

Supplemental Information

Identification of Antibiotics Using Variable Small Molecule Ligand Display on Gold Nanoparticles

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Table S1. List of nanoparticle conjugates synthesized.

Compound Number	Thiol A	Thiol B	Thiol C
1	1	2	3
2	1	2	4
3	1	2	5
6	1	2	8
7	2	3	4
8	2	3	5
10	2	3	7
11	2	3	8
12	3	4	5
14	3	4	7
15	3	4	8
16	4	5	6
17	4	5	7
18	4	5	8
19	5	6	7
20	5	6	8
21	2	7	8
22	3	7	8
23	4	7	8
24	5	7	8
25	2	5	8
26	1	3	8
27	1	4	8
28	1	5	8
29	1	6	8
30	1	5	6
31	2	5	6
32	3	5	6
33	2	6	8
34	3	5	8
35	1	3	4
36	1	4	5
37	2	4	5
38	3	5	7
39	1	6	7
40	2	6	7
42	4	6	7
43	3	6	8
44	2	5	7
45	1	3	5
47	1	3	7
49	1	4	7
50	1	5	7
51	2	4	6
52	2	4	7
53	2	4	8
54	4	6	8
55	6	7	8
56	1	7	8
57	1	2	9
58	1	3	9
59	1	4	9
60	1	5	9
61	1	6	9
62	1	7	9
64	2	3	9
66	2	5	9
67	2	6	9
68	2	7	9
69	2	8	9
70	3	4	9
71	3	5	9

Compound Number	Thiol A	Thiol B	Thiol C
73	3	7	9
74	3	8	9
75	4	5	9
77	4	7	9
78	4	8	9
80	5	7	9
81	5	8	9
82	6	7	9
83	6	8	9
84	7	8	9
88	1	5	10
93	2	3	10
94	2	4	10
95	2	5	10
96	2	6	10
97	2	7	10
98	2	8	10
100	3	4	10
101	3	5	10
103	3	7	10
104	3	8	10
105	3	9	10
106	4	5	10
108	4	7	10
109	4	8	10
111	5	6	10
112	5	7	10
113	5	8	10
114	5	9	10
115	6	7	10
116	6	8	10
118	7	8	10
119	7	9	10

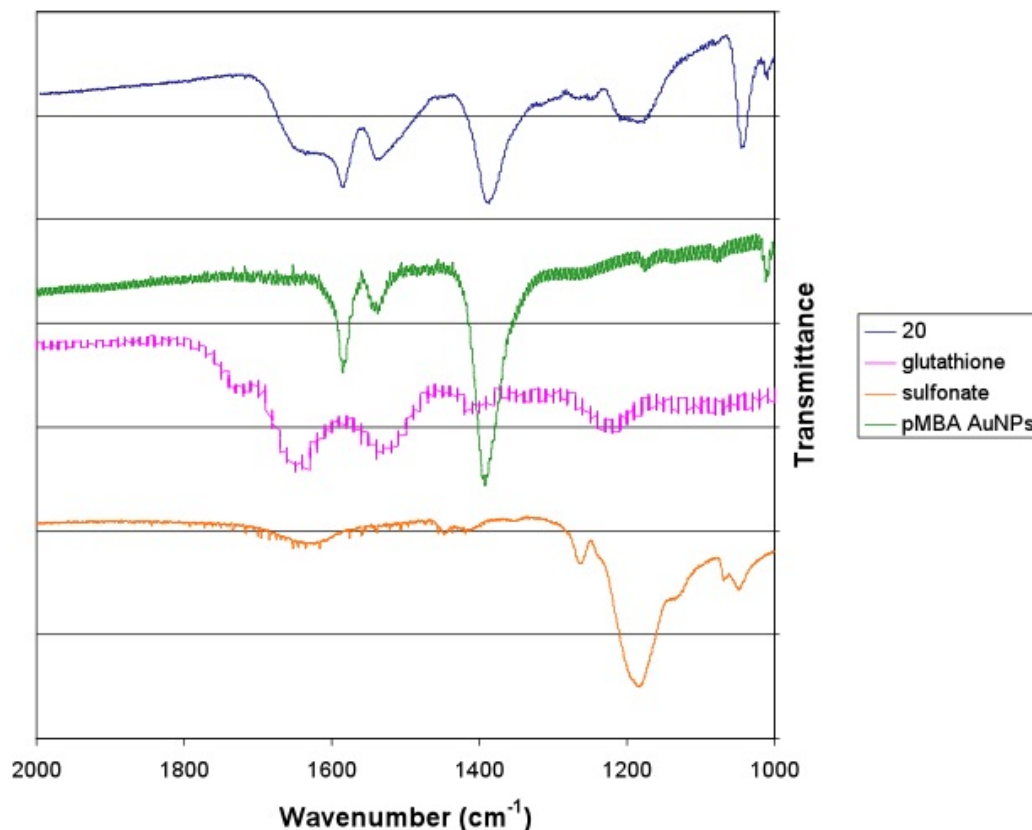


Figure S1. IR spectra of conjugate 20, pMBA-coated gold nanoparticles, and thiols **5** and **8**.

