

Supporting Information for

Synthesis of a Stimulus-responsive Processing Device and Its Application to a Nucleocytoplasmic Shuttle Peptide

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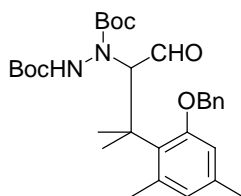
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[General Methods]

All reactions were carried out under a positive pressure of argon. For column chromatography, silica gel (KANTO KAGAKU N-60) was employed. All melting points are uncorrected. Exact mass spectra were recorded on Waters MICROMASS[®] LCT PREMIER[™] or Bruker Esquire200T. NMR spectra were recorded using a JEOL GSX400 spectrometer at 400 MHz frequency for ¹H and 100 MHz frequency for ¹³C in CDCl₃. Chemical shifts are calibrated to the solvent signal. For HPLC separations, a Cosmosil 5C₁₈-AR-II analytical column (Nacalai Tesque, 4.6 x 250 mm, flow rate 1 mL/min), a 5C₁₈-AR-II semi-preparative column (Nacalai Tesque, 10 x 250 mm, flow rate 2.5 mL/min) or a 5C₁₈-AR-II preparative column (Nacalai Tesque, 20 x 250 mm, flow rate 10 mL/min) was employed, and eluting products were detected by UV at 220 nm. A solvent system consisting of 0.1% TFA solution (v/v, solvent A) and 0.1% TFA in MeCN (v/v, solvent B) was used for HPLC elution. Elemental analysis was performed with Yanaco MT-3 CHN-Corder. Melting point was uncorrected. Photolysis was performed using Moritex MUV-202U with the filtered output (>365 nm) of a 3000 mW/cm² Hg-Xe lamp. The confocal microscopic cell images were observed with LSM510, Zeiss, Oberkochen, Germany. All chemicals were purchased from either Kanto Chemicals, Sigma Aldrich Japan, Tokyo Chemical Industry Kogyo, Wako Pure Chemical Industries or Watanabe Chemical Industry.

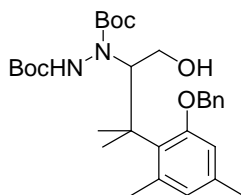
[Preparation of Photo-responsive Processing Device 7]

3-(2-Benzyloxy-4,6-dimethylphenyl)-2-(1,2-di-*tert*-butoxycarbonylhydrazinyl)-3,3-dimethylpropanal (**S1**).



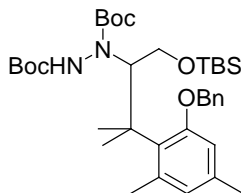
To a solution of aldehyde **2**^[S1] (5.25 g, 17.7 mmol) in dichloromethane (106 mL) were added di-*tert*-butyl azodicarboxylate (4.49 g, 19.5 mmol) and pyrrolidine (0.740 mL, 8.87 mmol), and the resulting solution was stirred for 24 h. After addition of saturated aqueous solution of NH₄Cl, the resulting solution was stirred for 30 min and then extracted with diethyl ether. Obtained organic phase was washed with brine, dried over Na₂SO₄, and concentrated in vacuo to give a crude product, which was purified by column chromatography (SiO₂, hexane/AcOEt = 20/1 then 10/1) and 7.89 g of alcohol **S1** (15.0 mmol, 85%) was obtained as a white amorphousness: ¹H NMR (CDCl₃, 400 MHz) δ = 1.14-1.26 (18H), 1.62-1.80 (6H), 2.18-2.23 (3H), 2.51-2.60 (3H), 5.00-5.41 (3H), 6.43-6.65 (2H), 7.28-7.45 (5H), 9.40-9.80 (1H); HRMS (ESI-TOF) calc. for C₃₀H₄₃N₂O₆ ([M + H]⁺): 527.3121, found: 527.3114.

3-(2-Benzyloxy-4,6-dimethylphenyl)-2-(1,2-di-*tert*-butoxycarbonylhydrazinyl)-3,3-dimethylpropanol (S2).



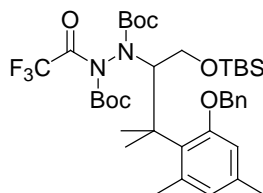
To a stirred solution of aldehyde **S1** (7.89 g, 15.0 mmol) in methanol (182 mL) was added sodium borohydride (0.681 g, 18.0 mmol) at 0 °C, and the resulting suspension was stirred at room temperature for 30 min. After addition of saturated aqueous solution of NH₄Cl, the reaction mixture was stirred for 30 min and then extracted with AcOEt. The combined organic layer was washed with brine, dried over Na₂SO₄ and concentrated in vacuo. The crude product was purified by reprecipitation from hexane and 7.93 g of diBoc alcohol **S2** (15.0 mmol, quant) was obtained as white powder: ¹H NMR (CDCl₃, 400 MHz) δ = 1.18-1.24 (9H), 1.40-1.43 (9H), 1.50-1.59 (6H), 2.19-2.23 (3H), 2.49-2.60 (3H), 3.60-3.79 (2H), 4.20-4.25 and 4.587-4.70 (1H), 4.86-5.15 (2H), 6.40-6.70 (2H), 7.30-7.50 (5H); HRMS (ESI-TOF) calc. for C₃₀H₄₄N₂NaO₆ ([M + Na]⁺): 551.3097, found: 551.3113; Anal. Calc. for C₃₀H₄₄N₂O₆: C, 68.15; H, 8.39; N, 5.30. Found: C, 67.93; H, 8.57; N, 5.26.

3-(2-Benzyloxy-4,6-dimethylphenyl)-2-(1,2-di-*tert*-butoxycarbonylhydrazinyl)-3,3-dimethylpropanol *tert*-butyldimethylsilyl ether (3).



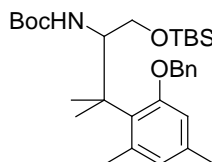
Triethylamine (6.16 mL, 44.2 mmol) and *tert*-butyldimethylsilyl trifluoromethanesulfonate (5.67 mL, 24.7 mmol) were added to a solution of alcohol **S2** (9.00 g, 17.0 mmol) in dichloromethane (100 mL) at 0 °C. The resulting mixture was stirred for 1 h and was quenched by the addition of water. After being extracted with dichloromethane, combined organic layer was washed with brine, dried over Na₂SO₄, and concentrated in vacuo to give a crude product, which was purified by column chromatography (SiO₂, chloroform) and 9.73 g of TBS ether **3** (15.1 mmol, 89%) was obtained as pale yellow oil: ¹H NMR (CDCl₃, 400 MHz) δ = -0.25-0.00 (6H), 0.70-0.90 (9H), 1.16-1.65 (24H), 2.08-2.18 (3H), 2.40-2.56 (3H), 3.30-3.40 (1H), 3.78-3.85 (1H), 4.90-5.10 (3H), 5.89-6.20 (1H), 6.40-6.52 (2H), 7.20-7.62 (5H); HRMS (ESI-TOF) calc. for C₃₃H₄₆NaN₄O₉ ([M + Na]⁺): 655.3162, found: 665.3143.

