Supporting Information

Recyclable Cu(I)/Melanin Dots for Cycloadditions, Bioconjugation and Cell Labeling

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|          | Diameter (nm) | Zeta potential (mV) |
|----------|---------------|---------------------|
| M-dots   | 7.45 ± 1.22   | -2.2 ± 2.43         |
| Cu(I)/M-dots | 8.55 ± 1.87 | +5.1 ± 3.26         |
Table S2. The recycle study of Cu(I)/M-dots catalytic system for two component [3+2] cycloaddition reaction.

| Entry | Alkyne     | Cycle | Yield (%) |
|-------|------------|-------|-----------|
| 1     | 10.2 mg (100 μmmol) | 1st   | 88        |
| 2     | 10.2 mg (100 μmmol) | 2nd   | 85        |
| 3     | 10.2 mg (100 μmmol) | 3rd   | 85        |
| 4     | 10.2 mg (100 μmmol) | 4      | 84        |
| 5     | 10.2 mg (100 μmmol) | 5      | 85        |
| 6     | 10.2 mg (100 μmmol) | 6      | 80        |
| 7     | 10.2 mg (100 μmmol) | 7      | 82        |

*Cu(I)/M-dots was removed by centrifugal-filtrate (MWCO=30K), and washed by water (3 times) for directly using in the next reaction.*
Table S3. Three-Component cyclization of alkyl halides, sodium azide, and alkynes.

| Entry | Alkyne | Alkyl Halides | Product | Yield (%) |
|-------|--------|---------------|---------|-----------|
| 1     | 1a     | 4a Br         | 3b      | 88        |
| 2     | 1a     | 4b Cl         | 3b      | 85        |
| 3     | 1b     | 4a            | 3c      | 78        |
| 4     | 1f     | 4a            | 3i      | 90        |
| 5     | 1g     | 4c Br Ph      | 3p      | 87        |

Conditions: 0.1 mmol alkyne, 0.12 mmol Halide and 0.12 mmol sodium azide, 2 nmol Cu(I)/M-dots in 1mL (containing 0.1% mol Cu(I))
General methods

All chemicals were purchased from commercial sources (such as Aldrich). NOTA-Azide was purchased from AREVA Med. Azido-PEG3-NHS and Alkyne-PEG3-NHS were purchased from Conju-Probe. Cy5.5 dye was purchased from Lumiprobe. RGDyK was purchased from peptide international. Dimethylthiazolyl-diphenyltetrazolium (MTT) was purchased from Biotium. Phosphate buffered saline (PBS) was purchased from Gibco. Amine-PEG5000-amine (NH₂-PEG₅₀₀₀-NH₂, 5kDa) was purchased from Laysan Bio. GelRed Nucleic Acid Gel Stain was purchased from Biotium. Tris base, Boric acid, Na₂EDTA, Acrylamide, bisacrylamide, Urea, ammonium persulfate (APS) and tetramethylethylenediamine (TEMED) purchased from Thermo Fisher. The ¹H and ¹³C NMR spectra were acquired on a Bruker 400 MHz magnetic resonance spectrometer. Data for ¹H NMR spectra are reported as follows: chemical shifts are reported as δ in units of parts per million (ppm) relative to chloroform-d (δ 7.26, s); multiplicities are reported as follows: s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), m (multiplet), or br (broadened); coupling constants are reported as a J value in Hertz (Hz); the number of protons (n) for a given resonance is indicated nH, and based on the spectral integration values. MALDI-TOF-MS spectrometric analyses were performed at the Mass Spectrometry Facility of Stanford University. HPLC was performed on a Dionex HPLC System (Dionex Corporation) equipped with a GP50 gradient pump and an in-line diode array UV-Vis detector. A reversed-phase C18 (Phenomenax, 5 μm, 4.6 × 250 mm, 5 μm, 10 × 250 mm or 21.2 × 250 mm) column was used for analysis and semi-preparation. UV absorbance of the probe was recorded on an Agilent 8453 UV spectrophotometer. Fluorescence was recorded on a Fluoromax-3 spectrofluorometer (Jobin Yvon). The hydrodynamic sizes of M-dots and Cu(I)/M-dots were measured by dynamic light scattering (DLS) instrument using a 90 Plus particle size analyzer (Malvern, Zetasizer Nano ZS90). Zeta potentials were measured using a zeta potential analyzer (Malvern, Zetasizer Nano ZS90). Transmission electron microscopy (TEM) images were recorded on a JEOL 2010 transmission electron microscope. X-ray photoelectron spectra was recorded by using Thermo ESCALAB 250Xi X-ray photoelectron spectrometer. The Cu ions analyses were
performed using inductively coupled plasma mass spectrometer (ICP-MS, Thermo Scientific Xseries 2 Quadrupole). PAGE gel was visualized by a UV transilluminator (UVP). The cells were imaged using fluorescence microscopy (Axiovert 200M fluorescence microscope).

**Preparation of M-dots**

Tyrosine-derived synthetic melanin (10 mg) was first dissolved in 5 mL of 0.1 N NaOH aqueous solution under vigorous stirring. After dissolving, HCl aqueous solution (0.1 N) was swiftly dropped into the obtained basic melanin solution to adjust the pH to 7.0 under sonication with output power = 10 W for 1 min. A bright black melanin aqueous solution was obtained. The neutralized solution was further centrifuged with a centrifugal-filter (MWCO = 30 kDa) and washed with deionized water and repeated several times to remove the produced NaCl. The aqueous solvent was removed by freeze-drying to obtain 7.5 mg black solid. 

NH\(_4\)OH solution (28 wt %) was added to 5 mL of above black solid aqueous solution (1 mg/mL of water) to adjust the pH of the solution to 9. This mixed solution was added dropwise into NH\(_2\)-PEG\(_{5000}\)-NH\(_2\) (25 mg) aqueous solution. After vigorous stirring for 12 h, PEG-modified melanin nanoparticle was retrieved by centrifugation with a centrifugal-filter (Amicon centrifugal filter device, MWCO = 30 kDa) and washed with deionized water several times by redispersion/centrifugation processes to remove the unreacted NH\(_2\)-PEG\(_{5000}\) \(-\) NH\(_2\). The aqueous solvent was removed by freeze-drying and obtained M-dots.

