Structure-Selective Modification of Aromatic Side Chains with Dirhodium Metallopepide Catalysts

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Supporting Information

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General Considerations.

Solvents and reagents were purchased from Fisher Scientific and used as received.

Experimental protocols. The term "aqueous buffer" refers to an aqueous solution of 0.1 M *t*-BuNHOH·HCl at pH = 6.2.

HPLC analysis. Reversed-phase HPLC (rp-HPLC) was performed on a Shimadzu CBM-20A instrument with Phenomenex Jupiter 4μ Proteo 90A (250 × 15 mm preparative) and Phenomenex Jupiter 4μ Proteo 90A (250 × 4.6 mm analytical) columns. Flow rates of 8 mL/min and 1 mL/min were used for preparative and analytical columns, respectively. Analytical spectra reported here were obtained using gradients of MeCN (10-90%) in water. Both solvents contained 0.1% trifluoroacetic acid (TFA).

Mass spectrometry. MALDI-MS and MS/MS analyses were performed on a Bruker Daltonics Autoflex MALDI-TOF/TOF mass spectrometer with CHCA matrix (10 mg/mL, Thermo Scientific Pierce). ESI-MS was performed on a Bruker Daltonics micrOTOF instrument.

Circular dichroism spectroscopy. CD spectra were obtained on Jasco-J810 spectropolarimeter. The spectra were acquired with a 0.1-nm interval in the range of 180–260 nm. The temperature was maintained by a Jacso PTC423S water bath. Job plot data were obtained by maintaining a total peptide concentration of 0.2 mM in aqueous buffer using a 0.01 cm cell. Temperature denaturation experiments (5 – 85 °C with a gradient of 20 °C/h) were performed on solutions of 0.1 mM $E3_gX$ and 0.1 mM $K3_{a,e}Rh_2$ in aqueous buffer in a 0.1 cm sealed cell. Ellipticity data were acquired at 222 nm. Full CD spectra were acquired during the temperature denaturation experiments at 10 °C intervals. All CD data results were converted to mean residual ellipticity by the equation

$$[\theta] = \theta_{obs} / (10 \times l \times C \times N)$$

where θ_{obs} is the ellipticity in millidegrees of rotation, l is the optical path length of the cell in cm, C is the concentration of the peptide in mol/L, and N is the number of residues in the peptide.

Peptide synthesis. All peptides were synthesized with an AAPPTEC APEX 396 Automated Multipeptide Synthesizer using standard solid-phase Fmoc protocols.[1] The purification was accomplished by reverse-phase HPLC with gradients of water-acetonitrile containing 0.1% trifluoroacetic acid, and peptides were isolated by lyophilization. Analysis and purity assessment was attained by mass spectrometry and analytical HPLC. Peptides were prepared using Rink amide MBHA resin (AAPPTEC) to afford the C-terminal amide and were acetylated at the N-terminus prior to cleavage from the resin.

Synthesis of known compounds. The dirhodium precursor *cis*-$Rh_2(tfa)_2(OAc)_2$[2] and diazo reagent [2-(2-methoxyethoxy)ethoxy]ethyl (*E*)-4-phenyl-2-diazo-3-butenoate (**1**)[3a] were prepared and purified according to published procedures.

Experimental.

Synthesis of $K3_{a,e}Rh_2$ complex from *cis*-$Rh_2(tfa)_2(OAc)_2$. Peptide **$K3_{a,e}EE$** (4.3 mg, 1.8 μmol) and *cis*-$Rh_2(tfa)_2(OAc)_2$ (1.1 mg, 1.8 μmol) were charged into a 1-dram vial equipped with a stir bar. A solution of MES buffer (2-(*N*-morpholino)ethanesulfonic acid, 1.6 mL, 0.1 M aq soln) was added and the pH adjusted to 4.5 with KOH (1 M aq soln). The reaction was heated to 50 °C for 3 h after which all reactants were consumed based on HPLC analysis. The dirhodium-peptide complex was purified by direct injection of the reaction mixture onto a preparative HPLC column. The complex was isolated by lyophilization to afford a fluffy light blue powder (3.7 mg, 79% yield).

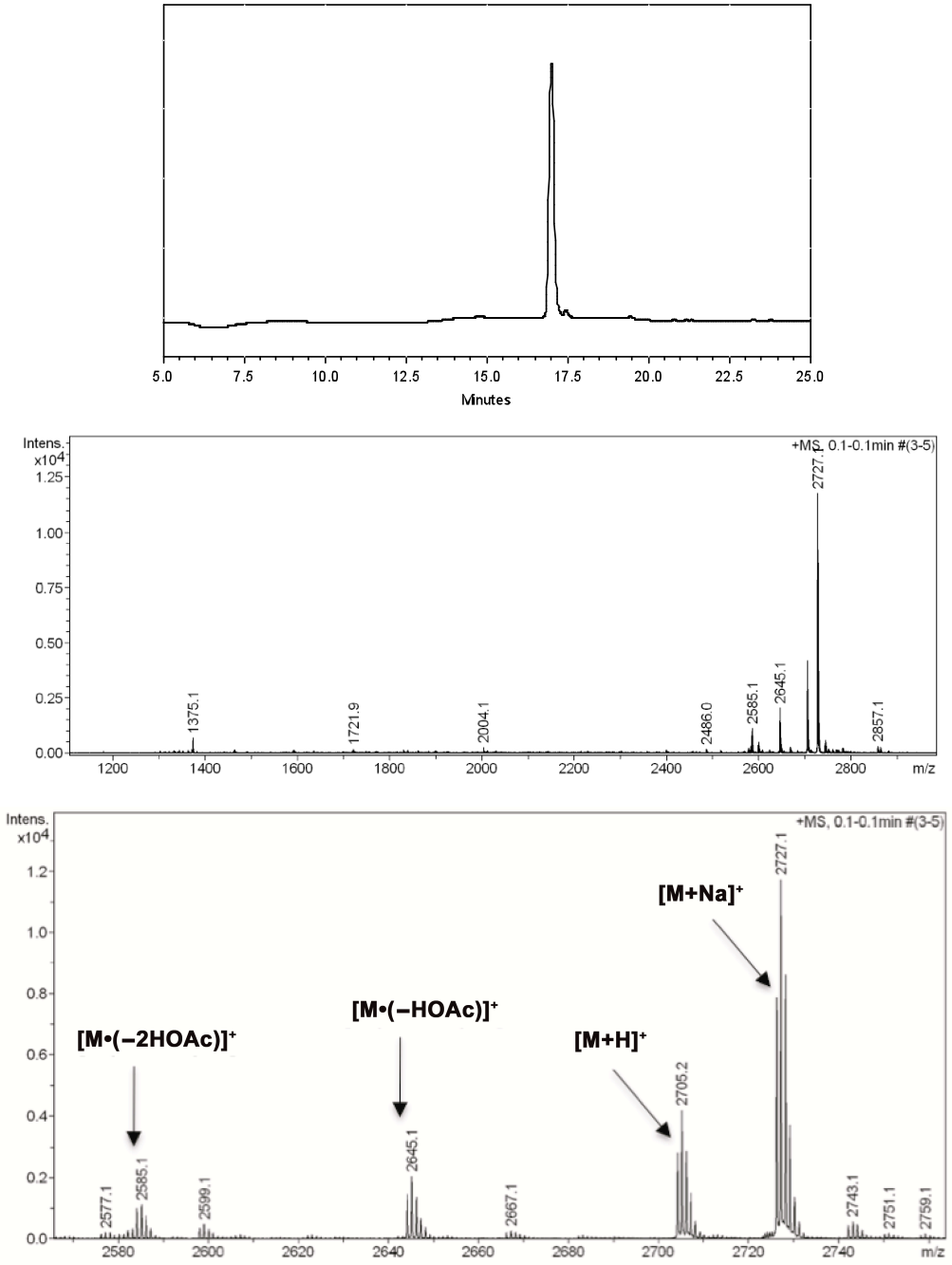


Figure S-1. HPLC trace and MALDI-TOF mass spectrum of isolated **$K3_{a,e}Rh_2$**. Calculated mass $[M+H]^+$: 2704.2; found: 2704.2.

Synthesis of $K3_{g,d}Rh_2$ complex.This complex was performed similarly to that of **$K3_{a,e}Rh_2$**, affording a dense aqua-green powder (5.3 mg, 98% yield).

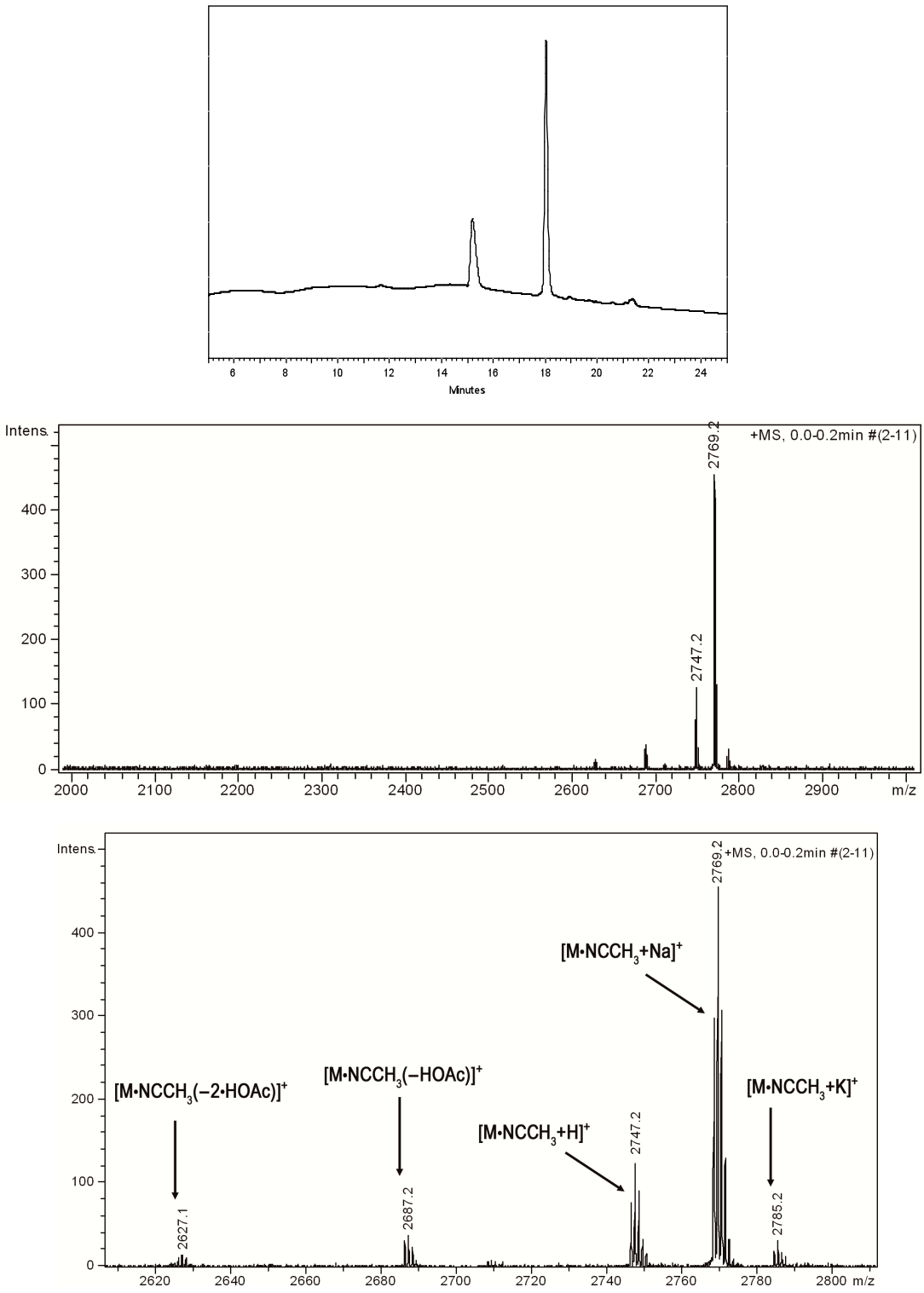


Figure S-2. HPLC trace and MALDI-TOF mass spectrum of isolated **$K3_{g,d}Rh_2$**. Calculated mass $[M•NCCH_3+H]^+$: 2747.3; found: 2746.2.

General procedure for catalytic tryptophan modification. reaction of E3gW with diazo 1 (70 eq) and $K3_{a,e}Rh_2$ (5 mol %). Stock solutions of $E3_gW$ (2.5 mM) and $K3_{a,e}Rh_2$ (0.25 mM) were prepared in aqueous buffer. A stock solution of diazo reagent **1** (36 mg in 81 mL *t*-BuOH) was also prepared. To a 0.6-mL Falcon tube, $E3_gW$ stock (5.0 μL, 50 μM final concn) was added to aqueous buffer (235 μL), followed by addition of $K3_{a,e}Rh_2$ stock (5 μL, 5 μM final concn). The reaction was initiated by addition of *t*-BuOH (2.3 μL) followed by diazo stock (2.7 μL, 3.5 mM final concn). The total reaction volume was 250 μL with 2% *t*-BuOH co-solvent. The reaction tube was initially mixed for ca. 30 s with a bench-top vortex mixer and was then placed in a bed shaker. Aliquots (5 μL) for time-course analysis of the reaction were removed at specific times and were quenched by diluting into a mixture of MeCN/H_2O (7:3) with 0.1% TFA.

Procedure for catalytic tryptophan modification of peptide $E3_gW$ with $Rh_2(OAc)_4$. A stock solution of $Rh_2(OAc)_4$ (2.7 mM in 1:1 aqueous buffer/*t*-BuOH) was prepared. To a 0.6-mL Falcon tube, **E3gW** stock (5.5 μL, 51 μM final concn) was added to aqueous buffer (237 μL), followed by addition of $Rh_2(OAc)_4$ stock (4.6 μL, 50 μM final concn). The reaction was initiated by addition of diazo stock (2.7 μL, 3.5 mM final concn). The total reaction volume was 250 μL with 2% *t*-BuOH cosolvent. The reaction tube was initially mixed for ca. 30 s with a bench-top vortex mixer and was then placed in a bed shaker at 275 rpm. Aliquots (4 μL) for time-course analysis of the reaction were removed at specific times and were quenched by diluting into a mixture of MeCN/H_2O (7:3) with 0.1% TFA.

Typical procedure for catalytic tryptophan modification competition experiments. Employing the metallopeptide procedure outlined above, an additional stock of control peptide soln (5 μL of a 2.5 mM soln in aqueous buffer, 50 μM final concn) was added prior to addition of the metallopeptides stock solution.

Modification analysis. All peptide conversion was determined from the ratio of modified to unmodified peptide using peak intensity from MALDI-TOF MS analysis and is uncorrected. Three spectra from different locations on the sample spot were acquired and averaged to obtain the reported conversion. This method is similar to that adopted by Antos and Francis.[3] Attempts were also made to analyze side-chain modification by HPLC, and conversion data obtained by HPLC is consistent with that from MS analysis (Figure S-4). The product of peptide modification can be observed by HPLC as well, the several modified products appear as overlapping peaks (Figure S-3). However in our hands, the presence of several inseparable modification products and the strong UV absorption of the diazo reagent, present in large excess, made quantification of the modification products by HPLC unreliable.

