Supplementary Material (ESI) for Chemical Communications

Supporting information

General

All synthetic manipulations were performed under dry argon atmosphere using standard techniques. ^{1}H and ^{13}C NMR spectra were recorded using a Varian 500 spectrometer (500 MHz for ^{1}H and 125 MHz for ^{13}C). Mass spectra were recorded using a Agilent 1100 series LC/MSD. Absorption spectra were recorded using a PerkinElmer Lambda35 UV/Vis spectrometer. A FluoroMax-2 fluorometer (HORIBA Jobin Yvon Inc.) was used for all of fluorescence measurements.

Synthesis and characterization

CrAsH: 5-carboxyfluorescein-bismercurictrifluoroacetate: 5-carboxyfluorescein (95 mg, 0.25 mmol) was added into trifluoroacetic acid (2mL) containing mercuric oxide (108 mg, 0.50 mmol) at room temperature. After overnight stirring, the reaction mixture was evaporated and diluted with water (20mL). Dark yellow precipitate (5-carboxy fluorescein bis-mercuric trifluoroacetate, 147 mg) was collected by filtration with 59% yield. Due to insolubility this compound was not further characterized.
After drying in high vacuum, 5-carboxy fluorescein bis-mercuric trifluoroacetate (108 mg) was suspended in dry N-methylpyrrolidinone (NMP) (2mL) with arsenic trichloride (135uL, 2.8 mmol), diisopropylethylamine (DIEA) (105 uL, 1.1 mmol) and palladium acetate (10 mg). The reaction was stirred at room temperature for 3 hours. After cooling, 5 mL 0.25M phosphate buffer pH 7 and 1,2-ethanedithiol (EDT) (0.4 mL) was added into reaction mixture. The solution was extracted with chloroform (3 × 30mL), dried over Na_2SO_4, evaporated and purified by chromatography on silica gel (MeOH: toluene= 1:2 v/v) with a 41% yield. ^{1}H NMR ($CDCl_3$): 3.55 (m, 8H), 6.15 (d, *J*=7.2, 2H), 6.81 (d, *J*=6.8, 2H), 7.08 (d, *J*=6.0, 1H), 8.03 (d, *J*=6.4, 2H), 8.60 (m, 1H). ^{13}C NMR ($CDCl_3$): 29.8, 48.1, 63.2, 69.8, 108.2, 114.3, 120.3, 130.1, 155.9, 159.8, 168.0, 168.4, 172.3, 174.1. ESI/MS: m/z calcd. for $[M+H]^+$ 708.84, found 709.12

Acetyl CrAsH: 55 mg CrAsH were added into 5 mL pyridine with 15 uL acetyl chloride at room temperature for 30 min. After reaction, pyridine was removed *in vacuo* and 10 ml H_2O and 15ml $CHCl_3$ were added to extract the product. After drying under high vacuum, crude acetyl CrAsH was purified by silica gel chromatography using 20:80 ethyl acetate/dichloromethane. The reaction afforded a white powder with 70.7% yield. ^{1}H NMR ($CDCl_3$): 2.35 (s, 6H), 3.60 (m, 8H), 6.89 (m, 4H), 7.42(d, *J*=5.6, 1H), 8.40(d, *J*=5.4, 1H), 8.82(s, 1H). ^{13}C NMR $CDCl_3$): 43.3, 43.8, 63.2, 69.8, 116.1, 120.3, 124.1, 125.7, 126.2, 130.1, 153.0, 153.8, 161.4, 171.3, 170.1. 176.1. ESI/MS: ESI/MS: m/z calcd. for $[M+H]^+$ 793.86 , found 794.13

