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A Modular Platform for the Development of Selective Fluorescent Metal Sensors

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Supporting Information

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General Procedures and Materials

The Tentagel MB NH_2 resin (140-170 μm, 0.3 mmol/g) was purchased from Rapp-Polymere (Tuebingen, Germany). Fritted disposable chromatography columns (Bio-Rad, Hercules, CA) were used for performing the library synthesis steps. The resin suspensions were stirred during the reaction steps using a nutator (Fisher Scientific, USA). The photolinker (Fmoc-(*R*)-3-amino-3-(2-nitrophenyl) propionic acid) and Fmoc-6-aminohexanoic acid were purchased from Chem-Impex (Wood Dale, IL). The 1PEG ((2-(Fmoc-amino)ethoxy)acetic acid) and 2PEG ((2-(2-(Fmoc-amino)ethoxy)ethoxy)acetic acid) linkers were purchased from Iris Biotech GMBH (Marktredwitz, Germany). Water (ddH_2O) used was deionized using a Barnstead NANOpure purification system (ThermoFisher, Waltham, MA). All other materials were purchased from commercial sources and used without further purification, except as noted below. Small scale centrifugation was performed in a Galaxy Mini Star (VWR, USA) and lyophilization was performed using a Labconco Freezone 4.5.

Library Synthesis

Piperonylamine, butylamine, and histamine submonomers were used as purchased and incorporated into the peptoids without additional protecting groups. *N*-boc-ethylenediamine was purchased and used as received. The glycine and β-alanine monomers (purchased as hydrochloric acid salts with *t*-butyl ester protecting groups) were neutralized with 1 M NaOH and extracted into a mixture of 15:85 v/v isopropanol-chloroform, which was then dried over sodium sulfate and concentrated, yielding the monomers as the free bases. Cysteamine was protected with a trityl group using a procedure previously reported by Maltese.[1] In order to confirm that each amine could be incorporated into a peptoid using the chemistry described below, test pentamers were synthesized with alternating benzylamine groups and test monomers.[2,3] The library was synthesized on Tentagel MB NH_2 resin, which was first swollen using dichloromethane (DCM). Standard Fmoc solid-phase synthesis techniques (using HCTU as a coupling agent) were used to incorporate the three residues in the linker via amide bond formation. For the first variable position, the resin was split evenly into seven separate fritted columns. Acylation and the addition of the first amine were performed according to the procedure previously reported by Zuckermann *et al.* for the incorporation of heterocyclic amines.[4] Acylation was achieved by gently stirring resin beads for 5 min with a solution of chloroacetic acid (6.8 eq, 0.4 mM) in dimethylformamide (DMF) and a solution of diisopropylcarbodiimide (8 eq, 2 M) in DMF. The acylation solution was removed via vacuum filtration and the resin beads were rinsed thoroughly with DMF. Next, the amine monomer were added as 2 M solutions in DMF. After 2-12 h of gentle stirring at room temperature, the resin samples were dried via vacuum filtration and rinsed with DMF. All 7 resin aliquots were then pooled and mixed in DCM for 5 min. Subsequently, the resin was dried via vacuum filtration and split into a new set of 7 fritted columns. The acylation and amination steps were then repeated to add amines at the second variable position. The library was once again pooled and split into three separate containers for the addition of the turn sequences. To insert the linker and proline monomers into the peptoid backbone, solutions of the linker (20 eq, 0.6 mM in DMF) and diisopropylcarbodiimide (19 eq, 3.2 mM in DMF) were incubated with the resin for 30 min with gentle agitation. The proline was attached using standard solid-phase peptide synthesis techniques (with HCTU as the coupling agent), after which the standard peptoid synthesis procedure was resumed to couple the remaining amines. After the final amination, the resin pools were recombined and agitated gently in a 20% solution of 4-methylpiperidine in DMF to remove any acylation adducts on the imidazole moieties. After isolation via filtration, the resin was rinsed with DMF and DCM, then dried under a vacuum. The protecting groups were removed by incubation with a cleavage solution (95:2.5:2.5) trifluoroacetic acid:water:triisopropylsilane) for 1.5 h. The resin was then rinsed with DCM, dried under vacuum, and stored, protected from light, at 4 °C until use.

