Electronic Supplementary Material (ESI) for Chemical Communications

Time-resolved photoluminescence spectroscopy for the detection of cysteine and other thiol containing amino acids in complex strongly autofluorescent media

Kewei Huang [a] Ireneusz W. Bulik[a] Angel A. Martí [a,b,c]*

[a] Department of Chemistry, [b] Department of Bioengineering, and [c] Rice Quantum Institute; Rice University, 6100 S. Main Street, Houston, TX 77005, USA.

Supporting Information

Table of contents

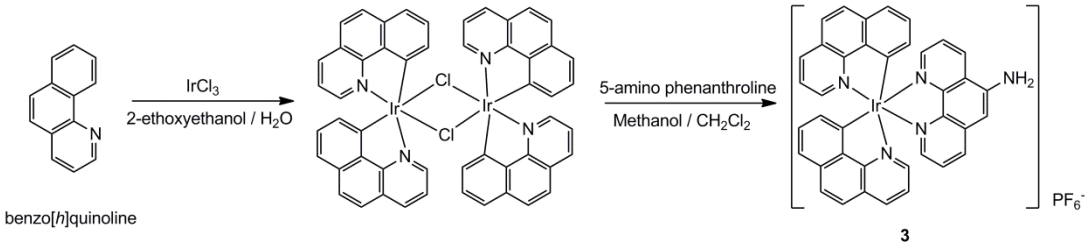
1. General instruments, spectroscopic and computational methods

^{1}H NMR spectra were recorded with a Bruker 400 MHz NMR spectrometer. Electrospray ionization mass spectra (ESI-MS) were measured on a Bruker MicroToF system.

UV-Visible spectra were recorded on a Shimadzu UV-2450 UV-Vis spectrophotometer. Steady-state photoluminescence spectra were obtained in a HORIBA JovinYvonFluorolog 3, with excitation at 370 nm. Time-resolved studies where performed using an Edinburgh Instruments OD470 single-photon counting spectrometer with a high speed red detector, and using a 370 nm picosecond pulse diode laser. Experiments in Dulbecco's Modified Eagle medium were peformed using high glucose, pyruvate DMEM obtained from Invitrogen (cat. no. 11995).

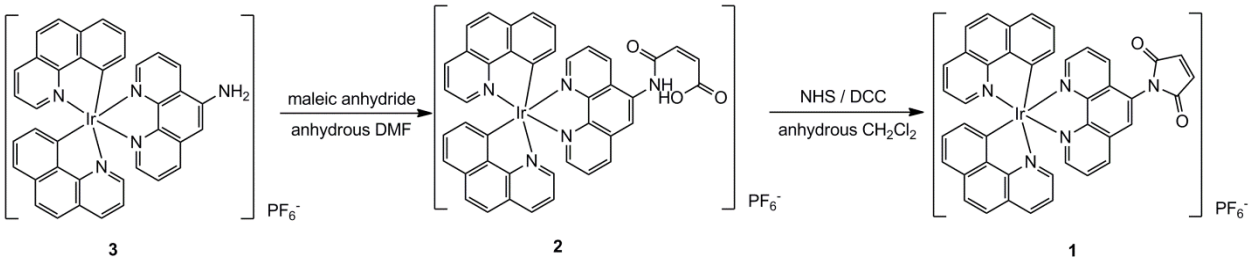
Density Functional Theory calculations were performed with the aid of the Gaussian 09 (revision B.01) suite of programs,[1] employing the PBE0 functional,[2] with a 6-31G* basis set for hydrogen, nitrogen, carbon and sulfur and the LANL2DZ pseudopotentials for Ir,[3] with an additional f-type function on the Ir atom with exponent $\alpha = 0.938$ bohr^{-2} similar to Zhao et al.[4] A conductive polarizable continuum model with water as a solvent was used. Vibrational frequency analysis was used to confirm the potential energy surface minima.

2. Synthesis procedures and characterization data



Scheme S1. Synthesis of iridium complex **3**.

Synthesis of complex 3: This compound was synthesized based on literature methods.[5] A mixture of 2-ethoxyethanol and water (3:1, v/v) was added to a flask containing $IrCl_3$ (0.30 g) and benzo[h]quinoline (0.34 g). The mixture was refluxed for 24 h. After cooling, the orange solid precipitate was filtered by vacuum to give crude cyclometalatediridium(III) chloro-bridged dimer. The solution of cyclometalatediridium(III) chloro-bridged dimer (0.1 g) and 5-amino phenanthroline (0.05 g) in CH_2Cl_2/MeOH [15mL, 1:1 (v/v)] was heated to reflux. After 4 h, the orange solution was cooled to room temperature and then potassium hexafluorophosphate (0.08 g) was added. The suspension was stirred for another 15 minutes and filtered to remove insoluble inorganic salts. The solution was evaporated to dryness under reduced pressure and the crude product was purified by silica gel chromatography with an acetone and dichloromethane mixture (1:5, v/v) as eluent resulting in 80% yield. Rf for compound **3** is 0.38. ^{1}H NMR (400 MHz, d_6-acetone) δ (ppm) 8.975 (d, J = 8.4, 1H), 8.457 (d, J = 8.0, 2H), 8.355 (m, 2H), 8.124 (m, 2H), 7.960 (d, J = 4.8, 1H), 7.934 (d, J = 8.8, 2H), 7.840 (m, 3H), 7.587 (m, 1H), 7.508 (m, 2H), 7.439 (m, 2H), 7.311 (s, 1H), 7.163 (m, 2H), 6.463 (m, 2H), 6.394 (s, 2H). ESI-MS: m/e 744.2 ($M - PF_6^-$).



