Macromolecular optical sensor arrays

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Abstract

Chemical sensors play an important role in our understanding of chemical and biological systems, providing sensitive and rapid detection of a variety of substrates. Array-based sensing approaches avoid the ongoing challenge of designing and synthesizing selective receptors for particular analytes, a labor-intensive process that can frustrate the development of sensors. Instead, cross-reactive sensor arrays utilize multiple sensing elements that interact uniquely with each analyte and produce a distinct pattern of responses, enabling identification. To date, there are a variety of strategies both to gain cross-reactivity and diversity of sensors required for array-based sensing, and to broaden the scope of analytes for detection. Sensor arrays constructed using macromolecular components, such as polymers and nanoparticles, offer an attractive route to the discrimination of multiple, similar analytes, particularly within the context of biological sensing, where recognition over large areas is often required. Here, we focus on macromolecular sensing arrays underpinned by optical detection methods, which can enable rapid, sensitive detection of a range of analytes. We discuss the current state-of-the art and explore the challenges to be overcome in translating exciting scientific advances to applications beyond the laboratory.

Keywords: polymer, sensor array, fluorophore, chromophore, discrimination

1. Introduction

Chemical sensors have enabled improvements in our understanding of many biological and natural processes.[1] There are a great number of sensors that have been developed to study cells and tissues, to understand and monitor cellular function[2] and to investigate metal and redox homeostasis,[3,4] drug uptake[5] and disease progression.[6] Furthermore, optical sensors are also a promising strategy for the detection of contaminants in the environment.[7] Natural receptor species such as enzymes, antibodies and other proteins are often a source of inspiration for chemists when designing sensors capable of analyte recognition, on account of their unparalleled selectivity.[8] Chemical sensors can also be categorized based on the sensing approach (specific lock-and-key[9] vs cross-reactive sensors),[10] the sensor material (e.g. small molecule vs macromolecule) and signal output (electrochemical, thermal and optical).[10–14] Judicious selection of the respective sensor elements can address the demand to create new sensors that enable rapid, robust, inexpensive and sensitive detection. The goal of this review is to provide a general overview of macromolecular optical sensor arrays and the current status of the field. Initially, we present a brief overview of each focus area, namely optical

sensing techniques, array-based sensing and polymeric sensing materials, followed by a detailed discussion of the statistical techniques which underpin array-based sensing methodologies. In subsequent sections we examine recent studies that utilize different strategies for array design. Finally, we discuss the future directions of macromolecular optical arrays in chemical sensing.

1.1 Optical sensing - A key function of a sensor, in addition to analyte recognition, is the generation of a detectable signal. In some cases, the recognition unit is attached to the reporter element (Figure 1a), while in other examples the sensing element can play the role of both recognition and signaling (Figure 1b).[15,16] For optical sensors, the reporter is either a chromophore or fluorophore, and detection involves the absorption and emission of infrared, visible or UV-light, and a change in either the intensity or wavelength of light upon analyte binding. The attractiveness of optical sensors lies in both their sensitivity, and the broad range of readily-accessible optical elements. Optical sensors using colorimetric[17] or fluorometric responses[18] generally enable rapid detection and can provide considerable flexibility as an analytical technique.[10,19] In some cases, fluorescence based-measurements allow for detection of analytes at the single-molecule level.[20] These types of responses can be used in a variety of systems, for example in indicator displacement assays[21] or analyte-directed aggregation techniques.[22] The properties of these materials have been well-studied and are tunable, and sensors can be selected based on desired properties such as wavelength of absorbance/emission, quantum yield, ease of synthesis or capacity for selective recognition.[23] Environmental sensitivity to aspects such as ionic strength, pH and polarity can cause aggregation and subsequent enhancement or quenching of a response.[24,25] Thus, the versatility and broad scope of optical reporters makes them ideal candidates to produce a detectable signal upon analyte binding.

1.2 Approaches to analyte detection - Sensors can be broadly defined as operating using one of two primary mechanisms for analyte detection: reactivity- and reaction- based sensing.[26] Reactivity-based sensors, or chemodosimeters, rely on a selective chemical reaction between the analyte and the sensing element to produce a dose-dependent response.[27] The process of analyte recognition is irreversible, with the sensor effectively consumed during detection.

Recognition-based sensors harness supramolecular interactions between the analyte and the recognition domain of the sensor to facilitate detection.[6] Interactions between the sensor and the analyte are reversible, and the sensitivity of the system is determined to a significant extent by the K_d of the sensor-analyte interaction.

Indicator displacement assays (IDAs) are a popular route to analyte detection, presenting a convenient method to obtain an optical read-out from a receptor.[28,29] In an IDA, an indicator is reversibly bound to a receptor, and competitive binding with an analyte will displace the indicator from the host, leading to an optical change of the indicator (Figure 1c). IDAs have been developed for a wide range of analytes, but it may be difficult to distinguish between high concentrations of weakly displacing analytes and low concentrations of strongly displacing analytes. IDAs lend themselves well to array-based sensing, since multiple receptor-indicator pairs of varying affinities can be used to detect analytes.[30–32] Multivariate analysis of a system takes into account multiple responses: for example, multiple absorbance and fluorescence wavelengths and fluorescence anisotropy. The combination of these responses may yield more information about displacing analytes and allow them to be discriminated.

The macromolecular optical sensor arrays reported to date have largely focussed on recognition-based sensing or indicator-displacement, and these systems will be the primary focus of this review.

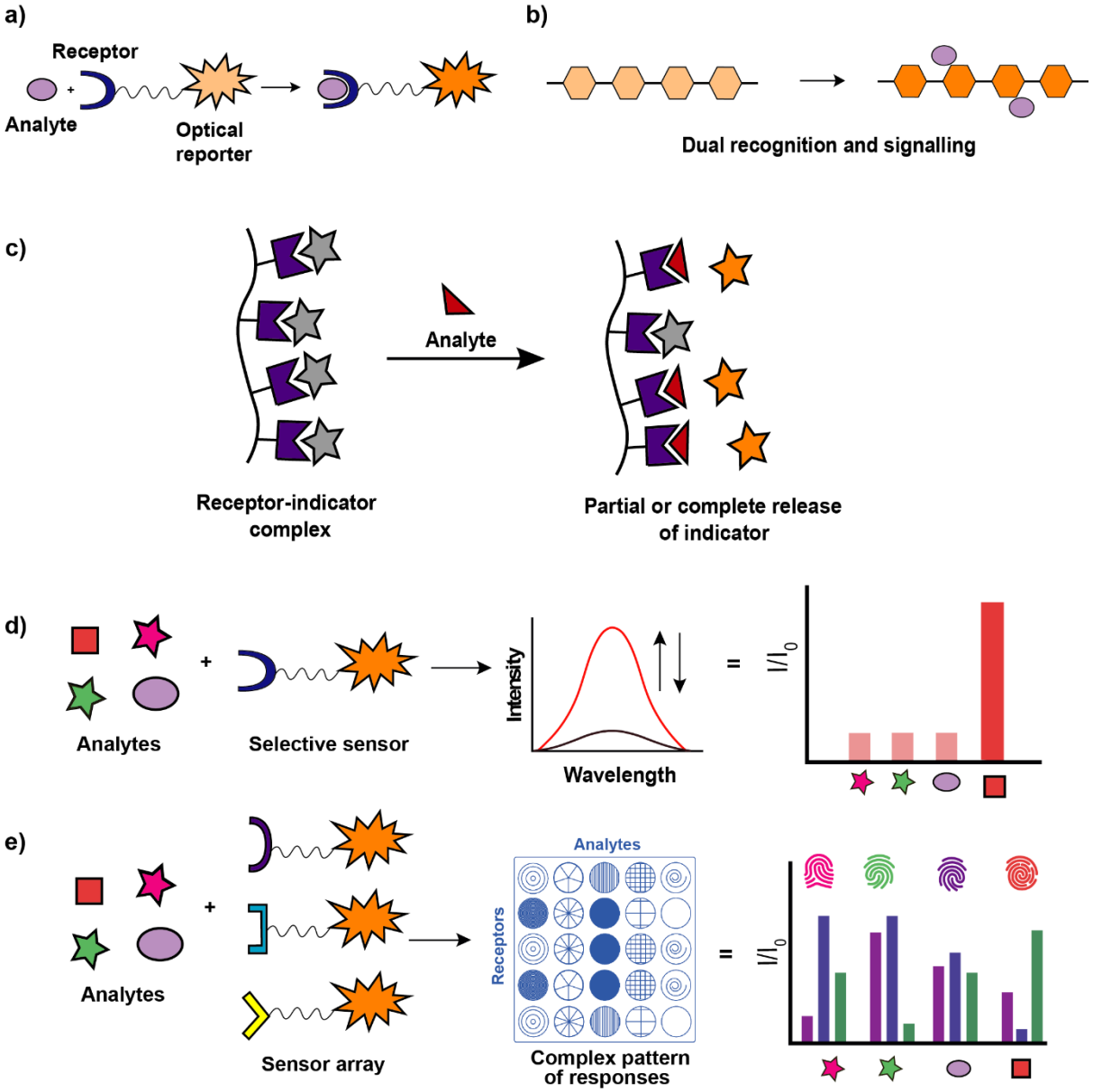


Figure 1. Schematic representation of a) a modular optical sensor containing a receptor with recognition ability, attached to an optical reporting element, b) a sensing element capable of both recognition and subsequent signaling, c) an indicator displacement assay (IDA), d) a 'lock-and-key' fluorescent sensor with selectivity for a particular analyte and e) an array of cross-reactive fluorescent sensors which produces a fingerprint response for each analyte.

1.3 Single sensors vs array-based sensors - The detection of analytes in complex systems is an ongoing challenge in chemical sensing due to the presence of interfering analytes. To date, the majority of sensing systems have been designed using the traditional 'lock-and-key' approach, in which a sensor is designed to be highly selective and specific for a single analyte of interest (Figure 1d).[3,6,9] Whilst presenting a valuable feature for analytical tools, highly specific analyte recognition is not always achievable. From a design standpoint, the creation of these highly selective sensors can often be time consuming and expensive, particularly for application in complex solutions such as biological or environmental samples. Additionally, when moving from analytical studies to practical applications, retaining this specificity is often challenging due to the presence of similar, competing analytes.[9] Furthermore, this sensor design requires the preparation of a unique sensor for each analyte. Recently, differential sensor arrays have gained attention, as they can address some of these difficulties.[33,34] These systems enable the discrimination of multiple, potentially similar, analytes through the analysis

of interactions with numerous cross-reactive sensing components.[33] Rather than being specific or selective for a particular analyte, interactions create a unique pattern, or 'fingerprint', of responses between all sensors and analytes (Figure 1e). If the combination of responses is sufficiently distinct, pattern-recognition strategies can be used to identify and distinguish multiple analytes. Thus, sensors with good cross-reactivity are beneficial in a sensor array approach and can avoid the issues associated with designing sensing elements with high selectivity.

Differential sensor arrays consider the simultaneous interactions of multiple analytes in an entire system and can therefore monitor overall changes in complex mixtures, a feature which is particularly well suited to analysis of biological systems.[35,36] Cross-reactive arrays are also advantageous in that they are hypothesis-free, meaning that individual interactions between sensors and analytes do not need to be understood to interpret results, facilitating the rapid development of sensor arrays to address emerging analytical challenges. Array-based sensing systems have been reported for a broad range of systems, including analysis of volatile organic compounds and explosives,[37–40] food and drink analysis,[36,41,42] environmental monitoring[43–45] and to probe biological systems.[46–48]

1.4 Polymeric receptors – The design of sensor arrays requires the careful selection of sensor materials to ensure stability, system compatibility and recognition ability. A range of sensor arrays have been reported using small molecules,[43,44] proteins,[47,48] nanomaterials,[49–52] quantum dots,[53] and polymers[18] as sensing elements. In this review we focus on the use of synthetic polymers as recognition elements. Macromolecules, in particular polymeric materials, often provide a robust and inexpensive method of producing sensing elements.[54] Advances in the controlled synthesis of polymers have enabled good control over the composition, dimensions and molecular architecture of the resultant materials, offering attractive candidates to act as sensor elements.[12,54,55] This synthetic control allows robust functionalization with reporter elements, such as chromophores or electrochemical elements. It also enables the fine-tuning of the hydrophobic and electrostatic properties of polymers, which proves useful for modifying these principal interaction mechanisms and controlling water solubility.[56] Furthermore, robust tunability of polymer size enables the construction of optimally-sized recognition surfaces with multiple interaction sites, presenting the necessary cross-reactivity for array-based detection.[8,57] Polymeric receptors are particularly appealing in the context of biological sensing, as they can enable multiple recognition events over large area scales, such as those encountered in proteins, bacteria and cell surfaces.[58,59] Incorporating these multivalent interactions into the design and synthesis of polymeric materials opens up an exciting opportunity to produce inexpensive diagnostic tools for biological macromolecules. In addition to presenting attractive receptor species, polymers can also offer a matrix to deposit or 'dope' small molecule recognition elements upon, allowing the construction of sensor arrays with desirable solubility and lipophilicity.

2. Analysis using multivariate statistical techniques

Cross-reactive sensor arrays generally contain multiple sensing elements and analytes, yielding large data-sets of high dimensionality.[19] In principle, each additional sensor contributes to the ability of the array to distinguish different analytes, but also leads to higher dimensionality, creating challenges in data visualization and interpretation.[60] Multivariate statistical techniques are commonly used to identify trends and predictability in data-sets and improve the analysis and presentation of multidimensional data-sets by reducing dimensionality (Figure 2a).

2.1 Multivariate statistical analysis

In general, analysis of cross-reactive arrays utilizes two classes of statistical methods: descriptive and classification techniques.[10,11] Descriptive analysis generally uses unsupervised techniques, meaning analyte class information is withheld and the analytical

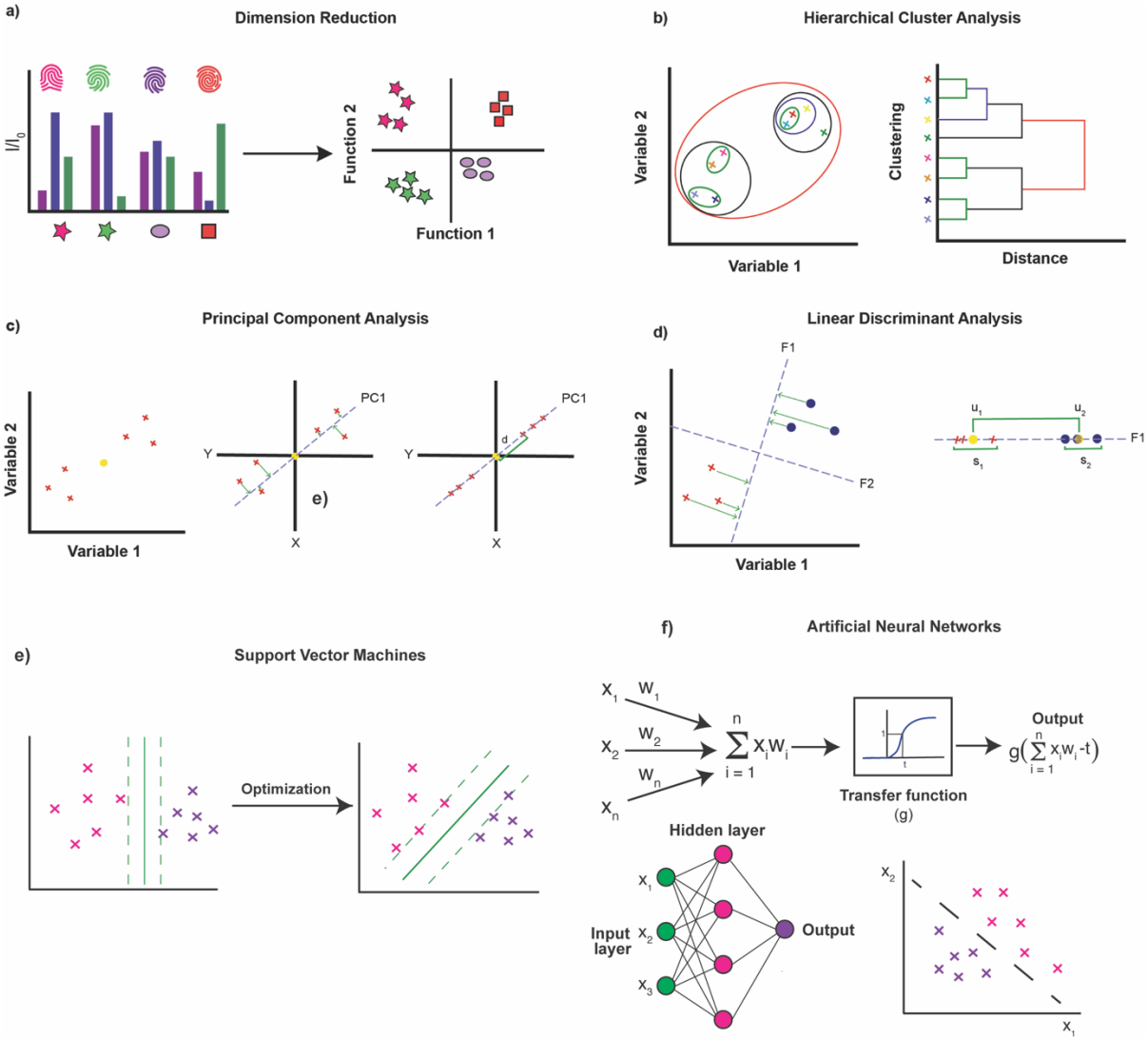


Figure 2. Schematic representation of a) a high dimensional data set (3× sensors, 4× analytes, 4× replicates) reduced to two dimensions through multivariate statistical analysis, b) a HCA bottom-up agglomerative approach and the resulting dendrogram illustrating the connectivity of data points, c) PCA method of determining the center of the data, projecting points onto a new vector and calculating the maximum variance and thus best-fitting line, d) LDA method of projecting points onto a new vector F1 that fulfils the criteria of maximizing the ratio of between-class to within-class variance, e) the SVM optimization process: the decision boundary (solid line) is optimized in an iterative process to discriminate classes while maximizing the size of the margin (dashed line), f) Use of an ANN for classification: the total input for the threshold unit is the weighted sum of all inputs. A transfer function is applied, and the output function is defined as 1 or 0 depending on whether or not it exceeds a threshold value. The threshold unit can classify points that are separated by a hyperplane (dashed line). Blue points correspond to data points assigned a value of 0, and red points to those assigned values of 1 by a threshold unit.

method is tasked with identifying trends and extracting elements that best cluster the data.[61] Since unsupervised techniques are unbiased towards analyte class, they use relatively simple algorithms to describe general trends in data sets, present a qualitative evaluation of whether classes are well-separable, and provide information about relationships amongst samples to identify redundant sensing elements. While descriptive techniques can provide useful information when evaluating datasets, they are less effective at predictive analysis, limiting the ability of the array to identify unknown interactions.

In contrast, classification analysis involves supervised techniques, which utilize analyte class information in order to distinguish data according to these classes. The ability to identify each replicate as the correct analyte is known as the classification accuracy. An algorithm is

developed during analysis of the 'training set', which can then be used to assign unknown samples into a class. The classification accuracy of both known and unknown samples indicates both the effectiveness of the algorithm and whether the system requires further training or additional sensing elements. Algorithms with accurate predictive power can be difficult to achieve and require a large number of samples to create an effective training set.

