Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Information

Label-Free C-Reactive Protein SERS Detection with Silver Nanoparticle Aggregates

Hyunmin Kim[1,*], Eunjoo Kim[1], Eunsook Choi[1], Chul Su Baek[1], Bokyung Song[2], Chang-Hee Cho[2,*], Sang Won Jeong[1]

[1]Division of Nano·Energy Convergence Research, Daegu Gyeongbuk Institute of Science and Technology, Daegu 711-873, Korea.

[2]Department of Emerging Materials Science, Daegu Gyeongbuk Institute of Science and Technology, Daegu 711-873, Korea.

***Corresponding authors:**

Dr. Hyunmin Kim and Prof. Chang-Hee Cho

email: hyunmin.kim@dgist.ac.kr, chcho@dgist.ac.kr

Synthesis of 11-azidoundecane-1-thiol

11-Bromo-1-undecanol (1 g, 3.98 mmol), sodium azide (285 mg, 4.38 mmol) and potassium iodide were dissolved in ethanol, and refluxed for 20 h. The solvent was removed under reduced pressure, and the residue was dissolved in diethyl ether. The mixture was washed with water, dried over anhydrous magnesium sulfate, and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel (R_f = 0.3, hexane:EtOAc=5:1) to give **1** (868 mg, 102.2%). IR: 3332, 2924, 2853, 2091; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.57 (t, 2H, OCH_2), 3.32 (s, 1H, OH), 3.25 (t, 2H, N_3CH_2), 1.57 (m, 4H, $HOCH_2CH_2(CH_2)_7CH_2$), 1.28 (m, 14H, $HOCH_2CH_2(CH_2)_7$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 62.4, 51.4, 32.6, 29.5, 29.4, 29.1, 28.8, 26.7, 25.7; Exact mass calcd for $C_{11}H_{23}N_3O$: 213.18, found: 236 $[M+Na]^+$.

Compound **1** (868 mg, 4.09 mmol), methanesulfonyl chloride (1.26 g, 11.0 mmol) and triethylamine (2.44 g, 24.1 mmol) were dissolved in THF. The reaction mixture was stirred for 2 h at room temperature. After the addition of ice-cold water, the organic phase was separated from the aqueous phase, and the aqueous phase was extracted twice with diethyl ether. Next, the organic phase was washed with 1 M HCl, deionized water, and saturated sodium bicarbonate. After drying over anhydrous magnesium sulfate, the solvent of organic phase was removed under reduced pressure, and the crude product was purified by column chromatography on silica gel (R_f = 0.4, hexane:EtOAc=5:1) to give **2** (1.17g, 98.2%). IR: 2925, 2854, 2092, 1180; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.21 (t, 2H, OCH_2), 3.26 (t. CH_2N_3), 3.00 (s, 3H, CH_3S), 1.74 (m, 2H, OCH_2CH_2), 1.59(m, 2H, $CH_2CH_2N_3$) 1.39-1.18 (m, 14H, $OCH_2CH_2(CH_2)_7$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 70.4, 51.4, 37.1, 29.4, 29.3, 29.11, 29.10, 29.0, 28.8, 26.7, 25.4; Exact mass calcd for $C_{12}H_{25}N_3O_3S$: 291.16, found: 314 $[M+Na]^+$.

Compound **2** (1.17 mg, 4.01 mmol) and potassium thioacetate (917 mg, 8.03 mmol) were dissolved in 90 mL of DMF. The reaction mixture was stirred for 1 h at room temperature. The solvent was removed under reduced pressure, and the residue was dissolved in diethyl ether. The organic phase was washed by

water, dried over anhydrous magnesium sulfate, and the solvent was removed under reduced pressure, and the crude product was purified by column chromatography on silica gel (R_f=0.7, hexane:EtOAc=9:1) to give **3** (831 mg, 76.4%). IR: 2924, 2853, 2092, 1690; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.25 (t, 2H, CH_2N_3), 2.85 (t, 2H, SCH_2), 2.31 (s, 3H, CH_2CO), 1.57 (m, 4H, $SCH_2CH_2(CH_2)_7CH_2$), 1.35-1.27 (m, 14H, $SCH_2CH_2(CH_2)_7$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.8, 51.4, 30.6, 29.5, 29.4, 29.15, 29.11, 28.86, 28.81, 26.7; Exact mass calcd for $C_{13}H_{25}N_3OS$: 271.17, found: 294 $[M+Na]^+$.

Compound **3** (588 mg, 2.17 mmol) was dissolved in 40 mL of methanol and 2 mL of concentrated HCl, and the reaction mixture was stirred for 3 h. The reaction mixture was quenched with water and the aqueous phase was extracted twice with diethyl ether. The organic phase was washed with water and dried over anhydrous magnesium sulfate. The solvent of the organic phase was removed under reduced pressure, and the crude product was purified by column chromatography on silica gel (R_f = 0.8, hexane:EtOAc=9:1) to give **4** (471 mg, 94.6%). IR: 2923, 2852, 2090; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.25 (t, 2H, CH_2N_3), 2.51 (q, 2H, SCH_2), 1.60 (m, 4H, $SCH_2CH_2(CH_2)_7CH_2$), 1.35-1.28 (m, 15H, $HSCH_2CH_2(CH_2)_7$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 51.4, 34.1, 29.5, 29.1, 29.0, 29.8, 28.4, 26.7, 24.6.

