SUPPORTING INFORMATION

Differentiating Between Fluorescence-Quenching Metal Ions with Polyfluorophore Sensors Built on a DNA Backbone

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Materials and General Methods

Solvents used for analysis were HPLC grade. DNAse and RNAse free ultrapure distilled water (Gibco) was used throughout the study. The metal salts used were $Co(NO_3)_2 \cdot 6H_2O$, $Ni(NO_3)_2 \cdot 6H_2O$, $Cu(NO_3)_2 \cdot xH_2O$, $Hg(NO_3)_2 \cdot H_2O$, $Pb(NO_3)_2$, $AgNO_3$, $Cr(NO_3)_2 \cdot 9H_2O$, and $Fe(NO_3)_3 \cdot 9H_2O$. For the metal salts with xH_2O, $1H_2O$ was used to calculate the molecular weight. Metal salts with ≥99.99+% purity were purchased from Sigma Aldrich and were used without further purification. *Sym*-collidine and nitric acid were purchased from Sigma Aldrich and Fisher Scientific respectively. Microplate assays were performed using white, flat bottom, 384-well polypropylene microplates from Greiner Bio-One.

General instruments

Applied Biosystems (ABI) 394 DNA/RNA synthesizer was used to prepare the oligonucleotides. MALDI-MS was performed by the University of California-Riverside mass spectrometry facility. Absorption measurements were carried out on a Cary 100 UV-Vis spectrophotometer, and fluorescence studies were performed on a FLEXstationII-384 microplate reader.

Procedures for metal ion sensing

Metal cation stock solutions (2 mM) were prepared in DNAse and RNAse free ultrapure distilled water for Co^{2+}, Ni^{2+}, Cu^{2+}, Hg^{2+}, Pb^{2+}, Ag^{+}, Cr^{3+}, Fe^{3+}. The metal solutions were further diluted to make 100 μM metal solutions that were used for the experiments. Stock solutions of the oligomers were prepared by dissolving the lyophilized HPLC fractions in DNAse and RNAse free ultrapure distilled water. The concentration of the oligomers were determined from the absorbance, and 10 μM oligomer solutions were then prepared from the stock solutions. Fluorescence studies were performed in 384-well microplates with the final total volume of 50 μL, 10 mM *sym*-collidine•HNO_3 pH ~7.3 buffer, 2 μM oligomers, and 10 μM metal ions.

To obtain the fluorescence response profiles of the oligomers for each metal ions, solutions containing 2 μM of sensor in 10 mM buffer were prepared in a series of wells; one for each metal

cation. Then the appropriate metal ion solutions were added to different wells to make 10 μM metal ion concentration in a final total volume of 50 μL. The solutions were mixed in a 1 min. shake-and-incubate cycle in a Thermo Electron Corp. Fluoroskan Ascent FL luminometer [3 x (10 s shake + 10 s incubate 25°C)]. Then the microplate was sealed with a ThermalSeal adhesive sealing film for PCR (Excel Scientific) and incubated in the dark for 60 min. at room temperature. Prior to acquiring the fluorescence spectra, the microplate without the sealing film was shaken three times for 5s and incubated for 5 min. inside the microplate reader open to air at 25°C. The microplate reader settings were $\lambda_{ex.}$ = 350 nm, $\lambda_{em.}$ = 390-700 nm at 10 nm intervals, cutoff = 420 nm, and temp. = 25°C.

Synthesis and Purification

5'-O-DMTr-protected 3'-2-cyanoethyl phosphoramidite derivatives of **L1**, **L2, D, H** and **E** were prepared according to references 1 and 2. 5'-O-DMTr-protected 3'-2-cyanoethyl phosphoramidite derivatives of **L3** and **S** were purchased from Glen Research. These building blocks were directly used on a DNA synthesizer (ABI 394 DNA/RNA synthesizer). The oligomers were synthesized on a 1.0 μmole scale (3' phosphate CPG column, Glen Research) using the standard (DMT off) protocol but with extended (999s) coupling times for **L1**, **L2 D**, **H**, and **E**. Stepwise coupling yields were ≥90% as determined by trityl monitoring. Cleavage and deprotection of the oligonucleotide followed the recommended protocol for the 3' phosphate CPG column and **L3** as indicated by Glen Research. The crude product was purified by HPLC (Jupiter 5u C5 column, 300A, 250 x 10 mm, 5 micron) using TEAA, pH ~ 7.2 and MeCN as the solvent system. The oligomers were characterized by MALDI-MS (Table S1), and the absorption and fluorescence spectra of the purified oligomers are shown in Fig. S1.

Table S1. MALDI-MS Data for Oligodeoxyfluoroside Sequences[a]

oligomer	sequence	calculated	found
1	5'-S-S-H-L2-D-H-S-S-3'	2422.6	2423
2	5'-S-S-E-L1-E-S-S-3'	2015.4	2015
3	5'-S-S-L1-L2-H-D-S-S-3'	2430.6	2431
4	5'-S-S-L3-L2-H-L1-S-S-3'	2374.5	2376
5	5'-S-S-L1-H-H-L3-S-S-3'	2316.5	2318
6	5'-S-S-L1-S-L2-D-S-S-3'	2202.5	2203

[a]All Sequences contain a phosphate group at the 3' terminus.

