Supporting Information

Selective Detection of RGD-Integrin Binding in Cancer Cells Using Tip Enhanced Raman Scattering Microscopy

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Supporting Information includes 5 supplementary figures and 2 supplementary tables.

Contents

1. Characterization of cRGD-GNPs

The cRGD peptides were conjugated onto gold nanoparticles through a ligand exchange mechanism. Strong covalent Au-S bonds replaced weak citrate-gold interaction after 24 h vortex mixing at room temperature (Figure S1a). UV-Vis spectra showed the localized surface plasma resonance (LSPR) of cRGD-GNPs was at 532 nm, a small red shift (~1 nm) from the LSPR of citrate-GNPs (531 nm), which is due to changes in local refractive index after ligand exchange. A small broad shoulder peak at 600-700 nm reflected an aggregation of the functionalized GNPs (Figure S1b). The extent of the aggregation is dependent on the peptide concentration, which has been reported in our previous study[1]. Raman spectrum of cRGD-GNPs exhibited an intense phenylalanine band at 1002 cm^{-1} (Figure S1c), which confirmed the successful coating of cRGD peptides onto GNPs.

The LSPR, characteristic Raman band and hydrodynamic diameter of citrate-GNPs and cRGD-GNPs were listed in Table S1. The hydrodynamic diameter, measured by dynamic light scattering (DLS), presented a subtle decrease after cRGD binding due to the small size of the peptide (~2 nm).

