Electronic Supplementary Material (ESI) for Analyst.

Mass spectrometry challenges for sequencing peptides modified by organo-iridium anticancer complexes: electron capture with an electron-quenching modification

Supporting information

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Synthesis of Ir1-Cl ligands and complexes

2-(4'-Formylphenyl)-5-pyridinecarboxaldehyde (ligand 1) 4-formylphenylboronic acid (250 mg, 1.67 mmol) and 6-bromo-3-pyridinecarboxaldehyde (222 mg, 1.19 mmol) were dissolved in 1:1:1 mixture of tetrahydrofuran:water:1 M sodium carbonate aqueous solution and stirred at ambient temperature under nitrogen for 1 h. Tetrakis(triphenylphosphine)-palladium (28 mg, 0.02 mmol) was added and the reaction mixture was refluxed at 100°C for 24 h. The reaction mixture was cooled to ambient temperature, extracted with dichloromethane, the organic layer was washed with saturated sodium hydrogen carbonate solution and brine, then dried with magnesium sulphate and concentrated to dryness, yielding the crude material. Purification by column chromatography was performed using 1:1 chloroform:ethyl acetate (Rf = 0.59), yielding an off-white solid (152 mg, 60 %). **^{1}H NMR** (400 MHz, dmso-d_6): δ 10.17 (s, 1H), 10.11 (s, 1H), 9.21 (d, 1H, J = 2.0 Hz), 8.41 (m, 2H), 8.38 (dd, 1H, J = 8.3, 2.0 Hz), 8.32 (d, 1H, J = 8.5 Hz), 8.07 (m, 2H). **^{13}C NMR** (100 MHz, dmso-d_6, DEPT135): δ 192.89 (C*H*O), 192.05 (C*H*O), 151.63, 137.49, 129.99, 127.93, 121.54. **ESI-MS** (MeCN) m/z = 212.1 $[M+H]^+$.

[(η^5-Cp*)Ir(2-(4'-formylphenyl)-5-pyridinecarboxaldehyde)Cl] (Ir1-Cl) 2-(4'-formylphenyl)-5-pyridinecarboxaldehyde (42 mg, 0.202 mmol) was dissolved in dichloromethane (30 mL) followed by the addition of sodium acetate (33 mg, 0.40 mmol) and the reaction mixture was stirred at ambient temperature under nitrogen for 30 min. $[(Cp^*)IrCl_2]_2$ (80 mg, 0.101 mmol) was added and the reaction mixture was heated under reflux for 46 h. The crude product was recrystallised from chloroform/hexane at 273 K to yield a red crystalline solid (65 mg, 57 %). **^{1}H NMR** (400 MHz, dmso-d_6): δ 10.17 (s, 1H, C*H*O), 10.13 (s, 1H, C*H*O), 9.20 (d, 1H, J = 1.5 Hz), 8.46 (d, 1H, J = 8.3 Hz), 8.34 (dd, 1H, J = 8.3, 2.0 Hz), 8.26 (d, 1H, J = 1.5 Hz), 8.17 (d, 1H, J = 8.0 Hz), 7.55 (dd, 1H, J = 8.0, 1.5

Hz), 1.67 (s, 15H). **ESI-MS** (MeCN) m/z = 538.1 $[M\text{-}Cl]^+$. Slow evaporation of a chloroform/hexane mixture at 273K led to crystals suitable for X-ray diffraction (below).

X-ray crystallography of Ir1-Cl

Data Collection and processing. Oxford Diffraction Gemini four-circle system with Ruby CCD area detector. The crystal was held at 150(2) K with the Oxford Cryosystem Cryostream Cobra. Maximum theta was 31.49 deg. The hkl ranges were -25/ 25, -11/ 11, -24/ 24. 55846 reflections measured, 6448 unique [R(int) = 0.0306]. Absorption correction by Semi-empirical from equivalents; minimum and maximum transmission factors: 0.66; 1.00.

Structure Analysis and Refinement. Systematic absences indicated space group P2(1)/c and shown to be correct by successful refinement. The structure was solved by direct methods using SHELXS (Sheldrick, 1990) (TREF) with additional light atoms found by Fourier methods. Hydrogen atoms were added at calculated positions and refined using a riding model with freely rotating methyl groups. Anisotropic displacement parameters were used for all non-H atoms; H-atoms were given isotropic displacement parameters equal to 1.2 (or 1.5 for methyl hydrogen atoms) times the equivalent isotropic displacement parameter of the atom to which the H-atom is attached. The weighting scheme was calc w=1/[\s^2^(Fo^2^)+(0.0243P)^2^+2.1339P] where P=(Fo^2^+2Fc^2^)/3. Goodness-of-fit on F^2 was 1.088, R1[for 5949 reflections with I>2sigma(I)] = 0.0195, wR2 = 0.0502. Data / restraints / parameters 6448/ 0/ 258. Largest difference Fourier peak and hole 1.114 and -0.545 e.A^-3 .

Refinement used SHELXL 97 (Sheldrick, 1997).

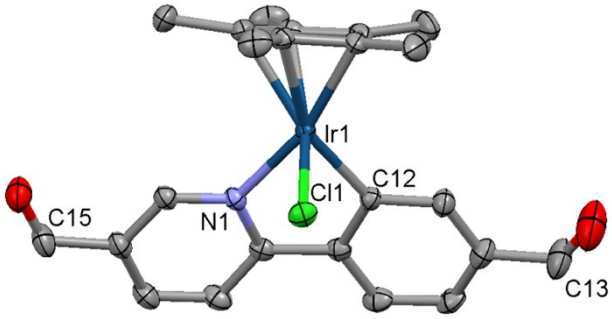
Additional material available from the Cambridge Crystallographic Data Centre comprises H-atom coordinates, thermal parameters and the

remaining bond lengths and angles. The crystal data for complex **Ir1-Cl** (am10) have been deposited at the Cambridge Crystallographic Data Centre under accession number CCDC CCDC 1817921.

Acknowledgement. The Oxford Diffraction Gemini XRD system was obtained through the Science City Advanced Materials project: Creating and Characterising Next Generation Advanced Materials, with support from Advantage West Midlands (AWM) and part funded by the European Regional Development Fund (ERDF)

References. For relevant information for the SHELXTL suite of programmes used to solve, refine and produce the files for this structure, please refer to

