

## Supporting Information

### Rational design of multi-functional gold nanoparticles with controlled biomolecule adsorption: a multi-method approach for in-depth characterization

*Isaac Ojea-Jiménez,<sup>1,#,\*</sup> Robin Capomaccio,<sup>1,#</sup> Inês Osório,<sup>1</sup> Dora Mehn,<sup>1</sup> Giacomo Ceccone,<sup>1</sup> Rohanah Hussain,<sup>2</sup> Giuliano Siligardi,<sup>2</sup> Pascal Colpo,<sup>1</sup> François Rossi<sup>3</sup>  
Douglas Gilliland,<sup>1</sup> Luigi Calzolari,<sup>1,\*</sup>*

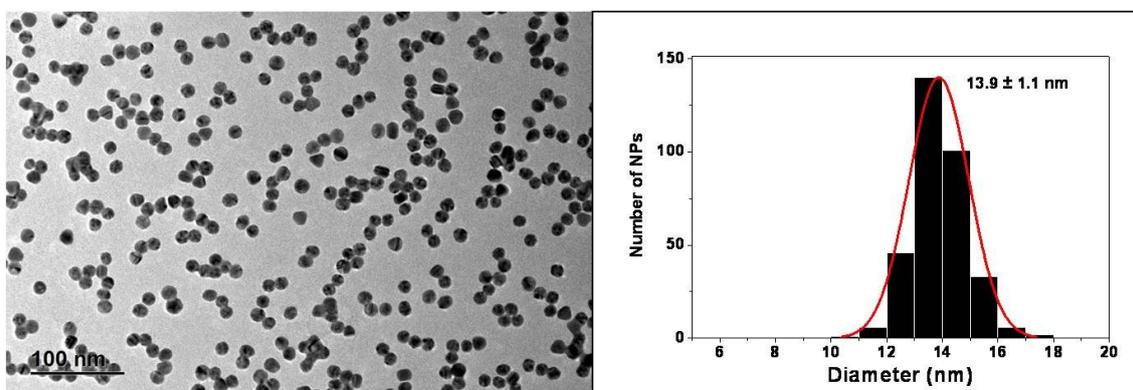
<sup>1</sup>European Commission, DG-Joint Research Centre, Via E. Fermi, 21027 Ispra, VA, Italy

<sup>2</sup>Diamond Light Source, Chilton, Didcot, OX11 0DE, United Kingdom

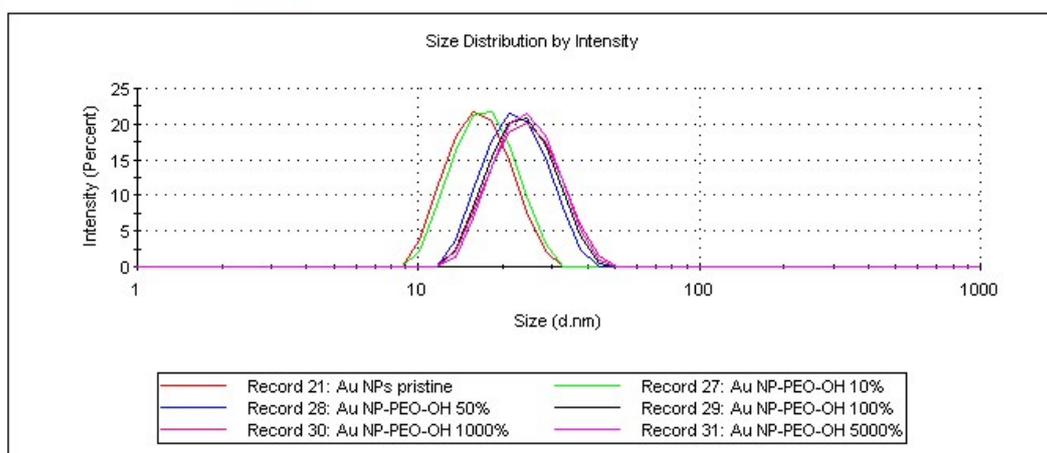
<sup>3</sup>European Commission, DG-Joint Research Centre, Westerduinweg 3, 1755ZG Petten, Netherlands.

