Electronic Supplementary Material (ESI) for Nanoscale


**Supplementary Information**

# Reactive Oxygen Species Production by Catechol Stabilized Copper Nanoparticles

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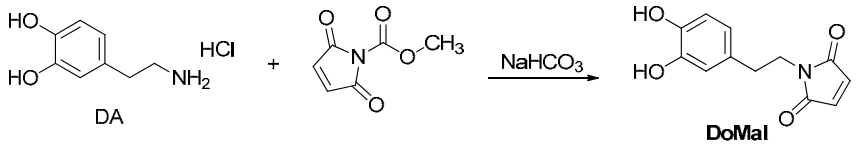
## 1. Chemicals and Instruments

All chemicals were purchased from Sigma Aldrich and used without further purification. Horseradish peroxidase (HRP) type VI-A was used for peroxidase activity tests. Milli-Q water was used throughout the experiments. The transmission electron microscopy (TEM) images and selected area electron diffraction (SAED) patterns were taken by a Philips CM200 FEG/ST electron microscope. Malvern Zetasizer Nanoinstrument (Malvern, UK) was used to perform zeta potential measurements. Fourier transform infrared spectra (FTIR) were recorded using Bruker ALPHA and performed in attenuated total reflection (ATR) mode. The peroxidase activity tests and degradation tests were performed on a Synergy H1 hybrid multi-mode microplate reader (BioTek).

## 2. Synthesis of Dopamine-Based Capping Agents

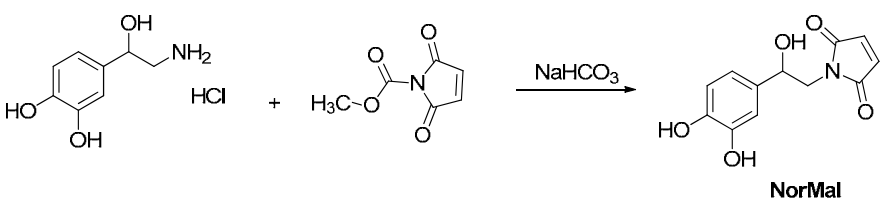
### *1-(3.4-dihydroxyphenethyl)-1H-pyrrole-2.5-dione (dopamine-maleimide, DoMal)*




**Scheme S1** Synthesis of DoMal

DoMal was synthesized according to a reported method.[1] 5.27 mmol dopamine hydrochloride (DA) was dissolved in 30 mL saturated $NaHCO_3$ solution was cooled to 0 °C. 5.27 mmol *N*-methoxycarbonyl maleimide was added under stirring. After 10 min the solution was diluted with 100 mL $H_2O$ and stirred at room temperature for 40 min. The solution was acidified to pH 1-2 with concentrated $H_2SO_4$ and extracted with 10 mL ethyl acetate (EtOAc) three times. The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure. The product was purified by silica gel chromatography ($CH_2Cl_2$/MeOH 20:1) to give the title compound DoMal as yellow solid (Yield: 65%). $^1$H NMR (250 MHz, $CD_3OD$): δ (ppm) 6.74 (s, 2H, C*H*=C*H*), 6.15-6.66 (m, 3H, Ar *H*), 3.60-3.67 (m, 2H, C$H_2$N), 2.70 (t, $^3J$ = 7.1 Hz, 2H, C$H_2$Ar); $^{13}$C NMR (100 MHz, $CD_3OD$), δ (ppm) 34.72, 40.35, 116.23, 116.94, 121.03, 130.73, 134.50, 145.01, 146.31, 172.42; HRMS (FAB) for $C_{12}H_{11}NO_4$ $[M+H]^+$: 234.0765.

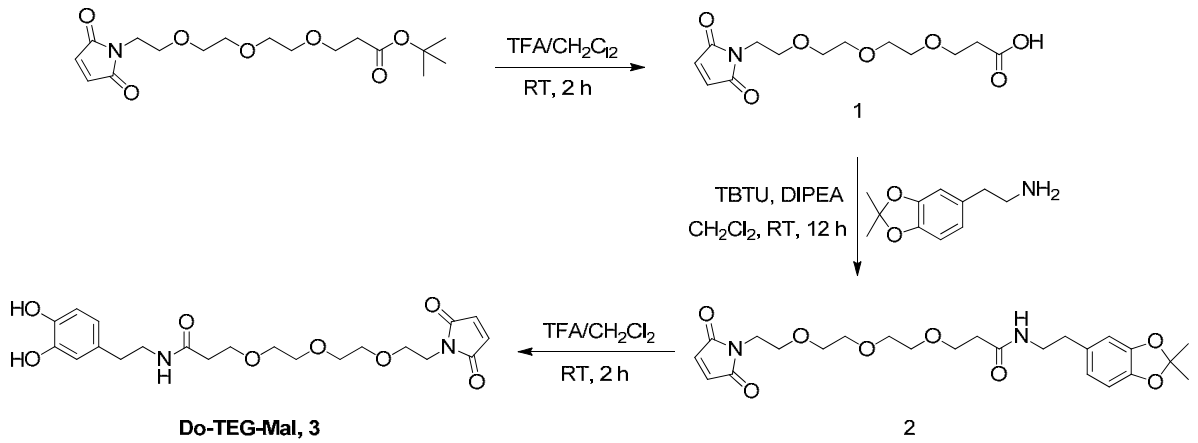
### *1-(2-(3,4-dihydroxyphenyl)-2-hydroxyethyl)-1H-pyrrole-2,5-dione (Norepinephrine-Maleimide, NorMal)*




**Scheme S2** Synthesis of NorMal

4.86 mmol norepinephrine hydrochloride was dissolved in a saturated aqueous solution of $NaHCO_3$ (25 mL) and cooled to 0 °C. 4.86 mmol *N*-methoxycarbonyl maleimide was added in three portions over 15 min. The reaction mixture was stirred at 0 °C for 40 min. Then it was diluted with 30 mL $H_2O$ and stirred at room temperature for 1 h. The solution was acidified to pH 1-2 with concentrated $H_2SO_4$ before extraction with EtOAc (3×10 mL). The organic layers were combined and the solvent was evaporated under reduced pressure. The product was purified by silica gel chromatography ($CH_2Cl_2$/MeOH 20:1) to give the title compound NorMal as yellow solid (Yield: 65%). $^1$H NMR (300 MHz, DMSO-$d_6$): δ (ppm) 3.34-3.51 (m, 2H), 4.59 (m, 1H), 5.34 (d, $J$ = 4.3 Hz, 1H), 6.50 (d, $J$ = 7.9, 2.3 Hz, 1H), 6.62 (d, $J$ = 7.9 Hz, 1H), 6.71 (d, $J$ = 2.3 Hz, 1H), 6.97 (s, 2H), 8.81 (s, 2H); $^{13}$C NMR (75 MHz, $CDCl_3$): δ (ppm) 45.0, 69.1, 113.2, 115.1, 116.7, 133.4, 134.3, 144.6, 144.9, 170.8; HRMS (FAB) for $C_{12}H_{12}NO_5$ $[M+H]^+$: 250.1133.

