Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

for

Multifunctional Linker for Orthogonal Decoration of Gold Nanoparticles with DNA and Protein

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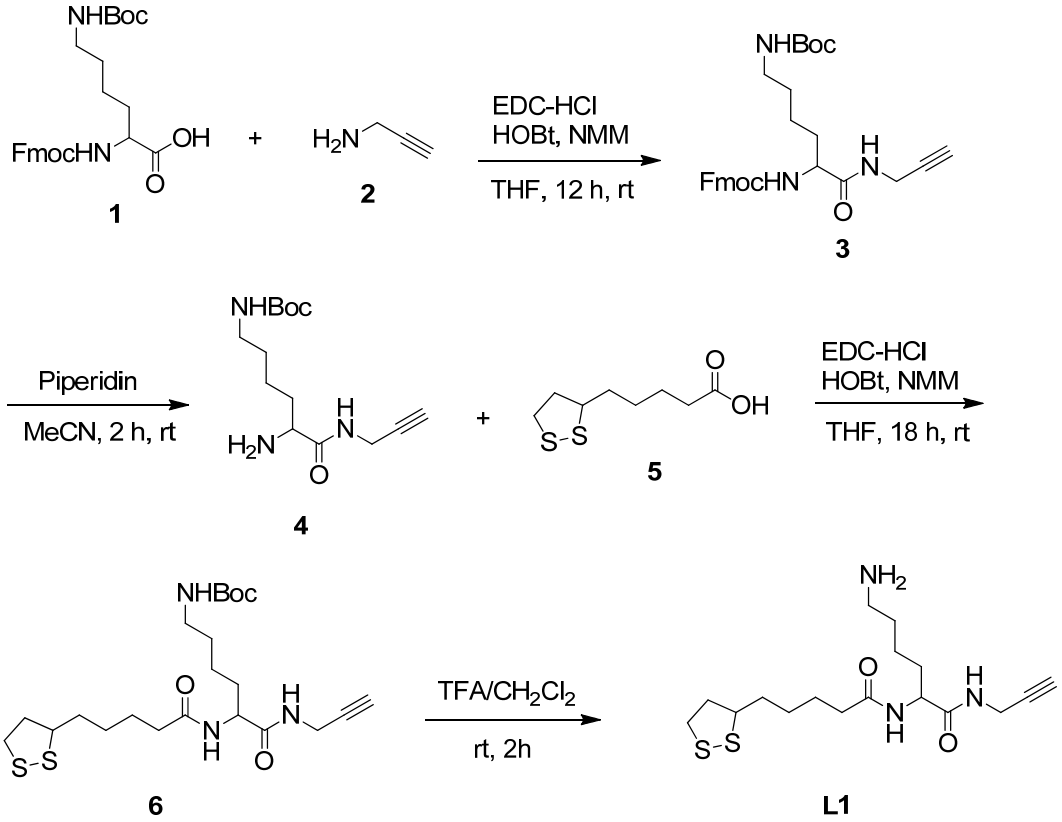
Content

(1) Synthesis of Trifunctional Linker L1



Scheme S1 Synthesis of trifunctional alkyne-linker **L1**.

(9*H*-Fluoren-9-yl)methyl tert-butyl (6-oxo-6-(prop-2-yn-1-ylamino)hexane-1,5-diyl)dicarbamate (3).

To a stirred solution of Nα-Fmoc-Nε-Boc-L-lysine (**1**) (5g, 10.60 mmol) in anhydrous THF (80 mL) was added EDC-HCl (2.45 g, 12.80 mmol) and HOBt (1.73 g, 12.80 mmol) at room temperature. The resulting mixture was stirred at room temperature for 10 min. Propargyl amine (**2**) (0.88 mL, 13.70 mmol) was added followed by N-methoxy morpholine (2.33 mL, 21.20 mmol) and the reaction mixture was allowed to stir at room temperature overnight. The mixture was neutralized by dilute HCl and extracted three times with ethyl acetate (3x60 mL). The organic layers were combined, washed with water (50 mL) and dried over anhydrous sodium sulfate. After filtration, the solvent was evaporated under reduced pressure to give brown residue which was purified by silica gel column chromatography eluting with cyclohexane, increasing to 40% ethyl acetate in cyclohexane to afford pure propargyl lysine derivative **3** as white solid (4.25, 79%)