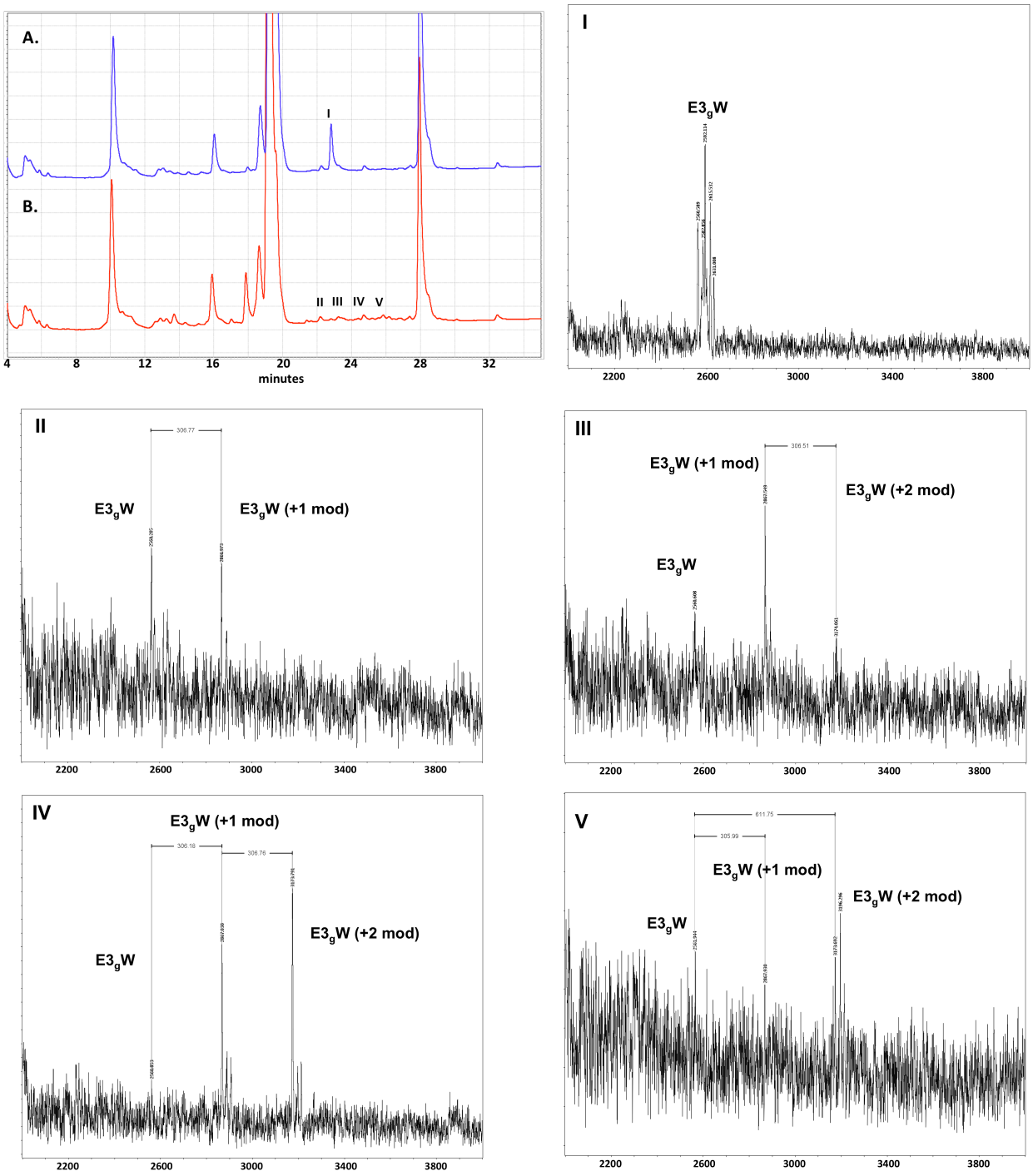


Figure S-3. HPLC traces for modification of peptide **E3gW** with $Rh_2(OAc)_2(tfa)_2$ (100 mol %) (**A**) and **K3$_{a,e}$Rh$_2$** (10 mol %) (**B**) after 24 h and MALDI-TOF MS spectra (**I**-**V**) from fractions isolated during the HPLC separations.

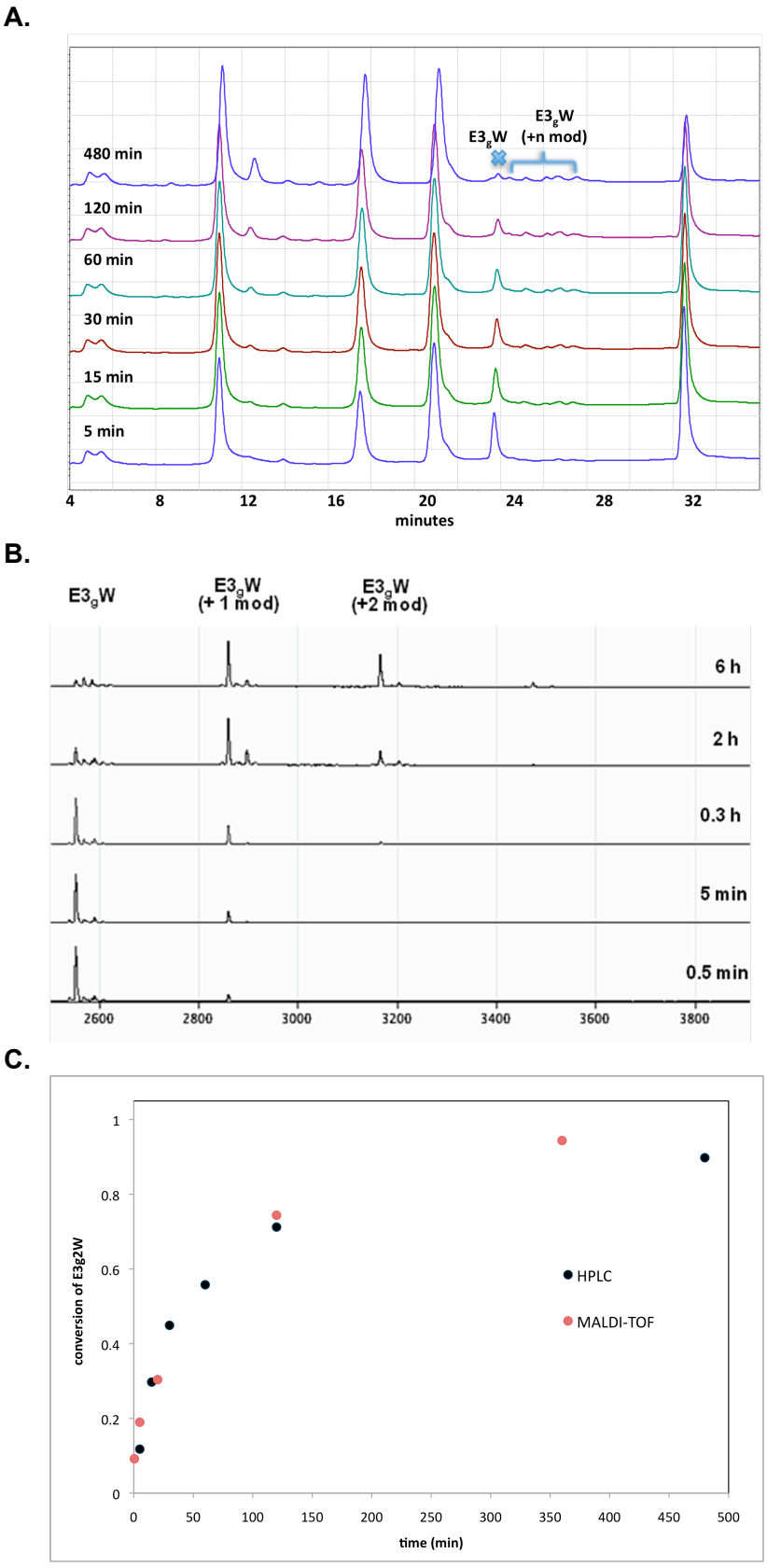


Figure S-4. HPLC (**A**) and MALDI-TOF MS (**B**) times courses of the modification of peptide **E3$_g$W** with **K3$_{a,e}$Rh$_2$** (2 mol %) and diazo **1**. (70 equiv). See Table 1 for MS conversion data. (**C**) Overlay of the conversion profile of **E3gW** from both analytical methods.

Table S-1. Modification Conversions from MALDI-TOF MS Analysis.

Entry	Catalyst (mol %)[a]	Reactants **A** + **B**[b]	Conversion **A** (h)	Conversion **B** (h)
1	$\mathbf{K3_{a,e}Rh_2}$ (10)	$\mathbf{E3_gW + W_{random}}$	84 (0.3); 94 (2); >95 (26)	< 5 (0.3); 5 (2); 7 (16)
2	$\mathbf{Rh_2(OAc)_4}$ (100)	$\mathbf{E3_gW + W_{random}}$	13 (2); 17 (26)	13 (2); 16 (26)
3	$\mathbf{K3_{a,e}Rh_2}$ (10)	$\mathbf{W_{random}}$	9 (16)	----
4	$\mathbf{K3_{a,e}Rh_2}$ (10)	$\mathbf{E3_gW + K3_gW}$	79 (0.3); 91 (1); 91 (2.5)	7 (0.3); 12 (1); 16 (2.5)
5	$\mathbf{K3_{a,e}Rh_2}$ (10)	$\mathbf{K3_gW}$	49 (16)	----
6	$\mathbf{K3_{a,e}Rh_2}$ (10)	$\mathbf{E3_gW + E3_eW}$	70 (0.3); 91 (1); 93 (2.5)	11 (0.3); 18 (1); 20 (2.5)
7	$\mathbf{Rh_2(OAc)_4}$ (100)	$\mathbf{E3_gW + E3_eW}$	10 (2); 31 (26)	8 (2); 23 (26)
8	$\mathbf{K3_{a,e}Rh_2}$ (10)	$\mathbf{E3_eW}$	23 (16)	----
9	$\mathbf{K3_{g,d}Rh_2}$ (10)	$\mathbf{E3_gW + E3_eW}$	20 (0.3); 57 (1); 68 (2.5)	77 (0.3); 84 (1); 84 (2.5)
10	$\mathbf{K3_{a,e}Rh_2}$ (20)	$\mathbf{E3_gY + W_{random}}$	25 (1); 44 (5); >95 (24)	< 5 (1); < 5 (5); < 5 (24)
11	$\mathbf{K3_{a,e}Rh_2}$ (20)	$\mathbf{E3_gF + W_{random}}$	22 (1); 46 (5); 94 (24)	< 5 (1); < 5 (5); 7 (24)
10	$\mathbf{Rh_2(OAc)_4}$ (200)	$\mathbf{E3_gY + W_{random}}$	0 (24)	< 5 (5); 5 (24)
11	$\mathbf{Rh_2(OAc)_4}$ (200)	$\mathbf{E3_gF + W_{random}}$	0 (24)	< 5 (5); 6 (24)

a) Mol % catalyst relative to the reactant A concn. b) [A] = [B] = 50 μM.

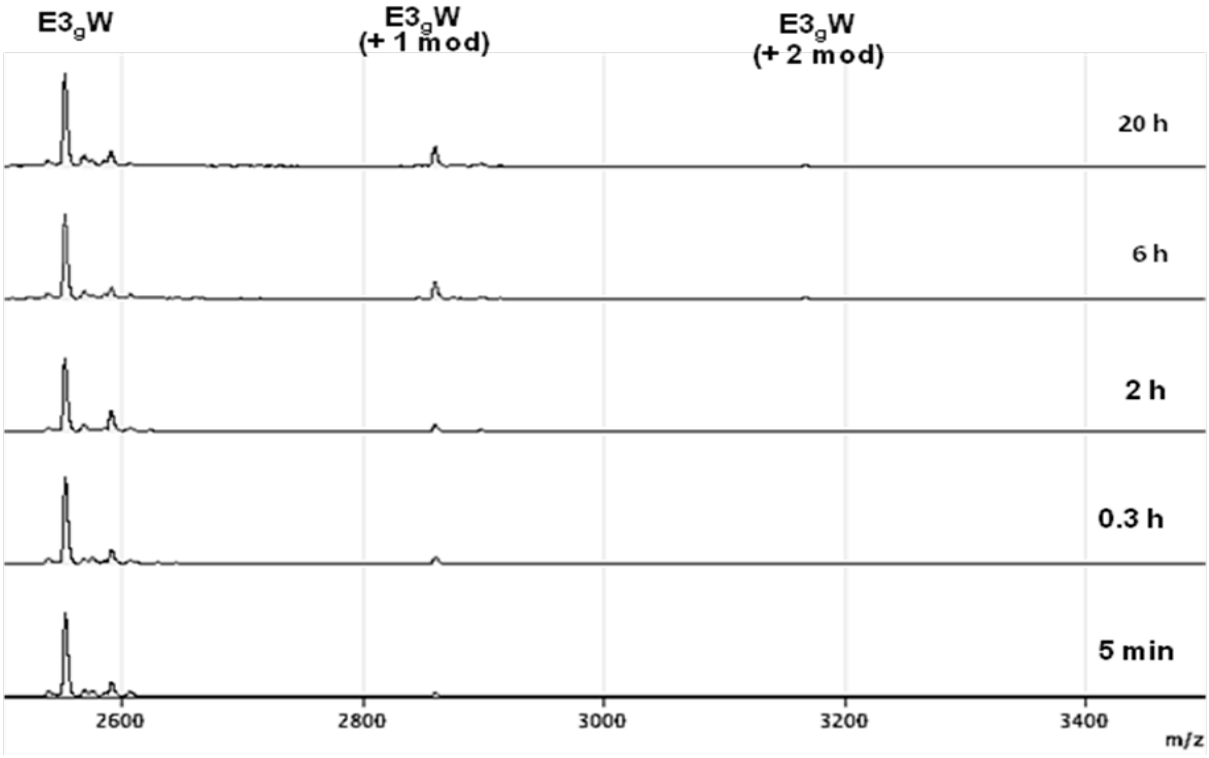


Figure S-5. MALDI-TOF MS spectra of the modification of peptide $\mathbf{E3_gW}$ with $\mathbf{Rh_2OAc_4}$ (100 mol %) and 70 equiv diazo **1**. See Table 1 for conversion data.

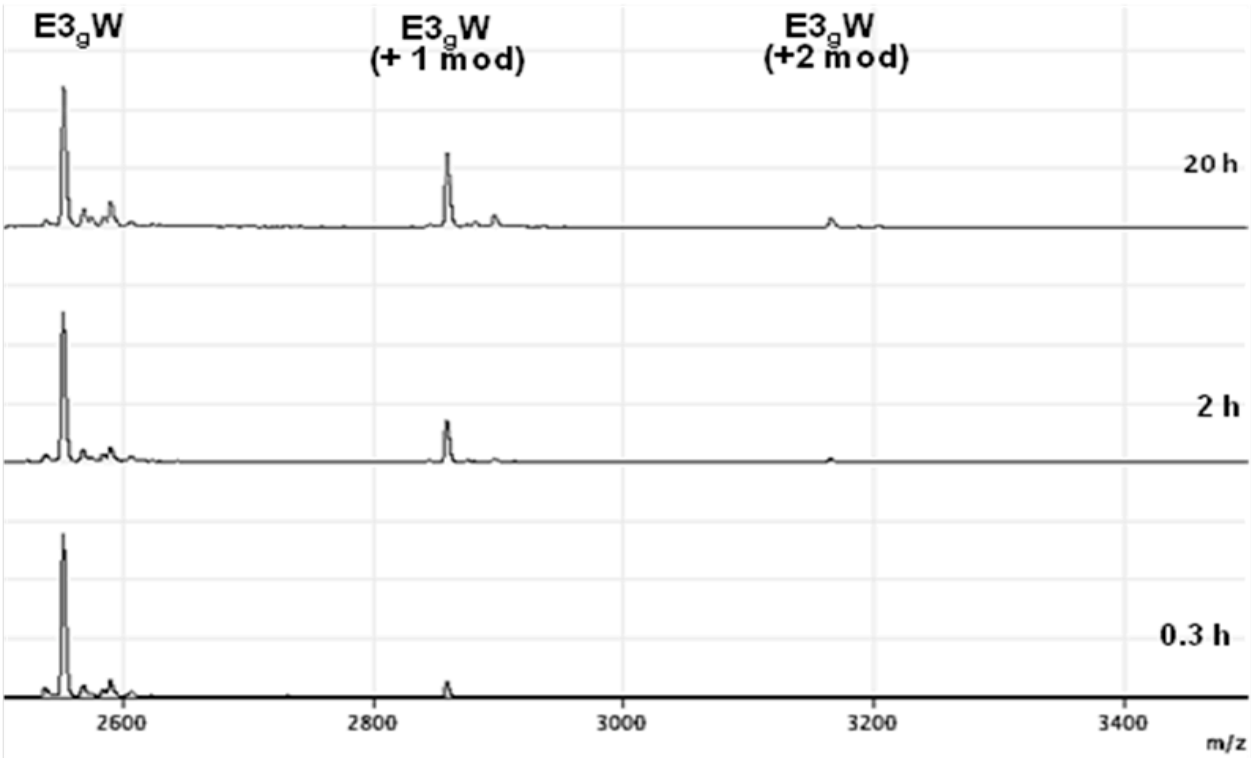


Figure S-6. MALDI-TOF MS spectra of the modification of peptide **E3$_g$W** with **K3$_{a,e}$Rh$_2$** (10 mol %) and 1 equiv diazo **1**. See Table 1 for conversion data.

