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High-Throughput Fabrication and Screening Improves Gold Nanoparticle Chemiresistor Sensor Performance

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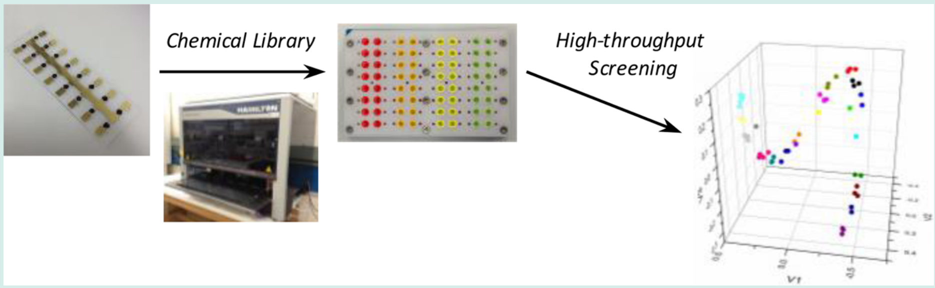
Ⓢ *Supporting Information*



ABSTRACT: Chemiresistor sensor arrays are a promising technology to replace current laboratory-based analysis instrumentation, with the advantage of facile integration into portable, low-cost devices for in-field use. To increase the performance of chemiresistor sensor arrays a high-throughput fabrication and screening methodology was developed to assess different organothiol-functionalized gold nanoparticle chemiresistors. This high-throughput fabrication and testing methodology was implemented to screen a library consisting of 132 different organothiol compounds as capping agents for functionalized gold nanoparticle chemiresistor sensors. The methodology utilized an automated liquid handling workstation for the in situ functionalization of gold nanoparticle films and subsequent automated analyte testing of sensor arrays using a flow-injection analysis system. To test the methodology we focused on the discrimination and quantitation of benzene, toluene, ethylbenzene, *p*-xylene, and naphthalene (BTEXN) mixtures in water at low microgram per liter concentration levels. The high-throughput methodology identified a sensor array configuration consisting of a subset of organothiol-functionalized chemiresistors which in combination with random forests analysis was able to predict individual analyte concentrations with overall root-mean-square errors ranging between 8−17 μg/L for mixtures of BTEXN in water at the 100 μg/L concentration. The ability to use a simple sensor array system to quantitate BTEXN mixtures in water at the low μg/L concentration range has direct and significant implications to future environmental monitoring and reporting strategies. In addition, these results demonstrate the advantages of high-throughput screening to improve the performance of gold nanoparticle based chemiresistors for both new and existing applications.

KEYWORDS: *gold nanoparticle, chemiresistor, sensor array, high-throughput, screening, benzene, BTEX, random forests, quantification, environmental monitoring, automation*

■ INTRODUCTION

Chemiresistor sensors are widely used in a variety of applications ranging from array detectors for analytical instrumentation[1] to homeland security.[2,3] Such sensors are based on arrays of chemiresistive materials that change their resistance in response to changes in their immediate chemical environment. However, in general, individual chemiresistors are at best only partially selective toward a particular analyte of interest. Hence, in order to detect the analyte of interest against a background of interfering substances, arrays of chemiresistor sensors, each with different partial selectivity for the analyte and interferents molecules are used. A variety of chemometric statistical methods are subsequently used to determine the presence or absence of the analyte from the "fingerprint" of the response of the sensor arrays.[4]

Chemiresistors based on organothiol-functionalized gold nanoparticle (Au_{NP}) thin films have been shown to be useful in different sensing areas both in the gas-phase[5−9] and the liquid-phase,[10−13] for applications ranging from environmental monitoring through to medical diagnostics. These types of chemiresistors consist of a Au_{NP} film, which changes its resistance through the partitioning of analytes into the organothiol caps surrounding the nanoparticles thus changing

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the electron tunneling current between the metal nanoparticle cores.[5,14,15]

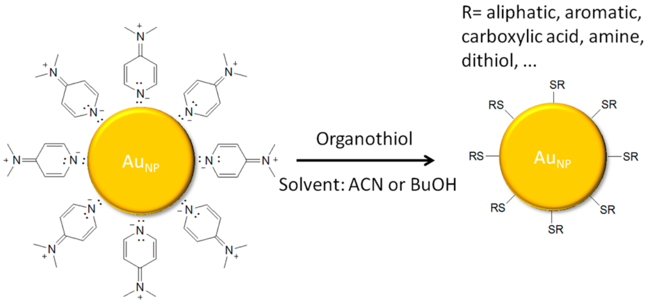
By changing the ligands forming the nanoparticle capping layer it is possible to impart different partial chemical selectivity and sensitivity to target analytes or groups of target analytes. Discovery of suitable nanoparticle capping layers for a particular application is typically through trial-and-error, or serendipity, based on a handful of candidates. However, in principle, this type of chemical sensor lends itself to being developed by high-throughput fabrication and screening methodologies because of the large number of synthetically and commercially available organothiol molecules. To the best of our knowledge, although a number of reports on high-throughput screening (HTS) methodologies for metal oxide[16,17] and conducting polymer[18] chemiresistor sensor arrays have been described, the development of functionalized Au_{NP} chemiresistors using HTS methodologies has not been reported. In the present work, we introduce using an automated liquid handling workstation as an in situ method for functionalizing Au_{NP} film arrays with 132 commercially available organothiol compounds and subsequent automated testing of the sensor arrays using a high-throughput screening system incorporating a flow-injection analysis (FIA) unit.