Screening to Identify Selective Mercury Ligands

In the first step of the screening process, the library was incubated with the chromium-containing solution (25 mM – NaOAc, $Mg(OAc)_2$, 2.5 mM – $Ca(C_3H_5O_6)_2$, CrO_3, $Cd(OAc)_2$, and $HgCl_2$ 250 μM in BisTris 50 mM pH 7). Sequences that exhibit binding to mercury were identified using 1,5 diphenylcarbazide dye (0.5 g in 100 mL of acetone) that turns purple when complexed with Hg^{2+}. The beads were selected and the ions were removed using the process described below. The sequences were then incubated with the lead-containing solution (25 mM – NaOAc, $Mg(OAc)_2$, 2.5 mM – $Ca(C_3H_5O_6)_2$, $Pb(NO_3)_2$, $Cd(OAc)_2$, and $HgCl_2$ 250 μM in BisTris 50 mM pH 7) and mercury

ligands were again visualized with diphenylcarbazide. The color of the beads were examined using a Leica S6D Microscope (Leica, Germany). The beads with the most intense colors were manually selected for ligand identification. All photographs were taken with an iPhone (model 4 or 5).

Metal Ion Removal and Photocleavage

Metal ion removal was previously found to be essential before interpretable MALDI-TOF MS sequencing data could be obtained. Beads selected from the library screen were first deposited onto the membrane of a 0.5 mL 0.2 μm centrifugal filter unit (Millipore, Billerica, MA). A mixture of Amberlite cation (Na^+ form, 5 mg) and anion (Cl^- form, 5 mg) exchange resins was then added to the filtrate collection tube of the centrifugal filter unit. The filter and the collection tube were then filled with 1 M HCl. This setup was incubated with gentle stirring on a nutator for 2 h, allowing diffusion to occur through the 0.2 μm filter. The selected library members were isolated from the exchange resins and acidic solution by centrifugation of the filtration unit. Water (500 μL) was added to the filter unit, and the unit was gently agitated on a nutator for 15 min to rinse the library members. The water was then removed by centrifugation. This process was repeated twice to ensure complete removal of residual HCl.

The beads containing hit sequences were removed from the filter unit and transferred to a Petri dish using ethanol (approx. 500 μL). The individual beads were then placed into individual 0.6 mL Posi-Click tubes (Denville Scientific, South Plainfield, NJ) using a pipette. The volume in each tube was brought to 5 μL with absolute ethanol. In order to cleave the peptoids from the resin beads, the tubes were placed in a computer controlled ICH-2 photoreactor with UVA bulbs (Luzchem, Ottawa, Canada) for 8 h. For mass verification of bulk peptoid samples, the beads were able to be directly cleaved with the nitrogen laser (337 nm) on the MALDI-TOF MS (Voyager-DE Pro, Applied Biosystems) in addition to the Nd:YAG laser (355 nm) on the MALDI-TOF-TOF MS/MS (4800 plus MALDI-TOF-TOF Analyzer, Applied Biosystems)

Single Bead Sequencing

After photocleavage of single beads, the ethanol was evaporated and replaced with a mixture of 1:1 water:acetonitrile containing tris(2-carboxyethyl)phosphine (0.5 mM). The sample (0.5 μL) was mixed with 0.5 μL matrix solution (5 mg α-cyano-4-hydroxycinnamic acid in 1:1 water:acetonitrile with 0.1% trifluoroacetic acid and 0.6 M ammonium phosphate) and spotted directly onto a stainless steel MALDI plate. MALDI-TOF MS (AB Sciex TF4800, Applied Biosystems) was then used to identify the mass of each selected sequence. MS/MS was used to fragment the peptoids for sequencing.

Mercury Depletion Analysis with ICP-OES

Sequences were synthesized on Tentagel MB NH_2 (0.3 mmol/g) using the procedures described above in the "library synthesis" section. A photolinker and two aminohexanoic acid residues were used to link the peptoid structures to the resin. The sequence identities were confirmed via MALDI-TOF MS by directly cleaving beads with the MALDI laser.

The selectivity of these sequences was evaluated by adding the sequences to solutions similar to those used in the selection process. (Lead: 25 mM – NaOAc, $Mg(OAc)_2$, 2.5 mM – $Ca(C_3H_5O_6)_2$, $Pb(NO_3)_2$, $Cd(OAc)_2$, and $HgCl_2$ 250 μM in BisTris 50 mM pH 7 and Chromium: CrO_3 instead of $Pb(NO_3)_2$). After a 2 h incubation, an aliquot from each sample was removed and diluted with nitric acid to a final concentration of 2% HNO_3. Each sample was measured five times in order to determine the concentration of mercury, cadmium, lead, and chromium remaining using a Perkin Elmer 5300 DV optical emission ICP using scandium as an internal standard.

