Supporting Information for

Understanding the good and poor cell targeting activity of gold nanostructures functionalized with molecular units for the epidermal growth factor receptor

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Materials and Methods.

9-Fluorenylmethoxycarbonyl (Fmoc)-amino acids and all other chemicals for the solid phase peptide synthesis. Rink Amide MBHA Resin (loading 0.64 mmol/g) was purchased from Novabiochem EMD Millipore and Fmoc-8-amino-3,6-dioxaoctanoic acid (Fmoc-O_2Oc-OH) from Iris Biotech GMBH. Analytical high-performance liquid chromatography (HPLC) separations were performed on a Dionex Summit dual-gradient HPLC instrument, equipped with four-channel UV–Vis detector, using a Vydac 218TP54 column (250 x 4.6 mm, 5 μm, flow rate at 1.5 mL min^{-1}). The mobile phase A (aqueous 0.1% Trifluoroacetic acid (TFA)) and B (90% aqueous acetonitrile containing 0.1% TFA) were used for preparing binary gradients. The elution condition was: isocratic 10% B for 3 min; linear gradient 10–90% B for 30 min. Preparative HPLC separations were performed on a Shimadzu series LC-6A chromatograph, equipped with two independent pump

units, a UV–vis detector, using a Vydac column 218TP54 (250 x 22 mm, 10 μm, flow rate at 15 mL min^{-1}) with the same mobile phases as described above. Mass spectral analysis was carried out on a Mariner API-TOF workstation (PerSeptiveBiosystemsInc), operating in positive mode with ESI technique. DLS and ζ-potential measurements were performed with a Malvern Nano-ZS instrument equipped with a 633 nm He–Ne Laser. SERRS spectra of cells were recorded with a micro-Raman spectrometer, inVia Renishaw, with a 20x objective, exciting at 633 nm for 1 s (1.5 mW). SERRS spectra of the colloidal solutions were recorded with a 5x objective for 10 s. An internal Si reference was used. UV–vis spectra were recorded with a Cary5000 spectrometer (Agilent) in 0.2 or 1 cm quartz cells.

Synthesis of Peptide GE11-C

The peptide sequence YHWYGYTPQNVI-O_2Oc-C-NH_2 (GE11-C) was prepared on an automated Advanced Chemtech 348 Ω peptide synthesizer, using Fmoc Chemistry and a Rink amide MBHA resin (0.64 mmol g^{-1}). The amino acid side chains were protected by the tert-butyl group (tyrosine and threonine), the tertbutyloxycarbonyl group (tryptophan) and the trityl group (histidine, glutamine, asparagine, and cysteine). Fmoc deprotection was achieved with 20% piperidine in DMF (5 + 15 min). Couplings were carried out in DMF, using an excess of the Fmoc-amino acid (4 eq) and in the presence of N-hydroxybenzotriazole, N,N,N′,N′-tetramethyl-O-(1Hbenzotriazol-1-yl) uroniumhexafluorophosphate, and N,N,N-ethyldiisopropylamine (4:4:12 eq) for 45 min. Cleavage and deprotection of peptides from the solid support was achieved by treatment with a mixture of TFA/triisopropylsilane/H_2O/1,2-ethanedithiol (94:1:2.5:2.5 v/v/v/v) for 1 h and 30 min at room temperature (RT). The resin was filtered off, the acid solution was reduced to a small volume and the crude peptide was precipitated by an addition of cold diethyl ether. Peptides were purified by semipreparative HPLC. The elution condition was: isocratic 27% B for 5 min: linear gradient 27–25% B for 20 min. The purified product was characterized by analytical HPLC and high resolution mass spectrometry (HRMS) electrospray ionization (ESI) (Table S1 and Fig. S1†).

Table S1. Analytical data of the peptide ligand.

Peptide	Sequence[a]	Yield %	HPLC[b] t_R (min)	Expected Mass $[M+H]^+$	Found Mass m/z[b]
GE11-C	YHWYGYTPQNVI-O_2Oc-C-NH_2	92	13.65	1787.886	894.911 $[M+2H]^{+2}$

[a] Abbreviations: O_2Oc, 8-amino-3,6-dioxoactanoic acid; In the sequence, the one-letter symbols for amino acids have been used.

[b] For Analytical HPLC and HRMS spectra see Fig. S1†.

