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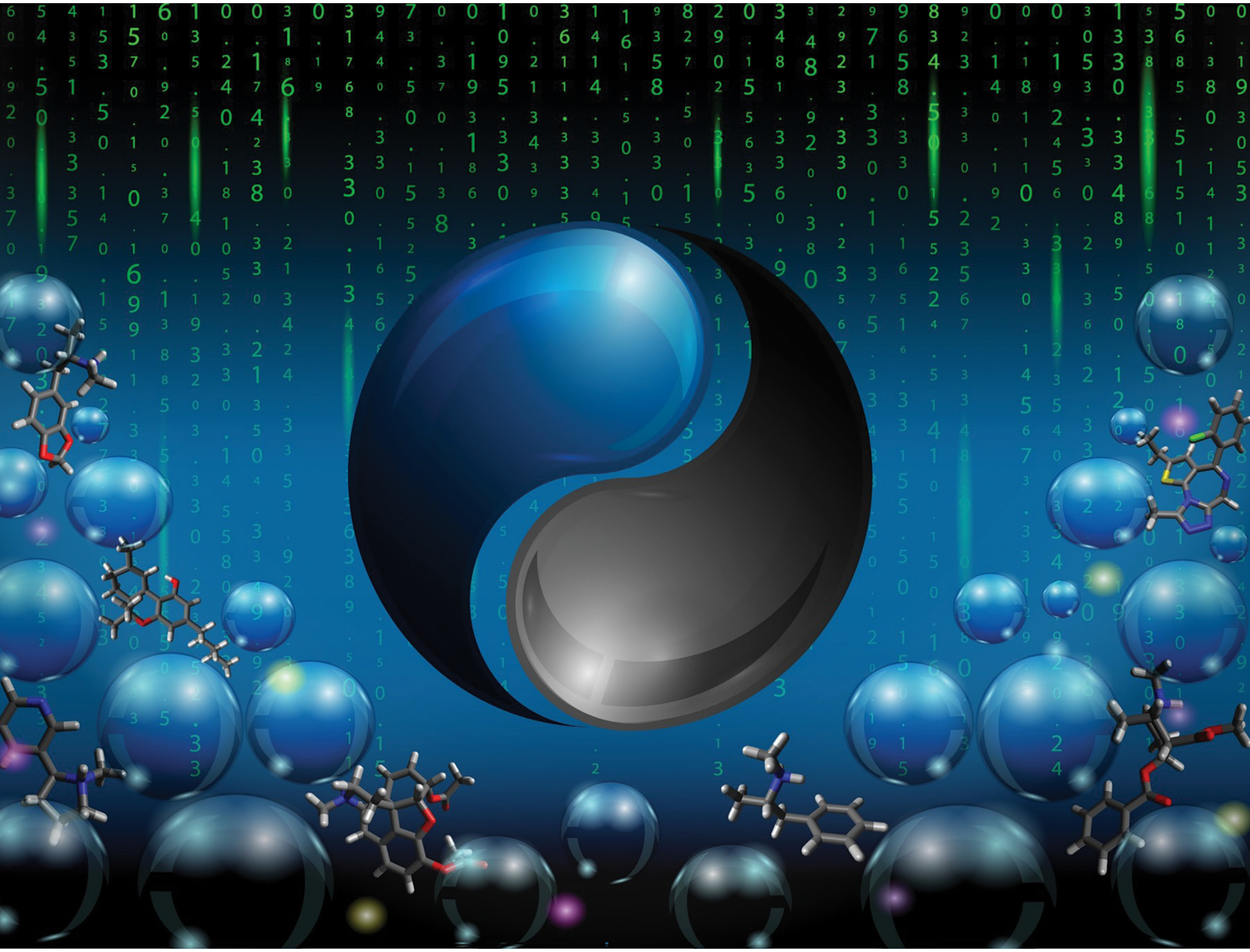

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Information-rich sensors by assembly: a review on the use of macrocyclic hosts in the context of complex chemical systems

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Host-based sensors that offer cross-reactivity to analytes can be valuable tools in the detection and differentiation of biologically relevant molecules. This review Feature Article provides a foundational background in developing macrocyclic host-based systems that harness chemical complexity to achieve sensing in biologically relevant solutions. We highlight our own contributions along with select examples from others, covering noncovalent self-assemblies, salt tolerant synthetic designs, and current multicomponent systems approaches to host-based sensing and differentiation. We emphasize our personal perspective on building complex systems, as an evolving strategy to obtaining emergent information-rich outputs that surpass the performance of conventional host-based sensor pairs. These recent developments are at the frontier of the field, involving more challenging sensing tasks, including the detection and identification of highly similar analytes, biomacromolecules, and complex mixtures.

1. Introduction

Supramolecular systems are fundamental to life. Molecules coming together on the molecular, meso- and nanoscale to achieve function are critical in biological processes. Although biology readily achieves this through the evolution of complicated systems, the design and development of synthetic systems represents an ongoing frontier in science.[1] In the field of systems chemistry, molecular interactions and chemical complexity are exploited to generate emergent properties, where a system takes on new characteristics beyond the sum of its individual parts.[2] Through the lens of "life" this can be viewed as systems that exist in transient dissipative states and supramolecular chemically fuelled processes that require the input of energy. In systems chemistry, chemical complexity can also be introduced through the construction of self-organizing "networks", containing multiple building blocks, simultaneously interacting within a web of interconnected equilibria. As synthetic supramolecular systems become more elaborate, chemists get closer to mimicking the complex systems that govern biology.[2]

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One subfield of supramolecular chemistry involves the construction of synthetic organic macrocyclic "host" molecules with concave motifs that recognize and bind targeted "guest" analytes. The molecular recognition of host·guest complexes is driven by supramolecular processes that occur beyond the molecule.[3] Such systems that function in water, buffer and biofluids have had many applications, ranging from detection to delivery, to *in vivo* binding and elimination of biologically relevant "guest" molecules.[4–8] In particular, the development of host-based sensors that function in aqueous conditions are valuable tools in areas of diagnostics, theranostics and biochemical research.[4]

This Feature Article provides a personal perspective on the progression of water-soluble macrocyclic host-based sensing systems, highlighting our own work, along with illustrative systems developed by other groups. The concepts of systems chemistry and complexity are interconnected. Comprehensive reviews are available covering the broader topics of systems chemistry, supramolecular analytical chemistry, and macrocyclic host·guest molecular recognition in water.[2,9–13] In this article we focus on the evolution of host-based complexity that involves multiple interacting components (combinations of hosts, dyes, analytes, and additional binding species), that achieve a sensing outcome. Such systems exist in an equilibrium position that can be shifted upon the addition of a new binding partner. First, we briefly introduce the basic concepts to consider in designing host-based systems. Then we highlight illustrative examples of macrocyclic sensors and their design strategies. Throughout this Feature Article the examples we include build in both chemical/structural complexity and in the complexity of the systems that are created. We arrive at a series of multicomponent systems that represent our latest approach to the acquisition of information-rich outputs. This is not a comprehensive review—work by others is included where it helps give critical context for our own progress.

1.1. Key considerations in designing host-based systems

Reversible interactions make up the foundation of self-assembled supramolecular systems. A molecular recognition event occurs when molecular building blocks reversibly come together, as driven and guided by noncovalent interactions, the hydrophobic effect, and in some cases dynamic covalent bonds (Fig. 1). When combined, individually weak noncovalent interactions can have a strong influence on molecular recognition (Fig. 1a).[14] Although not strictly defined as a noncovalent interaction the hydrophobic effect plays a central role in molecular recognition in water, with multiple different theories providing useful mechanistic frameworks.[15–23] Upon host·guest complexation, the release of interfacial and inner host cavity water into bulk solvent can occur *via* processes that display favourable enthalpic and/or entropic signatures (Fig. 1c).[21] Dynamic covalent bonds can also be applied in self-assembled systems to arrive at thermodynamically stable products (Fig. 1b).[24,25] To various extents, contributions from noncovalent interactions, the hydrophobic effect and/or dynamic covalent bonds, can achieve molecular recognition of targeted guest analytes.

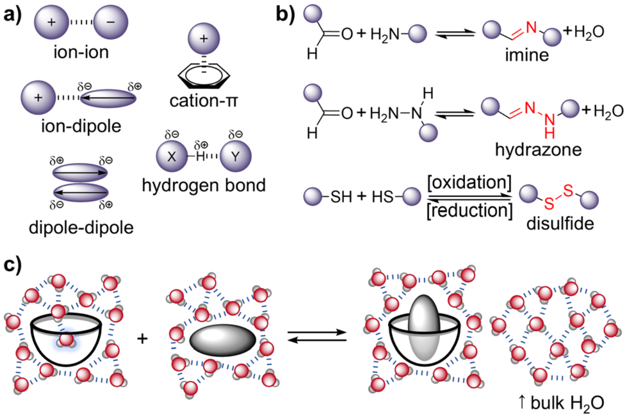

Fig. 1 Supramolecular self-assembly is driven by reversible processes. (a) Noncovalent interactions (electrostatics (ion–ion, ion–dipole, ion-induced dipole and dipole–dipole), cation–π, hydrogen-bonding and dispersive interactions (van der Waals or London dispersion forces)). (b) Select examples of dynamic covalent bonds in biological systems. (c) The hydrophobic effect contributes to reversible binding through release of interfacial and inner host cavity water, resulting in an increase of bulk water.

Host rigidity and host·guest shape complementarity play key roles in driving assemblies. A variety of host scaffolds offer different advantages and disadvantages in systems design (Fig. 2). Overall, hosts must have some form of rigidity to prevent hydrophobic collapse and provide beneficial preorganization. Deeper cavities can introduce more surface area for interactions by further extending the hydrophobic pocket, while maintaining scaffold rigidity. This can improve both enthalpic and entropic contributions to the hydrophobic effect.[26,27] The construction of wider host cavities can accommodate larger guests but often introduces more flexibility, consequently decreasing preorganization and often resulting in weaker binding affinities.[28] Guest binding properties can be further tuned through the incorporation of polar and non-polar functional groups inside and outside the host cavity.[29] Polar groups on the upper or lower rim of a host (*e.g.* carbonyl portals or polyethylene glycol tails) can be used to favour hydration, assisting in water solubility and binding of hydrophobic guests with interacting polar head groups. Functional groups that are anionic (*e.g.* sulfonate, sulfate, carboxylate and phosphonate) or cationic (*e.g.* ammonium, guanidinium and pyridinium) are often incorporated, favouring binding to oppositely charged guest molecules through electrostatic attraction. Alternatively, host cavities containing inwardly

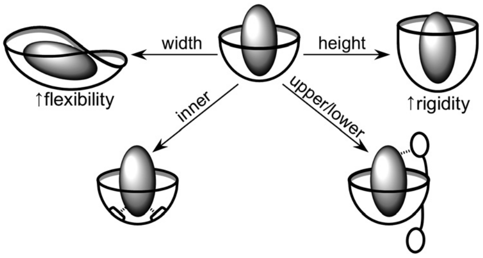

Fig. 2 Host scaffolds can be tuned to control conformation, binding interactions, and introduce new properties.

directed polar groups can assist in hydrogen bonding and electrostatic interactions within the cavity, binding hydrophilic polar guests in aqueous conditions.[30] Throughout this Feature Article, we introduce different host scaffolds and their characteristic binding properties as examples of sensing systems arise.

Traditional supramolecular systems are under thermodynamic control and exist at a stable energy minimum. In its simplest molecular form, this is a 1 : 1 host·guest complex. Thermodynamically stable systems can increase in complexity through the incorporation of multiple interacting host and guest components, to form a network of simultaneous reversible interactions within a single solution. Although the thermodynamically stable collection of species will be favoured, it is important to remember that self-assembly is not a static event, but a reversible process of exchanging components. Le Châtelier's principle applies even in very complex equilibrating systems. Changes in extrinsic factors, like relative component concentrations, solvent, polarity, pH and additive co-solutes or mediators, can be used to shift the equilibrium position of a system.[31] The selection of a buffer or addition of salts can also impact the binding affinity of a system, where buffers and salts can competitively bind to the host cavity.[32,33] In instances where the ionic strength of a solution is altered, electrostatic screening effects on charged hosts and guests also play a role, diminishing Coulombic interactions.[32] Overall, adaptation of a system in response to external or internal factors can impact molecular recognition and the strength of association events. This provokes careful consideration of the complete composition and design of host·guest systems, particularly under biologically relevant conditions or in biological media where salt content is higher and additional competitive species are present.