Scheme S2. Synthesis of iridium complex **1**.

Synthesis of complex 2: A mixture of complex 3 (0.10 g) and maleic anhydride (0.20 g) was stirred in anhydrous DMF (5 mL) at 60 ^{o}C for 48 hours. The solution was evaporated to dryness under reduced pressure and the crude product was purified by silica gel chromatography with an methanol and dichloromethane mixture (1:19, v/v) as eluent resulting in 60% yield. Rf for compound **2** is 0.28. ^{1}H NMR (400 MHz, CD3CN) 8.793 (d, J = 8.4, 1H), 8.565 (s, 1H), 8.544 (d, J = 8.4, 2H), 8.340 (m, 2H), 8.304 (d, J = 4.8, 1H), 8.205 (d, J = 4.8, 1H), 7.907 (d, J = 8.4,

2H), 7.875 (m, 2H), 7.768 (d, *J* = 8.4, 2H), 7.233(m ,1H), 7.640 (m, 1H), 7.516 (d, J = 8.0, 2H), 7.301 (m, 2H), 7.171 (t, *J* = 7.2, 2H), 6.770& 6.471 (both d, symmetric, *J* = 12.4, 1H&1H), 6.391 (t, *J* = 7.2, 2H). ESI-MS: m/e 842.2 ($M - PF_6^-$).

Synthesis of complex 1: A mixture of complex 2 (50 mg), N,N'-Dicyclohexylcarbodiimide (40 mg) and N-Hydroxysuccinimide (25 mg) was dissolved in anhydrous dichloromethane (10 mL) and stirred at room temperature under nitrogen overnight. The solution was filtered to remove the white precipitate and filtrate was evaporated to dryness under reduced pressure. The crude product was purified by silica gel chromatography with an acetone and dichloromethane mixture (1:5, v/v) as eluent resulting in 90% yield. Rf for compound **1** is 0.40. ^{1}H NMR (400 MHz, d_6-DMSO) δ (ppm) 8.918 (d, *J* = 8.4, 1H), 8.790 (d, *J* = 8.4, 1H), 8.536 (m, 3H), 8.236 (m, 2H), 7.966 (m, 8H), 7.594 (m, 2H), 7.486 (m, 2H), 7.432 (m, 2H), 7.236 (t, *J* = 7.2, 2H), 6.318 (t, *J* = 7.2, 2H). ^{13}C NMR (400 MHz, d_6-acetone) δ (ppm) 169.73, 157.15, 152.59, 152.38, 149.15, 147.98, 147.26, 146.43, 146.21, 140.91, 139.03, 137.57, 135.33, 135.27, 134.39, 130.48, 129.77, 129.49, 129.28, 129.12, 128.91, 127.40, 127.30, 127.06, 124.09, 122.43, 120.68. ESI-MS (high resolution): m/e 824.1636 ($M - PF_6^-$) (theoretical value: 824.16).

