

## SUPPORTING INFORMATION

### Fluorescent DNA Sensors: Identification of Bacterial Species by Their Volatile Metabolites

Chi-Kin Koo,<sup>a</sup> Shenliang Wang,<sup>a</sup> Rajiv L. Gaur,<sup>b</sup> Florent Samain,<sup>a</sup> Niaz Banaei,<sup>b</sup> and Eric T. Kool<sup>a \*</sup>

*Department of Chemistry, Stanford University, Stanford, California 94305(USA)*

*Department of Pathology, Stanford University School of Medicine, Palo Alto, CA 94304.*

\*to whom correspondence should be addressed: kool@stanford.edu

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## Experimental

**General Materials and Methods.** MALDI-MS was performed on a Voyager-DE RP Biospectrometer at the PAN facilities at Stanford University. Absorption and emission spectra were recorded on a Cary 1 UV-Vis spectrometer and a Jobin Yvon-Spex Fluorolog 3 spectrometer (excitation at 345 nm) respectively.

**Monomer and Library Syntheses.** Syntheses of the four deoxyriboside monomers **Y**, **E**, **B** and **K** were carried out as previously reported.<sup>1</sup> The spacer phosphoramidite (**S**), 5,6-dihydrodT-CE phosphoramidite (**H**), and 5-nitroindole-CE phosphoramidite (**I**) were purchased from Glen Research. Altogether, six fluorescent nucleosides (**Y**, **E**, **B**, **K**, **S**, **H** and **I**) were included to yield 7<sup>4</sup> tetramer-length ODF sequences in all combinations. The ODF library was constructed as previously described<sup>2</sup> on 130 μm amine-functionalized polyethylene glycol-polystyrene (PEG-PS) beads (NovaSyn TG amino resin (Novabiochem; average loading: 100 mmol/g) using standard tagging procedures<sup>3</sup> and split-and-mix methods<sup>4</sup>. Deprotection of the phosphate backbone was then performed in 0.05 M methanolic K<sub>2</sub>CO<sub>3</sub> solution (room temperature, 12 hours) to yield the library used for screening.

**Bacteria Cultures.** *E. coli* (ATCC 11175) and *P. putida* (ATCC 49128) were grown following the procedures provided by the commercial supplier. Briefly, *E. coli* strains were grown in lysogeny broth (LB) or on LB agar (Sigma-Aldrich) at 37 °C while *P. putida* was grown in nutrient broth (BD 213000) or nutrient agar (BD 234000) at 30 °C. *E. coli* and *P. putida* samples used for both screening and cross-screening were grown on agar plates for 18 hours. *M. tuberculosis* H37Ra (ATCC 25177) were grown on Middlebrock 7H9 agar supplemented with ADC at 37 °C for 14 days.

**Screening, Image Processing and Decoding.** The initial screening process was performed with bacteria grown on agar in closed Petri dishes as illustrated in Fig. S1. The beads from the library were placed onto a small microscope slide to prevent direct contact of the beads with the culture agar. The beads were first equilibrated in free agar plates (with no bacteria grown) for 30 minutes and then quickly transferred to another closed Petri dish with one of the selected bacteria cultures. Fluorescence was monitored under an epifluorescence microscope (Nikon Eclipse E800) with excitation at 340-380 nm, and all visible emission was observed (long-pass filter, >420 nm). Fluorescence images were taken with a Spot RT digital camera and Spot Advanced Imaging software before and after exposure to the bacteria (for 30 minutes) at room temperature.

To determine emission changes in response to the headspace volatile metabolites from each bacterial species, we constructed graphical 50% gray-based difference maps of the beads by inverting color/intensity of the image before exposure (i.e. making a photonegative) and merging it with the image taken after 30 minutes of exposure using 50% transparency (Adobe Photoshop, version 10.01). An example is shown in Fig. S2. Any part of the image that is 50% gray indicates no change, whereas beads that are darker than the 50% gray

