Supporting Information

**Design of oligoaziridine-PEG coatings for efficient nanogold cellular biotagging**

A. Sofia Silva§,†, Vasco D. B. Bonifácio‡*, Vivek P. Raje,§ Paula S. Branco§, Paulo F.B. Machado†, Ilídio J. Correia† and Ana Aguiar-Ricardo§,*

§REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Campus de Caparica, 2829-516 Caparica, Portugal.
†CICS-UBI, Health Sciences Research Center, Faculdade de Ciências da Saúde, Universidade da Beira Interior, Av. Infante D. Henrique, 6200-506 Covilhã, Portugal.
‡Instituto de Telecomunicações, Instituto Superior Técnico, Av. Rovisco Pais, 1049-001 Lisboa, Portugal.

* E-mail: air@fct.unl.pt; vasco.bonifacio@tecnico.ulisboa.pt
Estimation of the number of ligands per gold nanoparticle

According to TEM images and DLS measurements (Figure 2 and Table 1), the average diameter of the nanoparticles is 28 nm. Assuming the spherical shape for the particles, the volume of each particle is \((4/3)\pi(d/2)^3 = 1.15 \times 10^{-17} \text{ cm}^3\). Hence the mass of each particle \((M_{\text{Au}})\) is \((19.3 \times 10^3 \text{ mg/cm}^3) \times (1.15 \times 10^{-17} \text{ cm}^3) = 2.22 \times 10^{-13} \text{ mg}\). From TGA analysis of MPM coated AuNP (Figure S1A) the amount of gold core is 1.4 mg. So the number of gold nanoparticles \((N_{\text{Au}})\) is \((1.4 / 2.22 \times 10^{-13}) = 6.31 \times 10^{12}\). Also, from the TGA data, the amount of MPM \((M_w= 3400 \text{ g/mol})\) present is 0.10005 mg. Therefore, the number of MPM molecules \((N_{\text{MPM}})\) is \((0.100 \times 10^{-3}) \times (6.023 \times 10^{23}) / 3400 = 1.77 \times 10^{16}\) and the number of MPM molecules per one AuNP is \(N_{\text{MPM}}/N_{\text{Au}} = 2807\).

From TGA analysis of OA coated AuNP (Figure S1B) the amount of gold core is 2.63 mg. So the number of gold nanoparticles \((N_{\text{Au}})\) is \((2.63 / 2.22 \times 10^{-13}) = 1.19 \times 10^{13}\). Also, from the TGA data, the amount of OA \((M_w= 997.50 \text{ g/mol})\) present is 0.10005 mg. Therefore, the number of OA molecules \((N_{\text{OA}})\) is \((0.171 \times 10^{-3}) \times (6.023 \times 10^{23}) / 997.5 = 1.03 \times 10^{17}\) and the number of OA molecules per one AuNP is \(N_{\text{OA}}/N_{\text{Au}} = 8712\).

In order to understand how much OA and MPM are grafted onto particles’ surface in the sample Au-MPM-OA, an ICP analysis for the sulfur atom was performed. Considering that each mol of S origins 1 mol of OA, the amount of OA/AuNP (grams) is \(9.63 \times 10^{-3}\). Therefore, considering the amount of gold core \((1.6 - (0.04 \times 1.6)) = 1.536 \text{ mg}\), the amount of OA is \((1.54 \times 10^{-3}) \times (9.63 \times 10^{-3}) = 1.48 \times 10^{-5} \text{ g}\). Hence the mass of MPM in the weight loss from Au-MPM-OA (0.064 mg), is \((1.6 - 1.536 - 1.48 \times 10^{-5}) = 6.40 \times 10^{-5} \text{ g}\). Therefore, the number of molecules of OA would be \((1.48 \times 10^{-5}) \times 6.023 \times 10^{23} / 997.50 = 8.93 \times 10^{15}\) (results obtained from TGA analysis Figure S1C). Consequently, the number of molecules of MPM is \((6.40 \times 10^{-5}) \times 6.023 \times 10^{23} / 3400 = 1.13 \times 10^{16}\). Since the number of gold nanoparticles \((N_{\text{Au}})\) is \((1.536 / 2.22 \times 10^{-13}) = 6.93 \times 10^{12}\), the number of molecules of MPM per one AuNP is 1636 as the number of molecules of OA per one AuNP is 1289. Thus, the total number of molecules per one nanoparticle is 2925.
Figure ESI 1. TGA curves of A) Au-MPM, B) Au-OA and C) Au-MPM-OA nanoparticles.
Figure ESI 2. Fluorescence spectra of 1) OA, 2) Au-OA and 3) Au-MPM-OA biosensors.
Figure ESI 3. Image J color histograms: A) CLSM images of A549 cells transfected with Au-OA, green histogram; B) CLSM images of A549 cells transfected with Au-OA, blue histogram; C) CLSM images of A549 cells transfected with Au-MPM-OA, green histogram; CLSM images of A549 cells transfected with Au-MPM-OA, blue histogram. Green color – CellLight® Actin-GFP, BacMam 2.0; Blue color – GNP; Yellow color – region of interest selection.
Figure ESI 4. Optical micrographs of A549 cell line transfected with the produced biotags and respective precursors after 24 h and 48 h of incubation: Gold nanoparticles (AuNPs); Maleimide-PEG-Maleimide (MPM); Oligoaziridine Biosensor (OA); Gold nanoparticles coated with oligoaziridine (Au-OA); Gold nanoparticles capped with Maleimide-PEG-Maleimide (Au-MPM); Gold nanoparticles capped with Maleimide-PEG-Maleimide and oligoaziridine (Au-MPM-OA); Negative control (K-) and Positive control (K+). Original magnification x 100.