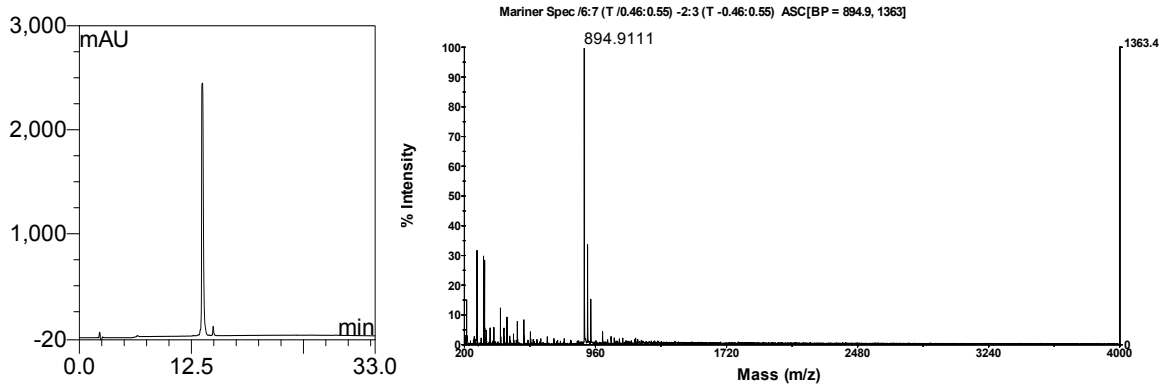
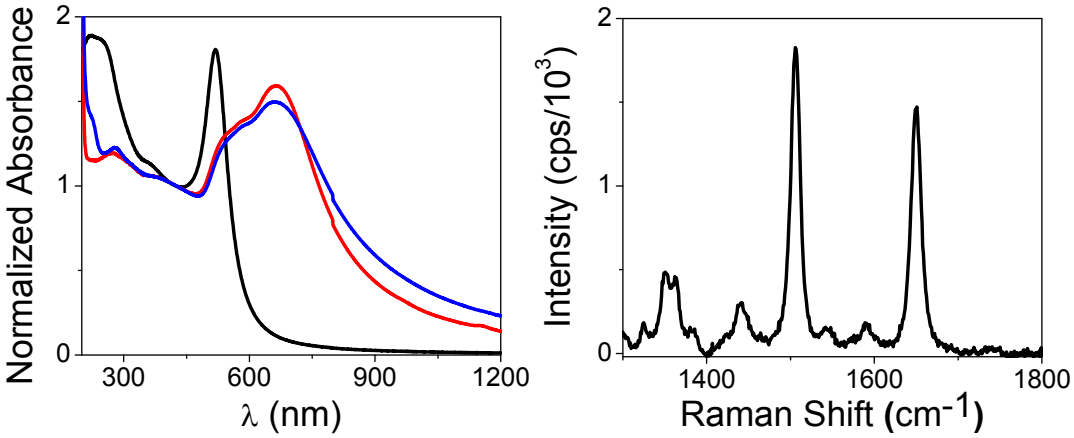


Fig. S1 Analytical HPLC (on the left) and HRMS spectra (on the right) of peptide GE11-C.

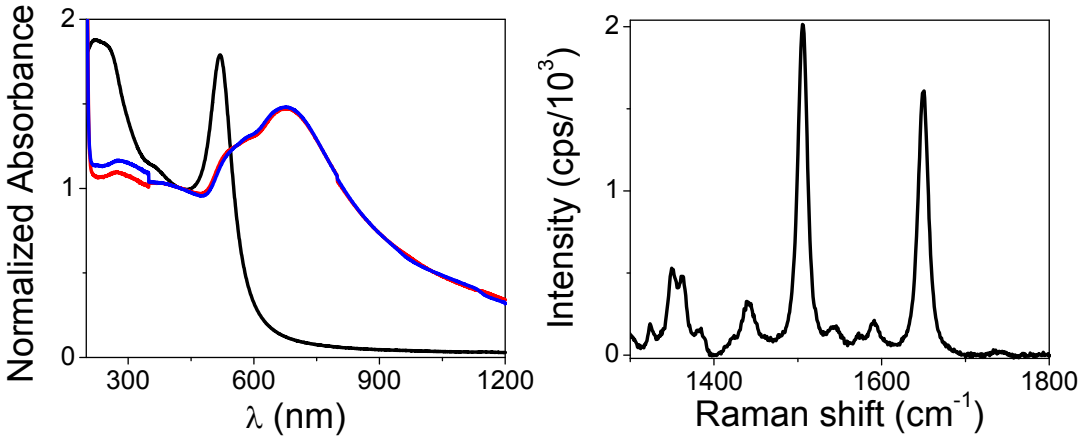
Preparation of SERRS Nanostructures

Synthesis of SERRS Nanoaggregates and of the Reference AuNP@PEG-SH Nanostructures.