Synthesis of 6-Propargylhexylphosphorylcholine (propargyl-PC)

A solution of 1,6-hexanediol (3 g, 25.4 mmol) in DMF (20 mL) was added drop-wise into a suspension of sodium hydride (1.52 g, 38.1 mmol) in DMF (20 mL) in an ice bath, and stirred for 30 min. Propargyl bromide (5.7 g, 38.1 mmol) in DMF (20 mL) was added to the mixture and then stirred for 20 h at room temperature. The solvent was removed under reduced pressure, and the crude product was dissolved in diethyl ether. The mixture was washed by water, dried over anhydrous magnesium sulfate, and purified by column chromatography on silica gel (R_f= 0.5, hexane/EtOAc=1:1) to give **5** (1.80 g, 45.4%). IR: 3373, 3292, 2933, 2858, 1093; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.13(s, 2H, HCC$CH2$O), 3.59 (t, 2H, CH_2OH), 3.52 (t, 2H, CH_2OCH_2), 3.03 (s, 1H, CH_2OH), 2.48 (s, 1H, $HCCCH_2O$), 1.58 (m, 4H,

$OCH_2CH_2CH_2CH_2CH_2$), 1.38 (m, 4H, $OCH_2CH_2CH_2CH_2CH_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 79.8, 74.3, 70.0, 62.3, 57.9, 32.5, 29.3, 25.8, 25.5; Exact mass calcd for $C_9H_{16}O_2$: 156.12, found: 179 $[M+Na]^+$.

Compound **5** (600 mg, 3.84 mmol), 2-chloro-1,3,2-dioxaphospholane 2-oxide (1.09 g, 7.68 mmol) and triethylamine (777 mg, 7.68 mmol) were dissolved in 30 mL DCM. The reaction mixture was stirred for 72 h at room temperature in the dark. The solvent was removed under reduced pressure, and the crude product was purified by column chromatography on silica gel (R_f = 0.3, hexane/EtOAc=1:4) to give **6** (700 mg, 69.5%).

Compound **6** (700 mg, 2.66 mmol) and trimethylamine (1.57 g, 26.6 mmol) were dissolved in 8 mL acetonitrile. The reaction mixture was stirred for 20 h at 60°C. The solvent was removed under reduced pressure, and the crude product was purified by column chromatography on silica gel (chloroform:methanol 2:1 and chloroform:methanol:water 50:50:4, R_f= 0.2).

The solvent was removed under reduced pressure and the residue was dissolved in anhydrous chloroform and filtered to give **7** (411 mg, 48.1%). IR: 3350, 3296, 2936, 2860, 1086, 1059; ^1H NMR (400 MHz, CD_3OD) δ 4.28 (m, 2H, $POCH_2CH_2N^+$), 4.15 (d, 2H, $HCCCH_2O$), 3.90 (q, 2H, $CH_2CH_2CH_2OP$), 3.69 (m, 2H, $POCH_2CH_2N^+$), 3.54 (t, 2H, $HCCCH_2OCH_2$), 3.27 (s, 9H, $N^+(CH_3)_3$), 2.85 (t, 1H, *HC*CCH2O), 1.64 (m, 4H, $OCH_2CH_2CH_2CH_2CH_2$), 1.44 (m, 4H, $OCH_2CH_2CH_2CH_2CH_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 79.7, 74.8, 69.6, 65.5, 59.0, 57.4, 53.5, 30.4, 29.2, 25.6, 25.3; Exact mass calcd for $C_{14}H_{28}NO_5P$: 321.17, found: 322 $[M+H]^+$.

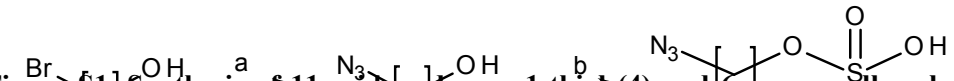
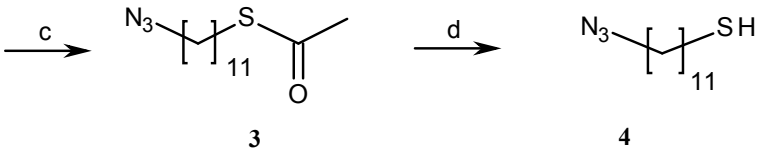
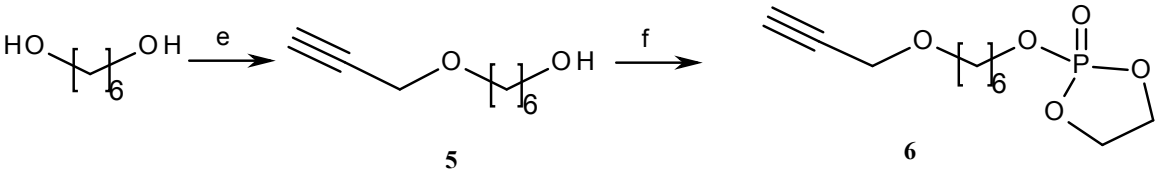


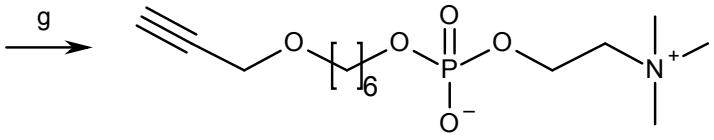
Figure S1 Synthesis of 11-azidoundecane-1-thiol (4) and 6-propargylhexyl phosphorylcholine (7). a-h Reagents and conditions: a, NaN_3, KI, ethanol, reflux; b, CH_3ClO_2S, TEA, THF, rt; c, KSAc, DMF, rt; d, methanol, HCl, rt; e, NaH in DMF at 0°C, and C_3H_3Br in DMF at rt; f, $C_2H_4ClO_3P$, TEA, DCM, rt; g, TEA, CH_3CN, 60 °C; h, NaH in DMF at 0 °C, and C_3H_3Br in DMF at rt.