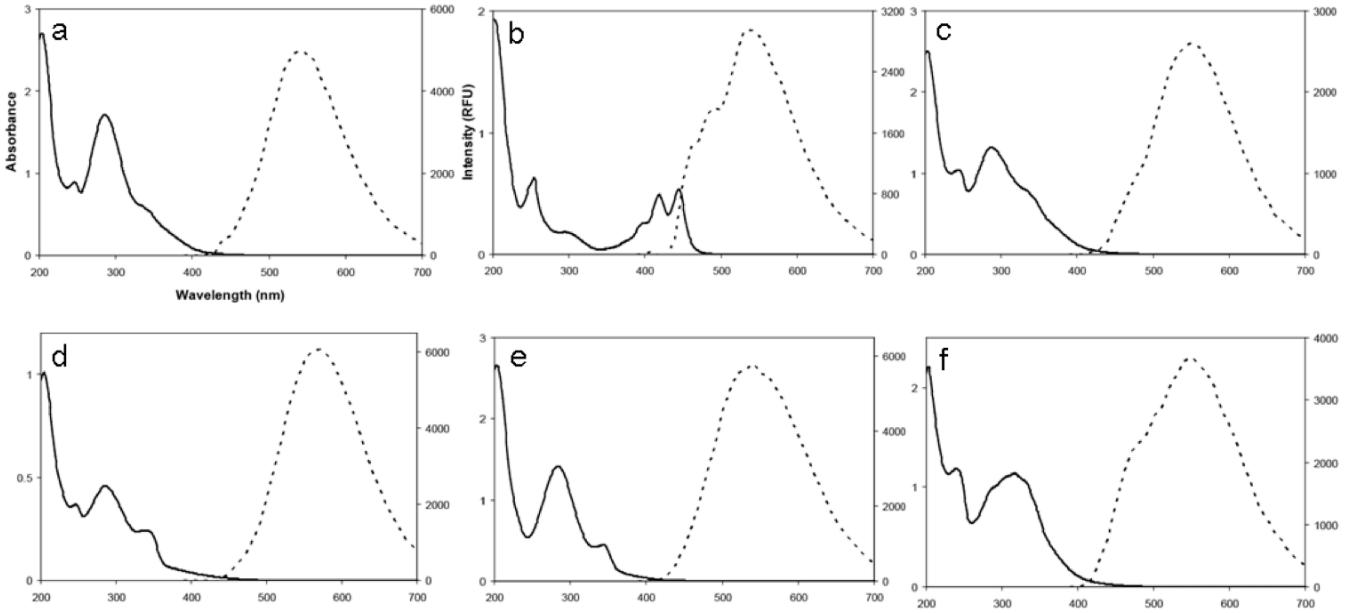

Figure S1. Absorption and fluorescence spectra of the purified sensor compounds **1**-**6** (solid line and dashed line respectively): (a) – (f) correspond to oligomers **1**-**6** respectively. All fluorescence spectra were measured in 10 mM *sym*-collidine•HNO_3 pH ~7.3 buffer ($\lambda_{ex.}$ = 350 nm) with 2 μM of the oligomer. Absorbance spectra were measure in deionized water; the oligomer concentrations were **1** (12 μM), **2** (6 μM), **3** (11.8 μM), **4** (4.5 μM), **5** (27.6 μM), and **6** (14.3 μM).

Library Synthesis and Screening

The tetramer library was prepared as previously described[2] using standard split and pool methods on PEG-polystyrene beads (NovaSyn® TG resin, NovaBiochem), but containing the monomers **L1**, **L2**, **L3**, **D**, **H**, **E**, and **S** (Fig. 1, main text). The tetramers were synthesized by automated DNA synthesis (1 μmol scale) via standard phosphoramidite chemistry. Binary encoding with molecular tags as described by Still et al.[3] was employed to record/decode the identity of the monomers of each sequence. The beads containing the tetramers were immobilized on a microscope slide via double sided tape, and then screened by imaging (Nikon Eclipse E800 equipped with a 4× objective, excitation 340-380 nm; emission >400 nm) in buffer alone and then in the presence of 100 μM metal ions. Beads that changed fluorescence most strongly in the presence of the metal ions were selected, decoded, and resynthesized for evaluation. This led to identification of oligomers **1**-**6**.