1.2. Pairwise sensing of biologically relevant molecules

Macrocyclic hosts are transformed into optical chemosensors through incorporation of an environmentally sensitive dye (Fig. 3). Dye structures that form noncovalent interactions with hosts are selected to encourage the formation of host–dye complexes. This can be attained through intermolecular binding of a dye present in solution (Fig. 3a), or intramolecular binding of a dye covalently tethered to the host (Fig. 3b). Alternatively, environmentally sensitive dyes can be covalently integrated into the host scaffold, eliminating the need for noncovalent dye complexation (Fig. 3c). We have developed a variation to these approaches, where synthetic integration of a dye into the host scaffold facilitates host dimerization (Fig. 3d), while others have created larger supramolecular arrangements that integrate hosts and dyes. Each of these frameworks relies on analyte binding to induce changes in the microenvironment of the dye, producing monitorable colourimetric and/or fluorescent responses. This can be achieved through several different mechanisms including static dye quenching complexes,[34–36] fluorescence enhancing complexes,[37] charge transfer arrangements that induce spectral shifts in absorbance and fluorescence,[36,38] and to a lesser extent dye pair arrangements that undergo Förster resonance energy transfer (FRET).[39] Through these mechanisms distinct host-based molecular and supramolecular arrangements can achieve analyte detection.

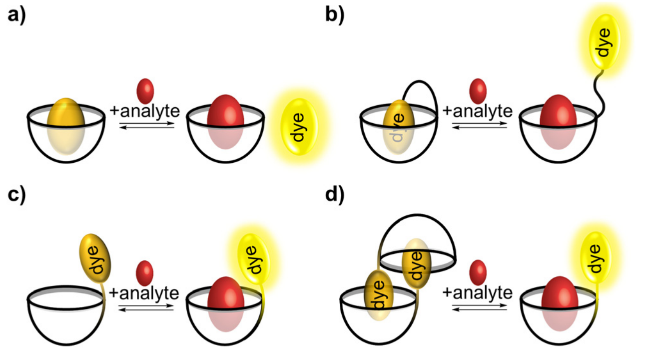

Fig. 3 Macrocyclic hosts are transformed into optical sensors through self-assembly or covalent incorporation of a reporter dye. (a) Intermolecular indicator displacement assay (IDA). (b) Intramolecular dye displacement assay of a covalently tethered/conjugated dye. (c) Direct assay of a covalently integrated environmentally sensitive dye. (d) DimerDye disassembly assay.

Noncovalent binding of a dye to a host is a facile approach to sensing. This conventional technique, known as an indicator displacement assay (IDA), relies on reversible binding of an indicator dye to the host scaffold. Upon addition of a competitive guest analyte, the indicator is displaced producing a monitorable response (Fig. 3a).[40–42] IDAs operate best under conditions where the host·guest affinity product is approximately equal to the host·dye affinity product, where affinity product is defined as the host·analyte or host·dye equilibrium binding constant (K_a) times the respective dye or analyte assay concentration.[43] This methodology is easily applied by mixing different indicator dyes with different host receptors containing favourable binding interactions and tailoring concentrations as needed.[37] Further adaptations include the guest displacement assay (GDA), where the guest analyte is first bound to the host and then subsequently displaced by an indicator dye, proving more suitable for insoluble or weaker binding guest analytes.[44]

IDAs can be used in biological applications where interferants (*e.g.* salts, buffer ions and metabolites) remain constant. An earlier example from Nau and co-workers paired the electron-rich anionic host *p*-sulfonatocalix[4]arene (**sCx4**) with the cationic reporter dye lucigenin (**LCG**), as a label-free method for continuous real-time enzyme assay monitoring (Fig. 4a and b).[43] In this system unbound free **LCG** produces an "on" fluorescence response, whereas complexed **sCx4·LCG** undergoes static quenching, producing an "off" response. Here a stronger host·dye binding constant is favourable, as it allows for lower host·dye concentrations to be used relative to the detected enzyme substrate and product. The hydrolysis of the neurotransmitter acetylcholine (**ACh**) to choline (**Ch**) produces the same "on" response, attributed to the same **sCx4** binding affinities ($K_a = 1.0 \times 10^5\ \mathrm{M}^{-1}$). However, the oxidation of **Ch** to the product betaine by a choline-specific enzyme results in an "off" response from betaine's weaker **sCx4** binding ($K_a \leq 500\ \mathrm{M}^{-1}$) (Fig. 4b). Through a domino enzyme-coupled supramolecular tandem assay, absolute concentrations of **Ch** followed by **ACh** are determined. The success of this assay relies on a difference in enzyme substrate *versus* product affinity by a factor of 10 or more.

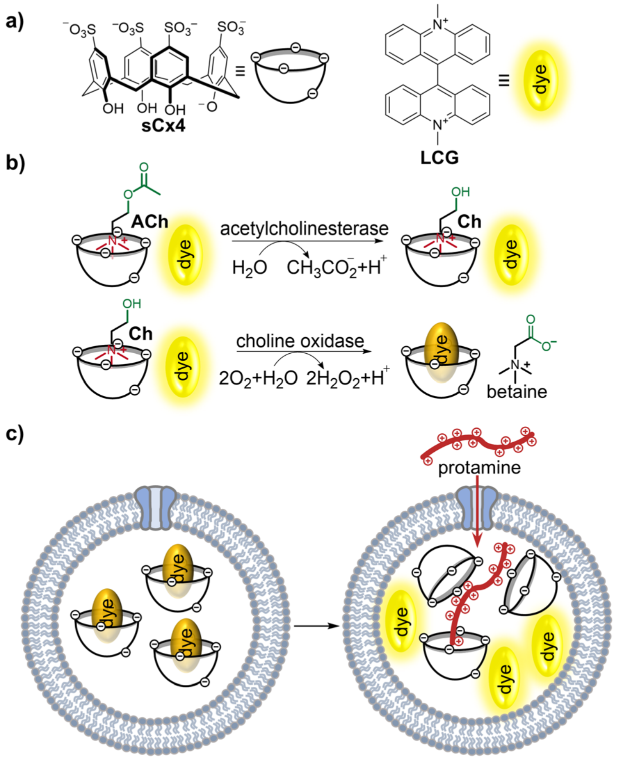

Fig. 4 IDAs provide a facile label free method for biological tandem assay monitoring. (a) Chemical structures of host·dye reporter pair **sCx4** and **LCG**. (b) Choline oxidase followed by acetylcholineseterase enzyme kinetic activity is monitored through a domino turn-off response, as the bound substrates **Ch** and **ACh** are converted to the weaker binding product betaine. (c) Peptide migration into liposomes is monitored through competitive binding of the cationic peptide protamine to **sCx4**, displacing **LCG** and inducing a turn-on fluorescence response.

Recent IDA applications show the use of noncovalent sensing pairs in increasingly complex biologically relevant systems, quantifying and monitoring guest transport through liposomes. In one example liposomes are formed containing the pre-assembled host·dye reporter pair **sCx4·LCG**, unable to permeate the membrane barrier. Crossing of the cationic peptide, protamine, *via* ion channels is monitored by competitive displacement of the host·dye complex inside the liposome, providing real-time fluorescence monitoring and kinetics of translocation (Fig. 4c).[45,46] A reported variation to this approach applies a pH gradient across the lipid bilayer to aid in controlling detected interior analyte concentrations, improving sensitivity.[47] This system design has been further advanced to functioning protocells, where host·dye IDAs are used to monitor both transmembrane uptake and intravesicular enzymatic reactions.[48]

Despite their ease of design, IDAs can be less effective in the presence of competitive interferants. IDAs are suitable in instances where relative concentrations of interferants do not change over the course of an experiment and the absolute change in signal is high. Although not always commented on, the presence of higher salt concentrations in biological assays and matrix effects among different biological samples can diminish IDA signal responses and reproducibility. This is a result of the free (unbound) dye being more susceptible to dynamic quenching events with salts and co-solutes in solution, decreasing the amplitude of response. In some instances, known quenchers can be avoided by salt substitutions, for instance known chloride quenching of **LCG** can be avoided by an alternative use of perchlorate in experiments that mimic salty biological conditions.[49] Competitive interferants can also bind host cavities and portals, blocking both host·dye and host·guest binding events, consequently diminishing apparent binding constants (K_{app}). The use of IDAs in imaging assays of tissue or whole cells is further complicated by unbound free dye being present everywhere in solution.

Synthetic attachment of a dye to a host scaffold is beneficial in cases where dynamic quenching and competition significantly influence apparent binding constants. Commonly used design strategies include a covalent flexible dye tether, allowing intramolecular host·dye complexation, or direct integration of an environmentally sensitive dye into the host scaffold (Fig. 3b and c). Although both these methods can be synthetically taxing, often requiring multistep syntheses, they can help mitigate competitive salt effects as well as dye dilution effects in biological solutions.

Covalently tethering a dye to a host at an optimal distance provides beneficial preorganization and prevents dye dilution. Intramolecular complexation *via* a flexible tether has been shown to produce increased intramolecular affinity in comparison to an equivalent intermolecular self-assembly.[50] The benefits of intramolecular tethered sensors are illustrated by a recent example from Biedermann and co-workers, who compared a noncovalent IDA of cucurbit[7]uril (**CB7**) and berberine chloride (**BC**) to equivalent host-linker-dye conjugates, CB7-hexaethylene glycol-berberine and CB7-tetraethylene glycol-berberine (Fig. 5).[51] The barrel-shaped host **CB7** consists of glycoluril units bridged by methylene pairs, forming a rigid hydrophobic cavity, lined with neutral polar carbonyl groups (Fig. 5b). Complexation of the cationic dye **BC** inside **CB7** induces confinement enhanced photostability, increasing fluorescence.[52] At 100 mM NaCl, complete disruption of the intermolecular **CB7·BC** complex occurs. This is a result of known cationic salt binding interactions with cucurbit[*n*]uril carbonyl portals,[53–55] blocking desired binding events (Fig. 5a). In comparison, intramolecular self-assembly of cationic **BC**, provides pre-organization and further repels cationic salt binding, functioning in the presence of $\sim 10^6$-fold excess NaCl (Fig. 5a). This tethered intramolecular sensor allows for the detection of Parkinson's drug amantadine (**$\mathrm{AdNH_3}^+$**) in solutions of human urine and saliva at medically relevant concentrations (Fig. 5b).[51] Tethered cucurbit[*n*]uril cationic dye sensors have also been explored in complex media with examples of cellular imaging in live and fixed HT22 neurons, where dye tethering alleviates dye dilution effects.[56]

Mechanical bonds can be used to further protect sensor function in complex media. In one study, the host·dye reporter pair, cucurbit[8]uril (**CB8**) and di-alkylated 2,7-diazapyrene (**DAP**), were embedded within a rotaxane structure by covalent polyethylene glycol linkages connecting β-cyclodextrin stoppers

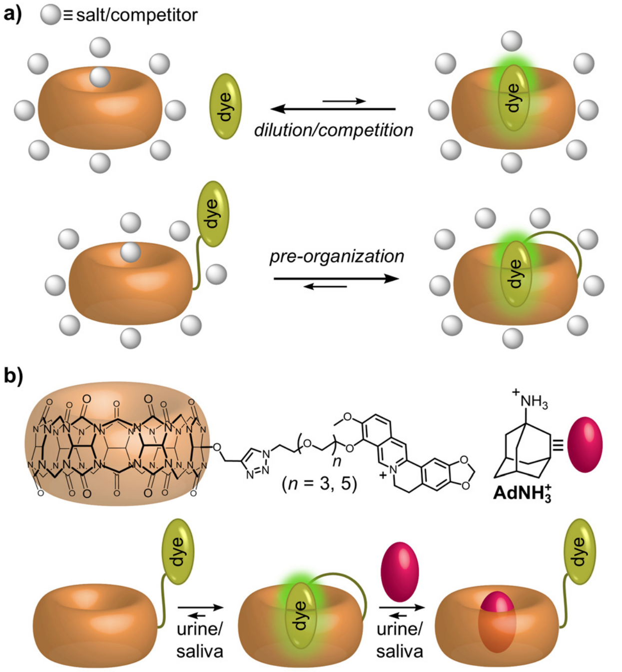

Fig. 5 Covalently tethered host-linker-dye conjugates help mitigate salt effects. (a) High concentrations of salts present in biofluids can competitively bind host portals. Synthetic host-linker-dye conjugates provide pre-organization, reducing competitive salt effects. (b) Chemical structures of unimolecular host-linker-dye conjugates that covalently tether the cationic dye **BC** to the host **CB7** by a hydrophilic flexible ethylene glycol linker. Chemical structure of target Parkinson's drug **$AdNH_3^+$**. Intramolecular self-assembled sensing detects **$AdNH_3^+$** in saline solution, urine and saliva.

