Selective Sensing of THC and Related Metabolites in Biofluids by Host:Guest Arrays

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Electronic Supplementary Information

Table of Contents

General Information.....*S*-2

Supporting Figures.....*S*-4

Sensor Calibrations.....*S*-4

1•3 Sensor Responses.....*S*-6

1•2 Sensor Responses.....*S*-9

Statistical Analysis.....*S*-12

NMR Spectra..... *S*-14

References..... *S*-15

General Information.

Cavitand **1**[1] and guest **3**[2] were synthesized and characterized according to literature procedures. Trans-[4-(dimethylamino)styryl]-1-methylpyridinium iodide (DSMI) **2** was purchased from Sigma Aldrich (St. Louis, MO). For detailed analysis of the fluorescence response of guests **3**[2] and **2**[3] in host **1**, please see the cited references. THC metabolite solutions were purchased as analytical standards from Sigma Aldrich (St. Louis, MO) and diluted in methanol to a final concentration of 300 μM. CBD was purchased from The CBDistillery (Denver, CO), and was used as received. Human saliva was purchased from Lee Biosolutions (Maryland Heights, MO), and human urine was purchased from Innovative Research (Novi, MI). Deuterated NMR solvents were obtained from Cambridge Isotope Laboratories (Andover, MA), and used without further purification. All other materials were purchased from Sigma Aldrich (St. Louis, MO) or Fisher Scientific (Fairlawn, NJ), and were used as received. NMR spectra were recorded on either a Bruker Avance 500 MHz, or a Bruker Avance Neo 400 MHz NMR spectrometer. All NMR spectra were processed using MestReNova by Mestrelab. Research S.L. Molecular modelling (Hartree-Fock) was performed using SPARTAN '06. Fluorescence measurements were performed in a Bio-Tek Synergy HT Multi-Detection Microplate Reader, with the Ex/Em wavelengths at 485/528 nm or 485/600 nm.

Differential Analysis and Multi-analyte sensing (description adapted from reference 4).[4] Differential sensing is possible using multiple arrayed sensors. Target recognition is achieved by analysing the total response of the target to the entire array of hosts. Each receptor variant causes a differential response, depending on binding affinity and, in this case, fluorescence quenching/enhancement mechanisms. The signals collected are analyzed by pattern recognition protocols, such as principal component analysis (PCA) and linear discriminant analysis (LDA).[5] Both PCA and LDA produce scores plots for the targets, which are shown in a multi-dimensional coordinate system by decomposing the raw data via a matrix technique.[4,5] The eigenvectors of the matrix produce axes in the coordinate system and the eigenvalues give a measure of the level of discrimination that exists in the data. PCA reduces the dimensionality of a data set, with the magnitudes of the eigenvalues representing the variances in the data. LDA is a supervised method, used for the classification of data as well as the assignment of unknown analytes.[5]

Fluorescence measurements. In general, the fluorescence assays were carried out by mixing 10 μL of the fluorescent guest **2** (15 μM) or **3** (30 μM), 10 μL of cavitand **1** (200 μM), 10 μL metal salts (500 μM in water), and 60 μL of the incubation buffer (Tris buffer HCl, pH 7.4, 20 mM, urine, or saliva) in the 96-well plate, adding 10 μL of the THC metabolite solution at 300 μM in methanol to bring the total volume up to 100 μL, then incubating with mild shaking for 15 mins at room temperature. Each experimental condition was repeated in quadruplicate, using identical sensor components across four

wells, and collecting fluorescence signals for each. The fluorescence signal (F) was recorded in a Bio-Tek Synergy HT Multi-Detection Microplate Reader with the Ex/Em wavelengths at 485/528 nm for guest **3** and 485/605 nm for guest **2**.

Data analysis. The quadruplicate raw fluorescence data sets were subjected to Linear Discriminant Analysis (LDA) and Principal Component Analysis (PCA) performed with RStudio (Version 1.0.136), an integrated development environment (IDE) for R (version 3.3.2). 2D scores plots and confidence intervals were graphed in RStudio using the packages ggplot2, ggpubr, and ggfortify. 3D PCA plots were graphed in OriginPro. All other fluorescence data charts were created in Microsoft Excel, with values representing the mean of the quadruplicate responses and error bars indicating their standard deviation.

Supporting Figures

Sensor Responses in Different Aqueous Fluids

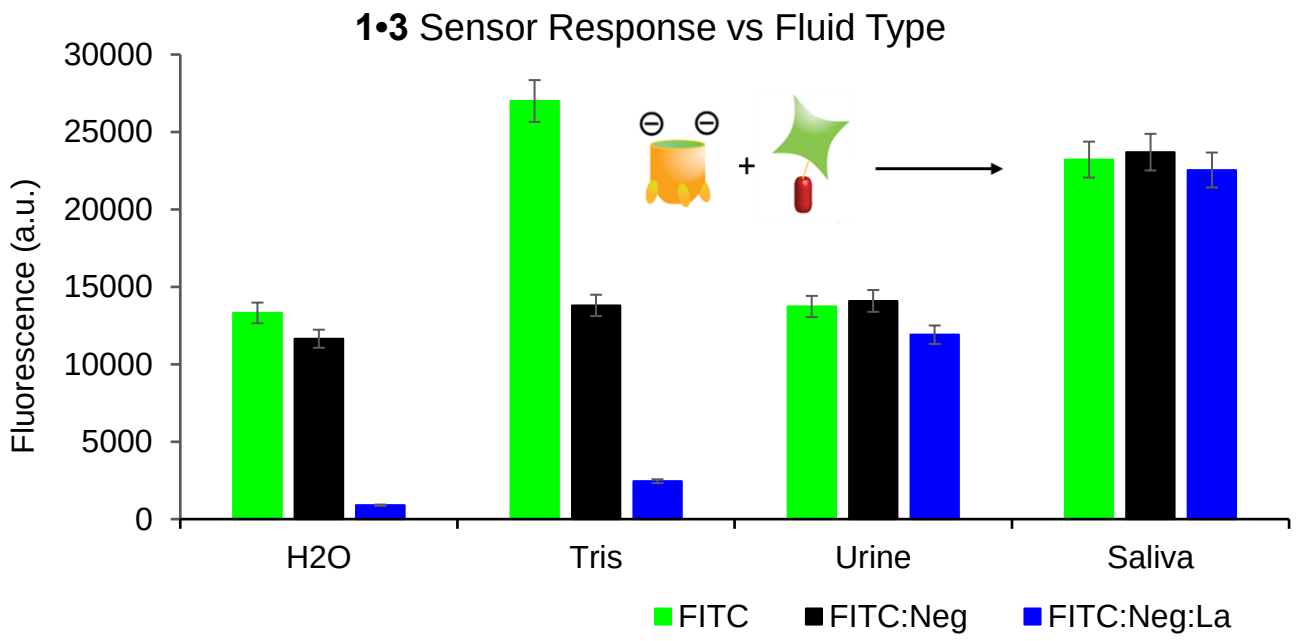

Figure S-1. Fluorescence responses of guest **3**, the **1•3** sensor, or the **1•3•La**$^{3+}$ sensor in either water, Tris buffer, urine or saliva. [**1**] = 20 μM, [**3**] = 3.0 μM, [La^{3+}] = 50 μM, [Tris] = 20 mM, pH 7.4.