A Short History of Shelx, G. M. Sheldrick, Acta Cryst. 2008, A64, 112-122

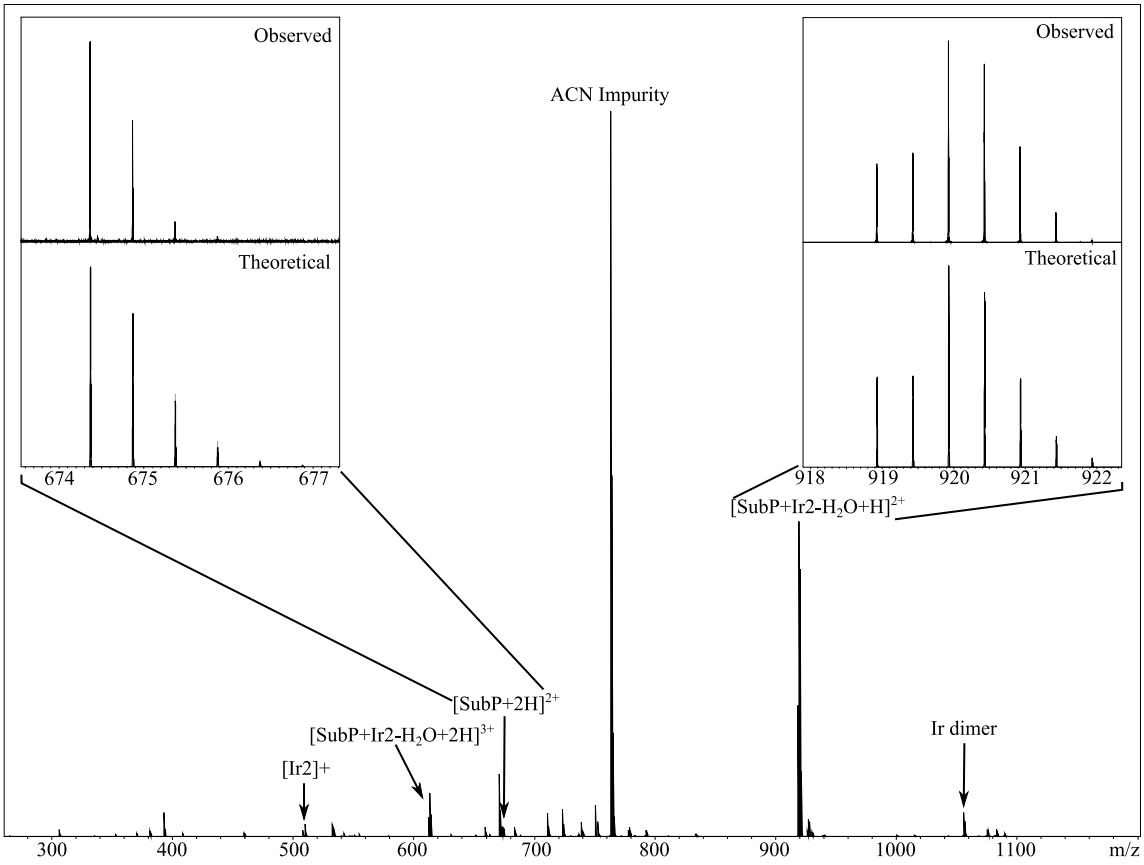


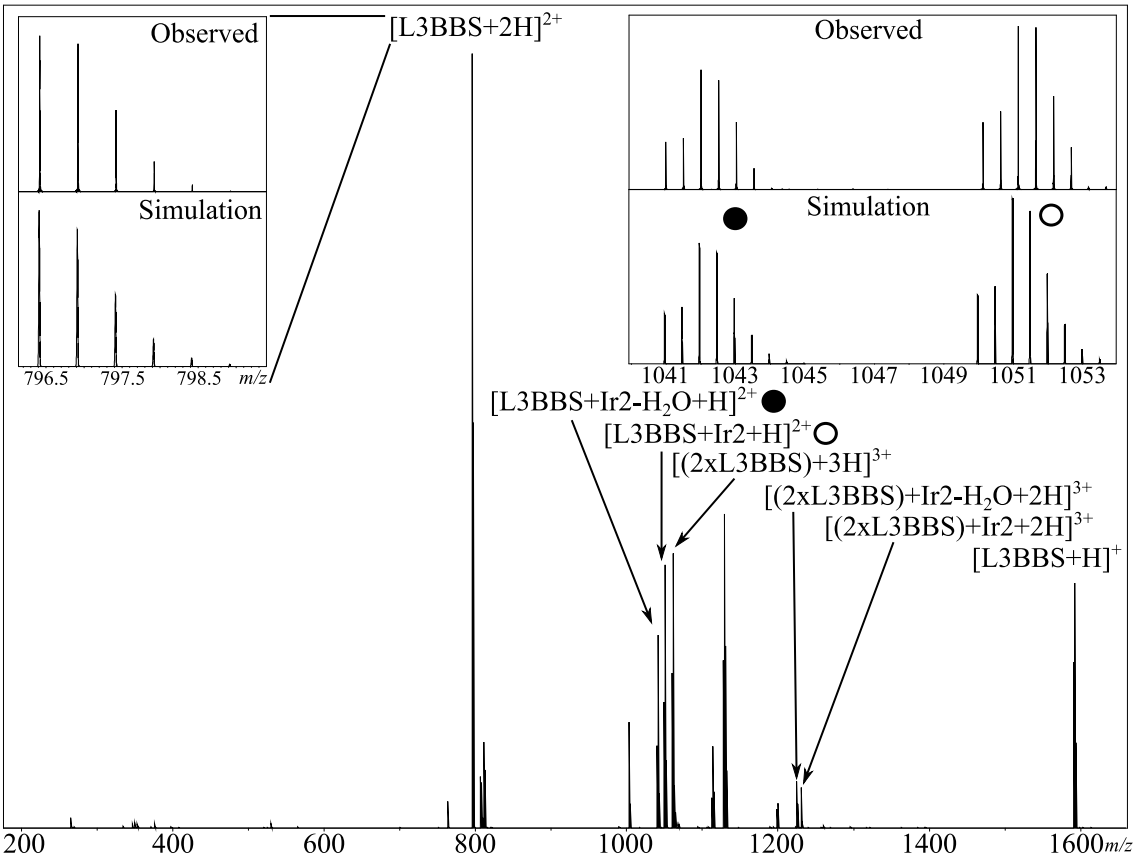
SI Figure SF1: X-ray crystal structure of the **Ir1-Cl** dialdehyde functionalised Iridium(III) piano-stool complex

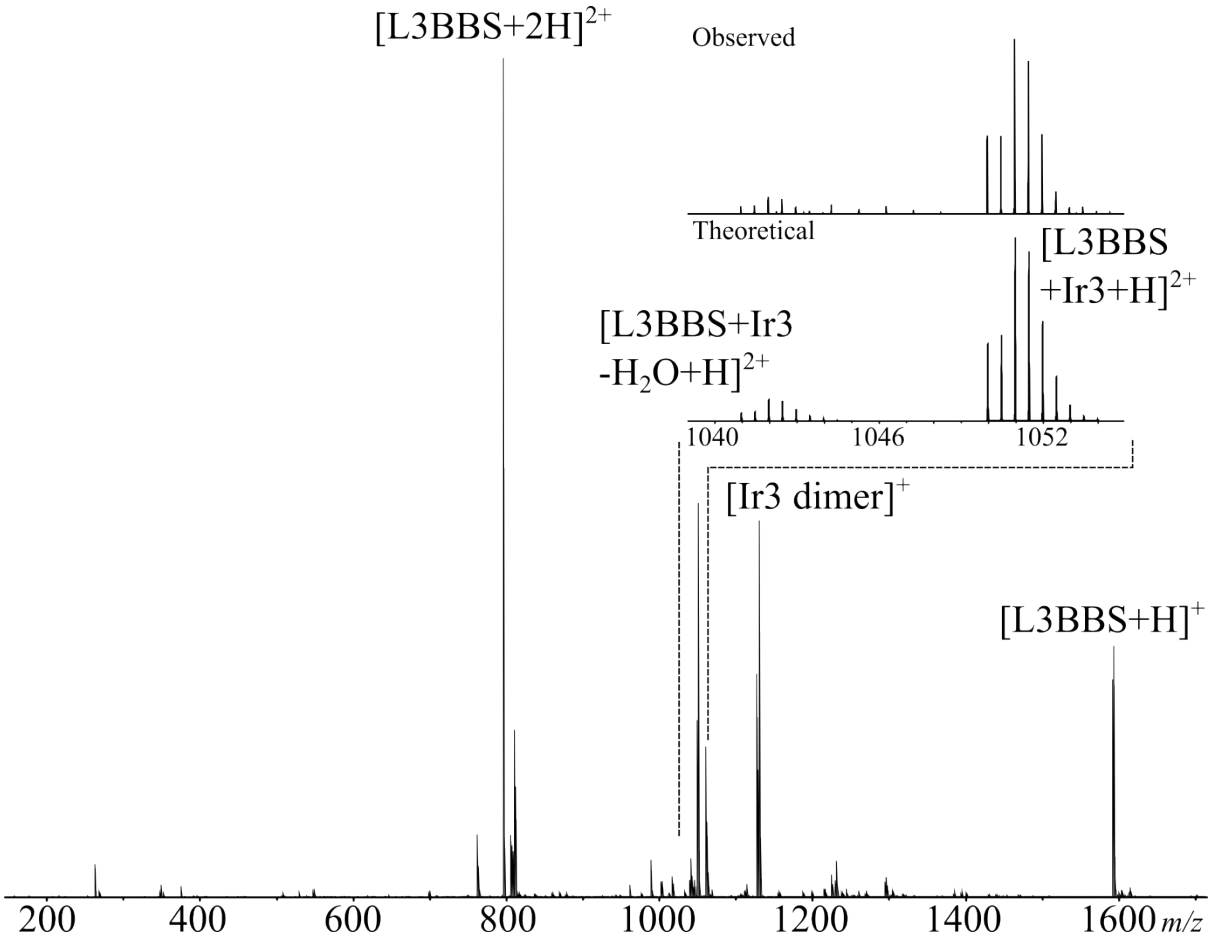
	2
Formula	$C_{23}H_{23}ClIrNO_2$
MW	573.07
Crystal Colour	Brown
Cryst size (mm)	0.25 x 0.20 x 0.15
λ (Å)	0.71073
Temp(K)	150
Cryst system	Monoclinic
Space group	P2(1)/c
a (Å)	17.3889(2)
b (Å)	7.66780(10)
c (Å)	16.5920(2)
α (°)	90
β (°)	113.945(2)
γ (°)	90
Vol (Å^3)	2021.89(4)
Z	4
$R(Fo^2)$	0.0195
$Rw(Fo^2)$	0.0502
GOF	1.088

	2
Ir-C (Cp* ring)	2.155(2)
	2.160(2)
	2.166(2)
	2.251(2
	2.259(2
Ir-C (centroid)	1.826
Ir-C	2.032(2)
Ir-N	2.0789(19)
Ir-Cl	2.4051(6)
C-Ir-N	77.86(9)
C-Ir-Cl	87.08(6)
N-Ir-Cl	84.91(6)

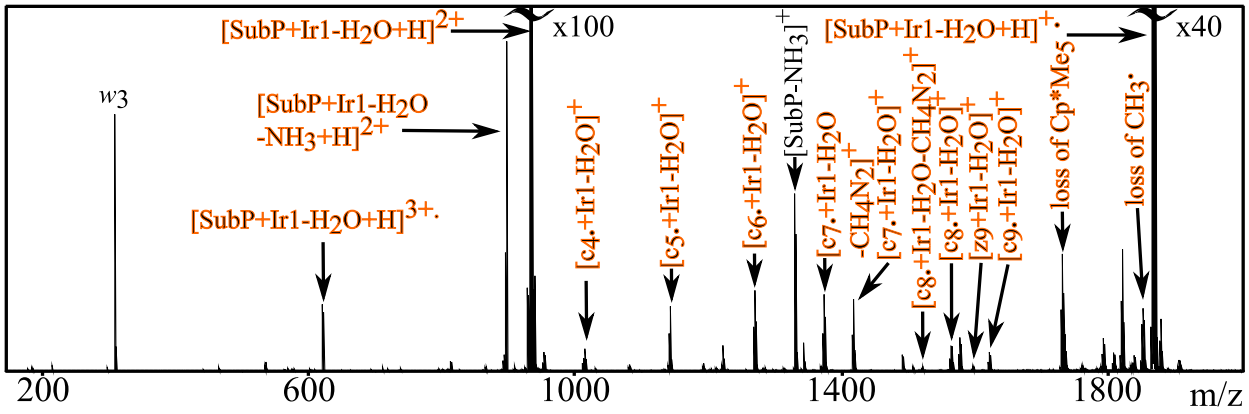
SI Table ST1: X-ray crystal structure information for the **Ir1-Cl** dialdehyde functionalised iridium(III) piano-stool complex