3. Supplementary spectra data

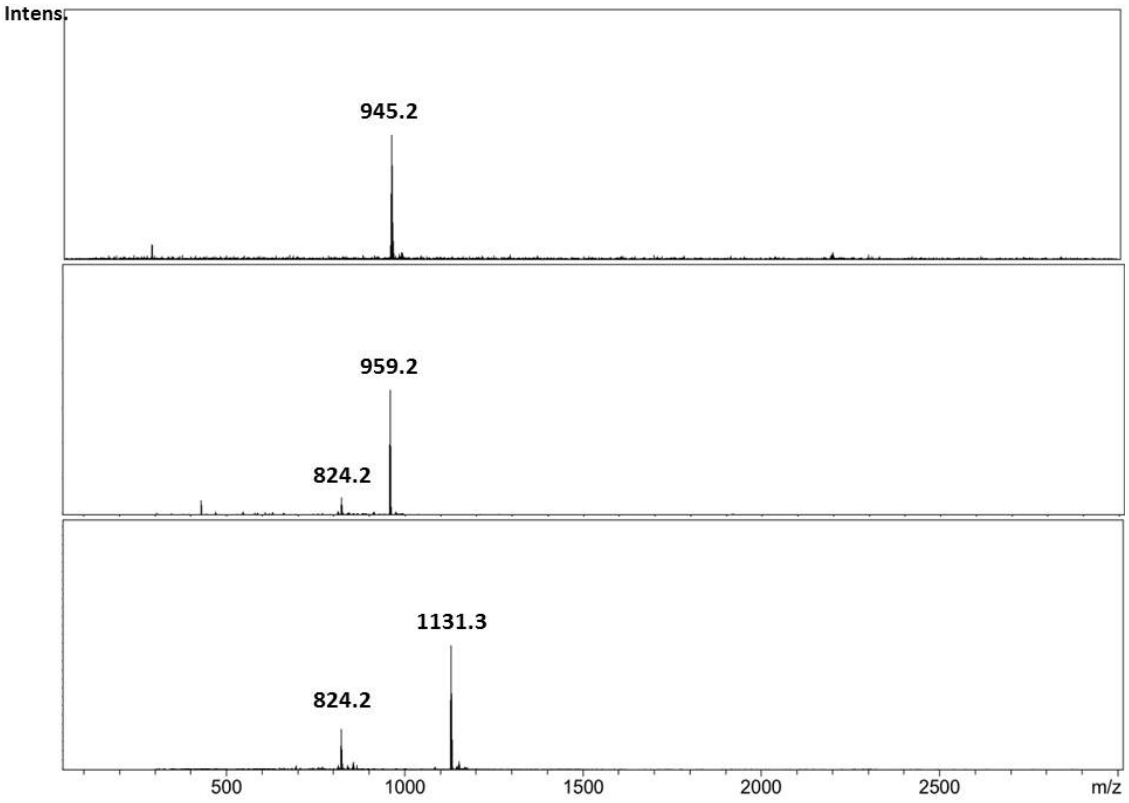


Figure S1. The ESI-Mass Spectrum of **1**-Cys, **1**-Hcy and **1**-GSH (from top to bottom).

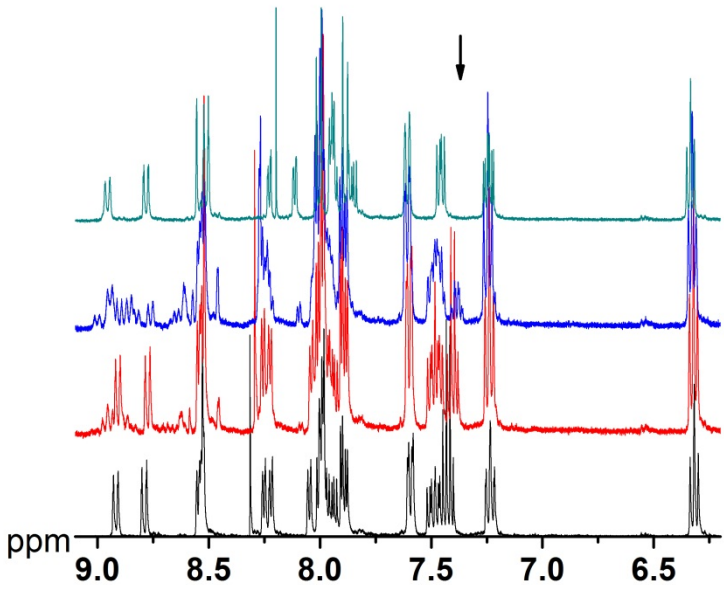


Figure S2. The ^{1}H NMR Spectra of 1.0 mM of **1** in d_6-DMSO upon addition of cysteine in D_2O (0, 0.33, 0.66 and 1.0 mM, from bottom to top). The quartet peak at 7.40 ppm disappeared

gradually after cysteine was added into the solution of **1**, which is attributed to the two protons of the maleimide.

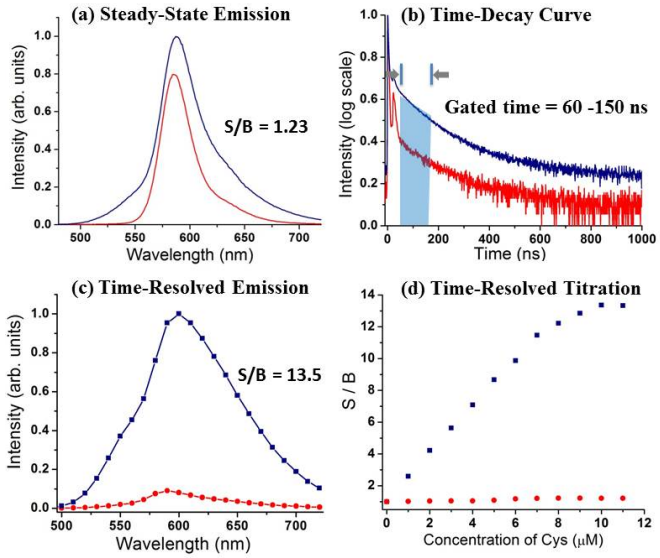


Figure S3. (a) Steady-state photoluminescence emission of 10 μM solution of **1** (PBS 6.7 mM, pH 7.2, 1% DMF) in sulforhodamine autofluorescent media before (red line) and after (blue line) addition of 10 μM cysteine; (b) Time-decay transients of the same solution before (red line) and after (blue line) addition of 10 μM cysteine (λ_{exc} = 370 nm, (λ_{em} = 590 nm); (c) Time-resolved emission spectra of the same solution time-gating from 60 to 150 ns after laser excitation; (d) Titration of cysteine to a solution of **1** in sulforhodamine autofluorescent media using a steady-state fluorometer (red points) and a time-resolved spectrometer with time-gating from 60 to 150 ns (blue points).

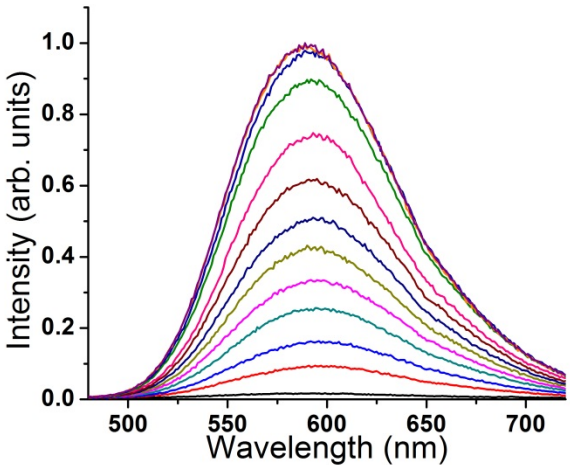


Figure S4. Fluorescence enhancement of 1 (10 μM) upon addition of homocysteine (0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 μM) in PBS buffer (6.7 mM, pH 7.2, 1% DMF).

