Supporting Information

# Orientation and Density Control of Bispecific Anti-HER2 Antibody on Functionalized Carbon Nanotubes for Amplifying Effective Binding Reactivity to Cancer Cells

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## List of the Contents of the Supporting Information

**Materials**

The chemical compounds such as CoMoCAT SWNT, 10kD dextran, 1,2-Epoxy-3-phenoxypropane, and 4-cotininecrboxylic acid, (Benzotrialzole-1-yloxy) tri (dimethylamihno) phosphonium, hexafluorophosphate (BOP), 4-(Dimethylamino)pyridine (DMAP) were purchased from Sigma Aldrich. All organic solvents were purchased from Daejung Chemical of Korea. A cellulose dialysis membrane (MWCO 8 kDa) was purchased from SPECTRUMLABS.COM. Amicon tube (MWCO 100 kDa, 4mL) was purchased from Millipore Inc. and the centrifugal filter device (MWCO 300 kDa) from Pall Life Sciences. A sensor chip, CM5, was purchased from GE Healthcare Life Sciences. The antigen, HER-2 Fc, immobilized on the CM5 was purchased from R&D Systems. All of bio reagents were purchased from Gibco® and a mounting solution was obtained from Prolong®Gold antifade reagents, Invitrogen.

**Synthesis of carboxymetylated phenoxy dextran (CM-PhO-dex)**

A 2 g portion of PhO-dex was dissolved in a 40 mL of 6M-NaOH solution in an ice bath, followed by addition of a 1.8 g portion of 2-chloroacetic acid. After the reaction proceeded for 1 h at 60 $^{o}$C, methanol was slowly added into the reaction mixture to precipitate the desired product. The obtained product was purified by washing it with methanol several times, and dried under high vacuum (40% yield).

$^{1}$H NMR (400 MHz, $D_2O$): δ (ppm) 3.479 (m, 1H), 3.513 (m, 1H), 3.678 (m, 1H), 3.856 (m, 1H), 3.939 (m, 2H), 4.115(m, 2H), 4.931(m, 1H), 7.006 (m, 1H), 7.333 (m, 1H), and 7.348 (m, 1H).

$^{13}$C NMR (100 MHz, $D_2O$): δ (ppm) 65.43, 68.77, 69.44, 70.11, 71.34, 71.75, 72.49, 73.33, 97.64, 114.81, 121.56, 129.89, 158.17, and 177.78.

**$^{1}$H NMR and $^{13}$C NMR results of PhO-dex:**

$^{1}$H NMR (400 MHz, $D_2O$): δ (ppm) 3.479 (m, 1H), 3.539 (m, 1H), 3.698 (m, 1H), 3.862 (m, 1H), 3.933 (m, 2H), 4.934 (s, 1H), 4.934 (s, 1H), 7.027 (m, 1H), 7.352 (m, 1H).

$^{13}$C NMR ( 100 MHz, $D_2O$): δ (ppm) 16.76, 57.40, 60.43, 65.49, 68.84, 69.49, 70.14, 71.37, 71.78, 73.05, 73.36, 97.66, 114.85, 121.59, and 129.92.

**$^{1}$H NMR and $^{13}$C NMR results of 1-Cot-PhO-dex:**

$^{1}$H NMR ( 400 MHz, DMSO-$d_6$): δ (ppm) 3.211 (m, 1H), 3.333 (m, 1H), 3.455 (m, 1H), 3.625 (m, 1H), 3.749 (m, 2H), 4.499 (m, 1H), 4.670 (s, 1H), 4.852 (m, 1H), 4.915 (m, 1H), 6.92 (m, 1H), 7.274 (m, 1H), 7.452 ( s, 1H), 7.754 (m, 1H), 8.584 (m, 1H).