3-(2-Benzyloxy-4,6-dimethylphenyl)-2-(1,2-di-*tert*-butoxycarbonyl-2-trifluoroacetylhydrazinyl)-3,3-dimethylpropanol *tert*-buthyldimethylsilyl ether (S3).



To a stirred solution of TBS ether **3** (9.65 g, 15.0 mmol) in dichloromethane (115 mL) were added pyridine (12.1 mL, 150 mmol) and trifluoroacetic anhydride (10.4 mL, 75.0 mmol) at 0 °C. After being stirred for 4 h, the reaction was quenched by the addition of saturated aqueous solution of NH₄Cl at 0 °C. The resulting mixture was extracted with AcOEt, and the combined organic layer was washed with brine, dried over Na₂SO₄ and concentrated in vacuo. The obtained trifluoroacetamide **S3** was unstable and was used without further purification: ¹H NMR (CDCl₃, 400 MHz) δ = -0.15-0.02 (6H), 0.86-0.98 (9H), 1.10-1.65 (24H), 2.06-2.12 (3H), 2.13-2.122 (3H), 3.20-4.32 (2H), 4.57-5.18 (3H), 6.50-6.62 (2H), 7.26-7.75 (5H); LRMS (ESI-TOF) calc. for C₃₈H₅₇N₂O₇F₃NaSi ([M + Na]⁺): 761.4, found: 761.3.

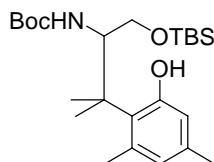
3-(2-Benzyloxy-4,6-dimethylphenyl)-2-*tert*-butoxycarbonylamino-3,3-dimethylpropanol *tert*-buthyldimethylsilyl ether (4).



Trifluoroacetamide **S3** (0.500 g, 0.640 mmol) in THF (1.29 mL) was treated with 0.1 M SmI₂ in THF (55.5 mL). It was prepared from Sm and CH₂I₂ in THF^[S21] - hexamethylphosphoramide (1.98 mL) in the presence of *tert*-BuOH (1.29 mL) for 3 h. The resulting mixture was quenched by the addition of saturated aqueous solution of NH₄Cl and was extracted with diethyl ether. The combined organic layer was washed with brine, dried over MgSO₄ and concentrated in vacuo. The crude product was purified by column chromatography (SiO₂, hexane/AcOEt = 40/1) and 0.250 g of Boc TBS ether **4** (0.470 mmol, 74%) was obtained as a pale yellow oil: ¹H NMR (CDCl₃, 400 MHz) δ = -0.04 (3H, s), -0.03 (3H, s), 0.92 (9H, s), 1.41 (9H, s), 1.48 (3H, s), 1.48 (3H, s), 2.22 (3H, s), 2.56 (3H, s), 3.49 (1H, dd, *J* = 10.0 and 5.2 Hz), 3.57 (1H, dd, *J* = 10.0 and 5.2 Hz), 4.59 (1H, dt, *J* = 9.0 and 5.2 Hz), 4.87 (1H, d, *J* = 9.0 Hz), 5.07 (1H, d, *J* = 12.0 Hz), 5.13 (1H, d, *J* = 12.0 Hz), 6.56 (1H, s), 6.62 (1H, s), 7.30 (1H, t, *J* = 8.0 Hz), 7.37 (2H, t, *J* = 8.0 Hz), 7.48 (2H, d, *J* = 8.0 Hz); ¹³C NMR (CDCl₃, 75 MHz) δ = -5.6 (CH₃), -5.5 (CH₃), 18.1 (C), 20.8 (CH₃), 25.9 (CH₃ x 3), 25.9 (CH₃ x 3), 27.7 (CH₃), 28.4 (CH₃), 29.3 (CH₃), 44.6 (C), 56.6 (CH), 63.7 (CH₂), 63.7 (C), 71.0 (CH₂), 112.7 (CH), 127.3 (CH x 4), 127.6 (CH), 128.5 (CH), 128.5 (C), 131.3 (C), 136.1 (C), 137.5 (C), 138.6 (C),

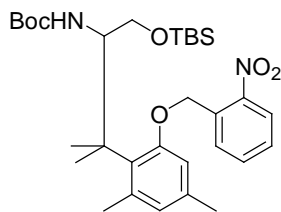
158.7 (C); HRMS (ESI-TOF) calc. for C₃₁H₄₉NO₄ NaSi([M + Na]⁺): 550.3329, found: 550.3326.

**2-tert-Butoxycarbonylamino-3,3-dimethyl-3-(4,6-dimethyl-2-hydroxyphenyl)propanol
tert-butyldimethylsilyl ether (S4).**



To a solution of benzyl ether **4** (3.37 g, 6.38 mmol) in methanol (32.9 mL) was added palladium on carbon (10%, 1.01 g), and the obtained mixture was stirred under H₂ for 24 h. Resulting suspension was filtered through the Celite, and the filtrate was concentrated in vacuo. Obtained residue was purified by column chromatography (SiO₂, hexane/AcOEt = 20/1 then 10/1) and 2.05 g of phenol **S4** (4.67 mmol, 73%) was obtained as a white amorphousness: ¹H NMR (CDCl₃, 400 MHz) δ = -0.06 (3H, s), -0.04 (3H, s), 0.85 (9H, s), 1.48 (9H, s), 1.52 (3H, s), 1.54 (3H, s), 2.15 (3H, s), 2.47 (3H, s), 3.51 (2H, d, *J* = 4.6 Hz), 4.83 (1H, br s), 5.06 (1H, br s), 6.43 (1H, s), 6.59 (1H, s), 7.56 (1H, br s); ¹³C NMR (CDCl₃, 100 MHz) δ = -3.7 (CH₃), -3.5 (CH₃), 20.1 (C), 22.2 (CH₃ x 2), 27.8 (CH₃ x 3), 28.5 (CH₃), 30.3 (CH₃), 30.5 (CH₃ x 3), 47.0 (C), 57.0 (CH), 66.0 (CH₂), 81.2 (C), 118.0 (CH), 127.8 (C), 129.6 (CH), 137.7 (C), 139.5 (C), 158.6 (C), 158.6 (C); HRMS (ESI-TOF) calc. for C₂₄H₄₃NNaO₄Si ([M + Na]⁺): 460.2859, found: 460.2859.