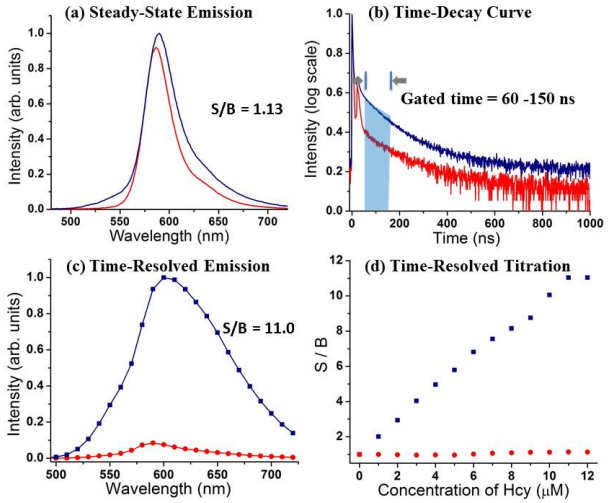


Figure S5. (a) Steady-state photoluminescence emission of 10 μM solution of **1** (PBS 6.7 mM, pH 7.2, 1% DMF) in sulforhodamine autofluorescent media before (red line) and after (blue line) addition of 10 μM homocysteine; (b) Time-decay transients of the same solution before (red line) and after (blue line) addition of 10 μM homocysteine (λ_{exc} = 370 nm, (λ_{em} = 590 nm); (c) Time-resolved emission spectra of the same solution time-gating from 60 to 150 ns after laser excitation; (d) Titration of homocysteine to a solution of **1** in sulforhodamine autofluorescent media using a steady-state fluorometer (red points) and a time-resolved spectrometer with time-gating from 60 to 150 ns (blue points).

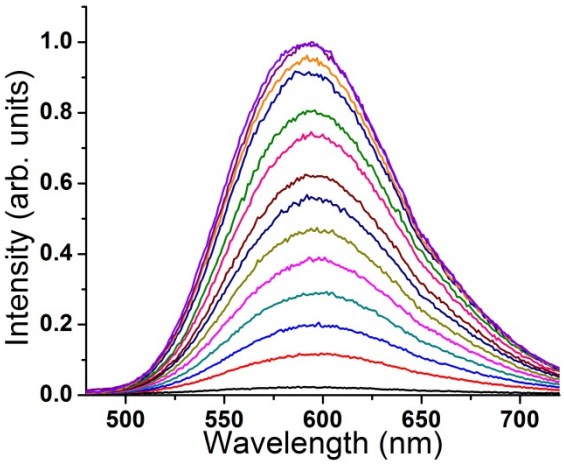


Figure S6. Fluorescence enhancement of 1 (10 μM) upon addition of glutathione (0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 μM) in PBS buffer (6.7 mM, pH 7.2, 1% DMF).

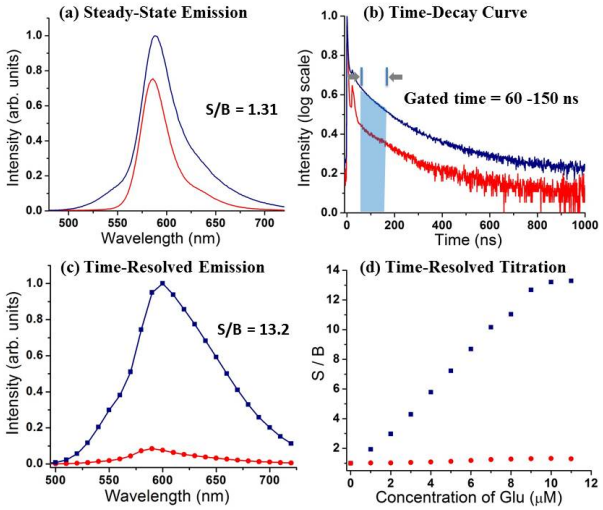


Figure S7. (a) Steady-state photoluminescence emission of 10 μM solution of **1** (PBS 6.7 mM, pH 7.2, 1% DMF) in sulforhodamine autofluorescent media before (red line) and after (blue line) addition of 10 μM glutathione; (b) Time-decay transients of the same solution before (red line) and after (blue line) addition of 10 μM glutathione (λ_{exc} = 370 nm, (λ_{em} = 590 nm); (c) Time-resolved emission spectra of the same solution time-gating from 60 to 150 ns after laser excitation; (d) Titration of glutathione to a solution of **1** in sulforhodamine autofluorescent media using a steady-state fluorometer (red points) and a time-resolved spectrometer with time-gating from 60 to 150 ns (blue points).

Table S1. Photoluminescence Properties of different species.

Name	Lifetime, ns (λ_{em} = 590 nm)[a]	Quantum Yield[b]
1	—	—
$[Ir(bq)_2(phen)]^+$	79.93 (62.37%) 213.51 (37.63%)	24.3%
1 + Cys	48.91 (61.45%) 179.39(38.55%)	11.1%
1 + Hcy	46.33 (58.38%) 188.94 (41.62%)	10.4%
1 + GSH	47.36 (64.00%) 193.99 (36.00%)	9.96%

a. In DMF/PBS (1/99, v/v)
b. In DMF/PBS (1/99, v/v); $Ru(bpy)_3^{2+}$ in H_2O was used as reference[6]

Table S2. Detection Limit of **1** to different Thiol molecules.