$^{13}$C NMR ( 100 MHz, DMSO-$d_6$): δ (ppm) 66.11, 70.127, 70.407, 71.873, 73.354, 98.251, 114.534, 120.478, 129.502, and 158.694

**$^{1}$H NMR and $^{13}$C NMR results of 3-Cot-PhO-dex:**

$^{1}$H NMR ( 400 MHz, DMSO-$d_6$): δ (ppm) 3.211 (m, 1H), 3.333 (m, 1H), 3.455 (m, 1H), 3.625 (m, 1H), 3.749 (m, 2H), 4.499 (m, 1H), 4.670 (s, 1H), 4.852 (m, 1H), 4.915 (m, 1H), 6.92 (m, 1H), 7.274 (m, 1H), 7.452 ( s, 1H), 7.754 (m, 1H), and 8.584 (m, 1H).

$^{13}$C NMR ( 100 MHz, DMSO-$d_6$): δ (ppm) 18.60, 27.28, 30.76, 56.13, 63.40, 66.08, 70.16, 70.45, 71.95, 73.42, 98.27, 114.56, 120.51, 124.14, 129.54, 134.88, 136.04, 148.84, 149.64, 158.74, 171.83, 172.42, 187.16, and 206.71.

**Synthesis of the SWNT bioconjugate prepared by EDC coupling.**

A 5 mg portion of SWNT was added into the aqueous solution containing a 50 mg portion of CM-PhO-dex, and then the resulting mixture was sonicated for 1.5 h in an ice bath using a probe tip sonicator (10-11 Watts). After centrifugation for 1.5 h at 15000 x g, the supernatent was obtained to get the CM-PhO-dex functionalized SWNT. In order to randomly conjugate the bispecific tandem antibody to the SWNT, a 1.96 mg of EDC and a 1.7 mg of NHS were added to 0.5 mL of CM-PhO-dex functionalized SWNT solution (7.6 mg $mL^{-1}$) to activate the carboxyl groups of the SWNT. After the reaction proceeded for 45 min at 25 $^{o}$C, the excessed reagents were washed out by filtration using a centrifugal filter device (MWCO 100 kDa, Amicon® Ultra-4 of Milipore Inc.). Then, a 44 μL porton of bispecific tandem antibody (570 μg $mL^{-1}$) was added to the solution of acticated CM-PhO-dex/SWNT (800 μg $mL^{-1}$) for the random conjugation of an antibody. After reaction for 4 h at 25 $^{o}$C, the 1.07 mg of ethanolamin was added to the reaction solution to deactivate the unreacted carboxyl-NHS ester. The unbound antibody and ethanolamin were removed from the SWNT bioconjugate solution by filtration using a centrifugal filter device (Pall Life Sciences, Nanosep® Centrifugal Devices, MWCO 300 kDa ) at 5,000 x g.

**Raman Instrument for the detection of cancer cells with SNAs**

Raman measurement was conducted using a confocal microscope Raman system (Alpha 300 R+, WITec, Germany). In the micro Raman system, the Raman system is equipped with a diode laser emitting at 633 nm. The diffraction grating limits the spectral range to 3000-240 $cm^{-1}$ with a spectral resolution of 2 $cm^{-1}$. The maximal output power of the diode laser at the source is 10 mW. Raman scattering signals were collected in a back-scattering geometry and detected using a spectrometer equipped with a thermo-electrically cooled (−60 $^{o}$C) EMCCD detector.

**NIR fluorescence spectrophotometer**

The SNAs in aqueous solution were excited at 632 nm for 5 sec using a light source (Xenon lamp) in a 1 cm optical path length quartz at room temperature and their nIR fluorescence was collected by an InGaAs detector cooled to 103 $^{o}$C using liquid nitrogen in a fluorescence spectrophotometer (Nano Logs, HORIBA) .