***N-(3,4-dihydroxyphenethyl)-3-(2-(2-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)ethoxy)ethoxy)propanamide (Do-TEG-Mal)***




**Scheme S3** Synthesis of Do-TEG-Mal

*3-(2-(2-(2-(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)ethoxy)ethoxy)propanoic acid* (**1**)

*Tert*-butyl 3-(2-(2-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)ethoxy)ethoxy)propanoate was firstly synthesized according to the literature methods.[2, 3] Then 5 mL trifluoroacetic acid (TFA) was added to 10 mL dry $CH_2Cl_2$ solution containing 1.40 mmol *tert*-butyl 3-(2-(2-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)ethoxy)ethoxy)propanoate. The reaction mixture was stirred at room temperature for 2 h. 15 mL $CH_2Cl_2$ was then added and the solvent was removed under reduced pressure. The procedure was repeated three times to remove traces of TFA to afford compound **1** as yellow thick oil (Yield: 88%). $^1$H NMR (300 MHz, $CDCl_3$): δ (ppm) 2.65 (t, $J$ = 6.4 Hz, 2H), 3.58-3.65 (m, 10H), 3.70-3.77 (m, 4H), 6.72 (s, 2H); $^{13}$C NMR (75 MHz, $CDCl_3$): δ (ppm) 34.6, 37.0, 66.2, 67.8, 69.9, 70.2, 70.3, 70.4, 134.1, 176.1; HRMS (FAB) for $C_{13}H_{20}NO_7$ $[M+H]^+$: 302.1236.

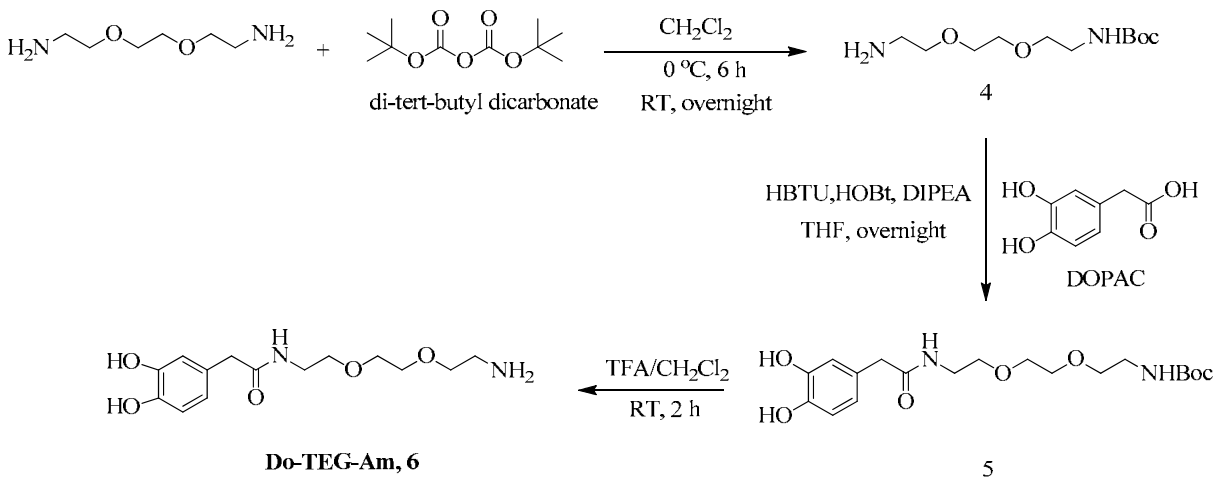
*N-(2-(2,2-dimethylbenzo[d][1,3]dioxol-5-yl)ethyl)-3-(2-(2-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)ethoxy)ethoxy)propanamide* (**2**)

1.19 mmol N,N,N′,N′-tetramethyl-O-(benzotriazol-1-yl)uronium tetrafluoroborate (TBTU) and 1.19 mmol N,N-diisopropylethylamine (DIPEA) were added to a solution of compound **1** (0.99 mmol) in dry $CH_2Cl_2$ (40 mL). The reaction mixture was cooled to 0 $^oC$, and then 1.19 mmol 2-(2,2-dimethylbenzo[d][1,3]dioxol-5-yl)ethanamine, which was synthesized by a reported method,[4] was dissolved in 10 mL $CH_2Cl_2$ and added to the above solution dropwise in 30 min. The as-obtained slurry was purged with argon and stirred at room temperature overnight. The volatiles were then removed under reduced pressure and the remaining semi-solid was partitioned between $CH_2Cl_2$ (100 mL) and $H_2O$ (3×30 mL). The combined organic phase was dried over anhydrous sodium sulfate and the volatiles were removed under reduced pressure to obtain an oily mass which was further purified by silica gel chromatography (EtOAc/cyclohexane 3:1) to afford compound **2** as yellow thick oil (Yield: 73%). $^1H$ NMR (300 MHz, $CDCl_3$): δ (ppm) 1.59 (s, 6H). 2.38 (m, 2H), 2.64 (m, 2H), 3.36 (m, 2H), 3.49-3.57 (m, 10H), 3.59-3.66 (m, 4H), 6.52-6.59 (m, 3H), 6.72 (s, 2H); $^{13}C$ NMR (75 MHz, $CDCl_3$): δ (ppm) 25.7, 35.3, 37.0, 38.9, 40.7, 66.5, 67.2, 69.6, 70.3, 108.0, 108.8, 117.6, 120.9, 128.4, 131.9, 134.1, 145,8, 147.4, 170.4, 171.4; HRMS (FAB) for $C_{24}H_{33}N_2O_8$ $[M+H]^+$: 477.4917.