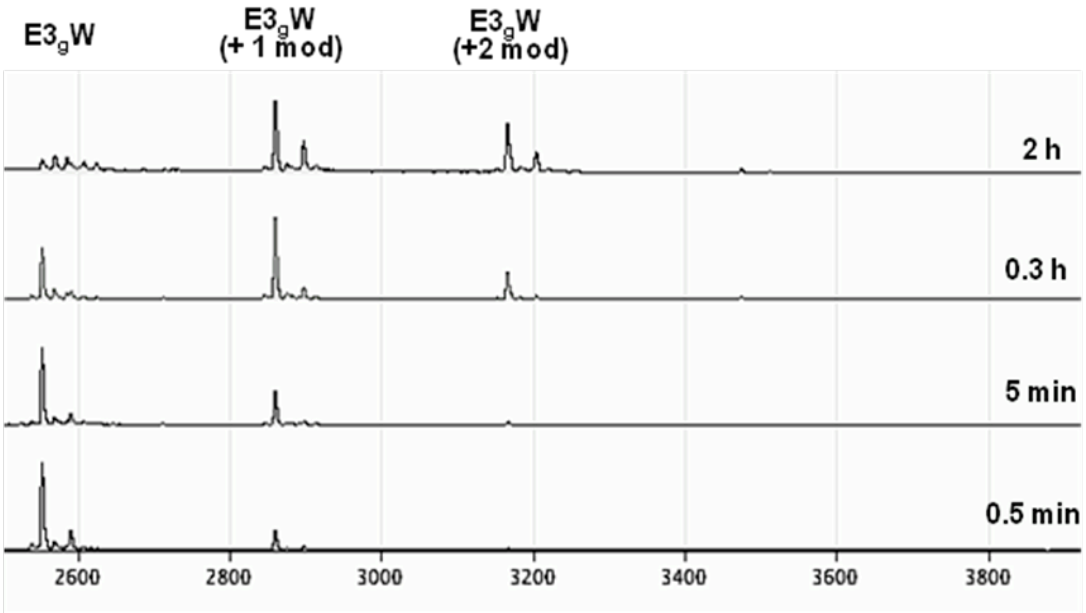


Figure S-7. MALDI-TOF MS spectra of the modification of peptide **E3$_g$W** with **K3$_{a,e}$Rh$_2$** (10 mol %) and 70 equiv diazo **1**. See Table 1 for conversion data.

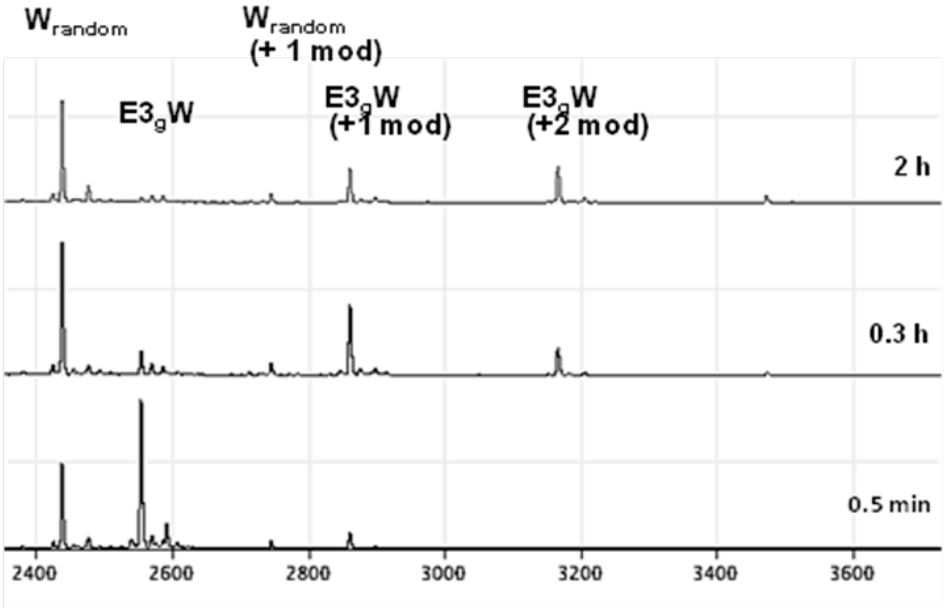


Figure S-8. MALDI-TOF MS spectra of the competitive modification of peptide **E3$_g$W** and **W$_{random}$** with **K3$_{a,e}$Rh$_2$** (10 mol %) and 70 equiv diazo **1**. See Table S-1 for conversion data.

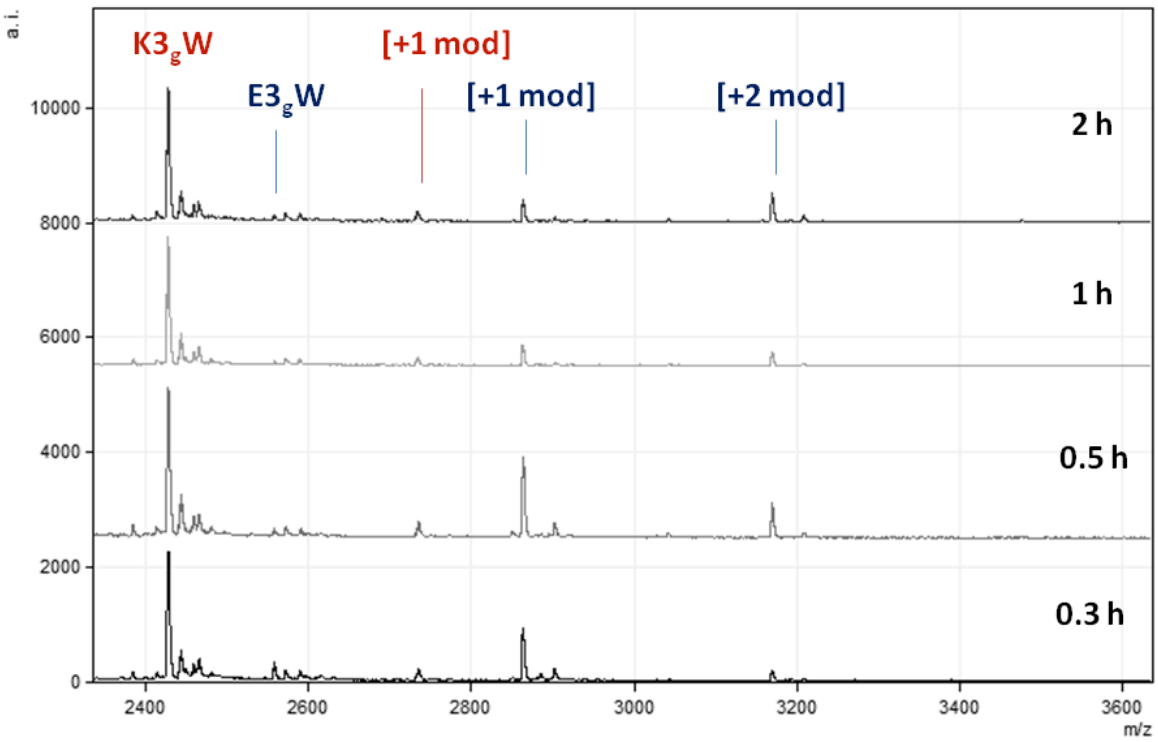


Figure S-9. MALDI-TOF MS spectra of the competitive modification of peptide **K3$_g$W** and **W$_{random}$** with **K3$_{a,e}$Rh$_2$** (10 mol %) and 70 equiv diazo **1**. See Table S-1 for conversion data.

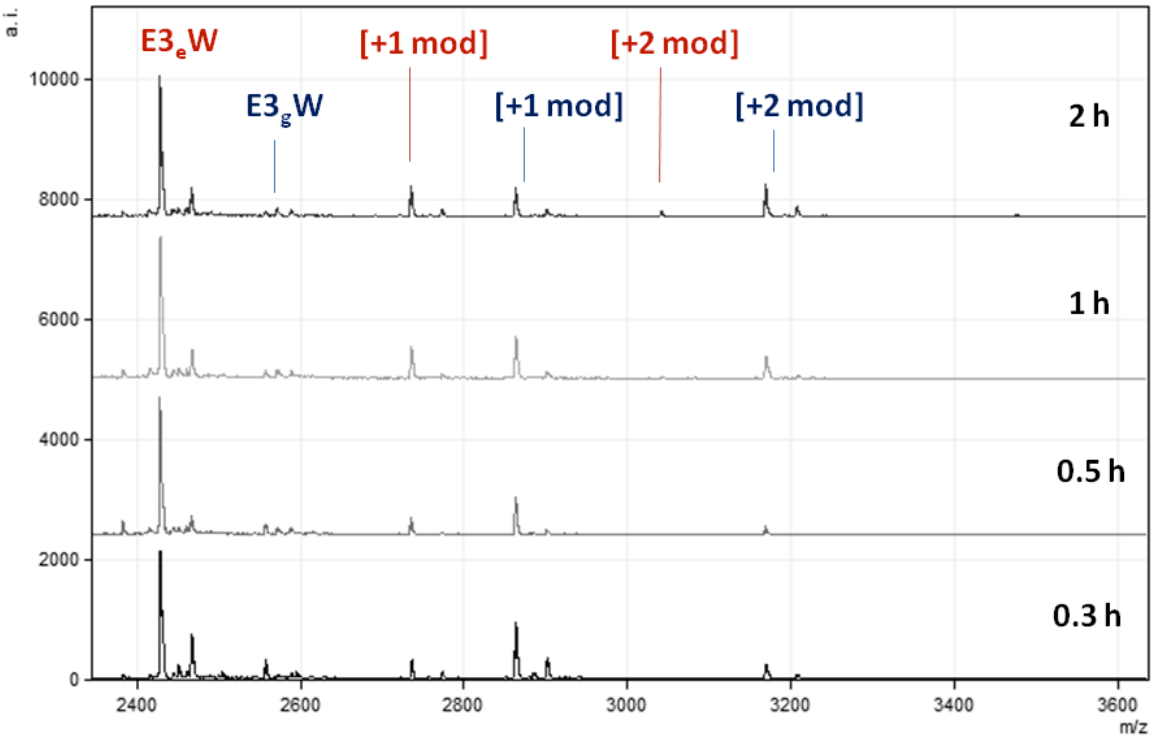


Figure S-10. MALDI-TOF MS spectra of the competitive modification of peptide **E3$_e$W** and **E3$_g$W** with **K3$_{a,e}$Rh$_2$** (10 mol %) and 70 equiv diazo **1**. See Table S-1 for conversion data.

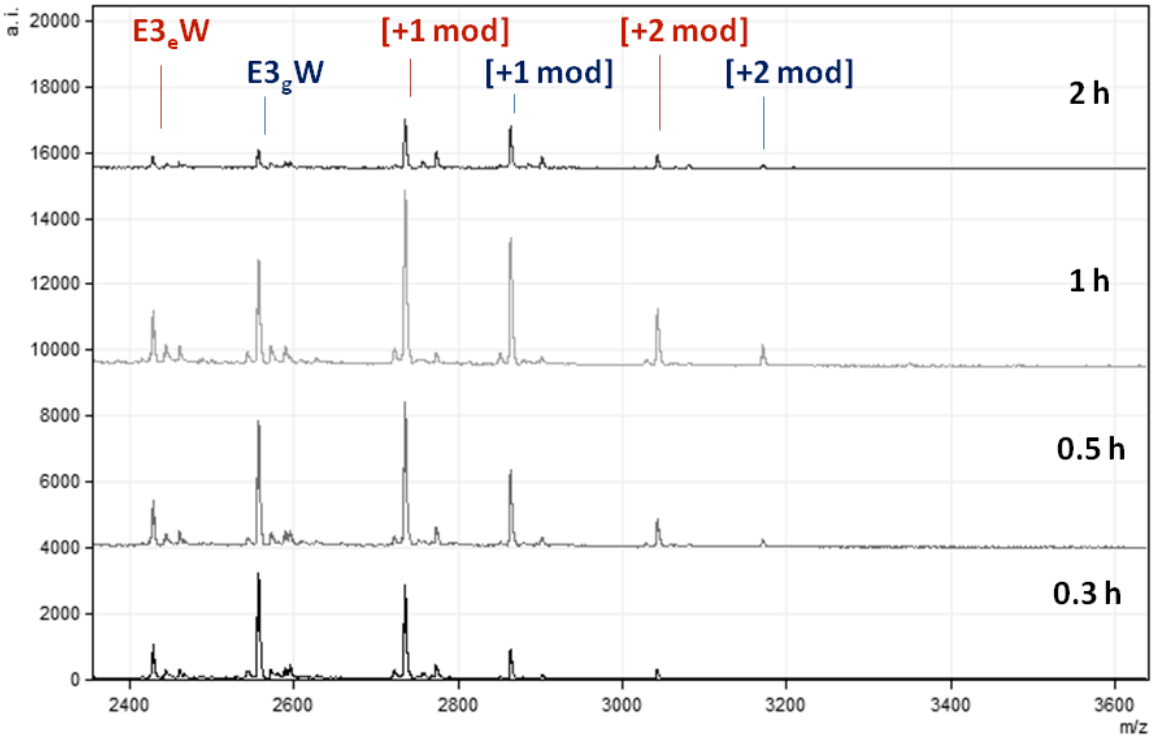


Figure S-11. MALDI-TOF MS spectra of the competitive modification of peptide **E3$_e$W** and **E3$_g$W** with **K3$_{g,d}$Rh$_2$** (10 mol %) and 70 equiv diazo **1**. See Table S-1 for conversion data.

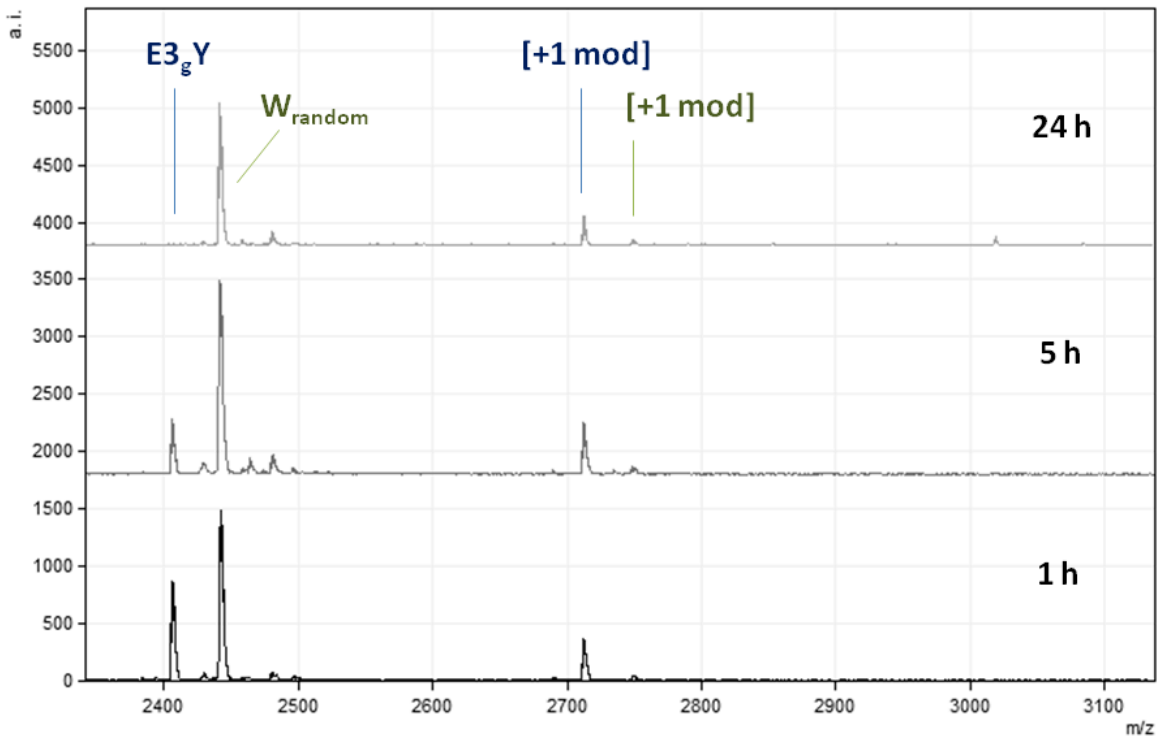


Figure S-12. MALDI-TOF MS spectra of the competitive modification of peptide **E3$_g$Y** and **W$_{random}$** with **K3$_{a,e}$Rh$_2$** (20 mol %) and 70 equiv diazo **1**. See Table S-1 for conversion data.

