Electronic Supplementary Material (ESI) for ChemComm.

Electronic Supplementary Information for:

Inactivation of myostatin by photo-oxygenation using catalyst-functionalized peptides

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General procedures

Myostatin was purchased from R&D Systems, Inc. (Minneapolis, MN, USA). Substance P and Aβ1-42 isopeptide were purchased from Peptide Institute, Inc. (Osaka, Japan). Aβ1-42 isopeptide, which contains an ester structure between the Gly25-Ser26 sequence, converts to intact Aβ1-42 *in situ* under a neutral pH through an *O*-to-*N* intramolecular acyl migration rapidly ($t_{1/2}$~10 s).[S1] Methylene blue was purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Other reagents and solvents were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan), Nacalai Tesque, Inc., Peptide Institute, Inc., Sigma-Aldrich Co. LLC (St. Louis, MO, USA), Tokyo Chemical Industries Co., Ltd. (Tokyo, Japan), Wako Pure Chemical Industries, Ltd. (Osaka, Japan), and Watanabe Chemical Industries, Ltd. (Hiroshima, Japan). All chemicals were used as received.

^{1}H NMR were measured in $CDCl_3$ or $(CD_3)_2CO$ solution, and referenced to tetramethylsilane (0.00 ppm) using Bruker AVANCE-III (400 MHz) spectrometer. ^{13}C NMR were measured in $CDCl_3$ solution, and referenced to residual solvent peaks of $CDCl_3$ (77.05 ppm) using Bruker AVANCE-III (400 MHz) spectrometer. LRMS and HRMS spectra were recorded on Shimadzu Biotech LCMS-2020 and Waters MICRO MASS LCT premier, respectively. MALDI-TOF MS spectra were recorded on Shimadzu Biotech AXIMA Assurance using α-cyano-4-hydroxy cinnamic acid as a matrix. Column chromatography was performed on silica gel 60N (spherical, neutral, 40–50 μm, Kanto Chemical Co., Inc.), and automatic flash chromatography system was performed on Biotage Isolera One with SNAP Ultra column. TLC was performed on Merck TLC silica gel 60 F245 (0.25 mm), and compounds were visualized with UV light, *p*-anisaldehyde stain, phosphomolybdic acid stain and ninhydrin stain. Analytical HPLC was carried out on a reverse-phase column (Nacalai Tesque COSMOSIL Protein-R or 5C18-AR-II, 4.6ID x 150 mm, or Hitachi LaChrom II C18, 4.6ID X 150 mm) using a linear gradient of acetonitrile in 0.1% aqueous trifluoroacetic acid with a flow rate of 1.0 mL min^{-1}, and detected at UV 230 or 200-800 nm. Preparative HPLC was carried out on a reverse-phase column (Waters SunFire Prep C18 OBD, 19ID X 150 mm) using a linear gradient of acetonitrile in 0.1% aqueous trifluoroacetic acid with a flow rate of 5.0 mL min^{-1}, and detected by absorbance at 230 or 700 nm. Infrared (IR) spectra were recorded on JASCO FT/IR 4100 spectrometer. Melting points were measured with Yanaco MP–500P melting point apparatus.

Peptide **4**

Fmoc-based solid-phase peptide synthesis was automatically performed following the protocol of a peptide synthesizer Prelude (Gyros Protein Technologies AB, Uppsala, Sweden). On a Fmoc-NH SAL Resin **9** (0.37 mmol/g, 108 mg, 0.04 mmol), Fmoc-amino acids or 3,3-diphenylpropionic acid (0.20 mmol) were sequentially coupled using an HATU (0.20 mmol)-HOAt (0.20 mmol)-DIPEA (0.40 mmol) method for 30 min in DMF after removing each Fmoc group with 20% piperidine-DMF for 10 X 2 min. The obtained protected peptide-bound resin **10** (278.3 mg) was treated with TFA/1,3-dimethoxybenzene[S2]/triisopropylsilane (92.5:5:2.5 v:v:v) for 90 min, concentrated, precipitated with diethyl ether, and followed by preparative RP-HPLC purification in a 0.1% aqueous TFA-CH_3CN system to obtain peptide **4** as a TFA salt (white amorphous, 34.1 mg, 24%).

HRMS *m/z* $[M+H]^+$ found 2930.7212 (calcd. for $C_{136}H_{225}N_{40}O_{32}$ 2930.7209); purity >95% (HPLC with a binary solvent system: a linear gradient of CH_3CN (5-85%, 40 min) in 0.1% aqueous TFA at a flow rate of 1.0 mL/min, detected at UV 230 nm, t_R = 19.0 min).

Compound **12a**

1,2,3,4-Tetrahydroquinoline (**11**, 1.0 mL, 8.0 mmol) and methyl 2-bromoacetate (0.9 mL, 9.6 mmol) were added to a solution of KI (3.2 g, 19.1 mmol) and K_2CO_3 (2.2 g, 15.9 mmol) in CH_3CN (4 mL) at room temperature under argon protection. The suspension was stirred for 38 h under reflux. After being cooled to room temperature, the reaction mixture was filtered, concentrated in vacuo, diluted with EtOAc, washed with water and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by flash column chromatography (hexane/EtOAc = 95/5 to 60/40) to afford **12a** as a yellow oil (1.5 g, 93%).

^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.05 (t, *J* = 8.5 Hz, 1H), 6.99 (d, *J* = 7.3 Hz, 1H), 6.64 (td, *J* = 7.3, 0.8 Hz, 1H), 6.42 (d, *J* = 8.0 Hz, 1H), 4.03 (s, 2H), 3.74 (s, 3H), 3.42 (t, *J* = 5.6 Hz, 2H), 2.82 (t, *J* = 6.4 Hz, 2H), 1.99-2.05 (m, 2H); LRMS (ESI): *m/z* calcd for $[M+H]^+$ 206.1, found 206.1.

Compound **12b**

Following the same procedure for compound **12a**, **11** (500 μL, 4.0 mmol), methyl 6-bromohexanoate (750 μL, 4.8 mmol), KI (1.6 g, 9.6 mmol) and K_2CO_3 (1.1 g, 8.0 mmol) afforded **12b** as a reddish brown oil (897 mg, 91%).

^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.06 (t, *J* = 7.8 Hz, 1H), 6.95 (d, *J* = 7.5 Hz, 1H), 6.55-6.58 (m, 2H), 3.70 (s, 3H), 3.21-3.30 (m, 4H), 2.77 (t, *J* = 6.3 Hz, 2H), 2.35 (t, *J* = 7.5 Hz, 2H), 1.93-1.99 (m, 2H), 1.58-1.72 (m, 4H), 1.35-1.43 (m, 2H); LRMS (ESI): *m/z* calcd for $[M+H]^+$ 262.1, found 262.1.

Compound **13a**

12a (2.2 g, 10.6 mmol) dissolved in THF (8 mL) was added to a suspension of LAH (501 mg, 13.2 mmol) in dry THF (2 mL) at 0 °C under argon protection. The mixture was then stirred for 20 h at room temperature. After stirring, the mixture was diluted with EtOAc, washed with saturated aqueous Rochelle salt (10 mL X 3), dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by silica gel flash column chromatography (hexane/EtOAc = 2/1) to afford **13a** as a yellow oil (1.6 g, 86%).

^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.07 (t, *J* = 8.5 Hz, 1H), 6.98 (d, *J* = 7.0 Hz, 1H), 6.71 (d, *J* = 8.0 Hz, 1H), 6.63 (td, *J* = 7.3, 0.9 Hz, 1H), 3.84 (q, *J* = 5.8 Hz, 2H), 3.47 (t, *J* = 5.8 Hz, 2H), 3.34 (t, *J* = 5.6 Hz, 2H), 2.80 (t, *J* = 6.4 Hz, 2H), 1.96-2.02 (m, 2H), 1.74 (t, *J* = 6.0 Hz, 1H); LRMS (ESI): *m/z* calcd for $[M+H]^+$ 178.1, found 178.0.

Compound **13b**

Following the same procedure for compound **13b**, **12b** (2 g, 7.7 mmol) and LAH (868.4 mg, 3.72 mmol) afforded **13b** as a yellow oil (2.4 mg, 65%).

^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.06 (t, *J* = 8.5 Hz, 1H), 6.96 (d, *J* = 7.3 Hz, 1H), 6.56 (t, *J* = 7.4 Hz, 2H), 3.67 (t, *J* = 6.7 Hz, 2H), 3.24-3.30 (m, 4H), 2.77 (t, *J* = 6.3 Hz, 2H), 1.93-1.99 (m, 2H), 1.58-1.64 (m, 5H), 1.38-1.46 (m, 4H); LRMS (ESI): *m/z* calcd for $[M+H]^+$ 234.1, found 234.2.

Compound **14a**
To a solution of **13a** (0.1 g, 0.6 mmol) in CH_2Cl_2 (10 mL) were added triethylamine (157 μL, 1.13 mmol) and methanesulfonyl chloride (66 μL, 0.85 mmol) at 0 °C under argon protection, and then stirred at room temperature for 2 h. The mixture was diluted with 0.1 M aqueous HCl at 0 °C and extracted with CH_2Cl_2. The organic layer was washed with water and brine, dried over Na_2SO_4, filtered, and concentrated in vacuo to obtain a crude mesylated compound.
To a solution of the crude (173 mg) in DMF was added sodium azide (183 mg, 2.8 mmol) at room temperature under argon protection, and then stirred for 2.5 h at 60 °C. After stirring, the mixture was cooled to room temperature and DMF was removed under reduced pressure. The residue was purified by silica gel flash column chromatography (hexane/EtOAc = 95/5 to 60/40) to afford **14a** as an orange oil (54.5 mg, 2 steps: 42%).
^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.08 (t, *J* = 7.7 Hz, 1H), 6.99 (d, *J* = 6.8 Hz, 1H), 6.58-6.65 (m, 2H), 3.47-3.52 (m, 4H), 3.38 (t, *J* = 5.6 Hz, 2H), 2.79 (t, *J* = 6.3 Hz, 2H), 1.96-2.02 (m, 2H); LRMS (ESI): *m/z* calcd for $[M+H]^+$ 203.1, found 203.1.

