# *Supporting Information*

## An Integrated Platform for Capture of Circulating Tumor Cells and In Situ SERS Profiling of Membrane Protein through Rationally Spatial Organization of Multi-functional Cyclic RGD Nanopatterns

*Hui Dong, Dazhi Yao, Qi Zhou, Limin Zhang * and Yang Tian**

*School of Chemistry and Molecular Engineering, East China Normal University, Dongchuan Road 500, Shanghai 200241, P.R. China.*

E-mail: ytian@chem.ecnu.edu.cn; lmzhang@chem.ecnu.edu.cn;

## 1. Experimental Section

**Regent and Chemicals.** All the reagents and materials were purchased from commercial suppliers, and are of analytical grade. All aqueous solutions were prepared with ultrapure water obtained with a Milli-Q System with a resistivity of 18 MΩ·cm. Gold(III) chloride trihydrate ($HAuCl_4•3H_2O$, 99%), 1-ethyl-3-(3-dimethylaminoprop-yl)-carbodiimide (EDC), N-hydroxy-succinimide (NHS), hexafluorophosphate (HBTU), Dimethylformamide (DMF), $H_2O_2$, Quercetin, 2,2-diphenyl-1-picrylhydrazyl radical, dicyclohexylcarrbodiimide (DCC) and all the amino acid (99%) were purchased from Sigma-Aldrich. α-Tocopherol was purchased from Calbiochem. KCl, $CaCl_2$, $MgCl_2$, $FeCl_3$, $ZnSO_4$, $CuSO_4$, glucose were obtained from Sinopharm Chemical Reagent Co. Ltd. HER2 was obtained from Abcam Co., Ltd (Cambridge, MA). Phosphate buffer solution (PBS) was prepared using $K_2HPO_4$ and $KH_2PO_4$, which was deaerated by purging with nitrogen gas. The galvinoxyl radical, trolox, and ascorbic acid were obtained from Acros Organics. Methanol was of HPLC grade and used directly, whereas ethyl acetate and acetic acid were of analytical grade and purified by standard techniques. The peptide, DNEYFYV(H2)-NH-C=O-CRGDKC(RGD)-acetyl (denoted as H2-RGD) were obtained by Shanghai Biotech Bioscience and Technology Co., Ltd., China. All experiments were carried out at room temperature, unless stated otherwise. TMS was synthesized according to previous report.[9b]

**Instruments and Characterization.** All electrochemical experiments were conducted with a computer-controlled CHI 660 (CHI, Shanghai) electrochemistry workstation. Working electrodes were gold-coated glass chips (5 mm × 20 mm size), used in conjunction with a Pt auxiliary electrode, and a saturated Ag/AgCl reference electrode. The electrodes for electrochemical experiments were purchased from Shanghai Chenhua Co., Ltd., China. Electrochemical activation of TMS/MGF was realized by potential scanning from −0.1 to 0.9 V with a scan rate of 100 mV $s^{-1}$ in 0.1 M $H_2SO_4$. SERS spectra were obtained using a confocal microscope Raman system (DXR ThermoFisher Confocal Raman Spectra) equipped with a Renishaw CCD camera. The microscope attachment is based on a system with a 50× long-working length objective, so that the objective will not be in contact with the electrolyte. A holographic notch filter was used to filter the exciting line, and two selective holographic gratings (1200 g/mm, 2400 g/mm) were employed, depending

on the spectral resolution required. The exciting wavelength was 633 nm. $^{1}H$, $^{13}C$, $^{31}P$ NMR experiments were performed on a Bruker Avance 500 MHz spectrometer and chemical shifts were referenced to the residue DMSO (2.50 ppm for $^{1}H$ and 39.52 ppm for $^{13}C$) and $H_2O$ (4.79 ppm). Mass spectrometry was carried out using a Perkin Elmer-Sciex API 365 instrument. SEM images were obtained using a Hitachi S4800 microscope at the accelerating voltage of 5 kV.

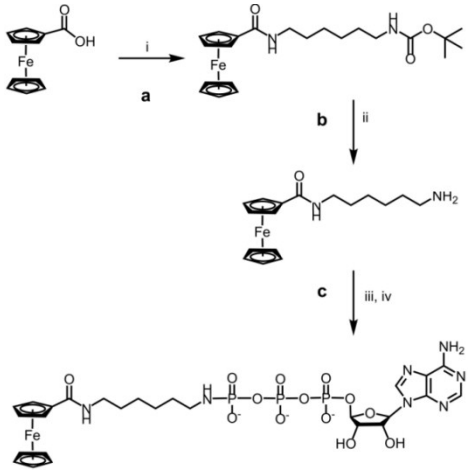
**Preparation of Au/ITO.** The Au-ITOs were prepared on glass chips (5 mm × 20 mm size) by thermal evaporation of a 500 Å gold layer (99.99%) with a 50 Å chromium as the adhesion layer. The chips were carefully pre-cleaned by repeated sonication in Millipore $H_2O$ and ethanol for at least three times before preparation of the mesoporous gold film (MGF).

