ELECTRONIC SUPPLEMENTARY INFORMATION

Investigating nanoparticles properties in plasmonic nanoarchitectures with DNA by Surface Plasmon Resonance imaging

Stefano Mariani,[a] Simona Scarano,[a] Maria Laura Ermini,[a] Massimo Bonini[a,b]* and Maria Minunni[a,b]*

[a]*Dipartimento di Chimica "Ugo Schiff", Università di Firenze, Via della Lastruccia 3-13, 50019 Sesto Fiorentino (FI), Italy; Tel: 0554573314;*

[b]*Consorzio dei Sistemi a Grande Interfase (CSGI), Università di Firenze, Via della Lastruccia 3-13, 50019 Sesto Fiorentino (FI), Italy; Tel: 0554573014;*

*massimo.bonini@unifi.it; bonini@csgi.it; maria.minunni@unifi.it

Nanoparticles synthesis

Gold nanospheres: tetrachloroauric acid (>99,9 %) and trisodium citrate have been purchased from Sigma Aldrich (Milan, Italy). The synthesis has been previously reported by Turkevich et al.,1951[1] while experimental details have been reported in Electronic Supplementary Materials in Mariani et al., 2013.[2] NPs concentration is theoretically estimated from the total amount of tetrachloroauric acid 9.85 x 10^{-4} g used for the synthesis in 25 mL MilliQ water, nanospheres dimension estimated from SEM microscopy (ø ~ 24 nm) and density (19.30 g/cm^3). As a result, the theoretical concentration is 2.8 x 10^{11} NPs/ml. Such NPs amount could be considered sufficient to perform a total spot surface saturation after injection in flow cell system of the NPs dispersion incubated with Probe2 (250 nM). Indeed 50 µL of dispersion reaching the spot surface contains 1.4 x 10^{10} NPs while we have estimated a theoretical amount of Au nanospheres equal to 1.1 x 10^9 NPs sufficient for surface saturation (ratios between spot area (~ 5.0 x 10^{11} nm^2) and section area of the nanospheres (~ 4.5 x10^2 nm^2).

Silver nanoprisms: trisodium citrate (>99 %), poly(sodium styrene sulfonate), sodiumtetrahydridoborate (>99 %) and silver nitrate (>99 %) have been purchased from Sigma Aldrich (Milan, Italy). Nanoprisms are prepared according to a seed-and-growth approach previously reported by Aherne et al., 2008[3] and the experimental details have been reported in Mariani et al., 2013.[2] NPs concentration is theoretically estimated as previously reported by the amount of Ag (2.05×10^{-4} g) in 10 mL of MilliQ water, dimension of nanoprisms estimated from SEM microscopy (side 37 nm x thickness 5 nm) and density (10.49 g/cm^3) and it is 6.6×10^{11} NPs/ml. Such amount could be considered sufficient to perform a total spot surface saturation after injection of the NPs dispersion incubated with Probe2 (250 nM) in flow cell system. Indeed 100 µL of dispersion injected contains 6.6×10^{10} NPs while we have estimated a theoretical amount of Ag nanoplates equal to 8.4×10^8 NPs sufficient for surface saturation (ratios between spot area (~ 5.0×10^{11} nm^2) and face area of the nanoplates (~ 5.9×10^2 nm^2).

Silica nanospheres: nanospheres are prepared through the condensation of 3.125 mM (3-Mercaptopropyl)triethoxysilane (MPES, from Sigma Aldrich) in 10 ml of ethanol with 28% aqueous ammonia and resulting in the formation of SiO_2 nanoparticles whose surface is characterized by the presence of thiol groups as reported by Nakamura et al., 2008.[4] Concentration is theoretically estimated from diameter (ø ~ 113 nm), amount of MPES (6.14×10^{-3} g) and silica density (2.36 g/cm^3) and it is 3.4×10^{11} NPs/ml. Such amount could be considered sufficient to perform a total spot surface saturation after injection of the NPs dispersion incubated with Probe2 (250 nM) in flow cell system. Indeed 100 µL of dispersion injected contains 3.4×10^{10} NPs while we have estimated a theoretical amount of SiO_2 nanospheres equal to 5.0×10^7 NPs sufficient for surface saturation (ratios between spot area (~ 5.0×10^{11} nm^2) and section area of the nanospheres (~ 1.0×10^4 nm^2)).

