Supporting Information for

Organometallic Ruthenium Anticancer Complexes Inhibit the Human Peroxiredoxin I Activity by Binding to and Inducing Oxidation of Its Catalytic Cysteine Residue

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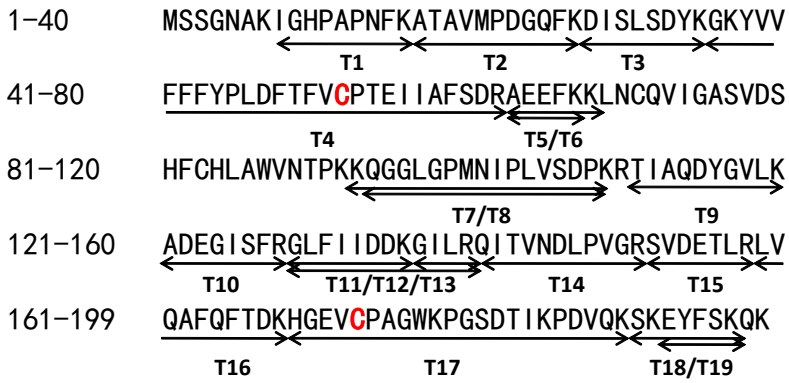

Scheme S1. Schematic representation of identified peptides by HPLC-ESI-MS from the tryptic digestion of Prx-I. The sequence coverage of the identified peptides is 82.4%. The catalytic sites Cys52 and Cys173 are in bold and red.

Table S1. Peptides identified in mass spectrometry arising from tryptic digestion of recombinant Prx-I.

Peptides	(position)sequence	charge	*m/z*	*m/z*	δ^b/ppm
T1	(8-16)IGHPAPNFK	1+	327.515	327.515	-0.31
		2+	490.769	490.771	3.06
		3+	980.531	980.545	13.97
T2	(17-27)ATAVMPDGQFK	1+	1164.571	1164.557	-12.88
		2+	582.791	582.783	-11.84
T3	(28-35)DISLSDYK	1+	940.462	940.452	-10.74
		2+	470.735	470.740	11.68
T4	(36-62)GKYVVFFFYPLDFTFVCPTEIIAFSDR	2+	1612.309	1612.318	5.46
		3+	1074.874	1074.893	17.11
T5	(63-67)AEEFK	1+	623.304	623.312	14.28
		2+	312.156	312.157	1.92
T6	(63-68)AEEFKK	1+	751.399	751.384	-19.43
		2+	376.203	376.197	-2.39
T7	(93-109)KQGGLGPMNIPLVSDPK	2+	875.980	875.972	-8.56
		3+	584.322	584.319	-5.31
T8	(94-109)QGGLGPMNIPLVSDPK	2+	811.932	811.927	-6.90
		3+	541.624	541.621	-5.72
T9	(111-120)TIAQDYGVLK	1+	1107.605	1107.596	-7.58
		2+	554.306	554.306	-0.36
T10	(121-128)ADEGISFR	1+	894.432	894.426	-6.26
		2+	447.719	477.728	14.23
T11	(129-136)GLFIIDDK	1+	920.509	920.504	-5.21
		2+	460.758	460.759	1.95
T12	(129-140)GLFIIDDKGILR	1+	1359.799	1359.778	-16.11
		2+	680.403	680.397	-9.11
		3+	453.938	453.935	-5.95
T13	(137-140)GILR	1+	458.309	458.301	-17.02
T14	(141-151)QITVNDLPVGR	1+	1211.674	1211.684	8.17
		2+	606.341	606.346	7.92
		3+	404.563	404.560	8.40
T15	(152-158)SVDETLR	1+	819.421	819.405	-18.79
		2+	410.214	410.206	-19.99
T16	(159-168)LVQAFQFTDK	1+	1196.631	1196.646	12.20
		2+	598.819	598.828	13.86
T17	(169-190)HGEVCPAGWKPGSDTIKPDVQK	2+	1175.588	1175.580	-6.90
		3+	784.061	784.057	-5.36
		4+	588.298	588.295	-4.59
T18	(191-197)SKEYFSK	1+	888.446	888.435	-12.38
		2+	444.727	444.716	-24.73
		3+	296.823	296.821	-3.37
T19	(193-197)EYFSK	1+	673.319	673.309	14.54
		2+	337.163	337.157	18.09

a. The theoretical (theor.) and observed (obs.) mass-to-charge ratio of the most abundant isotopomers.

b. $\delta = [(m/z)_{obs} - (m/z)_{theor}]/(m/z)_{theor.}$

Table S2. The minimized energies of different entities calculated by Sybyl X 1.1.