Thus, classification techniques are often supplemented by initial descriptive analysis to optimize the composition of the sensor array prior to classification analysis. The detailed calculations involved in statistical analysis techniques for cross-reactive sensing have been well described and we therefore include only a brief overview of the most commonly-used techniques.[11]

2.2 Hierarchical Cluster Analysis (HCA)

HCA is an unsupervised descriptive technique that describes a data-set using a discrete number of steps to forms clusters of data. [62] Cluster formation obeys a chosen metric of dissimilarity (Euclidean distance or Mahalanobis distance, which are distance metrics in multivariate space[63]), either by a top-down divisive approach which successively divides clusters based on differences until no clusters remain, or a bottom-up agglomerative approach which forms clusters by pairing nearest-neighbor points based on similarities until all clusters are merged.[64] The latter is more common in sensor arrays, where each data point is treated as its own cluster with no variance, and clusters form by pairing neighboring data points whilst minimizing the variance (Figure 2b).[62] Data is often presented as a tree-like dendrogram, with the metric for distance/dissimilarity on the x-axis, and clustering/connectivity on the y-axis. In array-based sensing, each data point represents an analyte, with clustering indicating which analytes are similar to one another and distance representing how similar they are to each other.

There are, however, a number of limitations to the method, primarily the fact that HCA has no predictive ability for unknown data. Furthermore, dendrograms need to be recalculated to include any additional data points and can be susceptible to misinterpretation (for example, the order of clusters already connected in a dendrogram may be swapped without any meaningful change to the dendrogram). HCA remains a useful technique for descriptive analysis, presenting a clear indication of similarities between data points in an easy to visualize manner and semi-quantitatively identifying trends in large-data sets.[65]

2.3 Principal Component Analysis (PCA)

PCA is another descriptive technique that is used as a tool for dimension reduction of datasets and for reducing the number of sensors in an array, whilst retaining discriminatory power. PCA involves the calculation of a set of orthogonal eigenvectors using linear combinations of the original dimensions. The new dimensions are known as principal components (PCs) and are calculated to retain the maximum variance within a data-set.[66] The first PC lies in the direction of the highest variance, meaning that data vary the greatest amount across that dimension. This process can be more easily explained in a simple two-dimensional case with six variables (Figure 2c).[67,68] Firstly, the center of the data is determined by calculating the average measurement of all variables and the data is shifted so this average becomes the origin. A best-fitting line is plotted that intersects the origin and each point is projected on to this new vector. The best-fitting line contains the maximum variance and is determined by calculating the maximum sum of the squared distances of each data point from the origin. Each subsequent PC is orthogonal and calculated, following the same criteria, as the dimension with the next largest variance.

PCA is a useful technique for reducing the dimensionality of multi-dimensional data-sets, as the first few PCs concentrate the majority of information and data can therefore be presented in lower dimensional space. Score plots of the first few PCs allow high dimensional data to be more easily visualized and interpreted. Typically, the number of PCs extracted will be adequate if they account for 95% of the variance.[19]

A closer interrogation of the PCA corresponding to a sensor array may also help to identify redundant or highly correlated sensors. Loading plots project the contribution of each sensing

element as a vector along each PC.[69] The sensing elements with the lowest contribution to the first few PCs can potentially be removed without significantly affecting the discriminatory power of the array. Additionally, sensing elements that contribute similar loadings to each PC and are highly correlated may also be redundant. Re-applying PCA to the reduced array will produce a new set of PCs and evaluating the variance across these PCs will indicate if the analysis is still adequate without these sensing elements. This ability to pre-screen larger libraries of sensor elements and subsequently minimize the number of elements to a simpler, more experimentally-convenient system is a major benefit of PCA.

PCA is an unsupervised technique, meaning that PCs are extracted without any bias towards analyte class. Thus, if the analysis shows obvious clustering of analyte classes, one may qualitatively say that a supervised technique is likely to have high classification accuracy.[10] The inverse, however, is not necessarily true, and data that is poorly separable by PCA may still have high classification and discrimination ability. Overall, PCA is useful for identifying optimal sensing elements and general trends in large data sets, but a better-optimized classification technique is preferable for further discrimination and classification studies.

2.4 Linear Discriminant Analysis (LDA)

LDA is a classification technique, and similar to PCA, is a dimension reduction method involving the construction of a set of orthogonal, linear combinations that represent the original data and concentrate this information in a minimal number of dimensions. In contrast to PCA, LDA is a supervised technique and retains information about analyte class during the construction of the classification algorithm.[70] Using an analogous two-dimensional case with six variables, a particular vector (F1) is calculated, and each data point is projected onto this vector (Figure 2d). This new dimension, F1, is optimized by a specific criterion using known class information. Specifically, the dimension is a linear combination of variables selected to maximize the distance between sample means (u_1 and u_2) and minimize the separation within classes (s_1 and s_2) (Figure 2d).[71] Each subsequent dimension or 'factor' is orthogonal to the previous dimensions and similarly obeys the same criteria; to maximize the ratio of between-class variance to within-class variance.

A major benefit of LDA as a classification technique for sensor arrays is its predictive ability.[62] Initially LDA creates a mathematical algorithm, constructed using a training set, that can be validated with unknown samples. A set of sample variables can be left out during initial analysis, and the accuracy of the model determined by how successfully it classifies these removed variables. Alternatively, a cross-validation method may be used to test the predictive power of an array. A common cross-validation technique is leave-one-out (or jack-knife) cross-validation, where one sample is removed, and the discriminant function is recalculated.[72] The removed sample is then used to test the model, to determine whether the algorithm can accurately identify the sample without knowing its analyte class. The process is repeated using all samples one at a time, to determine a cross-validation accuracy. Other cross-validation methods involve removing subsets of data and repeating a similar process.

LDA score plots allow for a visual evaluation of class clustering and the degree of discrimination. The majority of the information within a data-set can be presented by plotting the discriminant scores against the largest factors. The inclusion of 90-95% confidence intervals further highlights how well analyte classes are discriminated, and are often included in sensor array applications.[43,58,73] Other useful outputs from LDA include confusion matrices and loading scores.[74] A confusion matrix presents accurate classifications on the main diagonal, misclassifications on the off-diagonal, and allow easy identification of analytes that are being commonly misclassified. Loading plots illustrate how each sensing element in an array contributes to each discriminant factor. Sensing elements that have poor loading scores for the first few discriminant factors may be unnecessary, as they are only contributing a small amount to discrimination.[75] LDA is the most commonly utilized supervised technique in recent sensor array applications.

2.5 Support Vector Machines (SVMs)

Support vector machines are supervised machine learning models that enable classification within datasets.[10] The technique focusses on identifying decision boundaries that separate classes. These decision boundaries, or 'hyperplanes,' are defined and improved through multiple optimization cycles to maximize the distance between clusters and minimize misclassification errors. Optimization focuses on a small subset of data points, those close to the decision boundary, with these points defined as support vectors (Figure 2e). Data points that are not linearly separable can be mapped to higher dimensional feature space through the application of a weighting function, a process known as 'kerneling'.[76]

SVMs can offer advantages over discriminant analysis in that there is no requirement for large training sets in order to enable effective discrimination. SVMs can function with small sample populations, since only data points close to the decision boundary need to be considered. SVM models have been optimized to function with multivariate datasets, and can be used very effectively in array-based sensing applications.

2.6 Artificial Neural Networks (ANNs)

Artificial neural networks mimic early models of sensory processing, by simulating a network of model neurons.[77] Each model neuron, or 'threshold unit' processes information from multiple sources and reports a binary outcome based on the weighted sum of these inputs in relation to a programmed threshold value (Figure 2f). Each threshold unit defines a hyperplane, which separates input values which satisfy threshold conditions from those that do not. Classification problems where two classes can be separated by a hyperplane can be solved by a threshold unit, if the weighting factors and threshold have been appropriately set, usually through a machine learning process where the threshold unit is supplied with a training dataset for which classification is known. More complex linearly inseparable classification problems can be solved by the introduction of more threshold units in a layered arrangement known as a feed-forward network. These networks contain an additional layer of 'hidden' threshold units which partially classify inputs and direct their outputs to a third layer of threshold units that combine partial classifications to produce a final output.

ANNs present some advantages over other classification techniques for the processing of sensor array data. While more commonly-used methods like PCA and LDA provide descriptive analysis or classification, ANNs can be designed to provide similar descriptive outputs, or to provide regression results, enabling outputs consisting of continuous rather than discrete data. The type of output is specified by the function applied at the final layer: classification tasks use probability related functions, while regression task employ linear functions.[78] ANNs can also process non-linear input values more effectively than other techniques, by using a combination of threshold functions. This feature presents an advantage for chemical sensing systems where signal responses may not be linearly related to inputs (e.g. analyte concentration) over the range of input values.[10]

When using ANNs, however, the user must be vigilant towards the possibility of 'overfitting,' arising when small datasets are fitted using functions with too many parameters.[77] Overfitting can lead to large variations in output arising from small variations in input data. Strategies to avoid overfitting include regularisation of data, averaging over multiple networks and using Bayesian statistical tools.[79,80] ANNs should also be rigorously evaluated, either through testing on independent datasets, or through cross-validation procedures.

3. Strategies for the production of macromolecular optical sensor arrays

In this review, we have considered examples of sensor arrays that exhibit good analyte discrimination, as well as unique ways to achieve diversity in sensor elements and effective utilization of multivariate statistical techniques. Four main classes of polymeric materials that have been utilized frequently in optical sensor arrays are featured: conjugated polymers; polymers modulated with fluorophores and chromophores; molecularly imprinted polymers; and polymer films. Each of these polymer materials is discussed with a focus on recent studies

that utilize them effectively in sensor array systems, highlighting examples that move into applications beyond the laboratory.

3.1 Conjugated Polymers - Conjugated polymers are macromolecules that contain alternating single and double (or triple) bonds along their backbone, resulting in extended delocalization of p-electrons, which gives rise to the optical properties of these polymers.[81] Fluorescent conjugated polymers contain this extended conjugated chain, and interactions with analytes at one or more sites can be amplified across the entire polymer system, resulting in fluorescence quenching.[16,82] Many conjugated polymers with varying backbones have been utilized as sensing elements in arrays, including poly(p-phenylenevinylene)s,[83] poly(p-phenyleneethynylene)s,[18] polyfluorenes[84] and polythiophenes.[85] Poly(p-phenylenevinylene)s (PPVs) are synthesized via a number of methods, the predominant method being the Wessling polymerization route, involving the polymerization of p-xylylene precursors generated by the base-induced elimination of p-xylene derivatives.[86] Poly(p-phenyleneethynylene)s (PPEs) and poly(p-aryleneethynylene)s (PAEs) are synthesized by Sonogashira coupling,[55] and polyfluorenes are generally prepared by Suzuki coupling[75] or less commonly by Yamamoto coupling.[85] Polythiophenes can be accessed by chemical oxidation polymerization and metal-catalyzed polycondensation.[87]

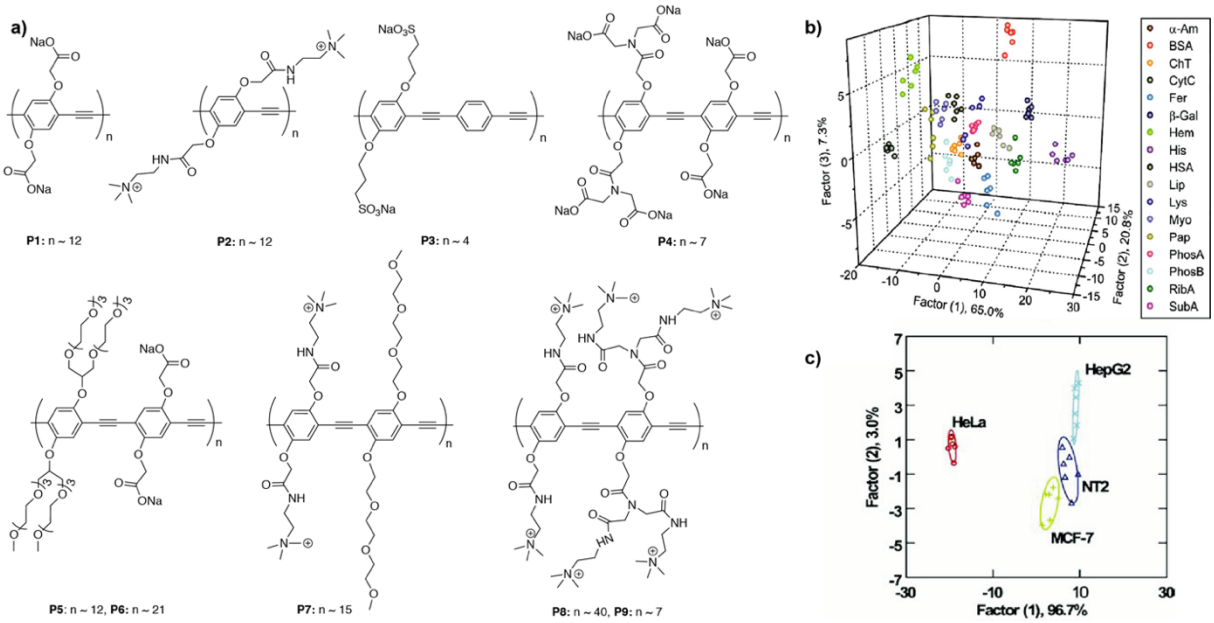


Figure 3. a) PPE sensors **P1-P9** with various pendant arms enabling diverse analyte recognition. b) Three-dimensional LDA score plot obtained from the fluorescence responses of **P1-P6** against 17 protein analytes. Reprinted (adapted) with permission from *J. Am. Chem. Soc.* **2007**, *129* (32), 9856–9857. Copyright (2007) American Chemical Society. c) Two-dimensional LDA score plot of the fluorescence responses of four different cancer cell lines using fluorescent polymers **P2, P5, P7** and **P8**. Reprinted (adapted) with permission from *J. Am. Chem. Soc.* **2010**, *132* (3), 1018–1022. Copyright (2010) American Chemical Society.

Conjugated polymer sensors can produce optical responses to analytes either by the incorporation of receptor groups or by relying on the inherent electrostatic and chemical properties of the polymers.[15,88] Cross-reactive response to analytes can be achieved by creating conjugated polymers that incorporate various pendant arms into the polymer scaffold, such as charged groups or polar side chains, to create diversity along the polymer backbone.[89] These diverse functional groups can participate in various electrostatic and hydrophobic interactions with analytes. This multivalent capacity of conjugated polymers makes them particularly suitable for sensing macromolecules with large recognition surfaces, such as proteins,[84] cell surfaces[73] and other biomolecules.[90,91] Furthermore, functionalization at

pendant sites along the backbone can modify the solubility properties of conjugated polymers, enabling detection in a range of media.

Elegant work by Rotello and co-workers demonstrates the potential of this approach, with their protein-sensor array comprising six PPE sensors, **P1-P6** (Figure 3a).[18,73] PPEs were functionalized with various charged residues to both increase water solubility and target binding of protein surfaces. This array could classify 17 proteins based on differences in their metal cofactors, molecular weight and isoelectric point (pI), with 100% accuracy using a LDA cross-validation routine (Figure 3b).[18]

Later work by the group utilized a similar PPE sensing system to discriminate healthy, cancerous and metastatic cell types.[73] This array contained most of the PPE sensors from their 2007 work, along with three additional PPE sensors with different cationic and polar side chains, **P7-P9** (Figure 3a). The environmentally-responsive polymers interact with various lipids, proteins, and polysaccharides on the cell surface, leading to variations in polymer fluorescence, attributed to differences in aggregation behaviour. Furthermore, by identifying which polymers contributed to the most discrimination in LDA, the complexity of the system could be reduced to just four polymers (**P2, P5, P7** and **P8**). Remarkably, using this optimized array, LDA could discriminate four phenotypically distinct cancer cell lines with 100% accuracy (Figure 3c), and 3 isogenic cell lines (normal, cancerous and metastatic) with 94% accuracy. This work highlights the versatility of array-based sensing: optimization of the sensing system enabled the discrimination of subtle differences within protein analytes and varied cell types.

Lavigne and co-workers utilized polythiophenes, another class of conjugated polymer, to discriminate various structurally similar amines.[22,41,92] The polythiophene sensor **P10** included a carboxylic acid functionality (Figure 4a), and produced a unique optical response across the visible spectrum upon addition of various amines. This single-sensor array achieved discrimination by using a combination of nine different absorption wavelengths, and initial studies were able to discriminate six structurally similar amines.[22] LDA classified each amine at five concentrations using leave-one-out cross-validation with 99% accuracy.

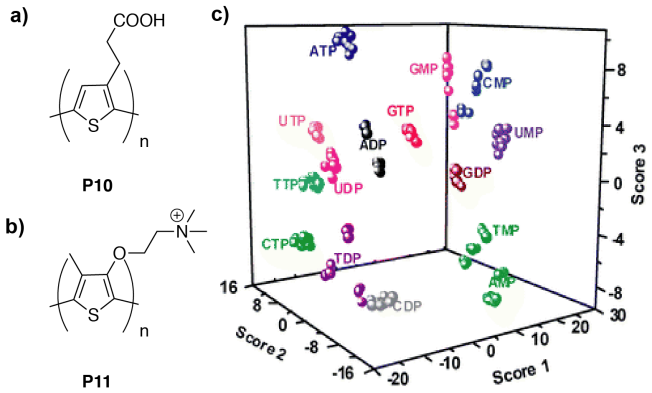


Figure 4. Polythiophene sensors a) **P10** with a carboxylic acid functionality used to discriminate various amines.[22,41] Reprinted in part with permission from *J. Am. Chem. Soc.* **2005**, *127* (15), 5695–5700, Copyright 2005 American Chemical Society; *Org. Lett.* **2007**, *9* (17), 3217–3220, Copyright 2007 American Chemical Society; and b) **P11** containing a quaternary ammonium side chain.[90] c) Three-dimensional LDA score plot of the absorbance responses of **P11** with 15 nucleotide phosphates. Reproduced with permission of the Royal Society of Chemistry, from *Chem. Commun.* **2009**, *405* (31), 4696–4698; permission conveyed through Copyright Clearance Center, Inc. Copyright 2009 Royal Society of Chemistry.

In subsequent studies, the group extended the range of analytes to include an additional 16 amines.[41] These amines included biologically relevant amines such as histamine and tryptamine, which are proposed neurotransmitters, or structurally similar amines like aniline, pyridine and 2-aminopyridine.[91] Impressively, LDA distinguished all 22 amines with 97% cross-validated accuracy. The change in absorbance profile was attributed to intramolecular interactions between polymers causing main-chain twisting, and analyte-directed aggregation causing scattering at longer wavelengths. These studies

highlight the benefits of using conjugated polymers in array-based sensing as they can exhibit multiple mechanisms of interaction.

Shi and co-workers also utilized polythiophenes in a sensor array, taking advantage of electrostatic and hydrophobic interactions of the conjugated polymer.[90] Interactions with various analytes led to changes in both the conformation and aggregation of the polymeric backbones, thus altering the absorbance profile. In this case, a single polythiophene sensor **P11** (Figure 4b), containing a quaternary ammonium side chain, could discriminate 15 nucleotide phosphates (adenine, uracil, guanine, cytosine and thymine with one, two and three phosphate groups.) Discrimination was proposed to arise from a combination of ionic-self-assembly processes between ammonium side chains and phosphates, leading to ordered phases within the poly(thiophene) structure, and aggregation facilitated by analyte addition. LDA of the resultant absorbance profiles allowed for the differentiation of 15 nucleotide phosphates with 100% accuracy, using a leave-one-out cross-validation strategy (Figure 4c).