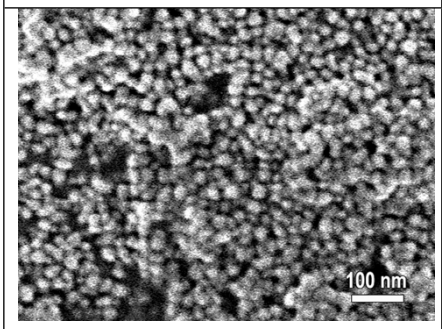
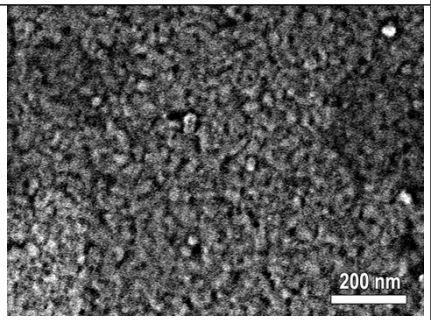
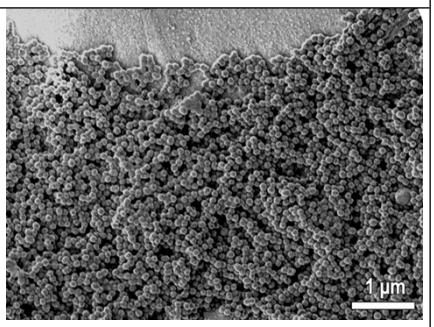
UV-vis absorption spectrum of the NPs dispersion

UV-vis absorption spectra of the metal noble NPs dispersion have been previously reported in Mariani et al., 2013.[2]

NPs characterization with SEM

Nanoparticles were deposited on gold SPRi-slide (Horiba, France) and characterized by SEM Scanning Electron Microscope (SEM) (Tab. 1). SEM experiments are performed with a ZEISS Sigma FEG-SEM.

Table 1. SEM of Au, Ag and SiO_2 nanostructures.

Au nanospheres	Ag nanoprisms	SiO_2nanospheres
		

Probe 2 labeling with nanoparticles

Metal noble NPs (gold nanospheres and silver nanoprisms) are covalently bound to thiolated Probe2 through 24 h incubation after dilution in PBS solution (300 mMNaCl, 20 mM Na_2HPO_4, 0.1 mM EDTA, aqueous solution pH 7.4 with 0.05% TWEEN® 20 (Polyethylene glycol sorbitan monolaurate)). In particular the dispersions have been incubated with Probe2 to obtain 250 nM concentration and the same surface density (1 Probe/nm^2) to homogenize the steric effect contribution to RV% during the assay for each Probe2-functionalized NPs type. The correct ratios to obtain the required surface density have been evaluated by theoretical calculating from Avogadro number, the Probe2 concentration and the volume of incubation, providing us the Probe2 amount in

the solution. This number was further divided by the overall NPs surface area in the dispersion. This latter was instead estimated using the sphere and triangular prism geometric formulas for surface (the radius of gold and silica nanospheres and the silver nanoprisms side were estimated by SEM images) and the amount of NPs in the incubated dispersion.

Silica nanoparticles are instead covalently bound to Probe2 using BMB (1,4-bis(maleimido)butane) purchased from Thermo Scientific (United States) and following a well known functionalization protocol (http://www.piercenet.com/instructions/2160649.pdf). 250 nM Probe 2 is incubated to silica nanoparticles to obtain the same surface density reported of DNA-labeled metal NPs (1 Probe/nm^2). In the Fig.2 the schematic functionalization steps for each NPs are summarized.

