Experimental Details

Peptide Synthesis

Peptide 1 (Cys-cysteamine): Cysteamine 4-methoxytrityl resin (AnaSpec, Inc.) was swelled in CH_2Cl_2 for 10 min, and then washed by DMF. The coupling reaction took place in DMF when adding another 2 equiv of Fmoc-L-Cys(Trt)-OH (AnaSpec, Inc.), 2 equiv of HCTU and 4 equiv of DIPEA for ~40 min. The resin was then washed with DMF to remove excess reagents. Fmoc deprotections were performed using 20% piperidine in DMF, followed by washing with CH_2Cl_2 and dried in Air. Resin cleavage was accomplished using 95% TFA containing 2.5% each of triisopropyl silane and water (TFA:TIS:water = 95:2.5:2.5). In the mean time, the thiol group of cysteine was deprotected with TFA. The resin was rinsed twice with TFA and then dried in vacuum.

Peptide 2 (Cys-Gly-cysteamine): The preparation was also started using cysteamine resin as described above. In the presence of HCTU and DIPEA, the resin was first reacted with Fmoc-Gly-OH, and then the amine group was deprotected with 20% piperidine in DMF. After thoroughly rinsed the resin with DMF, Fmoc-L-Cys(Trt)-OH was added and deprotected after the coupling reaction. The product was also cleaved from the resin using TFA as for peptide 1.

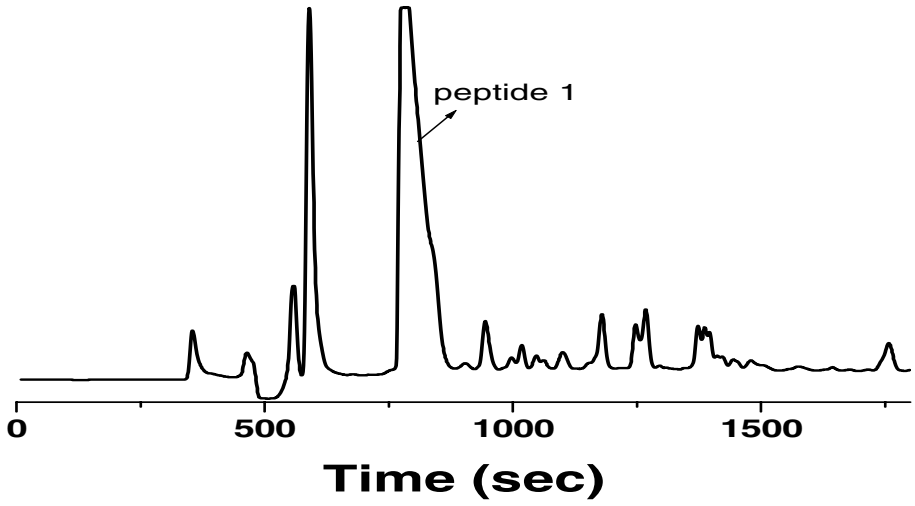
Peptide 3 (Cys-Gly-Cys): This peptide was sythesized using Fmoc-L-Cys(Trt)-PEG-PS resin (Applied Biosystems). The preparation procedure is similar to that of peptide 2.

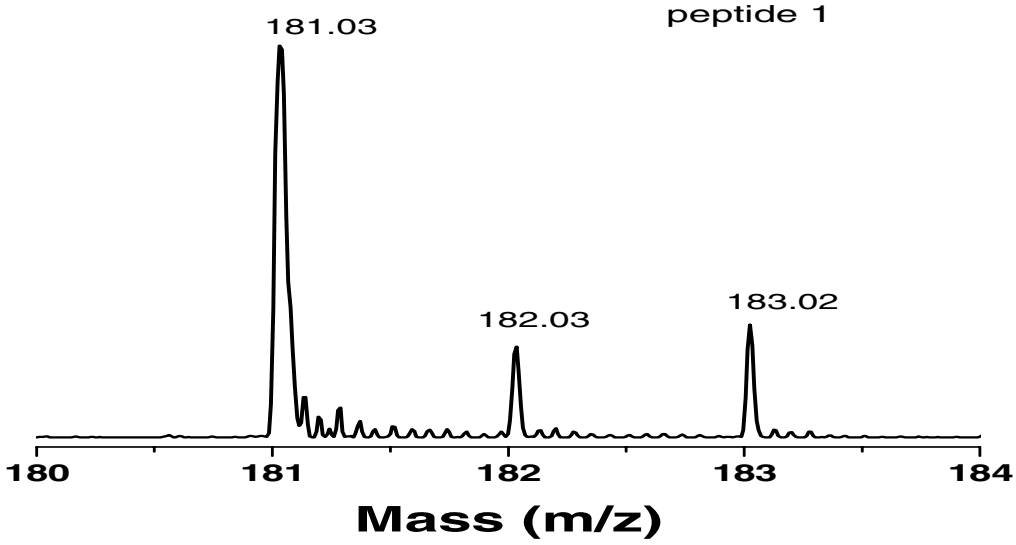
HPLC purification

Peptides synthesized using the procedures described above were re-dissolved in water and purified by running HPLC through C18 column (Alltech, Hyperprep PEP, 100Å, 8 μm) using a gradient of 5 to 20% acetonitile in water containing 10 mM TFA. Flow rate = 3.4 mL/min,

wavelength = 220 nm. After purification, the mass spectra of these peptides show peaks at m/z = 181, 238, and 282 respectively (see figures below), which are identical to their monoisotopic forms. No dimers and oligomers were detected in Mass spectra.

Chromatographic and Mass spectra of Peptide 1 are shown as an example:





Sample solutions

The supporting solutions of different pH value were prepared from perchloric acid, sodium perchlorate, sodium acetate, sodium carbonate/bicarbonate and sodium hydroxide, using 18 $M\Omega.cm^{-1}$ water (Nanopure system fed with campus distilled water). All have a concentration of ~0.1 M. The peptide samples were dissolved in water and diluted by each of the supporting solution to ~1 mM, and were used immediately in each experiment.

Substrate and tip preparations

The gold substrate was prepared by thermally evaporating 100 nm gold on mica in a UHV chamber. Before each experiment, the substrate was briefly annealed in a hydrogen flame and immediately immersed in one of above prepared solution. The cell was cleaned by boiling it in Piranha solution (98% H_2SO_4: 30% H_2O_2 = 3:1, v/v), and then sonicated in nanopure water three times. The STM tip was made of 0.25 mm gold wire (99.999%), and was coated with Apiezon wax in order to reduce ionic conduction and polarization currents. The measured leakage current is typically less than a few pA.