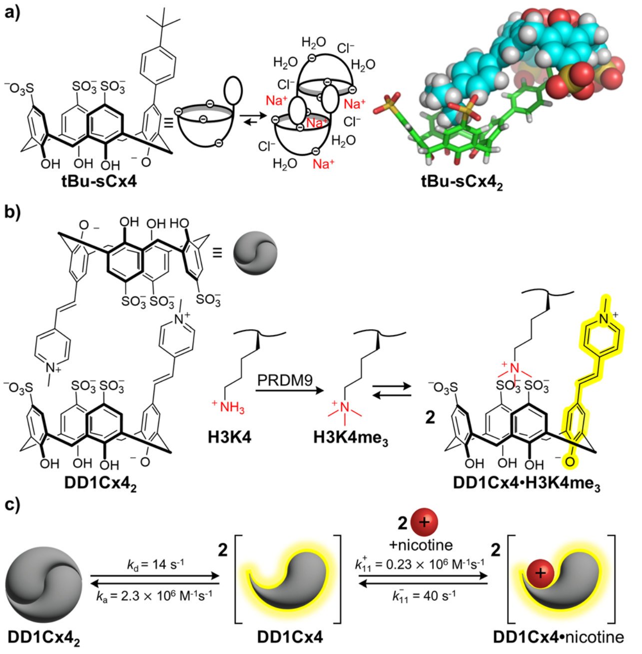

Fig. 6 Like-charged dimers provide a sensing motif that persists in biological solutions. (a) Mono-functionalized ***t*Bu-sCx4** forms a −8 homodimer that strengthens in the presence of salts. X-ray crystal structure of ***t*Bu-sCx4$_2$** homodimer, reproduced from ref. 59 with permission from the Royal Society of Chemistry. (b) Like-charged dimers are transformed into a DimerDye sensing system by synthetic integration of Brooker's merocyanine dye, forming **DD1Cx4$_2$**. DimerDye disassembly-driven turn-on sensing detects lysine methyltransferase PRDM9 conversion of the 21-mer histone tail peptide **H3K4** to **H3K4me$_3$** by a real-time turn-on fluorescence enzyme assay. (c) Stopped-flow kinetics studies determine stepwise mechanism of **DD1Cx4$_2$** disassembly followed by nicotine binding, occurring on the same timescale.

to either end of complexed **DAP**.[57] This elaborate interlocked construction only allows binding to select small molecules capable of forming a ternary complex with the **CB8·DAP** rotaxane, minimizing media interference and inhibiting host·dye dilution effects. This protection strategy successfully detected the biomarker tryptophan in human blood serum and urine, operating at physiologically relevant (low μM) concentrations associated with cardio-vascular and neurodegenerative diseases.

Like-charged assemblies provide an alternative binding motif to promote complexation in biological media. So far, we have touched on the competitive effects of salts in biological solutions, weakening binding events to neutral hosts. These effects, along with electrostatic interference, also apply to charged hosts whose binding of guests is often dominated by ion–ion electrostatic attraction. Contradictory to this behavior, assemblies that bring together highly negatively charged species can be further stabilized and strengthened in the presence of cationic salts.[58] Our lab stumbled upon an example of this phenomenon while tuning the molecular recognition properties of the anionic host **sCx4** *via* upper rim mono-functionalization. We found that incorporating a rigid hydrophobic *tert*-butyl arm facilitated the formation of a homodimer, ***t*Bu-sCx4$_2$**, in aqueous solution, in spite of each monomer bearing a –4 charge (Fig. 6a).[59] This homodimer is enthalpically driven ($\Delta H = -11.0$ kcal mol^{-1} and $T\Delta S = -5.9$ kcal mol^{-1}) by hydrophobic attraction of the *tert*-butyl pendant arm binding inside the hydrophobic calixarene cavity, despite the anionic repulsion between like-charged monomers. In the presence of higher salt concentrations the dimer strengthens, remaining assembled in high salt and denaturing conditions of real urine.[59] This persistent complexation is likely a result of salt screening effects, in which the increased amount of sodium cations reduces repulsion between the like-charged anionic monomers (Fig. 6a). We subsequently found that this general structural motif is routinely successful at achieving dimerization in salty water. Discovery of this privileged motif provided us with a promising basis for developing sensors that retain their function in biological media.

We transformed the general motif of like-charged homodimers into a novel sensing system by synthetic integration of chromophores, creating a class of sensors called DimerDyes (DDs). The first example, **DD1Cx4**, involved the solvatochromic styryl dye Brooker's merocyanine being installed into a sulfonatocalix[4]arene scaffold (Fig. 6b). In aqueous solution two copies of this dye-integrated host self-assemble into a homodimer, **DD1Cx4$_2$**, with the hydrophobic cationic dye pendant arm binding inside the hydrophobic pocket of another copy. This dimer-induced antiparallel stacking of two fluorophores results in quenched emission, characteristic of H-aggregate formation.[60]

Disassembly-driven turn-on fluorescent sensing is achieved by binding of a hydrophobic cationic analyte that outcompetes dimerization. We investigated the biological application potential of this system by a real-time enzyme assay, monitoring lysine methylation of the 21-mer histone tail peptide **H3K4**, as it is converted to **H3K4me$_3$** by methyltransferase PRDM9 (Fig. 6b).[61] In this assay, disassembly driven turn-on fluorescence is achieved in the presence of co-solutes, buffers, metal ions and cofactors.

We investigated the mechanism of DimerDye sensing by steady state and stopped-flow kinetic studies, using nicotine as a model cationic analyte at neutral pH.[62] The strength of **DD1Cx4** dimer formation in buffer ($K_a = 16 \times 10^4$ M^{-1}) allows for DimerDyes to achieve sensing at low μM concentrations. In this assay, disassembly of the dimer is followed by stepwise binding of the analyte nicotine ($K_a = 0.6 \times 10^4$ M^{-1}), occurring on the same timescale (Fig. 6c). When transitioning from buffer to artificial saliva, a slight decrease in dimer affinity occurs ($K_a = 0.13 \times 10^4$ M^{-1}). We suspect this is a result of **DD1Cx4** dimerization being partially driven by electrostatic attraction from the cationic pendant arm and sulfonates, influenced by the higher ionic strength of saliva. Despite this slight weakening of affinity, sensor output in artificial saliva is maintained with minimal effects on relative rate constants for **DD1Cx4$_2$** and **DD1Cx4**·nicotine formation. This set of results proved promising for our future development of more complex systems, consisting of multiple components in equilibrium, where fast off rates in more complex media are desirable.

Larger supramolecular aggregates introduce increased systems complexity and achieve detection of large biomacromolecules. Increasing the number of host·analyte interactions within a single multi-component system enables sensing mediated by multivalent interactions. A more recent multimacrocyclic adaptation to IDAs, reported by Guo and coworkers, introduced a method of self-adaptable detection through multiple peptide biotarget binding interactions.[63] In this example, co-assembly of amphiphilic calix[5]arene (**CA**) and amphiphilic cyclodextrin (**CD**) form heteromultivalent vesicle aggregates (140 nm in diameter), with many copies of the host receptor coating the outer surface (Fig. 7). This approach addresses multi-modal binding and site mismatching, through recognition of the amino acids tyrosine (**Y**) with host **CD**, and lysine (**K**) with host **CA**. The dye **LCG** is used as a noncovalent indicator for competitive **CA** binding (Fig. 7b). Dynamic self-assembly of the vesicle itself allows for surface mobility of the co-assembled hosts, providing detection of model peptides containing **Y** and **K** amino acid residues at different positions. The affinity-enhancing effects of multivalency is sufficient to allow binding and detection under competitive aqueous conditions.[64] Although this work operates on the premise of a conventional IDA, it represents a large jump in systems complexity, incorporating known molecular recognition events with larger dynamic aggregated self-assemblies.

1.3. Cross-reactive differential sensing

Hosts tend to be inherently selective towards a single class of analyte. Examples introduced thus far have incorporated pairwise scenarios where a single analyte is targeted for binding. Here, the distinction between specificity and selectivity is important. In the context of sensing systems, we define specificity as the detection of a single analyte by a single sensor, responding only to that analyte. This is based around the "lock-and-key" principle established by Emil Fischer,[65] where the structural properties of an enzyme binding pocket induce specific binding to one substrate.[66] Specific sensors are most valuable in cases where a single target needs to be identified within a complex mixture (*e.g.* antibodies). Conversely, we define selectivity as the ability to bind multiple structurally similar analytes to varying degrees. Unlike most protein binding pockets, which evolved to have specificity, macrocyclic hosts contain a high level of symmetry and are therefore selective (and cross-reactive) by nature. In the context of host-based systems, most macrocyclic hosts are good at binding certain classes of analytes but fall short when it comes to the specific binding of a single target molecule amongst structurally similar binding partners. This inherent selectivity (and corresponding lack of specificity) is advantageous in differential sensing platforms.

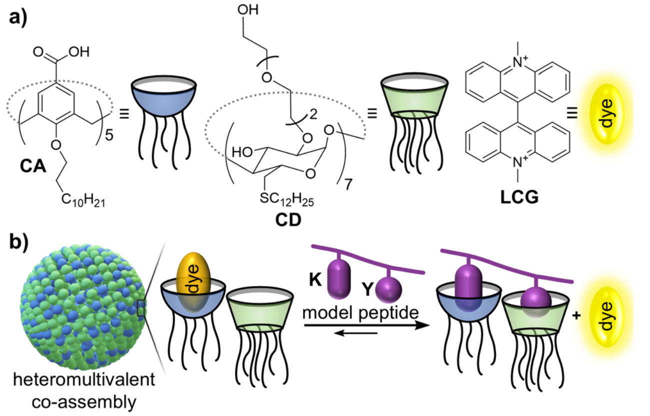

Fig. 7 Co-assembly of amphiphilic cyclodextrin and calix[5]arene hosts form heteromultivalent vesicles for heterotopic detection of **K** and **Y** containing peptides. (a) Chemical structures of amphiphilic calix[5]arene-based host **CA**, amphiphilic cyclodextrin-based host **CD**, and indicator dye **LCG**. (b) Co-assembly of amphiphilic hosts form heteromultivalent vesicles. Binding of **LCG** to **CA**, transforms the co-assembled heteromultivalent vesicle into an IDA. The mobile self-assembled nature of the system allows for flexible heterotopic binding to a variety of peptides containing **K** and **Y** amino acid residues that bind hosts **CA** and **CD** respectively.

Selective cross-reactive receptors are powerful tools in arrayed differential sensing schemes. Often referred to as a "chemical nose", sensor arrays use signal response patterns as a fingerprint to identify many different entities. Relying on cross-reactive selectivity, chemical nose sensors surmount the scaling limitations inherent to lock-and-key specific detection (*i.e.* *n* analytes require *n* specific sensors). This is likened to the mammalian olfactory system,[67] where the human nose can detect and discriminate over 10^{12} different olfactory stimuli, despite containing approximately 400 olfactory receptors.[68,69] The key to applying this approach in differential host-based sensing is obtaining reproducible unique response patterns for a variety of analytes, through fast stable equilibria varying in affinity. While signal processing in the human brain is complex, the signal processing for chemical nose sensors can be relatively straightforward. Through chemometric statistical analysis methods, multiple numerical absorbance and/or fluorescence responses from host·dye reporter pairs can be used as a fingerprint pattern to differentiate and identify analytes (Fig. 8). The true power in this approach lies within the ability to differentiate more analytes than the number of sensors required.

Differential sensing analysis is most often performed using multivariate statistical methods such as principal component analysis (PCA) and linear discriminant analysis (LDA). The aim of these techniques is to reduce large data sets – in this case multiple absorbance and fluorescence observations from the same or different sensing units – down to two- or three-dimensional space for visualization. PCA applies an unsupervised approach to retain the greatest variance while simplifying a complex dataset,[70] whereas LDA applies a supervised approach to identify classes (in this case target analytes) and maximize the distance between them.[71] Anslyn and co-workers have provided a helpful review describing the many factors involved in refining and improving differentiation plots.[72] More recently, machine learning algorithms like support vector machine (SVM),[73] and iterative recursive feature elimination (RFE)[74,75] are being applied as supervised techniques in supramolecular sensing.[76–78] This aids in both regression and classification to identify the most informative responses within an array, eliminate over-fitting data and building calibration models.

Differential sensing is easily applied to small molecules and has been extensively studied. We highlight a past example from our group to provide context for the advancement of sensing systems discussed later in this Feature Article. We applied traditional *p*-sulfonatocalix[*n*]arene IDA's in a sensor array, differentiating histone code post-translational modifications associated with gene regulation and many human diseases.[79] An array varying pH and organic co-solvent conditions of a single host·dye sensor pair distinguished methylation states of isolated lysine (mono-, di- and tri-) and arginine (mono-, asymmetric di- and symmetric di-) amino acids. This strategy of varying pH allows for changes in analyte affinity based on amino acid pK_a differences, however in this instance an organic co-solvent was required for discrimination. Fingerprint responses produced from a second array, varying a panel of *p*-sulfonatocalix[*n*]arene IDAs in buffer, enabled the differentiation of closely related synthetically modified peptides in isolation, including similar sequences **H3K9me3** and **H3K27me3** that contain the same trimethyllysine mark, sometimes misconstrued by antibodies.