Observed
Theoretical
674
675
676
677
ACN Impurity
Observed
Theoretical
918
919
920
921
922
[SubP+Ir2-H2O+H]2+
[SubP+2H]2+
[SubP+Ir2-H2O+2H]3+
[Ir2]+
Ir dimer
300
400
500
600
700
800
900
1000
1100
m/z


Observed
Simulation
796.5
797.5
798.5
m/z
[L3BBS+2H]2+
Observed
Simulation
1041
1043
1045
1047
1049
1051
1053
[L3BBS+Ir2-H2O+H]2+
[L3BBS+Ir2+H]2+
[(2xL3BBS)+3H]3+
[(2xL3BBS)+Ir2-H2O+2H]3+
[(2xL3BBS)+Ir2+2H]3+
[L3BBS+H]+
200
400
600
800
1000
1200
1400
1600m/z



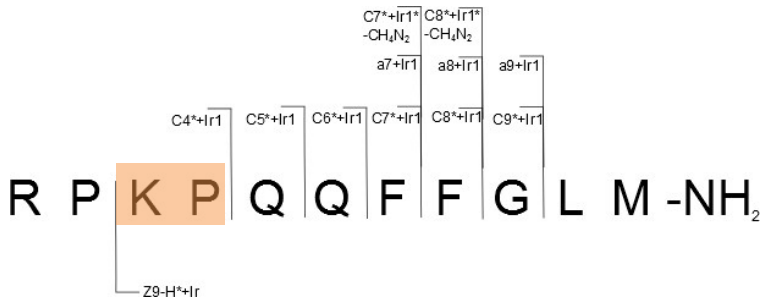
SI Figure SF2: nESI-FT-ICR mass spectra of Iridium complex+peptide mixtures, reacted for 24 h at 37°C prior to analysis. In the SubstanceP+**Ir2 -Cl**(top) only the condensation reaction product ([SubP+**Ir2**-H_2O+H]$^{2+}$) is observed outside of reagent peaks. In the [Lys]^3-Bombesin+**Ir2-Cl** spectrum 2 iridiated peptide species are observed (inset) both the condensation product ([L3BBS+**Ir2**-H_2O+H]$^{2+}$) and the non-condensation (metal bound) product ([L3BBS+**Ir2**+H]$^{2+}$). Inset: Observed iridiated peptide/Iridium containing peaks, showing the influence of the transition metal on the observed isotopic distribution. ACN impurity from solvent systems used during synthesis and dilution was confirmed through controls/blanks, but could not be readily separated from drug/mixture prior to analysis.



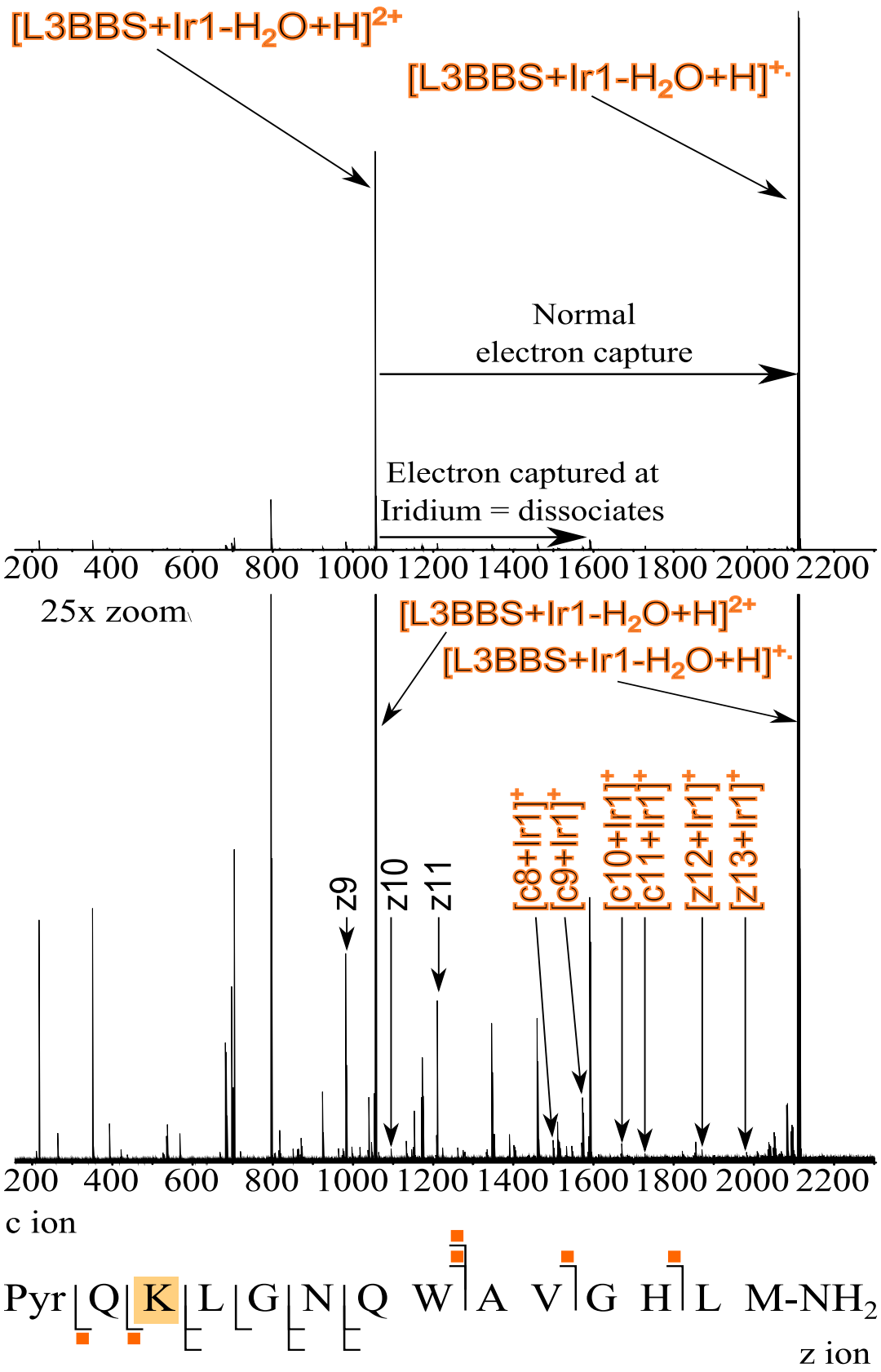
SI Figure SF3: ECD MS/MS spectrum of the [SubstanceP+**Ir1**-H2O+H]$^{2+}$ species.

SF Table ST2: Assignments from the analysis of the ECD MS/MS spectrum of the [SubstanceP+**Ir1**-H_2O+H]$^{2+}$ species.

Fragment	Observed mass	Exact mass	Error/ppm
[C4•+Ir1-H_2O]$^+$	1013.45049	1013.4506	-0.11
[C5•+Ir1-H_2O]$^+$	1141.50907	1141.50919	-0.11
[C6•+Ir1-H_2O]$^+$	1269.56765	1269.56763	0.02
[C7•+Ir1-H_2O]$^+$	1416.63606	1416.63635	-0.20
[C8•+Ir1-H_2O]$^+$	1563.70447	1563.70452	-0.03
[Z9-H•+Ir1-H_2O]$^+$	1595.67789	1595.67722	0.42
[C9•+Ir1-H_2O]$^+$	1620.72593	1620.72693	-0.62
[M+H+Ir1-H_2O]$^{+\bullet}$	1865.858328	1865.8583	0.02
[Sub P+Ir1-H_2O –NH3]$^+$	1330.70897	1330.70886	0.08
[C7•+Ir1-H_2O - CH_4N_2]$^+$	1372.62245	1372.598902	-0.19
[C8•+Ir1-H_2O - CH_4N_2]$^+$	1519.69438	1519.667072	-0.33
[SubP+H+Ir1-H_2O – $CpMe_5$]$^+$	1730.73857	1730.740386	-1.05
[SubP+Ir1-H_2O +H – Me]$^+$	1850.83652	1850.834835	0.91



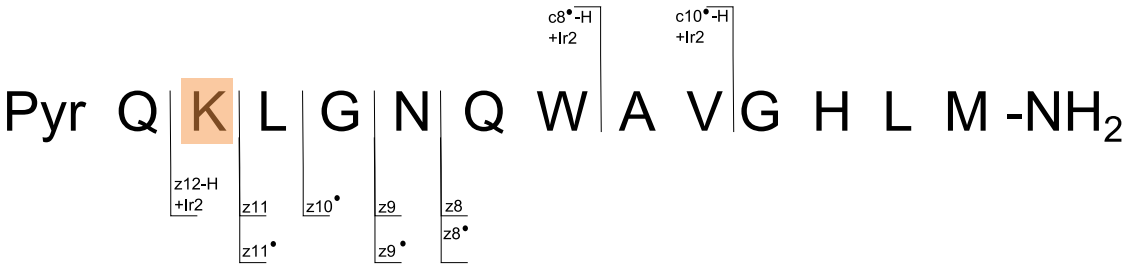
SI Figure SF4: Fragmentation map for the ECD MS/MS analysis of the [SubstanceP+**Ir1**-H_2O+H]$^{2+}$ species.