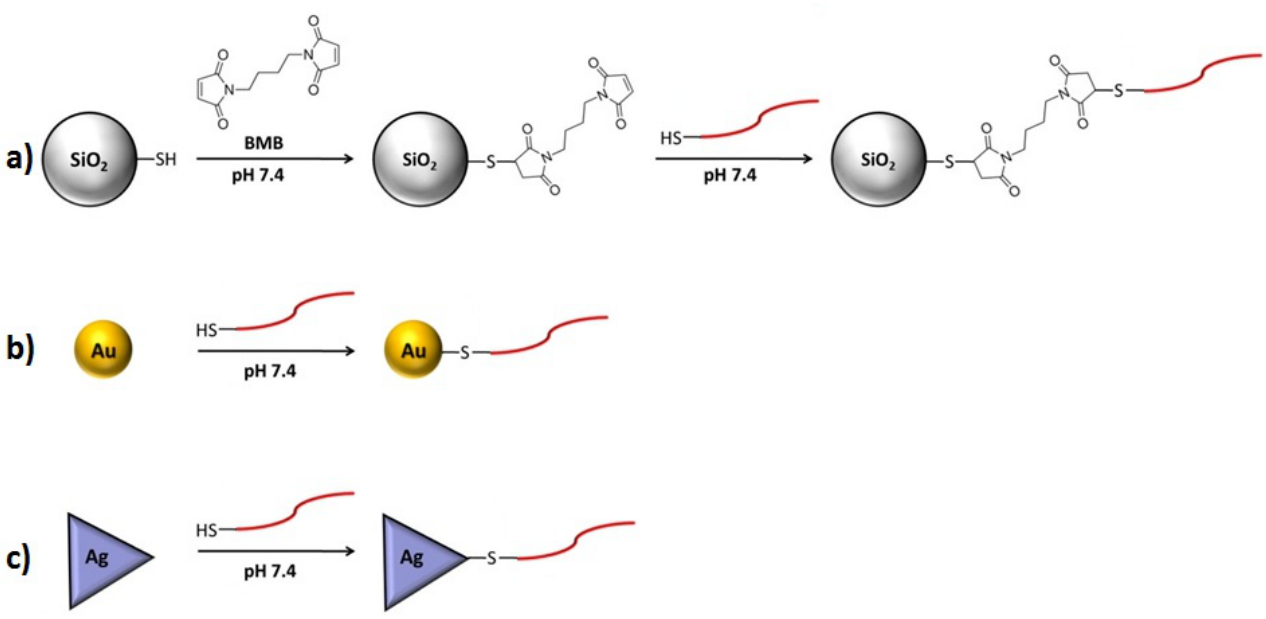


Fig. 2 Scheme of Probe2 (red) labeling NPs: a) silica nanospheres, b) gold nanospheres and c) silver nanoprisms.

The functionalization between Probe2 and silica nanospheres by BMB has been confirmed by the change in the surface charge and measuring the Zeta potential before and after the functionalization with Probe2 using a 90Plus instrument by Brookhaven Instruments.

Nucleotides sequences and solutions

Oligonucleotides sequences (Probe1, Target, Probe2 and CProbe) are purchased from Eurofins MWG Operon (Ebersberg, Germany) and reported in Tab.2. CProbe sequence was immobilized as Control Probe (CProbe, 18 mer) onto the bare gold to assess the selectivity of the assay.

Tab.2 Probe and Target sequences

Probe 1 (22 mer)	5' HS-$(CH_2)_6$-GTCACTGCCTAATGTAAGTCTC 3'
Target (84 mer)	5'GAGACTTACATTAGGCAGTGACTCGATGAAGGCATGTA TGTTGGCCTCCTTTGTGCCCTCACAATCTCTTCCTGTGACA CCAC 3'
Probe 2 (21 mer)	5' HS-$(CH_2)_6$-GTGGTGTCACAGGAAGAGATT 3'
CProbe (18 mer)	5' HS-$(CH_2)_6$- GAGGGCGATGCCACCTAC 3'

Immobilization Solution (IS, 1M NaH_2PO_4 aqueous solution pH 3.8) is used for Probe1 immobilization on gold chip surface, while PBS solution (300 mM NaCl, 20 mM Na_2HPO_4, 0.1 mM EDTA, aqueous solution pH 7.4 with 0.05% TWEEN® 20 (Polyethylene glycol sorbitanmonolaurate) is used for SPRi experiments as flowing buffer, and as solvent for Target and Probe2-labeled NPs dilution. Thiolic solution (1 μM 11-mercapto-1-undecanol (MU) and 1 μM 6-mercapto-1-hexanol (MCH) aqueous solution) is used to passivate the biochip surface before use. All reagents were purchased from Sigma-Aldrich (Milan, Italy). PBS solutions are prepared in MilliQ water (Millipore Corporation, Massachusetts, USA) and filtered using vacuum filter cups (Filtropur V50 0.2 μm, Sarstedt, Germany). Thiolic solutions are filtered by syringe filters (Puradisc, 0.2 μm Whatman GmbH, Dassel, Germany) before use. Immobilization and biochip preparation procedure have been performed as previously reported.[5]