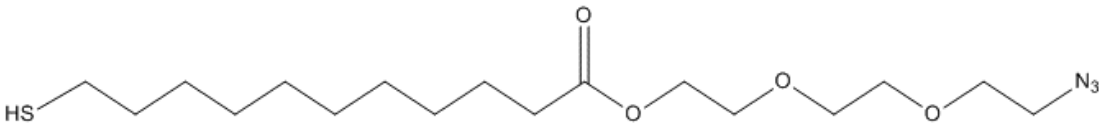
3 **4**



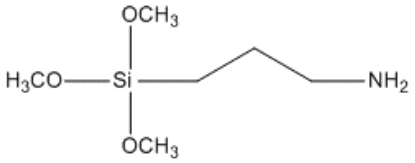
5 **6**



7

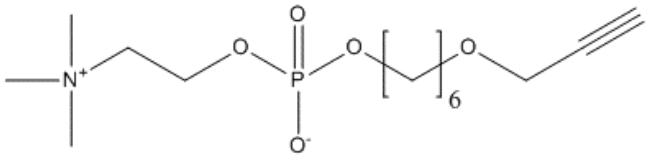


Chemical Formula: $C_{17}H_{33}N_3O_4S$ Thiol-(PEG)-azide
Exact Mass: 375.22
Molecular Weight: 375.53
m/z: 375.22 (100.0%), 376.22 (20.4%), 377.21 (4.5%), 377.23 (1.7%), 377.22 (1.2%)
Elemental Analysis: C, 54.37; H, 8.86; N, 11.19; O, 17.04; S, 8.54



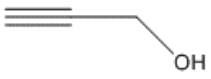
APTMS

Chemical Formula: $C_6H_{17}NO_3Si$
Exact Mass: 179.10
Molecular Weight: 179.29
m/z: 179.10 (100.0%), 180.10 (11.9%), 181.09 (3.4%), 181.10 (1.2%)
Elemental Analysis: C, 40.19; H, 9.56; N, 7.81; O, 26.77; Si, 15.66



Propargyl PC

Chemical Formula: $C_9H_{18}NO_5P$
Exact Mass: 251.09
Molecular Weight: 251.22
m/z: 251.09 (100.0%), 252.10 (10.1%), 253.10 (1.5%)
Elemental Analysis: C, 43.03; H, 7.22; N, 5.58; O, 31.84; P, 12.33



Propargyl alcohol

Chemical Formula: C_3H_4O
Exact Mass: 56.03
Molecular Weight: 56.06
m/z: 56.03 (100.0%), 57.03 (3.3%)
Elemental Analysis: C, 64.27; H, 7.19; O, 28.54

Figure S2. Chemical and structural information regarding the chemical moieties utilized in this work.

