Supporting Information for

Operando Characterization of Chemical Reactions in Single Living Cell Using Surface-Enhanced Raman Spectroscopy

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EXPERIMENTTAL SECTION

1. General procedure

The Raman spectra were detected by a Raman micro-spectrometer (Renishaw InVia) with a Peltier cooled charge-coupled device camera and a 633 and 785 nm diode laser. The laser power at the surface of sample was about 6.2 mW. The acquisition time for single spectral was 10 seconds with a long distance 50× objective lens. For time series experiment, the acquisition time was 1 second. The software package WiRE 4.3 was employed for spectral processing, including polynomial multipoint and curve fitting. Prior to each measurement, a silicon wafer with a Raman signal at 520 cm^{-1} was used for instrumental calibration. For each sample, SERS spectra (n = 5) were obtained. $Cp(W(CO)_3H$ was purchased from Sigma-aldrich.

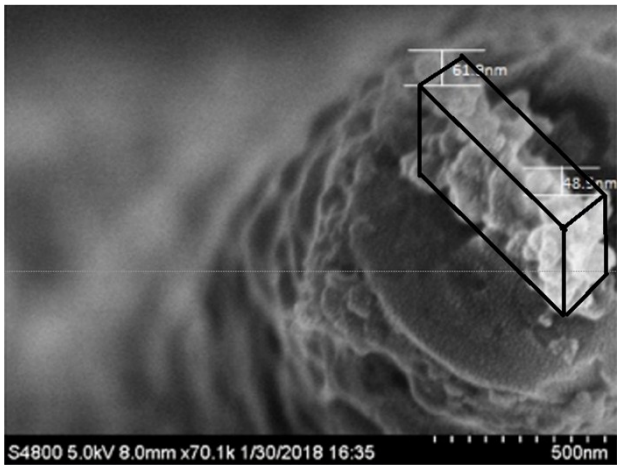
2. Preparation of $CpW(CO)_3$-Ag NP and estimation of number of $CpW(CO)_3$ on silver nanoparticles

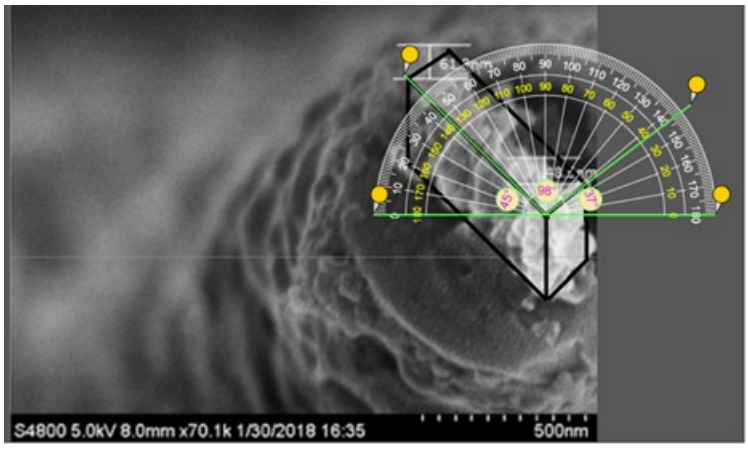
1 mM of ethanolic solution of $Cp(W(CO)_3H$ was prepared. 1 mL of Ag NP (2 x 10^2 mg/ml) was centrifugated and the pellet was collected (~ 100 uL). 10 uL of $CpW(CO)_3H$ was added into the Ag NP pellet and incubated for 1 hour. After that, the pellet was washed by water for three time (dispersed into 1 mL of water and re-centrifugated into pellet). The pellet was collected and performed SERS experiment. To ensure the quality of the $CpW(CO)_3$-Ag NP, every batch of $CpW(CO)_3$-Ag NP required to be characterized by IR to ensure all the unreacted $CpW(CO)_3H$ was removed.

We estimated the concentration of tungsten in $CpW(CO)_3$-Ag NP by using ICP-MS to measure the concentration of tungsten elements from unreacted $CpW(CO)_3H$ left behind in the process (supernatant). The unreacted amount was used to substrate the original amount of $CpW(CO)_3H$.