General Methods for UV-vis spectroscopy and Fluorometry

UV-vis and fluorescence spectra were measured using a Shimadzu 2501 Spectrophotometer (Shimadzu) and a Quantamaster Master 4 L-format scanning spectrofluorometer (Photon Technologies International), respectively. The fluorometer contains an LPS-220B 75W xenon lamp and power supply, an A-1010B lamp housing with an integrated igniter, a switchable 814 photon-counting/analog photomultiplier detector, and a MD5020 motor driver. The sam-

ples were generated by first preparing stock solutions of the fluoropeptoids (1 mM in ddH_2O), then diluting them to 1 μM with HBS (100 mM HEPES, pH 7.4, 1 mM NaCl, 1 mM $MgSO_4$). Samples were measured using 1-cm path length quartz cuvettes (Starna Cells).

Fluorometry Titrations with Metals in Buffer

Metal stock solutions (NaCl, $C_6H_{10}O_6Ca$, $CdCl_2$, $Co(C_2H_3O_2)_2$, CrO_3, $CuCl_2$, $Fe(CH_3COO)_2$, $Mn(CH_3COO)_2$, $Ni(CH_3CO_2)_2$, $PbCl_2$, $ZnCl_2$, and $Hg(CH_3CO_2)_2$) were prepared at 1 mM in either TBS (200 mM Bis-tris, pH 7) or HBS (10 mM HEPES, pH 7.4) depending on their solubility. These metal solutions were then added in 1 μL and 10 μL increments to freshly prepared fluoropeptoid solutions. The fluorescence spectrum was collected at each increment by exciting with 344 nm light and collecting light between 354 and 500 nm. The pyrene peak emission at 398 nm was used to plot fractional fluorescence (F/F_0) versus metal concentration. In the case of binding constant determinations for chromium and mercury, the plot was fit to a logistic binding curve using Origin software. The following equation was used to fit the data:

$$y=(A_1-A_2)/(1+x/x_0)^p+A_2$$

Equation 1

Where A_1 and A_2 are the asymptotes of the data, p refers to the slope of the curve, and x_0 is the inflection point used to calculate the dissociation constant.

Stream and Ocean Water Fluorometry and UV Visualization

Water samples were collected from Strawberry Creek in Berkeley, CA (Latitude: 37.871052, Longitude: -122.257214) and Ocean Beach in San Francisco (Latitude: 37.760232, Longitude: -122.512227). An appropriate amount of $Hg(CH_3CO_2)_2$ was added? to achieve desired concentrations (5 μM to 90 μM). Fluorescence spectra were once again collected exciting with 344 nm light and collecting light at 398 nm. For visualization, the solutions were pipetted into a transparent 96 well plate and placed in a Gel Doc EZ System (Bio-Rad) on a UV tray. The plate was then visualized using the Oriole channel. Images were analyzed using ImageJ by drawing regions of interest encompassing the inside of each well and the mean fluorescence intensity extracted. The fractional fluorescence values was plotted against mercury concentration to generate plots.

(1) Maltese, M. J. Org. Chem. 2001, 66, 7615–7625.

(2) Burkoth, T. S.; Fafarman, A. T.; Charych, D. H.; Connolly, M. D.; Zuckermann, R. N. J. Am. Chem. Soc. 2003, 125, 8841–8845.

(3) Knight AS, Zhou EY, Pelton JG, Francis MB. J. Am. Chem. Soc. 2013, 135, 17488–17493.

(4) Zuckermann, R. N.; Kerr, J. M.; Kent, S. B. H.; Moos, W. H. J. Am. Chem. Soc.1992, 114, 10646–10647.