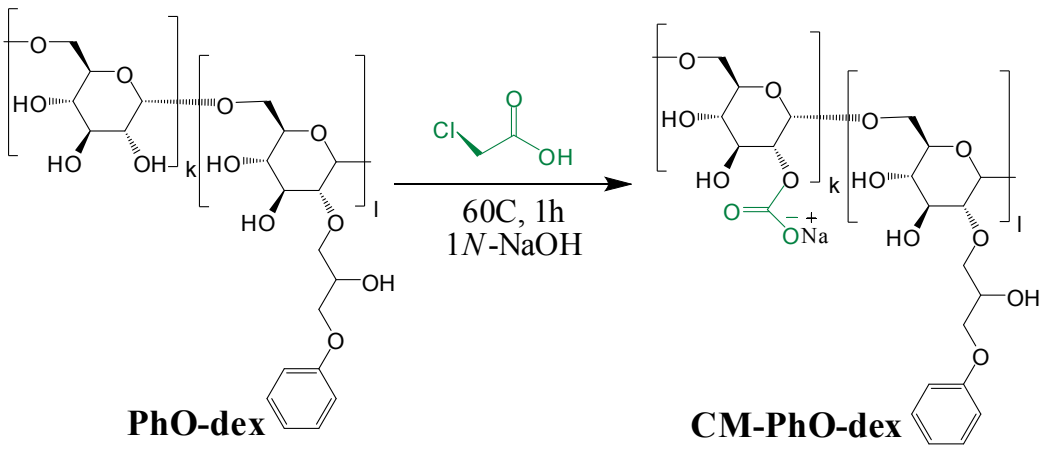
**Quantification of the bispecific tandem antibody bound to the SWNTs.**

After synthesis of SNAs, the unbound bispecific tandem antibody was filtered out using a centrifugal filter device (MWCO 300 kDa). Then, the filtrate was quantified by using a bicinchoninic adid (BCA) protein assay kit (Thermo Fisher Scientific Inc.). A 2 mg mL$^{-1}$ of IgG antibody was diluted to a specific range of 0 to 2 mg mL$^{-1}$, and the BCA Working Reagent(WR) was prepared by mixing 2.4 mL of reagent A with 48 μL of reagent B. A 100 μL of WR was added into a 96-wall plates containing 100 μL of the filtrate and diluted IgG antibody. The 96-wall plate was incubated at 60 $^{o}$C for 1 h, and then the absorbance of each sample was measured at 562 nm. A calibration curve was constructed based on the absorbance for the known concentrations of IgG antibody.

## Supplementary Figures:

***Table S1.*** Elemental analysis of Cot-PhO-dex polymers

| The added amount of Cot (mmol) | Nitrogen Contents in Cot-Phe-dex (wt%) | Content of Cotinine in Cot-Pho-dex (mmol/g) | Ratio of Cot to Glucose |
|---|---|---|---|
| **0.6** | **0.7** | **0.29** | **1 / 17** |
| **1.9** | **1.9** | **0.66** | **2.8 / 17** |
| **3.1** | **3.0** | **0.73** | **4.3 / 17** |