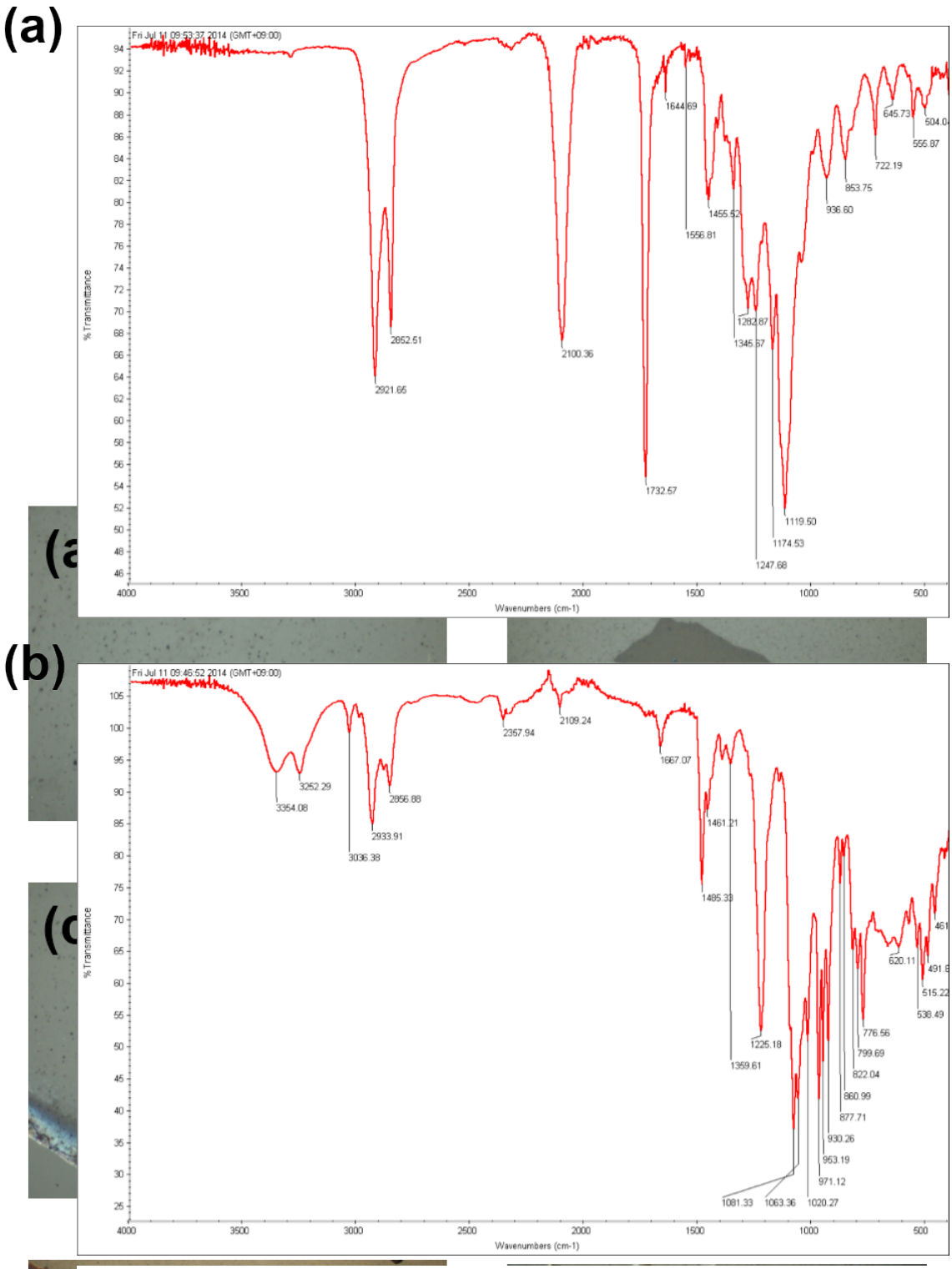


Figure S3. FTIR spectra of (a) Propargyl-$(CH)_6$-PC and (b) HS-(PEG)-N_3.

