

## Supporting information

### Competitive LC-MS/MS Assay to Investigate Protein Metalation Dynamics

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Table S1. Formulae and isotopologues throughout the analysis.

Table S2. Protein Sequences with potential binding sites highlighted.

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Table S1. Formulae and isotopologues throughout the analysis.

Protein	Formulae & isotopologues	complex	Formulae & isotopologues
<b>Cyt*</b>	C <sub>560</sub> H <sub>873</sub> N <sub>148</sub> O <sub>156</sub> S <sub>4</sub> <sup>56</sup> Fe <sub>1</sub>	<b>1</b> – Pt(NH <sub>3</sub> ) <sub>2</sub> Cl <sub>2</sub>	<sup>195</sup> Pt N <sub>2</sub> H <sub>6</sub> <sup>35</sup> Cl <sub>2</sub>
<b>Ub</b>	C <sub>378</sub> H <sub>629</sub> N <sub>105</sub> O <sub>118</sub> S <sub>1</sub>	<b>2</b> – Pt(ala)(ASA-But)Cl	<sup>195</sup> Pt C <sub>16</sub> H <sub>20</sub> N O <sub>6</sub> <sup>35</sup> Cl
<b>HEWL</b>	C <sub>613</sub> H <sub>951</sub> N <sub>193</sub> O <sub>185</sub> S <sub>10</sub>	<b>3</b> – Ru(HQ)(Cym)Cl	<sup>102</sup> Ru C <sub>19</sub> H <sub>20</sub> N O
<b>Myo</b>	C <sub>769</sub> H <sub>1212</sub> N <sub>210</sub> O <sub>218</sub> S <sub>2</sub>	<b>4</b> – Ru(NHC)(Cym)Cl <sub>2</sub>	<sup>102</sup> Ru C <sub>19</sub> H <sub>24</sub> N <sub>2</sub> <sup>35</sup> Cl <sub>2</sub>
<b>GSH</b>	C <sub>10</sub> H <sub>17</sub> N <sub>3</sub> O <sub>6</sub> S	<b>5</b> – Ir(HQ)(Cp*)Cl	<sup>193</sup> Ir C <sub>19</sub> H <sub>21</sub> N O <sup>35</sup> Cl
<b>Met</b>	C <sub>5</sub> H <sub>11</sub> N O <sub>2</sub> S	<b>6</b> – Ir(NHC)(Cp*)Cl <sub>2</sub>	<sup>193</sup> Ir C <sub>19</sub> H <sub>25</sub> N <sub>2</sub> <sup>35</sup> Cl <sub>2</sub>

\* (+ Ac + heme)

Table S2. Protein Sequences with potential binding sites highlighted.

Protein	Sequence
<b>Cyt*</b>	(Acet)GDVEKGKKIF <sub>10</sub> VQKC <b>C</b> <sub>14</sub> (heme)AQC <b>C</b> <sub>17</sub> (heme)HTV <sub>20</sub> EKGGKH <b>H</b> <sub>26</sub> KTGP <sub>30</sub> NLH <b>H</b> <sub>33</sub> GLFGRKT <sub>40</sub> GQAPGFTYTD <sub>50</sub> ANKNKGITWK <sub>60</sub> EETLM <b>M</b> <sub>65</sub> EYLEN <sub>70</sub> PKKYIPGTK <b>M</b> <sub>80</sub> IFAGIKKKTE <sub>90</sub> REDLIAYLKK <sub>100</sub> ATNE
<b>Ub</b>	<b>M</b> <sub>1</sub> QIFVKTLTG <sub>10</sub> KTITLEVEPS <sub>20</sub> <b>D</b> <sub>21</sub> TIENVKAKI <sub>30</sub> QDKEGIPPD <b>D</b> <sub>39</sub> Q <sub>40</sub> QRLIFAGKQL <sub>50</sub> <b>E</b> <sub>52</sub> GRTLSD <b>S</b> <sub>58</sub> YN <sub>60</sub> IQKESTL <b>H</b> <sub>68</sub> LV <sub>70</sub> LRLRGG
<b>HEWL</b>	KVFGRCELAA <sub>10</sub> AMKR <b>H</b> <sub>15</sub> GLDNY <sub>20</sub> RGYSLGNWVC <sub>30</sub> AAK <b>K</b> <sub>33</sub> FESNFNT <sub>40</sub> QATNRNTDGS <sub>50</sub> TDYGILQINS <sub>60</sub> RWWCNDGRTP <sub>70</sub> GSRNLCNIPC <sub>80</sub> SALLSSDITA <sub>90</sub> SVNCAKKIVS <sub>100</sub> <b>D</b> <sub>101</sub> GNGMNAWVA <sub>110</sub> WRNRCKGTDV <sub>120</sub> QAWIRGCRL
<b>Myo</b>	GLSDGEWQQV <sub>10</sub> LNVWGKVEAD <sub>20</sub> IAG <b>H</b> <sub>24</sub> GQEVLI <sub>30</sub> RLFTGH <b>H</b> <sub>36</sub> PETL <sub>40</sub> EKFDKFK <b>H</b> <sub>48</sub> LK <sub>50</sub> TEAEMKASED <sub>60</sub> LKK <b>H</b> <sub>64</sub> GTVVLT <sub>70</sub> ALGGILKKKG <sub>80</sub> <b>H</b> <sub>81</sub> <b>H</b> <sub>82</sub> EAELKPLA <sub>90</sub> QSH <b>S</b> <sub>93</sub> ATK <b>H</b> <sub>97</sub> KIP <sub>100</sub> IKYLEFISDA <sub>110</sub> I <b>H</b> <sub>113</sub> VL <b>H</b> <sub>116</sub> SK <b>H</b> <sub>119</sub> P <sub>120</sub> GDFGADAQGA <sub>130</sub> MTKALELFRN <sub>140</sub> DIAAKYKELG <sub>150</sub> FQG

\* (+ Ac + heme)

# 1) LC-MS data for the reactions of the protein mix with compounds 1 – 6:

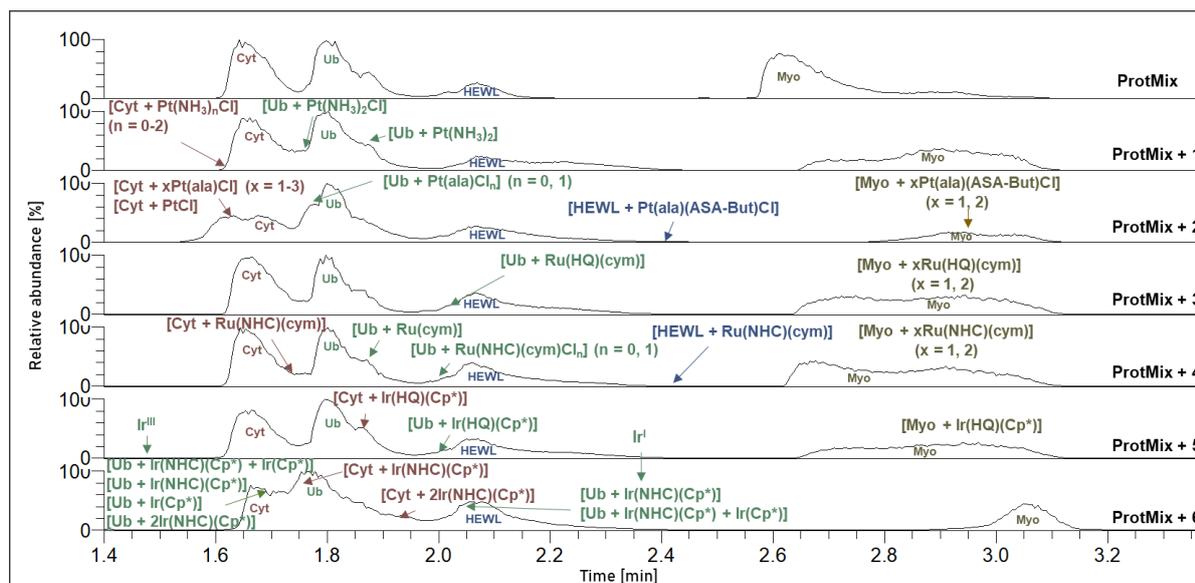


Figure S1. TICs after 24 h incubation of the protein mix and the protein-compound mixtures (1 – [Pt<sup>II</sup>(NH<sub>3</sub>)<sub>2</sub>Cl<sub>2</sub>]; 2 – [Pt<sup>II</sup>(ala)(ASA-But)Cl]; 3 – [Ru<sup>II</sup>(HQ)(cym)Cl]; 4 – [Ru<sup>II</sup>(NHC)(cym)Cl<sub>2</sub>]; 5 – [Ir<sup>III</sup>(HQ)(Cp<sup>\*</sup>)Cl]; 6 – [Ir<sup>III</sup>(NHC)(Cp<sup>\*</sup>)Cl<sub>2</sub>]). Identified adducts are indicated at their respective retention times.

Table S3. Identified protein adducts: m/z values, mass errors, RT, and signal intensity (Cyt – red, Ub – green, HEWL – blue, Myo – ocre; adducts considered for MS/MS inclusion list highlighted in grey). Both m/z<sub>exp</sub> and m/z<sub>theo</sub> refer to the basepeak in the respective cluster of isotopologues.

