Electronic Supplementary Information

Structure-dependent detection of polyphenols using crown ether-immobilized gold nanoparticles

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1. Experimental

1-1. Materials and instruments: Following materials were used in this study.

Hydrogen Tetrachloroaurate(III) Tetrahydrate (FUJIFILM Wako Pure Chemical Corporation)
Sodium Citrate Tribasic Dihydrate (Sigma-Aldrich)
Tannic acid (FUJIFILM Wako Pure Chemical Corporation)
Phosphate buffer（0.1M, pH7.4）（Nacalai Tesque, Inc.）
4’-Aminobenzo-18-Crown-6 (Sigma-Aldrich)
4’-Aminobenzo-15-Crown-5 (Tokyo Chemical Industry Co., Ltd.)
WSCD・HCl (Fujifilm Wako Pure Chemical Corporation)
DL-α-Lipoic Acid (Tokyo Chemical Industry Co., Ltd.)
Silica gel 60N, 63-210 μm (Kanto Chemical Co., Inc.)
Dichloromethane, Super Dehydrated and Methanol, Ethanol (Fujifilm Wako Pure Chemical Corporation)
(-)-Epicatechin gallate (Tokyo Chemical Industry Co., Ltd.)
(-)-Epicatechin (Tokyo Chemical Industry Co., Ltd.)
(-)-Epigallocatechin (Tokyo Chemical Industry Co., Ltd.)
(-)-Epigallocatechin Gallate Hydrate (Tokyo Chemical Industry Co., Ltd.)
Gallic acid hydrate (Tokyo Chemical Industry Co., Ltd.)
Theaflavin (Tokyo Chemical Industry Co., Ltd.)
L-Tyrosine (Tokyo Chemical Industry Co., Ltd.)
L-Ascorbic acid (Sigma-Aldrich)
Phenol reagent (Kanto Chemical Co. Inc.)

Sodium Carbonate (Na_2CO_3) (Fujifilm Wako Pure Chemical Corporation)

Ultrapure water was made by Smart2Pure, Thermo Scientific.

The hydrodynamic diameters and zeta potentials were measured at 25°C using an ELSZ-2000 analyzer (Otsuka Electronics, Japan). UV-Visible absorbance was monitored using a UV-2600 spectrophotometer (Shimadzu, Japan). ^{1}H-NMR spectra were recorded on an ECS-400 system (JEOL Ltd., 400MHz). ESI-MS measurements were performed by Exactive (Themo Fisher Scientific) at the Center for Analytical Instrumentation, Chiba University.

FTIR spectra of synthetic ligands were recorded on a FT/IR-4200 spectrometer (JASCO, Japan). The morphology and size of gold nanoparticles was examined by scanning transmission electron microscopy (STEM, HD-2000, Hitachi High-Tech Corporation, Japan) using a 200 kV beam. The sample was cast on a plasma-treated TEM grid (collodion membrane-coated Cu 400 mesh, Nisshin EM, Japan).

1-2. Synthesis of gold nanoparticles

We synthesized gold nanoparticles by a previously published method[1] with some modifications.

First, the following two aqueous solutions (a) and (b) were prepared in a plastic tube.

(a) Sodium citrate tribasic aqueous solution (10 mg/ml in pure water)

(b) Tannic acid aqueous solution (10 mg/ml in pure water)

Ultrapure water (8 ml), solution (a) 2 ml, and solution (b) 8 µl were added to a 15 ml plastic tube, and the mixture was stirred for 1 minute using a vortex mixer. This solution was used as the reducing solution. Ultrapure water (40 ml) was placed in a 100 ml Erlenmeyer flask and stirred while raising the temperature on a magnetic stirrer equipped with a heater. When the temperature reached 80 °C, 150 µl of $HAuCl_4$ aqueous solution (0.1 M) was added to the flask using a micropipette, then the reducing solution prepared above was added at once and stirring was continued for 15 min while keeping the temperature between 80 and 90 °C to give a red solution of gold nanoparticles.

1-3. Preparation of LA-AuNPs in the basic solution

We synthesized LA-AuNPs by a previously published method[2] with some modifications. To a 50 ml plastic tube was added 25 mg of DL-α-Lipoic Acid, 10 ml of ultrapure water, and

25 ml of the above synthesized gold nanoparticle aqueous solution. The solution pH was then adjusted to approx. pH 11.5 by adding sodium hydroxide aqueous solution (1.0 M). At this pH, the solution was transparent, and this was incubated at room temperature for 14 days to give LA-AuNPs.

1-4. Preparation of 18C6-AuNPs as a sensor solution

An aqueous solution of 18C6-AuNPs (sensor solution) was prepared by the following procedure.

(1) The above synthesized LA-AuNP aqueous solution was dispensed into 10 microtubes in 1 ml portions and then centrifuged at 12,000 rpm for 20 minutes.

(2) The supernatant was discarded, leaving 300 μl, and the residue (10 AuNP pellets) was combined into one tube and dispersed in the supernatant (300 μl) to produce a concentrated AuNP solution. This concentrated solution was stored in a refrigerator and sonicated using a sonication bus for 10 min before use.

(3) Next, 95 μl of concentrated solution (2) was transferred to a microtube and 5 μl of methanolic solution of 18C6-LA (25 mg/15 ml in methanol, 3.2 mM) was added and let stand for 10 minutes.

