Electronic Supplementary Material (ESI) for ChemComm.

Conformational control in a photoswitchable coiled coil

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Supporting Information

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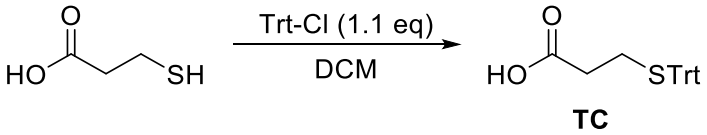
Experimental Details

Thiol-cap Synthesis



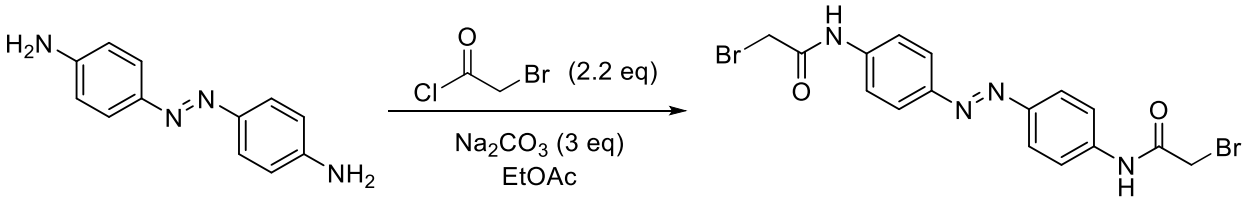
TC

3-mercaptopropionic acid (1 g, 9.42 mmol, 821 uL) and trityl chloride (1.1 eq, 2.89 g, 10.36 mmol) were dissolved in DCM and stirred 24 hr at room temperature. The white precipitate was collected by filtration and rinsed with DCM. Product was a white solid isolated in 81% yield.

^{1}H NMR (400 MHz, DMSO) δ 12.1 (bs, 1H), 7.2 (m, 15H), 2.18 (t, J = 6.72, 2H), 2.06 (t, J = 6.72, 2H); ^{13}C NMR (400 MHz, DMSO) δ 173.15, 144.8, 129.56, 127.2, 66.7, 33.4, 27.15; ESI-MS $[M+Na]^+$ calc: 371.11, found: 371.0.

Cross-linker Synthesis

Cross-linker was synthesized as described by Pfister et al.[1] beginning with acetanilide apart from the final step being modified as shown.



CL

Under N_2, 4,4'-azodianiline (13.5 mg, 63.6 umol) was dissolved in EtOAc on ice with Na_2CO_3 (21 mg, 190.8 umol, 3 eq). While stirring on ice, bromoacetyl chloride (12 uL, 139.9 umol, 2.2 eq) was added slowly over several minutes. The mixture was brought to room temperature and stirred for 3 hr before being quenched with water. The organic layer was set aside and the aqueous layer was extracted 3x with EtOAc. Organic layers were combined, rinsed with 3x dH_2O and 3x brine, dried over Na_2SO_4, filtrated, and concentrated *in vacuo* to afford a dark green solid in 82% yield.

^{1}H NMR (500 MHz, DMSO) δ 10.73 (s, 2H), 7.89 (d, J = 8.9 Hz, 4H), 7.81 (d, J = 8.95, 4H), 4.1 (s, 4H); ^{13}C NMR (400 MHz, DMSO) δ 165.7, 148.5, 141.8, 124, 120, 30.8; ESI-MS $[M+Na]^+$ calc: 476.94, found: 476.8.

Peptide Synthesis

Peptides were synthesized by Fmoc/t-Bu strategy on Rink Amide resin (Protein Technologies). Fmoc deprotection was done with 20% piperidine in DMF with 0.1 M HOBt, and activation for coupling was done with 5 equivalents each of DIC, HOBt, and amino acid. Peptides were N-acylated with S-trityl-3-mercaptoproionic acid (**TC**). Cleavage from resin and global deprotection was done with 90% TFA, 5% thioanisole, 3% ethanedithiol, and 2% anisole. Solvent was removed *in vacuo* and cold diethyl ether was added to triturate the solid peptide after which it was decanted. Peptides were purified with RP-HPLC on a C18 column with a gradient of 15-60% MeCN in water with 0.1% TFA followed by lyophilization yielding white powder.