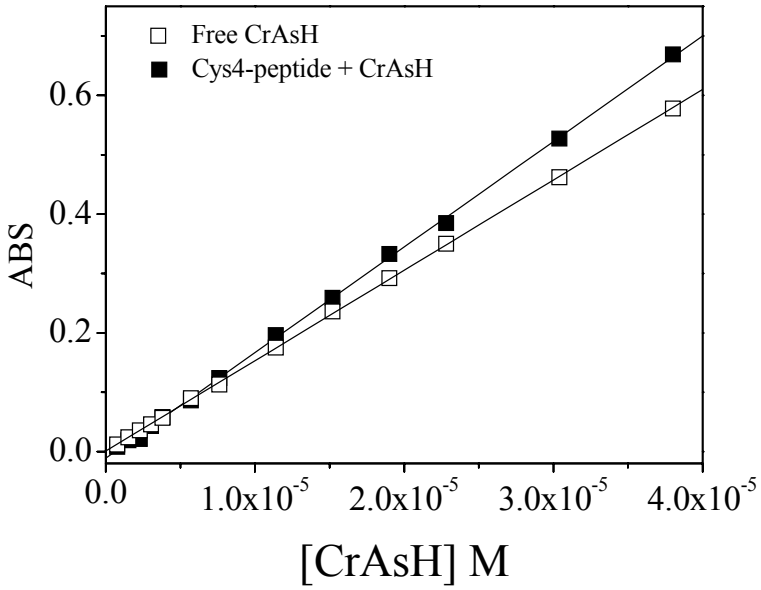


Figure 1s: The molar absorption coefficients for CrAsH, CrAsH-PG complex were measured in 50mM HEPES 140 mM KCl pH 7.5 buffer with 1mM TCEP and 1 mM BME. λ_{ex}= 495 nm, λ_{em}= 533 nm

Table Is: The binding properties of CrAsH to Cys4 peptide.

Peptide	ε ($mol^{-1}cm^{-1}$)	K_d (nM)
CCPGCC	17491±32	407±11
CCKACC	17780±29	486±7
No peptide	15221±23	-------

K_d calculated from equation $C_{bound}/C_{total}=C_{free}/(K_d+C_{free}) + C$

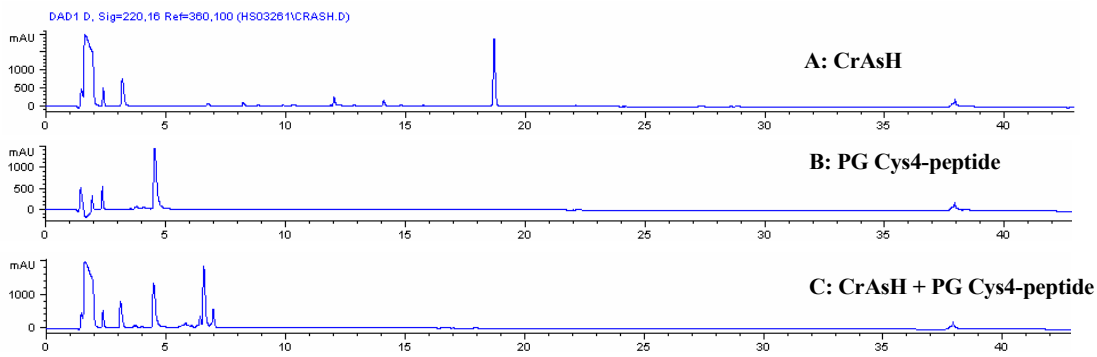


Figure2s: HPLC shows that CrAsH binds to PG Cys-4 peptide and forms a complex.
A: 1.89 mM CrAsH, 7.00 mM BME, 7.00 mM TCEP, HEPES buffer with pH 7.50
B: 0.32 mM PG, 7.00 mM BME, 7.00 mM TCEP, HEPES buffer with pH 7.50
C: 1.89 mM CrAsH, 0.32 mM PG, 7.00 mM BME, 7.00 mM TCEP, HEPES buffer with pH 7.50

