Supporting Information

[60]Fullerene-based Monolayers as Neuroprotective Biocompatible Hybrid Materials

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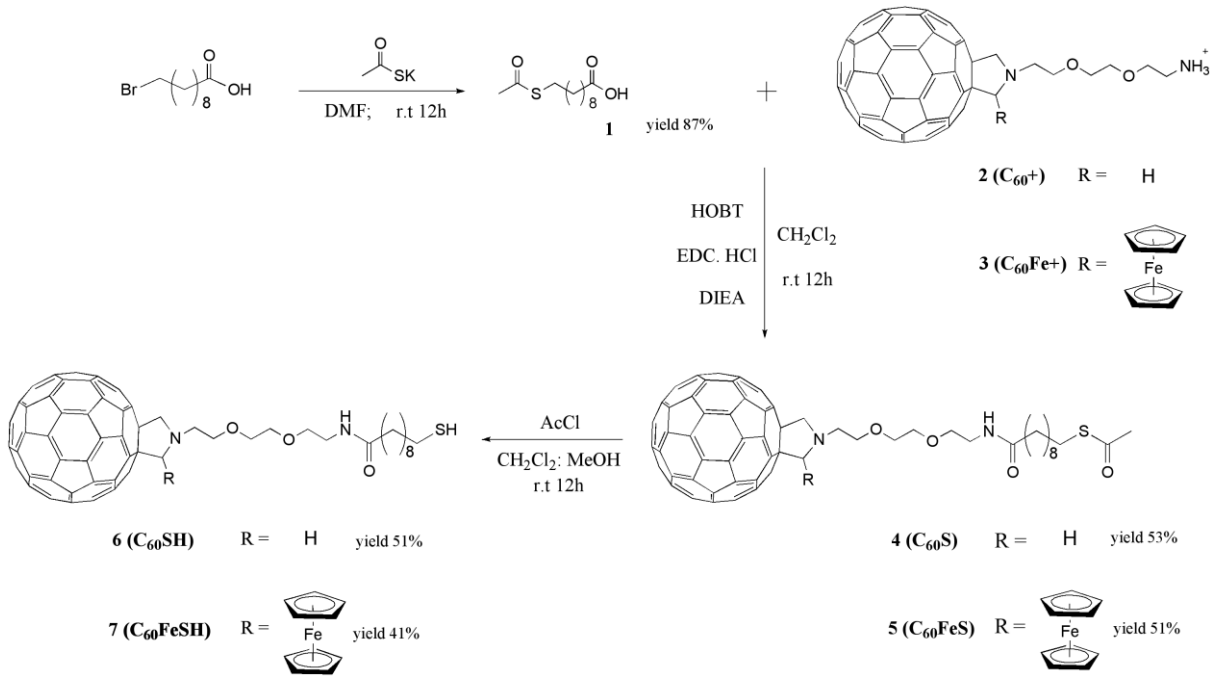
Detailed experimental procedures

Materials and methods: all reagents and solvents were used without further purification. Reactions were carried out under N_2 atmosphere and under anhydrous conditions. 1-dodecanethiol (used as reference compound) and MTT were purchased from Sigma Aldrich. Gold-coated mica (Au(111), 200 nm thickness) substrates (10 mm square) used for these experiment were purchased from Phasis (Switzerland). SH-SY5Y cells were purchased from ATCC, whereas DMEM medium and Trypsin Tryple Express from Gibco, USA. Petri dishes and 24 and 96-well plates were purchased from Nunc, Denmark. *Cell culture:* SH-SY5Y human neuroblastoma cells were grown in Dulbecco's Modified Eagle's Medium supplemented with 10% fetal bovine serum and 1% antibiotics (complete growth medium), under humidified atmosphere with 5% CO_2 at 37°C. SH-SY5Y cells were subcultured on 10 mL Petri dishes and when at confluence where detached by using 4 mL of trypsin, then centrifuged and re-suspended in complete growth medium. After that they were plated on to 24 wells and grown for 24h, to a final density of 2×10^5 cells well^{-1}.

Products were purified by flash chromatography on SiO_2 (particle size 0.032-0.063 mm).
Instrumentation: NMR spectra were obtained at 400 MHz (^{1}H NMR) and 100 MHz (^{13}C NMR) using $CDCl_3$ as solvent. IR-DRIFT spectra were recorded using KBr powder. UV-vis spectra were obtained using toluene as the reference solvent. SEM images were recorded on a Philips XL30 Scanning Electron Microscopy model. X-ray photoelectron spectroscopy analyses were performed with a SSX-100 system (Surface Science instrument). The photon source was a monochromatised Al Kα line (hν = 1486.6 eV) applied with a takeoff angle of 35°. In the spectrum analysis, the background signal was subtracted by Shirley's method. The C 1s core level peak position of carbon

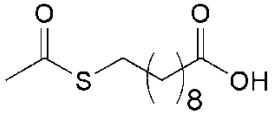
atoms was taken as the reference at 284.5 eV. The spectrum analysis was carried out by fitting the peak shape obtained in the same analysing conditions and other components with mixed (Gaussian + Lorentzian) line shapes. XPS atomic ratios have been estimated from the experimentally determined area ratios of the relevant core lines, corrected for the corresponding theoretical atomic cross-sections and for a square root dependence of the photoelectrons kinetics energies. Water contact angle of wetting measurements (WCA) were performed on a Dataphysic camera using SCA20 as caption software. Before each XPS and WCA measurement, Au(111) surfaces were rinsed with extra purified MILLI-Q water (Millipore, USA), following by several washes with distilled CH_2Cl_2 (HPLC Grade, Sigma-Aldrich, Germany), dried under N_2 flux and stored in capped dried vials until use. For WCA of C_{60}-bearing Au(111) surfaces, distilled water was used.