*#Both authors have equally contributed to this work.*

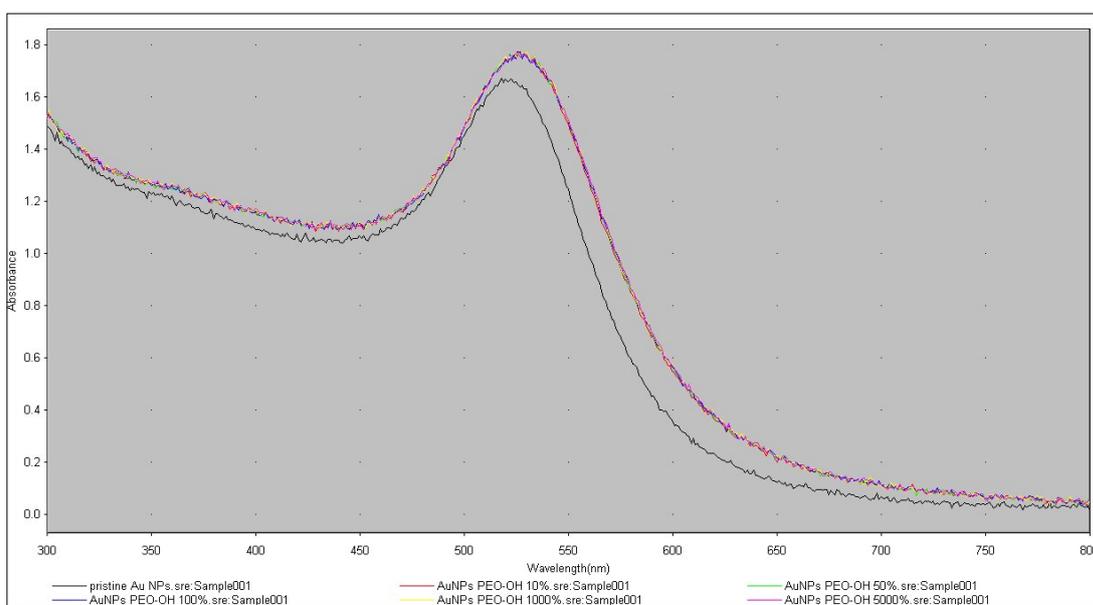
Email: *Isaac.OJEA-JIMENEZ@ec.europa.eu, Luigi.CALZOLAI@ec.europa.eu*



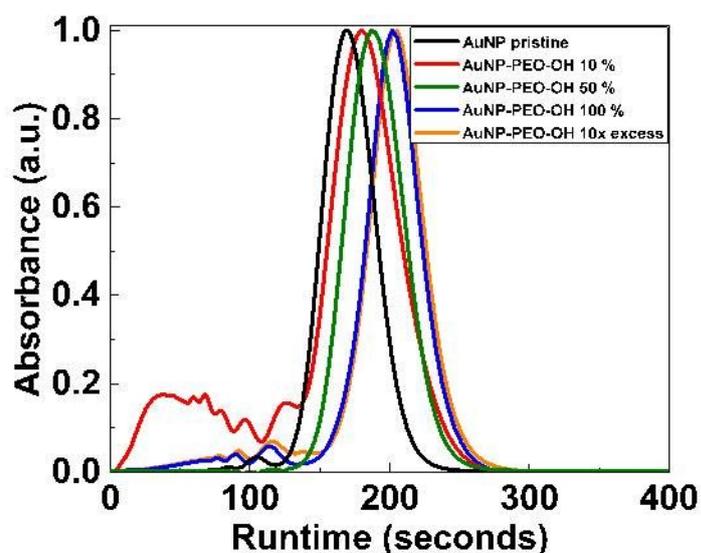
**Figure S1.** TEM characterization and size distribution analysis of pristine AuNPs showing uniform quasi-spherical morphology.



