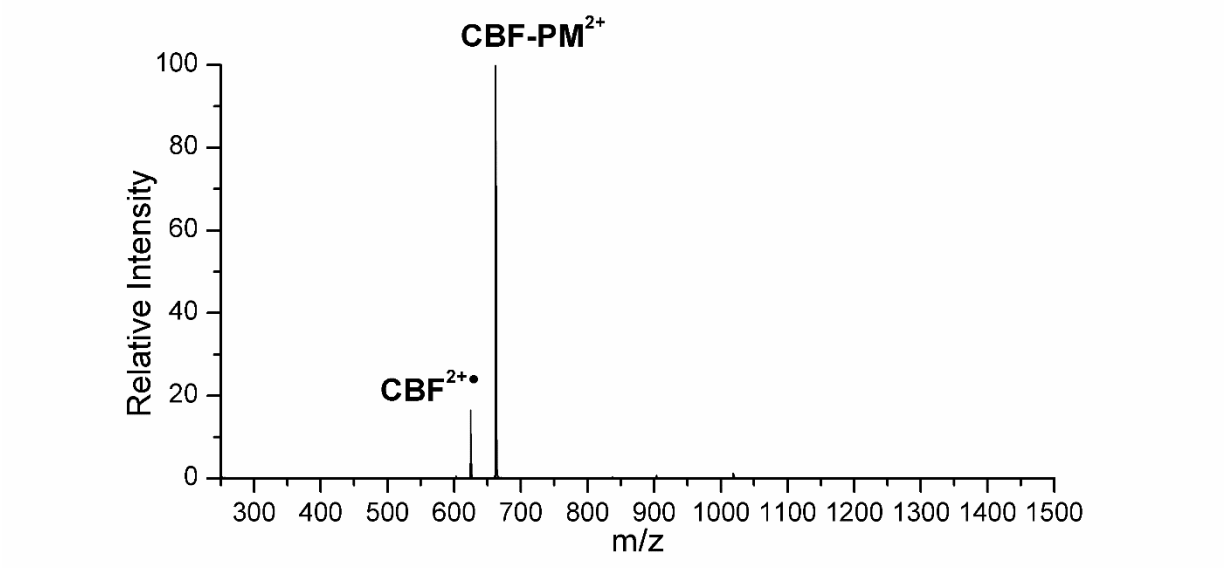
Supporting information

Materials and synthetic details

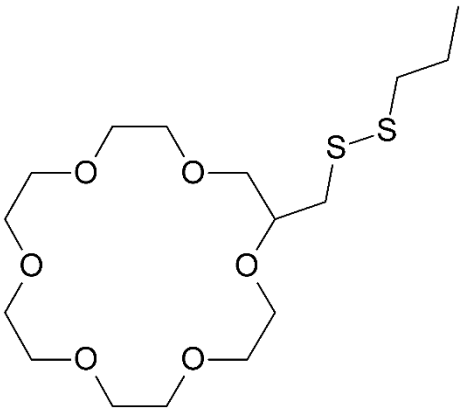
The Collagen Binding Fragment and CD36 139-155 peptides were purchased from American Peptide Company. The peptides YF and CQDSETRTVY were synthesized by standard solid-phase procedures detailed elsewhere.[1]

Peptides were reacted with propyl mercaptan (PM) by adding 1 µL of PM to 1 nmol of peptide in 50% dimethyl sulfoxide-water. The mixture was incubated at 40°C overnight before removing the solvent by lyophilisation.

Photodissociation (PD) yields were determined by calculating the percent product ion formed resulting from the loss of propyl mercaptan from the modified peptide. Only the homolytic disulfide cleavage was observed in any significant yield for these experiments, as shown in an example below with the MS^2 photodissociation spectra of the CBF-PM peptide in the 2+ charge state.



18-crown-6-methanethiol was synthesized from reacting 1.6mmol of 18-crown-6-alcohol with 1.5 equivalents of tosyl chloride and 2 equivalents of pyridine in 600 µL of dichloromethane for 1 hour. 2 equivalents of potassium thioacetate and 1 mL of methanol were added and the reaction mixture was heated to 50°C for 9.5 hours. The supernatant from the reaction mixture was removed and dried under nitrogen. The product was resuspended in 2 mL of methanol and 2 equivalents of potassium carbonate were added. The mixture was reacted for 2 hours with vigorous stirring. The supernatant was removed to obtain the product. Reaction progress at each step was monitored by mass spectrometry. The subsequent product was reacted with PM using the same procedure as in the case of peptides.