Supporting S1: synthesis of monopyrrolidino C_{60} derivatives bearing a thiol group



Scheme S1. Synthetic procedure for the production of C_{60} derivatives bearing a thiol group.

*Synthesis of compound **3** (10-(Acetylthio)decanoic acid)*

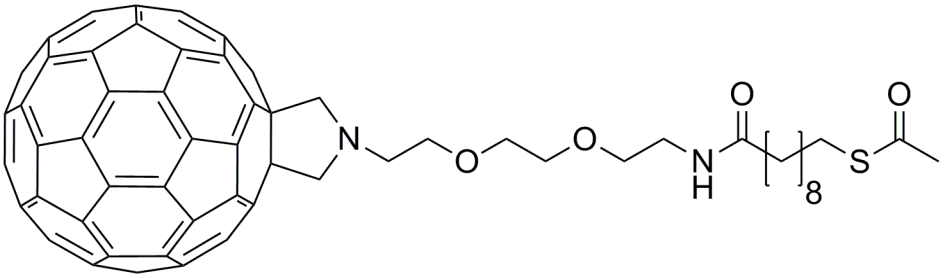


To a solution of 10-bromodecanoic acid (**2**, 1 g, 3.98 mmol) in 6 mL of DMF, potassium thioacetate (651 mg, 5.57 mmol) was added at 0 °C and the resulting mixture was stirred overnight at r.t. under

Ar. When the solution turned to deep orange, the reaction was stopped by solvent evaporation under reduce pressure. The residue was then dissolved in 15 mL of H_2O and the pH adjusted to 3 by addition of a 4 M HCl solution. The aqueous phase was then extensively extracted with CH_2Cl_2 (6 x 30 mL). The organic phase was then concentrated under reduced pressure, stirred over Na_2SO_4, filtered and dried under vacuum. Precipitation from hot diisopropylether afforded the product as a yellowish powder (880 mg, 87%).

M.p. 43-45 ºC; ^{1}H NMR (400 MHz, $CDCl_3$, δ): 2.86 (t, J = 7.3 Hz, 2H), 2.35 (t, J = 7.3 Hz, 2H), 2.32 (s, 3H), 1.67-1.52 (m, 4H), 1.38-1.25 (m, 10H); ^{13}C NMR (100 MHz, $CDCl_3$, δ): 196.29, 178.44, 44.31, 33.87, 30.80, 29.62, 29.35, 29.29, 29.26, 29.14, 28.89, 24.81; IR (KBr): ν = 3042, 2918, 2853, 1698, 1472, 1410, 1236, 1210, 1138, 630 cm^{-1}; HRMS (EI, m/z): [M^+] calcd for $C_{12}H_{22}O_3S$, 246.1290; found 246.1297.

Synthesis of compound ***6*** *(C_{60}S, S-10-Oxo-10-(2-(2-(2-(fulleropyrrolidin-1-yl)ethoxy)ethoxy)ethylamino)decyl ethanethioate)*

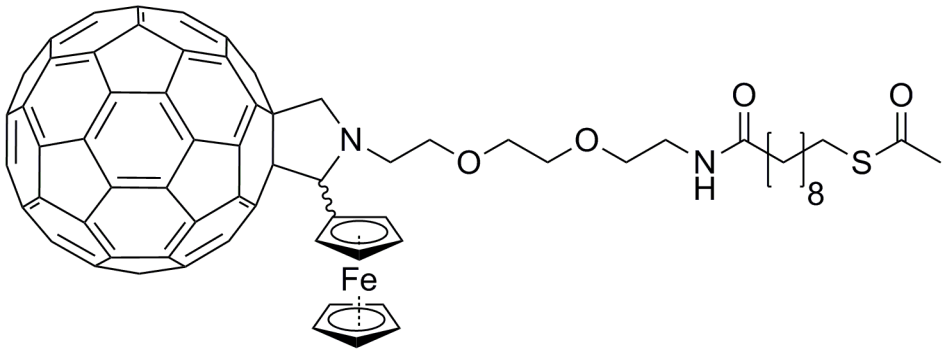


The fullerene derivative **4** (**C_{60}+**, (2-(2-(2-(fulleropyrrolidin-1-yl)ethoxy)ethoxy)ethanamonium trifluoroacetate) was synthesized and fully characterized according to literature.[1] 10-(Acetylthio)decanoic acid (31 mg, 0.225 mmol), N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (44 mg, 0.225 mmol) and 1-Hydroxybenzotriazole (25 mg, 0.225 mmol) were stirred under argon at r.t. in 7.5 mL of CH_2Cl_2 for 30 min. To this mixture, a solution of 2-(2-(2-(fulleropyrrolidin-1-yl)ethoxy)ethoxy)ethanamonium trifluoroacetate (123 mg, 0.110 mmol) and triethylamine (90 mg, 0.89 mmol) in 7.5 mL of CH_2Cl_2 was added. The resulting mixture was stirred overnight under Ar at r.t. and protected from light. The solvent was removed under reduced pressure and the precipitate was purified by silica gel column chromatography (toluene/AcOEt : 9/1), yielding the product as a black powder (63 mg, 53%).

