Journal of Materials Chemistry

Cite this: *J. Mater. Chem.*, 2011, **21**, 12588

www.rsc.org/materials

FEATURE ARTICLE

Mimicking tricks from nature with sensory organic–inorganic hybrid materials

Ramón Martínez-Máñez,*[abc] Félix Sancenón,[abc] Mustafa Biyikal,[d] Mandy Hecht[d] and Knut Rurack*[d]

Received 21st March 2011, Accepted 18th May 2011
DOI: 10.1039/c1jm11210d

Design strategies for (bio)chemical systems that are inspired by nature's accomplishments in system design and operation on various levels of complexity are increasingly gaining in importance. Within the broad field of biomimetic chemistry, this article highlights various attempts toward improved and sophisticated sensory materials that rely on the combination of supramolecular (bio)chemical recognition principles and nanoscopic solid structures. Examples range from more established concepts such as hybrid sensing ensembles with improved sensitivity and selectivity or for target analytes for which selectivity is hard to achieve by conventional methods, which were often inspired by protein binding pockets or ion channels in membranes, to very recent approaches relying on target-gated amplified signalling with functionalised mesoporous inorganic supports and the integration of native biological sensory species such as transmembrane proteins in spherically supported bilayer membranes. Besides obvious mimicry of recognition-based processes, selected approaches toward chemical transduction junctions utilizing artificially organized synapses, hybrid ensembles for improved antibody generation and uniquely colour changing systems are discussed. All of these strategies open up exciting new prospects for the development of sensing concepts and sensory devices at the interface of nanotechnology, smart materials and supramolecular (bio)chemistry.

[a]*Centro de Reconocimiento Molecular y Desarrollo Tecnológico (IDM), Unidad Mixta Universidad Politécnica de Valencia-Universidad de Valencia, Spain*
[b]*Departamento de Química, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022 Valencia, Spain. E-mail: rmaez@qim.upv.es*
[c]*CIBER de Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), Spain*
[d]*Div. 1.5, BAM Bundesanstalt für Materialforschung und -prüfung, Richard-Willstätter-Str. 11, D-12489 Berlin, Germany. E-mail: knut.rurack@bam.de*

Félix Sáncenón (left) and Ramón Martínez-Máñez (right)

Félix Sáncenón (left) studied Chemistry and was graduated in 1991. He received the PhD degree in 2003 with Professor R. Martínez-Máñez in the Polytechnic University of Valencia within the field of chromogenic and fluorogenic chemosensors for cations and anions. After this, he obtained a Marie-Curie contract from the E.U. and worked with Professor L. Fabbrizzi at the Universitá di Pavia on the synthesis of chromogenic receptors for ion-pairs. Then, he joined the Department of Chemistry at the Polytechnic University of Valencia with a Ramón y Cajal contract. He became Lecturer in 2006. His actual research interest comprises the use of hybrid materials for the development of sensors. Ramón Martínez-Máñez (right) studied Chemistry at the University of Valencia and received his PhD in 1990 with Prof. P. Lahuerta. He was a postdoctoral fellow at Cambridge University, UK, with Prof. E. C. Constable. He is full professor in the Department of Chemistry at the Polytechnic University of Valencia since 2002. At present he is the director of the IDM Research Institute at the Polytechnic University of Valencia. His actual research interest involves the development of new methods for sensing anions, cations and neutral molecules. He is also involved in the design of gated hybrid materials for on-command delivery applications.

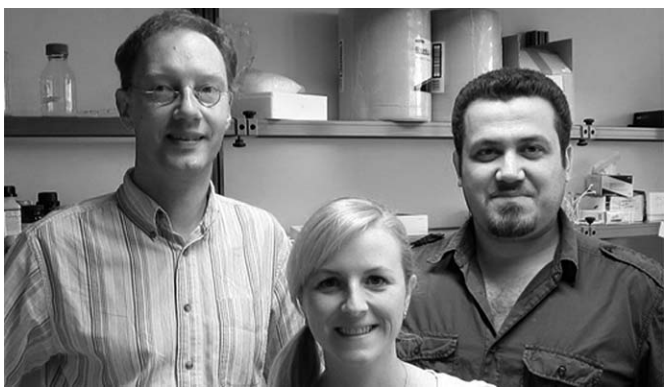
1 Introduction

Perhaps the best performing sensory systems today are much older than any attempt of a chemist to create an analytical test or device. Since millions of years, for instance, living organisms are able to differentiate between complex mixtures of compounds in different odours or to detect and respond to molecules such as pheromones almost at the single-molecule level.[1,2] Whereas differential recognition requires optimum complementarity of non- or less selective receptors, the ultrasensitive detection of pheromones relies on very specific receptors. At the cellular level, many vital functions are also connected to the ability of a particular receptor recognizing a specific guest, whether an effector or a second messenger,[3] or by mistake binding the wrong species which might block the receptor's activity,[4] eventually leading to malfunction. The latter feature, however, can also be used deliberately to inhibit certain cellular trafficking pathways.[5] In living organisms, the majority of these sensory elements does not exist as dissolved molecules, but is bound to a more or less flexible bio(in)organic skeletal backbone. The organizing support and supramolecular (bio)recognition element thus present a hybrid ensemble, often exerting synergistic effects and being implemented in signal transduction cascades.[6]

In analytical chemistry, the traditional approach to detect a guest through supramolecular recognition involves an indicator molecule that carries a suitable binding site and is able to undergo a change in a physico-chemical property upon target binding.[7] It is commonly employed in its molecular form in either a neat or processed sample. For many chemical species, however, there are inherent limitations with regard to indicator development, whether selectivity is difficult to reach by conventional supramolecular design, the interactions between host and guest to be expected are too weak or non-specific or the synthetic effort to create a suitable probe molecule is too high. In view of the perfection nature has reached in molecular and pattern recognition, chemists thus started to explore the possibilities of borrowing concepts and strategies from nature for the development of composite ensembles that mimic biological functions in the last decades. With regard to functional aspects, the combination of nanoscopic or nanostructured solid scaffoldings with additional (bio)organic molecules capable of supramolecular interactions is especially appealing.[8] Here, in particular the use of inorganic supports harbours distinct advantages, because these materials often possess a high physical and chemical stability, frequently combined with a high degree of order or periodicity, and because many of them can be functionalised in a straightforward manner. Over the last 20 years, various examples of such organic–inorganic hybrid functional materials were reported.[9–11] Within the scope of this article, responsive systems that incorporate addressable active units which can be triggered by a target species as an external stimulus and transduce a measurable analytical signal in return are especially attractive. The article will not discuss the synthesis and characterization of biomimetic hybrid ensembles in detail, but will present selected examples of hybrid materials that mimic biological functions with a special focus on chemical sensing. For all these examples, the combination of nanoscopic structures and supramolecular and/or bio-organic concepts leads to synergistic effects, enabling the ensembles to perform enhanced functions, which are hardly achievable by a use of the individual systems alone.