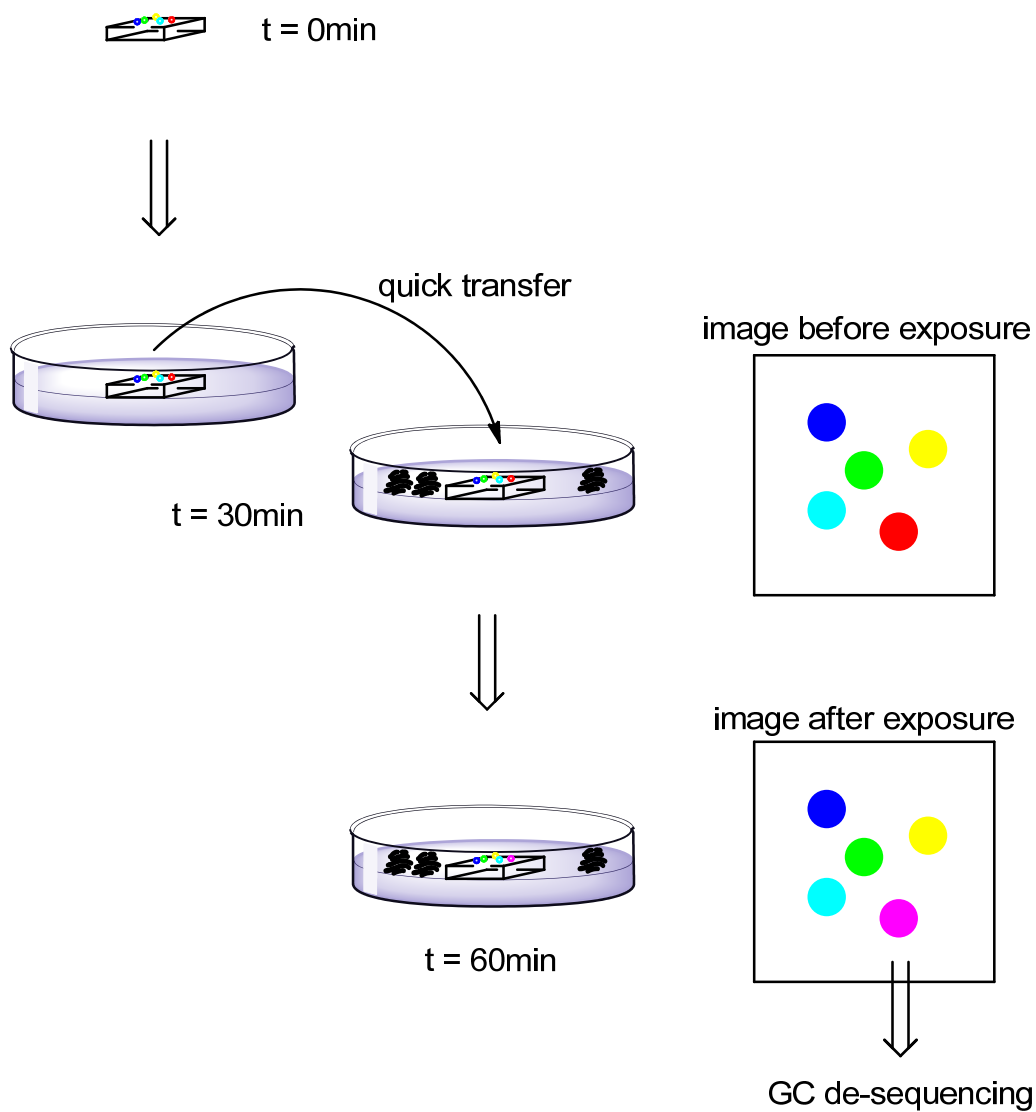
background reveal quenching, brighter beads show emission enhancement, and colors reflect a combination of the original ODF emission color and any wavelength shifts that occur during the sensing events. Beads that showed the strongest responses were picked up with a flame-pulled pipet and transferred into a capillary tube for sequence-decoding by electron-capture gas chromatography (EC-GC) (Fig. S3).

**Oligodeoxyfluoroside Synthesis.** The 14 sensor ODFs selected from the initial screening were individually re-synthesized on an Applied Biosystems 394 DNA/RNA synthesizer on a 1  $\mu$ mole scale and possessing a 3'-phosphate group for characterization off of the beads. Standard  $\beta$ -cyanoethyl phosphoramidite chemistry was employed for coupling, but with an extended coupling time (999 s) for the non-natural nucleotides. Overall coupling efficiencies exceeded 70%. Oligomers were then deprotected and cleaved from the solid support with 0.05 M methanolic  $K_2CO_3$  solution (room temperature, 12 hours). The oligomers were purified by HPLC (Shimadzu 10 Series with a Jupiter 5u C5 column) using water (TEAA, pH~7.2) and acetonitrile as the solvent system. The purified oligomers were characterized by MALDI-MS, UV-vis absorption and emission spectroscopy. Standard automatic DNA synthesis techniques were used except that 50/50 of cleavable controlled pore glass (CPG) and non-cleavable PEG-PS beads were included in the column, so that sensor beads and corresponding ODFs on standard cleavable CPG were made at the same time. This allowed the characterization of the ODFs off of the beads while making the corresponding sensors on beads in the same synthesis.

