Electronic Supplementary Material (ESI) for Dalton Transactions.

A Novel Binuclear Pd(II) Complex Displaying the Synergic Peptide Cleavage Behaviour

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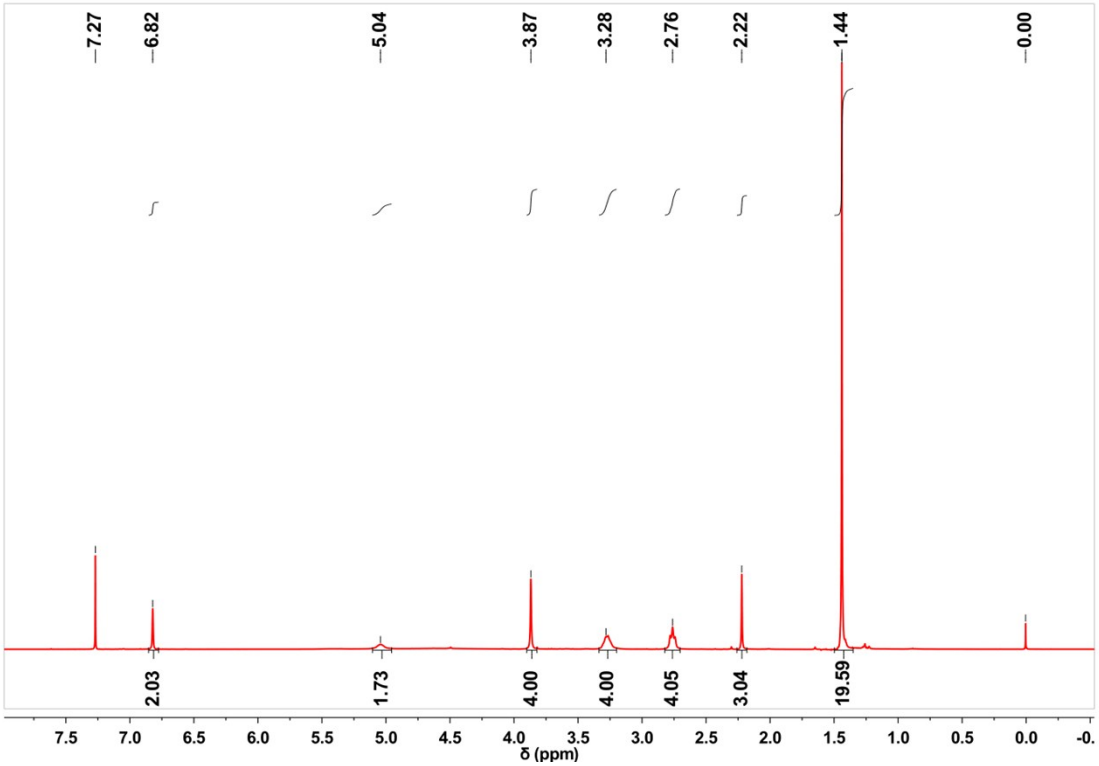

Figure S1. ^{1}H NMR of Boc-L in $CDCl_3$ (500 MHz).

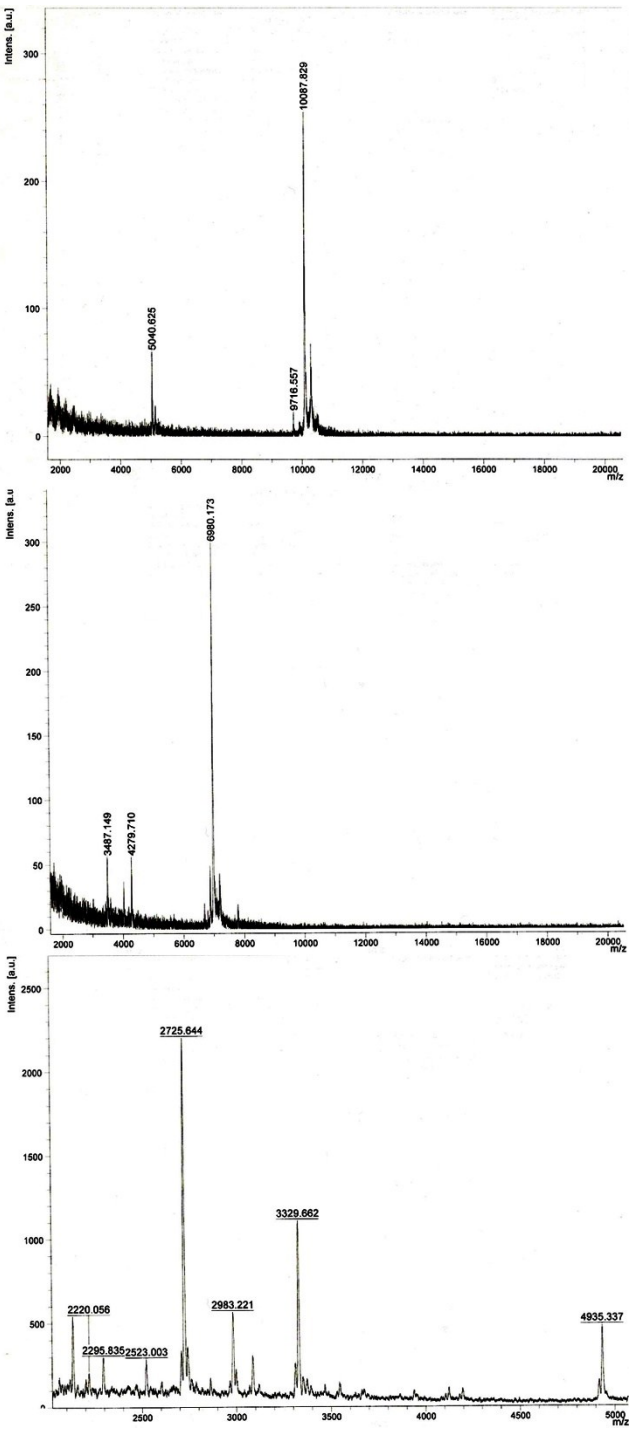

Figure S2. MALDI-TOF mass spectra of the extract of the tris-tricine SDS PAGE bands for myoglobin (Mb, 0.5 mM) incubated with $[Pd_2(\mu\text{-O-L-H})(\mu\text{-OH})]^{2+}$ (10 equiv) at 60°C in citric acid/Na_2HPO_4 buffer.