Instrumental setup and measurements

All SPR experiments are performed on the SPRi-Lab^{+} from Horiba Scientific (Orsay, France). The instrumental setup consists of opto-mechanics, fluidics and PC. Opto-mechanics consisted of a light source emitting red visible light (635 nm) converted in polarized electromagnetic radiation. Light exiting from the polarizer is reflected by a mirror into an optical system that collimates the incident beam on the prism where probes are immobilized. In resonance conditions, part of the incident energy is employed to excite surface plasmons (SPs) of gold layer; part is reflected to a CCD camera. Sensorgrams (i.e. Reflectivity Variation %, %RV vs. time), real-time images and digital images of each surface interaction are recorded. In particular in the first 30 minutes the interaction between Probe1 immobilized on the sensor surface and Target is monitored while in the next 30 minutes we have monitored the affinity interaction between Target and Probe2 linked to nanoparticles sampling %RVs signals at the 30th and 60th. This time was not optimized, being out of the main scope of the paper, and thus the signals were taken at the plateau since we deliberately have chosen to work in saturating conditions to normalize possible different binding activities among NPs types. %RVs signals reported in the study are averaged from three replies.

Fluidics consists of a flow-cell with connectors and PEEK tubes and prism is mounted in a support that is pushed on a cell by a piston. The whole instrumental setup has been previously reported.[6] All measurements are carried out at a fixed angle of incident light. Modifications that occur at the gold-solution interface (unspecific or specific due to affinity interaction) cause changes in plasmon resonance conditions, carrying to a modification of the light absorbed by plasmons and thus to %RV (signal recorded).

Fig. 3A Calibration curve with Target (0-100 nM)

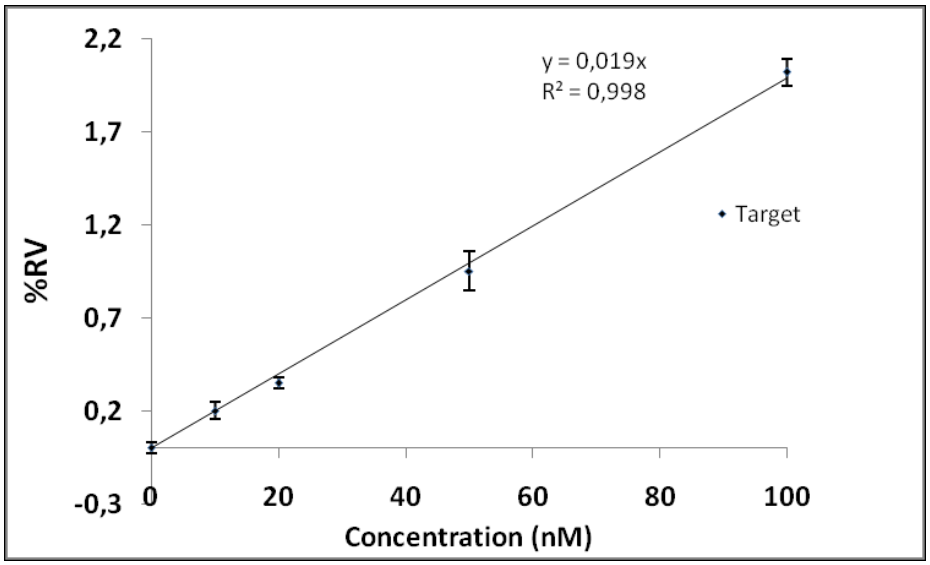


Fig. 3A Calibration curve of the Target (0-100 nM). The detection limit from the calibration curve sensitivity (0.019 %RV/nM) and the 3.3-fold instrumental noise (S = 0.092 %RV, N= 0.028 %RV) is evaluated, obtaining a DL = 4.8nM

Fig.4A (Zoomed from Fig.2 in the manuscript)