(4) After centrifugation (12000 rpm, 10 min), the supernatant was discarded, and 100 μl of pH 7.4 phosphate buffer (100 mM, Purchased from Nakalai Tesque. Japan) was added. This concentrated 18C6-AuNP solution is stable without aggregation and was used within 12 hours after preparation for Fig. 3~8. For the polyphenol assay in Figure 9, the sensor solution was prepared using a concentrated 18C6-AuNP solution that had been stored in a refrigerator for at least 2 days. The red color of the concentrated 18C6-AuNP solution was maintained without aggregation even after a longer period (at least 10 days).

（5）Finally, 50 μl of concentrated 18C6-AuNP solution was transferred into a disposable plastic cuvette, and 950 μl of buffer solution (pH 7.4, 100 mM phosphate buffer) was added. This diluted aqueous solution was used as a sensor for various polyphenols and beverages.

1-5. Preparation of 15C5-AuNPand LA-AuNP as a sensor solution (control)

For 15C5-AuNP, a methanolic solution of 15C5-LA (46 mg/30 ml in methanol, 3.3 mM) was used. Other procedures were the same as for the preparation of the 18C6-AuNP sensor solution. For LA-AuNPs, instead of a methanolic solution of 18C6-LA, the same volume of pure water was added before centrifugation. Other procedures were the same as for the preparation of the 18C6-AuNPs sensor solution.

1-6. Sensing of polyphenols, L-ascorbic acid and L-tyrosine

Sample solutions of the following polyphenol reagents were prepared and an aliquot was added to 1 ml of sensor solution in a PMMA cuvette to achieve the desired concentration. After pipetting, the cuvettes were left at room temperature for at least 20 min and the absorbance at 650 nm or spectra (400-700 nm) was measured. The volume added was 1 to 20 µL per sensor solution (1 ml). For beverage samples (green tea, red wine, barley tea), 20 µl of sample were added to each sensor solution and left for at least 20 min.

(1) Tannic acid: Aliquots of 1µl (0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 3.0 mg/mL in H_2O) was added to each cuvette (1 ml). At concentration 0(= [0 µg/ml]), 1 µl of H_2O only was added.

(2) (-)-Epicatechin gallate: Aliquots of 1µl (0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 3.0 mg/mL in ethanol) and aliquots of 1, 2, 3 µl (5mg/ml in ethanol) were added to each cuvette (1 ml). At concentration 0 (= [0 µg/ml]), 3 µl of ethanol only was added.

(3) (-)-Epicatechin: Aliquots of 1, 2, 3 µl (5mg/ml in ethanol) were added to each cuvette (1 ml). At concentration 0 (= [0 µg/ml]), 4 µl of ethanol only was added.

(4) (-)-Epigallocatechin: Aliquots of 1, 2, 3 µl (5mg/ml in ethanol) were added to each cuvette (1 ml). At concentration 0 (= [0 µg/ml]), 3 µl of ethanol only was added.

(5) (-)-Epigallocatechin Gallate Hydrate: Aliquots of 1µl (0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 3.0, 5.0, 10 mg/mL in H_2O) were added to each cuvette (1 ml). At concentration 0(= [0 µg/ml]), 1 µl of H_2O only was added.

(6) Gallic acid hydrate: Aliquots of 1µl (0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 3.0 mg/mL in H_2O) were added to each cuvette (1 ml). At concentration 0(= [0 µg/ml]), 1 µl of H_2O only was added.

(7) Theaflavin: Aliquots of 2, 4, 6 µl (2.5 mg/ mL in ethanol:H_2O = 50:50 v/v%) were added to each cuvette (1 ml). At concentration 0(= [0 µg/ml]), 6 µl of H_2O:ethanol (50:50 v/v%) only was added.

(8) L-Tyrosine: An aliquot of 1 µl (1.0 mg/ ml in 0.1M NaOHaq) and aliquots of 2, 4, 6, 8, 10 µl (10 mg/mL in 0.1M NaOHaq) were added to each cuvette (1 ml). At concentration 0(= [0 µg/ml]), 10 µl of 0.1 M NaOHaq only was added.

(9) L-Ascorbic acid: Aliquots of 1µl (1.0, 10, 20, 40, 60, 80, 100 mg/mL in H_2O) was added to each cuvette (1 ml). At concentration 0(= [0 µg/ml]), 1 µl of H_2O only was added.

1-7. Synthesis of surface ligands

1-7-1. Synthesis of 18C6-LA

In a 100-ml eggplant flask, 70 mg of DL-α-lipoic acid (Mw 206.3, 0.34 mmol) and 70 mg of WSC (Mw: 191.7, 0.37 mmol) were added to about 10 ml of dichloromethane and stirred. Then, 4'-aminobenzo-18-Crown6 was added and stirred for 1 day. The color of the solution after stirring was brown. The solvent was removed and dissolved in a 9:1 mixture of dichloromethane and methanol. Purification by silica gel column chromatography afforded a pale yellow solid (100 mg, 0.19 mmol) at 56% yield.

ESI-MS: m/z = 538.1889 (Observed) for Theoretical $[M+Na]^+$ 538.1904. ^{1}H-NMR (400MHz, $CDCl_3$), δ(ppm): 7.34 (d, 1H), 7.16(s, 1H), 6.89-6.77(m, 2H), 4.18-4.10(m, 4H), 3.94-3.88(m, 4H), 3.79-3.66(m, 12H), 3.62-3.52(m, 1H), 3.22-2.98(m, 2H), 2.52-2.42(m, 1H), 2.38-2.30(m, 2H), 1.98-1.86(m, 1H), 1.84-1.64(m, 4H),1.60-1.42(m, 2H)