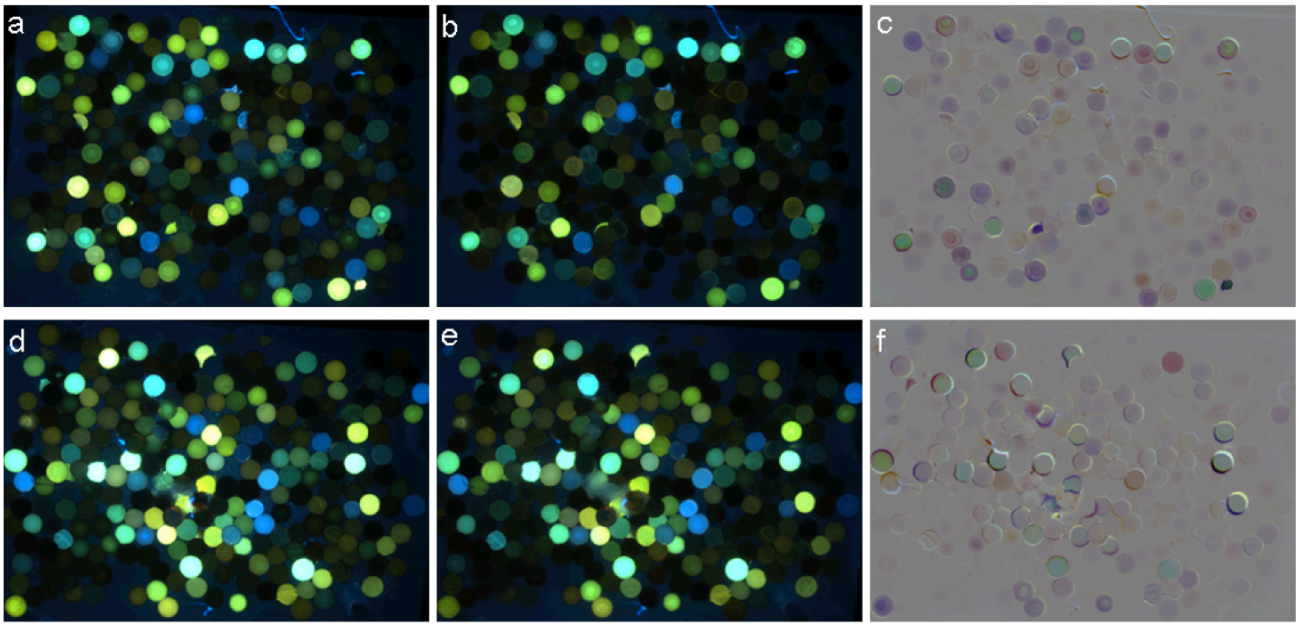

Figure S2. Examples of images from library screening with $Cu(NO_3)_2$ (a-c) and $Co(NO_3)_2$ (d-f). (a), (d) buffer alone; (b) and (e) in the presence of 100 μM metal ion, and (c), (f) 50% gray (metal – buffer) subtracted images. Gray color represents no change.