Compound **14b**
Following the same procedure for compound **14a**, **13b** (868 mg, 3.7 mmol), methanesulfonyl chloride (866 μL, 11.2 mmol) and triethylamine (2.1 mL, 14.9 mmol) afforded the crude mesylated compound as a brown oil (1.16 g). The crude and sodium azide (1.21 mg, 18.6 mmol) afforded **14b** as an orange oil (647 mg, 2 steps: 67%).
^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.06 (t, *J* = 8.4 Hz, 1H), 6.96 (d, *J* = 6.8 Hz, 1H), 6.55-6.58 (m, 2H), 3.24-3.31 (m, 6H), 2.77 (t, *J* = 6.4 Hz, 2H), 1.93-1.99 (m, 2H), 1.61-1.68 (m, 4H), 1.36-1.48 (m, 4H); LRMS (ESI): *m/z* calcd for $[M+H]^+$ 259.1, found 259.1.

Compound **15a**
To a solution of **14a** (875 mg, 4.3 mmol) in CH_2Cl_2 (5 mL) were added DMF (3.4 mL, 43.2 mmol) and $POCl_3$ (1.33 mL, 14.3 mmol), and the mixture was stirred under argon protection at room temperature for 90 min. The reaction mixture was diluted with water, neutralized to pH ca. 1 with aqueous NaOH on ice and extracted with EtOAc. The organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by flash column chromatography (hexane/EtOAc = 95/5 to 60/40) to afford **15a** as a yellow oil (304 mg, 31%).
^{1}H-NMR (400 MHz, $CDCl_3$) δ 9.72 (s, 1H), 7.59 (dd, *J* = 8.5, 2.0 Hz, 1H), 7.52 (s, 1H), 6.63 (d, *J* = 8.5 Hz, 1H), 3.54-3.62 (m, 4H), 3.50 (t, *J* = 5.6 Hz, 2H), 2.84 (t, *J* = 6.1 Hz, 2H), 1.99-2.05 (m, 2H); LRMS (ESI): *m/z* calcd for $[M+H]^+$ 231.1, found 231.0.

Compound **15b**
Following the same procedure for compound **15a**, **14b** (647 mg, 2.5 mmol), DMF (1.9 mL, 25 mmol) and $POCl_3$ (768 μL, 8.3 mmol) afforded **15b** as a yellow oil (284 mg, 40%).
^{1}H-NMR (400 MHz, $CDCl_3$) δ 9.67 (s, 1H), 7.56 (dd, *J* = 8.7, 1.9 Hz, 1H), 7.48 (s, 1H), 6.58 (d, *J* = 8.5 Hz, 1H), 3.41 (t, *J* = 5.6 Hz, 2H), 3.29-3.37 (m, 4H), 2.81 (t, *J* = 6.3 Hz, 2H), 1.95-2.01 (m, 2H), 1.61-1.71 (m, 4H), 1.39-1.51 (m, 4H); LRMS (ESI): *m/z* calcd for $[M+H]^+$ 287.1, found 287.1.

Compound **5a**
To a solution of **16**[S3] (103 mg, 0.224 mmol), **15a** (112 mg, 0.487 mmol), and tributyl borate (1.2 mL, 4.48 mmol) in toluene (7 mL) was added ^{n}BuNH$_2$ (17.8 μL, 0.179 mmol) under argon protection. The mixture was heated at 65 ˚C and stirred 20.5 h. After concentrated in vacuo, the obtained crude was purified by flash column chromatography (hexane/EtOAc =1/1) to afford **5a** as a dark blue solid (92.7 mg, 62%).
^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.98 (d, *J* = 14.8 Hz, 1H), 7.92 (d, *J* = 15.1 Hz, 1H), 7.42 (d, *J* = 8.3 Hz, 1H), 7.35 (s, 1H), 7.07-7.20 (m, 4H), 6.60 (d, *J* = 8.8 Hz, 1H), 3.50-3.61 (m, 6H), 3.37 (t, *J* = 5.6 Hz, 4H), 2.77-2.84 (m, 6H), 1.97-2.05 (m, 6H); ^{13}C NMR (100MHz, $CDCl_3$) δ 172.50, 171.16, 151.16, 148.65, 148.36, 131.18, 130.97, 130.72, 123.27, 123.01, 121.89, 121.44, 113.80, 111.40, 110.30, 77.24, 50.77, 50.59, 50.35, 48.44, 27.88, 27.56, 21.57, 21.16; LRMS(ESI): *m/z* calcd for $[M+H]^+$ 672.1, found 672.2; HRMS(ESI): *m/z* calcd for $[M+Na]^+$ 694.1583, found 694.1589; IR (film, cm^{-1}) 3433, 2927, 2852, 2101, 1571, 1516, 1452, 1430, 1314, 1237, 1204, 1152, 1077, 1011, 967; mp. decomp. 203.6 ˚C.

Compound **5b**
Following the same procedure for compound **5a**, **16** (444 mg, 1.0 mmol), **15b** (614 mg, 2.0 mmol), tributyl borate (4.5 mL, 16.8 mmol) and ^{n}BuNH$_2$ (128 μL, 1.3 mmol) afforded **5b** as a dark blue solid (348 mg, 45%).
^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.94 (d, *J* = 14.6 Hz, 2H), 7.42 (d, *J* = 9.0 Hz, 1H), 7.32 (s, 1H), 7.07-7.18 (m, 3H), 6.56 (d, *J* = 8.8 Hz, 1H), 3.43 (t, *J* = 5.5 Hz, 2H), 3.29-3.39 (m, 7H), 2.76-2.81 (m, 6H), 1.99-2.03 (m, 6H), 1.62-1.69 (m, 5H), 1.41-1.47 (m, 4H); ^{13}C NMR (100MHz, $CDCl_3$) δ 171.89, 171.37, 150.43, 149.33, 149.24, 147.51, 131.72, 130.95, 130.42, 122.50, 122.37, 121.91, 121.35, 112.66, 111.71, 110.36, 77.23, 51.44, 51.33, 50.30, 50.02, 28.82, 27.96, 27.58, 26.70, 26.63, 26.48, 21.55, 21.21; LRMS(ESI): *m/z* calcd for $[M+H]^+$ 728.2, found 728.2; HRMS(ESI): *m/z* calcd for $[M+Na]^+$ 750.2209, found 750.2216; IR (film, cm^{-1}) 3466, 2932, 2853, 2092, 1615, 1571, 1518, 1436, 1314, 1143, 1009, 960, 844, 803, 706; mp. 158.2-158.7 ˚C.

Compound **7a**
Following the same procedure for compound **5a**, **17**[S3] (26.8 mg, 0.1 mmol), **15a** (48.4 mg, 0.2 mmol), tributyl borate (771 μL, 663 mmol) and ^{n}BuNH$_2$ (11.4 μL, 0.2 mmol) afforded **7a** as a dark blue solid (29.9 mg, 63%).
1H-NMR (400 MHz, $(CD_3)_2CO$) δ 7.86 (t, *J* = 15.7 Hz, 2H), 7.68 (d, *J* = 9.0 Hz, 2H), 7.50 (dd, *J* = 8.8, 1.3 Hz, 1H), 7.44 (s, 1H), 6.82 (d, *J* = 7.5 Hz, 3H), 6.74 (d, *J* = 8.5 Hz, 1H), 6.70 (d, *J* = 8.3 Hz, 1H), 6.19 (s, 1H), 3.64-3.72 (m, 4H), 3.57 (t, *J* = 5.5 Hz, 2H), 3.12 (s, 6H), 2.79-2.83 (m, 2H), 1.99 (t, *J* = 5.6 Hz, 2H): ^{13}C NMR (100MHz, $CDCl_3$) δ 172.50, 171.16, 151.16, 148.65, 148.36, 131.18, 130.97, 130.72, 123.27, 123.01, 121.89, 121.44, 113.80, 111.40, 110.30, 77.24, 50.77, 50.59, 50.35, 48.44, 27.88, 27.56, 21.57, 21.16; LRMS(ESI): *m/z* calcd for $[M+H]^+$ 492.2, found 492.1; HRMS(ESI): *m/z* calcd for $[M+Na]^+$ 514.2198,

found 514.2202; IR (film, cm^{-1}) 3747, 2927, 2859, 2360, 2333, 2099, 1588, 1505, 1370, 1323, 1286, 1183, 1128, 1050, 1007, 817; mp. 113.0-113.6 ˚C.