**Preparation of MGF.** Our approach is based on an electrochemical method, which has been practically used for the general preparation of continuous metallic films.[8] In a typical synthesis, 10 mg of polystyrene-b-poly(oxyethylene) (PS-b-PEO) was dissolved in 3 mL THF completely at 40 ℃ and then 1.5 mL ethanol was added to the mixed solution. An aqueous solution of $HAuCl_4$ (5 mM) was added slowly to the clear PS-b-PEO solution and spherical micelles were formed by the presence of water. Gentle stirring for 30 min at room temperature is used to make sure that the dissolved Au species were well incorporated into the exterior PEO region of the micelles. Finally, a transparent bright-yellow colored electrolyte (pH 2.5) was obtained and directly used for electrodeposition. Electrochemical deposition from the precursor solutions was carried out by using an electrochemical machine (CHI 660D electrochemical station, CH Instrument, Shanghai) with a conventional three-electrode system, including a platinum wire as a counter electrode, an Ag/AgCl as a reference electrode and various conductive substrate as working electrode. The typical conducting substrate used is Au–ITO with a representative size of 0.45 $cm^2$ (0.3 cm × 1.5 cm), which was fabricated by a dicing cutter. The optimal electrodeposition of Au was carried out at a constant potential of - 0.5 V (versus Ag/AgCl) for 1000 s without stirring at room temperature. During the electrodeposition, a stable current was detected for the Au reduction. After the Au deposition, the micelles (used as soft-templates) were thoroughly removed by ultraviolet–ozone cleaner or low-powered $O_2$ plasma treatment. Calcination of the films in air, which has been

commonly used for complete removal of organic templates in mesoporous metal oxide films, led to removal of the templates from the films, but the mesoporous structures collapsed through the grain growth of Au.

**Synthesis of Fc-ATP**

Fc-ATP was prepared according to previous report.[9] The synthesis route was illustrated as shown in Scheme S1.




**Scheme S1.** Preparation routes of the Fc-ATP (Compound 3). Reagents and conditions: (i) TEA and HBTU; (ii) TFA and DCM, (iii) ATP, dicyclohexylcarrbodiimide (DCC), and DMF; (iv) compound 2 and methanol.

**Preparation of TMS/MGF.** TMS/MGF were constructed by immersing freshly TMS solution in EtOH and 2-(2-hydroxyethoxy) ethylethanethioate (DEG) in $10^{-4}$ mol $L^{-1}$ total mixed thiol solution (1 mol % TMS and 99 mol % DEG) for 48 h at room temperature.

**Detection limit.** This Detection limit value is calculated according to LOD = 3 N/S, where N represents the standard deviation of the $I_{320}/I_{1690}$ values obtained at the H2-cRGD/TMS/MGF within 20 measurements in the absence of HER2, and S represents the slope of the linear equation for HER2.

**Cell Culture.** Human breast cancer cells (SK-BR-3, MCF-7), human ovarian cancer cells (SK-OV-3) and Hela cells were purchased from the Chinese Academy of Medical Sciences& Perking Union Medical college (Beijing, China). SK-OV-3 represents one type of ovarian adenocarcinoma cells with the overexpressed HER2, Hela cells and MCF-7 cells are HER2-negative cells.[5b,S1] They were cultured in a flask containing complete medium supplemented with 10% heat-inactivated fetal bovine serum and 1% streptomycin/penicillin. The flask was placed in a humidified atmosphere with 5% $CO_2$ at 37 ℃ in a cell culture incubator. The media was replaced once every three days. Cells were cultured to 80-90% confluence before harvest. During harvest, the cells were washed twice with PBS buffer followed by trypsinization with 1 mL of trypsin-EDTA solution (0.25 w/v % trypsin, 2.5 g $L^{-1}$ EDTA) at 37 ℃ for 5 min to detach the cells from the flask. The trypsin was neutralized by adding 5 mL of fresh supplemented RPMI-1640 medium, and the harvested cells in medium suspension was transferred into a centrifuge tube and centrifuged at 1000 rpm for 5 min. The supernatant was discarded and the cells were re-suspended in fresh medium. White blood cells (WBCs) were obtained from lysed human blood.[3c] They were stained with 1,1-dioctadecy 1-3,3,3,3-tetramethylindocarbocyanine and kept at a concentration of $10^6$ cells/mL in PBS, respectively.