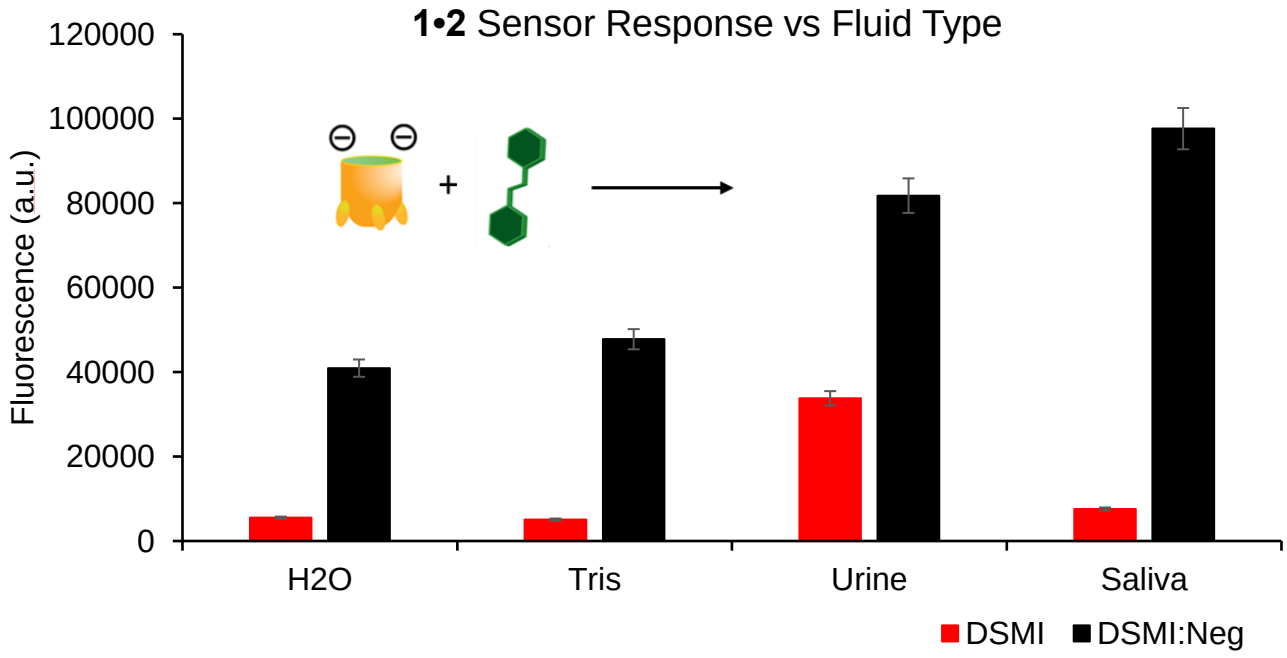

Figure S-2. Fluorescence responses of guest **2** or the **1•2** sensor in either water, Tris buffer, urine or saliva. [**1**] = 20 μM, [**3**] = 3.0 μM, [La^{3+}] = 50 μM, [Tris] = 20 mM, pH 7.4.

1•3 Sensor – Limits of Detection

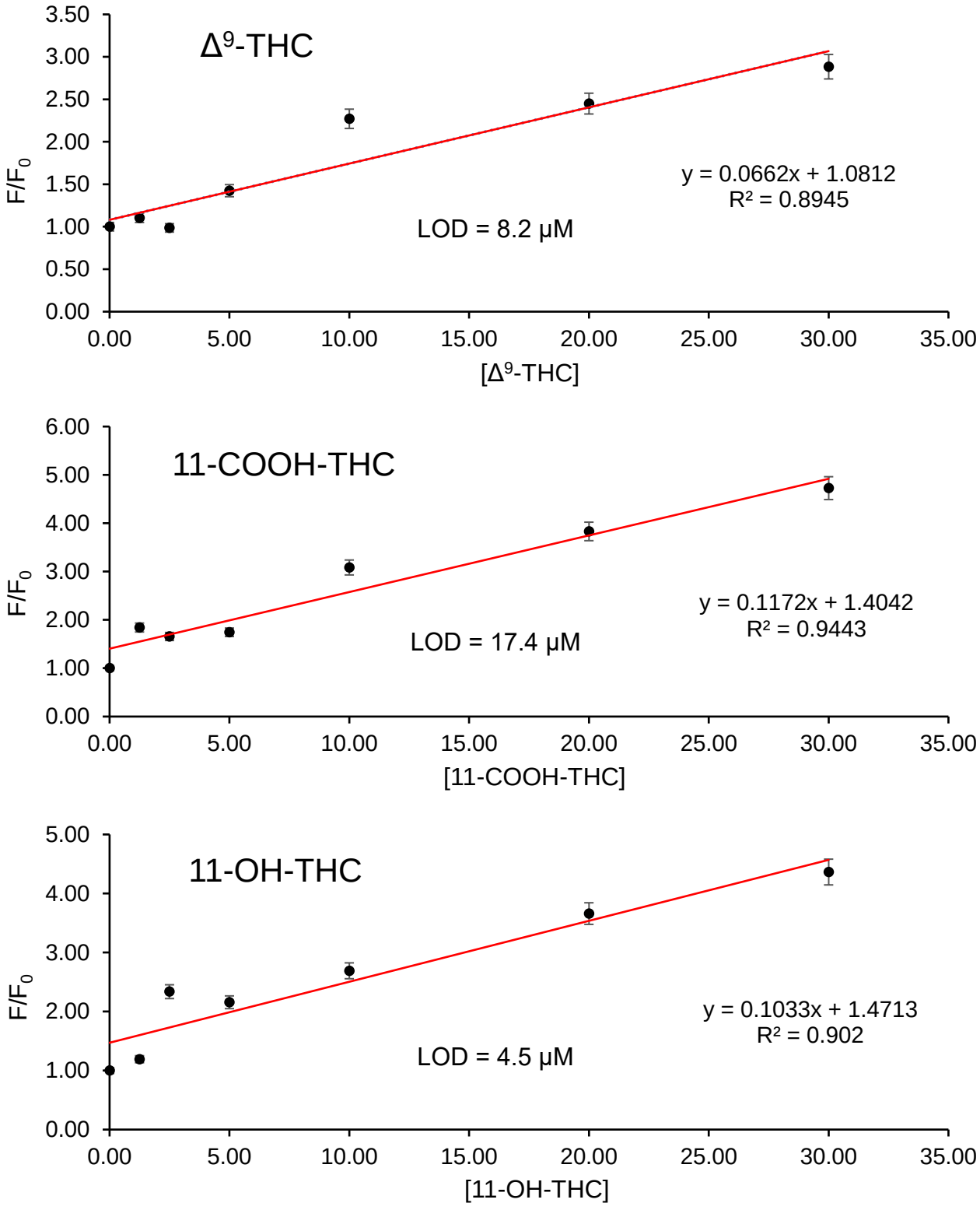

Figure S-3. Limits of Detection. Linear regression models for the relative fluorescence responses of the **1•3•La^{3+}** sensor with the 3 primary THC metabolites (Δ^9-THC, 11-COOH-THC, and 11-OH-THC) in 20 mM Tris buffer, pH 7.4. [**1**] = 20 µM, [**3**] = 3.0 µM, [La^{3+}] = 50 µM. F_0 = fluorescence response of the **1•3•La^{3+}** complex, F = fluorescence response of the **1•3•La^{3+}•Metabolite** complex.

Fluorescein Guest 3 Responses

1•3 Sensor – Tris Buffer

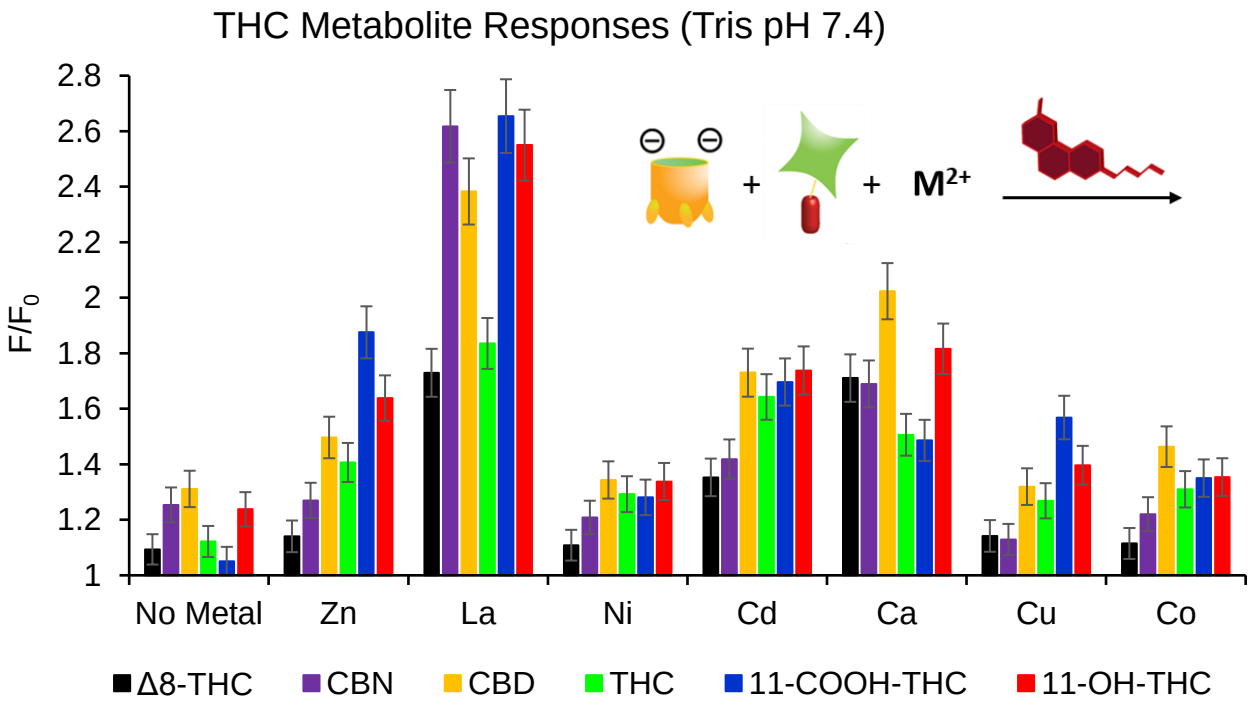

Figure S-4. Relative fluorescence responses of the **1•3•M**$^{2+}$ complex in 20 mM Tris buffer, pH 7.4. [**1**] = 20 μM, [**3**] = 3.0 μM, [Metal] = 50 μM, [Metabolites] = 30 μM. F_0 = fluorescence response of the **1•3•M**$^{2+}$ complex, F = fluorescence response of the **1•3•M**$^{2+}$**•Metabolite** complex.