Complex	Adduct	m/z <sub>exp</sub> /-	m/z <sub>theo</sub> /-	Error /ppm	RT /min	Intensity /-
1 Pt(NH <sub>3</sub> ) <sub>2</sub> Cl <sub>2</sub>	<b>[Cyt<sup>+</sup><sub>+15H</sub>]<sup>15+</sup></b>	<b>824.8951</b>	<b>824.8963</b>	<b>-1.45</b>	<b>1.66</b>	<b>9.28E7</b>
	[Cyt <sup>+</sup> <sub>+14H</sub> + Pt <sup>II</sup> Cl <sup>(1+)</sup> ] <sup>15+</sup>	840.0932	840.0914	2.14	1.62	1.88E6
	[Cyt <sup>+</sup> <sub>+14H</sub> + Pt <sup>II</sup> (NH <sub>3</sub> )Cl <sup>(1+)</sup> ] <sup>15+</sup>	841.3596	841.3599	-0.36	1.63	2.44E6
	[Cyt <sup>+</sup> <sub>+14H</sub> + Pt <sup>II</sup> (NH <sub>3</sub> ) <sub>2</sub> Cl <sup>(1+)</sup> ] <sup>15+</sup>	842.4291	842.4282	1.07	1.63	3.49E6
2 Pt(ala)(ASA-But)Cl	<b>[Cyt<sup>+</sup><sub>+15H</sub>]<sup>15+</sup></b>	<b>824.8964</b>	<b>824.8963</b>	<b>0.12</b>	<b>1.68</b>	<b>2.26E7</b>
	[Cyt <sup>+</sup> <sub>+14H</sub> + Pt <sup>II</sup> Cl <sup>(1+)</sup> ] <sup>15+</sup>	840.2245	840.2246	-0.12	1.58	4.20E6
	[Cyt <sup>+</sup> <sub>+15H</sub> + Pt <sup>II</sup> (ala)Cl <sup>(0)</sup> ] <sup>15+</sup>	846.0944	846.0943	0.12	1.64	2.20E7
	[Cyt <sup>+</sup> <sub>+15H</sub> + 2Pt <sup>II</sup> (ala)Cl <sup>(0)</sup> ] <sup>15+</sup>	867.3591	867.3588	0.35	1.61	2.27E7
4 Ru(NHC)(cym)Cl <sub>2</sub>	<b>[Cyt<sup>+</sup><sub>+15H</sub>]<sup>15+</sup></b>	<b>824.8956</b>	<b>824.8963</b>	<b>-0.85</b>	<b>1.64</b>	<b>1.22E8</b>
	[Cyt <sup>+</sup> <sub>+13H</sub> + Ru <sup>II</sup> (NHC)(cym) <sup>(2+)</sup> ] <sup>15+</sup>	850.2346	850.2352	-0.71	1.75	9.33E6
5 Ir(HQ)(Cp <sup>*</sup> )Cl	<b>[Cyt<sup>+</sup><sub>+15H</sub>]<sup>15+</sup></b>	<b>824.8962</b>	<b>824.8963</b>	<b>-0.12</b>	<b>1.65</b>	<b>1.09E8</b>
	[Cyt <sup>+</sup> <sub>+13H</sub> + Ir <sup>III</sup> (HQ)(Cp <sup>*</sup> ) <sup>(1+)</sup> ] <sup>14+</sup>	917.3965	917.3967	-1.22	1.86	7.75E6
6 Ir(NHC)(Cp <sup>*</sup> )Cl <sub>2</sub>	<b>[Cyt<sup>+</sup><sub>+15H</sub>]<sup>15+</sup></b>	<b>824.8958</b>	<b>824.8963</b>	<b>-0.61</b>	<b>1.66</b>	<b>6.05E7</b>
	[Cyt <sup>+</sup> <sub>+12H</sub> + Ir <sup>III</sup> (NHC)(Cp <sup>*</sup> ) <sup>(2+)</sup> ] <sup>14+</sup>	917.4700	917.4704	-0.44	1.76	6.75E7
	[Cyt <sup>+</sup> <sub>+13H</sub> + Ir <sup>I</sup> (NHC)(Cp <sup>*</sup> ) <sup>(0)</sup> + Ir <sup>III</sup> (NHC)(Cp <sup>*</sup> ) <sup>(2+)</sup> ] <sup>15+</sup>	887.9168	887.9174	-0.68	1.98	2.23E6
1 Pt(NH <sub>3</sub> ) <sub>2</sub> Cl <sub>2</sub>	<b>[Ub<sub>+10H</sub>]<sup>10+</sup></b>	<b>857.4679</b>	<b>857.4703</b>	<b>-2.80</b>	<b>1.80</b>	<b>2.74E8</b>
	[Ub <sub>+7H</sub> + Pt <sup>II</sup> (NH <sub>3</sub> ) <sub>2</sub> <sup>(2+)</sup> ] <sup>9+</sup>	977.8529	977.8551	-2.25	1.86	2.84E6
	[Ub <sub>+8H</sub> + Pt <sup>II</sup> (NH <sub>3</sub> ) <sub>2</sub> Cl <sup>(1+)</sup> ] <sup>9+</sup>	981.9622	981.9638	-1.63	1.77	8.19E6
2 Pt(ala)(ASA-But)Cl	<b>[Ub<sub>+10H</sub>]<sup>10+</sup></b>	<b>857.4694</b>	<b>857.4703</b>	<b>-1.05</b>	<b>1.80</b>	<b>2.09E8</b>
	[Ub <sub>+9H</sub> + Pt <sup>II</sup> (ala) <sup>(1+)</sup> ] <sup>10+</sup>	885.6696	885.6699	-0.34	1.78	1.22E8
	[Ub <sub>+10H</sub> + Pt <sup>II</sup> (ala)Cl <sup>(0)</sup> ] <sup>10+</sup>	889.2664	889.2679	-1.69	1.75	8.67E6
3 Ru(HQ)(cym)Cl	<b>[Ub<sub>+10H</sub>]<sup>10+</sup></b>	<b>857.4689</b>	<b>857.4703</b>	<b>-1.63</b>	<b>1.80</b>	<b>2.74E8</b>
	[Ub <sub>+9H</sub> + Ru <sup>II</sup> (HQ)(cym) <sup>(1+)</sup> ] <sup>10+</sup>	895.3746	895.3756	-1.12	1.98	4.22E6

<b>4</b> Ru(NHC)(cym)Cl <sub>2</sub>	<b>[Ub<sub>+10H</sub>]<sup>10+</sup></b>	<b>857.4690</b>	<b>857.4703</b>	<b>-1.52</b>	<b>1.80</b>	<b>2.38E8</b>
	[Ub <sub>+8H</sub> + Ru <sup>II</sup> (cym) <sup>(2+)</sup> ] <sup>10+</sup>	880.8690	880.8703	-1.48	1.83	4.18E6
	[Ub <sub>+8H</sub> + Ru <sup>II</sup> (NHC)(cym) <sup>(2+)</sup> ] <sup>10+</sup>	895.4777	895.4783	-0.67	1.90	7.83E6
	[Ub <sub>+9H</sub> + Ru <sup>II</sup> (NHC)(cym)Cl <sup>(1+)</sup> ] <sup>10+</sup>	899.0768	899.0763	0.56	1.98	2.03E6
<b>5</b> Ir(HQ)(Cp <sup>*</sup> )Cl	<b>[Ub<sub>+10H</sub>]<sup>10+</sup></b>	<b>857.4696</b>	<b>857.4703</b>	<b>-0.82</b>	<b>1.80</b>	<b>2.67E8</b>
	[Ub <sub>+9H</sub> + Ir <sup>III</sup> (HQ)(Cp <sup>*</sup> ) <sup>(1+)</sup> ] <sup>10+</sup>	904.4817	904.4819	-0.22	1.99	1.81E6
<b>6</b> Ir(NHC)(Cp <sup>*</sup> )Cl <sub>2</sub>	<b>[Ub<sub>+10H</sub>]<sup>10+</sup></b>	<b>857.4693</b>	<b>857.4703</b>	<b>-1.17</b>	<b>1.79</b>	<b>5.55E7</b>
	[Ub <sub>+8H</sub> + Ir <sup>III</sup> (Cp <sup>*</sup> ) <sup>(2+)</sup> ] <sup>10+</sup>	889.9761	889.9766	-0.56	1.71	1.49E7
	[Ub <sub>+8H</sub> + Ir <sup>III</sup> (NHC)(Cp <sup>*</sup> ) <sup>(2+)</sup> ] <sup>10+</sup>	904.5847	904.5850	-0.33	1.73	2.06E7
	[Ub <sub>+6H</sub> + Ir <sup>III</sup> (NHC)(Cp <sup>*</sup> ) <sup>(2+)</sup> + Ir <sup>III</sup> (Cp <sup>*</sup> ) <sup>(2+)</sup> ] <sup>10+</sup>	937.1918	937.1917	0.11	1.71	1.51E7
	[Ub <sub>+5H</sub> + 2Ir <sup>III</sup> (NHC)(Cp <sup>*</sup> ) <sup>(2+)</sup> ] <sup>9+</sup>	1057.4439	1057.4438	0.09	1.66	3.47E7
	[Ub <sub>+10H</sub> + Ir <sup>I</sup> (NHC)(Cp <sup>*</sup> ) <sup>(0)</sup> ] <sup>10+</sup>	904.7860	904.7867	-0.77	2.09	2.15E7
	[Ub <sub>+8H</sub> + Ir <sup>I</sup> (NHC)(Cp <sup>*</sup> ) <sup>(0)</sup> + Ir <sup>III</sup> (Cp <sup>*</sup> ) <sup>(2+)</sup> ] <sup>10+</sup>	937.3938	937.3933	0.53	2.00	2.61E6
<b>2</b> Pt(ala)(ASA-But)Cl	<b>[HEWL<sub>+10H</sub>]<sup>10+</sup></b>	<b>1431.3896</b>	<b>1431.3904</b>	<b>-0.56</b>	<b>2.06</b>	<b>4.45E7</b>
	[HEWL <sub>AcO</sub> +10H] <sup>10+</sup>	1435.6892	1435.6926	-2.37	2.06	1.00E7
	[HEWL <sub>+10H</sub> + Pt <sup>II</sup> (ala)(ASA-But)Cl <sup>(0)</sup> ] <sup>10+</sup>	1486.6990	1486.6970	1.35	2.35	1.06E6
<b>4</b> Ru(NHC)(cym)Cl <sub>2</sub>	<b>[HEWL<sub>+10H</sub>]<sup>10+</sup></b>	<b>1431.3883</b>	<b>1431.3901</b>	<b>-1.28</b>	<b>2.06</b>	<b>1.06E8</b>
	[HEWL <sub>+8H</sub> + Ru <sup>II</sup> (NHC)(cym) <sup>(2+)</sup> ] <sup>10+</sup>	1469.4006	1469.3991	1.02	2.39	5.61E5
<b>2</b> Pt(ala)(ASA-But)Cl	<b>[Myo<sub>+18H</sub>]<sup>18+</sup></b>	<b>942.7294</b>	<b>942.7291</b>	<b>0.32</b>	<b>2.94</b>	<b>2.08E5</b>
	[Myo <sub>+17H</sub> + Pt <sup>II</sup> (ASA-But)Cl <sup>(1+)</sup> ] <sup>18+</sup>	968.5075	968.5076	-0.10	2.92	6.66E5
	[Myo <sub>AcO</sub> +17H + Pt <sup>II</sup> (ASA-But)Cl <sup>(1+)</sup> ] <sup>18+</sup>	970.8416	970.8415	0.10	2.94	1.54E5
<b>3</b> Ru(HQ)(cym)Cl	<b>[Myo<sub>+18H</sub>]<sup>18+</sup></b>	<b>942.7286</b>	<b>942.7292</b>	<b>-0.64</b>	<b>2.75</b>	<b>8.57E6</b>
	[Myo <sub>+17H</sub> + Ru <sup>II</sup> (HQ)(cym) <sup>(1+)</sup> ] <sup>18+</sup>	963.7876	963.7877	-0.10	2.77	4.34E6
	[Myo <sub>+16H</sub> + 2Ru <sup>II</sup> (HQ)(cym) <sup>(1+)</sup> ] <sup>18+</sup>	984.7903	984.7901	0.20	2.89	3.44E6
<b>4</b> Ru(NHC)(cym)Cl <sub>2</sub>	<b>[Myo<sub>+18H</sub>]<sup>18+</sup></b>	<b>942.7304</b>	<b>942.7292</b>	<b>1.27</b>	<b>2.66</b>	<b>8.48E6</b>
	[Myo <sub>+16H</sub> + Ru <sup>II</sup> (NHC)(cym) <sup>(2+)</sup> ] <sup>18+</sup>	963.8461	963.8445	1.66	2.67	1.39E7
	[Myo <sub>+14H</sub> + 2Ru <sup>II</sup> (NHC)(cym) <sup>(2+)</sup> ] <sup>18+</sup>	984.9064	984.9048	1.62	2.68	3.41E6
<b>5</b> Ir(HQ)(Cp <sup>*</sup> )Cl	<b>[Myo<sub>+18H</sub>]<sup>18+</sup></b>	<b>942.7305</b>	<b>942.7291</b>	<b>1.49</b>	<b>2.71</b>	<b>8.78E6</b>
	[Myo <sub>+17H</sub> + Ir <sup>III</sup> (HQ)(Cp <sup>*</sup> ) <sup>(1+)</sup> ] <sup>18+</sup>	986.8486	968.8461	2.58	2.94	8.96E5

\* (+ Ac + heme)

## 2) Time-resolved adduct formation

Compound 2.