Entity	Prx-I	**1**	**2**	**3**
Minimized energy (kcal mol^{-1})	−2769.7	675.5	656.6	664.4
Entity		Prx-I + **1**	Prx-I + **2**	Prx-I + **3**
Minimized energy (kcal mol^{-1})		−2576.2	−2434.8	−2472.6
Binding energy (kcal mol^{-1})		−482.0	−321.7	−367.3

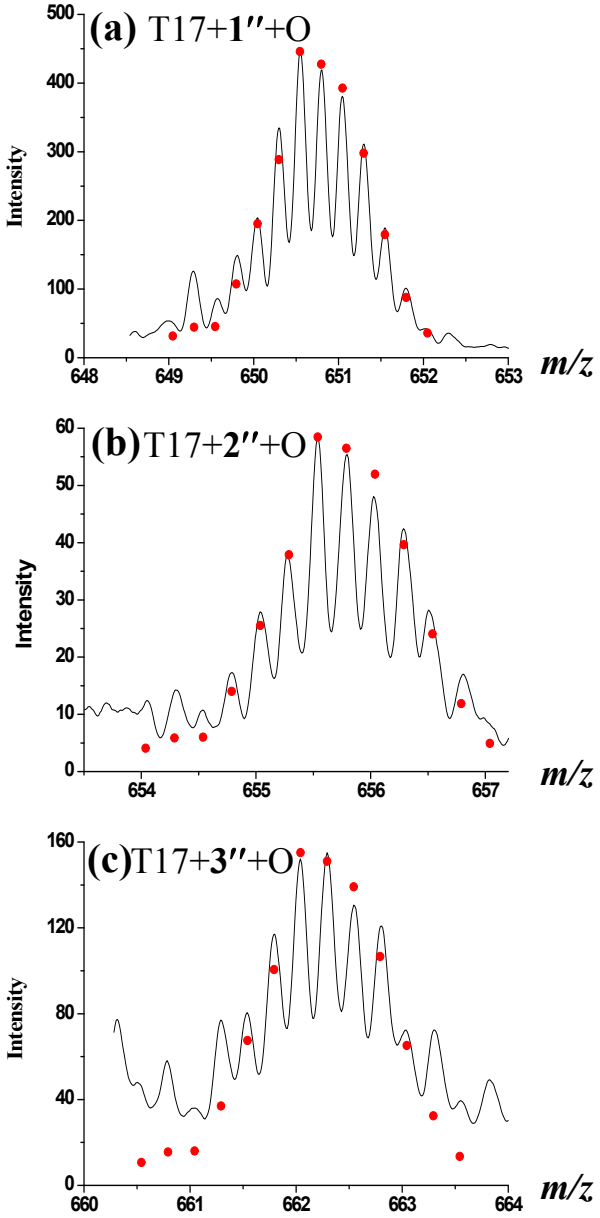

Figure S1. Mass spectra (lines) and isotopic models (red dots) for ruthenated peptides T17 arising from tryptic digestion of ruthenated Prx-I complexes, Prx-I + **1**, Prx-I + **2** and Prx-I + **3**, respectively. T17 = (aa169-aa190) HGEVCPAGWKPGSDTIKPDVQK; **1″** = $\{(\eta^6\text{-}p\text{-cym})Ru\}^{2+}$; **2″** = $\{(\eta^6\text{-bip})Ru\}^{2+}$; **3″** = $\{(\eta^6\text{-dhpa})Ru\}^{2+}$. The red dots correspond to the simulated isotopic peaks of ruthenated T17, respectively.