Compound **7b**
Following the same procedure for compound **5a**, **17** (27.2 mg, 0.1 mmol), **15b** (55.8 mg, 0.2 mmol), tributyl borate (783 μL, 2.9 mmol) and $^{n}BuNH_2$ (11.6 μL, 0.1 mmol) afforded **7b** as a blue solid (25.1 mg, 47%).
^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.94 (dd, *J* = 15.3, 10.0 Hz, 2H), 7.52 (d, *J* = 9.0 Hz, 2H), 7.34 (d, *J* = 6.5 Hz, 1H), 7.25 (s, 1H), 6.70 (d, *J* = 8.8 Hz, 2H), 6.55 (d, *J* = 8.8 Hz, 1H), 6.44 (dd, *J* = 20.7, 15.2 Hz, 2H) 5.88 (s, 1H), 3.29-3.43 (m, 6H), 3.10 (s, 6H), 2.77-2.80 (m, 2H), 2.00 (s, 2H), 1.62-1.70 (m, 4H), 1.39-1.50 (m, 4H); ^{13}C NMR (100MHz, $CDCl_3$) δ 177.80, 177.09, 152.50, 148.66, 147.12, 146.15, 131.23, 130.77, 130.31, 122.41, 122.37, 121.77, 115.11, 113.92, 111.86, 110.24, 100.91, 77.23, 51.33, 49.90, 40.13, 28.82, 28.02, 26.70, 26.63, 26.41, 21.58; LRMS(ESI): *m/z* calcd for $[M+H]^+$ 548.3, found 548.3; HRMS(ESI): *m/z* calcd for $[M+Na]^+$ 570.2828, found 570.2829; IR (film, cm^{-1}) 3436, 2924, 2855, 2090, 1588, 1502, 1367, 1322, 1282, 1183, 1123, 1044, 998, 972, 811; mp. 67.3-68.5 ˚C.

Peptide **6a**
To a solution of **4** (10 mM, 30 μL, 1 eq.), **5a** (11 mM, 30 μL, 1.1 eq.), tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (TBTA, 20 mM, 60 μL, 4 eq.), and L-ascorbic acid (120 mM, 60 μL, 24 eq.) in DMF/MeOH (1:1 v:v, 300 μL) was added tetrakis(acetonitrile)copper(I) hexafluorophosphate (1.44 mg, excess). The solution was stirred at room temperature for 1 h and purified by preparative RP-HPLC in a 0.1% aqueous TFA-CH_3CN system to obtain peptide **6a** as a TFA salt (dark blue amorphus, 0.9 mg, 35%).
HRMS *m/z* $[M+H]^+$ found 3601.8933 (calcd. for $C_{167}H_{256}N_{45}O_{34}BrF_4B$ 3601.8899); purity >95% (HPLC with a binary solvent system: a linear gradient of CH_3CN (5-85%, 40 min) in 0.1% aqueous TFA at a flow rate of 1.0 mL/min, detected at UV 230 nm, t_R = 25.5 min).

Peptide **6b**
Following the same procedure for compound **6a**, **4** (10 mM, 60 μL, 1 eq.) and **5b** (11 mM, 60 μL, 1.1 eq.) afforded **6b** as a TFA salt (dark blue amorphus, 0.8 mg, 32%).
HRMS *m/z* $[M+H]^+$ found 3657.9524 (calcd. for $C_{171}H_{264}N_{45}O_{34}$ 3657.9525); purity >95% (HPLC with a binary solvent system: a linear gradient of CH_3CN (5-85%, 40 min) in 0.1% aqueous TFA at a flow rate of 1.0 mL/min, detected at UV 230 nm, t_R = 27.3 min).

Peptide **8a**
Following the same procedure for compound **6a**, **4** (10 mM, 60 μL, 1 eq.) and **7a** (11 mM, 60 μL, 1.1 eq.) afforded **8a** as a TFA salt (violet amorphus, 1.2 mg, 48%).
HRMS *m/z* $[M+H]^+$ found 3421.9521 (calcd. for $C_{162}H_{253}N_{45}O_{34}$ 3421.9513); purity >95% (HPLC with a binary solvent system: a linear gradient of CH_3CN (5-85%, 40 min) in 0.1% aqueous TFA at a flow rate of 1.0 mL/min, detected at UV 230 nm, t_R = 23.0 min).

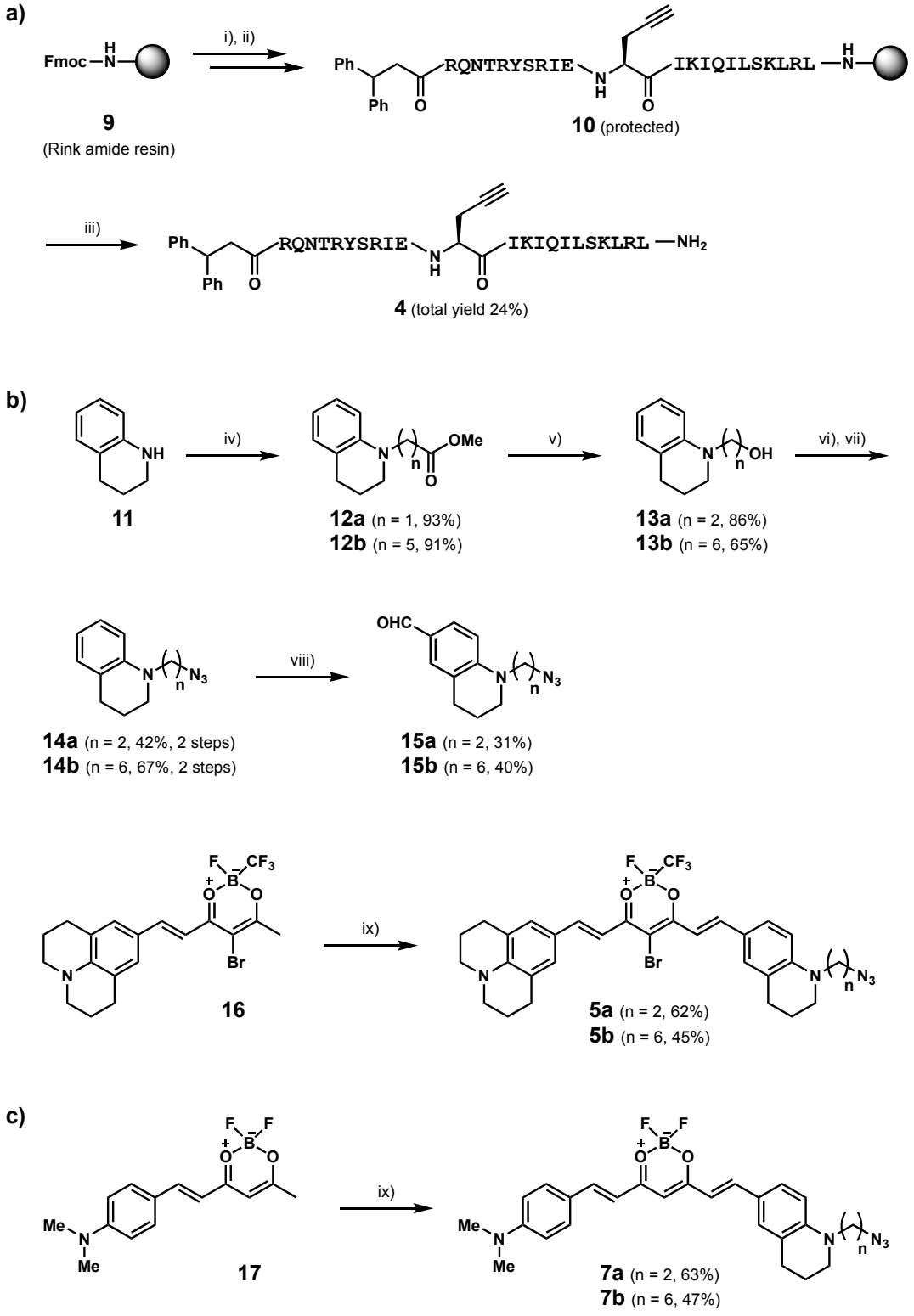
Peptide **8b**
Following the same procedure for compound **6a**, **4** (10 mM, 60 μL, 1 eq.) and **7b** (11 mM, 60 μL, 1.1 eq.) afforded **8b** as a TFA salt (dark violet amorphos, 0.7 mg, 55%).
HRMS *m/z* $[M+H]^+$ found 3478.0146 (calcd. for $C_{166}H_{261}N_{45}O_{34}$ 3478.0139); purity >95% (HPLC with a binary solvent system: a linear gradient of CH_3CN (5-85%, 40 min) in 0.1% aqueous TFA at a flow rate of 1.0 mL/min, detected at UV 230 nm, t_R = 24.3 min).

Absorbance and fluorescence measurements
Absorbance measurement was performed using a Shimadzu UV-1700 UV-visible spectrophotometer with a rectangular quartz cell (10 mm path-length). Fluorescence measurement was performed using a Hitachi F-7100 fluorescence spectrophotometer with a rectangular quartz cell (5 mm path-length).

Photo-oxygenation
A stock solution of myostatin (8 μM in 1 mM aqueous HCl) was diluted with 1 mM aqueous HCl and 20 mM phosphate buffer (pH 7.4) to 1 μM myostatin (10 mM phosphate, pH 7.4). A stock solution of substance P (100 μM in water) and Aβ1–42 isopeptide (250 μM in 0.1% aqueous trifluoroacetic acid, ultracentifuged)[S1] were diluted with water and 20 mM phosphate buffer (pH 7.4) to each 20 μM peptide (10 mM phosphate, pH 7.4). To each solution, **6a,b** or methylene blue (50 μM in CH_3CN/DMSO = 9/1) was added to 3 μM final concentration for the usual oxygenation studies. The mixture was irradiated with a light-emitting diode (LED) ISLM-150X150-FF (λ = 730 nm, 14 mW: CCS Inc., Kyoto, Japan) at 37 °C at approximately 3–5 cm distant away from samples. The reaction mixtures were reduced with 1,4-dithiothreitol (final 50 mM) at 37 ˚C for 0.5 h, digested with endoproteinase Lys-C, trypsin, or Glu-C (1/20 of protein by weight) at 37 ˚C for 2.5 h, de-salted with ZipTip U-C18 (Millipore Co.), analyzed using MALDI-TOF MS. For cell-based assay, the 10 mM phosphate buffered solution containing myostation (1 μM) and **6a** was diluted with serum-free DMEM (Nacalai Tesque) after or before irradiation (730 nm, 37 ˚C, 30 min) and then added to the cell culture (final 0.57 nM myostatin).