Polycyclic aromatic hydrocarbons (PAHs) are persistent environmental pollutants that damage human health, creating a need for reliable and sensitive detection methods.[75] Bonizzoni and co-workers recently reported a series of six polyfluorene copolymers to discriminate a library of PAHs. They attributed the differential recognition between the polymers and analytes to the inner filter effect (IFE). The IFE arises when excitation or emitted light is absorbed within the sample, either by the fluorophore itself, e.g. in a highly concentrated solution, or by another absorber. For example, the presence of a PAH with an absorbance spectrum that overlaps with the excitation spectrum of the polymer sensor reduces the amount of input excited light, and therefore the emission intensity of the fluorescent polymer (Figure 5a). Each polymer sensor in the series, **P12**-**P17**, has a unique spectral fingerprint due to the inclusion of a 2-phenylbenzimidazole optical modifier and either phenylene or thiophene co-monomers (Figure 5b). Each PAH differs slightly in its absorption spectrum and can act as an optically dense absorber through the IFE, causing slight differences in the fluorescence quenching of each polymer due to unique regions of spectral overlap. Taking advantage of this IFE, when the absorbance profile of the 6-series polymer system was subjected to LDA, the analysis could discriminate 16 different PAHs with 100% accuracy.

Subsequent work by the same group also used the IFE and conjugated copolymers to discriminate 12 azo dyes.[93] This system comprised 3 polyfluorene copolymers, **P18-P20**, with various conjugated comonomers of ethylene, thiophene and bithiophene, creating a diverse range of absorbance profiles (Figure 5c). The azo dyes act as dense absorbers, with sufficiently unique spectral overlap with each conjugated copolymer to create a differential response. Multiple absorbance and fluorescence measurements in the regions of greatest spectral overlap were examined using LDA, enabling complete discrimination of the dyes. The authors demonstrated the impressive sensitivity of the system by examining the dyes at a concentration commonly encountered in wastewater of 500 nM. At this concentration, the absorbance signals of the dyes alone are too low to effectively detect and discriminate them. However, when utilized in the array system at this concentration with the inclusion of both absorbance and fluorescence changes of the three polymers, all 12 dyes were successively discriminated. The sensitivity of these measurements highlights the advantage of fluorescence-based detection, which enables identification at concentrations beyond the limit of a colorimetric technique.

Schanze *et al.* used meta- and para-linked PPEs and polythiophenes in homopolymer and copolymer sensors to create initial structural diversity, and variant pendant arms to generate charge diversity.[84] The conjugated polymer array could discriminate seven proteins with varying pI and molecular weight by measuring the variation in protein induced aggregation of the six polymers, through fluorescence correlation spectroscopy. Jiang and co-workers used combinations of PPEs and polythiophenes as sensors, using interaction-based fluorescence responses to discriminate various nitroaromatics.[94] Polcha and co-workers introduced combinations of fluorene, vinylene, anthracene and benzothiadiazole units along the polymer backbone to obtain the sensor diversity necessary to discriminate various explosives.[83] The clear advantage of modifying the conjugated units along the polymer backbone is the variation in fluorescence properties of each polymer. In particular, the unique fluorescence and aggregation properties of different polymer backbones provides a useful platform for cross-reactivity and thus differential sensing applications.[88]

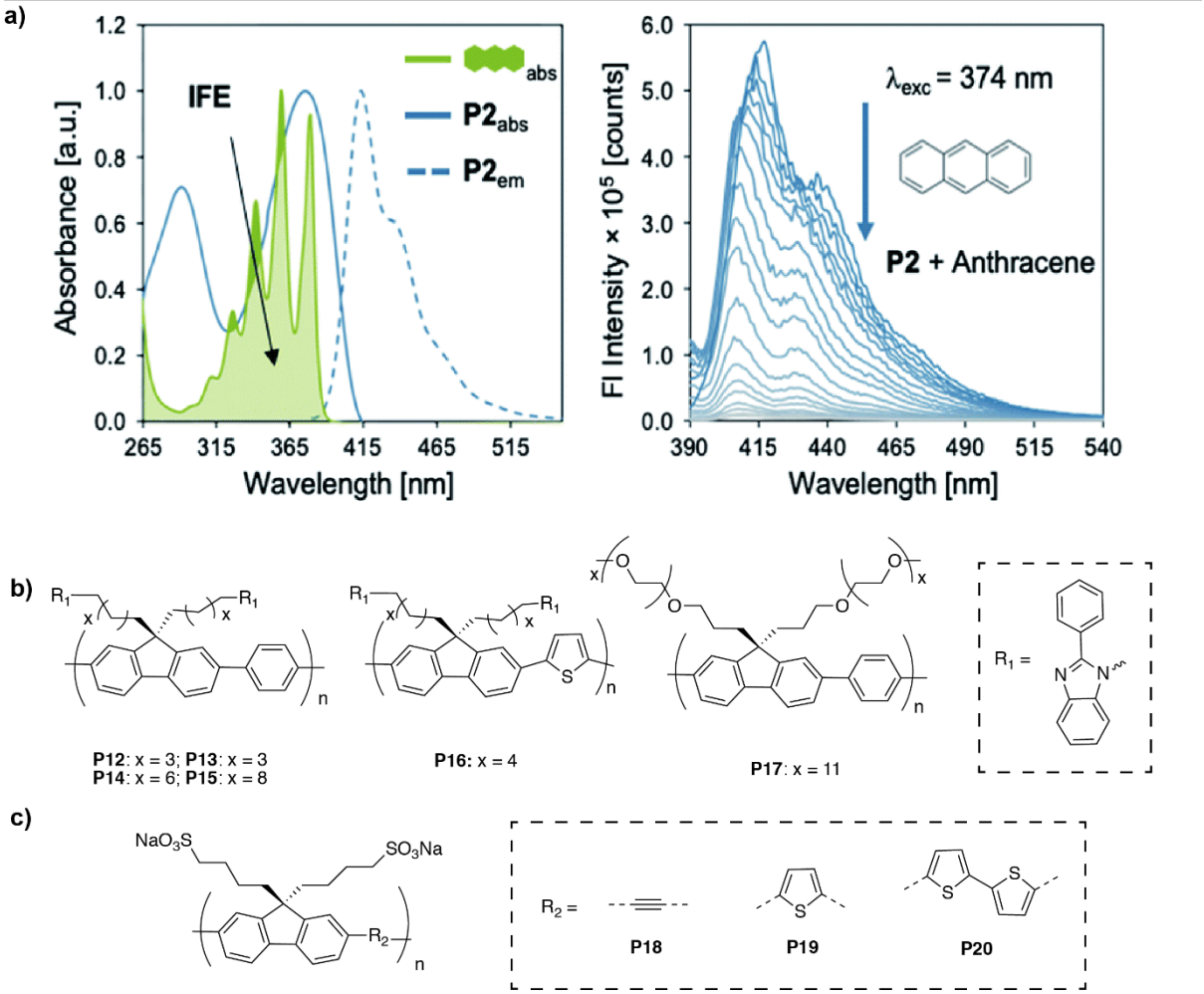


Figure 5. a) An illustration of the spectral overlap between analyte and a sensor necessary for the detection of PAHs through the IFE. Reproduced with permission of the Royal Society of Chemistry, from *Chem. Sci.* **2019**, *10* (44), 10247–10255; permission conveyed through Copyright Clearance Center, Inc. Copyright 2019 Royal Society of Chemistry. b) Conjugated polymer sensors **P12-17** used in an array to discriminate PAHs and c) Conjugated polymer sensors **P18-P20** with various conjugated co-monomers.[93] Reprinted (adapted) with permission from *ACS Sensors* **2020**, *5* (6), 1541–1547, Copyright 2020 American Chemical Society.

By far the most comprehensive studies using conjugated polymer sensors in optical arrays have been performed by Bunz and various co-workers. Since 2005, the Bunz group has published over 20 papers in the area, both with a variety of polymer systems and a diverse library of analyte systems. The most prevalent conjugated polymers to appear in their sensor arrays are PPEs and PAEs. With a range of polymer analogues, array studies have focused on sensing diverse analytes including organic acids,[95] carboxylic acids,[96] nitroaromatics,[97] flavonoids,[98] amino acids,[99,100] phosphates,[101] nitroarenes[40] and saccharides.[102] In addition to small molecule analytes, the group have also demonstrated progress towards using polyelectrolytes to study complex mixtures, with various sensor arrays able to discriminate antibiotics,[103] whiskies,[36] anti-inflammatory drugs,[56] fruit juices,[104] syrups and honeys.[105] It is remarkable to see such a diverse range of studies and the broad scope of analytes identified using small libraries of conjugated polymers. This success may be attributed to the fact that they have exploited the numerous interaction capabilities of conjugated polymers and balanced these interactions in finely optimized sensing libraries.

A key strategy in the work of Bunz and colleagues involves the use of a combination of positively-charged, neutral and negatively-charged functional arms. Ionic interactions between polymers and analytes can provide the cross-reactivity necessary for effective discrimination. For example, in 2017 the group reported a study to discriminate different whisky brands and

blends.[36] Initially they screened a library of 22 different PAEs, of which nine were positively-charged, four were neutral and nine were negatively-charged. Using PCA, the array could be reduced to just 3 elements: one positively-charged and two negatively-charged polymers, **P21-P23** (Figure 6a). Elimination of the charge-neutral polymers suggests that electrostatic interactions play an important role in discriminatory capacity. Using this small 3-element tongue, LDA was able to discriminate 24 different types of whisky with 99% cross-validation accuracy.

Electrostatic interactions can also be employed to distinguish analytes through the formation of complexes between different conjugated polymers. Additional work by Bunz and colleagues illustrated this approach using five complexes, comprised of one positively-charged PAE, **P24**, and five negatively-charged PAEs, **P25-P29** (Figure 6b).[95] Complexation of **P24** with each negative PAE caused a different degree of quenching. The addition of various organic acids either caused quenching by further complexation or enhanced the fluorescence by disrupting the PAE complex. With just five complexes, LDA could discriminate 13 different organic acids with 94% cross-validation accuracy.

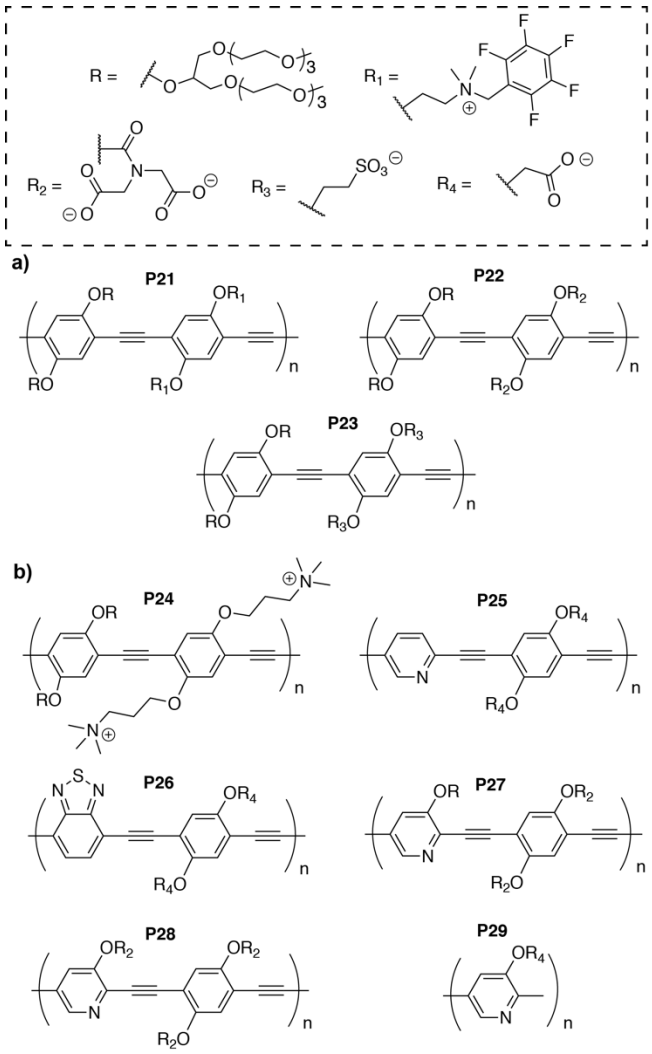


Figure 6. PAE sensors with various functionalities R-R4, a) **P21-23** used in an array to discriminate different whiskies;[36] reprinted (adapted) from *Chem* **2017**, *2* (6), 817–824, Copyright 2017, with permission from Elsevier; and b) **P24-P29** utilized in an array to discriminate different organic acids;[95] reprinted (adapted) with permission from *Chem. Eur. J.* **2016**, *22* (10), 3230–3233, Copyright 2016 John Wiley and Sons.

Another well-explored strategy by Bunz and co-workers to increase discrimination involves creating multiple pH channels for each sensing element. Many of the analytes that exist in biology and nature vary their behavior with pH. We can therefore also expect that sensor-analyte interactions will also be affected by environmental pH. This effect is highlighted by recent work in the Bunz group on distinguishing flavonoids, which are polyphenols abundant in many foods and drinks.[98] This study used three PPEs and two poly(tetraphenylethene)s (PTPEs) with diverse pendant functionalities (Figure 7a). The emission spectra of the five polymers were recorded at different pH values (pH 4, 7 and 10) in the presence of each analyte, effectively giving a 15-element array. LDA confirmed successful discrimination of 11 flavonoids. Through PCA, six key elements were identified as contributing most significantly to the discriminatory power of the array. LDA of the optimized 6-element array could effectively discriminate all 11 flavonoids, including clustering of sub-populations of structurally similar flavonoids, further illustrating the structural sensitivity of the array (Figure 7b). Notably, the work also includes structure-activity relationship studies of the hydrophobic and electrostatic behaviour of the conjugated polymers, exemplifying the important role both types of interactions play in the discrimination of analytes. These studies highlight how increasing understanding of the molecular recognition events underpinning complexation can enable effective optimization of sensor arrays.

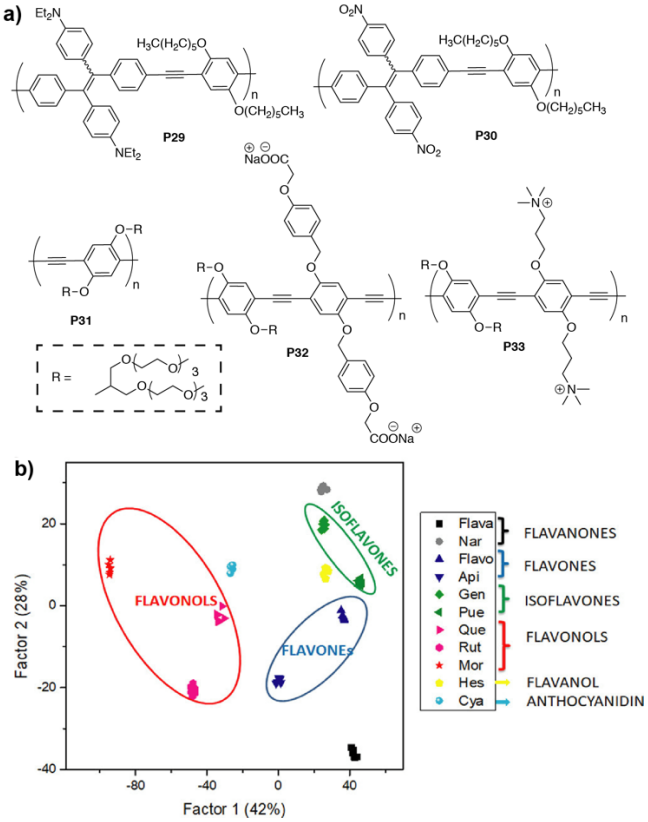


Figure 7. a) PPE and PTPE sensors **P29-P33.** b) Two-dimensional LDA score plot of the fluorescence responses with 11 flavonoids. Reprinted (adapted) with permission from *ACS Appl. Polym. Mater.* **2019**, *1* (6), 1301–130798. Copyright 2019, American Chemical Society.

The discriminatory power of conjugated polymer-based arrays can be increased by using other non-covalent interactions in addition to electrostatic interactions. PPEs contain aromatic units along the polymer backbone, capable of undergoing □-□ stacking interactions.[106] Bunz and co-workers explored these interactions in their recent work discriminating 13 nitroaromatic compounds, including known explosives or compounds used in their preparation.[97] They sought to increase recognition of these electron-poor aromatic species by developing an array of electron-rich PPEs. Sensors **P34-P37** exhibited conjugation that extended beyond the backbone through appendage of benzylic side chains to two of the polyelectrolytes (Figure

8a). Nitroaromatic compounds caused fluorescence quenching, proposed to be due to π-π stacking with the aromatic arms. Differential quenching responses for the 13 analytes, enabled classification using LDA with 99% cross-validation accuracy.

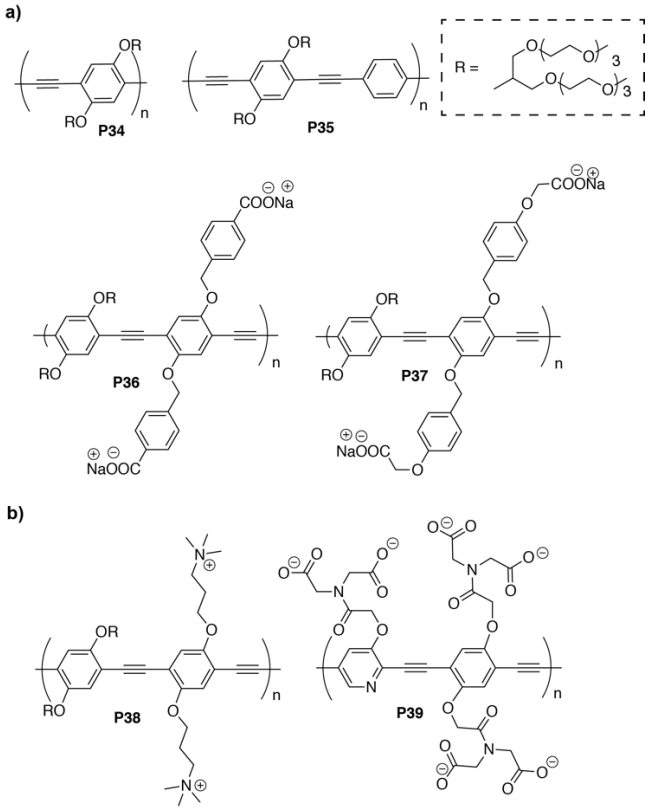


Figure 8. Conjugated polymer sensors a) **P34-P37** used to discriminate nitroaromatics;[97] . reprinted (adapted) with permission from *Macromolecules* **2017**, *50* (11), 4126–4131, Copyright 2019, American Chemical Society; and b) **P38-P39** used to study anti-inflammatory drugs;[56] reprinted (adapted) with permission from *ACS Appl. Mater. Interfaces* **2017**, *9* (1), 790–797, Copyright 2017, American Chemical Society.

Combining the strategies described above is likely to enhance the discriminatory capacity of a sensor array. This extra depth of discriminatory power is particularly beneficial when identifying analytes within a large library, or within complex mixtures, where individual interactions are difficult to discern. An elegant example of this approach was conducted by the Bunz group in their 2017 paper on detecting anti-inflammatory drugs.[56] Building on their previous work on the discrimination of structurally similar aromatic acids,[96] they selected a highly fluorescent cationic polymer (**P38**) and a complex of this polymer with a weakly fluorescent anionic polymer (**P39**) to form the two elements of their sensor array (Figure 8b). Different analytes disrupt the **P38-P39** complex and either enhance or quench the fluorescence. The experimentally determined binding constants of the complex are pH dependent, so measurements of each analyte-sensor combination were conducted at both pH 10 and pH 13. As expected, the interaction behaviour of sensors and analytes is also pH dependent. LDA of the 4-element array revealed 100% cross-validation classification accuracy of 11 different anti-inflammatory drugs.

