Electronic Supplementary Material (ESI) for Nanoscale.

Supplementary information for

Gold silver alloy nanoparticles (GSAN): an imaging probe for breast cancer screening with dual energy mammography or computed tomography

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Reactive oxygen species generation assay

The intracellular reactive oxygen species (ROS) generation of each GSAN formulation was examined in J774A.1 and Hep G2 cells using 5-(and 6) carboxy-2′,7′-dichloro-dihydroflourescein diacetate (carboxy H_2DCFDA, Invitrogen, CA, USA). Increased fluorescence as a result of intracellular oxidation of carboxy H_2DCFDA to 5-(and 6) carboxy-2′,7′-dichloro-dihydroflourescein (DCF) in cells is an indication of generation of ROS. The assay was performed in black 96 well flat bottom microplates (Nunc, Roskilde, Denmark), according to previously published methods.[1] In brief, 10,000 cells were seeded in each well and the 96 well plate was incubated at 37 °C for 24 hours in a 5% CO_2 humidified incubator. After 24 hours of incubation, the cell monolayer was washed with sterile PBS and then incubated with GSAN dispersed in cell culture media at a dose of 200 µg Ag/ml for 2 or 24 hours. Six replicate wells were used for each control and test concentration per microplate. After incubation with GSAN, the cell monolayer was washed gently with sterile PBS twice. Then 100 µl of 10 µM carboxy H_2DCFDA in PBS was added to each well and the plates subsequently incubated at 37 °C for 40 minutes. After this incubation, the fluorescence was measured at 485 nm excitation and 528 nm emission wavelengths using a microplate reader. Three independent experiments were performed for each GSAN formulation. The percentage of fluorescence compared to control was calculated and the data presented as mean ± standard deviation (n=3).

DNA damage assay

DNA isolation

DNA damage assay was performed in J774A.1 and Hep G2 cells after incubation with/without GSAN. In brief, J774A.1 or Hep G2 cells were seeded in 6 well plates at a density

of $1.5x10^6$ cells per well and the plate was incubated at 37 °C in a 5% CO_2 humidified incubator for 24 hours. After 24 hours, cells were washed with PBS and incubated with GSAN formulation at a dose 200 µg Ag/ml for 2 or 24 hours. After this incubation, the media was removed and the cell monolayer was washed twice with PBS. HepG2 cells were trypsinized, while scraping was used to remove the J774A.1 cells. The cells were then centrifuged at 600 rcf for 5 minutes at 4 °C. DNA from J774A.1 and HepG2 cells was isolated using a genomic DNA isolation kit (abcam, MA, USA) according to the manufacturer's instructions.

DNA damage quantification

The DNA damage assay was performed using DNA damage quantification kit (Oxford Biomedical Research, MI, USA), according to the manufacturer's instructions. This is a colorimetric assay, where increased absorbance in the sample as an indication of production of more apurinic/apyrimidinic (AP) sites in the DNA structure, which is a marker for DNA damage. All the reagents and buffers for this assay were provided in the kit. In brief, extracted DNA from J774A.1 and Hep G2 cells was tagged with biotin. 60 µl of each sample and standard was placed in each well of a 96 well plate. 100 µl of DNA binding buffer was added and the plate was incubated at room temperature for 16 hours. After this incubation, each well was washed five times with the multi-purpose solution provided in the kit. After washing, 100 µl of freshly prepared HRP-streptavidin was added to each well and then the plate was allowed to shake at 100 revolutions per minute for one hour. After 1 hour, each well was washed 5 times with multi-purpose solution. Then 100 µl of TMB substrate was added to each well and the plate was incubated at 37°C for one hour. After this incubation, absorbance was recorded at 650 nm using a plate reader. The percentage of DNA damage was calculated compared to control and the data presented as mean ± standard deviation (n=3).