Cell-based assay [S4]
HEK293 cells (provided from Prof. Y. Sunada in Kawasaki Medical School, Okayama, Japan) were cultured in DMEM (Nacalai Tesque) containing 10% FBS (Sigma-Aldrich) and non-essential amino acids (Life Technologies) at 37 °C under 5% CO_2. The cells were seeded at 2.0×10^4 cells per well in the 96-well plates. After 24 h, transfection of reporter (pGL4.48[luc2P/SBE/Hygro], Promega) and control (pGL4.74[hRluc/TK], Promega) vectors was carried out using FuGENE HD (Promega). After 24 h of

transfection, the culture medium was exchanged to serum-free DMEM and the cells were incubated at 37 °C under 5% CO_2 for 8 h. The medium was exchanged to each sample solution (0.57 nM myostatin). The cells were incubated at 37 °C under 5% CO_2 for 4 h, and washed with PBS. The preparation of cell lysates and the measurement of luciferase reporter activities were conducted according to manufacturer's protocol of a Dual-Luciferase Reporter Assay System (Promega). Each experiment was carried out in triplicate. Values represent means ± s.d. (n = 3).



Scheme S1. Synthetic schemes of a) peptide segment **4**, b) catalyst segments **5a,b** and c) fluorescent segments **7a,b**. i) 20% piperidine, DMF, ii) Fmoc-AA-OH or 3,3-diphenylpropionic acid (5 eq), HATU (5 eq), HOAt (5 eq), DIPEA (10 eq), DMF, iii) 92.5% TFA, 5% 1,3-dimethoxybenzene, 2.5% triisopropylsilane, RT, 1.5 h, iv) $Br(CH_2)_nCOOMe$ (n = 1 or 5, 1.2 eq), KI (2.4 eq), K_2CO_3 (2 eq), acetonitrile, reflux, 38 h, v) LAH (1.2 eq), THF, RT, 20 h, vi) MsCl (1.5 eq), TEA (2 eq), DCM, RT, 2 h, vii) NaN_3 (5 eq), DMF, 60 ˚C, 23 h, viii) $POCl_3$ (3.3 eq), DMF (10 eq), DCM, 0 ˚C to RT, 1.5 h, ix) **15a,b** (2.2 eq), $BuNH_2$ (0.8 eq), $(BuO)_3B$ (2 eq), toluene, 65 ˚C, 27 h.

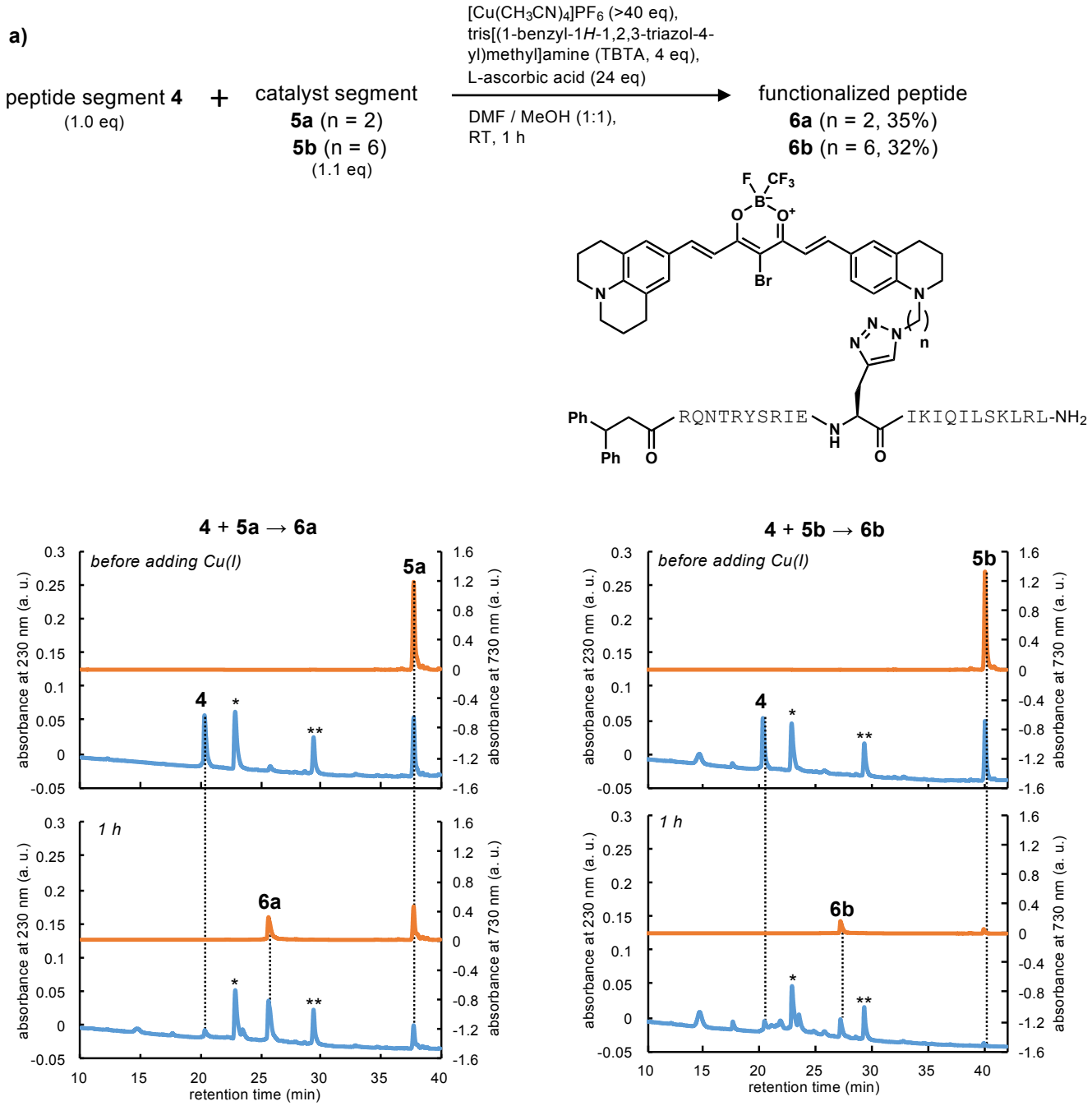
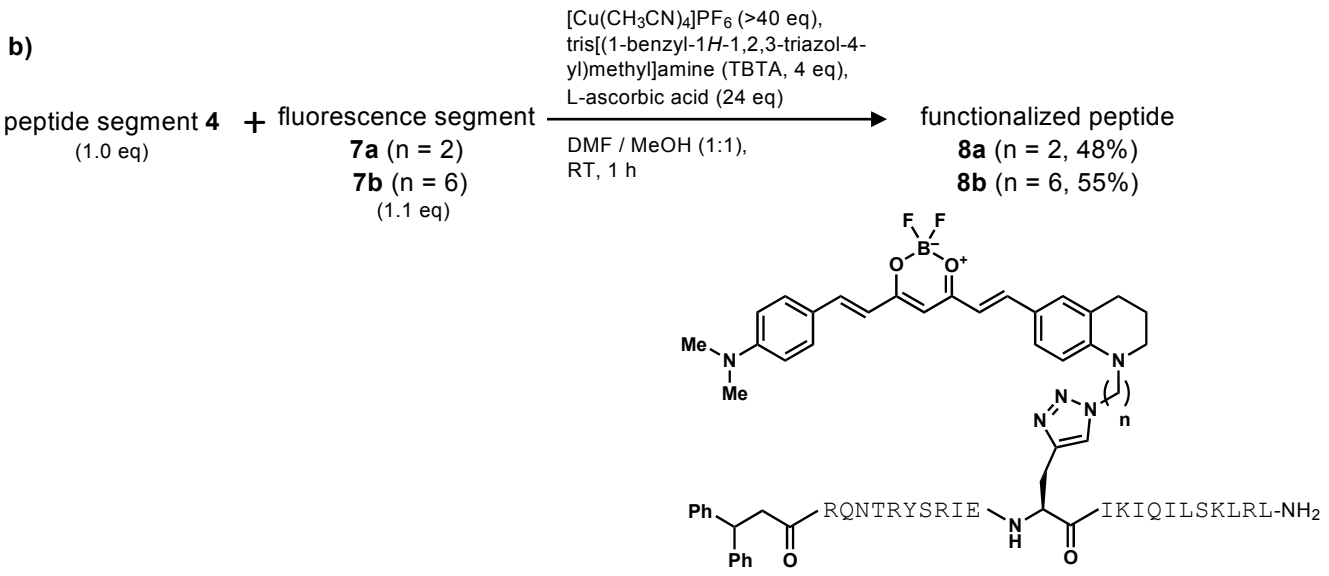
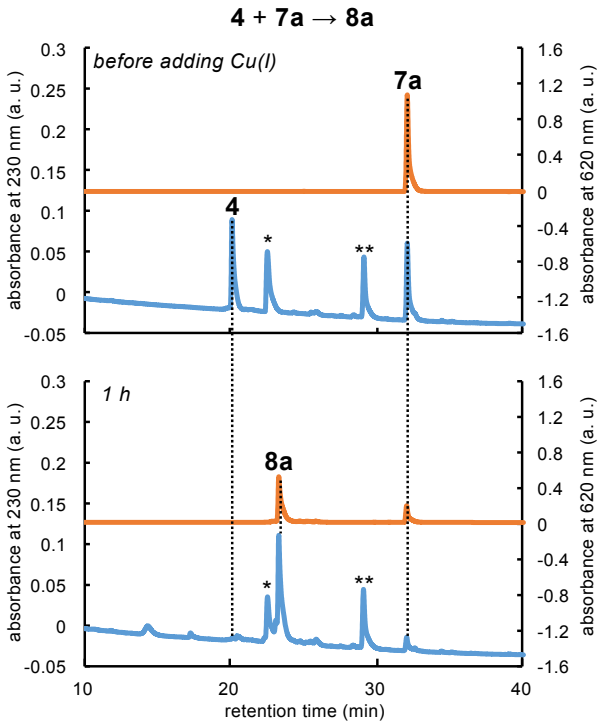