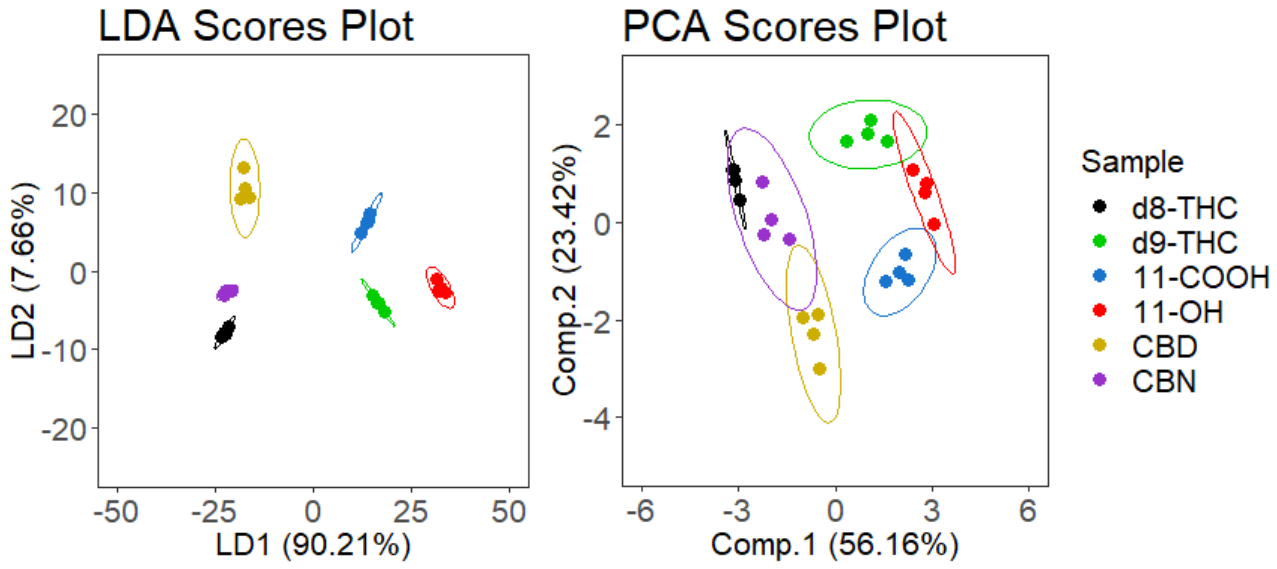

Figure S-5. LDA and PCA scores plots with 95% confidence intervals for the 8-factor **1•3•M**$^{2+}$**•Metabolite** sensor array in Tris buffer (obtained from statistical analysis of data in Figure S-4). [**1**] = 20 μM, [**3**] = 3 μM, [Metal] = 50 μM, [Metabolites] = 30 μM, [Tris] = 20 mM (pH 7.4).

1•3 Sensor – Urine

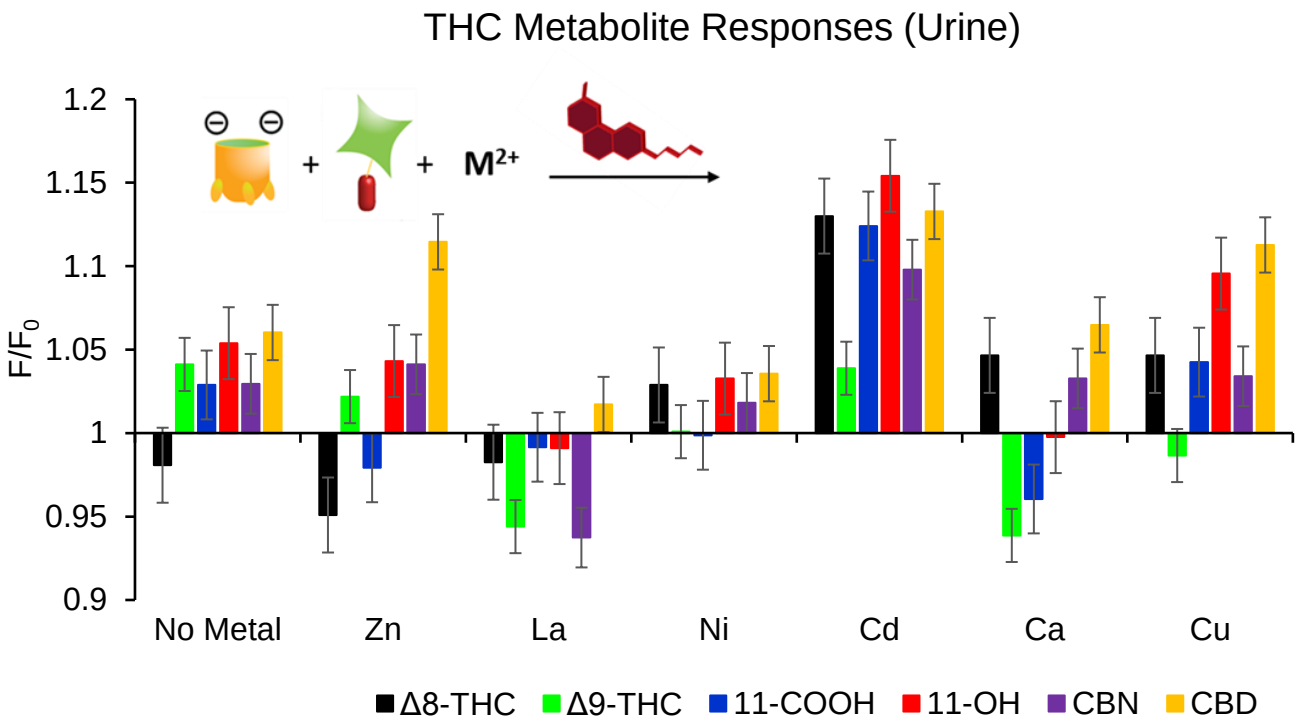

Figure S-6. Relative fluorescence responses of the **1•3•M^{2+}** complex in pooled human urine. [**1**] = 20 μM, [**3**] = 3.0 μM, [Metal] = 50 μM, [Metabolites] = 30 μM. F_0 = fluorescence response of the **1•3•M^{2+}** complex, F = fluorescence response of the **1•3•M^{2+}•Metabolite** complex.

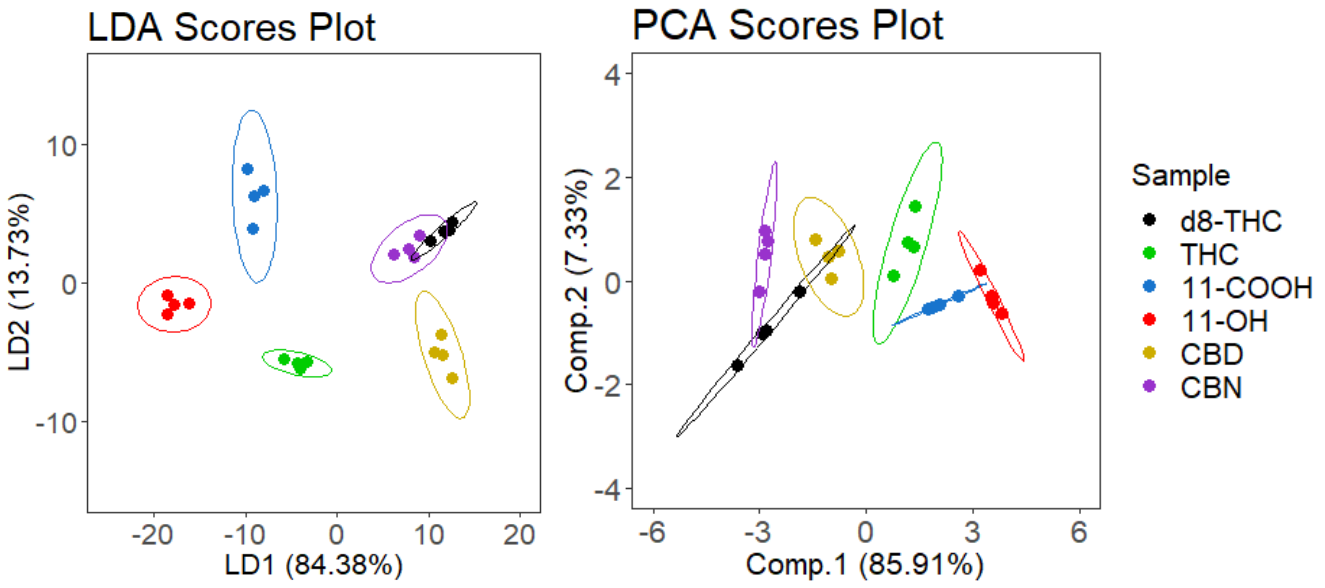

Figure S-7. LDA and PCA scores plots with 95% confidence intervals for the 7-factor **1•3•M^{2+}•Metabolite** sensor array in urine (obtained from statistical analysis of data in Figure S-6). [**1**] = 20 μM, [**3**] = 3 μM, [Metal] = 50 μM, [Metabolites] = 30 μM.

