Electronic Supplementary Information

Nanogold POxylation: Towards always-on fluorescent lung cancer targeting

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1. Synthesis of oligomers 2 and 3.

Cysteamine termination of living oligo-oxazoline was obtained through the addition of a tenfold excess of cysteamine, solubilized in anhydrous DMF, relatively to the initiator. The mixtures were kept at 70 ^{o}C using an oil bath under stirring for 24 hours. The oily oligomer solubilized in dry DMF was purified by dialysis against milli-Q water. The resulting mixture was dried under vacuum and the resulting oily polymer (OEtOx-SH, **2**) presented a yellow brownish color. For the synthesis of the oligo-oxazoline-*N*-chromylium PEI salt (OEI-CS, **3**), the living oligo-oxazoline was initially capped with water (OEtOx-OH, **1**),[1] (0.77 g, 7.8 mmol) and 2,4-dihydroxybenzaldehyde (1.07 g, 7.8 mmol) and $BF_3.OEt_2$ (4.0 mL) were added. The reaction mixture became red and was allowed to react for 24 h at room temperature. After this period, diethyl ether was added and the polymer precipitated as a dark red solid. The hygroscopic solid was washed several times with diethyl ether and dried under vacuum (quantitative yield). Oligomers **2** and **3** are both soluble in water and show a blue emission at 408 nm (λ_{ex}= 348 nm). The oligomers were characterized by IR, ^{1}H NMR and MALDI-TOF.

Next, GNPs were capped with **2** and **3** (GNP:OOx molar ratios of 1:5000 and 1:2500, respectively, at which no aggregation or flocculation occurs) producing Au-OEtOx-SH (**4**) and Au-OEI-CS (**5**), respectively. Afterwards, the mixtures were collected to 1.5 mL eppendorfs and centrifuged at 13500 X g, for 20 min, at room temperature. The supernatant was removed and the pellet was redispersed in milli-Q water. The resulting types of nanoparticles were further conjugated with the laminin fragment (YIGSR): different ratios of a stocking solution were added to the Au-OOXs producing Au-OEtOx-SH-YIGSR (**6**) and Au-OEI-CS-YIGSR (**7**). The mixtures

were allowed to stir during 16 hours at room temperature under dark conditions. The resulting mixtures were centrifuged at 13500 X g and ressuspended in milli-Q water.[2]

2. Cellular uptake

The uptake of the nanoprobes was assessed by confocal laser scanning microscopy (CLSM). A549 cell line was seeded at a density of $4x10^4$ cells/well and grown in Ham-F12 containing 10% FBS, on glass-bottomed coverslips coated with collagen during 24 h. Afterwards, nanoparticles were placed in contact with cells for 4 h to allow nanoparticles internalization. After this period of time, cells medium was replaced by Ham-F12 supplemented with FBS and antibiotics. Then, the cells' cytoplasm was marked with CellLight® Actin-GFP, BacMam 2.0 (GFP).[3] The proliferation of A549 cell line in the presence of nanogold POxylated probes was evaluated by seeding the cells in 96-well plates at a density of $4x10^4$ cells/well with nutrient mixture Ham-F12 supplemented with 10% fetal bovine serum (FBS), for 24 h. After that, the medium was removed; the nanoparticles were ressuspended in Ham F-12 at a concentration of 200 µg/mL and placed in contact with cells for 4 h to allow nanoparticles internalization. After this period of time, cells medium was replaced by Ham-F12 supplemented with FBS and antibiotics. Cell proliferation was evaluated at 24 and 72 h. Cell growth and adherence of the cells with internalized nanoparticles was monitored using an Olympus CX41 inverted light microscope equipped with an Olympus SP-500 UZ digital camera (Figure S1).