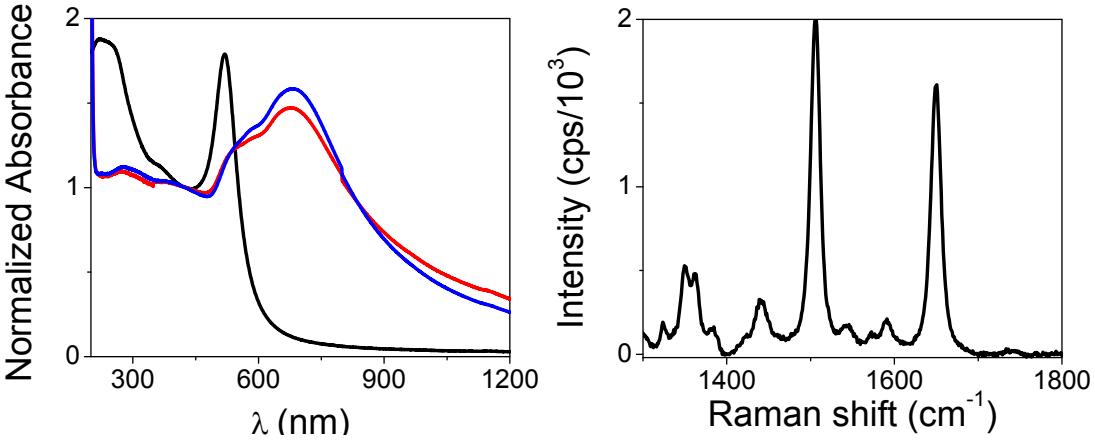
(a) AuNP@GE11-C



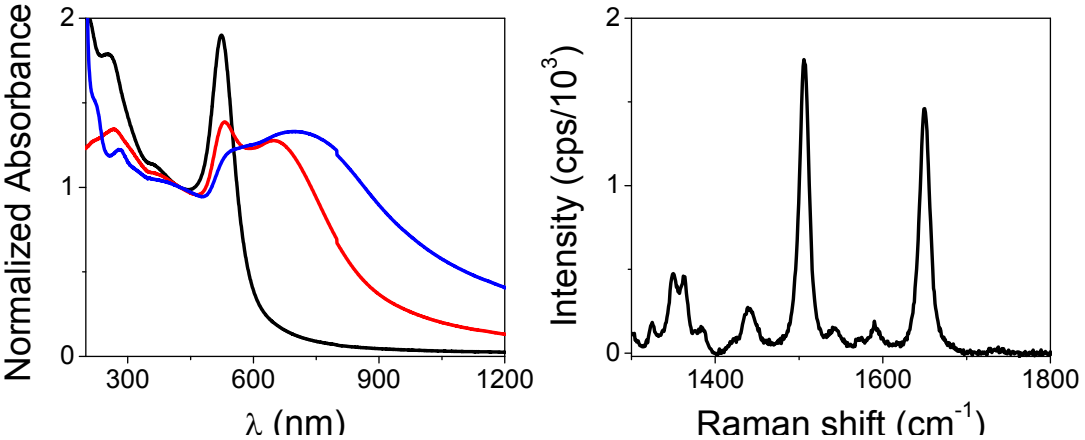
(b) AuNP@ GE11-C/PEG-SH_1



(c) AuNP@ GE11-C/PEG-SH_2



(d) AuNP@ GE11-C/PEG-SH_3



(e) AuNP@PEG-SH

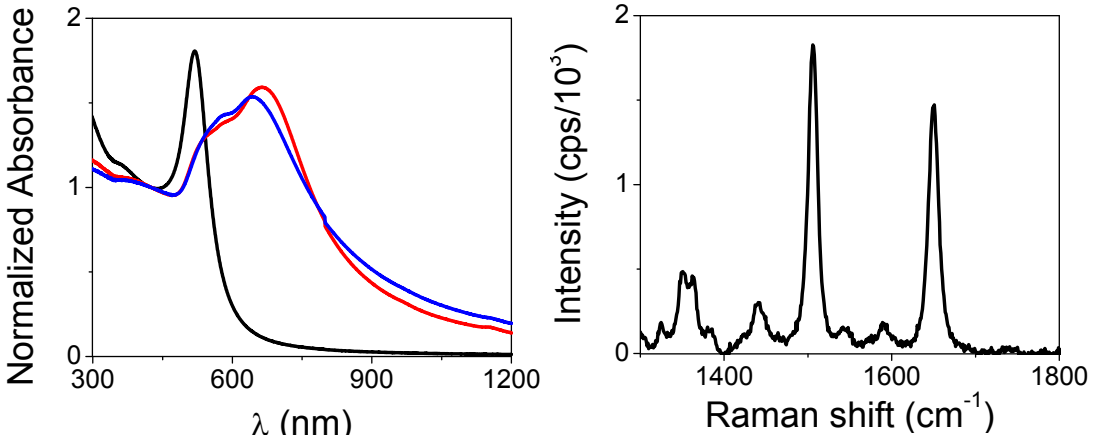


Fig. S2 On the left, evolution of the extinction spectra during the assembling steps of the nanostructures: Laser Ablation in Solution of gold nanoparticles (black line), aggregation and labelling with the SERRS reporter (red line) and conjugation of peptide ligands (blue line). On the right, SERRS spectra of the final nanostructures (a) AuNP@GE11-C (b) AuNP@GE11-C/PEG-SH_1 (c) AuNP@GE11-C/PEG-SH_2 (d) AuNP@ GE11-C/PEG-SH_3 (e) AuNP@PEG-SH

Molecular Dynamic Simulation

MD simulations performed considering a monolayer with only PEG-SH on an Au surface, showed that the polymers form a carpet, thus inhibiting any further specific binding on the gold surface (data not shown). In the case of GE11-C only, 36 peptides (maximum value due to steric hindrance) can be placed on