Difference Spectra Profiles

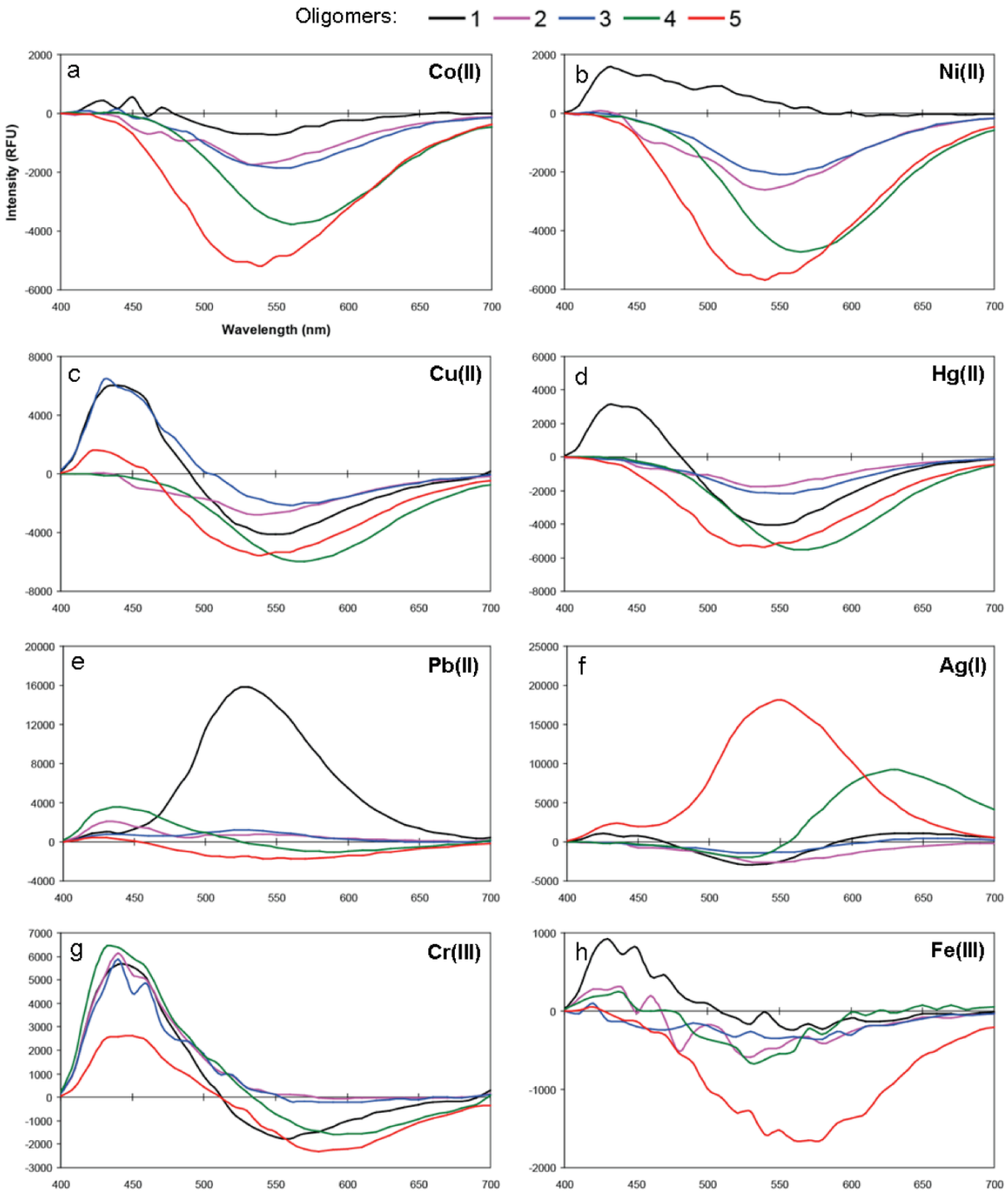

Figure S3. Difference spectra profiles for the various metal ions (10μM) with oligomeric sensors **1-5** (2μM) in 10mM buffer, pH ~7.3 ($\lambda_{ex.}$= 350nm). (a) – (h) correspond to Co(II), Ni(II), Cu(II), Hg(II), Pb(III), Ag(I), Cr(III), and Fe(III) respectively. Difference spectra were obtained by subtracting the oligomer's spectrum with no metal from the spectrum in the presence of the metal ion (difference = metal ion – no metal).