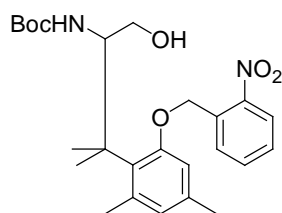
**2-tert-Butoxycarbonylamino-3,3-dimethyl-3-(2,4-dimethyl-6-(2-nitrobenzyloxy)phenyl)propano
I tert-butyldimethylsilyl ether (5).**



To a stirred solution of phenol **S4** (2.05 g, 4.67 mmol) in DMF (20.5 mL) were added K₂CO₃ (1.16 g, 8.41 mmol) and *o*-nitrobenzyl bromide (2.02 g, 9.35 mmol), and the resulting suspension was stirred for 24 h. After being added an aqueous ammonium hydroxide, the reaction mixture was stirred for 30 min, added H₂O and extracted with diethyl ether. Organic phase was washed with H₂O (x2) and brine, dried over MgSO₄ and concentrated in vacuo. The crude product was purified by column chromatography (SiO₂, hexane/AcOEt = 20/1) and 2.41 g of ether **5** (4.21 mmol, 90%) was obtained as a pale yellow oil: ¹H NMR (CDCl₃, 400 MHz) δ = -0.03 (3H, s), -0.02 (3H, s), 0.87 (9H, s), 1.44 (9H, s), 1.55 (3H, s), 1.59 (3H, s), 2.21 (3H, s), 2.57 (3H, s), 3.55 (1H, dd, *J* = 10.4 and 3.6 Hz), 3.60 (1H, dd, *J* = 10.4 and 3.6 Hz), 4.73 (1H, dt, *J* = 10.0 and 3.6 Hz), 4.91 (1H, d, *J* = 10.0 Hz), 5.49 (1H,

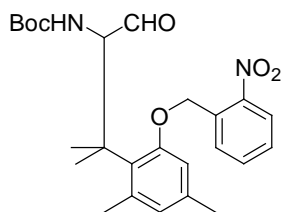
d, $J = 16.0$ Hz), 5.60 (1H, d, $J = 16.0$ Hz), 6.56 (1H, s), 6.61 (1H, s), 7.48 (1H, t, $J = 7.5$ Hz), 7.73 (1H, t, $J = 7.5$ Hz), 8.16 (1H, d, $J = 7.5$ Hz), 8.19 (1H, d, $J = 7.5$ Hz); ^{13}C NMR (CDCl_3 , 100 MHz) $\delta = -3.4$ (CH_3), -3.5 (CH_3), 20.1 (C), 22.6 (CH_3), 27.8 ($\text{CH}_3 \times 3$), 27.9 (CH_3), 29.3 (CH_3), 30.4 ($\text{CH}_3 \times 3$), 31.0 (CH_3), 47.0 (C), 57.9 (CH), 65.7 (CH_2), 70.7 (CH_2), 80.4 (CH_2), 115.2 (CH), 126.7 (CH), 129.8 (CH), 130.1 (CH), 131.2 (CH), 133.1 (C), 136.3 (CH), 138.2 (C), 140.5 (C), 148.2 (C), 157.8 (C), 160.0 (C); HRMS (ESI-TOF) calc. for $\text{C}_{31}\text{H}_{48}\text{N}_2\text{NaO}_6\text{Si}$ ($[\text{M} + \text{Na}]^+$): 595.3179, found: 595.3177.

2-*tert*-Butoxycarbonylamino-3,3-dimethyl-3-(2,4-dimethyl-6-(2-nitrobenzyloxy)phenyl)propanol (S5).



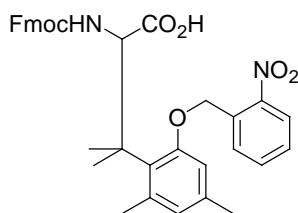
Glacial acetic acid (31.6 mL) and water (10.5 mL) were added to a solution of silyl ether **5** (2.41 g, 4.21 mmol) in THF (10.5 mL). The reaction mixture was stirred for overnight and was quenched by the addition of water. After being extracted with AcOEt, organic phase was washed with saturated aqueous solution of NaHCO_3 and brine, dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by column chromatography (SiO_2 , hexane/AcOEt = 5/1 then 2/1) and 1.45 g of alcohol **S5** (3.16 mmol, 75%) was obtained as a white amorphousness: ^1H NMR (CDCl_3 , 400 MHz) $\delta = 1.40$ (9H, s), 1.53 (3H, s), 1.55 (3H, s), 2.19 (3H, s), 2.54 (3H, s), 3.54 (1H, dd, $J = 10.0$ and 8.6 Hz), 3.68 (1H, d, $J = 10.0$ Hz), 4.67 (1H, t, $J = 8.6$ Hz), 4.91 (1H, d, $J = 8.6$ Hz), 5.50 (1H, d, $J = 16.0$ Hz), 5.58 (1H, d, $J = 16.0$ Hz), 6.52 (1H, s), 6.61 (1H, s), 7.50 (1H, t, $J = 7.6$ Hz), 7.70 (1H, t, $J = 7.6$ Hz), 7.93 (1H, d, $J = 7.6$ Hz), 8.19 (1H, d, $J = 7.6$ Hz); ^{13}C NMR (CDCl_3 , 100 MHz) $\delta = 20.6$ (CH_3), 25.9 (CH_3), 27.4 (CH_3), 28.3 ($\text{CH}_3 \times 3$), 28.5 (CH_3), 43.9 (C), 58.4 (CH), 63.7 (CH_2), 68.9 (CH_2), 79.0 (C), 113.5 (CH), 124.8 (CH), 128.0 (CH), 128.3 (CH), 129.0 (CH), 130.5 (C), 134.1 (CH), 136.4 (C), 138.0 (C), 146.3 (C), 156.8 (C), 158.1 (C); HRMS (ESI-TOF) calc. for $\text{C}_{25}\text{H}_{34}\text{N}_2\text{NaO}_6$ ($[\text{M} + \text{Na}]^+$): 481.2315, found: 481.2312.

2-tert-Butoxycarbonylamino-3,3-dimethyl-3-(2,4-dimethyl-6-(2-nitrobenzyloxy)phenyl)propanal (S6).



To a stirred solution of alcohol **S5** (1.45 g, 3.16 mmol) in dichloromethane (31.6 mL) was added PCC (2.73 g, 12.6 mmol), and resulting suspension was stirred for overnight. Reaction mixture was directly loaded on silica gel column chromatography (SiO₂, hexane/AcOEt = 1/0 then 5/1) and 1.13 g of aldehyde **S6** (2.48 mmol, 79%) was obtained as white amorphousness: ¹H NMR (CDCl₃, 400 MHz) δ = 1.41 (9H, s), 1.50 (3H, s), 1.62 (3H, s), 2.21 (3H, s), 2.52 (3H, s), 5.18 (1H, br d, *J* = 11.2 Hz), 5.42 (1H, d, *J* = 11.2 Hz), 5.54 (1H, d, *J* = 21.0 Hz), 5.62 (1H, d, *J* = 21.0 Hz), 6.57 (1H, s), 6.64 (1H, s), 7.50 (1H, t, *J* = 10.0 Hz), 7.70 (1H, td, *J* = 10.0 and 1.2 Hz), 7.94 (1H, br d, *J* = 10.0 Hz), 8.20 (1H, dd, *J* = 10.0 and 1.2 Hz), 9.48 (1H, s); ¹³C NMR (CDCl₃, 100 MHz) δ = 20.7 (CH₃), 25.9 (CH₃), 27.4 (CH₃), 28.2 (CH₃ x 3), 28.3 (CH₃), 44.0 (C), 65.5 (CH), 68.8 (CH₂), 79.5 (C), 113.5 (CH), 125.1 (C), 128.4 (CH), 128.7 (CH), 129.3 (C), 129.4 (C), 133.9 (C), 134.3 (CH), 134.3 (CH), 137.5 (C), 138.2 (C), 155.9 (C), 158.0 (C), 201.4 (CH); HRMS (ESI-TOF) calc. for C₂₅H₃₂N₂NaO₆ ([M + Na]⁺): 479.2158, found: 479.2145.