Name	Detection Limit (nM)[a]
Cys	16.4
Hcy	14.3

GSH	18.6

a. In DMF/PBS (1/999, (v/v))

Cartesian Coordinates and Parameter from Gaussian Computations

1

Structural Data [Å]					Harmonic Frequencies		
Atom	**Symbol**	**X**	**Y**	**Z**			
1	C	2.369621	1.610705	-2.296088	14.07	25.28	28.65
2	C	3.494683	1.110690	-1.651929	32.48	38.94	41.09
3	C	3.326053	0.271145	-0.523642	44.13	54.58	68.41
4	C	1.996381	0.008115	-0.091248	81.88	84.11	86.72
5	C	1.083897	1.282219	-1.831386	126.05	139.78	154.95
6	C	4.412556	-0.321278	0.230071	158.67	170.45	171.08
7	C	1.750948	-0.776201	1.080104	181.28	183.64	194.23
8	C	2.837451	-1.305209	1.827211	199.98	204.57	222.25
9	C	4.171816	-1.067023	1.364303	225.72	228.78	249.46
10	C	2.526427	-2.065085	2.983806	257.88	263.95	274.13
11	H	3.332521	-2.492652	3.587003	277.92	286.79	291.40
12	C	1.193534	-2.256359	3.329130	293.69	320.80	331.84
13	C	0.175219	-1.693887	2.531958	335.51	342.08	423.19
14	H	2.464284	2.261966	-3.167817	425.40	428.27	431.25
15	H	4.494239	1.371610	-2.003511	433.92	436.99	451.49
16	H	0.181549	1.647302	-2.324388	471.37	471.89	473.55
17	H	0.916633	-2.837890	4.211445	487.37	493.34	497.81
18	H	-0.880627	-1.826916	2.780712	502.48	502.83	523.67
19	N	0.441443	-0.966194	1.434622	532.69	534.16	547.96
20	N	0.890084	0.502547	-0.749821	550.79	553.75	563.61
21	C	-2.216043	-2.520552	-0.606030	564.55	566.37	585.09
22	C	-0.245755	-2.315043	-1.964639	594.08	620.22	620.77
23	C	-0.557010	-3.568548	-2.552066	625.59	635.23	640.00
24	C	-1.685942	-4.298786	-2.178387	642.07	645.80	647.69
25	C	-2.551666	-3.782116	-1.181415	674.93	710.68	711.67
26	C	-3.057724	-1.962703	0.398306	712.64	718.07	720.43
27	C	-3.404873	-0.155103	1.853743	728.35	739.80	741.31
28	C	-4.577891	-0.751525	2.352371	748.81	751.75	752.51
29	C	-4.997665	-1.984747	1.856907	769.95	770.19	773.05
30	C	-4.232205	-2.625051	0.852897	777.18	786.76	809.72
31	H	0.652464	-1.789358	-2.307013	812.12	823.59	824.74
32	H	0.109654	-3.971797	-3.322640	829.70	830.44	858.51
33	H	-1.908171	-5.264475	-2.644468	866.13	867.20	877.50
34	H	-3.052069	0.810073	2.221761	879.39	885.40	910.88
35	H	-5.145108	-0.229587	3.126610	912.81	916.11	916.75
36	H	-5.909014	-2.459008	2.234832	919.87	921.27	922.41
37	N	-2.654871	-0.744684	0.901683	932.13	940.20	941.30
38	C	-2.262539	2.405891	-0.833957	953.02	954.38	956.67

39	C	-2.634807	0.662530	-2.454214	958.50	960.25	966.76
40	C	-3.393804	1.573910	-3.231258	1005.14	1007.06	1022.73
41	C	-3.589657	2.892438	-2.824501	1025.43	1038.59	1058.69
42	C	-3.021269	3.340671	-1.605482	1060.65	1065.86	1078.69
43	C	-1.679224	2.838475	0.400542	1082.54	1090.44	1096.13
44	C	-0.398655	2.237371	2.266924	1098.21	1103.79	1110.25
45	C	-0.500734	3.537001	2.799986	1126.19	1139.06	1139.81
46	C	-1.217750	4.507989	2.105311	1149.20	1150.67	1155.34
47	C	-1.831875	4.174809	0.873715	1186.53	1187.73	1192.67
48	H	-4.176967	3.587038	-3.434399	1214.94	1216.29	1217.18
49	H	0.151390	1.451467	2.791362	1229.20	1229.84	1238.57
50	H	-0.016031	3.762503	3.752898	1239.68	1244.61	1257.97
51	H	-1.312130	5.524360	2.501306	1294.48	1319.36	1323.14
52	N	-0.971522	1.892060	1.100756	1331.20	1334.04	1339.90
53	H	-3.834607	1.230633	-4.173930	1341.82	1361.93	1362.52
54	H	-2.505773	-0.361146	-2.820524	1368.23	1372.54	1392.99
55	C	-2.046494	1.048867	-1.240237	1399.34	1401.99	1413.00
56	C	-1.067500	-1.754600	-0.975420	1415.14	1421.17	1423.18
57	Ir	-0.917239	-0.025766	0.056450	1427.38	1429.43	1447.20
58	N	5.749651	-0.100455	-0.189322	1456.83	1463.20	1490.58
59	C	6.780624	0.423935	0.642615	1494.46	1495.54	1502.88
60	C	6.302637	-0.527399	-1.427479	1504.28	1522.98	1566.40
61	C	8.034852	0.367858	-0.172469	1569.80	1576.32	1578.27
62	C	7.759413	-0.187549	-1.367871	1579.14	1584.69	1594.92
63	H	8.986431	0.734221	0.213556	1596.97	1600.42	1609.11
64	H	8.430119	-0.392928	-2.202506	1619.45	1622.45	1625.15
65	O	5.678131	-1.064144	-2.332953	1731.84	1785.85	3112.70
66	O	6.629806	0.826359	1.787189	3113.37	3120.29	3121.13
67	H	5.008176	-1.498322	1.919726	3126.34	3126.87	3135.91
68	C	-2.598610	5.091332	0.072681	3136.08	3137.20	3138.74
69	C	-3.167917	4.686705	-1.112587	3139.32	3140.27	3151.62
70	C	-3.743077	-4.446753	-0.716157	3154.47	3161.61	3162.21
71	C	-4.549082	-3.896389	0.255627	3165.33	3168.36	3168.95
72	H	-3.749460	5.397739	-1.709878	3176.16	3183.00	3184.15
73	H	-2.721106	6.119689	0.427735	3187.22	3198.32	3216.95
74	H	-5.449228	-4.419947	0.593140			
75	H	-4.004796	-5.415749	-1.155427			