Rotello and colleagues used an IDA strategy with conjugated polymers.[48,50] Their 2007 study used different nanoparticles to create six non-covalent gold nanoparticle-conjugated polymers with various surface functionality. These nanoparticles quench the fluorescence of the PPE polymer, which they previously reported for protein discrimination, **P1**.[50] Interaction with proteins disrupts the nanoparticle-polymer interaction and produces a change in polymer fluorescence. The six sensing elements enabled discrimination of seven proteins of differing size, charge and pI with 100% accuracy using LDA, and an additional study with 56 unknown

protein samples led to classification with 96.4% accuracy. Impressively, the study went on to achieve protein identification at varying concentrations, to meet the requirements for real-world applications.

More recently, Rotello, Bunz and colleagues designed an IDA-based sensor array comprised of conjugated polymers, and the green fluorescent protein (GFP) as the indicator, with Förster resonance energy transfer (FRET) quenching of the polymer by the protein. The FRET-based sensor array comprised the supramolecular complexes of GFP with four conjugated polymers, **P2, P7, P8** and **P33**, all of which had shown effective discrimination capacity in previous studies.[18,73,98] Multivalent binding of the polymers with cell surfaces disrupts the FRET signal of the polymer-GFP complexes to produce a ratiometric response, and LDA was able to differentiate and identify unknown cell lines. 16 different cell types were tested with this array, revealing 100% cross-validation accuracy of 3 different isogenic cell lines, 100% accuracy of four site-specific metastatic cell lines, 100% accuracy of four glycosaminoglycan (GAG)-modified cell lines and 94.5% accuracy of 128 unknown samples of all cell types. Importantly, using the conjugated polymers alone, only 63% and 72% cross-validated accuracy was achieved for GAG-modified cell lines and metastatic sublines respectively, highlighting the importance of the ratiometric response arising from the pairing between the polymers and GFP.

Another example by Bunz and colleagues utilized **P1** paired with four different antimicrobial peptides, which partially quench the polymer fluorescence, to discriminate bacteria in urine.[58] The interactions between the polymer complexes and each bacterial species were found to be dependent on the components on the bacterial surface. These interactions produced a pattern-based fluorescence response, due to either aggregation of complexes or displacement of the polymer, and responses for each unique bacterial species were classified using LDA with 100% accuracy.

The work addressed in this section highlights the broad scope of analytes that can be detected by conjugated polymers and has explored various interactions responsible for discrimination. Many systems tune the balance between two of the major interactions responsible for discrimination – hydrophobic interactions and electrostatic interactions – by varying elements such as the polymer backbone, the functionalization of ionic pendant arms, cationic/anionic complexes, pH, hydrophobicity of side chains, or various combinations of these factors.

3.2 Polymers decorated with chromophores and fluorophores

Another common method used to create optically active polymers involves appending both recognition motifs and optical reporters to a polymer scaffold in a modular approach. The modular strategy involves the synthesis of parent polymer architectures that can be functionalized with both a recognition unit and a chromophore/fluorophore in a post-polymerization modification step, enabling both components to be modified to achieve cross-reactivity and diversity of response. These systems have shown many successful applications for the discrimination and identification of various biomolecules, particularly for proteins.[57,59,107]

Kurita and co-workers utilized two copolymers, **P40-P41,** functionalized with a cationic lysine recognition site and two environmentally sensitive fluorophores (Figure 9a), to distinguish protein post-translational modifications (PTMs).[107] They postulated that the lysine would undergo multiple electrostatic and hydrophobic interactions with analytes due the charged amino group and the n-butyl group respectively.[108,109] Proteins with different PTMs would likely interact in a unique way to environmentally sensitive fluorophores and increase in fluorescence due to a decrease in polarity in the microenvironment surrounding the fluorophore upon protein binding. Both **P40** and **P41** demonstrated fingerprint responses to four different serum albumins, before PTM. **P40** was selected for further sensing experiments as it displayed a markedly larger fluorescence enhancement upon analyte addition, enabling improved precision and sensitivity for subsequent experiments. The fluorescence response of **P40** with various analytes was dependent on both the pH and ionic strength of the system, supporting the hypothesis that electrostatic and hydrophobic interactions between **P40** and proteins contributed to complexation. The researchers therefore optimized the array to one

comprising **P40** in six different buffer solutions of varying pH and ionic strength at four wavelength channels. The array could discriminate 12 protein analytes comprising six proteins in the presence and absence of various PTMs including phosphorylation, acetylation, methylation and glycation. LDA presented individual proteins in well-separated classes, and 100% accuracy in a leave-one-out classification procedure (Figure 9b).
.The data was also explored using HCA, with clustering of data indicating that ionic strength was the most important discriminating factor, followed by pH, supporting earlier observations about the important role of electrostatic and hydrophobic interactions in differentiation. While the system enables accurate discrimination of known protein samples, the reliance on ionic strength may limit its applicability for identifying unknown protein solutions, as the ionic strength of a solution is likely to be an unknown quantity.

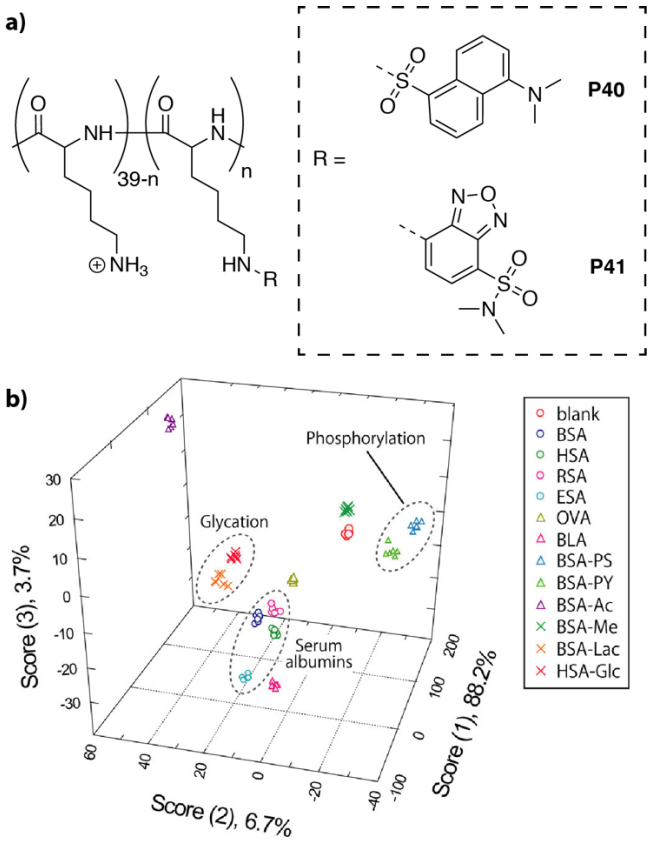


Figure 9. a) Poly-L-lysine copolymers **P40-P41** with environmentally sensitive fluorophores and b) three-dimensional LDA score plot of the fluorescence responses to various PTMs of different proteins. Reprinted (adapted) with permission from *ACS Appl. Mater. Interfaces* **2017**, *9* (27), 22970–22976. Copyright 2017, American Chemical Society

Subsequent work by the same group again utilized the dansyl-modified poly-L-lysine copolymer scaffold **P40**, in this case to discriminate different human cell lines.[110] As the scaffold had already displayed cross-reactive sensitivity to proteins and PTMs of proteins, the authors hypothesized that the sensor could also bind non-specifically to hydrophobic and charged components of cell surfaces, in a mechanism similar to that used by Rotello and co-workers, as described above.[18,73] By measuring emission at multiple wavelengths in buffers of varying pH and ionic strength, then employing LDA, they were able to discriminate eight human cancer cell lines from different tissue types, with 100% cross-validation accuracy. Applying their original protein sensor system to a more complex cell system highlights the benefits of array-based sensing. Translation of chemical sensors from single analytes to complex biological systems often results in considerable errors related to biocompatibility, arising particularly through the presence of interfering and competing species. Array-based sensing avoids this complication by characterizing the analyte based on the entire complex

system. Thus, by employing a small number of optimization steps and re-training the array in biological fluid, Kurita and colleagues were able to avoid the vast redesign efforts often required to apply sensors to biological systems.

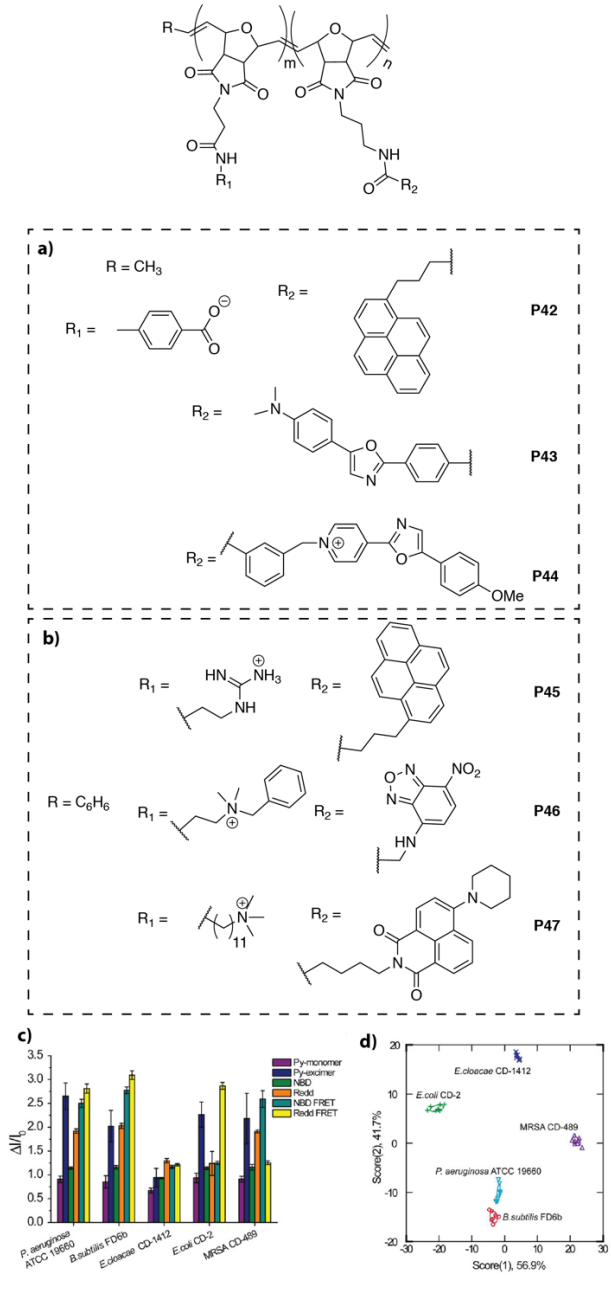


Figure 10. a) PONI random copolymers containing a benzoate solubilizing group and fluorophores pyrene (**P42**), dapoxyl (**P43**) and PyMPO (**P44**).[35] Reproduced (adapted) from *Adv. Mater.* **2018**, *30* (28), 2–7, under a Creative Commons CC BY licence. b) PONI random copolymers **P45-P47** containing different recognition groups (R_1) and fluorophores (R_2). c) Normalized fluorescence response of **P45-P47** and to five unique biofilms and d) two-dimensional LDA score plot of sensor responses to biofilm analytes. Reprinted (adapted) with permission from *ACS Appl. Mater. Interfaces* **2019**, *11* (12), 11202–11208. Copyright 2019, American Chemical Society.

Rotello and colleagues have presented another approach to the design of macromolecular sensor arrays, based upon polymer scaffolds decorated with optical elements.[35] Their approach involves a poly(oxanorborneneimide) (PONI) random copolymer produced via a ring-opening metathesis polymerization, enabling a high level of synthetic precision. The scaffold consists of a benzoate group that acts as a recognition element for proteins and either

a pendant pyrene, dapoxyl or PyMPO fluorescent dye, creating a library of three polymers, **P42-P44**, with distinct signal responses (Figure 10a). These environmentally responsive dyes were proposed to function as both cross-reactive recognition components and reporting groups for serum proteins implicated in liver fibrosis. Interestingly, the array design involved combining the three polymers in solution to enable a multichannel output from a single sample measurement. Initially, the array was tested against a number of common serum proteins to confirm the system was sensitive to fluctuations in protein levels in human samples. Five human serum proteins (human serum albumin, immunoglobulin, transferrin, fibrinogen and alpha-1-antitrypsin) were added to human serum and could be distinguished using LDA with 86% classification accuracy.

The ability of the array ability to classify fibrotic liver tissue was explored through a study of 65 clinical human serum samples. A set of 50 samples were tested and using LDA, the array could discriminate healthy and fibrotic samples with 80% accuracy. Using this as a training set, 15 additional samples were identified and correctly classified with 80% accuracy. Further tests examined whether classification accuracy was correlated to biomarkers linked to liver fibrosis, by comparing the concentration of each biomarker in the fibrotic tissue and any misclassification results. Ultimately, it was found that no single biomarker was responsible for classification results, indicating that multiple biomarkers were required to generate the signature response enabling discrimination between healthy and fibrotic samples. Importantly, the results of the sensor array were found to be comparable to other tests identifying these biomarkers, but with a faster and robust method that does not require specialist equipment.[111]

Subsequent work from the Rotello group used a similar PONI scaffold, with each polymer incorporating a cationic unit for recognition and an environmentally sensitive fluorophore as a signaling element.[57] Using various post-polymerization modification strategies, a guanidine unit, a benzyl unit and a trimethylammonium unit (R_1) were functionalized on the PONI scaffold with pyrene, NBD and naphthalimide derivatives respectively (R_2) (Figure 10b). The three resultant cationic fluorescent polymers **P45-P47** were again combined in solution, generating six distinct wavelength channels due to excimer formation and FRET-based interactions between different polymers. In this instance, the sensor array was used to identify different species of bacteria known to be present in biofilms. The polymers interact with the surfaces of biofilms, consisting of live and dead bacterial cells, proteins, DNA, polysaccharides and other biomolecules, depending on the species on bacteria present. Interaction with different biofilm matrices created a distinct fingerprint response for five different biofilm models containing a single species of bacteria (Figure 10c), and LDA produced 100% correct classification accuracy (Figure 10d).

The array was further validated with 40 ‘blind’ samples of biofilms which were correctly classified with 95% accuracy. Additional studies revealed the array was also able to discriminate biofilm models consisting of mixed species of bacteria and these were LDA-classified with 100% accuracy. The strategy of mixing all polymers together for biofilm classification not only simplifies sample preparation and measurement, but also increases the discriminatory power of the array. The two wavelength channels from the FRET-based interactions resulted from the mixing of polymers, and when LDA was performed without these channels, the prediction accuracy dropped to below 80%.

In a recent paper, Rotello and colleagues created an assembly between **P45** and GFP to create a FRET-based sensor array system to discriminate different macrophage polarization phenotypes.[112] Similar to their previous PONI polymer scaffolds, a single well containing both components of the sensor provided five unique fluorescence channels based on different interactions. The fluorescence responses provided enough discriminating power to identify five different macrophage polarization states using LDA with 100% classification accuracy.

In the context of new diagnostics, it is important to consider straightforward and robust sensor systems that are advantageous when compared to common protocols.[34] The PONI polymer systems discussed achieve this, as rather than including an individual measurement for each sensor, they mixed sensors together and decreased sample preparation and measurement time to produce more rapid and robust sensor systems. Despite this success of mixing polymer sensors and exploiting intermolecular interactions, this one-pot sensor array

concept has not been explored far beyond these key examples, presenting ample scope for the development of next-generation diagnostics for a wide range of diseases.

Recent work by Albrecht and co-workers has also explored the use of pyrene-labelled polymers, combining emission responses for monomer and excimer species to enable discrimination of proteins.[59] A co-polymer, **P48**, consisting of (2-(dimethylamino)ethyl methacrylate) (DMAEMA) and a pyrene-functionalized methacrylamide was synthesized *via* RAFT polymerization (Figure 11a), and presented an optical fingerprint response to various metallo- and non-metalloproteins (Figure 11b). The combination of hydrophobic pyrene and hydrophilic DMAEMA functionalities results in intermolecular assembly and aggregation. At neutral pH, hydrophilic PDMAEMA segments are partially protonated and form an outer shell that is able to interact with biomolecules, such as the charged surfaces of proteins. Pyrene units produce two distinct emission bands at 384 nm and 394 nm, and an excimer band at 488 nm, suggesting the presence of domains of aggregated pyrenes as well as isolated pyrenes. The molecular characteristics of each protein influenced its interactions with the polymer assembly and resulted in structure dependent disassembly of aggregates and either a fluorescence turn-on or quenching effect. These interactions resulted in an optical fingerprint of the monomer and excimer wavelengths for eight different proteins. Negatively-charged non-metalloproteins caused disassembly of aggregates due to strong Coulomb interactions between the analyte and partially protonated DMAEMA segments, resulting in disruption of the hydrophobic interactions in the pyrene domains and subsequent quenching of excimer fluorescence. Positively-charged non-metalloproteins were observed to have minimal effect on fluorescence, presumably due to their limited interactions with the partially positively-charged polymer subunits. Finally, metalloproteins quenched both the monomer and excimer fluorescence of the polymer aggregates, an observation which was attributed to electron or energy transfer effects.

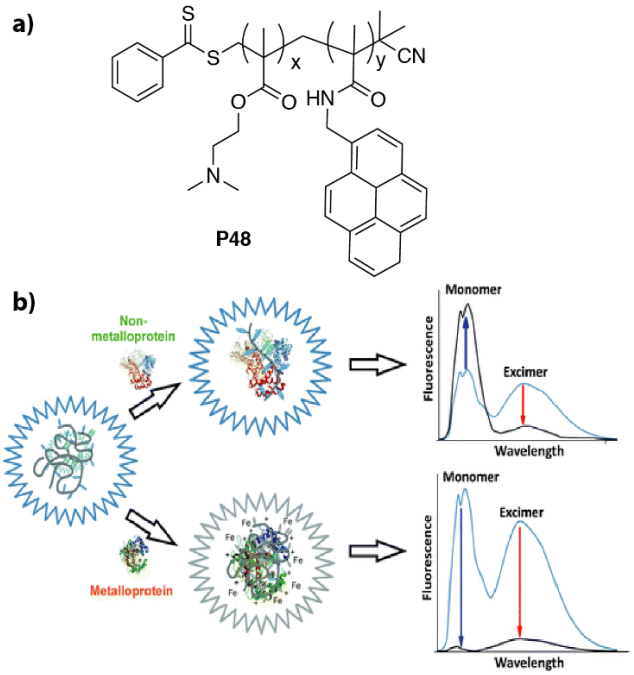


Figure 11. a) Copolymer **P48** b) Schematic representation of the interaction of **P48** with various non-metallo and metalloproteins.[59] Reproduced with permission of the Royal Society of Chemistry, from *J. Mater. Chem. B* **2018**, *6* (41), 6599–6606; permission conveyed through Copyright Clearance Center, Inc. Copyright 2018 Royal Society of Chemistry.

Despite establishing a fingerprint response towards multiple proteins, conditions and polymer wavelengths, the study did not include any multivariate analysis to classify them. In a simple, one-protein per well model with a specific pH and ionic strength, this is an adequate

system to distinguish proteins. However, any further studies in more complex mixtures will likely encounter issues with interfering species and system variation and some of these effects could be addressed by multivariate statistical analysis.