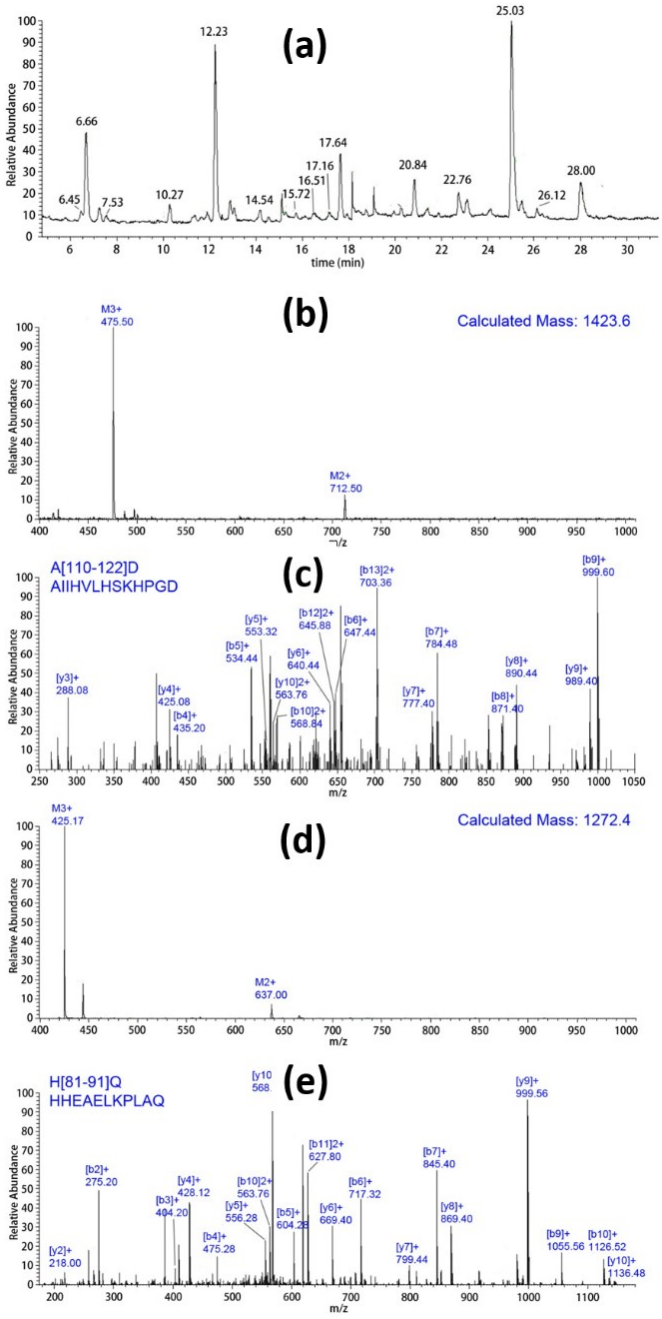

Figure S3. (a) LC chromatogram for Mb cleavage product after ultrafiltration. The cleavage reaction was carried out via incubating Mb (0.25 mM) with 10 equiv $[Pd_2(\mu\text{-O-L-H})(\mu\text{-OH})]^{2+}$ in citric acid/Na_2HPO_4 at 60°C (48 h). Gradient elution with water/acetonitrile (from 100% to 50%, v/v) was carried out in 40 min. The mobile phase was equilibrated with 1% acetic acid. (b-e) Mass spectra and assignments for the first two LC peaks in (a); (b) mass spectrum for the LC signal of retention time 6.45 min; (c) ms^2 spectrum of the signal m/z 712 in (b); (d) mass spectrum for the LC signal of retention time 6.66 min; (e) ms^2 spectrum of the signal m/z 637 in (d).

Table S1. Peptide sequence assignments of LC-MS peaks for Mb cleavage product promoted by $[Pd_2(\mu\text{-O-L-H})(\mu\text{-OH})]^{2+}$.

Retention time (min)	M.W.	Fragment peptide
6.45	1423.6	A[110-122]D
6.66	1272.4	H[81-91]Q
7.51	1272.4	H[81-91]Q
10.27	998.1	E[83-91]Q
12.23	1324.8	I[142-153]G
14.54	1206.4	T[132-141]D
15.72	1439.0	D[141-153]G
16.51	1820.2	K[63-80]G
17.16	2127.5	S[92-109]D
17.64	1408.6	A[130-141]D
20.84	1665.1	A[127-141]D
22.76	2513.1	T[132-153]G
25.03	2715.8	A[130-153]G
26.12	2055.3	F[123-141]D
28.00	2971.8	H[127-153]G

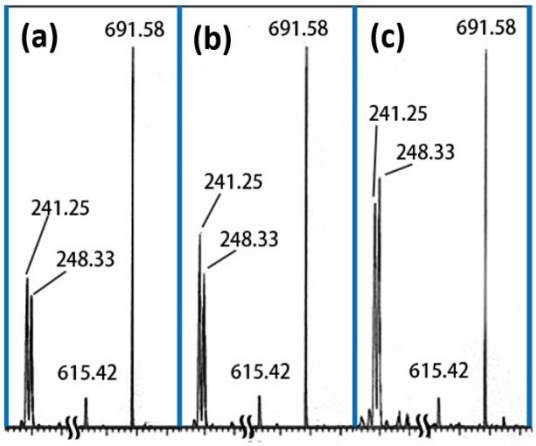

Figure S4. Major peaks in mass spectra of Seq-Asp (0.5 mM) incubated with equivalent $[Pd_2(\mu\text{-O-L-H})(\mu\text{-OH})]^{2+}$ at 60°C and pH 3.50. (a) Just after mixing; (b) after 4 h of incubation; (c) after 24 h of incubation.