Figure S1. Conjugation of peptide segment **4** with a) catalyst segment **5a,b** or b) fluorescent segment **7a,b** to functionalized peptide **6a,b** or **8a,b**, respectively. Upper HPLC chart shows the mixture before reaction (before adding Cu(I)), and bottom chart shows the mixture after reaction (1 h after adding Cu(I)). Red lines indicate absorbance at a) 730 and b) 620 nm. Blue lines indicate absorbance at 230 nm. *: TBTA, **: unknown noise.

b)





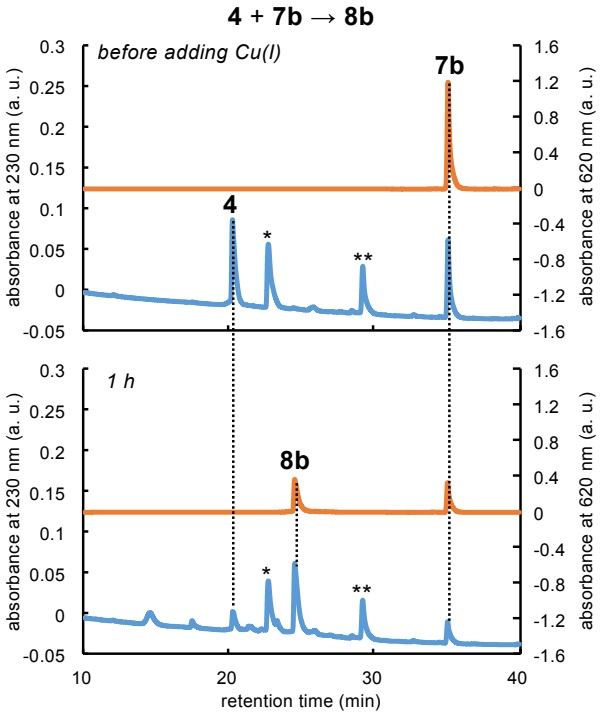


Figure S1. *Continued.*

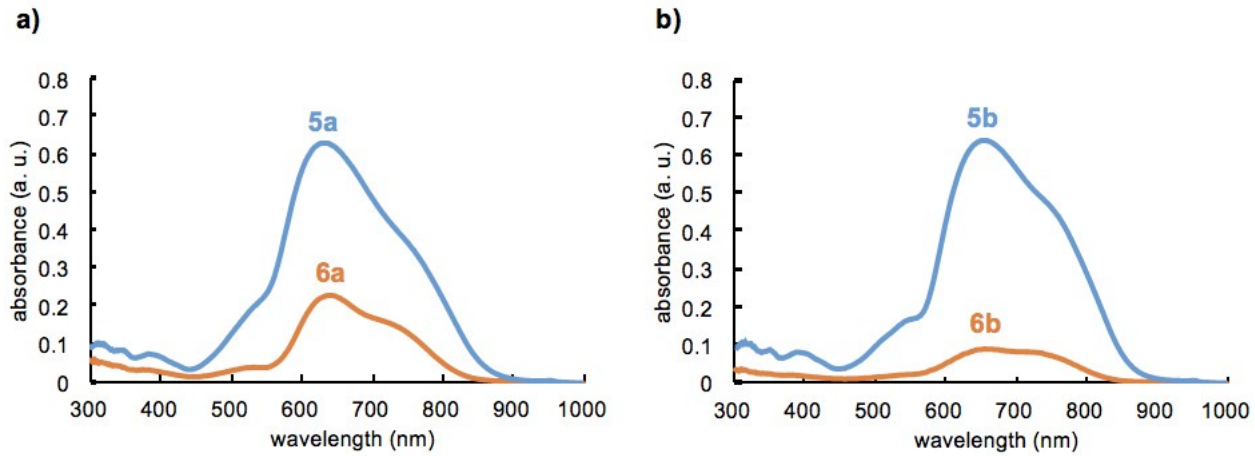


Figure S2. Absorbance spectra of a) functionalized peptide **6a** and catalyst segment **5a** and b) functionalized peptide **6b** and catalyst segment **5b**. 10 mM phosphate buffered solution (pH 7.4) containing **5a,b** or **6a,b** (10 μM) was used for the measurements.

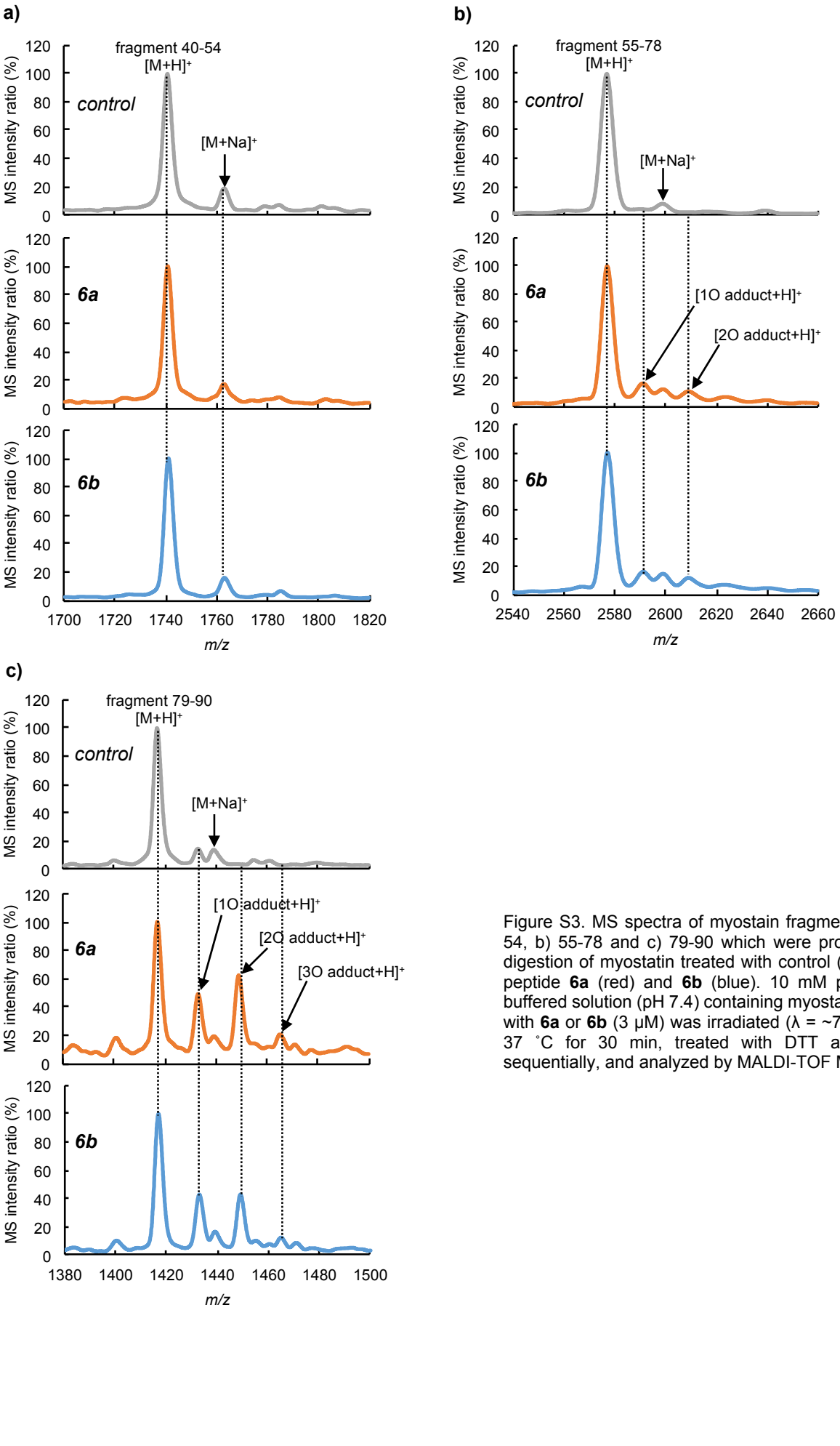


Figure S3. MS spectra of myostain fragments a) 40-54, b) 55-78 and c) 79-90 which were produced by digestion of myostatin treated with control (grey) and peptide **6a** (red) and **6b** (blue). 10 mM phosphate buffered solution (pH 7.4) containing myostatin (1 μM) with **6a** or **6b** (3 μM) was irradiated (λ = ~730 nm) at 37 ˚C for 30 min, treated with DTT and Lys-C sequentially, and analyzed by MALDI-TOF MS.

