Electronic Supplementary Information:

SERS assisted ultra-fast peptidic screening: A new tool for drug discovery

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Experimental Materials and Methods

Unless otherwise stated all chemicals were purchased from Aldrich and used without further purification.

Tentagel-Nanoparticle-NH_2 (TG-NP) hybrid beads were prepared by adsorbing a mixture of 350 mg of $AgNO_3$ and 50 mg of $KAuCl_4$ (Aldrich) onto 1 g of TentaGel^{TM} HL-NH_2 microspheres (Fluka), of about 110 μm, containing around 0.4 mmol g^{-1} of nitrogen. The mixture was stirred in 50 mL of water for three hours. Then the reducing agent, NaBH4 (Aldrich) 10 mL 0.5 M, was added. Instantaneity after the addition, the microspheres changed their colour from white to dark red. The mixture was stirred for another hour to ensure the complete degradation of $NaBH_4$. The resulting material was filtered, extensively washed with water and characterized. In order to check for the formation of single-metallic nanoparticles of silver or gold alone, the same experiments were carried out by using just one of the metals.

Chemical analysis and oxidation state of the silver and gold on the bead surface were investigated by x-ray photoelectron spectroscopy (XPS) in an Axis 165 XPS (Kratos Analytical). Gold and silver distribution on the surface was studied by Auger emission spectroscopy (AES) and mapping in a JAMP-9500F, scanning Auger microprobe for field emission-scanning electron microscopy and AES (JEOL). Crystallography of the bimetallic nanoparticles was obtained by x-ray diffraction on a Bruker D8 Discover. For transmission electron microscopy (TEM), the microbeads were thoroughly mixed with L.R. white resin medium in a gelatin capsule and were placed into oven at 60 ºC for 12 h. The resulting hardcore blocks were trimmed for TEM and sectioning was done with a Reichert

Jung ultramicrotome with sections at 50–70 nm. The sections were examined with A Morgagni 268 Phillips transmission electron microscopy. Raman and SERS spectra were carried out in a Renishaw Invia Raman system exciting the samples with a 633 nm laser line. For SERS characterization 10 µL 10-5 M of benzenethiol (BT, Aldrich) was added to 1 mL of 1mg mL^{-1} suspension of TG-NP giving rise to a final concentration of 10^{-7} M.

All peptides were synthesized using the Fmoc protocol in 3 mL polypropylene syringes fitted with a 20 µM polyethylene filter. TGNP-Amino resin (200 mg, 0.45 mmol g^{-1}) was pre-swelled in DMF for 1 h. and then washed several times with DMF. The corresponding Fmoc- amino acid (0.46 mmol, 5 equiv.), HOBT (70 mg, 5 equiv.) and DIPCDI (71 µL, 5 equiv.) were dissolved in 3 mL of DMF and left to stand for 10 min, then the mixture was added to the resin and allowed to react for 4-6 h. Subsequent amino acids were coupled under standard SPPS conditions, using HBTU/DIEA (AA/HBTU/DIEA-4/3,8/8) in DMF. Attachment of the peptides to the TG-NP resin was corroborated by LC/MS (m/z (EI) 350, M+H 100 %), after cleavage of a small sample of resin (10 mg) with TFA 95 %.

For SERS deconvolution, equal volumes of the three prepared tripeptides were combined and stirred for two hours to ensure a perfect mixture. 20 µL were then cast on a glass slide, air-dried and studied with a 633 laser line. Raman inelastic radiation was collected with a Renishaw Invia system, equipped with a 2-dimensional CCD detector and a confocal Leica microscope. The spectrographs have 1800 or 1200 g mm^{-1} gratings with additional band-pass filter optics. Spectra were collected in continuous mode with accumulation times of 2 s. To ensure spectral homogeneity, single beads were extensively mapped over the entire surface by focusing the laser line with a 50x objective. For the classification of the bead mixture, a single spectra was collected per bead, focusing the laser with a 5x objective.

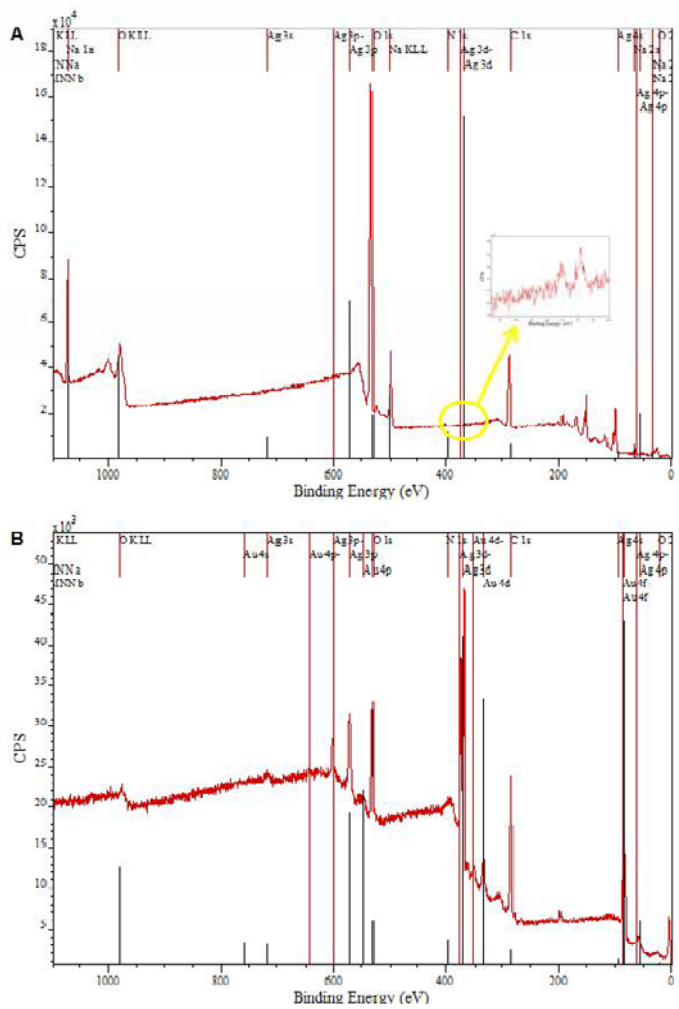

Figure S1. Survey XPS spectra for Tentagel microspheres (A) after the reduction of silver, and, (B) When silver is reduced in the presence of gold.

Table 1. SERS assignments for Phe, Ala and Leu (in cm^{-1}).

Phe	Ala	Leu	Assignment
	457		CC bending/amide bending
620			Ring deformation
		625	CONH vibraton
	707		NH deformation
749			Sym. benzene stretch
		802	CC skeletal vibration
	858	844	CC skeletal stretch/C-CH_3 stretching
	927		CC stretch/ C-COO- stretch
1001			Symmetric ring breathing
1034			In-plane CH def./ ring vibration
		1053	CC stretching/ CN stretching
	1139		CC stretching
1185			CH_2 twist and rock/CH-NH-
	1303		CH_2 twist and rock/CH_2 deformation
	1393		$C_{\alpha 2}H_2$ def./C-N stretch
1435	1476		CH and CH_3 deformation
1606			coupled C=C stretch
		1632	NH_2 scissors/NH_2 deformation 8/amide II band