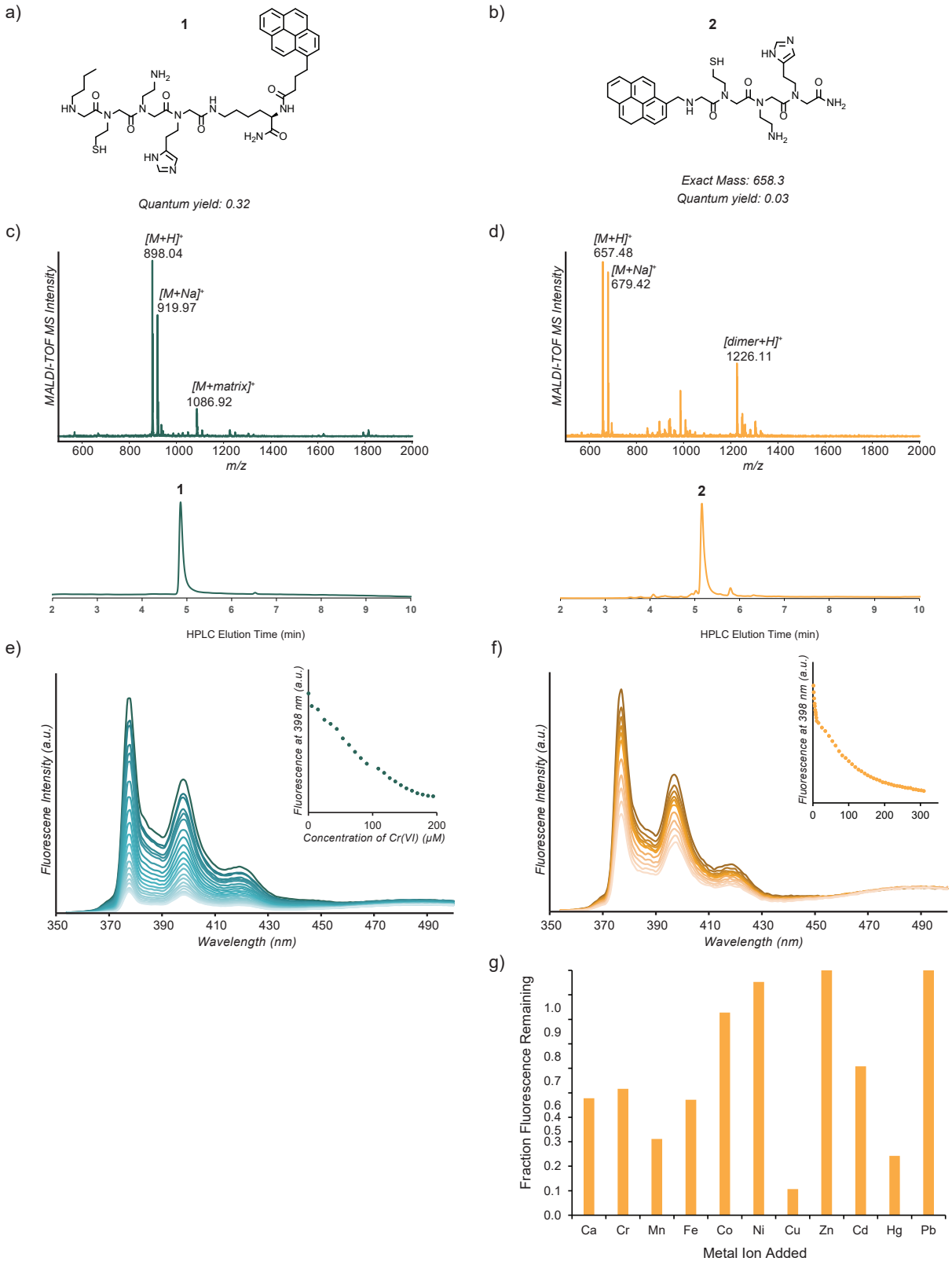
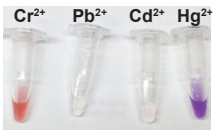
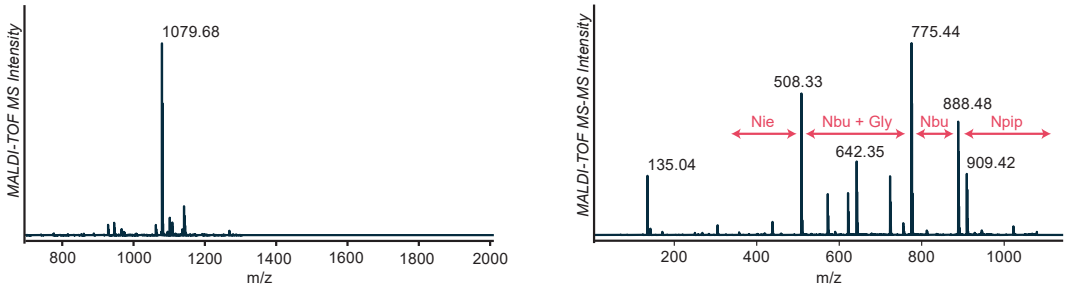


Figure S1. Chromium fluoropeptoids. a) Structure of the C-fluoropeptoid (**1**) based on Cr-peptoid. b) Structure of the *N*-fluoropepotid (**2**) based on Cr-peptoid. c) and d) MALDI-TOF and HPLC (220 nm) spectra of purified **1** and **2**, respectively. e) and f) Fluorescence spectra of the titration of Hg^{2+} (0 to 194 μM) into solutions of peptoids **1** and **2**, respectively (1 μM in buffer (100 mM HEPES, pH 7.4, 1 mM NaCl, 1 mM $MgSO_4$)). The inset graphs show the fluorescence at 398 nm. g) Evaluation of the fluorescence of **2** after the addition of metal ions (95 μM) in a buffered solution (HEPES, 100 mM, pH 7 with 1 mM NaCl). Each bar is the relative fluorescence of the fluoropeptoid with metal to the fluoropeptoid alone in the buffered solution.