1•3 Sensor – Saliva

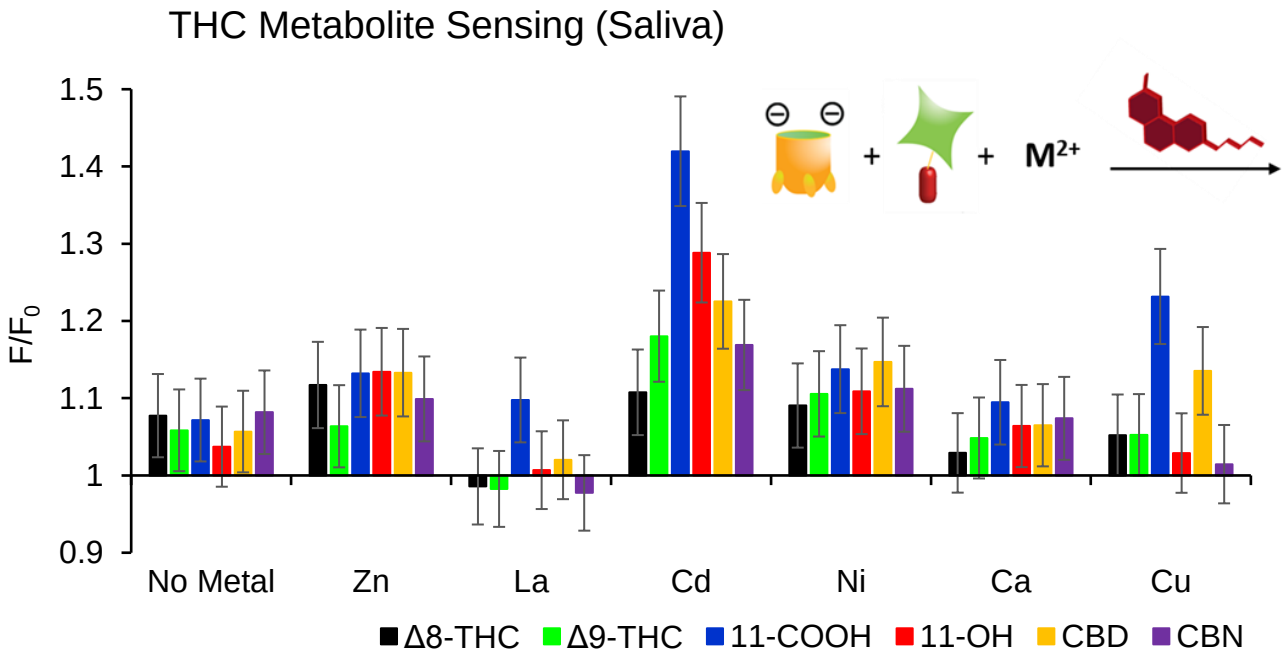

Figure S-8. Relative fluorescence responses of the **1•3•M^{2+}** complex in pooled human saliva. [**1**] = 20 μM, [**3**] = 3.0 μM, [Metal] = 50 μM, [Metabolites] = 30 μM. F_0 = fluorescence response of the **1•3•M^{2+}** complex, F = fluorescence response of the **1•3•M^{2+}•Metabolite** complex.

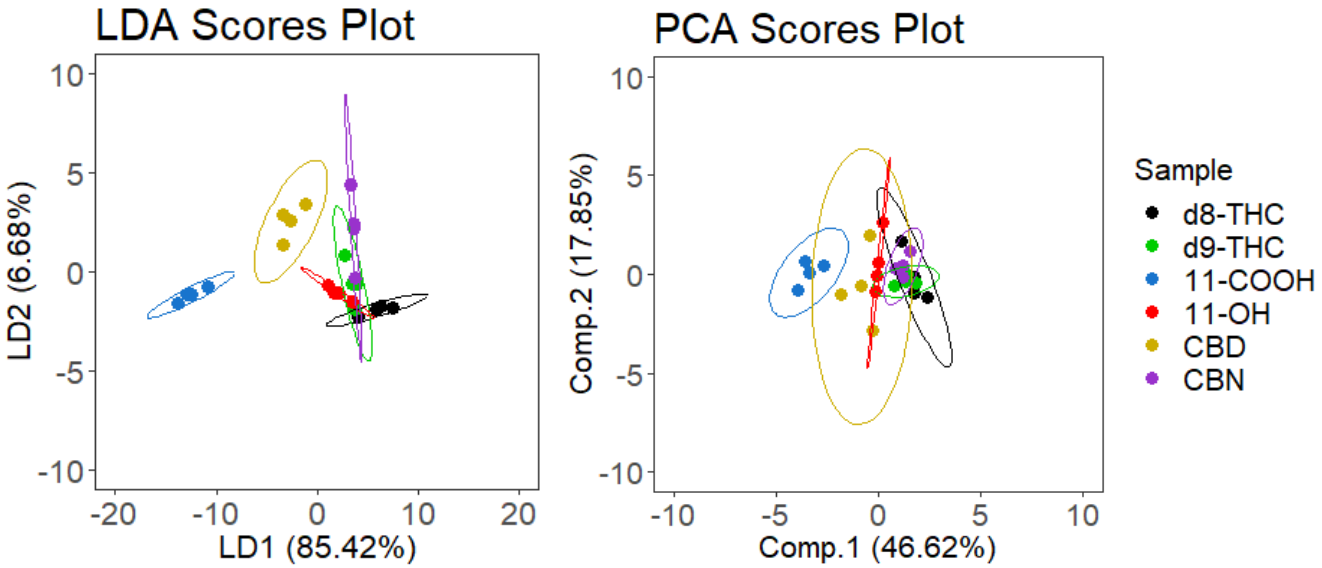

Figure S-9. LDA and PCA scores plots with 95% confidence intervals for the 7-factor **1•3•M^{2+}•Metabolite** sensor array in saliva (obtained from statistical analysis of data in Figure S-8). [**1**] = 20 μM, [**3**] = 3 μM, [Metal] = 50 μM, [Metabolites] = 30 μM.

