

Supporting Information

Water-Soluble Plasmonic Nanosensors with Synthetic Receptors for Label-Free Detection of Folic Acid

Randa Ahmad ^a, Nordin Félijdj ^a, Leïla Boubekeur-Lecaque^a, Stéphanie Lau-Truong ^a, Sarra Gam-Derouich ^a, Philippe Decorse ^a, Aazdine Lamouri ^a and Claire Mangeney^{*a}

I. EXPERIMENTAL PROCEDURE

I.1. Chemicals

Chloroauric acid (HAuCl₄) was purchased from Alfa Aesar, methacrylic acid (MAA), N,N'-methylbisacrylamide (MBAm), folic acid (FA), folinic acid (FnA), hexadecyltrimethylammoniumbromide CTAB (98%), sodium borohydride (99%), silver nitrate and L-ascorbic acid were purchased from Sigma-Aldrich. Deionized water (18 MΩ) was used in all the experiments. The bifunctional 2-(phenoxy)ethyldiethylcarbamidithionate diazonium chloride (Cl⁻, ⁺N₂-C₆H₄-O-CH₂.CH₂-DEDTC) was prepared following a procedure described in reference 24. For preparation of AuNRs, seed and growth solutions were made as described below.

I.2. Synthesis of gold nanorods (AuNRs).

AuNRs were prepared via the seed-mediated growth method, as described by el Sayed et al, ^[1] based on the reduction of HAuCl₄ with a weak reducing agent (ascorbic acid) on pre-made CTAB-stabilized Au nanoparticle seeds, in the presence of CTAB and AgNO₃. Briefly, the seed solution was prepared by mixing a CTAB solution (5 mL, 0.20 M) with aqueous HAuCl₄ (5 mL, 0.5 mM). To this mixture, 0.6 mL of ice-cold NaBH₄ solution (0.01 M) was added drop wise and the mixture was vigorously stirred for 2 min, resulting in a brownish yellow solution of small seed gold particles (2-5 nm) . The seed solution was kept at 25 °C. In order to grow AuNRs, the procedure was as follows. In a separate flask, 1 mL of AgNO₃ (4 mM) was added to CTAB solution (50 mL, 0.2 M) solution at 35° C. Then 50 mL of 1 mM of HAuCl₄ was added to the previous solution and mixed. To this solution, 0.7 mL of 78 mM ascorbic acid was added, changing the color of the growth solution from yellow brown to colorless. The final step consisted in the addition of 120 μL of the seed solution to initiate the NRs growth. A strong color change indicates the formation of the gold nanorods after about 10-20 min. NRs were purified by two centrifugation steps (20 min, 5000 rpm) for elimination of excess of surfactant and unreacted products from the growth solution and were redispersed in deionized water.

I.3. AuNRs surface modification

The AuNRs in aqueous solution ($[AuNRs] = 0.7 \times 10^{-9}$ M) were modified by adding 10^{-5} M of 2-(phenoxy)ethyldiethylcarbamadithionate diazonium chloride diazonium salt tetrafluoroborate. The mixture was left to incubate for 24 h at room temperature and then the nanorods were separated from solution via centrifugation at 4700 rpm for 10 min. Next, the AuNRs were redispersed in 1 mL of deionized water. The suspended modified particles were stable and stored at 7 °C until use.

I.4. Photopolymerization from functionalized-gold nanorods

The gold nanorod cores modified with DEDTC functions were submitted to polymerization by mixing the AuNR@DEDTC with methacrylic acid (MAA) (54 μ l, 0.63 mmol) as the functional monomer, N,N'-methylenebisacrylamide (MBAm) (50 mg, 0.344 mmol) as the cross-linker and folic acid (FA) (2 mg) as the template molecule before irradiating the deoxygenated mixture under UV light for varying time (4, 6 and 8 hours). Excess monomers were removed following several centrifugation/redispersion cycles. The nanorods were redispersed in water in order to maintain their colloidal stability. A reference non-imprinted polymer sample (AuNR@NIP) was prepared using the same procedure, but without addition of the FA template. The extraction of FA from AuNR@MIP was performed by using a mixture of water and acetic acid (9/1).