Cells were cultured on gold-coated glass slides in a 12-well plate under normal culture conditions. After a 48 h incubation period, medium was removed. Then cells were fixed with 4% polyfluoroalkoxy for 15 min and incubated with 0.01% Rhodamine for 5 min, followed by two washes with PBS. The mesoporous gold-coated ITO were viewed by confocal fluorescence microscopy. Then, the culture medium was removed, and cells was washed three times with PBS to remove unbonded samples. Finally, the dish was put on the microscope stage for cell imaging and spectroscopic detection. Confocal fluorescence images were taken using a laser scanning confocal microscope LEICA TCS SP8.

**Isolation of CTCs from Cancer Patients' Whole Blood.** The EDTA anticoagulated whole blood samples (4 cancer patients, including 3 breast cancer, 1 gastric cancer and 3 healthy individuals) were obtained from Zhongshan Hospital of Fudan University and used without any pretreatment. Before samples processing, the H2-cRGD/TMS/MGF was blocked by 5% BSA and 0.2% Tween-

20, to avoid the nonspecific binding of the blood cells onto the skeleton. Then 1 mL of whole blood samples were incubated with H2-cRGD/TMS/MGF for 30 min at 4 °C and washed three times with PBS to remove unbonded samples. Next, the H2-cRGD/TMS/MGF was incubated with 0.01% Rhodamine for 5 min and followed by two washes with PBS to remove the extra fluorescent dyes. Finally, the H2-cRGD/TMS/MGF electrode was observed under fluorescent microscope.

## 2. Pore Depth distributions of MGF

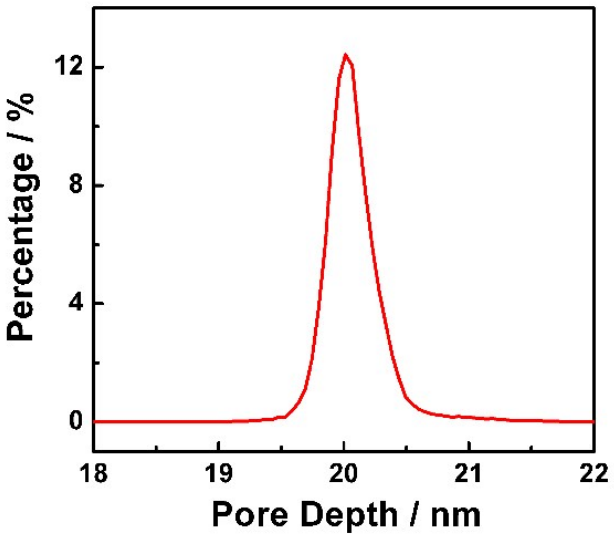



**Fig. S1** (a) Pore Depth distributions of MGF (n = 200) from AFM image as shown in Fig. 1e.

## 3. Characterization of TMS

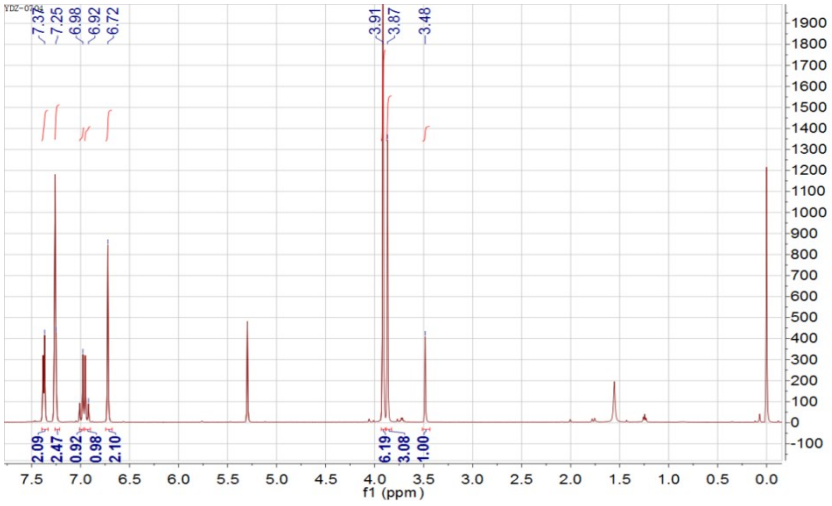



**Fig. S2** $^{1}$H NMR spectrum (500 MHz) of TMS molecule in $CDCl_3$.

$^{1}$H NMR (500 MHz, $CDCl_3$): $\delta$=3.48 (s, 1 H; SH), 3.87 (s, 3 H; $OCH_3$), 3.91 (s, 6 H; $OCH_3$), 6.72 (s, 2 H; H2′, H6′), 6.92 (d, *J*=16.0 Hz, 1 H; H8), 6.98 (d, *J*=16.0 Hz, 1 H; H7), 7.25 (d, *J*=8.0 Hz, 2 H; H3, H5), 7.37 ppm (d, *J*=8.0 Hz, 2 H; H2, H6); HRMS (ESI): m/z calc.$C_{17}H_{18}O_3S$ for [M+H]+: 303.1; found: 303.1.

