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Fingerprinting antibiotics with PAE-based fluorescent sensor arrays†

Jinsong Han^a, Benhua Wang^a, Markus Bender^a, Jessica Pfisterer^a, Wei Huang^a, Kai Seehafer^a, Mahdiah Yazdani^b, Vincent M. Rotello^b, Caren M. Rotello^c, and Uwe H. F. Bunz^{a,d}

^aOrganisch-Chemisches Institut, Ruprecht-Karls-Universität Heidelberg, Im Neuenheimer Feld 270, 69120 Heidelberg, Germany

^bDepartment of Chemistry, University of Massachusetts Amherst, 710 North Pleasant Street, Amherst, Massachusetts 01003, USA

^cDepartment of Psychology, University of Massachusetts Amherst, 710 N. Pleasant Street, Amherst, Massachusetts 01003, USA

^dCAM, Centre for Advanced Materials, Ruprecht-Karls-Universität Heidelberg, Im Neuenheimer Feld 225, 69120 Heidelberg, Germany

Abstract

We outline an evolution process for tongue elements composed of poly(*p*-aryleneethynylene)s (PAE) and detergents, resulting in a chemical tongue (24 elements) that discerns antibiotics. Cross-breeding of this new tongue with tongue elements that consist of simple poly(*p*-phenyleneethynylene)s (PPE) at different pH-values leads to an enlarged sensor array, composed of 30 elements. This tongue was pruned, employing principal component analysis. We find that a filial tongue featuring three elements from each original array (i.e. a six element tongue) is superior to either of the prior tongues and the composite tongue in the discrimination of structurally different antibiotics. Such a selection process should be general and give an idea how to successfully generate powerful low-selectivity sensor elements and configure them into discriminative chemical tongues.

Graphical Abstract

†Electronic Supplementary Information (ESI) available: General information, synthetic details and analytical data of the used polymers, screening and optimization process, LDA and PCA data, and NMR spectra. See DOI: 10.1039/x0xx00000x

Correspondence to: Uwe H. F. Bunz.

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Introduction

We describe the evolution of an efficient six-element, fluorescence-based optoelectronic tongue that discriminates antibiotics. This superior “filial tongue” results from combination of two starting tongues, followed by productive pruning of non-performing elements.

Sensing, detecting and discriminating of simple but also of complex analytes is an ever attractive and important issue for quality control of food,^{1,2} beverages³⁻⁷ and drugs;⁸⁻¹⁰ it is also critical for detecting fake malaria tablets,¹¹ and generally adulteration of prescription drugs. Antibiotics are among the most successful drugs used for human therapy.

A promising approach for the discrimination of complex (or simple) analytes are chemical tongues. These consist of 3-50 different sensor elements that are exposed towards an analyte of choice. Optical changes (color, fluorescence wavelength, or intensity etc.) are recorded, and the formed pattern is analyzed by statistical methods, including multivariate analysis of variance (MANOVA),^{12,13} principal component analysis (PCA), or linear discriminant analysis (LDA).¹⁴ The discrimination rests in the uniqueness of the formed pattern and not in the response of a single sensing element, which might display a rather low selectivity for any given analyte.

An important and not well understood aspect of this approach are the principles that guide the construction of such tongues, including what would be the minimum number of necessary tongue elements to identify a specific analyte or sample. In most of these problems, the classic issue of sensitivity is inoperative, as the analytes or samples for quality control are available on multi-gram or at least on a multi-100-mg scale. That is for sure true for (alcoholic) beverages and food-stuffs, but mostly also for prescription and non-prescription drugs.

Which concepts are currently available for the construction of successful tongues? 1) General-poorly fitting receptors that interact with the analytes of choice. This elegant concept, developed by Anslyn *et al.* as a variation of Fischer's lock-and-key principle,¹⁵⁻¹⁹ discriminates a variety of analytes with tongue elements of suitable shape/cavity/binding characteristic. 2) Suslick *et al.* developed a colorimetric assay, in which chemically different types of dyes (typically 16-36) are printed on a substrate and exposed towards gaseous or solution-phase analytes. Suslick stresses, that the chemical diversity of the elements of his tongue or nose (he calls the process smell-seeing) are critical for the success of the concept.²⁰⁻²³ 3) Rotello *et al.* discovered that binary complexes of positively charged gold nanoparticles and negatively charged conjugated polymers of the poly(*para*-

phenyleneethynylene) (PPE) type make for powerful chemical tongues that discriminate proteins, bacteria, but also cells and cell lysates.²⁴⁻²⁷ The functionalized gold nanoparticle is the protein-like recognition element but also a powerful quencher of the PPEs' fluorescence. Addition of the analytes releases the gold nanoparticle, and PPE fluorescence turn-on is observed. Yet, Bunz and Rotello found also that a library of simple, charged PPEs alone discriminates proteins, a critical discovery.²⁸