a)



b)



c)

Residue	Monomer Fragment Mass
Nce	129
Nbu	113
Nthe	117
Nae	100
Ncm	115
Nie	151
Npip	191

Residue	Mass of Turn plus Monomer Fragment	
	1PEG	2PEG
Nce	298	342
Nbu	311	355
Nthe	313	357
Nae	315	359
Ncm	327	371
Nie	349	393
Npip	389	433

Figure S2. Screening for selective mercury ligands. a) To identify a ligand with affinity for mercury in the presence of chromium, lead, and cadmium, diphenylcarbazide was identified to have a unique colorimetric response. b) Representative MALDI-TOF and MALDI-TOF-TOF mass spectra after screening and photocleavage. c) Masses of relevant monomers and monomer combinations used to identify structures .

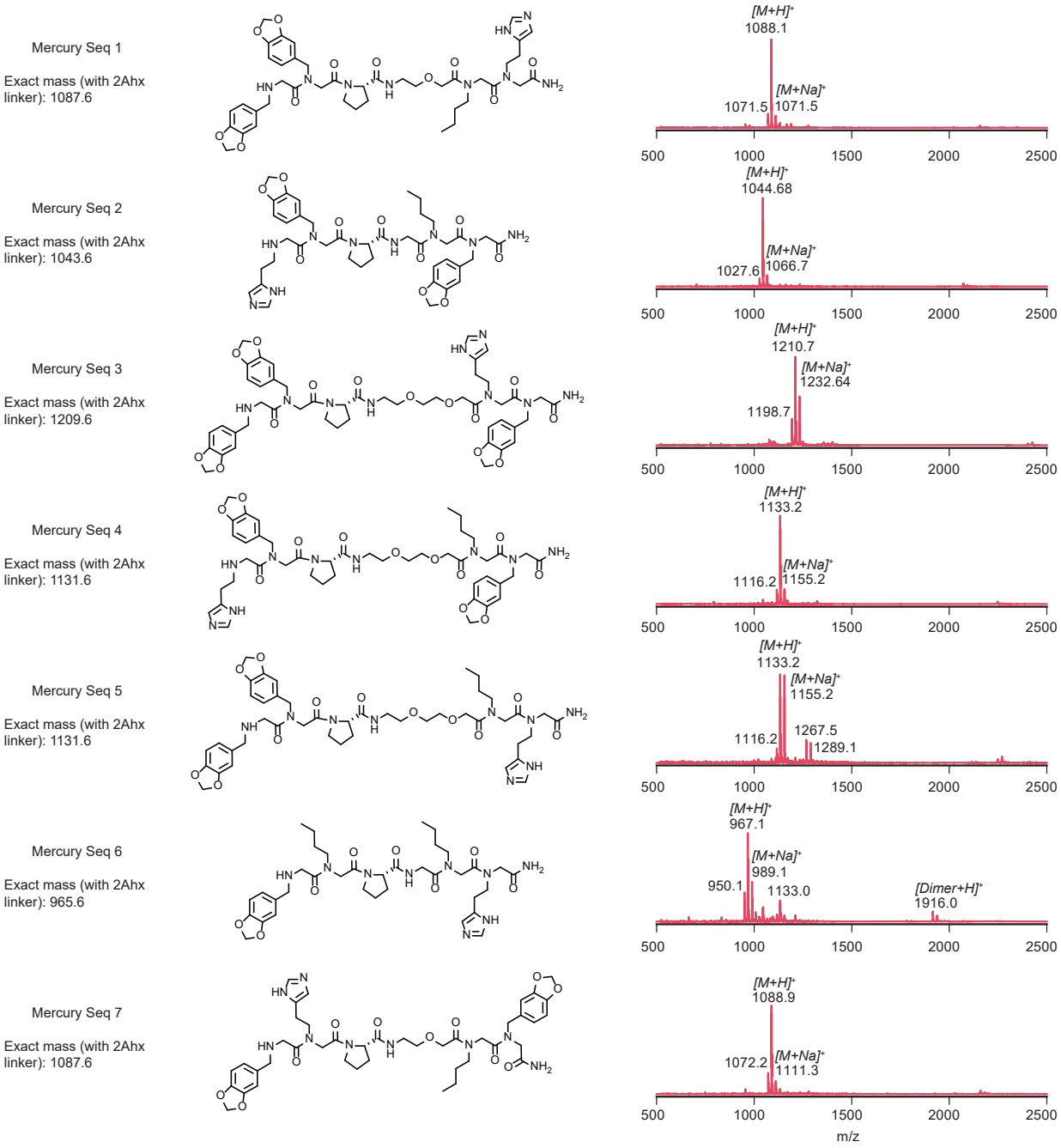


Figure S3. Identified mercury binding peptoids. Structures are shown in addition to the MALDI-TOF MS of the sequences resynthesized on Tentagel MB NH_2 resin with a linker of two aminohexanoic acids (2Ahx) after photocleavage from the resin.