DSMI Guest 2 Responses

1•2 Sensor – Tris Buffer

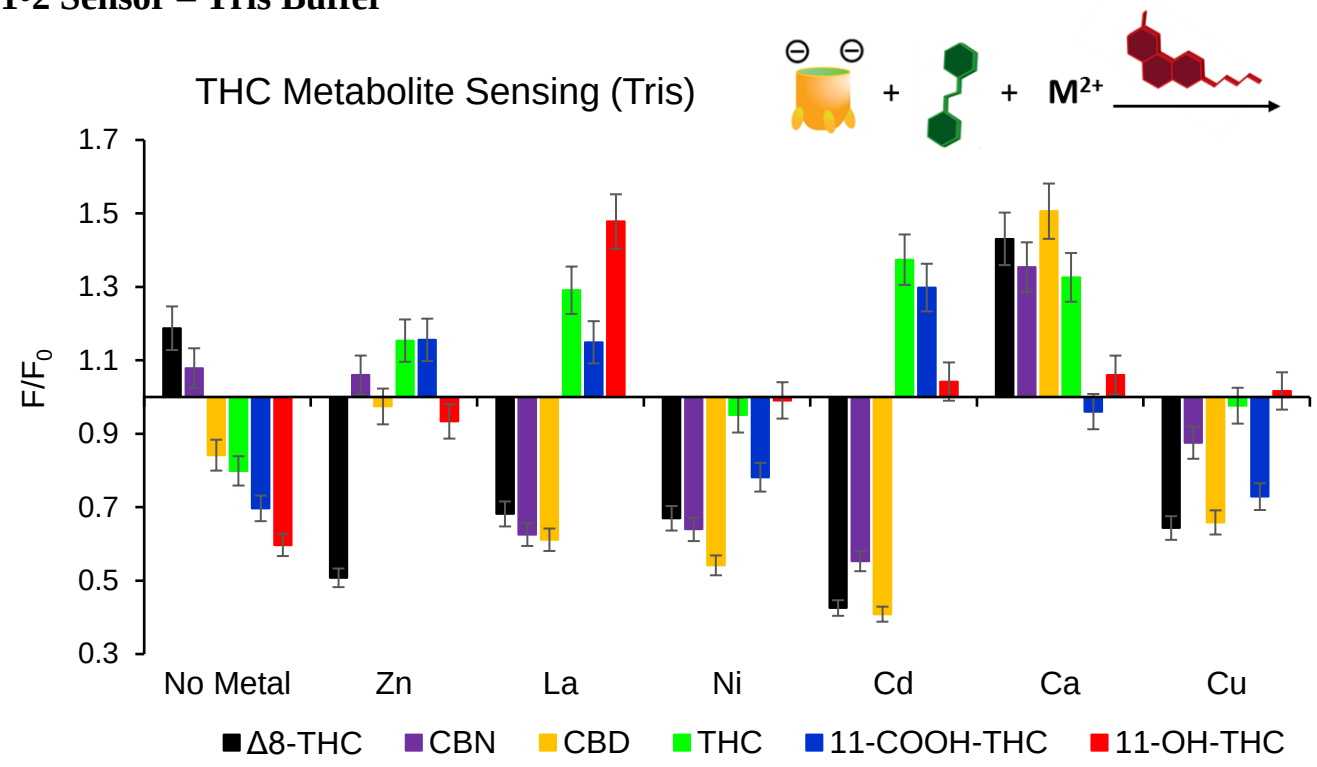

Figure S-10. Relative fluorescence responses of the **1•2•M^{2+}** complex in 20 mM Tris buffer, pH 7.4. [**1**] = 20 μM, [**2**] = 1.5 μM, [Metal] = 50 μM, [Metabolites] = 30 μM. F_0 = fluorescence response of the **1•2•M^{2+}** complex, F = fluorescence response of the **1•2•M^{2+}•Metabolite** complex.

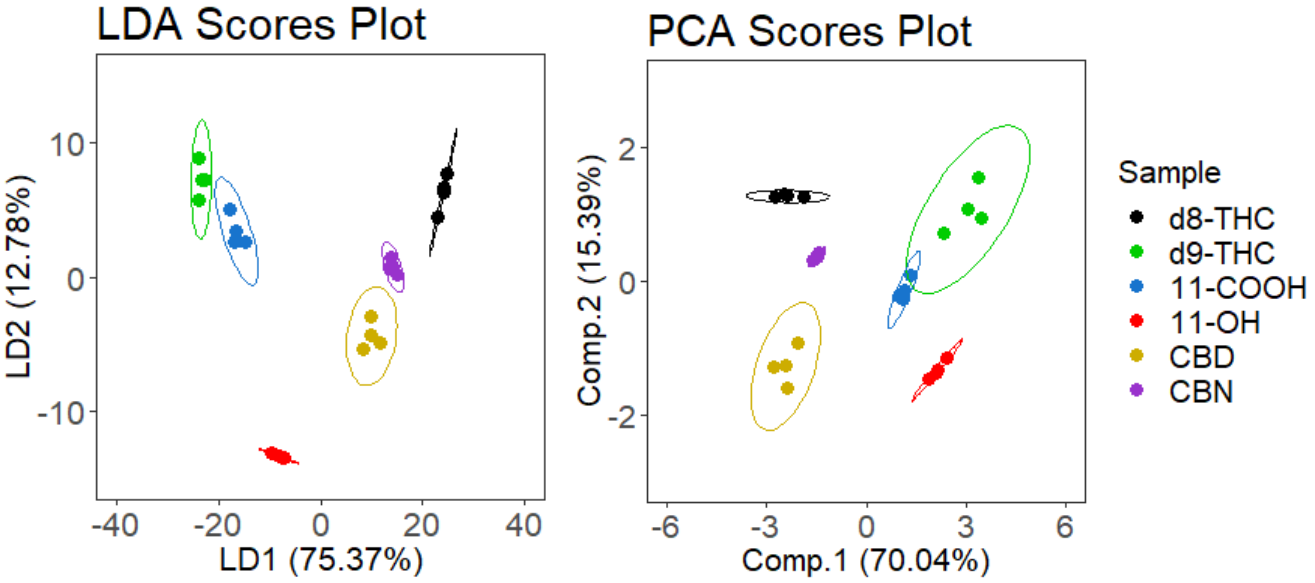

Figure S-11. LDA and PCA scores plots with 95% confidence intervals for the 7-factor **1•2•M^{2+}•Metabolite** sensor array in Tris buffer (obtained from statistical analysis of data in Figure S-10). [**1**] = 20 μM, [**2**] = 1.5 μM, [Metal] = 50 μM, [Metabolites] = 30 μM, [Tris] = 20 mM (pH 7.4).

1•2 Sensor – Urine

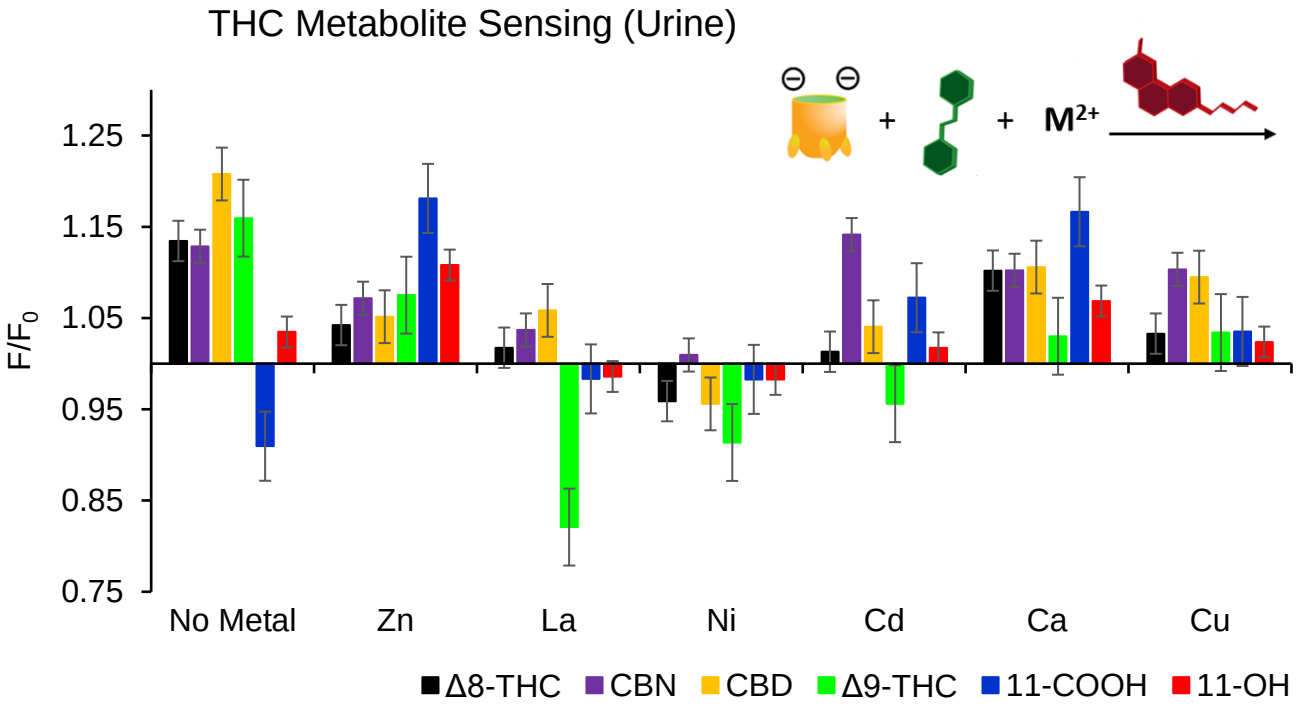

Figure S-12. Relative fluorescence responses of the **1•2•M**$^{2+}$ complex in pooled human urine. [**1**] = 20 μM, [**2**] = 1.5 μM, [Metal] = 50 μM, [Metabolites] = 30 μM. F_0 = fluorescence response of the **1•2•M**$^{2+}$ complex, F = fluorescence response of the **1•2•M**$^{2+}$**•Metabolite** complex.