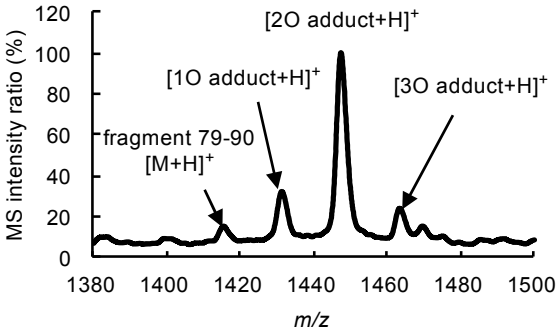


Figure S4. MS spectrum of myostatin fragment 79-90 which was produced by digestion of oxygenated myostatin treated with 0.3 eq of peptide **6a**. 10 mM phosphate buffered solution (pH 7.4) containing myostatin (1 μM) with **6a** (0.3 μM) was irradiated (λ = ~730 nm) at 37 ˚C for 3 h, treated with DTT and Lys-C sequentially, and analyzed by MALDI-TOF MS.

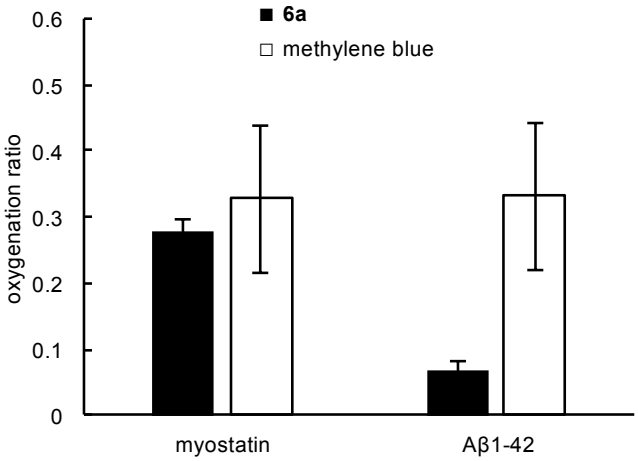


Figure S5. Myostatin-selective oxygenation under co-existence conditions of myostatin and Aβ1-42. 10 mM phosphate buffered solution (pH 7.4) containing **6a** or methylen blue (3 μM), myostatin (1 μM) and Aβ1-42 (1 μM) was irradiated (λ = ~730 nm) at 37 ˚C for 30 min, treated with DTT and Lys-C sequentially, and analyzed by MALDI-TOF MS. Oxygenation ratio was calculated from MS intensities of myostain 79-90 and Aβ1-16: Oxygenation ratio = (sum of MS intensities of oxygenated species) / [(MS intensity of native species) + (sum of MS intensities of oxygenated species)].

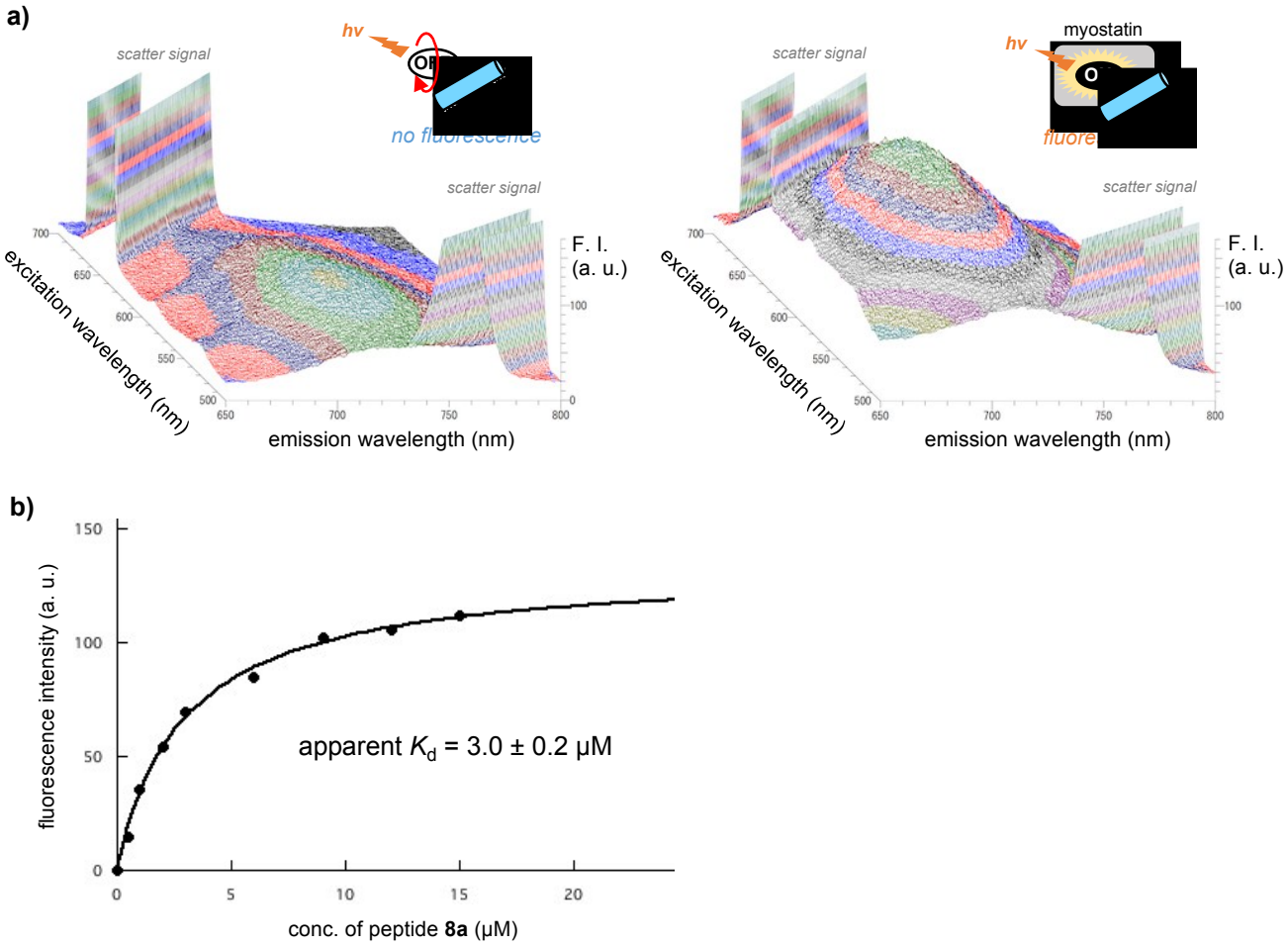
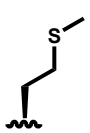
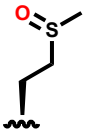
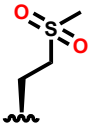
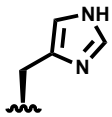
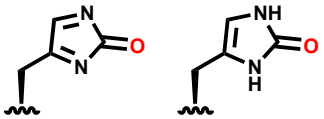
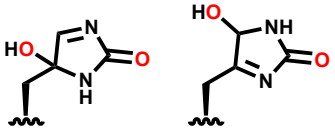
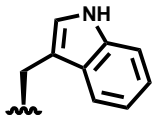
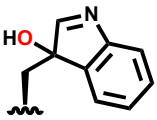
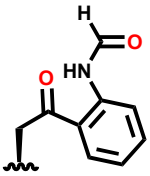


Figure S6. Fluorescence of **8a**. a) Excitation emission matrix (EEM) spectra of **8a** (3 μM) alone (left) or with myostatin (right). b) The fitting curve of the fluorescence intensity (ex: 626 nm, em: 680 nm) as a function of concentration of **8a**. 10 mM phosphate buffered solution (pH 7.4) containing **8a** (0-15 μM) with myostatin (1 μM) was used for the measurement. The binding curves were generated using KaleidaGraph 4.5 (Synergy Software, Reading, PA).

a)

amino acid	1[O] adducts	2[O] adducts
Met			
His			
Trp			

b)