Bonizzoni and colleagues have reported a number of IDA-based array systems based on polymers decorated with various dyes.[113–116] Early work employed a water-soluble poly-(amidoamine) dendrimer (PAMAM) that incorporated a fluorescein dye to discriminate physiological phosphates.[113] The fluorescence of 5(6)-carboxyfluorescein was quenched upon binding to the dendrimer host, and displacement of the dye by various phosphates revived the intense fluorescence. PCA resolved the four biological phosphates in well-separated clusters, which was not possible by a univariate approach. Including additional dyes in IDA systems is likely to increase variability within a dataset and thus improve discrimination capacity.

Subsequent work by Bonizzoni and co-workers demonstrated the advantage of incorporating additional dyes, by including both fluorescein and 4-methylumbelliferyl phosphate dyes in their dendrimer IDA to discriminate organophosphates, including environmental contaminants and compounds used in the preparation of chemical warfare agents.[114,117] The degree of displacement of each dye from the PAMAM dendrimer depends on the relative affinities of the dye and the analyte guest to the dendrimer host. The inclusion of a second guest dye with the dendrimer host increased the number of distinct displacement interactions between analytes and host, and therefore increased the discriminatory power of the array. The multi-dye IDA was able to discriminate four organophosphates and phosphate at 800 μM using LDA with a 100% cross-validation accuracy (Figure 12a). Remarkably, the array achieved additional concentration dependent discrimination of three phosphates from 10 μM to 2 μM with 96% jack-knifed cross-validation accuracy (Figure 12b).

Carbohydrates play important roles in biology as fuel sources,[118] and in cellular recognition,[119] and there is therefore much interest in the detection and distinction of sugars. This task is challenging, as most mono- and disaccharide species have minimal structural differences beyond the configuration of selected stereocentres. Sensing of carbohydrates is commonly achieved through a boronic acid-diol displacement strategy, as aromatic boronic acids have a strong affinity for the vicinal diols found in sugars.[120] This affinity can by harnessed in an IDA strategy, in which a dye is covalently bound to a boronic acid and subsequently displaced by competitive binding of the boronic acid to a carbohydrate, with release of the dye resulting in an optical signal change.

Bonizzoni and colleagues utilized this boronic acid-diol IDA approach in the development of a sensor array for sugars, comprising two boronic acid modified PAMAM dendrimers and two fluorescent dyes, 4-methylesculetin and alizarin red S.[115] The displacement of each dye from the two dendrimer complexes at two pH values (7.4 and 10) was monitored by absorbance and fluorescence spectroscopy and fluorescence anisotropy. The combined results were processed using LDA, discriminating four sugars into well-separable clusters.

Another recent example of sugar-sensing by Bonizzoni and colleagues is a polymer-dye complex involving a copolymer of poly(methacrylic acid) and 3-(acrylamido)phenylboronic acid (PMAA-co-AAPBA).[116] Both a hematoxylin and cyanidin chloride dye illustrated suitable binding to the boronic acid functionality on the polymer and were examined as sensing units in an IDA approach. The dyes and their polymer-dye complexes were exposed to eight common sugars, and spectra were collected across multiple absorbance and emission wavelength channels. LDA revealed distinct responses to all eight sugars. Further examination of the data indicated that the cyanidin chloride dye and complex presented a low contribution to the discriminatory capacity of the array. Re-analysis using LDA of the eight sugars with only the hematoxylin dye complex successfully resolved all analytes. The authors noted that whilst clustering is slightly tighter in the multi-dye array, the single dye array presents a more practical option. After reducing to a single sensor system, another factor to consider is whether multivariate analysis was necessary for analyte identification in this case, or if closer analysis of absorbance and emission profiles would have been adequate to identify analytes.

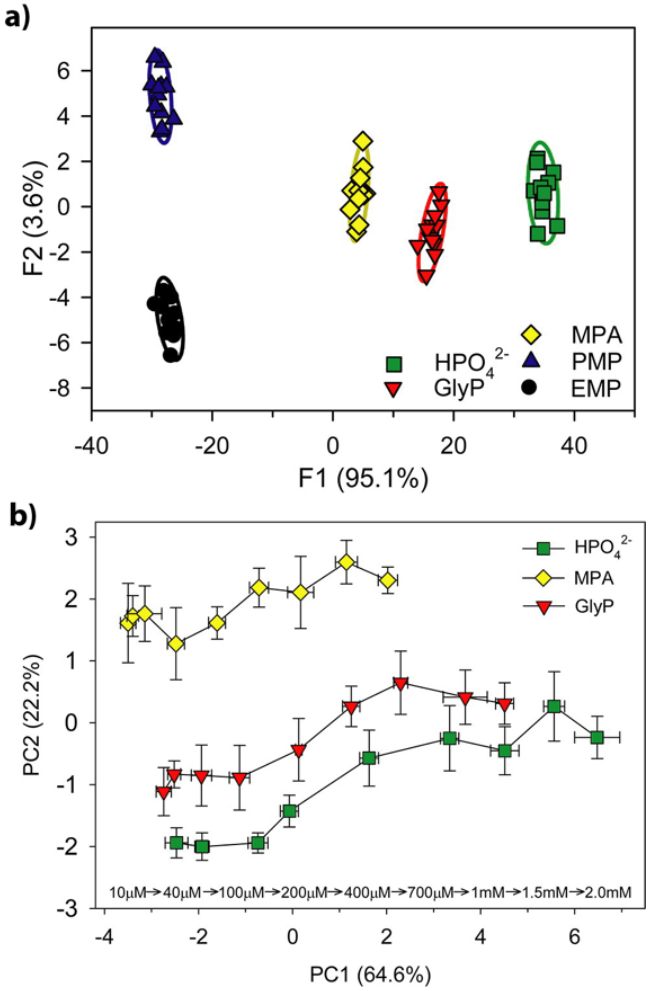


Figure 12. a) Two-dimensional LDA score plot for the response of five organophosphates, phosphate, glyphosate (GlyP), pinacolyl methylphosphonate (PMP), methylphosphonate (MPA) and ethyl methylphosphonate (EMP) at 800 μM. b) Two-dimensional LDA score plot for the analysis of three phosphates over a concentration range from 10 μM to 2 mM. Reprinted (adapted) with permission from *J. Am. Chem. Soc.* **2014**, *136* (40), 14223–14229. Copyright 2014, American Chemical Society.

The broad scope of analytes detected by the sensor arrays in this section highlights the benefit of using a modular approach between polymer and chromophore/fluorophore to create sensor diversity. A wide range of polymer architectures and optical elements are available, presenting an opportunity to produce large libraries of sensors by modifying either partner. The ability to choose different optical elements provides the flexibility to select and fine tune optimal features relating to wavelength, quantum yield and functionalization sites. Furthermore, the various polymer structures, namely dendrimers, homopolymers and copolymers, create different interaction surfaces, sizes and structures for sensors, allowing the detection of biomolecules as large as proteins and bacteria all the way down to small molecules such as phosphates and saccharides.

3.3 Molecularly imprinted polymer sensor arrays - Molecularly imprinted polymers[121] (MIPs) have, in recent years, been used as artificial receptors in a variety of sensing applications.[122,123] The molecular imprinting process involves the assembly of building blocks/monomers around a target template molecule through supramolecular interactions, and subsequent cross-linking polymerization to fix the spatial arrangement of these monomers.[8] Removal of the template yields a matrix containing cavities with specific functionality, geometry and size to enable selective recognition of the template molecule. MIP sensors provide access to the detection of analytes that may be difficult to sense selectively due to non-specific binding interactions. By including additional specifications regarding

orientation, shape and size, MIPs narrow the opportunity for non-selective binding. Upon initial consideration, it seems that MIP sensors would be poor candidates in a sensor array, where optimal sensing elements are preferentially cross-reactive rather than selective. It is therefore important to note that despite their successes, MIPs suffer from a few key limitations. In general, highly cross-linked materials have fairly rigid structures, which decrease the number of available binding sites.[122] Furthermore, these binding sites may not be uniform throughout the material and often consist of more lower affinity sites rather than high-affinity sites, because templating proceeds under kinetic rather than thermodynamic control.[8,124] Additionally, imprinted sites created by larger structures, such as proteins, may also have affinity to smaller biomolecules with similar binding sites.[123] These limitations generally lead to reduced selectivity and it is these shortcomings that often redirect these sensors for use in cross-reactive arrays.

Shimizu and colleagues conducted early work incorporating MIPs as sensing elements in cross-reactive arrays (Figure 13a).[17,21,124] Initially, they synthesized an eight polymer array, using seven different aryl amines as template molecules and one in the absence of a template.[17] The aryl amines were both pharmaceutically and biologically important amines such as propranolol, ephedrine and pseudoephedrine, as well as other structurally similar analytes (Figure 13b). The affinity of each polymer to each amine was examined by measuring the ratio of amine absorbance at 258 nm before and after shaking in acetonitrile. The imprinted polymers showed higher affinities than the non-imprinted polymer and also showed a significant change in absorbance not only for their imprinted analyte but also other structurally similar aryl amines. Responses for the first six aryl amines were analyzed using LDA, revealing 94% correct classification of analytes using a leave-one-out cross-validation method (Figure 13c). The study illustrated the effectiveness of using MIPs in the array, as each analyte showed the best response to its corresponding imprinted polymer, suggesting that selectivity within the array was likely due to the imprinting process. Whilst effective at discriminating these amines, each analyte required absorbance at the appropriate wavelength (258 nm), limiting the studies to UV-active analytes. Typically, MIPs contain no intrinsic signaling component, which is why this study utilized the absorbance of the unbound analyte and its modified response upon binding for discrimination of each analyte. An attractive strategy to address this limitation involves incorporating a chromophore as a signaling element within the MIP. Analyte binding will ideally cause a change in the spectral properties and signal response of the chromophore.[124]

Subsequent work by Shimizu and co-workers moved beyond sensing of UV-active analytes, by incorporating an IDA strategy into their MIP-based sensor array.[21] Similar to their previous study, they synthesized seven polymers, six imprinted with various aryl amines and one non-imprinted polymer. The final additional step in preparing the sensors involved the incorporation of a benzofurazan dye in each polymer scaffold. Benzofurazan is a small dye with similar functionality to aryl amines, so has sufficient binding affinity with each polymer, but strong absorption in a different region of the visible spectrum, reducing any potential interference from analyte absorbance. Each polymer was tested with seven amine analytes and the relative dye displacement response was measured. LDA was used to separate these responses into seven distinct clusters for each analyte, with 94% classification accuracy using leave-one-out cross-validation (Figure 13d).

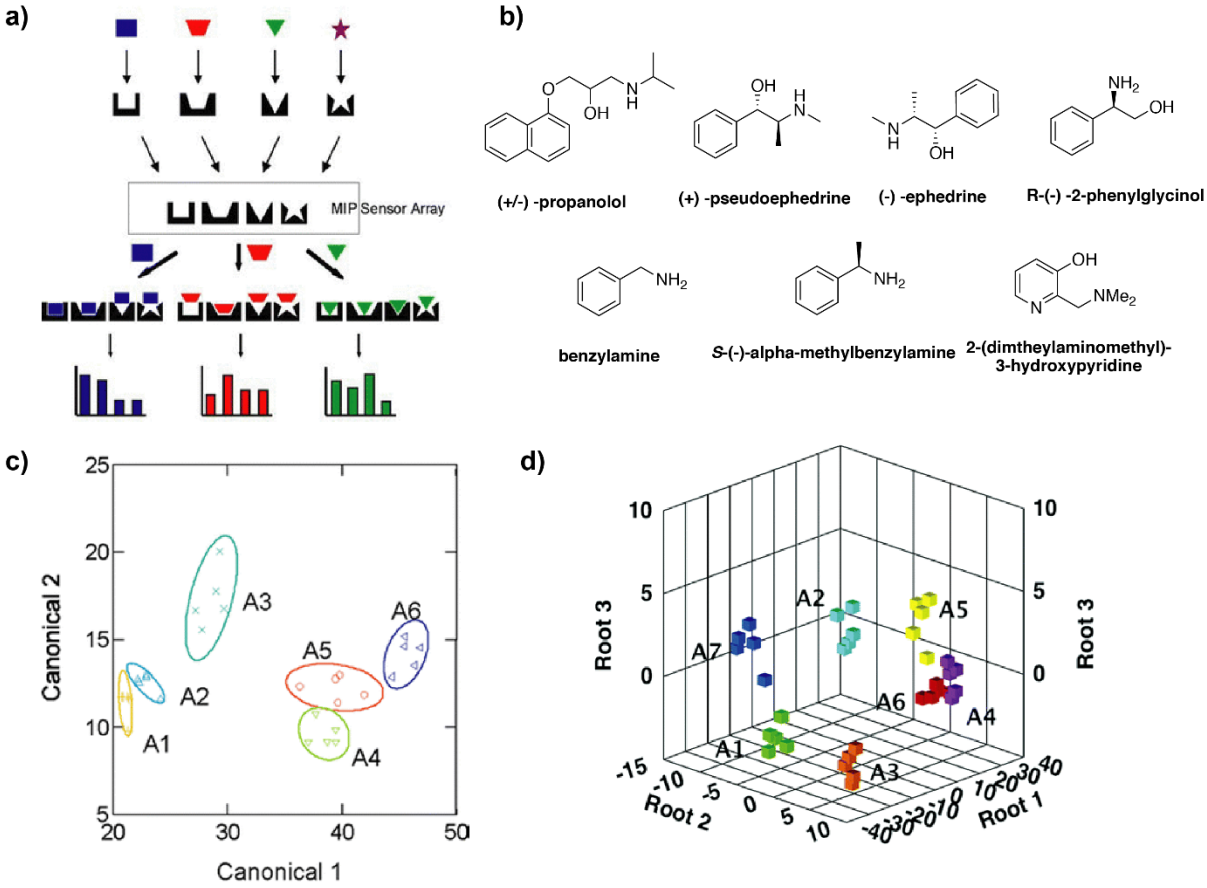


Figure 13. a) Schematic representation of MIP sensor arrays. b) Structures of seven aryl amines utilized as templates for MIPs. c) Two-dimensional LDA score plot of the responses of six amines tested against the MIP array. a)- c) Reproduced with permission of the Royal Society of Chemistry, from *Chem. Commun.* **2004**, 1172–117; permission conveyed through Copyright Clearance Center, Inc. Copyright 2004, Royal Society of Chemistry. d) Three-dimensional LDA score plot of the responses of seven amines against the MIP array. Reprinted (adapted) with permission from *J. Am. Chem. Soc.* **2005**, *127* (15), 5695–5700. Copyright 2005, American Chemical Society.

Yan and co-workers have constructed a number of sensor arrays using molecularly imprinted mesoporous silica as a sensing matrix.[125,126] They reported a metal ion sensor array system consisting of an 8-hydroxyquinoline (8-HQ) monomer covalently attached to the mesoporous silica, in the presence of two templates, Zn(II) and Cd(II), and in the absence of template during cross-linking.[125] 8-HQ was chosen as the fluorescent receptor as it forms fluorescent chelates with a number of metal ions. Unsurprisingly, the two imprinted sensors showed a higher affinity and fluorescence turn-on with each corresponding metal template than the non-imprinted sensor. The sensor array was subsequently tested with three additional metal analytes, Mg(II), Ca(II) and Al(III), at two different concentrations (10^{-4} M and 10^{-5} M). The characteristic response pattern for each metal ion was then analyzed using PCA to evaluate the discriminatory capabilities of the array. Each metal ion was clustered into a distinct group, as well as apparent sub-clustering of the two concentrations of each metal ion. The authors noted that the array was able to discriminate non-templated ions, together with templated ions, highlighting that using molecularly imprinted materials with high levels of cross-reactivity increases the number of analytes the array is capable of discriminating.

Subsequent work by the same group utilized a similar mesoporous silica matrix and an IDA strategy for saccharide discrimination.[126] The materials were constructed by covalently attaching a phenylboronic acid moiety to the mesoporous silica for saccharide discrimination, using D-fructose and D-xylose as templates, along with a nonimprinted material prepared without a carbohydrate template.[126] The two sugars selected as templates varied in size and structure, with different binding affinity to boronic acid, to create binding sites distinct enough

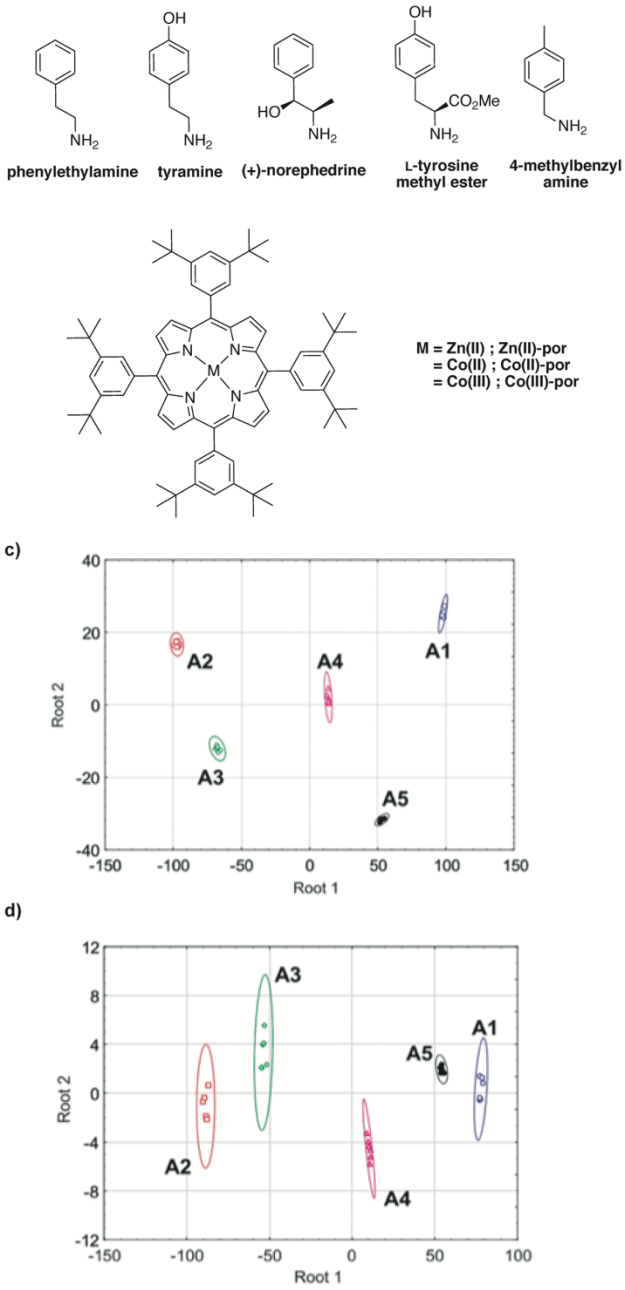


Figure 14. a) Structurally similar amine templates and analytes and b) metalloporphyrin dye structures with the metals Zn(II), Co(II) and Co(III). c) Two-dimensional LDA score plot for the response of 4 polymer channels combined with Zn(II)-porphyrin. d) Two-dimensional LDA score plot for the response of 4 polymer channels and Zn(II)-, Co(II)- and Co(III)- metalloporphyrins. Reprinted (adapted) from *Tetrahedron Lett.* **2013**, *54* (22), 2890–2893, Copyright 2013, with permission from Elsevier.

to capture a more diverse library of carbohydrates. Employing an IDA, the fluorescent dye Alizarin Red S binds to phenylboronic acid through adjacent diols present on the scaffold, resulting in a fluorescence response which is quenched upon displacement by various carbohydrates. Subsequent studies investigated a library of 10 saccharides: four five-carbon saccharides; five six-carbon saccharides and one disaccharide. PCA analysis illustrated tight non-overlapping clusters of all 10 saccharides, indicating both good reproducibility and great discriminatory power of the sensor array. The authors then tested their sensor array with three brands of orange juice, of which the major ingredients included fructose, sucrose and glucose. After confirming that other components of juice such as citric acid, sodium citrate and carotene, had no effect on the fluorescence response, the array was run and analyzed by PCA

to confirm successful discrimination of the three juices. Translation of this saccharide imprinted sensor array to real-world samples illustrates the advantages and feasibility of this type of system, particularly for future sensing systems.