The amount was divided by the number of silver nanoparticles, which we determined based on the peak optical density and Cytodiagnostics product data sheet. The number of $CpW(CO)_3$ per silver nanoparticles was ~3,598 ± 920. The measurement was repeated for every batch of $CpW(CO)_3$-Ag NP to ensure similar amount of the $CpW(CO)_3$ on silver nanoparticles.

As shown in the SEM Figure below,





Considering the shape of nanoparticles clustered on nanotip as a cuboid shape. We then estimate the dimension of the cuboid (Length, width and height) which is 600 nm, 146.7 nm and 266.7 nm. By considering the angle of the nanotip in SEM image, we could obtain the size of the cuboid is [600 nm*cos(45˚)] x [146.7 nm *cos(37˚)] x [266.7 nm] = 41,568,690nm^3

If we consider it cuboid only 70% filled, so the total number of nanoparticles on nanotip will be

41,568,690*70/(113,097)≒ 257.2 ~ 258

Each of nanoparticles was estimated to be 3,598. Thus, total $CpW(CO)_3$ on nanotip will be 3,598 x 258 = 928.284 $CpW(CO)_3$. Number of mole of $CpW(CO)_3$ will be $928.284/6.02x10^{23}$ = $1.5414516580189954\ x10^{-18}$ mole.

Thus, the number of CpW(CO)3 entering into cells is estimated to be $1.54\ x10^{-18}$ mole.

3. Anchoring of $CpW(CO)_3$–Ag NP on Nanotip.

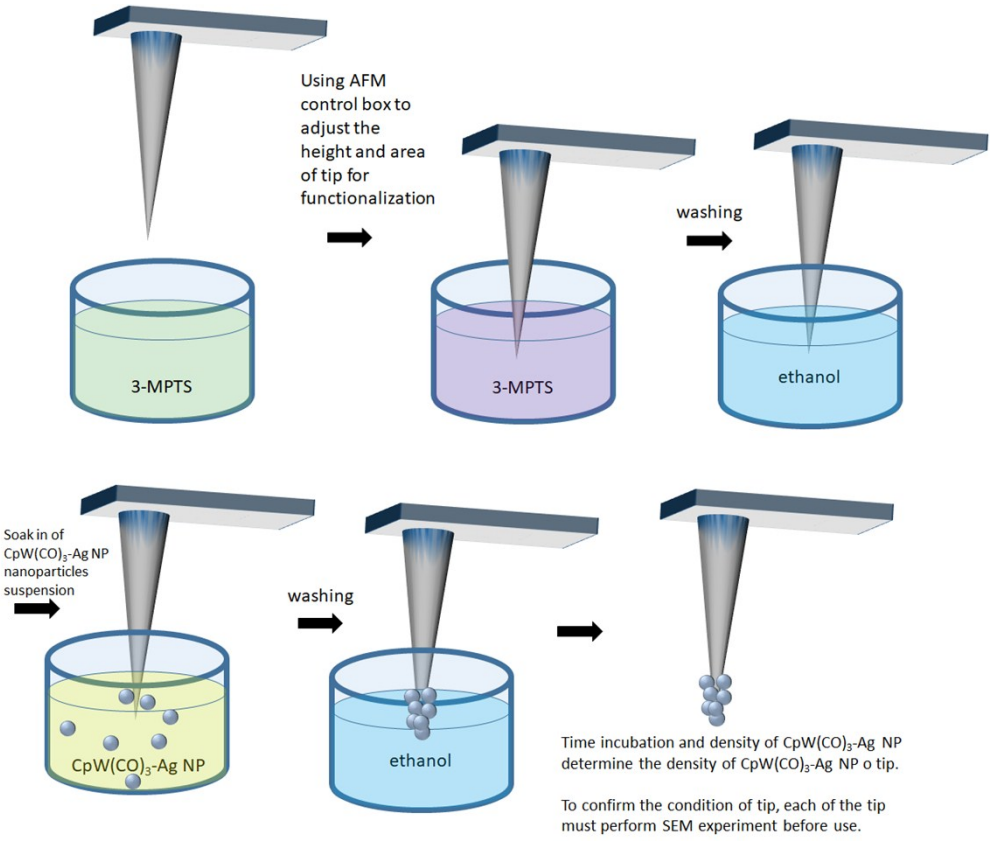


Figure S1. Fabrication of tip with $CpW(CO)_3$–Ag NP.

