Monitoring methotrexate in clinical samples of cancer patients during chemotherapy with a LSPR-based competitive sensor

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Electronic Supplementary Information

ESI 1. Folic acid functionalized gold nanoparticles spectroscopic characterization

Microscopic appearance of synthesized gold nanoparticles has been evaluated with Transmission Electron Microscopy (TEM, JEOL-TEM-1200-EX, Tokyo, Japan) where a drop of colloidal solution was dried on a copper mesh grip coated with amorphous carbon film (Electron microscopy Sciences). Absorption spectra were taken before and after gold nanoparticles functionalization by a Cary 100 Bio UV/Vis spectrophotometer (Varian Canada, Inc., Montréal, QC) for a qualitative test in 1 cm path length cell. Furthermore, mid-IR spectra were acquired for confirmation of sequential functionalization of AuNP by using FTIR-Reflectance Absorption Spectroscopy (Bruker optics). Samples were prepared by drying folic acid, AuNP and FANP solutions onto different gold coated coverslip. The FTIR measurements were taken at 4 cm^{-1} resolution with 1048 scans from 600 to 4000 cm^{-1}. Prior to Raman measurements, solutions of FA and FANP have been deposited onto glass coverslips and air-dried. The Raman spectrum was measured using a Renishaw InVia Raman microscope using 633 nm laser excitation. Spectra were acquired for 10 s at either 1% for the AuNP or 100 % of the laser power for pure folic acid. The Raman shift region covering 800-1800 cm^{-1} was monitored for the characteristic Raman peaks of folic acid.

ESI 2. Handling and activity of hDHFR

The hDHFR enzyme was prepared according to Volpato *et al.*[1]. The stock enzyme solution was quantified as 0.75 mg/mL with a purity of better than 95%. The activity test was performed by monitoring the absorbance decrease at λ = 340 nm due to consumption of substrate DHF and co-factor, NADPH with 100 ng/mL of hDHFR. The

specific activity of the enzyme was measured at 9 U/mg. The enzymes were stored at – 80°C and prior to analysis, they were thawed gradually to 0°C and diluted to desired concentration before analysis.

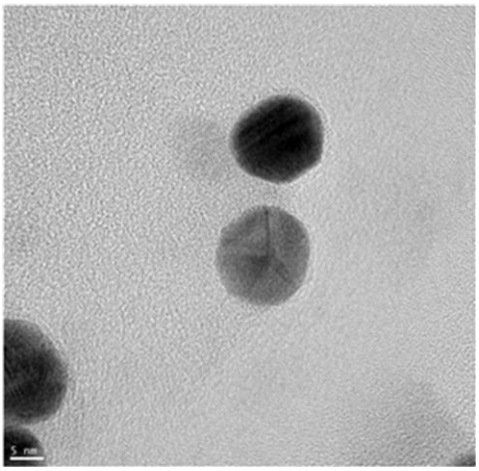

Figure S1: TEM image of synthesized citrate reduced AuNP of spherical shape of diameter 15 nm.

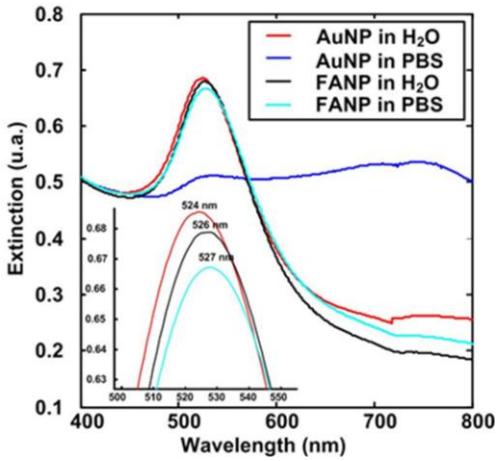

Figure S2. UV-Vis spectra of AuNP and FANP in water and in PBS. Inset shows the displacement of the LSPR band at each step of folic acid functionalization. The exchange of citrate by folate led to a 2 nm red shift is in accordance of folic acid attachment to the AuNP, where the local dielectric environment near the gold nanoparticles changes and further displaces the plasmon peak towards longer wavelength. The sharp peak in the UV-Vis spectrum of the folate-capped AuNP around 527 nm and the absence of a peak at high wavelengths were confirmatory for the stability of the FA-AuNP.

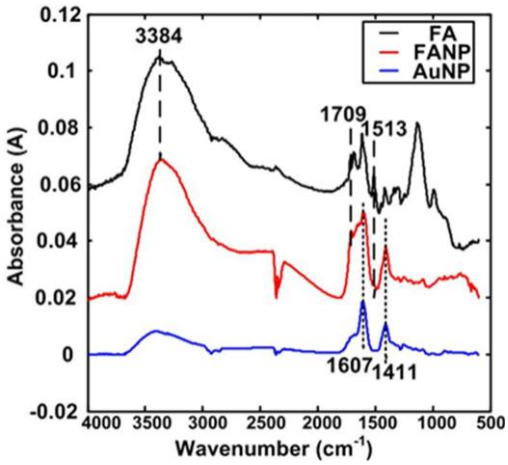

Figure S3. FTIR-RAS analysis of evaporated gold nanoparticles (AuNP) and folic acid functionalized gold nanoparticles (FA-AuNP) on gold surface in comparison to folic acid molecule. The vibrational bands of folate agree with the ones of FA-AuNP with characteristic peaks indicating C=O stretch peak at 1709 cm^{-1} from carboxylic acids, benzene ring vibration mode at 1513 cm^{-1} and a broad peak at 3384 cm^{-1}, representative of presence of N-H stretch in amide and primary amines [2]. Then by comparing FA-AuNP and AuNP spectra, both peaks at 1411 and 1607 cm^{-1} have corresponding –OH bending and C=O stretch vibrational modes respectively for the presence of carboxylic acids [3]. This demonstrates the presence of the reducing agent, citric acid for capping AuNP before and after the addition of folic acid.