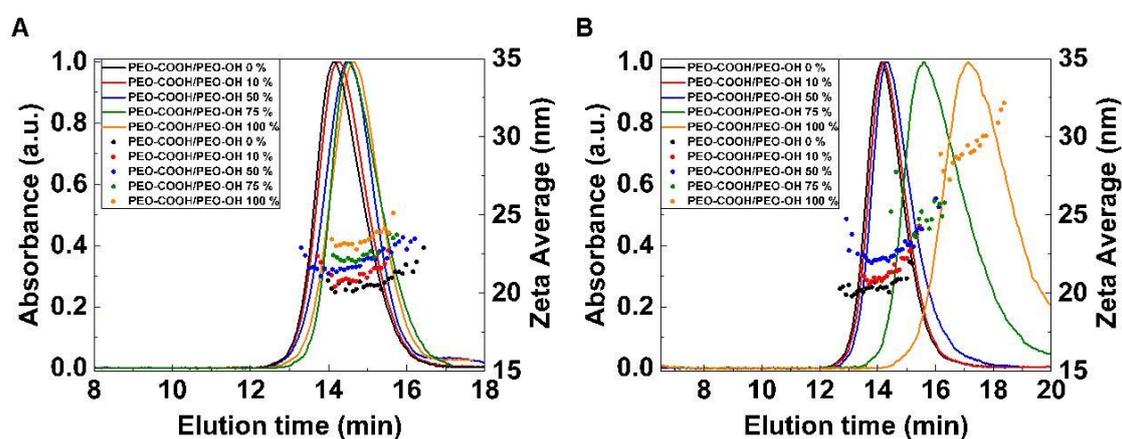
**Figure S2.** Hydrodynamic diameter by batch-mode DLS of pristine AuNPs and AuNP-PEO samples at different coating densities.



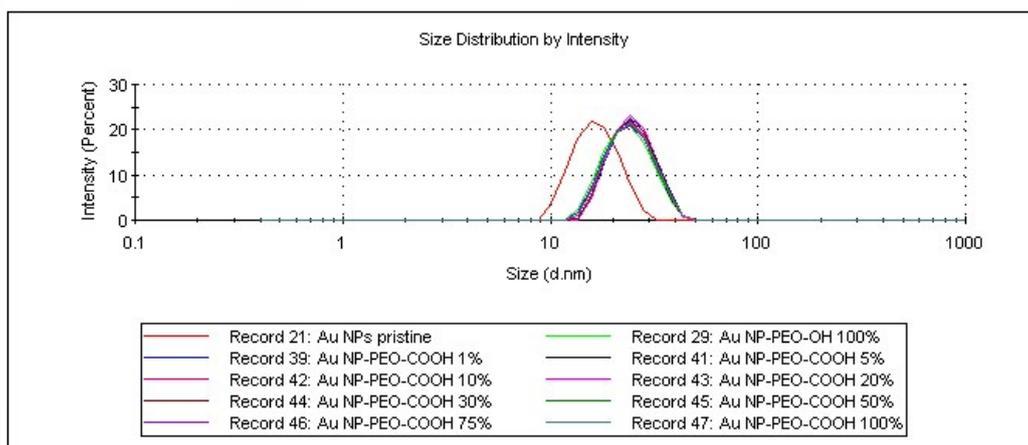
**Figure S3.** UV-vis absorption spectra of pristine AuNPs ( $\lambda_{\text{max}} = 523 \text{ nm}$ ) and AuNP-PEO samples ( $\lambda_{\text{max}} = 528 \text{ nm}$ ) at different coating densities.