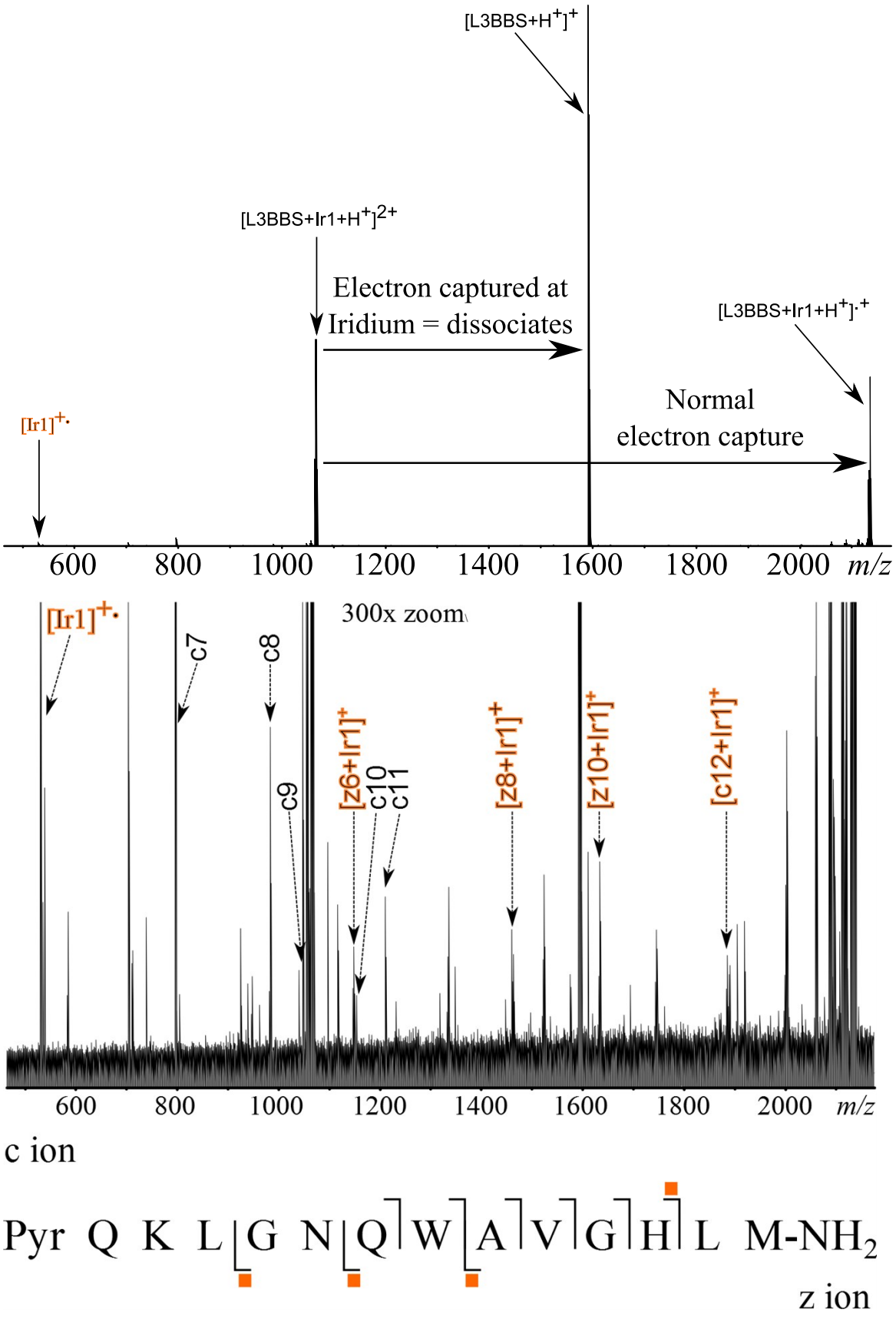
SI Figure SF5: IRECD MS/MS spectrum and fragmentation map of the [Ir1+L3BBS-H_2O+H]$^{2+}$ species

SI Table ST3: Assignments for the IRECD MS/MS analysis of the [L3BBS+**Ir1**-H_2O+H]$^{2+}$ species.

	Observed mass	Exact mass	Error/ppm
[z8]$^+$	924.46343	924.46346	-0.03
[z8•]$^+$	925.471255	925.47126	-0.01
[z9]$^+$	1038.50636	1038.50595	0.39
[z9•]$^+$	1039.514185	1039.51411	0.07
[z10•]$^+$	1096.535645	1096.5355	0.13
[z11]$^+$	1208.61188	1208.6119	-0.02
[z11•]$^+$	1209.619705	1209.62037	-0.55
[c8• -H+ Ir1-H2O]$^+$	1499.61298	1499.61322	-0.16
[c10• -H+ Ir1-H2O]$^+$	1669.7185	1669.71832	0.11
[z12-H$^+$ +Ir1-H2O]$^+$	1853.822465	1853.82123	0.67
[L3BBS+Ir1-H2O-NH_3]$^{+\bullet}$	2108.9307	2108.93071	-0.005
		Absolute average error	0.20
		Standard deviation	0.29



SI Figure SF6: Fragmentation map for the IRECD MS/MS analysis of the [L3BBS+**Ir1**-H_2O+H]$^{2+}$ species.



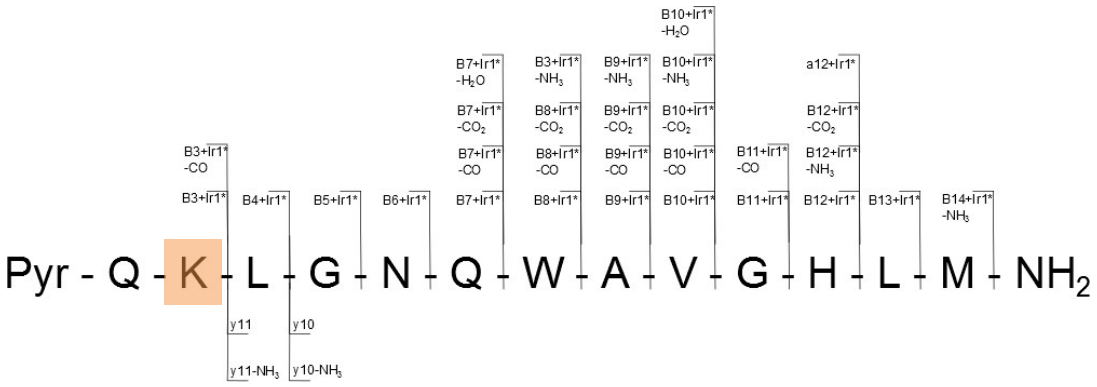
SI Figure SF7: ECD MS/MS spectrum and fragmentation map of the [**Ir1**+L3BBS+H]$^{2+}$ species

SI Table ST4: Assignments for the ECD MS/MS analysis of the [L3BBS+**Ir1**+H]$^{2+}$ species.

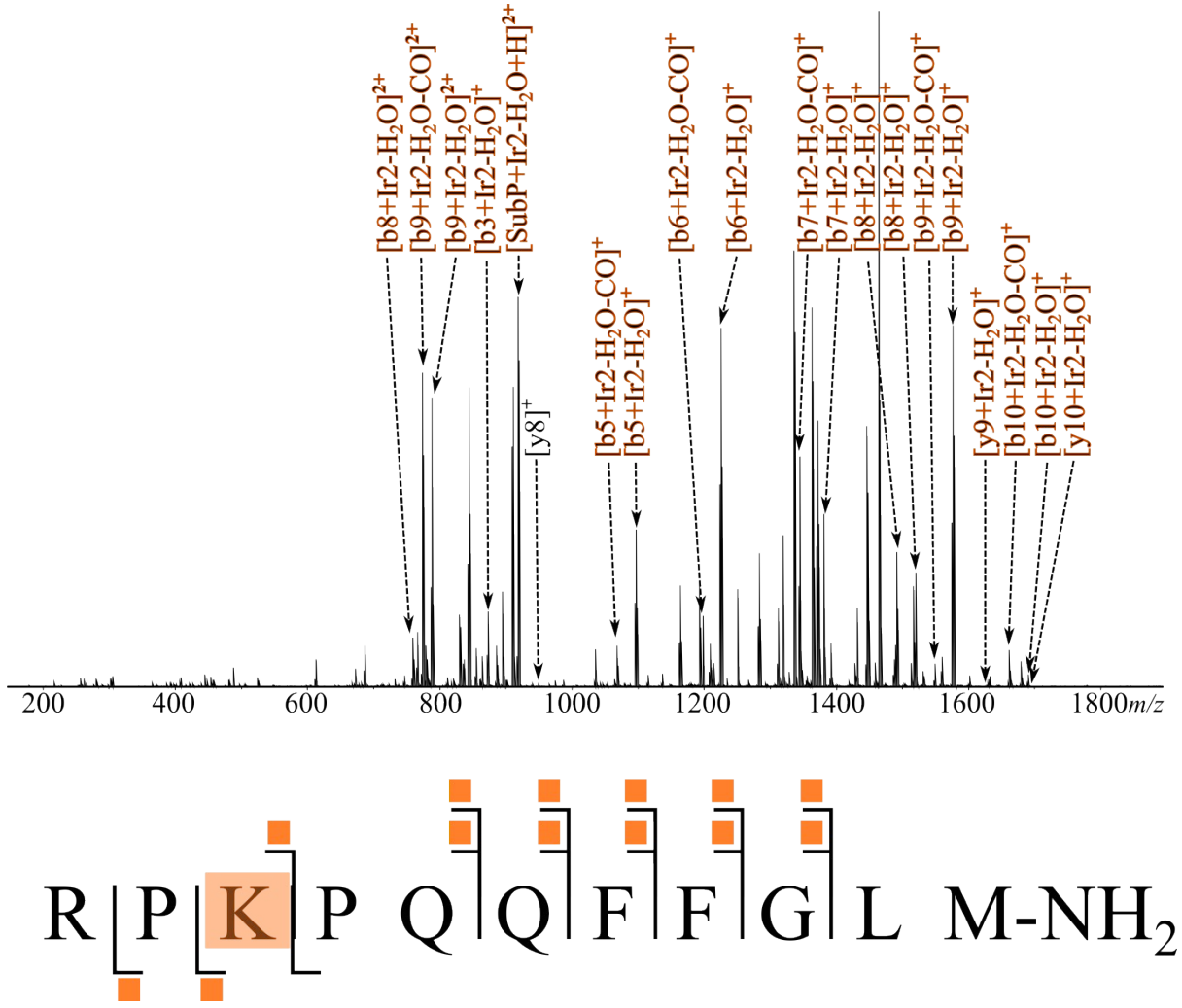
Fragment	Exact mass	Observed mass	Error/ppm
[c7]$^{+}$	797.42587	797.42588	-0.01
[c8]$^{+}$	983.50518	983.50515	0.03
[c9]$^{+}$	1054.54229	1054.54234	-0.05
[z6+Ir1]$^{+}$	1146.458461	1146.4578	0.58
[c10]$^{+}$	1153.6107	1153.61078	-0.07
[c11]$^{+}$	1210.63216	1210.63221	-0.04
[z8+Ir1]$^{+}$	1460.596351	1460.59634	0.01
[z10+Ir1]$^{+}$	1631.660741	1631.66003	0.44
[c12+Ir1]$^{+}$	1883.823991	1883.82326	0.39
		Absolute average	0.18
		Standard deviation	0.24