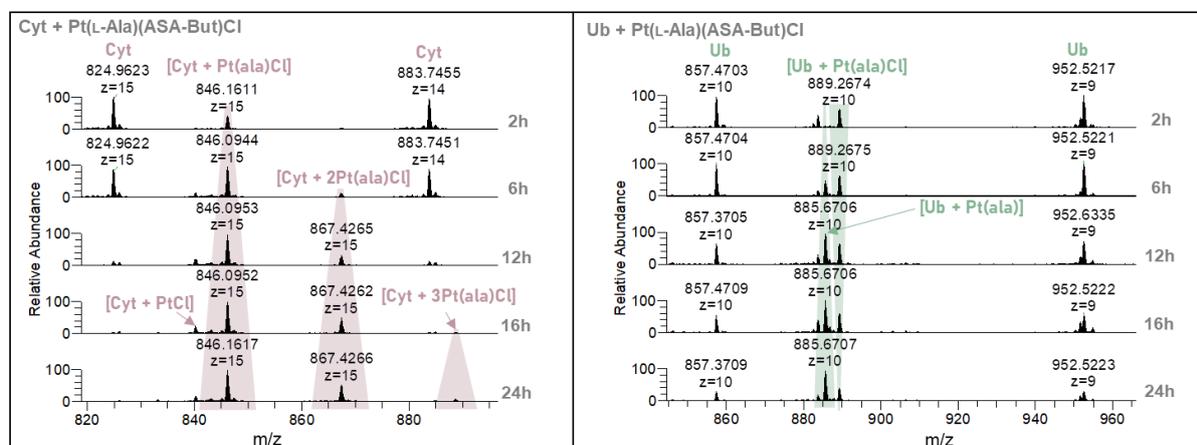


Figure S2. Time-resolved mass spectra of compound 2 with Cyt (left) and Ub (right).

### NHC-compounds (4 + 6):

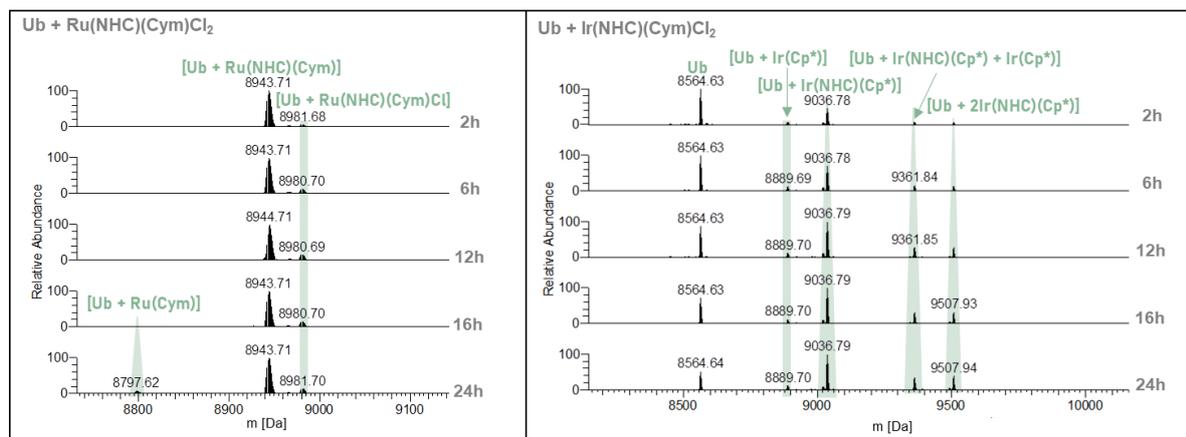


Figure S3. Time-resolved mass spectra of Ub with compound 4 (left) and compound 6 (right).

### 3) Complex and adduct speciation

#### Compound 6:

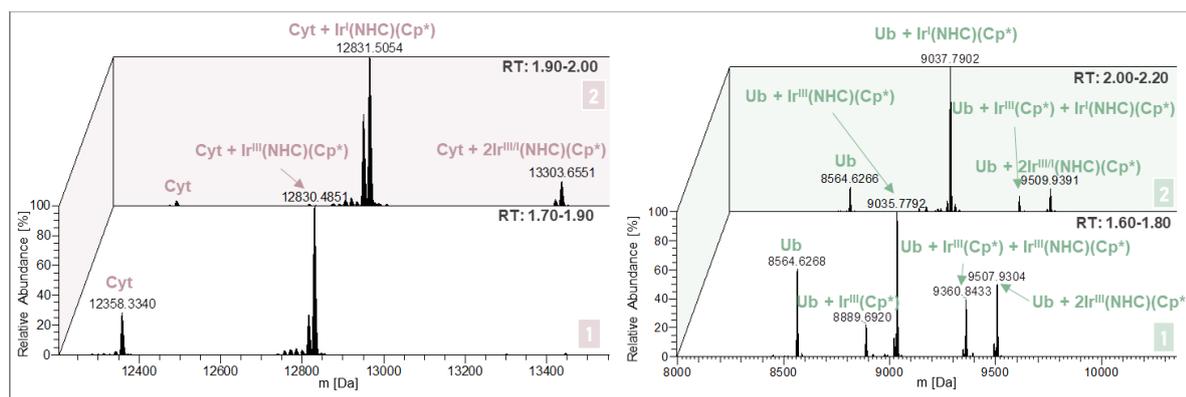


Figure S4. Deconvoluted mass spectra of the Cyt and Ub adducts with compound 6.

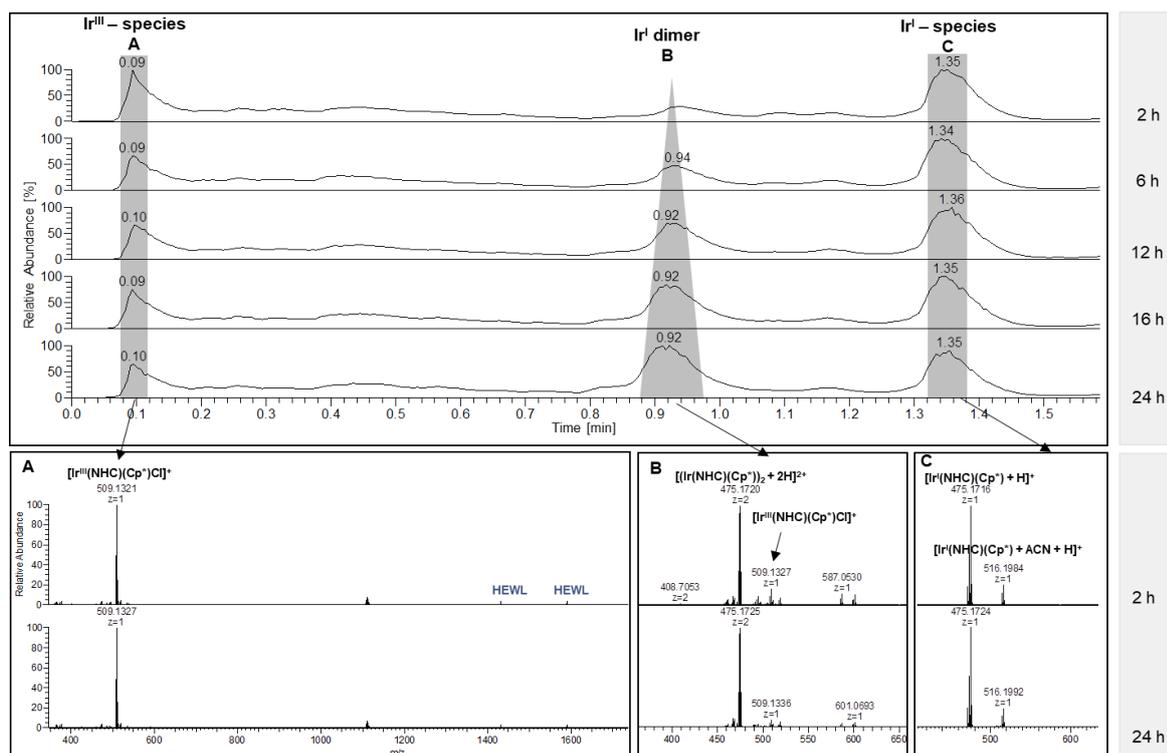


Figure S5. Top: TIC of the free Ir species of compound **6** after 2, 6, 12, 16, and 24 h incubation with the protein mixture. Bottom: Mass spectra of the Ir species of compound **6** after 2 and 24 h incubation with the protein mixture. Over time, a dimeric species formed (B) for which the oxidation state is unclear.

Table S4. Identified free complex species of compound **6** with sum formula, m/z, error and retention time (RT) in the chromatogram.

Spectrum	Species	Sum formula	m/z exp	m/z theo	Error /ppm	RT /min
<b>A + B</b>	$[\text{Ir}^{\text{III}}(\text{NHC})(\text{Cp}^*)\text{Cl}]^+$	$\text{Ir}(\text{C}_{10}\text{H}_{15})(\text{C}_9\text{H}_{10}\text{N}_2)\text{Cl}$	509.1327	509.1321	1.18	0.10
<b>B</b>	$[(\text{Ir}(\text{NHC})(\text{Cp}^*))_2 + 2\text{H}]^{2+}$	$(\text{Ir}(\text{C}_{10}\text{H}_{15})(\text{C}_9\text{H}_{10}\text{N}_2))_2\text{H}_2$	475.1725	475.1722	0.63	0.92
<b>C</b>	$[\text{Ir}^{\text{I}}(\text{NHC})(\text{Cp}^*) + \text{H}]^+$	$\text{Ir}(\text{C}_{10}\text{H}_{15})(\text{C}_9\text{H}_{10}\text{N}_2)\text{H}$	475.1724	475.172	0.84	1.35
	$[\text{Ir}^{\text{I}}(\text{NHC})(\text{Cp}^*) + \text{ACN} + \text{H}]^+$	$\text{Ir}(\text{C}_{10}\text{H}_{15})(\text{C}_9\text{H}_{10}\text{N}_2)\text{HCH}_3\text{CN}$	516.1991	516.1986	0.97	1.35

#### 4) LC-MS/MS-fragmentation of selected precursor ions:

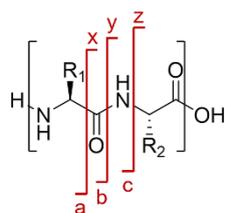


Figure S6. MS/MS fragment nomenclature of proteins.

Table S5. m/z values, mass errors, RT and signal intensity of the identified fragments (Cyt – red, Ub – green, HEWL – blue, Myo – ocre; precursor ions highlighted).