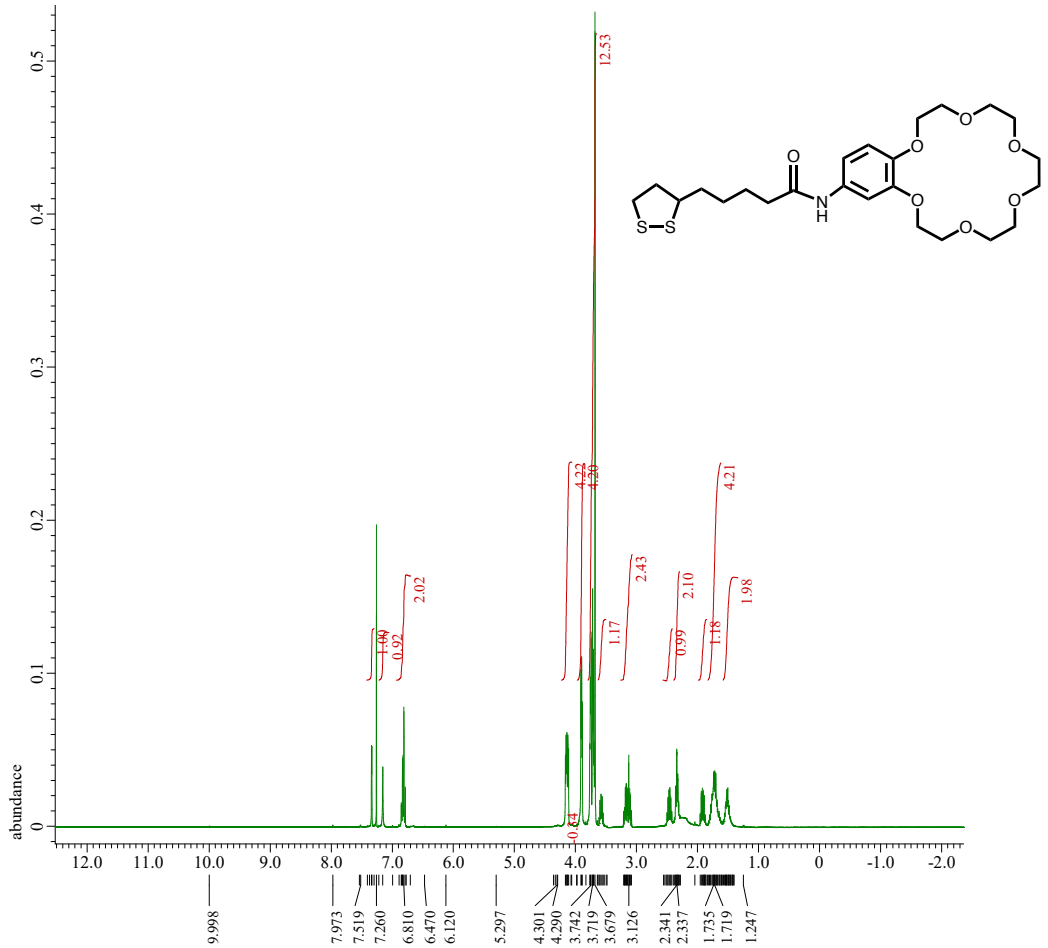


Fig. S1. ^{1}H-NMR spectrum for the 18C6-LA ligand in $CDCl_3$

1-7-2. Synthesis of 15C5-LA

In a 100-ml eggplant flask, 360 mg of DL-α-lipoic acid (Mw 206.3,1.74 mmol) and 204 mg of WSC (Mw: 191.7, 1.06 mmol) were added to about 15 ml of dichloromethane and stirred. Then, 311 mg of 4'-aminobenzo-15-Crown-5 (Mw:283.3, 1.10 mmol) was added and stirred for 3 days. The solvent was removed and dissolved in 8:2 mixture of dichloromethane and methanol. Purification by silica gel column chromatography afforded a blown solid (46 mg, 0.19 mmol) at 17 % yield.

ESI-MS: m/z = 494.1637 (Observed) for Theoretical $[M+Na]^+$ 494.1642. ^{1}H-NMR (400MHz, $CDCl_3$), δ(ppm): 7.35 (bs, 1H), 7.24 (bs, 1H), 6.85-6.75 (m, 2H), 4.16-4.07 (m, 4H), 3.92-3.86 (bs, 4H), 3.77-3.72 (bs, 8H), 3.62-3.52 (m, 1H), 3.22-3.07(m, 2H), 2.51-2.41(m, 1H), 2.36-2.3 (t, 2H), 1.95-1.86 (m, 1H), 1.82-1.63 (m, 4H), 1.56-1.41 (m, 2H)