3,3-Dimethyl-3-(2,4-dimethyl-6-(2-nitrobenzyloxy)phenyl)-2-(9-fluorenylmethylcarbonylamino) propionic acid (7).



2-Methyl-2-butene (1.77 mL, 16.7 mmol), NaH₂PO₄ (0.446 g, 3.72 mmol) and NaClO₂ (1.30 g, 14.4 mmol) were added to a solution of aldehyde **S6** (1.13 g, 2.48 mmol) in acetone/*tert*-BuOH/H₂O (6/4/1, 85.3 mL), and the resulting mixture was stirred for overnight. The reaction mixture was poured into saturated aqueous solution of NH₄Cl and extracted with diethyl ether. Organic phase was dried over Na₂SO₄ and concentrated in vacuo. Hydrogen chloride in AcOEt (4 M, 27.0 mL) was added to the crude product **6**, and the resulting mixture was stirred for 1.5 h. After being concentrated in vacuo, obtained residue was dissolved in acetonitrile/10% aqueous solution of Na₂CO₃ (1/1, 22.4 mL). To the resulting solution was added FmocOSu (0.878 g, 2.60 mmol), and the reaction mixture was stirred for 3 h. After being acidified by 5% aqueous solution of KHSO₄,

obtained mixture was extracted with diethyl ether. The organic phase was washed with brine and concentrated in vacuo. Obtained crude product was purified by column chromatography (SiO₂, chloroform/MeOH = 1/0 then 200/1) and 1.47 g of Fmoc derivative **7** (2.47 mmol, quant) was obtained as pale yellow amorphousness: ¹H NMR (CDCl₃, 400 MHz) δ = 1.67 (3H, s), 1.71 (3H, s), 2.16 (3H, s), 2.54 (3H, s), 4.15 (1H, t, *J* = 8.4 Hz), 4.24 (1H, t, *J* = 8.4 Hz), 4.39 (1H, t, *J* = 8.4 Hz), 5.48-5.70 (4H, m), 6.52 (1H, s), 6.59 (1H, s), 7.30 (2H, d, *J* = 7.2 Hz), 7.36-7.48 (3H, m), 7.50 (1H, d, *J* = 7.2 Hz), 7.56 (1H, d, *J* = 7.2 Hz), 7.62 (1H, t, *J* = 7.6 Hz), 7.77 (2H, d, *J* = 7.2 Hz), 7.97 (1H, d, *J* = 7.6 Hz), 8.18 (1H, d, *J* = 8.0 Hz), 10.60 (1H, br s); ¹³C NMR (CDCl₃, 100 MHz) δ = 20.7 (CH₃), 25.7 (CH₃), 27.7 (CH₃), 28.3 (CH₃), 44.4 (C), 47.0 (CH), 59.5 (CH), 66.9 (CH₂), 68.7 (CH₂), 113.1 (CH), 119.7 (CH x 2), 124.8 (CH x 2), 126.8 (CH), 126.8 (CH), 127.4 (CH x 2), 128.0 (CH x 2), 128.2 (C), 128.5 (CH), 128.8 (CH), 134.1 (CH), 136.9 (C), 137.8 (C), 141.0 (C x 2), 143.6 (C x 2), 146.4 (C), 155.8 (C), 158.0 (C), 176.9 (C); HRMS (ESI-TOF) calc. for C₃₅H₃₄N₂NaO₇ ([M + Na]⁺): 617.2264, found: 617.2266.

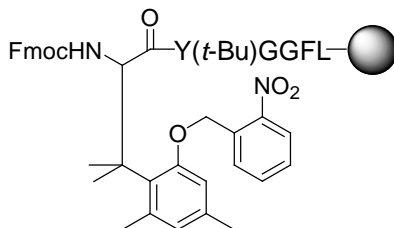
[Preparation of Photo-responsive Model Peptides **8**]

Peptide resin (**S7**).



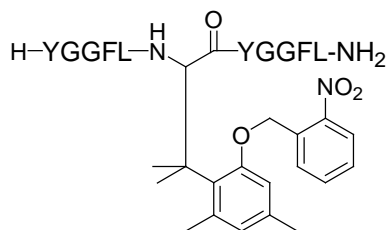
Fmoc-Leu-OH (0.14 g, 0.40 mmol) was coupled to Nova Syn TGR resin (loading: 0.25 mmol/g, 0.40 g, 0.10 mmol) in DMF/DMSO = 3/1^[S3] at room temperature for 1 h in the presence of DIC (62 μL, 0.40 mmol) and HOBt·H₂O (61 mg, 0.40 mmol). After deprotection of Fmoc group by the treatment with 20% piperidine in DMF, peptide elongation was performed by Fmoc chemistry (4.0 equiv of each amino acid) using DIC (62 μL, 4.0 eq.) / HOBt·H₂O (61 mg, 4.0 eq.) in DMF/DMSO = 3/1 to give the peptide resin **S7**.

Coupling of Fmoc derivative **7** with peptide resin **S7**.



Fmoc derivative **7** (0.12 g, 0.20 mmol) was attached to the amino group of the resin **S7** by DIC (31 μL, 0.20 mmol) and HOBt·H₂O (31 mg, 0.20 mmol) in DMF/DMSO = 3/1 for 24 h to give the **S8**.

Synthesis of photoresponsive peptide **8**.



Elongation of peptide on **S8** (0.20 mmol) was performed as described for peptide resin **S7**. The protected peptide resin was treated with TFA-Et₃SiH-H₂O (95:2.5:2.5 (v/v), 8.0 mL) at room temperature for 1.5 h. After the resin was filtrated off, cooled diethyl ether was added to the filtrate, and the resulting precipitate was collected by centrifugation. The obtained precipitate was washed with diethyl eshter and purified by RP-HPLC to give the desired photo-responsive peptide **8** (23 mg, 16%) as a diastereomeric mixture. Analytical HPLC condition: linear gradient of solvent B in solvent A, 10 to 60% over 30 min. Retention time = 27.4 or 29.7 min, respectively for each diastereomer. Preparative HPLC condition: linear gradient of solvent B in solvent A, 20 to 70% over 60 min. MS (ESI-TOF, reconstructed) calc. for C₇₆H₉₆N₁₃O₁₆ ([M + H]⁺): 1446.7, found; 1446.3 and 1446.2.

