Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

A Novel Near-infrared Fluorescence Chemosensor for Copper Ion Detection Using Click Chemistry and Energy Transfer

Aeju Lee[a], *Jungwook Chin*[b], *Ok Kyun Park*[c], *Hyunjin Chung*[d], *Jin Won Kim*[d], *Soo-Young Yoon*[a], *Kyeongsoon Park*[c,*]

[a]Department of Laboratory Medicine, College of Medicine, Korea University, Seoul 152-703, Korea, [b]Center for Marine Natural Products and Drug Discovery, School of Earth and Environmental Sciences, Seoul National University, NS-80, Seoul 151-747, South Korea, [c]Division of Bioimaging, Chuncheon Center, Korea Basic Science Institute, 192-1, Hyoja 2-dong, Chuncheon, Gangwon-do 200-701, Korea, [d]Multimodal Imaging and Theranostic Lab, Cardiovascular Center, Korea University Guro Hospital, 97, Guro-dong, Guro-gu, Seoul 152-703, Korea

Materials

Cy5.5-NHS ester, as a near-infrared (NIR) fluorophore, and BHQ3-NHS ester, as a dark quencher, were purchased from Lumiprobe Corporation (Hallandale Beach, FL) and Biosearch Technologies, Inc. (Novato, CA), respectively. Amine-PEG-azido, propargylamine, dichloromethane, all metal ions (i.e., $LiNO_3$, $CaCl_2$, NiCl, $HgCl_2$, $MgCl_2$, KCl, NaCl, $PdCl_2$, $CoCl_2$, $MnCl_2$, $CdCl_2$, $FeCl_2$, AgCl, and $CuSO_4$), L-cysteine, *N*-acetylcysteine, caffeic acid, tetraethylenepentamine, triethylenetetramine hydrate, ethylenediaminetetraacetic acid, and penicillamine were purchased from Sigma (St. Louis, MO). All reagents were of analytical grade and used without further purification.

Synthesis of azide-functionalized PEG-Cy5.5 (azido-PEG-Cy5.5)

Azido-PEG-Cy5.5 was synthesized as shown in Figure S1. In brief, Cy5.5-NHS ester (11 mg, 1.52×10^{-5} mol) and amine-PEG-azido (40 mg, 7.6×10^{-5} mol) were dissolved in 2 mL of dichloromethane and stirred in the dark overnight. The solvent was then completely removed from the reaction mixture via rotary evaporation. The product was purified using reverse-phase high-performance liquid chromatography (RP-HPLC, Agilent Technologies 1260 series HPLC, Wilmington, DE). The product was obtained using RP-HPLC with a Phenomenex Hydro-RP C18 column (250 × 10 mm, 4 μm, 80 Å, 2.0 mL/min, UV detection at 210 nm). The purification conditions were as follows: Solvent A = acetonitrile; solvent B = 0.1% trifluoroacetic acid (TFA);

gradient: from 40% A + 60% B to 50% A + 50% B over the first 10 min then to 80% A + 20% B over the next 30 min and further to 100% A for 10 min. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Bruker DPX 400 MHz spectrometer using CD_3Cl as the solvent with tetramethylsilane (TMS) as the internal standard. The product mass was determined to be 1133.7 using a MALDI-TOF mass spectrometer (Voyager-DE™ STR Biospectrometry Workstation, Applied Biosystems, Carlsbad, CA).