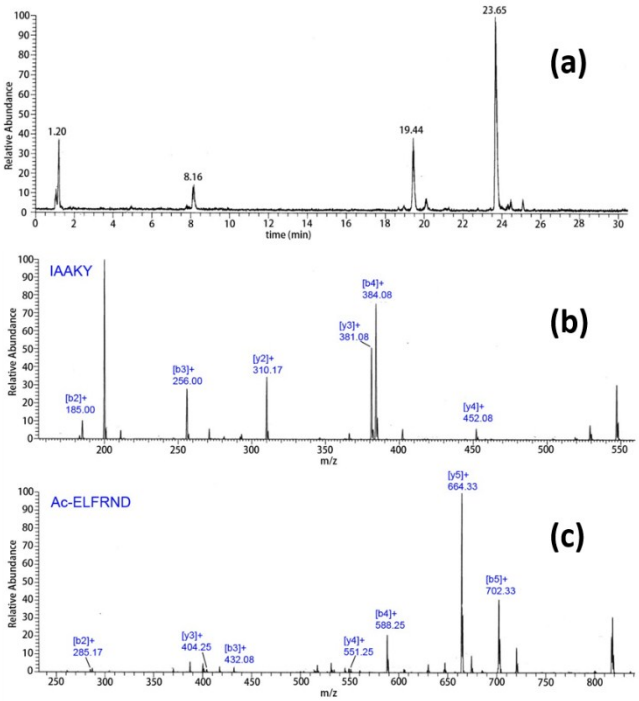

Figure S5. LC-MS-MS study of Seq-Asp incubated (4 days) with equivalent $[Pd_2(\mu\text{-O-L-H})(\mu\text{-OH})]^{2+}$ at 60°C in citric acid/Na_2HPO_4 buffer of pH 3.50.