2 Mimicking binding pockets

Binding pockets can be considered as the most crucial nanoscopic domains in biomacromolecules such as proteins and enzymes. Besides offering the relevant functional groups that facilitate binding of the desired substrate or class of substrates in an adequate topology, their construction also has to guarantee that the respective reaction between (commonly) organic compounds proceeds reliably and quantitatively in an aqueous environment. Exclusive supramolecular control is thus not sufficient for success, but usually a physico-chemical control is also of major importance. For instance, many proteins are only



Knut Rurack (**left**), **Mandy Hecht** (**middle**) **and Mustafa Biyikal** (**right**)

Mandy Hecht (middle) studied Chemistry at the Humboldt University Berlin (HUB) and received her diploma in 2009, focussing on dihydroperoxides as oxygen transfer reagents. She is currently pursuing a PhD degree in the group at BAM within the field of the synthesis, doping and assembly of nanoparticles for bioanalytical applications. Mustafa Biyikal (right) graduated in chemistry from Technical University Berlin (TUB) in 2005 and obtained his PhD in 2009 in the group of Siegfried Blechert at TUB, developing catalysts for hydroamination reactions. He joined the group at BAM later that year, working in the field of fluorescent molecularly imprinted polymers. Knut Rurack (left) studied chemistry/food chemistry at Kiel and Münster Universities. After obtaining his "Staatsexamen" at CVUA (Chemisches Landes- und Staatliches Veterinäruntersuchungsamt) Münster, he worked with Siegfried Dähne at BAM's Laboratory for Time-Resolved Spectroscopy and with Wolfgang Rettig at HUB, where he completed his PhD in 1999. He returned to BAM in 1999 and leads the Bioanalytical Sensor Materials group in BAM's Div. 1.5 Bioanalytics since 2008. His research interests encompass nanoparticle-based analytical platforms and methods, functional dyes, supramolecular chemistry and optical spectroscopy.

able to bind substrates by conventional weak forces (hydrogen bonding, hydrophobic interactions, π stacking, *etc.*) in a competitive aqueous environment because they extract the substrate into a hydrophobic pocket where the binding site–substrate interaction is shielded. Additionally, the active sites of proteins are usually embedded in a complex yet flexible (super) structure. Once a substrate enters, it "induces the fit",[12] *i.e.*, the binding site is reoriented, the pocket is closed and remaining water is squeezed out.[13] In this section we will show how the functionalization of pores of certain inorganic scaffoldings with various groups can lead to hybrids that mimic binding pockets and are able to perform a signalling reaction on this basis.

2.1 Confinement approach

Until today, the design of hybrid ensembles inspired by biological binding pockets prominently relies on pre-organized nanoscopic porous solid supports, the (inner) pore walls of which are functionalised with two types of organic moieties: (i) recognition centres and (ii) additional groups that fine-tune the polarity of the pore voids in which the (majority of the) recognition centres are located. The supramolecular recognition chemistry of the former is thus combined with a protective action by the second type of chemical group.

Within a biomimetic perspective, confinement of a responsive signalling site in a modified pore of a certain dimension allows discrimination by three factors: (i) selective recognition between signalling site and guest, (ii) hydrophilic/lipophilic partitioning or extraction and (iii) size exclusion phenomena (Fig. 1). However, system design is not limited to these features but, for instance, other forces such as electrostatic repulsion/attraction could also be invoked through the attachment of suitable functional groups.

Small-molecule sensing. One of the first examples of the use of hybrid materials with artificial binding pockets for sensory purposes was described by Lin and co-workers.[14] The authors functionalised the inner pores of a mesoporous MCM-41-type silica scaffolding with *o*-phthalic hemithioacetal moieties, acting as both binding and signalling sites. These chemical entities can react with amines to form highly fluorescent isoindole units. Additionally, in order to enhance selectivity, the pockets were also hydrophobized with different groups (propyl, phenyl and pentafluorophenyl). One of the prepared hybrids was able to selectively respond to the more lipophilic dopamine as substrate *versus* the more hydrophilic glucosamine. The fact that no selectivity was observed when non-porous silica functionalised with the same groups was used pointed toward the importance of the mesoporous structure in the recognition process. In further studies, the authors functionalised the external surface of similar mesoporous particles with poly(lactic acid) (PLA) that acted as a "molecular gate keeper". This PLA coating was able to regulate the penetration of certain amines into the nanoscopic pores by attractive/repulsive Coulomb forces, yielding selectivity for dopamine *versus* tyrosine and glutamic acid (Fig. 2).[15]

A closely related approach was used for the preparation of a hybrid material for the selective recognition of fatty acids in water. In this case, the mesoporous MCM-41 support was functionalised with a urea–phenoxazinone derivative that acted as both an anion receptor (through interaction of the urea group with the carboxylate head group of the fatty acids) and signalling unit (through colour and fluorescence changes, including fluorescence enhancement upon carboxylate binding).[16] Additionally, the inner walls of the pores were further functionalised with trimethylsilane in order to obtain a hydrophobic environment around the urea–phenoxazinone moiety. This solid displayed a remarkably selective response to fatty (long-chain) carboxylates. The hybrid material is highly selective and short-chain carboxylates, inorganic cations and anions and other biological species gave negligible responses. This example also shows an appealing characteristic usually found in biomimetic approaches, that is, an enhanced response of the hybrid compared with the individual parent indicator molecule. Thus, the molecular urea–phenoxazinone probe did not respond to any of the above mentioned guests in water, but bound only non-specifically to carboxylates and $H_2PO_4^-$ in polar organic solvents.

A very similar approach was successfully employed for the detection of amines. For instance, anilinopyrylium dyes (magenta coloured) are known to react with primary amines to yield the corresponding pyridinium salts (yellow coloured). Although this reaction is not selective in solution, a notable improvement in the chromogenic response was observed when the probe was anchored into the pores of MCM-41, which was then further trimethylsilylated to obtain highly lipophilic pores.[17] The sensing features of the support were tested with a family of

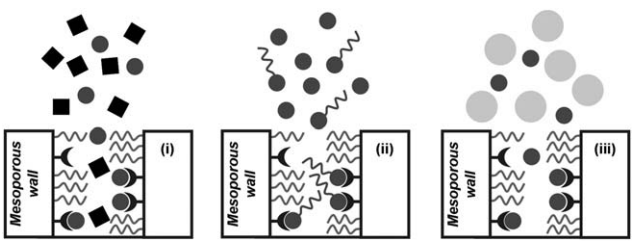


Fig. 1 Three main factors that govern supramolecular recognition chemistry with hybrid materials that mimic binding pockets: (i) selective recognition, (ii) extraction and (iii) size control.