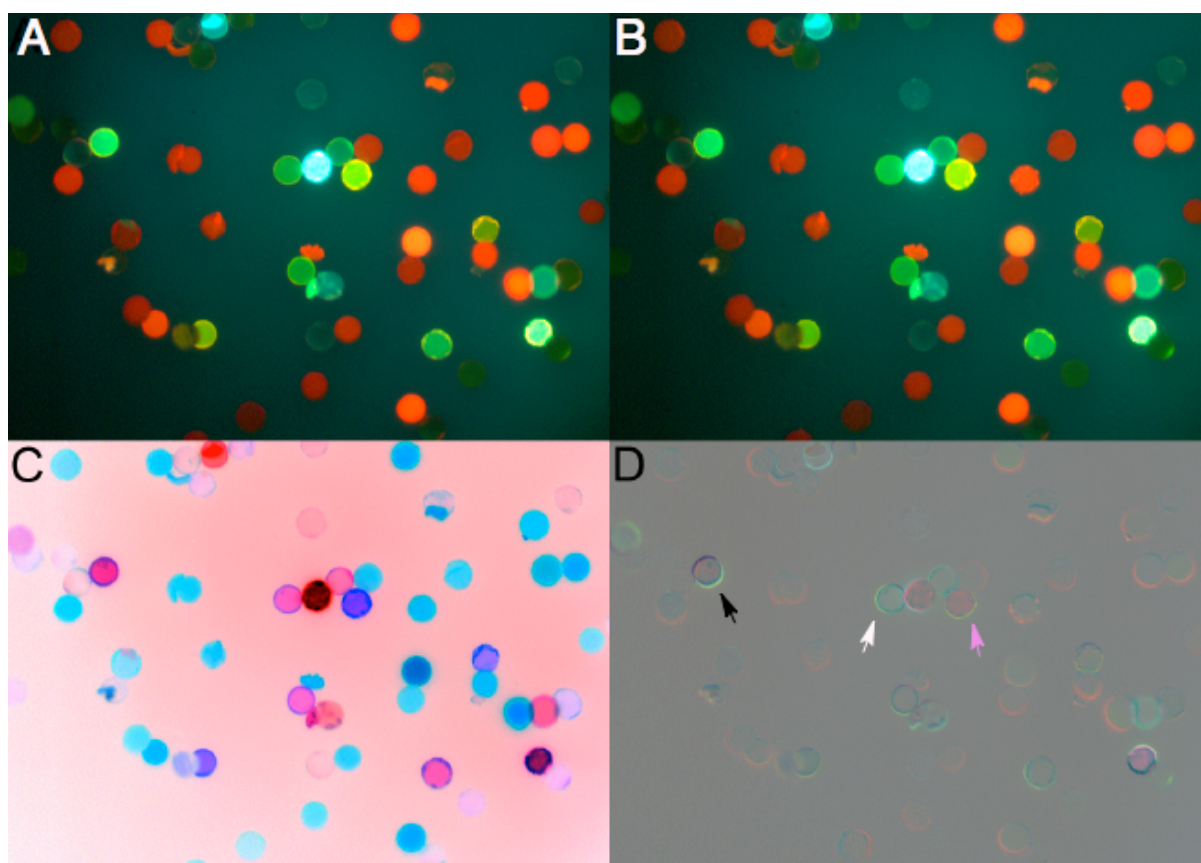
**Cross-screening and Statistical Analysis.** Since different bacterial species have their own constraints on the compositions of nutrients, it is important to grow a given bacterial species on a specific culture medium, to prevent any disturbance to their natural metabolic cycles. Indeed, our attempt to culture *M. tuberculosis* on LB agar, the most common culture medium for microbiology, was unsuccessful. The cross-screening experiments were done by using the bacteria grown on different medium agar plates (specified by the commercial suppliers). To account for any spectral changes due to the use of different culturing mediums, the cross-screening experiments were repeated with blank agar plates of the same media.

Cross-screening experiments were performed under an epifluorescence microscope with a 4X objective and fluorescence images were taken before and after the exposure of each analyte. The emission was then quantified as RGBL (red, green, blue, luma) values by using Adobe Photoshop. Average R/G/B/L values were determined within a 16 $\times$ 16-pixel box (256 pixels square from RGB 8 bits picture) in the center of each bead picked from the images recorded before and after exposure. Color change, expressed in  $\Delta R$ ,  $\Delta G$ ,  $\Delta B$  and  $\Delta L$ , was calculated and averaged (3 duplicates of 5 beads per bacteria for a given sensor). A set of quantitative color-change profiles was then constructed by plotting the  $\Delta R$ ,  $\Delta G$ ,  $\Delta B$  and  $\Delta L$  values for each experiment. Standard deviations were determined from the variance in the 15 beads to evaluate the accuracy and reproducibility of the responses.