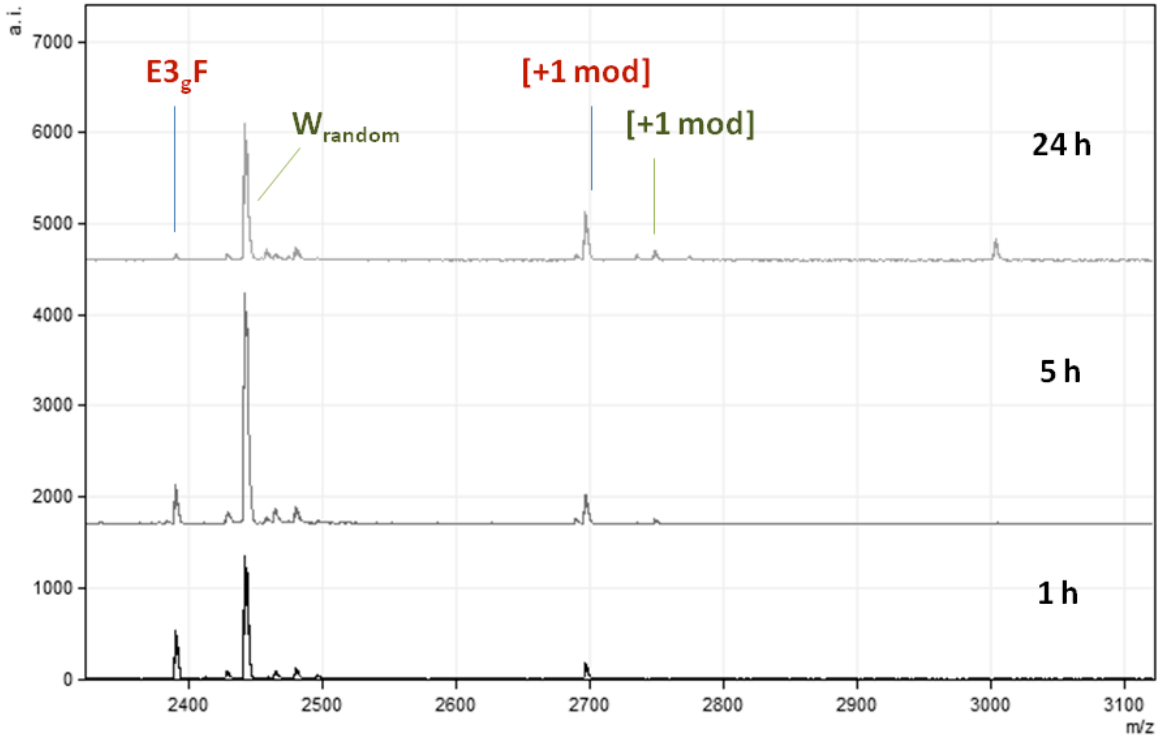


Figure S-13. MALDI-TOF MS spectra of the competitive modification of peptide **E3$_g$F** and **W$_{random}$** with **K3$_{a,e}$Rh$_2$** (20 mol %) and 70 equiv diazo **1**. See Table S-1 for conversion data.

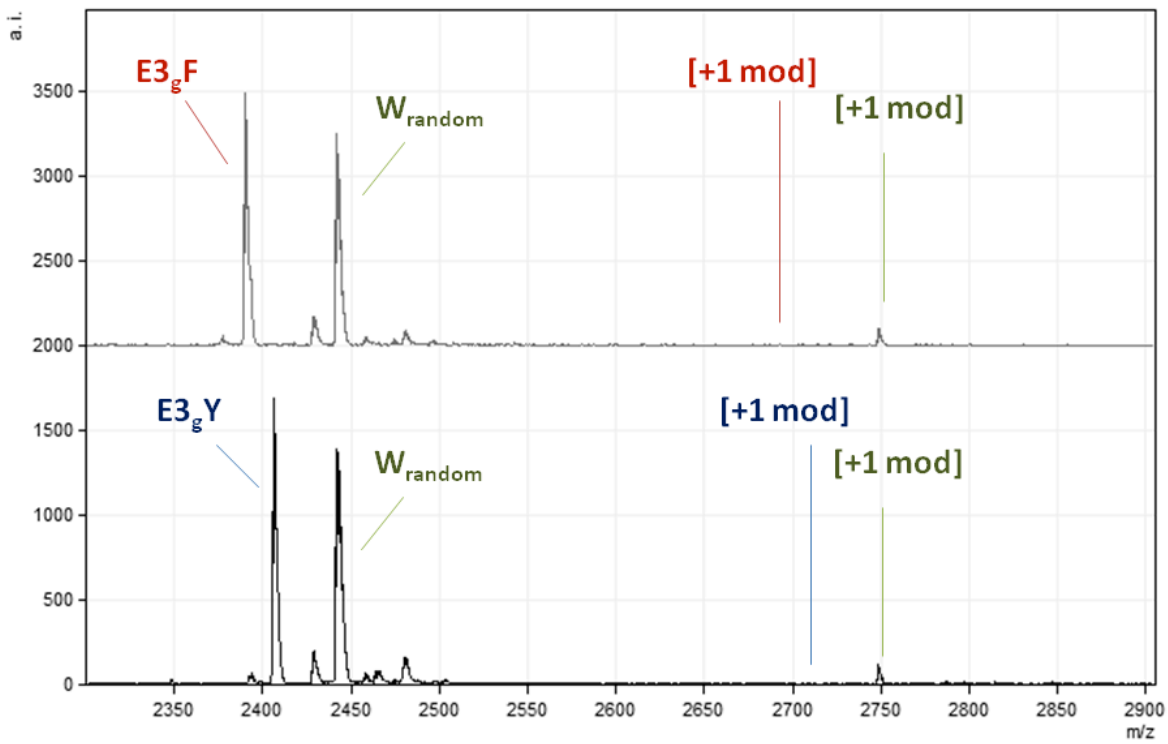
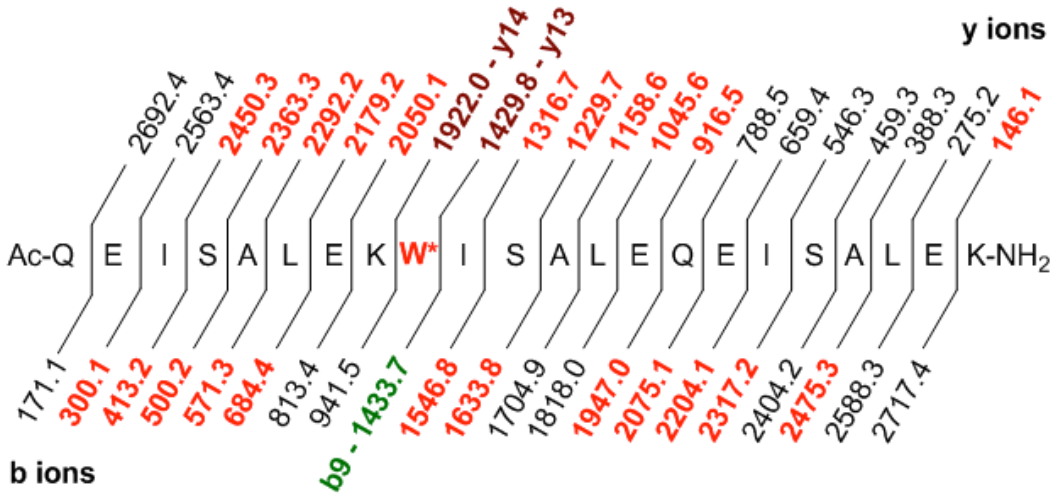


Figure S-14. MALDI-TOF MS spectra of the competitive modification of peptide **E3$_g$X** (X = Y,F) and **W$_{random}$** with the small molecule catalyst **Rh$_2$OAc$_4$** (200 mol %) and 70 equiv diazo **1**. See Table S-1 for conversion data.

MS/MS Modification Analysis: E3gW(+1mod)



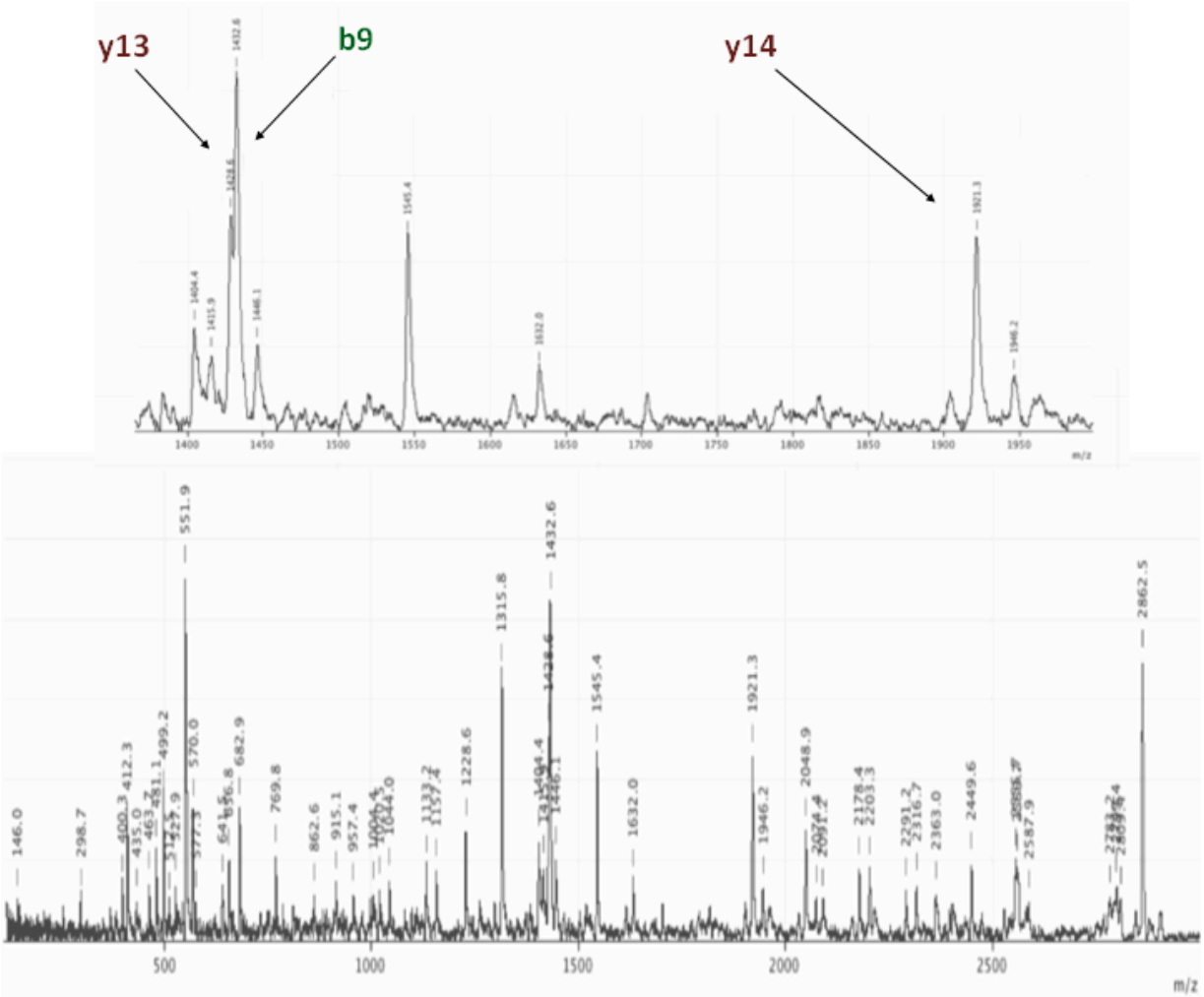


Figure S-15. MALDI-TOF MS/MS spectrum and y/b ion fragmentation diagram of singly modified peptide **E3$_g$W**. Located ions (2 Da tolerance) are highlighted in red or green in the fragmentation diagram. Fragmentation analysis was performed on a crude reaction aliquot removed at 2 h (see Figure S-5A). Data analysis was performed with the mMass program.[4]

MS/MS Modification Analysis: E3gW(+2mod)

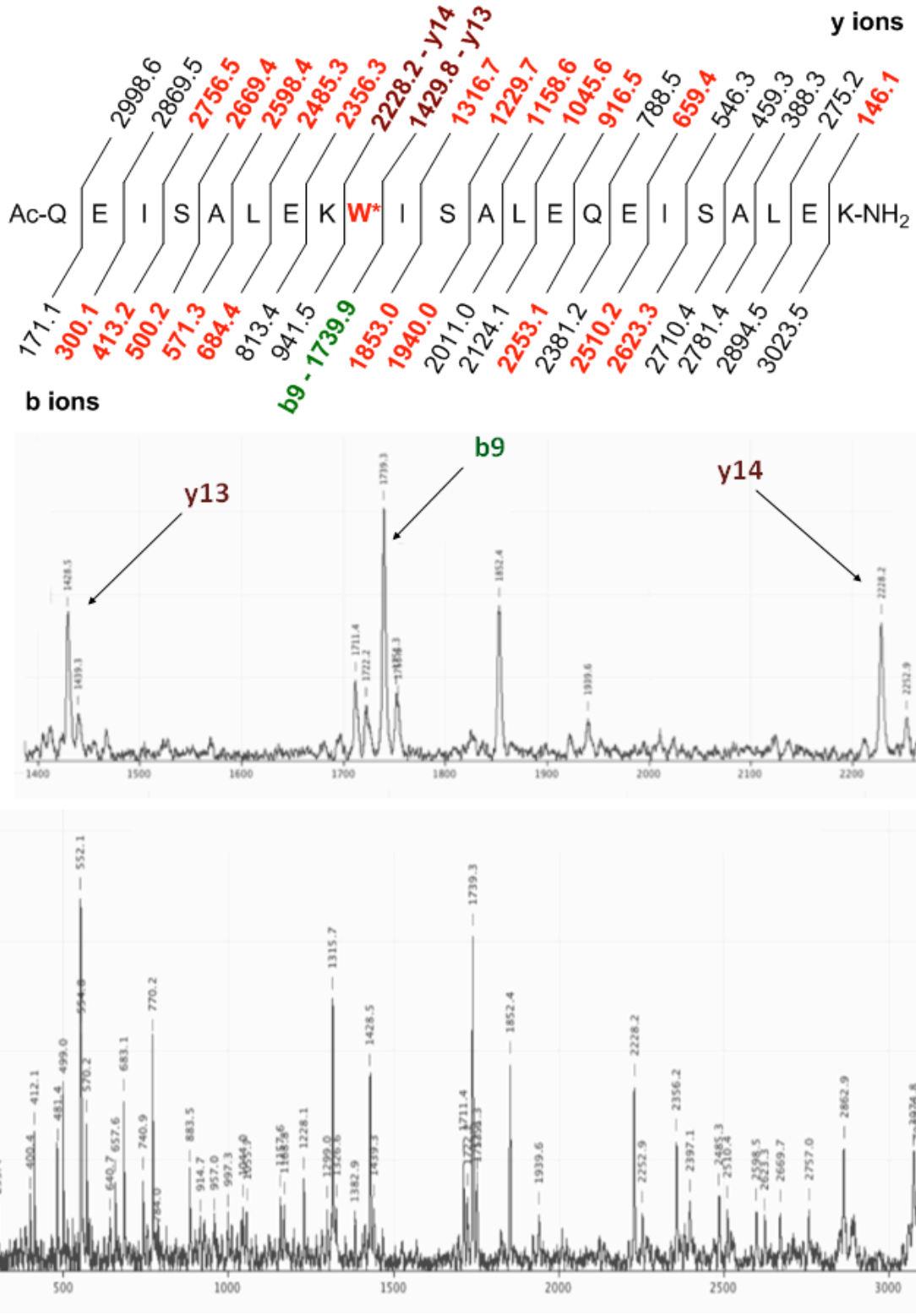


Figure S-16. MALDI-TOF MS/MS spectrum and y,b-ion fragmentation diagram of doubly modified peptide **E3$_g$W**. Located ions (2 Da tolerance) are highlighted in red or green in the fragmentation diagram. Fragmentation analysis performed on a crude reaction aliquot removed at 2 h (see Figure S-5A). Data analysis was performed with the mMass program.[4]