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.35 (s, 9 H), 1.15-1.47 (m, 4 H), 1.53-1.68 (m, 1 H) 1.69-1.85 (m, 1 H), 2.06 (br, 1 H), 2.90-3.01 (br, 2 H), 3.93 (br, 2 H), 4.11 (t, *J* = 6.7, 1 H), 4.32 (d, *J* = 6.7, 2 H), 4.62 (m, 1 H), 5.62 (br, 1 H), 6.75 (br, 1 H), 7.18-7.24 (m, 2 H, Ar-H), 7.29-7.33 (m, 2 H, Ar-H), 7.48 (d, *J* = 7.3 Hz, 2 H, Ar-H), 7.68 (d, *J* = 7.5 Hz, 2 H, Ar-H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 22.4, 28.4, 29.1, 29.5, 32.0, 39.9, 47.1, 54.6, 67.1, 71.7, 79.2, 120.0, 125.0, 127.1, 127.7, 141.3, 143.7, 143.8, 156.2, 171.5; IR (Film): ύ = 3294, 3067, 2972, 2940, 2858, 1686, 1646, 1526, 1447, 1364, 1342, 1229, 1085, 753, 737 cm^{-1}; MS (+FAB): *m/z* (%) = 528.2 (12) [M+Na], 506.2 (35) [M+H], 450 (20), 406 (43), 178 (100), 107 (15), 84 (20); HRMS (FAB) calcd. for $C_{29}H_{36}N_3O_5$ $[M+H]^+$: 506.2576; found: 506.2654.

tert-butyl (5-amino-6-oxo-6-(prop-2-yn-1-ylamino)hexyl)carbamate (4).

Propargyl lysine derivative **3** (100 mg, 0.19 mmol) was dissolved in acetonitrile (10 mL) and piperidine (2 mL) was then added. The mixture was stirred at room temperature for 2 h. The solvent (acetonitrile) and piperidine were removed at reduced pressure to give brown sticky residue which was used without further purification.

MS (+FAB): *m/z* (%) = 306.2 (10) [M+Na], 284.2 (32) [M+H], 254 (20), 228 (35), 210 (22), 184 (27), 165 (21), 128 (39), 84 (100); HRMS (FAB) calcd. for $C_{14}H_{25}N_3O_3$ $[M+H]^+$: 284.18959; found: 284.18967.

tert-butyl (5-(5-(1,2-dithiolan-3-yl)pentanamido)-6-oxo-6-(prop-2-yn-1-ylamino)hexyl)carbamate (6).

To a stirred solution of fmoc-deprotected lysine derivative **4** (300 mg, 0.593 mmol) in anhydrous THF (20 mL) was added lipoic acid (**5**) (122 mg, 0.593 mmol), EDC-HCl (136 mg, 0.712 mmol) and HOBt (96 mg, 0.712 mmol) at 0^{o}C. The resulting mixture was stirred at the same temperature for 10 min. followed by the addition of N-methoxy morpholine (0.13 mL, 1.18 mmol) and the reaction mixture was allowed to stir at room temperature overnight. The mixture was neutralized by dilute HCl and extracted three times by ethyl acetate (3x60 mL). The organic layers were combined, washed with water (50 mL) and dried over anhydrous sodium sulfate. After filtration, the solvent was evaporated under reduced pressure to give brown residue which was purified by silica gel column chromatography eluting with ichloromethane, increasing to 10% methanol in dichlromethane to afford pure propargyl-lysine-lipoic acid derivative **6** as white solid (412 mg, 82%).

