Published in final edited form as:

Chem Commun (Camb). 2011 November 07; 47(41): 11435–11437. doi:10.1039/c1cc14871k.

Fluorescent DNA chemosensors: identification of bacterial species by their volatile metabolites†

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Abstract

Polyfluorophores built on a DNA scaffold (ODFs) were synthesized and tested for fluorescence responses to the volatiles from *M. tuberculosis*, *E. coli* and *P. putida* in closed Petri dishes. Two sensors in a pattern-based response could distinguish the bacterial strains accurately, suggesting the use of ODFs in rapid identification of infectious agents.

Diverse and rapidly evolving bacterial pathogens are of primary concern to human health worldwide. For example, the World Health Organization reports that as much as one-third of the world population is infected with *Mycobacterium tuberculosis*,¹ leading to millions of deaths each year. In a different example, pathogenic strains of *E. coli*, including O104:H4 which caused a widespread disease outbreak in 2011, can cause rapidly lethal infections. To date, culturing and morphological identification remain the common ways of identifying bacteria for clinical diagnosis. DNA detection,² immunoassays³ and mass spectrometry⁴ are also employed but are considerably more expensive and time consuming, and so are not yet generally used to monitor infections around the world. In general, low-cost methods are needed that could identify bacteria rapidly with a minimum of handling and processing.

As an alternative to classical identification methods, the composition of volatile metabolites has recently proven to be useful as a fingerprint of bacterial species.⁵ These small molecules are generally volatile organic compounds (VOCs) including fatty acid esters, alcohols (*e.g.* geosmin, and 2-methylisoborneol), amines, aromatic hydrocarbons (*e.g.* pyrazine) and small sulfur compounds.⁶ In most studies, qualitative metabolite profiling was done by gas chromatography⁷ and/or mass spectrometry⁸ which can require additional sample preparations (*e.g.* pre-concentration by solid-phase extraction) and sophisticated instrumentation. Semiconductor gas sensors have also been employed to distinguish bacterial volatiles but yet additional instruments were needed for conductivity measurements.⁹

†Electronic supplementary information (ESI) available: Detailed experimental methods, characterization data, color-change and scattering plots. See DOI: 10.1039/c1cc14871k

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In contrast, optical sensing offers the possibility of simpler, faster and cheaper alternatives with high robustness. Rotello and Bunz¹⁰ recently reported the use of fluorescent nanoparticle-conjugated polymer sensor arrays for pattern-based identification¹¹ of several bacterial species in solutions. As compared with solution-based experiments, identification of bacteria by direct optical detection of headspace volatile metabolites may be preferred as it avoids the need for sample clean-up and pre-separation, and requires less contact with pathogens. In this light, a very recent study reported the use of absorbance-based changes in spotted pigments to distinguish bacterial types.¹² To date, the possibility of fluorescence sensing of bacterial volatiles has not been tested.

We have recently explored the use of fluorescent DNA as sensors for metal ions, organic vapors and toxic gases.¹³ The choice of DNA as a scaffold for chemosensing offers some useful advantages, including rapid synthesis on an automated instrument and large combinatorial diversity in sequence and properties. In our molecular design, DNA bases are replaced with known aromatic fluorophores (Fig. 1), creating potentially stacked arrays of electronically interacting components (termed ODFs). Association with small molecules can result in strong changes in fluorescence, including quenching, enhancement, and color shifts. Here, we report our initial efforts in testing whether these fluorescent DNA-like compounds can sense and distinguish bacteria by their metabolites in the vapor phase. Three bacteria species, namely *M. tuberculosis*, *E. coli* and *P. putida*, were chosen for this preliminary study.¹⁴ The first two medically important bacteria were mentioned previously; species of the third genus (*Pseudomonas*) are responsible for deaths due to pneumonia and sepsis in children and in patients with cystic fibrosis, and are highly susceptible to drug resistance.¹⁵

Our aim was to evaluate whether ODFs on PEG-polystyrene beads could be used to identify the bacterial species based on the composition of the volatiles released when they were grown on agar plates in closed Petri dishes. Fluorescence was monitored under an epifluorescence microscope (excitation at 340–380 nm, with all visible emission observed (long-pass filter, > 420 nm)). The responses were recorded by taking images before and after exposure for 30 min at room temperature. Any spectral changes (red, green and blue wavelengths) are converted into numerical expressions (R, G and B) as well as overall luminosity (L) on a standard ± 255 -unit RGB scale.

To find sequences of ODFs that could respond to vapor-phase metabolites, we constructed an ODF library as previously reported¹⁶ containing fluorophores, spacers and a hydrogen bonding element. The 7⁴-member library was first screened for useful sequences that gave the strongest responses. 14 sequences (**T1–T14**) were identified, sequenced and re-synthesized for characterization and cross-screening (see ESI[†]). These re-synthesized sequences were purified by reverse-phase HPLC and characterized by MALDI-MS, UV-vis absorption and fluorescence emission spectroscopy.

