Supporting Information:

A Structural Mass Spectrometry Strategy for the Relative Quantitation of Ligands on Mixed Monolayer-Protected Gold Nanoparticles

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Gold nanoparticles (AuNPs) with a protecting monolayer of gold-thiolate complexes have become an important nanomaterial for applications in medicine, catalysis, and electronics, among others. The use of heterogeneous ligand mixtures for precise functionality control has become commonplace, increasing the need for a fast and facile method for characterizing mixed monolayer-protected AuNPs. Using the recently developed methodology for the surface characterization of AuNPs by ion mobility-mass spectrometry (IM-MS), we have developed a new strategy for the measurement of the relative amounts of mixed ligand compounds in the protecting monolayer. This strategy was applied to AuNPs with three binary ligand mixtures: tiopronin:glutathione (avg. diam. 2.5 nm), octanethiol:decanethiol (avg. diam. 3.6 nm), and tiopronin:11-mercaptoundecyl(poly ethylene glycol) (avg. diam. 2.5 nm). For validation purposes, the results obtained for tiopronin:glutathione AuNPs were compared to parallel measurements using nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS) without ion mobility separation. Relative quantitation measurements for NMR and IM-MS were in excellent agreement, with an average difference of less than 1% relative abundance. IM-MS and MS without ion mobility separation were not comparable, due to a lack of ion signals for MS. The other ligand mixtures provide examples of measurements which cannot be performed using NMR spectroscopy.

I. Table S1. Sample peak list from 56:44 Tio:GS AuNPs.

Matched m/z	Au	Tio	GS	Ester	Na	H	Counts	Mass error (ppm)
711.04	1	1	1	0	2	0	384.5	6.9
718.99	2	2	0	0	0	1	58.97	3.7
721.99	2	0	1	0	1	-1	397.8	-2.4
725.06	1	1	1	1	2	0	92.76	27.5
733.03	1	1	1	0	3	-1	281.7	13.1
740.97	2	2	0	0	1	0	140	1.5
743.97	2	0	1	0	2	-2	268.4	-2.4
747.04	1	1	1	1	3	-1	56.78	17.2
755.01	1	1	1	0	4	-2	412.3	3.5
762.95	2	2	0	0	2	-1	184.2	-3.3
769.02	1	1	1	1	4	-2	77.61	2.7
776.99	1	1	1	0	5	-3	660.7	-4.1
784.93	2	2	0	0	3	-2	207.3	1.9
855.10	1	0	2	0	2	0	121.3	-8.9
863.04	2	1	1	0	0	1	287.3	-0.3
877.08	1	0	2	0	3	-1	212.7	-13.8
885.02	2	1	1	0	1	0	719.2	-2.5
899.06	1	0	2	0	4	-2	317.1	-7.0
907.00	2	1	1	0	2	-1	623.6	-2.3
921.04	1	0	2	0	5	-3	409	-4.9
925.98	2	3	0	0	2	0	33.98	8.8
928.99	2	1	1	0	3	-2	375.9	-0.3
940.00	2	3	0	1	2	0	61.39	1.9
943.03	1	0	2	0	6	-4	556.8	-8.3
950.97	2	1	1	0	4	-3	333.3	6.9
954.01	2	3	0	2	2	0	38.65	-4.0
958.91	3	2	0	0	2	-2	78.93	-5.4
961.98	2	3	0	1	3	-1	45.2	20.1
972.92	3	2	0	1	2	-2	37.53	18.6
975.99	2	3	0	2	3	-1	51.66	-6.6
983.96	2	3	0	1	4	-2	57.49	-8.6
991.93	2	3	0	0	5	-3	35.6	2.7
997.98	2	3	0	2	4	-2	26.97	24.0
1007.09	2	0	2	0	0	1	117.2	8.5
1019.96	2	3	0	2	5	-3	32.93	-27.1
1029.07	2	0	2	0	1	0	342	1.5
1051.06	2	0	2	0	2	-1	304.6	5.0
1059.00	3	1	1	0	0	0	228.5	4.8
1070.03	2	2	1	0	2	0	53.57	-16.0
1073.04	2	0	2	0	3	-2	234.9	5.2
1080.98	3	1	1	0	1	-1	344.7	0.9
1095.02	2	0	2	0	4	-3	185.7	6.2
1099.96	3	3	0	0	1	0	140.3	1.5
1102.96	3	1	1	0	2	-2	266.9	-1.9
1113.97	3	3	0	1	1	0	75.78	8.3
1117.00	2	0	2	0	5	-4	244.1	8.1
1124.94	3	1	1	0	3	-3	77.28	12.7
1128.01	2	2	1	1	4	-2	96.92	3.1
1135.98	2	2	1	0	5	-3	66.66	-1.0
1138.96	3	1	1	1	3	-3	28.19	-14.0
1157.96	2	2	1	0	6	-4	88.6	8.8
1203.05	3	0	2	0	0	0	445	6.2
1214.09	2	1	2	0	2	0	25.25	23.9
1225.03	3	0	2	0	1	-1	287.3	13.3
1236.07	2	1	2	0	3	-1	30.14	10.3
1244.01	3	2	1	0	1	0	196.8	8.4
1247.02	3	0	2	0	2	-2	171.9	10.9
1258.05	2	1	2	0	4	-2	99.28	-4.5
1265.99	3	2	1	0	2	-1	65.39	12.6
1269.00	3	0	2	0	3	-3	53.29	16.7
1273.93	4	3	0	0	0	0	129.2	11.5
1280.03	2	1	2	0	5	-3	36.99	20.6