Simulated annealing molecular dynamics simulations

Simulations to obtain lowest energy structures for the examined peptides were performed according to the same methods described in [1]. Simulations were performed in the Maestro software suite (Schrödinger Inc., Portland, Oregon). Simulations were done in 100 cycles using the OPLS 2005 force field, following with minimization using the Polak-Ribiere Conjugate Gradient (PRCG) method. Each cycle began with molecular dynamics runs were performed at 300 K (1.5 fs step interval, 1 ps duration), incrementally raising to 400, 500, and 1000 K (1.0 fs step interval, 10 ps duration) before lowering to 300 K and then 200 K (1.5 fs step interval, 10 ps duration). The structure is then minimized again by the PRCG method. Structures were saved at the end of each cycle to yield 100 structures after each simulation. Structures were subsequently sorted by energy to give the lowest-energy conformer. Constrained simulations were also performed in the same fashion, but while imposing a distance constraint between two atoms of interest (4±3 Å or 6±3 Å) to guide simulations to experimentally consistent structures. The constrained, annealed structures were then re-minimized by the PRCG method without constraints to yield a final structure and energy.

Energies were also calculated using PM6 semi-empirical calculations in the Gaussian 09 Rev A.1 software (Gaussian Inc., Wallingford, CT.). Initial structures were taken from the lowest-energy structures obtained from simulated annealing. Structures were optimized to a minimum without calculating force constants, and energies were calculated using the PM6 method in the ground state with the default spin. Charges were either +1 or +2, matching that of the CBF charge state. No counterion or solvation was present.

Disulfide dissociation energy calculations

Time dependent calculations to determine the energy of the dissociative transition for the disulfide bond were performed using RB3LYP 6-31G(d) in Gaussian 09 Rev A.1 program. The energy for the dissociation of methyldisulfide corresponded to the transition from the HOMO to the LUMO (n=25 to 26), which is clearly an antibonding orbital along the S-S bond.

Action-EET spectra and traditional action spectra

Action spectra were compiled by monitoring (as a percent) the loss of the propyl-mercaptan radical (for action-EET) or the largest product ion (for traditional action spectra) at varying wavelengths. Average laser power levels at each wavelength were recorded. In both action-EET and traditional action spectra the relative PD yields between wavelengths were corrected according to differences in laser power.

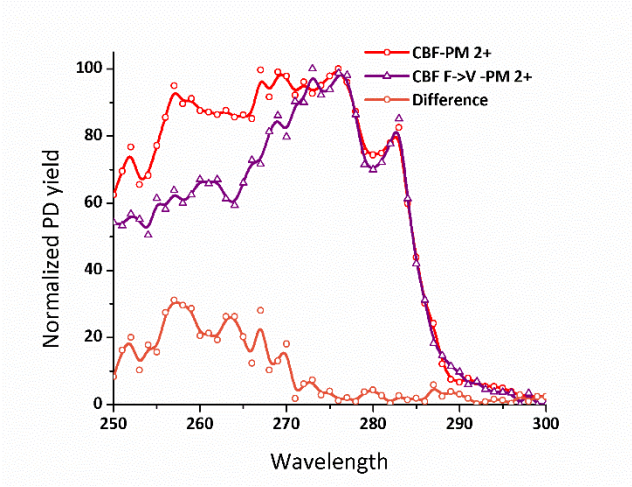


Figure S1

Action-EET spectra of the CBF peptide (red trace) and CBF F-to-V mutant (purple trace) in the 2+ charge state normalized to the highest PD yield in each data set. The difference is shown in orange.