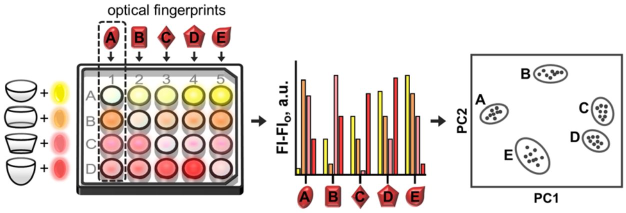

Fig. 8 An array of host–dye reporter pairs can discriminate multiple guest analytes through multivariate analysis.

In our more recent work, we were motivated to apply salt tolerant DimerDyes to differential sensing in biorelevant solutions. We were inspired by a combinatorial approach that reported rapid synthesis of a styryl dye library *via* a simple condensation reaction.[80] We emulated this approach by conducting rapid parallel synthesis and screening of 16 DimerDye sensors, each integrating a different styryl-based dye.[81] Parallel testing of nicotine turn-on fluorescence responses in buffer and saliva narrowed down the initial array to five hit DimerDye sensors; containing *N*-methylpyridinium (**DD1Cx4** and **DD8Cx4**), indolinium (**DD4Cx4**), bipyridinium (**DD12Cx4**) and *N*-phenylpyridinium (**DD13Cx4**) pendant arms (Fig. 9a). This assortment of integrated dyes introduces both photophysical diversity, covering a range of absorbance and emission wavelengths, as well as structural diversity, varying both dimer and analyte binding affinities. Each of these sensors operates through disassembly driven turn-on fluorescence, detecting cationic hydrophobic drugs, nicotine, cocaine, and 3,4-methylenedioxymethamphetamine (**MDMA**) in both buffer and saliva within relevant concentrations found after ingestion (low μM). Deployed in an array, PCA and LDA analysis of isolated DimerDye responses successfully differentiates between active illicit drugs and closely related metabolites within classes of amphetamines (Fig. 9b), anesthetics (Fig. 9c), and opioids (Fig. 9d).

Beyond data refinement, differential sensing falls short when analyte affinities for a set of hosts are too similar. Overlapping analyte clusters in differential plots are often an indication that sensor array response patterns are not unique among a given panel of analytes. Overlap of analyte confidence ellipses can also occur when sensing responses have high deviation within a set of replicates, relative to the amplitude of response. One strategy to overcome this is the incorporation of more hosts in an array that reinforce observed patterns, decreasing noise within clustered replicates. Alternatively, building sensor arrays that incorporate hosts with different binding properties, or different photophysical response mechanisms, can have a greater impact on multivariate analysis and improve analyte discrimination.[72]

Incorporating different modes of analyte interactions can aid in differential sensing. This is demonstrated by reported dual site upper- and lower-rim functionalized pillar[5]arene host, **CN-DFP5**, that combines noncovalent host·guest recognition with two reactive dynamic covalent coumarin and naphthyl fluorophore handles (Fig. 10).[82] This design strategy provides an alternative approach to a panel of host·dye self-assembled sensors. Instead, seven neurotransmitters are differentiated by a singular host through different modes of interaction, prompting different fluorescence response patterns. The pillar[5]arene's electron-rich host cavity contributes electrostatic noncovalent binding to the hydrophobic cationic neurotransmitter **ACh** (Fig. 10b). Appended fluorophores on **CN-DFP5** provide emission wavelength variables, corresponding to the chemical reactivity of the two attached dynamic covalent handles. Boric acid – naphthalimide allows for condensation reactions with catechol-containing

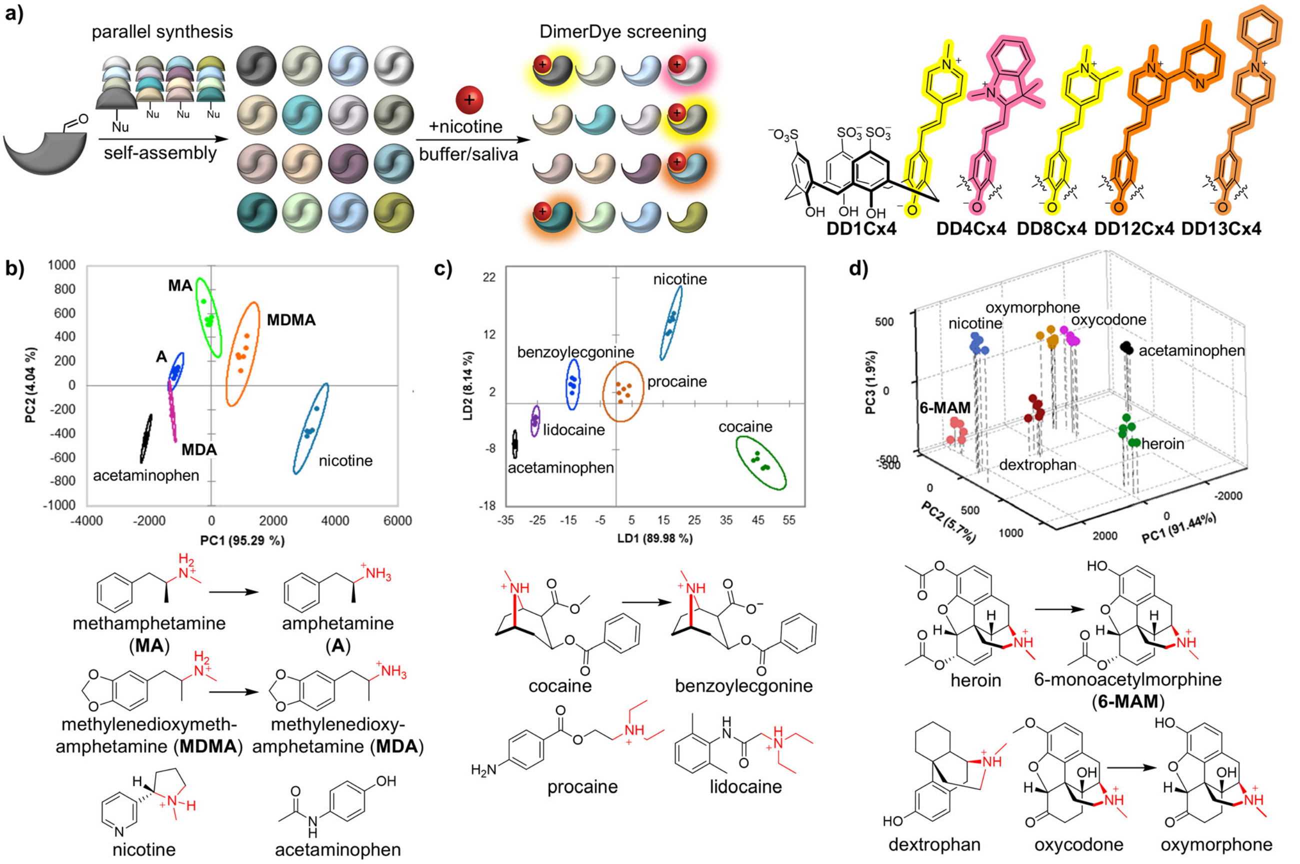

Fig. 9 Parallel synthesis and screening identifies an array of five hit DimerDye sensors that distinguish illicit cationic drugs and metabolites. (a) Parallel synthesis and screening of sixteen DimerDyes identifies five hit sensors, **DD1Cx4**, **DD4Cx4**, **DD8Cx4**, **DD12Cx4** and **DD13Cx4**. DimerDye sensors operate by a disassembly driven turn-on fluorescence sensing mechanism, detecting hydrophobic cationic drugs in buffer and saliva. PCA and LDA analysis of an array of five DimerDyes distinguishes drug classes (b) amphetamines (c) anesthetics and (d) opioids. Chemical structures of tested hydrophobic cationic drugs and their metabolites, red indicates binding motifs. Adapted with permission from *J. Am. Chem. Soc.*, 2019, **141**(42), 16763–16771. Copyright 2019 American Chemical Society.[81]

neurotransmitters (dopamine, epinephrine and norepinephrine), while aldehyde-coumarin undergoes Schiff base condensation reactions with monoamine containing neurotransmitters (dopamine, norepinephrine, serotonin, glutamate, and histamine) (Fig. 10b). This design strategy provides rapid access to detection, maintaining function in cerebrospinal fluids. Two photon long wavelength excitation of the napthlaimide and coumarin fluorophores enabled PCA differentiation within live neurons, through co-incubation of **CNF-DFP5** and neurotransmitters. Incubation of **CNF-DFP5** with brain tissue slices from the hippocampus Alzheimer's disease region of a mouse also enabled three-dimensional imaging.

Extending this concept further, sensor arrays that incorporate a range of binding interactions and photophysical mechanisms provide more diverse differential response patterns. In one example a library of six **CB7**-fluorophore conjugated hosts were applied to the detection and differentiation of twelve different model proteins (Fig. 11).[83] In this system the **CB7** cavity pan-selectively binds protein surface amino acid residues phenylalanine, tryptophan and tyrosine, with the conjugated dye providing additional secondary surface binding interactions. Diversity of response within this array arises from protein surface binding interactions, inducing different dye photophysical response mechanisms. This includes binding induced steric hindrance of the conjugated cyanine dyes in hosts **CB7-Cy3** and **CB7-Cy5** resulting in enhanced emission (Fig. 11b), **CB7-Cy5** also shows instances of absorbance/emission shifts resulting from environmental changes in dye polarity, along with decreased emission from dye aggregate formation. Hydrophobic binding interactions of conjugated silicon rhodamine in **CB7-SiR** results in ground state deactivated spirolactone formation (Fig. 11b). Decreased emission responses from conjugated **CB7-Coumarin** **CB7-Bodipy** and **CB7-TAMRA** are likely a result of amino acid static quenching events, as well as local pH effects on **CB7-Coumarin**. This sensitivity to a range of protein surface features linked to different photophysical responses was exploited in the differentiation of protein conformational changes, identifying native, misfolded, oligomeric and fibrillar protein states. Notably, this approach was able to distinguish amyloid-β folded aggregates associated with Alzheimer's disease.

So far, cross-reactive sensor arrays consisting of isolated host·dye reporter pairs or conjugated host sensors have proven effective at discriminating individual target analytes. In these examples solution complexity has progressed towards sensors

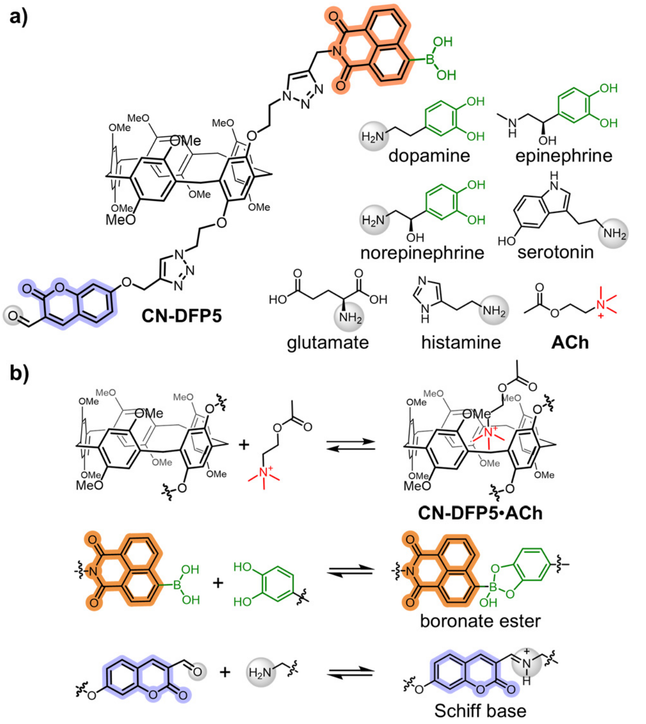

Fig. 10 Dual reactive host sensor **CN-DFP5** provides multimodal detection and differentiation of neurotransmitters in live neurons. (a) Chemical structure of **CN-DFP5** and differentiated neurotransmitters. The incorporated naphthalimide and coumarin fluorophores are indicated by orange and purple highlights respectively. Reactive boric acid and catechol handles are shown in green. Reactive aldehyde and primary amine handles are shown as grey circles. **ACh** quaternary amine binding motif is shown in red. (b) Neurotransmitter detection by **CN-DFP5** occurs through noncovalent self-assembly with cationic quaternary amines, and dynamic covalent reactions with catechol and primary amines.

that maintain function in biorelevant conditions, containing higher salt content and other competitive species. More recent examples in literature have demonstrated that incorporating diversity through a range of recognition properties and photophysical outputs can enhance differential applications, from biologically important small molecules, to larger peptides and proteins. Despite these advances, distinguishing highly similar analytes and complex mixtures of analytes is an ongoing challenge within the field. As application difficulty increases, more information-rich sensing outputs are required.