Peptide Cross-linking

Free-thiol peptides were dissolved in 50 mM tris pH 8.1 with 10% MeCN and 0.5 equivalents of TCEP for thirty minutes to ensure disulfide reduction. 0.3 equivalents of bis(bromoactetamide) cross-linker (**CL**) were dissolved in MeCN and added portion wise to the peptide solution and mixed overnight. The solution was acidified with dilute TFA, filtered, and injected on RP-HPLC for purification over a 15-60% MeCN gradient. Pure fractions were lyophilized yielding yellow powder.

UV-Vis Spectrophotometry

Peptides were dissolved at 300 μM in 50 mM sodium phosphate pH 5.8 (peptide **2** measured at 100 μM due to solubility). Spectra were collected on an Agilent Cary UV-Vis Compact Peltier in the 200 to 600 nm range with a blank of buffer subtracted. Samples were kept wrapped in aluminum foil until placed in the instrument for analysis to obtain dark-adapted spectra. Irradiation was performed with a 370 nm LED lamp (Kessil) before recording excited state spectra. Thermal reversion was observed by allowing the sample to relax in the instrument.

Circular Dichroism Spectroscopy

Peptides were dissolved at 30 μM in 50 mM potassium fluoride pH 7.4 (unless otherwise noted). Concentrations were determined by the azobenzene chromophore's absorption at 367 nm (ε = 28 000 $cm^{-1}M^{-1}$).[2] Spectra were collected on a Jasco J-1500 CD Spectrometer as an average of three accumulations with scanning speed of 50 nm/min and digital integration time of 4 sec. Samples were kept wrapped in aluminum foil until placed in the instrument for analysis to obtain dark-adapted spectra. Irradiation was performed with a 370 nm LED lamp (Kessil) before recording excited state spectra. Thermal reversion was observed by allowing the sample to relax in the instrument.

Size-exclusion Chromatography

Samples were dissolved in 0.1x PBS with 10% MeCN and injected onto a Superdex 30 increase 3.2/300 column (GE) with the same buffer flowing at 0.05 mL/min over a 50 minute run. Retention times of three standards (lysozyme, aprotinin, and vitamin B12) were recorded and plotted as ln(MW) vs. R_T. The equation to the resulting linear curve was used to extrapolate the apparent molecular weight of the analyte.

Dynamic Light Scattering

Peptide **1** was dissolved at 30 and 300 μM in 50 mM sodium phosphate pH 5.8 and filtered through a 0.45 μm syringe filter. Samples were analyzed with a Malvern Zetasizer Nano ZS.

Supplementary Figures

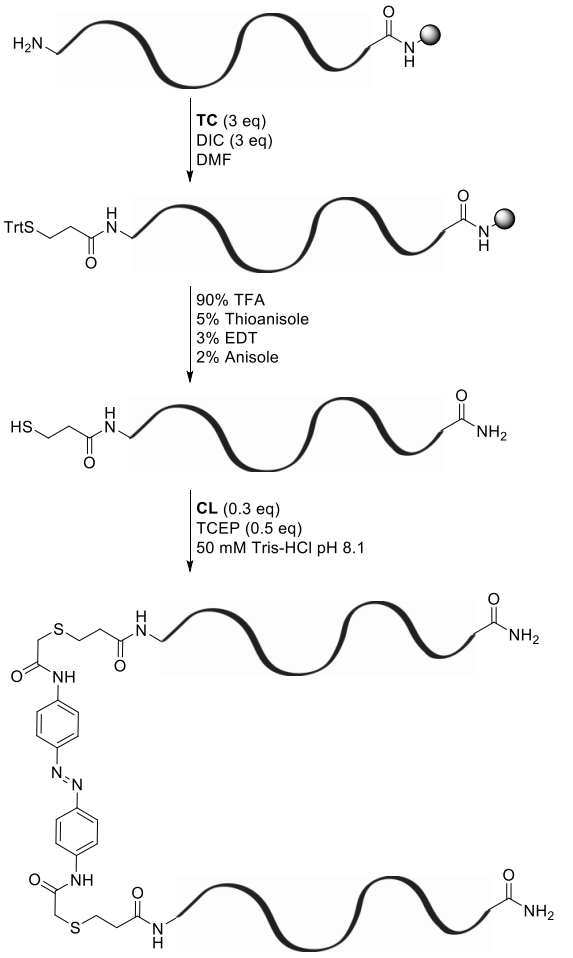


Figure S1. General scheme for synthesis of photoswitch peptides.