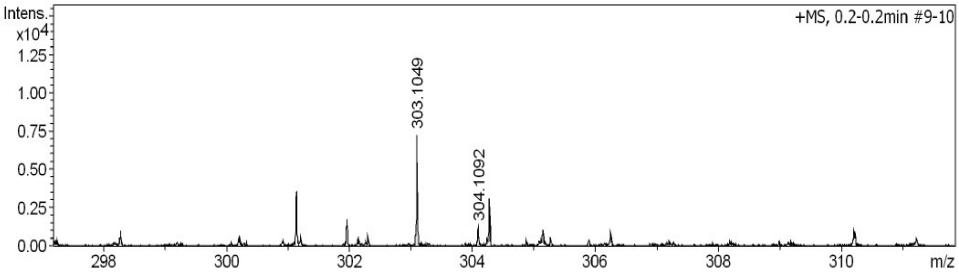



**Fig. S3** HRMS (ESI) for TMS.

## 4. Characterization of Fc-ATP

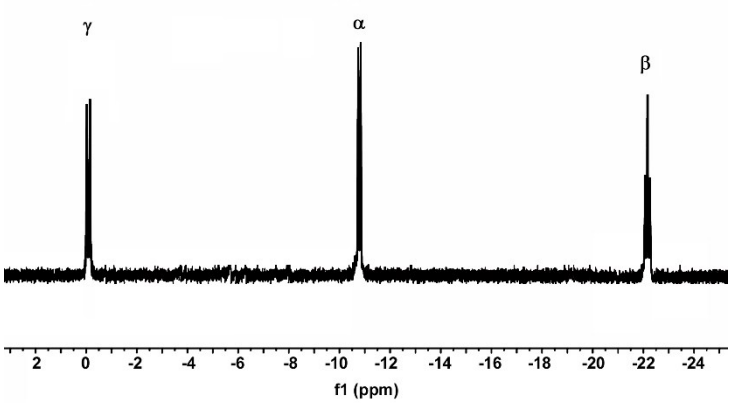



**Fig. S4** $^{31}P$ NMR spectrum (400 MHz) of Fc-ATP in $D_2O$.

$^{31}P\{^{1}H\}$-NMR (δ, $D_2O$): -0.07(γ) d, J = 21.1 Hz; -10.76(α) d, J = 19.9 Hz; -22.14(β) t, J = 19.9 Hz.

[H4·M](ESI$^+$) m/z: calc. for $C_{27}H_{39}FeN_7O_{13}P_3$: 818.1; found: 818.2.