**Preparation of Cu(I)/M-dots catalyst**

The M-dots solubilized in 1 mL of degassed water (1 mg in 1 mL H\(_2\)O) and 20 µL of fresh CuSO\(_4\) (10 mg/ mL) was successively added. The solution was stirred at 37 °C in 30 min under N\(_2\). Then, 20 eq sodium ascorbate solubilized in degassed water is added dropwise to the solution. After stirring 30 min under N\(_2\), the catalyst was then purified by a centrifugal-filter (MWCO = 30 kDa) and washed with water for three times to obtain Cu(I)/M-dots catalyst and directly used for further applications. The Cu(I) concentration of M-dots was measured by inductively coupled plasma-mass spectrometry (ICP-MS) analysis.

**Characterization of M-dots and Cu(I)/M-dots**
The hydrodynamic sizes of M-dots and Cu(I)/M-dots were measured by dynamic light scattering (DLS) instrument using a 90 Plus particle size analyzer (Malvern, Zetasizer Nano ZS90). Briefly, 200 µL of M-dots based samples were firstly passed through a 0.22 µm filter and then were filled with a 200 µL clean microcuvette for DLS analysis. We made 6 separate DLS measurements with each measurement consisting of 5 sub-runs with a 10 second duration. Zeta potential was measured using a zeta potential analyzer (Malvern, Zetasizer Nano ZS90). Zeta cells should be rinsed thoroughly and dried using nitrogen before use. The zeta cell was filled with 200 µL of samples by gently depressing the syringe plunger. In order to establish measurement repeatability, each sample was performed for three runs.

The $^1$H NMR spectra of M-dots was recorded on a 400 MHz NMR spectrometer (Bruker), using D$_2$O as solvent. Transmission electron microscopy (TEM) images were recorded on a JEOL 2010 transmission electron microscope at accelerating voltage of 100 kV. The TEM specimens were made by placing a drop of the M-dots and Cu(I)/M-dots aqueous solution on a carbon-coated copper grid, followed by plasma cleaning. Matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS) of M-dots was carried out by Stanford Protein and Nucleic Acid Biotechnology Facility, Stanford University. X-ray photoelectron spectra of Cu(I)/M-dots was recorded by using Thermo ESCALAB 250Xi X-ray photoelectron spectrometer. Monochromatic Al Kα X-rays (1486.68 eV) were utilized for excitation. The Cu(I)/M-dots dark powder (obtained by lyophilization) was used for the analysis and kept in a vacuum of 10$^{-8}$ mbar during measurements. For enhancing Cu signal intensities the measurements were recorded at 50 scans. Typically the hydrocarbon C 1s line was taken as an internal standard at 284.8 eV.

**Inductively coupled plasma-mass spectrometry (ICP-MS) analysis**

Thermo Scientific Xseries 2 Quadrupole was used to measure the concentration of Cu(I) in Cu(I)/M-dots. Standards (0.2 ppm, 0.5 ppm, 1 ppm, 4 ppm and 10 ppm of copper) were prepared in dilute nitric acid (2% w/w) solution and 2% nitric acid solution was used as the blank.

For freshly prepared Cu(I)/M-dots catalyst: 100 µL of detected samples were firstly heated to
evaporate the water solvent and then digested with 0.5 mL of trace metal grade concentrated nitric acid (HNO₃, 70% w/w) under heating until completely dissolved, then the residue was dissolved in 7 mL of dilute nitric acid (2% w/w) for final ICP-MS analysis. For recycled Cu(I)/M-dots catalyst: after each click reaction, Cu(I)/M-dots were separated immediately from the reaction mixture (30 kDa centrifugal-filter, washed with water for three times) and then stored in water. Recycled samples (100 µL) were prepared firstly and heated to evaporate the water solvent and then digested with 0.5 mL of trace metal grade concentrated nitric acid (HNO₃, 70% w/w) under heating until completely dissolved. Then the residue was dissolved in 7 mL of dilute nitric acid (2% w/w) for final ICP-MS analysis.

The stability of Cu(I)/M-dots catalyst

The stability of Cu(I)/M-dots was studied by incubating those Cu(I)/M-dots in PBS (pH = 7.4) at 37 °C. Those M-dots were placed in dialysis tube (MWCO 10K) with magnetic stirring, dialysis against 10 ml PBS. At a certain time, dialysate was removed for ICP-MS analysis and replaced with fresh PBS solution. For ICP-MS analysis, 100 µL of the detected sample was firstly heated to evaporate the water solvent and then digested with 0.5 mL of concentrated nitric acid (70% w/w) under heating. After the solvent was evaporated, the residue was then dissolved in 7 mL of dilute nitric acid (2% w/w) for final ICP-MS analysis.

Cell lines

U87MG glioblastoma and NIH3T3 cells were obtained from the American Type Culture Collection (Manassas, VA, USA) and culture media was obtained from Invitrogen Co. (Carlsbad, CA, USA). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal bovine serum and 1% (v/v) penicillin at 37°C and 5% CO₂.
Cell viability

In vitro cytotoxicity of Cu(I)/M-dots was determined in NIH3T3 and U87MG cells by the MTT assay. NIH3T3 and U87MG cells were incubated on 96-well plate in DMEM medium containing 10% FBS and 1% penicillin/streptomycin at 37 °C in 5% CO₂ humidified atmosphere for 24 h and 1×10⁴ cells were seeded per well. Cu(I)/M-dots and Cu(I)/THPTA (prepared by 1 equivalent of CuSO₄ was incubated with 1 equivalent of THPTA for 30 min and sodium ascorbate (30 eq) for 30 min) were added with copper concentrations of 50 μM, 100 μM and 200 μM for 1 h, 12 h, 24 h and 48 h (with n=3 per sample). After 1 h, 12 h, 24 h and 48 h of treatment, cells were washed with PBS and culture medium was replaced with DMEM. Addition of 10 μL of MTT (0.5 mg/mL) solution to each well and incubation for 4 h at 37 °C was followed to produce formazan crystals. Then, the supernatant was removed and the products were lysed with 100 μL of DMSO. The absorbance value was recorded at 490 nm using a microplate reader. The absorbance of the untreated cells was used as a control and its absorbance was as the reference value for calculating 100% cellular viability.