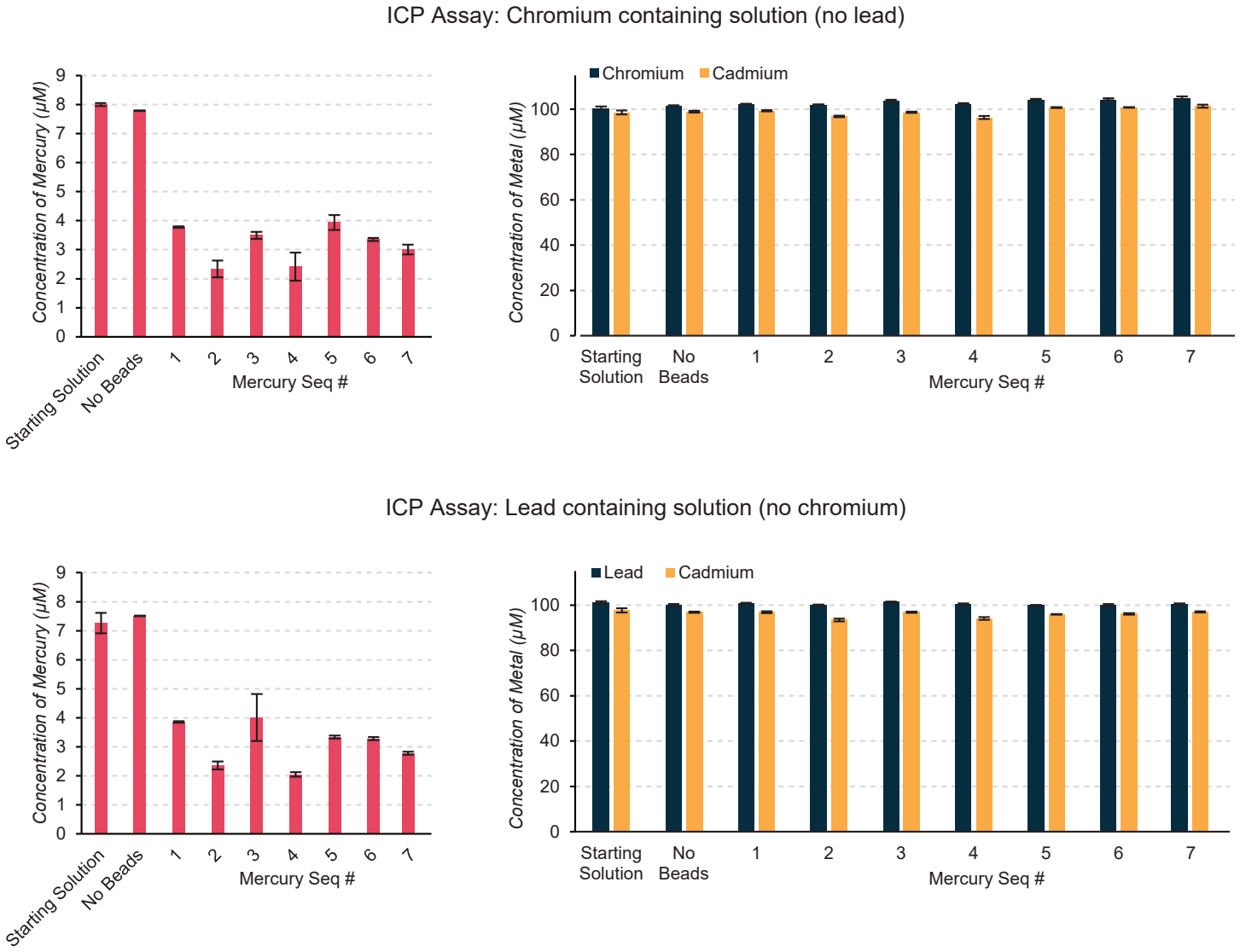


Figure S4. Depletion of toxic heavy metals by peptoid ligands. Immobilized peptoid ligands were added to solutions of Hg^{2+} (8 μM) with Cd^{2+} (100 μM) and either Pb^{2+} or Cr^{6+} (100 μM) in a mixture of innocuous ions (NaOAc and $Mg(OAc)_2$ (25 mM), $Ca(C_3H_5O_3)_2$ (2.5 mM) in BisTris (50 mM pH 7)).