SI Table ST5: Assignments for the CAD MS/MS analysis of the [L3BBS+**Ir1**-H_2O+H]$^{2+}$ species

Fragment	Exact mass	Observed mass	Error/ppm
[b3-CO+Ir1-H_2O]$^+$	857.313005	857.31314	0.16
[b3+Ir1-H_2O]$^+$	885.30792	885.30792	0.00
[b4+Ir1-H_2O]$^+$	998.39198	998.3919	-0.08
[b5+Ir1-H_2O]$^+$	1055.41344	1055.41309	-0.33
[b6+Ir1-H_2O]$^+$	1169.45637	1169.45623	-0.12
[b7-CO_2+Ir1-H_2O]$^+$	1253.52512	1253.52463	-0.39
[b7-CO+Ir1-H_2O]$^+$	1269.520035	1269.51992	-0.09
[b7-H_2O+Ir1-H_2O]$^+$	1279.504385	1279.50412	-0.21
[b7+Ir1-H_2O]$^+$	1297.51495	1297.51479	-0.12
[b8-CO_2+Ir1-H_2O]$^+$	1439.60443	1439.60517	0.51
[b8-CO+Ir1-H_2O]$^+$	1455.599345	1455.59912	-0.15
[b8-NH_3+Ir1-H_2O]$^+$	1466.567715	1466.56765	-0.04
[b8+Ir1-H_2O]$^+$	1483.59426	1483.59429	0.02
[b9-CO_2+Ir1-H_2O]$^+$	1510.64154	1510.64096	-0.38
[b9-CO+Ir1-H_2O]$^+$	1526.636455	1526.63648	0.02
[b9-NH_3+Ir1-H_2O]$^+$	1537.604825	1537.60477	-0.04
[b9+Ir1-H_2O]$^+$	1554.63137	1554.63148	0.07
[b10-CO_2+Ir1-H_2O]$^+$	1609.70995	1609.71108	0.70
[b10-CO+Ir1-H_2O]$^+$	1625.704865	1625.70489	0.02
[b10-H2O+Ir1-H_2O]$^+$	1635.689215	1635.6895	0.17
[b10-NH_3+Ir1-H_2O]$^+$	1636.673235	1636.67331	0.05
[b10+Ir1-H_2O]$^+$	1653.69978	1653.7002	0.25
[b11-CO+Ir1-H_2O]$^+$	1682.726325	1682.72685	0.31
[b11+Ir1-H_2O]$^+$	1710.72124	1710.72187	0.37
[b12-CO_2+Ir1-H_2O]$^+$	1803.79032	1803.79105	0.40
[b12-NH_3+Ir1-H_2O]$^+$	1830.753605	1830.75434	0.40
[b12+Ir1-H_2O]$^+$	1847.78015	1847.78068	0.29
[b13+Ir1-H_2O]$^+$	1960.86421	1960.86419	-0.01
[b12+Ir1-H_2O]$^{2+}$	924.393715	924.39371	-0.01
[L3BBS+Ir1-H_2O+H-NH_3]$^{2+}$	1046.455993	1046.45595	-0.04
[a12+Ir1-H_2O]$^+$	1803.78978	1803.79159	1.00
[y10]$^+$	1111.54654	1111.54641	-0.12
[y10-NH_3]$^+$	1094.519995	1094.51987	-0.11
[y11]$^+$	1224.6306	1224.63046	-0.11
[y11-NH_3]$^+$	1207.604055	1207.60385	-0.17
Average error	0.06	RMS	0.09
Standard deviation	0.29	RMSD	0.30



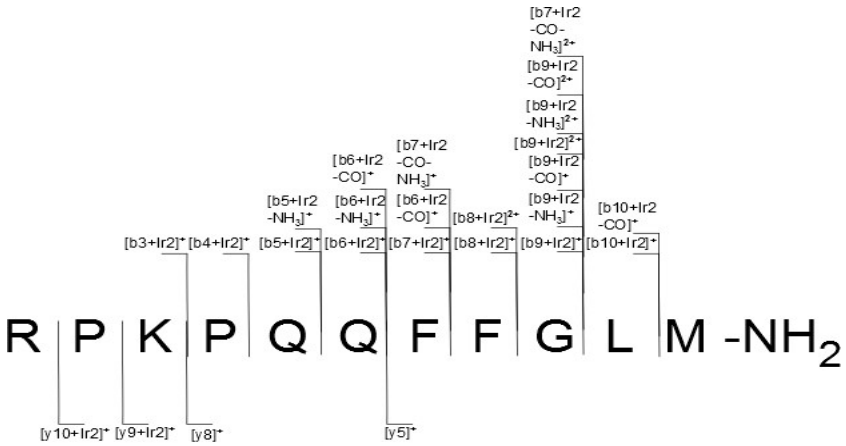
Si Figure SF8: Fragmentation map for the CAD MS/MS analysis of the [L3BBS+**Ir1**-H_2O+H]$^{2+}$ species



SI Figure SF9: CAD MS/MS spectrum and fragmentation map of the [**Ir2**+SubP-H2O+H]$^{2+}$ species

SI Table ST6: Assignments for the CAD MS/MS analysis of the [SubP+**Ir2**-H2O+H]$^{2+}$ species.

Assignment	Observed mass	Exact mass	Error/ppm
[b8+Ir2-H_2O]$^+$	1520.68602	1520.68804	1.328348
[b7+Ir2-H_2O]$^+$	1371.61563	1371.6146	-0.75094
[b6+Ir2-H_2O]$^+$	1224.54619	1224.54783	1.339272
[y10+Ir2-H_2O]$^+$	1684.76187	1684.76077	-0.65291
[b3+Ir2-H_2O]$^+$	970.43165	970.43348	1.885759
[b5+Ir2-H_2O]$^+$	1097.49058	1097.49037	-0.19135
[b9+Ir2-H_2O]$^+$	1575.70449	1575.70494	0.285587
[y9+Ir2-H_2O]$^+$	1589.71841	1589.71728	-0.71082
[y8]$^+$	966.486586	966.48503	-1.60996
[b8+Ir2-H_2O-CO]$^+$	1493.69368	1493.69495	0.850241
[b7+Ir2-H_2O-CO]$^+$	1346.62518	1346.62455	-0.46784
[b6+Ir2-H_2O-CO]$^+$	1199.55668	1199.55757	0.741941
[b5+Ir2-H_2O-CO]$^+$	1070.49536	1070.49613	0.719293
		Absolute average	0.88725
		standard deviation	0.989976



SI Figure SF10: Fragmentation map for the CAD MS/MS analysis of the [SubP+**Ir2**-H_2O+H]$^{2+}$ species