MS/MS Modification Analysis: E3gY(+1mod)

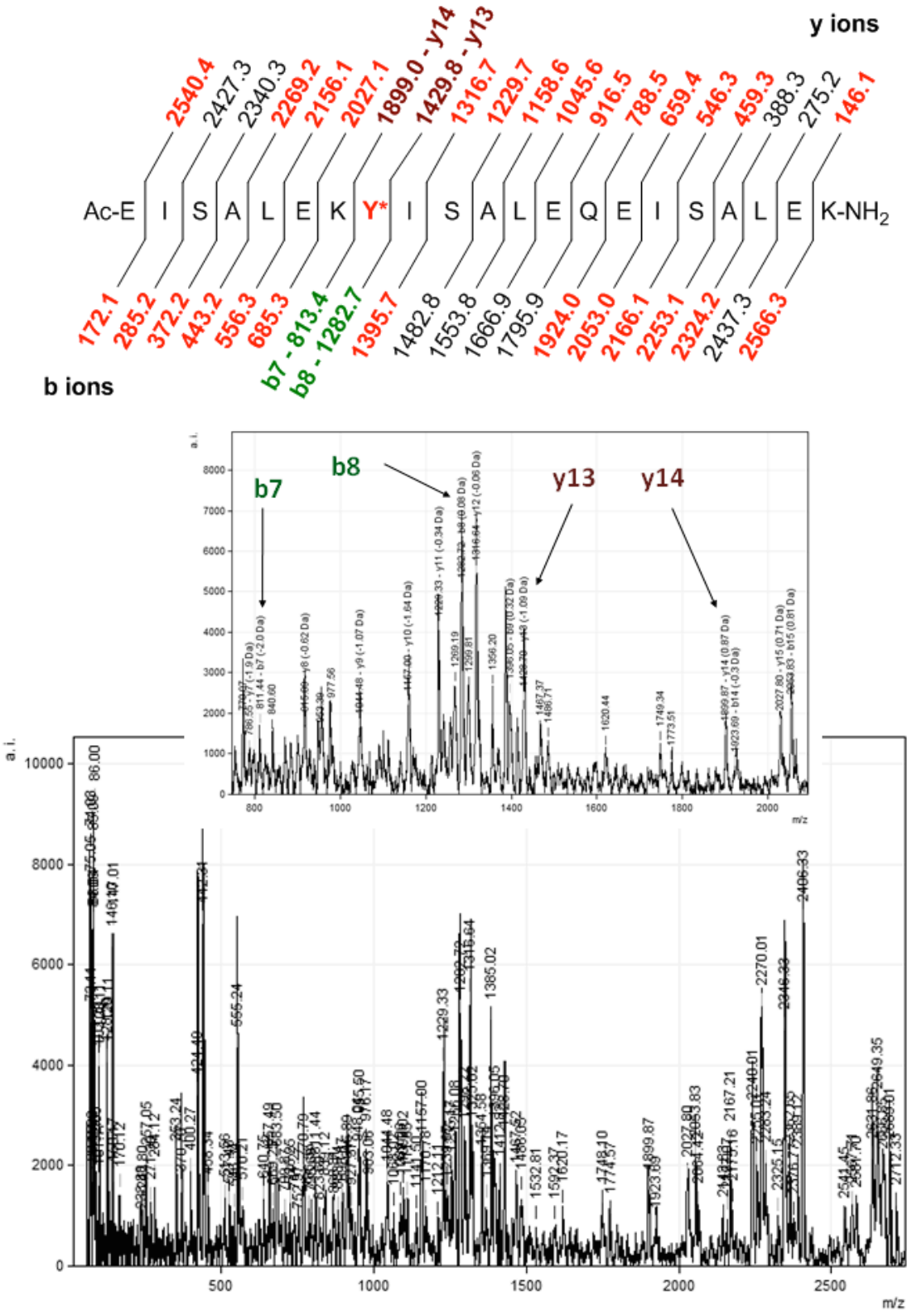


Figure S-17. MALDI-TOF MS/MS spectrum and y,b-ion fragmentation diagram of singly modified peptide **E3$_g$Y**. Located ions (2 Da tolerance) are highlighted in red or green in the fragmentation diagram. Fragmentation analysis performed on a crude reaction aliquot removed at 24 h (see Figure S-10). Data analysis was performed with the mMass program.[4]

MS/MS Modification Analysis: **E3gF(+1mod)**

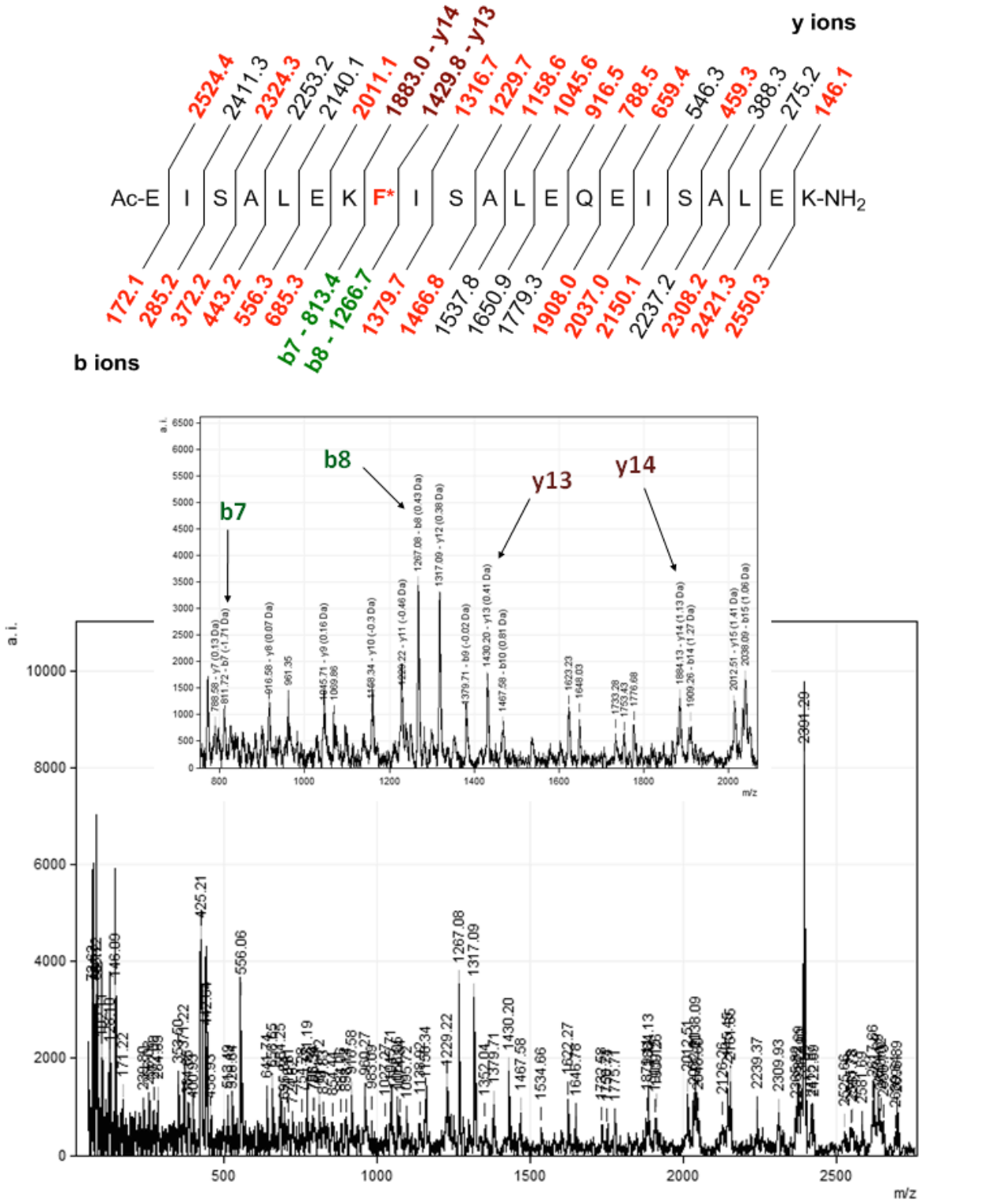
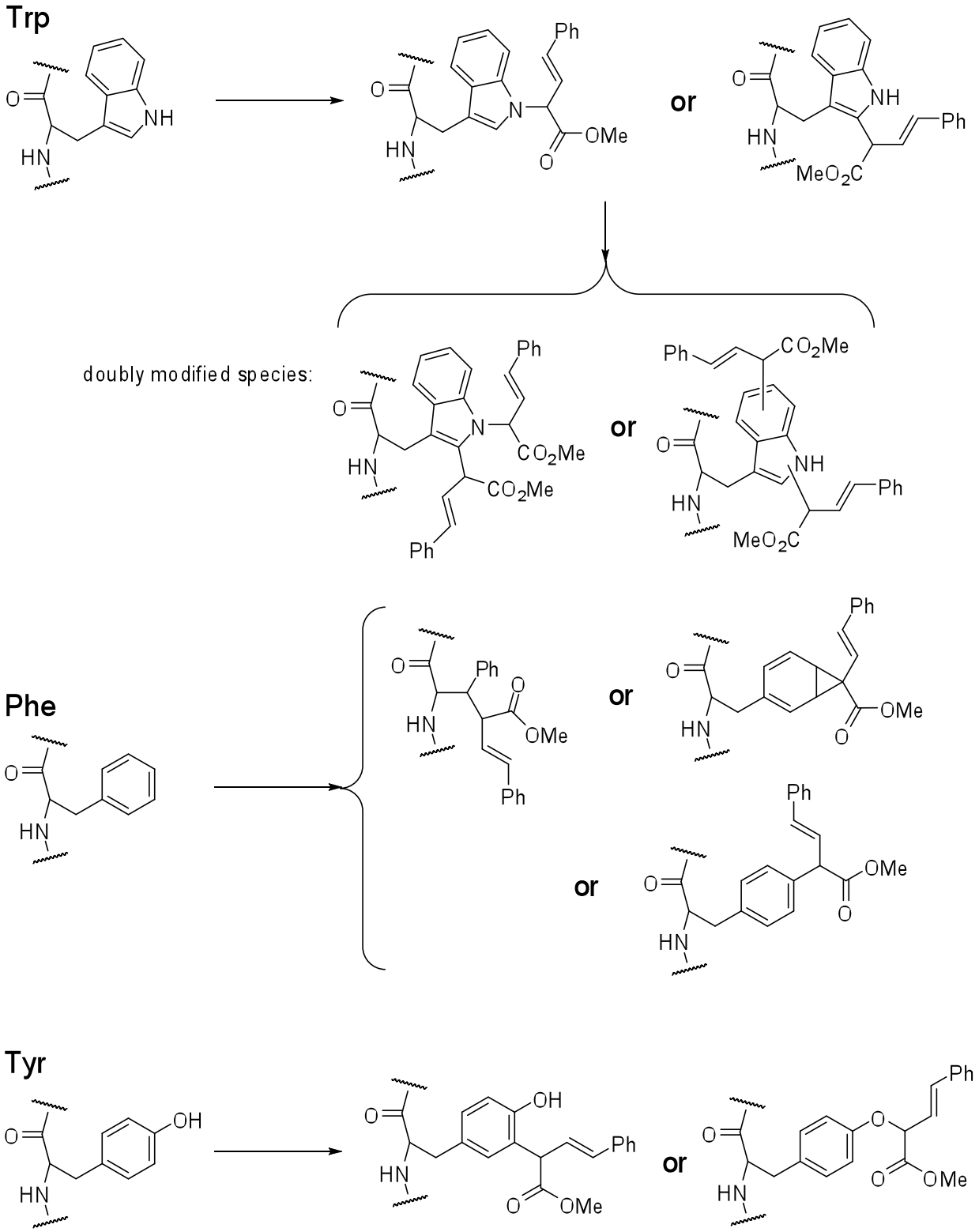


Figure S-18. MALDI-TOF MS/MS spectrum and y,b-ion fragmentation diagram of singly modified peptide **E3$_g$F**. Located ions (2 Da tolerance) are highlighted in red or green in the fragmentation diagram. Fragmentation analysis performed on a crude reaction aliquot removed at 24 h (see Figure S-11). Data analysis was performed with the mMass program.[4]

Scheme S-1. Plausible Chemical Structures for Aromatic Side Chain Modification



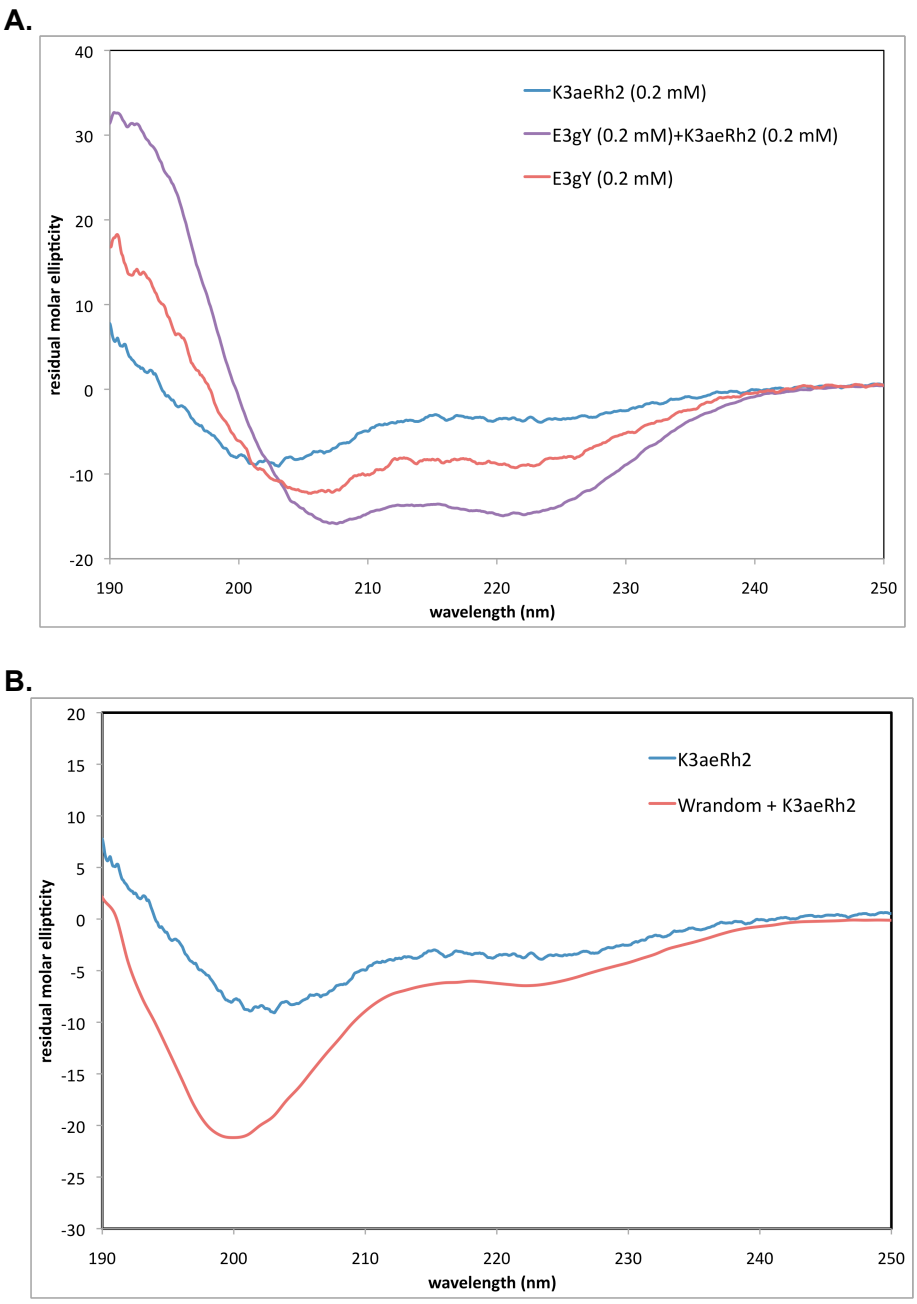


Figure S-19. CD spectra illustrating (**A**) induction of helical structure upon mixing equimolar amounts of $\mathbf{K3_{a,e}Rh_2}$ (200 μM) and **E3gY** (200 μM) and (**B**) the lack of helical structure upon mixing equivalent amounts of $\mathbf{K3_{a,e}Rh_2}$ (125 μM) and $\mathbf{W_{random}}$ (125 μM) in aq buffer. The features at 206 and 222 nm in the equimolar mixture in **A** are consistent with coiled-coil association of E3-K3 peptides.[5]