Unknown Difference Spectra Profiles

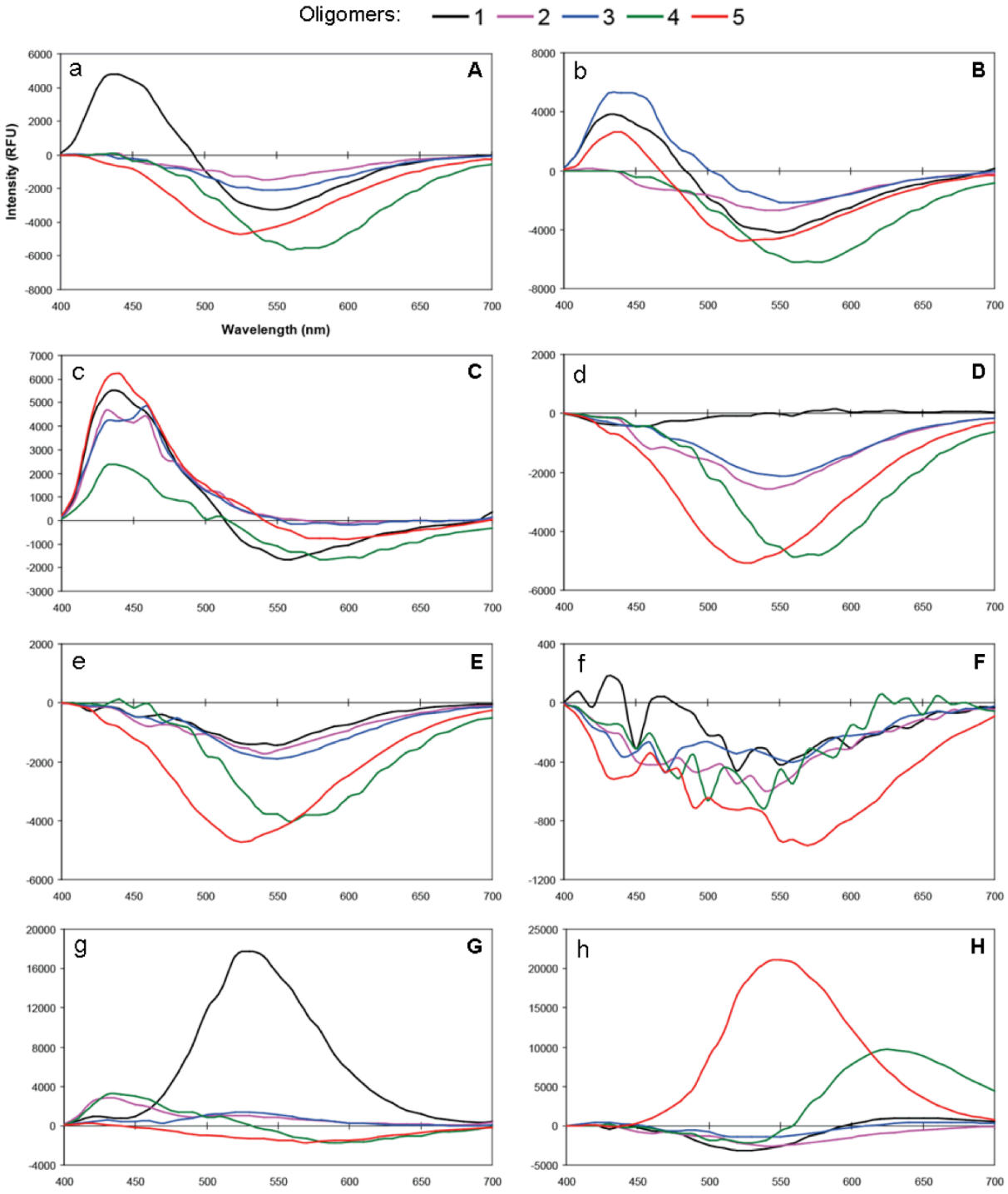

Figure S4. Difference spectra profiles of the unknown metal solutions (10μM) with sensors **1-5** (2μM) in 10mM buffer, pH ~7.3 ($\lambda_{ex.}$= 350nm). (a) – (h) correspond to the unknown metal solutions A-H respectively. Difference spectra were obtained by subtracting the oligomer's response with no metal from the response in the presence of the metal ion (difference = metal ion – no metal). Unknown solution identity: A = Hg(II), B = Cu(II), C = Cr(III), D = Ni(II), E = Co(II), F = Fe(III), G = Pb(II), H = Ag(I).

Titration Data

To obtain a preliminary measure of the sensitivity of sensors **1-6**, rough titration curves were obtained for selected oligomers and metal ions. For each metal ion, the oligomer that showed the strongest response was titrated against that metal ion. Each point on the graph (different metal ion concentration) represents the data from different wells. To a series of wells containing 2 μM of oligomer in 10 mM pH ~7.3 buffer was added the appropriate amounts of metal ion stock solution to make a final total volume of 50 μL. Incubation and fluorescence measurements followed procedures stated above (Procedures for metal ion sensing). The data were then fitted to a sigmoidal curve using GraphPad Prism[4] software to obtain the apparent dissociation constant (K_d).