M.p. > 300 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ): 6.00 (bs, 1H), 4.52 (s, 4H), 4.07 (t, J =5.5 Hz, 2H), 3.84 (m, 2H), 3.75 (m, 2H), 3.63 (t, J =6.7 Hz, 2H), 3.47 (t, J =3.2 Hz, 2H), 3.38 (t, J =4.0 Hz,

2H), 2.85 (t, *J* =7.3 Hz, 2H), 2.31 (s, 3H), 2.18 (t, *J* =7.6 Hz, 2H), 1.56 (m, 4H), 1.24 ppm (m, 10H); ^{13}C NMR (100 MHz, $CDCl_3$, δ): 196.22, 173.28, 155.11, 147.47, 146.41, 146.24, 146.16, 145.83, 145.57, 145.46, 144.71, 143.28, 142.81, 142.37, 142.22, 142.05, 140.33, 136.34, 70.95, 70.70, 70.60, 70.51, 70.26, 68.72, 54.51, 39.36, 36.99, 30.82, 29.63, 29.48, 29.30, 29.23, 28.94, 25.87, 23.01. Due to signal overlapping only the well-resolved peaks are reported; IR (KBr): ν = 3296, 2923, 2852, 1686, 1649, 1538, 1461, 1427, 1106, 526 cm^{-1}; UV/Vis (toluene): λ_{max} = 310, 329, 432, 703 nm; HRMS (ESI, m/z): [*M*+Na$^+$] calcd for $C_{80}H_{38}NaN_2O_4S$, 1145.24; found 1145.25.

Synthesis of compound ***7*** *(C_{60}FeS, S-10-Oxo-10-(2-(2-(2-(fulleropyrrolidin(ferrocene-1-yl)ethoxy)ethoxy)ethylamino)decyl ethanethioate)*

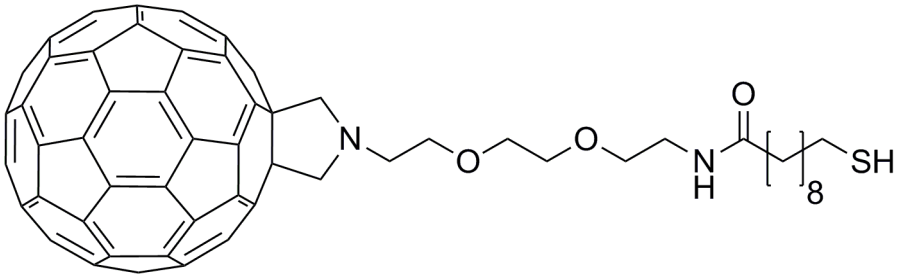


The fullerene derivative **5** (**C_{60}Fe+**, (2-(2-(2-(fulleropyrrolidin(ferocene)-1-yl)ethoxy)ethoxy)ethanamonium) used was synthesized and fully characterized according to literature.[1] 10-(Acetylthio)decanoic acid (31 mg, 0.225 mmol), N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (44 mg, 0.225 mmol) and 1-Hydroxybenzotriazole (25 mg, 0.225 mmol) were stirred under Ar at r.t. in 7.5 mL of CH_2Cl_2 for 30 min. To this mixture. a solution of 2-(2-(2-(fulleropyrrolidin(ferocene)-1-yl)ethoxy)ethoxy)ethanamonium trifluoroacetate (128 mg, 0.114 mmol) and triethylamine (45 mg, 0.445 mmol) in 7.5 mL of CH_2Cl_2 was then slowly added. The resulting mixture was stirred overnight under Ar at r.t. and protected from light. The solvent was then evaporated under reduced pressure and the precipitate was purified by silica gel column chromatography (toluene/AcOEt : 9/1) yielding the product as a black powder (63.5 mg, 51%).

M.p. > 300 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ): 5.93 (bs, 1H), 5.11 (m, 2H), 5.03 (s, 1H), 4.58 (s, 1H), 4.53 (m, 1H), 4.32 (s, 5H), 4.27-4.19 (m, 4H), 3.99 (m, 1H), 3.91 (m, 1H), 3.80 (t, *J* = 4.56 Hz, 2H), 3.65 (t, *J* = 5.04 Hz, 2H), 3.49 (t, *J* = 5.28 Hz, 2H), 3.18 (t, *J* = 5.96 Hz, 2H), 2.86 (t, *J* = 7.32 Hz, 2H), 2.33 (s, 3H), 2.13 (t, *J* = 7.76 Hz, 2H), 1.58 (m, 4H), 1.27 (m, 10H); ^{13}C NMR (100 MHz, $CDCl_3$, δ): 196.23, 173.28, 156.49, 156.47, 154.49, 154.43, 153.95, 153.49, 147.68, 147.41, 147.30, 146.46, 146.39, 146.35, 146.28, 146.20, 146.07, 146.20, 145.89, 145.70, 145.65, 145.61,

145.48, 145.38, 145.29, 144.84, 144.78, 144.68, 144.56, 144.42, 140.35, 140.31, 140.18, 140.10, 139.55, 138.97, 136.64, 136.41, 135.98, 135.81, 87.07, 71.07, 71.00, 70.67, 70.36, 69.55, 68.86, 68.54, 68.38, 67.75, 67.67, 67.32, 53.51, 39.37, 36.96, 30.83, 29.64, 29.47, 29.31, 29.23, 28.94, 25.86. Due to signal overlapping only the well-resolved peaks are reported; IR (KBr): ν = 3415, 2920, 2851, 1687, 1640, 1619, 1461, 1424, 1106, 527 cm^{-1}; UV/Vis (toluene): λ_{max} = 309, 325, 432, 706; HRMS (ESI, m/z): [*M*+Na^+] calcd for $C_{90}H_{46}NaFeN_2O_4S$, 1329.2421; found 1329.2353.