Matched m/z	Au	Tio	GS	Ester	Na	H	Counts	Mass error (ppm)
1287.97	3	2	1	0	3	-2	140.8	-7.4
1290.98	3	0	2	0	4	-4	71.65	0.0
1301.99	3	2	1	1	3	-2	96.53	2.6
1309.96	3	2	1	0	4	-3	25.49	-4.8
1317.90	4	3	0	0	2	-2	41.83	25.7
1323.97	3	2	1	1	4	-3	39.5	3.2
1331.94	3	2	1	0	5	-4	115.7	-3.7
1345.93	4	3	0	2	2	-2	45.98	7.7
1388.06	3	1	2	0	1	0	166.5	6.5
1402.08	3	1	2	1	1	0	31.09	1.2
1410.05	3	1	2	0	2	-1	69.58	18.8
1417.99	4	2	1	0	0	0	239	-3.3
1432.03	3	1	2	0	3	-2	142	-2.9
1436.96	4	4	0	0	0	1	59.47	-9.7
1439.97	4	2	1	0	1	-1	111.2	3.2
1454.01	3	1	2	0	4	-3	87.43	3.1
1458.95	4	4	0	0	1	0	1235	-1.8
1461.95	4	2	1	0	2	-2	82.71	-3.3
1472.96	4	4	0	1	1	0	811.3	3.6
1475.97	4	2	1	1	2	-2	201.7	1.8
1480.93	4	4	0	0	2	-1	471.6	6.5
1486.98	4	4	0	2	1	0	251.5	18.3
1490.01	3	1	2	1	5	-4	77.46	4.9
1494.94	4	4	0	1	2	-1	229.8	1.6
1497.95	4	2	1	1	3	-3	34.58	-10.4
1502.91	4	4	0	0	3	-2	242.3	12.8
1505.92	4	2	1	0	4	-4	38.94	19.1
1508.96	4	4	0	2	2	-1	64.49	-5.9
1516.93	4	4	0	1	3	-2	117.6	22.8
1524.89	4	4	0	0	4	-3	186.9	14.5
1530.94	4	4	0	2	3	-2	23.49	-22.1
1532.12	3	0	3	0	1	0	59.69	2.5
1538.91	4	4	0	1	4	-3	154.7	15.5
1562.04	4	1	2	0	0	0	519.9	5.5
1576.06	4	1	2	1	0	0	169	-19.0
1581.02	4	3	1	0	0	1	510.9	3.2
1584.02	4	1	2	0	1	-1	204.6	1.0
1595.03	4	3	1	1	0	1	215.8	11.2
1598.06	3	0	3	0	4	-3	76.65	3.2
1603.00	4	3	1	0	1	0	2135	-4.1
1606.00	4	1	2	0	2	-2	198.4	8.5
1617.02	4	3	1	1	1	0	1182	0.9
1620.02	4	1	2	1	2	-2	178	4.5
1624.98	4	3	1	0	2	-1	977.1	1.0
1627.99	4	1	2	0	3	-3	65.79	10.2
1631.03	4	3	1	2	1	0	204.6	9.9
1632.92	5	4	0	0	0	0	56.54	20.8
1639.00	4	3	1	1	2	-1	377.4	8.2
1642.00	4	1	2	1	3	-3	96	-0.6
1646.96	4	3	1	0	3	-2	558.3	4.1
1649.97	4	1	2	0	4	-4	44.17	13.3
1653.01	4	3	1	2	2	-1	48.73	9.1
1660.98	4	3	1	1	3	-2	207.4	10.6
1663.98	4	1	2	1	4	-4	76.24	24.4
1668.95	4	3	1	0	4	-3	317.3	6.7
1671.95	4	1	2	0	5	-5	72.9	-2.5
1676.89	5	4	0	0	2	-2	22.03	-15.8
1682.96	4	3	1	1	4	-3	177.5	7.9
1690.93	4	3	1	0	5	-4	197.9	21.4
1704.94	4	3	1	1	5	-4	106.7	28.1
1712.91	4	3	1	0	6	-5	116.4	12.2
1725.07	4	2	2	0	0	1	451.6	12.1