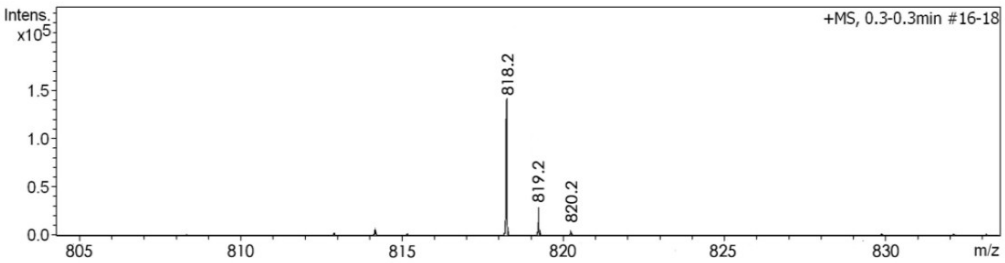



**Fig. S5** [H4·M] (ESI+) Mass Spectrum for Fc-ATP molecule.

## 5. Electrochemical characterization

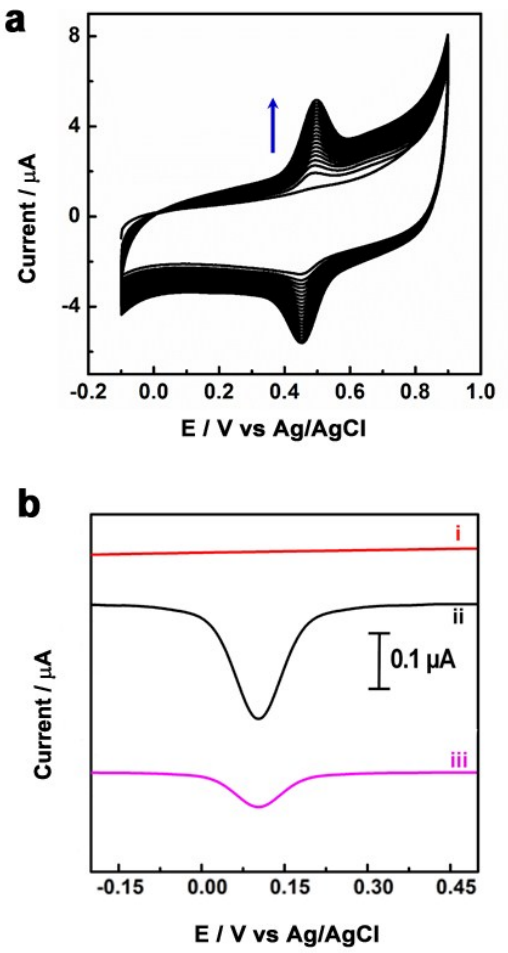



**Fig. S6** (a) CVs obtained at TMS/MGF electrode in 0.5 M $H_2SO_4$. Scan rate: 100 mV $s^{-1}$; (b) DPVs obtained at (i) MGF/Au, (ii) TMS/MGF after the first electrochemical activation, and (iii) formed H2-cRGD/TMS/MGF in 10 mM PBS (pH 7.4) after the second electrochemical activation.

## 6. SERS spectra characterization

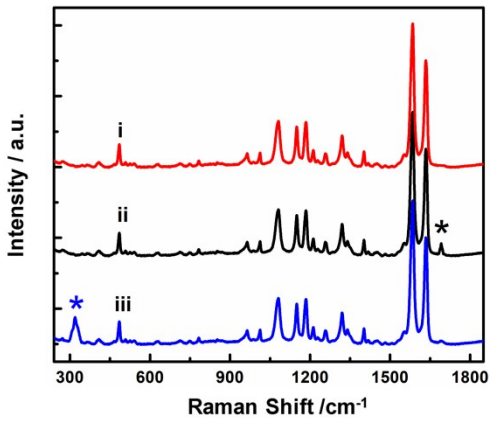



**Fig. S7** SERS spectra obtained at (i) electrochemically activated TMS/MGF, (ii) H2-cRGD/TMS/MGF, (iii) H2-cRGD/TMS/MGF in 10 mM PBS (pH 7.4) in the presence of 50 nM Fc-ATP and 40 fg $mL^{-1}$ HER2.

## 7. Concentration optimization of Fc-ATP

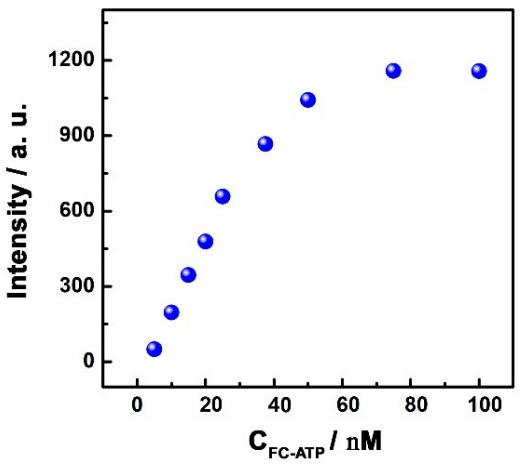



**Fig. S8** Responses in peak intensity at 1690 $cm^{-1}$ ($I_{1690}$) in Raman spectra with the addition of different concentrations of Fc-ATP and 40 fg $mL^{-1}$ HER2.

## 8. Response time for detection of HER2

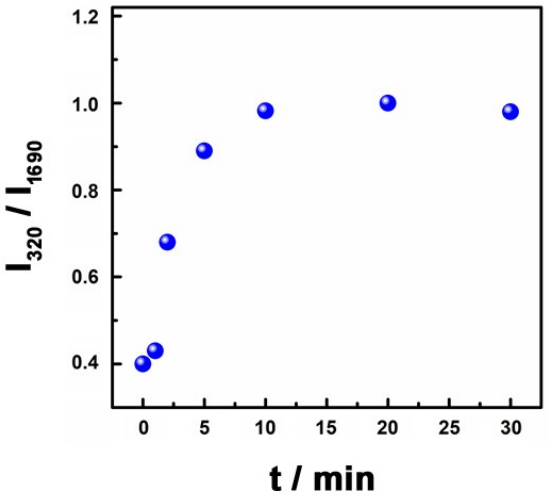



**Fig. S9** $I_{320}/I_{1690}$ values obtained at H2-cRGD/TMS/MGF substrate in 10 mM PBS (pH 7.4) containing 50 nM Fc-ATP and 40 fg $mL^{-1}$ HER2.