Synthesis of compound ***1H*** *(C_{60}SH, 10-mercapto-N-(2-(2-(2-(fulleropyrrolidin-1-yl)ethoxy)ethoxy)ethylamino)decanamide)*

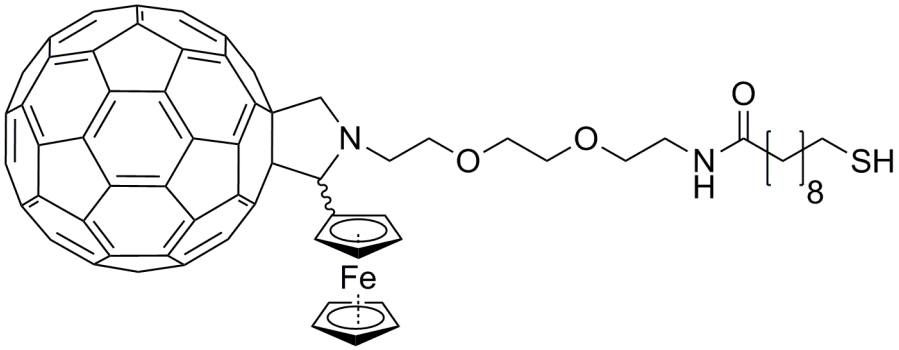


A solution of **6** **(C_{60}S)** (S-10-Oxo-10-(2-(2-(2-(fulleropyrrolidin-1-yl)ethoxy)ethoxy)ethylamino)decyl ethanethioate) (130 mg, 0.12 mmol) in distilled CH_2Cl_2 (15 mL): MeOH (5 mL) was stirred at r.t. under Ar. To this solution AcCl (250 µl, 3.6 mmol,) was added drop wise at 0° C and the resulting mixture was stirred at r.t. overnight under Ar. The reaction was stopped by drop wise addition of pyridine (200 µl, 2.5 mmol,) under stirring over Ar atmosphere. The mixture was then dried under vacuum, and the precipitate dissolved in 10 mL of $CHCl_3$ and washed with H_2O (3X 15 mL). The organic phase was then concentrated, stirred with $MgSO_4$, filtered, and then dried under vacuum. The residue was dissolved in a minimal amount of $CHCl_3$ to be subjected to column chromatography on fine silica using toluene/EtOAc 8:2 as the eluent. The residue was then dissolved in a minimal amount of $CHCl_3$ and precipitated with cyclohexane and subsequently triturated with pentane. The residue was dried under vacuum, affording the product as a brown solid (80 mg, 51%).

M.p. > 300 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ): 6.00 (bs, 1H), 4.52 (s, 4H), 4.07 (t, *J* = 5.52 Hz, 2H), 3.84 (m, 2H), 3.75 (m, 2H), 3.63 (t, *J* = 6.68 Hz, 2H), 3.47 (t, *J* = 3.16 Hz, 2H), 3.38 (t, *J* = 4.00 Hz, 2H), 2.85 (t, *J* = 7.32 Hz, 2H), 2.18 (t, *J* = 7.56 Hz, 2H), 1.56 (m, 4H), 1.24 (m, 10H). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 196.65, 173.64, 155.52, 147.86, 146.80, 146.55, 142.11, 142.62, 142.45, 71.08, 70.91, 69.11, 54.87, 39.76, 39.54, 37.39, 29.88, 29.69, 29.62. Due to signal overlapping only the well-resolved peaks are reported; IR (KBr): ν = 3288, 2921, 2358, 1644, 1538,

1427, 1341, 1230, 1103, 767, 526, 477 cm^{-1}. UV/vis (toluene) λ_{max} = 280, 320, 340, 436, 710. HRMS (ESI, *m/z*): [*M*+Na^+] calcd for $C_{78}H_{36}NaN_2O_3S$, 1079.20; found 1080.24.

Synthesis of compound ***2H*** *(C_{60}FeSH, 10-mercapto-N-(2-(2-(2 (fulleropyrrolidin(ferocene)-1-yl)ethoxy)ethoxy)ethyl)decanamide)*



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S-10-Oxo-10-(2-(2-(2-(fulleropyrrolidin(ferocene-1-yl)ethoxy)ethoxy)ethylamino)decyl ethanethioate (10 mg, 0.075 mmol) and acetyl chloride (88 mg, 1.125 mmol) were slowly added to a mixture of dry CH_2Cl_2 and MeOH at 0 °C and stirred overnight. The resulting solution was washed with H_2O (2 x 2 mL), stirred with Na_2SO_4, filtered and then concentrated under reduced pressure. After complete solvent removal the crude was purified by silica gel column chromatography, yielding the product as a black powder (5 mg, 41%).