Full cross-screening of **T1–T14** on beads was performed with the three bacterial species grown on three different agar media. Plates with media alone were also tested as

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backgrounds to be subtracted to yield responses to bacteria alone. The differential spectral responses of six selected sequences are depicted as color-change plots in Fig. 2 (see ESI for all results[†]). These subtracted profiles reflect the actual spectral changes in response only to the headspace volatile metabolites. Significantly, a wide diversity of responses, from lighting up to quenching as well as color shifts, is seen. Simple visual inspection of the responses shows that a number of single sensors can be used qualitatively to identify bacteria. For example, **T3** (5'-**IYSH**) gives a light-up response in the blue with *M. tuberculosis* but a decrease in blue and red for *P. putida* relative to medium alone. In another example, **T2** (5'-**EHYE**) gives a light-up response in the green channel, an increase in the red channel, and moderate quenching for *M. tuberculosis*, *E. coli*, and *P. putida*, respectively.

To evaluate the discriminating power of the selected sensors quantitatively, discriminant analysis (DA, a form of Principal Component Analysis)¹⁷ was performed with **XLSTAT** software (Addinsoft). DA is a classical statistical technique that evaluates combinations of variables in multidimensional data sets and then characterizes groupings of objects (classification) within the sets.¹⁸ The analysis was carried out on the six types of analytes: three bacteria plus media, and three media alone (Fig. 3). Results showed that a combination of six sensors was able to clearly separate the responses in the bacterial cultures from those of the media alone despite a large excess mass of media. Not surprisingly, the three media responses overlapped, as they share many components in common.

We found that much clearer separation of the bacterial data was achieved by subtracting media responses prior to the DA analysis. The analysis of background-subtracted responses revealed that highly effective identification of bacteria could be achieved by a pair of sensors. Results for the two most effective sensor pairs (**T2** & **T3**; **T2** & **T4**) are shown in Fig. 4. From the resulting canonical score plots, all three bacterial species occupy their own space in the plot with good separation as indicated by the confidence ellipses drawn at 95% confidence level. The analysis also revealed a correct classification rate of 100% for each of the three bacteria tested in this study. Cross-validation (by the leave-one-out approach)¹⁹ confirmed that over 95% of the single measurements could be classified correctly; of these **TB** was 100% correctly classified.

It has been reported that the headspace concentrations of different VOCs released from various bacteria range from 300–50 000 ppb.²⁰ This fits well with previous findings that ODF sensors gave reproducible responses to selected VOCs at least down to low ppm levels.²¹ More studies will be needed to evaluate how few bacteria can be detected by this approach.

Interaction of the ODF oligomers with the volatile metabolites likely occurs by multiple mechanisms. Electrostatic attractions to the bases and backbones of the ODFs are possible, as are van der Waals interactions with low-polarity compounds. The planar hydrocarbons pyrene (Y) and perylene (E) provide excellent platforms for stacking interactions while dihydrothymidine (H) can act as both donor and acceptor for hydrogen bonds. The detection may be aided by the lipophilic polystyrene solid support which likely enhances local concentrations of metabolites, as slight swelling of the beads was observed.

In summary, our experiments have established the use of fluorescent ODFs for identifying bacterial species based on their optical response patterns towards the volatile metabolites released. The solid-supported ODF sensors are sensitive enough to respond to headspace VOCs emitted inside standard closed Petri dishes. The approach is non-invasive and simple: no pre-separation of bacteria or pre-concentration of gaseous metabolites are required, minimizing handling and lowering the risk of contamination. Furthermore, multiple culture media can be used with simple data subtraction; this is important because some bacterial species (*e.g. M. tuberculosis*) require specific media for culturing. We speculate that such chemosensors might be developed into reliable, inexpensive and fast reporters for early identification of bacteria from patient samples and from other medically important sources such as contaminated food.

Acknowledgments

This work was supported by the U.S. National Institutes of Health (GM067201). C.K.K. acknowledges the Croucher Foundation for a postdoctoral fellowship, and F.S. was supported by the Swiss National Science Foundation.

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Fig. 1.

Structures of fluorescent monomers and DNA-like sensors in this study. Monomer nucleosides are shown with their one-letter abbreviations. Sensors are tetramers (5'-EHYE is shown as an example) attached by an amide linkage to PEG-polystyrene beads.

Fig. 2.

Responses of ODF sensors to headspace volatile metabolites released by bacteria after subtraction of backgrounds from growth media. Shown are differential color-change profiles of **T2**, **T3**, **T4**, **T6**, **T7** and **T10** upon exposure to the three bacterial species (*E. coli* (EC), *P. putida* (PP), and *M. tuberculosis* (TB)) after subtracting the signals from those with the corresponding culture agars alone. Red, green, blue bars represent changes in R/G/B (on a scale of ± 255); Yellow represents change in Luma.

Fig. 3. Discrimination of six types of specimens (three media (denoted by lowercase m) and three media plus bacteria) as a pattern-based response. 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.

Fig. 4. DA canonical score plots and cross-validations for the spectral responses to the three bacteria species from two selected sensor pairs: (A) **T2 & T3**; (B) **T2 & T4**. Scattering data for individual sequences are shown in the ESI[†].