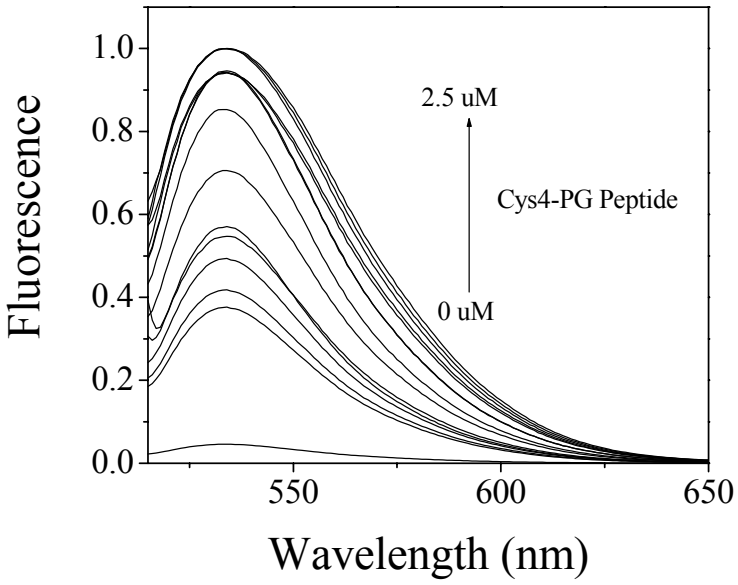


Figure 3s. CrAsH shows 1:1 binding with PG peptides.1uM CrAsH in DMSO titrated by PG peptides from 0 to 2.5 uM in 50mM HEPES ,140 mM KCl pH 7.5 buffer With 1mM TCEP, 1 mM BME and 1 uM EDT. λex= 495 nm, λem= 533 nm

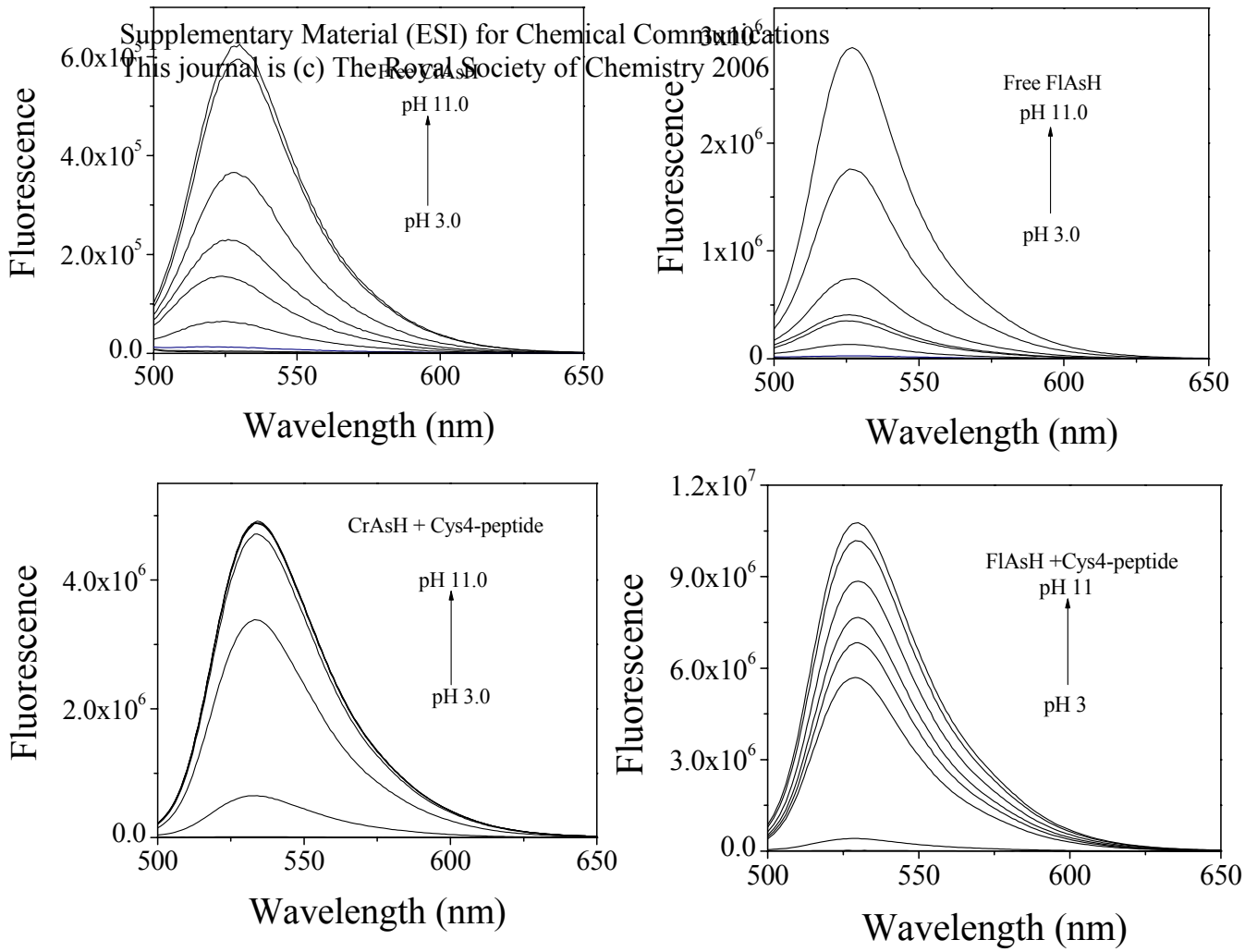
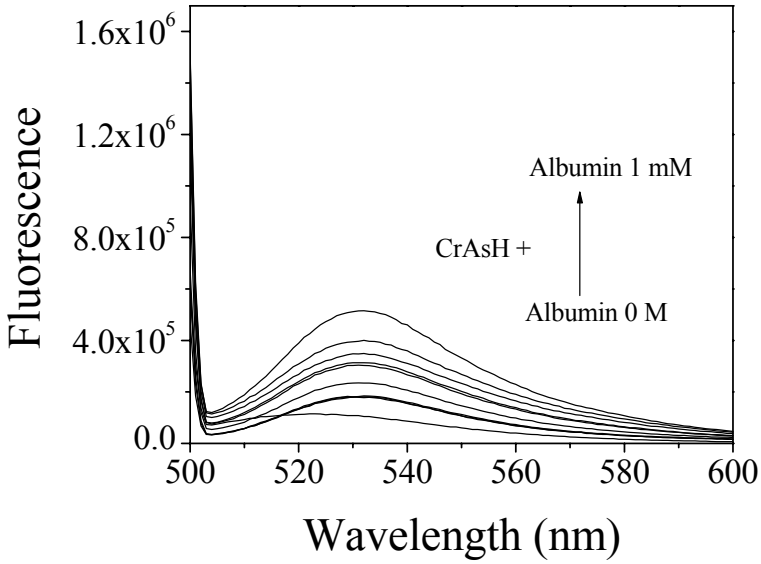