M.p. > 300 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ): 5.92 (bs, 1H), 5.13 - 5.03 (m, 3H), 4.57 (s, 1H), 4.52 (s, 1H), 4.31 (s, 5H), 4.26 - 4.17 (m, *4*H), 3.98 (mult. 1H), 3.92 (mult., 1H), 3.80 (t, *J* = 4.6 Hz, 2H), 3.65 (t, *J* = 5.1, 2H), 3.49 (mult., 2H), 3.17 (mult., 2H), 2.51 (mult., 2H), 2.13 (t, *J* = 9.2 Hz, 2H), 1.56 (m, 4H), 1.24 (m, 10H); ^{13}C NMR (100 MHz, $CDCl_3$, δ): 173.25, 156.52, 154.49, 153.99, 153.49, 153.42, 147.68, 147.59, 147.41, 147.30, 146.57, 146.52, 146.39, 146.32, 146.28, 146.19, 146.08, 145.88, 145.69, 145.64, 145.61, 145.48, 145.38, 145.29, 144.84, 144.78, 144.56, 143.27, 143.15, 142.85, 142.75, 142.29, 142.21, 141.95, 141.92, 141.78, 141.56, 140.82, 140.39, 140.34, 140.18, 139.56, 138.97, 136.64, 136.41, 135.98, 132.82, 131.76, 87.08, 71.09, 70.99, 70.67, 70.34, 69.54, 68.87, 68.54, 68.38, 67.75, 67.66, 67.34, 54.76, 53.51, 39.38, 39.33, 36.97, 34.18, 29.49, 29.39, 29.20, 28.70, 28.51, 25.87, 24.83. Due to signal overlapping only the well-resolved peaks are reported; IR (KBr): ν = 2923, 2852, 1740, 1649, 1541, 1461, 1378, 1262, 1106, 1023 cm^{-1}; UV/Vis (toluene): λ_{max} = 309, 325, 432, 706 nm; HRMS: (ESI, m/z): [M+Na^+] calcd for $C_{88}H_{44}FeNaN_2O_3S$, 1285.2366; found 1285.2374.

Supporting S2: preparation of [1•Au(111)] and [2•Au(111)]

Monopyrrolidino-[60]fullerene derivatives **1H** and **2H** were prepared according to literature.[1] SAMs [**1•Au(111)**] and [**2•Au(111)**] were prepared by immersion technique of the Au(111) surfaces in a solution of the pyrrolidino[60]fullerene according to literature protocols.[2] Briefly, Au(111)-coated mica substrates were subjected to UV light and extensively washed with H_2O, EtOH, conditioned with dry CH_2Cl_2 and dried under Ar flux. The obtained cleaned substrates were introduced in round bottom flasks containing 1 mM dry CH_2Cl_2 solutions of **1H** or **2H** and kept at room temperature for 24h protected from light. After thorough washing with CH_2Cl_2 and drying under high vacuum, SAMs [**1•Au(111)**] and [**2•Au(111)**] were stored at 4 °C and under protected atmosphere, until used for the biological experiments. As a reference substrate used to evaluate the effect of fullerene, $CH_3(CH_2)_{11}SH$ was self-assembled on the cleaned Au(111) mica substrates under the same adsorption conditions, affording WCA values in agreement with literature reports.[3]

Supporting S3: XPS analysis of C_{60}-modified Au(111) surfaces.

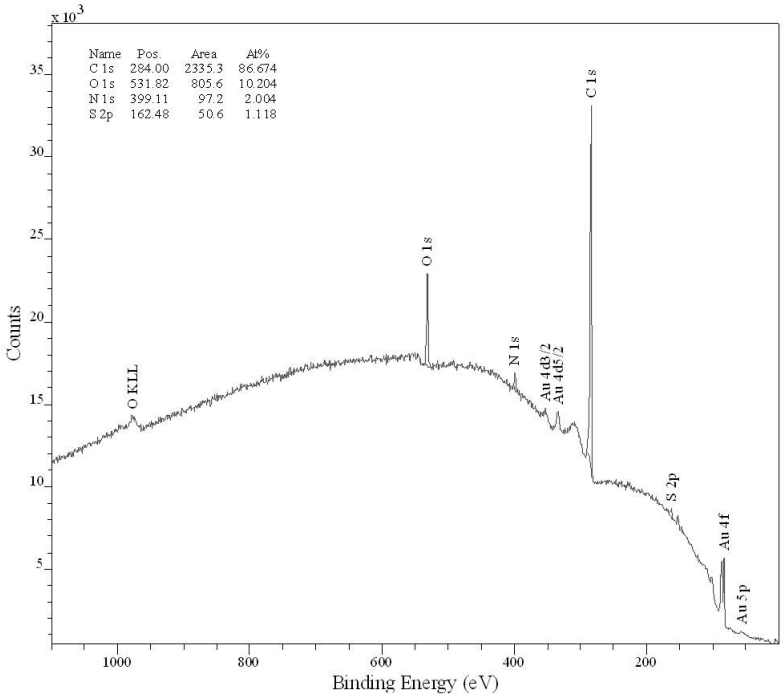

Figure S3-1. XPS survey spectrum of [**1•Au(111)**], reporting both qualitative and quantitative elemental composition.