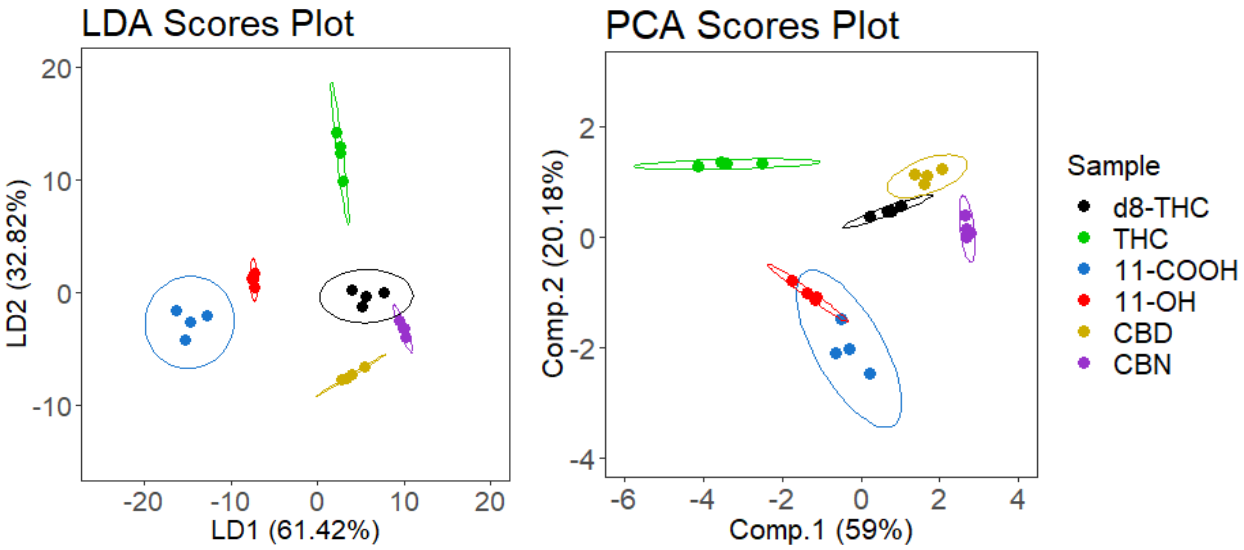

Figure S-13. LDA and PCA scores plots with 95% confidence intervals for the 7-factor **1•2•M**$^{2+}$**•Metabolite** sensor array in urine (obtained from statistical analysis of data in Figure S-12). [**1**] = 20 μM, [**2**] = 1.5 μM, [Metal] = 50 μM, [Metabolites] = 30 μM.

1•2 Sensor – Saliva

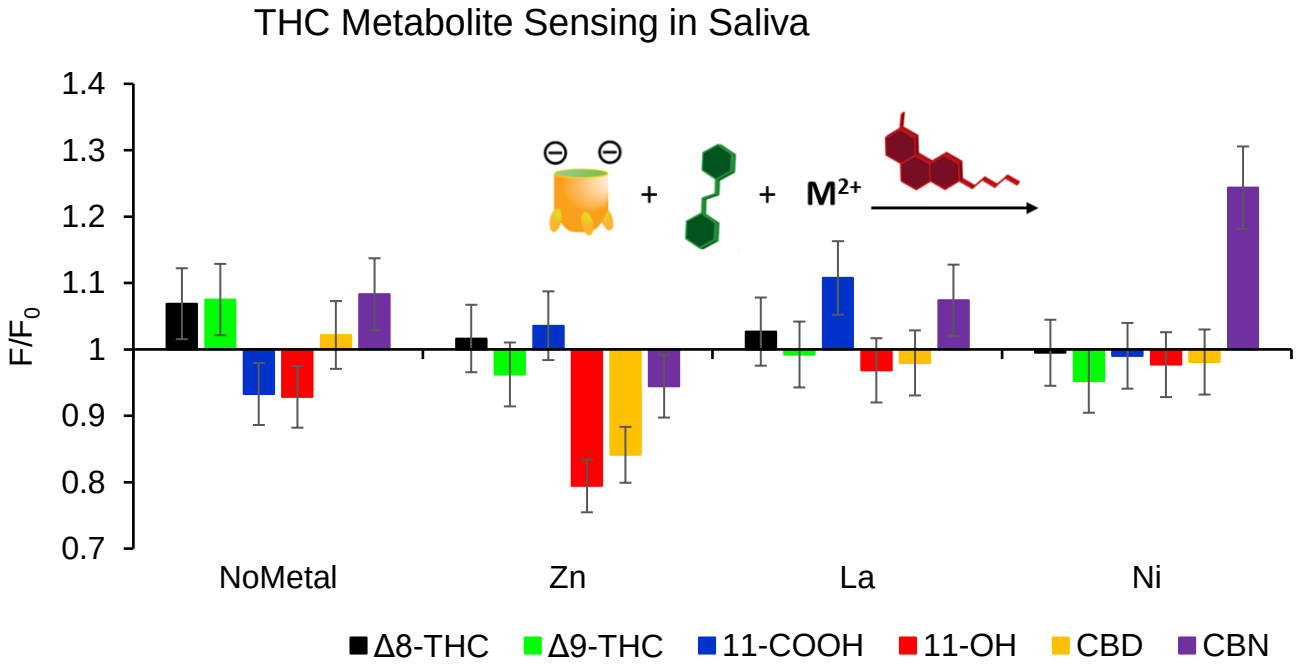

Figure S-14. Relative fluorescence responses of the **1•2•M**$^{2+}$ complex in pooled human saliva. [**1**] = 20 μM, [**2**] = 1.5 μM, [Metal] = 50 μM, [Metabolites] = 30 μM. F_0 = fluorescence response of the **1•2•M**$^{2+}$ complex, F = fluorescence response of the **1•2•M**$^{2+}$**•Metabolite** complex.

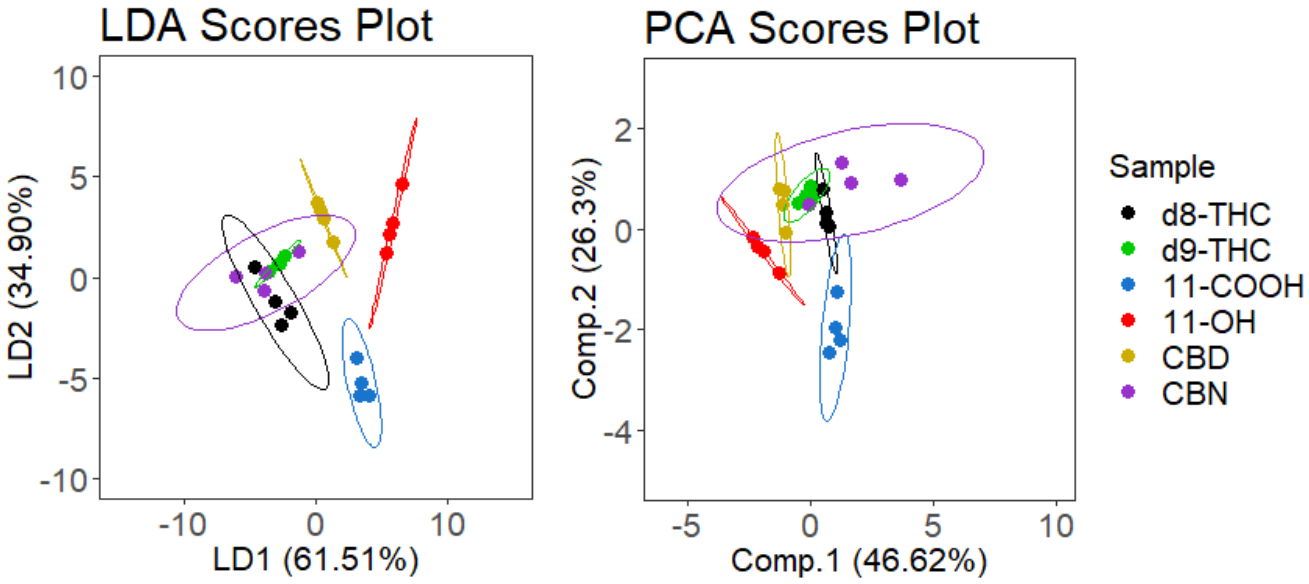

Figure S-15. LDA and PCA scores plots with 95% confidence intervals for the 4-factor **1•2•M**$^{2+}$**•Metabolite** sensor array in saliva (obtained from statistical analysis of data in Figure S-14). [**1**] = 20 μM, [**2**] = 1.5 μM, [Metal] = 50 μM, [Metabolites] = 30 μM.