**9. Fluorescence microscope images of SK-OV-3, MCF-7 and Hela cells captured at H2-cRGD/TMS/MGF platform.**

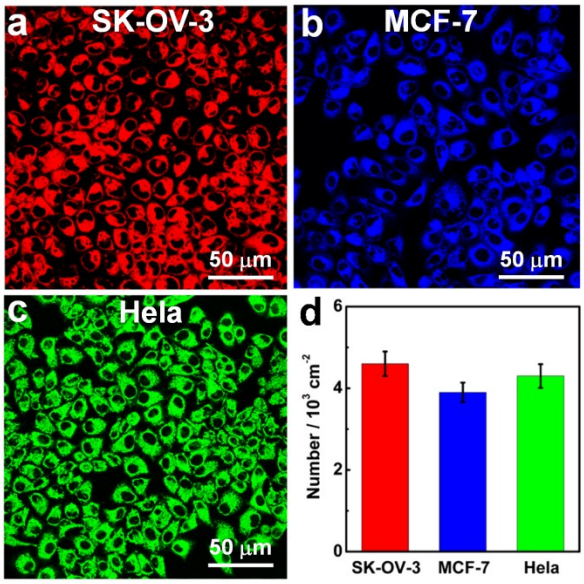



**Fig. S10** (a-c) Fluorescence microscope images of SK-OV-3, MCF-7 and Hela cells captured by H2-cRGD/TMS/MGF. (d) Cell capture efficiencies for SK-OV-3, MCF-7 and Hela cells at H2-cRGD/TMS/MGF. Error bars equal to the standard deviation.