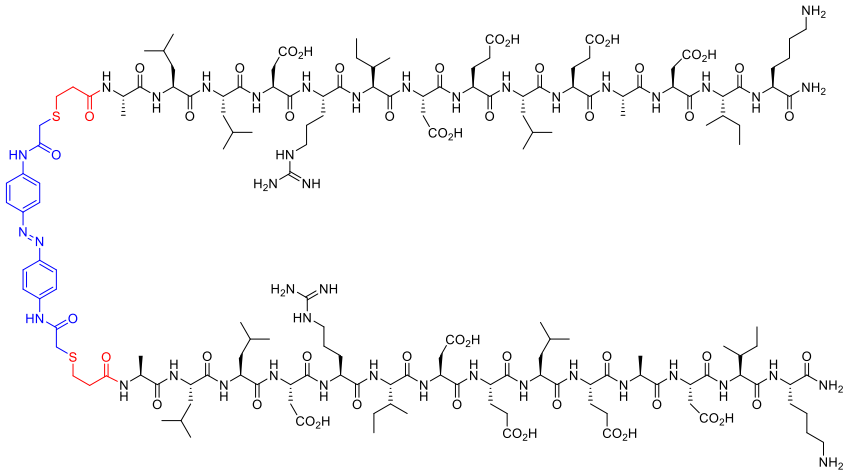


Figure S2. Chemical structure of peptide **1**. Azobenzene photoswitch shown in blue, 3-mercaptopropionate residue shown in red, and peptide chains shown in black.

	c	*d*	*e*	*f*	*g*	*a*	*b*	*c*	*d*	*e*	*f*	*g*	*a*	*b*	*c*	*d*	*e*	*f*	**Connectivity**	**N-termini distance (Å)**	**Change in θ_{222}**
1	A	L	L	D	R	I	D	E	L	E	A	D	I	K					Outer Diagonal	13.1	50%
2				A	R	I	D	E	L	E	A	D	I	K	A	L	E		Across	12.5	-
3					A	I	D	E	L	E	A	R	I	K	A	L	E	D	Inner Diagonal	11.2	-
4		A	L	D	R	I	D	E	L	E	A	D	I	K	A				Adjacent	8.6	-

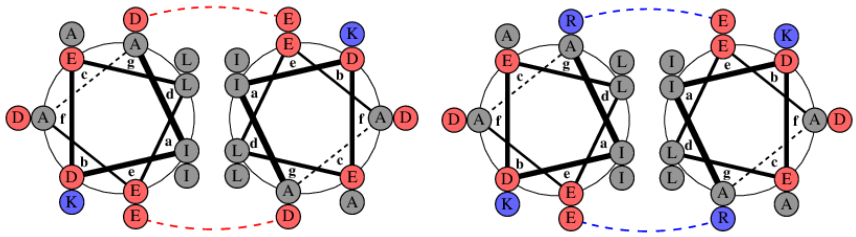


Figure S3. (Top) Peptide sequences aligned by heptad repeat position. Connectivities, N-terminal nitrogen distances, and CD results are listed. (Bottom) Helical wheel of **3** showing ionic clash/pair before [left] and after [right] D8R mutation