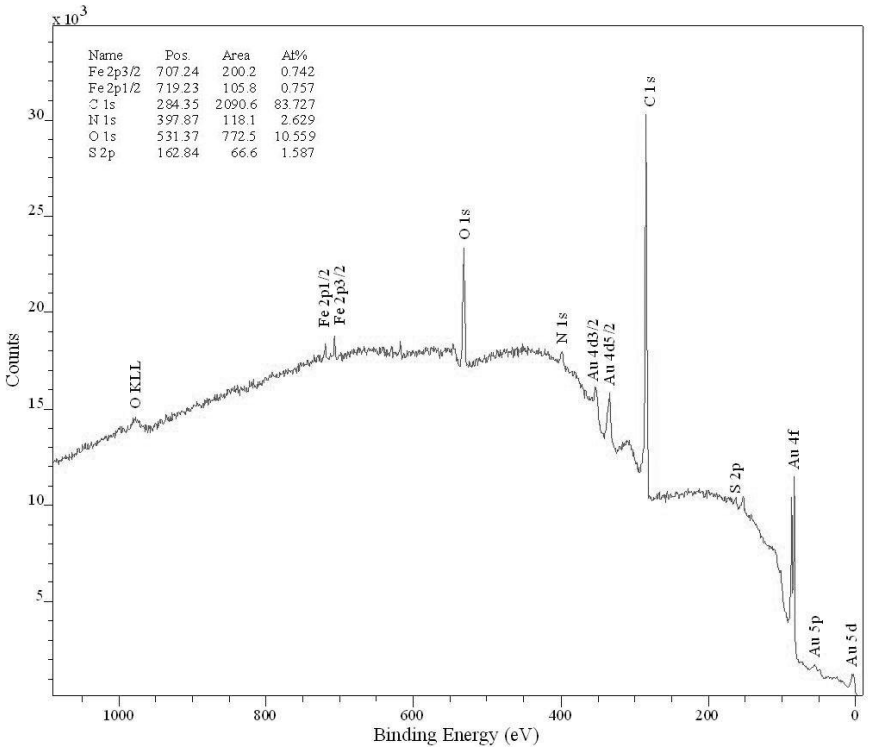

Figure S3-2. XPS survey spectrum of [**2•Au(111)**], reporting both qualitative and quantitative elemental composition.

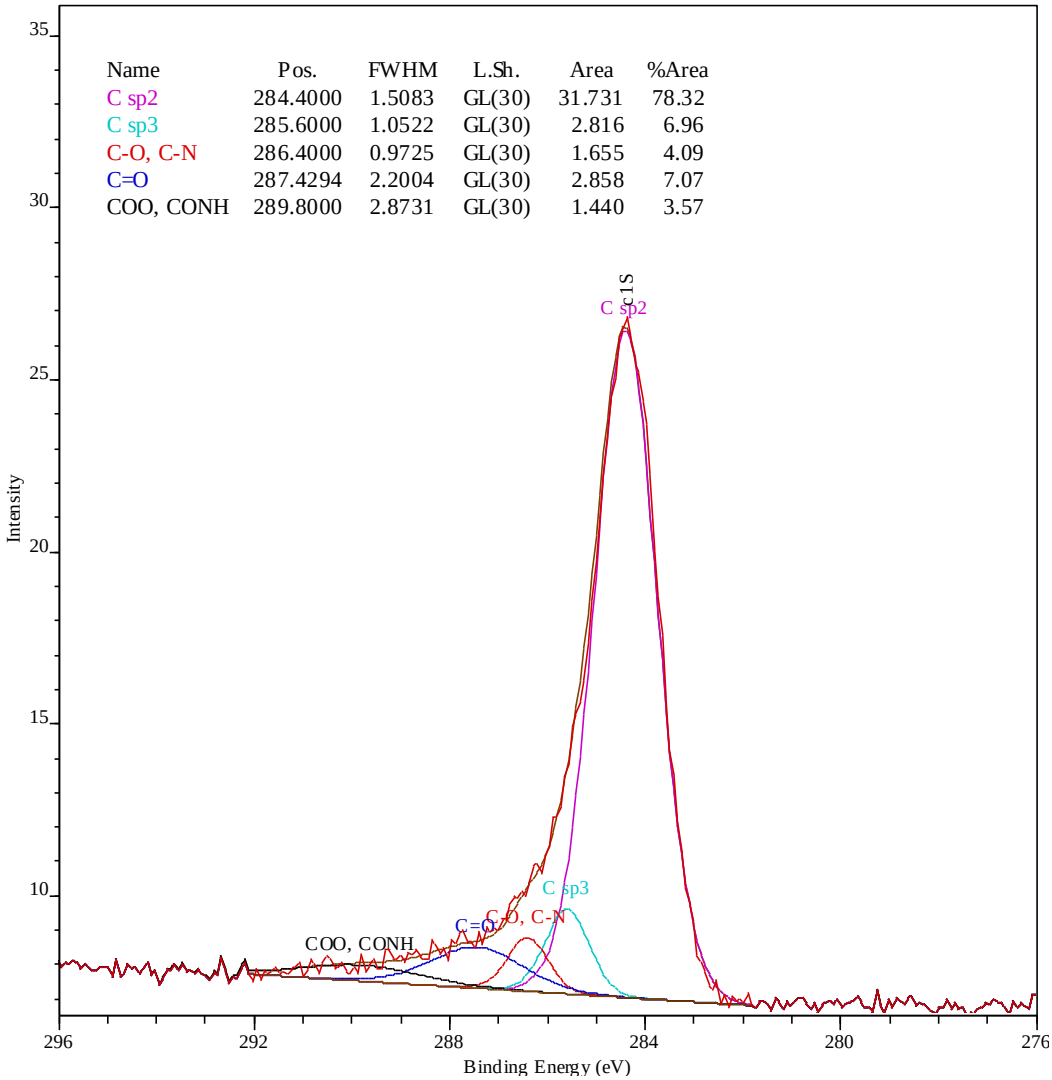

Figure S3-3. High-resolution C 1s XPS spectrum of [**1•Au(111)**].