The main steps of the nanoparticle attachment procedure are schematically presented in Figure above. We used standard fused silica AFM tip. The surface of the tip was made chemically functionalized for 3-MPTS deposition. The nanoparticles were subsequently attached by first silanizing the tips with 3-MPTS and finally dipping them in a $CpW(CO)_3$–Ag NP suspension for

a certain period of time. The tips were then washed by gradually diluting the suspension with pure water to mitigate nonspecific nanoparticle deposition. The tip arrangements were subsequently stored in a clean vacuum chamber for future use. After optimizing the parameters in each step, we were able to achieve a success rate of as high as 60% for attaching $CpW(CO)_3$–Ag NP to the end of the probe. For a batch of ten tips that was usually prepared, we could obtain 6 $CpW(CO)_3$–Ag NP functionalized tips for experiments. The fail functionalized tips were due to tip broken during mounting tips or washing.

Figure S2. SEM images of functionalized tip and tip fail to functionalized.

We used commercially available colloidal silver from the following suppliers: Cytodiagnostics. These colloids are produced by laser ablation which can devoid of any contaminants. Most importantly, they do not have any stabilizing molecules on the surface, which makes them ideal for further functionalization. Fused silica sharpened AFM tip cantilevers (Nanonics) were used. The tips have a nominal radius of 20 nm. The levers were initially plasma cleaned for 3 minutes combined with a plasma generator. This left the tip surface free of organic contaminations, and reach on silanol groups, thus facilitating the silanization. The TCTS silanization was performed by placing the cantilevers in a 3×10^{-3} M TCTS in anhydrous toluene solution at 70 ^{o}C for 30 minutes. The tips were then consecutively washed in toluene, acetone, ethanol and water.

Silanization with 3-MPTS was done by placing the levers in a solution containing 10 ml of 2-propanole, 200 µl of 3-MPTS and 200 µl of water for 20 minutes. The tips were then washed by placing the tips consecutively in containers of 2-propanole and water. Functionalized tips were dipped in a 0.01 OD colloidal silver suspension for 360 minutes to allow the nanoparticles adsorption. Washing was then done by gradually dissolving the suspension with pure water. Each tip was examined by SEM before proceed for subsequent experiment.

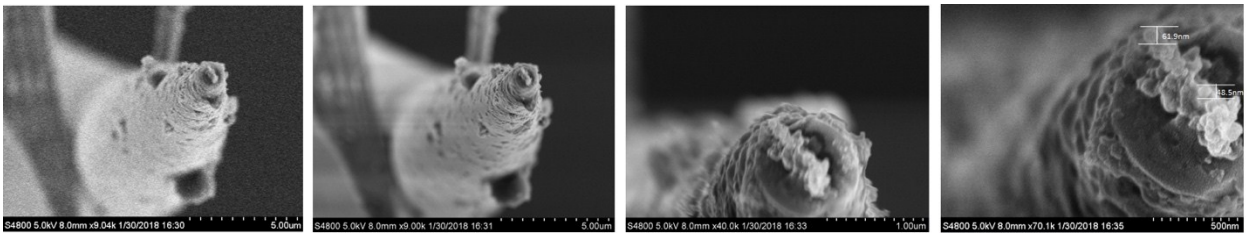


Figure S3. SEM images after processing AFM tip.