Figure 4s. Spectra of FlAsH and CrAsH in different pH solutions.



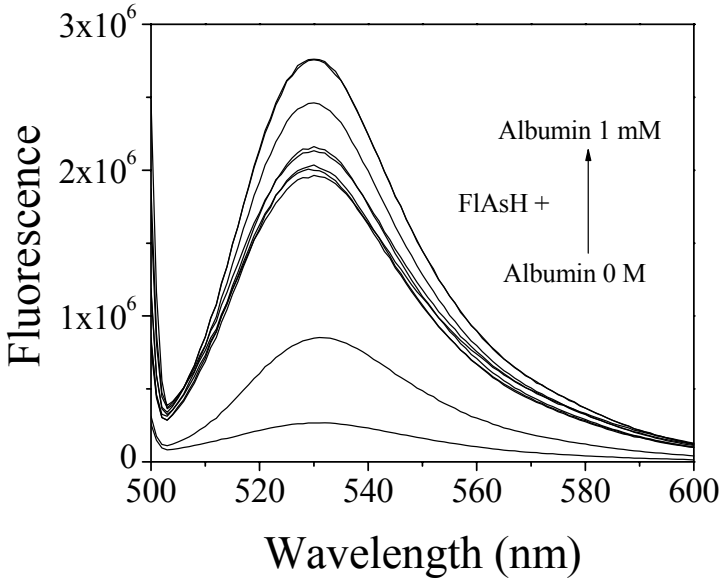


Figure 5s: The spectra of FlAsH and CrAsH during albumin addition.