The above concepts state rules *sufficient* for the construction of tongues; do these rules formulate conditions that are *necessary* for the construction of a successful tongue? We found simple, ionic, PPE-based chemical tongues without any discernable sensory properties to recognize useful analytes. A small PPE-based tongue easily discriminates white wines⁷ but also aliphatic and aromatic acids.^{29,30} An important aspect of this approach is the combination of different tongue elements into new complexes that work as sensor elements. Additionally, the change of the pH-value empowers one PPE to act in several independent sensor elements with modulated responses. Complex formation and pH-control are powerful yet simple strategies as they do not entail the (work intensive) synthesis of new tongue elements. As a consequence, an efficient approach towards development of tongues will include complexation and pH-changes. The modulation of the inherent fluorescence response by (commercially available) adjuvants such as cationic or anionic surfactants should also modulate the fluorescence response of tongue elements towards analytes. In this contribution we discriminate 19 different antibiotics (seven different families) as test-bed to train and develop our tongues; antibiotics belong to different structure types for the different families, yet are structurally similar within their families, an ideal test bed. There are aromatic (sulfonamides, quinolones, tetracyclines) antibiotics, then, antibiotics that have at least one aromatic substituent (β -lactams) and sugar-based antibiotics, such as the macrolides and the aminoglycosides. The desired antibiotic-sensitive tongue could help to uncover potential drug fraud or falsification, as the price differences in penicillin can reach a factor of >300 per prescribed unit (amoxicillin as tablet is cheap, vs. penicillin G-benzathin complex as injectable solution); that, even though the penicillin G-benzathin complex is not patent-protected anymore. A working optical tongue for antibiotics is also of potential interest if one wishes to perform quality and activity control of these antibiotics as tablet or any other formulation, some of which are quite sensitive towards degradation.

Results and discussion

Fig. 1 shows the selection of the four PAEs employed in the construction of tongue #1. Their fluorescence quantum yield is fairly low. As surfactants (additives) we employed cetyltrimethylammoniumchloride (**CTMA**) and sodium dodecylbenzenesulfonate (**SDBS**). The analytes consist of seven families of commercially available antibiotics. Of each type two or three examples, structurally similar to one another, were selected as member of the analyte pool for discrimination (Fig. 2). Fig. 3 highlights the construction of the fluorescent chemical tongues. Tongue #1 was prepared by treating the almost non-fluorescent solutions of the PAEs **P1-P4** with counter charged surfactants; a significant fluorescence increase is observed. Fig. 4a shows an example titration of **P1** ($c = 2 \mu\text{M}$) with **CTMA** at different pH values. Upon addition of the 100-fold amount of the **CTMA** at pH 7 ($c = 200 \mu\text{M}$), below the critical micelle concentration (CMC) of **CTMA** (1.85 mM), the fluorescence intensity of **P1**

increased by a factor of 16. The increase is observed at pH 3, pH 7 and pH 13, even though the end quantum yields are lower, particularly when working at pH 3. That is not surprising, as the carboxylate units of **P1** must be protonated at pH 3, and the positively charged **CTMA** can not interact as strongly with the carboxylic acid as it does with the carboxylate.