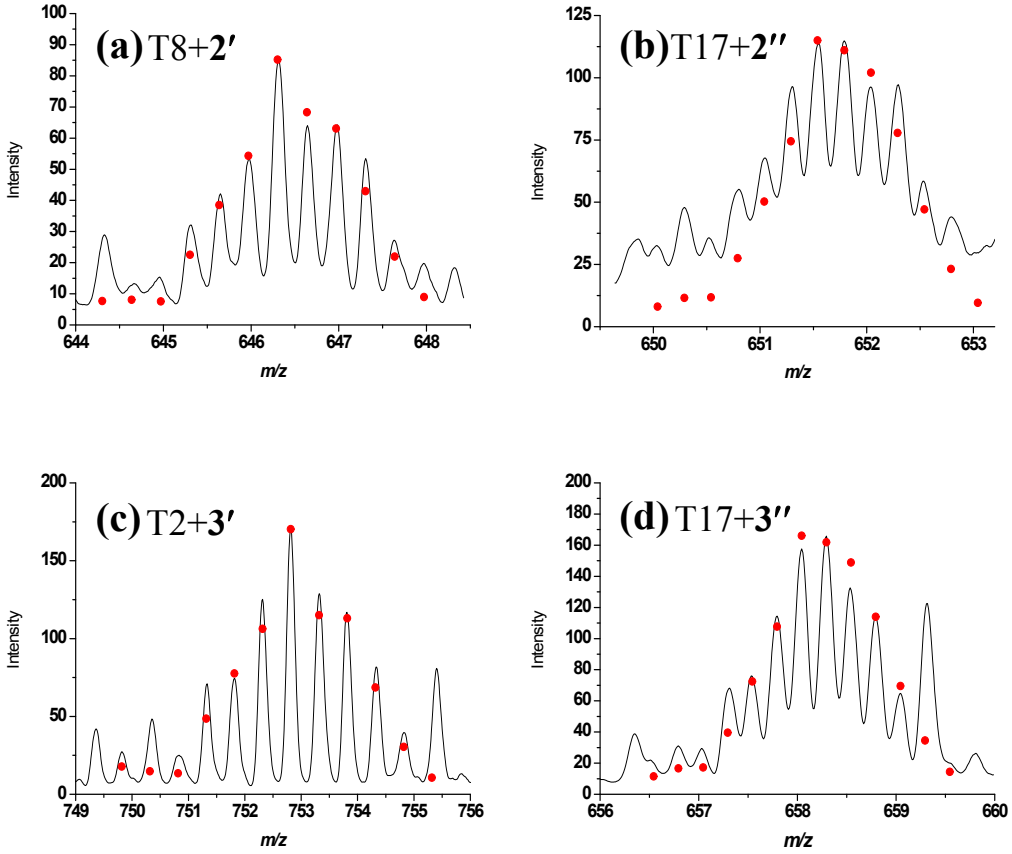

Figure S2. Mass spectra (lines) and isotopic models (red dots) for ruthenated peptides arising from tryptic digestion of ruthenated Prx-I complexes Prx-I + **2** and Prx-I + **3**, respectively. T2 = (aa17-aa27) ATAVMPDGQFK; T8 = (aa94-aa109) QGGLGPMNIPLVSDPK; T17 = (aa169-aa190) HGEVCPAGWKPGSDTIKPDVQK; **2′** = $\{(\eta^6\text{-bip})\text{Ru(en)}\}^{2+}$; **3′** = $\{(\eta^6\text{-dhpa})\text{Ru(en)}\}^{2+}$; **2″** = $\{(\eta^6\text{-bip})\text{Ru}\}^{2+}$; **3″** = $\{(\eta^6\text{-dhpa})\text{Ru}\}^{2+}$. The red dots correspond to the simulated isotopic peaks of ruthenated peptides, respectively.