Experimental Methods

Synthesis of 2.0 nm gold nanoparticles

Two nanometer diameter [Au₁₄₄(SC₆H₄COOH)₆₀] gold nanoparticles were synthesized as previously described.¹ In short, a solution of 11.1 mM HAuCl₄ (SigmaAldrich), 37.8 mM p-mercaptobenzoic acid (p-MBA) (TCI-America), 178 mM NaOH in 55.6 % (v/v) aqueous methanol was prepared and allowed to equilibrate for 24 hours with constant stirring. Fifty milliliters of this solution (0.556 mmoles of Au³⁺) were diluted to a final Au³⁺ concentration of 0.48 mM with the addition of 260 mL methanol and 740 mL water. The Au³⁺ was reduced with the addition of 10 mL of 0.25 M NaBH₄ (SigmaAlrich). The final methanol concentration was adjusted to 24.8 % with the addition of 100 mL of water. The reduction of gold was allowed to proceed for 48 hours at room temperature with constant stirring. Gold nanoparticles were precipitated with the addition of 68 mmoles of NaCl and 500 mL of methanol (final methanol concentration of 47 % v/v) followed by centrifugation at 3200 RCF for five minutes. The precipitated nanoparticles were reconstituted in water. The concentration was measured by UV-visible spectroscopy, using the $\epsilon_{510\text{ nm}}$ of 409, 440 M⁻¹cm⁻¹. Further, it was noted that the source of reagents for this synthesis is important to the preparation. Ensuring that reagents were not stored with other chemicals that could react or contaminate them was also critical.

Place exchange reactions

One pot place exchange reactions were conducted with the addition of 740 μM total thiol added in 1:1:1 molar ratio to 7.4 μM gold nanoparticles in 20 mM sodium phosphate buffer, pH 9.5. Reactions were placed on a plate shaker and agitated for 24 hours at room temperature. The exchange product was harvested through the addition of 40 mmoles of NaCl and a volume of methanol equal to that of phosphate buffer and added salt. Reactions were centrifuged at 3200 RCF for 30-60 minutes. Precipitated nanoparticles were resuspended and precipitated with the addition of NaCl and methanol two times to wash out excess unreacted thiol. Particles were allowed to dry to completion overnight at room temperature and resuspended in 20 mM sodium phosphate buffer, pH 9.5. Resuspended nanoparticles were washed with 20 mM sodium phosphate buffer, pH 9.5 over a 30K MWCO centricon filter to remove excess salt and thiol followed by buffer exchange into Mueller-Hinton broth for assay.

In order to optimize exchange reactions, the molar ratio of thiols added into the reaction can be altered. For example, as nanoparticle compounds 28 and 50 were relatively insoluble after place exchange reactions in aqueous media and had low yields, the amount of (3-nitrobenzyl)mercaptan was reduced to $\frac{2}{3}$ the original feed.

Bacterial growth inhibition assays

Inoculation of *E. coli* into 3 mL of Mueller-Hinton broth (Fisher) was carried out by touching the top of 4 well isolated colonies of *E. coli* (ATCC 25922) from a Mueller-Hinton agar (Fisher) plate with an inoculation loop. The culture was allowed to grow at 37 °C, 225 rpm for 7 hours after which it was diluted to 1×10^6 CFU/ml in Mueller-Hinton broth. Equal volumes of diluted inoculum and nanoparticle sample (adjusted to the correct assay concentration in Mueller-Hinton broth) were mixed to make the final inoculum concentration 5×10^5 CFU/ml. Samples were incubated at 37 °C, 225 rpm for 18 hours. End points were determined by colony counting on Mueller-Hinton agar after dilution of each sample in PBS and incubation of the plates at 37 °C for 24 hours

Infrared spectroscopy

Nanoparticle samples were reconstituted and washed of contaminants over a 30K MWCO centricon filter with water. Samples were then spotted onto potassium bromide Real Crystal IR cards (International Crystal Laboratories) in their appropriate solvent and allowed to dry. IR analysis was carried out on a Thermo Nicolet Avatar 360 FT-IR E.S.P.

IM-MS

IM-MS was performed as described previously.² Average values of thiol/nanoparticle were based upon 60 ligands total that each nanoparticle can accommodate.

Reference List

1. Bowman, M. C.; Ballard, T. E.; Ackerson, C. J.; Feldheim, D. L.; Margolis, D. M.; Melander, C. J. *Am. Chem. Soc.* **2008**, *130*, 6896.
2. Harkness, K. M.; Fenn, L. S.; Cliffler, D. E.; McLean, J. A., *Anal. Chem.* 2010, *82*, 3061.