[Photo-processing Reaction of Model Peptides **8**]

The photo-responsive peptide **8** (0.50 mg, 0.35 μmol) in acetonitrile (316 μL) was added to phosphate buffer (20 mM, pH 7.6, 1.26 mL), and the resulting mixture was irradiated by UV (λ > 365 nm) for 3 min. The reaction solution was incubated at 37 °C and reaction was monitored by analytical HPLC. Analytical HPLC conditions: linear gradient of solvent B in solvent A, 10 to 60% over 30 min. **9**: retention time = 23.7 or 25.3 min, respectively for each diastereomer, MS (ESI-TOF, reconstructed) calc. for C₆₉H₉₁N₁₂O₁₄ ([M + H]⁺): 1311.7, found 1311.7 and 1311.6; **10**: retention time = 25.3 or 25.6 min, respectively for each diastereomer, MS (ESI-TOF) calc. for C₄₁H₅₃N₆O₈ ([M + H]⁺): 757.4, found 757.1 and 757.1. **11**: retention time = 13.7 min, MS (ESI-TOF) calc. for C₂₈H₃₉N₆O₆ ([M + H]⁺): 555.3, found 555.3.

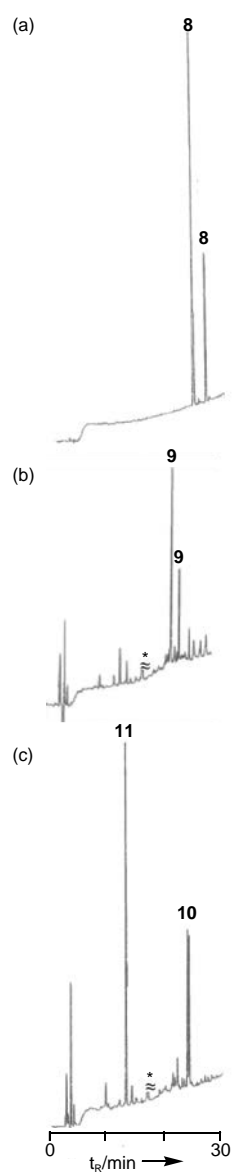
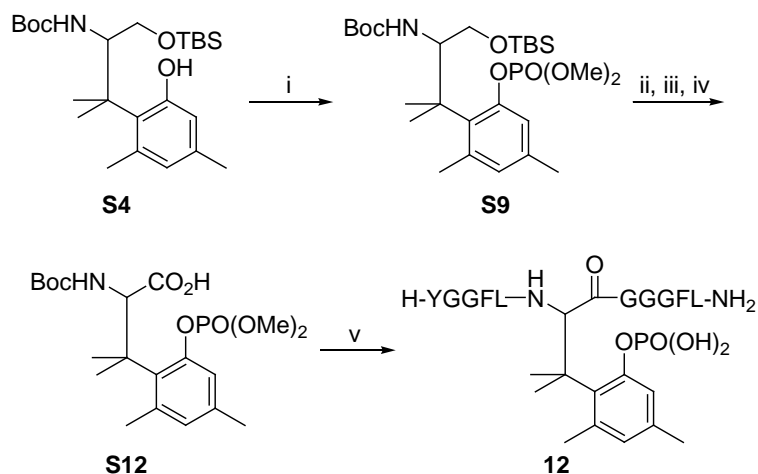


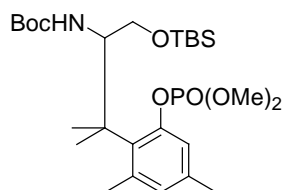
Figure S1. HPLC profiles (a) before UV irradiation (b) after 3 min of UV irradiation, and (c) after 3 min of UV irradiation followed by 2 h of incubation at 37 °C. Peptides were detected by UV absorbance at 220 nm. Asterisked peaks are not peptidic compounds. **8**, **9**, and **10** are diastereomeric mixture.

[Preparation of Phosphatase-responsive Model Peptide 12]



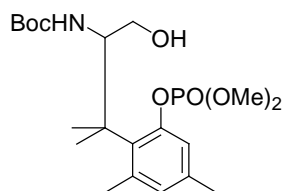
Reagents and conditions. (i) dimethyl-*N,N*-diethylphosphoramidite, 1*H*-tetrazole, THF, then OXONE, water, 84%; (ii) AcOH, THF, H₂O, 91%; (iii) PCC, CH₂Cl₂, 79%; (iv) NaClO₂, NaH₂PO₄, *tert*-BuOH, H₂O, acetone, 2-methyl-2-butene, 63%; (v) Boc SPPS.

2-*tert*-Butoxycarbonylamino-3,3-dimethyl-3-(2,4-dimethyl-6-dimethoxyphosphoryloxyphenyl)propanol *tert*-butyldimethylsilyl ether (S9).



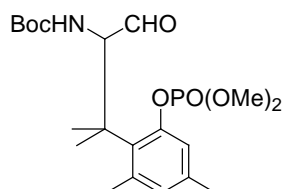
To a stirred solution of phenol **S4** (0.500 g, 1.14 mmol) in THF (5.00 mL) were added 1*H*-tetrazole (0.340 g, 4.85 mmol) and dimethyl-*N,N*-diethylphosphoramidite^[S4] (0.440 g, 2.66 mmol). After being stirred for 4.5 h, OXONE (1.75 g, 2.85 mmol) in water (10.0 mL) was added to the reaction mixture at 0 °C. Resulting mixture was stirred for 10 min at same temperature and was extracted with AcOEt. Organic phase was washed with saturated aqueous solution of Na₂S₂O₃ and brine, dried over Na₂SO₄ and concentrated in vacuo. The crude product was purified by column chromatography (SiO₂, hexane/AcOEt = 3/1 then 2/1) and 0.545 g of phosphonate **S9** (0.990 mmol, 87%) was obtained as a colorless oil: ¹H NMR (CDCl₃, 400 MHz) δ = -0.05 (3H, s), -0.05 (3H, s), 0.84 (9H, s), 1.41 (9H, s), 1.48 (3H, s), 1.53 (3H, s), 2.23 (3H, s), 2.58 (3H, s), 3.50 (1H, dd, *J* = 10.4 and 5.0 Hz), 3.51 (1H, dd, *J* = 10.4 and 5.0 Hz), 3.88 (3H, d, *J* = 11.2 Hz), 3.90 (3H, d, *J* = 12.0 Hz), 4.42 (1H, dt, *J* = 10.4 and 5.0 Hz), 4.85 (1H, br d, *J* = 10.4 Hz), 6.75 (1H, s), 7.09 (1H, s); ¹³C NMR (CDCl₃, 100 MHz) δ = -5.7 (CH₃), -5.6 (CH₃), 18.0 (C), 20.3 (CH₃), 25.6 (CH₃), 25.7 (CH₃ x 3), 26.9 (CH₃), 28.3 (CH₃ x 3), 28.9 (CH₃), 44.2 (C), 54.7 (CH₃, d, *J* = 5.8 Hz), 54.8 (CH₃, d, *J* = 5.8 Hz), 56.5 (CH), 63.4 (CH₂), 78.4 (C), 118.3 (CH, d, *J* = 7.0 Hz), 131.0 (CH), 131.7 (C, d, *J* = 9.0 Hz), 136.4 (C), 139.0 (C), 150.2 (C, d, *J* = 6.0 Hz), 155.8 (C).; HRMS (ESI-TOF) calc. for C₂₆H₄₉NO₇PSi ([M + H]⁺): 546.3016, found: 546.3019.