DNA bioconjugation

Oligonucleotide synthesized by Protein and Nucleic Acid (PAN) facility at Stanford. The 3’-position modified oligonucleotide (S1, 5 nmol, MW=6317.2) in 20 μL of water was reacted with an excess of NHS ester V1 (50 eq) in 30 μL of water at room temperature for 12 h. Unreacted V1 was removed by a centrifugal-filter (3 kDa), and then lyophilized to obtain alkyne-labeled DNA S2. A click reaction of S2 (20 μM) was then performed with Cy5.5-azide (2f, 80 μM) mediated by 2 μM Cu(I)/M-dots (containing about 100 μM Cu⁺) in 0.1 M PBS at pH 7 for 2h. Unreacted Cy5.5 dye was removed by centrifugal-filter (MWCO = 3 kDa). Then the resulting fluorescent DNA S3 was separated from the catalyst by centrifugal-filter (MWCO = 10 kDa).

DNA denaturing PAGE Gel for Electrophoresis

The product S3 was analyzed on a 16% PAGE gel (PAGE gel was prepared by following the standard protocol). The GelRed Nucleic Acid Gel Stain (Biotium) was used to visualize the DNA. Samples were electrophoresed for 3 h at 15 mA, using TBE buffer. The DNA PAGE
gel was imaged under 365 nm UV light (UVP, UV imager) In addition, MALDI-TOF-MS showed the expected molecular weight for the corresponding cyclo-adduct (MW=7299.9).

**Cell Labeling with CuAAC probes for fluorescence microscopy imaging.**

U87MG cells (1×10^5) were suspended in 500 µL of DMEM seeded in 12-well tissue culture plates and incubated at 37°C for overnight. Then, U87MG cells were incubated first with RGD-alkyne (10 µM) for 30 min at 37°C. After several washing steps to remove unbound alkyne, the Cy5.5-azide (2f, 30 µM) and 4 µM Cu(I)/M-dots (containing about 200 µM Cu^+) were added to the cell culture medium for incubation for 60 min at 37°C. After 1h click reaction for labeling live cells, the U87MG cells were washed three times with PBS and then fixed with fresh 4% paraformaldehyde (PFA) for 5 min at room temperature. Cell nuclei were stained by adding 4',6-diamidino-2-phenylindole (DAPI). The cells were then imaged using fluorescence microscopy (Axiovert 200M fluorescence microscope).

**Chemical synthesis and characterization**

**General procedure for the two-componets [3+2] cyclo-addition reaction.**

\[
\text{Cu}(I)/M\text{-dots} + \text{PEG} - \text{NH}_2 + \text{alkyne} \rightarrow \text{cyclo-alkyne}
\]

1H NMR (400 MHz, \[\text{D}_2\text{O}\])

**Synthesis of 3a:** To a solution of 1a (10.2 mg, 0.1 mmol) and compound 2a (26.2 mg, 0.12 mmol, 1.2 equiv) in PBS (1 mL) at room temperature was added 2 nmol Cu(I)/M-dots (containing 0.1% mol Cu^+). The reaction mixture was stirred at this temperature for 0.5 h. The catalyst was separated by a centrifugal-filter (MWCO = 30 kDa), and the residue solvent was lyophilized to obtain the crude product. The crude product was purified by flash chromatography affording the desired product 3a (30.0 mg, 94% yield). 1H NMR (400 MHz,
D$_2$O): $\delta$ = 8.16 (s, 1H), 7.63 (s, 2H), 7.37-7.31 (m, 3H), 4.49 (s, 2H), 3.84 (s, 2H), 3.56-3.34 (m, 12H), 2.91 (s, 2H); $^{13}$C NMR (101 MHz, D$_2$O) $\delta$ = 147.20, 129.25, 129.17, 128.79, 69.60, 69.43, 69.35, 69.20, 68.47, 66.14, 50.12, 38.88; HRMS (ESI) Calcd. for: C$_{16}$H$_{25}$N$_4$O$_3$$^+$ ([M+H]$^+$): 321.1921, found: 321.1917.

Synthesis of 3b: To a solution of 1a (10.2 mg, 0.1 mmol) and compound 2b (15.9 mg, 0.12 mmol, 1.2 equiv) in PBS/DMSO (9:1) at room temperature was added 2 nmol Cu(I)/M-dots (containing 0.1% mol Cu$^+$). The reaction mixture was stirred at this temperature for 0.5 h. The catalyst was separated by a centrifugal-filter (MWCO = 30 kDa), and the residue solvent was lyophilized to obtain the crude product. The crude product was purified by flash chromatography affording the desired product 3b (21.1 mg, 90% yield). $^1$H NMR (400 MHz, MeOD): $\delta$ = 8.30 (s, 1H), 7.79 (s, 2H), 7.38 (m, 7H), 5.63 (s, 2H); $^{13}$C NMR (101 MHz, MeOD) $\delta$ = 147.80, 135.38, 130.20, 128.63, 128.53, 128.18, 127.94, 127.65, 125.24, 120.79, 109.99, 53.62; HRMS (ESI) Calcd. for: C$_{15}$H$_{14}$N$_3$$^+$ ([M+H]$^+$): 236.1182, found: 236.1185.