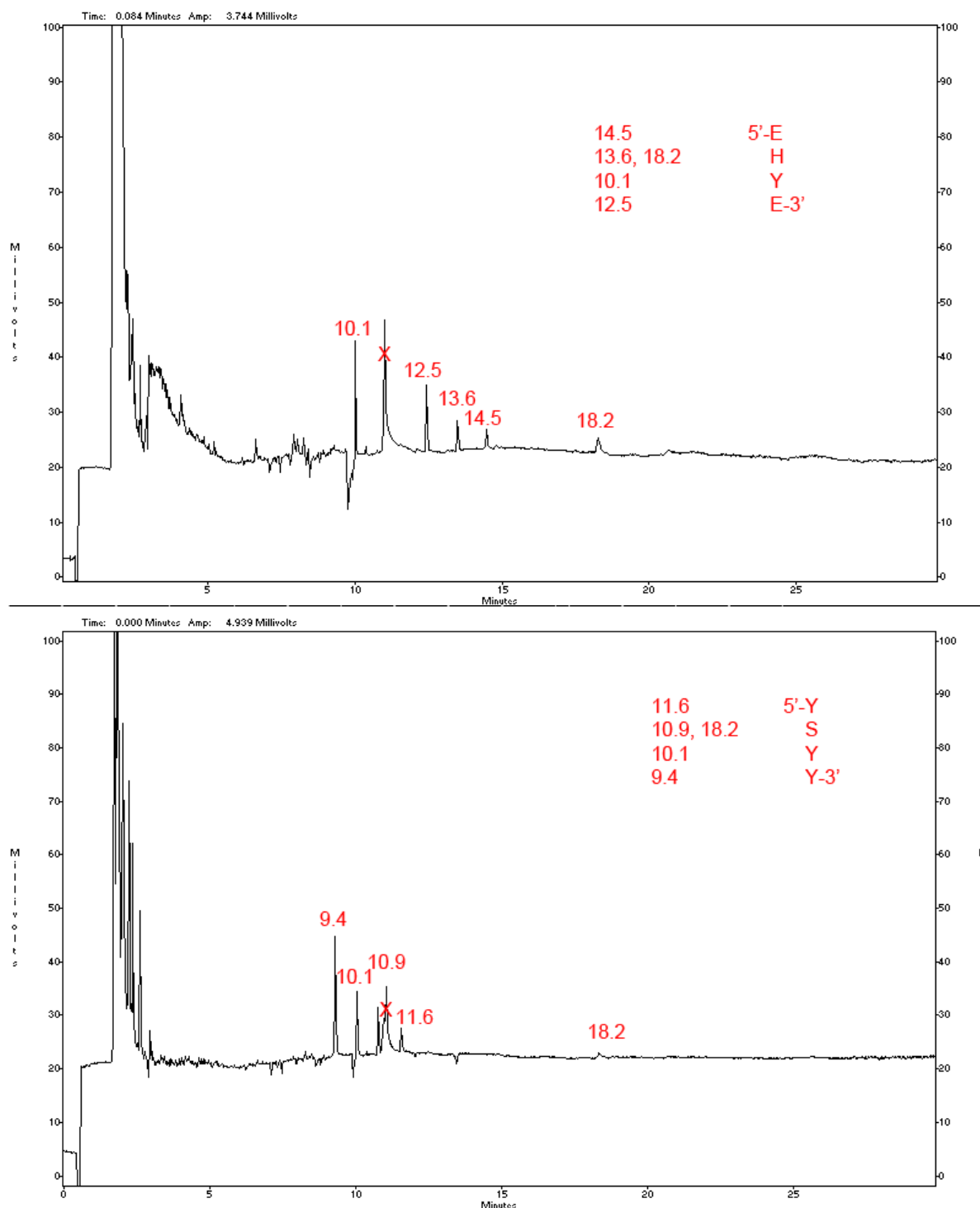
To analyze the response data quantitatively, discriminant analysis (DA) was performed with XLSTAT software (Addinsoft) using the  $\Delta R$ ,  $\Delta G$ ,  $\Delta B$  and  $\Delta L$  values as input. DA, a form of Principal Component Analysis, is a supervised statistical technique to characterize or separate two or more classes of data sets and has been widely used in pattern-based sensing<sup>5</sup>. The analysis generates 2-dimensional canonical score plots by plotting the responses on new (non-Euclidean) axes that represent the most orthogonal components. For each category of the dependent variable, confidence ellipses correspond to a 95% confidence interval for a bivariate normal distribution with the same means and the same covariance matrix as the factor scores. Another important feature of DA is the cross-validation of the classification by a leave-one-out approach which is done by randomly selecting 33% of the training set, so as to verify the predictive ability of a given sensor array.<sup>6</sup>



**Figure S1.** Graphical illustration of the screening procedure for selecting responsive sequences from the ODF library.



**Figure S2.** Fluorescence images of the ODF library before and after exposure to *M. tuberculosis*: (a) blank (equilibrated with culture agar alone); (b) after 30 min exposure to *M. tuberculosis* in a closed Petri dish; (c) inverted image of the blank; (d) difference image by blending images (b) and (c) at 50% transparency. The difference image allows subtle changes from before and after exposure to become apparent. In the difference image (d), 50% gray represents no change after exposure; beads lighter than 50% gray (e.g. near white arrow) indicate a lighting-up response, while darker represents a quenching response (black arrow). Colors represent a combination of the original bead color and shifts in emission wavelength that occur upon exposure (pink arrow). Scale: beads are 130 microns in diameter.