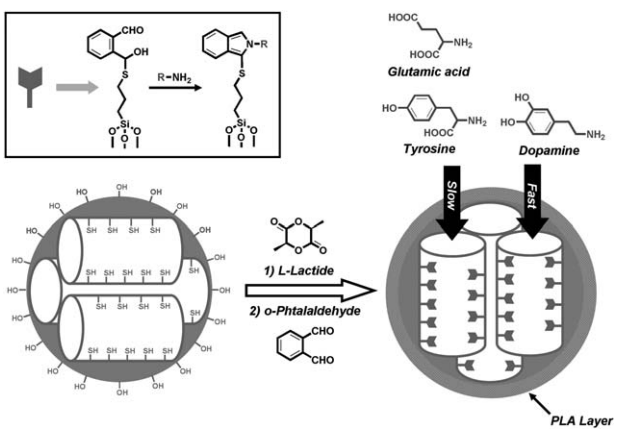


Fig. 2 Schematic representation of the synthesis of a PLA-coated fluorescent sensor material for the detection of amine-containing neurotransmitters (dopamine, glutamic acid and tyrosine). Box: A fluorescent isoindole moiety is formed in the signalling reaction.

linear primary aliphatic amines. Only relatively short but sufficiently hydrophobic medium-chain amines (such as *n*-octylamine) induced a chromogenic reaction in water. Short amines (*e.g.*, *n*-propylamine) were too hydrophilic to enter into the mesopores—see (ii) in Fig. 1—and long-chain or fatty amines (*e.g.*, *n*-dodecylamine) closed the pores after reaction with the pyrylium units close to the pore openings, thus hampering diffusion of following analytes by steric crowding (Fig. 3). In analogy to Fig. 1 (iii), this system shows self-sorting features in the sense that a small number of fatty amines bound at the pore openings blocks the way for further long chain competitors. Similar sensing features were observed for MCM-41-type solids containing other signalling units (dicyanomethylene-2-chloro-3-amino-indenes, styrylpyrylium dyes)[18,19] and trimethylsilyl-passivated walls. In addition, anchoring of anilinopyrylium dyes into the pores of zeolite β also yielded a chromogenic sensing material with specific size discrimination features.[20]

Recently, artificial binding pockets were also employed in selective colorimetric indicator displacement assays (IDAs). The sensing protocol involves several steps: (i) the pores of an inorganic support are functionalised with adequate binding sites, (ii) the latter are loaded with a dye that coordinates to these anchored sites with moderate strength and (iii) arrival of the target species, which forms a stronger complex with the binding sites, leads to a displacement of the dyes and their diffusion into the bulk solution, (iv) enabling the indirect colorimetric detection of the guest. Following this approach, an MCM-41-type solid functionalised with guanidinium binding sites within the pores and methylthymol blue as the indicator dye was prepared. This hybrid material showed a remarkable response to the tricarboxylate citrate (Fig. 4).[21] Similar protocols were developed for borate sensing using a mannose-functionalised MCM-41 solid with a boronate-functionalised dye and for phosphate detection with amino binding sites attached to an MCM-41 support and carboxyfluorescein as indicator. In the latter case, addition of phosphate to neutral aqueous suspensions of the hybrid induced a displacement of the dye to the bulk solution because of preferential interaction of the phosphate anion with the grafted protonated amines.[22,23]

Metal ion sensing. The regulation of metal ion uptake, storage and distribution is a critical task for organisms. Many transition metal ions are essential for cell functions, *e.g.*, iron, zinc, copper or manganese, but also most of them, at elevated concentrations, can exert toxic effects. In cells, metal cations are stored and regulated by a series of proteins such as metallothioneins[24] which are rich in cysteine and can bind a considerable amount of metals, especially when forming sulfur-based protein–metal clusters. These systems have the important function of sequestering toxic trace metals such as mercury or cadmium. A complementary strategy that biological systems employ for detoxification is to create bio-inorganic clusters of reduced metals (Au^0, Ag^0, *etc.*) with single amino acids or small peptides that contain a high amount of histidine, cysteine, glutathione and imidazole sites, diminishing the free concentration of these harmful species in an organism.[25] Apart from these examples of metal storage/removal with small peptides and metallothioneins, another class of metalloproteins like the ferric uptake regulator (Fur) are especially involved in regulation.[26] Within this crucial function the proteins are able to sense the concentration of a metal (ion) before starting to activate either its release or sequestration.

From a biomimetic point of view, metalloregulatory proteins can inspire the preparation of nanoscopic ensembles containing a large number of coordination sites that can be powerful sensing materials for metal ions when coupled with a signalling mechanism. In this regard, the equipment of hybrid mesoporous silicas with chromogenic or fluorogenic groups for metal ion sensing has received considerable attention in recent years. Most of the examples reported so far rely on mesoporous MCM-41, MCM-48, SBA-15 or silica nanotube matrices functionalised with organic moieties, *i.e.*, binding sites and signalling units. The binding sites were incorporated either through covalent bonds or electrostatic and hydrogen bonding interactions.

The covalent functionalization approach is very similar to the strategy described above for small-molecule sensory hybrids, *i.e.*, either a suitable indicator molecule or the binding and signalling units as two separate components are derivatized with alkoxysilane moieties and subsequently grafted onto the surface of the selected inorganic support. According to this design principle, sensory materials for the detection of Hg^{2+} employing grafted rhodamine derivatives,[27,28] dansyl-functionalised calixarenes,[29] azo-coupled macrocycles,[30] ruthenium dyes,[31] and pyrene derivatives[32] were described. Dealing with the optical recognition of Cu^{2+}, hybrids functionalised with 5-methoxy-2-thiazole[33] and Schiff bases[34,35] were investigated. A typical response curve is

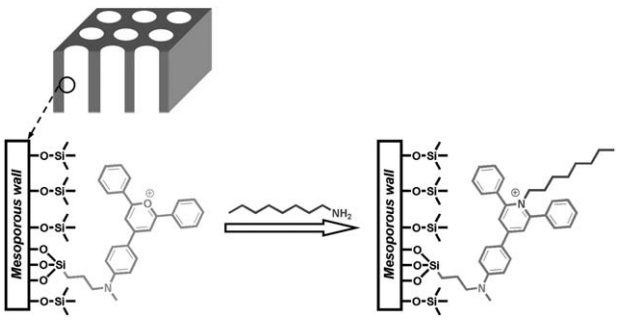


Fig. 3 Schematic model of a biomimetic hybrid for amine signalling. Changes in colour are due to the reaction between the pyrylium ring and the primary amine. Short amines are too hydrophilic to enter and long-chain amines clog in the pore openings.