1.4. Expanding differential sensing through increased systems complexity

Systems chemistry is a powerful tool at the forefront of information-rich sensing. In complex systems, adaptive behaviour arises through self-organized networks of multiple interconnected equilibria. Information-rich outputs are produced from induced shifts in the overall networked equilibria, and emergent properties can arise that are otherwise unobtainable from the isolated system components.[84] Earlier work by Anslyn[85] and Severin[86] applied systems chemistry in sensing, showing that a single mixture of multiple interacting receptors and indicators can differentiate analytes through unique networked equilibria. The recent application of systems chemistry in macrocyclic host-based sensing has broadened perspectives from isolated pairwise molecular recognition events, towards building more complex systems. A simple way to increase complexity is through a co-complexation agent, an additional component (*e.g.* metal ion or salt) that interacts with sensor building blocks to promote the formation of additional complexes and/or binding geometries in equilibrium. Complexity can also be introduced by increasing the number of interacting building blocks (*e.g.* multiple dynamic covalent building blocks, hosts and/or dyes in a single solution), this can be further extended into multicomponent systems that form higher order complexation events.

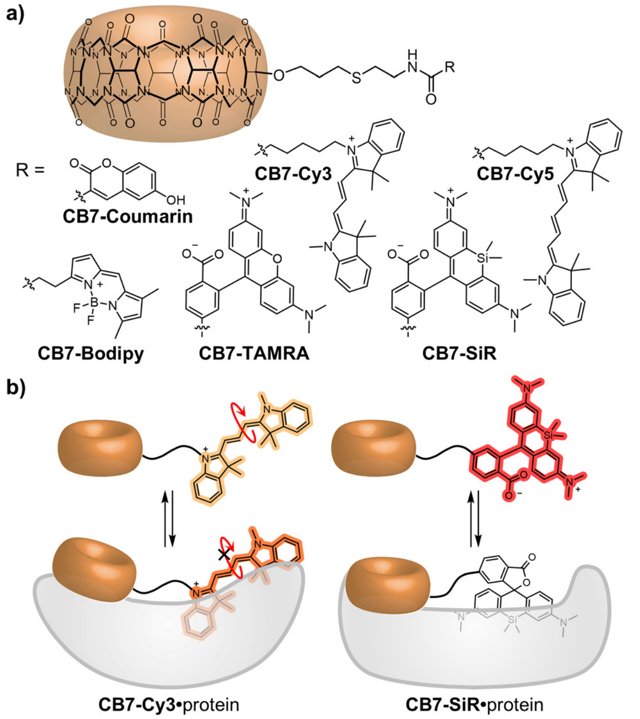

Fig. 11 A diverse array of conjugated **CB7**-fluorophores provides different mechanisms for probing protein surfaces, identifying a range of proteins and conformational folded states. (a) Chemical structures of an array of six **CB7**-fluorophore conjugates. (b) Sensing scheme illustrations of enhanced emission of **CB7-Cy5** through protein induced restricted rotation and decreased emission of **CB7-SiR** from spirolactone formation in hydrophobic protein environments. Grey shapes represent protein surface interactions.

Co-complexation agents in host-based sensing systems can modulate multiple binding geometries, enhancing sensing outputs for differentiation. In one example the known adverse effects of cationic salt interactions binding cucurbit[*n*]uril carbonyl portals was exploited as a method of introducing adaptive sensing (Fig. 12).[87] A unimolecular host-linker-dye was synthesized, tethering the polarity responsive dye nitrobenzoxadiazole (**NBD**) to form **CB7-NBD**. This synthetic design encourages co-complexation interactions with cationic salts (M^{n+}), facilitated by the conjugation of a neutral weak binding dye, **NBD** (as apposed to the previously introduced **CB7-BC**

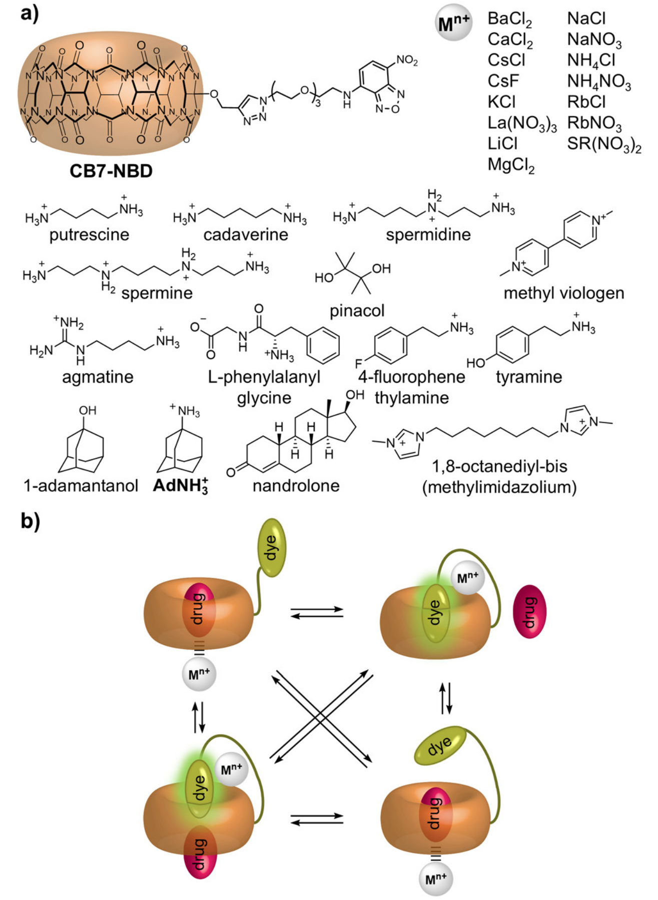

Fig. 12 Salts can be used as binding modulators, providing adaptive differential sensing of biorelevant compounds. (a) Chemical structures of the unimolecular dye conjugated host **CB7-NBD**, modulating salts (M^{n+}) and biorelevant analytes for differentiation. (b) Salt-induced analyte binding effects can cause displacement of the dye or induce different modes of binding in equilibrium, creating unique analyte sensing outputs.

cationic dye conjugates that repelled cationic salts). **CB7-NBD** forms an equilibrium of multiple analyte binding geometries modulated by secondary salt interactions (Fig. 12b). It is important to note that this salt induced complexity differs from previously mentioned interference effects that can prevent a change in signal. These salt interactions induce distinctive spectral shifts in absorbance and emission, contributing to a unique analyte fingerprint. Through alterations in fixed salt concentration and salt type, PCA differentiation of 14 bioorganic analytes (Fig. 12a) in buffer and biofluids (human urine, deproteinized human serum, artificial saliva and artificial synthetic urine) was achieved.

Combining dynamic combinatorial libraries with multicomponent noncovalent networks introduces a new method of *in situ* host formation and differential sensing. Waters and co-workers described an "imprint-and-report" dynamic combinatorial library, in which exchangeable dithiol building blocks form template-driven macrocycle IDA's (Fig. 13).[88] First the formation of disulfide containing macrocycles are templated by a guest (analyte or dye), amplifying a mixture of thermodynamically favoured hosts, relative to an untemplated system. Key to the operation of this approach is complete disulfide oxidation, "imprinting" the formed macrocycles and inhibiting building block re-equilibration. Subsequent addition of a secondary guest (dye or analyte) provides a unique signal based on a network of noncovalent interactions with the mixture of imprinted macrocycles (Fig. 13b and c). Combinations of 3–5 dynamic combinatorial libraries consisting of different macrocycle building blocks (**E**, **G** and **N**), dyes (**LCG**, acridine orange (**AO**), **Nme_3^+**-tethered fluorescein (**FAM-Nme_3^+**), thioflavin T (**ThT**), and dansyl choline), and analytes (methylated states of histone peptides) are applied in an array towards the discrimination of arginine (**R**) and lysine (**K**) post translational modifications on histone peptides **H3R8me$_x$** and **K9me$_y$**. This dynamic combinatorial and noncovalent systems approach avoids the need for host synthesis optimization, purification and characterization, achieving analyte differentiation through a network of dynamic covalent and noncovalent equilibria. Further advancements of "imprint-and-report" dynamic combinatorial libraries have been applied to the differentiation of trimethylamine *N*-oxide dietary metabolites and precursors,[89] as well as per- and polyfluoroalkyl contaminant mixtures in water.[90]

Highly similar biomacromolecules pose a challenge in differential sensing. Identifying small variations between biomacromolecules with conserved sequences and relative charge (*e.g.* differences in 3D folding and orientation) is a nontrivial task, often requiring techniques like crystallography and multidimensional NMR spectroscopy. In host-based sensing differentiation by hosts is limited by the similarity of occurring recognition events and binding affinity. This becomes a challenge for distinguishing biomacromolecules when there is little to no change in the direct host binding event (e.g. an anionic host can bind similar numbers of exposed cationic surface **K** and **R** amino acid residues on different proteins). Systems chemistry approaches that introduce multiple interacting components in equilibrium with one another and with other analyte surface features can overcome these limitations. Through perturbations of the overall interconnected competing equilibria, the effects of relatively small differences between samples can be enhanced.

Multi-complexation systems can distinguish identical biomacromolecule sequences that have adopted different folded structures. Work presented by Hooley and co-workers demonstrated that an array of sensing systems differentiated noncanonical folded DNA motifs by increasing the number of competing equilibria.[76,91,92] In this approach, cationic styryl pyridinium dyes (**DSMI**, **PSMI**, **MSMI** and **DQMI**) act as the primary sensing motif, mimicking known oligonucleotide ligands that directly bind DNA through aromatic nucleotide π–π stacking and phosphate backbone ionic interactions. The addition of cationic deep cavitand hosts (**CHI**, **CHP** or **AMI**) can interact with the dye, DNA, or both, in a mixture of competing binary and ternary fluorescent complexes (Fig. 14b). In one report a 16-element sensor array (4 styryl pyridinium dyes × 3 cavitand hosts and 4 isolated dyes) were applied to the

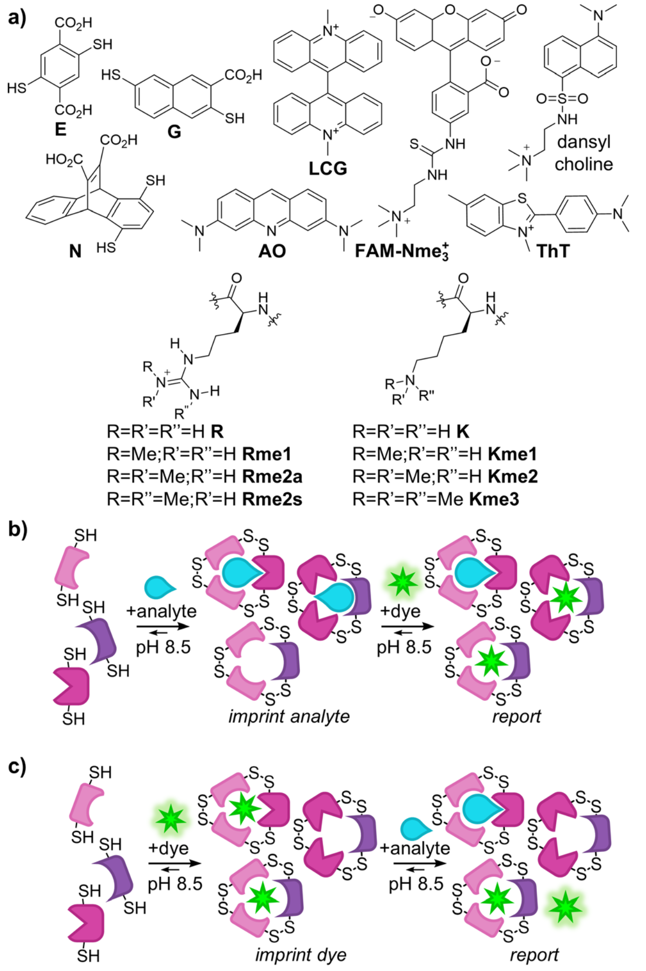

Fig. 13 Dynamic combinatorial libraries are combined in an "imprint-and-report" sensor array, differentiating methylation states of **R** and **K** containing peptides. (a) Chemical structures of exchangeable building blocks (**E**, **G** and **N**), fluorescent dyes (**LCG**, **AO**, **FAM-Nme$_3^+$**, **ThT** and dansyl choline) and methylation states of arginine and lysine residues of histone peptides **H3R8me$_x$** and **K9me$_y$**. (b) Dynamic combinatorial library components are imprinted by an analyte and then competitively displaced by a reporter fluorophore. (c) Dynamic combinatorial library components are imprinted by a fluorophore and then competitively displaced by an analyte.

differentiation of 18 different nonclassical DNA motifs (DNA G-quadruplexes with bulges and vacancies, Hoogsteen triplexes, hairpin and i-motifs).[76] This large data set illustrates an example where hand-selecting array responses for multivariate analysis becomes challenging. Fluorescence array outputs analyzed by an SVM–RFE training algorithm followed by PCA, not only allowed for the success in this challenging differentiation, but also the classification of unknown folded DNA samples. Most notably, differentiation is not possible with a traditional sensor array consisting of a set of DNA binding dyes. Instead, differentiation is achieved through host interactions that don't bind the target DNA directly but compete with DNA for dye binding. Overall, this work demonstrates that an increase in information can be achieved through additional competing equilibria, improving both the performance and capabilities of a sensing system.

