METHODS

General Procedure. All chemical synthesis manipulations were carried out using standard Schlenk techniques under an argon or nitrogen atmosphere. Ruthenium compounds were prepared according to the reported procedure.[1-2] All other chemicals were purchased from other commercial sources and used as supplied. We obtained the IR spectra using a Thermo Scientific Nicolet iS5N FT-NIR spectrometer. The Raman spectral measurements were carried out using a Renishaw *In Via* Raman (UK) microscope with a Peltier cooled CCD detector and an excitation wavelength at 633 nm, where the laser excitation was directed onto the sample *via* a 50× objective lens (with a confocal pinhole 25 μm in diameter), and the exposure time was set at 10 s for all of the measurements. All Raman spectra were processed using WiRE 4.3 software. Before each measurement, the instrument was calibrated using the standard Raman spectrum of silicon, whose Raman peak is centered at 520 cm^{-1}. For each sample, 20 SERS spectra were acquired over each sample (3.5-mW laser power); the average of the 20 spectra was used for analysis. The CO spectra were presented at baseline using a polynomial multipoint fitting function and curve fitting function provided by the Renishaw WiRE 4.3 software. The Raman intensities of the peaks were taken as the height above the baseline. Transmission electron microscope (TEM) and scanning electron microscope (SEM) images were recorded on a Hitachi H-7100 and KEOL-JSM-7600F, respectively. The TEM samples were prepared by placing a drop of the nanoparticles onto a carbon coated Cu grid.

Preparation of CO Nanotags. 100 μM (1 μL) of freshly prepared solutions of metal carbonyl compounds in ethanol were mixed with 40 nm gold colloids (2.6×10^{10} particles/mL, BBInternational UK) in ethanol. After a 2-day incubation period, the excess metal carbonyl was

removed by centrifugation (10,000 rpm, 2 min). Please note that shorter incubation periods, incomplete reactions and excess metal carbonyl typically interfere with the consistency of the SERS CO signal (*e.g.*, site reactions with subsequent incubation material protein or PEG to generate other CO species, which have different CO signals that complicate SERS measurement). Thus, to ensure quality control of the CO nanotags, we quantified the number of metal carbonyls bound on the nanoparticles (refer to the "Estimation of CO peak enhancement" section). We performed Raman scan to confirm the binding of the ruthenium compounds after washing procedure. The CO nanotag pellet was re-suspended in 1 mL of DI water for subsequent bioconjugation. The nanotags were then incubated with 10 μM of peptide solution for 2 hours customized by Pepmic Co., Ltd as follows:

(1) QpYDHPNI-$CONH_2$

(2) QYDHPNI-$CONH_2$

(3) EAIpYAAPFAKKKC-$CONH_2$

(4) SRVGEEEHVpYSFPNKQKSAEC-$CONH_2$

(5) SRVGEEEHVYSFPNKQKSAEC-$CONH_2$

(6) SRVGEEEHVFSFPNKQKSAEC-$CONH_2$

(7) EETPpYSYPTGNH for Tyr31

(8) GNHTpYQEIAVPP for Tyr40

(9) EEHVpYSFPNKQK for Tyr118

(10) LSPLpYGVPETNS for Tyr181.

Electromagnetic Simulation. A Lenovo desktop computer with an Intel(R) Core$(TM)_2$ Quad CPU Q9650 at 3.00 GHz with 8 GB RAM was used to perform high-mesh-density

simulations of the nanoparticles. The operating platform was 64-bit Windows 7 Professional. To simulate the plasmonic properties of the gold nanoparticles, we used an RF extended module under COMSOL Multiphysics. The desired particle size and 3D shape were drawn in draw mode using the Cartesian coordinate system. The boundary conditions and perfectly matched layer were also defined in draw mode. Simulation duration for a single nanoparticle took about 4 hours.

Estimation of CO Peak Enhancement. The enhancement of the CO vibration peak intensity was estimated by comparing the intensity of the 2045 cm^{-1} peak for the $CpRu(CO)_2$ and the $CpRu(CO)_2$ on gold nanoparticles (Au-$CpRu(CO)_2$) as: Enhancement = $(C_{CpRu(CO)2}$ x $I_{(Au\text{-}CpRu(CO)2)}) / (C_{(Au\text{-}CpRu(CO)2}$ x $I_{CpRu(CO)2})$ where $C_{CpRu(CO)2}$ and $C_{(Au\text{-}CpRu(CO)2}$ are the concentration, and $I_{CpRu(CO)2}$ and $I_{(Au\text{-}CpRu(CO)2}$ the corresponding normal Raman and SERS intensities, for the $CpRu(CO)_2$ and Au-$CpRu(CO)_2$, respectively.

The concentration of $CpRu(CO)_2$ on gold nanoparticles was determined using ICP-MS as follows: A 10 ml sample of a 4.3 x 10^{-5} μM* suspension of gold nanoparticle was pelleted by centrifugation. This was dispersed in 1.0 ml of a 10 μM solution of $CpRu(CO)_2I$ in ethanol, incubated for overnight and then centrifuged again.