Hong and co-workers have reported a system involving doping MIPs with metalloporphyrins in an array to discriminate a variety of structurally similar amines.[127] These amines comprised both primary and secondary amines and amines with other functional groups including a pharmaceutical agent. The four MIPs are synthesized using three amines as templates, phenethylamine, tyramine and (+)-norephedrine as well as a non-imprinted version (Figure 14a). The three metalloporphyrin dyes were prepared using 5,10,15,20-tetrakis-(3,5-di-tert-butylphenyl)-21H, 23H-porphine and the metals Zn(II), Co(II) and Co(III) (Figure 14b). The array was constructed by mixing amine analytes with each MIP, allowing the mixture to equilibrate, removing the MIPs and testing the supernatant containing all unbound amines with a solution of a metalloporphyrin. Absorbance measurements with different concentrations of MIP established that the spectral change of the dyes was a consequence of binding to analytes remaining in the supernatant, and therefore presented an indirect measure of the binding affinity of each analyte to the MIP. An initial test array utilized all four polymer channels combined with Zn(II)-porphyrin, against the three imprinted amines and two additional amines, L-tyrosine methyl ester and 4-methylbenzyl amine. LDA enabled accurate classification of all analytes, including the two amines not used during the imprinting process (Figure 14c). A 12-channel array consisting of the four polymers and all three metalloporphyrins was also tested with the five amines and resulted in a higher degree of visual discrimination using LDA, specifically tighter clusters and smaller confidence intervals (Figure 14d). The combination of MIPs and metalloporphyrins in this study capitalized upon the advantages of both materials, without the addition of costly design and synthesis processes. Specifically, the pattern recognition capabilities of MIPs and the useful absorbance properties of metalloporphyrins upon binding to analytes were together able to discriminate structurally similar amines, with improved distinguishability compared to one system alone.

It is evident that molecularly imprinted sensors are useful in array-based sensing applications on account of their capacity for molecular recognition, and the facile production of sensors. MIPs have the benefit of presenting large interaction surfaces to produce general differential responses to analytes, as well as containing regions tailored towards selective recognition created during the imprinting process.[21] Furthermore, this optimal combination of general and specific recognition properties in MIP arrays is accessed through minimal synthetic effort compared to other types of receptor, and large sensor libraries can be generated relatively quickly through modification of the template molecule only.

Since first reported in the early 1970s by Sarhan and Wulff,[128] MIPs have been extensively explored as receptor species in the academic literature, but their commercial exploitation has rarely been demonstrated. This gap in their development may be a consequence of limitations in production capabilities, such as challenges associated with preparing MIPs on a large scale, or issues with reproducibility arising from the kinetically-controlled nature of the templating process. More recent research developments, including ‘smart’ molecular imprinting approaches may accelerate the development of MIP-based receptors and sensors for commercial use.[122]

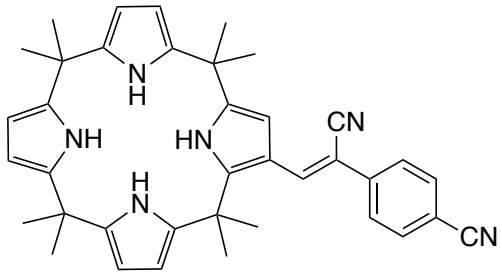
3.4 Sensor arrays constructed on polymer films

In addition to functioning as macromolecular receptor species, polymers can also present a convenient matrix upon which small molecule sensors can be deposited, or ‘doped’ to facilitate convenient fabrication of sensor arrays. A solid-state array is generally prepared by inclusion of sensing dyes within a polymer matrix such as polyurethane, or by the deposition of a pigment onto a porous polymer film, such as polyethylene terephthalate (PET). These polymer film sensor arrays are beneficial as they can improve the compatibility of aqueous systems, and in some cases are more user-friendly as they often do not require the dissolution or pipetting of sensors.[129]

Anzenbacher and colleagues have demonstrated the success of a doping strategy in an anion sensor array.[130] Their system incorporated a fluorescent calix[4]pyrrole probe (Figure

15a) with affinity to various anions, into ten poly(ether-urethane) hydrogel copolymer matrices (Figure 15b). The proportions of ethylene glycol ether and butylene glycol ether were varied to create different hydrophilic environments, with the aim to exploit the effect this would have on the transport of anions within the gel after exposure to water. Eight anions were tested with the ten polymer films doped with the fluorescent probe, namely acetate, benzoate, chloride, fluoride, hydrogen sulfide, cyanide, hydrogen phosphate, and hydrogen pyrophosphate. LDA of the fluorescence response illustrated 100% cross-validated classification accuracy. The sensor array was also applied to eight urine samples, providing a complex multi-electrolyte system to validate the array's versatility. The 10-sensor array was able to correctly classify the eight urine samples with 100% accuracy.

a)



b)

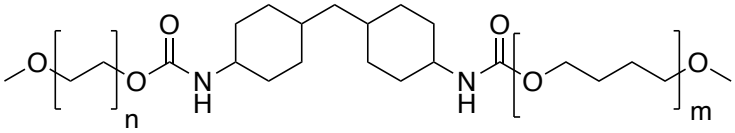


Figure 15 a) Structure of calix[4]pyrrole fluorescent probe. b) General structure of the 10 poly(ether-urethanes) hydrogel copolymer films used. Reprinted (adapted) with permission from *Chem. Eur. J.* **2013,** *19* (26), 8497-8506, Copyright 2013 John Wiley and Sons.

Another example utilizing a polymer matrix by Anzenbacher and colleagues is their sensor array for metal ion discrimination.[131] The system involves six sensors, **P49-P54**, made up of an extended conjugated fluorenes and a pyrene moiety attached to an 8-hydroxyquinoline (8-HQ) metal receptor (Figure 16), which are immobilized in a polyurethane film. The conjugated chromophores are partially quenched by 8-HQ, depending on the length of the fluorene bridges, with emission changes resulting from coordination to various metal ions. The hydrophilic film draws in the aqueous solution of metal ions, assisting complexation to the 8-HQ receptor and overcoming issues with solubility and lipophilicity. The responses to 9 metal cations were investigated with the six sensors and LDA classified all replicates with 100% accuracy. The authors performed an additional study to reduce the number of sensors required for metal ion discrimination.[132] PCA identified the key sensors responsible for the most variance within the dataset, and the original set of six sensors could be reduced to two sensors (**P50** and **P52**) that enabled accurate discrimination of 10 metal ions using LDA. The array's versatility was explored by determining its ability to discriminate different complex mixtures based on different concentrations of metal cations. Further studies of electrolyte drinks identified the best combination of sensors (**P50**, **P51** and **P53**) to achieve 100% classification accuracy in these complex samples.

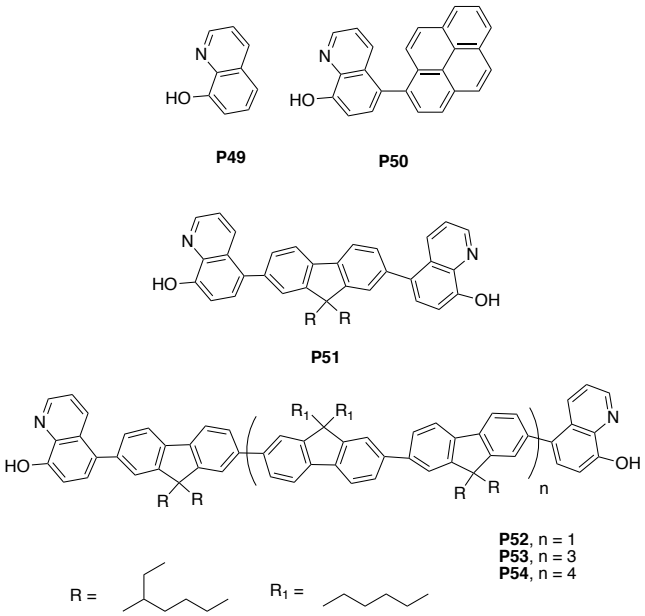


Figure 16. Structures of fluorene and pyrene sensors, **P49-P54**, modified with 8-hydroxyquinoline. Reproduced with permission of the Royal Society of Chemistry, from *Chem. Commun.* **2007**, *7345* (36), 3708–3710; permission conveyed through Copyright Clearance Center, Inc. Copyright 2007 Royal Society of Chemistry.

Suslick and colleagues have developed numerous colorimetric sensor arrays based on metalloporphyrins and other colorimetric dyes to discriminate diverse libraries of analytes.[10] A notable strategy involves the immobilization of a range of chemically responsive dyes onto an ormosil matrix and printing onto polyethylene terephthalate (PET) films.[32,133–135] The printed array is exposed to each analyte and a before and after image taken using a flatbed scanner. A difference map using RGB values can be generated from the difference between these images, creating a fingerprint response for each analyte. This approach has been demonstrated to discriminate a wide range of analytes, using a combination of different dye classes, namely metalloporphyrins, pH indicators, vapochromic dyes and redox responsive metal salts. A 36-element sensor array using these dye classes was printed and tested against 19 different toxic industrial chemicals (Figure 17).[133,134] HCA of the color-difference responses correctly classified all 19 toxins with 100% accuracy. The sensitivity of the array system was further investigated in a study testing a similar library of toxic, retaining 100% classification accuracy with estimated limits of detection in the ppb range.[135]

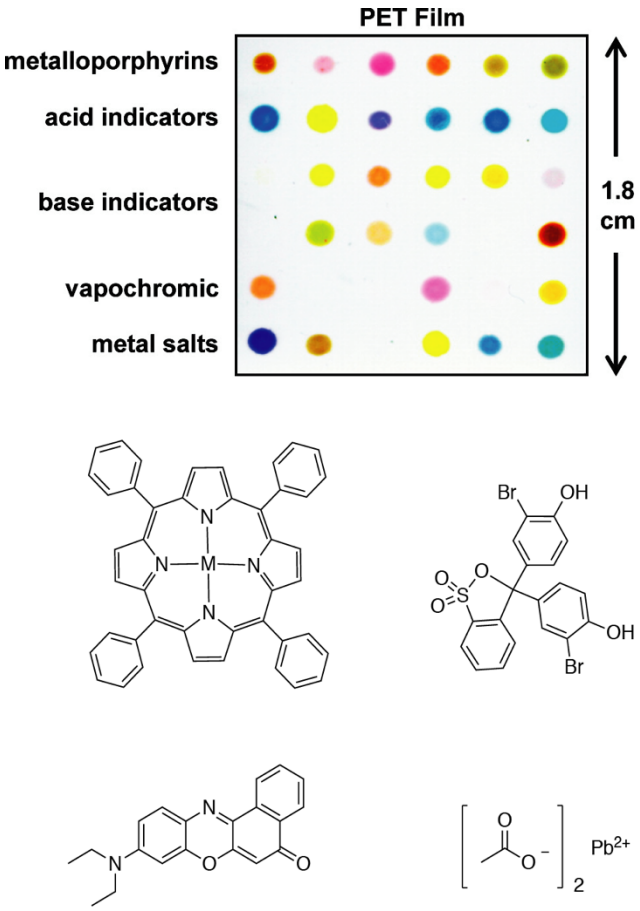


Figure 17. Example of a colorimetric sensor array with various dyes and pigments printed on a polyethylene terephthalate (PET) film. Reprinted (adapted) with permission from *Anal. Chem.* **2010**, *82* (22), 9433–9440. Copyright 2010, American Chemical Society.

Suslick and colleagues also used their sensor array system to discriminate various natural sugars and artificial sweeteners.[32,136] In this case, an IDA strategy was utilized, harnessing boronate ester formation between carbohydrate diols and boronic acids, which reduces the pH of the solution. 16 chemically responsive dyes were printed onto a PET film, and 3-nitrophenylboronic acid was included in the buffer solution. The addition of sugars altered the pH of the solution, causing changes in the colors of pH indicators in the array. The responses of 15 different sugars and sweeteners were processed using HCA and all replicates were classified correctly with 100% accuracy.[136] A subsequent study was also able to accurately discriminate different teas infused with sweeteners, demonstrating a potential real-world application for the sensor array.[32]

Recent work by Suslick and colleagues has yielded a hand-hand device reader containing colorimetric sensor array cartridges to improve the speed and sensitivity of gaseous analyte detection.[137] Sensors are printed onto a polypropylene (or similar) film, mounted to the cartridge and sealed. The cartridge is designed to be compatible for gaseous analytes by allowing a low-volume flow path for exposure. A cartridge of this design was subsequently utilized for the discrimination of aldehydes and ketones.[138] Exploiting the rapid reactivity between gaseous analytes and the solid-state sensor array elements, three amine-containing colorimetric dyes were printing onto the sensor array under various acidic conditions. The reaction of the printed anilines and phenylhydrazines with aldehydes and ketones led to imine formation and a subsequent change in UV-absorption. Color difference maps illustrated a cross-reactive response between the dyes and analytes. Seven aldehydes and eight ketones were measured at concentrations of both 25 and 0.5 ppm, and were easily discriminated using HCA, with 99.4% classification accuracy. Analysis also showed that aliphatic aldehydes were clustered separately from aromatic aldehydes. The same sensor array was also tested with various complex alcoholic liquid samples to demonstrate its potential in food inspection and security screening. After pre-oxidation of the alcohol analytes and exposure to the array, SVM analysis was performed, demonstrating effective discrimination of 6 liquor samples with 100% cross-validated accuracy. Additional work by Suslick and colleagues utilizing their rapid, gaseous detecting colorimetric

sensor array includes discrimination of air pollutants at risk of damaging sensitive artworks, other alcoholic liquors and volatiles emitted during fruit ripening.[139–141]

Bueno et al. have recently developed a colorimetric sensor array immobilized on cellulose acetate film to discriminate volatile amines.[142] The amines studied were identified as present during food spoilage and have the potential to give early warning signs for rotting food that may lead to food poisoning. Five pH sensitive dyes were immobilized by mixing with cellulose acetate and drying, before a small sample of each polymer-dye sensor was placed on a dish in a closed chamber. Exposure to each analyte caused a pattern-based colorimetric response of the sensor array and a before and after color map was captured. RGB values generated a unique pattern to discriminate between three amines (triethylamine, isobutylamine, isopentylamine). PCA and HCA of these responses revealed distinct clusters of each amine and no misclassifications, with successful discrimination achieved of samples at concentrations of 5 ppm, 2.5 ppm and 1 ppm. The applicability of the system for food quality applications was also demonstrated by testing in meat samples contaminated with each amine, again illustrating separate clusters of each amine contaminant.

Polymer films provide clear benefits for the development of optical sensor arrays. Primarily, the polymer matrix allows properties such as hydrophilicity and lipophilicity to be easily adjusted to suit the application without affecting the choice of indicator. Additionally, solid-state arrays enable convenient detection of both solution-phase and gaseous analytes and often avoid time-consuming solvent handling and pipetting of sensors required for traditional solution-based sensor systems. Finally, compared to previous polymetric materials, polymer film sensor arrays have seen the greatest progress towards the development of sensor array cartridges and portable and accessible devices to detect and analyze responses.

4. Conclusion and future directions

Whilst still a developing field, macromolecular optical sensor arrays have already been utilized in a number of successful sensing applications. In particular, significant progress has been made in sensing challenging biological systems such as bacteria, proteins and cellular surfaces. This review has highlighted the benefits of the cross-reactive array technique for these systems, specifically the different strategies that have been used to achieve cross-reactive recognition and the interpretation of these results using multivariate statistical analysis. We have focused on the use of polymers as a sensing element to emphasize the benefits of polymeric materials, both for their robust functionalization and their tunability of size, allowing for large diverse recognition surfaces and thus a capacity for good cross-reactivity. Finally, we have explored the optical detection strategies utilized in these systems, through either the inherent absorption or emission properties of the material, or the covalent attachment of chromophores/fluorophores. Importantly, it is the optimal combination of these aspects that we believe is responsible for the successful discrimination capacity within these sensor systems and the broad scope of analytes scrutinized in this review.

Unsurprisingly, in most cases the addition of more sensors gave better discrimination of analytes. However, a new generation of cross-reactive arrays has begun to emerge, involving other strategies to achieve diverse recognition capabilities. A common strategy involves using the same sensor in multiple channels and altering the pH, solvent, ionic strength and wavelengths of absorbance, excitation and emission. These array simplification strategies often improve the applicability of the system, by reducing the sensor-to-analyte ratio and decreasing the time spent designing and synthesizing additional sensors. Whilst a seemingly desirable strategy, it is crucial that these simplification steps do not limit the compatibility of the system in end-user applications. For example, a benefit of optical sensors within array systems lies in the ability to select a number of wavelengths for a single sensing element and has been successfully demonstrated in a number of systems. However, if the absorbance and emission wavelengths of these arrays do not match the common filters in optical instruments, it will be difficult to translate these systems beyond high-specification laboratory instruments. Similarly, altering the ionic strength of a solution to identify different proteins may be useful for training a sensor array, but would be of limited use for identifying unknown analytes in the likely event that the ionic strength of the protein solution is also unknown. IDAs are a popular approach in sensing applications and the examples addressed in all sections of the review highlight the benefits of including them in an array-based sensing approach. In particular, the marriage of the techniques provides added advantages for IDAs by discriminating between strongly and weakly displacing analytes at multiple concentrations.

While good discrimination was achieved using purely polymeric materials as sensing elements, there are numerous examples of systems that incorporate composite materials such as proteins, nanoparticles, peptides, mesoporous silica and photonic crystals, together with polymeric materials. In many of these instances, very good discrimination was achieved and allowed for more flexibility during

the design process. The success of these systems suggests that polymer composite materials present an exciting future direction for macromolecular sensor arrays.

The field of optical sensing, both selective sensors and sensor arrays, is dominated by the use of sensors decorated with only one recognition/reporter system. More recent work has highlighted the promise of tethering multiple sensors to a single scaffold, with small molecule systems reported that distinguish different drug molecules,[143] a broad range of inorganic and organic analytes[144] and β-amyloid aggregates.[145] Since macromolecular systems lend themselves to multifunctionalization, this strategy is likely to be promising for the future development of sensor arrays that comprise fewer discrete sensing elements.

Multivariate statistical techniques are the standard approach to analyze and interpret array responses to analyte addition. PCA, and more recently LDA, are commonly used to evaluate systems and classify analytes. Most examples display data in reduced dimensionality 2D and 3D score plots, allowing for easy interpretation of the results. To date, there are few examples of macromolecular sensor arrays harnessing more sophisticated multivariate techniques such as SVMs and ANNs, presenting exciting opportunities for progress in array development. A few key examples have presented further interrogation of the analysis by looking at loading plots, building an understanding of how sensors are responding to analytes. Whilst the hypothesis-free approach to the generation of sensing arrays allows for a more general interpretation of results, further examination of the data output and better understanding of sensor-analyte interactions may pave the way for the next generation of sensors.

Despite the benefits of macromolecular optical sensing arrays, there remain other challenges and limitations in the studies discussed above that must to be addressed to access the true potential of these systems, as is the case for all sensor arrays. Primarily, when designing and evaluating an array system, it is crucial that the end-user and application of the system are considered. Whilst all the studies we examined identified a key application for the sensing system, array systems which move out of the lab, and conduct analysis in clinical and environmental samples are the most impressive and highlight the necessity for the successful translation of these systems into the real world. Generally, sensing platforms based on optical detection strategies may require multiple sample preparation or clean-up steps, which increase the complexity of the analytical protocol, and may be sensitive to background interferants. Systems that rely on colorimetric responses have the advantage of easy interpretation by untrained users, as demonstrated by the success of lateral flow testing for pregnancy. Other optical-based detection strategies, such as fluorescence, typically display far greater sensitivity but require a more complex experimental setup and an increased level of user skill in interpretation of results. Consideration of these factors is important if the platform is to be used beyond the confines of the laboratory.