Statistical Analysis

Full Combined Sensor – Tris Buffer

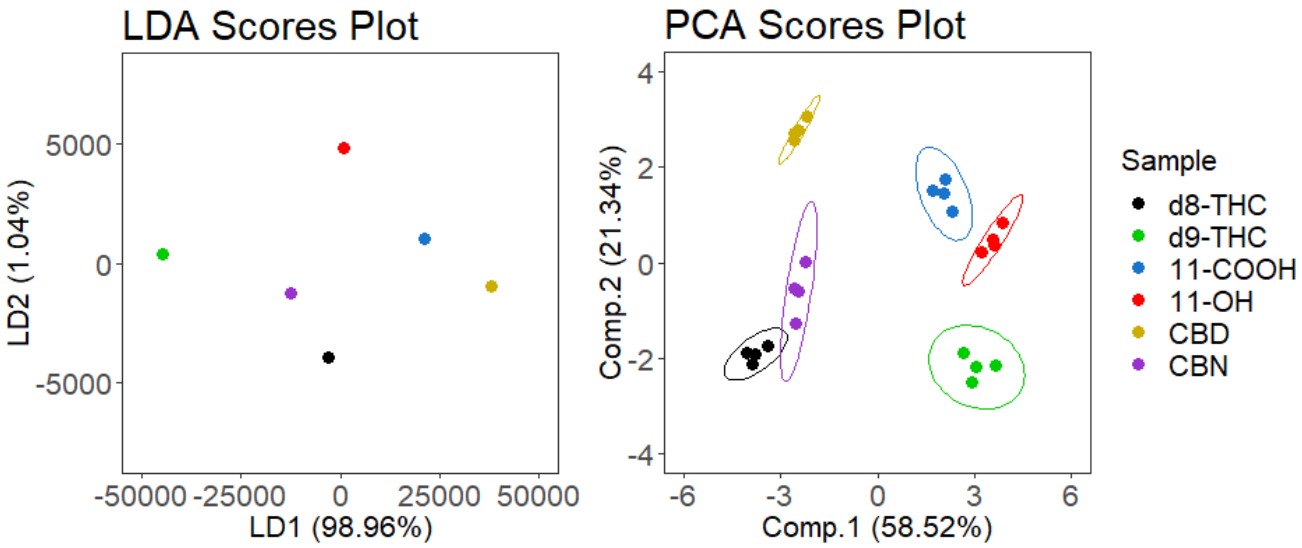

Figure S-16. LDA and PCA scores plots with 95% confidence intervals for the full 15-factor **1•2•M**$^{2+}$ and **1•3•M**$^{2+}$ sensor array in Tris buffer (obtained from statistical analysis of data in Figures S-4 and S-10). [**1**] = 20 μM, [**2**] = 1.5 μM, [**3**] = 3 μM, [Metal] = 50 μM, [Metabolites] = 30 μM, [Tris] = 20 mM (pH 7.4).

Minimal Combined Sensor – Tris Buffer

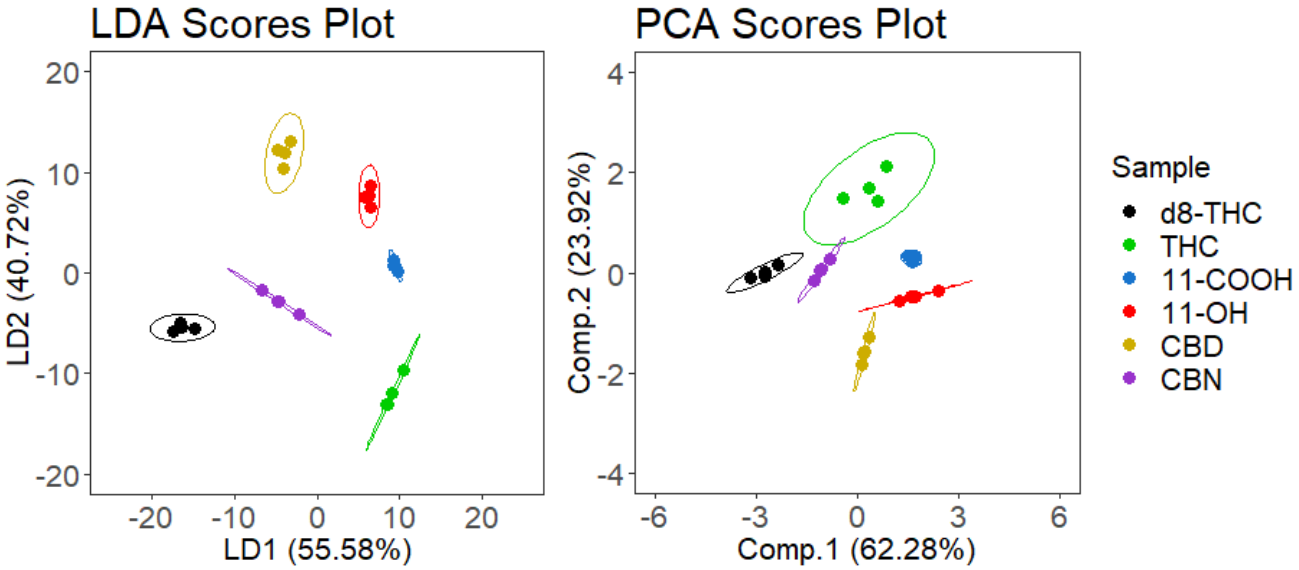

Figure S-17. LDA and PCA scores plots with 95% confidence intervals for a minimal 4-factor array with **1•2** or **1•3** and either no metal or Zn^{2+} in Tris buffer (obtained from statistical analysis of selected data from Figures S-4 and S-10). [**1**] = 20 μM, [**2**] = 1.5 μM, [**3**] = 3 μM, [Metal] = 50 μM, [Metabolites] = 30 μM, [Tris] = 20 mM (pH 7.4).

Full Combined Sensor – Urine

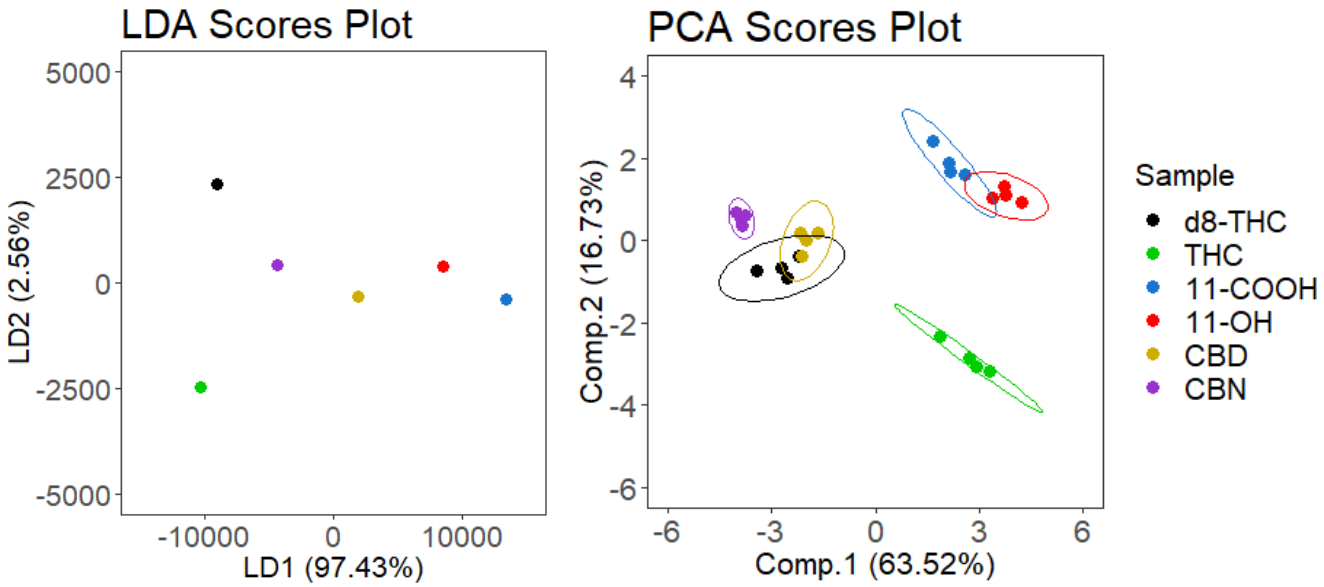

Figure S-18. LDA and PCA scores plots with 95% confidence intervals for the full 14-factor **1•2•M**$^{2+}$ and **1•3•M**$^{2+}$ sensor array in urine (obtained from statistical analysis of data in Figures S-6 and S-12). [**1**] = 20 μM, [**2**] = 1.5 μM, [**3**] = 3 μM, [Metal] = 50 μM, [Metabolites] = 30 μM.