3. Laminin fragment quantification

YIGSR was previously quantified using RP-HPLC and a ubondapack C18 10 µm 3.9x100 mm with a gradient of acetonitrile: water (0.05% TFA + 5% TFA respectively) at 220 nm based on standard curves (R^2> 0.99). Briefly, the produced nanoparticles containing the oxazolines and the peptide sequence were centrifuged twice. The supernatant was removed, lyophilized and ressuspended in 1mL of mili-Q water in order to recover possible traces of unbounded YIGSR.[4]

4. MTS assay

Briefly, $4x10^4$ cells/well were seeded in a 96-well plate and cultured with Ham-F12 at 37ºC under a 5% CO_2 humidified atmosphere. Then, 200 µg/mL of nanoparticles were added, and the mitochondrial redox activity of the viable cells was assessed through the reduction of the MTS into a water-soluble brown formazan product as previously described. Wells containing cells in the culture medium without materials were used as negative control. EtOH 96% was also added to some wells, to be used as a positive control.[3,5]

5. IR, ^{1}H NMR and MALDI-TOF spectra

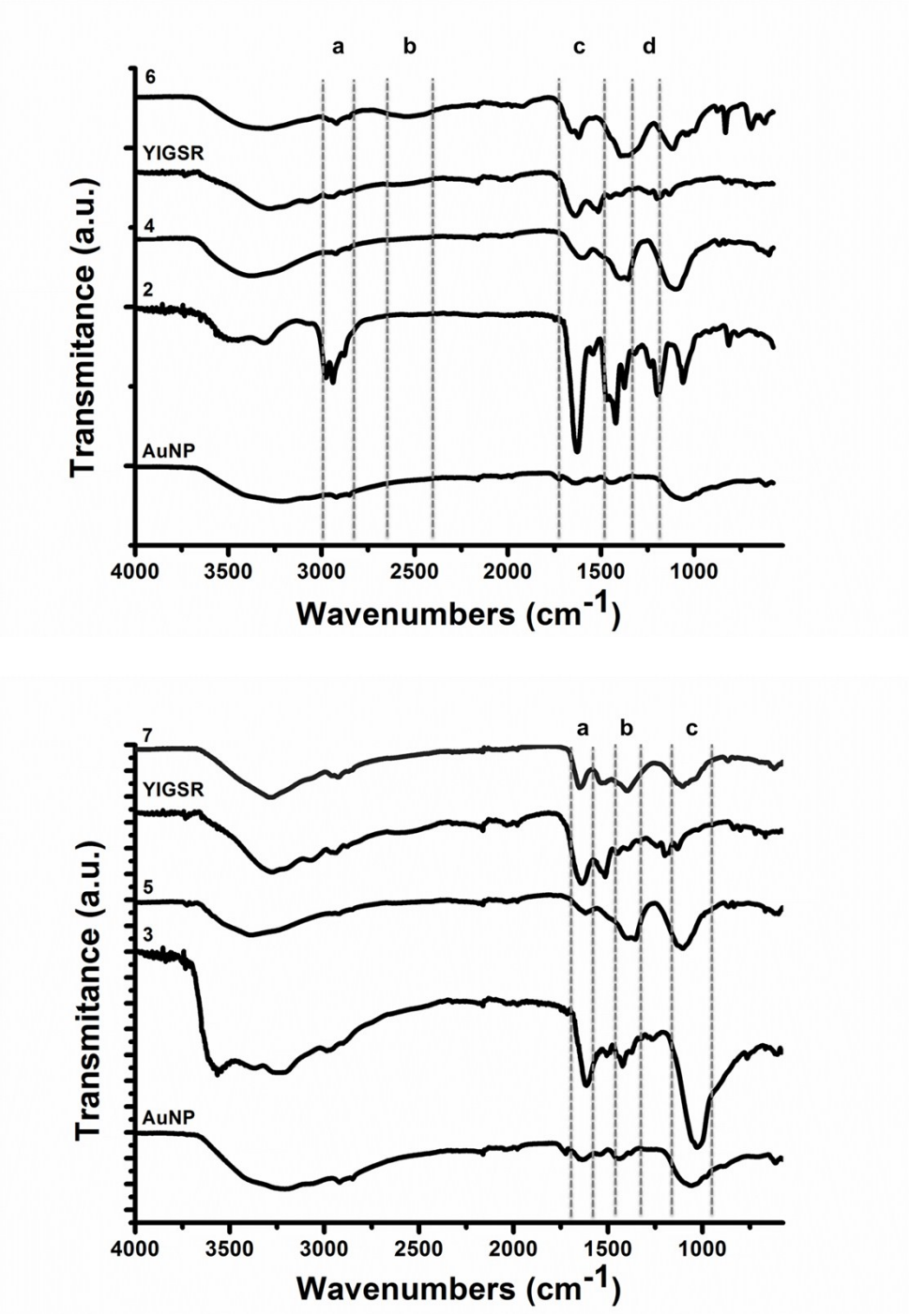

Figure S1. FTIR spectra (top and bottom) of the synthetic peptide (YIGSR), oligomers (**2** and **3)**, gold nanoparticles (AuNPs), nanogold POxylated probes (**6** and **7**) and their intermediates (**4** and **5**).

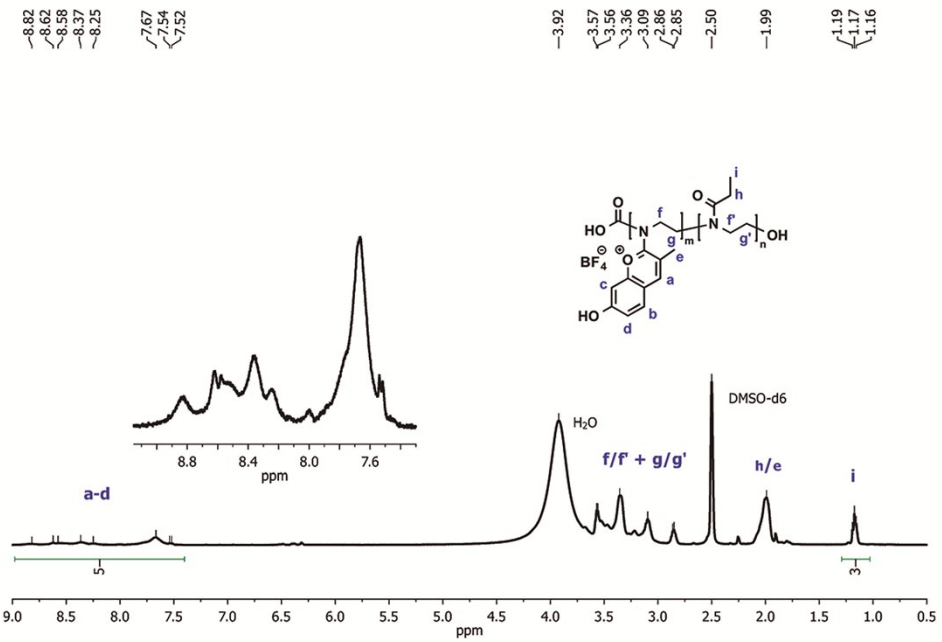

Figure S2. ^{1}H NMR spectra of oligomer **3**.

From the MALDI-TOF analysis of oligomer **3**, eight different distributions were found: four with CO_2 incorporation (M_w= 974 g.mol-1) and four without (M_w= 1549 g.mol-1). The incorporation of CO_2 in the polymer chains is ~ 48%.