1-Cys

Structural Data [Å]					Harmonic Frequencies		
Atom	**Symbol**	**X**	**Y**	**Z**			
1	C	0.809494	1.597660	-2.255770	8.61	13.39	15.32
2	C	1.917775	1.038273	-1.632146	24.73	26.79	29.22
3	C	1.721345	0.173852	-0.527387	29.93	37.38	40.02
4	C	0.384718	-0.056672	-0.098661	44.16	46.06	54.47
5	C	-0.485917	1.301755	-1.795543	63.16	70.44	81.30

6	C	2.784572	-0.479978	0.204659	84.31	84.68	104.02
7	C	0.115443	-0.873377	1.045462	113.60	121.91	148.63
8	C	1.183924	-1.468098	1.768684	158.82	161.77	164.58
9	C	2.525575	-1.256036	1.312140	171.54	176.22	181.91
10	C	0.850054	-2.258116	2.898080	188.63	196.51	199.59
11	H	1.642425	-2.735268	3.481881	203.94	223.63	225.83
12	C	-0.487934	-2.414744	3.241444	229.04	248.12	254.11
13	C	-1.487560	-1.788483	2.468594	259.09	263.22	274.58
14	H	0.924462	2.269507	-3.109227	279.79	287.28	292.06
15	H	2.925480	1.269445	-1.981782	298.40	309.24	321.30
16	H	-1.375761	1.712866	-2.274818	330.42	332.59	335.72
17	H	-0.783174	-3.017750	4.103110	347.35	391.80	425.71
18	H	-2.546769	-1.894629	2.716024	426.25	428.97	433.60
19	N	-1.199169	-1.031306	1.397194	436.59	440.04	451.95
20	N	-0.705081	0.495370	-0.738589	470.92	472.16	474.22
21	C	-3.882211	-2.449441	-0.689051	487.55	489.29	492.40
22	C	-1.903971	-2.251334	-2.037341	495.27	499.14	502.89
23	C	-2.240496	-3.482076	-2.658005	508.33	524.29	532.96
24	C	-3.385931	-4.197190	-2.306105	534.49	548.35	550.80
25	C	-4.243315	-3.687921	-1.298219	553.96	556.15	564.18
26	C	-4.714897	-1.899970	0.327416	565.45	571.32	577.91
27	C	-5.028801	-0.123094	1.827656	586.66	620.67	625.03
28	C	-6.217963	-0.704990	2.304636	626.43	628.89	634.07
29	C	-6.662391	-1.915626	1.776059	640.53	641.15	644.89
30	C	-5.906118	-2.547248	0.759686	648.32	701.77	710.84
31	H	-0.993053	-1.737383	-2.363751	711.28	711.88	718.09
32	H	-1.580348	-3.879513	-3.437145	721.16	724.33	728.77
33	H	-3.627594	-5.145407	-2.797676	740.36	744.33	748.82
34	H	-4.656571	0.824024	2.222630	752.35	752.91	756.90
35	H	-6.777742	-0.189998	3.088874	770.45	774.04	776.88
36	H	-7.586184	-2.378655	2.137340	778.26	789.03	811.91
37	N	-4.286941	-0.705189	0.864627	823.87	824.93	829.61
38	C	-3.794165	2.491662	-0.768900	830.52	834.49	858.57
39	C	-4.208463	0.814449	-2.447802	865.55	869.00	872.24
40	C	-4.931395	1.774584	-3.200255	879.39	885.67	886.67
41	C	-5.087853	3.085034	-2.752454	911.28	912.93	913.57
42	C	-4.516118	3.474763	-1.515079	917.23	917.78	920.71
43	C	-3.209033	2.864997	0.484039	923.40	935.50	939.12
44	C	-1.964900	2.163371	2.339919	941.03	941.30	953.34
45	C	-2.035378	3.446208	2.916780	955.22	956.90	958.66
46	C	-2.716083	4.461220	2.249110	958.83	960.84	967.26
47	C	-3.326406	4.188675	1.000877	1005.42	1007.22	1015.72
48	H	-5.647069	3.817819	-3.343791	1025.36	1047.85	1058.00
49	H	-1.441264	1.345131	2.841432	1059.34	1061.88	1072.18
50	H	-1.554359	3.624582	3.881481	1080.06	1083.16	1091.73
51	H	-2.784630	5.465782	2.679141	1095.97	1099.17	1105.13
52	N	-2.536129	1.874934	1.157478	1117.86	1129.50	1136.52
53	H	-5.375159	1.476162	-4.156674	1139.29	1139.88	1149.03
54	H	-4.109620	-0.200232	-2.847138	1149.78	1150.77	1153.60