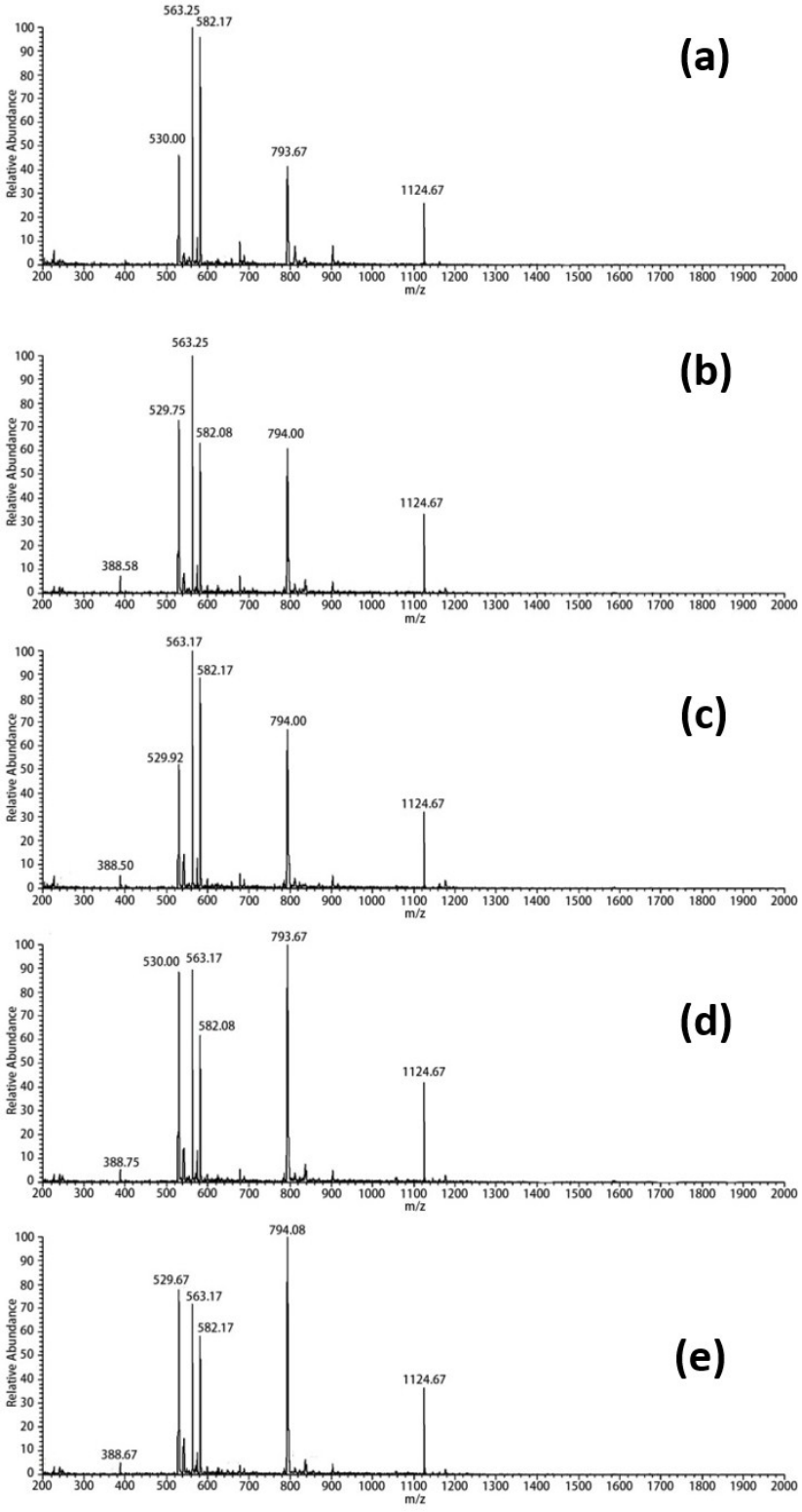

Figure S6. Mass spectroscopic monitoring of peptide Seq-His (0.5 mM) incubated with equivalent $[Pd_2(\mu\text{-O-L-H})(\mu\text{-OH})]^{2+}$ in citric acid/Na_2HPO_4 buffer of pH 3.50 at 60°C. (a) Mass spectrum obtained just after mixing; (b) spectrum after 4 h of incubation; (c) spectrum after 8 h of incubation; (d) spectrum after 12 h of incubation; (e) spectrum after 24 h of incubation. The signals of m/z 529 and m/z 793 can be assigned as $[Pd_2L+Seq\text{-}His\text{-}H]^{3+}$ and $[Pd_2L+Seq\text{-}His\text{-}2H]^{2+}$,

respectively. The signals of m/z 563, 583 and 1124 can be assigned as $[Seq\text{-}His+2H]^{2+}$, $[Seq\text{-}His+H_2O+Na]^{+}$, and $[Seq\text{-}His+H]^{+}$, respectively.

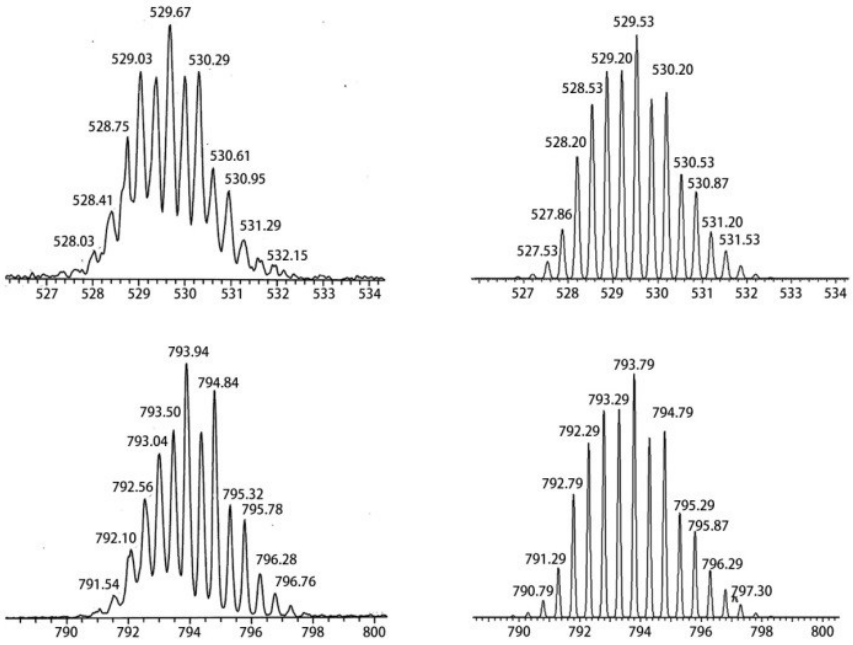

Figure S7. Upper: the detected (Left) and simulated (right) isotopic distribution patterns (IDPs) of $[Pd_2L+Seq\text{-}His\text{-}H]^{3+}$ in Figure S5. Lower: the detected (Left) and simulated (right) IDPs of $[Pd_2L+Seq\text{-}His\text{-}2H]^{2+}$ in Figure S6.

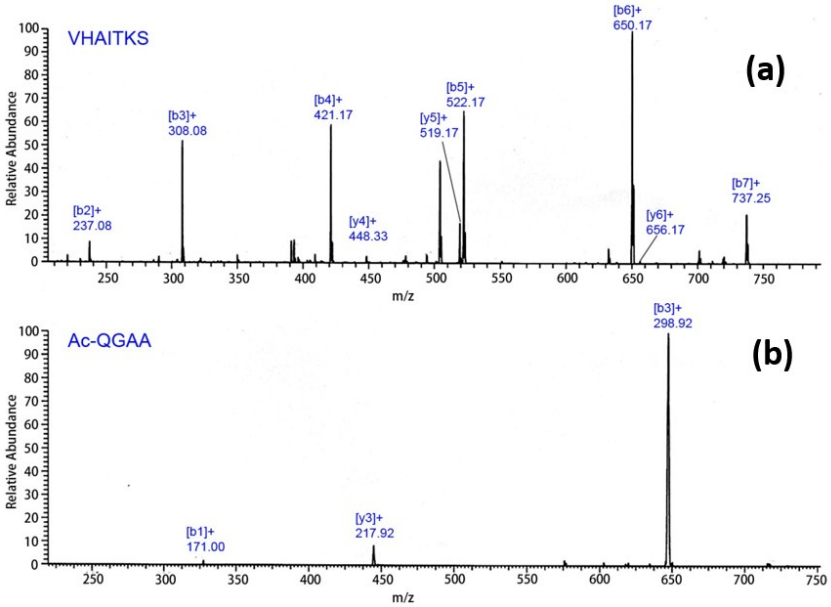

Figure S8. LC-MS-MS study of peptide Seq-His (0.5 mM) incubated (24 h) with equivalent $[Pd_2(\mu\text{-}O\text{-}L\text{-}H)(\mu\text{-}OH)]^{2+}$ at 60°C in citric acid/Na_2HPO_4 buffer (pH 3.50). (a) ms^2 spectrum of the fragment of RT 4.10 min and mass signal m/z 754; (b) ms^2 spectrum of the fragment with RT 3.17 min and mass signal of m/z 387.