I. Sample peak list (cont.)

Matched m/z	Au	Tio	GS	Ester	Na	H	Counts	Mass error (ppm)
1728.08	4	0	3	0	1	-1	116.5	-29.3
1739.09	4	2	2	1	0	1	164.2	-15.9
1747.05	4	2	2	0	1	0	1888	-3.8
1750.06	4	0	3	0	2	-2	185.9	1.8
1753.10	4	2	2	2	0	1	25.52	3.5
1761.07	4	2	2	1	1	0	757.1	4.5
1769.03	4	2	2	0	2	-1	951.2	1.7
1772.04	4	0	3	0	3	-3	80.19	2.7
1775.08	4	2	2	2	1	0	73.25	7.8
1776.98	5	3	1	0	0	0	25.69	-17.8
1783.05	4	2	2	1	2	-1	308.8	-0.2
1791.02	4	2	2	0	3	-2	587.6	3.3
1798.96	5	3	1	0	1	-1	28.62	3.9
1805.03	4	2	2	1	3	-2	159.4	2.9
1813.00	4	2	2	0	4	-3	318.6	4.0
1816.00	4	0	3	0	5	-5	21.29	-3.2
1817.94	5	5	0	0	1	0	67.48	6.2
1820.94	5	3	1	0	2	-2	40.16	-10.6
1827.01	4	2	2	1	4	-3	94.41	21.7
1831.95	5	5	0	1	1	0	35.06	7.4
1834.98	4	2	2	0	5	-4	138.5	6.0
1839.92	5	5	0	0	2	-1	23.49	5.4
1845.97	5	5	0	2	1	0	33.41	-7.8
1853.93	5	5	0	1	2	-1	30.33	-14.8
1856.96	4	2	2	0	6	-5	40.76	26.3
1861.90	5	5	0	0	3	-2	22.91	20.2
1869.12	4	1	3	0	0	1	180.7	15.2
1883.14	4	1	3	1	0	1	51.68	-12.0
1891.11	4	1	3	0	1	0	890.8	2.9
1897.90	5	5	0	1	4	-3	27.65	29.8
1905.12	4	1	3	1	1	0	210.7	2.9
1913.09	4	1	3	0	2	-1	440.4	8.4
1921.03	5	2	2	0	0	0	80.66	-10.9
1927.10	4	1	3	1	2	-1	86.27	12.2
1935.07	4	1	3	0	3	-2	263.4	0.7
1957.05	4	1	3	0	4	-3	130.4	7.5
1961.99	5	4	1	0	1	0	148.9	7.0
1976.00	5	4	1	1	1	0	110.1	10.6
1983.97	5	4	1	0	2	-1	66.09	6.3
2001.02	4	1	3	0	6	-5	55.38	3.2
2005.95	5	4	1	0	3	-2	63.98	22.9
2019.97	5	4	1	1	3	-2	30.86	2.3
2035.16	4	0	4	0	1	0	201.2	-3.0
2057.14	4	0	4	0	2	-1	64.86	-16.0
2065.08	5	1	3	0	0	0	29.51	17.3
2079.10	5	1	3	1	0	0	76.51	4.5
2079.84	6	5	0	0	4	-4	21.92	-12.5
2084.06	5	3	2	0	0	1	29.31	7.7
2098.08	5	3	2	1	0	1	24.73	18.6
2101.11	4	0	4	0	4	-3	24.52	22.1
2106.04	5	3	2	0	1	0	135.8	-7.9
2109.05	5	1	3	0	2	-2	35.59	9.6
2120.06	5	3	2	1	1	0	81.07	9.8
2123.09	4	0	4	0	5	-4	33.86	15.2
2128.02	5	3	2	0	2	-1	102.1	8.6
2145.07	4	0	4	0	6	-5	31.75	20.9
2150.01	5	3	2	0	3	-2	68.95	0.6
2164.02	5	3	2	1	3	-2	29.85	-0.3
2167.03	5	1	3	1	4	-4	37.23	20.6
2190.94	6	6	0	1	1	0	20.96	17.1
2193.97	5	3	2	0	5	-4	29.49	26.4
2196.97	5	1	3	0	6	-6	19.16	0.8