I.5. Instrumentations

XPS spectra were recorded using a Thermo VG Scientific ESCALAB 250 system fitted with a microfocused, monochromatic Al K α X-ray source ($h\nu = 1486.6$ eV; spot size = 650 μ m; power = 15 kV \times 200 W). The pass energy was set at 150 and 40 eV for the survey and the narrow regions, respectively. An electron flood gun was used, under a 2.10^{-8} mBar partial pressure of argon, for static charge compensation. These conditions resulted in negative but uniform static charge. Spectral calibration was determined by setting the main C1s component at 285 eV. The surface composition was determined using the integrated peak areas and the corresponding Scofield sensitivity factors corrected for the analyzer transmission function. The LSPR resonance of these systems was probed by using far-field visible–NIR extinction microspectroscopy in the range of 500–900 nm (LOT-ORIEL spectrometer). Transmission electron microscopy (TEM) characterizations were performed using a Jeol 100-CX II microscope, operating at 100 kV. The concentrated AuNRs were first dispersed in water and one drop of this dispersion was deposited on a carbon grid. Raman spectra were recorded using a Jobin-Yvon LABRAM HR 800 microspectrometer, using a He–Ne laser excitation (632.8 nm, 100 μ W power) in backscattering mode. All spectra, recorded after having deposited a drop of the samples on a glass plate, were taken with a 3 s integration time and recorded within the 120–1750 cm^{-1} spectral range. For GNRs@DEDTC, the recorded spectra were baseline corrected. Photopolymerization was performed using the commercial ultraviolet processor Spectrolinker XL

1500 UV (Spectronics Corp.). This processor was equipped with 6 tubes (8 W) with a wavelength range of 365 nm and intensity of 17.6 mW cm^{-2} .

I.6. Computational details

All calculations were carried out using the Gaussian 09 suite of programs^[2] using the B3LYP exchange and correlation functional^[3] along with the 6-311++G(d,p) basis set for all atoms but gold.^[4] The LANL2DZ basis set consisting of *Effective Core Potential* (ECP) and double- ζ quality functions for valence electrons was employed for Au.^[5] The structures were optimized without symmetry constraint (see Supporting Information). The vibrational frequencies and normal modes were calculated within the harmonic approximation and a scaling factor of 0.976 was chosen on the basis of previously published work.^[6]