4. Intracellular experiment using nanotip

The cell line was cultured at 37 °C under a 5% CO_2 atmosphere (D-6450 incubator; Heraeus) in Dulbecco's modified Eagle's medium (DMEM) containing 1.0 g/L glucose and sodium pyruvate supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin. The medium, serum and antibiotics were purchased from Invitrogen Life Technologies. 18 to 24 h before Raman studies, cells were transferred at 5-10% confluence in a 60-mm tissue culture dish (Falcon) to obtain essentially isolated single cells. Just prior to the experiments, adherent cells were rinsed with PBS at pH 7.4 and, when it mattered, immersed in PBS at pH 7.

A plastic 60-mm culture dish with isolated living cells was mounted on the horizontal stage of Axiovert-S100 microscope (Zeiss) that was set on an optical table. The tip position relative to the target cell could be precisely monitored. The experiments were carried out at room temperature

(25 ± 2 °C). Tips moved down to the living cell surface so that the platinized tip first penetrated through the membrane. To avoid tip crashing, the whole process was monitored under a microscope (Supporting Information, Video 1). The laser beam was focused at the tip (using internal function provided by Renishaw In Via Raman to observe the laser beam on samples and adjust with the control panel on WiRE 4.2 software.) and spectrum were recorded at different tip positions either outside the cell or inside it. The tip tuning was always performed by positioning the tip far away from the cell before and after each experiment. The spectrum baseline was then normalized from the recorded response to yield the final spectrum.

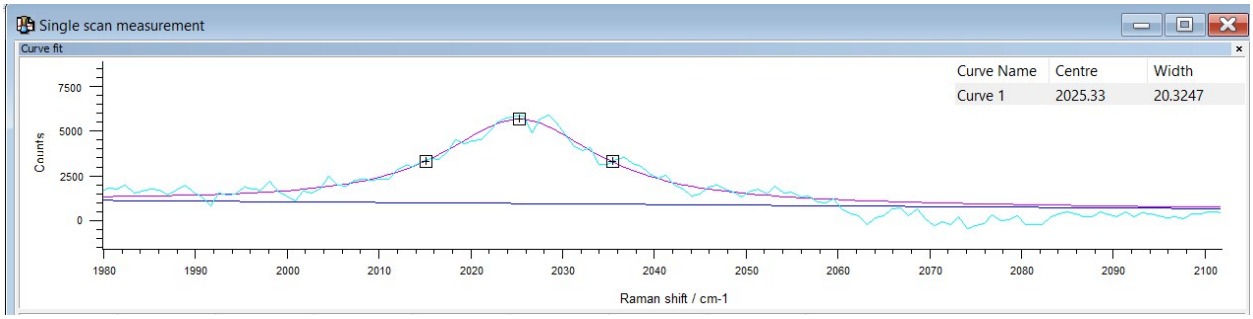


Figure S4. All the peak assignment was based on Polynomial multipoint and curve fitting.

For GSH and AAPH experiments in Figure 3, the adding of GSH into cell was performed after extracellular and membrane measurement. The tip was retracted and dithiothreitol (DTT, 1 mM) added into cell samples to induce reductive changes. After 30 min, the cell sample was washed three times with PBS and placed on the measurement platform and tip was positioned to intracellular of cell for measurement. After that, tip was retracted again and added 2,2'-azobi-2-amidinopropane (AAPH 30 mM) for 30 min to induce oxidative stress. The same measurement was performed. The force constant of nano-tip was 5 N/m. The depth of indentation was calculated by subtracting the deflection of the cantilever from the displacement of the piezo-actuator for a given spring constant. The nano-tip was initially made

contact to the cell with the applied force of 100 pN as the baseline. The indentation depth of cell is approximately 0.6μm.

5. CO detection in cells using COP-1

To assess CO inside cells, cells were inoculated (5×10^5 cells per mL) in 6-well plates, containing a sterile coverslip, and incubated for 24 h. After the removal of the medium, the cells were washed three times with PBS buffer and exposed, for 15 min, to $CpW(CO)_3$-Ag NP (200 μg/mL) and next incubated, for 30 min, with 0.1 μM COP-1, which was prepared in DMSO as previously described.[1] After treatment with COP-1 (1 μM), for 15 min, the cells were collected by centrifugation, washed with PBS, and resuspended in 1/10 of their initial volume in PBS.