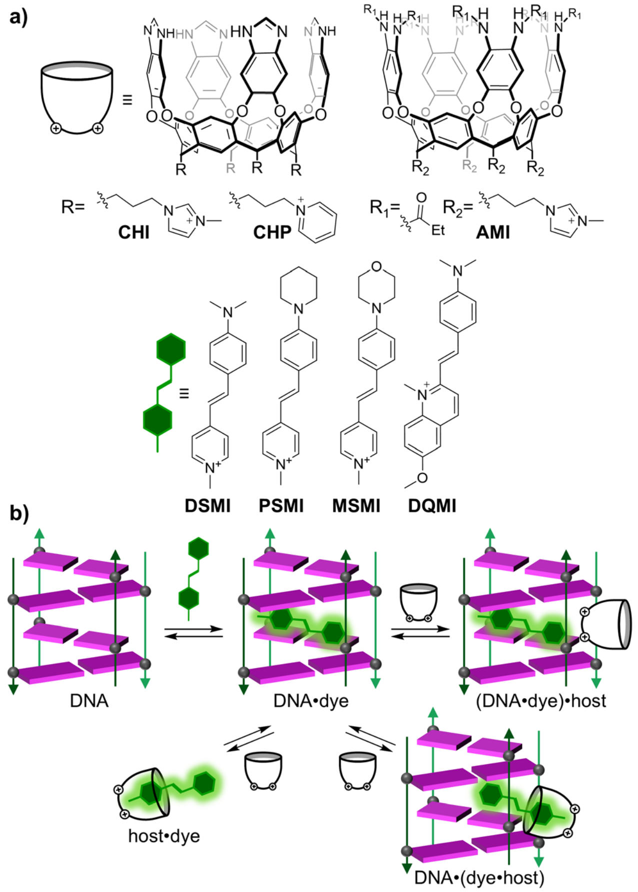

Fig. 14 An array of cationic deep cavitand hosts and styryl pyridinium dyes discriminate highly similar noncanonical DNA folded motifs. (a) Chemical structures of cavitand hosts (**CHI**, **CHP** and **AMI**) and reporter styryl pyridinium dyes (**DSMI**, **PSMI**, **MSMI** and **DQMI**). (b) Schematic illustration of the multiple binary and ternary complex equilibria between DNA i-motif, host, and reporter styryl dye leading to differential sensing.

We were intrigued by the recent advancements of systems-oriented strategies capable of more challenging sensing tasks. The prospect of emergent properties compelled us to combine multiple DimerDye hosts in a single one-pot solution. This initially began as a fundamental study driven by curiosity. Would mixed DimerDyes undergo self-sorting or would they form a network of homodimers and heterodimers in equilibrium? We selected three previously explored styryl pendant arm dyes (*N*-methylpyridinium, indolinium and *N*-phenylpyridinium) and integrated them into different *p*-sulfonatocalix[*n*]arene scaffolds, forming **hemiDD1Cx4**, **DD4Cx4**, and **DD13Cx5**.[93] These three DimerDyes cover a range of absorbance and emission properties while encouraging a range of binding affinities through differences in upper-rim negative charge and host cavity size. When combining these hosts in solution, heterodimers formed and non-additive changes in absorbance profiles indicated that complex photophysical perturbations were encoded in the equilibrating mixture. This network is primed to shift in distinct ways when an analyte is introduced, with the overall shifts in multiple linked equilibria providing a unique information-rich photophysical fingerprint

(Fig. 15a). As a fundamental study we selected mammalian serum albumin proteins as a challenging set of model analytes with highly conserved sequence identity and charge (Fig. 15b). From a one pot DDNetwork, easily discernible absorbance profile responses were produced (Fig. 15c) resulting in complete PCA differentiation of serum albumin proteins, including some that are up to 92% identical to each other (Fig. 15d). To further test the DDNetwork differentiation capabilities, we applied it to whole protein extracts from fish tissue (Fig. 15e). We found that the systems-based adaptive DDNetwork outperformed an analogous traditional DimerDye sensor array, providing us with an easy method to enhance the capabilities of sensors currently in our repertoire.

Co-assembled systems of different host scaffolds introduce diverse sensing of dissimilar analytes. Motivated by the enhanced performance of the DDNetwork, we decided to address DimerDye binding limitations through a co-assembled systems approach (*i.e.* *p*-sulfonatocalix[*n*]arenes exhibit limited binding to cationic analytes). We selected cucurbit[*n*]uril hosts (**CB7** and **CB8**) to promote co-assembly with DimerDyes (**DD8Cx4**, **DD4Cx4** and **DD13Cx4**), through binding of the pendant arm in the hydrophobic cucurbit[*n*]uril cavity.[94] By co-assembling two different host types *via* an integrated environmentally sensitive fluorophore, we create a single sensor that detects analyte binding interactions to either host, including binding interactions to the non-fluorophore-containing host (Fig. 16a). We showed that an array of mixed host co-assembled sensors can distinguish a range of bulky, hydrophobic, cationic, neutral and anionic drugs and adulterants (Fig. 16b). Not only does this approach increase the variety of analytes that are detectable, but it also introduces the chance for responses to analytes that are not canonical binders of either host to emerge. This is observed by the differentiation of highly similar Δ^8- and Δ^9-THC isomers (Fig. 16b), which are not known to bind either to DimerDye sensors or to **CB7**, and yet generate strong and useful responses to multiple co-assembled DD·CB sensors. This binding is an unpredicted emergent property. We rationalize it after the fact as possibly resulting from THC analogs forming ternary or higher-order complexes with the co-assembled hosts, that influence the reporter fluorophore. Whatever the origin of this emergent behaviour, the ability to detect and discriminate such incredibly similar analytes stands out as a remarkable outcome of chemical complexity that would not have been discovered from traditional single-sensor approaches. We further challenged mixed host co-assembled systems by applying them to multi-component illicit drug samples obtained directly from people who use drugs in Victoria, BC as part of a harm reduction project. These samples are incredibly complex, in that each individual drug sample can be made up of multiple hydrophobic, neutral and/or cationic molecules in varying amounts (Table 1), and can contain drugs that are very similar to each other (*e.g.* fentanyl and fluorofentanyl). In our hands, the mixed host co-assembled sensor responses provided a range of absorbance and fluorescence responses that enabled differentiation of all samples, apart from two samples that were reported as the same from the same street source (H and I). We found that the systems chemistry approach again outperformed the same DimerDye sensors when used in isolation from each other, as part of a traditional sensor array (Fig. 16c).

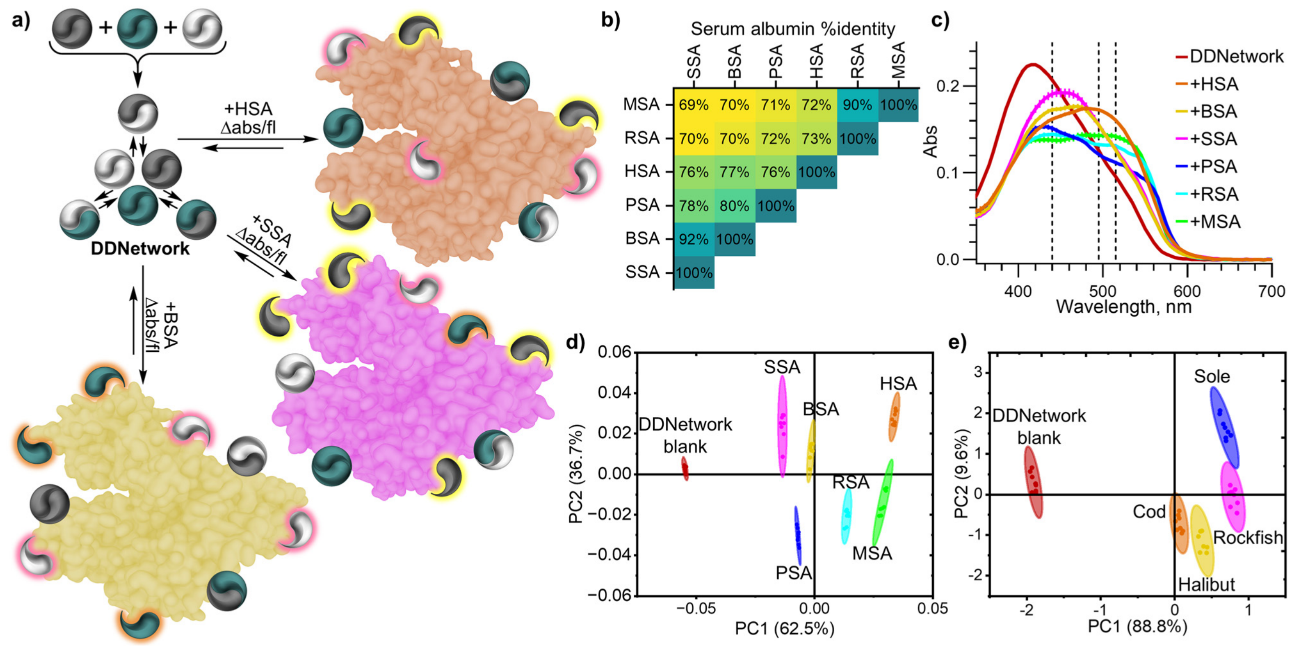

Fig. 15 A one-pot adaptive network of DimerDye sensors discriminates model proteins and complex protein mixtures. (a) Schematic of DDNetwork adaptive sensing responses to serum albumin proteins. (b) Serum albumin (human (HSA), bovine (BSA), sheep (SSA), porcine (PSA), rat (RSA) and mouse (MSA)) protein sequences are highly conserved across different species. (c) DDNetwork produces distinct absorbance profile responses to highly similar serum albumin proteins, (c) distinguishable by PCA analysis. (d) PCA analysis of DDNetwork responses distinguishes complex protein mixtures extracted from the tissue of different fish varieties. Reproduced/adapted from ref. 93. Copyright 2023 with permission from John Wiley and Sons/Wiley-VCH.

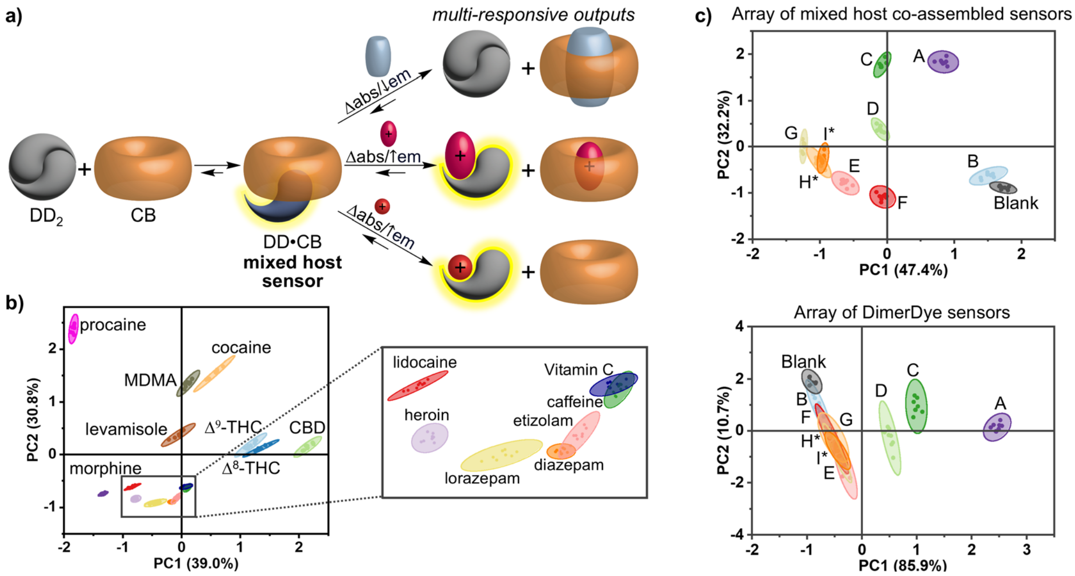

Fig. 16 Mixed host co-assembled sensors produce multi-responsive outputs expanding the scope of analyte sensing and differentiation. (a) Schematic of mixed host co-assembly of DimerDye (DD) and cucurbit[*n*]uril (CB) producing moderate changes in absorbance and fluorescence. Addition of an analyte disrupts co-assembly through binding to either host, producing distinct changes in absorbance and/or fluorescence. (b) PCA analysis of an array of mixed host co-assembled DD·CB sensors distinguishes cationic and neutral illicit drugs and adulterants. (c) PCA analysis of an array of mixed host co-assembled sensors discriminates multi-component real-world street drug samples A–I (Table 1). PCA analysis of an equivalent array of DimerDye sensors does not discriminate. Reproduced from ref. 94 with permission from the Royal Society of Chemistry.