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.29-1.38 (m, 2 H), 1.41 (s, 9 H), 1.43-1.51 (m, 2 H), 1.56-1.73 (m, 6 H), 1.77-1.93 (m, 2 H), 2.07 (br, 1 H), 2.20-2.24 (m, 3 H), 2.40-2.48 (m, 1 H),

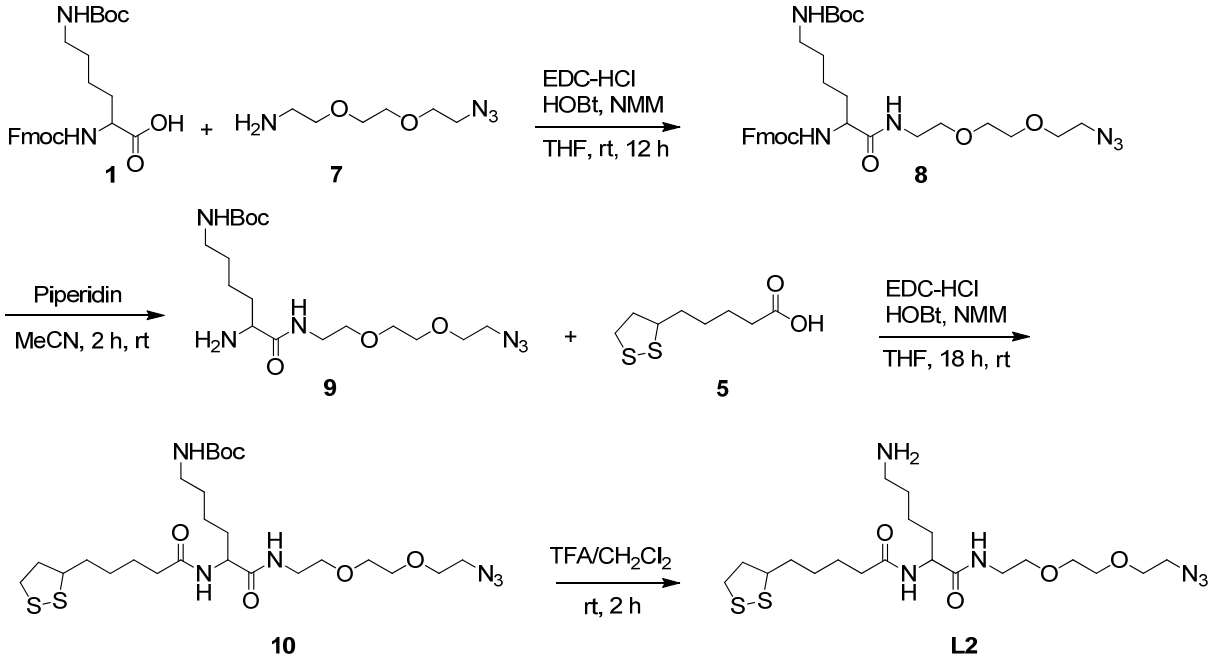
2.97-3.22 (m, 4 H), 3.51-3.58 (m, 1 H), 3.98-4.01 (m, 2 H), 4.43-4.53 (m, 1 H), 4.74-4.80 (m, 1 H), 6.50-6.56 (m, 1 H), 7.23 (br, 1 H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 22.5, 25.2, 25.3, 28.4, 28.8, 29.0, 29.6, 31.9, 34.5, 36.1, 38.4, 40.2, 52.6, 56.3, 71.5, 79.3, 156.2, 171.6, 173.2; IR (Film): ύ = 3329, 3231, 2932, 2858, 1678, 1653, 1625, 1529, 1434, 1390, 1367, 1307, 1170, 783 cm^{-1}; MS (+FAB): *m/z* (%) = 494.2 (8) [M+Na], 472.2 (45) [M+H], 471.2 (28) [M^+], 416 (18), 372 (38), 361 (20), 189 (25), 165 (15), 136 (70), 128 (37), 84 (100);); HRMS (FAB) calcd. for $C_{22}H_{38}N_3O_4S_2$ [M+H]$^+$: 472.2225; found: 472.2305.

2-(5-(1,2-dithiolan-3-yl)pentanamido)-6-amino-N-(prop-2-yn-1-yl)hexanamide (L1).