Complex	Fragments	Site	m/z <sub>exp</sub> /-	m/z <sub>theo</sub> /-	Error /ppm	NCE	Intensity /-
<b>1</b> Pt(NH <sub>3</sub> ) <sub>2</sub> Cl <sub>2</sub>	[Cyt* <sub>+14H</sub> + Pt <sup>II</sup> (NH <sub>3</sub> ) <sub>2</sub> Cl <sup>(+1)</sup> ] <sup>15+</sup>	-	-	842.4951	-	20	-
	[y <sub>39+6H</sub> ] <sup>6+</sup>	S <sub>Met65</sub>	763.0913	763.0894	2.49	20	2.20E5
	[y <sub>39+6H</sub> - H <sub>2</sub> O] <sup>6+</sup>	S <sub>Met65</sub>	760.0901	760.0877	3.16	20	3.45E5
	[y <sub>39+5H</sub> ] <sup>5+</sup>	S <sub>Met65</sub>	915.5077	915.5059	1.97	20	3.23E5
	[y <sub>29+4H</sub> ] <sup>4+</sup>	-	824.4660	824.4634	3.15	20	2.30E5
	[b <sub>45*+5H</sub> ] <sup>6+</sup>	-	903.1239	903.1215	2.66	20	1.64E5
	[b <sub>46*+5H</sub> ] <sup>6+</sup>	-	927.6343	927.6334	0.97	20	1.36E5
	[b <sub>47*+5H</sub> ] <sup>6+</sup>	-	944.4762	944.4745	1.80	20	9.35E4
	[b <sub>47*+6H</sub> ] <sup>7+</sup>	-	809.6958	809.6936	2.72	20	7.45E4
	[y <sub>43+6H</sub> + Pt <sup>II</sup> Cl <sup>(+1)</sup> - H <sub>2</sub> O] <sup>6+</sup>	S <sub>Met65</sub>	877.4456	877.4443	1.48	20	1.08E5
	[y <sub>43+7H</sub> + Pt <sup>II</sup> Cl <sup>(+1)</sup> - H <sub>2</sub> O] <sup>7+</sup>	S <sub>Met65</sub>	752.2398	752.2392	0.80	20	5.39E4
	[a <sub>65*+7H</sub> + Pt <sup>(+2)</sup> ] <sup>9+</sup>	S <sub>Met65</sub>	884.3205	884.3182	2.60	20	1.99E5
	[a <sub>65*+7H</sub> + Pt <sup>(+2)</sup> - H <sub>2</sub> O] <sup>9+</sup>	S <sub>Met65</sub>	882.3202	882.3164	4.31	20	1.11E5
	[b <sub>65*+7H</sub> + Pt <sup>(+2)</sup> ] <sup>9+</sup>	S <sub>Met65</sub>	887.4294	887.4282	1.35	20	9.73E4
	[b <sub>65*+8H</sub> + Pt <sup>II</sup> Cl <sup>(+1)</sup> ] <sup>9+</sup>	S <sub>Met65</sub>	891.5396	891.5368	3.14	20	4.98E4
<b>2</b> Pt(ala)(ASA-But)Cl	[Cyt* <sub>+15H</sub> + Pt <sup>II</sup> (ala)Cl <sup>(0)</sup> ] <sup>15+</sup>	-	846.0974	846.0944	3.55	20	1.22E5
	[y <sub>39+6H</sub> ] <sup>6+</sup>	S <sub>Met65</sub>	763.0917	763.0894	3.01	20	3.29E5
	[y <sub>39+6H</sub> - H <sub>2</sub> O] <sup>6+</sup>	S <sub>Met65</sub>	760.0910	760.0877	4.34	20	6.20E5
	[y <sub>39+5H</sub> ] <sup>5+</sup>	S <sub>Met65</sub>	915.5078	915.5059	2.08	20	3.38E5
	[y <sub>29+4H</sub> ] <sup>4+</sup>	-	824.4669	824.4634	4.25	20	2.25E5
	[b <sub>45*+6H</sub> ] <sup>6+</sup>	-	902.9567	902.9544	2.55	20	1.24E5
	[b <sub>46*+6H</sub> ] <sup>6+</sup>	-	927.6349	927.6329	2.16	20	6.61E6
	[b <sub>47*+6H</sub> ] <sup>6+</sup>	-	944.4763	944.4745	1.91	20	1.30E5
	[b <sub>47*+7H</sub> ] <sup>7+</sup>	-	809.6963	809.6932	3.83	20	1.01E5
	[Cyt* <sub>+15H</sub> + Pt <sup>II</sup> (ala)Cl <sup>(0)</sup> - H <sub>2</sub> O] <sup>15+</sup>	-	844.9629	844.9606	2.72	20	8.18E4
	[Cyt* <sub>+13H</sub> + Pt <sup>(+2)</sup> ] <sup>15+</sup>	-	837.7618	837.7598	2.39	20	2.03E4
	[y <sub>56+8H</sub> + Pt(ala)Cl <sup>(0)</sup> ] <sup>8+</sup>	S <sub>Met65</sub>	857.5643	857.5628	1.75	20	1.00E5
	[y <sub>57+9H</sub> + Pt(ala)Cl <sup>(0)</sup> ] <sup>9+</sup>	S <sub>Met65</sub>	780.5091	780.5079	1.54	20	2.74E4
	[a <sub>65*+7H</sub> + Pt <sup>(+2)</sup> ] <sup>9+</sup>	S <sub>Met65</sub>	884.3206	884.3177	3.27	20	1.96E5
	[a <sub>65*+7H</sub> + Pt <sup>(+2)</sup> - H <sub>2</sub> O] <sup>9+</sup>	S <sub>Met65</sub>	882.3206	882.3165	4.65	20	8.29E4
	[b <sub>56*+7H</sub> + Pt <sup>(+2)</sup> ] <sup>9+</sup>	S <sub>Met65</sub>	887.4306	887.4282	2.70	20	1.02E5
	[b <sub>56*+8H</sub> + Pt <sup>II</sup> Cl <sup>(+1)</sup> ] <sup>9+</sup>	S <sub>Met65</sub>	891.5393	891.5368	2.80	20	3.99E4
	[Cyt* <sub>+15H</sub> + 2Pt <sup>II</sup> (ala)Cl <sup>(0)</sup> ] <sup>15+</sup>	-	867.2923	867.2924	-0.12	20	2.54E4
	[y <sub>24+3H</sub> ] <sup>3+</sup>	S <sub>Met80</sub>	927.5306	927.5297	0.97	20	1.27E5
	[y <sub>24+4H</sub> ] <sup>4+</sup>	S <sub>Met80</sub>	695.9002	695.8988	2.01	20	9.27E4
[b <sub>45*+6H</sub> ] <sup>6+</sup>	-	902.9552	902.9544	0.89	20	6.14E6	
[b <sub>46*+6H</sub> ] <sup>6+</sup>	-	927.6337	927.6334	0.32	20	6.78E4	
[b <sub>47*+6H</sub> ] <sup>6+</sup>	-	944.4755	944.4742	1.38	20	6.00E4	
[b <sub>47*+7H</sub> ] <sup>7+</sup>	-	809.6944	809.6936	0.99	20	2.51E4	
[y <sub>39+5H</sub> + Pt <sup>II</sup> (ala)Cl <sup>(0)</sup> ] <sup>5+</sup>	S <sub>Met80</sub>	979.3016	979.3006	1.02	20	3.01E4	

	[y56+8H + 2Pt <sup>II</sup> (ala)Cl <sup>(0)</sup> ] <sup>8+</sup>	S <sub>Met65</sub> , S <sub>Met80</sub>	897.4351	897.4342	1.00	20	2.66E4
	[y86+11H + Pt <sup>II</sup> (ala)Cl <sup>(0)</sup> ] <sup>11+</sup>	-	911.7461	911.7444	1.86	20	3.60E4
	[a65*+7H + Pt <sup>(+2)</sup> ] <sup>9+</sup>	S <sub>Met65</sub>	884.3203	884.3177	2.94	20	1.96E5
	[a65*+7H + Pt <sup>(+2)</sup> - H <sub>2</sub> O] <sup>9+</sup>	S <sub>Met65</sub>	882.3185	882.3165	2.27	20	2.47E4
	[b56*+7H + Pt <sup>(+2)</sup> ] <sup>9+</sup>	S <sub>Met65</sub>	887.4300	887.4282	2.03	20	2.46E4
	[b56*+8H + Pt <sup>II</sup> Cl <sup>(+1)</sup> ] <sup>9+</sup>	S <sub>Met65</sub>	891.5375	891.5361	1.57	20	1.19E4
4 Ru(NHC) (Cym)Cl <sub>2</sub>	[Cyt*+13H + Ru <sup>II</sup> (NHC)(Cym) <sup>(2+)</sup> ] <sup>15+</sup>	-	850.1686	850.168	0.71	20	6.12E4
	[y29+4H] <sup>4+</sup>	-	824.464	824.4634	0.73	25	4.70E4
	[y29+3H] <sup>3+</sup>	-	1098.9488	1098.9488	0.00	25	3.59E4
	[y25+2H] <sup>3+</sup>	-	971.2099	971.2094	0.51	25	2.25E4
	[y24+2H] <sup>3+</sup>	-	927.5294	927.5292	0.22	25	2.00E4
	[y40+4H] <sup>5+</sup>	-	941.7142	941.7139	0.32	25	1.64E4
	[y22+2H] <sup>3+</sup>	-	840.8116	840.8121	-0.59	25	1.83E4
	[y56+7H] <sup>8+</sup>	-	817.8169	817.8161	0.98	20	6.12E4
	[y57+7H] <sup>8+</sup>	-	838.0741	838.0737	0.48	20	9.14E4
	[y58+7H] <sup>8+</sup>	-	850.7047	850.705	-0.35	20	7.04E4
[b43*+4H + Ru(NHC)(Cym) <sup>(+2)</sup> ] <sup>6+</sup>	N <sub>His26/33</sub>	940.7907	940.7907	0.00	20	2.65E4	
[b46*+4H + Ru(NHC)(Cym) <sup>(+2)</sup> ] <sup>6+</sup>	N <sub>His26/33</sub>	990.9828	990.9817	1.11	20	1.96E4	
[b45*+4H + Ru(NHC)(Cym) <sup>(+2)</sup> ] <sup>6+</sup>	N <sub>His26/33</sub>	966.3026	966.3034	-0.83	20	1.91E4	
[b47*+5H + Ru(NHC)(Cym) <sup>(+2)</sup> ] <sup>7+</sup>	N <sub>His26/33</sub>	863.8488	863.8489	-0.12	20	1.79E4	
1 Pt(NH <sub>3</sub> ) <sub>2</sub> Cl <sub>2</sub>	[Ub+8H + Pt <sup>II</sup> (NH <sub>3</sub> ) <sub>2</sub> Cl <sup>(+1)</sup> ] <sup>9+</sup>	-	981.8522	981.8521	0.10	20	1.04E5
	[Ub+7H + Pt <sup>(+2)</sup> ] <sup>9+</sup>	-	974.0727	974.0716	1.13	20	4.60E5
	[Ub+7H + Pt <sup>II</sup> (NH <sub>3</sub> ) <sup>(+2)</sup> ] <sup>9+</sup>	-	975.8526	975.8520	0.61	20	1.94E5
	[Ub+7H + Pt <sup>II</sup> (NH <sub>3</sub> ) <sub>2</sub> <sup>(+2)</sup> ] <sup>9+</sup>	-	977.7433	977.7438	-0.51	20	1.09E5
	[Ub+8H + Pt <sup>II</sup> (NH <sub>3</sub> )Cl <sup>(+1)</sup> ] <sup>9+</sup>	-	979.9616	979.9607	0.92	20	1.91E5
	[y58+4H + Pt <sup>(+2)</sup> ] <sup>6+</sup>	N <sub>His68</sub>	1121.5836	1121.5816	1.69	25	4.60E4
	[y53+4H + Pt <sup>(+2)</sup> ] <sup>6+</sup>	N <sub>His68</sub>	1036.0430	1036.0412	1.74	25	1.81E4
	[y40+3H + Pt <sup>(+2)</sup> ] <sup>5+</sup>	N <sub>His68</sub>	952.2910	952.2885	2.63	25	9.01E3
	[y52+4H + Pt <sup>(+2)</sup> ] <sup>6+</sup>	N <sub>His68</sub>	1014.5361	1014.5339	2.17	25	7.49E3
	[y40+2H + Pt <sup>(+2)</sup> ] <sup>4+</sup>	N <sub>His68</sub>	1190.1108	1190.1088	1.68	25	6.43E3
2 Pt(ala)(ASA -But)Cl	[Ub+9H + Pt <sup>II</sup> (ala) <sup>(+1)</sup> ] <sup>10+</sup>	-	885.6719	885.6699	2.26	20	1.90E6
	[y58+7H] <sup>7+</sup>	-	933.9383	933.9355	3.00	20	2.07E6
	[y58+8H] <sup>8+</sup>	-	817.3224	817.3193	3.79	20	1.28E6
	[y59 - H <sub>2</sub> O+7H] <sup>7+</sup>	-	949.7995	949.7972	2.42	20	2.67E5
	[y59+7H] <sup>7+</sup>	-	952.3724	952.3701	2.42	20	2.55E5
	[y40+5H] <sup>5+</sup>	-	913.7023	913.6987	3.94	20	3.09E5
	[b2 + Pt <sup>II</sup> (ala) <sup>(+1)</sup> ] <sup>+</sup>	S <sub>Met1</sub>	542.1047	542.1032	2.77	20	5.60E4
	[b14+2H + Pt <sup>II</sup> (ala) <sup>(+1)</sup> - H <sub>2</sub> O] <sup>3+</sup>	S <sub>Met1</sub>	609.9683	609.9664	3.11	20	5.56E4
	[a15+2H + Pt <sup>II</sup> (ala) <sup>(+1)</sup> ] <sup>3+</sup>	S <sub>Met1</sub>	644.3359	644.3333	4.04	20	9.89E4
	[b15+2H + Pt <sup>II</sup> (ala) <sup>(+1)</sup> ] <sup>3+</sup>	S <sub>Met1</sub>	653.6680	653.6650	4.59	20	8.33E4
	[b16+2H + Pt <sup>II</sup> (ala) <sup>(+1)</sup> ] <sup>3+</sup>	S <sub>Met1</sub>	696.6816	696.6796	2.87	20	6.39E5
	[a17+2H + Pt <sup>II</sup> (ala) <sup>(+1)</sup> ] <sup>3+</sup>	S <sub>Met1</sub>	720.3732	720.3706	3.61	20	1.21E5
	[b17+2H + Pt <sup>II</sup> (ala) <sup>(+1)</sup> ] <sup>3+</sup>	S <sub>Met1</sub>	729.7043	729.7020	3.15	20	7.23E5
[b18+2H + Pt <sup>II</sup> (ala) <sup>(+1)</sup> ] <sup>3+</sup>	S <sub>Met1</sub>	772.7189	772.7162	3.49	20	9.37E5	
4 Ru(NHC) (Cym)Cl <sub>2</sub>	[Ub+8H + Ru <sup>II</sup> (NHC)(cym) <sup>(+2)</sup> ] <sup>10+</sup>	-	895.4787	895.4787	0.00	25	2.33E3
	[y40+5H] <sup>5+</sup>	-	913.6993	913.6987	0.66	25	2.95E4
	[y40+4H] <sup>4+</sup>	-	1141.8718	1141.8711	0.61	25	3.60E4
	[y58+6H] <sup>6+</sup>	-	1089.4229	1089.4231	-0.18	25	2.11E4
	[y14+2H] <sup>2+</sup>	-	789.9756	789.9756	0.00	25	3.06E4
	[y13+2H] <sup>2+</sup>	-	725.9283	725.9281	0.28	25	3.14E4
	[y15+2H] <sup>2+</sup>	-	854.0049	854.0048	0.12	25	8.54E2
	[y24+3H] <sup>3+</sup>	-	909.8403	909.8411	-0.88	25	1.42E4
	[b16+2H] <sup>2+</sup>	-	903.0155	903.0157	-0.22	25	1.76E4
	[y12+2H] <sup>2+</sup>	-	661.4072	661.4068	0.60	25	1.89E4
	[y11+2H] <sup>2+</sup>	-	617.8911	617.8908	0.49	25	1.54E4
	[Ru <sup>II</sup> (cym)(NHC) <sup>(+2)</sup> ] <sub>-H</sub> <sup>+</sup>	-	381.0897	381.0905	-2.10	25	7.06E4