Adjustments in building block combinations, ratios and environment is a facile way to introduce a greater range of complexity that enhances differential sensing. Work presented by Guo and co-workers reports a straightforward construction of tunable sensor array libraries with heteromultivalent self-assemblies.[95] Co-assembly of amphiphilic calixarene (**GCnAS**, **QCnAS**, and **sCxnAS**) and amphiphilic cyclodextrin (**CD**) hosts leads to the formation of self-assembled vesicle aggregates that vary in recognition ability (Fig. 17a). The dynamic noncovalent self-assembly of vesicles allows for the generation of several different co-assembled sensors, where binding properties are modified simply by varying relative concentrations. Cationic calixarene hosts (**GCnAS** and **QCnAS**) were combined with fluorescent dyes that quench upon binding (**AlPcS$_4$** and **TPPS**) and enhance upon binding (**2,6-TNS**, **1,8-ANS** and **PTPE**). Anionic calixarene hosts (**sCxnAS**) were combined with **LCG**, inducing fluorescence quenching. This work incorporates a multitude of sensor arrays with different host co-assemblies, dye combinations, component ratios and solution pH, while fixing one of these variables within each array (Fig. 17b). Through this library strategy, thirteen model proteins and their mixtures were differentiated using LDA analysis. Array-based sensing was further applied to the differentiation of honeys from different floral origins, brands and adulterated mixtures representing more complex sample compositions (containing sugars, enzymes, amino acids, vitamins, minerals and aromatic compounds). The discrimination ability of these system sensor arrays arises from the different intertwined host·dye, host·analyte, and dye·analyte affinities, as well as the nonlinear relationship between signal response and concentration. This work represents a very robust path to quickly and easily obtaining information-rich libraries that can be extended to many building blocks and targets. Further development of heteromultivalent amphiphilic co-assemblies have been applied in recognition and discrimination of normal, cancerous and cross-contaminated cell lines.[96]

Table 1 Multi-component illicit street drug sample composition. Reproduced from ref. 94 with permission from the Royal Society of Chemistry

Street drug sample	Composition
A	Cocaine (90%), sorbitol
B	Bromazolam (>80% single component)
C	Methylenedioxymethamphetamine (MDMA, >80% single component)
D	Methylenedioxyamphetamine (MDA, 50%), dimethyl sulfone
E	Fentanyl (20%), caffeine, erythritol
F	Fentanyl (13%), fluorofentanyl (1%), caffeine
G	Fentanyl (6%), bromazolam (5%), chloroisobutyryl fentanyl (0.1%), caffeine
H[a]	Fentanyl (16%), fluorofentanyl (14%), 4-anilino-*N*-phenethyl-piperidine (ANPP, 3.7%), erythritol, caffeine
I[a]	Fentanyl (18%), fluorofentanyl (16%), ANPP (3.5%), erythritol, caffeine

[a] Samples H and I were provided by two different people who use drugs reporting the same drug from the same batch and supplier.

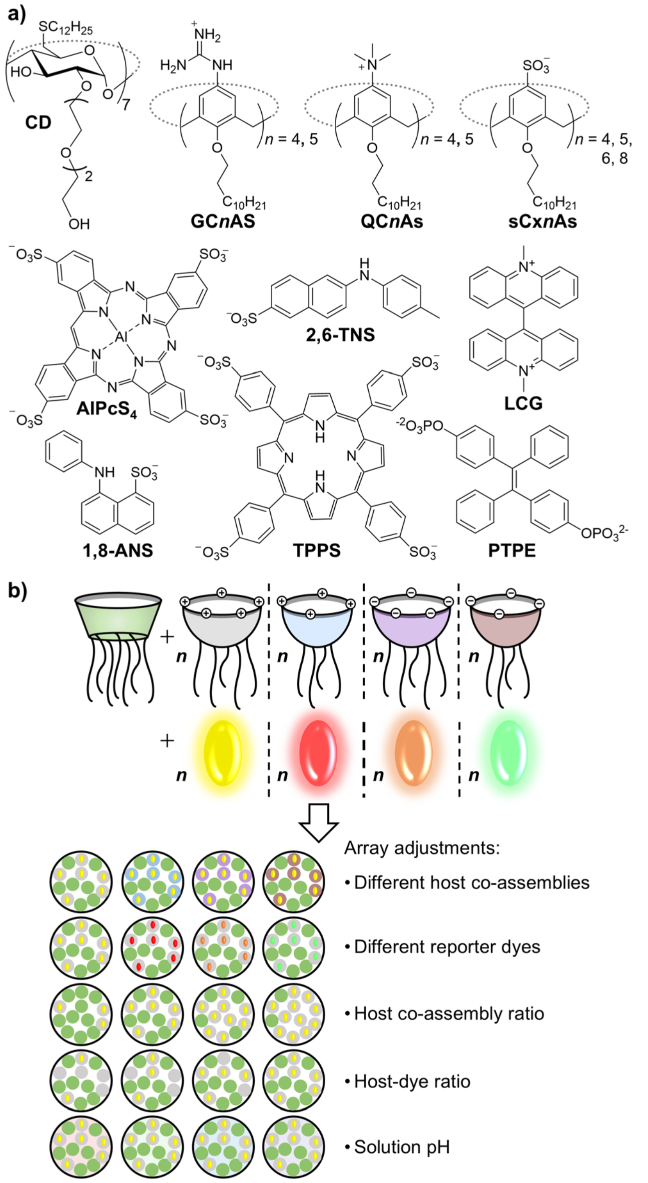

Fig. 17 Muli-complexation sensing systems provide a robust and facile method for building differential sensor arrays. (a) Chemical structures of amphiphilic hosts (**CD**, **GCnAS**, **QCnAS**, and **sCxnAS**) and reporter dye (**AlPcS$_4$**, **2,6-TNS**, **LCG**, **1,8-ANS**, **TPPS**, **PTPE**) building blocks explored in the construction of multiplexing sensor arrays. (b) Schematic representation of different host co-assemblies, reporter dye combinations, ratios and environmental pH conditions used to construct sensor arrays towards the differentiation of protein model analytes and complex honey mixtures.

2. Conclusions

Overall, the recent application of systems chemistry represents a paradigm shift in the field towards increasingly complex, information-rich sensing. Initial work done by our group on host-based sensor arrays, much like others in the field, was built around isolated sensors detecting individual small molecules in aqueous solution. This typical array approach can be effective when operating in relatively simple conditions with analytes that vary in binding affinity. However, when distinguishing analytes that are more challenging by virtue of being highly similar to each other, large biomacromolecules, mixtures of analytes, or all of the above, new information-rich approaches are needed.

As the field has progressed, rational synthetic design of salt tolerant sensors has also developed alongside it. Our group contributed to salt-tolerant sensors through the development of like-charged host self-assemblies that persist and strengthen in the presence of salts. Others have contributed clever covalent host-dye conjugation designs that repel salt interference as well as harness salt interactions to further modulate binding. These advances have provided host-based design strategies that maintain function in salty biological solutions.

More recently, complex systems are being applied to differential sensor arrays, achieving more challenging sensing tasks that overcome limitations in isolated pairwise sensor arrays. Our work made contributions to this area through the design of multi-host systems capable of distinguishing highly similar proteins, tissue extracts and real-world samples containing complex mixtures of similar and dissimilar analytes. Systems-oriented strategies introduced by others, incorporating multiple hosts, dyes, or co-complexation agents have proved invaluable, providing more information-rich sensing outputs.

The concepts of systems complexity that are touched on in this Feature Article can be readily extended to build more powerful sensing tools. Most importantly, the design of new complex systems should promote cross-reactivity amongst multiple building blocks, to facilitate information-rich outputs as well as to create the potential for beneficial emergent properties. To achieve this, we must let go of the classical view that one can design the perfect host for each guest and each associated function. Instead, we encourage a shift towards designing systems that are likely to generate the desired functions as an expression of their complexity. In short, we must embrace the promiscuous binding that symmetrical macrocycles have to offer.

To build systems complexity, mixtures of three or more cross-reactive building blocks can be combined. This could include a range of accessible water-soluble macrocyclic hosts that differ in binding properties and affinities (*e.g.* calixarenes, cucurbiturils, pillararenes, calixpyrroles, and cyclodextrins), the incorporation of interacting dyes that undergo diverse photophysical responses (*e.g.* dyes that undergo static quenching, fluorescence enhancement, charge transfer, FRET pairs, solvatochromic sensitivity, pH sensitivity) and/or the incorporation of additional components that facilitate co-complexation or higher-order self-assemblies. Reported examples thus far include metal ions or salts that co-complex with host assemblies, the incorporation of dynamic covalent building blocks, host dimerization and host aggregation. When selecting reporter pairs it is important to consider the influence of salts in biological solutions. Noncovalent pairs must provide a monitorable change in signal or be resilient to unwanted dye quenching or interference. Further systematic variations in the construction of system arrays can be explored through changes in component combinations, ratios and pH. By combining building blocks that interact with one another and operate in a non-linear binding region, small differences in multiple interconnected equilibria can be extrapolated. Through systems approaches the ability to distinguish highly similar analytes along with diverse classes of dissimilar analytes can arise, pushing differential sensing further.

As larger data sets are produced, the community is adopting more advanced data science strategies such as machine learning. This offers efficient routes to determine the most valuable variables within complex data sets, and also can offer new ways to derive enhanced sensing functions. The development of such approaches will further enable the community to overcome challenging tasks like identifying small changes in complex biological mixtures, or identifying multiple components within complex samples. As the field progresses, we believe that increasingly powerful sensing tools will emerge from efforts that use some elements of rational molecular design while allowing for complexity to give rise to non-designed sensing outcomes.

Author contributions

A. J. S. wrote the original draft. F. H. acquired funding and contributed review and editing.

Conflicts of interest

There are no conflicts to declare.

Data availability

No primary research results have been included, and no new data were generated or analyzed as part of this Feature Article.