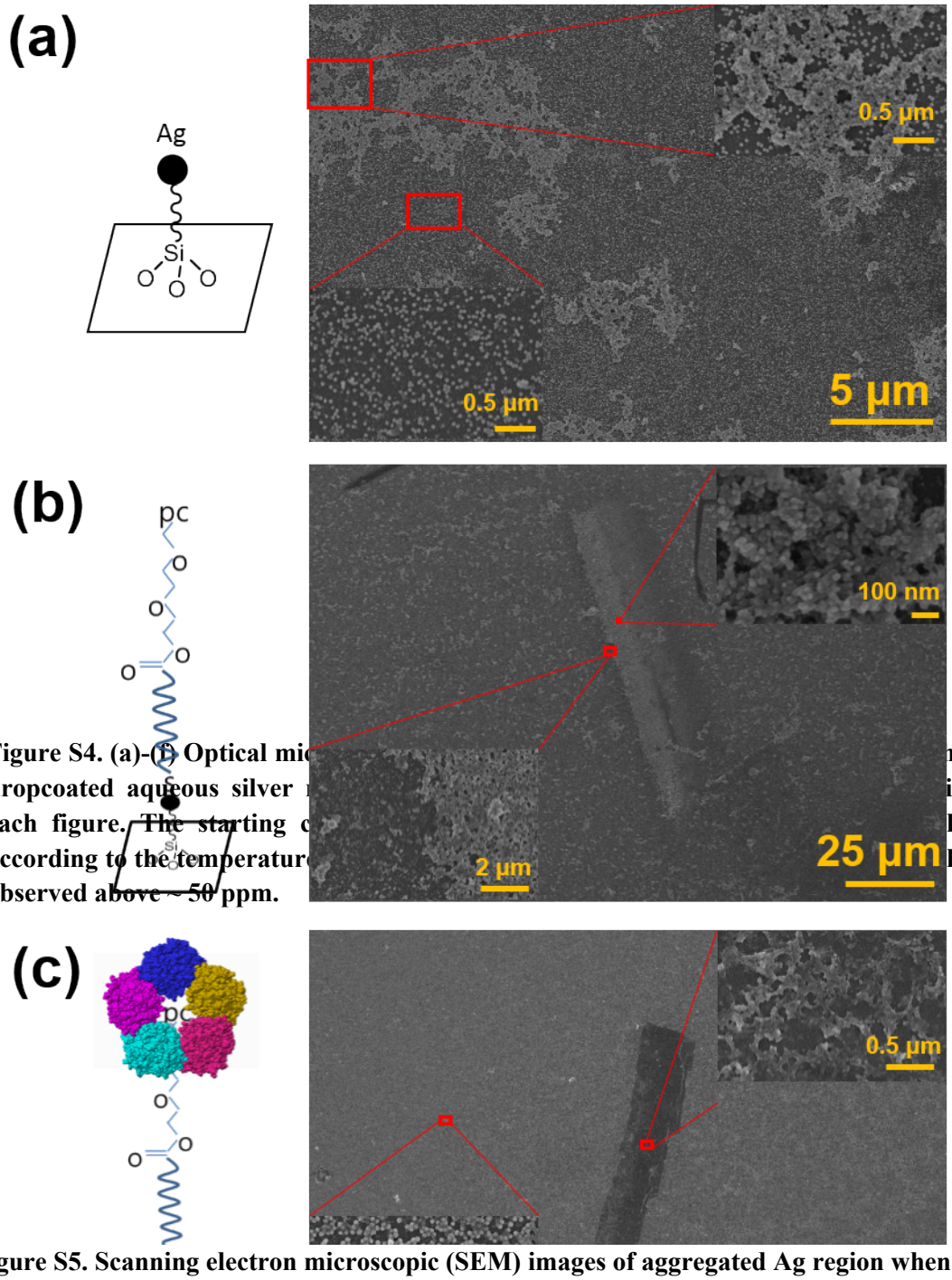


Figure S4. (a)-(f) Optical mic ne dropcoated aqueous silver n in each figure. The starting c ly according to the temperatur ly observed above ~ 50 ppm.

Figure S5. Scanning electron microscopic (SEM) images of aggregated Ag region when the silanized surfaces were treated with (a) Ag nanoparticles only, (b) phosphocholine (PC) and subsequently "clicked' with an azide group, and (c) c-reactive protein (CRP).

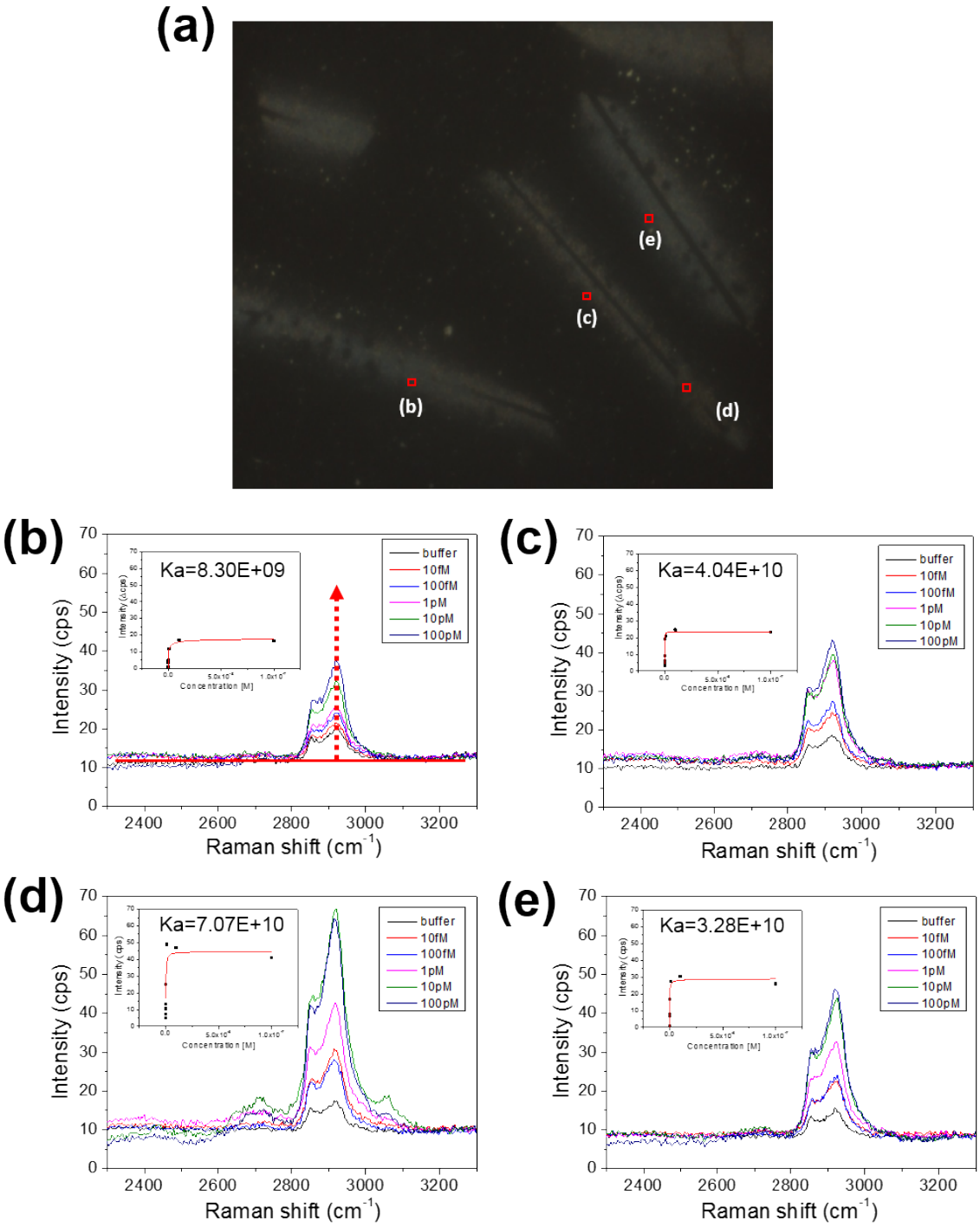


Figure S6. (a) Optical microscopic image of AgNAs. (b), (c), (d), and (e) are high resolution Raman spectra for the marked points in (a). Insets are the binding constants calculated from the traced points at ~ 2930 cm^{-1} (along the dotted arrow) in the Raman spectra.