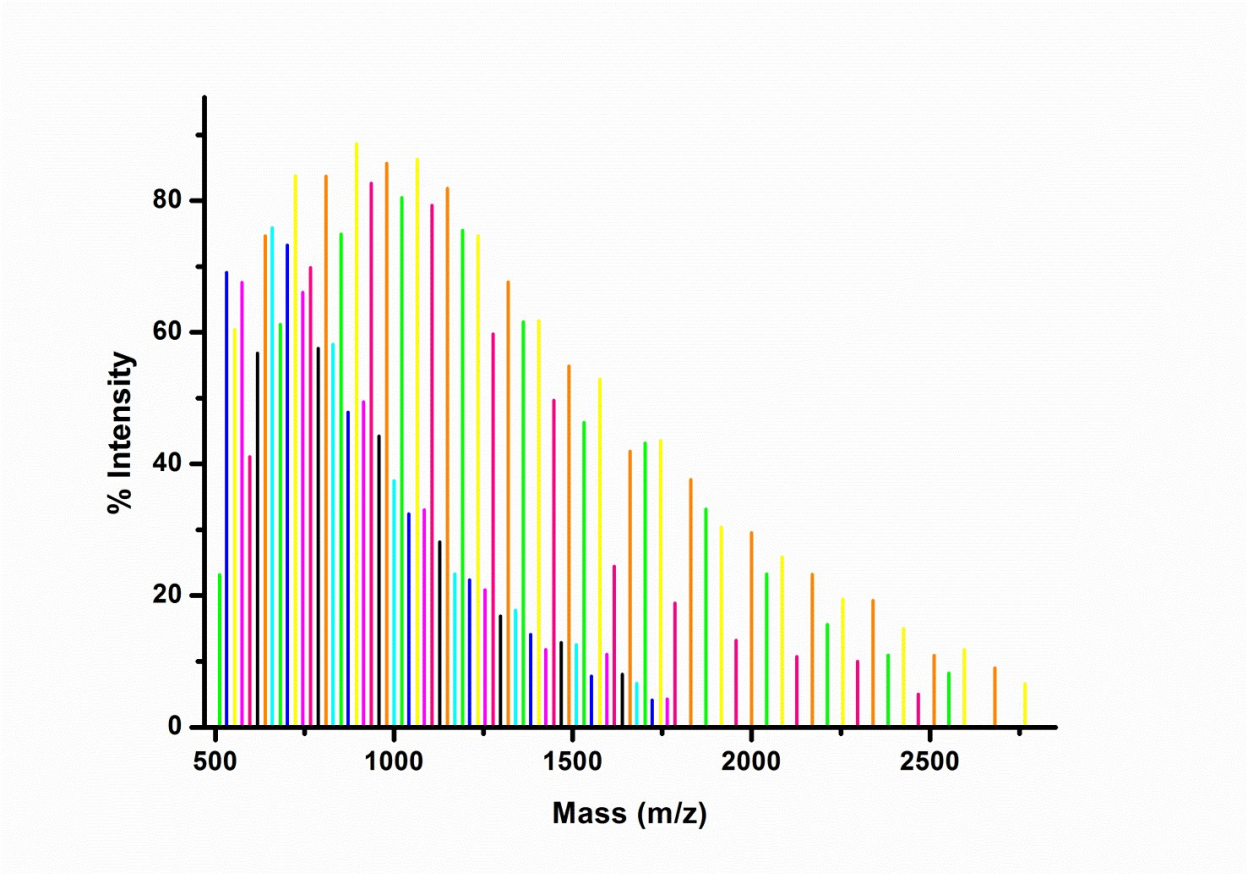

Figure S3. MALDI-TOF spectra of oligomer **3**. Matrix: DHB+Na. **CO_2 insertion –** blue, black, cyan and red; **No CO_2 insertion** – yellow, green, orange and pink.

6. Estimation of the number of ligands *per* gold nanoparticle

According to the DLS measurements, the average diameter of the gold nanoparticles is 28 nm. Assuming the spherical shape for the particles, the volume of each particle is $(4/3)\pi(d/2)^3$= 1.14882×10^{-17} cm^3. Hence the mass of each particle (M_{Au}) is (19.3×10^3 mg/cm^3) x (1.14882×10^{-17} cm^3)= 2.21723×10^{-13} mg.

The amount of YIGSR added to that was 5.17×10^{-5} M and the detection limit for the YIGSR was found to be 5.17×10^{-8} M (which is 0.1% of the original concentration). Considering both values

and considering that no trace of peptide was found in the HPLC chromatogram and that the thiol groups from the peptide have high affinity to the GNPs core, the binding of the peptide was considered to be 100%.

