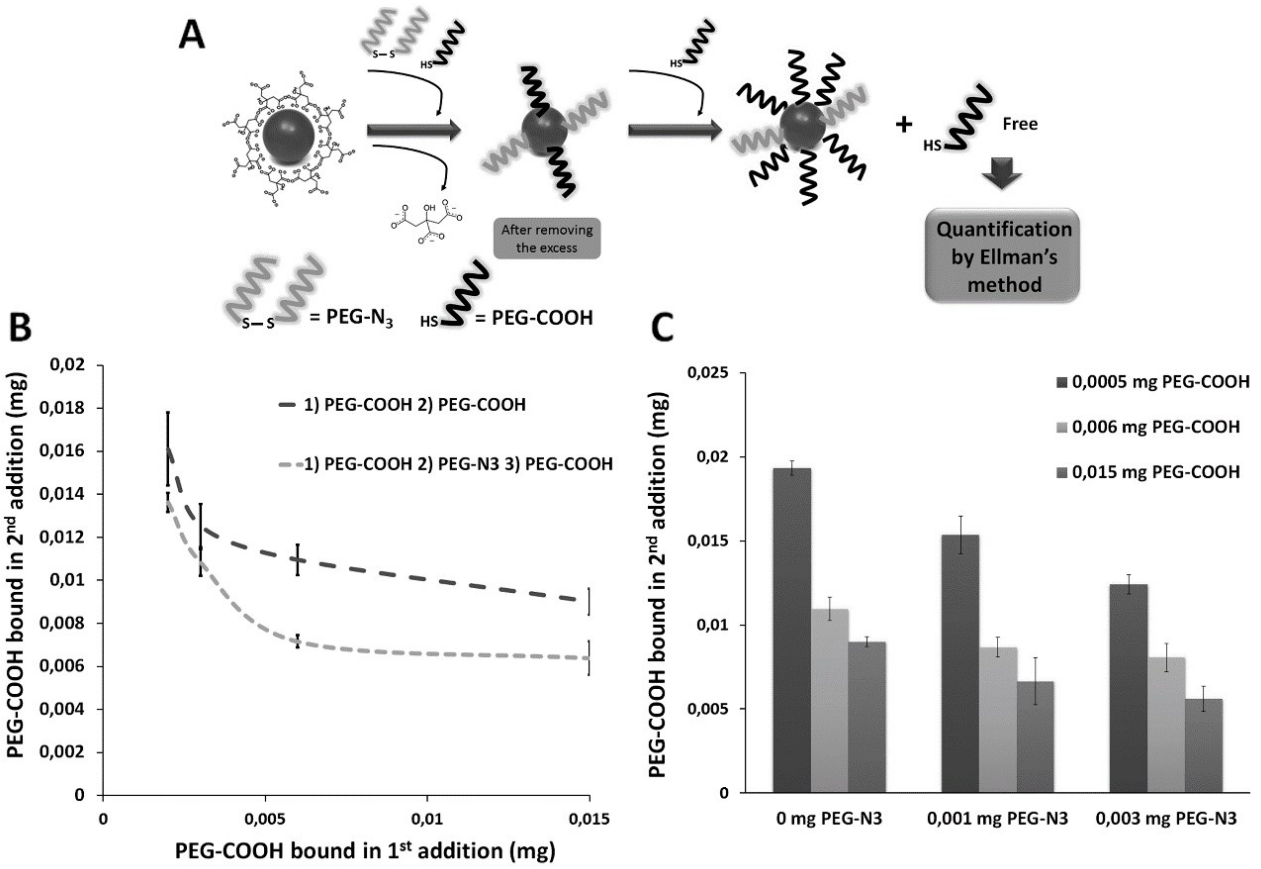

Figure S1. Determination of the PEG-N_3 attached to the AuNPs. (**A**) Scheme of the experiment, functionalizing the AuNPs in a first step with different amounts of PEG-COOH and PEG-N_3, followed by removing the excess of reactants and a final addition of a constant concentration of PEG-COOH that will be quantified by Ellman's method. (**B**) Different amount of PEG-COOH attached in the second addition with or without PEG-N_3 and (**C**) experiment varying the concentration of both the PEG-N_3 and the PEG-COOH added.