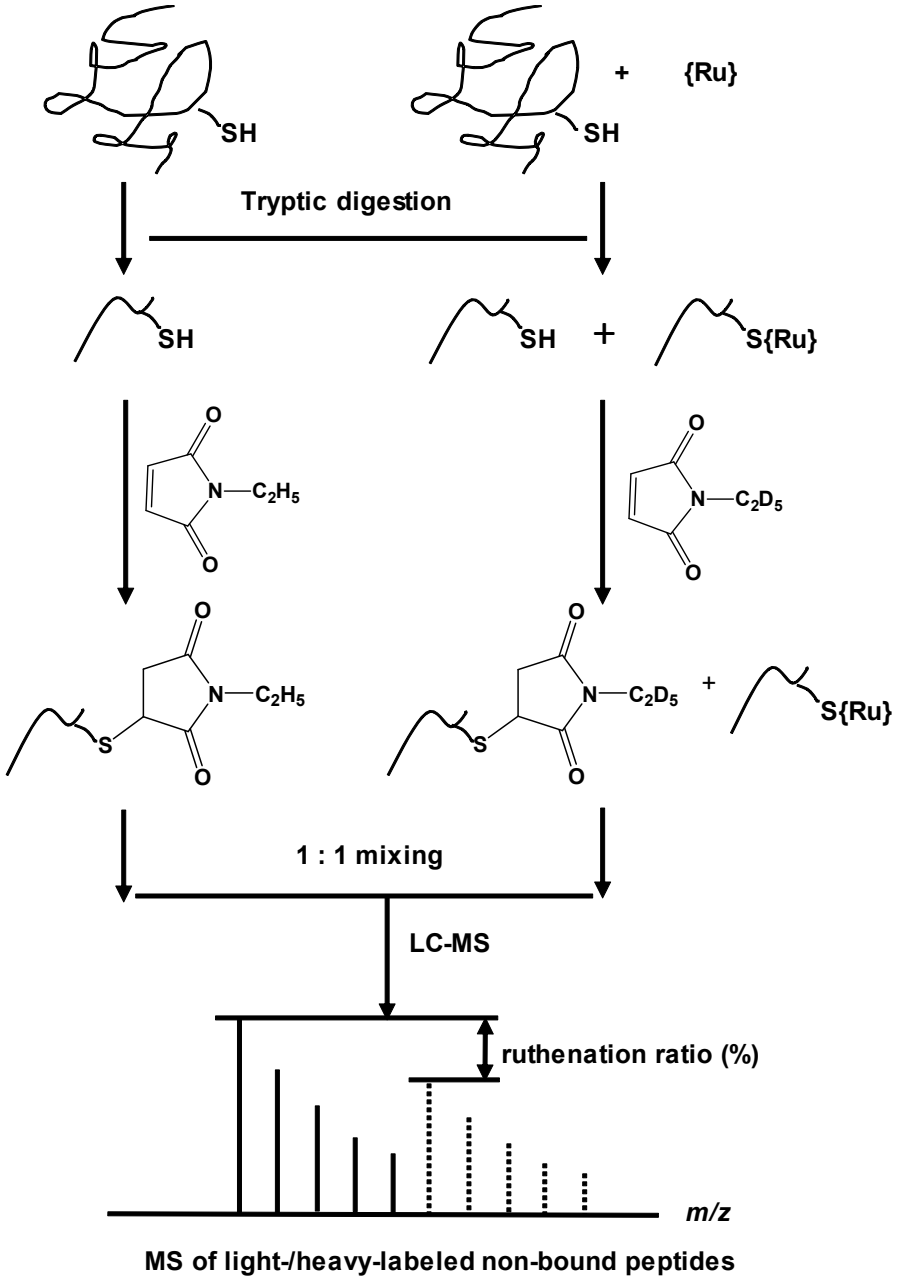

Figure S3. Diagram for the measurement of binding stoichiometry of the ruthenium complexes to a cysteine thiol of protein. {Ru}= $[(\eta^6\text{-arene})Ru(en)]^{2+}$ or $[(\eta^6\text{-arene})Ru]^{2+}$.

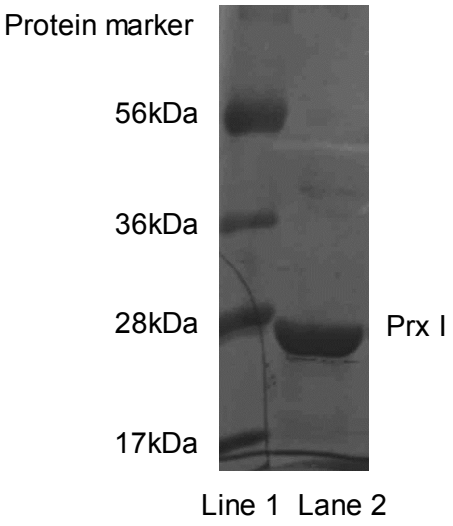

Figure S4. SDS-PAGE of recombinant Prx-I eluted by buffer C (Lane 2), indicating the purity of recombinant Prx-I is about 95%.