A great challenge of detection within clinical samples is the presence of different background interferents. For systems to be practically useful, it is important to explore discrimination beyond ‘spiked’ lab samples in a controlled environment and investigate if the system is robust enough to function in the presence of these contaminants and across the variable nature of clinical samples.

It is key that the sensing ability matches the application, for example having adequate limit of detection that is relevant to real samples, such as concentration levels of toxic metal contaminants.[89] Furthermore, the ability to distinguish not only different analytes, but high concentrations from low concentrations is often relevant, and a few examples impressively illustrate this kind of testing.[50,114]

More broadly, the development of functional sensing platforms requires a broad range of expertise, from fundamental (bio)chemical research to device and interface design, along with the need to establish rigorous quality analysis and ensure compliance with regulatory frameworks. An interesting discussion on the challenges of bringing biosensing devices to the market can be found in an excellent article by Sia and coworkers.[146]

Finally, improvements in technology will certainly play a role in the future of array-based sensing. The development of smart phone technology may provide a scope for improving the accessibility of sensor array technology, however it is important to ensure the system is compatible with constantly evolving technology. Instead, the development of portable devices for testing methods may be more applicable for array-based sensing and have already shown some promise for colorimetric sensor arrays.

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References:

(1) Hare, D. J.; New, E. J. On the Outside Looking in: Redefining the Role of Analytical Chemistry in the Biosciences. *Chem. Commun.* **2016**, *52* (58), 8918–8934.

(2) Pendin, D.; Greotti, E.; Lefkimmiatis, K.; Pozzan, T. Exploring Cells with Targeted Biosensors. *J. Gen. Physiol.* **2016**, *149* (1), 1–36.

(3) Carter, K. P.; Young, A. M.; Palmer, A. E. Fluorescent Sensors for Measuring Metal Ions in Living Systems. *Chem. Rev.* **2014**, *114* (8), 4564–4601.

(4) Kaur, A.; New, E. J. Bioinspired Small-Molecule Tools for the Imaging of Redox Biology. *Acc. Chem. Res.* **2019**, *52* (3), 623–632.

(5) Ong, J. X.; Lim, C. S. Q.; Le, H. Van; Ang, W. H. A Ratiometric Fluorescent Probe for Cisplatin: Investigating the Intracellular Reduction of Platinum(IV) Prodrug Complexes. *Angew. Chem. Int. Ed.* **2019**, *58* (1), 164–167.

(6) Wu, D.; Sedgwick, A. C.; Gunnlaugsson, T.; Akkaya, E. U.; Yoon, J.; James, T. D. Fluorescent Chemosensors: The Past, Present and Future. *Chem. Soc. Rev.* **2017**, *46* (23), 7105–7123.

(7) Ibañez, G. A.; Escandar, G. M. Fluorescence and Phosphorescence Chemical Sensors Applied to Water Samples. In *Smart Sensors for Real-Time Water Quality Monitoring. Smart Sensors, Measurement and Instrumentation*; Springer Berlin Heidelberg, 2013; pp 45–65.

(8) Mahon, C. S.; Fulton, D. A. Mimicking Nature with Synthetic Macromolecules Capable of Recognition. *Nat. Chem.* **2014**, *6* (8), 665–672.

(9) New, E. J. Harnessing the Potential of Small Molecule Intracellular Fluorescent Sensors. *ACS Sensors* **2016**, *1* (4), 328–333.

(10) Li, Z.; Askim, J. R.; Suslick, K. S. The Optoelectronic Nose: Colorimetric and Fluorometric Sensor Arrays. *Chem. Rev.* **2019**, *119* (1), 231–292.

(11) Anzenbacher, P.; Lubal, P.; Buek, P.; Palacios, M. A.; Kozelkova, M. E. A Practical Approach to Optical Cross-Reactive Sensor Arrays. *Chem. Soc. Rev.* **2010**, *39* (10), 3954–3979.

(12) You, L.; Zha, D.; Anslyn, E. V. Recent Advances in Supramolecular Analytical Chemistry Using Optical Sensing. *Chem. Rev.* **2015**, *115* (15), 7840–7892.

(13) Huynh, T. P.; Kutner, W. Molecularly Imprinted Polymers as Recognition Materials for Electronic Tongues. *Biosens. Bioelectron.* **2015**, *74*, 856–864.

(14) Ramanathan, K.; Danielsson, B. Principles and Applications of Thermal Biosensors. *Biosens. Bioelectron.* **2001**, *16* (6), 417–423.

(15) Tan, C.; Pinto, M. R.; Schanze, K. S. Photophysics, Aggregation and Amplified Quenching of a Water-Soluble Poly(Phenylene Ethynylene). *Chem. Commun.* **2002**, *2* (5), 446–447.

(16) Alvarez, A.; Costa-Fernández, J. M.; Pereiro, R.; Sanz-Medel, A.; Salinas-Castillo, A. Fluorescent Conjugated Polymers for Chemical and Biochemical Sensing. *Trends Anal. Chem.* **2011**, *30* (9), 1513–1525.

(17) Greene, N. T.; Morgan, S. L.; Shimizu, K. D. Molecularly Imprinted Polymer Sensor Arrays. *Chem. Commun.* **2004**, 1172–1173.

(18) Miranda, O. R.; You, C. C.; Phillips, R.; Kim, I. B.; Ghosh, P. S.; Bunz, U. H. F.; Rotello, V. M. Array-Based Sensing of Proteins Using Conjugated Polymers. *J. Am. Chem. Soc.* **2007**, *129* (32), 9856–9857.

(19) Askim, J. R.; Mahmoudi, M.; Suslick, K. S. Optical Sensor Arrays for Chemical Sensing: The Optoelectronic Nose. *Chem. Soc. Rev.* **2013**, *42* (22), 8649–8682.

(20) Gooding, J. J.; Gaus, K. Single-Molecule Sensors: Challenges and Opportunities for Quantitative Analysis. *Angew. Chem. Int. Ed.* **2016**, *55* (38), 11354–11366.

(21) Greene, N. T.; Shimizu, K. D. Colorimetric Molecularly Imprinted Polymer Sensor Array Using Dye Displacement. *J. Am. Chem. Soc.* **2005**, *127* (15), 5695–5700.

(22) Nelson, T. L.; O'Sullivan, C.; Greene, N. T.; Maynor, M. S.; Lavigne, J. J. Cross-Reactive Conjugated Polymers: Analyte-Specific Aggregative Response for Structurally Similar Diamines. *J. Am. Chem. Soc.* **2006**, *128* (17), 5640–5641.

(23) New, E. J. Harnessing the Potential of Small Molecule Intracellular Fluorescent Sensors. *ACS Sensors* **2016**, *1*, 328–333.

(24) Albert, K. J.; Lewis, N. S.; Schauer, C. L.; Sotzing, G. A.; Stitzel, S. E.; Vaid, T. P.; Walt, D. R. Cross-Reactive Chemical Sensor Arrays. *Chem. Rev.* **2000**, *100* (7), 2595–2626.

(25) Svechkarev, D.; Sadykov, M. R.; Bayles, K. W.; Mohs, A. M. Ratiometric Fluorescent Sensor Array as a Versatile Tool for Bacterial Pathogen Identification and Analysis. *ACS Sensors* **2018**, *3* (3), 700–708.

(26) Aron, A. T.; Ramos-Torres, K. M.; Cotruvo, J. A.; Chang, C. J. Recognition- and Reactivity-Based Fluorescent Probes for Studying Transition Metal Signaling in Living Systems. *Acc. Chem. Res.* **2015**, *48* (8), 2434–2442.

(27) Bruemmer, K. J.; Crossley, S. W. M.; Chang, C. J. Activity-Based Sensing: A Synthetic Methods Approach for Selective Molecular Imaging and Beyond. *Angew. Chem. Int. Ed.* **2020**, *59* (33), 13734–13762.

(28) Nguyen, B. T.; Anslyn, E. V. Indicator-Displacement Assays. *Coord. Chem. Rev.* **2006**, *250* (23–24), 3118–3127.

(29) Sedgwick, A. C.; Brewster, J. T.; Wu, T.; Feng, X.; Bull, S. D.; Qian, X.; Sessler, J. L.; James, T. D.; Anslyn, E. V.; Sun, X. Indicator Displacement Assays (IDAs): The Past, Present and Future. *Chem. Soc. Rev.* **2020,** DOI:10.1039/C9CS00538B.

(30) Buryak, A.; Severin, K. A Chemosensor Array for the Colorimetric Identification of 20 Natural Amino Acids. *J. Am. Chem. Soc.* **2005**, *127* (11), 3700–3701.

(31) Schiller, A.; Wessling, R. A.; Singaram, B. A Fluorescent Sensor Array for Saccharides Based on Boronic Acid Appended Bipyridinium Salts. *Angew. Chem. Int. Ed.* **2007**, *46* (34), 6457–6459.

(32) Musto, C. J.; Lim, S. H.; Suslick, K. S. Colorimetric Detection and Identification of Natural and Artificial Sweeteners. *Anal. Chem.* **2009**, *81* (15), 6526–6533.

(33) Lavigne, J. J.; Anslyn, E. V. Sensing a Paradigm Shift in the Field of Molecular Recognition: From Selective to Differential Receptors. *Angew. Chem. Int. Ed.* **2001**, *40*, 3118–3130.

(34) Geng, Y.; Peveler, W. J.; Rotello, V. M. Array-Based "Chemical Nose" Sensing in Diagnostics and Drug Discovery. *Angew. Chem. Int. Ed.* **2019**, 5190–5200.

(35) Peveler, W. J.; Landis, R. F.; Yazdani, M.; Day, J. W.; Modi, R.; Carmalt, C. J.; Rosenberg, W. M.; Rotello, V. M. A Rapid and Robust Diagnostic for Liver Fibrosis Using a Multichannel Polymer Sensor Array. *Adv. Mater.* **2018**, *30* (28), 2–7.

(36) Han, J.; Ma, C.; Wang, B.; Bender, M.; Bojanowski, M.; Hergert, M.; Seehafer, K.; Herrmann, A.; Bunz, U. H. F. A Hypothesis-Free Sensor Array Discriminates Whiskies for Brand, Age, and Taste. *Chem* **2017**, *2* (6), 817–824.

(37) Keshri, G.; Magan, N. Detection and Differentiation between Mycotoxigenic and Non-Mycotoxigenic Strains of Two Fusarium Spp. Using Volatile Mycotoxigenic Strains of Two Production Profiles and Hydrolytic Enzymes. *J. Appl. Microbiol.* **2000**, *89* (5), 825–833.

(38) Severin, E. J.; Doleman, B. J.; Lewis, N. S. An Investigation of the Concentration Dependence and Response to Analyte Mixtures of Carbon Black/Insulating Organic Polymer Composite Vapor Detectors. *Anal. Chem.* **2000**, *72* (4), 658–668.

(39) Huang, W.; Smarsly, E.; Han, J.; Bender, M.; Seehafer, K.; Wacker, I.; Schröder, R. R.; Bunz, U. H. F. Truxene-Based Hyperbranched Conjugated Polymers: Fluorescent Micelles Detect Explosives in Water. *ACS Appl. Mater. Interfaces* **2017**, *9* (3), 3068–3074.

(40) Huang, W.; Bender, M.; Seehafer, K.; Wacker, I.; Schröder, R. R.; Bunz, U. H. F. A Tetraphenylethene-Based Polymer Array Discriminates Nitroarenes. *Macromolecules* **2018**, *51* (4), 1345–1350.

(41) Maynor, M. S.; Nelson, T. L.; O'Sullivan, C.; Lavigne, J. J. A Food Freshness Sensor Using the Multistate Response from Analyte-Lnduced Aggregation of a Cross-Reactive Poly(Thiophene). *Org. Lett.* **2007**, *9* (17), 3217–3220.

(42) Arrieta, Á. A.; Rodríguez-Méndez, M. L.; de Saja, J. A.; Blanco, C. A.; Nimubona, D. Prediction of Bitterness and Alcoholic Strength in Beer Using an Electronic Tongue. *Food Chem.* **2010**, *123* (3), 642–646.

(43) Smith, D. G.; Mitchell, L.; New, E. J. Pattern Recognition of Toxic Metal Ions Using a Single-Probe Thiocoumarin Array. *Analyst* **2019**, *144* (1), 230–236.

(44) Bowyer, A. A.; Shen, C.; New, E. J. A Fluorescent Three-Sensor Array for Heavy Metals in Environmental Water Sources. *Analyst* **2020**, *145* (4), 1195–1201.

(45) Mallet, A. M.; Davis, A. B.; Davis, D. R.; Panella, J.; Wallace, K. J.; Bonizzoni, M. A Cross Reactive Sensor Array to Probe Divalent Metal Ions. *Chem. Commun.* **2015**, *51* (95), 16948–16951.

(46) Kim, I. B.; Dunkhorst, A.; Bunz, U. H. F. Nonspecific Interactions of a Carboxylate-Substituted PPE with Proteins. A Cautionary Tale for Biosensor Applications. *Langmuir* **2005**, *21* (17), 7985–7989.

(47) De, M.; Rana, S.; Akpinar, H.; Miranda, O. R.; Arvizo, R. R.; Bunz, U. H. F.; Rotello, V. M. Sensing of Proteins in Human Serum Using Conjugates of Nanoparticles and Green Fluorescent Protein. *Nat. Chem.* **2009**, *1* (6), 461–465.

(48) Rana, S.; Elci, S. G.; Mout, R.; Singla, A. K.; Yazdani, M.; Bender, M.; Bajaj, A.; Saha, K.; Bunz, U. H. F.; Jirik, F. R.; Rotello, V. M. Ratiometric Array of Conjugated Polymers-Fluorescent Protein Provides a Robust Mammalian Cell Sensor. *J. Am. Chem. Soc.* **2016**, *138* (13), 4522–4529.

(49) Sener, G.; Uzun, L.; Denizli, A. Colorimetric Sensor Array Based on Gold Nanoparticles and Amino Acids for Identification of Toxic Metal Ions in Water. *ACS Appl. Mater. Interfaces* **2014**, *6* (21), 18395–18400.

(50) You, C. C.; Miranda, O. R.; Gider, B.; Ghosh, P. S.; Kim, I. B.; Erdogan, B.; Krovi, S. A.; Bunz, U. H. F.; Rotello, V. M. Detection and Identification of Proteins Using Nanoparticle-Fluorescent Polymer "Chemical Nose" Sensors. *Nat. Nanotechnol.* **2007**, *2* (5), 318–323.

(51) Fahimi-Kashani, N.; Hormozi-Nezhad, M. R. Gold Nanorod-Based Chrono-Colorimetric Sensor Arrays: A Promising Platform for Chemical Discrimination Applications. *ACS Omega* **2018**, *3* (2), 1386–1394.

(52) Bigdeli, A.; Ghasemi, F.; Golmohammadi, H.; Abbasi-Moayed, S.; Nejad, M. A. F.; Fahimi-Kashani, N.; Jafarinejad, S.; Shahrajabian, M.; Hormozi-Nezhad, M. R. Nanoparticle-Based Optical Sensor Arrays. *Nanoscale* **2017**, *9* (43), 16546–16563.

(53) Abbasi-Moayed, S.; Hormozi-Nezhad, M. R.; Maaza, M. A Multichannel Single-Well Sensor Array for Rapid and Visual Discrimination of Catecholamine Neurotransmitters. *Sensors Actuators, B Chem.* **2019**, *296* (June), 126691.

(54) Anslyn, E. V. Supramolecular Analytical Chemistry. *J. Org. Chem.* **2007**, *72* (3), 687–699.

(55) Bunz, U. H. F. Poly(Aryleneethynylene)s: Syntheses, Properties, Structures, and Applications. *Chem. Rev.* **2000**, *100* (4), 1605–1644.

(56) Han, J.; Wang, B.; Bender, M.; Kushida, S.; Seehafer, K.; Bunz, U. H. F. Poly(Aryleneethynylene) Tongue That Identifies Nonsteroidal Anti-Inflammatory Drugs in Water: A Test Case for Combating Counterfeit Drugs. *ACS Appl. Mater. Interfaces* **2017**, *9* (1), 790–797.

(57) Ngernpimai, S.; Geng, Y.; Makabenta, J. M.; Landis, R. F.; Keshri, P.; Gupta, A.; Li, C. H.; Chompoosor, A.; Rotello, V. M. Rapid Identification of Biofilms Using a Robust Multichannel Polymer Sensor Array. *ACS Appl. Mater. Interfaces* **2019**, *11* (12), 11202–11208.

(58) Han, J.; Cheng, H.; Wang, B.; Braun, M. S.; Fan, X.; Bender, M.; Huang, W.; Domhan, C.; Mier, W.; Lindner, T.; Seehafer, K.; Wink, M.; Bunz, U. H. F. A Polymer/Peptide Complex-Based Sensor Array That Discriminates Bacteria in Urine. *Angew. Chem. Int. Ed.* **2017**, *56* (48), 15246–15251.

(59) Kapf, A.; Albrecht, M. Discrimination of Proteins through Interaction with Pyrene-Labelled Polymer Aggregates. *J. Mater. Chem. B* **2018**, *6* (41), 6599–6606.

(60) Smith, D. G.; Topolnicki, I. L.; Zwicker, V. E.; Jolliffe, K. A.; New, E. J. Fluorescent Sensing Arrays for Cations and Anions. *Analyst* **2017**, 3549–3563.

(61) Cunningham, P.; Cord, M.; Delany, S. J. *Machine Learning Techniques for Multimedia*, Springer, Berlin, Heidelberg, 2008.

(62) Hair, J. F.; Black, B.; Babin, B.; Anderson, R. E.; Tatham, R. L. *Mutivariate Data Analysis*, 6th ed.; Pearson, 2006.

(63) Flury, B. K.; Riedwyl, H. Standard Distance in Univariate and Multivariate Analysis. *Am. Stat.* **1986**, *40* (3), 249–251.

(64) Frades, I.; Matthiesen, R. *Overview on Techniques in Cluster Analysis*; Humana Press, 2010.

(65) Landau, S.; Chis Ster, I. Cluster Analysis: Overview. *Int. Encycl. Educ.* **2010**, No. December, 72–83.

(66) Jollife, I. T.; Cadima, J. Principal Component Analysis: A Review and Recent Developments. *Philos. Trans. R. Soc. A Math. Phys. Eng. Sci.* **2016**, *374* (2065), 20150202.

(67) Wold, S.; Esbensen, K.; Geladi, P. Principal Component Analysis. *Chemom. Intell. Lab. Syst.* **1987**, *2* (1–3), 37–52.

(68) Ringnér, M. What Is Principal Component Analysis? *Nat. Biotechnol.* **2008**, *26* (3), 303–304.

(69) Stewart, S.; Ivy, M. A.; Anslyn, E. V. The Use of Principal Component Analysis and Discriminant Analysis in Differential Sensing Routines. *Chem. Soc. Rev.* **2014**, *43* (1), 70–84.

(70) Izenman, A. J. Linear Discriminant Analysis. In *Modern Multivariate Statistical Techniques: Regression, Classification, and Manifold Learning*; Springer New York: New York, NY, 2008; pp 237–280.