II. CHARACTERIZATION OF THE AuNR@MIP SAMPLES

II.1. TEM images

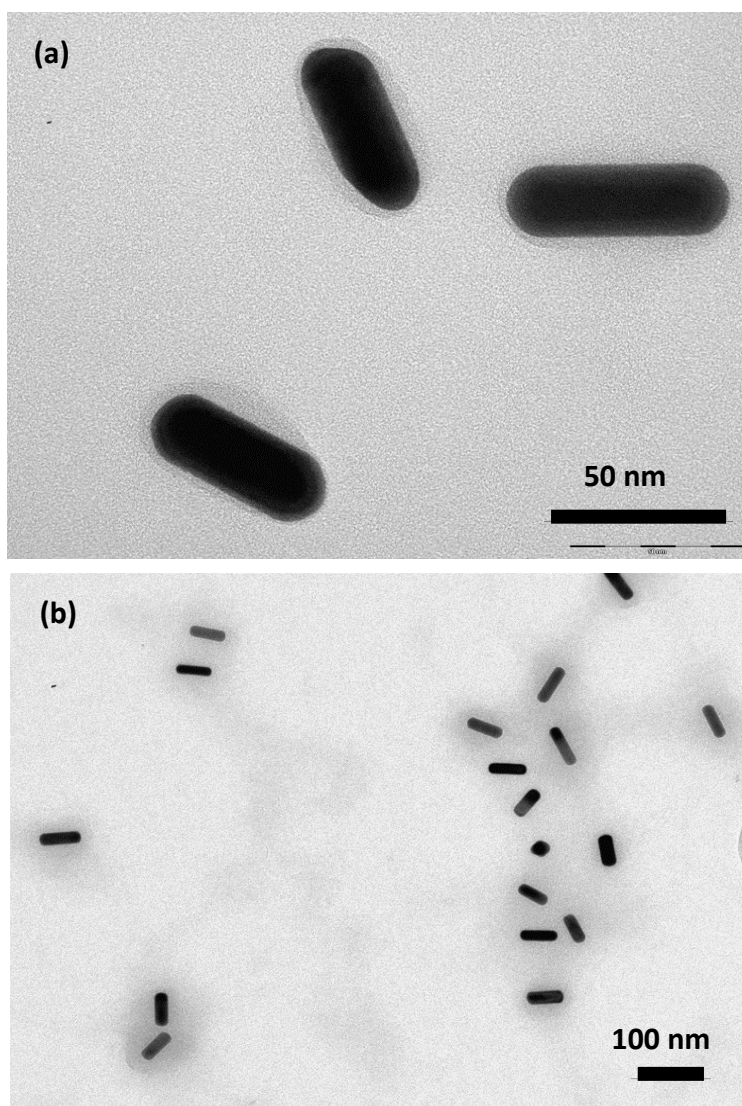


Figure S1. TEM images of (a) AuNR@MIP_{4h} and (b) AuNR@MIP_{8h}.

II.2. UV-visible spectra

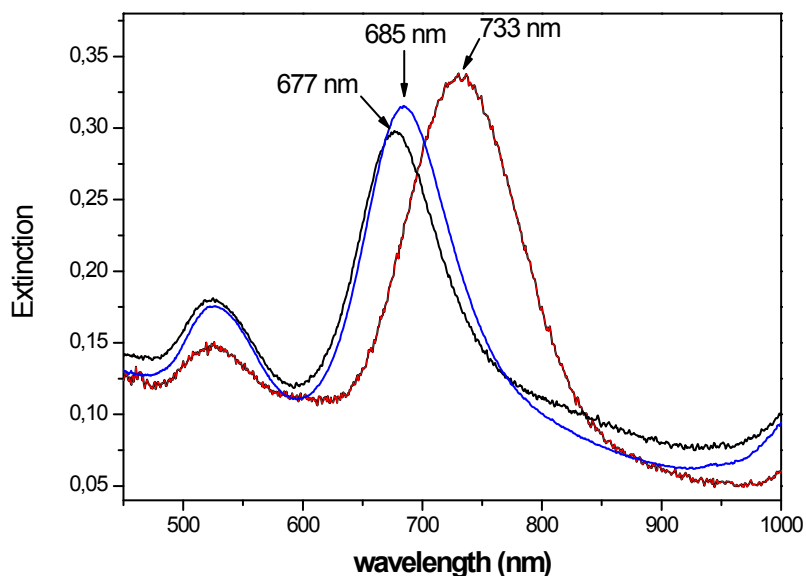


Figure S2. Extinction spectra of colloidal solutions of initial CTAB-coated AuNRs (black curve), AuNR@DEDTC (blue curve), and AuNR@MIP_{8h} obtained after 8 h polymerization (red curve).