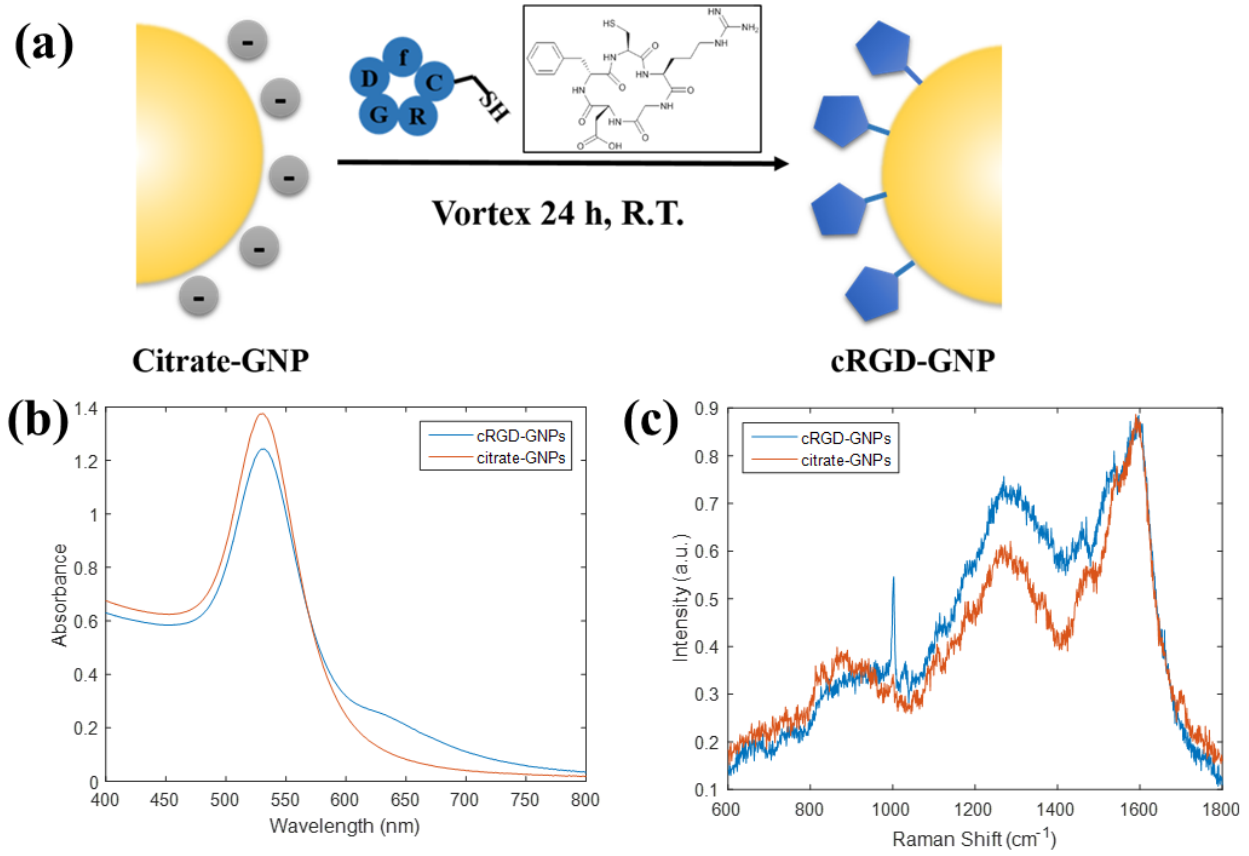

Figure S1. (a) Schematic illustration of gold nanoparticles conjugating with cRGD peptide. Inset: chemical structure of cRGD peptide. (b) UV-Vis spectra and (c) average Raman spectra of gold nanoparticles with (cRGD-GNPs) and without (citrate-GNPs) peptide conjugation. The strong Raman band at 1002 cm^{-1} corresponds to the phenylalanine residue present in the cRGD-conjugated nanoparticles.

2. SERS spectra of integrin α5β1- and αvβ3-incubated cRGD-GNPs

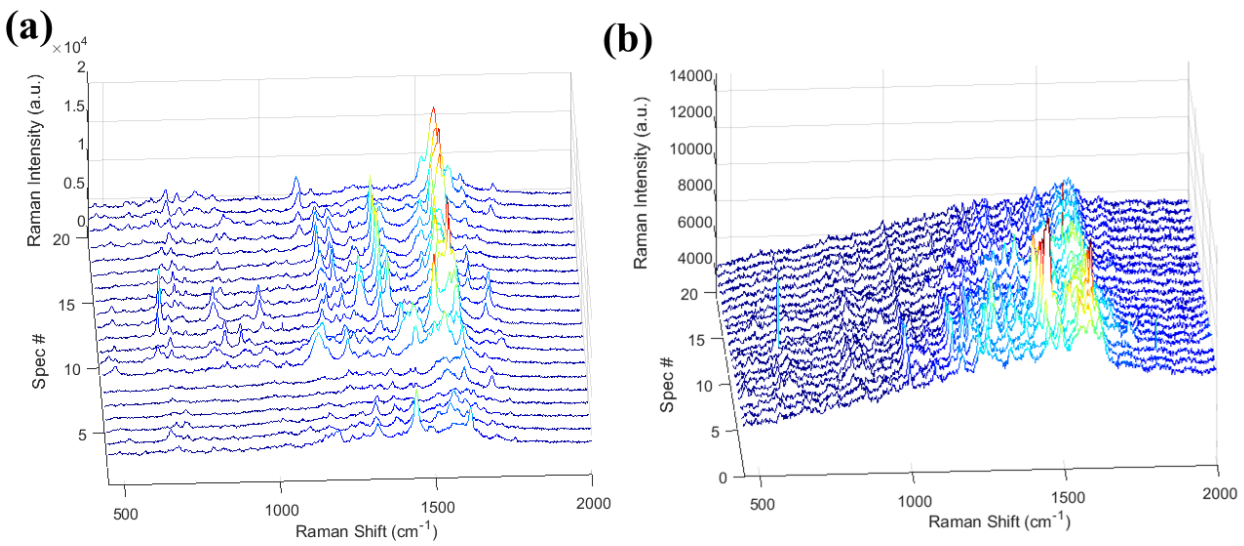

Figure S2 Consecutive Raman acquisitions of (a) α5β1-bound cRGD-GNPs (n=20) and (b) αvβ3-bound cRGD-GNPs (n=20). Changes in spectral pattern and intensity suggest the functionalized nanoparticles moving in and out of the Plasmon-enhanced "hot spots".