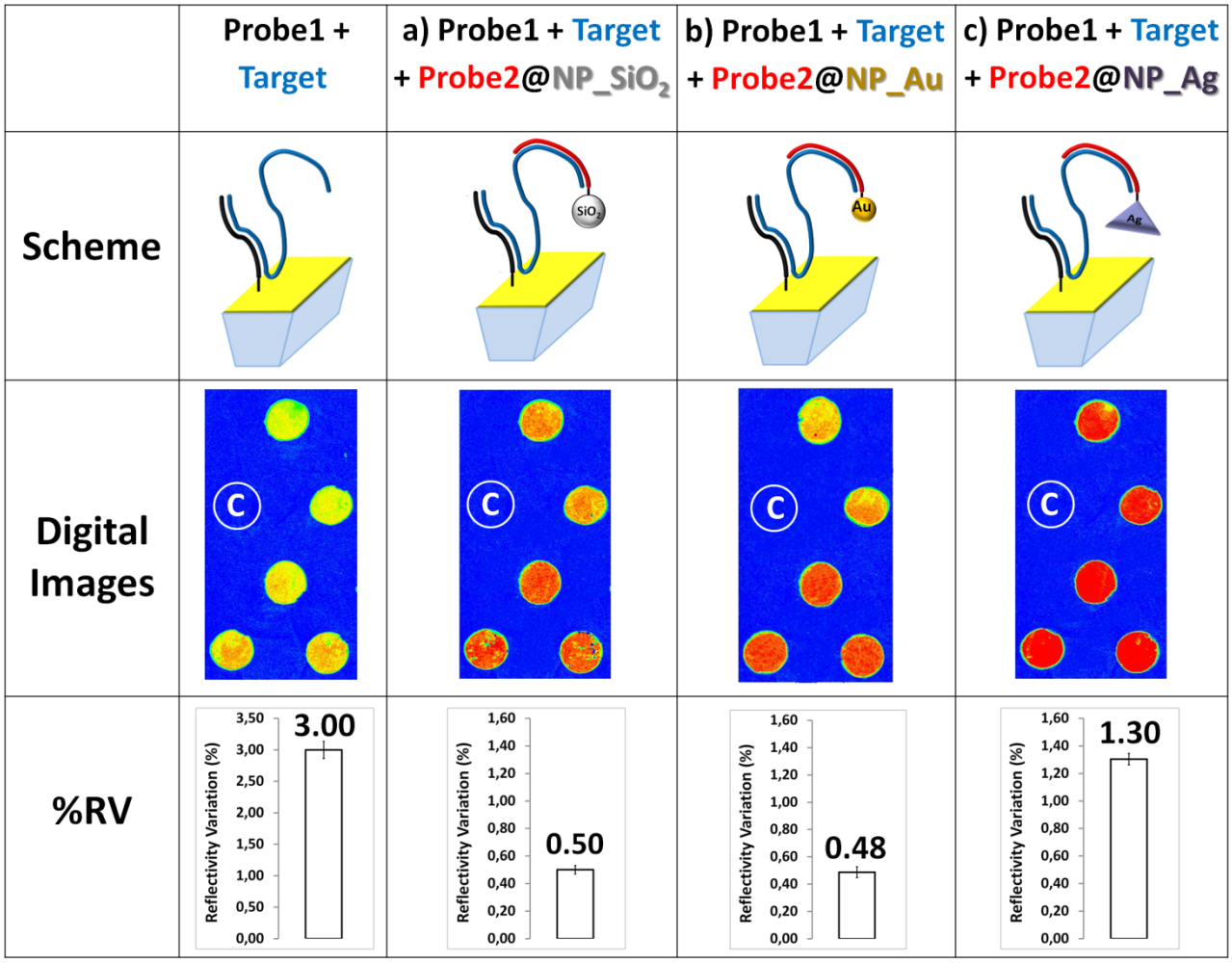


Fig. 4A Scheme, digital images and reflectivity variation % (%RV) of the target hybridization and a) Probe2-functiolized gold nanospheres, b) Probe2-functionalized silver nanoplates, and c) Probe2-functionalized silica nanospheres hybridizations.

Reference

J. Turkevich, P. C. Stevenson, and J. Hillier, *Discuss. Faraday Soc*., 1951, **11**, 55.

S. Mariani, M. L. Ermini, S. Scarano, F. Bellissima, M. Bonini, D. Berti, and M. Minunni, *Microchim. Acta*, 2013, **180**, 1093.

D. Aherne, D. M. Ledwith, M. Gara, and J. M.Kelly, *Adv. Funct. Mater.*, 2008,**18**, 2005.

M. Nakamura and K. Ishimura, *Langmuir*, 2008,**24**, 5099.

M. L. Ermini, S. Mariani, S. Scarano, and M. Minunni, *Biosens. Bioelectron.*, 2013, **40**, 193.

S. Scarano, M. Mascini, A. P. F. Turner, M. Minunni, *Biosens.Bioelectron.*, 2010, **25**, 957.