To a stirred solution of tert-butyl (5-(5-(1,2-dithiolan-3-yl)pentanamido)-6-oxo-6-(prop-2-yn-1-ylamino)hexyl)carbamate (**6**) (200 mg, 0.42 mmol) in dry CH_2Cl_2 (15 mL) was added trifluoroacetic acid (5 mL). The reaction mixture was stirred for 2 h at room temperature. Dichloromethane (15 mL) was then added and the solvent was removed under reduced pressure. The procedure was repeated three times to remove the traces of trifluoroacetic acid to afford 2-(5-(1,2-dithiolan-3-yl)pentanamido)-6-amino-N-(prop-2-yn-1-yl)hexanamide (**L1**) as yellow thick oil (147 mg 93%)

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.27-1.34 (m, 4 H), 1.35-1.70 (m, 10 H), 2.02 -2.29 (m, 3 H), 2.40-2.63 (m, 2 H), 2.76-2.85 (m, 2 H), 313-3.55 (m, 2 H), 3.84 (br, 2 H), 4.19 (br. 1 H), 7.88 (m, 3 H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 22.0, 24.9, 26.3, 28.4, 29.3, 31.0, 36.3, 38.9, 52.6, 58.2, 58.3, 70.9, 78.8, 172.3, 174.2; IR (Film): ύ = 3439, 2938, 2121, 1681, 1537, 1434, 1205, 1136, 1043, 1205, 1136, 1043, 840, 802, 723 cm^{-1}; MS (+FAB): *m/z* (%) = 372.2 (4) [M+H], 340.2 (8) [M^+], 317 (18), 189 (13), 165 (10), 129 (29), 115 (26), 102 (22), 84 (100); HRMS (FAB) calcd. for $C_{17}H_{30}N_3O_2S_2$ [M+H]$^+$: 372.1701; found: 372.1779.

(2) Synthesis of Trifunctional Linker L2



Scheme S2 Synthesis of trifunctional azide-linker **L2**.

(9*H*-fluoren-9-yl)methyl tert-butyl (6-((2-(2-(2-azidoethoxy)ethoxy)ethyl)amino)-6-oxohexane-1,5-diyl)dicarbamate (8).

To a stirred solution of Nα-Fmoc-Nε-Boc-L-lysine (**1**) (2g, 4.27 mmol) in anhydrous THF (60 mL) was added EDC-HCl (0.98 g, 5.12 mmol) and HOBt (0.69 g, 5.12 mmol) at room temperature. The resulting mixture was stirred at room temperature for 10 minutes. Amine-TEG-azide (**7**) (0.96 g, 5.55 mmol) followed by N-methoxy morpholine (0.93 mL, 8.54 mmol) were added and the reaction mixture was allowed to stir at room temperature overnight. The mixture was neutralized by dilute HCl and extracted three times by ethyl acetate (3x60 mL). The organic layers were combined, washed with water (50 mL) and dried over anhydrous sodium sulfate. After filtration, the solvent was evaporated under reduced pressure to give brown residue which was purified by silica gel column chromatography eluting with dichloromethane, increasing to 5% methanol in dichloromethane to afford pure lysine azide product **8** as pale yellow solid (2.10 g, 79%).

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.35-140 (m, 2 H), 1.44 (s, 9 H), 1.48-1.50 (m, 2 H), 1.60-1.68 (m, 1 H), 1.76-1.90 (m, 1 H), 3.10-3.13 (m, 2 H), 3.34-3.37 (m, 2 H, CH_2), 3.42-3.47 (m, 2 H, CH_2), 3.54-3.57 (m, 2 H, CH_2), 3.60-3.63 (m, 4 H), 3.64-3.66 (m, 2 H), 4.14-4.23 (m, 2 H), 4.35-4.43 (m, 1 H), 4.72 (br, 1 H), 5.69 (br, 1 H), 6.60 (br, 1 H), 7.32 (t, *J* = 7.4 Hz, 2 H,