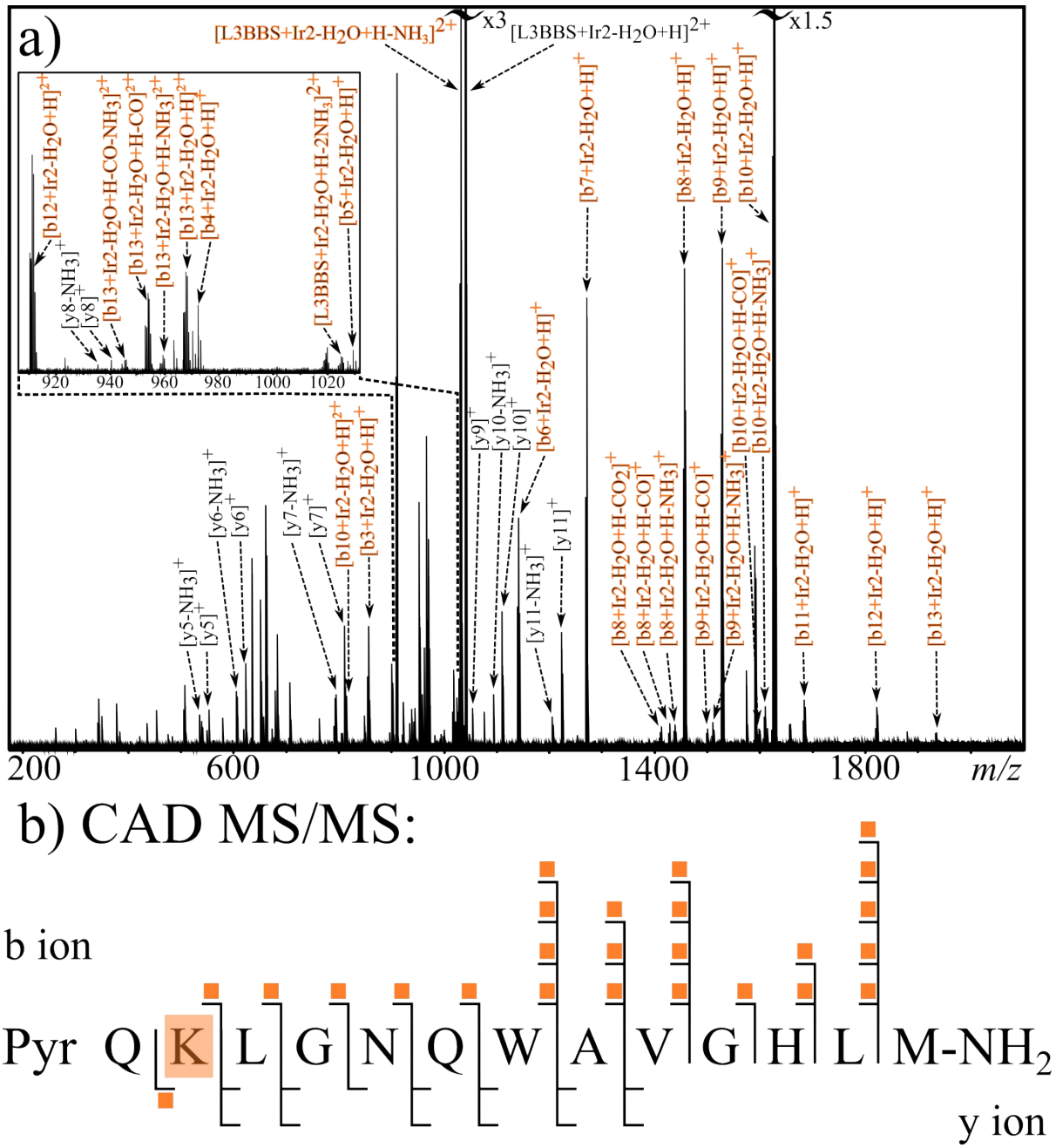
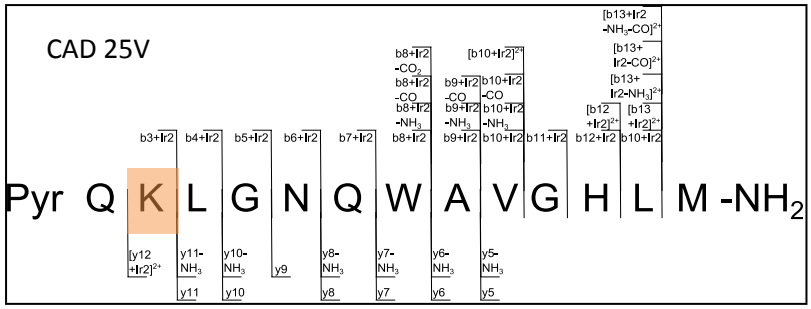


Figure SF11: CAD MS/MS spectrum and fragmentation map of the [L3BBS+**Ir2**-H2O+H]$^{2+}$ species.

SI Table ST7: Assignments and fragmentation map for the CAD MS/MS analysis of the [L3BBS+**Ir2**-H_2O+H]$^{2+}$ species

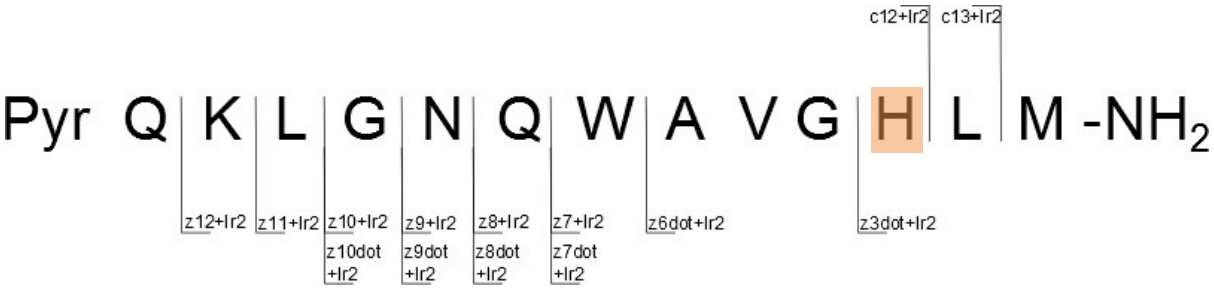
Assignment	Observed mass	Exact mass	Error/ppm
[b3+(Ir2-H_2O)-H]$^+$	857.313	857.313005	-0.01
[b4+(Ir2-H_2O)-H]$^+$	970.39702	970.397065	-0.05
[b5+(Ir2-H_2O)-H]$^+$	1027.41868	1027.418525	0.15
[b6+(Ir2-H_2O)-H]$^+$	1141.46129	1141.461455	-0.14
[b7+(Ir2-H_2O)-H]$^+$	1269.52003	1269.520035	0.00
[b8+(Ir2-H_2O)-H]$^+$	1455.59958	1455.599345	0.16
[b9+(Ir2-H_2O)-H]$^+$	1526.63602	1526.636455	-0.28
[b10+(Ir2-H_2O)-H]$^+$	1625.70466	1625.704865	-0.13
[b11+(Ir2-H_2O)-H]$^+$	1682.72557	1682.726325	-0.45
[b12+(Ir2-H_2O)-H]$^+$	1819.78479	1819.785235	-0.24
[b13+(Ir2-H_2O)-H]$^+$	1932.86903	1932.869295	-0.14
[b8+(Ir2-H_2O)-H+-NH_3]$^+$	1438.57279	1438.5728	-0.01
[b9+(Ir2-H_2O)-H+-NH_3]$^+$	1509.60922	1509.60991	-0.46
[b10+(Ir2-H_2O)-H+-NH_3]$^+$	1608.67813	1608.67832	-0.12
[b8+(Ir2-H_2O)-H+-CO]$^+$	1427.60488	1427.60443	0.32
[b9+(Ir2-H_2O)-H+-CO]$^+$	1498.64119	1498.64154	-0.23
[b10+(Ir2-H_2O)-H+-CO]$^+$	1597.71107	1597.70995	0.70
[b8+(Ir2-H_2O)-H+-CO_2]$^+$	1411.60967	1411.609516	0.11
[y5]$^+$	555.30719	555.30715	0.07
[y6]$^+$	626.3443	626.34426	0.06
[y7]$^+$	812.42356	812.42357	-0.01
[y8]$^+$	940.48218	940.48215	0.03
[y9]$^+$	1054.52498	1054.52508	-0.09
[y10]$^+$	1111.54644	1111.54654	-0.09
[y11]$^+$	1224.63048	1224.6306	-0.10
[y5-NH_3]$^+$	538.2806	538.280605	-0.01
[y6-NH_3]$^+$	609.31776	609.317715	0.07
[y7-NH_3]$^+$	795.39692	795.397025	-0.13
[y8-NH_3]$^+$	923.45555	923.455605	-0.06
[y10-NH_3]$^+$	1094.51992	1094.519995	-0.07
[y11-NH_3]$^+$	1207.60419	1207.604055	0.11
[b10+Ir2-H_2O-H]$^{+2}$	813.35599	813.3560725	-0.10
[b12+Ir2-H_2O-H]$^{+2}$	910.39616	910.3962575	-0.11
[b13+Ir2-H_2O-H]$^{+2}$	966.93832	966.9382875	0.03
[b13+Ir2-H_2O -H-NH_3]$^{+2}$	958.42511	958.425013	0.10
[b13+Ir2-H_2O-H-CO]$^{+2}$	952.94074	952.94083	-0.09
[b13+Ir2-H_2O -H-CO-NH_3]$^{+2}$	944.42731	944.4275555	-0.26
[L3BBS+(Ir2-H_2O)+ H+]2+	1040.97159	1040.971808	-0.21
[(L3BBS+(Ir2-H_2O)+ H+-NH_3]$^{+2}$	1032.45839	1032.458533	-0.14
[L3BBS+(Ir2-H_2O)+ H+ -2NH_3]$^{+2}$	1023.94508	1023.945259	-0.17
[y12+(Ir2-H_2O)-H]$^{+2}$	921.4265	921.426775	-0.30
	Absolute average =		0.15
	Standard deviation =		0.20



SI Figure SF12: Fragmentation map for the CAD MS/MS analysis of the [L3BBS+**Ir2**-H2O+H]$^{2+}$ species.

SI Table ST8: Assignments for the ECD MS/MS analysis of the [L3BBS+**Ir2**+H]$^{2+}$ species.

Fragment	Observed mass	Exact mass	Error/ppm
[z3•+Ir2]$^{+}$	891.33643	891.33657	-0.16
[z6•+Ir2]$^{+}$	1118.46426	1118.464091	0.15
[z7+Ir2]$^{+}$	1303.53558	1303.53558	0.00
[z7•+Ir2]$^{+}$	1304.54389	1304.543401	0.37
[z8+Ir2]$^{+}$	1431.59435	1431.59416	0.13
[z8•+Ir2]$^{+}$	1432.60186	1432.601981	-0.08
[z9+Ir2]$^{+}$	1545.63665	1545.63709	-0.28
[z9•+Ir2]$^{+}$	1546.64474	1546.644911	-0.11
[z10+Ir2]$^{+}$	1602.65836	1602.65855	-0.12
[z10•+Ir2]$^{+}$	1603.66588	1603.666371	-0.31
[z11+Ir2]$^{+}$	1715.7425	1715.74261	-0.06
[z12+Ir2]$^{+}$	1843.83686	1843.83757	-0.39
[c12+Ir2]$^{+}$	1854.82214	1854.821796	0.19
[c13+Ir2]$^{+}$	1967.90497	1967.905856	-0.45
		Absolute average	0.20
		Standard deviation	0.23



SI Figure SF13: Fragmentation map for the ECD MS/MS analysis of the [L3BBS+**Ir2**+H]$^{2+}$ species.