*N-(3,4-dihydroxyphenethyl)-3-(2-(2-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)ethoxy)ethoxy)propanamide* (**Do-TEG-Mal**, **3**)

6 mL TFA was added to a solution of compound **2** (0.84 mmol) in 15 mL dry $CH_2Cl_2$. The reaction mixture was stirred at room temperature for 2 h. Then 15 mL $CH_2Cl_2$ was added and the solvent was removed under reduced pressure. The procedure was repeated three times to remove traces of TFA to afford the title compound Do-TEG-Mal (**3**) as yellow oil (Yield: 89%). $^1H$ NMR (300 MHz, $CDCl_3$): δ (ppm) 2.41 (m, 2H), 2.57 (m, 2H), 3.34 (m, 2H), 3.43-3.47 (m, 4H), 3.50-3.54 (m, 8H), 3.59 (m, 2H), 6.46 (br, 1H), 6.59 (s, 2H), 6.60-3.69 (m, 2H), 8.94 (br, 2H); $^{13}C$ NMR (75 MHz, $CDCl_3$): δ (ppm) 34.1, 35.8, 37.0, 38.7, 41.1, 66.6, 67.8, 69.8, 69.9, 70.0, 70.1, 115.5, 115.8, 120.7, 130.7, 134.0, 142.9, 144.0, 170.9, 174.1; HRMS (FAB) for $C_{21}H_{29}N_2O_8$ $[M+H]^+$: 437.4759.

***N-(2-(2-(2-aminoethoxy)ethoxy)ethyl)-2-(3,4-dihydroxyphenyl)acetamide (Do-TEG-Am)***




**Scheme S4** Synthesis of Do-TEG-Am

*N-Boc-2,2'-(ethylene-l,2-dioxy)bisethylamine* (**4**)

Compound **4** was firstly synthesized according to a reported method with slight modification.[5] A solution of di-*tert*-butyl dicarbonate (16.8 mmol) in 50 mL $CH_2Cl_2$ was added to a solution of 2,2′-(ethylene-l,2-dioxy)bis(ethylamine) (33.7 mmol) in 100 mL dry $CH_2Cl_2$ at 0 $^oC$ under nitrogen atmosphere over a period of 4 h. The reaction mixture was stirred at 0 $^oC$ for 6 h and then at room temperature overnight. After removal of the solvent in *vacuo*, the residue was dissolved in 50 mL of chloroform and washed twice with aqueous sodium bicarbonate solution. The organic layer was dried over anhydrous $MgSO_4$ and concentrated to give compound **4** (yield: 62%). $^1H$ NMR (300 MHz, $CDCl_3$): δ (ppm) 1.41 (s, 9H). 2.61 (t, $J$ = 5.3 Hz, 2H), 3.02 (m, 2H), 3.24-3.36 (m, 4H), 3.38-3.44 (m, 4H), 5.41 (br, 1H); HRMS (FAB) for $C_{11}H_{24}N_2O_4$ $[M+H]^+$: 249.1.

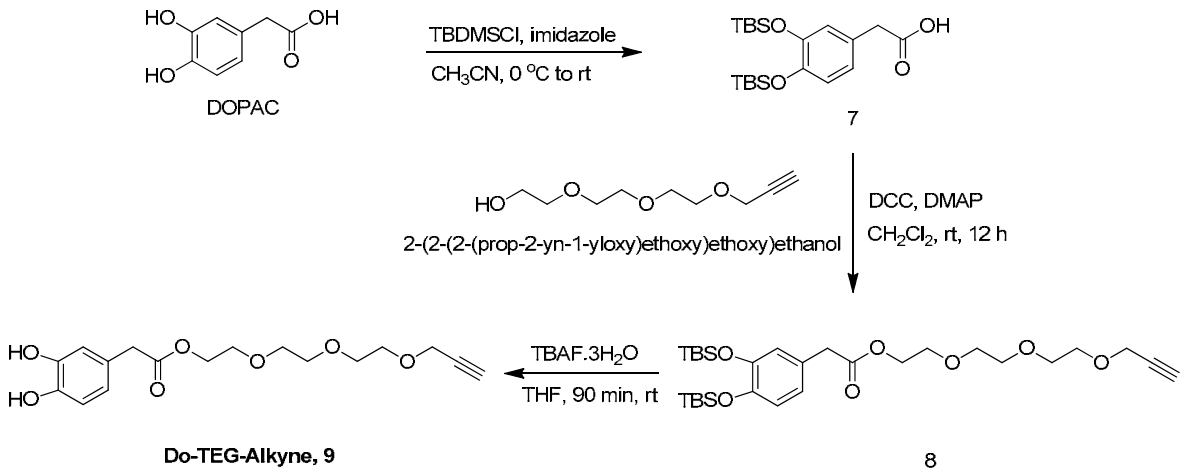
*Tert-butyl (2-(2-(2-(2-(3,4-dihydroxyphenyl)acetamido)ethoxy)ethoxy)ethyl)carbamate* (**5**)

11.9 mmol DIPEA was added to a solution of 2-(3,4-dihydroxyphenyl)acetic acid (DOPAC, 5.95 mmol) in 20 mL tetrahydrofuran (THF). The reaction mixture was cooled to 0 $^oC$ and a solution of compound **4** (7.14 mmol) in 20 mL THF was added dropwise. The reaction mixture was stirred at room temperature overnight. The solvent was removed and the residue was dissolved in 30 mL $CH_2Cl_2$ and washed with water (2×20 mL), brine (20 mL) and dried over anhydrous sodium sulfate. The combined organic layers was concentrated to give yellow oil which was purified by silica gel chromatography eluting with $CH_2Cl_2$ to $CH_2Cl_2$/methanol (10:1) to afford compound **5** as pale yellow liquid (yield: 77%). $^1H$ NMR (300 MHz, $CDCl_3$): δ (ppm) 1.41 (s, 9H). 3.21-3.61 (m, 14H), 5.60 (br, 1H), 6.58 (m, 1H), 6.71-6.77 (m, 2H), 7.74 (br, 1H); $^{13}C$ NMR (75 MHz, $CDCl_3$): δ (ppm) 28.0, 39.5, 40.2, 42.8, 43.3, 53.4, 55.3, 67.9, 69.4, 70.0, 70.2, 79.7, 115.6, 116.2, 121.2, 126.4, 143.8, 144.6, 156.4, 162.9, 172.7; FT-IR (ATR) $\upsilon_{max}$: 3329, 2977, 2933, 2875, 1648, 1522, 1447, 1284, 1252, 1169, 969, 845, 737 $cm^{-1}$; HRMS (FAB) for $C_{19}H_{31}N_2O_7$ $[M+H]^+$: 399.2131.