II. Characterization of AuNPs: TEM, UV-Vis, and TGA analysis

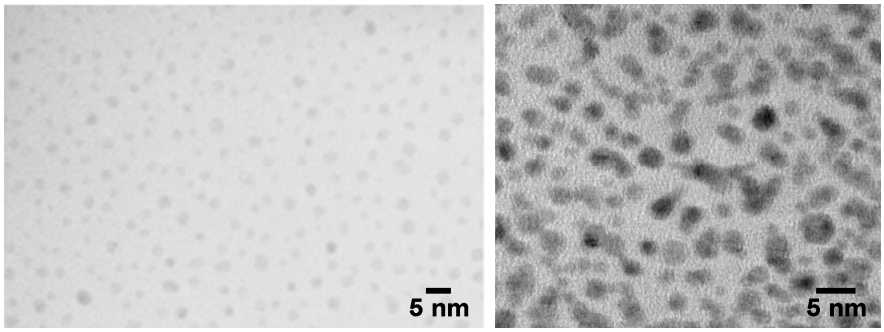

Figure S1. Transmission electron microscopy images of Tio and OT AuNPs. Average core diameters were measured to be 2.5 ± 0.6 and 3.6 ± 1.5 nm, respectively.

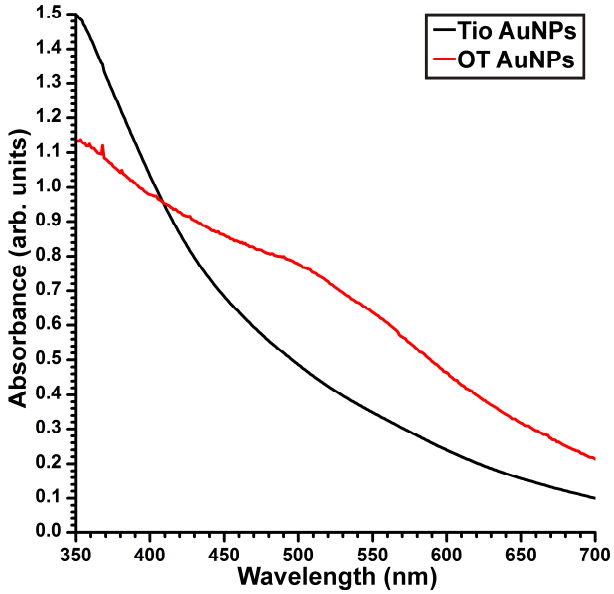

Figure S2. UV-Vis spectrum of Tio and OT AuNPs. A surface plasmon band (centered at approximately 520 nm) was observed for OT AuNPs, but not for Tio AuNPs.