***Scheme S1.*** Synthesis of carboxylmethyl-PhO-dex (CM-PhO-dex).

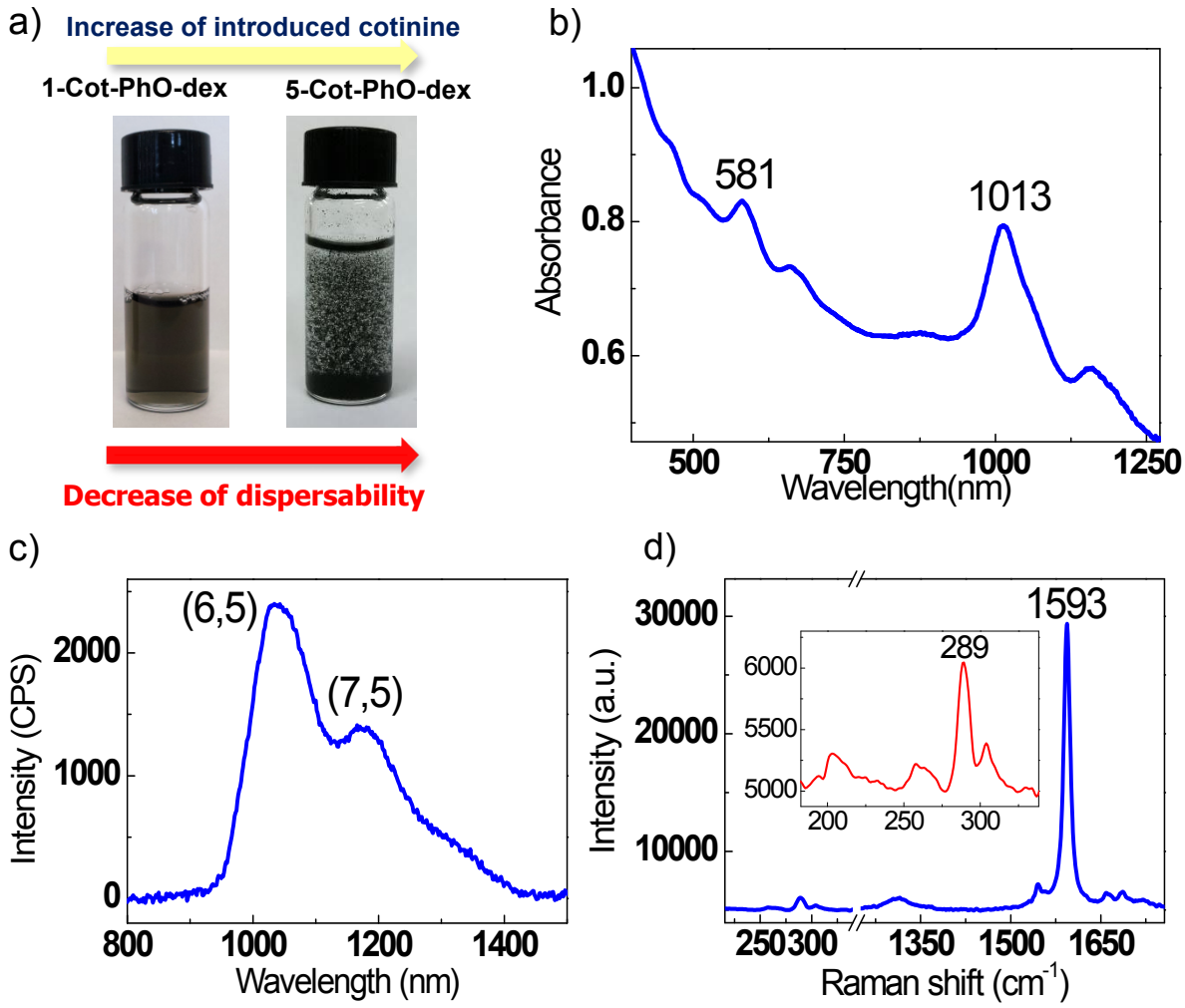



***Figure S1***. Analysis of the Cot-PhO-dex functionalized SWNTs. (a) Photographs of the SWNTs functionalized with 1-Cot-PhO-dex and 5-Cot-PhO-dex. (b) Absorption spectrum of the SWNT functionalized with 3-Cot-PhO-dex. (c) nIR fluorescence of the SWNT functionalized with 3-Cot-PhO-dex. (d) Raman spectrum of the SWNT functionalized with 3-Cot-PhO-dex. The inset is the Raman spectrum for the RBM-mode of SWNT.