2-tert-Butoxycarbonylamino-3,3-dimethyl-3-(2,4-dimethyl-6-dimethylphosphoryloxypropanol (S10).



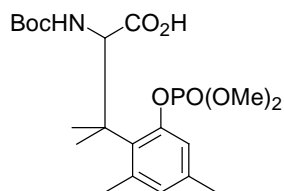
To a solution of silyl ether **S9** (0.599 g, 1.10 mmol) in THF (2.70 mL) and H₂O (2.70 mL) was added glacial acetic acid (8.20 mL). The resulting mixture was stirred for overnight and then quenched by the addition of water. After being extracted with AcOEt, organic phase was washed with saturated aqueous solution of NaHCO₃ and brine, dried over Na₂SO₄ and concentrated in vacuo. The crude product was purified by column chromatography (SiO₂, hexane/AcOEt = 1/1 then 1/100) and 0.431 g of alcohol **S10** (1.00 mmol, 91%) was obtained as a colorless oil: ¹H NMR (CDCl₃, 400 MHz) δ = 1.42 (9H, s), 1.47 (3H, s), 1.55 (3H, s), 2.24 (3H, s), 2.58 (3H, s), 2.90 (1H, br s), 3.44-3.64 (2H, m), 3.88 (3H, d, J = 11.6 Hz), 3.89 (3H, d, J = 11.2 Hz), 4.48 (1H, m), 5.04 (1H, d, J = 9.2 Hz), 6.79 (1H, s), 7.01 (1H, s); ¹³C NMR (CDCl₃, 100 MHz) δ = 20.3 (CH₃), 25.6 (CH₃), 26.9 (CH₃), 28.3 (CH₃ x 3), 28.7 (CH₃), 44.0 (C), 54.9 (CH₃, d, J = 9.0 Hz), 55.0 (CH₃, d, J = 6.0 Hz), 57.8 (CH), 63.4 (CH₂), 79.1 (C), 119.1 (CH), 131.5 (CH), 131.8 (C, d, J = 7.0 Hz), 136.7 (C), 139.0 (C), 149.9 (C, d, J = 6.0 Hz), 156.7 (C); HRMS (ESI-TOF) calc. for C₂₀H₃₅NO₇P ([M + H]⁺): 432.2151, found: 432.2145.

2-tert-Butoxycarbonylamino-3,3-dimethyl-3-(2,4-dimethyl-6-dimethoxyphosphoryloxyphenyl)propanal (S11).



Pyridinium chlorochromate (1.10 g, 4.00 mmol) was added to a solution of alcohol **S10** (0.431 g, 1.00 mmol) in dichloromethane (17.2 mL). After being stirred for overnight, resulting mixture was directly loaded on silica gel column chromatography (SiO₂, hexane/AcOEt = 1/1) and 0.341 g of aldehyde **S11** (0.790 mmol, 79%) was obtained as white amorphousness: ¹H NMR (CDCl₃, 400 MHz) δ = 1.43 (9H, s), 1.46 (3H, s), 1.56 (3H, s), 2.26 (3H, s), 2.62 (3H, s), 3.87 (3H, d, J = 11.6 Hz), 3.90 (3H, d, J = 11.2 Hz), 5.15 (1H, d, J = 7.6 Hz), 5.30 (1H, d, J = 7.6 Hz), 6.83 (1H, s), 7.14 (1H, s), 9.40 (1H, s); ¹³C NMR (CDCl₃, 100 MHz) δ = 20.4 (CH₃), 25.5 (CH₃), 26.8 (CH₃), 28.2 (CH₃ x 3), 29.7 (CH₃), 43.3 (C), 54.9 (CH₃, d, J = 6.0 Hz), 55.0 (CH₃, d, J = 5.0 Hz), 65.3 (CH), 79.6 (C), 119.0 (CH), 129.3 (C, d, J = 8.0 Hz), 131.5 (CH), 137.6 (C), 138.8 (C), 150.0 (C, d, J = 7.0 Hz), 155.8 (C), 200.7 (CH); HRMS (ESI-TOF) calc. for C₂₀H₃₃NO₇P ([M + H]⁺): 430.1995, found: 430.1974.

2-*tert*-Butoxycarbonylamino-3,3-Dimethyl-3-(2,4-dimethyl-6-dimethoxyphosphoryloxyphenyl) propionic acid (S12).



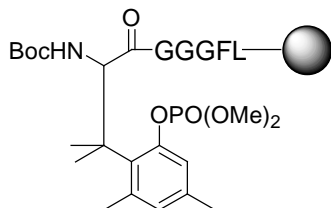
To a solution of aldehyde **S11** (0.020 g, 0.047 mmol) in acetone/*tert*-BuOH/H₂O (6/4/1, 1.10 mL) were added 2-methyl-2-butene (0.034 mL, 0.32 mmol), NaH₂PO₄ (0.0085 g, 0.071 mmol) and NaClO₂ (0.022 g, 0.24 mmol), and the reaction mixture was stirred for 3.5 h. The resulting mixture was poured into saturated aqueous solution of NH₄Cl and was extracted with diethyl ether. Organic phase was dried over Na₂SO₄ and concentrated in vacuo. Obtained crude product was purified by column chromatography (SiO₂, hexane/AcOEt = 1/2, then AcOEt) and 0.013 g of carboxylic acid **S12** (0.030 mmol, 63%) was obtained as colorless oil: ¹H NMR (CDCl₃, 400 MHz) δ = 1.40 (9H, s), 1.56 (3H, s), 1.60 (3H, s), 2.24 (3H, s), 2.53 (3H, s), 3.89 (3H, d, *J* = 11.6 Hz), 3.99 (3H, d, *J* = 10.0 Hz), 5.04 (1H, d, *J* = 9.2 Hz), 5.35 (1H, d, *J* = 9.2 Hz), 6.79 (1H, s), 6.94 (1H, s); ¹³C NMR (CDCl₃, 100 MHz) δ = 20.4 (CH₃ x 3), 25.4 (CH₃), 27.1 (CH₃), 28.2 (CH₃), 28.3 (CH₃), 43.9 (C), 55.1 (CH₃, d, *J* = 6.0 Hz), 55.2 (CH₃, d, *J* = 6.0 Hz), 59.0 (CH), 79.5 (C), 118.6 (CH), 130.5 (C, d, *J* = 6.0 Hz), 137.0 (CH), 139.2 (C), 150.1 (C), 155.3 (C, d, *J* = 5.0 Hz), 174.1 (C); HRMS (ESI-TOF) calc. for C₂₀H₃₃NO₈P ([M + H]⁺): 446.1934, found: 446.1963.