For the other polymers, **P2-P4** (at pH 3, pH 7, and pH 13) a similar increase in fluorescence intensity observed (details see the ESI†). **P4**'s fluorescence quantum yield is vanishingly small in aqueous solution at pH 13. Upon addition of a 100 fold excess of the **SDBS**, the quantum yield is significant. Surfactochromic behavior, an effect described by Lavigne *et al.*, is operative.³¹ In the following experiments we treated the surfactant-PAE-complexes with the different antibiotics (**AT1-AT19**, 5 mM) and obtained a response pattern (see Fig. S2 in the ESI†). We measured the fluorescence intensity upon addition of the analytes at two different wavelengths (463 and 503 nm for **C1**, 470 and 505 nm for **C2**, 533 and 565 nm for **C3**, 531 and 569 nm for **C4**, typical example see Fig. 4b), as the addition of the 5 mM solution of the antibiotics does not only modulate the fluorescence intensity but also has ratiometric elements. Differential quenching results at different wavelengths. We employed a second tongue (details see the ESI†), consisting of **P5** and its complex **C5** at three different pH-values, a simple six-element control tongue that does not have any surfactants added. Similar fluorescence responses result for structurally similar antibiotics within each family. Especially for tetracyclines (**AT9-AT11**), strong fluorescence quenching was found for *all* of the sensor elements (**S1-S30**). That is reasonable because the extended aromatic system makes these species yellow and non-fluorescent in water and quenched the fluorescence of *all* of the sensor polymers. The raw fluorescence intensity change data were evaluated by the statistical method of linear discriminant analysis (LDA) and by principal component analysis (PCA, see Fig. S7 in the ESI†). Both methods are widely used for the workup of data from sensor-fields. Fig. 5 (top) shows the LDA plots of the two different tongues.

Depicted in grey is the control, i.e. if only water is added as analyte. Either of the two tongues is reasonably well capable of discriminating the antibiotics, even though they result in different LDA-plots. Surprisingly, the quality of the separation and discrimination does not change much upon the combination of the two different tongues into a larger tongue containing 30 elements. We performed principal component analysis (see Fig. S7 in the ESI†) on the data and also find a reasonable separation with the single tongues but also with the combined tongue, even though the result seems more like the one gleaned from the first tongue; both PCA and LDA work well.

Contrary to LDA, PCA allows analysis of the discriminating factors, which in this heterogeneous yet well-defined analyte library does not correspond to an easily explainable physicochemical property. Some of the sensor elements are much better at discriminating the analytes than others. The fluorescence response of the antibiotic analytes towards 24 sensing elements (**S1-S24**, tongue #1) was evaluated using PCA (see Fig. S8 in the ESI†); the first three principal components (PC1-PC3) represent 74.4% (43.8%+18.7%+11.9%) of the total variance. For each principal component (PC1-PC3), **S12** contributes the most to PC1, **S18**

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contributes the most to PC2, and **S2** contributes the most to PC3. Thus, **S2**, **S12** and **S18** of the new tongue are the most responsive elements. Similarly, for tongue #2 (**S25-S30**, see Fig. S8 in the ESI†), PCA was applied, **S25**, **S28** and **S29** make the most contribution to the first three PCs, respectively, which are also selected into the new, pruned tongue #2.

Both pruned tongues give a somewhat reasonable discrimination, tongue #2 more so than tongue #1. Once we performed data analysis (PCA) with the six best elements from both parental tongues we see (Fig. 6) that all of the antibiotics are discriminated. When the same data are processed using LDA, the result is a bit different (Fig. 6c). The penicillins and the sulfonamides are not well separated, particularly amoxicillin and sulfacetamide are almost non-separable, and sulfaguanidine is in the area where one would expect penicillins. PCA resolves the data. The pruned tongue is better than the tongue in which *all* elements of both of the original tongues are present. Removal of the low responding sensor elements improves the quality of the overall tongue by weeding out elements that contribute to the noise but not to the signal. Which of the elements are most successful for the construction of the pruned tongue? From tongue #1 **S2** (**P1** complexed with **CTMA**, pH 3), **S12** (**P2** complexed with **SDBS**, pH 13) and **S18** (**P3** complexed with **CTMA**, pH 13). From the tongue#2 **S25** (**P5**, pH3), **S28** (**P5**, pH 13) and **S29** (**C5** from **P5/P6**, pH 7) are the elements with the most discriminatory power. We observe that the anionic polymers unfold their discriminatory prowess at strongly basic conditions. Under those conditions some of the analytes might be not stable but hydrolyze, such as the lactam antibiotics. That however is not an issue; the hydrolyzed species are discriminated. As we have no problems with reproducibility, the hydrolysis is either very fast or too slow to interfere with the measurements.