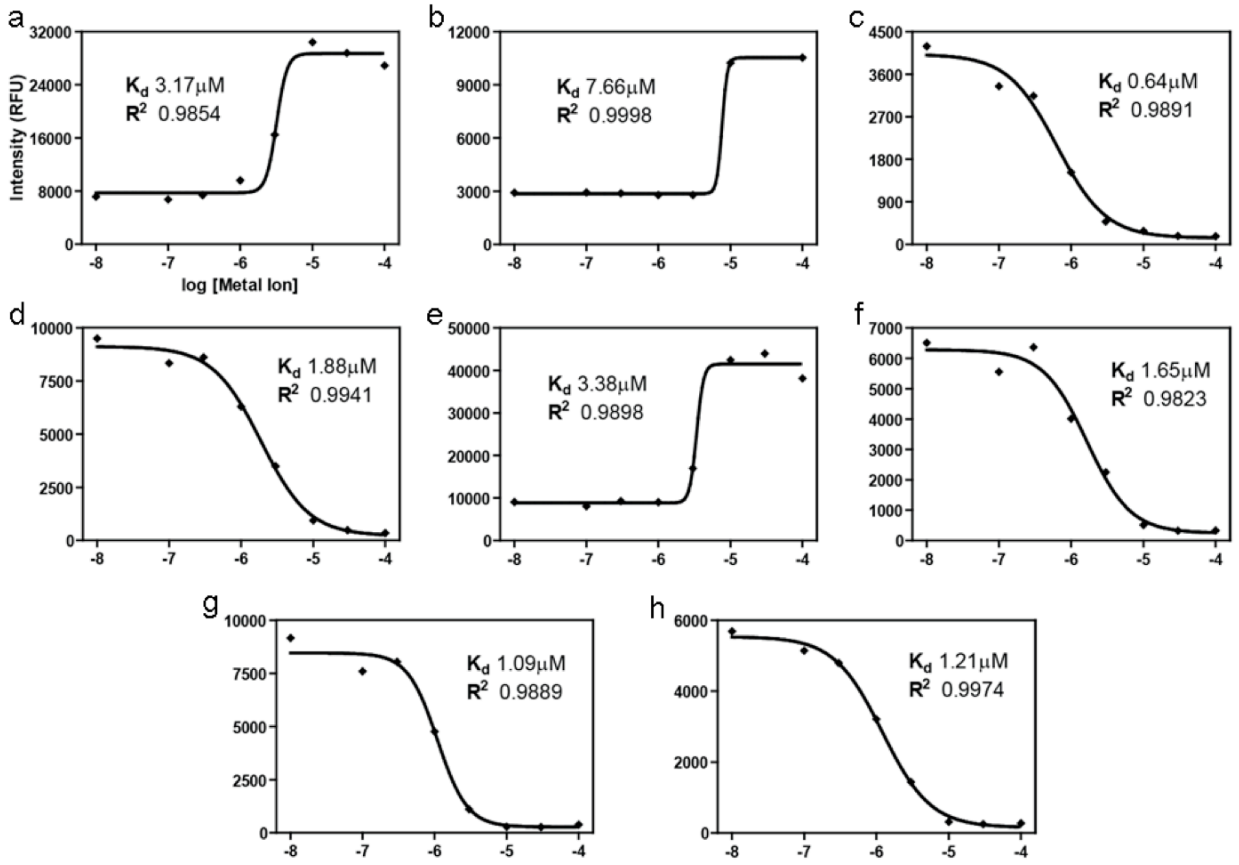

Figure S5. Titration data for selected oligomers (2 μM) and metal ions in 10 mM buffer, pH ~7.3 ($\lambda_{ex.}$ = 350 nm). For each metal ion, the oligomer that showed the strongest response was titrated against that metal ion. Metal ion concentrations were 0, 0.1, 0.3, 1.0, 3.0, 10, 30, and 100 μM. (a) Oligomer **1** titrated with Pb^{2+}, $\lambda_{em.}$ = 540 nm; (b) **2** with Cr^{3+}, $\lambda_{em.}$ = 450 nm; (c) **3** with Cu^{2+}, $\lambda_{em.}$ = 570 nm; (d) **4** with Hg^{2+}, $\lambda_{em.}$ = 560 nm; (e) **5** with Ag^{+}, $\lambda_{em.}$ = 540 nm; (f) **5** with Co^{2+}, $\lambda_{em.}$ = 570 nm; (g) **5** with Ni^{2+}, $\lambda_{em.}$ = 540 nm; and (h) **6** with Cu^{2+}, $\lambda_{em.}$ = 550 nm.

Principal Component Analysis

Principal component analysis (PCA) was performed on the difference spectra data using XLSTAT Version 2010.4.01.[5] The PCA method used for all the analyses is the Pearson's correlation matrix. In the PCA for oligomers **1-6** (Fig. 5 and S6), the entire difference spectra data of each sensor for all eight metal ions (48 total) were included in the analysis.