II.3. XPS analysis

a) Survey scans and C1s high resolution spectra

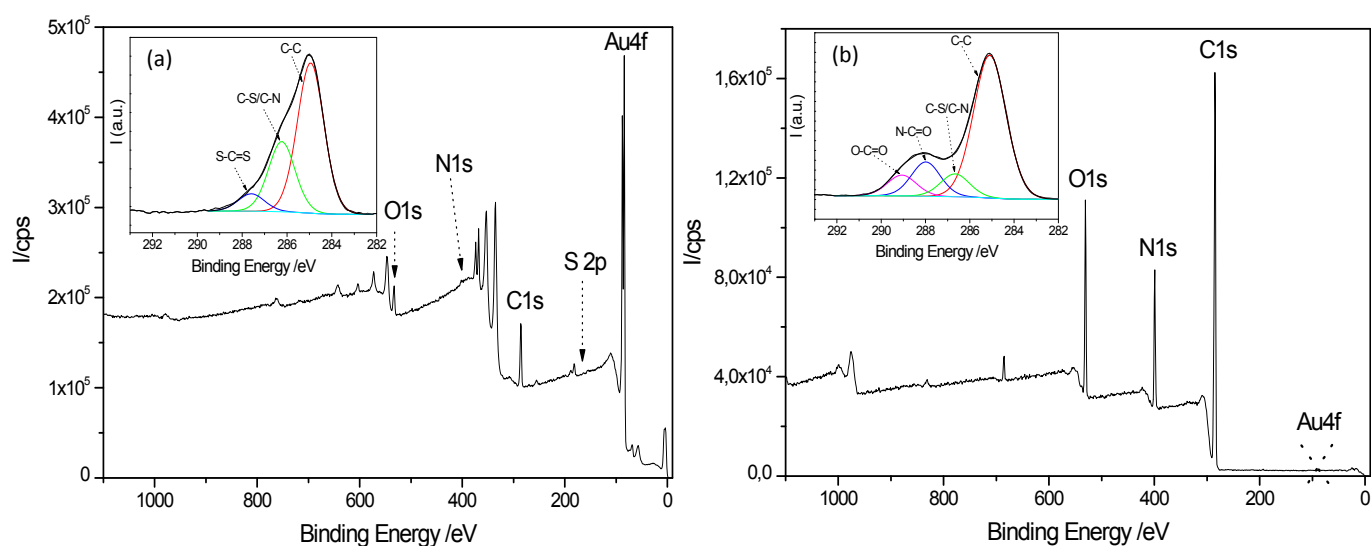


Figure S3. XPS survey scans of (a) AuNR@DEDTC and (b) AuNR@MIP_{8h}. The insets show the corresponding C1s high resolution spectra.