Synthesis of 3c: To a solution of 1b (10.3 mg, 0.1 mmol) and compound 2a (26.2 mg, 0.12 mmol, 1.2 equiv) in PBS at room temperature was added 2 nmol Cu(I)/M-dots (containing 0.1% mol Cu$^+$). The reaction mixture was stirred at this temperature for 0.5 h. The catalyst was
separated by a centrifugal-filter (MWCO = 30 kDa), and the residue solvent was lyophilized to obtain the crude product. The crude product was purified by flash chromatography affording the desired product 3c (28.9 mg, 90% yield). $^1$H NMR (400 MHz, D$_2$O): $\delta = 8.96$ (s, 1H), 8.70 (s, 1H), 8.33-7.93 (m, 3H), 4.70 (s, 2H), 4.02-3.97 (m, 2H), 3.36-3.61 (m, 9H), 3.11 (s, 2H); $^{13}$C NMR (101 MHz, D$_2$O) $\delta = 123.91$, 70.03, 69.93, 69.91, 69.78, 68.78, 66.40, 50.31, 39.19; HRMS (ESI) Calcd. for: C$_{15}$H$_{24}$N$_5$O$_3^+$ ([M+H]$^+$): 322.1874, found: 322.1871.

**Synthesis of 3d:** To a solution of 1b (10.3 mg, 0.1 mmol) and compound 2b (15.9 mg, 0.12 mmol, 1.2 equiv) in PBS/DMSO (9:1) at room temperature was added 2 nmol Cu(I)/M-dots (containing 0.1% mol Cu$^+$). The reaction mixture was stirred at this temperature for 0.5 h. The catalyst was separated by a centrifugal-filter (MWCO = 30 kDa), and the residue solvent was lyophilized to obtain the crude product. The crude product was purified by flash chromatography affording the desired product 3d (21.2 mg, 90% yield). $^1$H NMR (400 MHz, MeOD): $\delta = 9.22$ (s, 1H), 8.81-8.62 (m, 3H), 7.96 (s, 1H), 7.40 (s, 4H), 5.69 (s, 2H); $^{13}$C NMR (101 MHz, MeOD) $\delta = 141.74$, 141.34, 140.64, 139.33, 134.97, 130.53, 128.72, 128.39, 127.89, 126.99, 123.20; HRMS (ESI) Calcd. for: C$_{14}$H$_{13}$N$_4^+$ ([M+H]$^+$): 237.1135, found: 237.1135.

**Synthesis of 3e:** To a solution of 1c (24.5 mg, 0.1 mmol) and compound 2b (15.9 mg, 0.12 mmol, 1.2 equiv) in PBS/DMSO (9:1) at room temperature was added 2 nmol Cu(I)/M-dots (containing 0.1% mol Cu$^+$). The reaction mixture was stirred at this temperature for 0.5 h. The catalyst was separated by a centrifugal-filter (MWCO = 30 kDa), and the residue solvent was lyophilized to obtain the crude product. The crude product was purified by flash chromatography affording the desired product 3e (28.9 mg, 90% yield). $^1$H NMR (400 MHz, D$_2$O): $\delta = 8.96$ (s, 1H), 8.70 (s, 1H), 8.33-7.93 (m, 3H), 4.70 (s, 2H), 4.02-3.97 (m, 2H), 3.36-3.61 (m, 9H), 3.11 (s, 2H); $^{13}$C NMR (101 MHz, D$_2$O) $\delta = 123.91$, 70.03, 69.93, 69.91, 69.78, 68.78, 66.40, 50.31, 39.19; HRMS (ESI) Calcd. for: C$_{15}$H$_{24}$N$_5$O$_3^+$ ([M+H]$^+$): 322.1874, found: 322.1871.
mmol, 1.2 equiv) in PBS at room temperature was added 2 nmol Cu(I)/M-dots (containing 0.1% mol Cu\(^{+}\)). The reaction mixture was stirred at this temperature for 0.5 h. The catalyst was separated by a centrifugal-filter (MWCO = 30 kDa), and the residue solvent was lyophilized to obtain the crude product. The crude product was purified by flash chromatography affording the desired product 3e (35.9 mg, 95% yield). \(^1\)H NMR (400 MHz, D\(_2\)O): \(\delta = 7.96\) (s, 1H), 7.39-7.32 (m, 5H), 5.59 (s, 2H), 4.62 (s, 2H), 3.70-3.57 (m, 20H), 3.31 (d, \(J = 4.0\) Hz, 2H), 3.07-3.04 (m, 2H); \(^{13}\)C NMR (101 MHz, D\(_2\)O) \(\delta = 144.67, 135.32, 128.62, 128.23, 127.74, 123.50, 70.01, 69.81, 69.76, 69.73, 69.40, 69.33, 66.36, 63.40, 53.54, 38.99\); HRMS (ESI) Calcd. for: C\(_{19}\)H\(_{31}\)N\(_4\)O\(_4\)\(^{+}\) ([M+H]\(^{+}\)): 379.2340, found: 379.2336.

**Synthesis of 3f**: To a solution of 1d (5.5 mg, 0.1 mmol) and compound 2b (15.9 mg, 0.12 mmol, 1.2 equiv) in PBS at room temperature was added 2 nmol Cu(I)/M-dots (containing 0.1% mol Cu\(^{+}\)). The reaction mixture was stirred at this temperature for 0.5 h. The catalyst was separated by a centrifugal-filter (MWCO = 30 kDa), and the residue solvent was lyophilized to obtain the crude product. The crude product was purified by flash chromatography affording the desired product 3f (16.9 mg, 90% yield). \(^1\)H NMR (400 MHz, MeOD): \(\delta = 8.04\) (s, 1H), 7.35 (s, 5H), 5.61 (s, 2H), 4.42 (s, 2H); \(^{13}\)C NMR (101 MHz, MeOD) \(\delta = 140.15, 135.13, 128.63, 128.29, 127.82, 123.97, 53.63, 34.00\); ESI-MS Calcd. for: C\(_{10}\)H\(_{13}\)N\(_4\)\(^{+}\) ([M+H]\(^{+}\)): 189.1135, found: 189.1132.