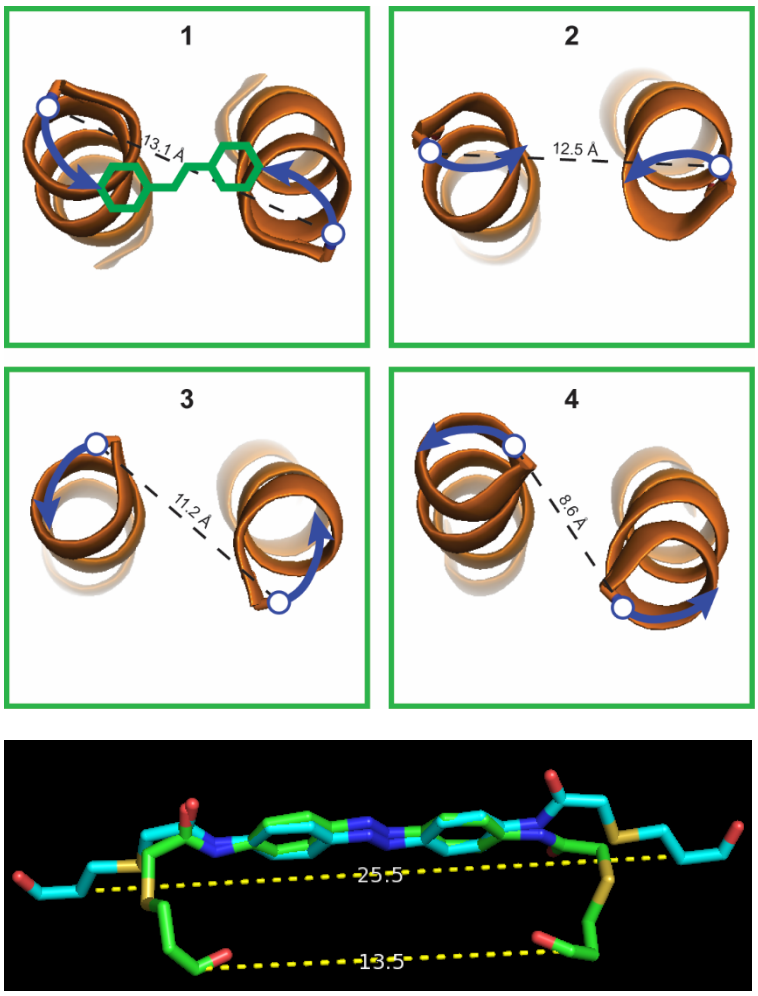


Figure S4. (Top) Top-down view of excised regions of tropomyosin (PDB 1c1g[3]) used in this study. N-terminal nitrogens represented by white circles with arrows indicating main chain trajectory. (Bottom) Model of azobenzene photoswitch linker. Distance measurements are between carbonyl carbons to be linked to peptide N-termini. Cyan structure is fully extended and green structure is with folding to accommodate linkage in **1**.