3. Integrin immobilization on glass slides

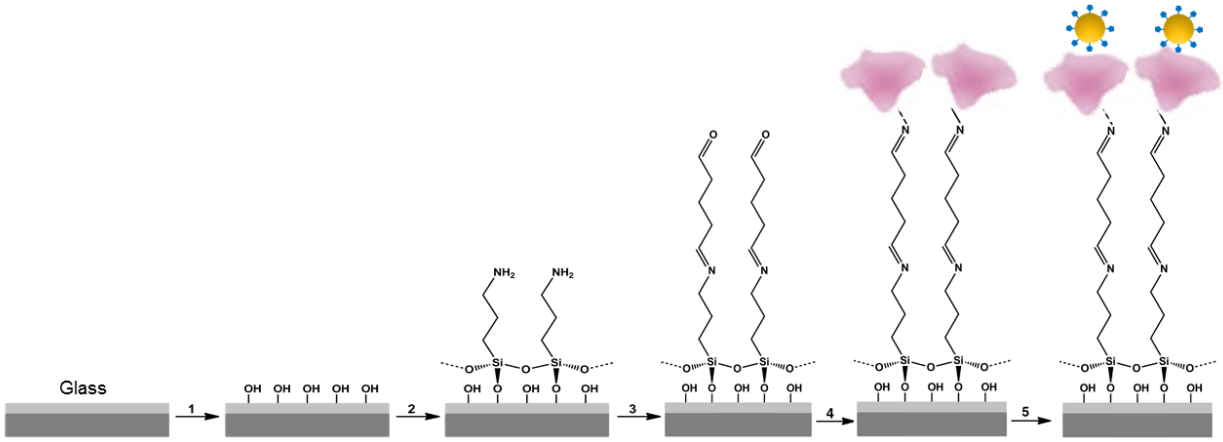

Figure S3. Immobilization procedure of integrin receptors on glass slides. (1) Glass slides were cleaned and oxidized by air plasma. (2) Silanization by APTES. (3) Glutaraldehyde linking. (4) Integrin receptor immobilization. (5) Binding of the immobilized integrin receptors with cRGD-GNPs.

4. Near field TERS vs. far field SERS

Near field TERS mapping of a single SW480 cell incubated with cRGD-GNPs showed Raman bands corresponding to integrin α5β1 and αvβ3 (Figure S4c), while far field SERS mapping didn't show any Raman signal (Figure S4d).

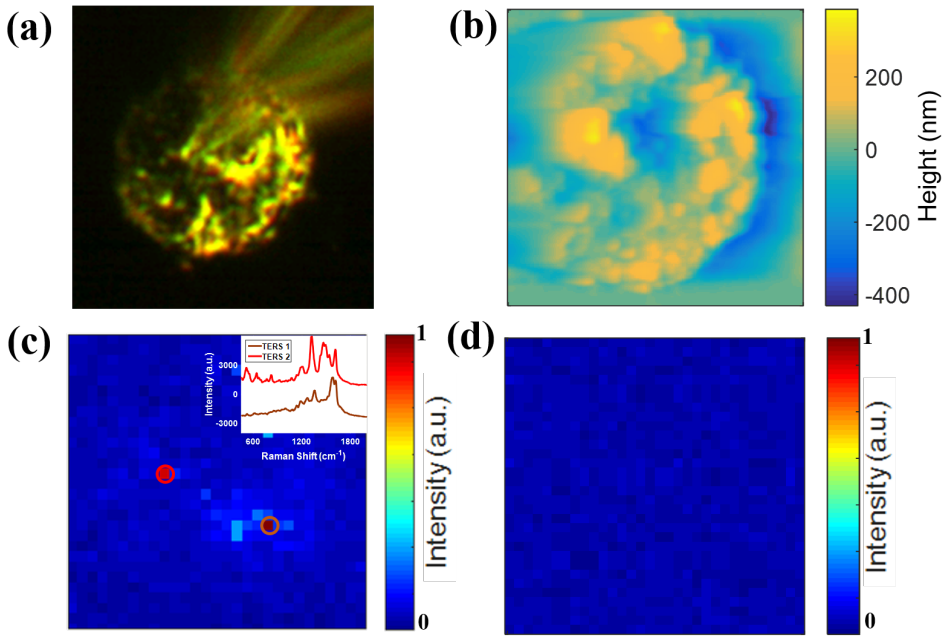

Figure S4. (a) Dark field image of a single SW480 cell. (b-d) Corresponding AFM, near field TERS, and far field SERS images of the same single SW480 cell. Color bar of the AFM image (b) represents height in nanometers. TERS (c) and SERS (d) maps were generated using peak intensity at 1003 cm^{-1}. Inset (c): the spectra of the circled pixels in (c). Scan area (b-d): 9×9 μm^2.