Figure S1

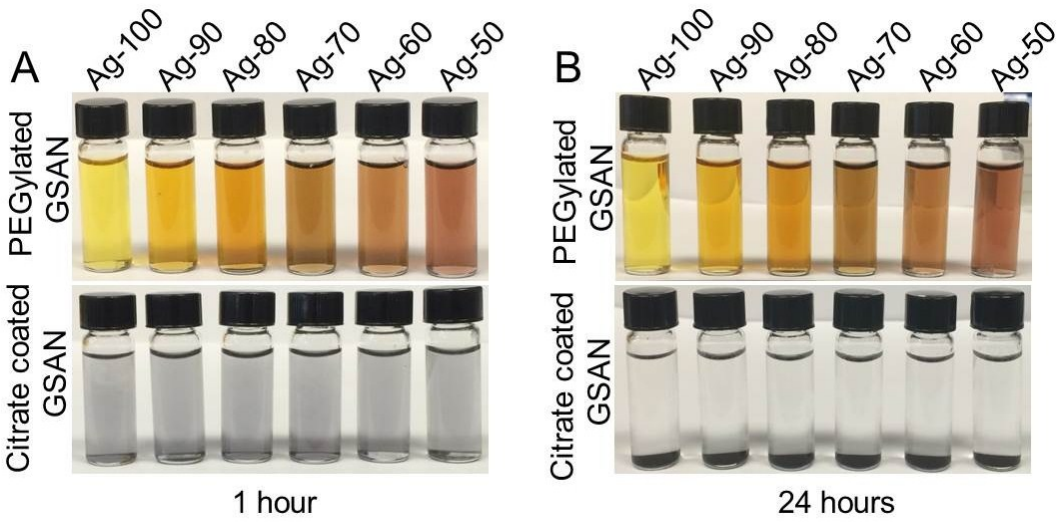

Figure S1. Photographs of GSAN (citrate coated and PEGylated) incubated in PBS for 1 hour (A) and 24 hours (B). After 1 hour and 24 hours incubation of GSAN formulations (PEG coated) in PBS, no aggregation or settling was visible. On the other hand, citrate coated GSAN were not stable in PBS, and precipitated and settled to the bottom of the vial.

Figure S2

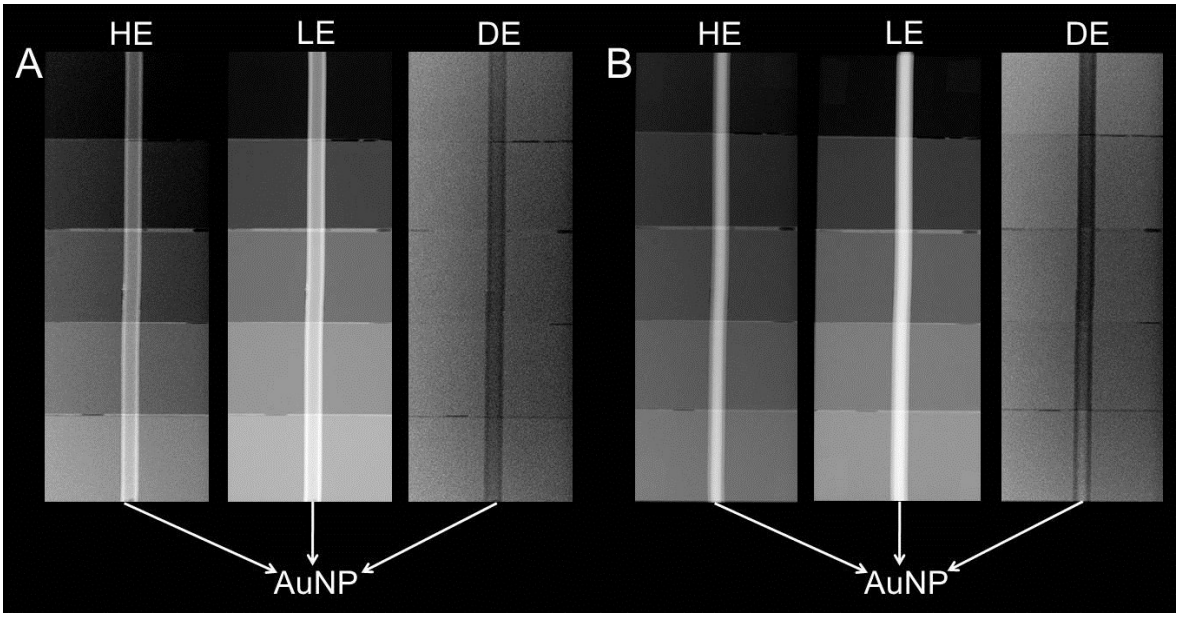

Figure S2. Dual energy mammography phantom images of AuNP at concentrations of 16 mg/ml (A) and 50 mg/ml (B). HE: high energy image; LE: low energy image and DE: dual energy subtraction image.