After 24hr, incubation at 37^0C in a 5% CO_2 atmosphere, cells were washed with cold 1 X PBS and immediately placed at -20^0C for 5 min to allow rapid cooling. This procedure is essential to maintain phosphorylation status, if any, of the proteins of interest. The cells were then scraped in the presence of 200ul of lysis buffer (10mM HEPES (pH7.9) (Sigma), 10mM KCL (Merk), 0.1mM EDTA, 10% NP40) supplemented with 1 x protease inhibitor (Pierce Biotechnology), and phosphatase inhibitors – 50 uM okadaic acid (Sigma) and 200 mM sodium vanadate (Sigma). The mixture was vortexed 1 min every half hour for 2 hours and kept on ice at all times. The lysate was precleared by centrifugation at 13,000g; 4^oC, and subsequently stored at -20^oC until evaluation by SDS-PAGE. 5 μl of protein albumin standards with known protein concentrations (PIERCE) or 1 μl of protein sample was added to 150 μl of Comassie Plus™ Protein Assay Reagent (PIERCE). Absorbance was measured at a wavelength of 595 nm and the absorbance intensities of the protein standards were plotted to obtain a standard curve. Absorbance intensities of protein samples were then read off the standard curve to determine the amount of protein within the

sample. Equal amount of samples were subjected to 15% SDS-PAGE electrophoresis and then electrophoretically transferred to nitrocellulose membranes (Bio-Rad Laboratories) using a wet transfer apparatus (Bio-Rad Laboratories). Precision Plus Protein^{TM} Dual Color Standards (Bio-Rad laboratories) were used as a molecular mass standard. After transfer, the membranes were washes in distilled water to remove trances of transfer buffer, and then air dried fro a few hours prior to blocking. Membranes were blocked with 1xTris-Buffered Saline with Tween-20 (TBST) containing 5% (w/v) non fat milk for overnight at 4^{o}C. The membranes were then washed with TBST before exposure to appropriate primary antibodies overnight at 4^{o}C (Table 1). Washes with TBST were carried out again before incubation with the appropriate horseradish peroxidase-labeled secondary antibodies for 1h at room temperature. After secondary body incubation, membranes were washed again with TBST. The antibody-reactive bands were revealed by chemiluminescence based detection using West Pico Substrate (Pierce Biotechnology). COP-1 was excited using a 488 nm Ar laser, and the emission was collected between 500 and 650 nm. For ROS measurement, we are using 2′,7′-Dichlorodihydrofluorescein diacetate (DCFH2-DA) were obtained from Sigma-Aldrich for ROS measurement for RAW264.7 cells (treated with LPP) and NHDF cells. Cells were seeded in 24-well plates with a density of 1.0×10^5 cells per well. For Raw264.7, cells were treated with LPP (1 μg/ml). We then incubated ROS probe DCFH2-DA (10 μM, 60 min) for RAW246.7 and NHDF cells. After probes incubation, cells were collected and tested by flow cytometry (Becton-Dickinson, Franklin Lakes, NJ, USA) at the FITC channel.

6. Cell viability study.

Five thousand cells were seeded in a 96-well plate for 24 hours before $CpW(CO)_3$-Ag NP (2−

200 μg/mL) in DMEM were introduced into each well. The cells were then allowed to incubate for another 24 hours, after which 10 μL of CCK8 (cell counting proliferation kit, Sigma-Aldrich) was added to each well. Cell absorbance was measured with a SpectraMax 384 Plus spectral analyser after 4 hours, at 450 nm excitation.