9. Determination of the biotin-(PEG)n-NH_2 attached

The effective functionalization of the AuNPs with biotin was tested by addition of the protein streptavidin because of the strong interaction between these two compounds (the streptavidin used for the assays was conjugated with fluorescein). In first place it was observed the aggregation of the AuNPs after 30 minutes of incubation as each molecule of streptavidin is able to interact with 4 molecules of biotin. The aggregation was also confirmed by analyzing the samples by UV-Vis spectroscopy obtaining a broader peak as the amount of streptavidin added was increased. Lastly, after incubation with the streptavidin, the samples were centrifuged with Amicon-Ultra® of 100KDa in order to avoid the aggregation as much as possible, and the supernatants were kept and tested for protein concentration by Bradford assay (Thermo Fisher). This way it was possible not only to determine the effective functionalization of the AuNPs with biotin but to quantify the streptavidin attached per AuNP (**Figure S2**).

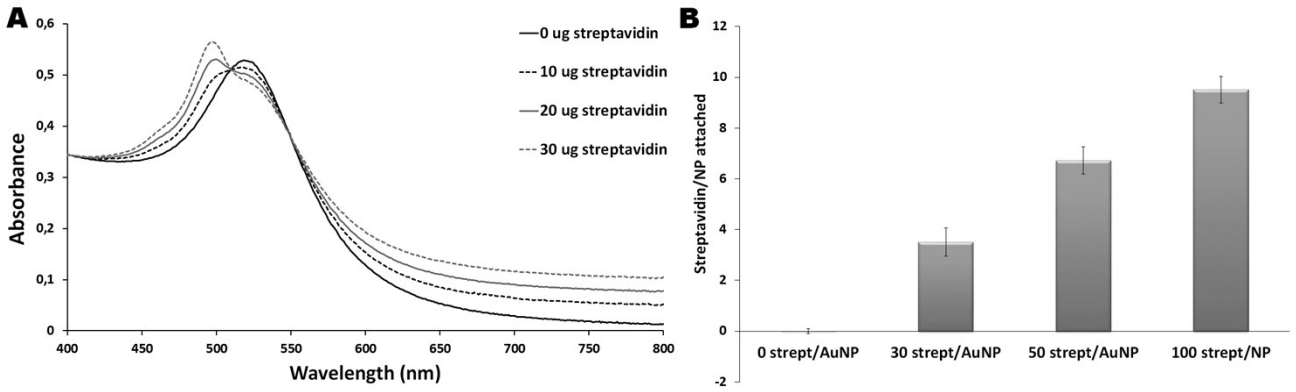

Figure S2. Determination of the biotin-(PEG)n-NH_2 attached. (A) UV-Vis spectroscopy showing how the peak of absorbance broadens as the amount of streptavidin-fluorescein added to the Au@PEG@Biot. **(B)** Quantification of the streptavidin attached to the AuNPs by indirect determination by Bradford's assay, depending on the concentration of streptavidin used.

10. Determination of the 4-aminophenyl-β-D-glucopyranoside bound

In first place, the samples were analyzed by zeta potential and electrophoresis in agarose gels because of the different net charge of the AuNPs once functionalized with either glucose or galactose. To test the effective functionalization, the AuNPs were incubated with lectin Concanavalin A, known because of its strong affinity for glucose (but not for galactose, used as control). As well as the streptavidin, the Con A possesses 4 points of interaction, being able to attach to 4 molecules of glucose, inducing the aggregation of the AuNPs if glucose is covalently attached. To this aim, 10 nM AuNP@glucose@biotin were incubated with 2.5 μM Concanavalin A in a solution of phosphate buffer 10 mM pH 7, sodium chloride 0.1 M, calcium chloride 1 mM and magnesium chloride 1 mM, for 2 hours before centrifuging the samples. The aggregation was analyzed by UV-Vis spectroscopy, the unfunctionalized AuNPs and the ones with galactose showed a narrow peak at 519 nm while AuNP@glucose@biotin had a broad peak indicating its aggregation (**Figure S3**).