SI Table ST9: Assignments for the CAD MS/MS analysis of the [SubP+**Ir3**-H2O+H]$^{2+}$ species.

Assignment	Observed mass	Exact mass	Error/ppm
[b7+Ir3-H_2O]$^+$	1374.62011	1374.61864	-1.07
[b6+Ir3-H_2O]$^+$	1226.54893	1226.54744	-1.21
[y10+Ir3-H_2O]$^+$	1682.75743	1682.76002	1.54
[y8]$^+$	966.486586	966.48669	0.11
		Absolute average	0.98
		Standard deviation	1.11

SI Figure SF14: CAD MS/MS fragmentation map of the [**Ir3**+SubP-H2O+H]$^{2+}$ species

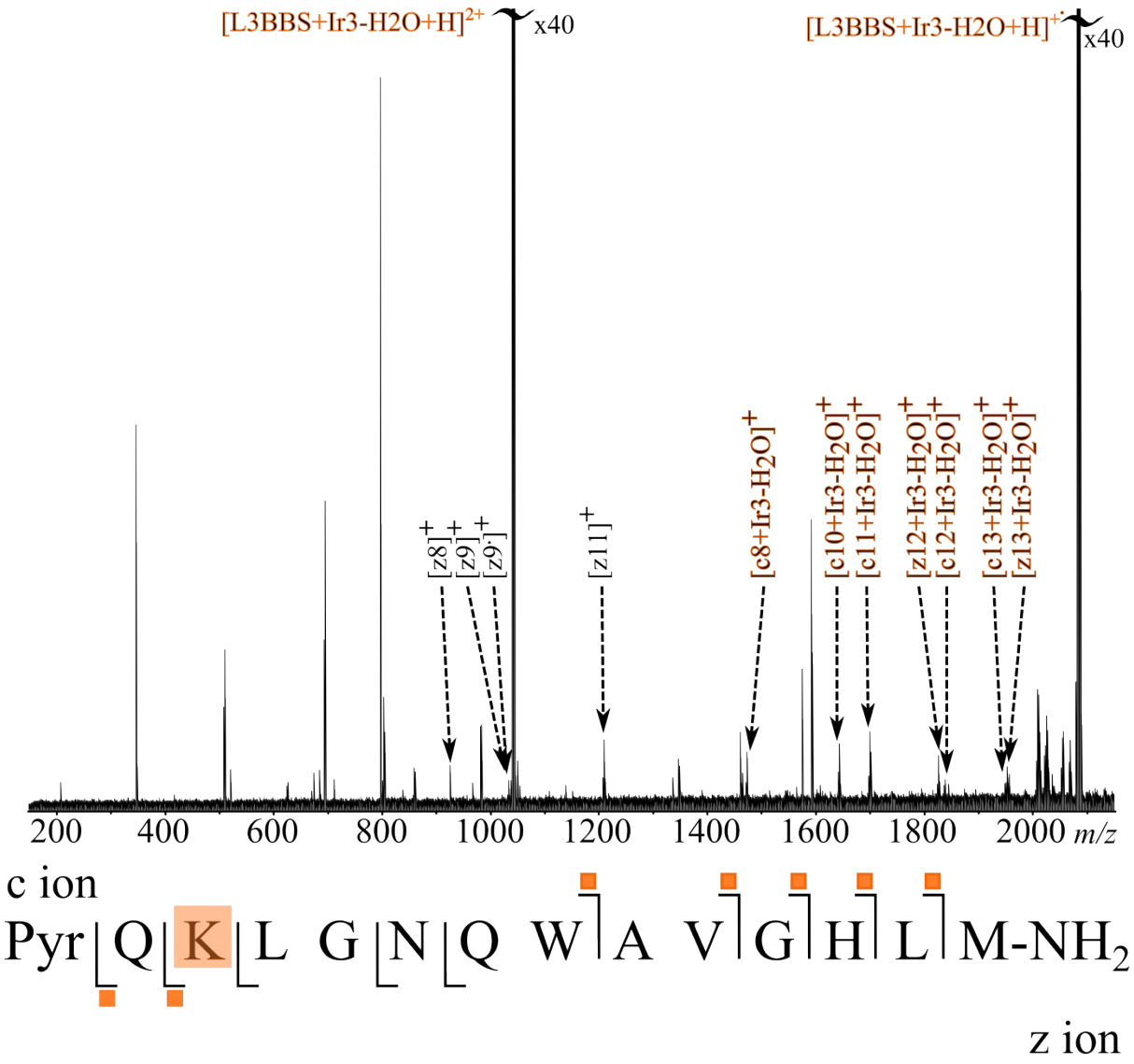


Figure SF15: IRECD MS/MS spectrum and fragmentation map of the [L3BBS+**Ir3**-H2O+H]$^{2+}$ species.

SI Table ST10: Assignments for the IRECD MS/MS analysis of the [L3BBS+**Ir3**-H2O+H]$^{2+}$ species.

Assignment	Observed mass	Exact mass	Error/ppm
[c8+Ir3-H_2O]$^+$	1471.618065	1471.61869	0.42
[c10+Ir3-H_2O]$^+$	1641.723585	1641.72294	-0.39
[c11+Ir3-H_2O]$^+$	1698.745045	1698.74583	0.46
[c13·+Ir3-H_2O]$^+$	1949.895295	1949.89416	-0.58
[z9]$^+$	1038.50636	1038.5057	-0.64
[z11]$^+$	1208.61188	1208.61188	0.00
[z12+Ir3-H_2O]$^+$	1825.827005	1825.82735	0.19
[z13+Ir3-H_2O]$^+$	1954.89341	1954.8955	1.07
[z8·]$^+$	925.471255	925.47126	0.01
[z9·]$^+$	1039.514185	1039.51419	0.00
[c12+Ir3-H_2O]$^+$	1839.81737	1839.81477	-1.41
		Absolute average	0.47
		Standard deviation	0.63

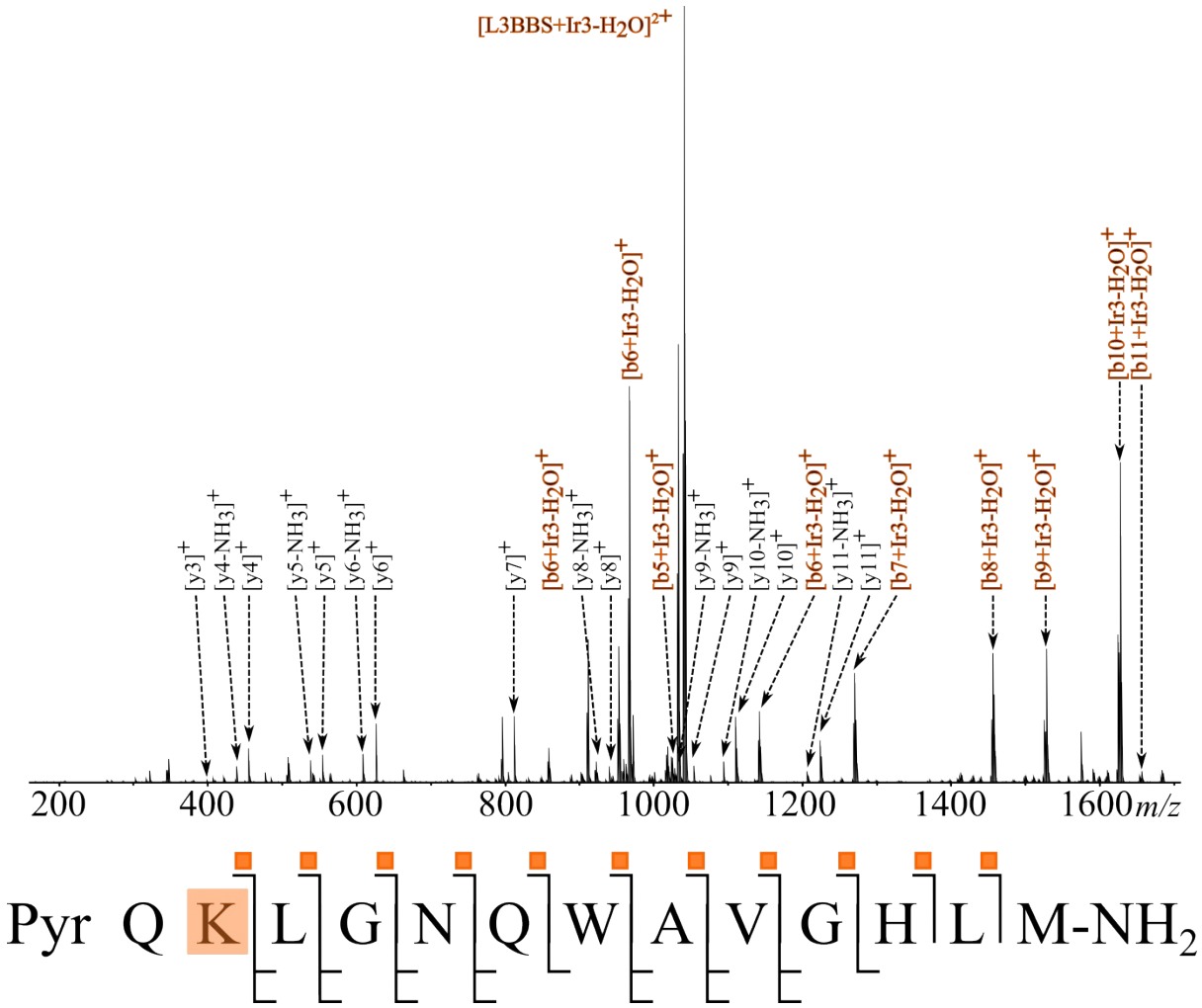


Figure SF16: CAD MS/MS spectrum and fragmentation map of the [L3BBS+**Ir3**-H2O+H]$^{2+}$ species.