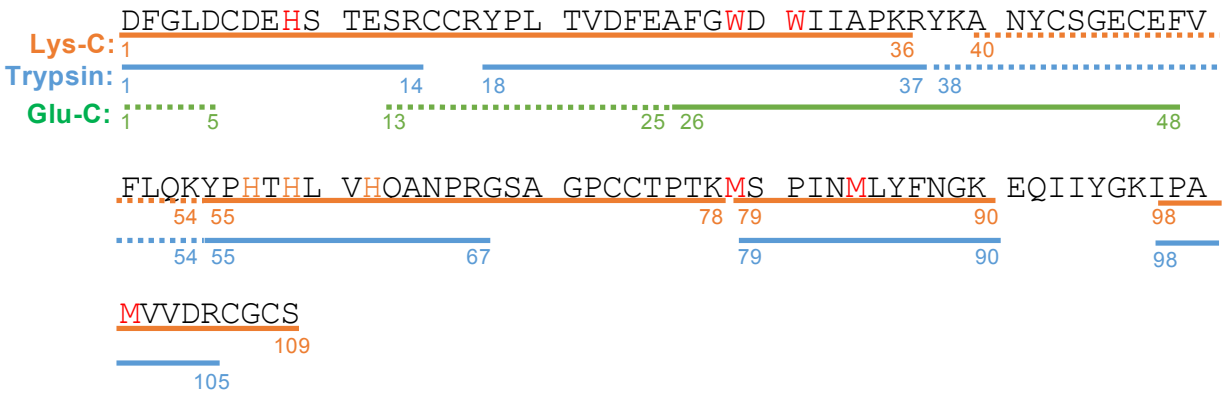
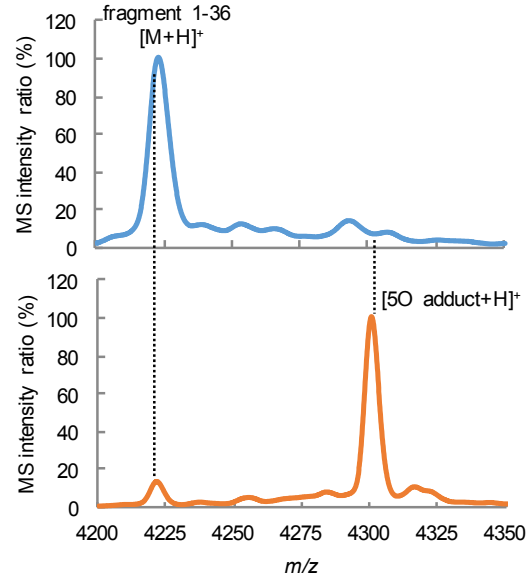
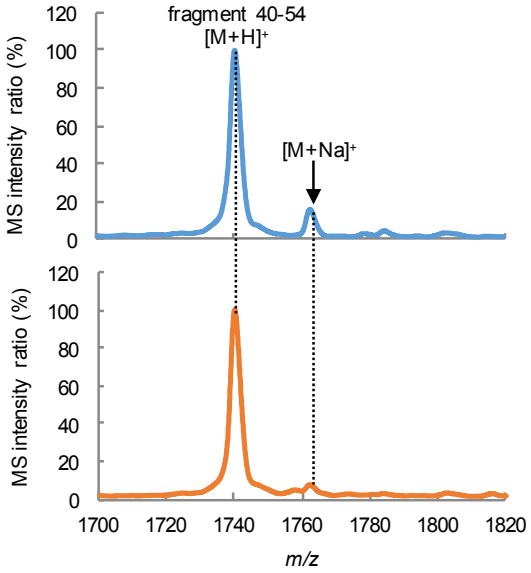


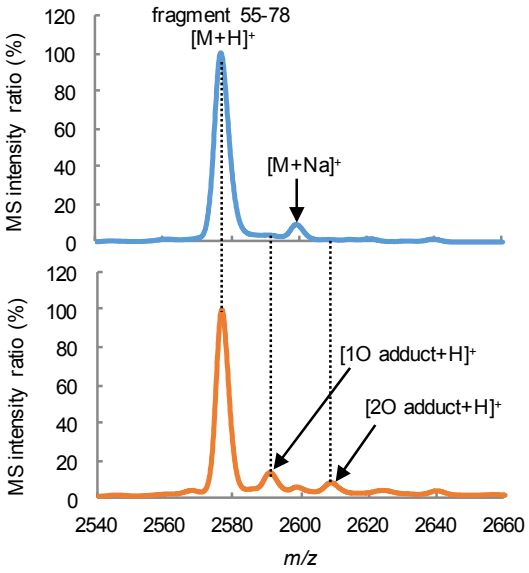
Figure S7. Oxygenated amino acid residues. a) Plausible structures of oxygenated amino acid residues. b-d) 10 mM phosphate buffered solution (pH 7.4) containing myostatin (1 μM) with **6a** (3 μM) was irradiated (λ = ~730 nm) at 37 ˚C for 30 min, treated with DTT and Lys-C, trypsin or Glu-C, sequentially, and analyzed by MALDI-TOF MS. b) Amino acid sequence of myostatin. Solid under lines indicate the digested fragments in which oxygenated product was observed, and dot under lines indicate the fragments in which non-oxygenated form was observed but oxygenated form was not. Oxygenated amino acid residues are shown in red, and all or any of His residues shown in orange were oxygenated. c) MS spectra of the fragments which were produced by digestion of the oxygenated myostatin with Lys-C, trypsin or Glu-C. d) Expected and observed masses of the detected myostatin fragments.

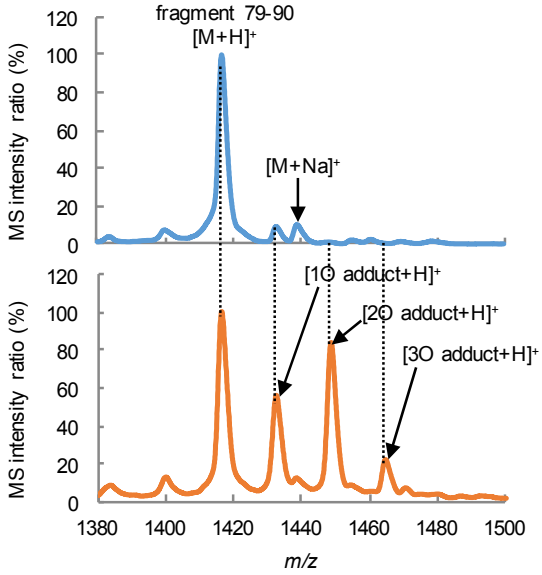
Generally, the major oxygenation-sensitive amino acid residues are considered to be Met, His, Trp, Tyr and Cys.[S5] In the case of myostatin, all Cys residues have already been oxidized to cystines. In addition, the Tyr residue was not oxygenated by previously reported photo-oxygenation catalyst **3**. In contrast, the catalyst predominantly oxygenated the Met, His and Trp residues. Therefore, we focused on those residues in this study (Fig. S7a). The oxygenated myostatin prepared with functionalized peptide **6a** was digested by endoproteinase Lys-C, trypsin or Glu-C following the disulfide cleavage with DTT, and then analysed by MALDI-TOF MS. Several oxygenated or non-oxygenated fragments were observed in the MS analyses (Figs. S7c and S7d). The results are summarized in Fig. S7b. No oxygenation species was observed in fragments 13-25, 38-54 and 40-54 including Tyr residues. Those results were supported by the previous results that Tyr residue was not oxygenated by photo-oxygenation by catalyst **3**. This might be attributed to the poor reactivity of Tyr with singlet oxygen rather than the residues mainly react with radical oxygen species.[S8] In contrast, fragments 98-105 and 98-109 including one Met showed 1 or 2 oxygen atom adducts, suggesting that Met101 was oxygenated to sulfide and sulfone. Fragment 1-14 including one His showed 1 oxygen atom adduct, suggesting that His9 was oxygenated. Similarly, fragments 18-37 and 26-48 including two Trp showed maximum 4 oxygen atom adducts, suggesting that Trp29 and 31 were oxygenated to 1 or 2 oxygen atom adducts. These results are supported by the result that fragment 1-36 including the His and Trp residues showed 5 oxygen atom adduct. Fragment 79-90 including two Met showed maximum 3 oxygen atom adducts, suggesting the oxygenation of Met79 and 84. Fragments 55-67 and 55-78 showed maximum 2 oxygen atom adducts. From this result, all or any of His57, 58 and 62 could be oxygenated.

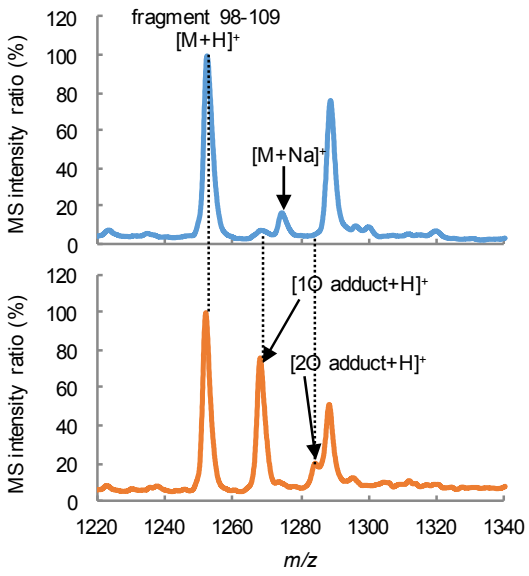
c-i) MS spectra of the fragments which were produced by digestion of the oxygenated myostatin with Lys-C.