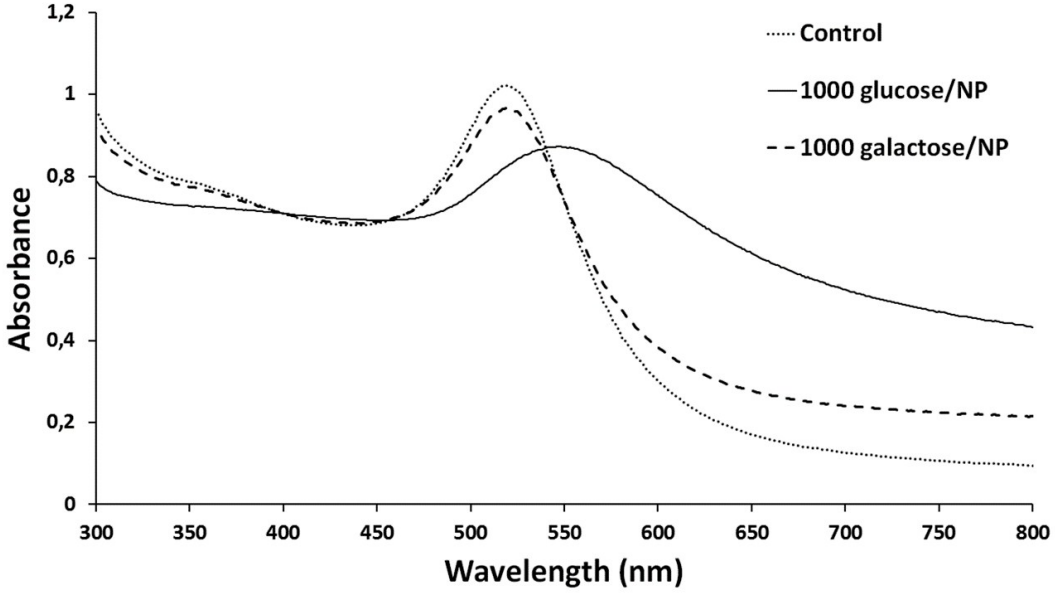

Figure S3. Determination of the 4-aminophenyl-β-D-glucopyranoside bound. Analysis by UV-Vis spectroscopy of the stability of AuNPs in the presence of the lectin Concanavalin A. The control (AuNP@PEG unfunctionalized) and AuNP@PEG@Galactose@Biotin remained stable with the maximum of the peak of absorbance at 519 nm,while the AuNPs functionalized with glucose showed a peak displaced to higher wavelengths indicating strong aggregation.

11. Determination of siRNA

The quantification was made using a nucleic acid intercalator, the GelRed™ (Biotium) and measuring the fluorescence once in the presence of the siRNA. The supernatants were incubated with GelRed 1× and phosphate buffer 10 mM pH 8, and fluorescence was measured with emission at 602 nm. A calibration curve was first establish with the thiolated siRNA used (Emission at 602 nm = 7328.3×[siRNA, nmol] + 39.366).

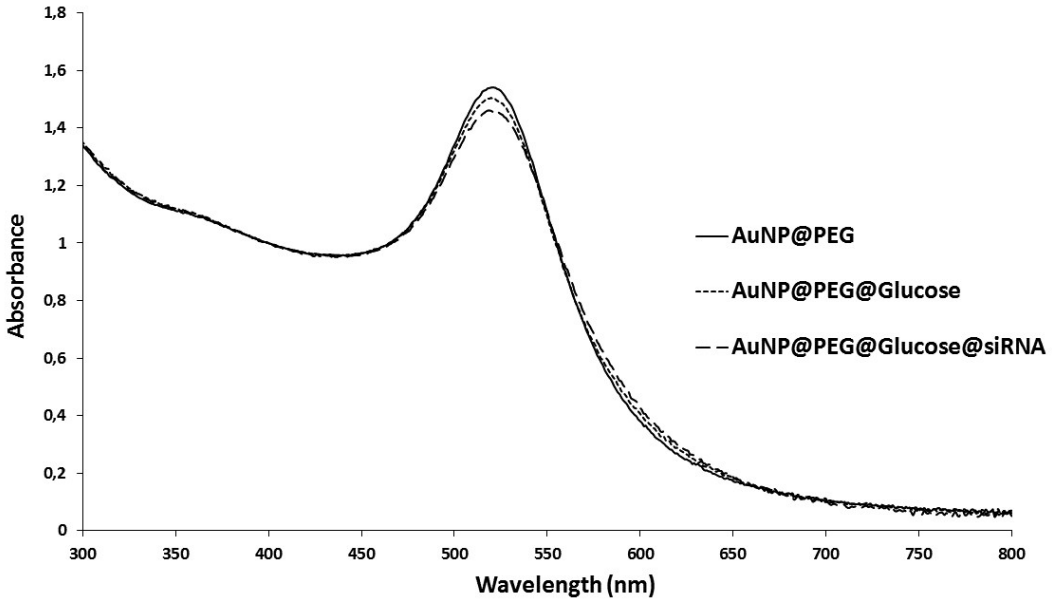

Figure S4. Analysis by UV-Vis spectroscopy of the stability of AuNPs functionalized with PEG, glucose and siRNA. All NPs remained stable with the maximum of the peak of absorbance at 519 nm.