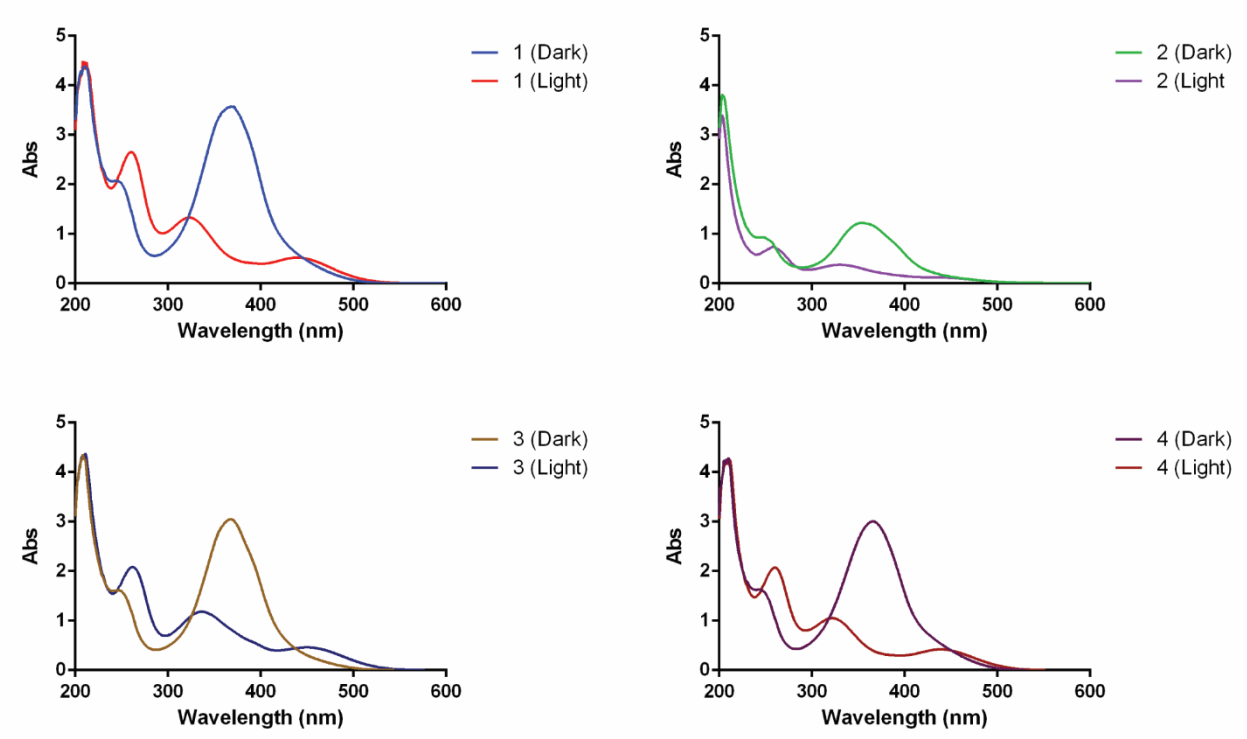


Figure S5. UV-vis spectra of dark-adapted and irradiated peptides **1**-**4**

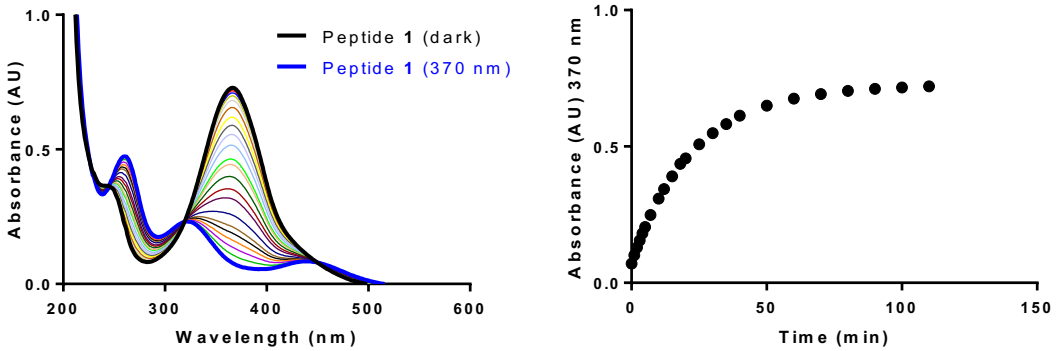


Figure S6. (Left) UV-vis spectrum of peptide **1** showing thermal reversion after photoisomerization (Right) Absorbance of peptide **1** at 370 nm over time during thermal reversion in the dark which was complete after 110 min.