SI Table ST11: Assignments for the CAD MS/MS analysis of the [L3BBS+**Ir3**-H2O+H]$^{2+}$ species.

Assignment	Observed mass	Exact mass	Error/ppm
$[y3]^+$	399.217286	399.21701	-0.69
$[y4\text{-}NH_3]^+$	439.212201	439.21203	-0.39
$[y4]^+$	456.23875	456.23866	-0.20
$[y5\text{-}NH_3]^+$	538.280615	538.28066	0.08
$[y5]^+$	555.307164	555.30721	0.08
$[y6\text{-}NH_3]^+$	609.317729	609.31779	0.10
$[y6]^+$	626.344278	626.34446	0.29
$[y7]^+$	812.423591	812.42401	0.52
$[y8\text{-}NH_3]^+$	923.45562	923.45591	0.31
$[y8]^+$	940.482169	940.48262	0.48
$[y9\text{-}NH_3]^+$	1037.498548	1037.49937	0.79
$[y9]^+$	1054.525097	1054.52557	0.49
$[y10\text{-}NH_3]^+$	1094.520012	1094.52036	0.32
$[y10]^+$	1111.546561	1111.54699	0.36
$[y11\text{-}NH_3]^+$	1207.604076	1207.60457	0.41
$[y11]^+$	1224.630625	1224.63118	0.45
$[b3+Ir3\text{-}H_2O]^+$	857.31246	857.3129	0.51
$[b4+Ir3\text{-}H_2O]^+$	970.39652	970.3972	0.71
$[b5+Ir3\text{-}H_2O]^+$	1027.41798	1027.41857	0.57
$[b6+Ir3\text{-}H_2O]^+$	1141.46091	1141.4613	0.34
$[b7+Ir3\text{-}H_2O]^+$	1269.51949	1269.51999	0.39
$[b8+Ir3\text{-}H_2O]^+$	1455.5988	1455.59976	0.66
$[b9+Ir3\text{-}H_2O]^+$	1526.63591	1526.63635	0.23
$[b10+Ir3\text{-}H_2O]^+$	1625.70432	1625.70465	0.20
$[b11+Ir3\text{-}H_2O]^+$	1682.72578	1682.7263	0.31
$[b12+Ir3\text{-}H_2O]^+$	1819.78469	1819.78554	0.47
	Absolute average		0.30
	Standard deviation		0.32

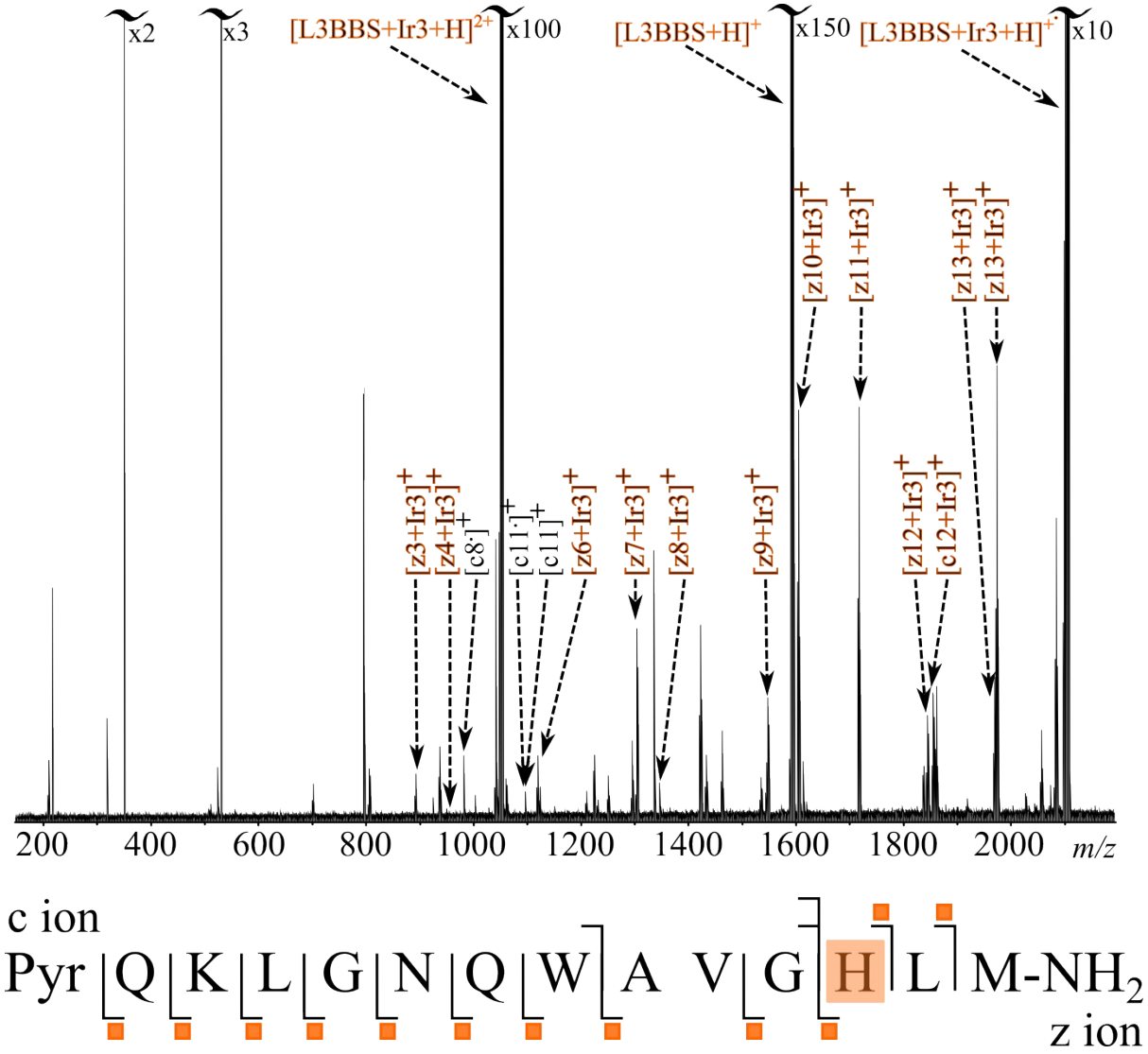


Figure SF17: ECD MS/MS spectrum and fragmentation map of the [L3BBS+**Ir3**+H]$^{2+}$ species.

SI Table ST12: Assignments for the ECD MS/MS analysis of the [L3BBS+**Ir3**+H]$^{2+}$ species.

Assignment	Observed mass	Exact mass	Error/ppm
[z3+Ir3]$^{+}$	891.336566	891.33626	-0.34
[z4+Ir3]$^{+}$	950.361	950.36084	-0.17
[z6+Ir3]$^{+}$	1118.463546	1118.46352	-0.02
[z7+Ir3]$^{+}$	1304.542856	1304.54213	-0.56
[z8+Ir3]$^{+}$	1432.601436	1432.5991	-1.63
[z9+Ir3]$^{+}$	1546.644366	1546.64274	-1.05
[z10+Ir3]$^{+}$	1603.665826	1603.66467	-0.72
[z11+Ir3]$^{+}$	1715.742061	1715.74143	-0.37
[z12+Ir3]$^{+}$	1843.837021	1843.83695	-0.04
[z13+Ir3]$^{+}$	1971.895601	1971.89396	-0.83
[c8·]$^{+}$	982.497355	982.49736	0.01
[c11]$^{+}$	1210.63216	1210.63212	-0.03
[c11·]$^{+}$	1209.624335	1209.62541	0.89
[c13·+Ir3]$^{+}$	1854.821251	1854.82103	-0.12
[c14·+Ir3]$^{+}$	1967.905311	1967.90582	0.26
		Absolute average	0.47
		Standard deviation	0.57

SI Table ST13: Assignments for the ECD MS/MS analysis of the [2XL3BBS+**Ir1**-H2O+2H]$^{3+}$ species.

Assignment	Measured	Theoretical	ppm
[Ir1+H2O+ACN]$^{+}$	597.17247	597.172469	0.00
[L3BBS]$^{2+}$	796.4121	796.411734	0.46
[L3BBS]$^{+}$	1591.81729	1591.816192	0.69
[z12+L3BBS+Ir1]$^{2+}$	1724.32212	1724.32198	0.08
[c12+L3BBS+Ir1]$^{2+}$	1729.81343	1729.814427	-0.58
[c13+L3BBS+Ir1]$^{2+}$	1786.35632	1786.356489	-0.09
[z13+L3BBS+Ir1]$^{2+}$	1788.35188	1788.351288	0.33
[c7+L3BBS+Ir1]$^{+}$	2908.3526	2908.354328	-0.59
[z8+L3BBS+Ir1]$^{+}$	3036.40779	3036.405942	0.61
[c8+L3BBS+Ir1]$^{+}$	3094.43573	3094.435558	0.06
[z9+L3BBS+Ir1]$^{+}$	3149.44393	3149.446068	-0.68
[z10+L3BBS+Ir1]$^{+}$	3206.47192	3206.473544	-0.51
[c11+L3BBS+Ir1]$^{+}$	3320.55927	3320.556808	0.74
[z12+L3BBS+Ir1]$^{+}$	3448.64472	3448.644509	0.06
		Absolute average	0.39
		Standard deviation	0.49