Based on the successful selection process of pruned tongue #4 and positive results of antibiotics discrimination with such sensor array, we further carried out a semi-quantitative assay to identify antibiotics with various concentrations (from 0.05 mM to 5 mM). The fluorescence modulation data of **AT11**, **AT12** and **AT15** were recorded and calculated with LDA, which converts the training matrix (6 factors $\times$ 7 concentrations $\times$ 3 replicates) into canonical scores. The first three canonical factors represent 93% of the total variation. The jackknifed classification matrix with cross-validation reveals 100% accuracy. As shown in Fig. 7, the concentration is linearly mapped in the LDA plot, clear discrimination dependence on the concentration of **AT11**, **AT12** and **AT14** were observed. The results suggesting that the array should allow for a rigorous quantitative detection.

So far, we have established different tongues (tongue #1 with 24 sensing elements; tongue #2 with 6 sensing elements; tongue #3, combination of tongue #1 and #2; and tongue #4, the most responsive elements of tongue #1 and #2), each of which generates unique responses for the studied antibiotics.

Effectively, combination of these responses in each data set (i.e order of entry into the data matrix) represents the structure of data used to generate the desired classifications. Within each tongue, a large number of unique responses (i.e., diverse data orderings) are possible.³² The small number of replicates in these data sets, coupled with the possibility of having large distortions caused by potential outliers and different data orders, raises questions about

the robustness of the LDA classification results. Bootstrapping³³ is a statistical re-sampling method that can be used to explore these concerns by measuring the variability of the LDA solution spaces. In bootstrapping analyses, each data set is randomly sampled (with replacement) numerous times; each resulting sample is treated as another data set that could reasonably be obtained in the experiment. Overall, this statistical technique provides insights into the robustness of the LDA results from different combinations of observations in the data set.

An analysis of 20,000 stratified bootstrapped samples was conducted for each separate tongue. With stratified sampling, each sample has the same size as the original data, as well as the same number of samples within each training class. For each bootstrapped sample, the best-fitting LDA solution was obtained and the proportion of correctly identified unknowns (CIU) was calculated using a specially written R script. When identifying the unknowns, we used only the first three discriminants because they account for more than 95% of the variance in our original data sets. Fig. 8 shows the histograms of the classification accuracies of the unknowns across the 20,000 bootstrapped samples for each different tongue. The red line represents the CIU for the original data set and the blue lines represent the CIUs of the 2.5th and 97.5th percentile for which 95% of the bootstrapped data is covered. These values have been tabulated in Table 1. The bootstrapping results reveal that the accuracy of unknown identification is highly dependent on the structure of the training set. In effect, bootstrapped data sets can be obtained across all tongues with substantial variability in the CIU values. The probability of obtaining specific CIU values varies across the 20,000 bootstrapped samples, as shown by the heights of the bars in Fig. 8. Therefore, the bars with the highest density reflect the most frequent outcomes of the system and thus they can provide a test bed for recognizing the most reliable and consistent combinations. Accordingly, original CIU values that fall in high-density regions of the histogram are results similar to those that would be expected in replication studies; original CIU values that fall in low-density regions would not necessarily replicate. The effect of noise in the measurements is to increase the range of possible CIU values, resulting in wide histograms. Overall, this strategy could be considered as a potential route for substantially improving the classification performance reliability of array-based sensors.

Conclusions

Simple surfactants modulate and increase the fluorescence of ionic PAEs and PPEs. The formed constructs are sensor elements for opto-electronic tongues and discriminate antibiotics. Important is a) six different polymers create a library of 30 different elements. Changing the investigated emission wavelength, the pH-value, and the addition of oppositely charged surfactants modulates the response of the sensor elements into an efficient tongue. b) Using PCA, the six most important contributing elements were selected to give a pruned filial tongue with an improved overall response towards all of the investigated antibiotics.

Quo vadis lingua optoelectronica?