*N-(2-(2-(2-aminoethoxy)ethoxy)ethyl)-2-(3,4-dihydroxyphenyl)acetamide* (**Do-TEG-Am, 6**)

6 mL TFA was added to a solution of compound **5** (0.753mmol) in 15 mL dry $CH_2Cl_2$. The reaction mixture was stirred at room temperature for 2 h. 15 mL $CH_2Cl_2$ was then added and the solvent was removed under reduced pressure. The procedure was repeated three times to remove traces of TFA to afford the title compound Do-TEG-Am (**6**) as brown semi-solid (yield: 82%). $^1H$ NMR (300 MHz, $CD_3OD$): δ (ppm) 3.07 (m, 2H), 3.35-3.42 (m, 4H), 3.52 (m, 2H), 3.58-3.72 (m, 6H), 6.59-6.75 (m, 3H), 7.99 (br, 1H); $^{13}C$ NMR (75 MHz, $CDCl_3$): δ (ppm) 39.2, 40.6, 42.3, 43.4, 66.7, 67.8, 69.4, 70.1, 70.2, 70.5, 71.3, 116.4, 117.3, 121.6, 128.3, 144.1, 146.7, 173.8; FT-IR (ATR) $\upsilon_{max}$: 3353, 2937, 1781, 1654, 1498, 1449, 1167, 981, 798 $cm^{-1}$; HRMS (FAB) for $C_{14}H_{23}N_2O_5$ $[M+H]^+$: 299.1606.

***2-(2-(2-(prop-2-yn-1-yloxy)ethoxy)ethoxy)ethyl 2-(3,4-dihydroxyphenyl)acetate (Do-TEG-Alkyne)***




**Scheme S5** Synthesis of Do-TEG-Alkyne

*2-(3,4-bis((tert-butyldimethylsilyl)oxy)phenyl)acetic acid* (**7**)

Compound 7 was synthesized following literature method for TBS protection.[6, 7] To a stirred solution of *tert*-butyldimethylsilyl chloride (TBDMS-Cl) (0.036 mol) in 50 mL anhydrous acetonitrile was added 0.012 mol DOPAC. The colorless suspension was cooled on an ice bath for 10 min prior to the addition of 0.036 mol imidazole over 30 min. The reaction mixture was left stirring in an ice bath for 2 h followed by further stirring at room temperature for additional 12 h. The volatiles were then removed under reduced pressure and the residue obtained was partitioned between EtOAc (100 ml) and water (3×40 ml). The combined organic phase was washed with 100 mL brine, dried over anhydrous sodium sulfate, filtered and the solvent was removed under reduced pressure to obtain an oily mass which was further chromatographed over silica gel ($CH_2Cl_2$/MeOH 20:1) to afford compound **7** as white solid (Yield: 94%). $^1$H NMR ($CDCl_3$, 300 MHz): δ (ppm) 0.21 (s, 12H), 1.01 (s, 18H), 3.52 (s, 2H), 6.71-6.80 (m, 3H), 9.94 (br, 1H); $^{13}$C NMR ($CDCl_3$, 75 MHz): δ (ppm) -4.1, 25.9, 40.4, 120.9, 122.2, 122.3, 126.2, 146.1, 146.7 178.0; HRMS (FAB) for $C_{20}H_{37}O_4Si_2$ $[M + H]^+$: 397.2231.

*2-(2-(2-(prop-2-yn-1-yloxy)ethoxy)ethoxy)ethyl-2-(3,4-bis((tert-butyldimethylsilyl)oxy)phenyl)acetate* (**8**)

The Steglich esterification was done according to literature involving *N,N*-dicyclohexylcabodiimide (DCC) activation and *N,N*-dimethylaminopyridine (DMAP) as catalyst.[8] To a solution of TBS protected DOPAC and 0.48 mmol compound **7** in 20 mL $CH_2Cl_2$was added 0.026 mmol DMAP and 0.26 mmol 2-(2-(2-(prop-2-yn-1-yloxy)ethoxy)ethoxy)ethanol, which was prepared using a reported protocol.[9] After stirring the reaction mixture for 10 min. the solution of 0.69 mmol DCC in 8 mL $CH_2Cl_2$ was added dropwise over a 45-minute period at room temperature. The reaction mixture was stirred at room temperature for 12 h. The solvent was then evaporated under reduced pressure and urea was removed by repeated precipitation with EtOAc/cyclohexane (2:8). The filtrate was concentrated under reduced pressure and the product was purified by silica gel flash chromatography starting with

cyclohexane to cyclohexane/EtOAc (10:3), affording compound **8** as colorless oil (Yield: 89%). $^{1}$H NMR ($CDCl_3$, 300 MHz): δ (ppm) 0.17 (s, 12H), 0.97 (s, 18H), 2.41 (t, *J* = 2.3 Hz, 1H), 3.50 (br, 2H), 3.62 (br, 4H), 3.64-3.71 (m, 6H), 4.18 (d, *J* = 2.3 Hz, 2H), 4.20-4.24 (m, 2H), 6.68 (dd, *J* = 8.0, 2.2 Hz, 1H,), 6.54 (d, *J* = 8.0, 1H), 6.57 (d, *J* = 2.2 Hz, 1H); $^{13}$C NMR ($CDCl_3$, 75 MHz): δ (ppm) -4.1, 25.3, 40.4, 58.3, 63.8, 68.0, 70.5, 70.6, 72.6, 74.5, 79.6, 120.8, 122.1, 126.8, 145.9, 146.6, 171.7; FT-IR (ATR) $\upsilon_{max}$: 3312, 2928, 2856, 2118, 1736, 1606, 1577, 1360, 1291cm$^{-1}$; HRMS (FAB) for $C_{29}H_{51}Si_2O_7$ $[M + H]^+$: 567.3171.

*2-(2-(2-(prop-2-yn-1-yloxy)ethoxy)ethoxy)ethyl 2-(3,4-dihydroxyphenyl)acetate* (**Do-TEG-Alkyne**, **9**)