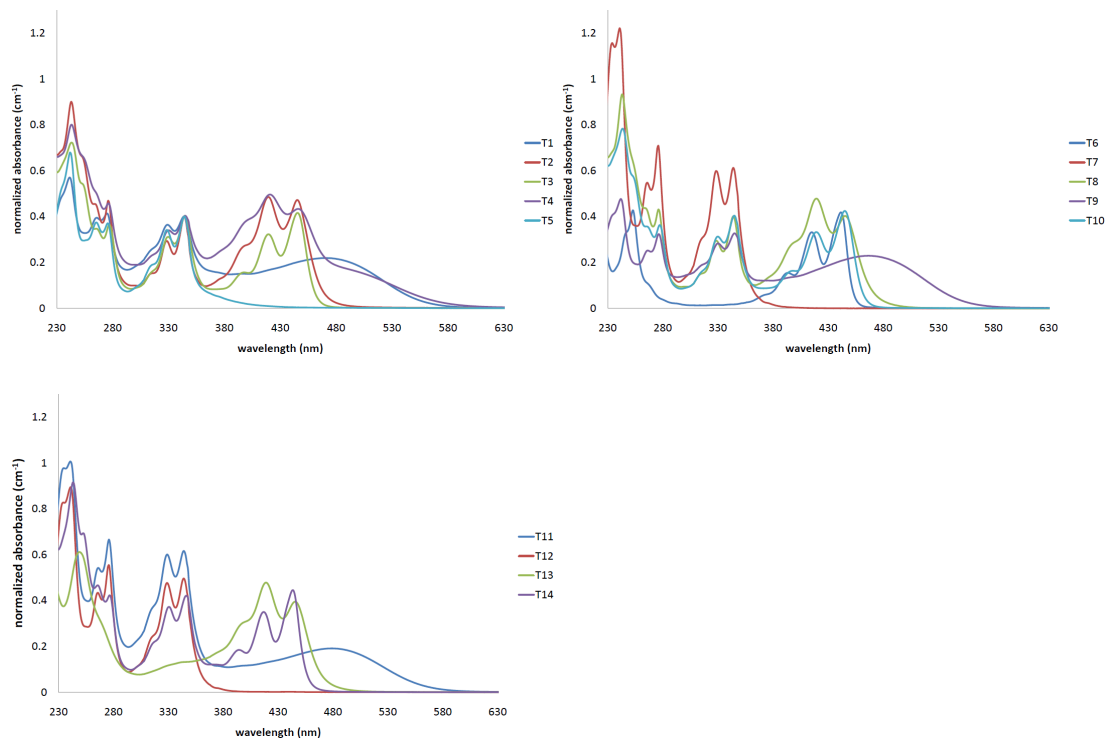


**Figure S3.** Sequencing of ODFs selected from library. Shown are two examples of chromatographic traces (by EC-GC) of tags oxidatively cleaved from single beads (upper: 5'-EHYE (T2); bottom: 5'-YSYY (T7)).

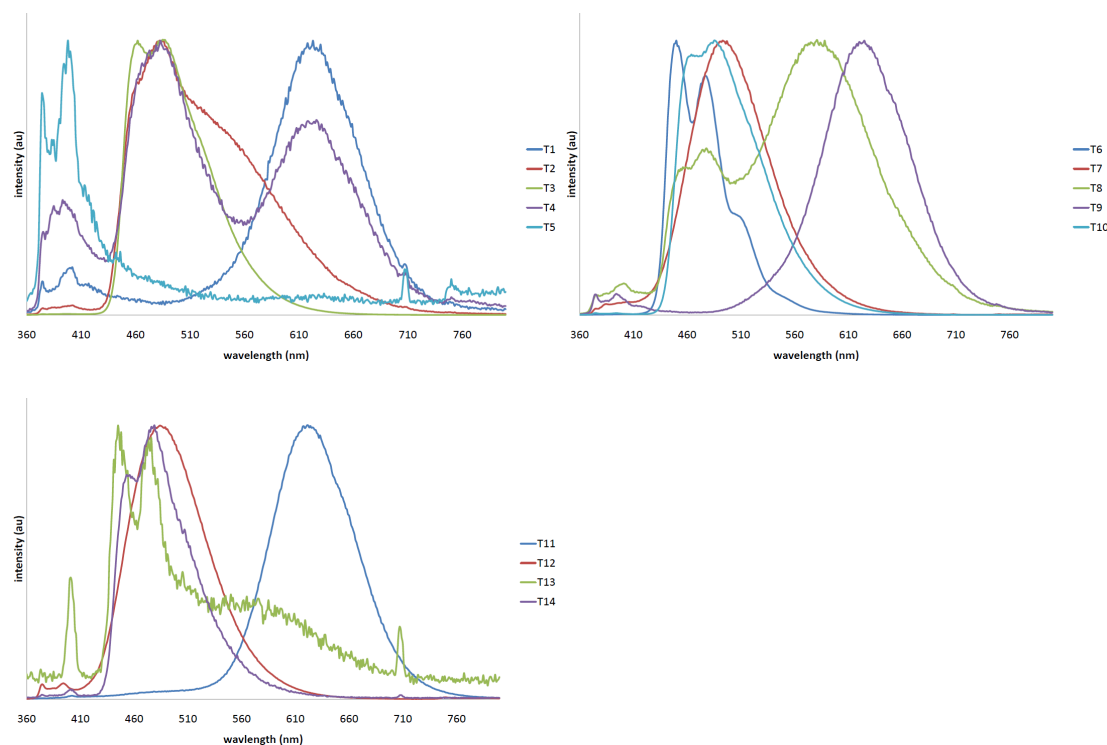
**Table S1.** MALDI-MS characterization of re-synthesized ODF sensors in this study.

	<b>Sequence</b>	<b>Calculated Mass</b>	<b>Observed Mass</b>
<b>T1</b>	5'-YSKI-3'	1431.137	1429.108
<b>T2</b>	5'-EHYE-3'	1566.340	1565.385
<b>T3</b>	5'-HYES-3'	1316.046	1314.864
<b>T4</b>	5'-KEEY-3'	1771.595	1770.150
<b>T5</b>	5'-IYSH-3'	1225.883	1224.471
<b>T6</b>	5'-HHES-3'	1240.918	1240.171
<b>T7</b>	5'-YSYY-3'	1342.125	1338.685
<b>T8</b>	5'-YEES-3'	1440.227	1438.876
<b>T9</b>	5'-SYHK-3'	1397.121	1396.624
<b>T10</b>	5'-HSYE-3'	1316.046	1314.991
<b>T11</b>	5'-YYYK-3'	1673.492	1670.474
<b>T12</b>	5'-YSYS-3'	1140.881	1139.057
<b>T13</b>	5'-ISEE-3'	1399.114	1398.994
<b>T14</b>	5'-IYHE-3'	1476.177	1475.704