	[y58 <sub>+5H</sub> + Ru <sup>II</sup> (cym)(NHC) <sup>(+2)</sup> ] <sup>7+</sup>	N <sub>His68</sub>	988.2342	988.2333	0.91	25	5.43E4
	[y58 <sub>+6H</sub> + Ru <sup>II</sup> (cym)(NHC) <sup>(+2)</sup> ] <sup>8+</sup>	N <sub>His68</sub>	864.8302	864.8298	0.46	25	1.70E4
	[y57 <sub>+5H</sub> + Ru <sup>II</sup> (cym)(NHC) <sup>(+2)</sup> ] <sup>7+</sup>	N <sub>His68</sub>	974.3693	974.3684	0.92	25	1.40E4
	[y40 <sub>+4H</sub> + Ru <sup>II</sup> (cym)(NHC) <sup>(+2)</sup> ] <sup>6+</sup>	N <sub>His68</sub>	824.9313	824.9309	0.48	25	1.26E4
	[y42 <sub>+3H</sub> + Ru <sup>II</sup> (cym)(NHC) <sup>(+2)</sup> ] <sup>5+</sup>	N <sub>His68</sub>	1023.7372	1023.7369	0.29	25	1.04E4
	[y37 <sub>+3H</sub> + Ru <sup>II</sup> (cym)(NHC) <sup>(+2)</sup> ] <sup>5+</sup>	N <sub>His68</sub>	927.8893	927.8887	0.65	25	9.56E3
	[y43 <sub>+3H</sub> + Ru <sup>II</sup> (cym)(NHC) <sup>(+2)</sup> ] <sup>5+</sup>	N <sub>His68</sub>	1049.5442	1049.5454	-1.14	25	8.85E3
	[y24 <sub>+2H</sub> + Ru <sup>II</sup> (cym)(NHC) <sup>(+2)</sup> ] <sup>4+</sup>	N <sub>His68</sub>	777.6541	777.6541	0.00	25	1.03E4
	[y56 <sub>+4H</sub> + Ru <sup>II</sup> (cym)(NHC) <sup>(+2)</sup> ] <sup>7+</sup>	N <sub>His68</sub>	961.9354	961.9354	0.00	25	6.99E3
	[y50 <sub>+3H</sub> + Ru <sup>II</sup> (cym)(NHC) <sup>(+2)</sup> ] <sup>6+</sup>	N <sub>His68</sub>	1010.2048	1010.2051	-0.30	25	9.59E3
<b>6</b> Ir(NHC) (Cp*) <sub>2</sub> Cl <sub>2</sub>	[Ub <sub>+6H</sub> + Ir <sup>III</sup> (NHC)(Cp*) <sup>(+2)</sup> + Ir <sup>III</sup> (Cp*) <sup>(+2)</sup> ] <sup>10+</sup>	-	937.1917	937.1917	0.00	20	3.82E4
	[y75 <sub>+8H</sub> ] <sup>8+</sup>	-	1055.0786	1055.0807	-1.99	20	8.78E5
	[Ub <sub>+7H</sub> + Ir(Cp) <sup>(+2)</sup> ] <sup>9+</sup>	-	988.7495	988.7510	-1.52	20	3.82E5
	[b16 <sub>+H</sub> + Ir(Cp*) <sup>(+2)</sup> ] <sup>3+</sup>	S <sub>Met1</sub>	711.0341	711.0347	-0.84	20	2.01E4
	[b17 <sub>+H</sub> + Ir(Cp*) <sup>(+2)</sup> ] <sup>3+</sup>	S <sub>Met1</sub>	744.0568	744.0573	-0.67	20	2.58E4
	[b18 <sub>+H</sub> + Ir(Cp*) <sup>(+2)</sup> ] <sup>3+</sup>	S <sub>Met1</sub>	787.0714	787.0718	-0.51	20	3.71E4

\* (+ Ac + heme)

### Compound 1:

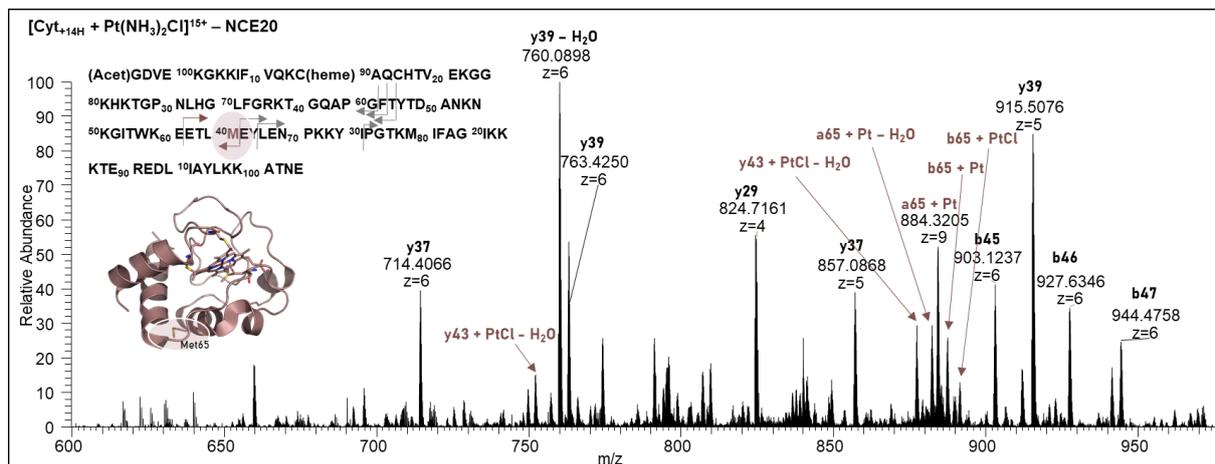


Figure S7. HCD fragmentation spectrum of [Cyt + Pt(NH<sub>3</sub>)<sub>2</sub>Cl] at NCE 20 with corresponding protein sequence and 3D structure of the protein (platination sites highlighted; metalated fragments are indicated in red and non-metalated fragments in grey).

