

Supplementary information:

High sequence-coverage detection of proteolytic peptides using bis(terpyridine)ruthenium(II) complex

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Table S1. Predicted tryptic fragment ions of myoglobin and their mass number. The shading characters indicate basic amino acid residues. The underline indicates acidic amino acid residues. K* in the amino acid sequences represents a homoarginine residue. The pI values were estimated by pI/MW prediction tool in Proteome of *Xylella fastidiosa*.^a The all mass numbers are monoisotopic mass.

	Sequence	pI	M	<Ru>-CO-labeled
f ₁	Y <u>LE</u> FISD <u>AI</u> IHV <u>L</u> HSK*	5.99	1927.2	2596.9
f ₂	GLSD <u>G</u> E <u>W</u> QQV <u>L</u> NVWGK*	4.37	1858.0	2527.7
f ₃	V <u>E</u> AD <u>I</u> AGHGQ <u>E</u> VLIR	4.65	1606.8	2260.5
f ₄	H <u>P</u> GNFGAD <u>A</u> QGAMTK*	6.74	1543.7	2213.4
f ₅	H <u>G</u> TVVLTALGGILK*	8.76	1404.7	2074.4
f ₆	LFTGHP <u>E</u> TL <u>E</u> K*	5.40	1313.5	1983.2
f ₇	PLAQSHATK*	9.18	994.10	1663.8
f ₈	GHHE <u>A</u> ELK*	6.00	962.02	1631.7
f ₉	TE <u>A</u> EMK*	4.53	749.83	1419.5
f ₁₀	AL <u>E</u> LFR	6.05	747.88	1417.6
f ₁₁	AS <u>E</u> DLK*	4.37	703.74	1373.4
f ₁₂	ND <u>I</u> AAK*	5.84	672.73	1342.4
f ₁₃	<u>E</u> LG <u>F</u> QG	4.00	649.69	1319.4
f ₁₄	I <u>P</u> IK*	8.75	511.66	1181.4
f ₁₅	F <u>D</u> IK*	5.84	450.49	1120.2
f ₁₆	H <u>L</u> IK*	8.76	438.52	1108.2
f ₁₇	Y <u>K</u> *	8.59	351.40	1021.1
f ₁₈	F <u>K</u> *	8.75	335.40	1005.1
f ₁₉	H <u>K</u> *	8.76	325.40	995.06
f ₂₀	K*	8.75	188.22	857.91

^a<http://proteome.ibi.unicamp.br/tools/pimw/index.htm>

Table S2. Observed tryptic fragment ions of myoglobin in Figure 9a.

Monoisotopic mass number of observed ions (m/z)	Calcd. mass number	Fragment ion
1965.9	1966.2	$f_1 + K^+$
1858.8	1859.0	$f_2 + H^+$
1747.7	1747.0	$f_1 - Y - OH$
1607.4	1607.8	$f_3 + H^+$
1545.3	1544.7	$f_4 + H^+$
1405.5	1405.7	$f_5 + H^+$
1314.9	1314.5	$f_6 + H^+$
1170.7	1170.3	$f_7 + H + K^+$
985.33	985.02	$f_8 + Na^+$
771.34	770.88	$f_{10} + Na^+$
705.62	704.74	$f_{11} + H^+$
439.71	439.52	$f_{16} + H^+$
357.11	358.40	$f_{18} + Na^+$
348.92	348.40	$f_{19} + Na^+$

Table S3. Observed ^{Ru}-CO-labeled fragment ions of tryptic myoglobin in Figure 10. Superscription “Ru” indicates ^{Ru}-CO-labeled fragment. K* in the fragment ions represents a homoarginine residue.

Monoisotopic mass number of observed ions (<i>m/z</i>)	Calcd. mass number	Fragment ion
2597.2	2596.9	f ₁ ^{Ru}
2527.6	2527.7	f ₂ ^{Ru}
2427.4	2426.7	f ₁ ^{Ru} – K*
2259.9	2260.5	f ₃ ^{Ru}
2213.8	2213.4	f ₄ ^{Ru}
2104.9	2104.3	F ₃ ^{Ru} – R
2074.2	2074.4	f ₅ ^{Ru}
1904.1	1904.3	f ₅ ^{Ru} – K*
1812.4	1813.0	f ₆ ^{Ru} – K*
1664.6	1663.8	f ₇ ^{Ru}
1632.5	1631.7	f ₈ ^{Ru}
1493.7	1493.6	f ₇ ^{Ru} – K*
1419.3	1419.5	f ₉ ^{Ru}
1373.8	1373.4	f ₁₁ ^{Ru}
1341.8	1342.4	f ₁₂ ^{Ru}
1319.7	1319.4	f ₁₃ ^{Ru}
1260.3	1260.4	f ₁₀ ^{Ru} – R
1203.3	1203.2	f ₁₁ ^{Ru} – K*
1181.6	1181.4	f ₁₄ ^{Ru}
1120.5	1120.2	f ₁₅ ^{Ru}
1108.0	1108.2	f ₁₆ ^{Ru}
1022.0	1021.1	f ₁₇ ^{Ru}
995.08	995.06	f ₁₉ ^{Ru}
950.37	950.00	f ₁₅ ^{Ru} – K*
857.91	857.91	f ₂₀ ^{Ru}
834.50	834.90	f ₁₈ ^{Ru} – K*

Table S4. Predicted tryptic fragment ions of lysozyme and ubiquitin with their mass number. The shading characters indicate basic amino acid residues. The underline indicates acidic amino acid residues. K* in the amino acid sequences represents a homoarginine residue. The all mass numbers are monoisotopic mass.