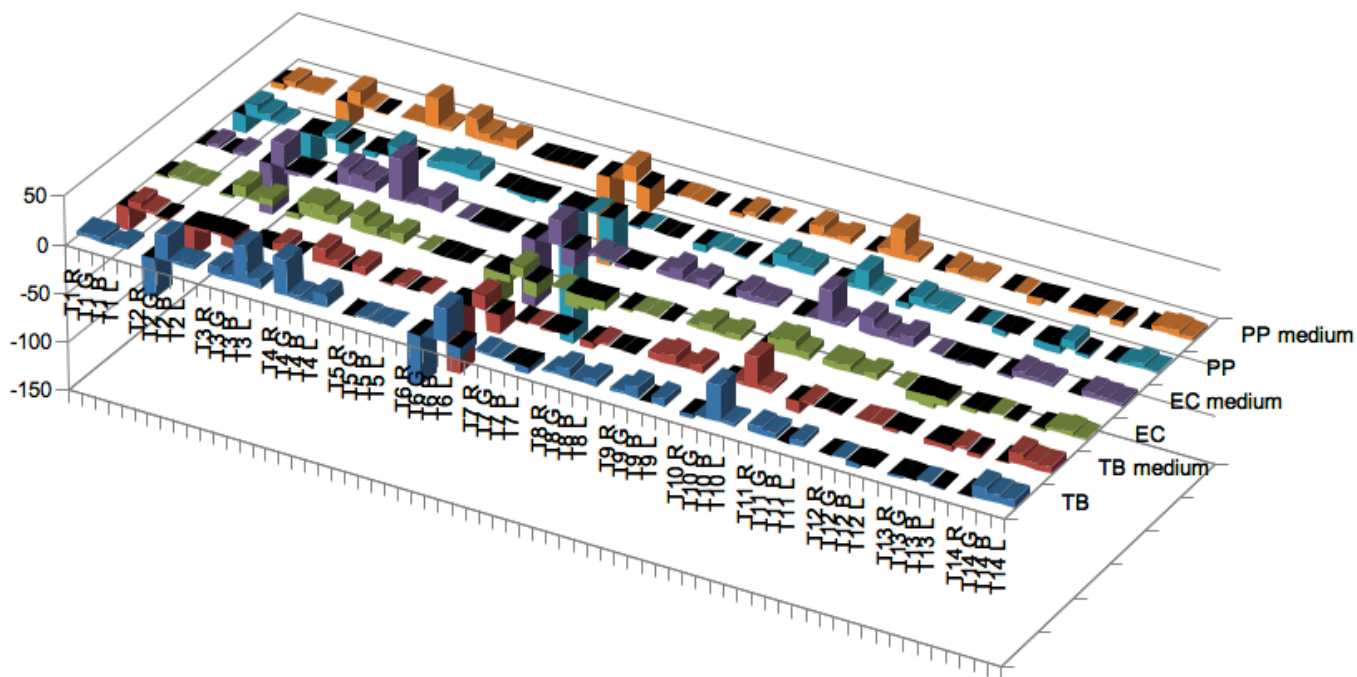




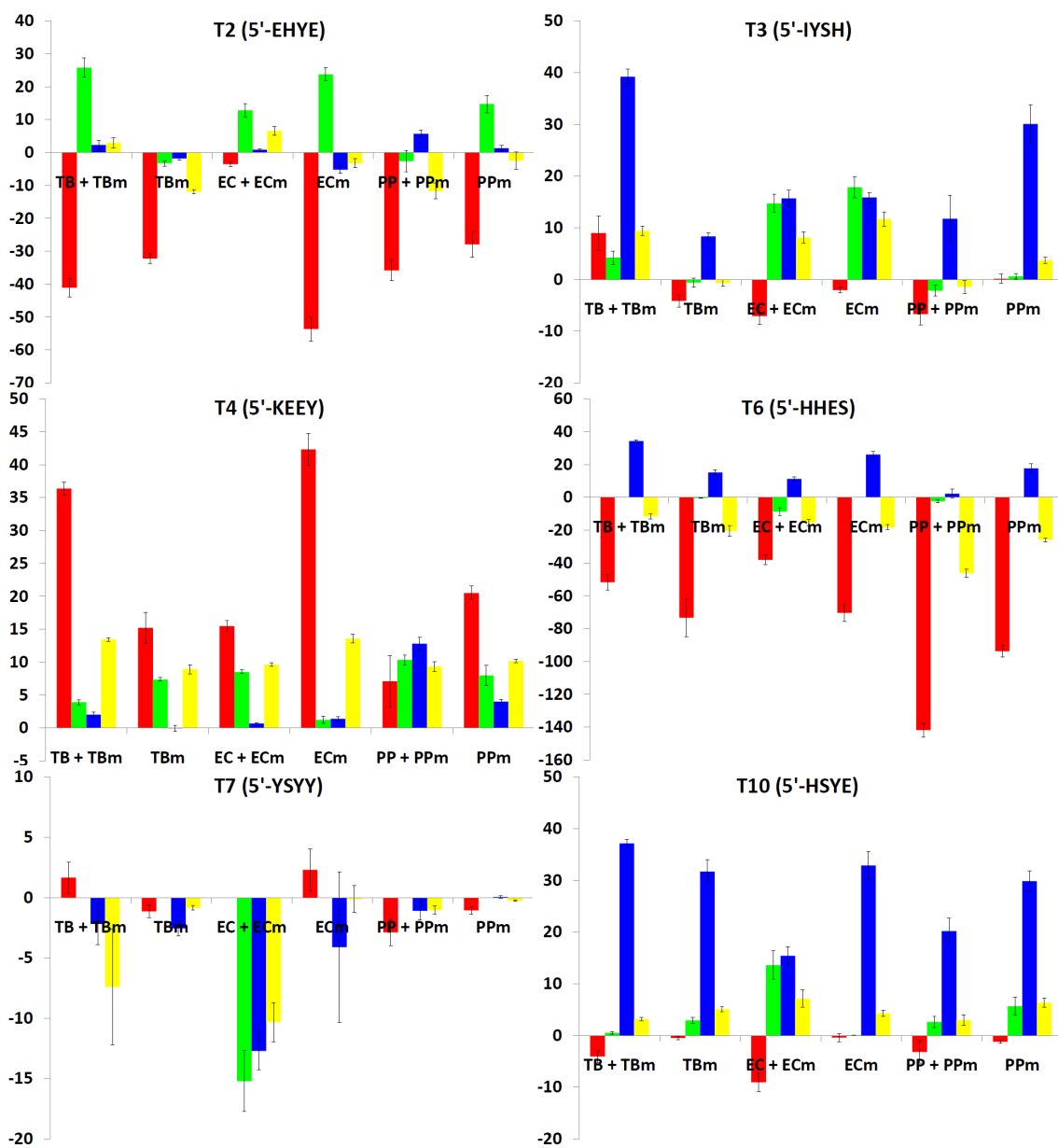
**Figure S4.** Normalized absorption spectra of the 14 ODFs (T1 to T14) from Table S1 in water.