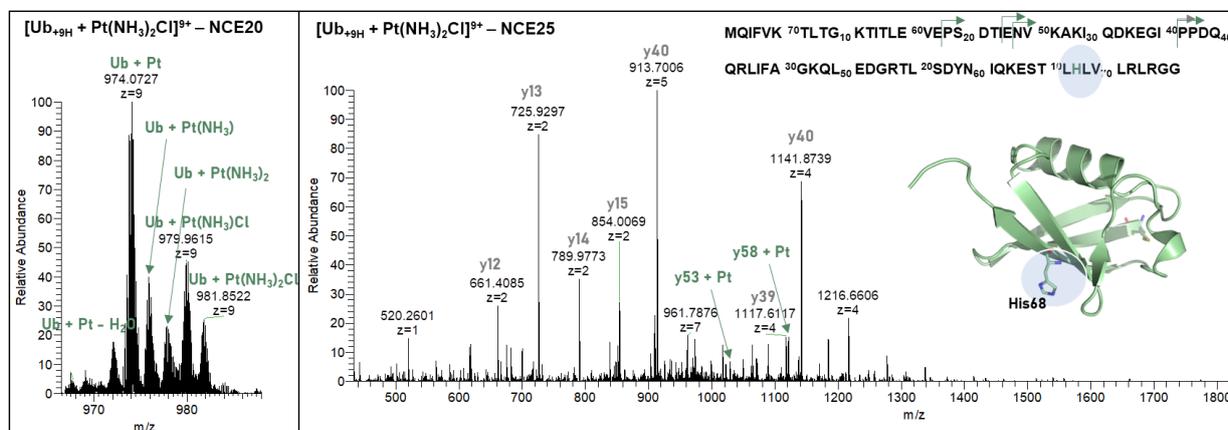


Figure S8. Left: HCD fragmentation spectrum of [Ub + Pt(NH<sub>3</sub>)<sub>2</sub>Cl] at NCE 20. Right: HCD fragmentation spectrum of [Ub + Pt(NH<sub>3</sub>)<sub>2</sub>Cl] at NCE 25 with corresponding protein sequence and 3D structure (platination sites highlighted; metalated fragments are indicated in green and non-metalated fragments in grey).

### Compound 4:

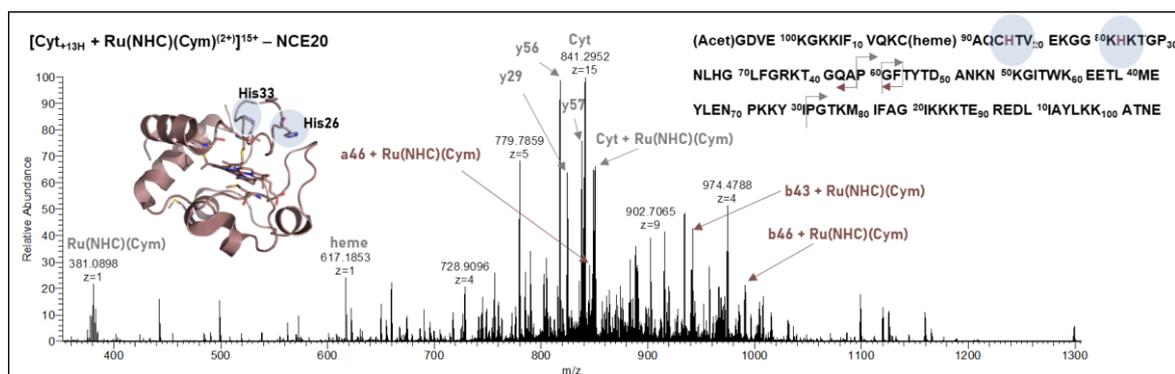


Figure S9: HCD fragmentation spectrum of [Cyt + Ru(NHC)(Cym)] at NCE 20 with corresponding protein sequence and 3D structure (platination sites highlighted; metalated fragments are indicated in red and non-metalated fragments in grey).

### Compound 6:

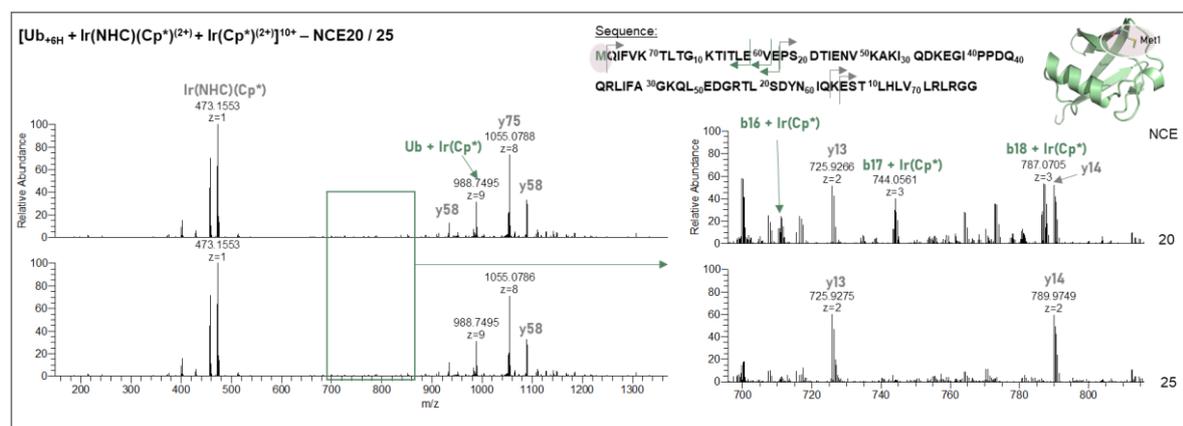


Figure S10: Left: HCD fragmentation spectra of [Ub + Ir(NHC)(Cp\*) + Ir(Cp\*)] at NCE 20 and 25. Right: Zoom into fragment spectra to show low abundant metalated fragments. Top: Protein Sequence with metalated fragments indicated in green and non-metalated fragments in grey; primary binding site for Ir(Cp\*) highlighted.

## Compounds without metalated fragments in MS/MS Spectra (3, 5, and 6)

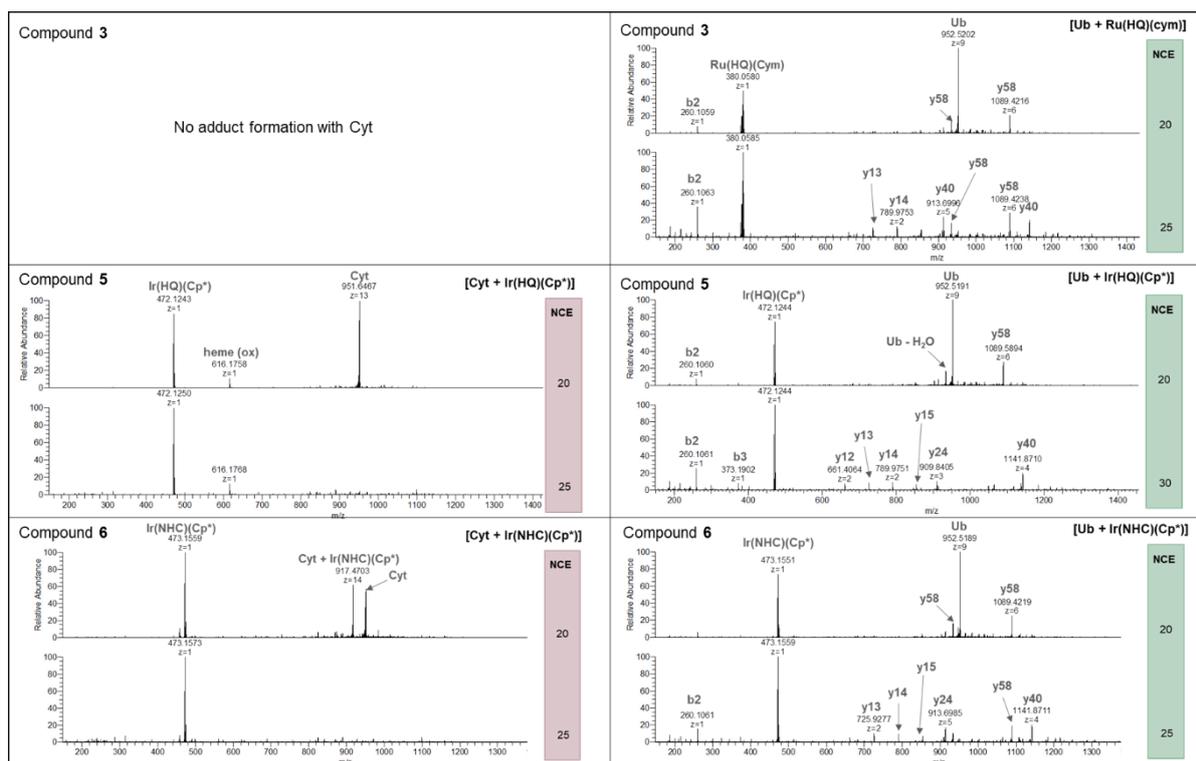


Figure S11. Left: HCD fragmentation spectra of [Cyt + 5 and 6] at NCE 20 and 25. Right: HCD fragmentation spectra of [Ub + 3, 5, and 6] at NCE 20 and 25 or 30.

## 5) Nucleophile exchange of compound 2 and 6:

Table S6. Adduct cleavage of compound 2 and 6 with Cyt and Ub after 0, 2, and 24 h nucleophile exposure with m/z values, mass errors, RT and signal intensity (Cyt – red, Ub – green).

Complex	Nuc exp. /h	Adduct	m/Z <sub>exp</sub> /-	m/Z <sub>theo</sub> /-	Error /ppm	Intensity /-
2 Pt(ala)(ASA-But)Cl	0	[Cyt*]	12358.3392	12358.3334	0.47	2.59E6
		[Cyt* <sub>-H</sub> + PtCl(1 <sup>+</sup> )]	12588.2691	12588.2595	0.76	3.35E5
		[Cyt* + Pt(ala)Cl]	12677.3109	12677.3031	0.62	1.85E6
		[Cyt* + 2Pt(ala)Cl]	12995.2824	12995.2767	0.47	1.54E6
		[Cyt* + 3Pt(ala)Cl]	13314.2585	13314.2566	0.14	1.90E5
	2	[Cyt*]	12358.3461	12358.3329	1.07	2.89E5
		[Cyt* <sub>-H</sub> + PtCl(1 <sup>+</sup> )]	-	12588.2595	-	-
		[Cyt* + Pt(ala)Cl]	12677.3131	12677.3026	0.83	1.09E5
		[Cyt* + 2Pt(ala)Cl]	-	12995.2767	-	-
	24	[Cyt* + 3Pt(ala)Cl]	-	13314.2566	-	-
		[Cyt*]	12358.3546	12358.3329	1.76	2.01E5
		[Cyt* <sub>-H</sub> + PtCl(1 <sup>+</sup> )]	-	12588.2595	-	-
[Cyt* + Pt(ala)Cl]		12677.3209	12677.3026	1.44	3.29E4	
6 Ir(NHC)(Cp*)Cl <sub>2</sub>	0	[Cyt*]	12358.3342	12358.3329	0.11	4.97E6
		[Cyt* <sub>-2H</sub> + Ir(NHC)(Cp*) <sup>(2+)</sup> ]	12830.4872	12830.4855	0.13	7.05E6
		[Cyt* <sub>-4H</sub> + 2Ir(NHC)(Cp*) <sup>(2+)</sup> ]	13301.6424	13301.6296	0.96	2.26E5
	2	[Cyt*]	12358.3272	12358.3385	-0.91	5.65E5
		[Cyt* <sub>-2H</sub> + Ir(NHC)(Cp*) <sup>(2+)</sup> ]	12830.4808	12830.4855	-0.37	7.02E5
		[Cyt* <sub>-4H</sub> + 2Ir(NHC)(Cp*) <sup>(2+)</sup> ]	-	13301.6360	-	-