As part of our ongoing research interest in environmental monitoring applications, in the current work, we focus on sensor array development for the detection and discrimination of benzene (B), toluene (T), ethylbenzene (E), *p*-xylene (X), and naphthalene (N). Monitoring of BTEXN in groundwater near petrochemical facilities and storage areas is in fact mandated in several countries[19] and is also of general interest in environmental monitoring. In the current study, we explore the use of HTS methodology to search for thiol components that produce improved selectivity and sensitivity toward the BTEXN components compared to our previous work.[20]

■ RESULTS AND DISCUSSION

Previously we have shown that inkjet printing of aqueous solutions of 4-(dimethylamino)pyridine (DMAP) functionalized gold nanoparticles, and subsequent exposure of the dry nanoparticle film to solutions of thiols, results in the facile replacement of the weakly bound DMAP on the gold nanoparticle with the thiol (Scheme 1). An advantage of this approach is that different thiol functionalized gold nanoparticle films can be prepared without the need to individually synthesize each different thiol functionalized gold nanoparticle separately. In addition, the inkjet printing conditions only have to be optimized for the DMAP functionalized gold nanoparticle solution rather than for each thiolated gold nanoparticle solution. In our previous work,[10−13,20−22] we have successfully used this approach with approximately 10−15 different thiols. The current work tests this methodology for application to automated high-throughput fabrication and screening using an expanded library of 132 commercially available thiols (Supporting Information Table S1).

Scheme 1. DMAP-Au_{NP} Replacement Reaction (in Situ) with a Variety of Different Organothiol Compounds



The automated functionalization of the Au_{NP} chemiresistors is based on a liquid handling workstation commonly used in conjunction with standard 96-well SBS footprint well plates. For convenience, standard microscope glass slides (25 × 75 mm) were used to form 16 chemiresistor microelectrodes per glass slide using standard photolithography lift-off techniques. An aqueous solution of DMAP functionalized gold nanoparticles, 4−6 nm in diameter, was deposited onto each microelectrode and allowed to dry. The microscope slides were then fitted into a customized 64-well plate, designed to fit the constraints of the SBS footprint well plates (four glass slides per well plate).

A liquid handling workstation (Microlab STARlet, Hamilton Robotics, Reno, USA) was utilized to automate the sensor functionalization process. Such workstations are capable of multichannel, multiliquid aspiration/dispensing, and robotic manipulation of well plates. An application specific method was programmed for the workstation to control every step of the sensor functionalization process, from chemical barcode identification and dispensing of the 132 thiol solutions; incubation of the DMAP functionalized nanoparticle films with thiol solution; through to final solvent washing.

After functionalization the sensor arrays were placed in a custom flow cell integrated with a flow injection analysis system (GlobalFIA Inc., USA) that is based on precision milliGAT pumps (Figure 1). The sensor arrays were connected to an in-house built 32-channel potentiostat. The high-throughput sensor screening setup allows for the programming of sequential analyte delivery to the flow cell, under similar conditions between analytical runs.

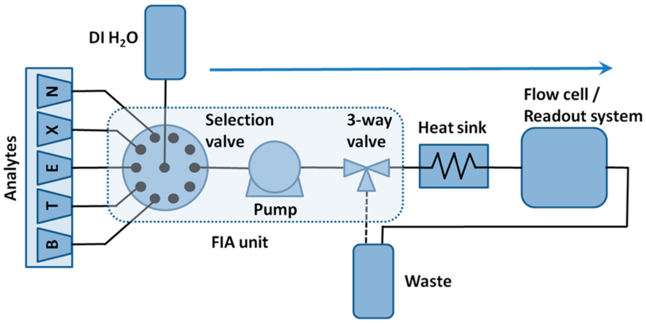


Figure 1. Schematic of the high-throughput sensor screening setup, consisting of a flow-injection analysis (FIA) unit, sensor array flow cell integrated with 32-channel potentiostat, and readout electronics.

Chemiresistor arrays formed with 132 different thiols were subsequently tested for their response toward BTEXN at a relatively high concentration of 5 mg/L in water. Figure 2 shows a typical response of a 2-amino-4-chlorobenzenethiol-functionalized Au_{NP} chemiresistor sensor toward T, B, E, X, and N. From this data two figures of merit were obtained for each thiol and analyte combination. First the initial sensor resistance (R_o) and second the % sensor response was calculated (i.e., $\Delta R_{(\infty)}/R_o$ (%) = $((R_{\text{maximum}} - R_{\text{initial}})/R_{\text{initial}}) \times 100$) (see Supporting Information Figure S1).

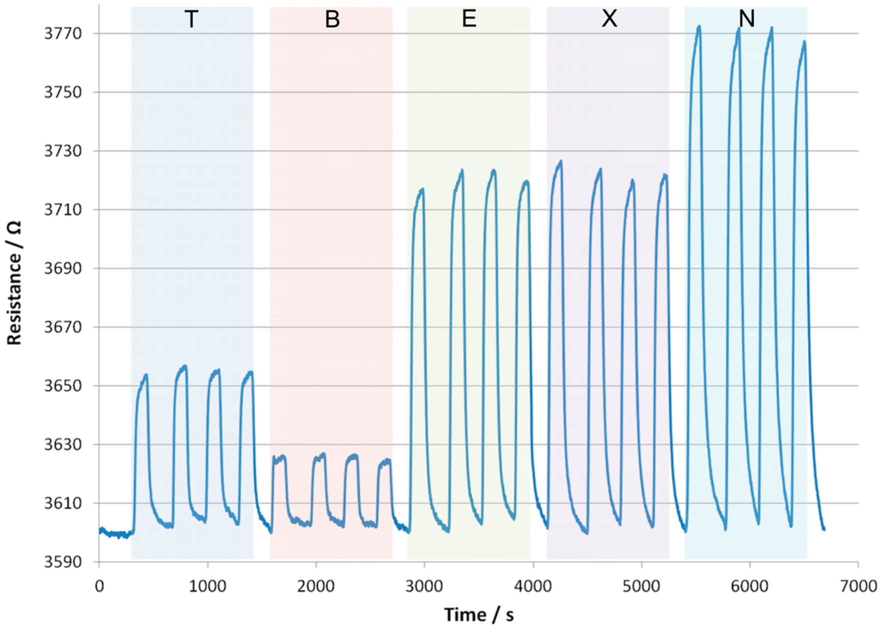


Figure 2. Typical chemiresistor response obtained for a T, B, E, X, and N (5 mg/L) testing profile using the high-throughput sensor screening setup; data for a 2-amino-4-chlorobenzenethiol-Au$_{NP}$ (2-A-4-CBT; No. 100) chemiresistor sensor with sensor data baseline corrected.