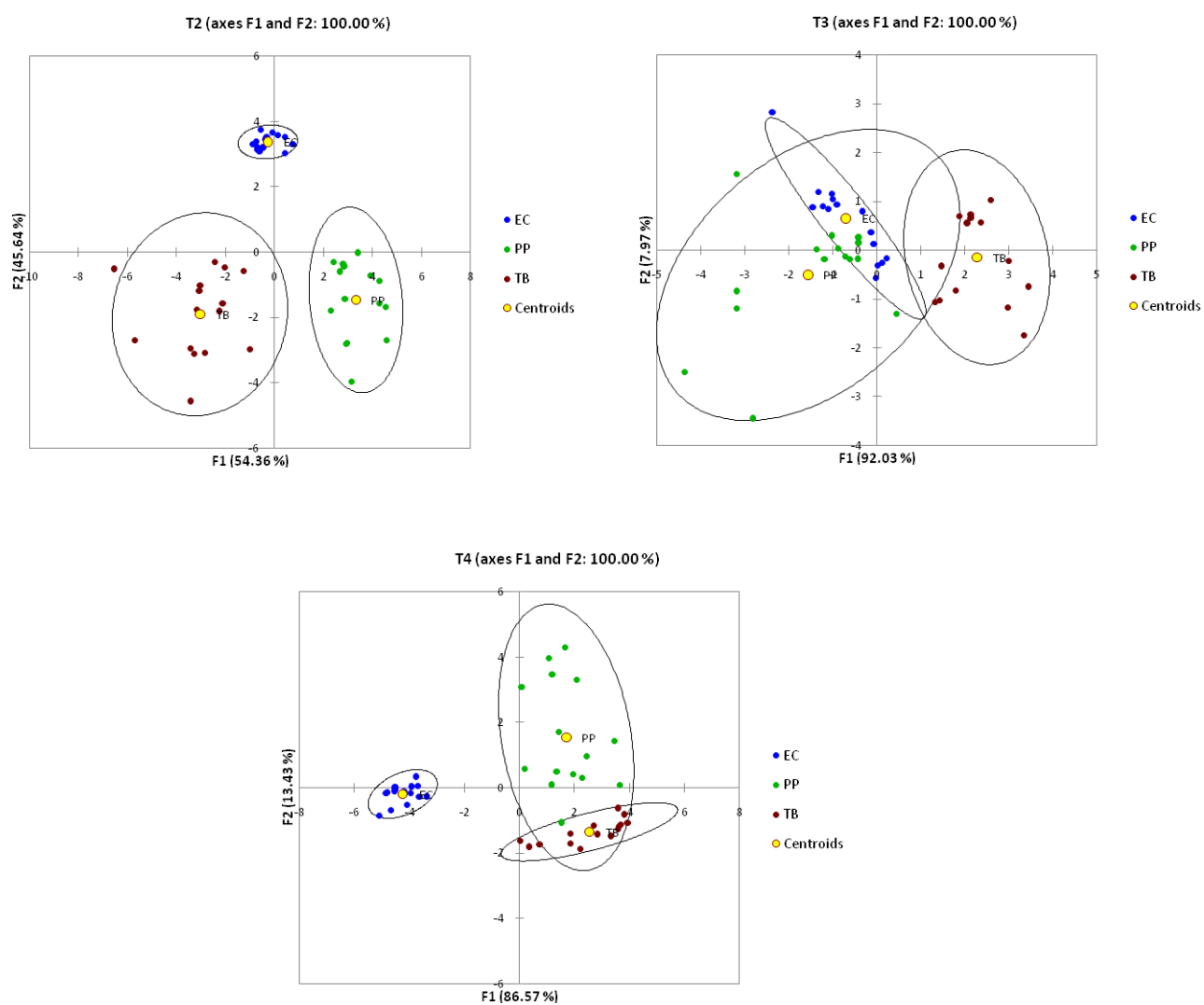
**Figure S5.** Normalized emission spectra of the 14 ODFs (T1 to T14) from Table S1 in water (excitation at 350nm).