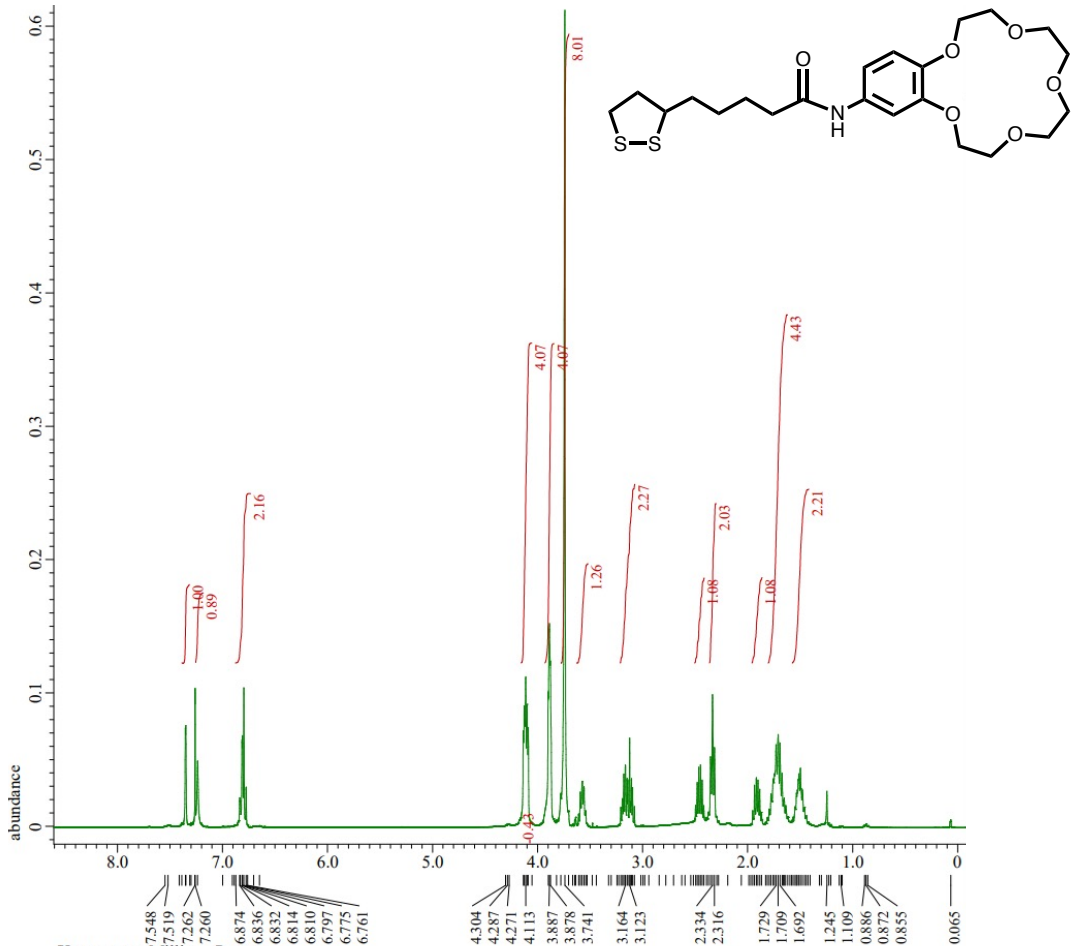


Fig. S2. ^{1}H-NMR spectrum for the 15C5-LA ligand in $CDCl_3$

1-7-3. FT-IR Spectra of synthetic ligands

The methanol solution of each sample was cast on a silicon wafer, and their FTIR spectra were measured by the transmission method (Fig. S3). The FTIR spectrum of lipoic acid (LA) was measured as a reference (Fig.S3c). 18C6-LA and 15C5-LA gave almost similar signals. The broad carboxylic acid peak in LA disappeared and a new amide-derived peak appeared at 3300 cm. Signals derived from the benzene ring and crown ether were also observed, supporting the structure of the target product. Reference [3] was referred for peak identification.