To a solution of 1.76 mmol *tert*-butyldimethylsilyl (TBS)-protected compound 8 in 25 mL anhydrous THF was added 1.59 mmol tetra-n-butylammonium fluoride ($TBAF{\cdot}3H_2O$). The reaction mixture was stirred at room temperature for 1.5 h. After complete conversion (monitored by TLC), the reaction mixture was quenched by addition of 3 mL saturated $NaHCO_3$ solution. The reaction mixture was diluted with 50 mL water and extracted with EtOAc (3×40 mL). The combined organic layer was washed with 25 mL brine and 25 mL water. The organic layer was then dried over anhydrous sodium sulfate and concentrated under reduced pressure to obtain a brown residue which was purified by flash chromatography on a silica gel column starting with cyclohexane to cyclohexane/EtOAc (1:1) to obtain the title compound Do-TEG-Alkyne (**9**) as colorless oil (Yield: 91%). $^{1}$H NMR ($CDCl_3$, 300 MHz): δ (ppm) 2.42 (t, *J* = 2.3 Hz, 1H), 3.42 (br, 2H), 3.52-3.56 (m, 2H), 3.57-3.60 (m, 4H), 3.62 (br, 4H), 4.10 (d, *J* = 2.3 Hz, 2H), 4.14-4.18 (m, 2H), 6.56 (dd, *J* = 8.1, 1.9 Hz, 1H,), 6.70 (d, *J* = 8.1 Hz, 1H), 6.75 (d, *J* = 1.9 Hz, 1H); $^{13}$C NMR ($CDCl_3$, 75 MHz): δ (ppm) 40.5, 58.3, 63.8, 68.8, 68.9, 70.0, 70.2, 70.4, 74.9, 79.3, 115.3, 116.2, 121.3, 125.6, 143.5, 144.1, 172.3; FT-IR (ATR) $\upsilon_{max}$: 3279, 2874, 2115, 1730, 1607, 1520, 1351, 1286 cm$^{-1}$; HRMS (FAB) for $C_{17}H_{23}O_7$ $[M + H]^+$: 339.1292.

**3. Preparation of Cu NPs**

In a typical procedure, 0.5 mmol ligand was dissolved in 5 mL $H_2O$ or the mixture of $H_2O$ and acetonitrile, and then heated to 80 $^{o}$C in an oil bath with magnetic stirring. 5 mL aqueous solution of $CuCl_2{\cdot}2H_2O$ (0.1 M) was injected into the solution of ligand drop by drop. The reaction was kept at 80 $^{o}$C until the color of the solution became dark brown (3 ~ 8 h depending on which ligand was used). The as-prepared aqueous solutions of Cu NPs were stable against oxidation over months of storage at 4 $^{o}$C. The selected area electron diffraction was performed on DA-coated Cu NPs (Fig. S1), and the calculated interface distance d is 0.360 nm indicating the presence of fcc Cu ($d_0$ = 0.3613 nm).

The stability of the Cu NPs after 2 months of storage was confirmed by TEM (Fig. S2) and zeta potential analysis (Table S1). No significant changes were observed in the morphologies and the zeta potential values of the Cu NPs.

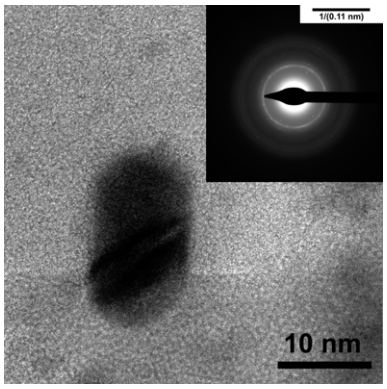



**Fig. S1** Selected area electron diffraction pattern of representative DA-Cu NP

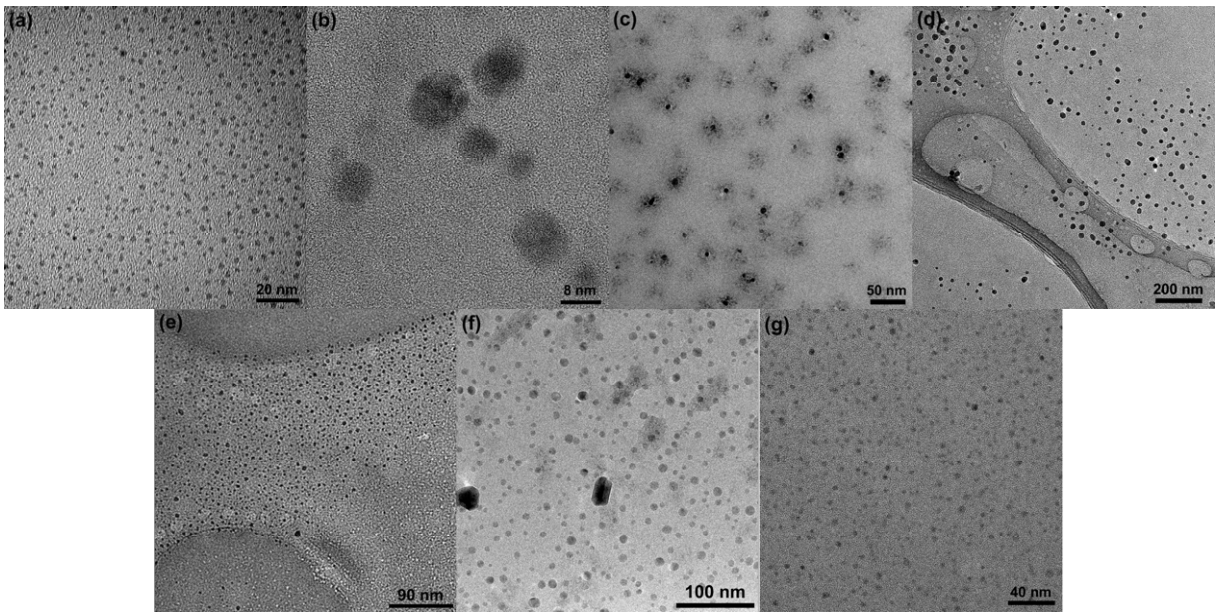



**Fig. S2** TEM images of (a) AA-Cu, (b) DA-Cu, (c) DoMal-Cu, (d) NorMal-Cu, (e) Do-TEG-Mal-Cu, (f) Do-TEG-Am-Cu and (g) Do-TEG-Alkyne-Cu after two months of storage

**Table S1** Zeta potential ($\zeta$) of Cu NPs after two months of storage

| | $\zeta$ / mV |
|---|---|
| AA-Cu | -5.47±0.18 |
| DA-Cu | 32.1±0.5 |
| DoMal-Cu | 5.63±0.27 |
| NorMal-Cu | 1.91±0.32 |
| Do-TEG-Mal-Cu | 1.91±0.46 |
| Do-TEG-Am-Cu | 50.4±2.0 |
| Do-TEG-Alkyne-Cu | -4.38±0.31 |

FTIR was used to analyze the dopamine linkers before and after the synthesis of NPs (Fig. S3 shows the spectra of DA, DA-Cu, DoMal and DoMal-Cu). The stretching band of catechol groups around 3400 $cm^{-1}$ disappeared after the synthesis of Cu NPs indicating the coordination between catechol groups and Cu.