Ar-H), 7.40 (t, *J* = 7.3 Hz, 2 H, Ar-H), 7.59 (d, *J* = 7.4 Hz, 2 H, Ar-H), 7.76 (d, *J* = 7.5 Hz, 2 H, Ar-H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 22.5, 28.4, 29.6, 32.5, 38.6, 39.2, 40.0, 47.1, 50.6, 54.8, 66.9, 70.0, 70.2, 70.4, 79.1, 119.9, 125.0, 127.1, 127.7, 141.2, 143.8, 143.9, 156.1, 171.7; IR (Film): ύ = 3352, 3303, 2919, 2851, 2102, 1682, 1650, 1527, 1449, 1389, 1364, 1272, 1243, 1168, 1119, 765, 735 cm^{-1}; MS (+FAB): *m/z* (%) = 647.3 (18) [M+Na], 625.3 (25) [M+H], 525 (38), 342 (17), 195 (20), 191 (24), 178 (100), 84 (15); HRMS (FAB) calcd. for $C_{32}H_{45}N_6O_7$ $[M+H]^+$: 625.3271; found: 625.3370.

tert-butyl (5-amino-6-((2-(2-(2-azidoethoxy)ethoxy)ethyl)amino)-6-oxohexyl)carbamate (9).

Lysine derivative **8** (200 mg, 0.320 mmol) was dissolved in acetonitrile (10 mL) and piperidine (4 mL) was then added. The mixture was stirred at room temperature for 3 h. Acetonitrile and piperidine were removed at reduced pressure to give brown sticky residue which was used without further purification.

MS (+FAB): *m/z* (%) = 425.2 (7) [M+Na], 403.2 (10) [M+H], 345 (20), 239 (43), 192 (6), 107 (15), 102 (12), 86 (100); HRMS (FAB) calcd. for $C_{17}H_{34}N_6O_5$ $[M+H]^+$: 403.25907; found: 403.25915.

tert-butyl (5-(5-(1,2-dithiolan-3-yl)pentanamido)-6-((2-(2-(2-azidoethoxy)ethoxy)ethyl)amino)-6-oxohexyl)carbamate (10).

To a stirred solution of fmoc-deprotected lysine derivative **9** (1 g, 2.48 mmol) in anhydrous THF (30 mL) was added lipoic acid (**5**) (512 mg, 2.48 mmol), EDC-HCl (572 mg, 2.98 mmol) and HOBt (402 mg, 2.98 mmol) at 0^{o}C. The resulting mixture was stirred at the same temperature for 10 min. and *N*-methoxy morpholine (0.546 mL, 4.97 mmol) was added and the reaction mixture was allowed to stir at room temperature overnight. The mixture was neutralized by dilute HCl and extracted three times by ethyl acetate (3x60 mL). The organic layers were combined, washed with water (50 mL) and dried over anhydrous sodium sulfate. After filtration, the solvent was evaporated under reduced pressure to give brown residue which was purified by silica gel column chromatography eluting eluting with dichloromethane, increasing to 10% methanol in dichlromethane to afford pure azide-lysine-lipoic acid derivative **10** as brown oil (1.12 g, 76%).

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.29-1.30 (m. 2 H), 1.40 (s, 9 H), 1.41-1.46 (m, 2 H), 1.56-1.69 (m, 5 H), 1.70-1.75 (m, 1 H), 1.83-1.92 (m, 1 H), 2.21 (t, 1 H, *J* = 7.4 Hz), 2.35-2.48 (m,

1 H), 2.97-3.18 (m, 4 H), 3-32 (br, 1 H), 3.38-3.43 (m, 4 H), 3.50.3.57 (m, 3 H), 3.60-3.63 (m, 4 H), 3.66 (t, 2 H, *J* = 4.5 Hz), 4.39 (m. 1 H), 4.80 (m, 1 H), 6.62 (br, 1 H), 6.82 (br, 1 H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 22.5, 25.3, 28.4, 28.8, 32.1, 34.5, 36.1, 38.4, 39.3, 40.2, 50.6, 52.9, 56.4, 69.6, 70.0, 70.2, 70.4, 79.1, 156.2, 172.0, 173.2; IR (Film): ύ = 3299, 3076, 2930, 2863, 2106, 1692, 1643, 1530, 1453, 1390, 1365, 1250, 1170, 1137, 933, 780 cm^{-1}; MS (+FAB): *m/z* (%) = 613.2 (35) [M+Na], 591.3 (65) [M+H], 565 (10), 535 (20), 491 (58), 361 (15), 284 (12), 189 (21), 175 (54), 89 (26), 84 (100); HRMS (FAB) calcd. for $C_{25}H_{47}N_6O_6S_2$ $[M+H]^+$: 591.2999; found: 591.3000.