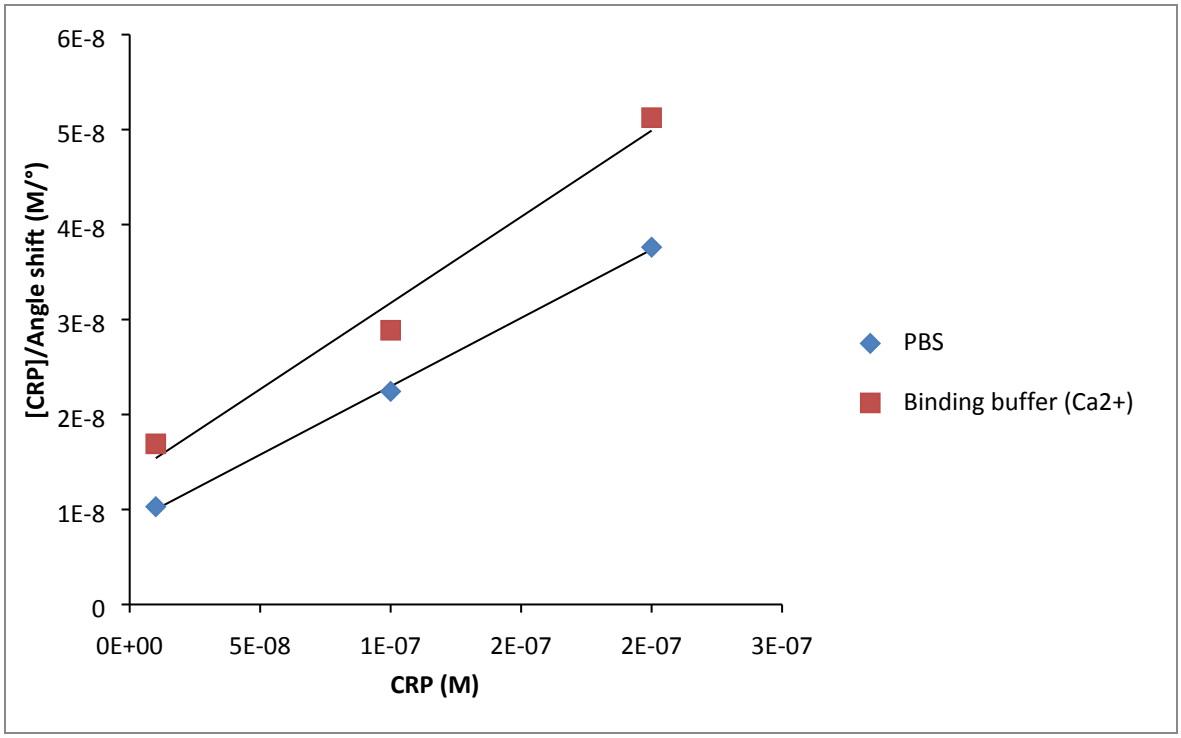


Figure S7. Binding constants of the CRP with regards to the pc-functionalized surface measured by SPR in the phosphate buffer (blue rhombus) and the binding buffer used in this experiment (red rectangle) for various concentrations. The analysis was performed at a flow rate of 20 µL/min and 25 °C. The samples were passed over the SPR chip by injecting of CRP solution (300 µL), starting with 1 pM and increasing the concentration in 10-fold increments until the binding was saturated. Protein binding was recorded by the reflectance change (%) at a fixed angle.