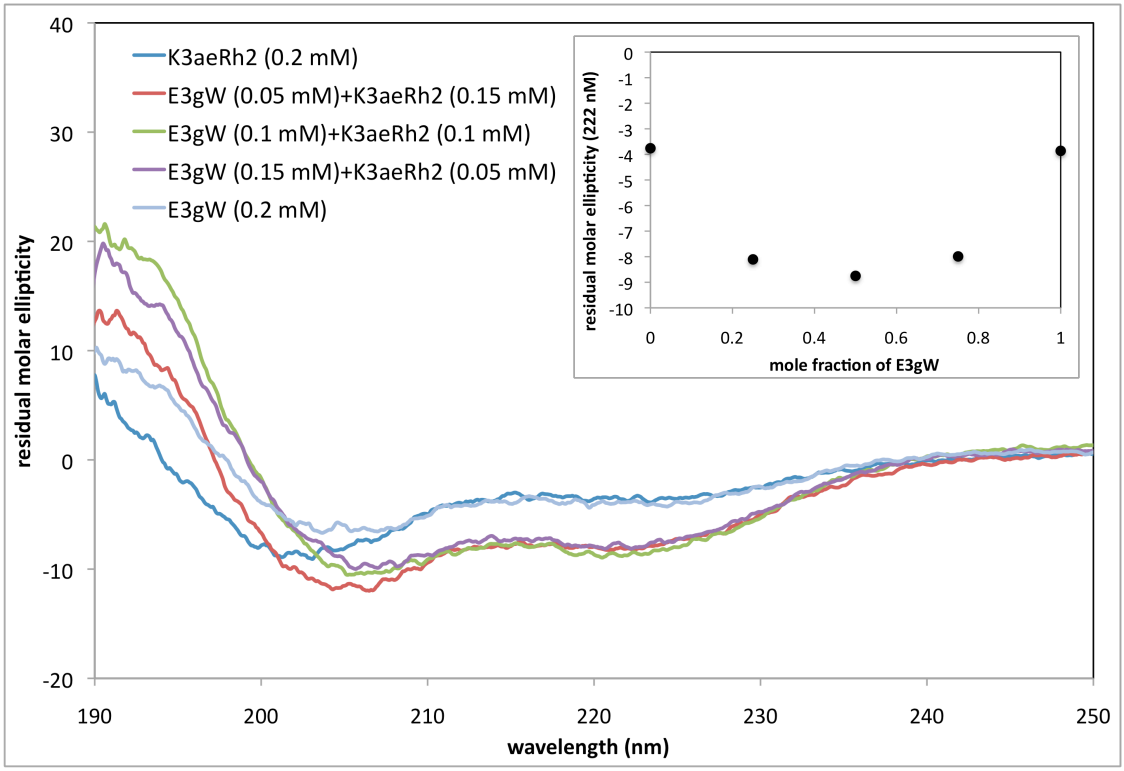


Figure S-20. CD spectra of mixtures of $\mathbf{K3_{a,e}Rh_2}$ and **E3gW** maintained at constant concentration (200 μM) in aqueous buffer. Inset: Job plot based on residual molar ellipticity at 222 nm.

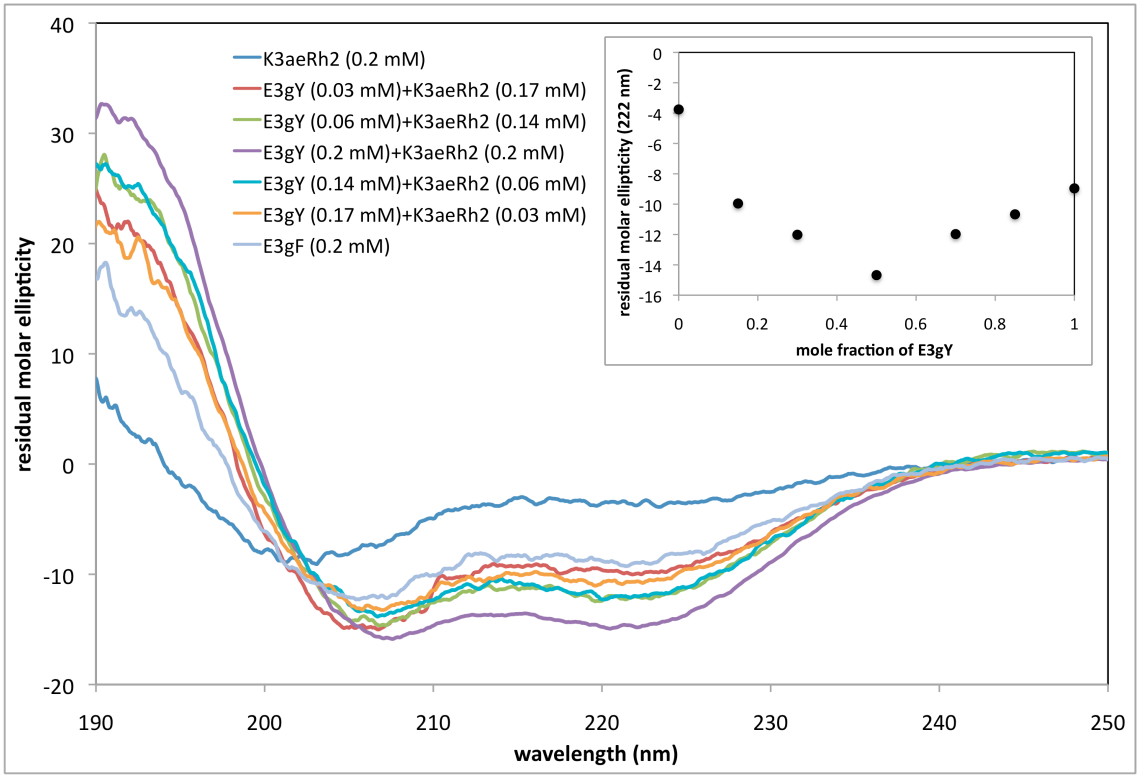


Figure S-21. CD spectra of mixtures of $\mathbf{K3_{a,e}Rh_2}$ and **E3gY** maintained at constant concentration (200 μM) in aqueous buffer. The equimolar spectrum was acquired at 400 μM total peptide concentration. Inset: Job plot based on residual molar ellipticity at 222 nm.

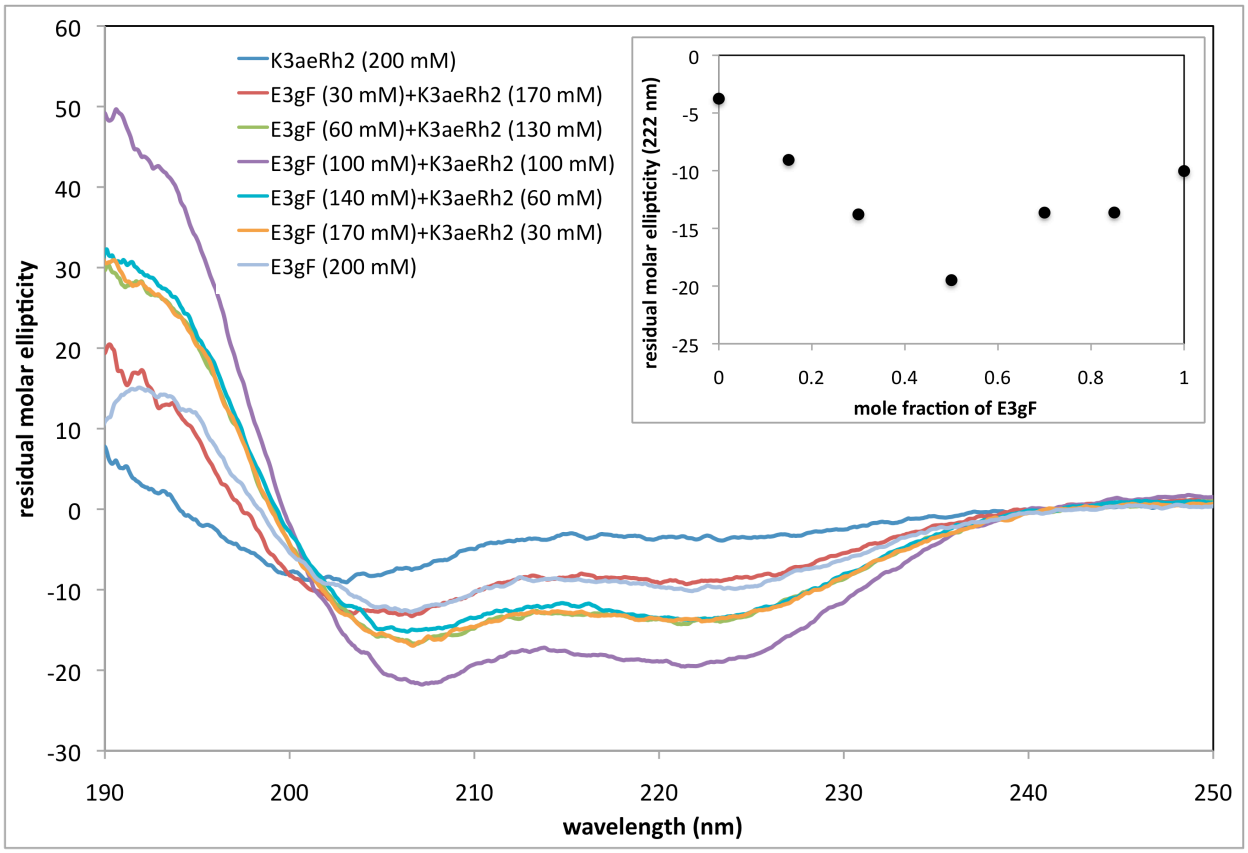


Figure S-22. CD spectra of mixtures of $\mathbf{K3_{a,e}Rh_2}$ and **E3gF** maintained at constant concentration (200 μM) in aqueous buffer. Inset: Job plot based on residual molar ellipticity at 222 nm.

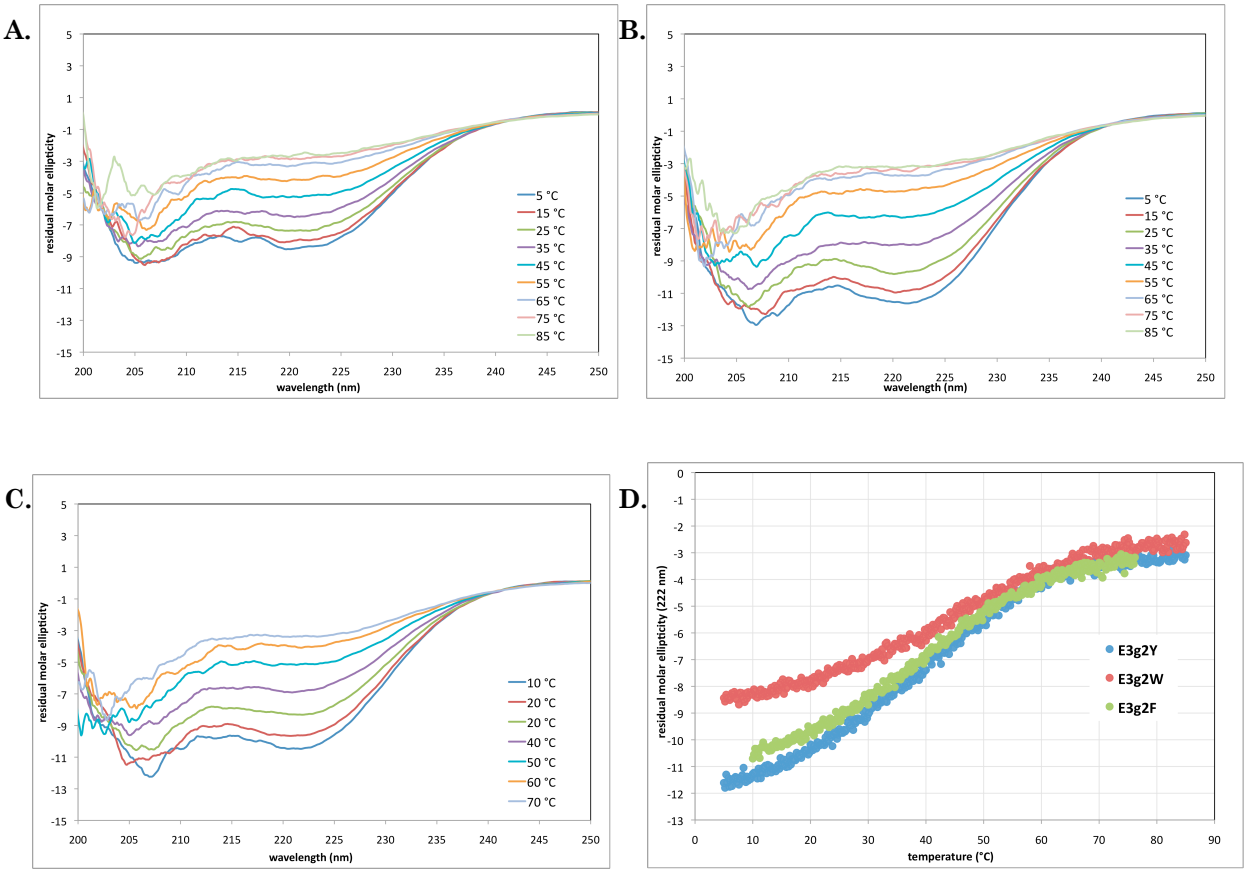


Figure S-23. Thermal denaturation CD spectra for equimolar mixtures of **K3$_{a,e}$Rh$_2$** (100 μM) and **E3$_g$X** (X = W (**A**), Y (**B**), F (**C**)) (100 μM) in aq buffer. (**D**) The melting temperature (T_m) measured at 222 nm for all coiled-coil assemblies is approximately 43 °C. The thermal denaturation was not reversible, presumably due to dirhodium metallopeptide decomposition, via irreversible ligand substitution, at elevated temperatures.

Analytical data for peptides.