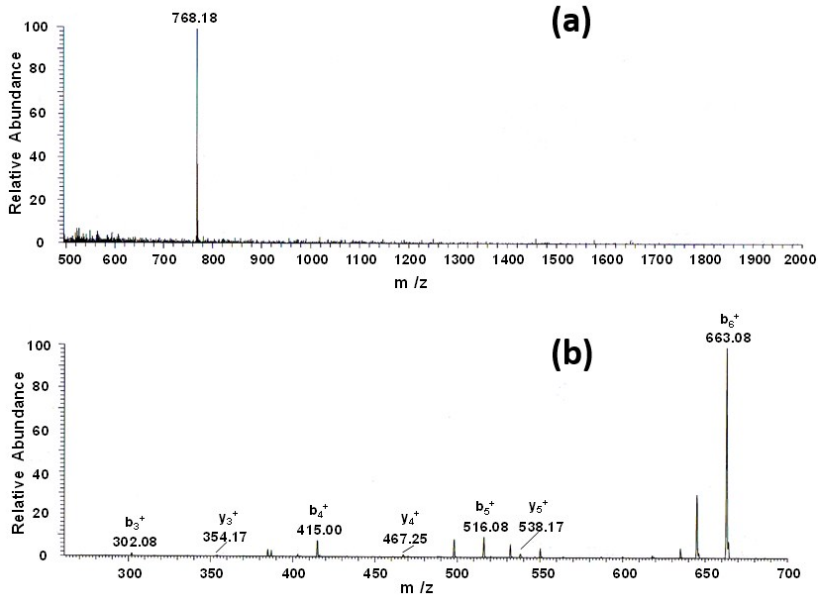

Figure S9. (a) ESI-MS spectrum for the Seq-Met cleavage product ([Val5-Ser11+H]$^+$) induced by incubation with equivalent $[Pd_2(\mu\text{-O-L-H})(\mu\text{-OH})]^{2+}$; (b) ms^2 spectrum with the signal in (a) as mother signal.

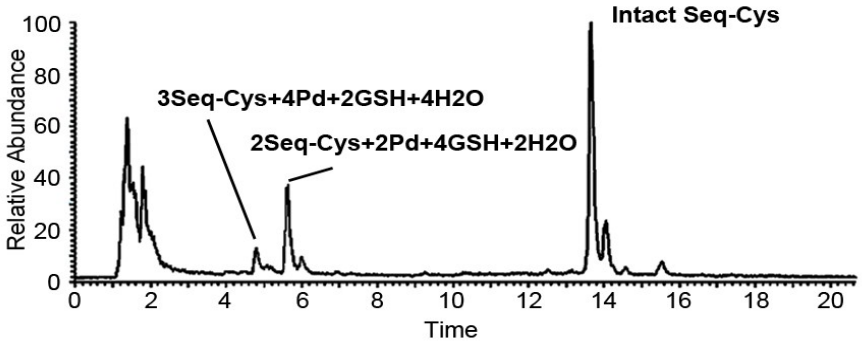

Figure S10. LC chromatogram of LC-MS determination for Seq-Cys (0.5 mM) incubated (4 days) with equivalent $[Pd_2(\mu\text{-O-L-H})(\mu\text{-OH})]^{2+}$ at 60°C and pH 3.50 in citric acid/Na_2HPO_4 buffer. The determination was carried out after GSH treatment.

Table S2. LC-MS identification of MP-11 solution upon incubation with 2 equiv $[Pd_2(\mu\text{-O-L-H})(\mu\text{-OH})]^{2+}$.

Peak	Scan Mode	Mother Peak	Assignment
Retention time=1.18 min			
234.81	Full Scan	N/a	$[2DTCO+Pd]^{2+}$
366.96	Full Scan	N/a	$[DTCO+Pd+Ac^{-}+H2O]^{+}$
468.86	Full Scan	N/a	$[2DTCO+Pd-H]^{+}$
Retention time=1.56 min			
606.62	Full Scan	N/a	$[MP11+2Pd+2DTCO-H]^{3+}$
546.42	ms^2	606.62	$[MP11+2Pd+DTCO-H]^{3+}$
348.08	ms^3	545.42	$MP11\ y3^{+}$
485.75	ms^3	545.42	$[MP11+2Pd-H]^{3+}$
645.00	ms^3	545.42	$MP11\ b8^{+}$
Retention time=6.40 min			
597.84	Full Scan	N/a	$[2MP11+3Pd+DTCO-H]^{5+}$
747.47	Full Scan	N/a	$[2MP11+3Pd+DTCO-2H]^{4+}$
996.31	Full Scan	N/a	$[2MP11+3Pd+DTCO-3H]^{3+}$
935.67	ms^2	996.31	$[2MP11+3Pd-3H]^{3+}$
1228.67	ms^3	935.67	$[Mp11+b8^{+}+3Pd-5H]^{2+}$
1054.67	ms^3	935.67	$[2b8^{+}+3Pd-6H]^{2+}$
Retention time=7.89 min			
415.41	Full Scan	N/a	$[MP11+3H]^{3+}$
622.41	Full Scan	N/a	$[MP11+2H]^{2+}$
Retention time=26.65 min			
652.18	Full Scan	N/a	Heme