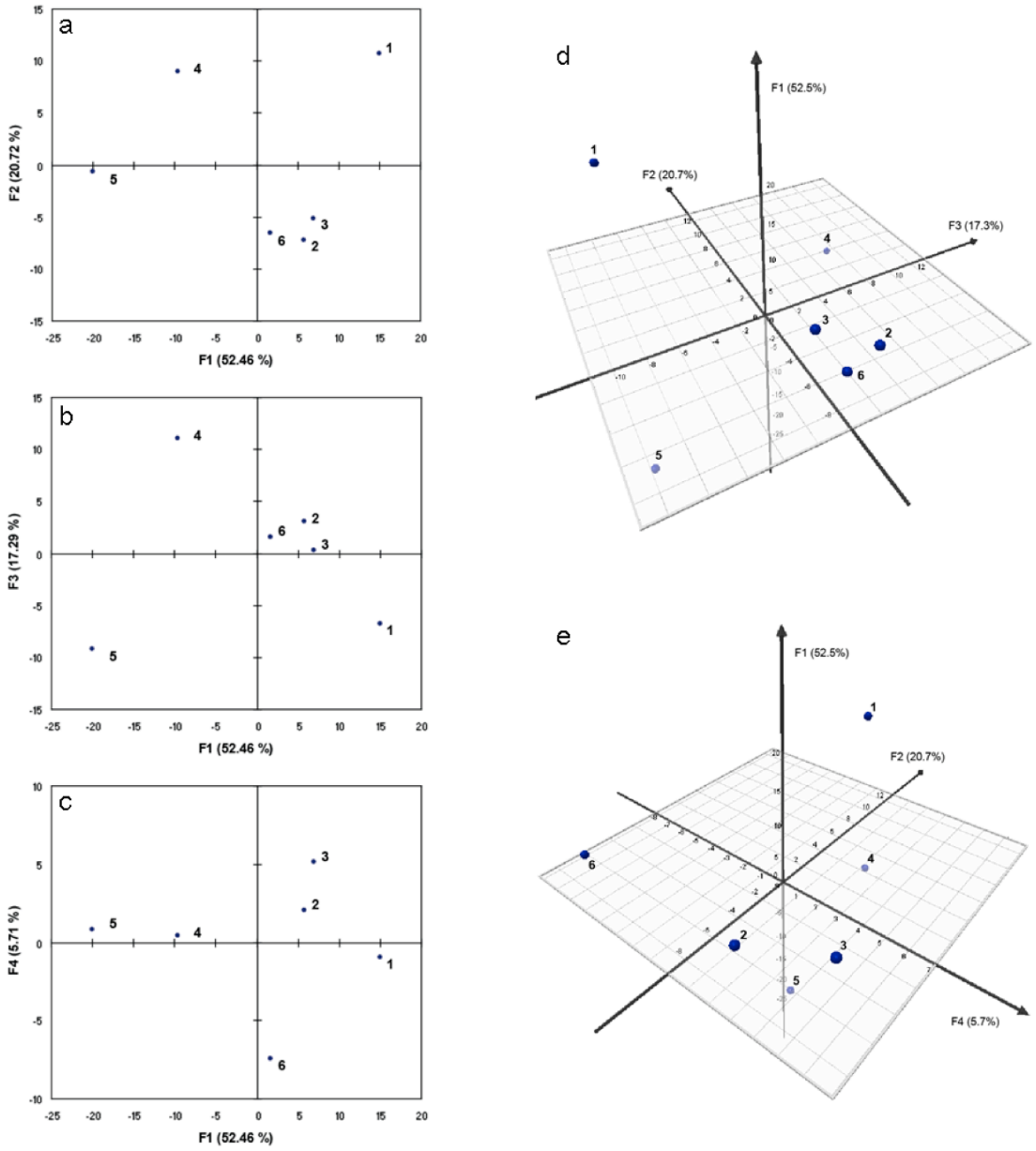

Figure S6. PCA plots of oligomers **1-6** based on their responses (difference profile data) to all the metal ions. (a), (b) and (c) Two-dimensional plots for the different principal components axes: F1 vs. F2, F1 vs. F3 and F1 vs. F4. Three-dimensional plots using principal components F1, F2 and F3 (c), and F1, F2 and F4 (d).

In the PCA for the metal ions (Fig. 7 and S7), the entire difference spectra data (Fig. S3) were included in the analysis except the data for oligomer **6** because the metal ions induce similar responses from **6**. The difference spectra data for the unknown solutions (Fig. S4) were also included in the PCA in order to facilitate comparison with the known solutions, simplifying identification of the unknown solutions.