Peptide resin S13



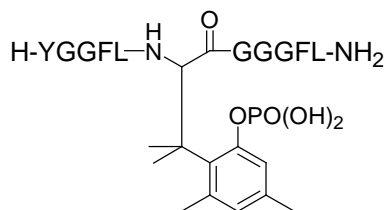
Pre-activated Boc-Leu-OH monohydrate (0.13 g, 0.50 mmol) in the presence of DIC (77 μ L, 0.50 mmol) and HOBt·H₂O (76 mg, 0.50 mmol) in DMF (1.0 mL) for 30 min was coupled to MBHA LL resin·HCl (loading: 0.70 mmol/g, 0.14 g, 0.10 mmol, pre-washed with 20% piperidine in DMF) for 3 h. After deprotection of Boc group by 50% TFA with 2% anisole in toluene, peptide elongation was performed by Boc chemistry (5.0 equiv of each amino acid) using DIC (77 μ L, 0.50 mmol) / HOBt·H₂O (76 mg, 0.50 mmol.) / DIEA (35 μ L, 0.20 mmol) in DMF (1.0 mL) to give the peptide resin **S13**.

Coupling of Boc derivative **S12** with peptide resin **S13**.



Boc derivative **S12** (0.25 mmol) was attached to the amino group of the resin **S13** by DIC (39 μL , 0.25 mmol), HOBt·H₂O (38 mg, 0.25 mmol) and DIEA (35 μL , 0.20 mmol) in DMF for 40 h to give the **S14**.

Synthesis of phosphate responsive peptide **12**.



Elongation of peptide on **S14** (0.025 mmol) was performed as described for peptide resin **S13**. Boc-Tyr(2-Br-Z)-OH was used as Tyr building block. For the global deprotection, our two-step protocol was used.^[S5] To the protected peptide resin (48 mg, 0.016 mmol) were added *m*-cresol (120 μL), ethanedithiol (120 μL), and thioanisole (281 μL). After being stirred for 5 min at room temperature, reaction mixture was cooled in an ice-chilled bath, and then precooled TFA (1.66 mL) and TMSOTf (434 μL) were successively added. After stirring for 1.5 h at 4 °C, dimethylsulfide (960 μL) and TMSOTf (240 μL) were added to the reaction mixture. The reaction was carried out for 3 h at 4 °C. After filtration of the resin, filtrate was poured into pre-cooled diethyl ether and extracted with water. Aqueous phase was washed by cooled diethyl ether and purified by RP-HPLC to give the desired peptide **12** (2.6 mg, 13%) as a diastereomeric mixture. Analytical HPLC condition: linear gradient of solvent B in solvent A, 10 to 60% over 30 min. Retention time = 23.6 or 24.2 min, respectively for each diastereomer. Semi-preparative HPLC condition: linear gradient of solvent B in solvent A, 25 to 55% over 30 min. MS (ESI-TOF, reconstructed) calc. for C₆₂H₈₆N₁₂O₁₆P ([M + H]⁺): 1285.6, found; 1285.5 and 1285.6.

[Phosphatase-induced Processing Reaction of Model Peptide **12**]

Alkaline phosphatase solution purchased from Wako Pure Chemical Industries (0.2 μL ; 10 units/ μL alkaline phosphatase, 10 mM Tris-HCl (pH 8.2), 50 mM KCl, 1 mM MgCl₂, 0.1 mM ZnCl₂, 50% glycerol) was added to the peptide **12** (64 μg , 0.050 μmol) in Tris-HCl buffer (88.9 μL ; 10 mM Tris-HCl (pH 7.9), 50 mM NaCl, 10 mM MgCl₂, 1 mM DTT), and the resulting mixture was

incubated at 37 °C for 24 h. Reaction was monitored by analytical HPLC and peptides were characterized by ESI-MS. Analytical HPLC condition: linear gradient of solvent B in solvent A, 10 to 60% over 30 min. **13**; Retention time = 12.5 min. MS (ESI-TOF) calc. for C₂₁H₃₃N₆O₅ ([M + H]⁺): 449.3, found; 449.2.

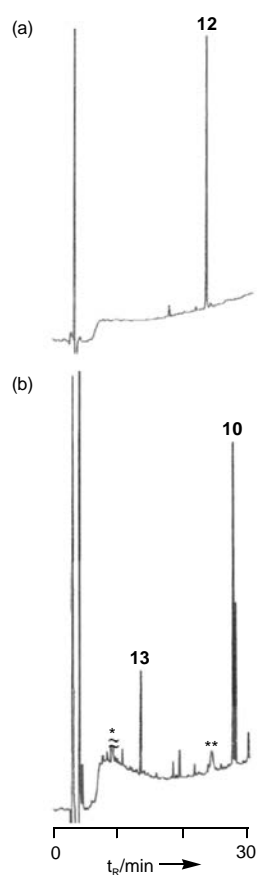
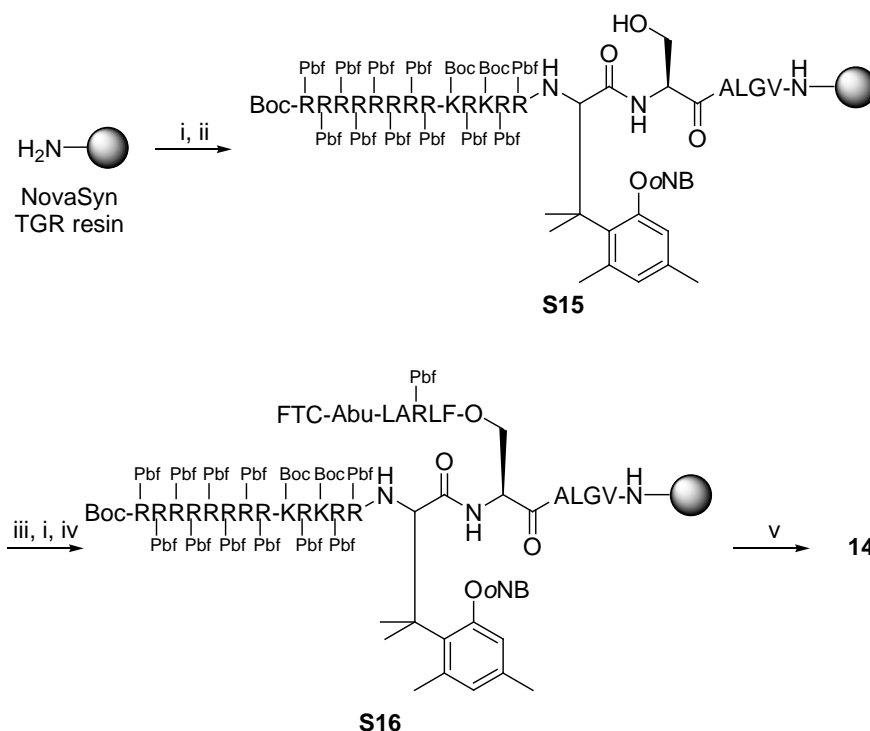


Figure S2. HPLC profiles (a) before treatment with alkaline phosphatase and (b) after 24 h of incubation with alkaline phosphatase at 37 °C. Peptides were detected by UV absorbance at 220 nm. *Non-peptidic compound. **Alkaline phosphatase.