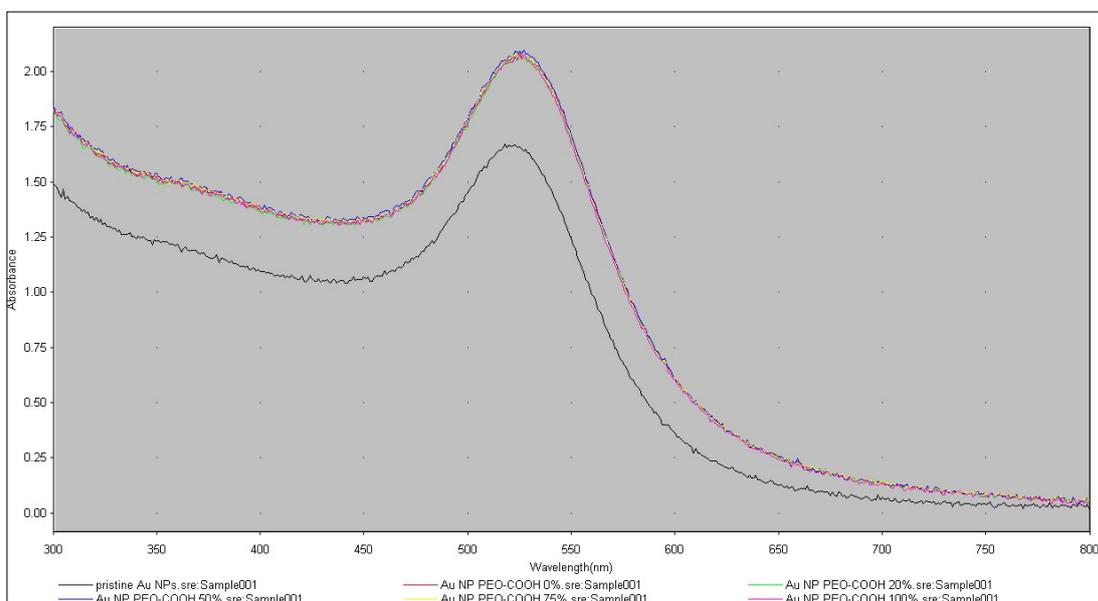
**Figure S4.** Sedimentation time by CLS of pristine AuNPs and AuNP-PEO samples with different surface densities (10, 50, 100 % theoretical coverage and 10x excess).



**Figure S5.** Separation by AF4 and size measurement by DLS in flow mode for AuNP-PEO samples with different ratios of PEO-COOH/PEO-OH ligands (from 0 % to 100 %) in the absence (A) and reacted (B) with HSA protein after pre-activation with EDC/NHS. Selected parts of the AF4 fractograms (UV-Vis traces)



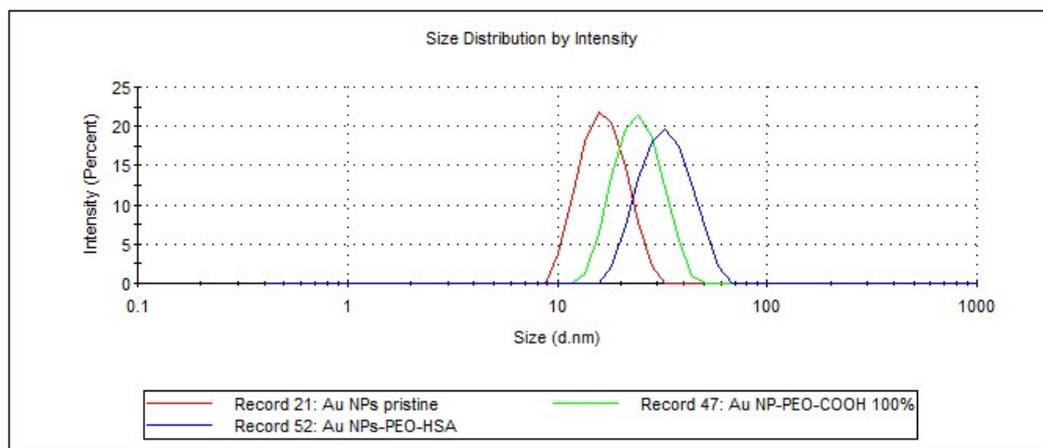
**Figure S6.** Hydrodynamic diameter by batch-mode DLS of pristine AuNPs and AuNP-PEO samples with different ratios of PEO-COOH/PEO-OH ligands (from 0 % to 100 %).