## 10. Fluorescence microscope images of White blood cells

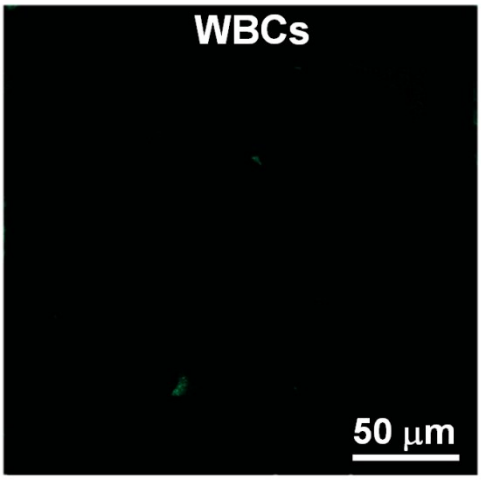



**Fig. S11** Fluorescence microscope images of White blood cells captured by H2-cRGD/TMS/MGF.

## 11. Selectivity

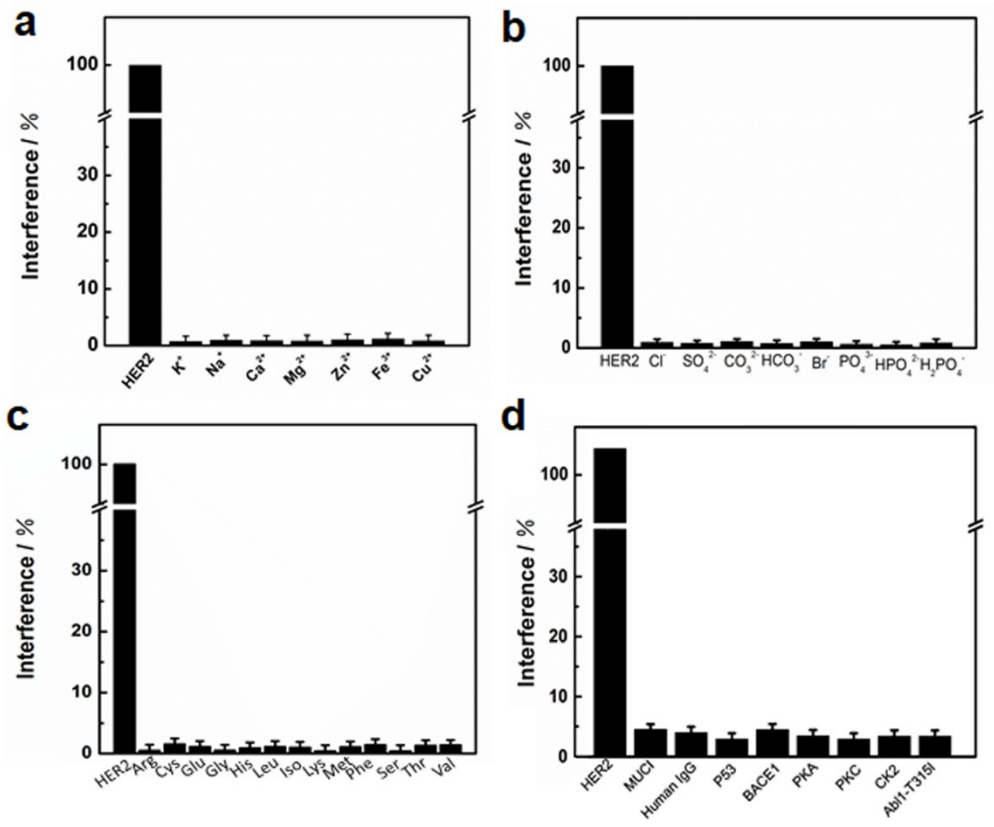



**Fig. S12** Interference on peak intensity ratio ($I_{320}/I_{1690}$) obtained at 320 $cm^{-1}$and 1690 $cm^{-1}$ in addition of (a) cations: (i) 10 fg $mL^{-1}$ HER2, (ii) $K^+$ (150 mM), (iii) $Na^+$ (150 mM), (iv) $Ca^{2+}$ (2.5 mM), (v) $Mg^{2+}$ (2.5 mM), (vi) $Zn^{2+}$ (1 mM), (vii) $Fe^{3+}$ (10 μM), (viii) $Cu^{2+}$ (10 μM); (b) Anions: (i) 10 fg $mL^{-1}$ HER2 (ii) $Cl^-$, (iii) $SO_4^{2-}$, (iv) $CO_3^{2-}$, (v) $HCO_3^-$, (vi) $Br^-$, (vii) $PO_4^{3-}$, (viii) $HPO_4^{2-}$, (ix) $H_2PO_4^-$ (from ii to ix concentrations are 100 μM); (c) amino acids: (i) 10 fg $mL^{-1}$ HER2, (ii) Arg, (iii) Cys, (iv) Glu, (v) Gly, (vi) His, (vii) Leu, (viii) Iso, (ix) Lys, (x) Met, (xi) Phe, (xii) Ser, (xii) Thr, (xiv) Val (10 μM from ii to xiv) and (d) proteins: (i) 10 fg $mL^{-1}$ HER2, (ii) MUC1 (1 ng $mL^{-1}$), (iii) human IgG (10 μg $mL^{-1}$), (iv) p53 (0.1 ng $mL^{-1}$), (v) BACE1 (1 U $mL^{-1}$), (vi) PKA (1 U $mL^{-1}$), (vii) PKC (1 U $mL^{-1}$), (viii) Abl1-T315l (1 ng $mL^{-1}$). Herein, the interference % was estimated according to $(I_{320}/I_{1690})'/(I_{320}/I_{1690})$, in which $(I_{320}/I_{1690})'$ means the $I_{320}/I_{1690}$ after addition of various interferences.

## 12. Anti-biofouling performance of H2-cRGD/TMS/MGF platform

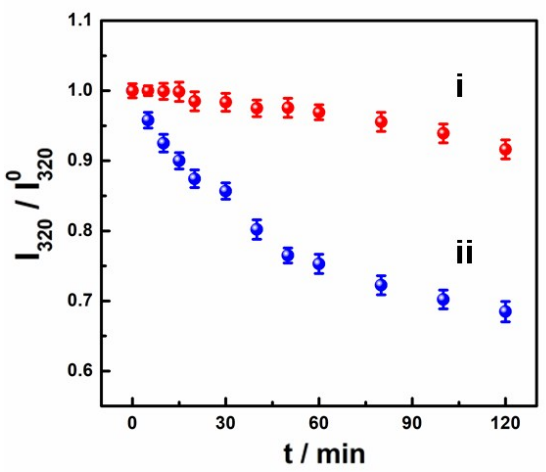



**Fig. S13** $I_{320}/I_{320}^0$ values obtained at (i) H2-cRGD/TMS/MGF platform and (ii) H2-cRGD/TMS/Au in SK-BR-3 cell lysate containing 40 fg $mL^{-1}$ HER2 under different time.

## 13. Comparison of capture efficiency in PBS and whole blood

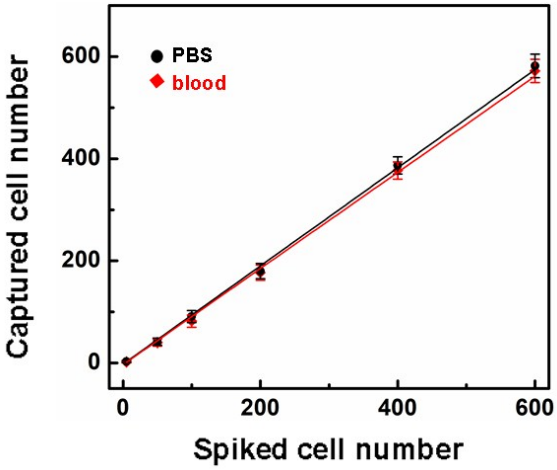



**Fig. S14** Comparison of capture efficiency for SK-BR-3 cells in 10 mM PBS buffer (pH 7.4) and whole blood using H2-cRGD/TMS/MGF platform.