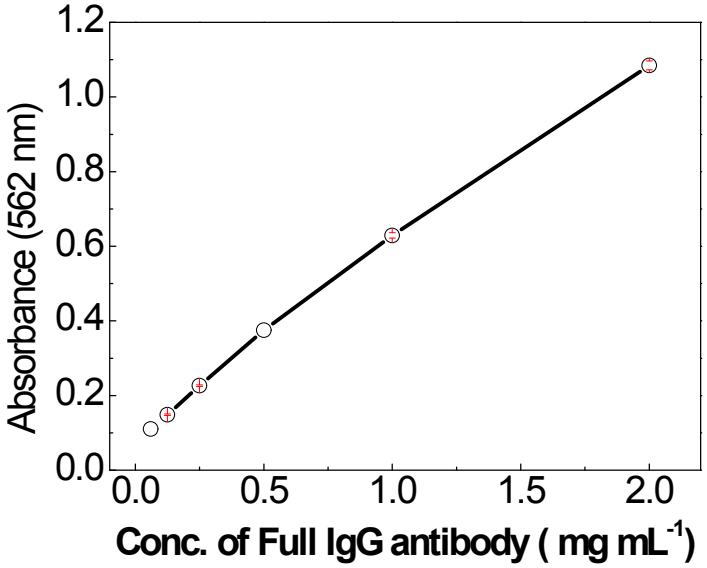



***Figure S2.*** A calibration curve based on a known concentration of Full IgG antibody.

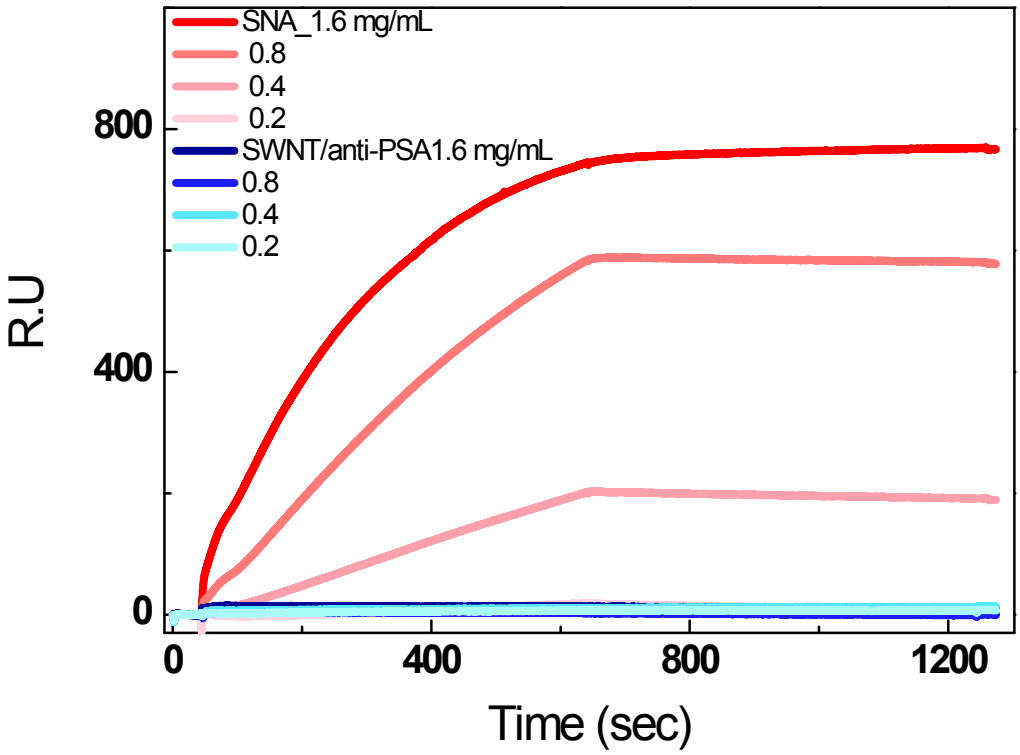



***Figure S3.*** SPR sensorgrams for the binding of SNA-3 and SWNT/anti-PSA to HER2.

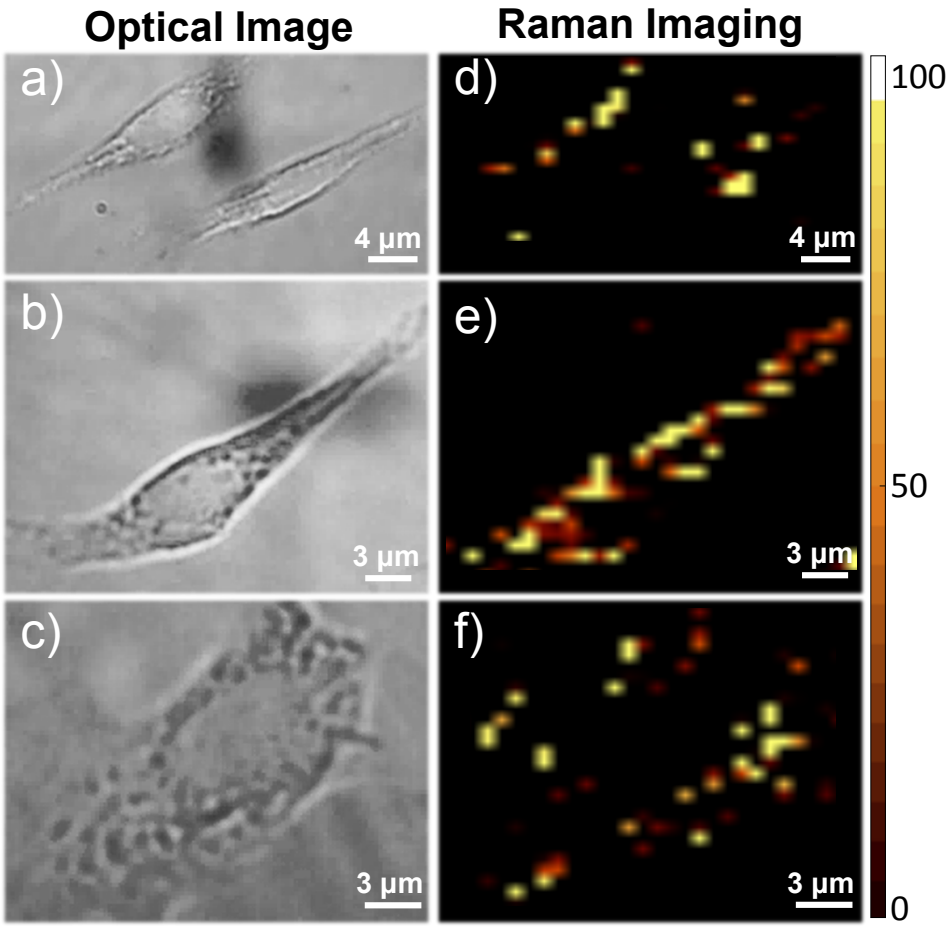