55	C	-3.617471	1.142215	-1.217995	1163.58	1182.98	1185.88
56	C	-2.716220	-1.699252	-1.035630	1188.03	1192.32	1213.99
57	Ir	-2.527503	-0.006098	0.047842	1216.01	1217.25	1229.57
58	N	4.133974	-0.296121	-0.217114	1229.99	1234.35	1239.29
59	C	5.135566	0.259075	0.614989	1239.63	1240.91	1248.19
60	C	4.637616	-0.768308	-1.456707	1258.40	1262.89	1274.73
61	C	6.425915	0.322721	-0.208269	1304.71	1319.54	1323.02
62	C	6.137149	-0.514481	-1.466057	1328.31	1334.18	1337.49
63	H	6.642940	-1.495601	-1.431622	1340.58	1341.65	1349.72
64	O	3.953404	-1.299492	-2.317480	1361.65	1368.54	1372.40
65	O	4.961575	0.621329	1.767610	1375.98	1392.17	1399.51
66	H	3.349707	-1.731002	1.850034	1402.11	1414.35	1415.13
67	C	-4.056044	5.154827	0.224110	1419.58	1421.19	1423.95
68	C	-4.625350	4.807891	-0.979273	1427.43	1429.20	1447.79
69	C	-5.450482	-4.338361	-0.854007	1453.38	1456.72	1462.39
70	C	-6.248276	-3.795576	0.128801	1491.21	1494.71	1495.79
71	H	-5.178526	5.556155	-1.557684	1503.28	1504.37	1523.14
72	H	-4.150026	6.174145	0.612302	1566.73	1569.80	1576.38
73	H	-7.160982	-4.307680	0.449724	1578.51	1579.20	1584.79
74	H	-5.731432	-5.289750	-1.319117	1594.88	1597.52	1601.01
75	H	6.582880	1.384882	-0.472351	1619.79	1622.42	1626.49
76	H	6.430270	-0.021185	-2.405681	1646.07	1741.31	1754.34
77	S	7.832976	-0.219534	0.849966	1803.88	2985.81	2999.58
78	C	9.195860	0.121741	-0.337891	3004.88	3018.77	3073.60
79	H	9.261949	-0.694720	-1.078739	3079.01	3112.50	3113.16
80	H	8.997572	1.063399	-0.873528	3120.77	3121.31	3125.82
81	C	10.510847	0.244824	0.449281	3127.07	3135.73	3135.94
82	H	10.425966	1.132652	1.105221	3137.64	3139.43	3139.94
83	C	11.635901	0.585901	-0.548702	3140.36	3152.90	3153.72
84	N	10.700761	-0.919050	1.310865	3162.53	3163.64	3165.87
85	H	10.880286	-1.749041	0.732003	3168.18	3169.07	3177.07
86	H	11.544919	-0.790231	1.879925	3181.54	3186.96	3187.30
87	O	11.559623	1.467428	-1.396108	3394.00	3480.71	3547.22
88	O	12.734073	-0.188562	-0.370255			
89	H	13.396546	0.115534	-1.036106			

$[Ir(bq)_2(phen)]^+$

Structural Data [Å]					Harmonic Frequencies		
Atom	**Symbol**	**X**	**Y**	**Z**			
1	C	-3.051647	1.242143	2.845909	25.42	27.92	37.15
2	C	-4.192814	0.604351	2.373539	40.76	42.94	43.75
3	C	-4.097410	-0.212640	1.219725	81.60	82.14	84.28
4	C	-2.822203	-0.334088	0.601961	152.88	162.46	165.34
5	C	-1.821911	1.071966	2.182055	171.06	175.69	182.84
6	C	-5.210280	-0.920541	0.645359	190.99	201.31	203.62
7	C	-2.663515	-1.146443	-0.564945	223.52	224.16	228.47