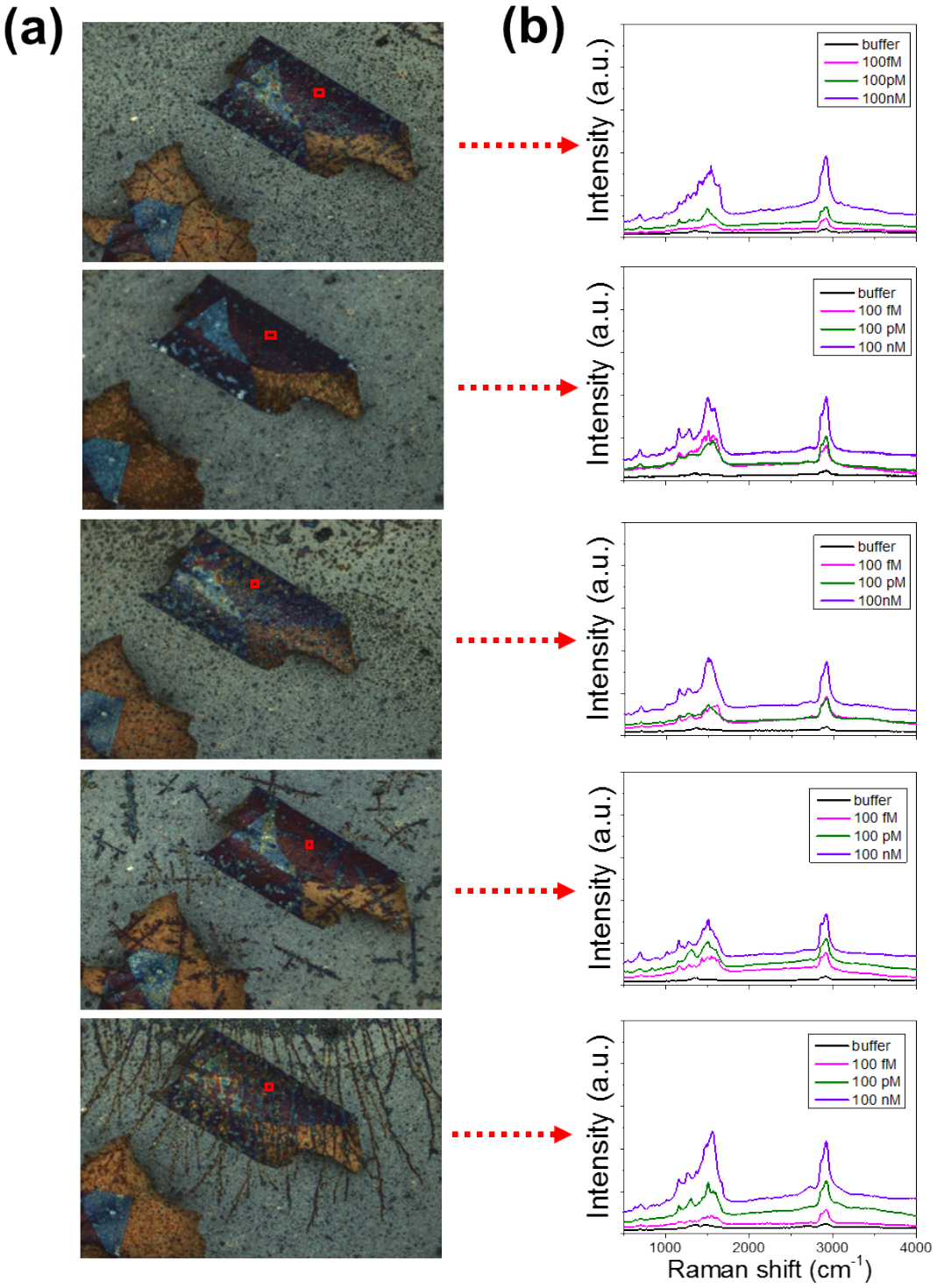


Figure S8. Reproducibility of SERS experiments. (a) A serial optical microscopic image of AgNAs after CRP solution was blown off with N_2 gun. Remnant salts exhibited various morphologies. (b) Raman spectroscopy on CRP reaction test after images were taken on red square marker in (a). Background correction was not performed.