Manipulation and modulation of the response of tongue elements reaches far beyond changes in chemical structure and sequence of the employed polymers. Changes of pH,

observation wavelength, and addition of surfactants modulate the response of the sensor elements towards analytes, here, antibiotics. The “naive” tongue, i.e. one where the polymers **P1-P4** are employed at physiological pH displays large error bars and does not reliably discriminate the antibiotics (see Fig. S11 in the ESI[†]); *modulation unlocks the full potential of the sensor elements*. We have only started to scratch at the surface of a multidimensional space, where observation wavelength, temperature, pressure, pH, simple additives and change of solvents and/or a combination of all of the above render small libraries of conjugated polymers all-powerful and omni-capable of discerning and discriminating any analyte available in more than mg-quantities. Questions of sparse data and big data as well as data processing are increasingly critical to answer the question of the definition of minimally necessary structural changes of the sensor elements to discriminate analytes. Prediction of the pattern observed in LDA is currently not possible, and the axes of variation cannot be attributed to simple properties (electrostatic interactions + hydrogen bonding + hydrophobicity + nucleophilicity + π - π stacking + ... + ...) that are operative. Consequently, construction of suitable minimalist tongues is purely empirical. When larger data amounts are amassed and different concepts are explored, further analysis shall allow to formulate rules for construction of these highly interesting and ultimately powerful optoelectronic tongues.

Experimental

Materials

Polymers **P1**,³⁰ **P2**,³⁴ **P5**³⁰ and **P6**³⁰ were synthesized according to previously reported procedures. Chemicals were purchased from commercial laboratory suppliers. Reagents were used without further purification unless otherwise noted. Solvents were purchased from commercial laboratory suppliers and if necessary distilled prior use. Absolute solvents were dried by a MB SPS-800 using drying columns. For dialysis regenerated cellulose tubular membranes (ZelluTrans, Carl Roth[®]) with a molecular weight cut-off of 3500 Da were used against deionized (DI) water.

Measurements

¹H- and ¹³C-NMR spectra were recorded at room temperature on the following spectrometers: Bruker Avance III 300 MHz, Bruker Avance III 400 MHz, Bruker Avance III 500 MHz and Bruker Avance III 600 MHz. The data were interpreted in first order spectra. The spectra were recorded in CDCl₃, D₂O or DMSO-d₆ as indicated in each case. Chemical shifts are reported in δ units relative to the solvent residual peak or TMS. High resolution mass spectra (HR-MS) were either recorded on a Bruker ApexQehybrid 9.4 T FT-ICR-MS (ESI+, DART+), a Finnigan LCQ (ESI+) or a JEOL JMS-700 (EI+) mass spectrometer. IR spectra were recorded on a JASCO FT/IR-4100. Substances were applied as solid. The obtained data was processed with the software JASCO Spectra Manager[™] II. Quantum yields (ϕ) were measured by using the comparative method with quinine sulfate in 0.1 N sulfuric acid as a reference ($\phi = 0.54$, the average values of three measurements were calculated for each sample. Gel permeation chromatography (GPC): Number- (M_n) and weight-average (M_w) molecular weights and polydispersities (PDI, M_w/M_n) were determined by GPC versus polystyrene standards. Measurements were carried out at room

temperature in CDCl₃ or THF with PSS-SDV columns (8.0 mm × 30.0 mm, 5 μm particles, 10²-, 10³- and 10⁵- Å pore size) on a Jasco PU-2050 GPC unit equipped with a Jasco UV-2075 UV- and a Jasco RI-2031 RI-detector.

Synthesis of P3 and P4

Polymer synthesis of the neutral precursor was achieved through standard Sonogashira cross-coupling reaction with the following general procedure: The two monomers (details see ESI†) were dissolved in a solvent mixture consisting of degassed THF/CHCl₃/NEt₃ (0.1 M). Pd(PPh₃)₄ (2 mol%) and CuI (4 mol%) were added as catalyst system and the mixture was stirred at 50 °C for 5 d. Saturated aqueous NH₄Cl and CH₂Cl₂ were added, the aqueous layer was separated and extracted with CH₂Cl₂. The combined organic layers were dried over MgSO₄, filtered and concentrated *in vacuo*. Two times, the crude product was dissolved in a small amount of CHCl₃ and precipitated by slow addition to an excess of pentane to give the neutral precursor polymer (details see ESI†).