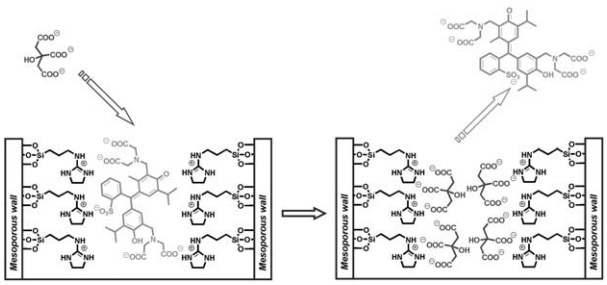


Fig. 4 Protocol for citrate signalling in water by indicator displacement with a mesoporous solid containing guanidinium-functionalised nanoscopic binding pockets and methylthymol blue as the indicator.

shown in Fig. 5. Besides these two popular target metal ions, hybrid materials were also developed for the detection of Fe^{3+} and Zn^{2+}.[36,37] Almost all the sensory hybrid materials cited here contain MCM-41 and SBA-15 mesoporous silicas as inorganic scaffoldings.

Incorporation of indicator molecules into an inorganic scaffolding by electrostatic and hydrogen bonding interactions has a clear advantage, *i.e.*, it can dispense with the tedious synthesis of a reactive silylated probe derivative. For instance, Matsunaga *et al.*[38] and El-Safty *et al.*[39,40] used a mesoporous SBA-15 support that was first functionalised with trimethylammonium groups. In the second step, 4-(2-pyridylazo)-resorcinol (PAR) probe molecules were electrostatically anchored at the pore walls. Coordination of Cd^{2+} to the PAR units then resulted in a colour change from yellow-orange to violet.[38] In analogy, mesoporous trimethylammonium-decorated cavities hosting anionic dyes such as tetraphenylporphine tetrasulfonic acid (TPPS) and pyrogallol red (PR) were also prepared along the electrostatic fixation route (Fig. 6). In a complementary procedure, cationic α,β,γ,δ-tetrakis (1-methylpyridinium-4-yl)porphine (TMPyP) was electrostatically loaded into nano-cages which were previously grafted with SO_3^--terminated spacers (Fig. 6). Neutral dyes such as dithizone were attached to the surface through hydrogen bonding interactions with the silanol groups (Fig. 6). These hybrids were used for the selective detection of Sb^{3+}, Hg^{2+}, Cd^{2+} and Pb^{2+} at sub-nanomolar levels.[39,40] The versatility of the non-covalent approach is apparent from a recent work in which various probe molecules and matrices were combined and led to the development of a family of nanoscale hybrid sensor materials for the indication of multiple metal ions.[41]

Going beyond simple analyte detection, several recent works aimed at a combination of two different functions such as metal ion storage and sensing by designing hybrid materials that can do both, (i) report the concentration of a (harmful) metal ion and (ii) sequester it. In a biomimetic sense, such systems resemble typical regulator proteins that are able to notice the amount of ingested Cd^{2+} or Hg^{2+}, while at the same time they are active components of a detoxification mechanism.

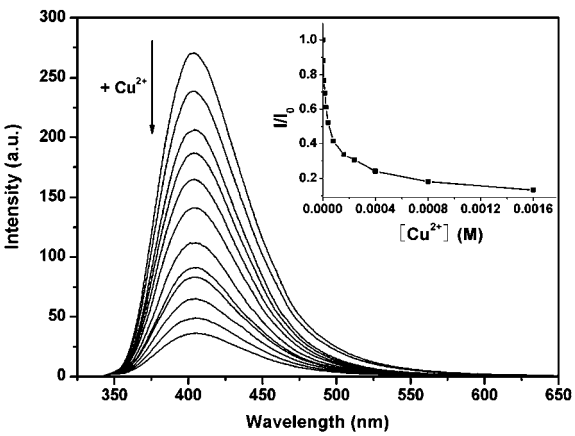


Fig. 5 Fluorescence changes of a hybrid SBA-based sensor material as a function of increasing Cu^{2+} concentration in water at pH 6.0. Inset: titration curve. (Reproduced with permission from ref. 33. Copyright Royal Society of Chemistry.)

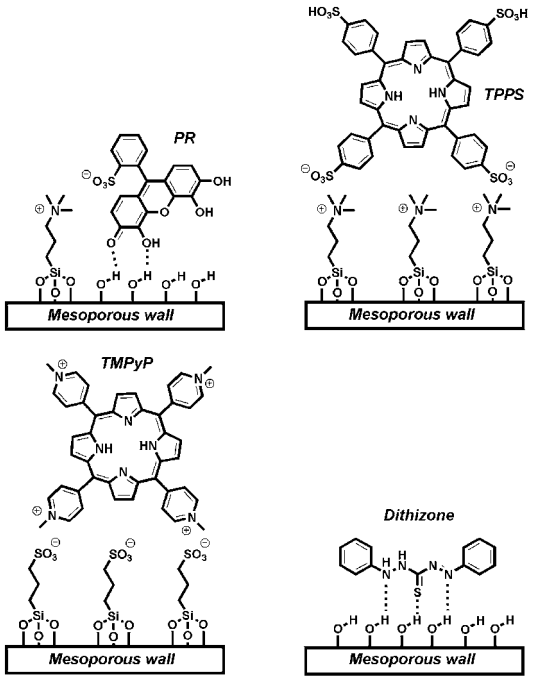


Fig. 6 Nanocaged monoliths containing selected dyes for the colorimetric signalling of various metal ions.

An example of such a sorption and sensing material for Hg^{2+} was reported by Martínez-Máñez, Rurack and co-workers. They synthesized a mesoporous silica support containing covalently attached thiol groups. In a subsequent step, the material was reacted with a squaraine (SQ) derivative. Bleaching of the solution that contained the dye indicated quantitative uptake of the dye into the pores and nucleophilic attack of the central cyclobutane ring of the SQ dye by the deprotonated grafted thiolate groups. This reaction led to the formation of a 2,4-bis(4-dialkylaminophenyl)-3-hydroxy-4-alkylsulfanylcyclobut-2-enone (APC) derivative that is covalently anchored to the silica matrix.

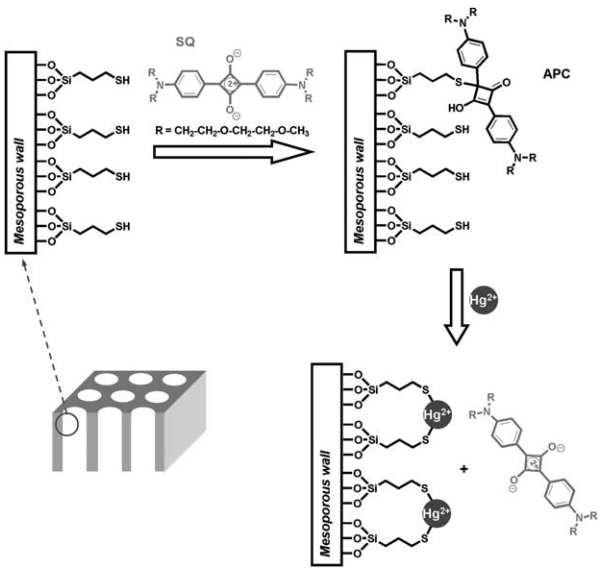


Fig. 7 Hybrid system with sensing and sorption functions for Hg^{2+}.