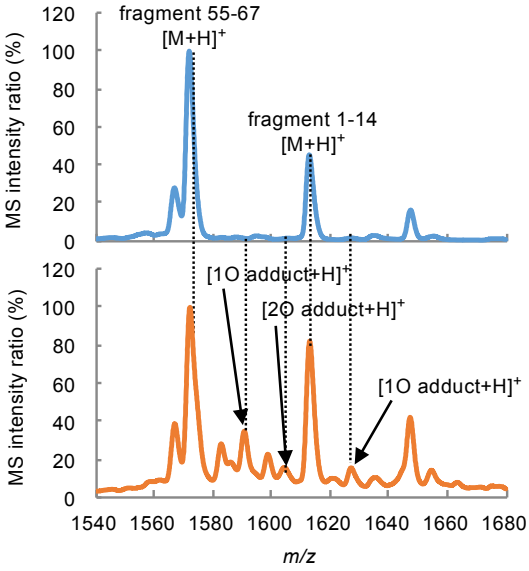


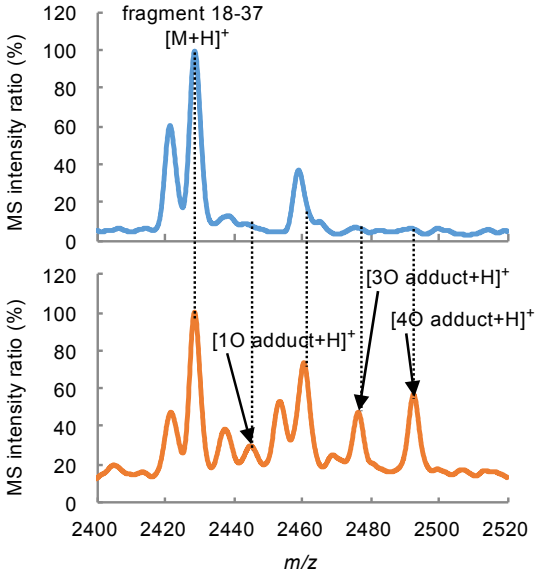


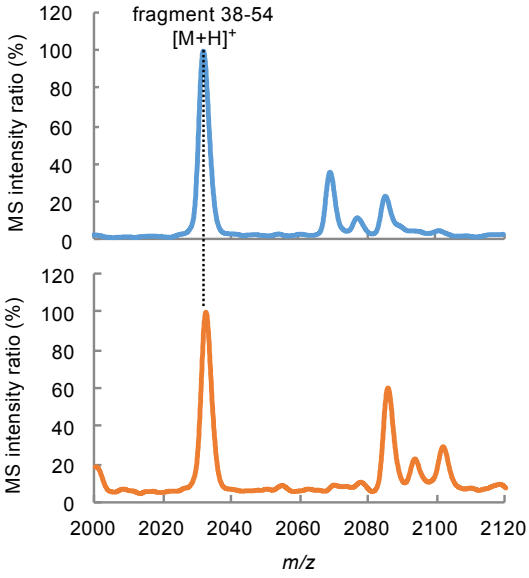


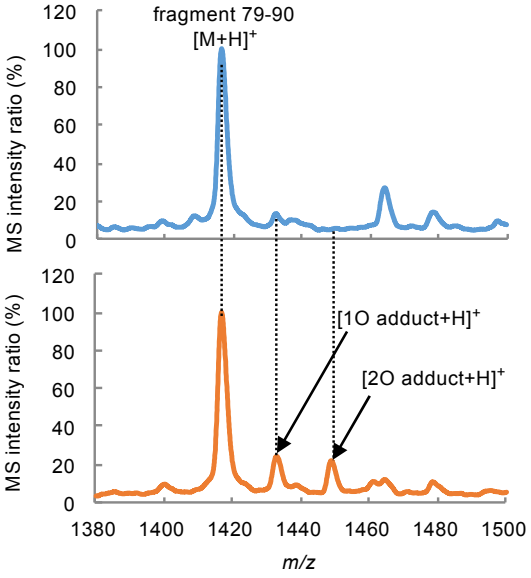


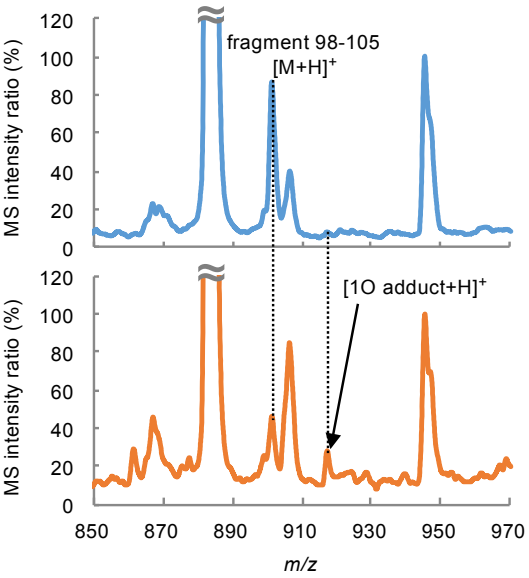
c-ii) MS spectra of the fragments which were produced by digestion of the oxygenated myostatin with trypsin.



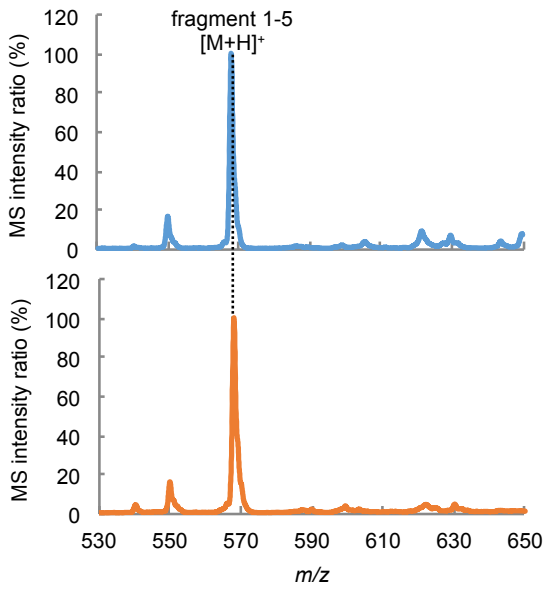


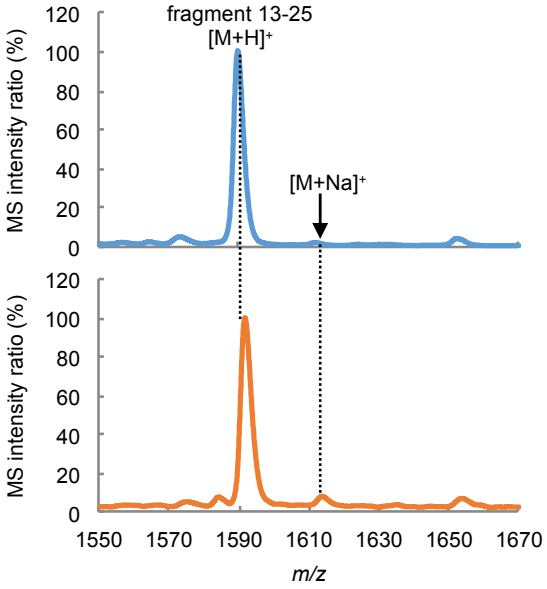


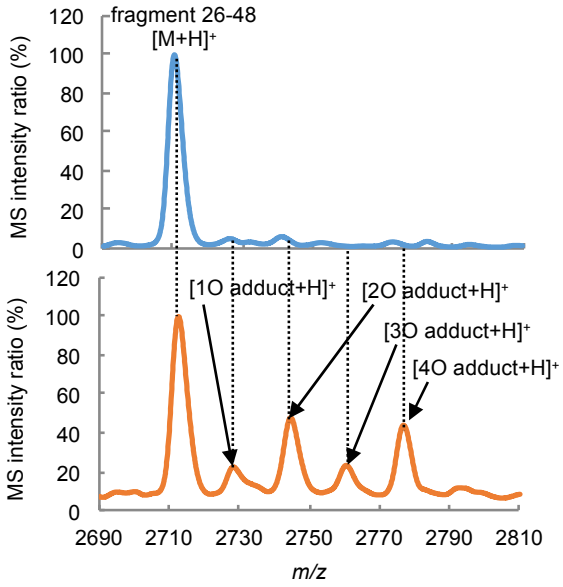




c-iii) MS spectra of the fragments which were produced by digestion of the oxygenated myostatin with Glu-C.







d)

enzyme	fragment		expected mass $[M+H]^+$		observed mass $[M+H]^+$		sequence[c]
		oxygen adduct		mass increase in oxygen adduct[a]		mass increase in oxygen adduct[a]	
Lys-C	1-36		4222.9		4223.1		DFGLDCDEHS TESRCCRYPL TVDFEAFGWD WIIAPK
		+5[O]		+80.0		+81.2	
	40-54		1738.8		1739.4		WIIAPKRYKA NYCSGECEFV FLQK
	55-78		2573.2		2575.6		YPHTHL VHQANPRGSA GPCCTPTK
		+1[O]		+14.0[b]		+13.9[b]	
		+2[O]		+32.0		+31.7	
	79-90		1415.7		1416.4		MS PINMLYFNGK
		+1[O]		+16.0		+16.5	
		+2[O]		+32.0		+31.9	
		+3[O]		+48.0		+48.0	
	98-109		1251.7		1253.0		IPA MVVDRCGCS
		+1[O]		+16.0		+15.9	
		+2[O]		+32.0		+31.7	
trypsin	1-14		1611.6		1613.4		DFGLDCDEHS TESR
		+1[O]		+14.0[b]		+13.9[b]	
	18-37		2425.2		2427.1		YPL TVDFEAFGWD WIIAPKR
		+1[O]		+16.0		+16.2	
		+3[O]		+48.0		+47.6	
		+4[O]		+64.0		+63.8	
	38-54		2029.9		2032.3		YKA NYCSGECEFV FLQK
	55-67		1570.8		1572.5		YPHTHL VHQANPR
		+1[O]		+14.0[b]		+13.4[b]	
		+2[O]		+32.0		+31.7	
	79-90		1415.7		1417.1		MS PINMLYFNGK
		+1[O]		+16.0		+15.9	
		+2[O]		+32.0		+31.8	
	98-105		901.5		901.3		IPA MVVDR
		+1[O]		+16.0		+15.8	
Glu-C	1-5		567.2		567.7		DFGLD
	13-25		1589.7		1590.4		SRCCRYPL TVDFE
	26-48		2708.2		2709.8		AFGWD WIIAPKRYKA NYCSGECE
		+1[O]		+16.0		+16.5	
		+2[O]		+32.0		+31.8	
		+3[O]		+48.0		+47.7	
		+4[O]		+64.0		+63.6	

[a] [mass of oxygen adduct fragment] – [mass of native fragment]

[b] +14 Da products were observed in His-including fragments. Those results agree with ref. S5-S7. His could be converted to +14 Da product, as shown in 1[O] adduct of His (left structure) in Fig. S7a, via adduction of O_2 (+32 Da) and then elimination of H_2O (–18 Da).

[c] Oxygenated amino acid residues are shown in red, and all or any of His residues shown in orange were oxygenated.

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