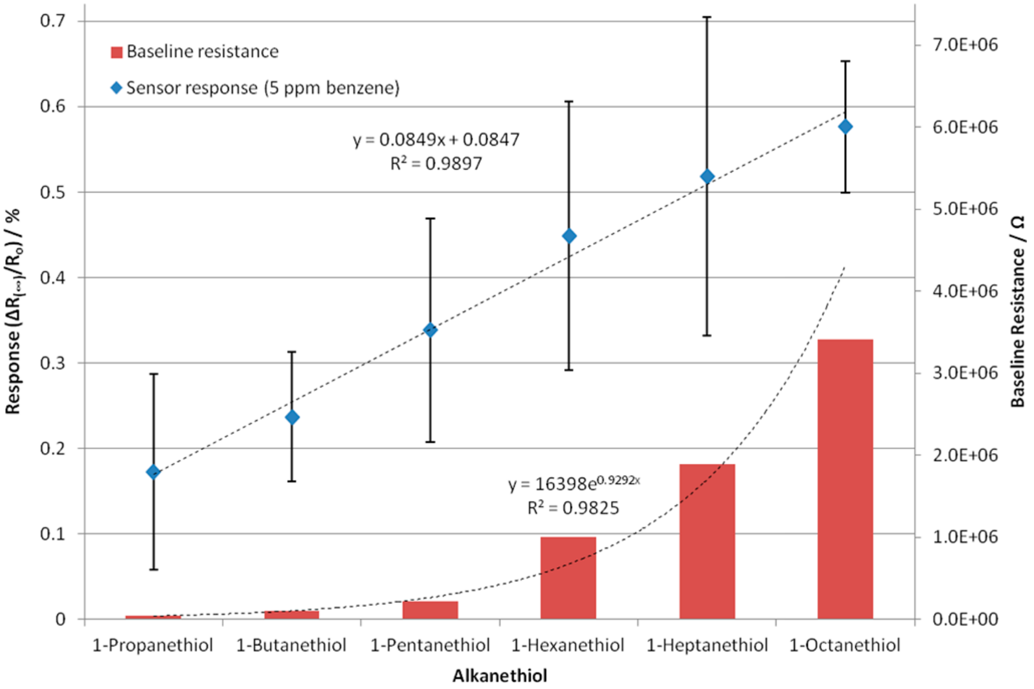


Figure 3. Chemiresistor sensor baseline resistance and sensor response to benzene (5 mg/L in water) for increased alkanethiol-capping agent chain length; each set of error bars are the standard deviation of triplicate measurements from at least 12 individual chemiresistors; the dotted lines are linear (sensor response) and exponential (baseline resistance) fits to the data.

The initial sensor resistance was used as a first pass screen of the chemiresistors. Thus, chemiresistors that had resistances <100 Ω were not analyzed further as such low resistance indicated that the nanoparticle film had partially sintered, possibly because of a lack of proper thiol coverage during functionalization or because the instability or thinness of the thiol self-assembled monolayer does not protect the mobile gold nanoparticle surface sufficiently to prevent interparticle contacts from forming.

Similarly, chemiresistors with resistances >10 MΩ were also not pursued further as such high resistances indicated that either the nanoparticle film had dissolved in the water or in the case of long alkanethiols decreased the signal-to-noise (*S*/*N*) to unacceptable levels. It was typically found that chemiresistors with high resistances were formed from thiols with alkyl chains >12 carbons in length. Improvements in the electronics (i.e., reducing instrument noise) or redesign of the microelectrodes with smaller interelectrode gaps, thus reducing the overall

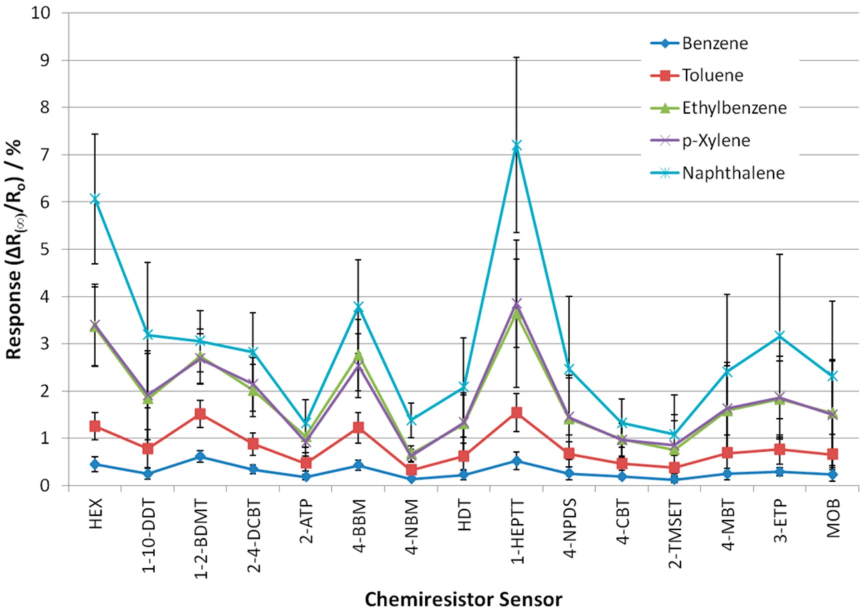


Figure 4. Average responses to B, T, E, X, and N (5 mg/L in water) for a subset of chemiresistors; each set of error bars are the standard deviation of triplicate measurements from at least seven individual chemiresistors.

chemiresistor resistance, could be used in the future to improve the *S*/*N*.