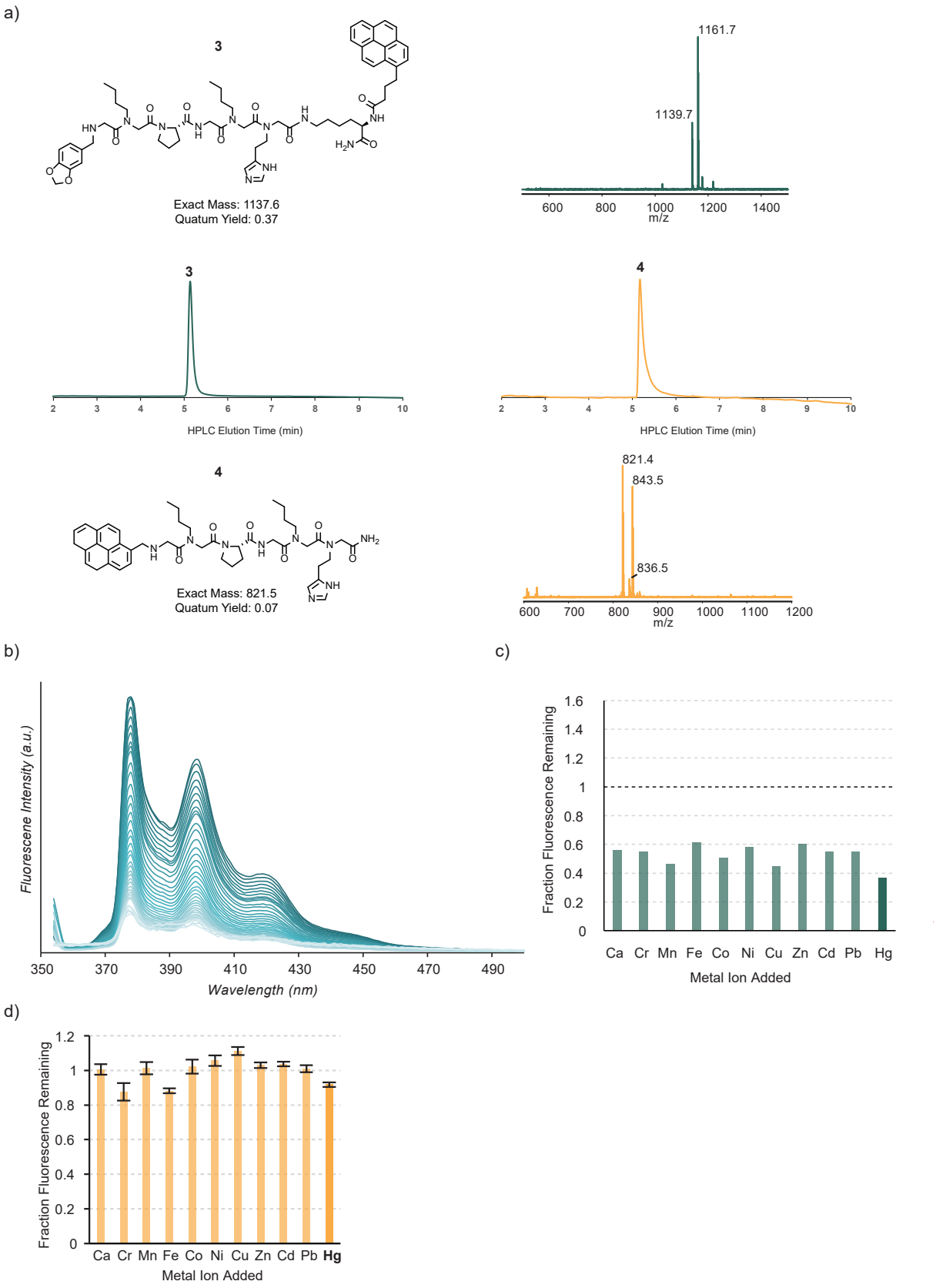


Figure S5. Fluoropeptoid based on Mercury Seq 6. a) Structures and HPLC (220 nm) and MALDI-TOF MS spectra of purified **3** and **4**. b) Fluorescence spectrum of the titration of Hg^{2+} (0 to 315 μM) into a solution of **3** (1 μM in HBS (100 mM HEPES, pH 7.4, 1 mM NaCl, 1 mM $MgSO_4$)). c) The fluorescence signal of **3** in the presence of 95 μM of the indicated ions in addition to 95 μM of Hg^{2+} in HBS. The values are normalized to the fluorescence of **3** in HBS. d) The fluorescence signal of **4** in the presence of 95 μM of the indicated ions in HBS. The values are normalized to the fluorescence of **4** in HBS.