Because of its high thiophilicity, reaction of Hg^{2+} with the hybrid resulted in the ion's binding to the sulfur atoms, allowing for a removal of the support-bound contaminant from the solution by simple centrifugation, and release of the restored SQ chromophore. The latter is delivered into the solution and can be used for the quantification of Hg^{2+} concentration (Fig. 7).[42]

A second example of a hybrid system with sensing and sorption functionalities used only a single organic function, a reactive derivative of phenanthroline, and attached it to the surface of a porous silica support.[43] The hybrid material showed a selective response to Cu^{2+}, revealing a promising discrimination against a series of other metal ions. In a recent report, the same research group prepared magnetic $Ni@SiO_2$ core–shell nanoparticles that were additionally functionalised with a 4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (BODIPY) derivative able to selectively detect Pb^{2+} in water through distinct fluorescence enhancement.[44] The magnetic properties of the nanoparticles allowed removal of the nanoparticle-bound Pb^{2+} from the solution in a facile manner by using a small magnet. The authors demonstrated simultaneous Pb^{2+} detection and removal from human blood.

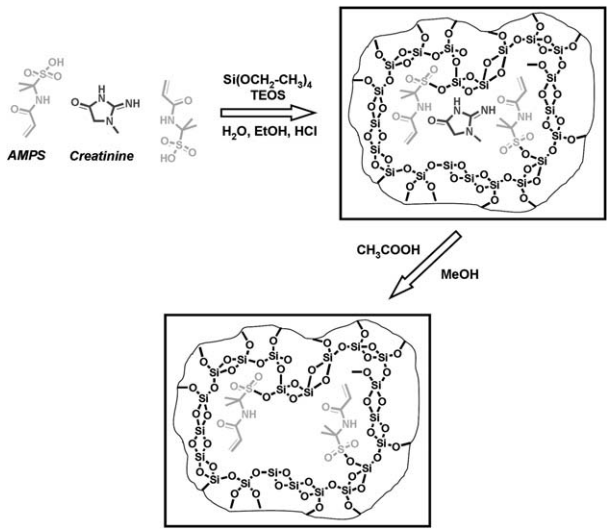


Fig. 8 Schematic preparation of the hybrid organic–inorganic sol–gel creatinine-imprinted polymer.

2.2 Templated approach

In the examples discussed above, the lack of flexibility of the artificial binding pockets compared with the active centres of enzymes is balanced by offering an excess of receptor units to reduce the entropic freedom of the resulting complexes and thus to enhance binding. An approach to prepare more suitable cavities adapted in shape and size to the target molecule relies on the so-called molecular imprinting strategy. In molecular imprinting, the desired analyte is used as a template and is mixed with polymerizable monomers that express suitable sites which can bind to functional groups of the template, forming a pre-organized complex in the reaction mixture. Polymerization of the latter then yields a polymeric material which contains imprinted cavities in which the template is bound. After removal of the template, binding pockets result which are, in the ideal case, optimally adapted to the shape, electronic structure and interacting entities of the guest and which are embedded in a (more or less flexible, depending on the polymer backbone chosen) superstructure.[45,46] The cavities of these potentially highly selective materials are thus also termed "artificial antibodies" with the difference that the supporting polymer skeleton usually possesses a higher thermal, chemical and mechanical stability than the corresponding biological antibodies.[47] Therefore, the application of molecularly imprinted polymers (MIPs) is widespread in separation techniques, solid phase extraction, catalysis and sensory materials. Apart from the use of traditional organic polymer chemistry for the preparation of MIPs, the trend to imprinted functionalised silica supports containing suitable binding sites for signalling applications increased lately.[48,49] This is basically due to the richness and versatility of sol–gel chemistry, for instance, allowing the use of mild temperatures and processing conditions and offering the preparation of MIPs in a wealth of shapes and formulations, ranging from bulks and powders through fibres, tubes and thin films to micro- and nanoparticles. The mild processing conditions also permit the synthesis of organic–inorganic hybrid sol–gel materials with advanced properties. In addition, recent developments have shown that not only bulk imprinting holds promises for advanced chemosensors, but also that surface imprinting offers many exciting prospects for recognition and signal transduction.[50]

Bulk imprinting. Imprinted bulk materials are commonly designed for the recognition of small molecules, offering a robust material ideally suited for applications such as solid-phase extraction and chemical sensing. Macromolecular imprinting in bulks is a challenge, because of the often limited accessibility of binding sites for both template removal and rebinding. In most cases, bulk sol–gel materials are employed in high-performance liquid chromatography (HPLC) coupled with optical or other detection methods. The sol–gel silica network alone naturally has limited imprinting possibilities, because only silanol and siloxane groups are available to interact with the template. The addition of organosilanes or other organic aids that can be covalently integrated into the sol–gel network is thus required. For instance, Syu and co-workers showed the advantages of hybrid organic–inorganic imprinted sol–gel bulks compared to entirely inorganic (tetraethyl orthosilicate- or TEOS-derived) sol–gel materials with the recognition of creatinine.[51] For the preparation of the hybrid material, the template creatinine was mixed with 2-acrylamido-2-methylpropane-sulfonic acid (AMPS) as coordinating ligand and TEOS (Fig. 8). Under the reaction conditions employed, AMPS does not only form a complex with creatinine but also reacts with silanol groups of the sol–gel network to be covalently integrated through sulfonic anhydride linkages. The sensitivity of the MIPs toward creatinine was investigated by comparison of the organic–inorganic hybrid (H-MIP) and the inorganic sol–gel MIP (S-MIP) with the respective non-imprinted materials (H-NIP, S-NIP), which were prepared in the conventional way by carrying out the synthesis under identical conditions but in the absence of template. In all cases, the non-imprinted polymers showed a low affinity to creatinine. Moreover, compared with the S-MIP, the H-MIP was characterized by a threefold higher binding capacity for creatinine, because of the favourable