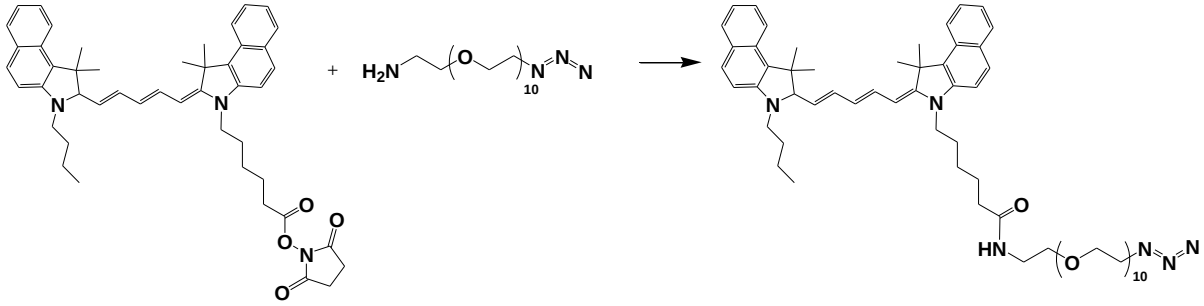

Figure S1. Synthesis of azide-functionalized PEG-Cy5.5 (azido-PEG-Cy5.5).

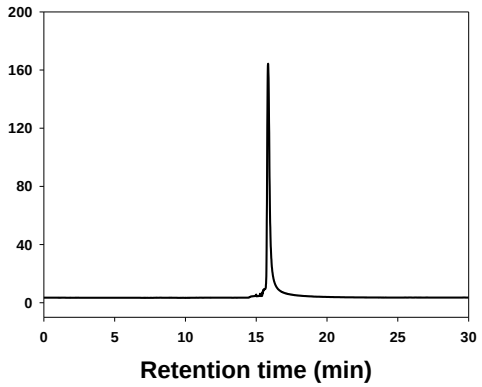

Figure S2. HPLC profile of azido-PEG-Cy5.5 (ex: 675 nm, em: 690 nm).

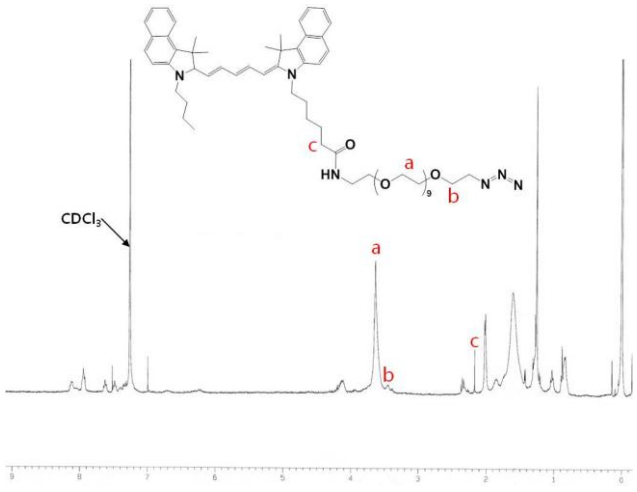

Figure S3. ^{1}H NMR spectrum of azido-PEG-Cy5.5.

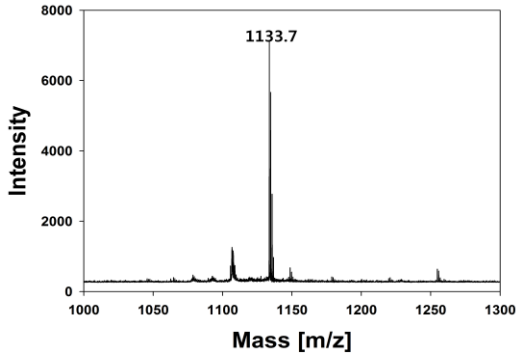

Figure S4. MALDI-TOF mass spectrum of azido-PEG-Cy5.5.

Synthesis of alkyne-functionalized BHQ3 (alkyne-BHQ3)

Alkyne-BHQ3 was synthesized as follows: Briefly, BHQ3-NHS ester (11 mg, 1.39×10^{-5} mol) was dissolved in 1.5 mL of dichloromethane followed by the addition of propargylamine (8.9 μL, 2.79×10^{-4} mol); the mixture was then stirred in the dark overnight. The reaction mixture was completely dried by rotary evaporation and the product was purified with RP-HPLC using the same conditions as for azido-PEG-Cy5.5. ^{1}H NMR spectra were recorded on a Bruker DPX 400 MHz spectrometer using D_2O as the solvent. The molecular weight of the product determined via MALDI-TOF mass spectrometry was 584.3.