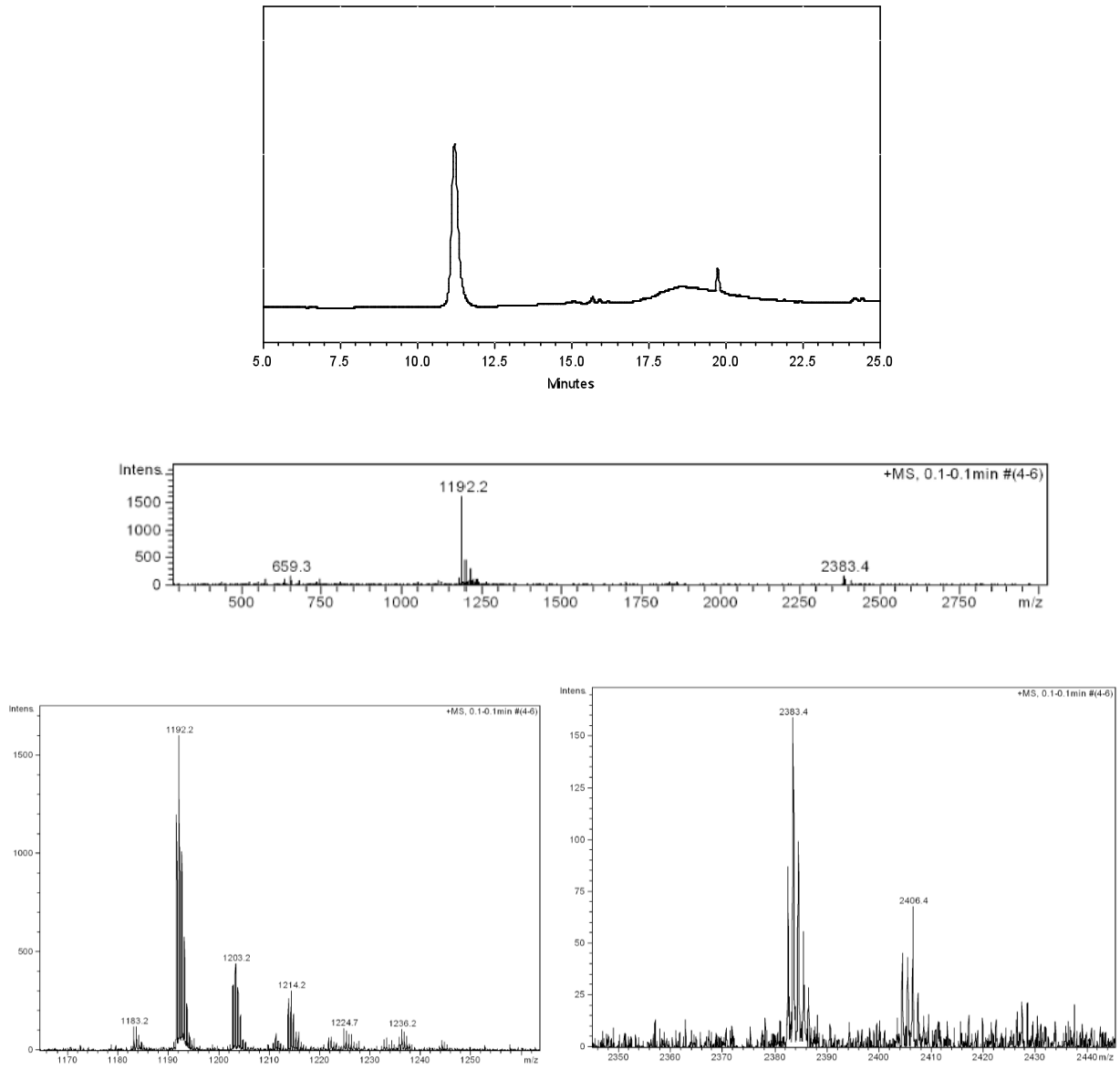


Figure S-24. HPLC trace and ESI-MS spectrum of the peptide **K3$_{a,e}$EE** (sequence: Ac-KISALKQKESALEQKISALKQ-NH_2). Calculated mass $[M+H]^+$: 2382.4 and $[M+2H]^{2+}$: 1191.7; found: 2382.4 and 1191.7.

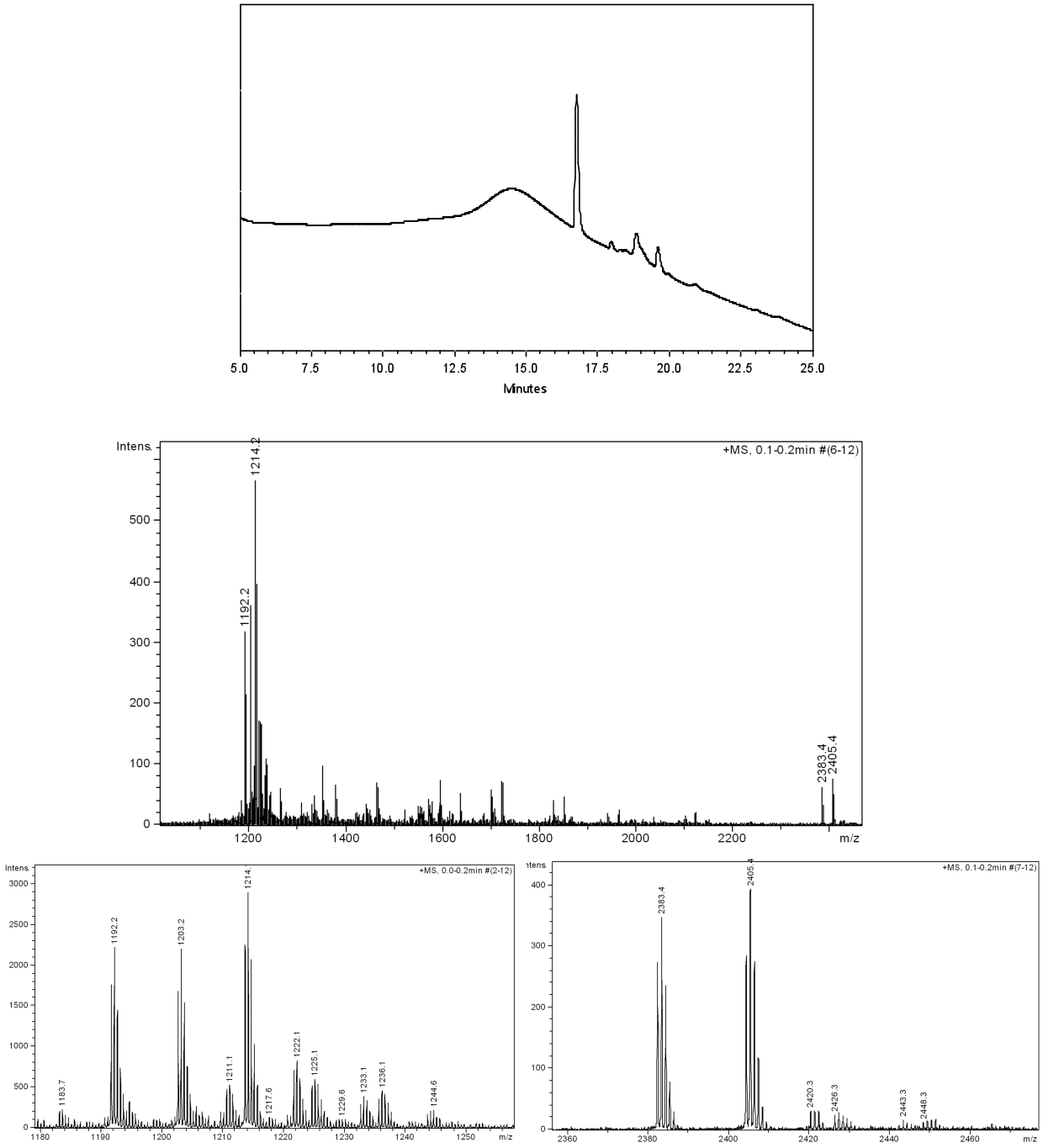


Figure S-25. HPLC trace and ESI-MS spectrum of the peptide **K3$_{g,d}$EE** (sequence: Ac-KISALKQEISAEKQKISALKQ-NH_2). Calculated mass $[M+H]^+$: 2382.4 and $[M+2H]^{2+}$: 1191.7; found: 2382.4 and 1191.7.

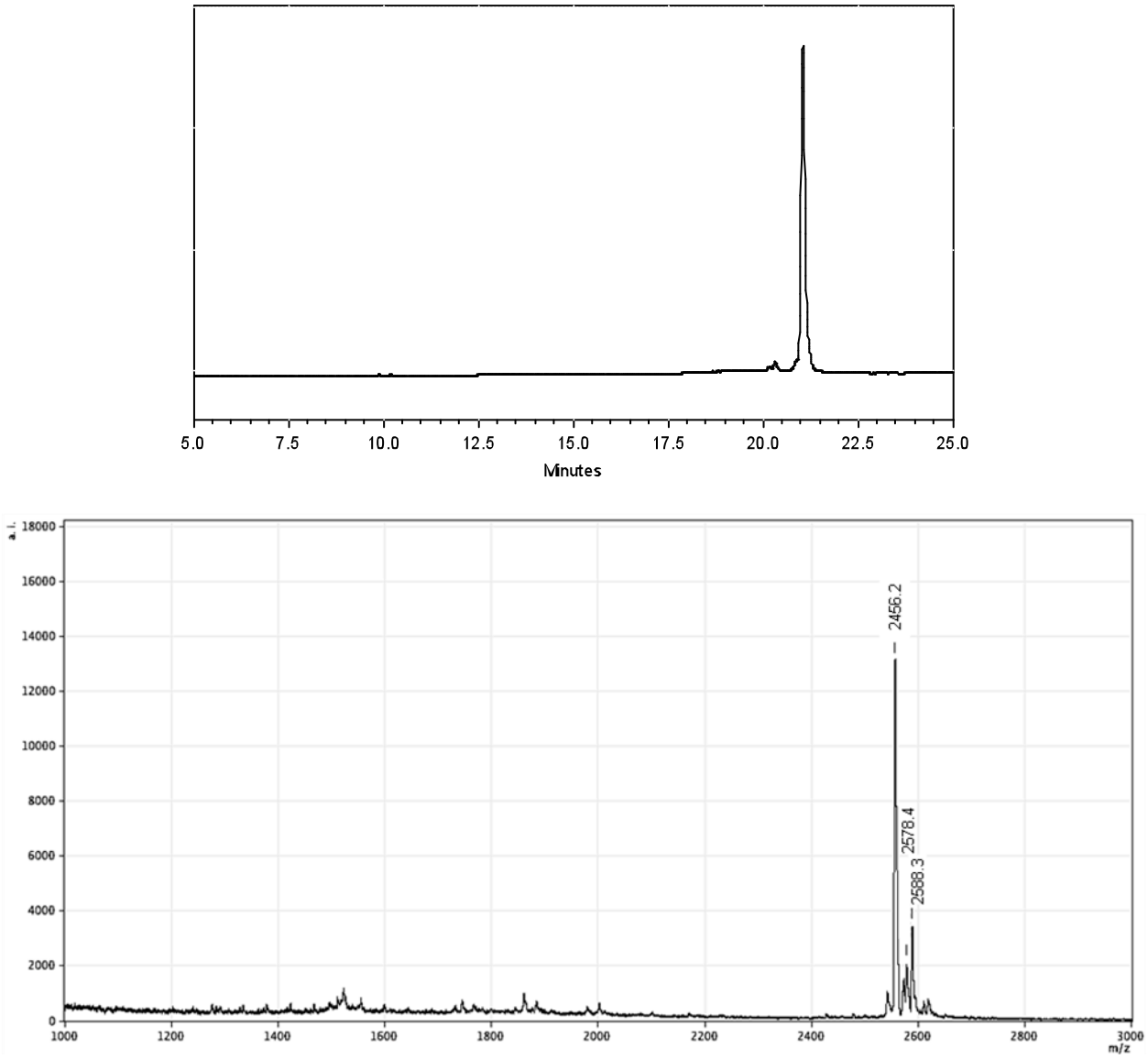
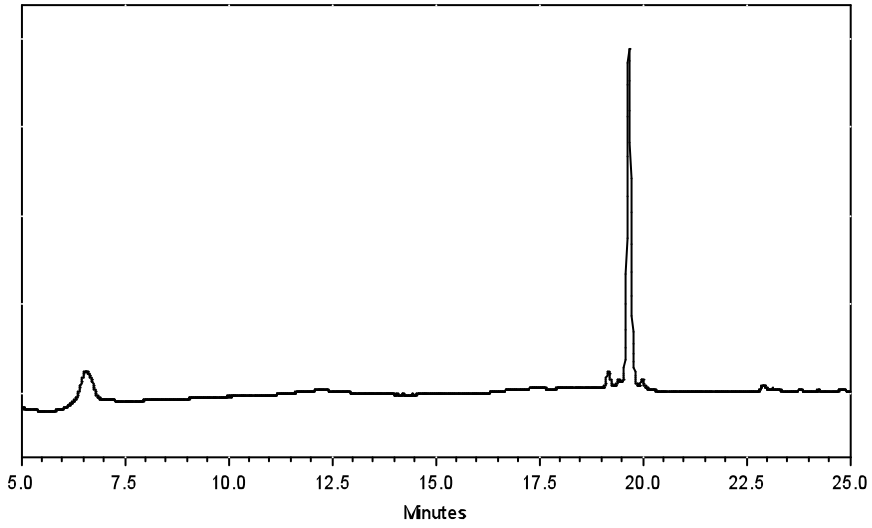


Figure S-26. HPLC trace and MALDI-TOF MS spectrum of peptide **E3$_g$W**. Calculated mass $[M+H]^+$: 2556.4; found: 2556.2.



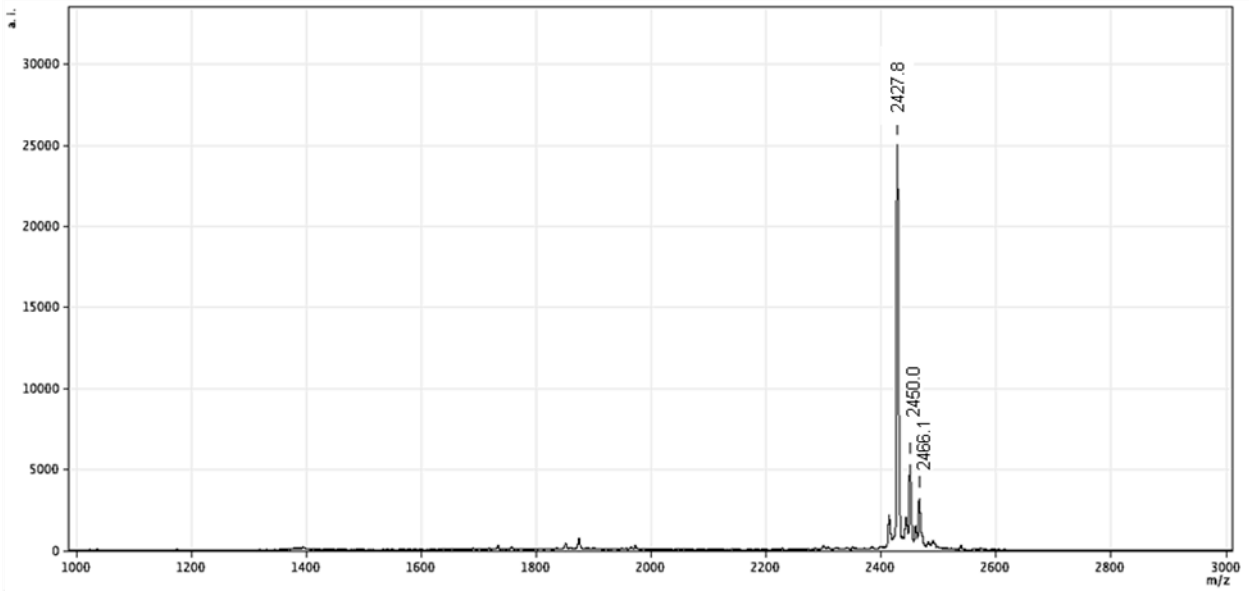


Figure S-27. HPLC trace and MALDI-TOF MS spectrum of peptide **E3$_e$W**. Calculated mass $[M+H]^+$: 2428.3; found: 2427.8.