	24	<b>[Cyt*]</b> [Cyt* <sub>-2H</sub> + Ir(NHC)(Cp*) <sup>(2+)</sup> ] [Cyt* <sub>-4H</sub> + 2Ir(NHC)(Cp*) <sup>(2+)</sup> ]	<b>12358.3390</b> 12830.4919 -	<b>12358.3329</b> 12830.4855 13301.6360	<b>0.04</b> 0.50 -	<b>6.02E5</b> 3.77E5 -
<b>2</b> Pt(ala)(ASA- But)Cl	0	<b>[Ub]</b> [Ub <sub>-H</sub> + Pt(ala) <sup>(1+)</sup> ] [Ub + Pt(ala)Cl]	<b>8564.6244</b> 8846.6232 8882.6004	<b>8564.6308</b> 8846.6265 8882.6016	<b>-0.75</b> -0.37 -0.14	<b>1.56E7</b> 5.86E6 8.52E5
	2	<b>[Ub]</b> [Ub <sub>-H</sub> + Pt(ala) <sup>(1+)</sup> ] [Ub + Pt(ala)Cl]	<b>8564.6250</b> 8846.6254 -	<b>8564.6302</b> 8846.6212 889.2679	<b>-0.61</b> 0.47 -	<b>2.02E6</b> 5.57E5 -
	24	<b>[Ub]</b> [Ub <sub>-H</sub> + Pt(ala) <sup>(1+)</sup> ] [Ub + Pt(ala)Cl]	<b>8564.6281</b> 8846.6281 -	<b>8564.6302</b> 8846.6212 8882.6010	<b>-0.25</b> 0.78 -	<b>2.12E6</b> 5.52E5 -
<b>6</b> Ir(NHC)(Cp*)Cl <sub>2</sub>	0	<b>[Ub]</b> [Ub <sub>-2H</sub> + Ir(Cp*) <sup>(2+)</sup> ] [Ub <sub>-2H</sub> + Ir(NHC)(Cp*) <sup>(2+)</sup> ] [Ub <sub>-4H</sub> + Ir(NHC)(Cp*) <sup>(2+)</sup> + Ir(Cp*) <sup>(2+)</sup> ] [Ub <sub>-4H</sub> + 2Ir(NHC)(Cp*) <sup>(2+)</sup> ]	8889.6932 9035.7822 9361.8457 9506.9283	8889.6931 9035.7772 9361.8378 9506.9198	0.01 0.55 0.84 0.89	4.30E6 1.10E6 5.55E6 2.01E6
	2	<b>[Ub]</b> [Ub <sub>-2H</sub> + Ir(Cp*) <sup>(2+)</sup> ] [Ub <sub>-2H</sub> + Ir(NHC)(Cp*) <sup>(2+)</sup> ] [Ub <sub>-4H</sub> + Ir(NHC)(Cp*) <sup>(2+)</sup> + Ir(Cp*) <sup>(2+)</sup> ] [Ub <sub>-4H</sub> + 2Ir(NHC)(Cp*) <sup>(2+)</sup> ]	<b>8564.6205</b> 8889.6819 9035.7721 9361.8361 9507.9201	<b>8564.6250</b> 8889.6931 9035.7810 9361.8438 9507.9274	<b>-0.53</b> -1.26 -0.98 -0.82 -0.77	<b>6.10E5</b> 2.55E5 9.75E5 2.97E5 3.50E5
	24	<b>[Ub]</b> [Ub <sub>-2H</sub> + Ir(Cp*) <sup>(2+)</sup> ] [Ub <sub>-2H</sub> + Ir(NHC)(Cp*) <sup>(2+)</sup> ] [Ub <sub>-4H</sub> + Ir(NHC)(Cp*) <sup>(2+)</sup> + Ir(Cp*) <sup>(2+)</sup> ] [Ub <sub>-4H</sub> + 2Ir(NHC)(Cp*) <sup>(2+)</sup> ]	<b>8564.6261</b> 8889.6943 9035.7781 9361.8438 9507.9314	<b>8564.6250</b> 8889.6931 9035.7810 9361.8438 9507.9274	<b>0.13</b> 0.13 -0.32 0.00 0.42	<b>1.40E6</b> 4.29E5 8.39E5 1.24E5 9.91E4

\* (+ Ac + heme)

## 6) Nucleophile adducts of compounds 1 – 6:

Table S7. Identified nucleophile adducts with the compounds (m/z -values, mass errors, RT and signal intensity.)

Complex	Adduct	m/z <sub>exp</sub> /-	m/z <sub>theo</sub> /-	Error /ppm	RT /min	Intensity /-
<b>3</b> Ru(HQ)(cym)Cl	[Ru <sup>II</sup> (HQ)(cym)] <sup>+</sup>	380.0576	380.0588	-3.16	0.24	1.80E7
	[GS <sup>(-1)</sup> <sub>+H</sub> + Ru <sup>II</sup> (HQ)(cym) <sup>(+1)</sup> + 2O] <sup>+</sup>	719.1308	719.1319	-1.53	0.21	7.11E4
<b>4</b> Ru(NHC)(cym)Cl <sub>2</sub>	[Ru <sup>II</sup> (NHC)(cym) <sup>(+2)</sup> - H] <sup>+</sup>	381.0890	381.0905	-3.94	0.11	2.27E6
	[GS <sup>(-1)</sup> <sub>+H</sub> + Ru <sup>II</sup> (NHC)(cym) <sup>(+2)</sup> ] <sup>+</sup>	688.1725	688.1748	-3.34	0.20	2.00E4
<b>5</b> Ir(HQ)(Cp*)Cl	[Ir <sup>III</sup> (HQ)(Cp*)] <sup>+</sup>	472.1242	472.1247	-1.06	0.29	5.93E7
	[GS <sup>(-1)</sup> + Ir <sup>III</sup> (Cp*) <sup>(+2)</sup> ] <sup>+</sup>	634.1552	634.1557	-1.58	0.09	2.34E6
	[2(GS <sup>(-1)</sup> + Ir <sup>III</sup> (Cp*) <sup>(+2)</sup> )] <sup>2+</sup>	634.1576	634.1557	3.00	0.65	1.10E5
<b>6</b> Ir(NHC)(Cp*)Cl <sub>2</sub>	[Ir <sup>III</sup> (NHC)(Cp*) <sup>(+2)</sup> <sub>+H</sub> ] <sup>+</sup>	473.1567	473.1565	0.42	3.08	8.14E5
	[GS <sup>(-1)</sup> <sub>+H</sub> + Ir <sup>III</sup> (NHC)(Cp*) <sup>(+2)</sup> ] <sup>2+</sup>	390.6230	390.6237	-1.79	0.22	1.23E8
	[GS <sup>(-1)</sup> + Ir <sup>III</sup> (NHC)(Cp*) <sup>(+2)</sup> ] <sup>+</sup>	780.2386	780.2401	-1.92	0.22	4.38E7
	[GS <sup>(-1)</sup> + Ir <sup>III</sup> (NHC)(Cp*) <sup>(+2)</sup> ] <sup>+Na</sup>	802.2206	802.2220	-1.75	0.22	1.92E7
	[GS <sup>(-1)</sup> + Ir <sup>III</sup> (NHC)(Cp*) <sup>(+2)</sup> ] <sup>+2Na</sup>	824.2025	824.2041	-1.94	0.22	3.17E6
	[GS <sup>(-1)</sup> <sub>-2H</sub> + 2Ir <sup>III</sup> (NHC)(Cp*) <sup>(+2)</sup> ] <sup>+</sup>	1252.3907	1252.3895	0.96	1.28	9.15E4
	[GS <sup>(-1)</sup> <sub>-H</sub> + 2Ir <sup>III</sup> (NHC)(Cp*) <sup>(+2)</sup> ] <sup>2+</sup>	625.6977	625.6970	1.12	1.28	3.52E6
	[GS <sup>(-1)</sup> <sub>-H</sub> + 2Ir <sup>III</sup> (NHC)(Cp*) <sup>(+2)</sup> ] <sup>2+Na</sup>	636.6881	636.6881	0.00	1.28	8.46E5
[Met <sup>(-1)</sup> <sub>-H</sub> + Ir(NHC)(Cp*) <sup>(+2)</sup> ] <sup>+</sup>	622.2065	622.2073	-1.29	0.11	2.56E7	

**Ru compounds (3 + 4):**

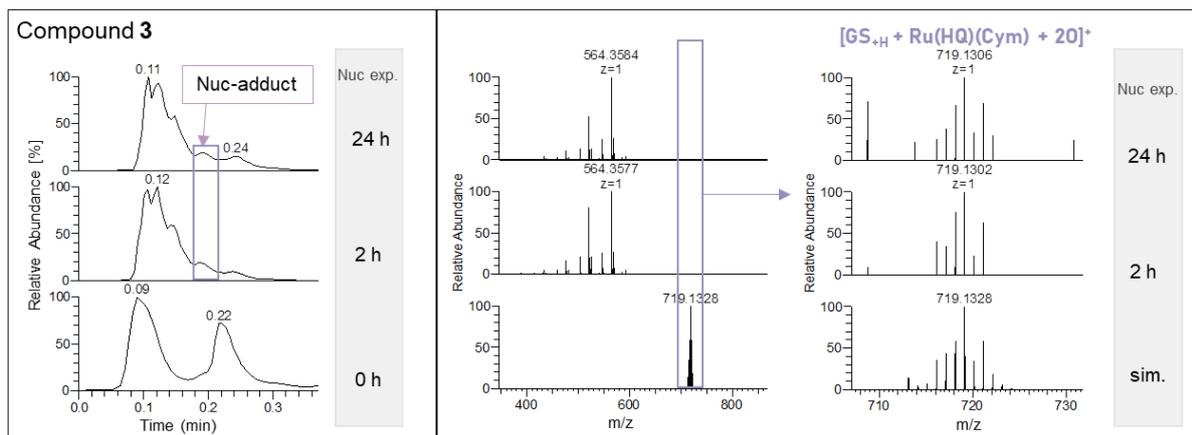


Figure S12. Nucleophile adduct with compound **3**. Left: TIC of the incubation mixture with compound **3** after 0, 2, and 24 h nucleophile exposure. Right: mass spectrum at RT = 0.19 min (nucleophile adduct elution) and comparison of experimental and simulated isotopic pattern.

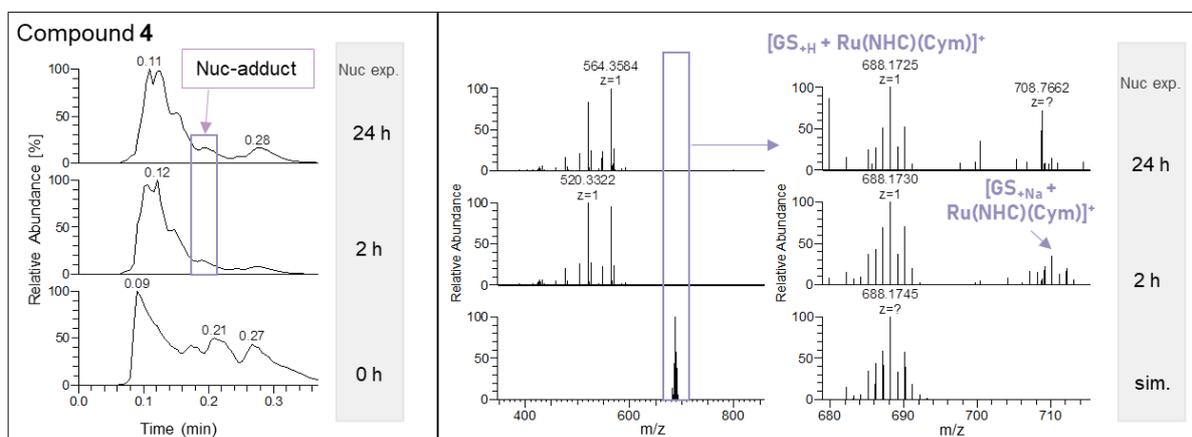


Figure S13. Nucleophile adduct with compound **4**. Left: TIC of the incubation mixture with compound **4** after 0, 2, and 24 h nucleophile exposure. Right: mass spectrum at RT = 0.19 min (nucleophile adduct elution) and comparison of experimental and simulated isotopic pattern.