**Synthesis of 3g**: To a solution of 1d (5.5 mg, 0.1 mmol) and compound 2a (26.16 mg, 0.12 mmol, 1.2 equiv) in PBS at room temperature was added 2 nmol Cu(I)/M-dots (containing 0.1% mol Cu\(^{+}\)). The reaction mixture was stirred at this temperature for 0.5 h. The catalyst was separated by a centrifugal-filter (MWCO = 30 kDa), and the residue solvent was lyophilized to obtain the crude product. The crude product was purified by flash chromatography affording the desired product 3g (22.1 mg, 85% yield). \(^1\)H NMR (400 MHz, D\(_2\)O): \(\delta = 7.98\) (s, 1H), 7.39-7.32 (m, 5H), 5.59 (s, 2H), 4.62 (s, 2H), 3.70-3.57 (m, 20H), 3.31 (d, \(J = 4.0\) Hz, 2H), 3.07-3.04 (m, 2H); \(^{13}\)C NMR (101 MHz, D\(_2\)O) \(\delta = 144.67, 135.32, 128.62, 128.23, 127.74, 123.50, 70.01, 69.81, 69.76, 69.73, 69.40, 69.33, 66.36, 63.40, 53.54, 38.99\); HRMS (ESI) Calcd. for: C\(_{19}\)H\(_{31}\)N\(_4\)O\(_4\)\(^{+}\) ([M+H]\(^{+}\)): 379.2340, found: 379.2336.
mol Cu\(^{+}\)). The reaction mixture was stirred at this temperature for 0.5 h. The catalyst was separated by a centrifugal-filter (MWCO = 30 kDa), and the residue solvent was lyophilized to obtain the crude product. The crude product was purified by HPLC affording the desired product 3g (24.6 mg, 95% yield). \(^1\)H NMR (400 MHz, MeOD): \(\delta = 8.10 \ (s, \ 1H), \ 4.61 \ (s, \ 2H), \ 4.24 \ (s, \ 2H), \ 3.90 \ (s, \ 2H), \ 3.79-3.61 \ (m, \ 10H), \ 3.12 \ (s, \ 2H)\); \(^{13}\)C NMR (101 MHz, MeOD) \(\delta = 124.65, \ 76.08, \ 70.05, \ 69.94, \ 69.86, \ 69.81, \ 68.91, \ 66.43, \ 50.02, \ 39.21, \ 34.03, \ 28.48\); ESI-MS Calcd. for: C\(_{10}\)H\(_{22}\)N\(_5\)O\(_3\)\(^+\) ([M+H]\(^+\)): 260.1717, found: 260.1714.

**Synthesis of 3h:** To a solution of compound 1e (7.0 mg, 0.1 mmol) and compound 2b (15.9 mg, 0.12 mmol, 1.2 equiv) in PBS at room temperature was added 2 nmol Cu(I)/M-dots (containing 0.1% mol Cu\(^{+}\)). The reaction mixture was stirred at this temperature for 0.5 h. The catalyst was separated by a centrifugal-filter (MWCO = 30 kDa), and the residue solvent was lyophilized to obtain the crude product. The crude product was purified by flash chromatography affording the desired product 3h (18.2 mg, 90% yield). \(^1\)H NMR (400 MHz, MeOD): \(\delta = 8.48 \ (br, \ 1H), \ 7.36 \ (s, \ 5H), \ 5.65 \ (s, \ 2H)\); \(^{13}\)C NMR (101 MHz, MeOD) \(\delta = 161.86, \ 140,03, \ 134.88, \ 128.68, \ 128.34, \ 128.08, \ 127.81, \ 53.70\); ESI-MS Calcd. for: C\(_{10}\)H\(_{10}\)N\(_3\)O\(_2\)\(^+\) ([M+H]\(^+\)): 204.0768, found: 204.0765.

**Synthesis of 3i:** To a solution of compound 1f (5.6 mg, 0.1 mmol) and compound 2b (15.9 mg, 0.12 mmol, 1.2 equiv) in PBS at room temperature was added 2 nmol Cu(I)/M-dots (containing 0.1% mol Cu\(^{+}\)). The reaction mixture was stirred at this temperature for 0.5 h. The catalyst was separated by a centrifugal-filter (MWCO = 30 kDa), and the residue solvent was lyophilized to obtain the crude product. The crude product was purified by flash
chromatography affording the desired product 3i (17.4 mg, 92% yield). $^1$H NMR (400 MHz, MeOD): $\delta = 7.96$ (s, 1H), 7.38-7.31 (m, 5H), 5.58 (s, 2H), 4.68 (s, 2H); $^{13}$C NMR (101 MHz, MeOD) $\delta = 135.15, 128.66, 128.28, 127.76, 54.86, 53.74$; ESI-MS Calcd. for: C$_{10}$H$_{12}$N$_3$O$^+$ ([M+H$^+$]): 190.0975, found: 190.0978.

**Synthesis of 3j**: To a solution of compound 1g (13.1 mg, 0.1 mmol) and compound 2c (36.4 mg, 0.36 mmol, 3.6 equiv) in PBS/DMSO (9:1) at room temperature was added 6 nmol Cu(I)/M-dots (containing 0.3% mol Cu$^+$). The reaction mixture was stirred at this temperature for 0.5 h. The catalyst was separated by a centrifugal-filter (MWCO = 30 kDa), and the residue solvent was lyophilized to obtain the crude product. The crude product was purified by flash chromatography affording the desired product 3j (38.2 mg, 90% yield). $^1$H NMR (400 MHz, MeOD): $\delta = 7.98$ (s, 3H), 4.52-4.49 (m, 6H), 3.74 (s, 6H), 3.58-3.55 (m, 6H), 2.14-2.07 (m, 6H); $^{13}$C NMR (101 MHz, MeOD) $\delta = 143.78, 124.26, 57.89, 32.54$; ESI-MS Calcd. for: C$_{18}$H$_{31}$N$_{10}$O$_3^+$ ([M+H$^+$]): 435.2575, found: 435.2575.