[Preparation of Shuttle Peptide 14]



Reagents and conditions. (i) Fmoc SPPS; (ii) Boc₂O, HOBT, DIEA, DMF; (iii) Fmoc-Phe-Cl, DIEA, DMAP, CH₂Cl₂; (iv) FITC, DIEA, DMF; (v) TFA/triethylsilane/H₂O = 95/2.5/2.5.

Peptide elongation.

Peptide was elongated by Fmoc SPPS in a manner similar to that of photo-responsive model peptide **8**. As a serine building block, side chain unprotected one was used.

Boc protection of *N*-terminal amine group.

Peptide resin (0.0375 mmol) was treated with Boc₂O (34.5 μL, 4 eq.), HOBT (20.0 mg, 4 eq.) and DIEA (26.4 μL, 4 eq.) in DMF for 1 h to give peptide resin **S15**.

Acylation of Ser side chain.

Pre-mixed Fmoc-Phe-Cl^[S6] (133 mg, 10 eq.) and DIEA (129 μL, 22 eq.) in dichloromethane (833 μL) and DMAP (0.80 mg, 0.2 eq.) were added to peptide resin **S15** (0.0340 mmol). Reaction mixture was shaken for overnight. Reaction progress was monitored by ESI-MS after test cleavage.

Introduction of fluorescein thiocarbamoyl group.

To peptide resin (0.0340 mmol) were added FITC (32.2 mg, 2.4 eq.) and DIEA (15.0 μL, 2.5 eq.) in DMF (270 μL), and reaction mixture was shaken for 5 h to give FTC derivative **S16**.

Global deprotection and purification.

Global deprotection was performed in a manner similar to that of photo-responsive model peptide **8**. The shuttle peptide was diastereomerically purified to give peptide **14** (19%) and its diastereomer (8%). Analytical HPLC condition: linear gradient of solvent B in solvent A, 10 to 60% over 30 min. Retention time = 21.0 (**14**) and 21.6 (diastereomer of **14**) min. Preparative HPLC condition: linear gradient of solvent B in solvent A, 13 to 23% over 30 min. MS (ESI, reconstructed) calc. for $C_{172}H_{281}N_{66}O_{34}S$ ($[M + H]^+$): 3849.6, found; 3849.4 (**14**) and 3849.4 (diastereomer of **14**).

[Photo-responsive Processing Reaction of Shuttle Peptide 14]

Shuttle peptide **14** (0.10 mg, 0.026 μmol) in acetonitril (126 μL) was added to phosphate buffer (0.02 M, pH 7.6, 632 μL) and was irradiated by UV light ($\lambda > 365$ nm) for 4 min. Resulting mixture was incubated at 37 $^{\circ}\text{C}$, and reaction was monitored by analytical HPLC. Analytical HPLC conditions: linear gradient of solvent B in solvent A, 10 to 60% over 30 min. **15**: retention time = 12.8 min, MS (ESI, reconstructed) calc. for $C_{91}H_{174}N_{49}O_{15}$ ($[M + H]^+$): 2194.7, found; 2194.9. **17**: retention time = 28.3 min, MS (ESI, reconstructed) calc. for $C_{74}H_{103}N_{16}O_{17}S$ ($[M + H]^+$): 1520.8, found; 1521.0. **S17**: retention time = 24.4 min, MS (ESI, reconstructed) calc. for $C_{74}H_{103}N_{16}O_{18}S$ ($[M + H]^+$): 1536.8, found; 1537.0. **S18**: retention time = 27.7 min, MS (ESI, reconstructed) calc. for $C_{74}H_{103}N_{16}O_{18}$ ($[M + H]^+$): 1504.7, found; 1505.0.

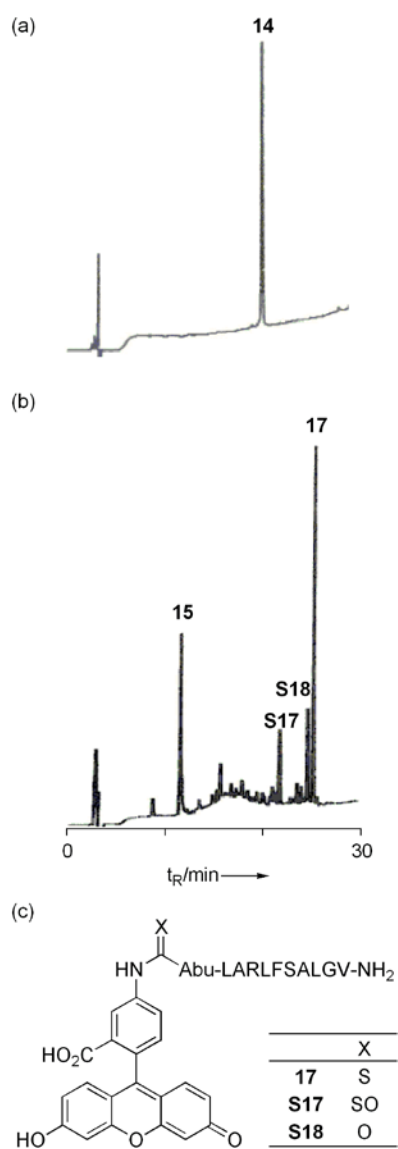


Figure S3. HPLC profiles (a) before UV irradiation and (b) after 4 min of UV irradiation followed by 1.5 h of incubation at 37 °C. Peptides were detected by UV absorbance at 220 nm. (c) Structure of processing products.

[Localization Assay of Shuttle Peptide 14]

CHO-K1 cells were seeded on an eight-well Lab-Tek-II chamber slide (Nalge Nunc International, Naperville, IL) at a density of 1×10^4 cells/well in Ham's F-10 containing 10% fetal bovine serum (FBS). To examine the localization of the shuttle peptide, cells were incubated with shuttle peptide **14** (10 μ M) in serum free medium for 1 h. After washing three times with ice-cold phosphate-buffered saline (PBS) and replacement with fresh medium containing FBS, the cells were irradiated by UV light (>365 nm) for 4 min. After additional 1 h of incubation, the cells were fixed by 4% paraformaldehyde (PFA), stained with Hoechst 33258, and then examined with confocal fluorescent microscopy. In some experiments, leptomycin B (LMB), an inhibitor of NES-mediated nuclear export, was added to a final concentration of 200 nM, after UV irradiation.

References

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