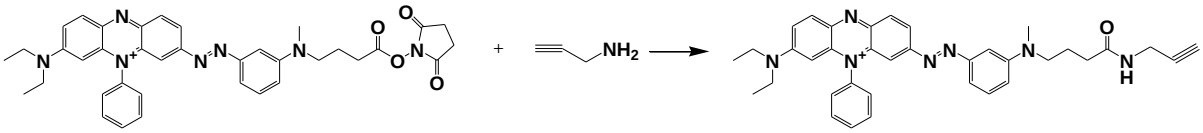

Figure S5. Synthesis of alkyne-functionalized BHQ3 (alkyne-BHQ3).

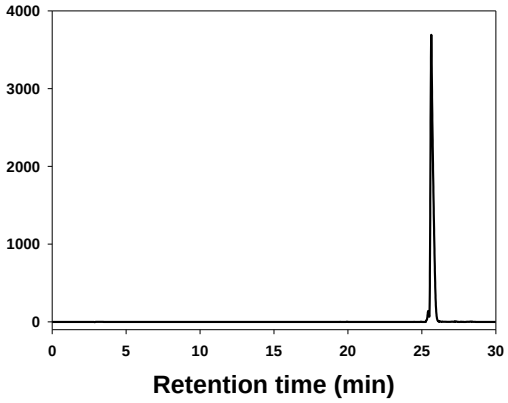

Figure S6. HPLC profile of alkyne-BHQ3 (Absorbance at 600 nm).

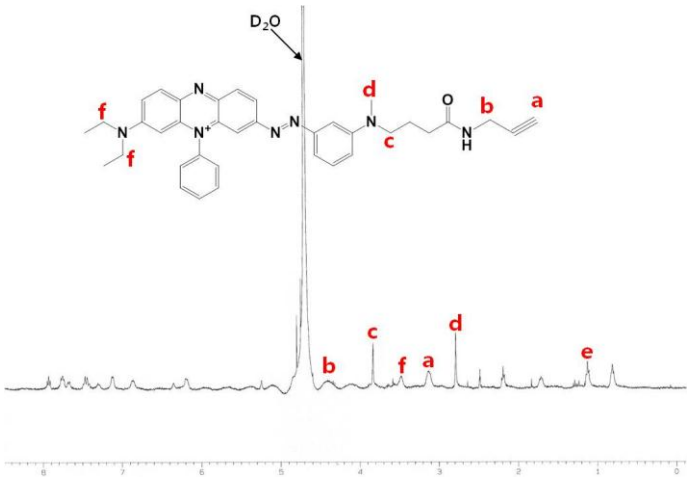

Figure S7. ^{1}H NMR spectrum of alkyne-BHQ3.

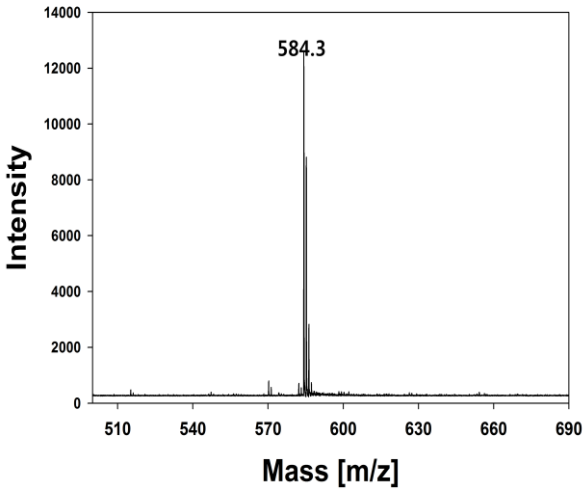

Figure S8. MALDI-TOF mass spectrum of alkyne-BHQ3

Copper ion detection assays

Selectivity: To evaluate the Cu^{2+} detection, various metal ions were added to mixtures of azido-PEG-Cy5.5 (25 μM) and alkyne-BHQ3 (25 μM) in PBS buffer (1× PBS, pH 7.0, Biosesang, Korea) containing sodium ascorbate (1 μM) followed by incubation for 2 h. The final concentrations of the metal ions were as follows: 0.5 mM Li^{+}, Ca^{2+}, Ni^{+}, Hg^{2+}, Mg^{2+}, K^{+}, Na^{+}, Pd^{2+}, Co^{2+}, Mn^{2+}, Cd^{2+}, Fe^{2+}, and Ag^{+}, and 50 nM Cu^{2+}.