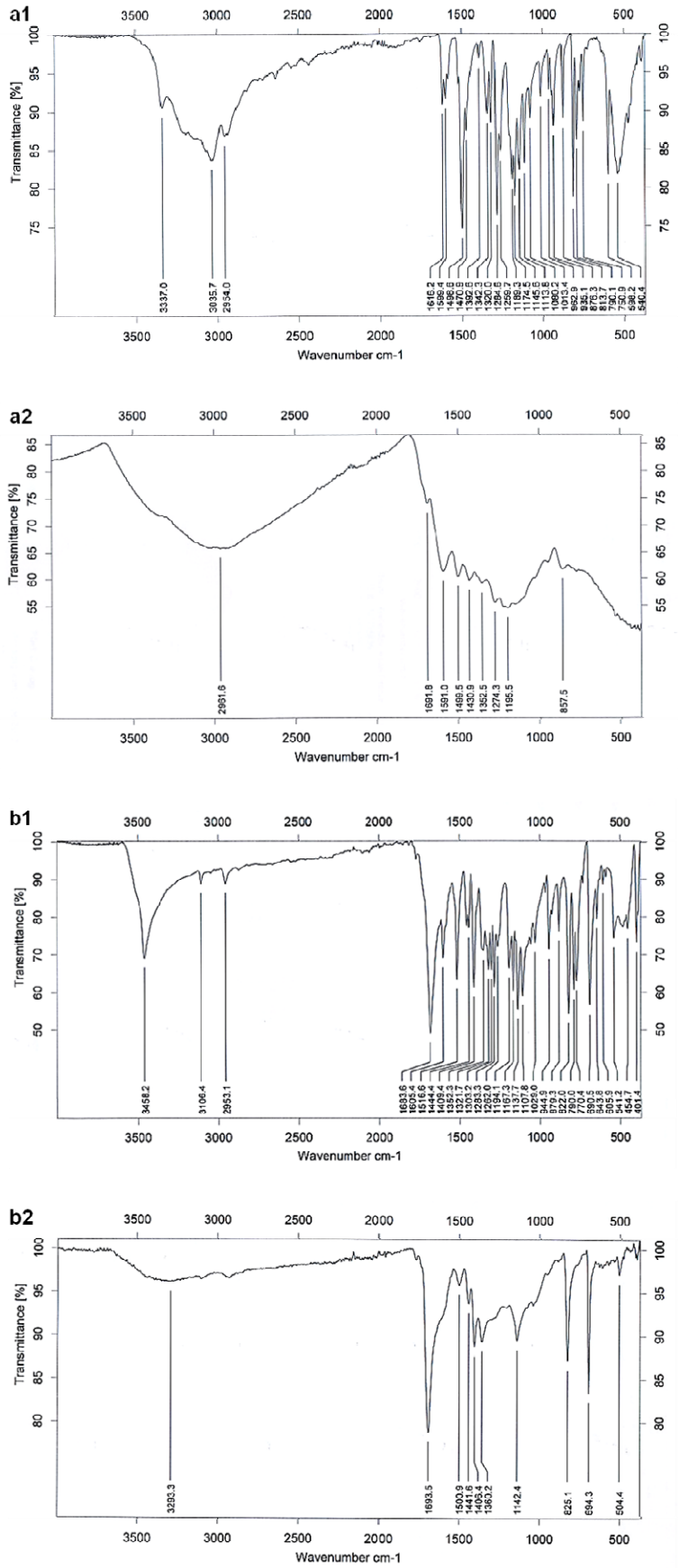



**Fig. S3** FTIR spectra of (a1) DA, (a2) DA-Cu NPs, (b1) DoMal and (b2) DoMal-Cu NPs

## 4. Peroxidase Activity Tests for Cu NPs (Amplex Red Assay)

Peroxidase activity tests were performed by adding different volumes of as-prepared Cu NPs (0 ~ 7 µL) to the mixture of 10 µL Amplex Red (AR, 1 mM) and 37.6 µL horseradish peroxidase (HRP, 50 U/mL). Phosphate buffer (pH = 6) was used to adjust the total volumes to 150 µL. A microplate reader was used to record the fluorescence signals of resorufin ($\lambda_{ex}$ = 540 nm, $\lambda_{em}$ = 585 nm, sensitivity: 95), which was formed by the oxidation of AR. Control experiments were done in the absence of Cu NPs or HRP. Fig. S4 shows that the fluorescence intensity of resorufin first increased and then began to decrease upon addition of larger volume of Cu. We hypothesize that after a certain saturation point there would be large amount of resorufin presented in the solution which was then in turn oxidized by ROS.