II. Characterization of AuNPs: TEM, UV-Vis, and TGA analysis (cont.)

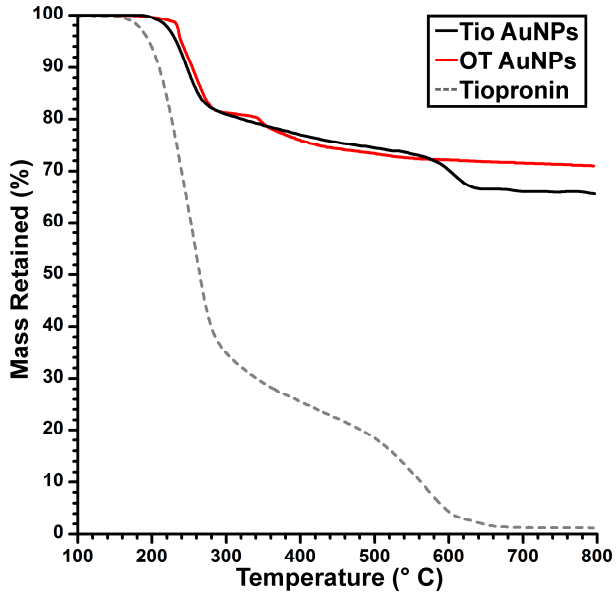

Figure S3. Thermal gravimetric analysis of Tio and OT AuNPs. The organic mass fraction of the two samples were measured to be 35.3% and 29.0%, respectively. The results for pure tiopronin are shown for comparison. The two-stage decomposition of the tiopronin and AuNPs do not represent impurity; each AuNP sample was inspected for purity by NMR prior to usage.