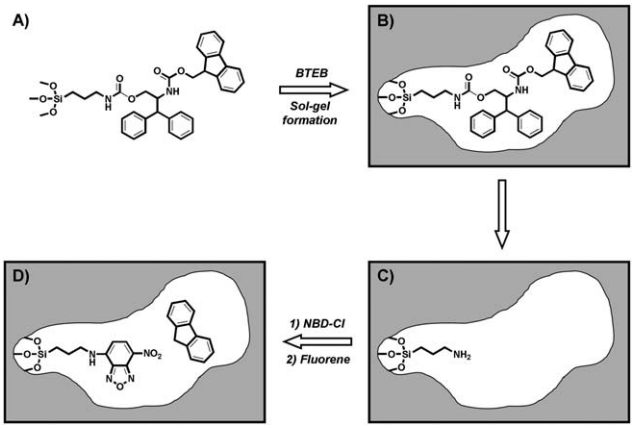


Fig. 9 Molecular imprinting and detection scheme for fluorene. (A, B) The dye mimic silane template is reacted with a 60-fold molar excess of bis(trimethoxysilylethyl)benzene (BTEB), incorporating it into a sol–gel matrix. (C) The fluorene analogue is cleaved from the sol–gel matrix *via* the carbamate linkage, leaving a free amine group. (D) The amine group is used as an attachment site to localize a NBD fluorophore near the binding pocket, which can be used to detect the presence of the target after rebinding.

hydrogen bonding interactions between template and the organic co-monomer AMPS. A high selectivity of 85.4% was obtained for creatinine against creatine, 2-pyrrolidinone and *N*-hydroxysuccinimide for the hybrid MIP. The authors also showed that subsequent passivation of residual silanol groups in the MIPs has a negative influence on guest binding for the H-MIP, but no significant effect in the case of S-MIP, stressing the importance of optimum cavity formation (with the aid of AMPS) and the availability of a sufficient amount of hydrophilic groups to interact with a polar guest such as creatinine.

The importance of controlling the polarity of the binding environment in the cavities was also shown for imprinted bulk materials with multiple primary amine functions in the imprinted cavities.[52] Aiming to study the reactivity of primary amines like lysine toward polar species such as 1,3,5-trinitrobenzenesulfonic acid (TNBS) as a function of the hydrophilicity of a protein's binding pocket, the authors prepared two types of sol–gel MIPs, one with primary amine residues in a polar, silanol-rich environment and a second type for which the amine-functionalised, hydrophilic silica material was hydrophobized by end-capping the silanol groups with trimethylsilyl functionalities. The arylation tendency of the imprinted amines with TNBS was characterized with diffuse reflectance UV/Vis spectroscopy. Whereas the hydrophilic materials showed significant TNBS binding, the corresponding passivated MIPs showed no reaction between amines and TNBS. These results demonstrate that a primary amine-containing residue in a hydrophobic pocket is inaccessible for polar intruders and support the fact that entities like lysine are effectively shielded against unspecific reactions in highly competitive environments by integration into hydrophobic binding pockets of proteins.

Approaches covalently incorporating a fluorophore as a signalling element in close proximity to the imprinted cavity of silica-based MIPs were also reported recently. For the detection of fluorene, the template was covalently attached to an organoalkoxysilane dye mimic and thus introduced to the sol–gel matrix for covalent imprinting (Fig. 9).[53] The dye mimic serves as a placeholder for the actual signalling unit to be used. After chemical removal of the template-carrying dye mimic, an amine group was left, which was reacted with the fluorescent unit of choice, 7-nitrobenz-2-oxa-1,3-diazole (NBD), covalently attaching the dye to the cavity. Fluorene binding was then detected by a change in NBD fluorescence when the resulting cavity was occupied by fluorene. The material showed a good selectivity for fluorene over analogues such as naphthalene, fluoranthene, and anthracene.

A related approach again relying on NBD as the signalling unit of a MIP xerogel was developed for the detection of 9-anthrol.[54] Here, a triethoxysilyl derivative of 9,10-anthracenediol served as both, the template and dye mimic and was polymerized together with tetramethoxyorthosilicate (TMOS). 9,10-Anthracenediol was then removed with $LiAlH_4$, leaving two closely placed primary amines. The solid was subsequently immersed in a solution containing 9-anthrol as the target and 4-chloro-NBD as the tentative signalling unit. Further processing led to a covalent attachment of the NBD fluorophore to one amino group and coordination of 9-anthrol with the other. Final removal of 9-anthrol resulted in the desired sensing solid. The authors demonstrated that further rebinding of 9-anthrol in the amino group- and NBD-containing cavities led to an enhancement of the NBD fluorescence as the analytically measurable response. Although all these examples utilized optical detection, recent works on the electrochemical detection of 2,4,6-trinitrotoluene (TNT)[55] and dopamine[56,57] showed that hybrid silica MIPs are also suitable sensor materials for non-optical detection.

Particularly attractive from a biomimetic point of view is chiral imprinting and the enantioselective detection or separation of targets. In a recent example, Marx and co-workers reported chirally imprinted thin-film chemosensors for the enantioselective discrimination of the enantiomer pairs (*R*)- and (*S*)-propranolol, (*R*)- and (*S*)-2,2,2-trifluoro-1-(9-anthryl) ethanol, and D- and L-3,4-dihydroxyphenylalanine (D- and L-dopa).[58] The selective adsorption properties of the prepared 540 nm thin films toward the imprinted molecules were assessed by direct or indirect fluorescence analysis and radioactive assays. In all cases, the non-specific adsorption was low and the preferred adsorption of one enantiomer was observed. In a subsequent work, the authors targeted parathion and explored other detection techniques.[59] Imprinted sol–gel thin films were coated on glassy carbon electrodes and quartz crystal microbalance (QCM) resonators to detect parathion in aqueous solutions and in the gas phase (Fig. 10). The imprinted films functionalised with phenyltrimethoxysilane (PTMOS) for the interaction with the nitrophenyl group of parathion through π–π interactions, and 3-aminopropyltriethoxysilane (APTES), which binds parathion *via* hydrogen bonds, showed high selectivity toward parathion in comparison to similar organophosphates. Specific binding in the gas phase however proved to be less sensitive to the imprinting effect and exhibited relatively high non-specific binding.

Thin film MIPs and surface imprinting. A common disadvantage of bulk imprinted materials is the lack in accessibility of the cavities. Usually, after preparation of a bulk MIP, extraction of

the template is not quantitative so that a certain degree of the cavities is permanently occupied. Moreover, because of the disordered nature of the pore structure, the fact that the structure of the sol–gel network can continue to change with aging and potentially long diffusion paths, not all empty cavities are available for guest rebinding, often reducing a MIP's efficiency and performance. A way to overcome these drawbacks is to reduce the thickness of the active layer, for instance, by using MIP nanoparticles or thin films. Moreover, the larger the analyte is the more important the reduction of the sensing layer's thickness becomes, especially when biomacromolecules with slow diffusion coefficients such as proteins or even larger objects such as bacteria are concerned. For these targets, surface imprinting has become the technique of choice. Surface-imprinted materials typically include thin films that are coated on (porous) particles or on planar substrates and various ways of arranging the anchor points or "stamping" (a part of) the 3D structure of a macromolecule.[60] Modern approaches utilize nanostructured materials as the support structure.