8	C	-3.778556	-1.836840	-1.116490	247.16	256.67	265.63
9	C	-5.058327	-1.700774	-0.475745	283.11	286.28	291.78
10	C	-3.547725	-2.620662	-2.275265	321.37	322.52	335.71
11	H	-4.375923	-3.167947	-2.735044	424.16	425.80	428.35
12	C	-2.265910	-2.681430	-2.810728	429.20	434.48	436.30
13	C	-1.215915	-1.966449	-2.198416	457.53	470.29	471.31
14	H	-3.085668	1.879710	3.732317	487.26	487.40	489.95
15	H	-5.156221	0.724432	2.877635	497.38	502.91	506.11
16	H	-0.906810	1.552323	2.532123	523.01	524.47	532.57
17	H	-2.053405	-3.278058	-3.700883	548.20	550.13	552.29
18	H	-0.198435	-2.000236	-2.595909	554.49	563.33	564.71
19	N	-1.404517	-1.210746	-1.103824	612.63	620.09	624.98
20	N	-1.697981	0.303072	1.081896	636.89	640.82	647.64
21	C	1.728742	-2.298221	0.541186	710.79	711.40	711.94
22	C	-0.008786	-2.268029	2.199604	716.27	717.27	720.73
23	C	0.561847	-3.432560	2.775057	729.68	740.32	749.59
24	C	1.706053	-4.031755	2.247206	750.91	753.44	760.63
25	C	2.322052	-3.468776	1.100946	769.76	774.29	781.88
26	C	2.317768	-1.696158	-0.607947	821.10	823.01	824.44
27	C	2.184446	0.056604	-2.163167	828.63	830.55	835.54
28	C	3.336315	-0.410397	-2.823291	858.50	864.06	870.40
29	C	3.992527	-1.549082	-2.358980	876.69	887.06	905.65
30	C	3.486884	-2.225568	-1.222436	911.76	913.22	915.87
31	H	-0.904005	-1.843242	2.667025	921.84	922.57	925.93
32	H	0.088730	-3.871571	3.660677	939.39	941.69	952.13
33	H	2.131003	-4.929807	2.707782	953.44	955.36	955.64
34	H	1.650863	0.947548	-2.500591	959.33	966.04	969.52
35	H	3.700361	0.136919	-3.695950	1005.20	1006.43	1024.98
36	H	4.890391	-1.922054	-2.861979	1058.39	1059.25	1064.71
37	N	1.678805	-0.571077	-1.083008	1078.68	1082.01	1091.86
38	C	1.141743	2.596334	0.574730	1098.04	1098.81	1108.56
39	C	1.989720	1.014808	2.182225	1139.07	1140.19	1140.51
40	C	2.728823	2.060271	2.791296	1149.36	1149.58	1150.79
41	C	2.680979	3.365966	2.306086	1187.37	1191.18	1207.08
42	C	1.878694	3.663976	1.175654	1215.30	1217.15	1225.01
43	C	0.322160	2.876616	-0.566354	1229.49	1231.73	1233.68
44	C	-1.143444	2.008879	-2.173066	1239.56	1240.22	1253.27
45	C	-1.297691	3.276040	-2.768480	1319.92	1323.24	1335.64
46	C	-0.616118	4.369045	-2.239229	1340.51	1342.87	1347.19
47	C	0.221351	4.190038	-1.111574	1361.66	1369.06	1374.03
48	H	3.256327	4.164583	2.786205	1399.88	1401.80	1410.36
49	H	-1.661463	1.130575	-2.567018	1413.36	1415.93	1423.18
50	H	-1.950666	3.381271	-3.638180	1426.79	1428.29	1430.13
51	H	-0.719851	5.363270	-2.685954	1447.10	1457.52	1457.79
52	N	-0.354766	1.808893	-1.103079	1494.06	1495.38	1498.41
53	H	3.351822	1.833290	3.663780	1503.21	1504.54	1520.70
54	H	2.058524	0.005954	2.602801	1566.39	1569.89	1576.54
55	C	1.174125	1.250398	1.064323	1578.32	1579.34	1585.23
56	C	0.559207	-1.666050	1.066508	1595.38	1597.33	1601.39

57	Ir	0.014635	-0.034751	0.009733	1619.83	1623.31	1630.32
58	H	-5.914084	-2.234130	-0.900286	3108.65	3113.23	3120.34
59	C	0.976396	5.241590	-0.484201	3120.87	3122.01	3126.89
60	C	1.770105	4.984358	0.609345	3134.77	3136.06	3137.41
61	C	3.505262	-3.999498	0.472113	3139.30	3140.21	3140.82
62	C	4.064672	-3.407843	-0.638542	3141.11	3150.75	3150.87
63	H	2.339283	5.797042	1.074227	3154.24	3155.22	3160.96
64	H	0.900308	6.254859	-0.891613	3162.00	3169.15	3170.39
65	H	4.960842	-3.832939	-1.101590	3173.01	3174.99	3184.15
66	H	3.963506	-4.899691	0.896304			
67	H	-6.188824	-0.824740	1.124918			

References

1 M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, T. Keith, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, O. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski, D. J. Fox, Gaussian, Inc., Wallingford CT, **2010**.

2 a) M. Ernzerhof, M. and G. E. Scuseria *J. Chem. Phys.* **1999**, *110*, 5029-5036; b) C. Adamo and V. Barone *J. Chem. Phys.* **1999,** *110,* 6158-69

3 a) P. J. Hay, W. R. Wadt, *J. Chem. Phys.* **1985**, *82*, 270; b) P. J. Hay, W. R. Wadt, *J. Chem. Phys.* **1985**, *82*, 284; c) P. J. Hay, W. R. Wadt, *J. Chem. Phys.* **1985**, *82*, 299.

4 N. Zhao, Y.-H. Wu, L.-X. Shi, Q.-P. Lin, Z.-N. Chen, *Dalton Trans.* **2010**, *39*, 8288.

5 Q. Zhao, C. Y. Jiang, M. Shi, F. Y. Li, T. Yi, Y. Cao, C. H. Huang, *Organometallics*, **2006**, *25*, 3631.

6 K. Nakamaru, *Bull. Chem. Soc. Jpn.* **1982**, *55*, 2697.