III. NMR spectra

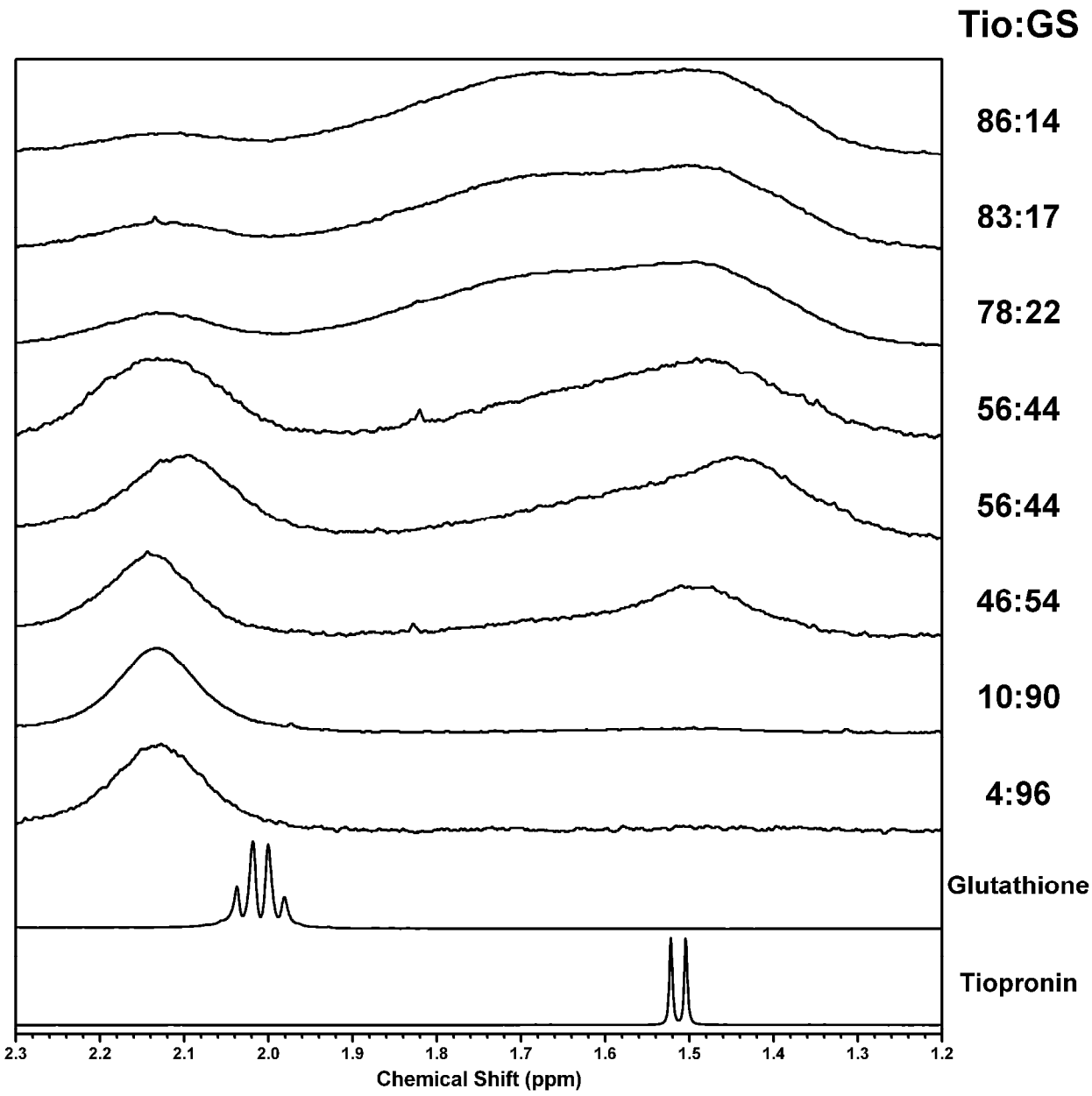

Figure S4. NMR spectra of Tio:GS AuNPs, free glutathione, and free tiopronin. This portion of the NMR spectrum was used for relative quantitation of tiopronin and glutathione on the monolayer-protected AuNP surface. The peak between 1.9 and 2.3 corresponds to glutathione, the peak below 1.9 corresponds to tiopronin. Integration was performed for each peak starting from the outside edge of the peak to the minimum between the two.