2-(5-(1,2-dithiolan-3-yl)pentanamido)-6-amino-N-(2-(2-(2-azidoethoxy)ethoxy)ethyl)hexanamide (L2).

To a stirred solution of tert-butyl (5-(5-(1,2-dithiolan-3-yl)pentanamido)-6-((2-(2-(2-azidoethoxy)ethoxy)ethyl)amino)-6-oxohexyl)carbamate (**10**) (200 mg, 0.33 mmol) in dry CH_2Cl_2 (15 mL) was added trifluoroacetic acid (5 mL). The reaction mixture was stirred for 2 h at room temperature. Dichloromethane (15 mL) was then added and the solvent was removed under reduced pressure. The procedure was repeated three times to remove the traces of trifluoroacetic acid to afford 2-(5-(1,2-dithiolan-3-yl)pentanamido)-6-amino-N-(2-(2-(2-azidoethoxy)ethoxy)ethyl)hexanamide (**L2**) as yellow thick oil (152 mg 91%)

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.10-1.32 (m, 6 H), 1.36-1.50 (m, 8 H), 1.67-1.69 (m, 1 H), 2.10 (br, 1 H), 2.32-2.44 (m, 1 H), 2.74 (br, 3 H), 2.85-2.94 (m, 1 H), 3.32-3.36 (m, 11 H), 3.82 (br. 1 H), 4.24 (m, 1 H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 22.1, 25.6, 26.4, 27.9, 31.4, 33.7, 34.9, 35.0, 35.1, 36.6, 39.0, 50.4, 52.6, 56.8, 69.2, 69.7, 69.9, 70.2, 172.3, 173.8; IR (Film): ύ = 3392, 1733, 1666, 1461, 1191, 1132, 843, 799, 604, 517 cm^{-1}; MS (+FAB): *m/z* (%) = 491.2 (5) [M+H], 425 (13), 339 (23), 175 (68), 153.8 (100), 135.8 (82), 106 (41), 94 (48); HRMS (FAB) calcd. for $C_{20}H_{39}N_6O_4S_2$ $[M+H]^+$: 491.2396; found: 491.2531.