***Figure S4.*** Selective detection of HER2-overexpressing cancer cells, SK-OV-3, with SNA-3. (a)-(c) Optical images (left column) and Raman imaging (right column) of SK-OV-3 cells.

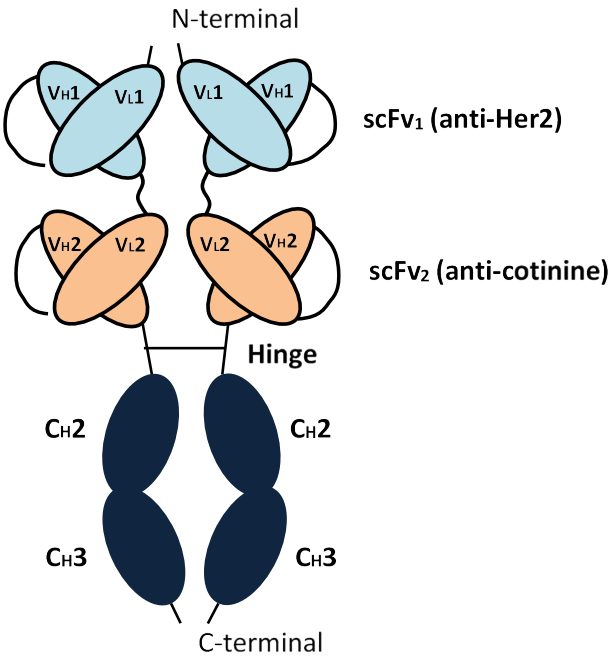



***Figure S5***. Structure of bispecific anti-HER2 × cotinine tandem scFv Fc fusion protein.