## 14. ELASA kit detection of HER2 activity from different passages of SK-BR-3 cells

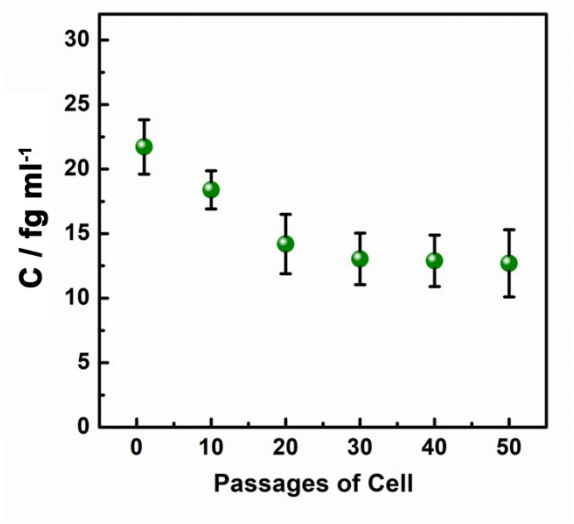



**Fig. 15** HER2 activity detected by ELISA kit for cell extracts collected from different passages of SK-BR-3 cells.

## 15. SERS images of SK-OV-3, MCF-7 and Hela cells captured by H2-cRGD/TMS/MGF platform

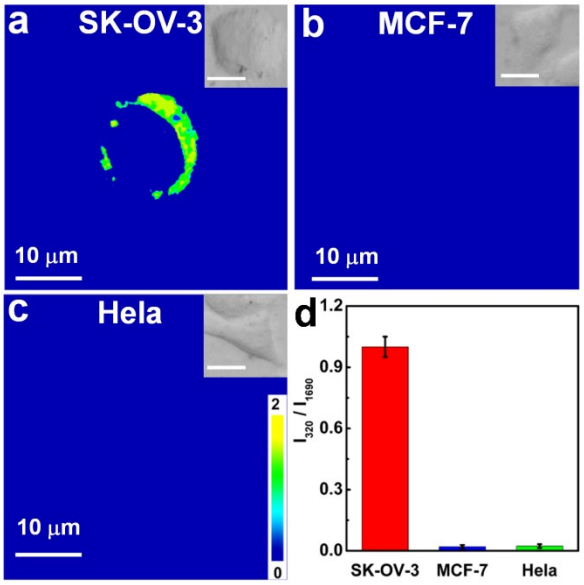



**Fig. S16** (a-c) The SERS images of SK-OV-3, MCF-7 and Hela cells captured at H2-cRGD/TMS/MGF. Inset: their corresponding dark field images of cells, the scale bar is 5 μm. (d) The averaged $I_{320}/I_{1690}$ value for SK-BR-3, MCF-7 and Hela cells captured by H2-cRGD/TMS/MGF. Error bars equal to the standard deviation.

**Table S1.** Comparison of SERS detection at H2-cRGD/TMS/MGF with ELISA Kit for detection of HER2 in human serum.

| Sample no. | Type | Number of CTCs | $C_{HER2\text{-}b}$ / ng ml$^{-1}$ (n = 3) | ELISA kit / ng ml$^{-1}$ (n = 3) |
|---|---|---|---|---|
| 1 | Normal | 0 | 0 | - |
| 2 | Normal | 0 | 0 | - |
| 3 | Normal | 0 | 0 | - |
| 4 | Breast | 100 | 22.18 ± 1.52 | 23.62 ± 4.85 |
| 5 | Breast | 255 | 35.75 ± 2.31 | 38.05 ± 4.23 |
| 6 | Breast | 280 | 27.09 ± 3.25 | 29.10 ± 3.86 |
| 7 | Gastric | 457 | 48.05 ± 2.84 | 50.00 ± 6.96 |

* The level of HER2 in whole blood was estimated through the following equation:

$$C_{HER2\text{-}b} = C_{HER2\text{-}c} \times N$$

in which $C_{HER2\text{-}b}$ represents the total HER2 activity in the blood sample, $C_{HER2\text{-}c}$ is the HER2 activity obtained from averaged values for 10 measurements at one CTC. N is the number of CTCs captured at H2-cRGD/TMS/MGF. n = 3 represents three blood samples from the same individual.

## References

**S1.** D. W. Rusnak, K. J. Alligood, R. J. Mullin, G. M. Spehar, C. Arenas-Elliott, A. M. Martin, Y. Degenhardt, S. K. Rudolph, T. F., Jr. Haws, B. L. Hudson-Curtis, T. M. Gilmer, *Cell Proliferat.* 2007, **40**, 580.