Supporting Information for Publication

Predictive value of the SERRS-based MTT assay: a rapid and ultrasensitive method for cell viability in situ

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**Figure S-1**  
(a) UV−Vis spectra of formazan solution with different concentration (0.04−0.0001 mg/mL). (b) Plot of $A_{499}$ absorbance of the band at 499 nm against the concentration of formazan.
Figure S-2 Time-dependence of formazan-Au mixtures with the formazan concentration of 10 ng/mL: the SERRS intensity of band at 1290 cm$^{-1}$ (excited at 632.8 nm) as a function of time after mixed. The laser was off during each time interval.
Figure S-3 SERRS spectra observed from the MTT-formazan mixtures with the same concentration of (a1) 1 μg/mL, (b1) 10 ng/mL, and (c1) 1 ng/mL, compared with pure SERS of MTT solution (a0) 1 μg/mL, (b0) 10 ng/mL, and (c0) 1 ng/mL in Au NPs suspension. The excitation wavelength is 632.8 nm. The volume ratio is MTT:formazan = 1:1.
Figure S-4 SERRS spectra observed from the addition of (a0) 0, (a1) 0.1%, (a2)1% BSA in 100 ng/mL formazan-Au NPs suspension, and (b0) 0, (b1) 0.1%, (b2)1% BSA in 1ng/mL formazan-Au NPs suspension. The excitation wavelength is 632.8 nm.
Figure S-5 SERRS-based MTT assay were performed for human lung cancer cell line A549. When the MTT was just added to the drug (5-Fu) treated cell solution, we observed clear SERRS signal of formazan after Au staining due to the trace formazan from reductive cleavage of MTT by living cell. (a) SERRS spectra observed from the Cell MTT assay. (b) SERRS spectra of formazan-Au mixtures with the formazan concentration of 50 ng/mL. The excitation wavelength is 632.8 nm.
Figure S-6 Photomicroscopy of A549 human lung cancer cells stained with MTT. Cells were incubated with MTT solution for 4 hours at 37°C; note “hairy” extensions representing long-shaped formazan crystals.
Figure S-7

Dose-response curves showing the cell viability taken at incubation conditions for 30 min. The results are calculated from the data of Figure 6 and expressed as percentage of the untreated controls. The datum points shown in each figure represent the mean±SD for the 8 replicate determinations normalized to the control value.

Equation: \[ Y = 98.41 - 1.83X \]

\[ R^2 = 0.995 \]
Figure S-8

a) Dose-response curves showing the OD value at 490 nm (expressed as the OD$_{490}$) measured by conventional MTT assay. b) The cell viability is calculated from the data of a) and expressed as percentage of the untreated controls. The datum points shown in each figure represent the mean±SD for the 8 replicate determinations normalized to the control value.