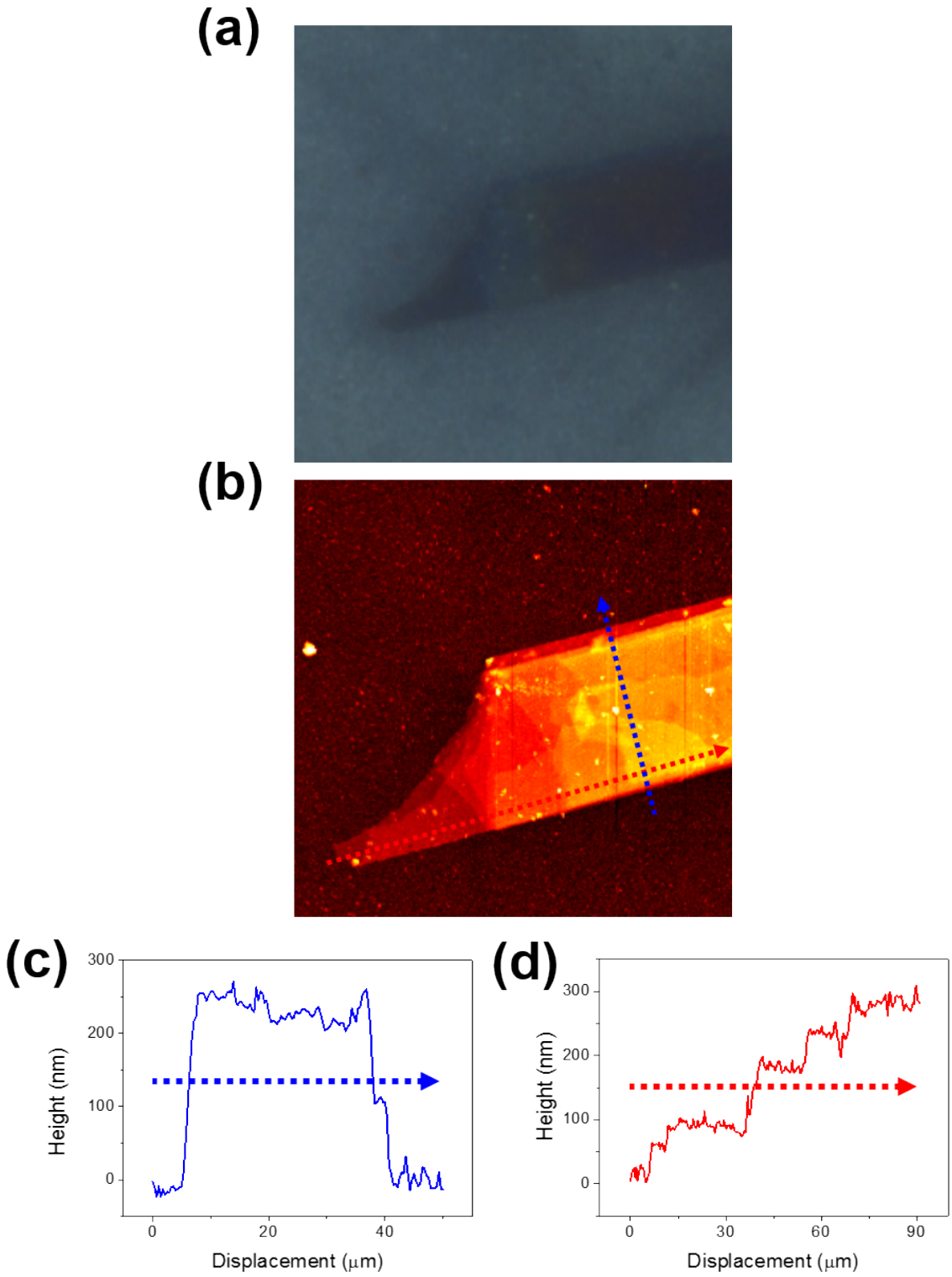


Figure S9. Correlation between (a) optical and (b) AFM image for an arbitrary AgNA. AFM height profile scanned along (c) blue and (d) red arrows, respectively.

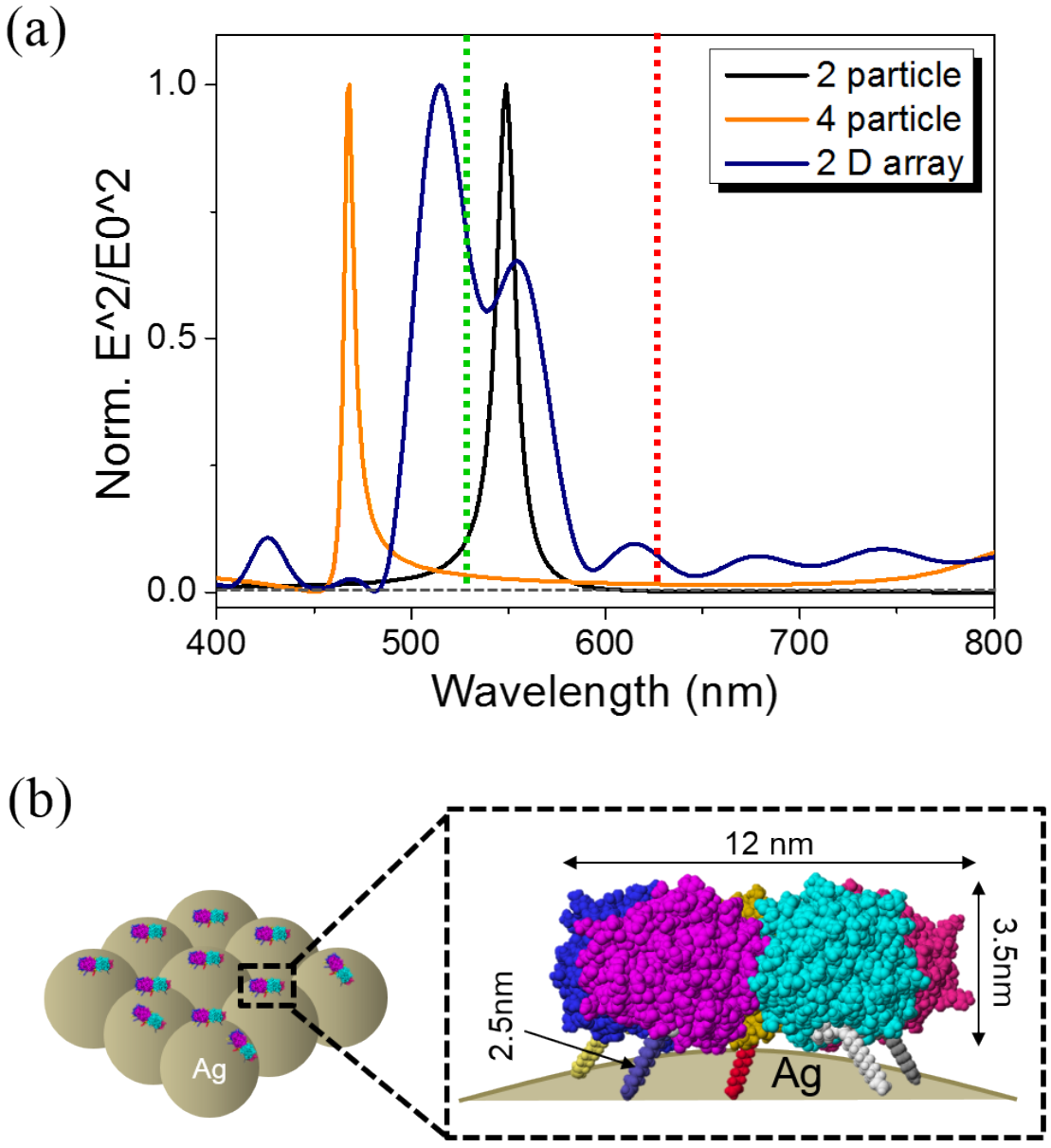


Figure S10. (a) Spectral dependence of the normalized relative light absorption with regards to attached substrate as for a few array conditions. Green dotted line: 532 nm, Red dotted line: 630 nm. (b) Illustration of the CRP tethered to AgNA with being PC as *the bridge*. The magnified image of one CPR in the dashed square box shows detailed geometrical information of the CRP.