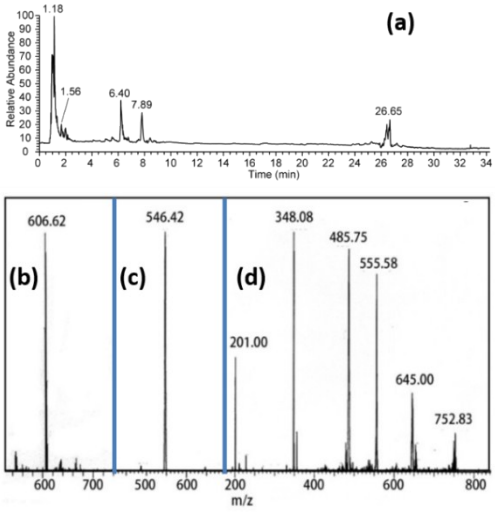

Figure S11. LC-MS-MS study of MP-11 (0.5 mM) incubated with 2 equivalent $[Pd_2(\mu\text{-O-L-H})(\mu\text{-OH})]^{2+}$ for 6 days. (a) LC chromatogram determined after DTCO treatment; (b-d) mass spectroscopic results for fraction of RT 1.54 min: (b) main mass signal in ESI-MS spectrum; (c) ms2 spectrum with m/z 606.62 as mother peak; (d) ms3 spectrum with m/z 546.42 as mother peak

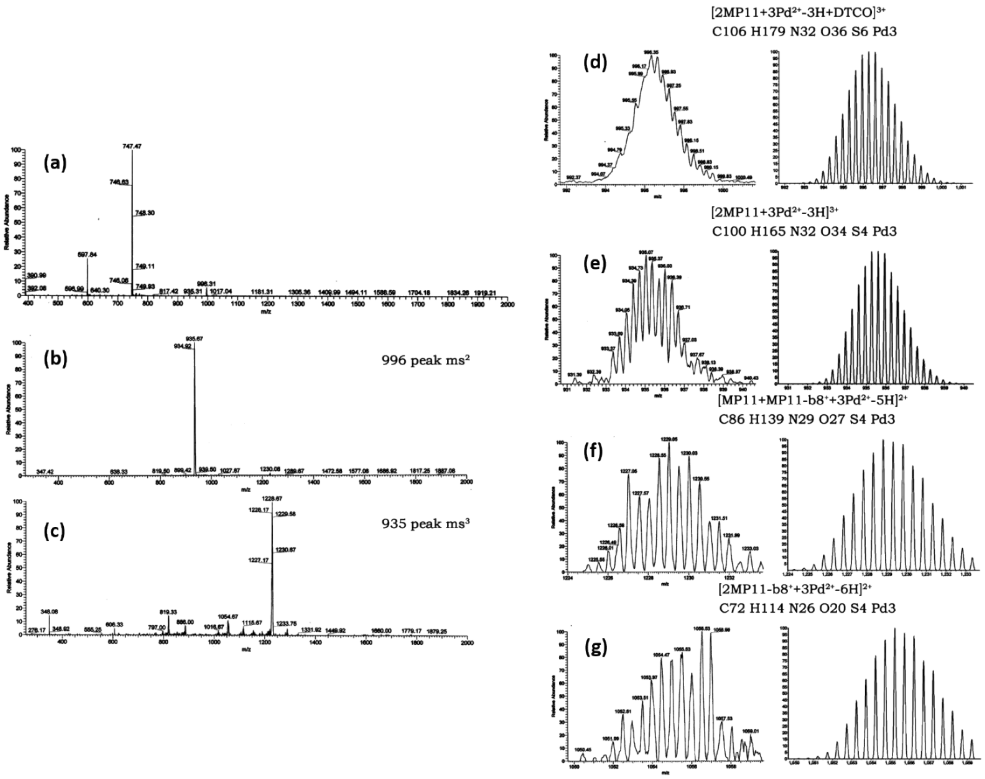

Figure S12. LC-MS-MS study of MP-11 (0.5 mM) incubated with 2 equivalent $[Pd_2(\mu\text{-O-L-H})(\mu\text{-OH})]^{2+}$ for 6 days. The determination was carried out after DTCO treatment. (a) ESI-MS spectrum for fraction of RT 6.40 min; (b) ms^2 spectrum with signal m/z 996 as mother peak; (c) ms^3 spectrum with ms^2 signal m/z 935 as mother peak; (d-g) detected (left) and simulated (right) IDPs for the assigned species.

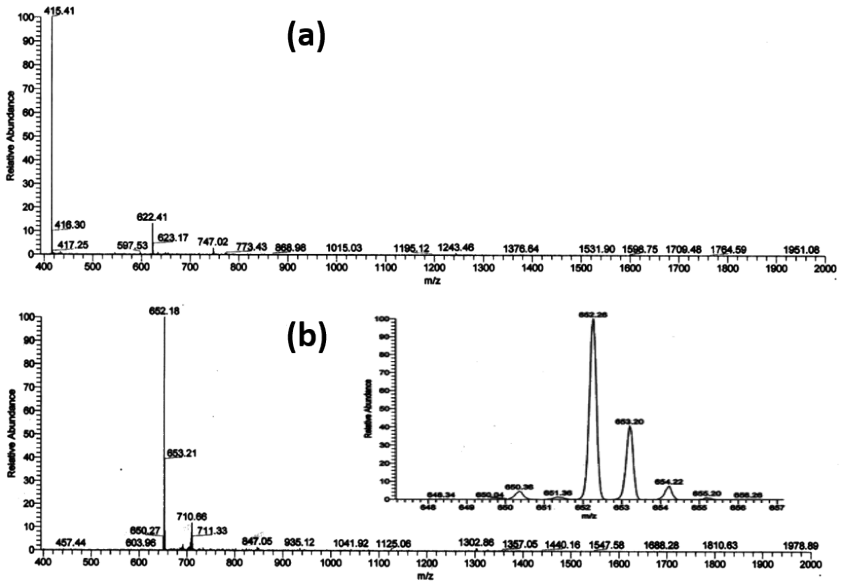

Figure S13. LC-MS-MS study of MP-11 (0.5 mM) incubated with 2 equivalent $[Pd_2(\mu\text{-O-L-H})(\mu\text{-OH})]^{2+}$ for 6 days. The determination was carried out after DTCO treatment. (a) ESI-MS spectrum for LC fraction of RT 7.89 min; (b) ESI-MS spectrum for LC fraction of RT 26.65 min. Inset in (b): the detected IDP of signal m/z 652.