P3—The neutral precursor polymer (114 mg, 83 μmol) was dissolved in H₂O (20 mL), NaOH (65 mg, 1.66 mmol) was added and the resulting mixture was stirred at 70 °C for 2 d. After adjusting a pH of 7 (HCl) the aqueous mixture was dialyzed against DI H₂O for 3 d. Freeze-drying gave **P3** as spongy red solid (28 mg, 25%). The M_n was estimated to be 2.1×10^4 with a PDI of 1.5 (results from precursor). ¹H NMR (500 MHz, D₂O): δ = 7.72-8.15 (m, 4 H), 7.41-7.68 (m, 2 H), 6.36-7.09 (m, 4 H), 3.05-4.03 (m, 74 H) ppm. IR (cm⁻¹): ν 3429, 3263, 3038, 2871, 1668, 1604, 1532, 1510, 1474, 1445, 1411, 1380, 1348, 1296, 1246, 1179, 954, 924, 841, 661, 577, 528. Due to low solubility, ¹³C NMR spectrum could not be obtained.

P4—The neutral precursor polymer (113 mg, 81.9 μmol) was dissolved in *N,N*-dimethylethylenediamine (15 mL) and stirred at 70 °C for 12 h. The reaction mixture was evaporated to dryness and washed with copious amounts of *n*-pentane. The crude product was dissolved in CH₂Cl₂ (10 mL). After addition of MeI (5 mL) the reaction mixture was stirred for 1 d at ambient temperature. All volatiles were removed under reduced pressure. The residue was dissolved in H₂O and dialyzed against DI H₂O for 3 d. Freeze-drying afforded **P4** as spongy red solid (141 mg, 99%). The M_n was estimated to be 2.1×10^4 with a PDI of 1.5 (results from precursor). ¹H NMR (500 MHz, D₂O): δ = 6.91-7.77 (m, 10 H), 7.63-7.75 (m, 2 H), 4.41-4.74 (m, 6 H), 3.10-3.84 (m, 4 H) ppm. IR (cm⁻¹): ν 3392, 2921, 2874, 1720, 1646, 1603, 1511, 1444, 1348, 1297, 1200, 1175, 1073, 944, 878, 838, 775, 675, 647, 601, 576, 529. Due to low solubility, ¹³C NMR spectrum could not be obtained.

Fluorescence response patterns

Emission spectra were recorded and analyzed on a CLARIOstar (firmware version 1.13) Platereader (BMG Labtech, built in software, version 5.20 R5). Data were analyzed by CLARIOstar MARS Data Analysis Software (version 3.10 R5) from BMG Labtech. The specific response for each analyte was measured six times, the peak values acquired. These were used as the observables for the subsequent linear discriminant analysis (LDA).

Linear discriminant analysis (LDA) and principal component analysis (PCA)

Both methods were carried out by using SYSTAT (version 13.0). For LDA, all variables were used in the model (complete mode) and the tolerance was set as 0.001. The fluorescence response patterns were transformed to canonical patterns. The Mahalanobis distances of each individual pattern to the centroid of each group in a multidimensional space were calculated and the assignment of the case was based on the shortest Mahalanobis distance. PCA is a mathematical transformation used to extract variance between entries in a data matrix by reducing the redundancy in the dimensionality of the data. It takes the data points for all analytes and generates a set of orthogonal eigenvectors (principal components, PCs) for maximum variance.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Notes and references