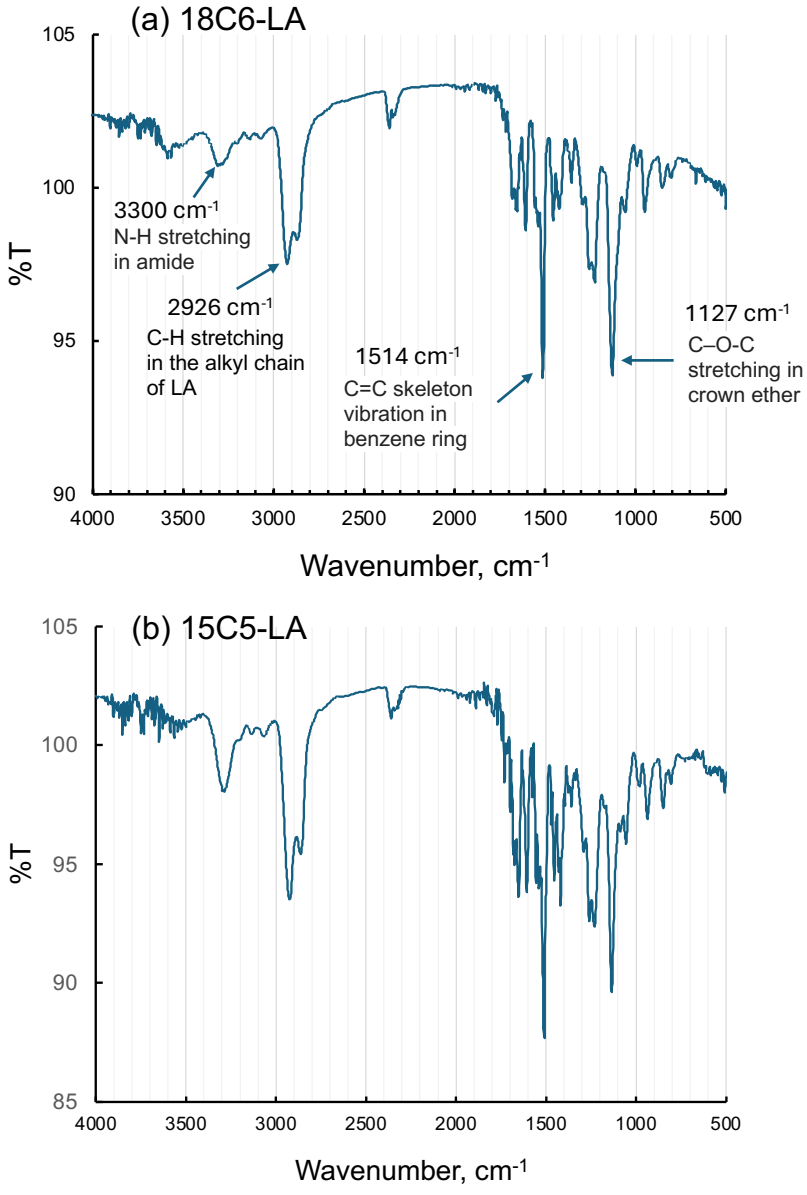


Fig. S3 (a) and (b). FTIR spectra for 18C6-LA and 15C5-LA

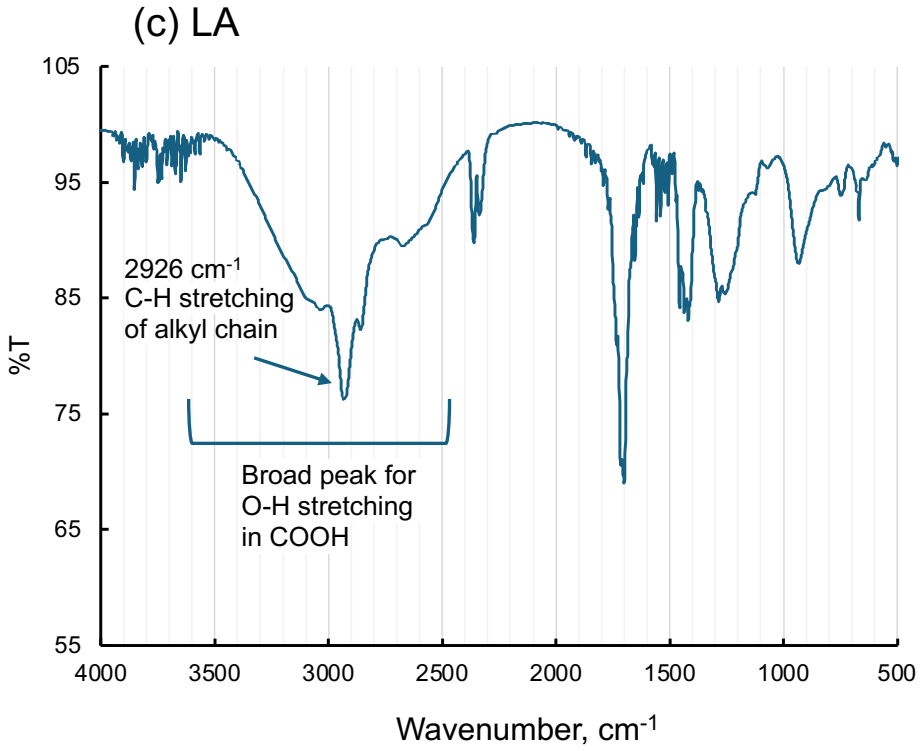
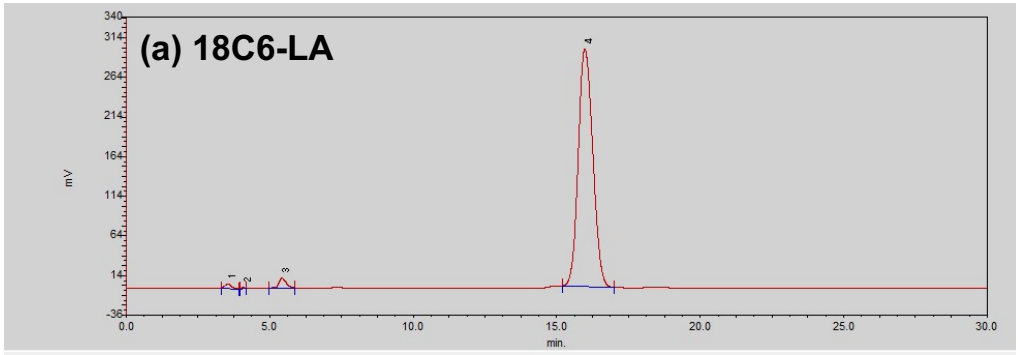


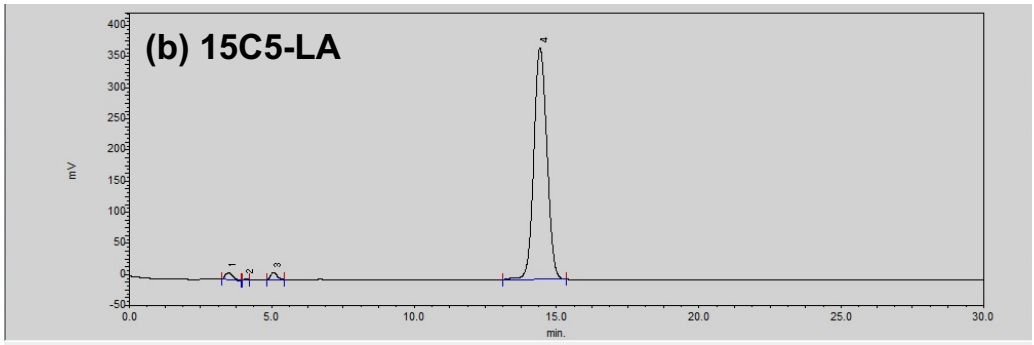
Fig. S3 (c). FTIR spectrum for lipoic acid (LA)

1-7-4. Purity measurement by high performance liquid chromatography (HPLC)

Purity of each compound was analysed by UV absorption at 254 nm using HPLC (Gulliver of JASCO Co.) with a C18 column (Osaka soda Inc., Capcell pak C18 UG-120, f 4.6–250 mm) using an elution mixture of 40% MeCN in 10 mM phosphate buffer; pH 6.8. The purity of the two synthesized compounds (15C5-La and 18C6-LA) was confirmed to be approximately 97% by HPLC.