*Ir Compound (5):*

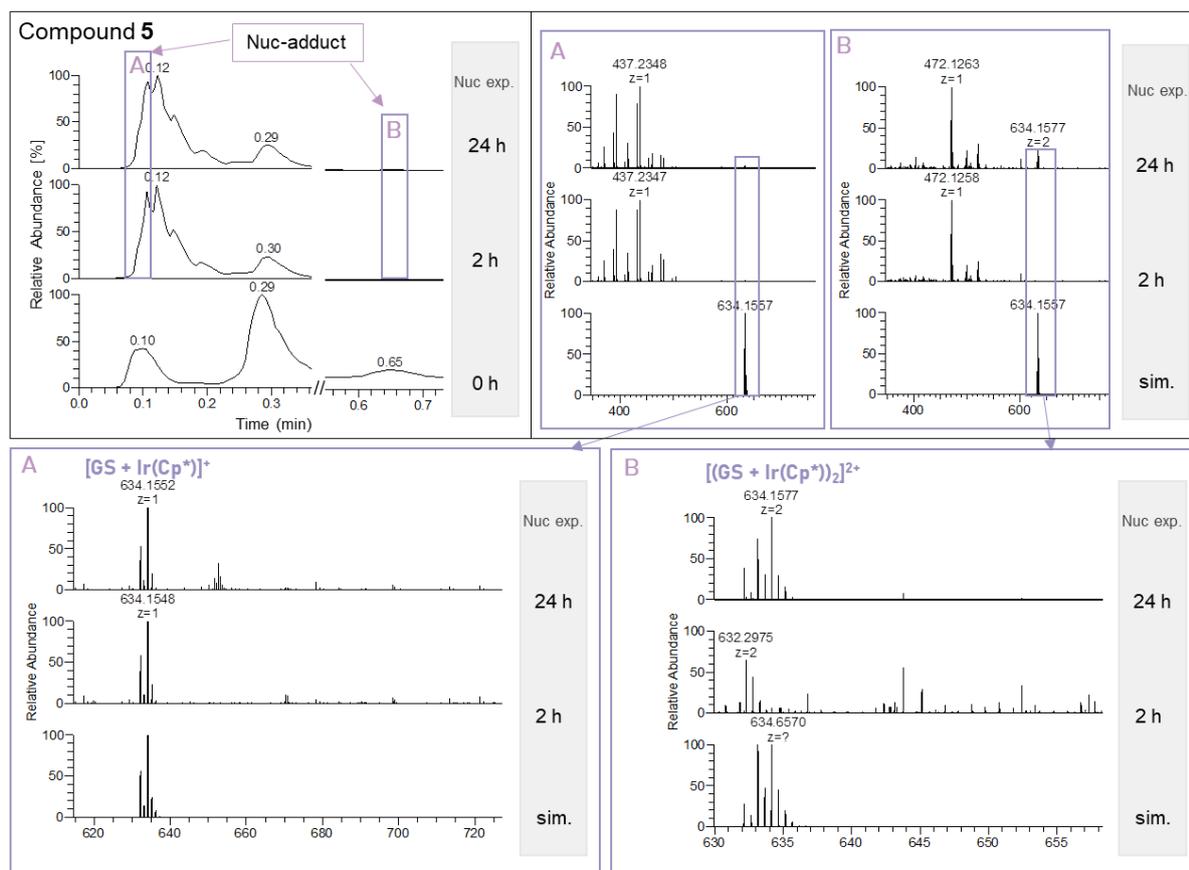


Figure S14. Nucleophile adduct with compound **5**. Top, left: TIC of the incubation mixture with compound **5** after 0, 2, and 24 h nucleophile exposure. Top, right: mass spectra at RT = 0.09 min and 0.65 min (nucleophile adduct elution). Bottom: comparison of experimental and simulated isotopic patterns.

## 6) Experiments in buffered solution

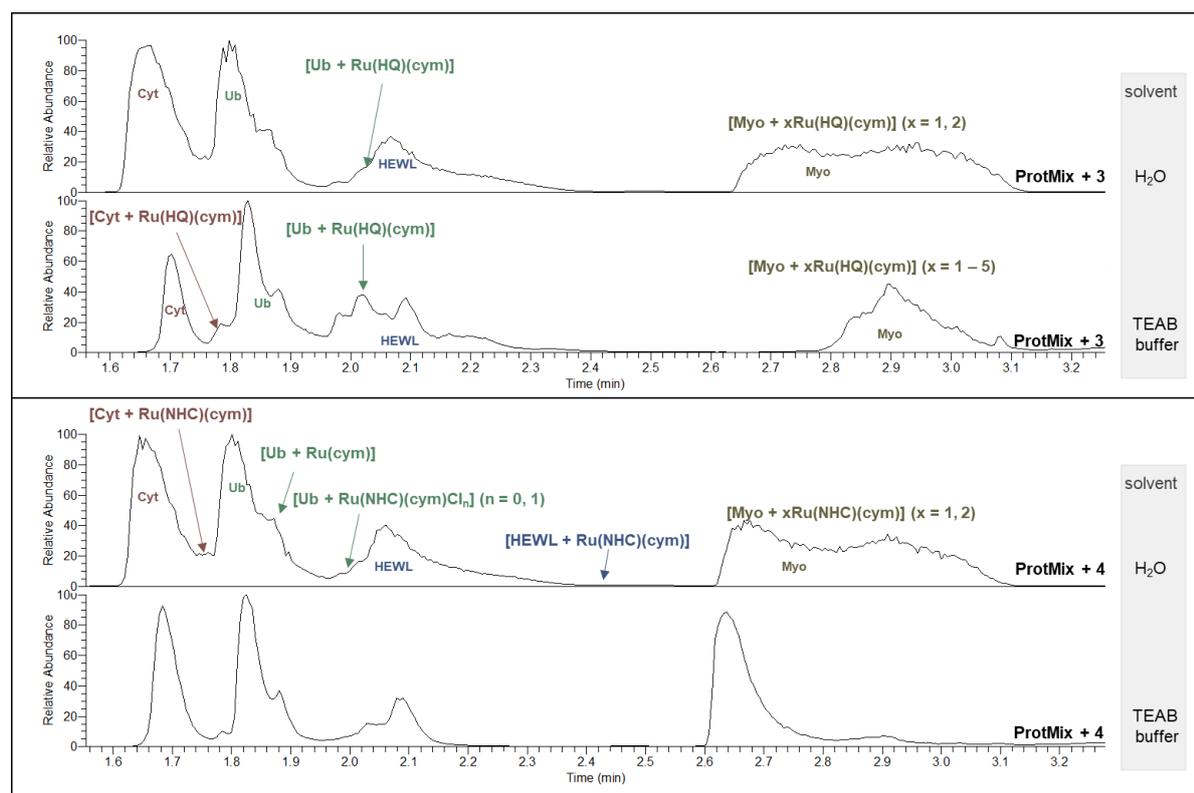


Figure S15. TICs after 24 h incubation of the protein mixtures with **3** – [Ru<sup>II</sup>(HQ)(cym)Cl] and **4** – [Ru<sup>II</sup>(NHC)(cym)Cl<sub>2</sub>] in aqueous solution and 20 mM tetraethylammonium bicarbonate.

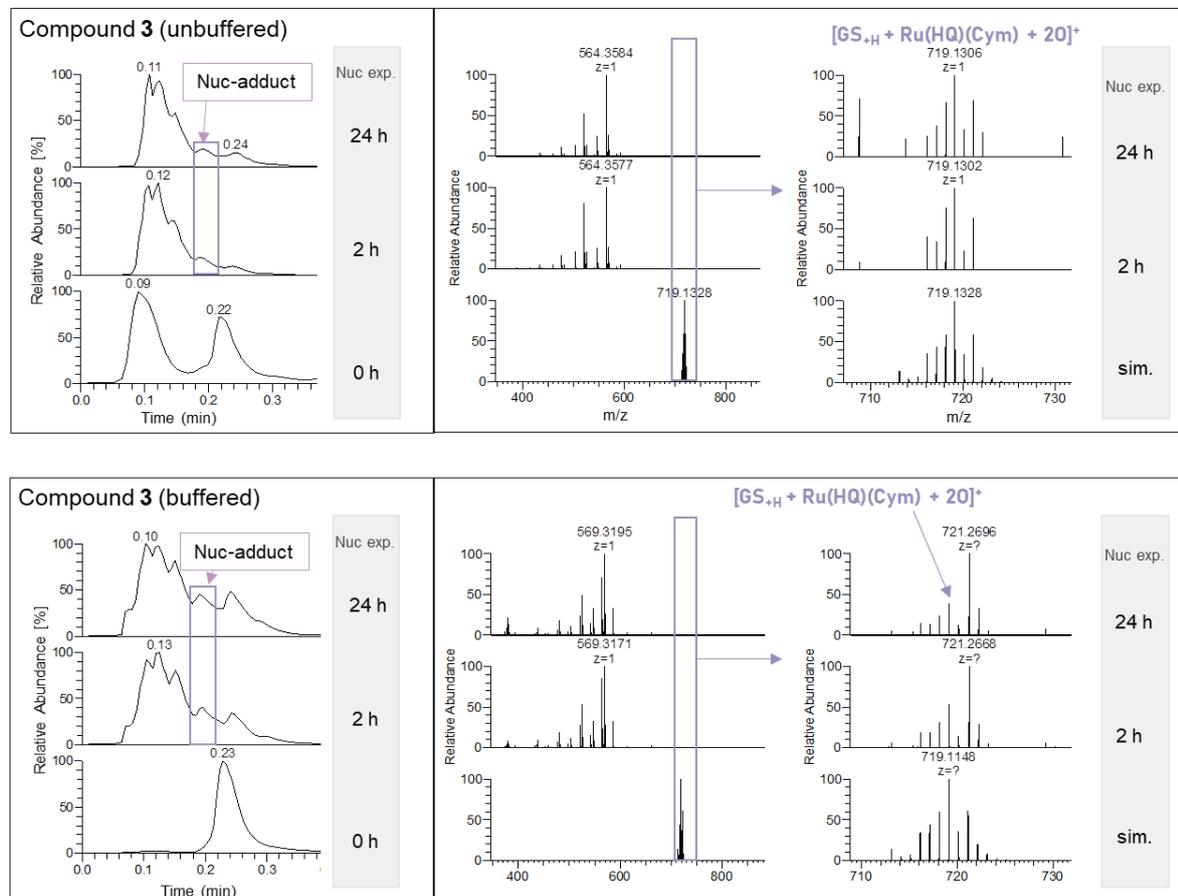


Figure S16. Comparison of the nucleophile adduct with compound **3** formed in aqueous solution (top) and 20 mM tetraethylammonium bicarbonate (bottom).