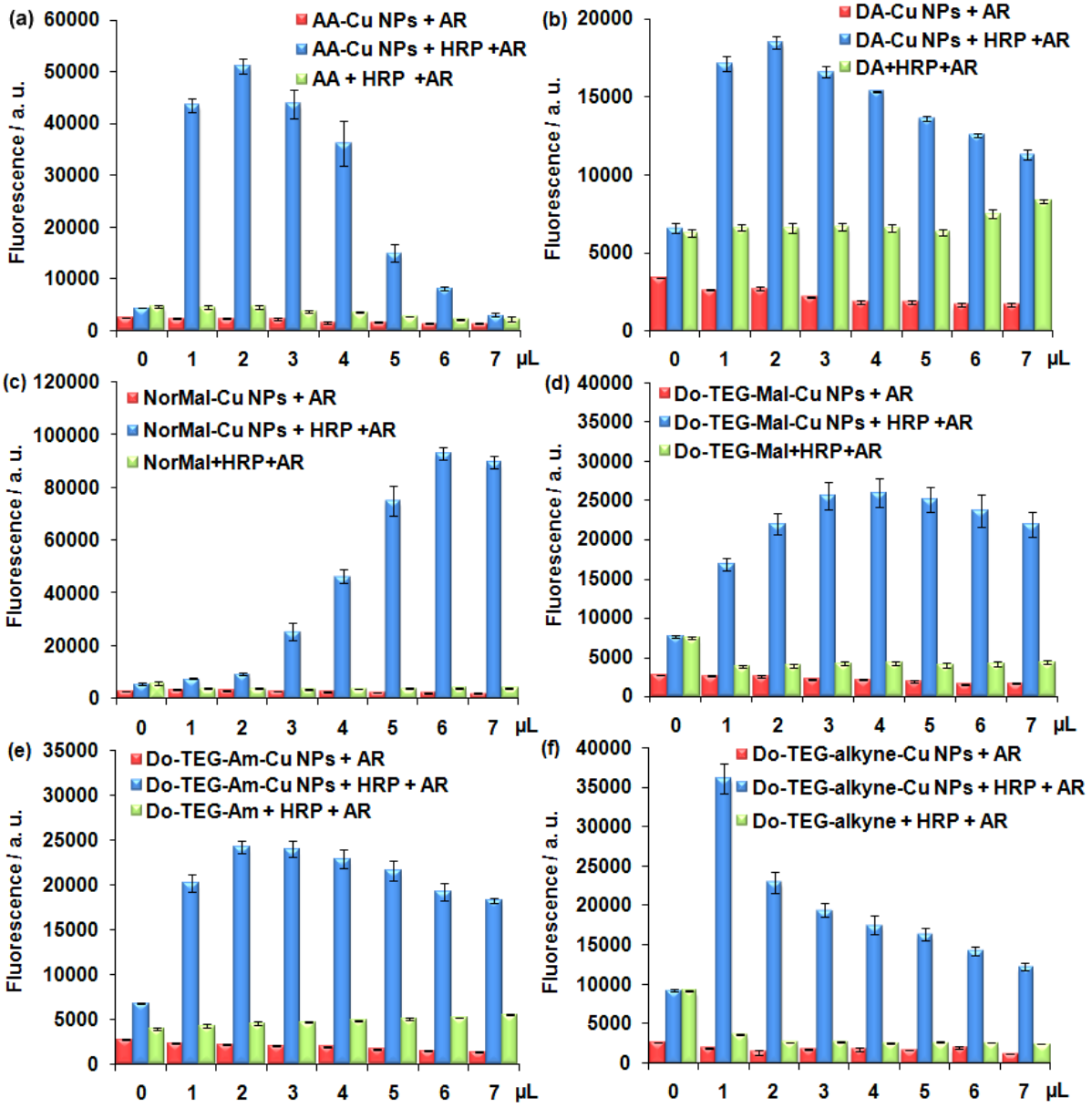



**Fig. S4** Peroxidase activity tests by using different volumes of (a) AA-Cu NPs, (b) DA-Cu NPs, (c) NorMal-Cu NPs, (d) Do-TEG-Mal-Cu NPs, (e) Do-TEG-Am-Cu NPs, (f) Do-TEG-Alkyne-Cu NPs and corresponding controls in the absence of Cu NPs or HRP

Peroxidase activity of the Cu NPs was checked again after several months of storage, and the results (Fig. S5b) are comparable with that of the freshly made Cu NPs (Fig. S5a), which indicate that the NPs are stable.

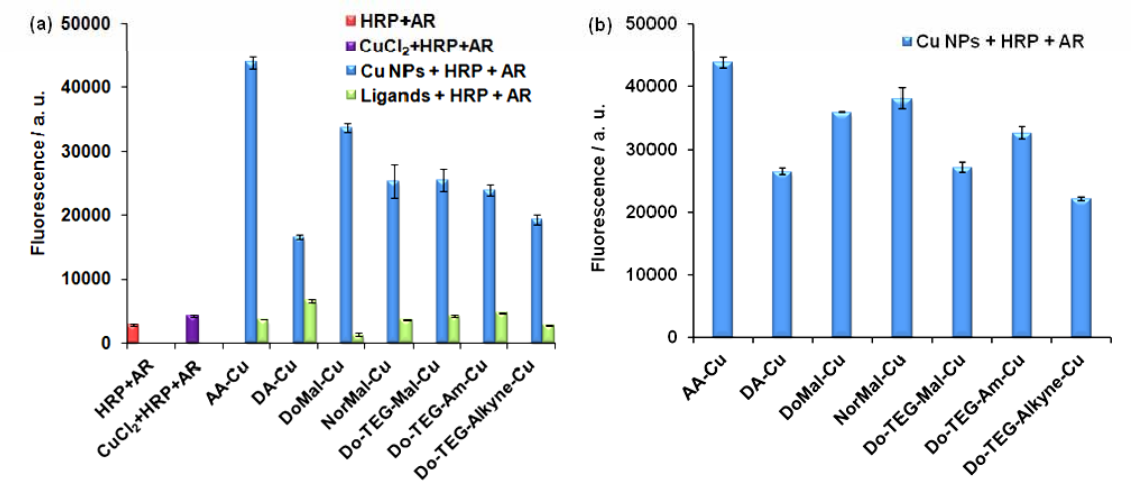



**Fig. S5** Amplex red assay using (a) freshly made Cu NPs (3 µL) and (b) Cu NPs after months of storage (3 µL)

## 5. Influence of Cu NPs on the Fluorescence of Resorufin

10 µL aqueous solutions of different ligands coated Cu NPs were mixed with 10 µL resorufin (100 µM). Phosphate buffer (pH = 6) was used to adjust the total volumes to 150 µL. Fluorescence signals of resorufin were recorded on a microplate reader (Fig. S6, sensitivity: 95). Control experiments were done by adding different ligands instead of Cu NPs to resorufin.

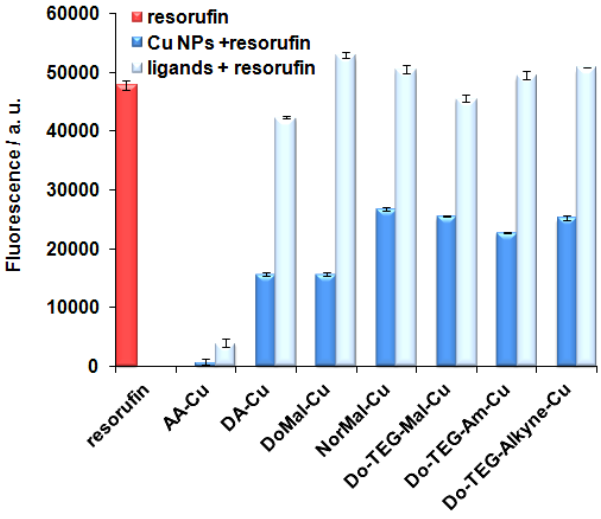



**Fig. S6** Influence of different Cu NPs and their corresponding ligands on the fluorescence of resorufin

## 6. ROS Inhibition by Catalase

2 µL aqueous solutions of different ligands coated Cu NPs were mixed with 10 µL catalase (2,000 U/mL), then 37.6 µL HRP (50 U/mL) and 10 µL AR (1 mM) were added. Phosphate buffer (pH = 6) was used to adjust the total volumes to 150 µL. Fluorescence signals of the formed resorufin were

recorded on a microplate reader (sensitivity: 95). Control experiments were done in the absence of catalase.