Figure S3

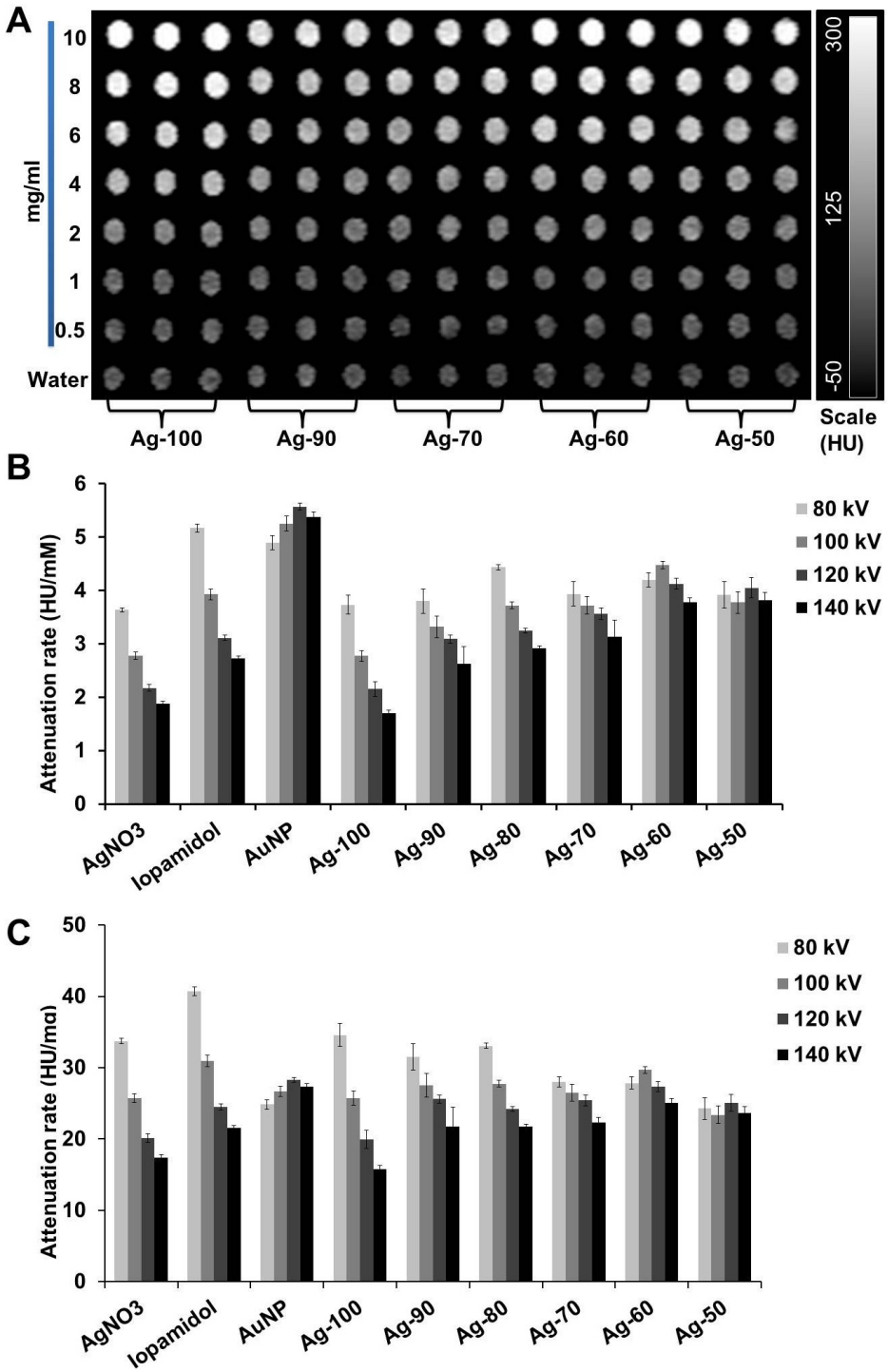

Figure S3: CT phantom images of GSAN (Ag-100, Ag-90, Ag-70, Ag-60 and Ag-50) scanned at 80 kV (A); CT attenuation rates HU/mM (B) and HU/mg (C) of different agents.

Figure S4.