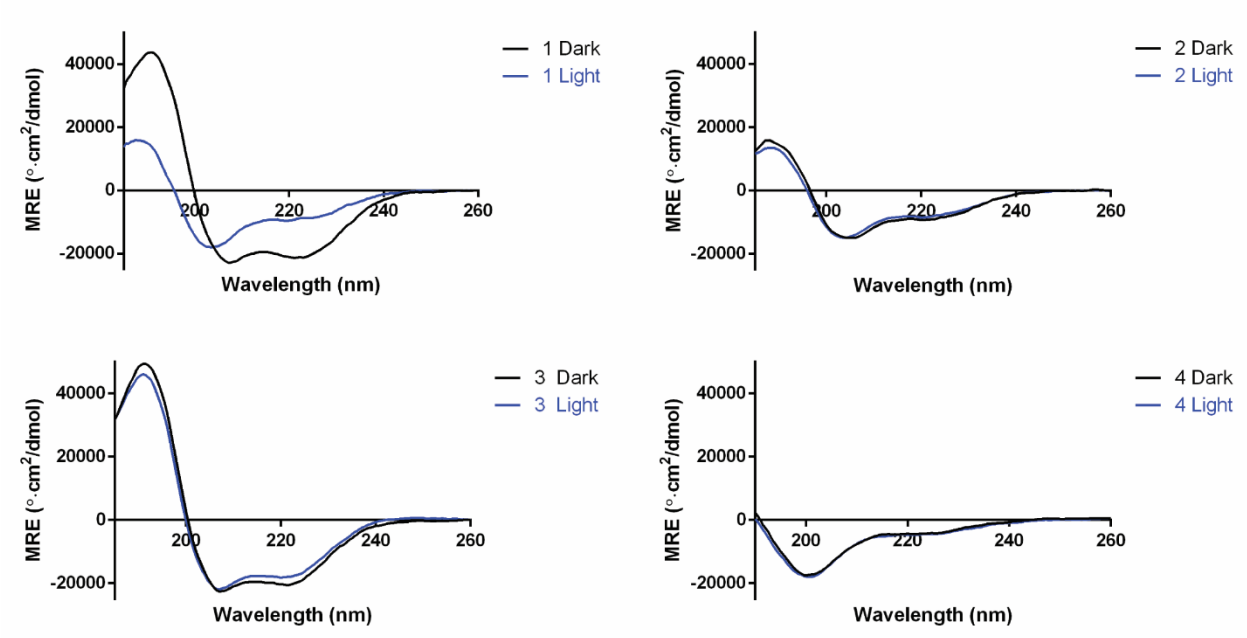


Figure S7. CD spectra of dark-adapted and irradiated peptides **1**-**4**

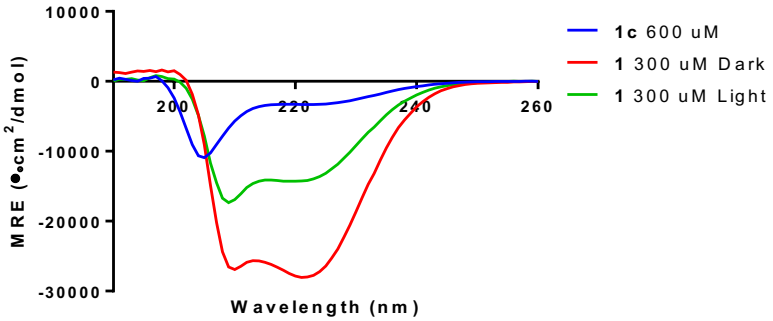


Figure S8. CD spectra of peptide **1** dark-adapted (red) and after 370 nm irradiation (green). Control peptide **1c** with no photoswitch linker (blue) is remarkably less structured highlighting the critical role of the linker. Spectra were recorded in 50 mM sodium phosphate pH 5.8.