IV. Analysis of relative quantitation using only Au_4L_4 ions

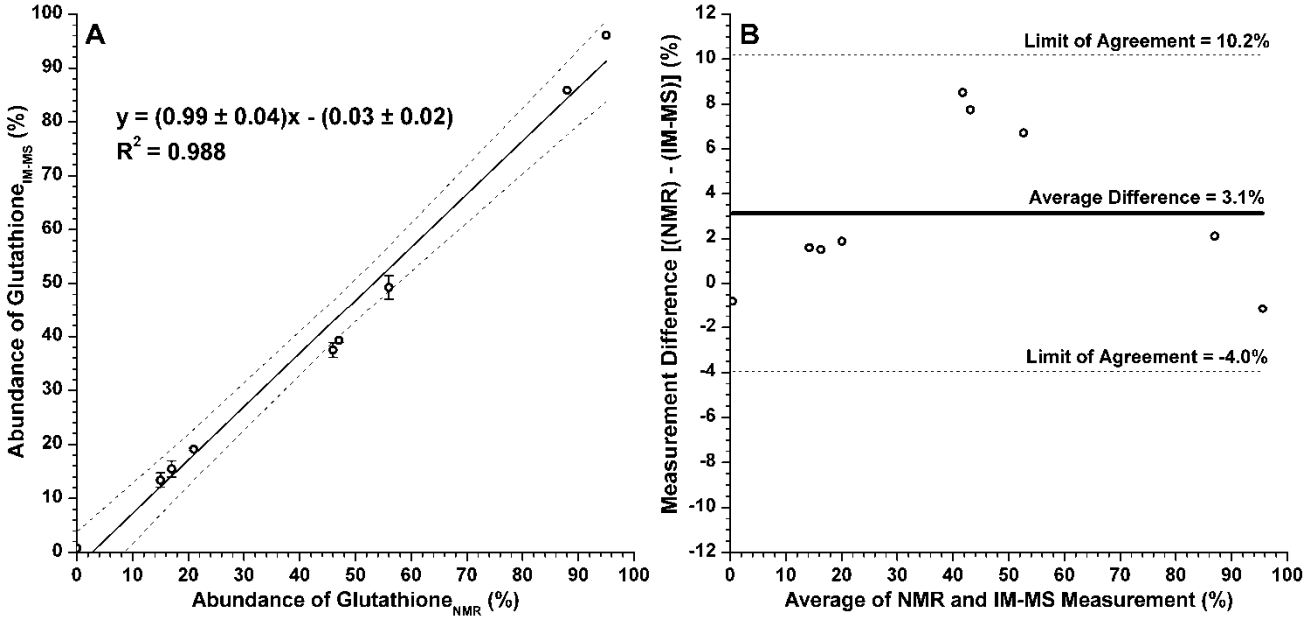

Figure S5. Regression (left) and Bland-Altman (right) plots comparing NMR and MALDI-IM-MS measurements of Tio:GS ratios using only Au_4L_4 ions, expressed as a molar percentage of glutathione. Using only Au_4L_4 ions makes the quantitation measurement quicker and more facile, but yields poorer results than using all gold-thiolate ions (Figure 3).

V. Other Mass Spectra and Ion Mobility-Mass Spectra

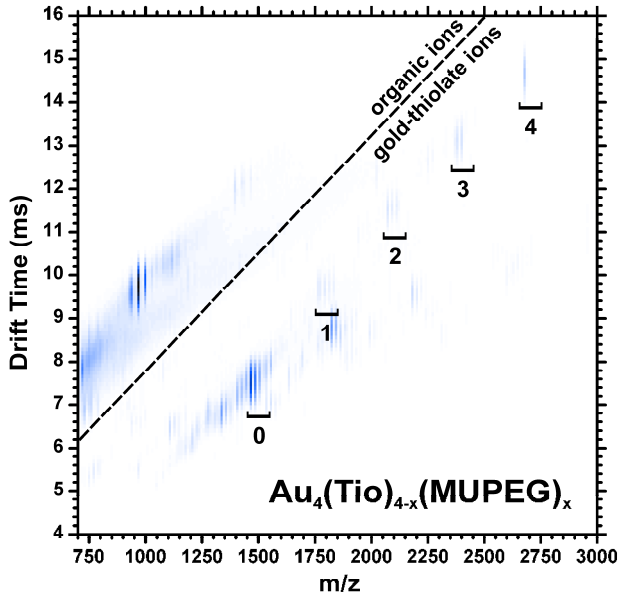

Figure S6. Ion mobility-mass spectrum of 86:14 Tio:MUPEG AuNPs.

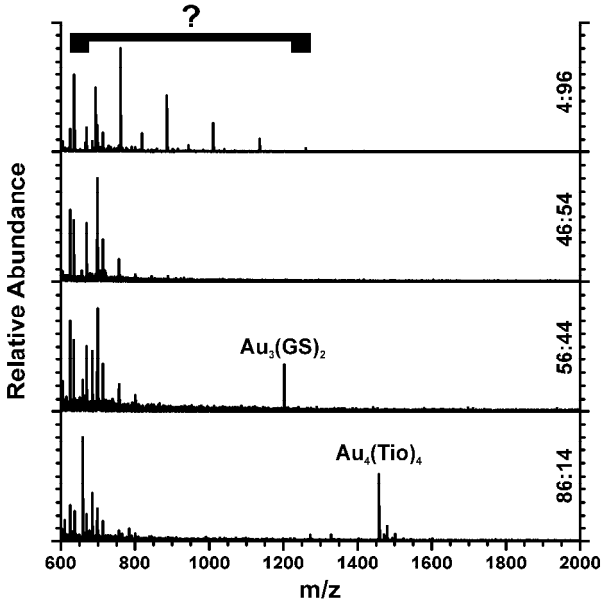

Figure S7. Mass spectra of selected Tio:GS AuNPs, with ratios shown on the right. AuNPs with low amounts of glutathione tend to yield only the most dominant ion from the equivalent IM-MS spectrum. Peaks below 1200 m/z are unidentified ions, likely organic cluster ions or fragmented and rearranged gold-thiolate ions.