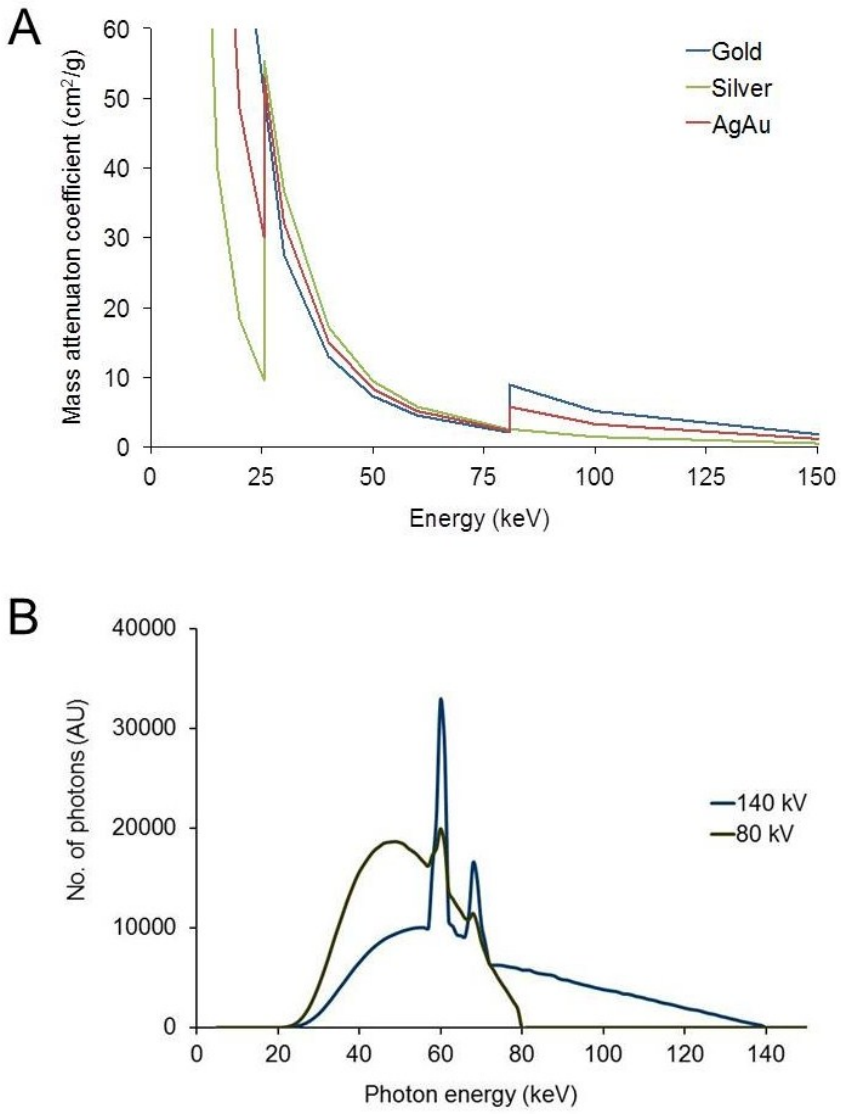

Figure S4: (A) Mass attenuation coefficients of gold, silver and 1:1 mixture of gold and silver. The data was obtained from the NIST website. (B) Photon energy distribution generated from the X-ray tube of a CT scanner run at 80 or 140 kV.

Figure S5.

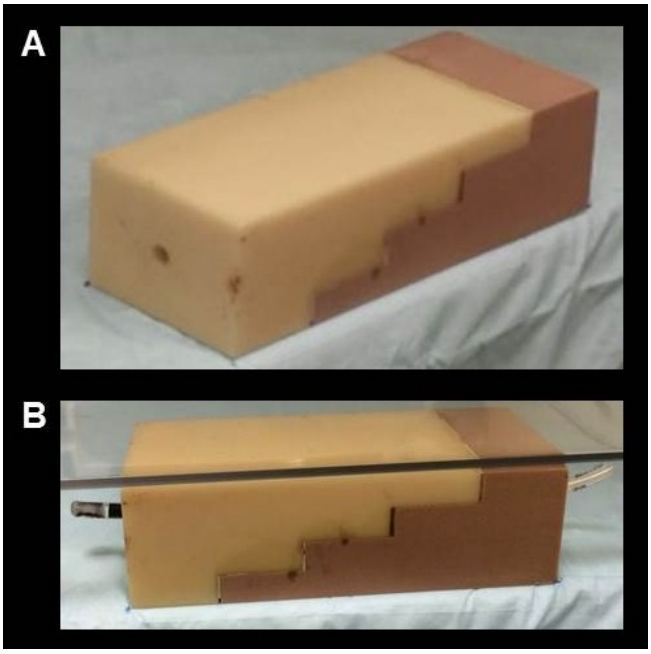

Figure S5. DE Mammography step phantom image, A) Empty phantom; B) Tube containg GSAN inserted into the step phantom.

References

1. Naha, P. C.; Davoren, M.; Lyng, F. M.; Byrne, H. J. Reactive oxygen species (ROS) induced cytokine production and cytotoxicity of PAMAM dendrimers in J774A.1 cells. *Toxicol Appl Pharm.* **2010**, *246*, 91-9.