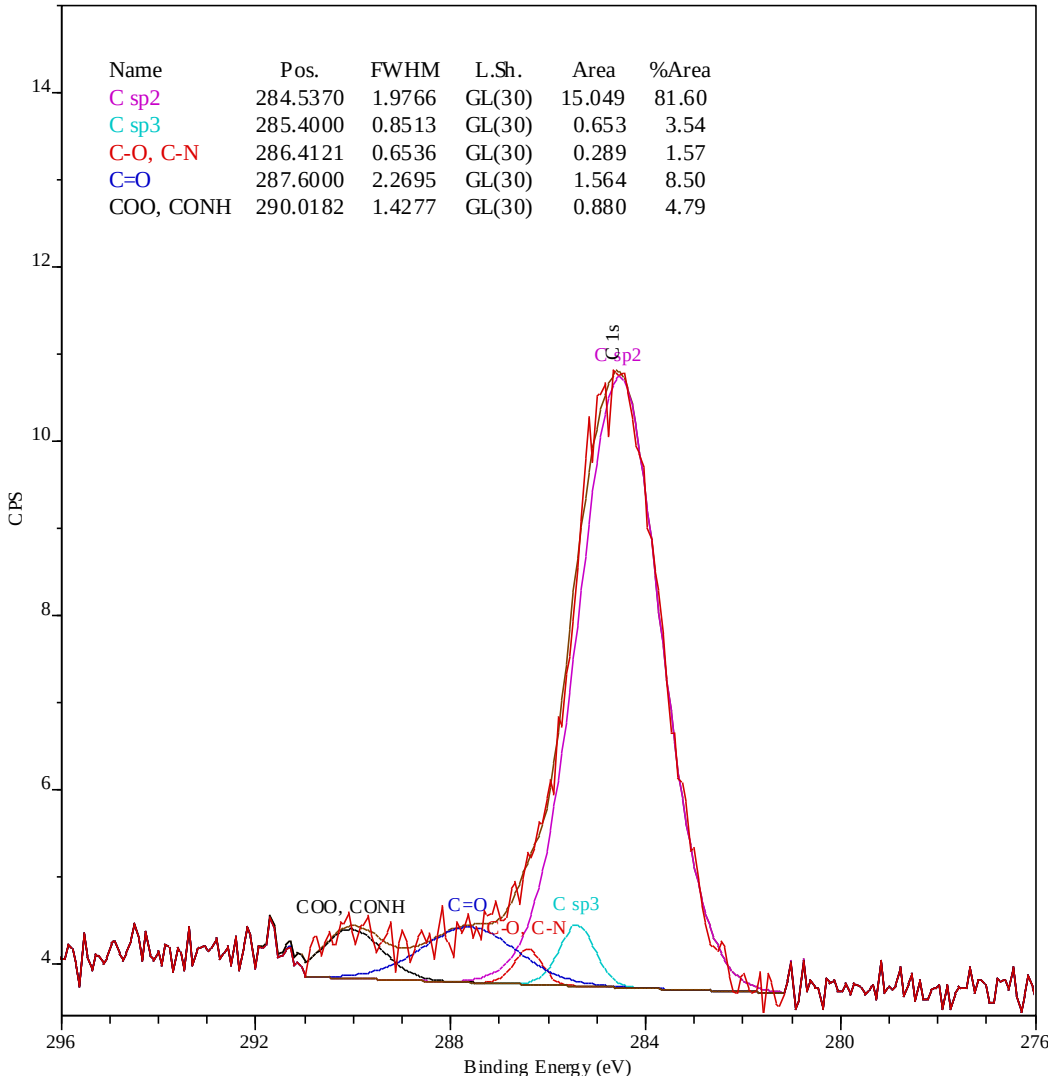

Figure S3-4. High-resolution C 1s XPS spectrum of [**2•Au(111)**].

Supporting S4: Water contact angle measurements of C_{60}-modified Au(111) surfaces.

	blank	**[1•Au(111)]**	**[2•Au(111)]**	**[$CH_3(CH_2)_{11}S$•Au(111)]**
Angle q	37.7	76.9	83.6	99.5
Angle q	41.6	81.4	83.3	101.3
Angle q	37.6	78.1	83.5	100
Angle q	42.0	78.3	87.7	99.8
Angle q	34.0	72.6	83.7	99.7
Angle q	41.2	75.2		
Angle q	38.5			
Angle q				
Average angle	**38.9**	**77.1**	**84.4**	**100.1**
Counts	7	6	5	5
ST dev	2.9	3.0	1.9	0.7

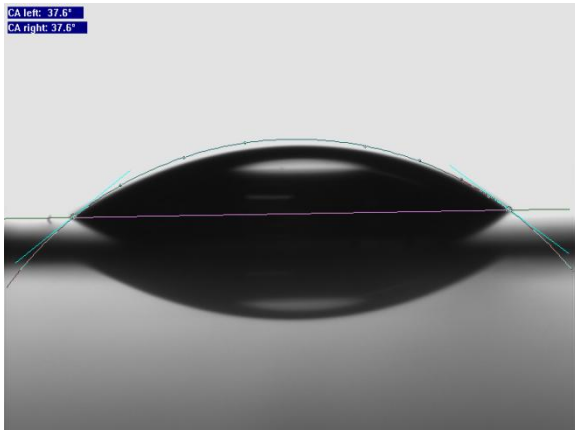

Figure S4-1. Water drop formation on cleaned gold surface (blank).