(71) Webb, A. R.; Copsey, K. D. Linear Discriminant Analysis. In *Statistical Pattern Recognition*; John Wiley & Sons, 2011; pp 221–273.

(72) Molinaro, A. M.; Simon, R.; Pfeiffer, R. M. Prediction Error Estimation: A Comparison of Resampling Methods. *Bioinformatics* **2005**, *21* (15), 3301–3307.

(73) Bajaj, A.; Miranda, O. R.; Phillips, R.; Kim, I. B.; Jerry, D. J.; Bunz, U. H. F.; Rotello, V. M. Array-Based Sensing of Normal, Cancerous, and Metastatic Cells Using Conjugated Fluorescent Polymers. *J. Am. Chem. Soc.* **2010**, *132* (3), 1018–1022.

(74) Leech, N. L.; Barrett, K. C.; Morgan, G. A. *SPSS for Intermediate Statistics: Use and Interpretation.*; Psychology Press, 2005.

(75) Tropp, J.; Ihde, M. H.; Williams, A. K.; White, N. J.; Eedugurala, N.; Bell, N. C.; Azoulay, J. D.; Bonizzoni, M. A Sensor Array for the Discrimination of Polycyclic Aromatic Hydrocarbons Using Conjugated Polymers and the Inner Filter Effect. *Chem. Sci.* **2019**, *10* (44), 10247–10255.

(76) Kung, S. Y. *Kernel Methods and Machine Learning*; Cambridge University Press: Cambridge, 2014.

(77) Krogh, A. What Are Artificial Neural Networks? *Nat. Biotechnol.* **2008**, *26* (2), 195–197.

(78) Burns, J. A.; Whitesides, G. M. Feed-Forward Neural Networks in Chemistry: Mathematical Systems for Classification and Pattern Recognition. *Chem. Rev.* **1993**, *93* (8), 2583–2601.

(79) Priddy, K. L.; Keller, P. E. *Artificial Neural Networks: An Introduction*; SPIE press: Belingham, Washington USA, 2005.

(80) Bishop, C. M. *Neural Networks for Pattern Recognition*; Oxford University Press: Oxford, 1995.

(81) Pei, Q. Light-Emitting Polymers. *Mater. Matters* **2007**, *2.3* (26), 1–9.

(82) Thomas, S. W.; Joly, G. D.; Swager, T. M. Chemical Sensors Based on Amplifying Fluorescent Conjugated Polymers. *Chem. Rev.* **2007**, *107* (4), 1339–1386.

(83) Woodka, M. D.; Schnee, V. P.; Polcha, M. P. Fluorescent Polymer Sensor Array for Detection and Discrimination of Explosives in Water. *Anal. Chem.* **2010**, *82* (23), 9917–9924.

(84) Wu, D.; Schanze, K. S. Protein Induced Aggregation of Conjugated Polyelectrolytes Probed with Fluorescence Correlation Spectroscopy: Application to Protein Identification. *ACS Appl. Mater. Interfaces* **2014**, *6* (10), 7643–7651.

(85) Freudenberg, J.; Hinkel, F.; Jänsch, D.; Bunz, U. H. F. Chemical Tongues and Noses Based upon Conjugated Polymers. *Top. Curr. Chem.* **2017**, *375* (4), 67.

(86) Blayney, A. J.; Perepichka, I. F.; Wudl, F.; Perepichka, D. F. Advances and Challenges in the Synthesis of Poly(p-Phenylene Vinylene)-Based Polymers. *Isr. J. Chem.* **2014**, *54* (5–6), 674–688.

(87) Perepichka, I. F.; Perepichka, D. F.; Meng, H.; Wudl, F. Light-Emitting Polythiophenes. *Adv. Mater.* **2005**, *17* (19), 2281–2305.

(88) Lee, S. H.; Kömürlü, S.; Zhao, X.; Jiang, H.; Moriena, G.; Kleiman, V. D.; Schanze, K. S. Water-Soluble Conjugated Polyelectrolytes with Branched Polyionic Side Chains. *Macromolecules* **2011**, *44* (12), 4742–4751.

(89) Wu, Y.; Tan, Y.; Wu, J.; Chen, S.; Chen, Y. Z.; Zhou, X.; Jiang, Y.; Tan, C. Fluorescence Array-Based Sensing of Metal Ions Using Conjugated Polyelectrolytes. *ACS Appl. Mater. Interfaces* **2015**, *7* (12), 6882–6888.

(90) Yao, Z.; Feng, X.; Hong, W.; Li, C.; Shi, G. A Simple Approach for the Discrimination of Nucleotides Based on a Water-Soluble Polythiophene Derivative. *Chem. Commun.* **2009**, *405* (31), 4696–4698.

(91) Cooper, J. R.; Bloom, F. E.; Roth, R. H. *The Biochemical Basis of Neuropharmacology*; Oxford University Press, USA, 2003.

(92) Nelson, T. L.; Tran, I.; Ingallinera, T. G.; Maynor, M. S.; Lavigne, J. J. Multi-Layered Analyses Using Directed Partitioning to Identify and Discriminate between Biogenic Amines. *Analyst* **2007**, *132* (10), 1024–1030.

(93) Tropp, J.; Ihde, M. H.; Crater, E. R.; Bell, N. C.; Bhatta, R.; Johnson, I. C.; Bonizzoni, M.; Azoulay, J. D. A Sensor Array for the Nanomolar Detection of Azo Dyes in Water. *ACS Sensors* **2020**, *5* (6), 1541–1547.

(94) Wu, J.; Tan, C.; Chen, Z.; Chen, Y. Z.; Tan, Y.; Jiang, Y. Fluorescence Array-Based Sensing of Nitroaromatics Using Conjugated Polyelectrolytes. *Analyst* **2016**, *141* (11), 3242–3245.

(95) Han, J.; Bender, M.; Hahn, S.; Seehafer, K.; Bunz, U. H. F. Polyelectrolyte Complexes Formed from Conjugated Polymers: Array-Based Sensing of Organic Acids. *Chem. - A Eur. J.* **2016**, *22* (10), 3230–3233.

(96) Han, J.; Wang, B.; Bender, M.; Seehafer, K.; Bunz, U. H. F. Water-Soluble Poly(p-Aryleneethynylene)s: A Sensor Array Discriminates Aromatic Carboxylic Acids. *ACS Appl. Mater. Interfaces* **2016**, *8* (31), 20415–20421.

(97) Wang, B.; Han, J.; Bender, M.; Seehafer, K.; Bunz, U. H. F. Array-Based Sensing of Explosives by Water-Soluble Poly(p-Phenyleneethynylene)s. *Macromolecules* **2017**, *50* (11), 4126–4131.

(98) Huang, W.; Seehafer, K.; Bunz, U. H. F. Discrimination of Flavonoids by a Hypothesis Free Sensor Array. *ACS Appl. Polym. Mater.* **2019**, *1* (6), 1301–1307.

(99) Wang, B.; Han, J.; Bojanowski, N. M.; Bender, M.; Ma, C.; Seehafer, K.; Herrmann, A.; Bunz, U. H. F. An Optimized Sensor Array Identifies All Natural Amino Acids. *ACS Sensors* **2018**, *3* (8), 1562–1568.

(100) Wang, B.; Han, J.; Ma, C.; Bender, M.; Seehafer, K.; Herrmann, A.; Bunz, U. H. F. A Simple Optoelectronic Tongue Discriminates Amino Acids. *Chem. - A Eur. J.* **2017**, *23* (51), 12471–12474.

(101) Kim, I. B.; Han, M. H.; Phillips, R. L.; Samanta, B.; Rotello, V. M.; Zhang, Z. J.; Bunz, U. H. F. Nano-Conjugate Fluorescence Probe for the Discrimination of Phosphate and Pyrophosphate. *Chem. - A Eur. J.* **2009**, *15* (2), 449–456.

(102) Bojanowski, N. M.; Bender, M.; Seehafer, K.; Bunz, U. H. F. Discrimination of Saccharides by a Simple Array. *Chem. - A Eur. J.* **2017**, *23* (50), 12253–12258.

(103) Han, J.; Wang, B.; Bender, M.; Pfisterer, J.; Huang, W.; Seehafer, K.; Yazdani, M.; Rotello, V. M.; Rotello, C. M.; Bunz, U. H. F. Fingerprinting Antibiotics with PAE-Based Fluorescent Sensor Arrays. *Polym. Chem.* **2017**, *8* (17), 2723–2732.

(104) Han, J.; Wang, B.; Bender, M.; Seehafer, K.; Bunz, U. H. F. Poly(p-Phenyleneethynylene)-Based Tongues Discriminate Fruit Juices. *Analyst* **2017**, *142* (3), 537–543.

(105) Bojanowski, N. M.; Hainer, F.; Bender, M.; Seehafer, K.; Bunz, U. H. F. An Optical Sensor Array Discriminates Syrups and Honeys. *Chem. - A Eur. J.* **2018**, *24* (17), 4255–4258.

(106) Kim, J.; Swager, T. M. Control of Conformational and Interpolymer Effects in Conjugated Polymers. *Nature* **2001**, *411* (6841), 1030–1034.

(107) Tomita, S.; Ishihara, S.; Kurita, R. Environment-Sensitive Turn-On Fluorescent Polyamino Acid: Fingerprinting Protein Populations with Post-Translational Modifications. *ACS Appl. Mater. Interfaces* **2017**, *9* (27), 22970–22976.

(108) Kim, J.; Mosior, M.; Chung, L. A.; Wu, H.; McLaughlin, S. Binding of Peptides with Basic Residues to Membranes Containing Acidic Phospholipids. *Biophys. J.* **1991**, *60* (1), 135–148.

(109) Mislick, K. A.; Baldeschwieler, J. D. Evidence for the Role of Proteoglycans in Cation-Mediated Gene Transfer. *Proc. Natl. Acad. Sci. U. S. A.* **1996**, *93* (22), 12349–12354.

(110) Sugai, H.; Tomita, S.; Ishihara, S.; Kurita, R. One-Component Array Based on a Dansyl-Modified Polylysine: Generation of Differential Fluorescent Signatures for the Discrimination of Human Cells. *ACS Sensors* **2019**, *4* (4), 827–831.

(111) Castera, L.; Yuen Chan, H. L.; Arrese, M.; Afdhal, N.; Bedossa, P.; Friedrich-Rust, M.; Han, K. H.; Pinzani, M. EASL-ALEH Clinical Practice Guidelines: Non-Invasive Tests for Evaluation of Liver Disease Severity and Prognosis. *J. Hepatol.* **2015**, *63* (1), 237–264.

(112) Geng, Y.; Hardie, J.; Landis, R. F.; Mas-Rosario, J. A.; Chattopadhyay, A. N.; Keshri, P.; Sun, J.; Rizzo, E. M.; Gopalakrishnan, S.; Farkas, M. E.; Rotello, V. M. High-Content and High-Throughput Identification of Macrophage Polarization Phenotypes. *Chem. Sci.* **2020**, *10*, 8231–8239.

(113) Mallet, A. M.; Liu, Y.; Bonizzoni, M. An Off-the-Shelf Sensing System for Physiological Phosphates. *Chem. Commun.* **2014**, *50* (39), 5003–5006.

(114) Liu, Y.; Bonizzoni, M. A Supramolecular Sensing Array for Qualitative and Quantitative Analysis of Organophosphates in Water. *J. Am. Chem. Soc.* **2014**, *136* (40), 14223–14229.

(115) Liang, X.; Bonizzoni, M. Boronic Acid-Modified Poly(Amidoamine) Dendrimers as Sugar-Sensing Materials in Water. *J. Mater. Chem. B* **2016**, *4* (18), 3094–3103.

(116) Liang, X.; Trentle, M.; Kozlovskaya, V.; Kharlampieva, E.; Bonizzoni, M. Carbohydrate Sensing Using Water-Soluble Poly(Methacrylic Acid)-Co-3(Acrylamido)Phenylboronic Acid Copolymer. *ACS Appl. Polym. Mater.* **2019**, *1* (6), 1341–1349.

(117) Sambrook, M. R.; Notman, S. Supramolecular Chemistry and Chemical Warfare Agents: From Fundamentals of Recognition to Catalysis and Sensing. *Chem. Soc. Rev.* **2013**, *42* (24), 9251–9267.

(118) Sun, X.; Zhai, W.; Fossey, J. S.; James, T. D. Boronic Acids for Fluorescence Imaging of Carbohydrates. *Chem. Commun.* **2016**, *52* (17), 3456–3469.

(119) Bernardi, A.; Jiménez-Barbero, J.; Casnati, A.; De Castro, C.; Darbre, T.; Fieschi, F.; Finne, J.; Funken, H.; Jaeger, K. E.; Lahmann, M.; Lindhorst, T. K.; Marradi, M.; Messner, P.; Molinaro, A.; Murphy, P. V; Nativi, C.; Oscarson, S.; Penadés, S.; Peri, F.; Pieters, R. J.; Renaudet, O.; Reymond, J. L.; Richichi, B.; Rojo, J.; Sansone, F.; Schäffer, C.; Bruce Turnbull, W.; Velasco-Torrijos, T.; Vidal, S.; Vincent, S.; Wennekes, T.; Zuilhof, H.; Imberty, A. Multivalent Glycoconjugates as Anti-Pathogenic Agents. *Chem. Soc. Rev.* **2013**, *42* (11), 4709–4727.

(120) Oshovsky, G. V.; Reinhoudt, D. N.; Verboom, W. Supramolecular Chemistry in Water. *Angew. Chem. Int. Ed.* **2007**, *46* (14), 2366–2393.

(121) Wulff, G. Molecular Imprinting in Cross-Linked Materials with the Aid of Molecular Templates - A Way towards Artificial Antibodies. *Angew. Chem. Int. Ed.* **1995**, *34* (17), 1812–1832.

(122) Chen, L.; Wang, X.; Lu, W.; Wu, X.; Li, J. Molecular Imprinting: Perspectives and Applications. *Chem. Soc. Rev.* **2016**, *45* (8), 2137–2211.

(123) Selvolini, G.; Marrazza, G. MIP-Based Sensors: Promising New Tools for Cancer Biomarker Determination. *Sensors (Basel)* **2017**, *17* (4), 718.

(124) Zimmerman, S. C.; Lemcoff, N. G. Synthetic Hosts via Molecular Imprinting- Are Universal Synthetic Antibodies Realistically Possible? *Chem. Commun.* **2004**, *4* (1), 5–14.

(125) Tan, J.; Wang, H. F.; Yan, X. P. A Fluorescent Sensor Array Based on Ion Imprinted Mesoporous Silica. *Biosens. Bioelectron.* **2009**, *24* (11), 3316–3321.

(126) Tan, J.; Wang, H. F.; Yan, X. P. Discrimination of Saccharides with a Fluorescent Molecular Imprinting Sensor Array Based on Phenylboronic Acid Functionalized Mesoporous Silica. *Anal. Chem.* **2009**, *81* (13), 5273–5280.

(127) Lee, J. D.; Hong, J. I. Two-Dimensional Sensor Array for Discrimination of Amines. *Tetrahedron Lett.* **2013**, *54* (22), 2890–2893.

(128) Wulff, G. The Role of Binding-Site Interactions in the Molecular Imprinting of Polymers. *Trends Biotechnol.* **1993**, *11* (3), 85–87.

(129) Kangas, M. J.; Burks, R. M.; Atwater, J.; Lukowicz, R. M.; Williams, P.; Holmes, A. E. Colorimetric Sensor Arrays for the Detection and Identification of Chemical Weapons and Explosives. *Crit. Rev. Anal. Chem.* **2017**, *47* (2), 138–153.

(130) Anzenbacher, P.; Liu, Y.; Palacios, M. A.; Minami, T.; Wang, Z.; Nishiyabu, R. Leveraging Material Properties in Fluorescence Anion Sensor Arrays: A General Approach. *Chem. Eur. J.* **2013**, *19* (26), 8497–8506.

(131) Palacios, M. A.; Wang, Z.; Montes, V. A.; Zyryanov, G. V.; Hausch, B. J.; Jursíková, K.; Anzenbacher, P. Hydroxyquinolines with Extended Fluorophores: Arrays for Turn-on and Ratiometric Sensing of Cations. *Chem. Commun.* **2007**, *7345* (36), 3708–3710.

(132) Palacios, M. A.; Wang, Z.; Montes, V. A.; Zyryanov, G. V.; Anzenbacher, P. Rational Design of a Minimal Size Sensor Array for Metal Ion Detection. *J. Am. Chem. Soc.* **2008**, *130* (31), 10307–10314.

(133) Lim, S. H.; Feng, L.; Kemling, J. W.; Musto, C. J.; Suslick, K. S. An Optoelectronic Nose for the Detection of Toxic Gases. *Nat. Chem.* **2009**, *1* (7), 562–567.

(134) Kemling, J. W.; Suslick, K. S. Nanoscale Porosity in Pigments for Chemical Sensing. *Nanoscale* **2011**, *3* (5), 1971–1973.

(135) Feng, L.; Musto, C. J.; Kemling, J. W.; Lim, S. H.; Zhong, W.; Suslick, K. S. Colorimetric Sensor Array for Determination and Identification of Toxic Industrial Chemicals. *Anal. Chem.* **2010**, *82* (22), 9433–9440.

(136) Lim, S. H.; Musto, C. J.; Park, E.; Zhong, W.; Suslick, K. S. A Colorimetric Sensor Array for Detection and Identification of Sugars. *Org. Lett.* **2008**, *10* (20), 4405–4408.

(137) Askim, J. R.; Suslick, K. S. Hand-Held Reader for Colorimetric Sensor Arrays. *Anal. Chem.* **2015**, *87* (15), 7810–7816.

(138) Li, Z.; Fang, M.; LaGasse, M. K.; Askim, J. R.; Suslick, K. S. Colorimetric Recognition of Aldehydes and Ketones. *Angew. Chem. Int. Ed.* **2017**, *56* (33), 9860–9863.

(139) LaGasse, M. K.; McCormick, K.; Li, Z.; Khanjian, H.; Schilling, M.; Suslick, K. S. Colorimetric Sensor Arrays: Development and Application to Art Conservation. *J. Am. Inst. Conserv.* **2018**, *57* (3), 127–140.

(140) Li, Z.; Suslick, K. S. A Hand-Held Optoelectronic Nose for the Identification of Liquors. *ACS Sensors* **2018**, *3* (1), 121–127.

(141) Li, Z.; Suslick, K. S. Colorimetric Sensor Array for Monitoring CO and Ethylene. *Anal. Chem.* **2019**, *91* (1), 797–802.

(142) Bueno, L.; Meloni, G. N.; Reddy, S. M.; Paixão, T. R. L. C. Use of Plastic-Based Analytical Device, Smartphone and Chemometric Tools to Discriminate Amines. *RSC Adv.* **2015**, *5* (26), 20148–20154.

(143) Rout, B.; Unger, L.; Armony, G.; Iron, M. A.; Margulies, D. Medication Detection by a Combinatorial Fluorescent Molecular Sensor. *Angew. Chem. Int. Ed.* **2012**, *51* (50), 12477–12481.

(144) Sarkar, T.; Selvakumar, K.; Motiei, L.; Margulies, D. Message in a Molecule. *Nat. Commun.* **2016**, *7* (May), 1–9.

(145) Hatai, J.; Motiei, L.; Margulies, D. Analyzing Amyloid Beta Aggregates with a Combinatorial Fluorescent Molecular Sensor. *J. Am. Chem. Soc.* **2017**, *139* (6), 2136–2139.

(146) Chin, C. D.; Linder, V.; Sia, S. K. Commercialization of Microfluidic Point-of-Care Diagnostic Devices. *Lab Chip* **2012**, *12* (12), 2118–2134.