b) N1s and S2p high resolution spectra

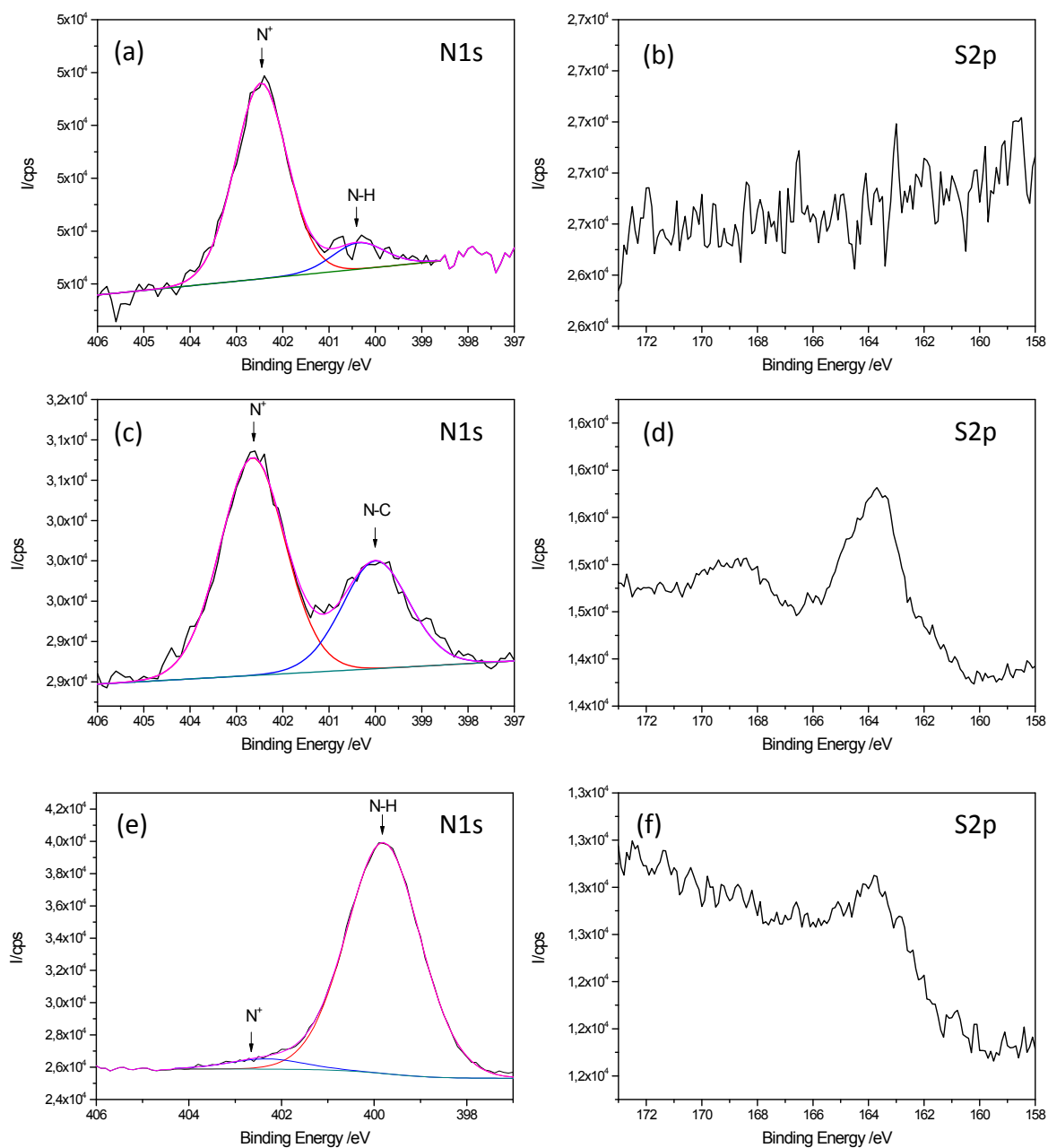


Figure S4: high resolution XPS spectra of N1s and S2p for bare CTAB-coated AuNRs (a and b); AuNR@DEDTC (c and d) and AuNR@MIP_{8h} (e and f).

II.4. Raman and DFT analysis

a) Normal Raman spectrum of the pure DEDTC-diazonium salt and SERS spectrum of AuNR@DEDTC

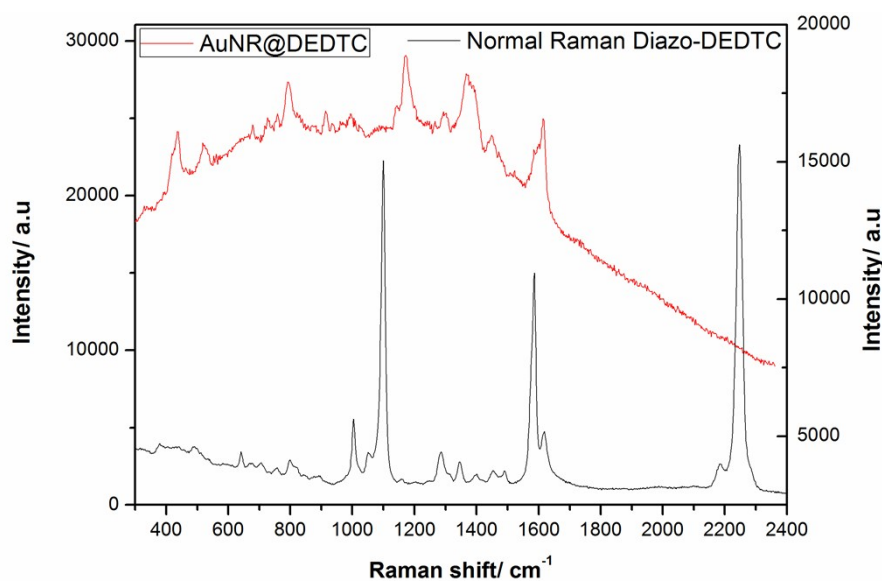


Figure S5. Normal Raman spectrum of the pure DEDTC-diazonium salt (black) and SERS spectrum for AuNR@DEDTC (red).