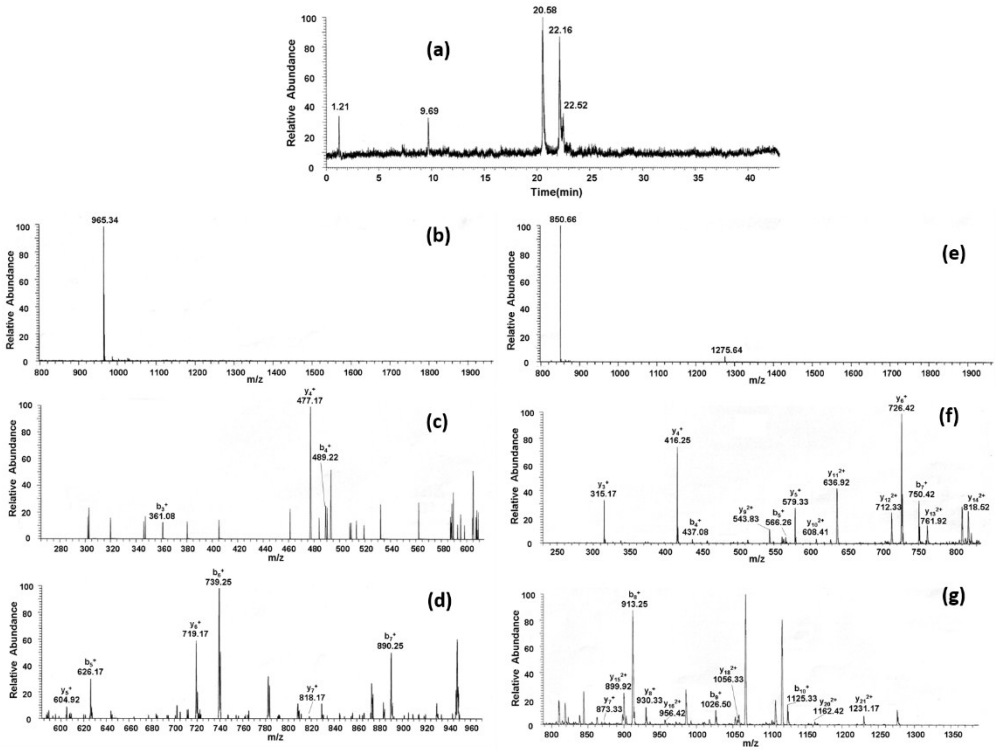

Figure S14. LC-MS-MS study of the oxidized insulin B chain (0.5 mM) incubated (4 days) with 2 equiv $[Pd_2(\mu\text{-O-L-H})(\mu\text{-OH})]^{2+}$ at 60°C in citric acid/Na_2HPO_4 buffer (pH 3.50) . (a) LC chromatogram; (b) ESI-MS spectrum for fraction of RT 9.69 min; (c) and (d) ms^2 spectra with mass signal m/z 965 in (b) as mother peak (for Phe1-Gly8); (e) ESI-MS spectrum for fraction of RT 20.58 min; (f) and (g)

ms^2 spectrum with mass signal of m/z 850 as the mother peak (Ser9-Ala30).

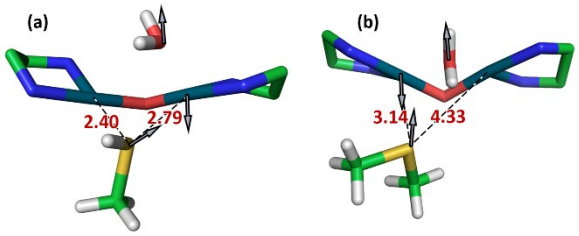

Figure S15. Optimized geometries of TS1 (a) and TS4 (b) showed in Figures 6 and 7. The arrows show the vibration direction of the imaginary frequency which leads to the subsequent reaction.

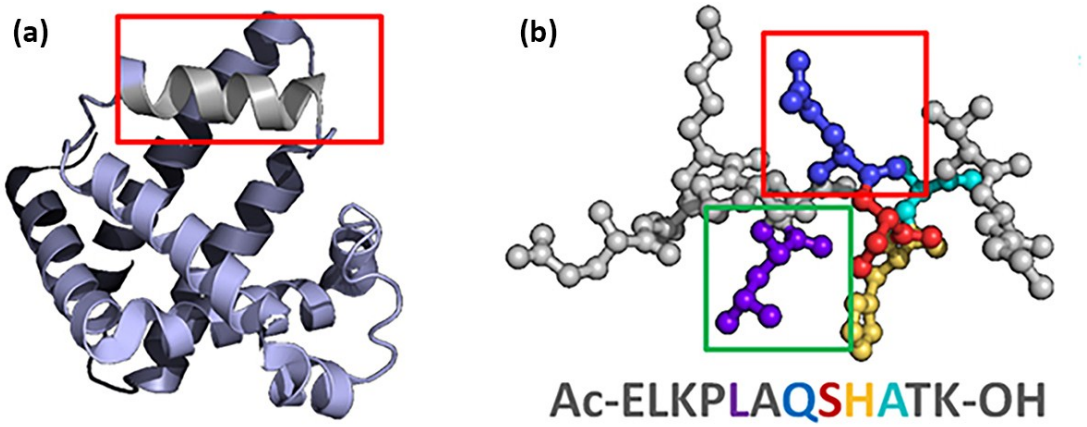

Figure S16. (a) The selected substrate peptide in myoglobin crystal structure; (b) the structure of this selected substrate peptide in ball and stick view. Different residues were labelled with the color as labelled in the sequence. Green box highlighted the leucine which is on the side of histidine, whereas red box highlighted glutamine on the opposite side.