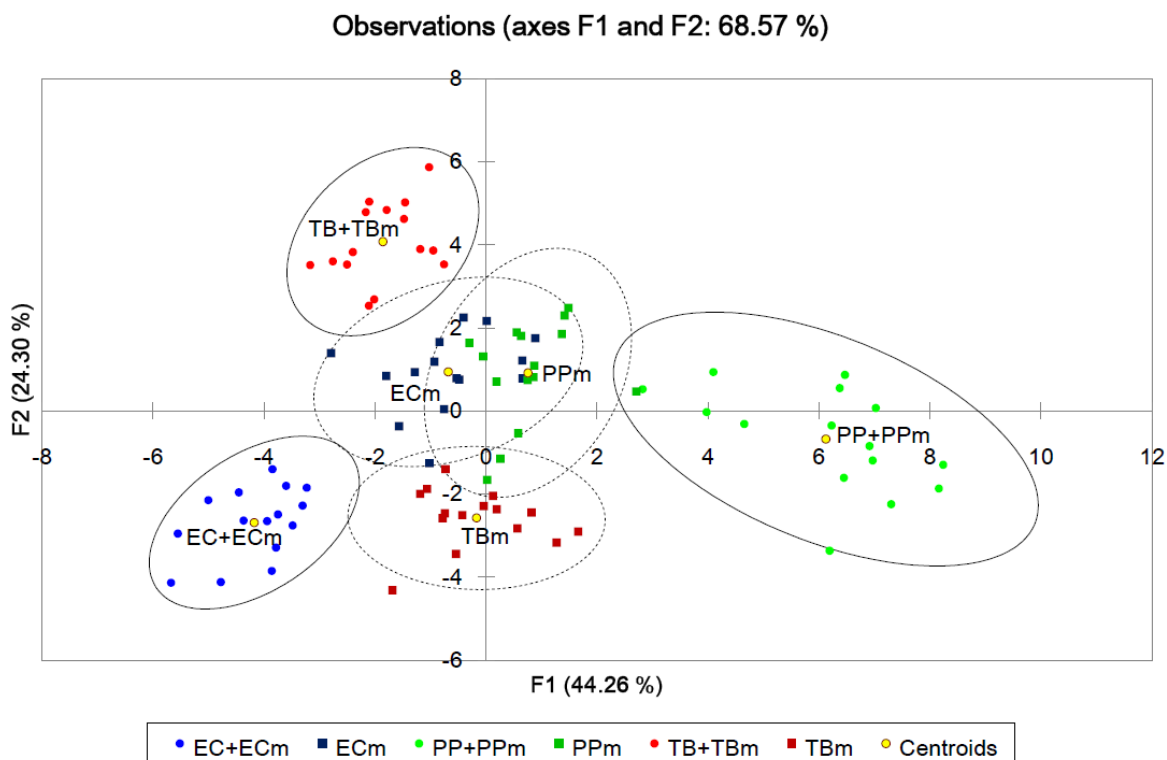
**Figure S6.** Quantitative color-change profiles of all 14 sensor sequences, showing spectral changes upon exposure to the three bacterial species on media and corresponding culture agars alone. Each bar shows the averaged value over 15 sensor beads (from 3 duplicates).



**Figure S7.** Quantitative color-change profiles in  $\Delta R$  (red bar),  $\Delta G$  (green),  $\Delta B$  (blue) and  $\Delta L$  (yellow) of all sensor sequences (T2, T3, T4, T6, T7 and T10) upon exposure to different bacteria (TB+TBm, EC+ECm and PP+PPm) and the corresponding culture medium (TBm, ECm and PPm) for 30 minutes (x-axis: digital difference value,  $\pm 255$  units). Values are averaged from 3 duplicates of at least 5 measurements.



**Figure S8.** Bacteria discrimination as a pattern-based response from single sensors. Shown are individual DA canonical score plots of R/G/B/L responses from T2, T3 and T4 for bacteria with media subtracted. Data for 15 beads (from 3 duplicates) of each type are shown. Ellipses are drawn at 95% confidence level.



**Figure S9.** Scattering data for six analytes (three media plus bacteria; three media alone) as a pattern-based response (enlarged version of Fig. 3 in main paper). Shown is a DA canonical score plot of the R/G/B/L responses from a group of six sensors (T2, T3, T4, T6, T7 and T10). Data for 15 beads (from 3 duplicates) of each type are shown. Ellipses are drawn at 95% confidence level. Note that this is a 2-D representation of multidimensional data.

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