b) Simulated Raman spectra

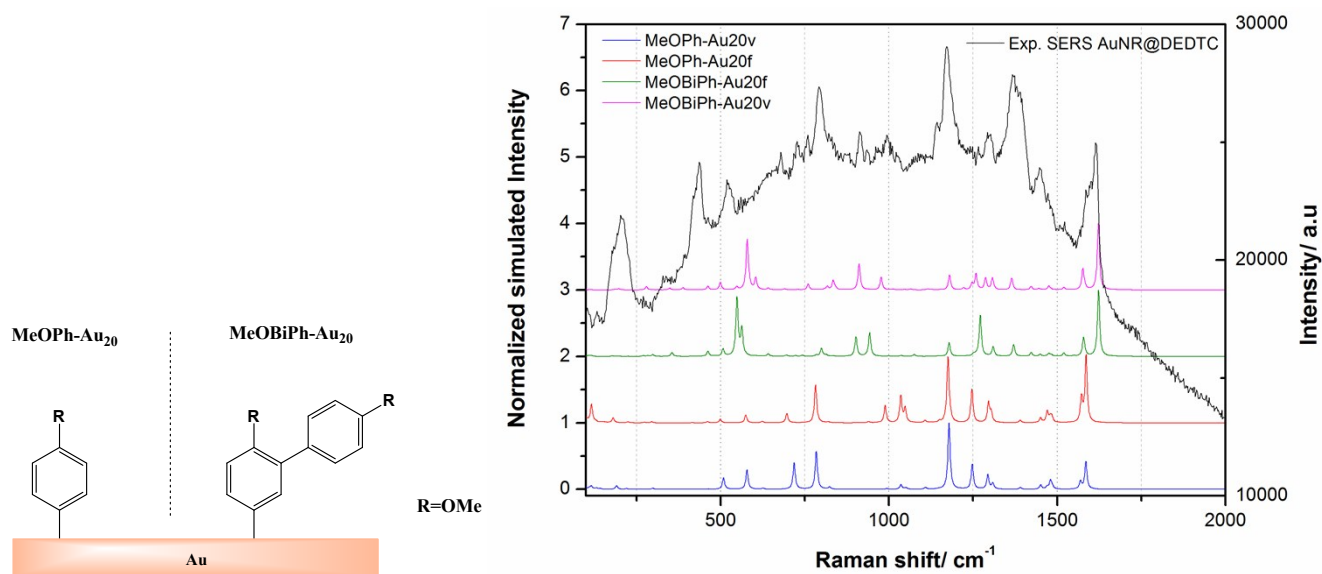


Figure S6. (left) MeOPh adducts considered for DFT modeling. (right) Corresponding Raman spectra simulated by DFT for vertex and face configurations.