V. Other Mass Spectra and Ion Mobility-Mass Spectra (cont.)

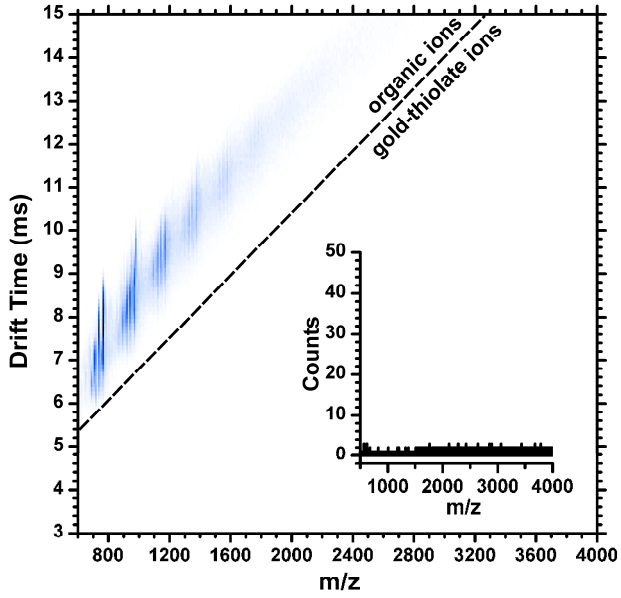

Figure S8. Ion mobility-mass spectrum of a mixture of 2 nm bare gold AuNPs and free tiopronin, with the mass spectrum of the extracted gold-thiolate region shown inset. The solution was spotted and dried immediately after combination to prevent gold-thiol bonding in solution. No signals are seen in the gold-thiolate ion region, establishing that the gold-thiolate complexes investigated here do not form in the gas phase.

V. Other Mass Spectra and Ion Mobility-Mass Spectra (cont.)

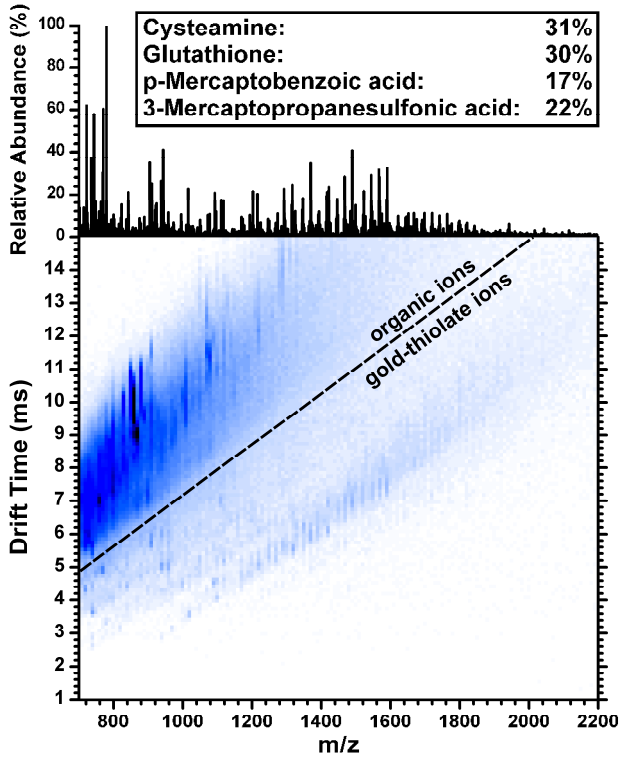

Figure S9. Ion mobility-mass spectrum of AuNPs with a quaternary ligand system. Measured molar percentages of the four ligands are shown above.

VI. Place-Exchange Reaction Parameters

Octanethiol (OT): Decanethiol (DT)		Tiopronin (Tio): Glutathione (GS)		Tiopronin (Tio): (MUPEG)	
Feed Ratio	Time	Feed Ratio	Time	Feed Ratio	Time
1:1	8h	7:1	1h	4:1	3d
1:1	24h	5:1	1h	1:1	3d
1:2	24h	3:1	1h	1:4	3d
1:5	8h	1:1	1d		
1:5	24h	1:1	1d		
1:10	24h	1:2	3d		
		1:60	7d		
		1:60	7d		

Table S2. Feed ratio (original ligand:alternate ligand, as listed in headings) and time elapsed for place exchange reactions in this report. Amount of original ligand estimated from TEM and TGA data. Listings are ordered from least exchange (top) to most exchange (bottom), for comparison to Table 1. A comparison of feed ratios to measured ratios on the final AuNP product reveals a generally incomplete exchange, with the original ligand being favored in the final product. The only exceptions are Tio:GS AuNPs at low amounts of glutathione added.