(3) Optimization of AuNP synthesis by using different linker to precursor ratios

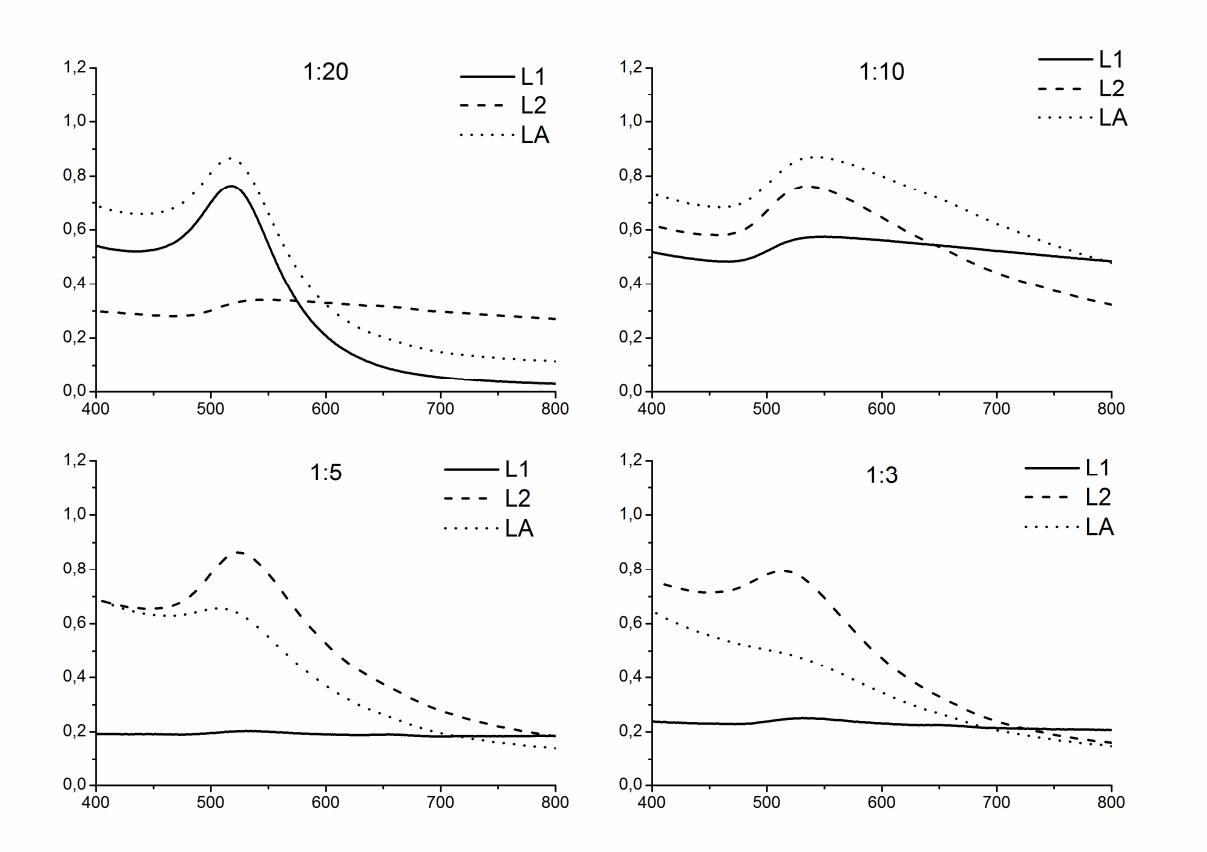


Fig. S2 UV-Vis spectra of AuNP synthesized with different linker to gold ratios.

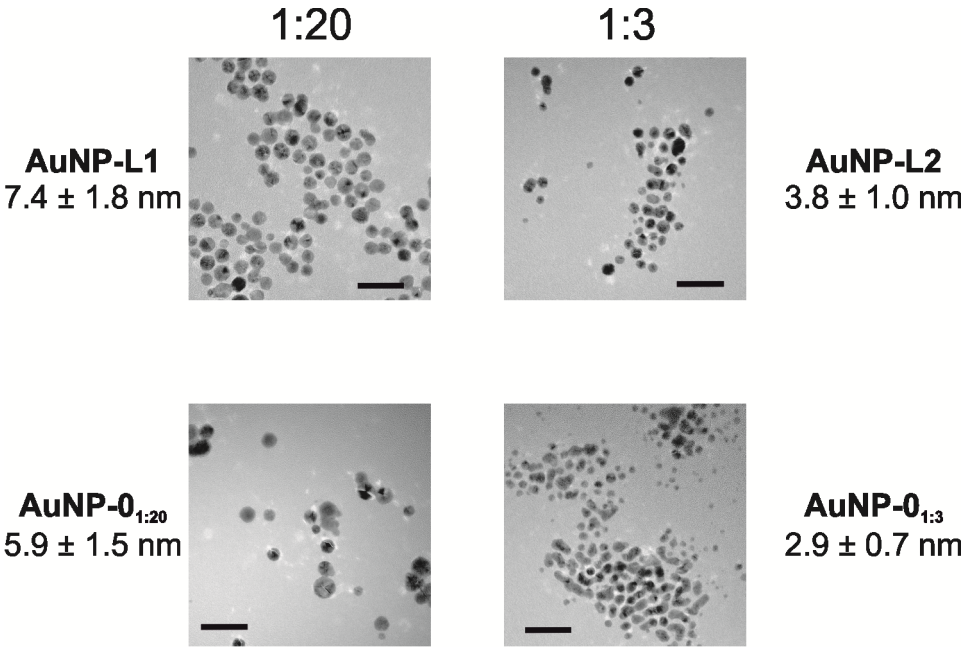


Fig. S3 TEM images of AuNP synthesized with different linker to gold ratios.