Full Combined Sensor – Saliva

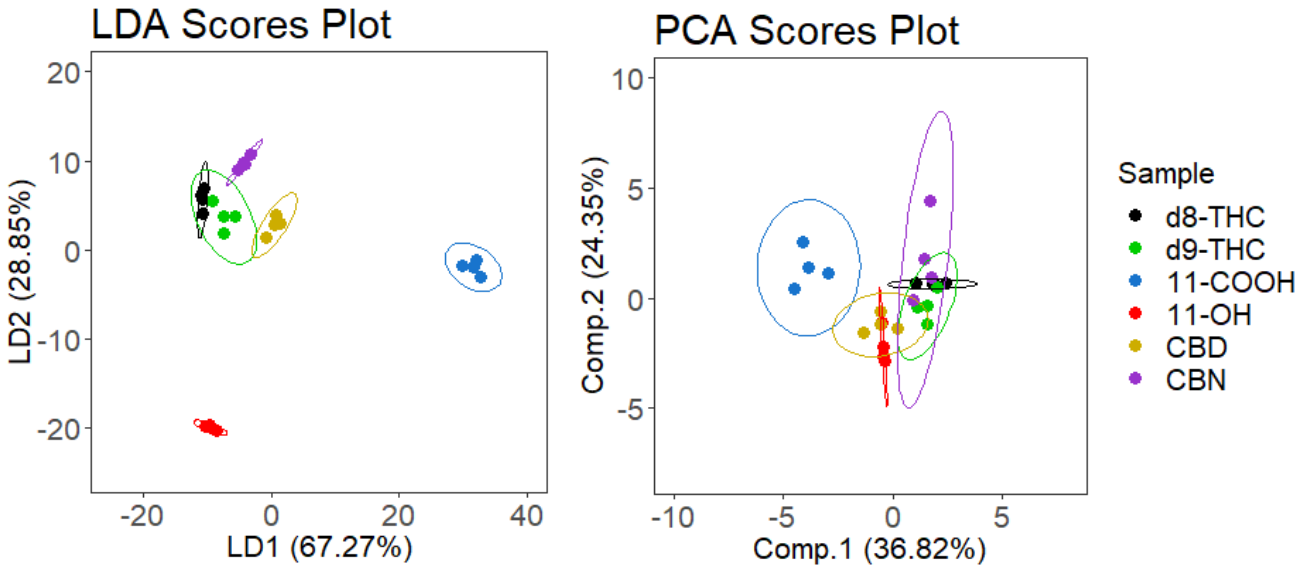

Figure S-19. LDA and PCA scores plots with 95% confidence intervals for the full 11-factor **1•2•M**$^{2+}$ and **1•3•M**$^{2+}$ sensor array in saliva (obtained from statistical analysis of data in Figures S-8 and S-14). [**1**] = 20 μM, [**2**] = 1.5 μM, [**3**] = 3 μM, [Metal] = 50 μM, [Metabolites] = 30 μM.

NMR Spectra

Host 1•CBD Complex

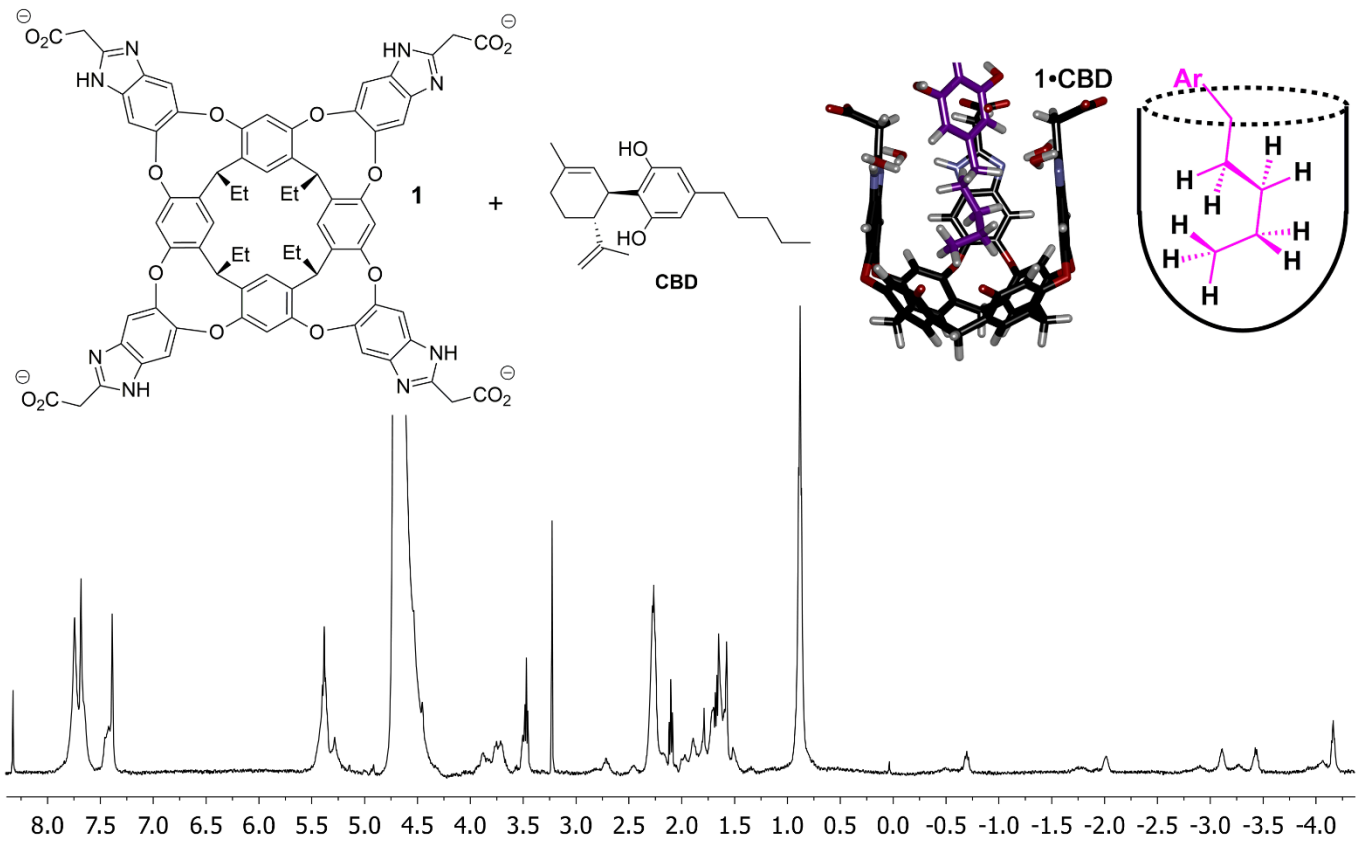

Figure S-20. ^{1}H NMR spectrum (500 MHz, D_2O) of the **1•CBD** complex.

CBD

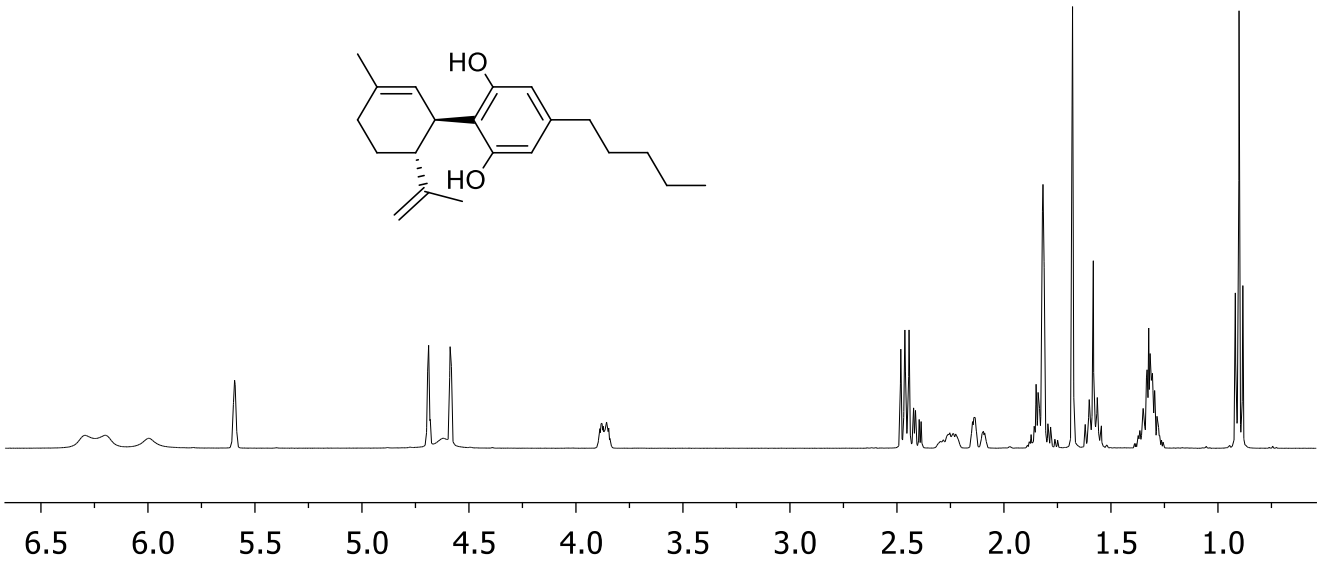

Figure S-21. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **CBD** as received.

References

1. S. M. Biros, E. C. Ullrich, F. Hof, L. Trembleau, J. Rebek, Jr. *J. Am. Chem. Soc.*, 2004, **126**, 2870.
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3. Y. Liu, M. Mettry, A. D. Gill, L. Perez, W. Zhong, R. J. Hooley, *Anal. Chem.*, 2017, **89**, 11113.

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