Although biomacromolecules are the major target for surface imprinting approaches, this technique also gained increasing popularity in small-molecule imprinting. For these small analyte molecules it is less likely that all the cavities are actually lying at the surface of the MIP layer. Nonetheless, we refer here to surface imprinting when monolayers of active interaction sites or functional groups are used and the sol–gel network formed around and between these sites is only a few nanometres thick. For instance, Yang *et al.* prepared surface-imprinted silica nanotube membranes which were synthesized within the cylindrical pores of alumina nanopore membranes for the selective detection of estrone.[61] Recently, Xie *et al.* used the same method to prepare highly uniform molecularly imprinted silica nanotubes for the recognition of TNT.[62] In the first step, a monolayer

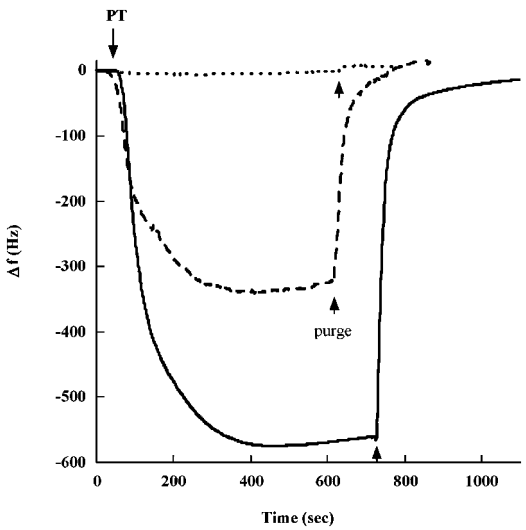


Fig. 10 Frequency changes of QCM resonators coated with parathion-imprinted (solid line) and non-imprinted (dashed line) sol–gel films in response to parathion-saturated nitrogen. Upward arrow indicates the time of introduction of clean N_2 stream into the chamber to desorb bound parathion. Dotted line: response of a bare, uncoated QCM resonator. (Reproduced with permission from ref. 59. Copyright American Chemical Society.)

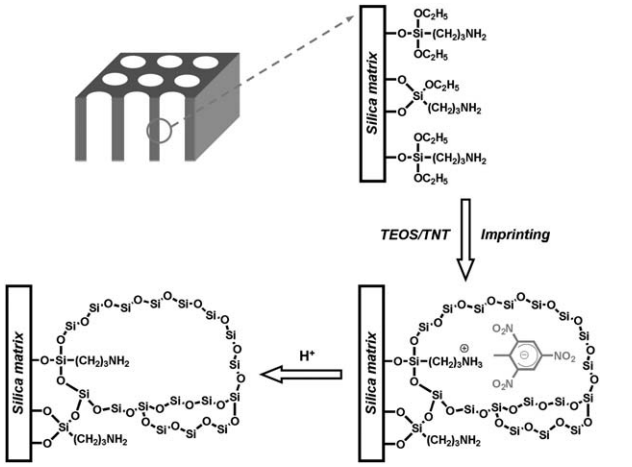


Fig. 11 Fabrication and formation mechanism of TNT-imprinted silica nanotubes within alumina membrane pores (adapted from ref. 62).

of APTES was grafted onto the walls of the nanotubes of 70 nm diameter. Second, TNT template molecules and TEOS as the cross-linking and cavity-forming agent were reacted with the activated tube walls. The imprinting occurs through strong acid–base pairing interactions between TNT and the amino moieties of APTES. Because a monolayer of APTES is used and strict control of the polymerization process is maintained, a uniform coating of 15 nm thickness is achieved and most of the recognition sites are situated close to the surface, providing a better accessibility and lower mass-transfer resistance (Fig. 11).

As revealed by absorption spectroscopy, the maximum uptake capacity of the nanotubes is almost 3.6-times higher than that of bulk microparticles with 2–3 μm diameter. This result shows that the density of effectively imprinted sites is much higher at the nanotube walls than deep in the bulk of particles. For the microparticles, apparently only the imprinted sites in the proximity of the particle surface are available for rebinding of the target analyte.

Ordered mesoporous silicas are also a particularly suitable type of support for thin film-imprinting, offering high specific surface areas and large pore volumes thus enabling a much faster mass transport to the recognition sites. In this case, the MIP films are usually coated on the pore walls and various optical sensors

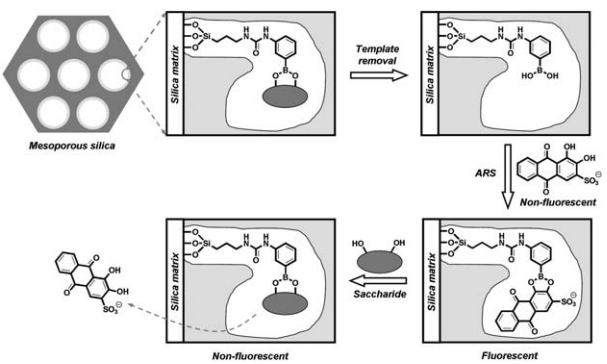


Fig. 12 Schematic representation of molecularly imprinted mesoporous silica as an indicator-displacement sensor for saccharides.

were designed according to this strategy. For instance, Tan *et al.* developed an IDA with the fluorescent dye Alizarin Red S (ARS) and D-fructose- and D-xylose-imprinted mesoporous silicas (FruIM, XylIM) utilizing phenylboronic acid as the recognition centres for detection (Fig. 12).[63]

For the preparation of the sensor materials, a linker-appended phenylboronic acid derivative was prepared from 3-isocyanatopropyltriethoxysilane and 3-aminophenylboronic acid. The respective silane precursor was then reacted with the templates D-fructose or D-xylose to form the corresponding saccharide ester, carrying the templates for covalent imprinting. Co-condensation of these conjugates with TEOS in the presence of the surfactant cetyltrimethylammonium bromide (CTAB) resulted in a mesoporous silica matrix, the pores of which were decorated with a thin MIP film as revealed by a reduction of the average pore size from 2.1 to 1.8 nm. After removing the templates through ester cleavage, FruIM and XylIM were loaded with the indicator dye. Depending on the template used, the MIPs showed selective displacement reactions in the presence of the designated guests. Because of their smaller molecular size, the pentoses such as D-xylose, D-arabinose and D-ribose showed higher cross-reactivities for FruIM than hexoses did for XylIM. Although the authors found that the sensitivity of the MIPs was not extremely high, they could successfully employ the MIPs in a sensor array for the pattern recognition of saccharides in beverages in a rapid assay.