### 7. $H_2O_2$ Calibration

The calibration curve was made by using $H_2O_2$ (50 μM) for the Amplex Red assay. Different volumes of $H_2O_2$ (50 μM, 0 ~ 5 μL) were added to the mixture of 10 μL AR (1 mM) and 37.6 μL HRP (50 U/mL). Phosphate buffer (pH = 6) was used to adjust the total volumes to 150 μL. A microplate reader was used to record the fluorescence signals of resorufin (sensitivity: 95). By comparing the fluorescence intensity of resorufin formed in the experiments using Cu NPs (3 μL) with that formed in the experiments using $H_2O_2$, the ROS production ability of Cu NPs could be calculated (Fig. S7).

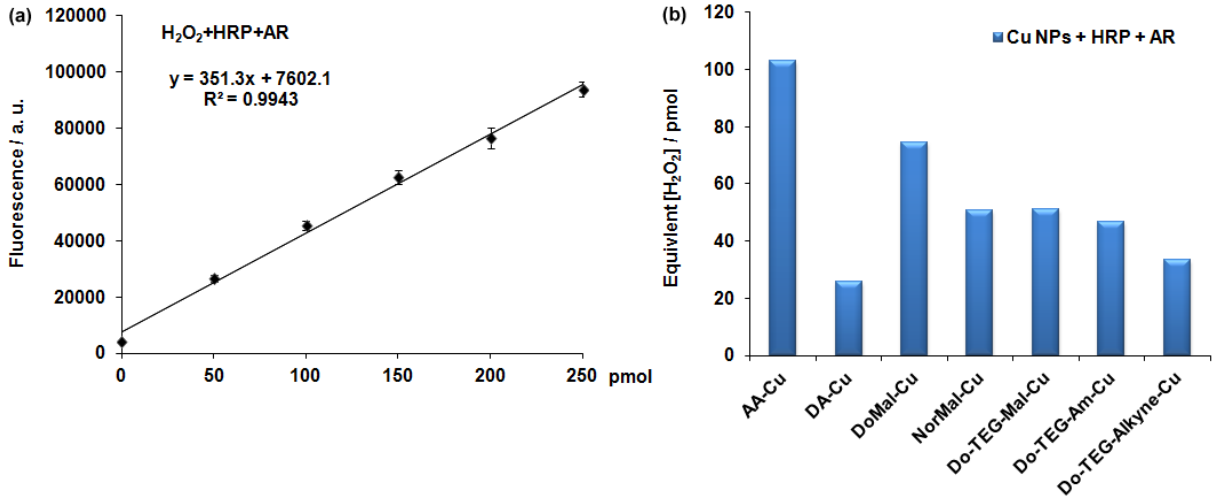
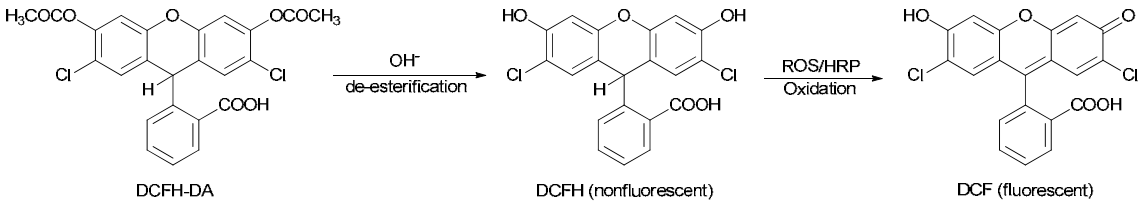



**Fig. S7** (a) $H_2O_2$ calibration curve and (b) the ROS production ability of Cu NPs (3 μL)

### 8. ROS Production of Cu NPs (DCFH Assay)

The ROS production ability of Cu NPs was confirmed again using 2,7-dichlorodihydrofluorescein (DCFH), which could be oxidized to fluorescent 2,7-dichlorofluorescein (DCF) by $H_2O_2$ in the presence of peroxidase (Scheme S6). Firstly, 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA) was de-esterificated by NaOH (0.01 N), and then the reaction was stopped by adding sodium phosphate buffer (pH = 7.2)[10]. 3 μL aqueous solutions of different ligands coated Cu NPs were added to the mixture of 100 μL DCFH (20.8 μM) and 25 μL HRP (50 U/mL) (Fig. S8). Fluorescence signals of DCF ($\lambda_{ex}$ = 498 nm, $\lambda_{em}$ = 535 nm, sensitivity: 80) were recorded on a microplate reader. Control experiments were done in the absence of Cu NPs or using Cu (II) salt precursor instead of Cu NPs, which indicate that the presence of Cu NPs is necessary for the oxidation of DCFH.




**Scheme S6** Schematic illustration of DCFH-DA de-esterification to DCFH and then oxidation to DCF

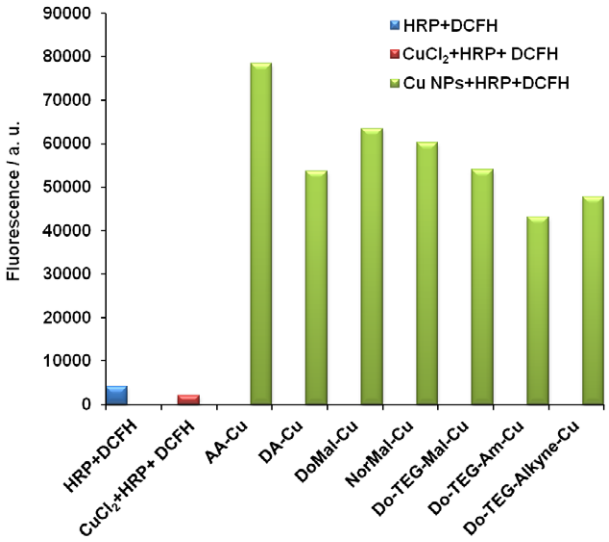



**Fig. S8** DCFH assay using 3 μL solutions of different Cu NPs

## 9. Degradation of Fluorescent Dyes by Cu NPs

Degradation tests were performed by adding 10 μL aqueous solutions of different ligands coated Cu NPs to fluorescent dyes Rhodamine B (RB, 20.8 μM) or methylene blue (MB, 31.2 μM). Phosphate buffer (pH = 6) was used to adjust the total volumes to 150 μL. Fluorescence signals of RB ($\lambda_{ex}$ = 540 nm, $\lambda_{em}$ = 625 nm) and MB ($\lambda_{ex}$ = 650 nm, $\lambda_{em}$ = 685 nm) were recorded on a microplate reader. Control experiments were carried out by adding different ligands instead of Cu NPs to RB or MB.

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