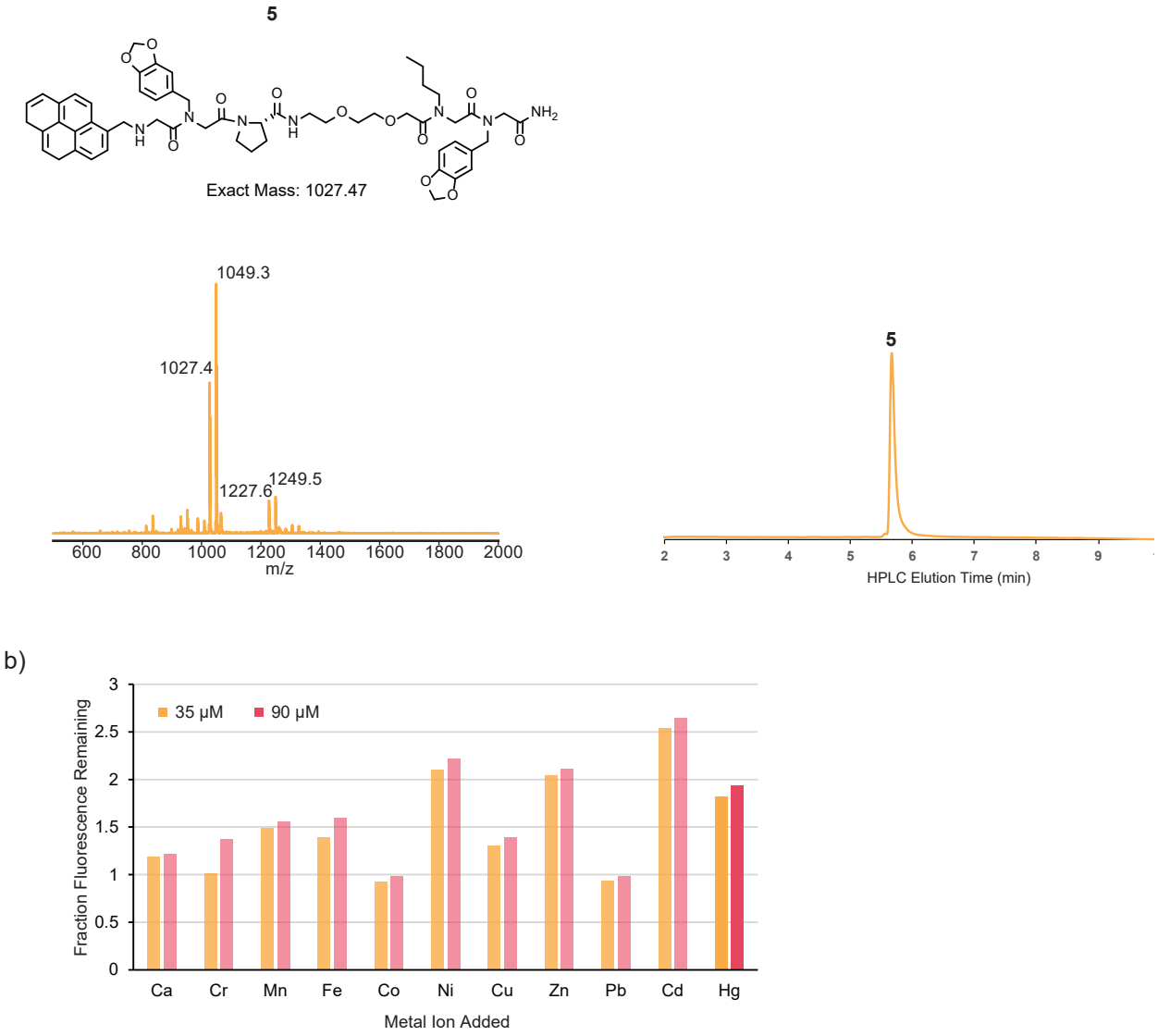


Figure S6. Control fluoropeptoid based on Mercury Seq **4**. a) Structure and HPLC (220 nm) and MALDI-TOF MS spectra of purified 5 b) The fluorescence signal of 5 in the presence of 90 µM of the indicated ions in HBS.