Although thiols that resulted in chemiresistors of high resistance (>10 MΩ) because of long alkyl chain lengths were thus not further investigated in the present study, it should be noted that an advantage of such thiols is their increased signal response. Figure 3 shows the baseline resistance data of alkanethiol-capped Au_{NP} films with increasing alkanethiol chain length (C3−C8). The baseline resistance follows an exponential dependency with increased alkanethiol chain length. This exponential relationship is expected as the conductivity of the nanoparticle film is reliant on electron tunneling through the film.[14] As the alkanethiol chain length increases the average nanoparticle separation gap within the film increases. This increased nanoparticle separation gap leads to a decrease in tunneling current between the nanoparticle cores and a decreased conductance within the nanoparticle film. The data in Figure 3 also shows a linear correlation between the chemiresistor maximum relative sensor response ($\Delta R_{(\infty)}/R_o\%$) to benzene in water and increasing alkanethiol chain length. A similar effect has been previously observed for thiol functionalized gold nanoparticle sensors in vapor-phase studies by Lewis and co-workers.[23]

After application of the resistance criteria described above ($100\ \Omega < R < 10\ \mathrm{M}\Omega$), to the 132 thiols initially tested, 56 thiols remained as suitable candidates for further investigation in terms of the sensor response and selectivity. Figure 4 shows the $\Delta R_{(\infty)}/R_o(\%)$ values toward BTEXN for a range of typical chemiresistors tested (data for the remaining chemiresistors are shown in Supporting Information Figure S1a-b). As expected, with semiselective chemiresistors, most sensors responded to each of the target analytes. However, the magnitude of response varied for different chemiresistors and this allows for arrays of semiselective sensors to be utilized for discrimination and quantification of analytes.[24] The data in Figure 4 also shows that the organothiol-functionalized Au_{NP} chemiresistors respond more strongly to analytes with larger octanol/water partition coefficients (log $P_{\mathrm{octanol/water}}$), for example, log $P_{\mathrm{octanol/water}}$ naphthalene ≫ log $P_{\mathrm{octanol/water}}$ benzene.[10,21] In addition, the data shows the similar responses observed for different organothiol-functionalized Au_{NP} chemiresistors toward ethylbenzene and *p*-xylene, suggesting these analytes interact similarly with the chemiresistors.

Each chemiresistor sensor was next assessed for its comparative sensitivity performance based on its response toward benzene; the most challenging analyte in terms of detection level. Figure 5 shows the sensitivity ranking of the 56 different organothiol-functionalized chemiresistors in relation to the average responses toward benzene in water (5 mg/L). The organothiol with the highest average benzene response was 3-mercaptohexyl hexanoate (3-MHH; no. 27) with an average sensor response of 0.63 ± 0.19%, with cyclohexylmercaptan (CHM; no. 34) resulting in the lowest average sensor response of 0.10 ± 0.06%. Of the 56 thiols, the top four chemiresistors in terms of benzene sensitivity are 3-mercaptohexyl hexanoate (3-MHH; no. 27), 1,2-benzenedimethanethiol (1−2-BDMT; no. 121), 1-octanethiol (OCT; no. 19); 1-heptanethiol (1-HEPTT; no. 36).

The HTS data for the chemiresistor sensors was next used to compare the B, T, E, X, and N selectivity of the different chemiresistor sensors. This selectivity assessment allows sensor arrays to be configured which maximize the discrimination power toward B, T, E, X, and N compounds. In addition, this analysis also allows the identification of colinear chemiresistor sensors which will in turn reduce the amount of redundant sensors when configuring sensor arrays.

To assess selectivity of the chemiresistor sensors to BTEXN, the HTS data was calibrated as per Cooper et al.[20] (Supporting Information Figure S2) and analyzed using a subset selection algorithm using the (R) programming language.[25] This algorithm utilized the chemiresistor HTS data and attempts to maximize the between group (analyte) variation relative to the within group (analyte) variation and indicate a suitable subset of chemiresistor sensors. This subset of chemiresistors can be configured into a sensor array for subsequent testing for BTEXN mixture discrimination and quantification. The algorithm starts by analyzing the BTEXN discriminatory power of individual chemiresistor sensors and then assesses

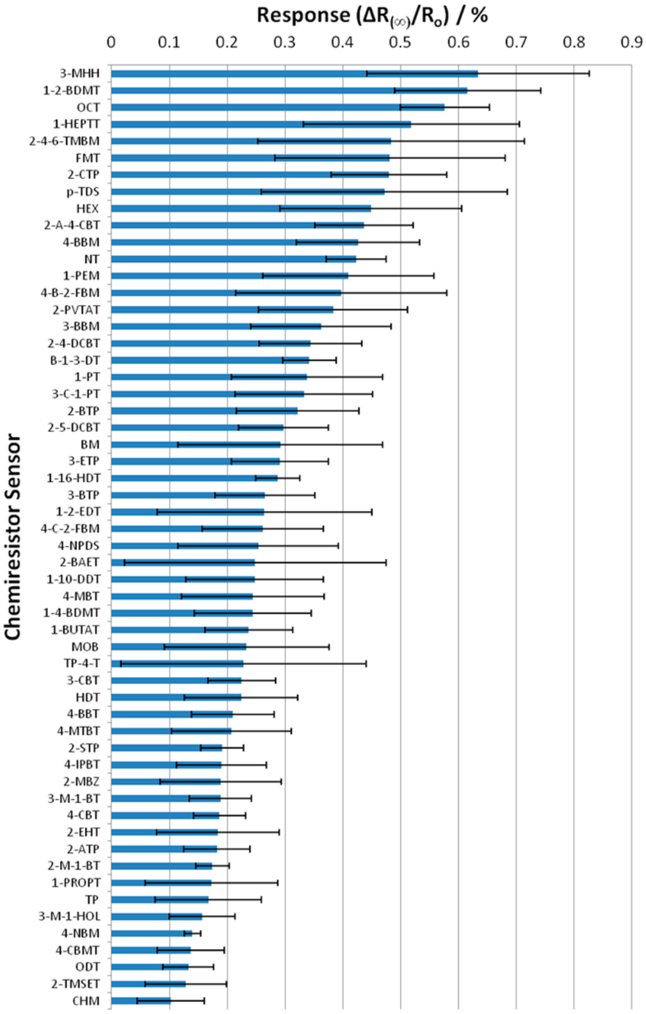


Figure 5. Average responses for benzene in water (5 mg/L) for all 56 chemiresistors, sensors are ranked based on increasing maximum sensor response; each set of error bars are the standard deviation of triplicate measurements from at least four individual chemiresistors.