Bearing this, the amount of YIGSR *per* GNP could be estimated. For this experiment, the amount of peptide added to the GNP was 1.36×10^{-6} g or 1.41×10^{-9} mol; based on the molar ratio 100:1 (peptide:GNP). From TGA analysis of OEtOx-SH-YIGSR (**6**) (Figure S5) the amount of gold core is 1.47104 mg. So the number of gold nanoparticles (N_{Au}) is ($1.47104 / 2.21723 \times 10^{-13}$)= 6.6346×10^{12}. Also, the weight of organic molecules was 0.12896 mg (1.6 mg of the initial sample – 1.47104 mg from the gold core). If the amount of peptide in the organic part is 1.36×10^{-6} g (or 1.36×10^{-3} mg) than, the amount of OEtOx-SH (M_w= 1449.15 g/mol, estimated through ^{1}H NMR) lost was ($1.6 - 1.47104 - 1.36 \times 10^{-3}$)= 1.28×10^{-4} g. Therefore, the number of OEtOx-SH molecules ($N_{OEtOx-SH}$) is (1.28×10^{-4}) x (6.023×10^{23}) / 1449.15 = 5.30×10^{16} and the number of OEtOx-SH molecules *per* one GNP is $N_{OEtOx-SH}/N_{Au}$= 7993.49. As for the YIGSR sequence, the amount of YIGSR molecules (N_{YIGSR}) *per* GNP is N_{YIGSR}/N_{Au}= 127.64. Thus the total number of organic molecules *per* nanoparticle is 8121.13.

The same study was performed for Au-OEI-CS-YIGSR (**7**). Briefly, from TGA analysis (Figure S5) the amount of gold core is 2.447 mg. So the number of GNPs (N_{Au}) is ($2.447 / 2.21723 \times 10^{-13}$)= 1.10363×10^{13}. Again, the amount of peptide added to the GNP was $1.36 \times 10_{-6}$ g or 1.41×10^{-9} mol; based on the molar ratio 100:1 (peptide:GNP). Therefore, the amount of OEI-CS (M_w= 1549 g/mol, estimated through MALDI-TOF) in the organic material is ($3 - 2.447 - 1.36 \times 10^{-3}$)= 9.46403×10^{-2} mg. Therefore, the number of OEI-CS molecules (N_{OEI-CS}) is (9.46403×10^{-5}) x (6.023×10^{23}) / 1549= 3.38×10^{16} and the number of OEI-CS molecules *per* one GNP is N_{OEI-CS}/N_{Au}=

2809.64. The amount of YIGSR molecules (N_{YIGSR}) *per* GNP is N_{YIGSR}/N_{Au}= 64.66. Thus the total number of organic molecules *per* nanoparticle is 2874.3.

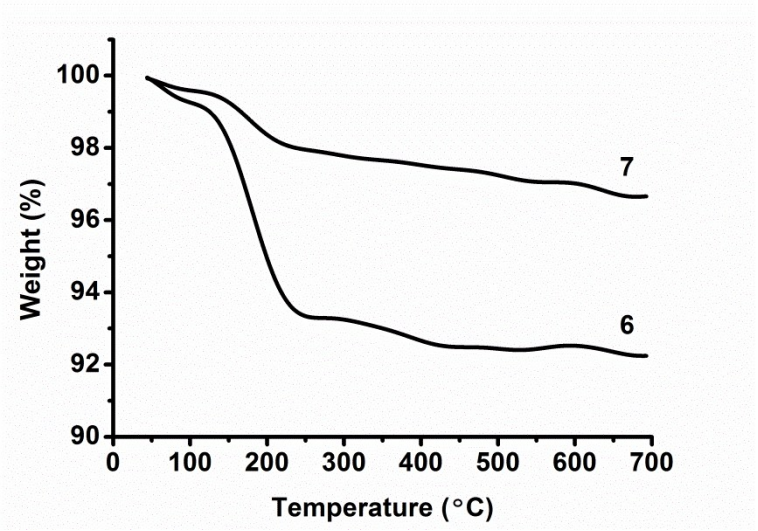

Figure S4. TGA curves of Au-OEtOx-SH-YIGSR (**6**) and Au-OEI-CS-YIGSR (**7**).

References

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