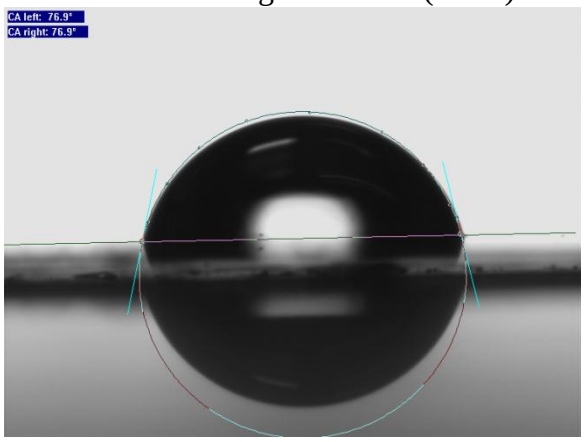

Figure S4-2. Water drop formation on **1H** ($C_{60}SH$) modified gold surface [**1•Au(111)**].

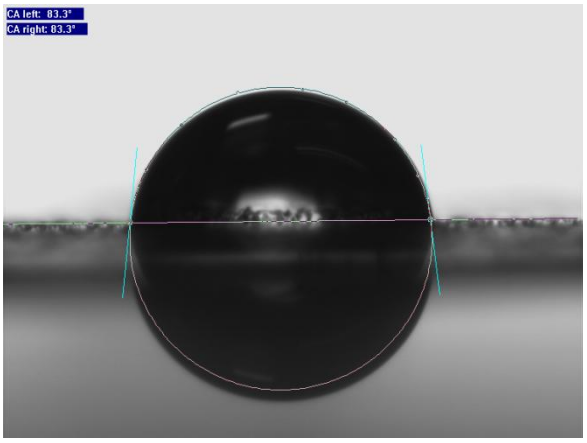

Figure S4-3. Water drop formation on **2H** (C_{60}FeSH) Modified surface [**2•Au(111)**].

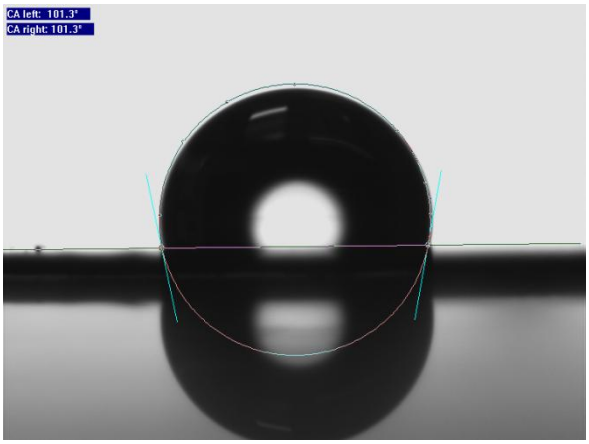

Figure S4-4. Water drop formation on $CH_3(CH)_{11}SH$ modified gold surface.

Supporting S5: biological tests

*SH-SY5Y cells growth on [**1•Au(111)**] and [**2•Au(111)**]:* before cells seeding, SAMs [**1•Au(111)**] and [**2•Au(111)**] were extensively washed with phosphate saline buffer 1M (PBS) and conditioned for 30 min with complete growth medium at 37°C. SH-SY5Y cells were then seeded to the final concentration of 2×10^5 cells on the different Au(111) substrates in a 24-wells plate. Cells were grown for 48h on Au(111) surfaces prior L-Glu treatment and before fixation for SEM imaging. Differentiation[4] of SH-SY5Y on Au(111) substrates and their fixation for SEM imaging[5] were performed as reported in literature.

Cells viability using MTT reduction assay on modified Au(111) substrates: cell viability was determined using an in vitro toxicology assay kit based on the reduction of 3-[4,5-dimetylthiazol-2-yl]-2,5-dipheniltetrazolium bromide (MTT), according to literature.[6] Briefly, SH-SY5Y

neuroblastoma cells were seeded at 2×10^5 cells well in 24 well plates containing Au(111) surfaces. After 6h 100 μM L-Glu treatment in 250 μL of complete medium, then 25 μL of MTT solution (5 mg mL^{-1}) were added and incubated at 37 ºC for 3h. After incubation, 250 μL of MTT solubilisation solution (10% Triton X-100 plus 0.1 N HCl in anhydrous isopropanol) were added to the wells to dissolve formazan crystals. The resulting suspension from each substrate was thus well homogenised by pipetting the entire volume, which was then split in 5 fraction of 100 μL each and transferred to a 96-wells plate to be spectrophotometrically measured. The plates were then thoroughly shaken and the absorbance of each well was measured at 570 nm.

Statistical and data analysis: statistical data analysis was performed using Student's t-test. Differences between mean values obtained in MTT assays were considered statistically significant at $P < 0.05$.

Supporting S6: cell morphology after growth on [60]fullerene-bearing SAMs

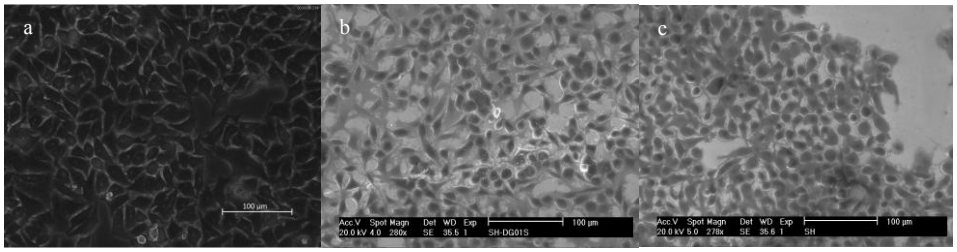

Figure S6. SEM images of undifferentiated SH-SY5Y cells after 48h growth on a (a) Petri dish, (b) [**1•Au(111)**] and (c) [**2•Au(111)**] SAMs.

References

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