(*gold nanoparticles concentration was obtained from BBInternational UK product data sheet.)

A pellet collected from 10 mL of Au-$CpRu(CO)_2$ solution was mineralized with 65% HNO_3 at 120 °C, and the dried digested material was redissolved in 2% HNO_3. The ruthenium content was then determined by ICP-MS on an ELAN 6100 instrument. This gave 81.7 μg/10 mL, which is equivalent to $CpRu(CO)_2$ concentration of 43 μM, if at the same concentration as that above.

The intensity of the 2025 cm^{-1} peak in the Raman spectrum of a 20 mM solution of $CpRu(CO)_2I$ in ethanol, and of the Au-$CpRu(CO)_2$, were measured at 312 and 6600 counts, respectively. From this, enhancement of the CO signal is estimated to be: (20 mM x 6600) / (43μM x 312) = 98389 ~ 9.8 x 10^4.

TERS experiments. TERS measurement was conducted by a commercial AFM system (nanonics MV 2000) and a Renishaw Raman microscope. A 785 nm excitation laser was used to illuminate the sample. The TERS tip was purchased from supplier and used as received. There is a gold nanoparticle (diameter of ~ 150 nm) attached to the apex of a transparent glass cantilever (Nanonics Imaging Ltd). A long working distance objective (x50; Olympus) was used. The TERS tip was position to a liquid cell with AFM controller boxes provided by Nanonics. The laser light was focusing on the tip apex region for excitation. TERS tip was incubated with 500 μL SH-PEG-NHS for 2 hours in water. The tip was subsequently incubated with 500 μL of YOP 51 solution in 50 mM HEPESl, pH 7.0, 100 mM NaCl, 5 mM DTT, 0.01% Brij 35, 50% glycerol, and 2 mM Na_2EDTA. The YOP51-TERS tip was incubated with nanotags in a buffer solution (500 μL, 20 mM Tris-HCl pH7.5, 20 mM NaCl, 0.4 mM Na2EDTA, 2 mM DTT, 0.004% Brij 35). The aggregation of nanotags (500 μL) on YOP51-TERS tip was examined by Raman scan and SEM as well. By focusing on the tip, the association and dissociation of nanotag on tip can be monitored by the CO signal over time. To redirect dissociated nanotag to assembly on YOP51-TERS tip, nanotag was incubated with a kinase reaction buffer (500 μL, 20 mM Tris-HCl, pH7.5, 2 mM MgCl2, 20 mM NaCl, 0.2 mM EGTA, 0.4 mM DTT, 0.004% Brij 35, 0.2 mM ATP) in a total volume of 200 μL. Kinase reaction was initiated by adding 50 U $μL^{-1}$ amount of Abl kinase (New

England Biolabs). After two hours, the nanotag solution was incubated with tip for 6 hours. Nanotag assemblies were characterized by Raman scan over time.

For the detection of Crk binding of nanotag, GST-Crk (Creative Biomart) was purchased commercially. GST-Crk was added into nanotag solution for 6 hours prior incubation with YOP51-TERS tip. The effect of Crk can be evaluated by collecting CO signal from tip. GST-Crk was recovered and the binding of nanotag to Crk was examined by the presence of CO signal.

For cell lysate study, cells were washed with cold 1XPBS and immediately placed at -20^{0}C 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 (per 3 x 10^6 cells) 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 – 50uM okadaic acid (Sigma) and 200mM 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^{o}C, and subsequently mixed with nanotag for 6 hours. These nanotags were then incubated with YOP51-TERS tip.

Cell Migration Assay. Experimental cultures of the breast carcinoma cell line MCF7 were obtained from the American Type Culture Collection (ATCC) (Rockville, MD) and maintained in RPMI 1640 (Sigma) supplemented with 10% fetal bovine serum (FBS), 1% L-glutamate (GIBCO Laboratories) and 1% Penicillin-streptomycin (GIBCO Laboratories) at 37^{o}C in a 5% CO2 atmosphere. Cultures (passages 8-16) were grown in T-75 flasks and maintained by the addition of fresh medium and subcultured (1:8) once every week. Cell concentration was allowed to exceed 1x 106 cells per ml, as recommended by ATCC.

Cells (5 x 10^4) were counted and seeded directly onto 6.5 mm Corning Costar trans-wells coated with 100 μg/cm_2 matrigel (BD Biosciences, San Jose, CA) for the invasion assays. Complete media was added to both the top and bottom wells and cells were incubated at 37°C overnight. Following the overnight incubation, cells on both sides of the transwells were fixed using formalin phosphate for 20 minutes at room temperature. After washing with double-distilled water, cells were stained with 0.1% crystal violet in 20% methanol for 20 minutes at room temperature. Cells on the top layer were scraped and membranes were left to dry overnight. Images were captured using a cytation 5 (Biotek).

Statistical Data Analysis. In cases where multiple duplications were possible, data are represented as mean ±SEM. Individual groups were compared using the Student's *t* test with a two-tailed *p* value. A value of $P < 0.05$ was taken as significant. Trends between groups were compared using ANOVA analysis. A value of $P < 0.05$ was taken as significant.

REFERENCES

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