Lysozyme Sequence	M
f _{L1R} ^u NLCNIPCSALLSSDITASVNC A K*	3220.6
f _{L2} ^{Ru} NT <u>D</u> GST <u>D</u> YGILQINSR	2423.5
f _{L3} ^{Ru} IV <u>S</u> <u>D</u> GNGMNAWVAVR	2345.6
f _{L4} ^{Ru} FESNFNTQATNR	2098.7
f _{L5} ^{Ru} GYSLGNWVCA A K*	2037.2
f _{L6} ^{Ru} GT <u>D</u> VQAWIR	1704.9
f _{L7} ^{Ru} WWCN <u>D</u> GR	1662.8
f _{L8} ^{Ru} CE <u>L</u> AA A M K *	1604.8
f _{L9} ^{Ru} HGL <u>D</u> NYR	1543.6
f _{L10} ^{Ru} TPGSR	1185.2
f _{L11} ^{Ru} VFGR	1147.3
f _{L12} ^{Ru} GCR	1051.1
f _{L13} ^{Ru} C K *	1048.1
f _{L14} ^{Ru} NR	957.99
f _{L15} ^{Ru} K *	857.91
f _{L16} ^{Ru} R	843.90
f _{L17} ^{Ru} L	800.87
Ubiquitine Sequence	M
f _{U1R} ^u TIT <u>L</u> EV <u>E</u> PSD <u>T</u> IENV K *	2499.7
f _{U2} ^{Ru} T <u>L</u> S <u>D</u> YNIQ K *	1792.9
f _{U3} ^{Ru} <u>E</u> STLHLVLR	1736.9
f _{U4} ^{Ru} <u>E</u> GIP <u>P</u> <u>D</u> QQR	1708.8
f _{U5} ^{Ru} MQIFV K *	1476.7
f _{U6} ^{Ru} Q <u>L</u> EDGR	1386.4
f _{U7} ^{Ru} LIFAG K *	1359.5
f _{U8} ^{Ru} TLTG K *	1230.3
f _{U9} ^{Ru} IQD K *	1214.3
f _{U10} ^{Ru} LR	957.05
f _{U11} ^{Ru} A K*	929.00
f _{U12} ^{Ru} GG	801.81

Table S5. Observed <Ru>-CO-labeled tryptic fragment ions of protein mixture in Figure 11. Superscription “Ru” indicates <Ru>-CO-labeled fragment. Subscriptions “M, L, U” indicate the initial letter of each protein. K* in the fragment ions represents a homoarginine residue.

Monoisotopic mass number of observed ions (m/z)	Calcd. mass number	Fragment ion
3219.9	3220.6	f _{L1}
3179.3	3178.6	f _{L1} – guanidyl
2595.8	2596.9	f _{M1}
2528.9	2527.7	f _{M2}
2499.3	2499.7	f _{U1}
2422.3	2423.5	f _{L2}
2344.7	2345.6	f _{L3}
2276.5	2276.5	f _{M3}
2213.3	2213.4	f _{M4}
2171.7	2171.4	f _{M4} – guanidyl
2120.0	2120.5	f _{M3} – R
2098.2	2098.2	f _{L4}
2073.9	2074.4	f _{M5}
2036.7	2037.2	f _{L5}
1983.3	1983.2	f _{M6}
1904.5	1904.4	f _{M5} – K*
1866.8	1867.2	f _{L5} – K*
1816.5	1816.2	f _{M6} – K*
1792.7	1792.9	f _{U2}
1737.2	1736.9	f _{U3}
1708.8	1708.8	f _{U4}
1705.2	1704.9	f _{L6}
1663.6	1663.8	f _{M7}
1631.8	1631.7	f _{M8}
1604.3	1604.8	f _{L8}
1543.8	1543.6	f _{L9}
1506.2	1506.6	f _{L7} – R
1476.3	1476.7	f _{U5}

Table S5. (Continued) .

Monoisotopic mass number of observed ions (m/z)	Calcd. mass number	Fragment ion
1458.7	1458.7	f _{U5} – H ₂ O
1419.6	1419.5	f _{M9}
1417.0	1417.6	f _{M10}
1373.4	1373.4	f _{M11}
1359.4	1359.5	f _{U7}
1300.8	1300.4	f _{M12} – guanidyl
1277.9	1277.4	f _{M13} – guanidyl
1261.2	1261.4	f _{M10} – R
1230.2	1230.3	f _{U8}
1214.1	1214.3	f _{U9}
1184.9	1185.2	f _{L10}
1147.6	1147.3	f _{L11}
1120.7	1120.2	f _{M15}
1077.8	1078.1	f _{M15} – guanidyl
1061.0	1061.1	f _{L12}
1021.4	1021.1	f _{M17}
1005.5	1005.1	f _{M18}
994.92	995.06	f _{M19}
976.50	976.10	f _{L13} – guanidyl
957.05	957.05	f _{U10}
929.03	929.00	f _{U11}
857.92	857.91	f _{M20} , f _{L15}
843.36	843.90	f _{L16}
824.75	824.85	f _{M19} – K*
800.50	800.87	f _{L17}
744.64	744.76	f _{U12} – G
687.33	686.73	c ₀
644.52	644.13	a ₀