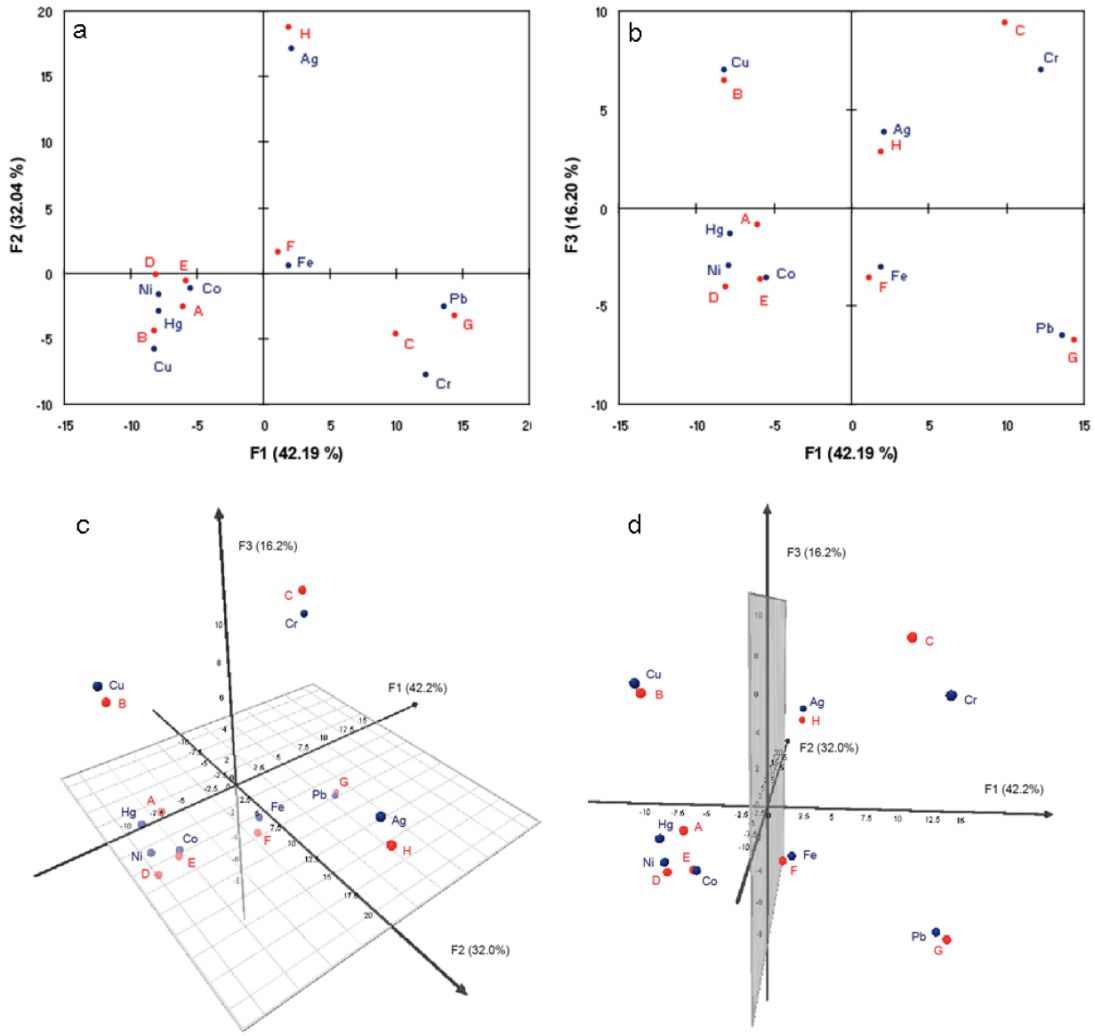

Figure S7. PCA plots of the known and unknown quenching metal ions based on the difference profile data of sensors **1**-**5**. (a) and (b) Two-dimensional plots for F1 vs. F2 and F1 vs. F3; and two different views of a three-dimensional plot (c) and (d). Blue color = known metal solutions and red color = unknown metal solutions A-H.

Agglomerative Hierarchical Clustering

Agglomerative hierarchical clustering (AHC) was also performed on the difference spectra data using XLSTAT Version 2010.4.01.[5] The analysis is based on Euclidean distance, employing Ward's agglomeration method. In the AHC for the metal ions (Fig. S8), two sets of data were analyzed. The first set of data includes only the difference spectra profiles of the known metal ion solutions (Fig. S8a), inputting intensity at each 10 nm of the spectrum. The second set of data includes the difference spectra profiles of both the known and unknown metal ion solutions (Fig. S8b) in order to allow direct comparison of the unknowns with the known solutions.

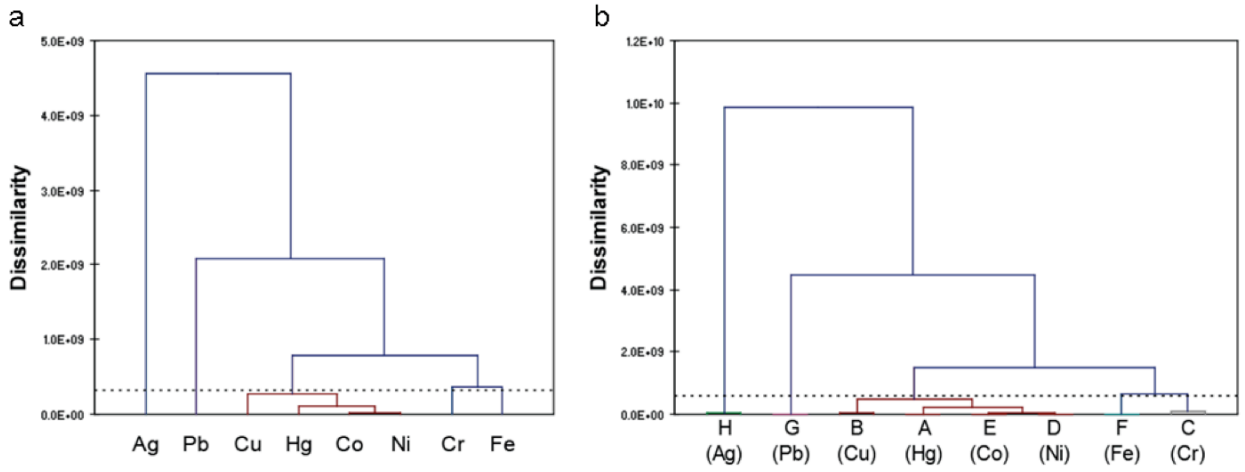

Figure S8. AHC dendrograms of the quenching metal ions based on the difference spectra profiles of sensors **1**-**5** without (a) and with (b) the unknown solution data. The metal cations are categorized according to the dissimilarity of the overall responses they induce on sensors **1**-**5**. They are grouped together based on the level of dissimilarity; the metal ions inducing mostly similar response pattern (low level of dissimilarity, non-blue colored lines) from all five sensors can be grouped into one class. The vertical lines correspond to the different classes, and the horizontal lines to the dissimilarity values between the classes. For each known-unknown solution pair in (b), the dissimilarity level is very low, making the horizontal line for each pair difficult to see near the zero dissimilarity level.

References:

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[4] Sigmoidal dose-response (variable slope) nonlinear regression (curve fit) was performed using GraphPad Prism version 4.03 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com.

[5] (a) Kitamura, M.; Shabbir, S. H.; Anslyn, E. V. *J. Org. Chem.* **2009**, 74, 12, 4479-4489. (b) Zhang, T.; Edwards, N. Y.; Bonizzoni, M.; Anslyn, E. V. *J. Am. Chem. Soc.* **2009**, 131, 33 11976-11984. (c) Hewage, H. S.; Anslyn, E. V. *J. Am. Chem. Soc.* **2009**, 131, 36, 13099-13106.