increasing size chemiresistor subsets to determine the optimal subset that should afford maximum analyte selectivity. The subset selection algorithm results were further analyzed with the computation of a similarity matrix. This similarity matrix data can be utilized to cluster similarly performing chemiresistors through hierarchical cluster analysis (HCA) (Supporting Information Figure S3), a multidimensional scaling plot provides further visual demonstration of the chemiresistor clustering (Figure 6). These analyses identify chemiresistor sensors which have similar responses to B, T, E, X, and N and express very similar discriminatory power, with grouped chemiresistors indicating sensor redundancy, Supporting Information Table S2. Ideally, sensors from different identified groups will be utilized to configure a sensor array to have maximum analyte discrimination.

The sensitivity and selectivity analysis of the chemiresistor HTS data allows for the configuration of a chemiresistor sensor array for the discrimination and quantification of BTEXN mixtures at low concentration. The selected subset of chemiresistors consists of a mixture of the identified top ranking sensitivity chemiresistors (Figure 5), and a wide range of chemiresistors covering groups identified through the selectivity analysis (Supporting Information Table S2). A total of 15 different organothiol-functionalized chemiresistors were selected for the subset sensor array. Two of the four top ranking benzene sensitivity chemiresistors were selected, with the remaining chemiresistors selected based on the selectivity analysis, with 11 of the 22 chemiresistor groups represented on the subset chemiresistor array. This subset of chemiresistors (Table 1) was fabricated into a sensor array and used to test its ability to discriminate and quantitate BTEXN (100 μg/L in water) mixtures.

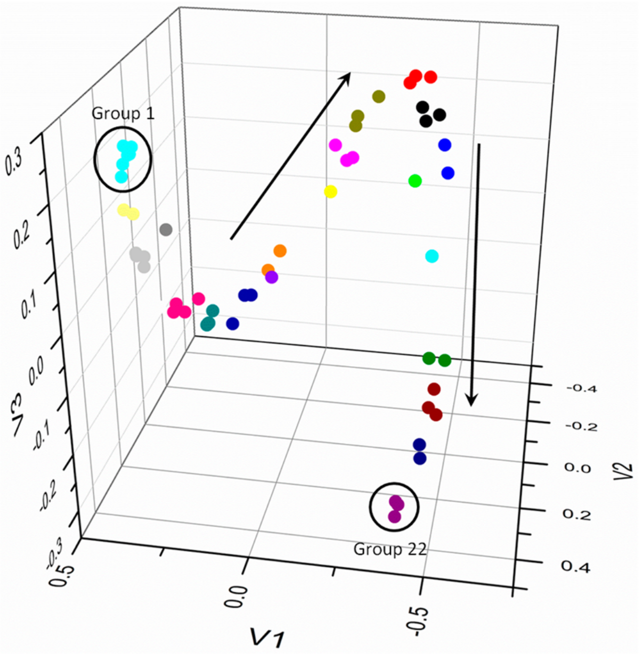


Figure 6. Multidimensional scaling plot illustrating sensor similarity for the chemiresistors which passed the selection criteria; each datum point represents a chemiresistor type with similar chemiresistors identified by the same color resulting in 22 individual group classifications; chemiresistor group assignment classified by hierarchical cluster analysis is shown in Supporting Information Figure S3, with specified group assignment detailed in Supporting Information Table S2.

Previously, we demonstrated that Au_{NP} chemiresistor sensor arrays could be used to successfully discriminate and quantify individual components from BTEXN mixtures in water,[20] albeit at relatively high analyte concentrations (10 mg/L and 1 mg/L) and therefore with limited practical utility. This was achieved by using a full factorial experimental design of all possible B, T, E, X, and N combinations, at a set concentration, in combination with random forests analysis.[20] We applied this same experimental design and analysis to assess our new sensor array configured from the selected subset of chemiresistors (Table 1), however, this study attempts to achieve quantification at much lower concentration levels (100 μg/L in water) of each individual analyte, which to the authors' knowledge has not been previously demonstrated in the literature using a simple chemical sensor.

Figure 7 shows two representative chemiresistors on the sensor array and their response profiles across the BTEXN (100 μg/L in water) full factorial mixture experiment. It can clearly be seen that these two sensors have varied response profiles which is advantageous for individual analyte discrimination and the quantification of mixtures using random forests analysis. Each individual mixture was also analyzed by an

Table 1. Organothiol-Functionalized Chemiresistor Sensor Array Configured for the BTEXN (100 μg/L in Water) Mixtures Full Factorial Experiments

organothiol	experimental ID	organothiol	experimental ID
1-hexanethiol	HEX	1,10-decanedithiol	1−10-DDT
1,2-benzenedimethanethiol	1−2-BDMT	2,4-dichlorobenzenethiol	2−4-DCBT
2-aminothiophenol	2-ATP	4-bromobenzyl mercaptan	4-BBM
(4-nitrobenzyl)mercaptan	4-NBM	1,6-hexanedithiol	HDT
1-heptanethiol	1-HEPTT	4-nitrophenyl disulfide	4-NPDS
4-chlorobenzenethiol	4-CBT	2-(trimethylsilyl)ethanethiol	2-TMSET
4-methylbenzenethiol	4-MBT	3-ethoxythiophenol	3-ETP
4-methoxybenzylmercaptan	MOB		