c) SERS analysis of the uptake of FA by the hybrids AuNR@MIP_{8h}

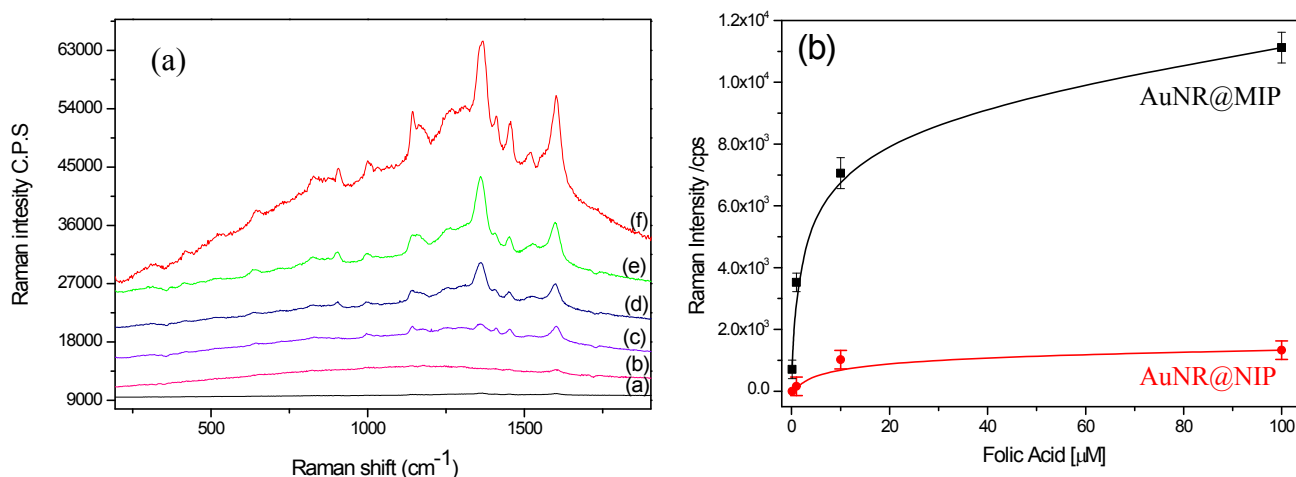


Figure S7. (a) SERS spectra of AuNR@MIP_{8h} after incubation with different concentrations of FA: (a) before incubation (dark); (b) 10⁻⁸ M (pink); (c) 10⁻⁷ M (violet); (d) 10⁻⁶ M (blue); (e) 10⁻⁵ M (green); (f) 10⁻⁴ M (red). (b) Evolution of the intensities of the SERS bands characteristic of FA for AuNR@MIP (square) and AuNR@NIP (circle) as a function of FA concentration.

d) Selectivity of AuNR@MIP_{8h}

In order to demonstrate the selective character of the AuNR@MIP sensor systems, the latter were incubated with folic acid (FnA), a structurally related molecule having molecular structure similar to FA, at concentrations varying from 10⁻⁸M to 10⁻⁴M. As a reference, the spontaneous Raman spectra of FA and FnA were recorded using the corresponding pure powders (see Fig. S8(a)). It is worth mentioning that the spontaneous Raman spectra of these two molecules dissolved in aqueous solution at 10⁻³ M do not exhibit any detectable vibrational bands.

In contrast, the adsorption of FA by the nano hybrids allows revealing perfectly well the vibrational bands of FA, even at very low concentrations (see Figure S8(b)). The absence of any signature from the analogue molecule FnA after incubation with the nano hybrids shows that the AuNR@MIP sensors are highly selective for the detection of folic acid.

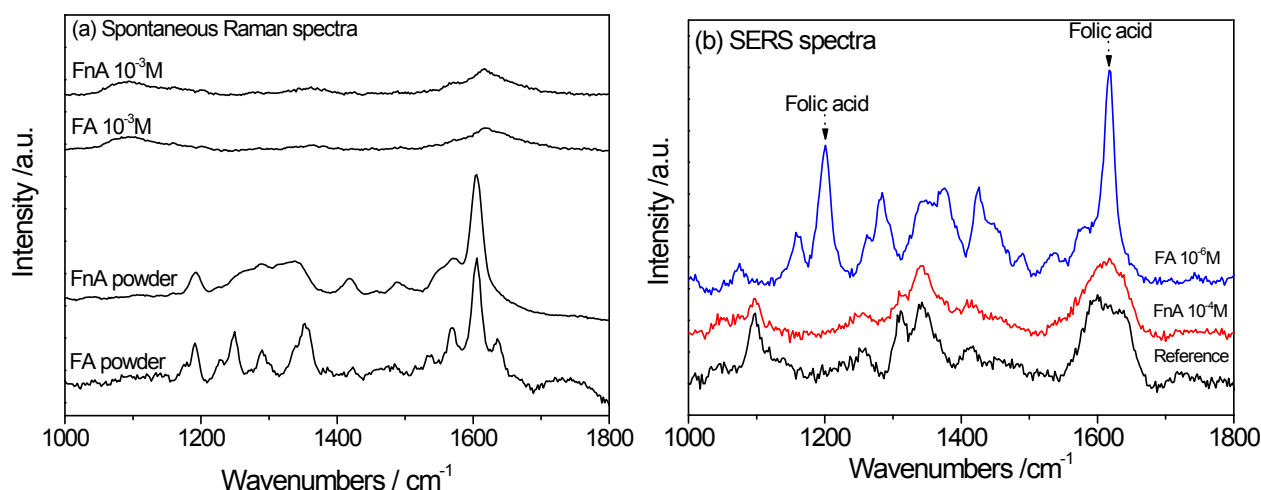


Figure S8. (a) Spontaneous Raman spectra of FA and FnA powders and corresponding aqueous solution at 10^{-3} M; (b) SERS spectra of AuNR@MIP_{8h} after incubation with FA (10^{-6} M) and FnA (10^{-4} M). The reference spectrum corresponds to the SERS spectrum recorded with AuNR@MIP_{8h} in pure water, free from any template molecules or analogues.

II. 5. Bibliography

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