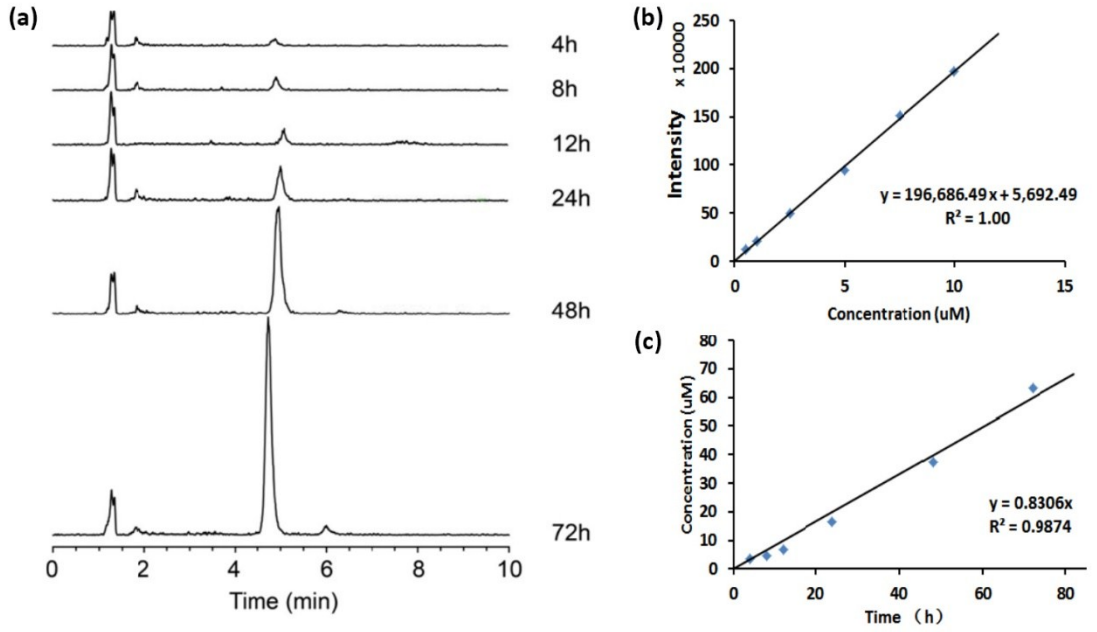

Figure S17. An example of the reaction rate measurement for the cleavage reaction of peptides in Table 2 promoted by $[Pd_2(\mu\text{-O-L-H})(\mu\text{-OH})]^{2+}$. (a) Ion current intensity of cleavage product of Ac-ELKPLAKSHATK measured by LC-MS at different cleavage time; (b) standard curve of integral intensity to Ac-ELKPLAK peptide concentration; (c) reaction rate curve for the cleavage of u2k (Ac-ELKPLAKSHATK) peptide incubated with 5 equiv $[Pd_2(\mu\text{-O-L-H})(\mu\text{-OH})]^{2+}$ at pH 3.5 and 60°C.

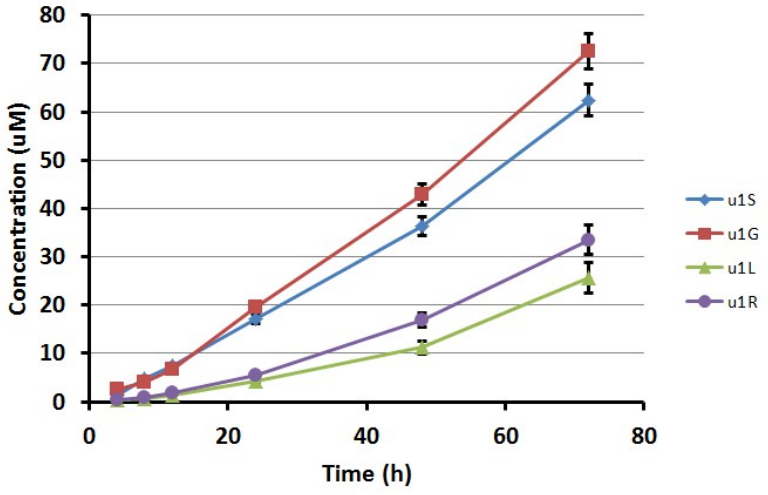

Figure S18. Cleavage rate curves for his-containing peptides u1S, u1G, u1L and u1R upon incubation with 5 equiv $[Pd_2(\mu\text{-O-L-H})(\mu\text{-OH})]^{2+}$ at pH 3.5 and 60°C.

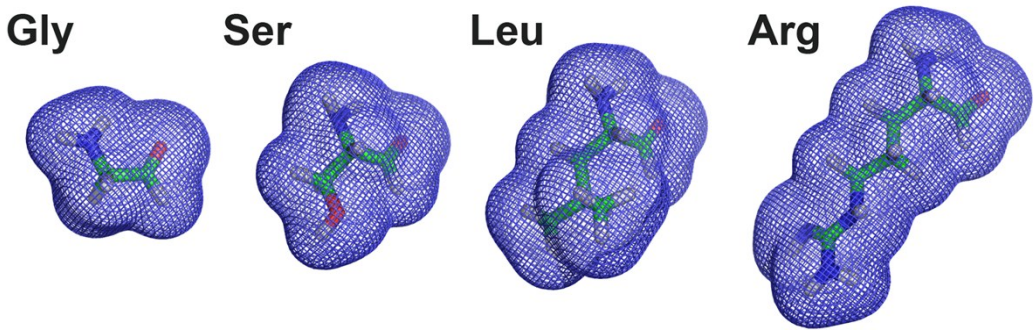

Figure S19. Molecular surface shape calculated by quantum mechanics depicted the relative size difference of the four residues: Gly, Ser, Leu, and Arg.