**Synthesis of 3k**: To a solution of 1b (5.2 mg, 0.05 mmol) and compound 2d (26.64 mg, 0.06 mmol, 1.2 equiv) in PBS at room temperature was added 2 nmol Cu(I)/M-dots (containing 0.1%
mol Cu\(^{+}\)). The reaction mixture was stirred at this temperature for 0.5 h. The catalyst was separated by a centrifugal-filter (MWCO = 30 kDa), and the residue solvent was lyophilized to obtain the crude product. The crude product was purified by HPLC affording the desired product \(3k\) (28.9 mg, 90% yield). \(^1\)H NMR (400 MHz, MeOD): \(\delta = 8.37\) (s, 1H), 7.83-7.81 (d, \(J = 8.0\) Hz, 2H), 7.54-7.33 (m, 9H), 4.48-4.45 (m, 1H), 4.25-4.22 (m, 1H), 3.63-3.60 (m, 10H), 3.19-3.16 (m, 1H), 2.70-2.67 (m, 1H), 2.19-2.15 (m, 2H), 1.73-1.66 (m, 4H), 1.54-1.38 (m, 2H)\(^{13}\)C NMR (101 MHz, MeOD) \(\delta = 174.66, 164.69, 130.28, 128.62, 127.97, 125.25, 121.77, 70.10, 70.03, 69.78, 69.09, 68.89, 61.93, 60.20, 55.56, 50.16, 39.61, 38.86, 35.27, 28.32, 28.06, 25.50;\) ESI-MS Calcd. for: C\(_{25}\)H\(_{38}\)N\(_7\)O\(_7\)S\(_5\) + (\([\text{M}+\text{H}]^+\)): 548.2650, found: 548.2654.

### Synthesis of 1h

To a solution of RGDyK (3.10 mg, 5 µmol) and \(\text{V1}\) (2.34 mg, 7.5 µmol, 1.5 equiv) in DMF at room temperature was added DIPEA (0.1 equiv). The reaction mixture was stirring at this temperature for 4 h. The crude product was purified by HPLC. Lyophilization of the purified material gave 3.19 mg (78%) of \(1h\). \(^1\)H NMR (400 MHz, D\(_2\)O): \(\delta = 8.67\) (d, \(J = 8.0\) Hz, 1H), 8.53-8.49 (m, 1H), 7.98-7.95 (m, 1H), 7.27-7.12 (m, 7H), 4.52-4.48 (m, 2H), 4.25-4.21 (m, 2H), 4.12-4.10 (m, 3H), 4.06 (s, 2H), 3.76-3.72 (m, 2H), 3.68-3.65 (m, 3H), 3.63-3.61 (m, 3H), 3.58-3.55 (m, 12H), 3.39 (s, 1H), 3.35 (s, 1H), 3.11-2.93 (m, 8H), 2.87-2.74 (m, 5H), 2.62-2.53 (m, 3H), 2.41-2.38 (m, 3H), 1.78-1.69 (m, 2H), 1.58-1.48 (m, 3H), 1.44-1.33 (m, 4H), 1.27-1.20 (m, 3H), 0.98 (d, \(J = 8.0\) Hz, 1H), 0.87-0.79 (m, 3H), ESI-MS Calcd. for: C\(_{37}\)H\(_{56}\)N\(_9\)O\(_{12}\) + (\([\text{M}+\text{H}]^+\)): 818.4, found: 819.2.

### Synthesis of 1i

To a solution of AE105 (1.45 mg, 1 µmol) and \(\text{V1}\) (0.47 mg, 1.5 µmol, 1.5 equiv) in DMF at room temperature was added DIPEA (0.1 equiv). The reaction mixture was stirring at this temperature for 4 h. The crude product was purified by HPLC. Lyophilization
of the purified material gave 1.28 mg (78%) of 1h. ESI-MS Calcd. for: C_{80}H_{116}N_{17}O_{21}^+ ([M+H]^+): 1650.8, found: 1651.2.

$$\text{RGDyK} + \text{NHS-(PEG)}_3\text{-azide} \xrightarrow{\text{DIPEA}} \text{RGDyK-(PEG)}_3\text{-azide}$$

**Synthesis of 2e** To a solution of RGDyK (3.10 mg, 5 µmol) and V2 (2.58 mg, 7.5 µmol, 1.5 equiv) in DMF at room temperature was added DIPEA (0.1 equiv). The reaction mixture was stirring at this temperature for 4 h. The crude product was purified by HPLC. Lyophilization of the purified material gave 3.02 mg (71%) of 2e. $^1$H NMR (400 MHz, D$_2$O): $\delta$ = 8.67 (d, $J$ = 4.0 Hz, 1H), 8.53-8.49 (m, 1H), 7.98-7.95 (m, 1H), 7.27-7.12 (m, 7H), 4.52-4.48 (m, 2H), 4.25-4.21 (m, 2H), 4.12-4.10 (m, 3H), 4.06 (s, 2H), 3.76-3.72 (m, 2H), 3.68-3.65 (m, 3H), 3.63-3.61 (m, 3H), 3.58-3.55 (m, 12H), 3.39 (s, 1H), 3.35 (s, 1H), 3.11-2.93 (m, 8H), 2.87-2.74 (m, 5H), 2.62-2.53 (m, 3H), 2.41-2.38 (m, 3H), 1.78-1.69 (m, 2H), 1.58-1.48 (m, 3H), 1.44-1.33 (m, 4H), 1.27-1.20 (m, 3H), 0.98 (d, $J$ = 8.0 Hz, 1H), 0.87-0.79 (m, 3H). ESI-MS Calcd. for: C$_{36}$H$_{57}$N$_{12}$O$_{12}^+$ ([M+H]^+): 849.4, found: 849.1.