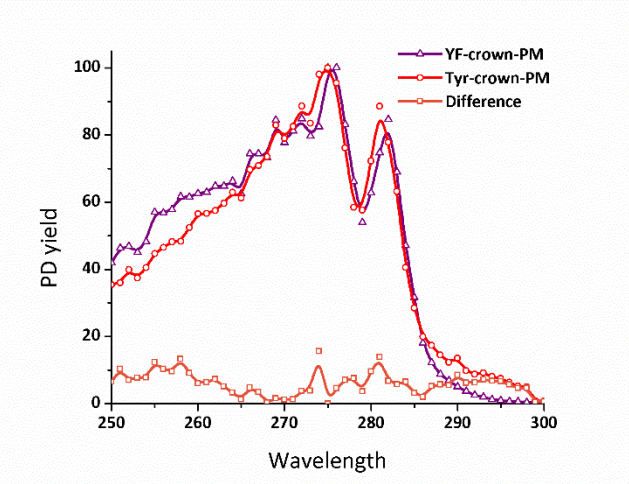
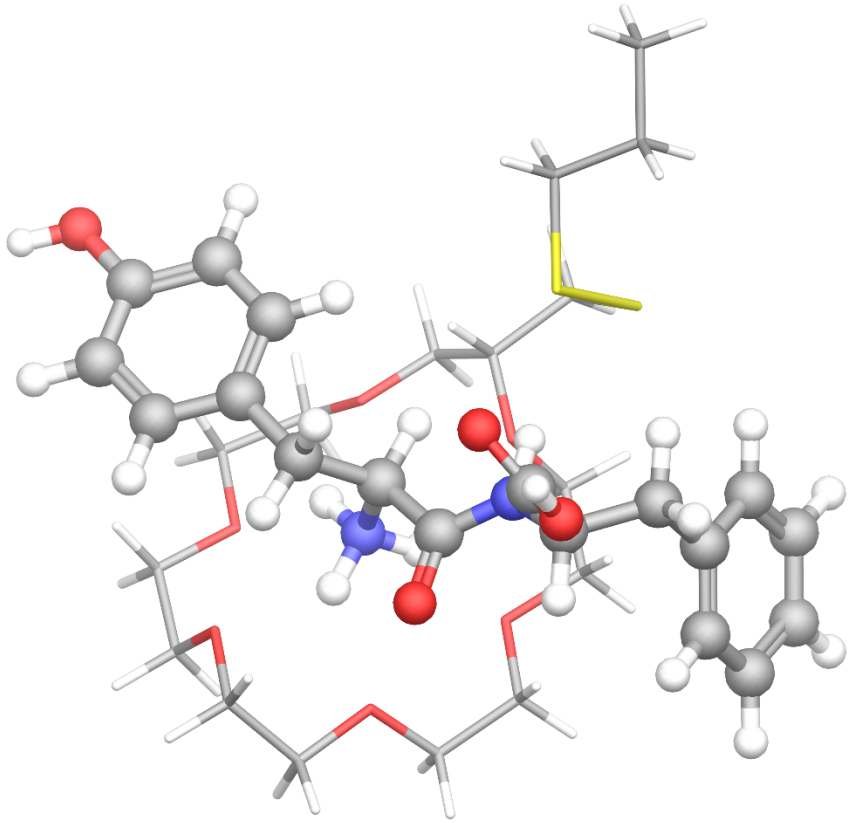


Figure S2

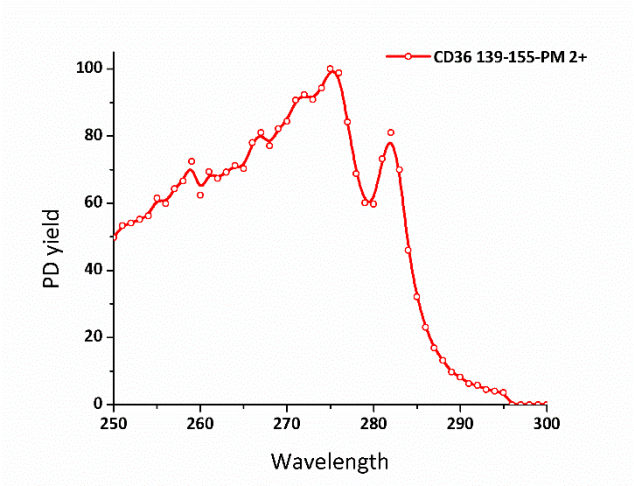
Action-EET spectra of Tyrosine (red) and the YF peptide (purple) attached to crown-PM. The red trace is representative of tyrosine absorbance spectra. The difference is shown in orange.

Figure S3



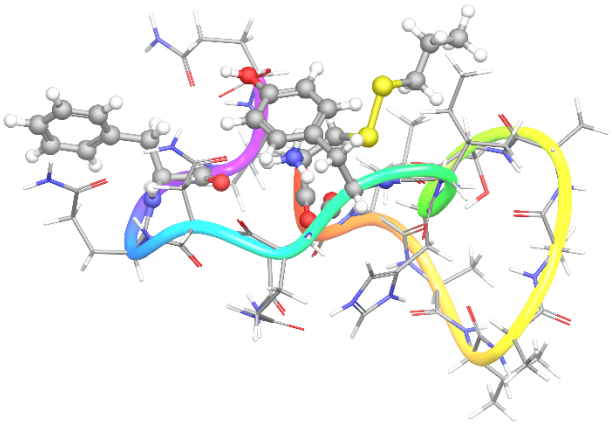
Lowest energy structure of the YF, crown-PM complex. The distance between Phe and Tyr is 7.7 Å. The simulated annealing maximum temperature was reduced to 650K (from 1000K) to preserve the noncovalent complex. All other parameters were the same as other calculations.

Figure S4



Action-EET spectrum of the 2+ CD36 139-155 peptide (CNLAVAAASHIYQNQFVQ, purchased from American Peptide Company) modified with PM.

Figure S5



The lowest energy conformation of the CD36 139-155 peptide modified with PM (charges on N-terminus, His) as obtained by the previously mentioned simulated annealing procedure. Distance between Tyr and the disulfide is 3.6 Å, and the Phe-to-Tyr distance is 8.0 Å.

[1] N. G. Hendricks, N. M. Lareau, S. M. Stow, J. A. McLean, R. R. Julian, *J. Am. Chem. Soc.* **2014**, *136*, 13363–13370.