the simulated box of 5 x 5 x 12 nm^3, with the thiol group constrained near the Au surface. Some regions of the amino acid sequence can adopt parallel beta sheets (Fig. S3). These structural arrangements can be considered responsible for the targeting activity but not selectivity of GE11-C coated AuNPs (AuNP@GE11-C). Simulations of monolayer containing both PEG-SH and GE11-C (molar ratio 1:1) showed that the addition of PEG-SH destabilizes the GE11-C assembly above described, causing a decrease in the population of parallel beta sheet conformation and a consequent formation of an irregular mixture on the Au surface.

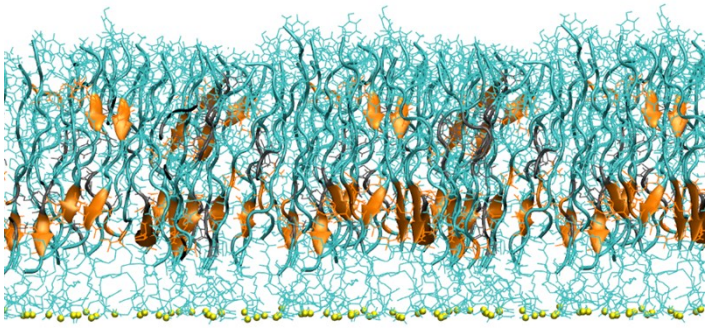


Fig. S3. Compact monolayer of GE11-C only, chemisorbed on the Au surface. S atoms are colored in yellow, while all the other atoms are colored in cyan. β-sheet conformation is represented in New Cartoon and colored in orange.

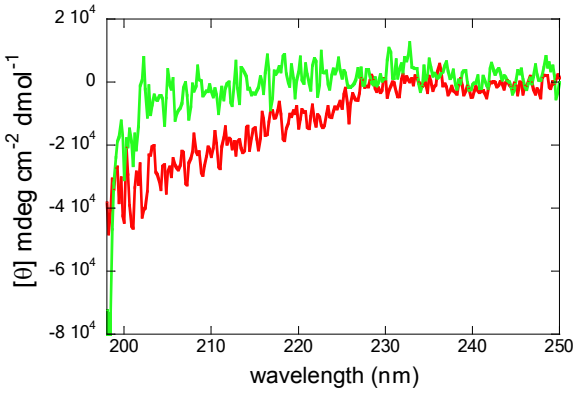


Fig. S4. CD spectra of GE11-C (*red*) and of GE11-C/PEG-SH =19:1 (*green*). [GE11-C] = 50 μM.