No.	Rt(min)	area	area (%)	高さ	定量結果	NTP	対称性	分離度
1	3.57	96925.200	0.8902	5698	-------	902.7	1.174	1.602
2	4.08	9563.800	0.0878	1412	-------	9153.1	0.880	4.303
3	5.43	212142.000	1.9484	12374	-------	2260.5	1.250	15.232
4	15.98	10569110.000	97.0735	299661	-------	4739.9	1.122	-----
		10887741.000	100.0000	319145				



No.	Rt(min)	area	area (%)	高さ	定量結果	NTP	対称性	分離度
1	3.49	195754.200	1.5939	10808	-------	922.6	1.298	1.862
2	4.10	16913.200	0.1377	2118	-------	7075.1	0.892	3.210
3	5.06	172070.800	1.4011	11655	-------	2547.8	1.282	15.101
4	14.42	11896528.800	96.8673	372420	-------	4730.3	1.062	-----
		12281267.000	100.0000	397001				

Fig. S4. HPLC analyses of synthetic ligands

2. Supplementary Figures

2-1. Dynamic light scattering analyses of gold nanoparticles

The following two DLS samples are analyzed.

1. LA-AuNP solution prepared in section **1-3** above: Dispersed in lipoic acid-containing aqueous solution (pH 11.5).

2. 18C6-AuNP solution prepared in section **1-4** (5) above: Dispersed in phosphate buffer (pH 7.4, 100 mM).

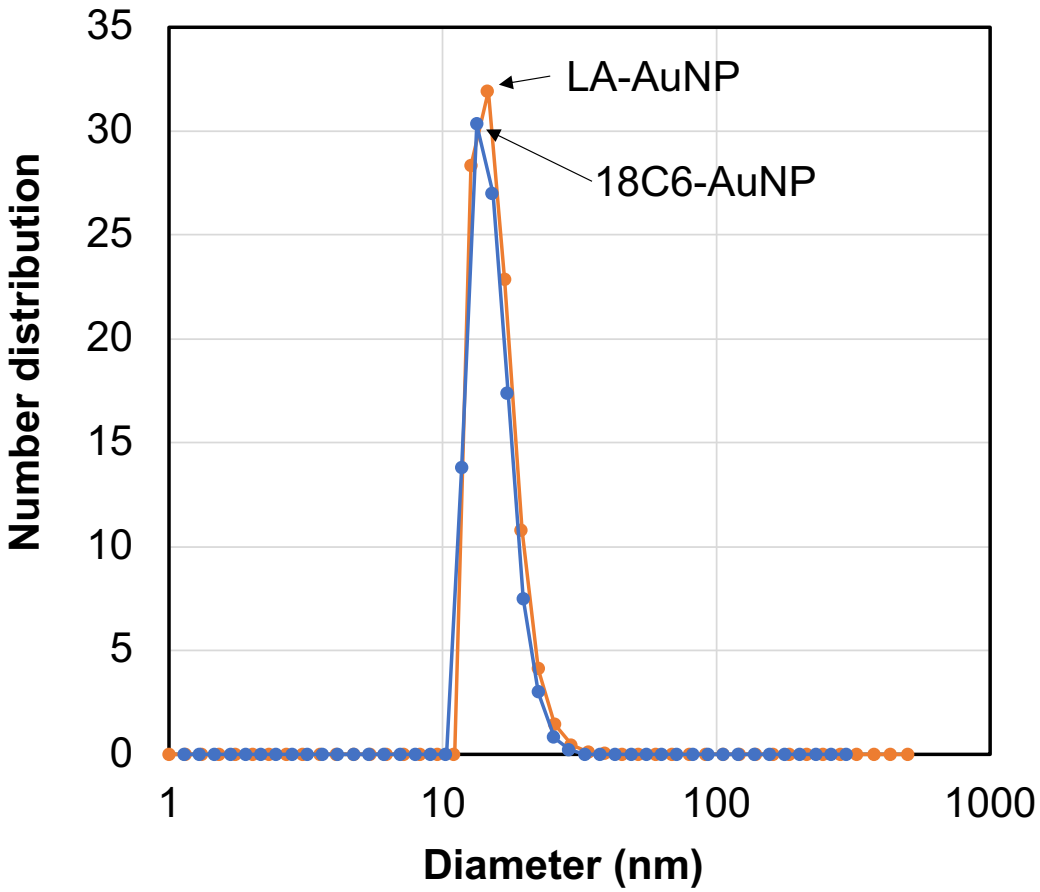


Figure S5. DLS analysis of LA-AuNPs and 18C6-AuNPs

Blue line: 18C6-AuNP, Orange line: LA-AuNP

2-2 Synthesis of Lipoic acid (LA)-coated AuNPs

The untreated AuNPs (citric acid-coated AuNPs) immediately aggregated to give a blue solution in the phosphate buffer used in this study, whereas the LA-AuNPs dispersed well in the same buffer and remain red color indicating that the coating was successful.