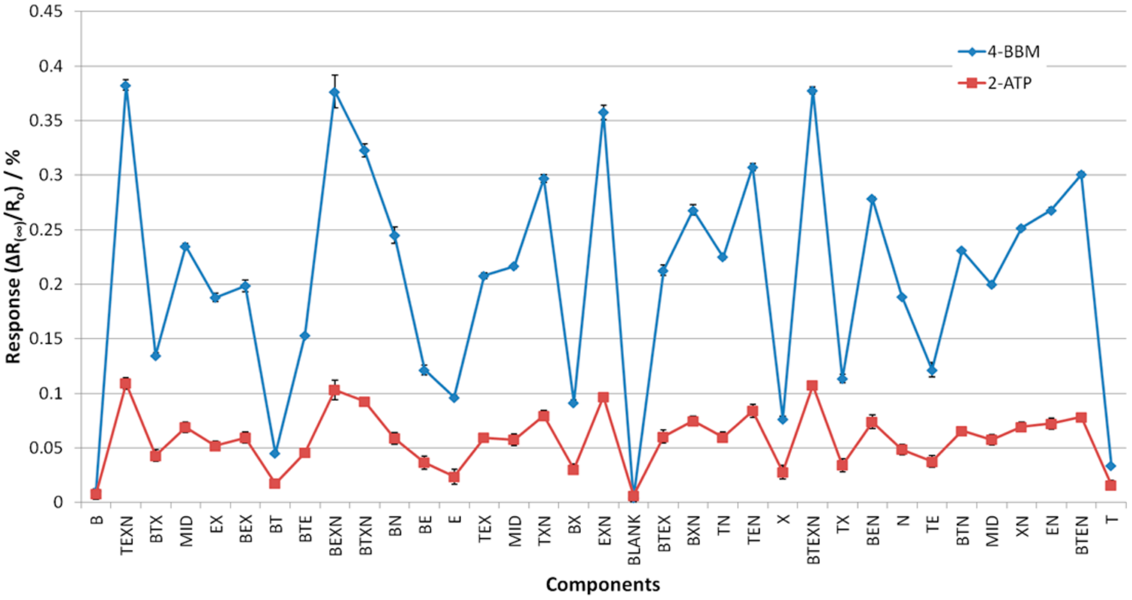


Figure 7. Maximum sensor responses for two representative chemiresistor sensors: 4-bromobenzyl mercaptan-Au$_{NP}$ (4-BBM; no. 102) and 2-aminothiophenol-Au$_{NP}$ (2-ATP; no. 28) over the BTEXN (100 μg/L in water) mixtures full factorial experiment; each set of error bars are the standard deviation of 12 measurements from each chemiresistor; each mixture is represented by the B, T, E, X, N combination with BLANK indicating no BTEXN components present, and MID represents 50 μg/L of each BTEXN component in a mixture.

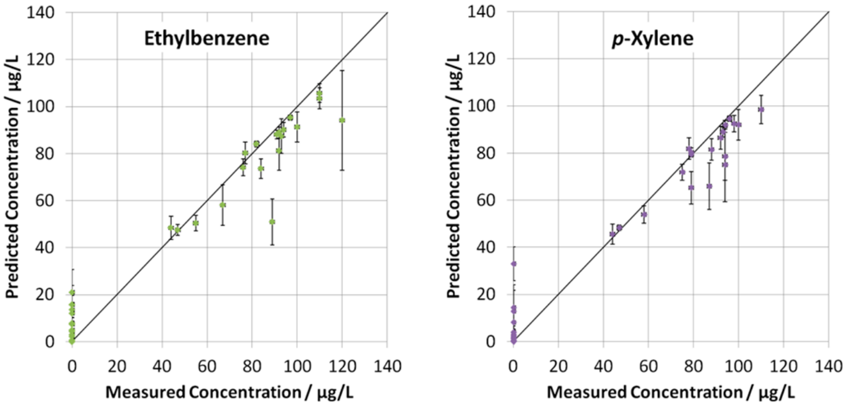


Figure 8. Measured concentration versus the concentration predicted with the random forests model utilizing sensor array responses from the BTEXN (100 μg/L) mixtures in water experiment: (a) ethylbenzene, (b) *p*-xylene; comparable B, T, and N data in Supporting Information Figures S5a-c.

independent analytical measurement laboratory using purge and trap GC-MS to measure and verify the component concentrations (Supporting Information Figure S4).

Random forests analysis was next applied to the chemiresistor sensor array response data for the BTEXN (100 μg/L in water) mixtures. We utilized the nonlinear multiple regression version of random forests which is an ensemble method that uses bootstrap samples and random feature selection to build a large set of regression trees which are averaged to produce predictions.[20,26] Briefly, random forests analysis builds a model based on the BTEXN (100 μg/L) mixtures in water experiment data from the sensor array and uses this to give predictions of the individual B, T, E, X, and N component concentrations; the predictions are based on “out of bag” data, that is, data not used in training.

Using the sensor array data the random forests analysis model demonstrated the ability to predict the concentrations of each individual component for the different BTEXN (100 μg/L in water) mixtures, Figure 8a, b and Supporting Information Figure S5a−c. Figure 8 shows the measured concentration versus the random forests predicted concentration for both E and X (see Supporting Information Figures S5a−c for B, T, and N data). This demonstrates that the HTS derived chemiresistor sensor array has the required sensitivity to detect these compounds at the 100 μg/L concentration level. Importantly, the results also demonstrate that the selected chemiresistor sensor array has the required analyte selectivity to discriminate the five analytes when in mixtures as well as determine their individual concentrations in water at this low level.