**Synthesis of 2f** To a solution of Cy5.5 NHS ester (0.70 mg, 1 µmol) and 2a (0.26 mg, 1.2 µmol, 1.2 equiv) in DMF at room temperature was added DIPEA (0.1 equiv). The reaction mixture was stirring at this temperature for 4 h. The crude product was purified by HPLC. Lyophilization of the purified material gave 0.42 mg (50%) of 2f. ESI-MS Calcd. for: C$_{48}$H$_{60}$N$_{6}$O$_{4}$ ([M+H]^+): 784.4, found: 784.2.

**Synthesis of 3l**: To a solution of 1h (1.23 mg, 1.5 µmol) and compound 2e (1.87 mg, 2.2 µmol, 1.5 equiv) in PBS at room temperature was added 0.03 nmol Cu(I)/M-dots (containing 0.1% mol Cu$^+$). The reaction mixture was stirred at this temperature for 0.5 h. The catalyst was separated by a centrifugal-filter (MWCO = 30 kDa), and the residue solvent was lyophilized to obtain the crude product. The crude product was purified by HPLC affording the desired
product 3l (1.87 mg, 75% yield); ESI-MS Calcd. for: C_{73}H_{112}N_{21}O_{24}^+ ([M+H]^+): 1666.8, found: 1666.5.

Synthesis of 3m: To a solution of 1h (1.23 mg, 1.5 µmol) and compound 2f (1.73 mg, 2.2 µmol, 1.5 equiv) in PBS at room temperature was added 0.03 nmol Cu(I)/M-dots (containing 0.1% mol Cu^+). The reaction mixture was stirred at this temperature for 0.5 h. The catalyst was separated by a centrifugal-filter (MWCO = 30 kDa), and the residue solvent was lyophilized to obtain the crude product. The crude product was purified by HPLC affording the desired product 3m (1.87 mg, 78% yield); ESI-MS Calcd. for: C_{85}H_{115}N_{15}O_{16}^+ ([M+H]^+): 1601.8, found: 1602.5.

Synthesis of 3n: To a solution of 1i (1.65 mg, 1.0 µmol) and compound 2f (1.17 mg, 1.5 µmol, 1.5 equiv) in PBS at room temperature was added 0.02 nmol Cu(I)/M-dots (containing 0.1% mol Cu^+). The reaction mixture was stirred at this temperature for 0.5 h. The catalyst was separated by a centrifugal-filter (MWCO = 30 kDa), and the residue solvent was lyophilized to obtain the crude product. The crude product was purified by HPLC affording the desired product 3n (1.86 mg, 75% yield); MALDI-TOF-MS: C_{128}H_{175}N_{23}O_{25}^+ ([M+H]^+): 2434.3, found: 2435.1.

Synthesis of 3o: To a solution of 1h (0.82 mg, 1.0 µmol) and compound 2g (0.575 mg, 1.5 µmol, 1.5 equiv) in PBS at room temperature was added 0.02 nmol Cu(I)/M-dots (containing 0.1% mol Cu^+). The reaction mixture was stirred at this temperature for 0.5 h. The catalyst was separated by a centrifugal-filter (MWCO = 30 kDa), and the residue solvent was lyophilized to obtain the crude product. The crude product was purified by HPLC affording
the desired product 3o (1.04 mg, 84% yield); ESI-MS Calcd. for: C_{25}H_{38}N_{16}O_{17}^+ ([M+H]^+): 1203.6, found: 1203.3.

**Synthesis of AE105:** Peptide AE105 (Ac-Lys-Gly-Asp-Cha-Phe-(D)Ser-(D)Arg-Tyr-Leu-Trp-Ser-NH₂) was synthesized on Tentagel S RAM resin using traditional Fmoc solid-phase peptide chemistry. After deprotection and cleavage from the resin using 93% TFA, 5% Tips, and 2% H₂O for 2 h, the peptide was precipitated in cold Et₂O and washed with Et₂O three times. The dried peptide was purified by prep-HPLC. MS Calcd for: C_{70}H_{102}N_{17}O_{17}^+ ([M+H]^+): 1452.8, found: MALDI-MS: m/z 1452.3.

**General Procedure for the Three-Component [3+2] Cyclo-addition Reaction.**

**Synthesis of 3b:** To a solution of 1a (10.2 mg, 0.1 mmol), Compound 4a (20.4 mg, 0.12 mmol, 1.2 equiv) and sodium azide (7.8 mg, 0.12 mmol) in PBS/DMSO (8:2) at room temperature was added 2 nmol Cu(I)/M-dots (containing 0.1% mol Cu⁺). The reaction mixture was stirred at this temperature for 1 h. The catalyst was separated by a centrifugal-filter (MWCO = 30 kDa), and the residue solvent was lyophilized to obtain the crude product. The crude product was purified by flash chromatography affording the desired product 3b (20.6 mg, 88% yield).

**Synthesis of 3b:** To a solution of 1a (10.2 mg, 0.1 mmol), Compound 4b (15.1 mg, 0.12 mmol, 1.2 equiv) and sodium azide (7.8 mg, 0.12 mmol) in PBS/DMSO (8:2) at room temperature...
was added 2 nmol Cu(I)/M-dots (containing 0.1% mol Cu\(^{+}\)). The reaction mixture was stirred at this temperature for 1 h. The catalyst was separated by a centrifugal-filter (MWCO = 30 kDa), and the residue solvent was lyophilized to obtain the crude product. The crude product was purified by flash chromatography affording the desired product \(3\textbf{b}\) (19.9 mg, 85% yield).

\[
\text{1b} + \text{4a} \xrightarrow{\text{Cu(I)/M-dots, NaN}_3, \text{PBS/DMSO=8:2/1 h}} \text{3d}
\]