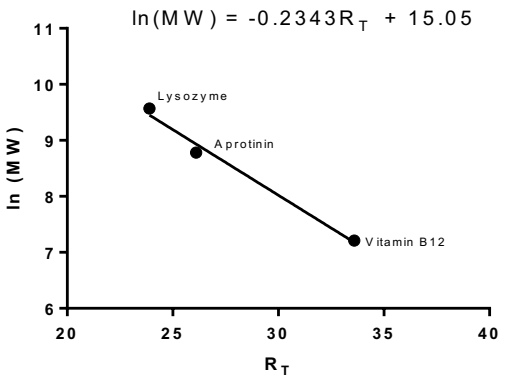


Figure S9. SEC calibration curve plotting ln(MW) vs. R_T

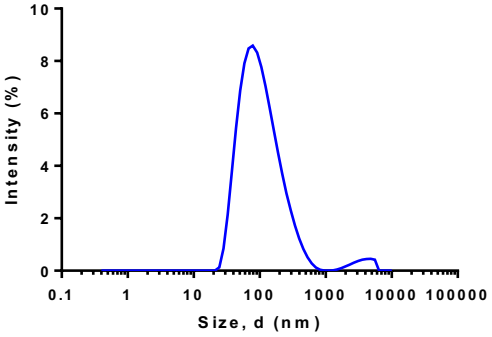
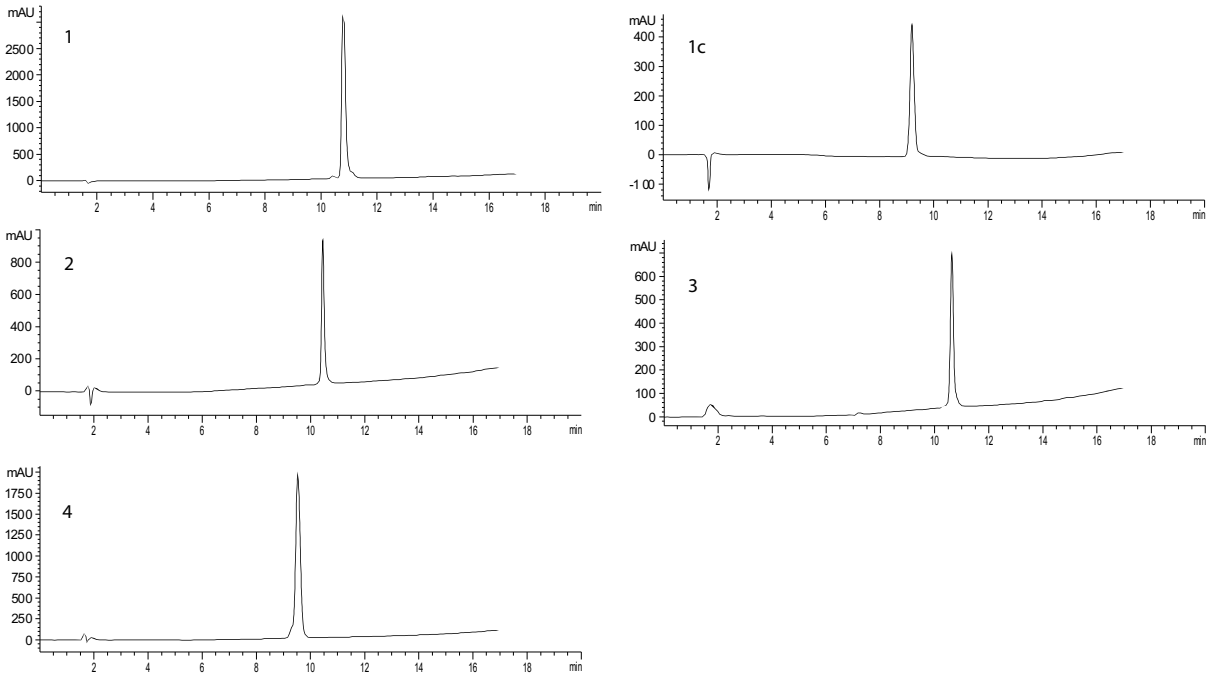


Figure S10. DLS plot of dark-adapted peptide **1** at 300 µM in 50 mM sodium phosphate pH 5.8. No large particle was observed at 30 µM.

Analytical Data

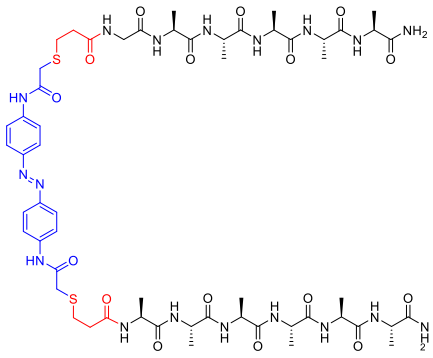
Peptide HPLC chromatograms



Peptide MALDI-TOF MS

Peptide	Sequence	Calc. Mass	Obs. Mass
1	[PS](XALLDRIDELEADIK)$_2$	3692.90	3693.199
1c	Ac-ALLDRIDELEADIK	1654.90	1655.059
2	[PS](XARIDELEADIKALE)$_2$	3636.81	3637.915
3	[PS](XAIDELEARIKALED)$_2$	3636.81	3636.969
4	[PS](XALDRIDELEADIKA)$_2$	3608.78	3609.492

[PS] = photoswitch, 4,4'-diacetamide(azobenzene); X = 3-mercaptopropionic acid



References

1. R. Pfister, J. Ihalainen, P. Hamm and C. Kolano, *Organic & Biomolecular Chemistry*, 2008, **6**, 3508-3517.
2. D. G. Flint, J. R. Kumita, O. S. Smart and G. A. Woolley, *Chemistry & Biology*, 2002, **9**, 391-397.
3. F. G. Whitby and G. N. Phillips Jr., *Proteins: Structure, Function, and Bioinformatics*, 2000, **38**, 49-59.