The overall root-mean-square (RMS) error of the random forests model was used to obtain the prediction accuracy for each of the individual analytes within the mixtures in water (Table 2). When compared to our previous work (pre-HTS),[20] before the introduction of a HTS methodology (Table 2), we observe comparable prediction accuracies for the analytes, however, the pre-HTS mixture analysis was undertaken at much higher concentrations (i.e., 1000 and 10000 μg/L) and were not capable of achieving low microgram per liter level quantification at the level of error seen with the improved chemiresistor array.

Table 2. BTEXN Mixture Analysis of Organothiol-Functionalized Au_{NP} Chemiresistor Sensor Arrays Demonstrating the Discrimination Power and Prediction Accuracies for Individual B, T, E, X, and N Components[a]

	Pre-HTS BTEXN (1000 μg/L) mixture analysis[b]		Post-HTS BTEXN (100 μg/L) mixture analysis (this study)	
analyte	overall RMS error (μg/L)	prediction accuracy (%)	overall RMS error (μg/L)	prediction accuracy (%)
benzene	±230	77	±17	83
toluene	±70	93	±9	91
ethylbenzene	±150	85	±12	88
p-xylene	±140	86	±11	89
naphthalene	±60	94	±8	92

[a] The comparison is between sensor arrays configured pre- and post-HTS methodology. [b] Refer to ref 20 for pre-HTS screening chemiresistor sensor array configuration.

■ CONCLUSIONS

A high-throughput screening methodology has been outlined to produce, test, and select functionalized-Au_{NP} chemiresistor semiselective sensor arrays. This high-throughput methodology uses an automated liquid handling workstation for the in situ functionalization of Au_{NP} films and the subsequent automated analyte testing of sensor arrays using a flow injection analysis system. This methodology was utilized to produce and screen new chemiresistor materials from an organothiol library containing 132 different compounds and could, in principle, be readily adapted to screen larger libraries. To test the utility of the HTS methodology, a chemiresistor array for improved BTEXN discrimination and quantification was investigated.

Analysis of the HTS results for individual B, T, E, X, and N solutions in water, based on sensitivity and selectivity performance of the chemiresistors, allowed for a suitable semiselective chemiresistor array to be configured and tested. The selected subset sensor array was subsequently subjected to a BTEXN (100 μg/L in water) mixtures full factorial experiment to assess its discrimination and quantification ability. Random forests analysis demonstrated that the individual analytes from the BTEXN mixtures could be discriminated and quantified at the 100 μg/L level, with an overall RMS error range of 8−17 μg/L. The post-HTS chemiresistor array and previously reported pre-HTS chemiresistor array random forests results indicated similar BTEXN prediction accuracies yet operating at an order of magnitude lower analyte concentration. To the authors knowledge the discrimination and quantification of the five component BTEXN mixtures in water at this concentration level using a simple chemical sensor has not been reported previously in the literature. Although the sensitivity of the chemiresistor array is still an order of magnitude too low in order to determine benzene in water at the 5 μg/L level mandated by regulatory authorities, these results demonstrate the potential of HTS to improve the performance of gold nanoparticle based chemiresistors for specific applications.

■ EXPERIMENTAL PROCEDURES

Materials. Gold(III) chloride trihydrate ($HAuCl_4 \cdot 3H_2O$), tetraoctylammonium bromide (TOAB), 4-(dimethylamino)-pyridine (DMAP), 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate hydrate (CHAPS), bovine serum albumin (BSA), acetonitrile, sodium borohydride, sodium carbonate, sulfuric acid, benzene, toluene, ethylbenzene, *p*-xylene, and naphthalene were purchased from Sigma-Aldrich, Australia; All organothiol compounds comprising the chemical library (132 compounds in total) were commercially purchased from either Sigma-Aldrich, Australia, Fluka, Australia, or Alfa Aesar, UK; (3-mercaptopropyl)triethoxysilane (MPTES) was from Fluka, Australia; All reagents are of analytical grade and were used as received unless otherwise stated. All B, T, E, X, and N individual analyte solutions and mixtures were prepared using a Milli-Q deionized water (>18.0 MΩ cm, Millipore, Australia) unless otherwise stated.

Nanoparticle Synthesis. Gold nanoparticles were synthesized according to the process detailed by Brust et al.,[27] and transferred to the aqueous phase upon the addition of DMAP previously demonstrated by Gittens and Caruso.[28] The diameter range of the DMAP-Au_{NP} nanoparticles was 4−6 nm, as determined by dynamic light scattering.

Electrodes. Gold interdigitated circular microelectrodes were fabricated as previously described,[10] using a modified photolithography mask to afford interdigitated circular electrodes (outer diameter of 900 μm, finger width of 5 μm) with an interelectrode spacing of 9 mm by 9 mm (SBS footprint for a 96 well plate) with 16 microelectrodes per glass slide (Borofloat 33, Schott, Australia). Following this, the surface of the glass slide was modified with MPTES.[10] Prior to nanoparticle film deposition the electrode surface was modified using BSA.

Nanoparticle Film Deposition. A solution containing 1.0% w/v DMAP-Au_{NP} with 0.01% CHAPS as a cosolvent was drop-cast (2 μL) at the center of each circular microelectrode on the patterned glass slide, with solvent evaporation at ambient temperature in a N_2 atmosphere.

High-Throughput Sensor Fabrication. Our previous sensor functionalization method[10,11] was adapted to an automated liquid handling workstation (Microlab STARlet, Hamilton Robotics, Reno, USA). This workstation handles SBS footprint well plates and customized 64-well plates were designed to fit the constraints of the glass slides (four glass slides per well plate). The 64-well plates consisted of an aluminum base-plate and a top-plate milled to specification from Acetal (Delrin). The glass slides containing the microelectrodes with the deposited DMAP-Au$_{NP}$/CHAPS films were secured into the well plate, with a seal created by a molded silicon gasket, and then sealed with aluminum foil in order to minimize cross-contamination during the functionalization process.