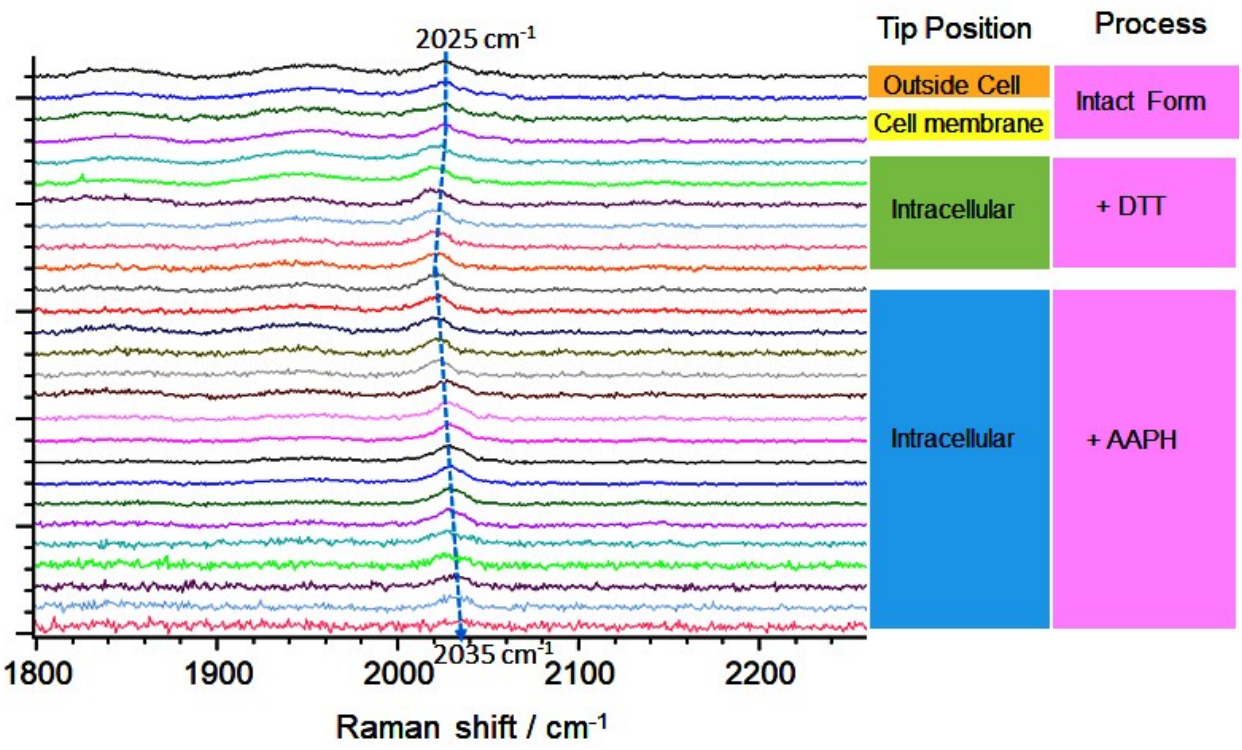


Figure S5. Direct detection of DTT and AAPH induce charge-transfer process as reflected by CO band position.

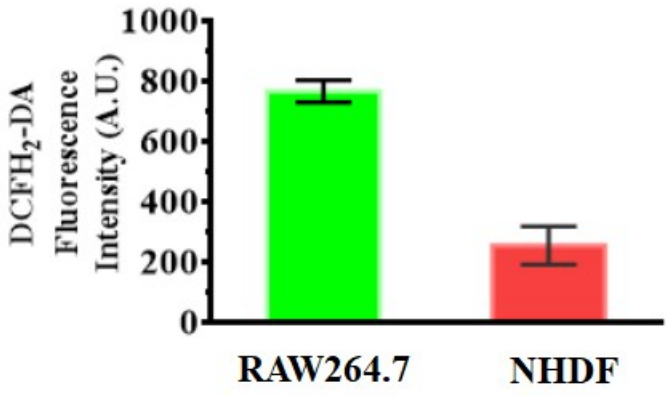


Figure S6. RAW264.7 cells (treated LPP) and NHDF were stained with $DCFH_2$-DA (by $DCFH_2$-DA (2',7'-Dichlorodihydrofluorescein diacetate) (0.5 μM) for 60 min.

Reference

Michel, B. W.; Lippert, A. R.; Chang, C. J. *J. Am. Chem. Soc.*, **2012**, *134*, 15668–15671.