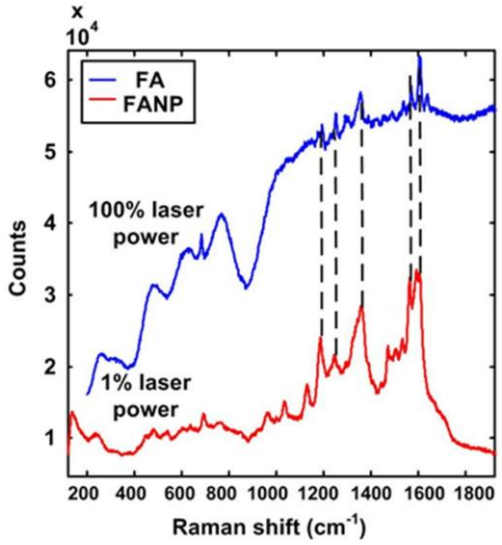

Figure S4. Raman spectrum of folic acid in comparison to SERS spectrum of folic acid adsorbed onto AuNP by using a 632.8 nm laser. Note that the SERS spectrum of FA-AuNP was acquired using laser power 100X less than the acquisition of FA spectrum. The local electromagnetic field is amplified due to resonant oscillation of free electrons found on closely associated NPs, the Raman signal of FA attached on the AuNP surface was amplified. The confirmation of FA-AuNP was mainly due to the presence of an intense peak around 1610 cm^{-1}, which is characteristic of the C=O stretch of carboxylic acids [4]. Furthermore, the second most intense peak around the region of 1250-1380 cm^{-1} is significant for amines in aromatic systems.

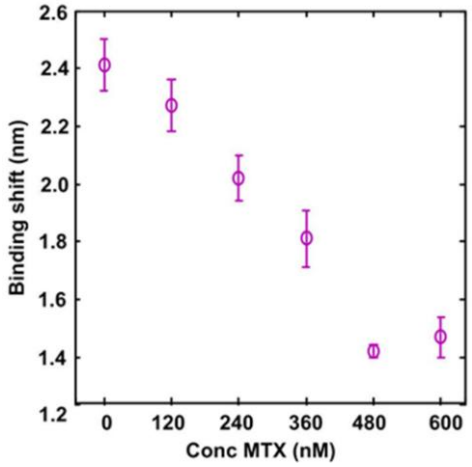

Figure S5. Calibration curve of MTX using FANP and 100 nM hDHFR in direct treated FBS after cartridge extraction. Note that the x-axis is scaled with respect to the initial spiked concentrations of MTX. Linear regression $y = -0{,}0018x + 2.43$ $R^2 = 0.95$.

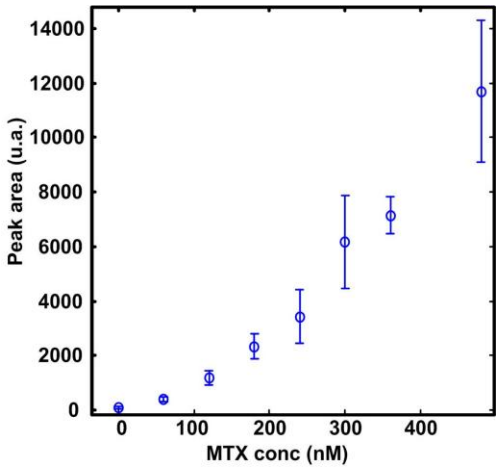

Figure S6. LC-MS/MS calibration curve of MTX spiked in human serum followed by cartridge treatment. Note that the x-axis is scaled with respect to the initial spiked concentrations of MTX. Linear regression: $y = 27x - 2130$ $R^2 = 0.97$.

Table S1. Recovery test conducted by spiking a specific concentration of MTX into FBS followed by treatment by cartridge. The concentrations of MTX have been quantified before spiking into FBS and after elution from cartridge treatment by UV-vis spectroscopy using ε_{258nm} = 22.1 mM^{-1} cm^{-1}.

	Spiked MTX concentration (uM)	Collected MTX concentration (uM)	Recovery (%)
1	102	98.1	96.6
2	82.4	88.0	107
3	82.4	86.0	104
		average	103
		SD	5.3
		relative SD	5.2

References:

1. J. P. Volpato, E. Fossati and J. N. Pelletier, *Journal of molecular biology*, 2007, **373**, 599-611.
2. J. M. Chalmers and P.R. Griffiths in *Handbook of Vibrational spectroscopy*, John Wiley, Chichester, vol. 1, pp. 189-199.
3. G. Li, D. Li, L. Zhang, J. Zhai and E. Wang, *Chemistry*, 2009, **15**, 9868-9873.
4. R. J. Stokes, E. McBride, C. G. Wilson, J. M. Girkin, W. E. Smith and D. Graham, *Applied spectroscopy*, 2008, **62**, 371-376.