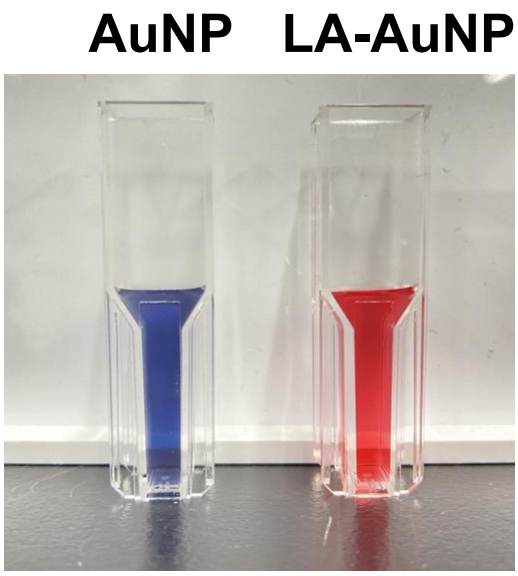
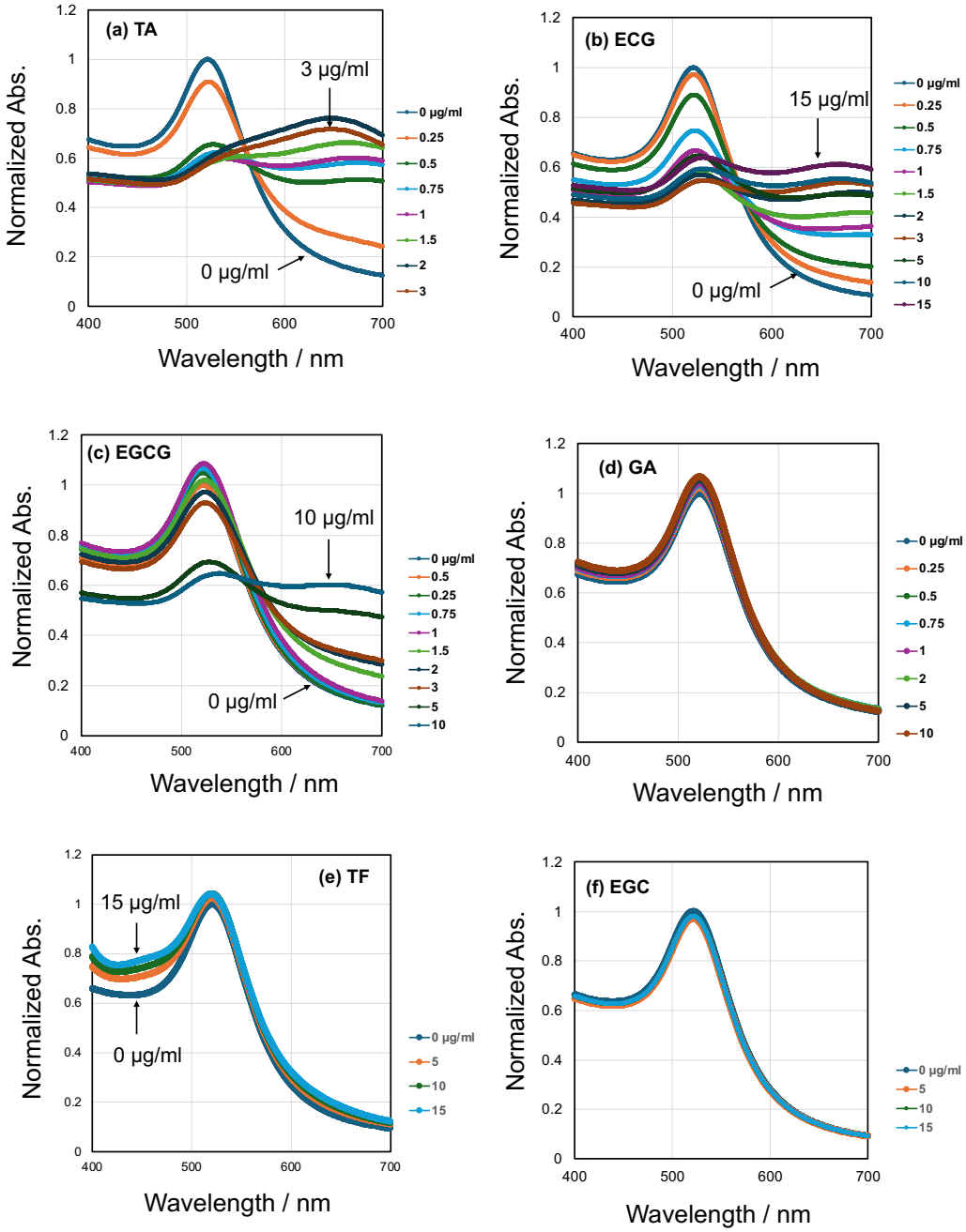


Figure S6. Photo of gold nanoparticle solution with or without lipoic acid coating.

2-3. UV-Vis spectra of 18C6-AuNPs upon addition of various polyphenols, L-tyrosine and L-ascorbic acid.