Response time: To investigate the speed of the detection of Cu^{2+} using the NIR fluorescence chemosensor, mixtures of azido-PEG-Cy5.5 (25 μM), alkyne-BHQ3 (25 μM), and Cu^{2+} (50 nM) in PBS buffer (pH 7.0) containing sodium ascorbate (1 μM) were incubated and the fluorescence intensity was recorded after 5, 10, 20, 30, 60, and 120 min of incubation. Increases of the molecular weight after Cu(I)-catalyzed cycloaddition reaction between azido-PEG-Cy5.5 and alkyne-BHQ3 were confirmed by MALDI-TOF mass spectrometry.

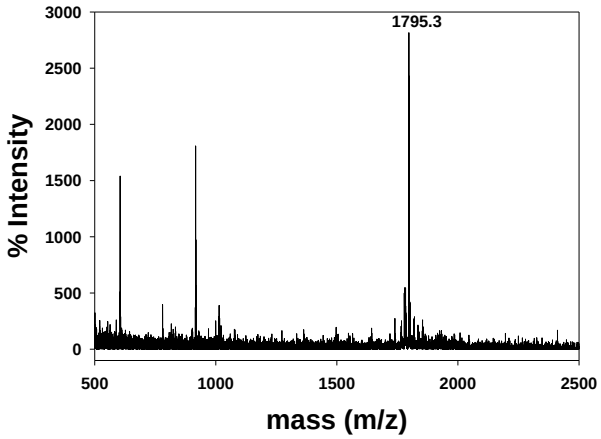

Figure S9. MALDI-TOF mass spectrum after Cu(I)-catalyzed cycloaddition reaction between azido-PEG-Cy5.5 and alkyne-BHQ3

Specificity: Next, the specificity of the detection of Cu^{2+} was studied as follows: Three combinations of four different types of metal ions (group 1: Li^{+}, Ca^{2+}, Ni^{+}, Hg^{2+}; group 2: Mg^{2+}, K^{+}, Pd^{2+}, Co^{2+}; group 3: Mn^{2+}, Cd^{2+}, Fe^{2+}, Ag^{+}; final metal ion concentration = 0.5 mM) were reacted with solutions (PBS, pH 7.0) containing azido-PEG-Cy5.5 (25 μM), alkyne-BHQ3 (25 μM), and sodium ascorbate (1 μM) in the absence and presence of Cu^{2+} (50 nM) for 30 min.

Detection limitation: The detection limits of the NIR fluorescence chemosensor were determined by incubating solutions containing azido-PEG-Cy5.5 (25 μM), alkyne-BHQ3 (25 μM), and sodium ascorbate (1 μM) with different concentrations of Cu^{2+} (from 5 fM to 1 nM) for 30 min.

Screening of the copper chelating effect: To investigate whether the NIR fluorescence chemosensor can determine the efficacy of copper chelating agents for high-throughput screening in practical applications, seven chemicals (i.e., non–copper-chelators: cysteine (CYS), *N*-acetylcysteine (NAC), and caffeic acid (CA); and copper chelators: tetraethylenepentamine (TEPA), triethylenetetramine (TETA), ethylenediaminetetraacetic acid (EDTA), and penicillamine (PA)) were tested. Each compound, which was present at a final concentration of 0.5 nM, was reacted for 30 min with solutions (PBS, pH 7.0) containing azido-PEG-Cy5.5 (25 μM), alkyne-BHQ3 (25 μM), sodium ascorbate (1 μM), and Cu^{2+} (50 nM).