\textit{Synthesis of 3d:} To a solution of \(1\textbf{b}\) (10.3 mg, 0.1 mmol), Compound \(4\textbf{a}\) (20.4 mg, 0.12 mmol, 1.2 equiv) and sodium azide (7.8 mg, 0.12 mmol) in PBS/DMSO (8:2) at room temperature was added 2 nmol Cu(I)/M-dots (containing 0.1% mol Cu\(^{+}\)). The reaction mixture was stirred at this temperature for 1 h. The catalyst was separated by a centrifugal-filter (MWCO = 30 kDa), and the residue solvent was lyophilized to obtain the crude product. The crude product was purified by flash chromatography affording the desired product \(3\textbf{d}\) (18.4 mg, 78% yield).

\[
\text{1f} + \text{4a} \xrightarrow{\text{Cu(I)/M-dots, NaN}_3, \text{PBS, 1h}} \text{3i}
\]

\textit{Synthesis of 3i:} To a solution of \(1\textbf{f}\) (0.56 mg, 0.1 mmol), Compound \(4\textbf{a}\) (20.4 mg, 0.12 mmol, 1.2 equiv) and sodium azide (7.8 mg, 0.12 mmol) in PBS at room temperature was added 2 nmol Cu(I)/M-dots (containing 0.1% mol Cu\(^{+}\)). The reaction mixture was stirred at this temperature for 1 h. The catalyst was separated by a centrifugal-filter (MWCO = 30 kDa), and the residue solvent was lyophilized to obtain the crude product. The crude product was purified by flash chromatography affording the desired product \(3\textbf{i}\) (17.1 mg, 90% yield).
Synthesis of 3p: To a solution of compound 1g (13.1 mg, 0.1 mmol), compound 4c (55.8 mg, 0.36 mmol, 3.6 equiv) and sodium azide (23.4 mg, 0.36 mmol) in PBS/DMSO (7:3) at room temperature was added 6 nmol Cu(I)/M-dots (containing 0.3% mol Cu⁺). The reaction mixture was stirred at this temperature for 1.5 h. The catalyst was separated by a centrifugal-filter (MWCO = 30 kDa), and the residue solvent was lyophilized to obtain the crude product. The crude product was purified by flash chromatography affording the desired product 3p (32.7 mg, 67% yield). ¹H NMR (400 MHz, CDCl₃): δ = 8.17 (s, 3H), 7.37-7.26 (m, 15H), 5.53 (s, 6H), 4.14 (s, 6H); ¹³C NMR (101 MHz, CDCl₃) δ = 134.05, 129.19, 128.92, 128.92, 128.09, 54.49, 46.17 ¹H NMR (400 MHz, MeOD): δ = 7.98 (s, 3H), 4.52-4.49 (m, 6H), 3.74 (s, 6H), 3.58-3.55 (m, 6H), 2.14-2.07 (m, 6H); ¹³C NMR (101 MHz, MeOD) δ = 143.78, 124.26, 57.89, 32.54; ESI-MS Calcd for: C₂₇H₂₅N₁₀⁺ ([M+H]⁺): 489.2258, found: 489.2254.

In vitro Kinetic study

General conditions: 50 µM 1A, 25 µM benzyl azide and Cu(I)/M-dots in 10 ml PBS:DMSO (8:2). The reaction mixture was purified by HPLC to obtain the desired product 3A. ESI-MS Caled. for: C₁₈H₁₄N₃O₂⁺ ([M+H]⁺): 304.1008, found: 304.1015. Coumarin fluorescence was recorded on a Tecan SAFIRE microplate reader at 2-min intervals for 30 min with excitation at 320 nm and emission detected at 400 nm.

The free radical quenching ability of M-dots in CuAAC conditions.

The ascorbate-driven, metal-induced hydroxyl radical production was monitored by HPLC
using the hydroxyl radical scavenging compound 3-CCA (sigma-aldrich). The hydroxyl radical reaction was carried out with or without M-dots (100 µM) in the presence of 100 µM 3-CCA and ascorbate (5 mM) after 12h.

**Recycle study for the Two-Components [3+2] cyclo-addition reaction.**

![Chemical structure](image)

General conditions: To a solution of 1a (10.2 mg, 0.1 mmol) and compound 2a (26.2 mg, 0.12 mmol, 1.2 equiv) in PBS at room temperature was added 2 nmol Cu(I)/M-dots (containing 0.1% mol Cu⁺). The reaction mixture was stirred at this temperature for 0.5 h. The catalyst was separated by a centrifugal-filter (MWCO = 30 kDa), and washed with water for three times. Then the recycle catalyst was reused directly in the next reaction.

**Recycle study for the Three-Components [3+2] cyclo-addition reaction.**

![Chemical structure](image)

General conditions: To a solution of 1f (5.6 mg, 0.1 mmol), Compound 4a (20.4 mg, 0.12 mmol, 1.2 equiv) and sodium azide (7.8 mg, 0.12 mmol) in PBS at room temperature was added 2 nmol Cu(I)/M-dots (containing 0.1% mol Cu⁺). The reaction mixture was stirred at this temperature for 1.5 h. The catalyst was separated by a centrifugal-filter (MWCO = 30 kDa), and washed with water for three times. Then the recycle catalyst was reused directly in the next reaction.
Radiochemistry

The $^{64}\text{Cu-3o}$ was radiosynthesized as previously described. Probe precursor $3\text{o}$ (20 µg) was incubated with 1 mCi $^{64}\text{CuCl}_2$ at room temperature in 0.1 M sodium acetate buffer (pH 5.5) for 30 min and was analyzed using RP-HPLC with a C18 column.
NMR Spectra

(a). $^1$H NMR and $^{13}$C NMR for 3a
(b). $^1$H NMR and $^{13}$C NMR for 3b
(c). $^1$H NMR and $^{13}$C NMR for 3c
(d). $^1$H NMR and for 3d
(e) $^1$H NMR and for 3e
(f) $^1$H NMR and for 3f
(g). $^1$H NMR and for 3g
(h). $^1$H NMR and for 3h
(i). $^1$H NMR and for 3i
(j) $^1$H NMR and for 3j
(k). $^1$H NMR and for 3k
(l). $^1$H NMR and for 3p
(m). $^1$H NMR and for 1h

(n). $^1$H NMR and for 2e