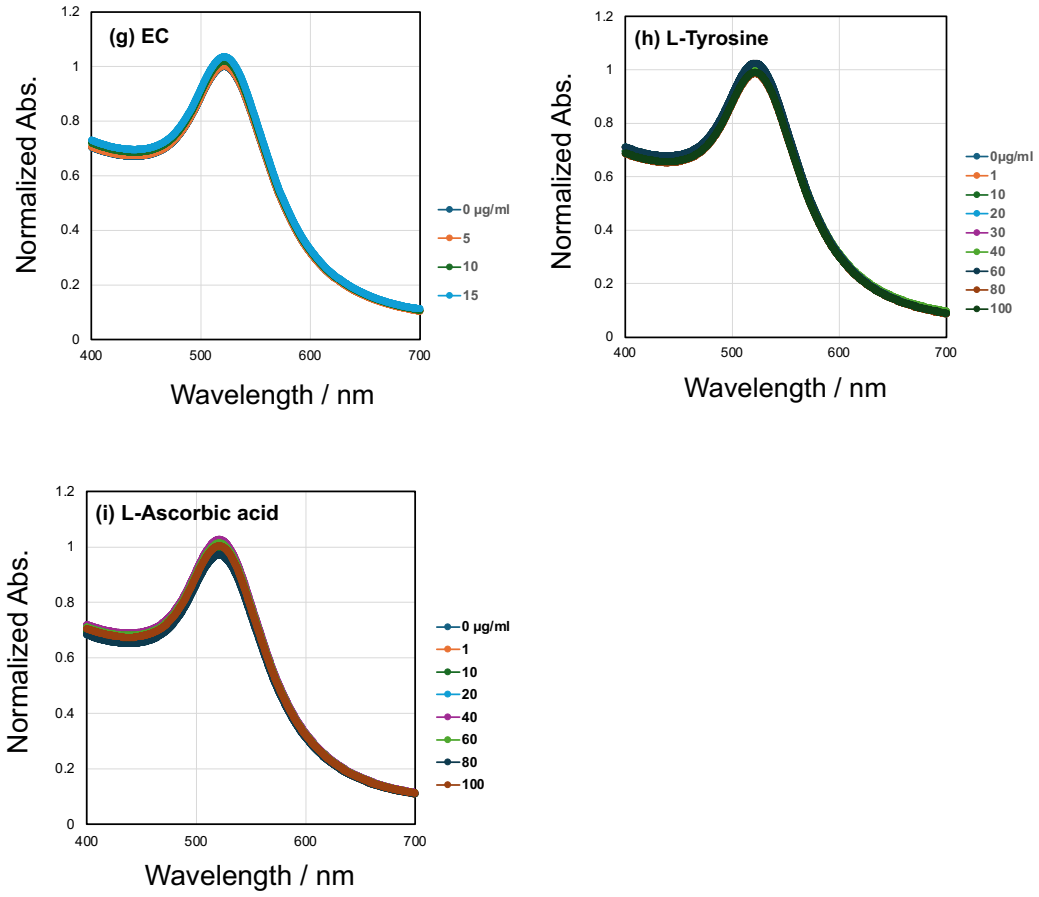


Fig. S7. Absorption spectra when various polyphenols, L-tyrosine, and L-ascorbic acid were added to 18C6-AuNP solution (sensor solution). The absorbance at 520 nm without additive (0 μg/ml) was normalized to 1.

2-4. Folin-Ciocalteu assay

Various concentrations of gallic acid, ascorbic acid and tyrosine were assayed with Phenol reagent according to the ISO protocol[4]. The absorbance at 765 nm was plotted.

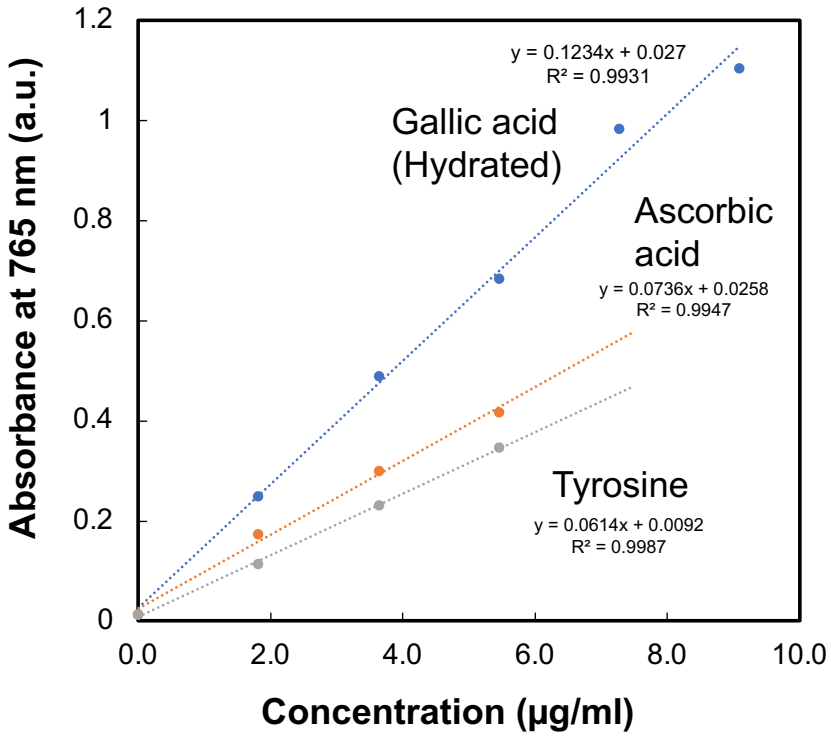


Figure S8. Folin-Ciocalteu assay for gallic acid, L-ascorbic acid and L-tyrosine

References:

[1] G. Tsutsui, S. Huang, H. Sakaue, S. Shingubara, T. Takahagi, *Jpn. J. Appl. Phys.,* 2001, **40**, 346. DOI 10.1143/JJAP.40.346

[2] J. Tournebize, A. Boudier, A. Sapin-Minet, P. Maincent, P. Leroy, R. Schneider, *ACS Appl. Mater. Interfaces* 2012, ***4***, 5790. https://doi.org/10.1021/am3012752

[3] X. Wang, X. Zheng, X. Liu, B. Zeng, Y. Xu, C. Yuan, L. Dai, *Polymers* 2022, 14, 406; https://doi.org/10.3390/polym14030406

[4] ISO 14502-1:2005. Determination of substances characteristic of green and black tea —Part 1: Content of total polyphenols in tea — Colorimetric method using Folin- Ciocalteu reagent.