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References

1 G. M. Whitesides and R. F. Ismagilov, *Science*, 1999, **284**, 89–92.
2 G. Ashkenasy, T. M. Hermans, S. Otto and A. F. Taylor, *Chem. Soc. Rev.*, 2017, **46**, 2543–2554.
3 J. M. Lehn, *Chem. Soc. Rev.*, 2007, **36**, 151–160.
4 J. Krämer, R. Kang, L. M. Grimm, L. De Cola, P. Picchetti and F. Biedermann, *Chem. Rev.*, 2022, **122**, 3459–3636.
5 J. Zhou, G. Yu and F. Huang, *Chem. Soc. Rev.*, 2017, **46**, 7021–7053.
6 A. S. Braegelman and M. J. Webber, *Theranostics*, 2019, **9**, 3017–3040.
7 H. Yin, X. Zhang, J. Wei, S. Lu, D. Bardelang and R. Wang, *Theranostics*, 2021, **11**, 1513–1526.
8 C.-L. Deng, S. L. Murkli and L. D. Isaacs, *Chem. Soc. Rev.*, 2020, **49**, 7516–7532.
9 M. A. Beatty and F. Hof, *Chem. Soc. Rev.*, 2021, **50**, 4812–4832.
10 L. Escobar and P. Ballester, *Chem. Rev.*, 2021, **121**, 2445–2514.
11 E. Mattia and S. Otto, *Nat. Nanotechnol.*, 2015, **10**, 111–119.
12 W.-L. Guan, J.-F. Chen, J. Liu, B. Shi, H. Yao, Y.-M. Zhang, T.-B. Wei and Q. Lin, *Coord. Chem. Rev.*, 2024, **507**, 215717.
13 Z.-D. Tang, X.-M. Sun, T.-T. Huang, J. Liu, B. Shi, H. Yao, Y.-M. Zhang, T.-B. Wei and Q. Lin, *Chin. Chem. Lett.*, 2023, **34**, 107698.
14 K. Daze and F. Hof, *Encyclopedia of Physical Organic Chemistry*, ed Z. Wang, U. Wille and E. Juaristi, Wiley, 2016, pp. 1–51.
15 H. S. Frank and M. W. Evans, *J. Chem. Phys.*, 1945, **13**, 507–532.
16 C. Tanford, *Proc. Natl. Acad. Sci. U. S. A.*, 1979, **76**, 4175–4176.
17 D. B. Smithrud, E. M. Sanford, I. Chao, S. B. Ferguson, D. R. Carcanague, J. D. Evanseck, K. N. Houk and F. Diederich, *Pure Appl. Chem.*, 1990, **62**, 2227–2236.
18 D. Chandler, *Nature*, 2005, **437**, 640–647.
19 E. V. Anslyn and D. A. Dougherty, *Modern physical organic chemistry*, University Science, Sausalito, Calif., 2006.
20 F. Biedermann, W. M. Nau and H. J. Schneider, *Angew. Chem., Int. Ed.*, 2014, **53**, 11158–11171.
21 S. Kubik, *Supramolecular Chemistry in Water*, Wiley, 2019.
22 S. Kubik, *ChemistryOpen*, 2022, **11**, e202200028.
23 G. W. Gokel, L. Barbour and J. L. Atwood, *Comprehensive supramolecular chemistry II*, Elsevier Science, 2017.
24 S. Otto, R. L. E. Furlan and J. K. M. Sanders, *Drug Discovery Today*, 2002, **7**, 117–125.
25 P. T. Corbett, J. Leclaire, L. Vial, K. R. West, J. L. Wietor, J. K. Sanders and S. Otto, *Chem. Rev.*, 2006, **106**, 3652–3711.
26 M. V. Rekharsky and Y. Inoue, *Chem. Rev.*, 1998, **98**, 1875–1918.
27 K. N. Houk, A. G. Leach, S. P. Kim and X. Zhang, *Angew. Chem., Int. Ed.*, 2003, **42**, 4872–4897.
28 Y.-C. Pan, J.-H. Tian and D.-S. Guo, *Acc. Chem. Res.*, 2023, **56**, 3626–3639.
29 F.-Y. Chen, W.-C. Geng, K. Cai and D.-S. Guo, *Chin. Chem. Lett.*, 2024, **35**, 109161.
30 L. Escobar, Q. Sun and P. Ballester, *Acc. Chem. Res.*, 2023, **56**, 500–513.
31 J. Quílez-Pardo and J. J. Solaz-Portolés, *J. Res. Sci. Teach.*, 1995, **32**, 939–957.
32 J. H. Jordan, H. S. Ashbaugh, J. T. Mague and B. C. Gibb, *J. Am. Chem. Soc.*, 2021, **143**, 18605–18616.
33 A. J. Selinger and D. H. Macartney, *RSC Adv.*, 2017, **7**, 42513–42518.
34 J. S. Kim and D. T. Quang, *Chem. Rev.*, 2007, **107**, 3780–3799.
35 A. P. De Silva, T. S. Moody and G. D. Wright, *Analyst*, 2009, **134**, 2385–2393.
36 B. Valeur, *Coord. Chem. Rev.*, 2000, **205**, 3–40.
37 R. N. Dsouza, U. Pischel and W. M. Nau, *Chem. Rev.*, 2011, **111**, 7941–7980.
38 J.-R. Wu, G. Wu, D. Li and Y.-W. Yang, *Angew. Chem., Int. Ed.*, 2023, **62**, e202218142.
39 X.-Y. Lou, N. Song and Y.-W. Yang, *Molecules*, 2017, **22**, 1640.
40 B. T. Nguyen and E. V. Anslyn, *Coord. Chem. Rev.*, 2006, **250**, 3118–3127.
41 A. C. Sedgwick, J. T. Brewster, T. Wu, X. Feng, S. D. Bull, X. Qian, J. L. Sessler, T. D. James, E. V. Anslyn and X. Sun, *Chem. Soc. Rev.*, 2021, **50**, 9–38.
42 S. L. Wiskur, H. Ait-Haddou, J. J. Lavigne and E. V. Anslyn, *Acc. Chem. Res.*, 2001, **34**, 963–972.
43 D.-S. Guo, V. D. Uzunova, X. Su, Y. Liu and W. M. Nau, *Chem. Sci.*, 2011, **2**, 1722–1734.
44 S. Sinn, J. Krämer and F. Biedermann, *Chem. Commun.*, 2020, **56**, 6620–6623.
45 G. Ghale, A. G. Lanctot, H. T. Kreissl, M. H. Jacob, H. Weingart, M. Winterhalter and W. M. Nau, *Angew. Chem., Int. Ed.*, 2014, **53**, 2762–2765.
46 S. Pangeni, J. D. Prajapati, J. Bafna, M. Nilam, W. M. Nau, U. Kleinekathofer and M. Winterhalter, *Angew. Chem., Int. Ed.*, 2021, **60**, 8089–8094.
47 M. Nilam, S. Karmacharya, W. M. Nau and A. Hennig, *Angew. Chem., Int. Ed.*, 2022, **61**, e202207950.
48 R. Jiang, M. Nilam, C. Piselli, M. Winterhalter, D. S. Guo, S. Y. Yu, A. Hennig and W. M. Nau, *Angew. Chem., Int. Ed.*, 2025, **64**, e202425157.
49 K. D. Legg and D. M. Hercules, *J. Phys. Chem.*, 1970, **74**, 2114–2118.
50 V. M. Krishnamurthy, V. Semetey, P. J. Bracher, N. Shen and G. M. Whitesides, *J. Am. Chem. Soc.*, 2007, **129**, 1312–1320.
51 C. Hu, L. Grimm, A. Prabodh, A. Baksi, A. Siennicka, P. A. Levkin, M. M. Kappes and F. Biedermann, *Chem. Sci.*, 2020, **11**, 11142–11153.

52 M. Megyesi, L. Biczók and I. Jablonkai, *J. Phys. Chem. C*, 2008, **112**, 3410–3416.
53 S. S. Thomas, H. Tang and C. Bohne, *J. Am. Chem. Soc.*, 2019, **141**, 9645–9654.
54 H. Tang, D. Fuentealba, Y. H. Ko, N. Selvapalam, K. Kim and C. Bohne, *J. Am. Chem. Soc.*, 2011, **133**, 20623–20633.
55 H.-J. Buschmann, E. Cleve, K. Jansen, A. Wego and E. Schollmeyer, *J. Incl. Phenom. Macrocycl. Chem.*, 2001, **40**, 117–120.
56 A. T. Bockus, L. C. Smith, A. G. Grice, O. A. Ali, C. C. Young, W. Mobley, A. Leek, J. L. Roberts, B. Vinciguerra, L. Isaacs and A. R. Urbach, *J. Am. Chem. Soc.*, 2016, **138**, 16549–16552.
57 J. Krämer, L. M. Grimm, C. Zhong, M. Hirtz and F. Biedermann, *Nat. Commun.*, 2023, **14**, 518.
58 M. B. Hillyer, H. Gan and B. C. Gibb, *ChemPhysChem*, 2018, **19**, 2285–2289.
59 G. A. Garnett, K. D. Daze, J. A. Pena Diaz, N. Fagen, A. Shaurya, M. C. Ma, M. S. Collins, D. W. Johnson, L. N. Zakharov and F. Hof, *Chem. Commun.*, 2016, **52**, 2768–2771.
60 N. J. Hestand and F. C. Spano, *Chem. Rev.*, 2018, **118**, 7069–7163.
61 M. A. Beatty, J. Borges-González, N. J. Sinclair, A. T. Pye and F. Hof, *J. Am. Chem. Soc.*, 2018, **140**, 3500–3504.
62 C. Gallo, S. S. Thomas, A. J. Selinger, F. Hof and C. Bohne, *J. Org. Chem.*, 2021, **86**, 10782–10787.
63 Z. Xu, S. Jia, W. Wang, Z. Yuan, B. Jan Ravoo and D.-S. Guo, *Nat. Chem.*, 2019, **11**, 86–93.
64 E. Zumbro and A. Alexander-Katz, *ACS Omega*, 2020, **5**, 10774–10781.
65 E. Fischer, *Ber. Dtsch. Chem. Ges.*, 1984, **27**, 2985–2993.
66 J.-P. Behr, *The Lock-and-Key Principle: The State of the Art-100 Years On*, Wiley, Chichester [England], 1994.
67 Y. Geng, W. J. Peveler and V. M. Rotello, *Angew. Chem., Int. Ed.*, 2019, **58**, 5190–5200.
68 C. Bushdid, M. O. Magnasco, L. B. Vosshall and A. Keller, *Science*, 2014, **343**, 1370–1372.
69 J. D. Mainland, Y. R. Li, T. Zhou, W. L. Liu and H. Matsunami, *Sci. Data*, 2015, **2**, 150002.
70 S. Wold, K. Esbensen and P. Geladi, *Chemom. Intell. Lab. Syst.*, 1987, **2**, 37–52.
71 J. H. Friedman, *J. Am. Stat. Assoc.*, 1989, **84**, 165–175.
72 S. Stewart, M. A. Ivy and E. V. Anslyn, *Chem. Soc. Rev.*, 2014, **43**, 70–84.
73 C. Cortes and V. Vapnik, *Mach. Learn.*, 1995, **20**, 273–297.
74 I. Guyon, J. Weston, S. Barnhill and V. Vapnik, *Mach. Learn.*, 2002, **46**, 389–422.
75 R. G. Brereton and G. R. Lloyd, *Analyst*, 2010, **135**, 230–267.
76 J. Chen, A. D. Gill, B. L. Hickey, Z. Gao, X. Cui, R. J. Hooley and W. Zhong, *J. Am. Chem. Soc.*, 2021, **143**, 12791–12799.
77 T. Minami, N. A. Esipenko, B. Zhang, M. E. Kozelkova, L. Isaacs, R. Nishiyabu, Y. Kubo and P. Anzenbacher, *J. Am. Chem. Soc.*, 2012, **134**, 20021–20024.
78 J. Chen, J. L. Moreno, W. Zhang, L. J. Gibson-Elias, R. Lian, S. Najafi, H. Zhang, W. Zhong and R. J. Hooley, *Chem. Commun.*, 2024, **60**, 5598–5601.
79 S. A. Minaker, K. D. Daze, M. C. Ma and F. Hof, *J. Am. Chem. Soc.*, 2012, **134**, 11674–11680.
80 G. R. Rosania, J. W. Lee, L. Ding, H. S. Yoon and Y. T. Chang, *J. Am. Chem. Soc.*, 2003, **125**, 1130–1131.
81 M. A. Beatty, A. J. Selinger, Y. Li and F. Hof, *J. Am. Chem. Soc.*, 2019, **141**, 16763–16771.
82 Y. Mei, Q. W. Zhang, Q. Gu, Z. Liu, X. He and Y. Tian, *J. Am. Chem. Soc.*, 2022, **144**, 2351–2359.
83 N. Das Saha, S. Pradhan, R. Sasmal, A. Sarkar, C. M. Berač, J. C. Kölsch, M. Pahwa, S. Show, Y. Rozenholc, Z. Topçu, V. Alessandrini, J. Guibourdenche, V. Tsatsaris, N. Gagey-Eilstein and S. S. Agasti, *J. Am. Chem. Soc.*, 2022, **144**, 14363–14379.
84 J. R. Nitschke, *Nature*, 2009, **462**, 736–738.
85 S. L. Wiskur, P. N. Floriano, E. V. Anslyn and J. T. McDevitt, *Angew. Chem., Int. Ed.*, 2003, **42**, 2070–2072.
86 A. Buryak and K. Severin, *Angew. Chem., Int. Ed.*, 2005, **44**, 7935–7938.
87 C. Hu, T. Jochmann, P. Chakraborty, M. Neumaier, P. A. Levkin, M. M. Kappes and F. Biedermann, *J. Am. Chem. Soc.*, 2022, **144**, 13084–13095.
88 E. E. Harrison, B. A. Carpenter, L. E. Louis, A. G. Mullins and M. L. Waters, *J. Am. Chem. Soc.*, 2021, **143**, 14845–14854.
89 E. E. Harrison and M. L. Waters, *Angew. Chem., Int. Ed.*, 2022, **61**, e202205193.
90 E. E. Harrison and M. L. Waters, *Chem. Sci.*, 2023, **14** 928–936.
91 J. Chen, B. L. Hickey, L. Wang, J. Lee, A. D. Gill, A. Favero, R. Pinalli, E. Dalcanale, R. J. Hooley and W. Zhong, *Nat. Chem.*, 2021, **13**, 488–495.
92 J. Chen, B. L. Hickey, Z. Gao, A. A. P. Raz, R. J. Hooley and W. Zhong, *ACS Sens.*, 2022, 7, 2164–2169.
93 A. J. Selinger and F. Hof, *Angew. Chem., Int. Ed.*, 2023, **62**, e202312407.
94 A. J. Selinger, J. Kramer, E. Poarch, D. Hore, F. Biedermann and F. Hof, *Chem. Sci.*, 2024, **15**, 12388–12397.
95 J.-H. Tian, X.-Y. Hu, Z.-Y. Hu, H.-W. Tian, J.-J. Li, Y.-C. Pan, H.-B. Li and D.-S. Guo, *Nat. Commun.*, 2022, **13**, 4293.
96 X.-Y. Hu, Z.-Y. Hu, J.-H. Tian, L. Shi, F. Ding, H.-B. Li and D.-S. Guo, *Chem. Commun.*, 2022, **58**, 13198–13201.