5. SEM imaging of SW480 cells incubated with cRGD-GNPs

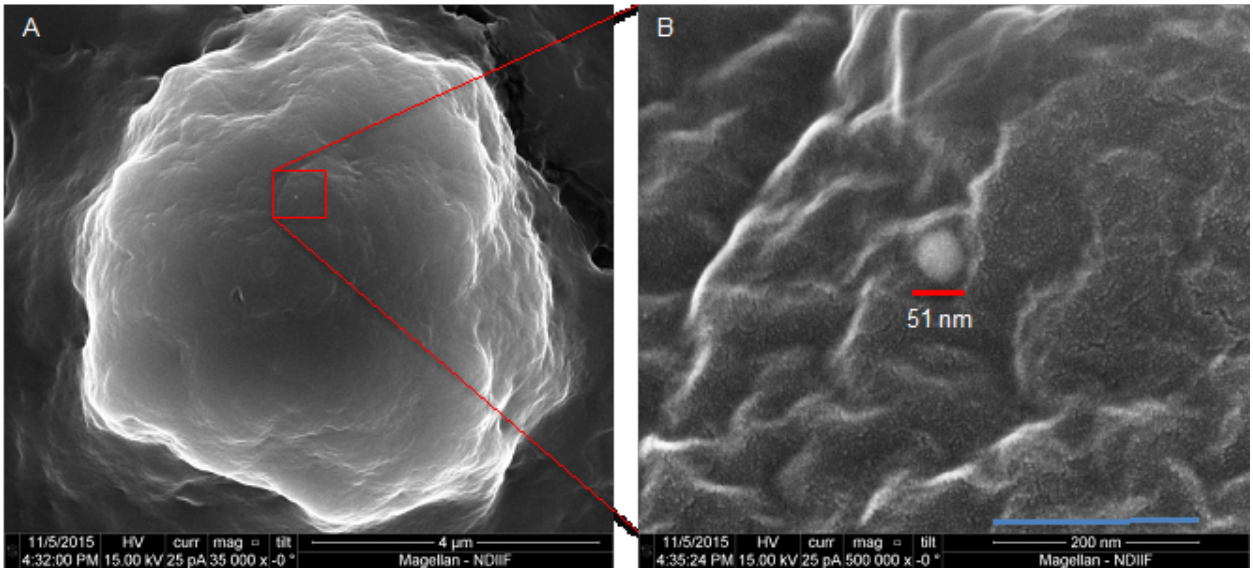

Figure S5. SEM images of SW480 cells incubated with cRGD-GNPs. (a) whole cell; (b) red box area in (a). Nanoparticle size is found to be about 50 nm.

Table S1. LSPR, characteristic Raman band and hydrodynamic diameter of citrate-GNPs and cRGD-GNPs.

Sample	LSPR (nm)	Raman band (cm^{-1})	Dia. (nm)
Citrate-GNPs	531	--	52.57±0.92
cRGD-GNPs	532	1002	49.21±1.52

Table S2. Raman band assignments based on Refs [2, 3].

SERS band (cm^{-1})	TERS band (cm^{-1})	Assignment
-	668	Cys
831, 1261	832 ,1269	Tyr
852, 1351	1304, 1349	Ala, Leu
980, 1323	-	Arg
995	991	Thr
1002, 1210, 1603	1003, 1211, 1580	Phe
1054	-	C-O, C-N stretching
1076, 1143	1144	Lys
1121, 1251	-	Asp
1167, 1290, 1452	1285, 1453~1457	C-H bending
-	1525	C=C vibration
1546	1545, 1560	Trp
1562	1560-1600	Amide

REFERENCES

(1) Wang, H.; Schultz, Z. D. *ChemPhysChem.* **2014**, *15*, 3944-3949.

(2) Movasaghi, Z.; Rehman, S.; Rehman, I. U. *Appl.Spectrosc.Rev.* **2007**, *42*, 493-541.

(3) Zhu, G.; Zhu, X.; Fan, Q.; Wan, X. *Spectrochim.Acta.A.* **2011**, *78*, 1187-1195.