1. Nelson TL, O'Sullivan C, Greene NT, Maynor MS, Lavigne JJ. J Am Chem Soc. 2006; 128:5640–5641. [PubMed: 16637623]
2. Maynor MS, Nelson TL, O'Sullivan C, Lavigne JJ. Org Lett. 2007; 9:3217–3220. [PubMed: 17637024]
3. Zhang C, Suslick KS. J Agric Food Chem. 2007; 55:237–242. [PubMed: 17227048]
4. Piatek AM, Bomble YJ, Wiskur SL, Anslyn EV. J Am Chem Soc. 2004; 126:6072–6077. [PubMed: 15137773]
5. Umali AP, LeBoeuf SE, Newberry RW, Kim S, Tran L, Rome WA, Tian TA, Taing D, Hong J, Kwan M, Heymann H, Anslyn EV. Chem Sci. 2011; 2:439–445.
6. Gallagher LT, Heo JS, Lopez MA, Ray BM, Xiao J, Umali AP, Zhang A, Dharmarajan S, Heymann H, Anslyn EV. Supramol Chem. 2012; 24:143–148.
7. Han J, Bender M, Seehafer K, Bunz UHF. Angew Chem Int Ed. 2016; 55:7689–7892.
8. Weaver AA, Reiser H, Barstis T, Benvenuti M, Ghosh D, Hunckler M, Joy B, Koenig L, Raddell K, Lieberman M. Anal Chem. 2013; 85:6453–6460. [PubMed: 23725012]
9. Kovacs S, Hawes SE, Maley SN, Mosites E, Wong L, Stergachis A. PLoS One. 2014; 9:e90601. [PubMed: 24671033]
10. Newton PN, Fernandez FM, Plancon A, Mildenhall DC, Green MD, Ziyong L, Christophel EM, Phanouvong S, Howells S, McIntosh E, Laurin P, Blum N, Hampton CY, Faure K, Nyadong L, Soong CWR, Santoso B, Zhiguang W, Newton J, Palmer K. PLoS Med. 2008; 5:e32. [PubMed: 18271620]
11. Newton PN, Green MD, Fernandez FM, Day NPJ, White NJ. Lancet Infect Dis. 2006; 6:602–613. [PubMed: 16931411]
12. Patze C, Brödner K, Rominger F, Trapp O, Bunz UHF. Chem Eur J. 2011; 17:13720–13725. [PubMed: 21956686]
13. Davey EA, Zuccherro A, Trapp O, Bunz UHF. J Am Chem Soc. 2011; 133:7716–7718. [PubMed: 21520966]
14. Stewart S, Ivy MA, Anslyn EV. Chem Soc Rev. 2014; 43:70–84. [PubMed: 23995750]
15. You L, Zha DJ, Anslyn EV. Chem Rev. 2015; 115:7840–7892. [PubMed: 25719867]
16. Umali AP, Anslyn EV. Curr Opin Chem Biol. 2010; 14:685–692. [PubMed: 20801075]

17. Kitamura M, Shabbir SH, Anslyn EV. *J Org Chem*. 2009; 74:4479–4489. [PubMed: 19459597]
18. Nguyen BT, Anslyn EV. *Coord Chem Rev*. 2006; 250:3118–3127.
19. Lavigne JJ, Anslyn EV. *Angew Chem Int Ed*. 2001; 40:3118–3130.
20. Rakow NA, Suslick KS. *Nature*. 2000; 406:710–713. [PubMed: 10963592]
21. Janzen MC, Ponder JB, Bailey DP, Ingison CK, Suslick KS. *Anal Chem*. 2006; 78:3591–3600. [PubMed: 16737212]
22. Suslick KS, Rakow NA, Sen A. *Tetrahedron*. 2004; 60:11133–11138.
23. Askim JR, Mahmoudi M, Suslick KS. *Chem Soc Rev*. 2013; 42:8649–8682. [PubMed: 24091381]
24. You CC, Miranda OR, Gider B, Ghosh PS, Kim IB, Erdogan B, Krovi SA, Bunz UHF, Rotello VM. *Nat Nanotechnol*. 2007; 2:318–323. [PubMed: 18654291]
25. Bunz UHF, Rotello VM. *Angew Chem Int Ed*. 2010; 49:3268–3279.
26. Bajaj A, Miranda OR, Phillips R, Kim IB, Jerry DJ, Bunz UHF, Rotello VM. *J Am Chem Soc*. 2010; 132:1018–1022. [PubMed: 20039629]
27. Moyano DF, Rana S, Bunz UHF, Rotello VM. *Faraday Discuss*. 2011; 152:33–42. [PubMed: 22455037]
28. Miranda OR, You CC, Phillips R, Kim IB, Ghosh PS, Bunz UHF, Rotello VM. *J Am Chem Soc*. 2007; 129:9856–9857. [PubMed: 17658813]
29. Han J, Wang B, Bender M, Seehafer K, Bunz UHF. *ACS Appl Mater Interfaces*. 2016; 8:20415–20421. [PubMed: 27415439]
30. Han J, Bender M, Hahn S, Seehafer K, Bunz UHF. *Chem Eur J*. 2016; 22:3230–3233. [PubMed: 26748536]
31. Lavigne JJ, Broughton DL, Wilson JN, Erdogan B, Bunz UHF. *Macromolecules*. 2003; 36:7409–7412.
32. Jonathan P, McCarthy WV, Roberts AMIJ. *Chemometrics*. 1996; 10:189–213.
33. Henderson AR. *Clin Chim Acta*. 2005; 359:1–26. [PubMed: 15936746]
34. Rana S, Elci SG, Mout R, Singla AK, Yazdani M, Bender M, Bajaj A, Saha K, Bunz UHF, Jirik FR, Rotello VM. *J Am Chem Soc*. 2016; 138:4522–4529. [PubMed: 26967961]

Fig. 1.