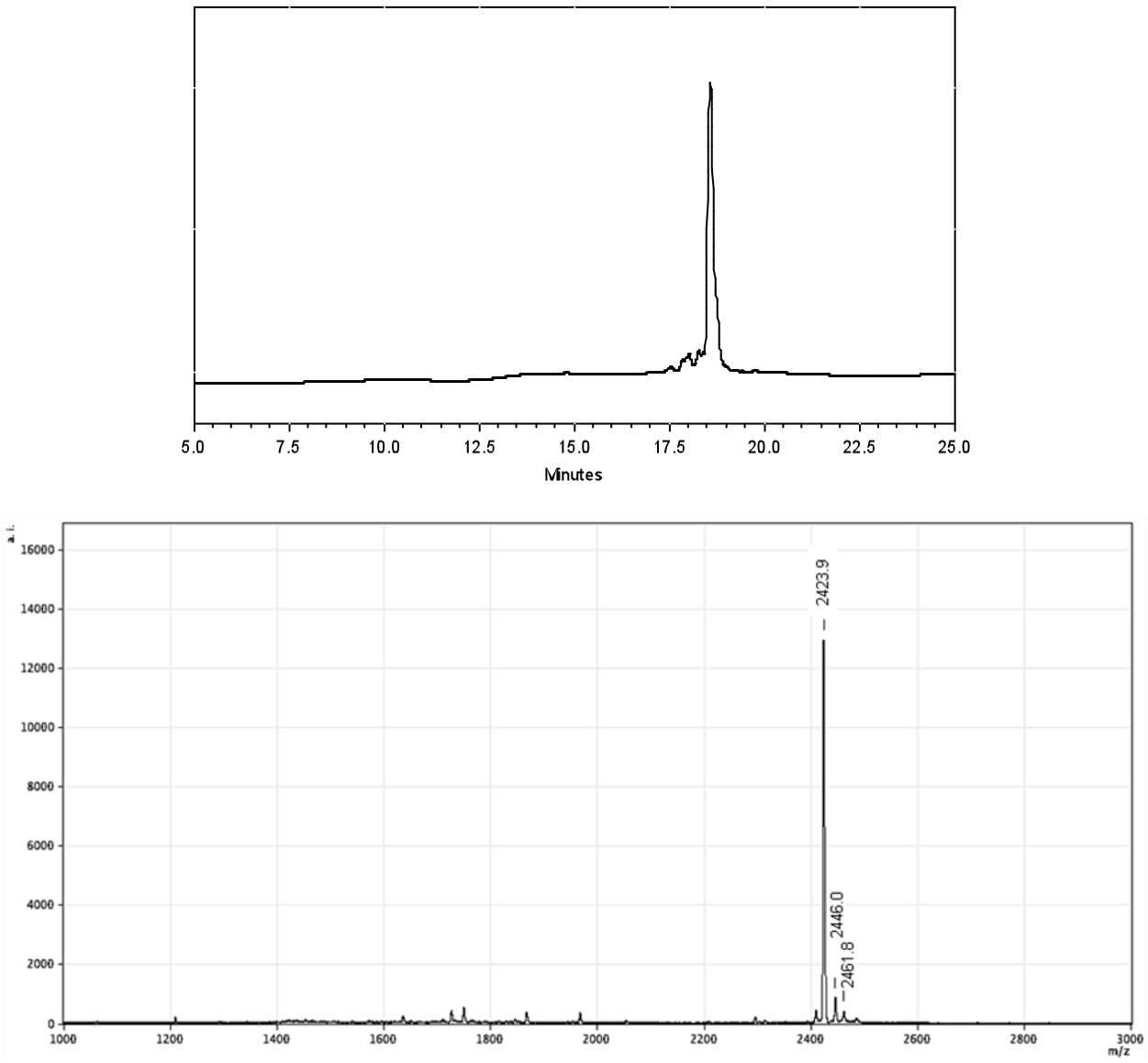
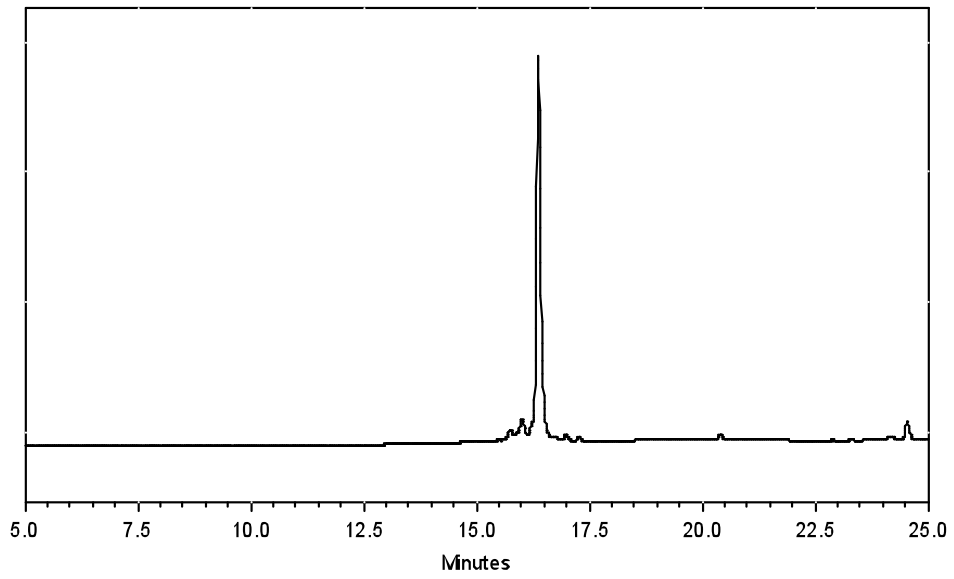


Figure S-28. HPLC trace and MALDI-TOF MS spectrum of peptide **K3$_g$W**. Calculated mass $[M+H]^+$: 2425.5; found: 2423.9



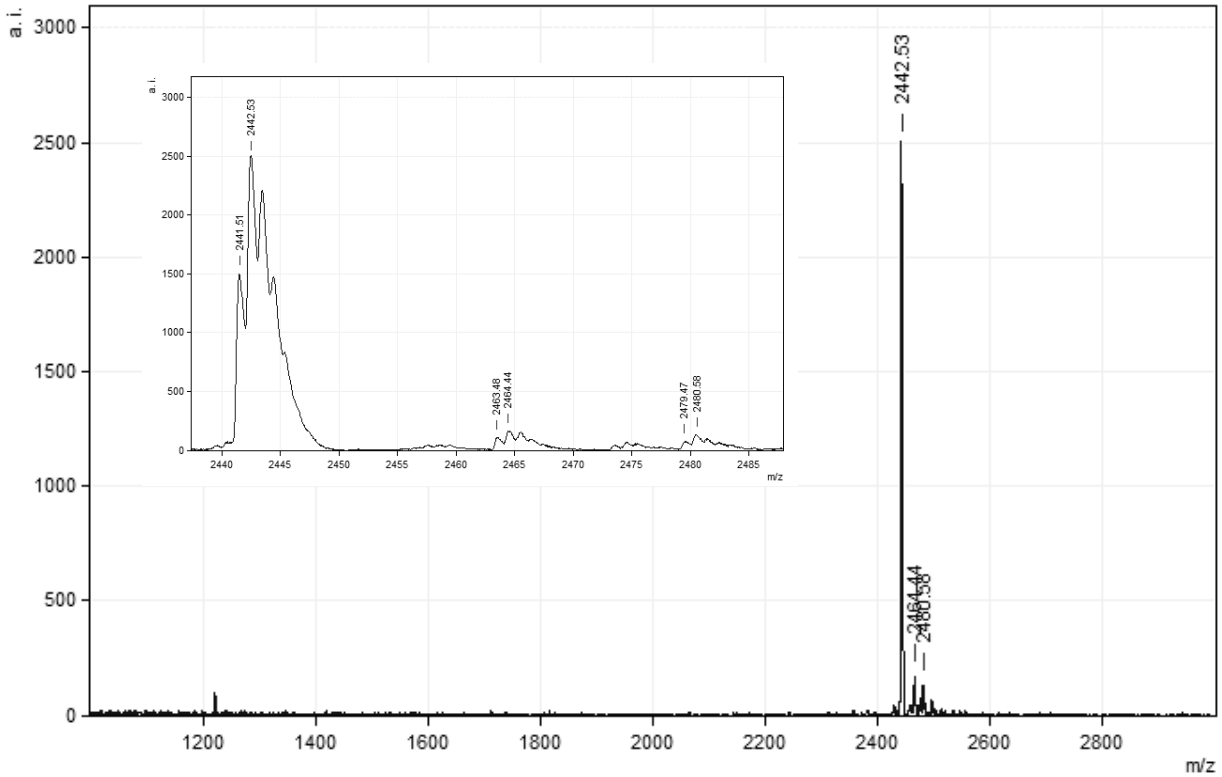


Figure S-29. HPLC trace and MALDI-TOF MS spectrum of peptide $\mathbf{W}_{\mathbf{random}}$. Calculated mass $[M+H]^+$: 2441.2; found: 2441.5.

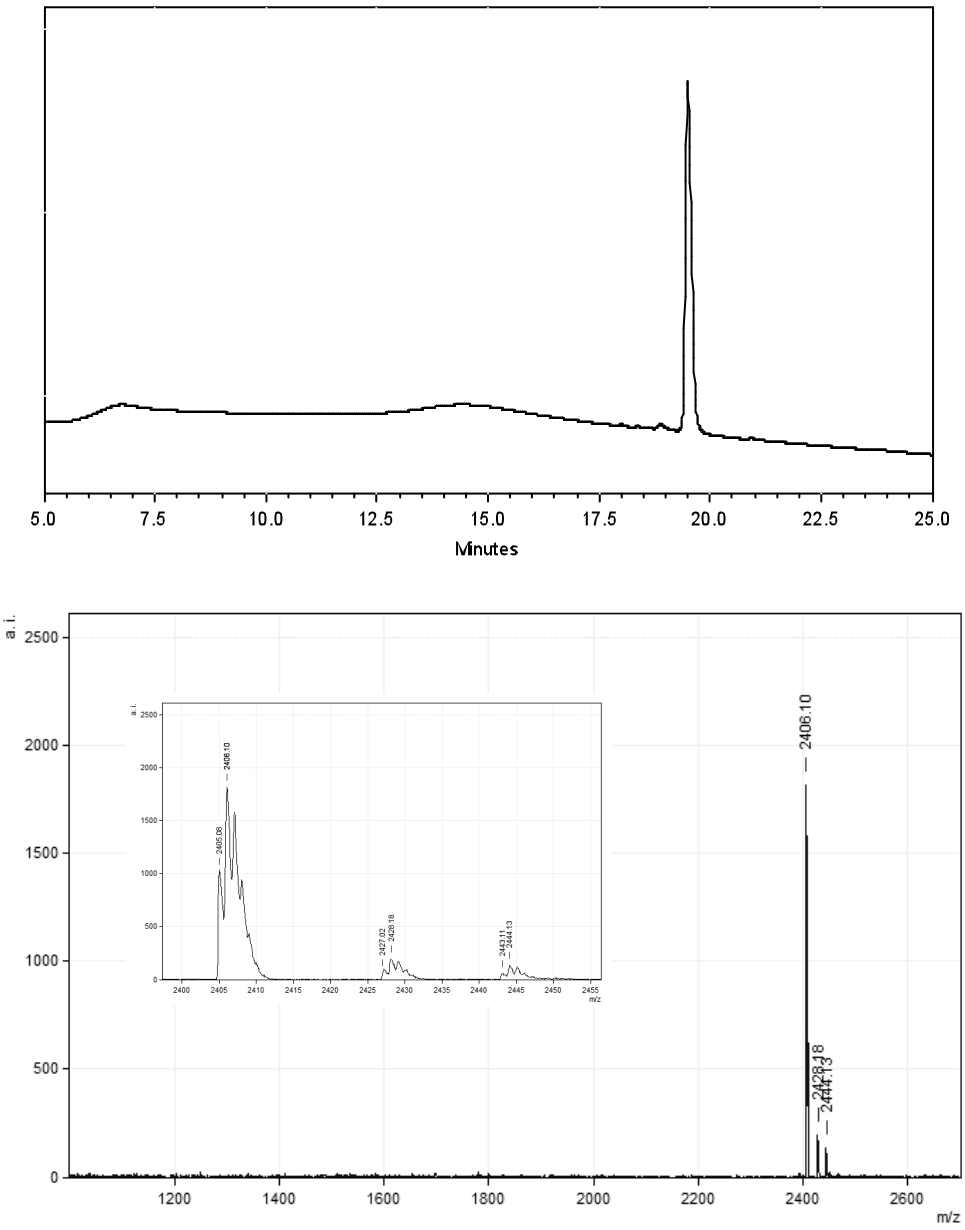


Figure S-30. HPLC trace and MALDI-TOF MS spectrum of peptide **E3$_g$Y**. Calculated mass $[M+H]^+$: 2405.3; found: 2405.1.

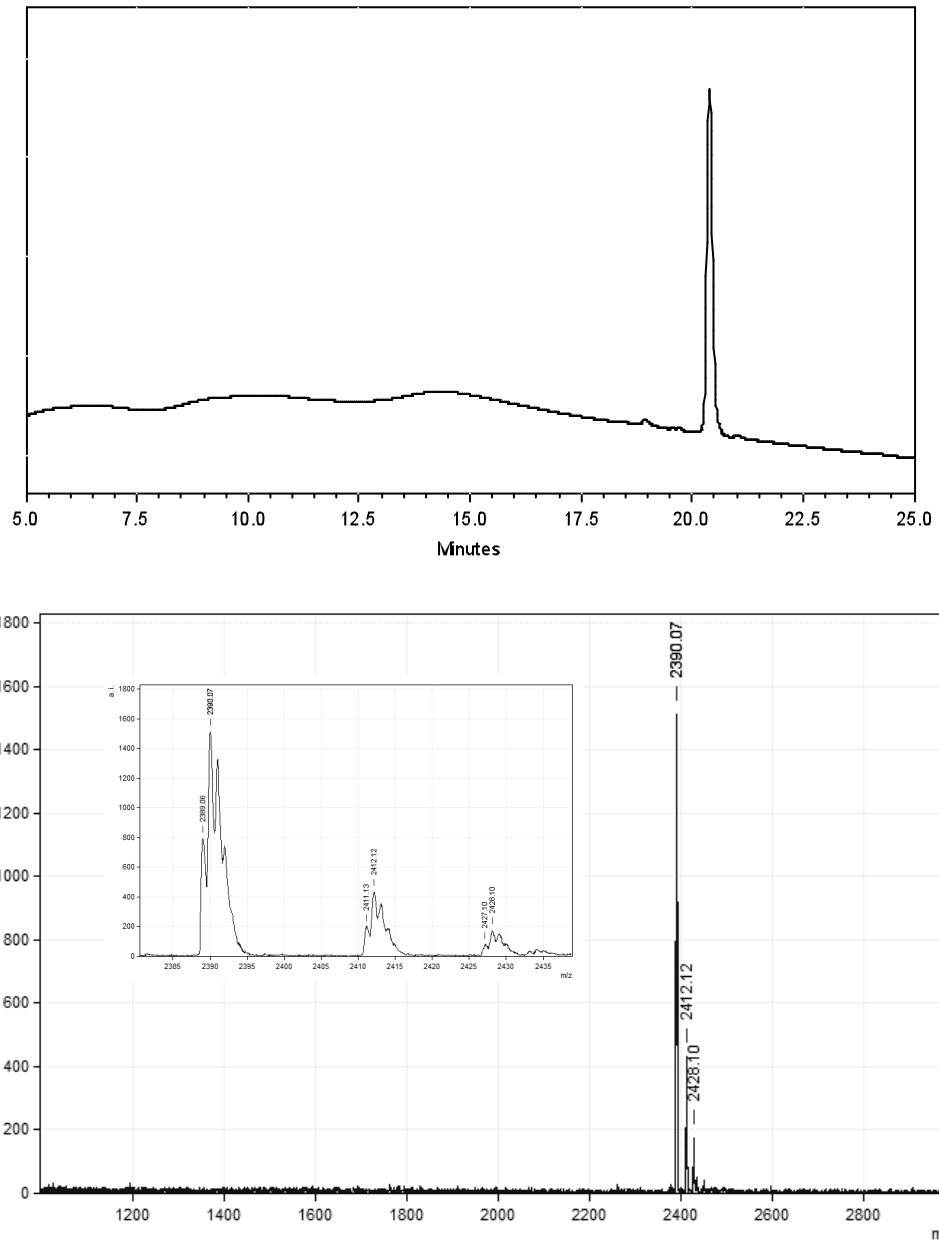


Figure S-31. HPLC trace and MALDI-TOF MS spectrum of peptide **E3$_g$F**. Calculated mass $[M+H]^+$: 2389.3; found: 2389.1.

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