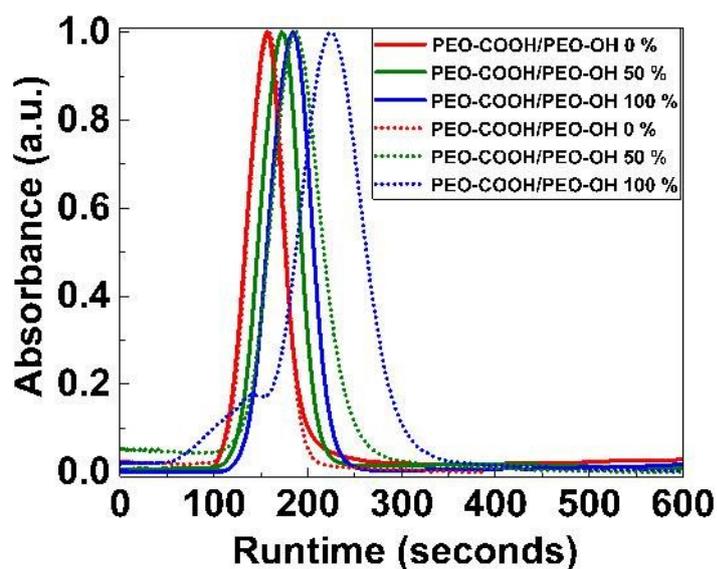
**Figure S7.** UV-vis absorption spectra of pristine AuNPs ( $\lambda_{\text{max}} = 523 \text{ nm}$ ) and AuNP-PEO samples ( $\lambda_{\text{max}} = 526 \text{ nm}$ ) with different ratios of PEO-COOH/PEO-OH ligands (from 0 % to 100 %).

PEO-COOH/PEO-OH (%)	0		1		5		10		20		30		50		75		100	
HSA	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+
<b>density</b>	6.4	6.5	6.4	6.3	6.0	6.1	5.7	5.9	5.8	5.6	5.5	5.6	5.3	5.2	4.9	4.2	4.8	2.6
<b>DLS Size</b>	20.8	20.5	20.8	20.8	21.3	21.0	21.7	21.5	21.6	21.8	22.2	22.1	22.6	22.6	23.5	24.9	23.4	30.0
<b>stdev</b>	0.6	0.5	0.7	0.5	0.6	0.5	0.5	0.8	0.6	0.6	0.7	0.5	0.6	0.6	0.6	0.8	0.5	1.3

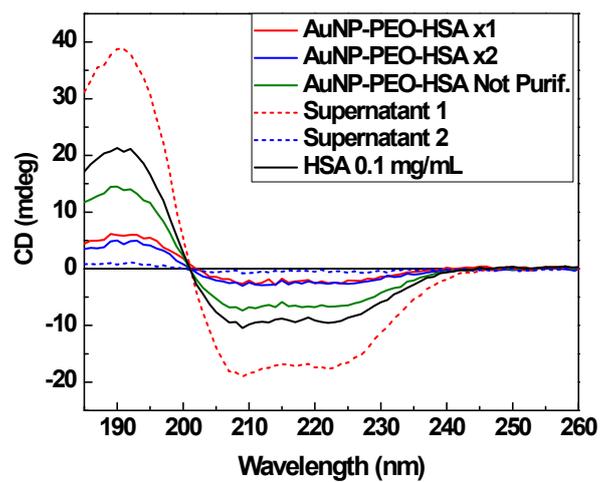
**Table S1.** Determination of the densities of AuNP-PEO complexes with different ratios of PEO-COOH/PEO-OH ligands in the absence and presence of covalently bound HSA by the combination of hydrodynamic diameters obtained by AF4-DLS with the CLS data. The standard deviation is reported as stdev.



**Figure S8.** Hydrodynamic diameter by batch-mode DLS of pristine AuNPs, AuNP-PEO-COOH (100 % PEO-COOH coverage) and AuNP-PEO-HSA samples (100 % PEO-COOH coverage).



**Figure S9.** Sedimentation time by CLS of AuNP-PEO complexes with different ratios of PEO-COOH/PEO-OH ligands in the absence (continuous line) and presence (dashed line) of covalently bound HSA molecules.



**Figure S10.** Selected CD spectra from Chirascan of AuNP-PEO-HSA complexes and their supernatants after centrifuge purification steps (either once or twice).