Detection of copper ion in living cells. HEK 293T cells were cultured in DMEM supplemented with 10% FBS, glutamine (2 mM) and 1% penicillin-streptomycin. Two days before imaging, cells were passed and plated on cover glass bottom dishes (SPL). For copper ion detection experiments, 100 μM $CuSO_4$ was added to the cells and incubated for 12 h. Then, the copper sensor [azido-PEG-Cy5.5 (25 μM), alkyne-BHQ3 (25 μM)] was added to the cells in serum free medium condition. After 20 min incubation, the cells were washed with culture medium and further incubated 4 h in DMEM. The copper ion detection in living cells was performed by using LSM 510 META NLO (Carl Zeiss, Germany) at chuncheon center in Korea Basic Science Institute. Excitation of the copper sensor-loaded cells at 633 nm was carried out with He/Ne laser, and emission was collected in a band pass (650~710 nm).

Detection of copper ion in serum: To determine copper detection in serum, mice (BALB/c, 7 week-old) were treated with 5 mg/kg $CuSO_4$. After 2 h, blood was collected, clotted for 1 h at room temperature, and centrifuged for 10 min at 13,000 rpm. Serum was stored at 80°C until assayed before use. To determine the level of Copper ion in serum, serum was diluted 50-fold in PBS (pH 7.0) containing azido-PEG-Cy5.5 (25 μM) and alkyne-BHQ3 (25 μM) and reacted for 30 min. Then, fluorescence intensities of each sample were detected. The quantitative analysis of copper ion in serum was calculated from the standard curve of copper ion as shown in the below figure.

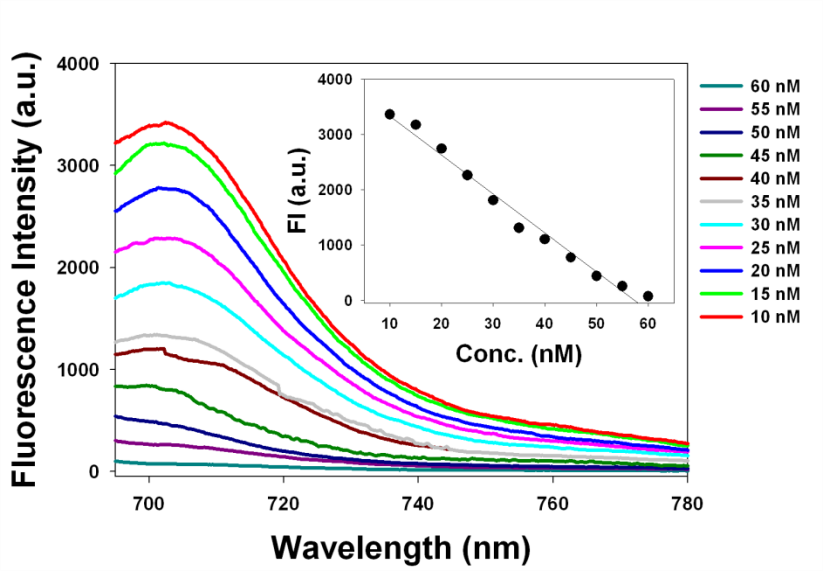

Figure S10. Fluorescence emission spectra of the copper probe in the presence of various concentrations of copper ion. Inset: Copper ion standard curve.

Fluorescence spectra and image: In copper-ion detection assays, fluorescence spectra were acquired using a Hitachi F-4500 spectrofluorometer (Hitachi, Japan). The excitation was fixed at 675 nm and the emission spectra were recorded from 690 to 840 nm at room temperature using a 1 mL cuvette. The NIR fluorescence image and signal on a 96-well microplate were obtained using a Kodak Image Station 4000MM Digital Imaging System (Kodak, New Haven, CT) consisting of a light-tight box equipped with a 150 W halogen lamp and an excitation filter system for Cy5.5 (680 nm to 720 nm, Omega Optical, Battleboro, VT).