The liquid handling workstation was utilized to automate the sensor functionalization process. An application specific method was programmed for the workstation, where specific thiol solutions were dispensed into designated wells. ***Caution:*** *suitable air extraction from the liquid handling workstation is advised due to the pungent and noxious nature of the organothiol solutions.* With the thiol solution in direct contact with the DMAP-Au$_{NP}$ film the well plate is incubated for 1 h at ambient temperature.[22] Following incubation the films are repeatedly rinsed with acetonitrile followed by water. Once the workstation-based functionalization is completed the organothiol-functionalized Au$_{NP}$ chemiresistor sensors are exposed to 1% w/v BSA for 1h, before being water rinsed and stored in Milli-Q water until use.

High-Throughput B, T, E, X, and N Screening. Initial screening experiments were carried out on individual component (B, T, E, X, and N) solutions. The individual analyte solutions were prepared from the dilution of a 0.01 g/mL stock solution in methanol, to a nominal concentration of 5 mg/L in Milli-Q water.

The high-throughput sensor screening setup consisted of a flow injection analysis system from GlobalFIA Inc. (Fox Island, WA, USA) with sensor array flow cells which were integrated with a 32-channel potentiostat and readout electronics, Figure 1. The FIA system has been configured to allow up to seven analyte input feeds to a selection valve, with a further background buffer line (Milli-Q deionized water). The FIA unit is programmed to switching between the analytes and buffer solutions, to control the on/off times when exposing the chemiresistor sensors to the target analytes. All analyte flow line tubing is fabricated from stainless steel (1/16 in.), the diluent flow line is Teflon tubing. The output tubing from the FIA unit was passed through a coiled heat sink to ensure temperature equilibration of the solution before entering the flow cell.

The two flow cells consists of a milled aluminum plate to accommodate a glass slide, with gold-coated pins for electrical contacts between the chemiresistors and the in-house built 32 channel potentiostat.[11] A molded silicon gasket was fitted onto the aluminum plate to provide a sealed flow channel, with and a Perspex top plate used to secure the glass slide in place.

Using the potentiostat, chemiresistor sensors were biased at +100 mV DC. The resultant amplified potential output was recorded using two 16-channel e-corder units (1621, eDAQ, Australia) at a rate of 10 points s^{-1} with eChart software (v5.5.11, eDAQ, Australia).

In a typical HTS experiment, two glass slide arrays containing the functionalized chemiresistors (2 × 16 sensors) are loaded into the flow cells. The background buffer (Milli-Q deionized water) is pumped through the flow cells until stable baselines are observed. Once the sensor baseline is stable the FIA run sequence is initiated with a set exposure time (120 s) and flushing time (180 s) and repeated four times, then switched to the next analyte to screen the analyte set. The entire screening experiment is carried out at a flow rate of 50 $\mu L\ s^{-1}$ (3 $mL\ min^{-1}$). All sensor testing experiments were undertaken at ambient temperature (20.5 °C).

High-Throughput Screening Data Preprocessing and Data Analysis. The B, T, E, X, and N (5 mg/L) screening data was smoothed (10-point) and the individual chemiresistor maximum relative responses ($\Delta R_{(\infty)}/R_o(\%)$) for each analyte exposure were extracted and collated (Microsoft Office Excel 2007, Microsoft Co., USA). Benzene sensitivity analysis was completed through sensor response rankings based on HTS data. Chemiresistor selectivity analysis was carried out on calibrated HTS data (Supporting Information Figure S2) using a subset selection algorithm using R (v2.13.2, The R Foundation for Statistical Computing), with output data converted to a similarity matrix. The similarity matrix data was further analyzed through hierarchical cluster analysis (HCA) to illustrate chemiresistor group clustering based on the selectivity analysis using JMP (JMP 10.0.0, SAS Institute Inc., USA).

Discrimination and Quantitation of BTEXN (100 μg/L in water) Mixtures. The BTEXN mixtures in water full factorial experiment was undertaken as previously described,[20] but at a lower concentration (100 μg/L in water). Each mixture was tested in a randomized order against the chemiresistor sensor array composed of the selected subset of chemiresistors (Table 1). Once the sensor array baseline is stable the FIA run sequence is initiated with a set exposure time (120 s) and flushing time (180 s), and this is repeated 12 times, then switched to the next mixture in the sequence. A sample (44 mL) of each tested mixture was independently analyzed by purge and trap GC-MS to verify concentration (National Measurement Institute, Organics Laboratory, Sydney, Australia).

Random forests analysis was carried out using the R programming language[25] using tree = 3000 trees and with the number of variables randomly sampled as candidates at each split set to the default value of 5.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Organothiol compound chemical library information, average responses to B, T, E, X, and N (5 mg/L) for chemiresistors, which passed the selection criteria, calibration of HTS data for subset selection algorithm, similarity matrix analysis groupings of chemiresistors, hierarchical cluster analysis (HCA) dendrogram of similarity matrix data, overall B, T, E, X, N nominal concentration versus measured concentration for the BTEXN (100 μg/L) mixtures in water, measured concentration versus the concentration predicted with the random forests algorithm utilizing sensor array responses from the BTEXN (100 μg/L) mixtures in water for B, T, and N. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

ACN = acetonitrile
Au_{NP} = gold nanoparticle
B = benzene
BSA = bovine serum albumin
BuOH = butanol
CHAPS = 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate hydrate
DMAP = 4-(dimethylamino)pyridine
E = ethylbenzene
FIA = flow-injection analysis
HCA = hierarchical cluster analysis
HTS = high-throughput screening
$\log P_{\text{octanol/water}}$ = octanol/water partition coefficient
MPTES = (3-mercaptopropyl)triethoxysilane
N = naphthalene
ppb = parts per billion
$\Delta R_{(\infty)}/R_0$ = maximum relative sensor response
RMS = root-mean-square
SBS = Society for Biomolecular Sciences
T = toluene
TOAB = tetraoctylammonium bromide
X = *p*-xylene

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