(a) Structures and quantum yields (ϕ) of the poly(*para*-aryleneethynylene)s (PAE) **P1-P4** and surfactants **CTMA** and **SDBS** employed for construct PAE/surfactant tongue. (b) Structures and quantum yields (ϕ) of the poly(*para*-aryleneethynylene)s PAEs **P5-P6** used to construct PAE/PAE tongue elements. Detailed information about the used polymers and the screening results to evaluate the applied surfactants can be found in the ESI†.

Fig. 2.
(a) Timeline of antibiotics development and **(b)** structures, classification of the investigated antibiotics (AT1-AT19).

Fig. 3.

(a) Components of PAE/surfactant tongue and PAE/PAE tongue. (b) Systematic illustration of PAE/surfactant tongue and fluorescence modulation after adding antibiotics. The contents of the polymer and surfactant are: **C1** = **P1** (2 μ M) + **CTMA** (200 μ M), **C2** = **P2** (2 μ M) + **SDBS** (300 μ M), **C3** = **P3** (2 μ M) + **CTMA** (100 μ M); **C4** = **P4** (2 μ M) + **SDBS** (200 μ M), **C5** = **P5** (0.5 M) + **P6** (0.25 μ M).

Fig. 4.

(a) **P1** (2 μM , black line) titrated with **CTMA** at pH 3, pH 7, and pH 13. Inserted graph shows the change of I_{F1} (463 nm) with increasing **CTMA** concentration (similar titrations of the other PAE **P2-P4** can be found in the ESI[†]). Applying higher concentrations of surfactant than indicated did not elevate the fluorescence further. (b) Fluorescence intensity properties of PAE, PAE/surfactant and PAE/surfactant + antibiotics are shown; two wavelengths for detection were selected (pH 13). (c) Quantum yield of **P1-P4** before and after adding the surfactant (pH 3, pH 7, pH 13), each value is from the average of two measurements.

Fig. 5. 2D LDA canonical score plot for the first two factors obtained with an array of **S1-S24** (left, PAE/surfactant tongue #1), **S25-S30** (right, PAE/PAE tongue #2) and the combined tongue of **S1-S30** (bottom, tongue #3) treated with antibiotics **AT1-AT19** ($c = 5$ mM) with 95% confidence ellipses. Each point represents the response pattern for a single antibiotic to the array. Each kind of (seven kinds) antibiotic is marked by an individual shape (triangle, square, circle etc.) and similar color. After combining the two tongues, the result looks similar to the result gathered from the first tongue (left), and inefficient but somewhat improved discrimination endures.

Fig. 6.

(a) Fluorescence response pattern I obtained by the pruned tongue (S2, S12, S18, S25, S28, S29). (b) Combined PCA plot from the optimized six sensing factor. (c) Combined LDA plot from the optimized six sensing factor. All antibiotics can be classified and clustered depend on the antibiotics types. Cross-validated LDA showed 100% correct accuracy for all antibiotics.

Fig. 7. 3D canonical score plot for the semi-quantitative assay of antibiotics (AT11, AT12 and AT15) with the pruned tongue #4, cross-validated LDA showed 100% accuracy.

Fig. 8.

Distribution of proportion of correctly identified unknowns (CIU) obtained through the analysis of 20,000 bootstrapped samples of each data set from different tongues. **(a)** Tongue #1; **(b)** tongue #2; **(c)** tongue #3 (tongue #1 & #2); **(d)** tongue #4 (most responsive elements of tongue #3). The red line shows the CIU for the original data set without bootstrapping. The blue lines represent the CIUs of the 2.5th and 97.5th percentile of the data.

Table 1
Proportion of CIU of the 2.5th and 97.5th percentile of the bootstrapped results along with the CIU of the original data set (without bootstrapping)

	original data set	2.5 th percentile	97.5 th percentile
Tongue #1	89.47	85.53	89.47
Tongue #2	98.68	90.79	100
Tongue #3	88.16	84.21	89.47
Tongue #4	89.47	86.84	97.37