Recently, Wang *et al.* reported a new type of surface imprinted hybrid, Mn-doped ZnS quantum dots (QDs) for the detection of pentachlorophenol (PCP) in water based on room-temperature phosphorescence (RTP).[64] To anchor the MIP layer on the surface of the QDs, the nanoparticles were first capped with 3-mercaptopropyltriethoxysilane (MPTS) and TEOS. Subsequently, APTES, PCP and TEOS were employed to generate the MIP matrix (Fig. 13). The size of the QDs employed amounted to *ca.* 12 nm, that of the MPTS-capped QDs to *ca.* 40 nm and the MIP-capped QDs finally reached *ca.* 50 nm, indicating that the actually active MIP coating is approximately 10 nm thin.

The synergistic effect of the surface imprinted recognition layer and the RTP properties of the Mn-doped ZnS QDs improved the selectivity of the Mn-doped ZnS QDs and allowed the development of an optosensor for the selective detection of non-phosphorescent analytes without the need for a further transducer. The authors invoked charge transfer interactions between the excited QD and the PCP anion, which is formed in the presence of APTES (Fig. 13), to be responsible for the RTP quenching response. A comparison of panels (b) and (c) in Fig. 13 shows how the imprinting process facilitates the response toward the target analyte. Moreover, the benefit of utilizing RTP- instead of fluorescence-based detection is evident from Fig. 13d, leading to a detection limit of 86 nM for PCP.

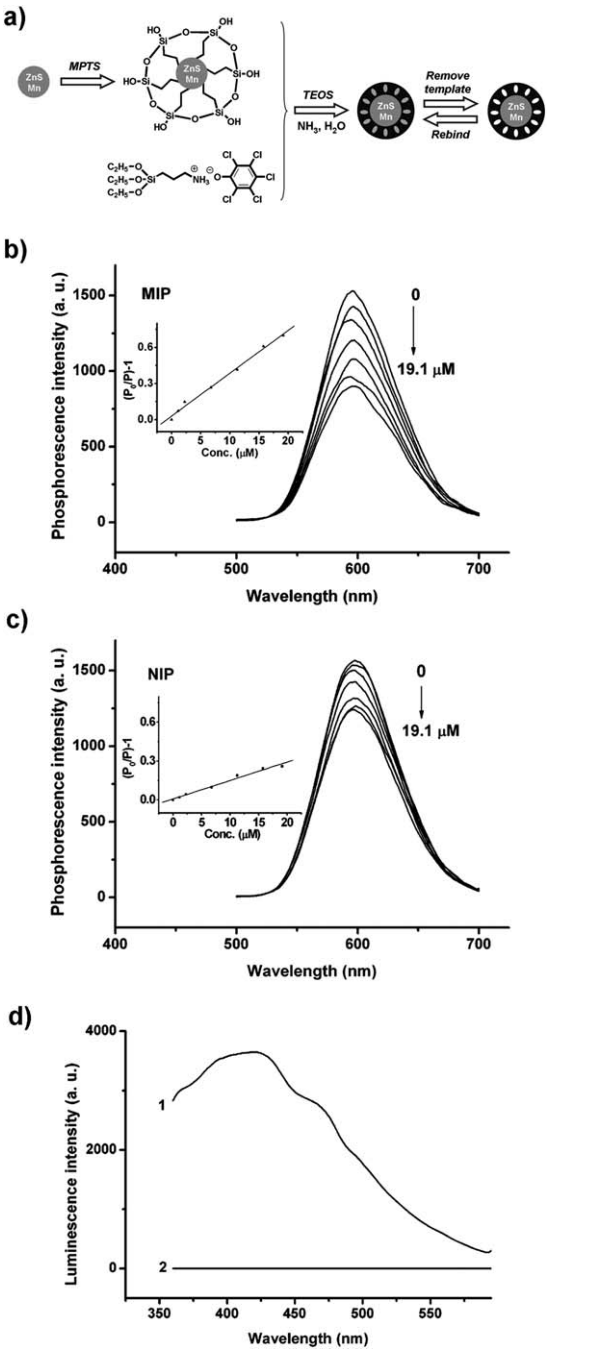


Fig. 13 a) Schematic illustration for the fabrication of MIP-capped Mn-doped ZnS QDs; RTP emission spectra of (b) MIP- and (c) NIP-capped QDs after addition of the indicated concentrations of PCP in pH 5 acetate buffer; (d) fluorescence (curve 1) and RTP (curve 2) spectra of a real water sample. (Reproduced in part with permission from ref. 64. Copyright American Chemical Society.)

3 Mimicking ion channels

Whereas binding pockets as biochemical processing systems are well-defined entities in biomacromolecules, ion channels of biological origin are ensembles of varying macromolecular composition, exerting vital control and unique transport functions for an organism. Ion channels are ubiquitous biological structures important for inter- and intracellular signalling and transport and are found in virtually any cell or organelle membrane.[65–67] Many efforts were made in the last 20 years to exploit their principle of operation for technological applications, ranging from the use of (modified) biological channels[68] or protein pores[69] *via* natural ionophores such as valinomycin in conjunction with biomimetic membranes[70,71] or under selected artificial conditions[72] to the combination of artificial ionophores

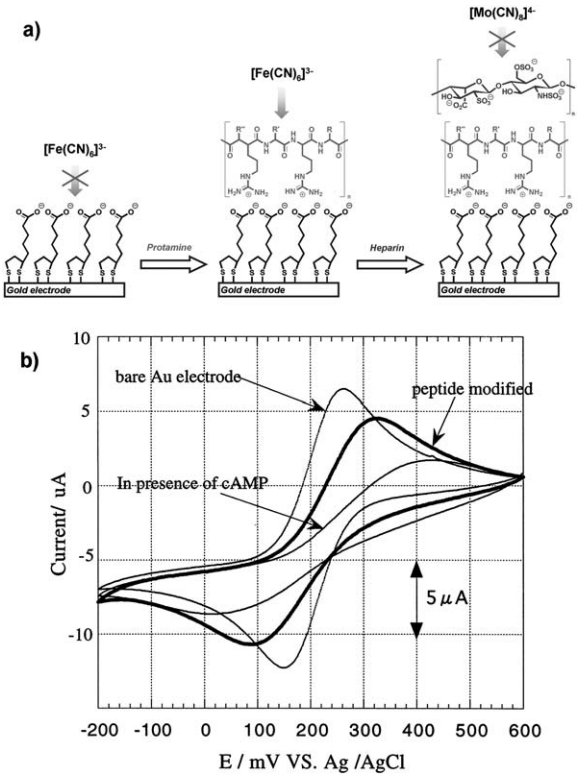


Fig. 14 a) Architectures of ion channel sensor materials for protamine and heparin detection; (b) cyclic voltammograms of the ferrocyanide/ferricyanide redox couple with a peptide-modified gold electrode in the absence and presence of cAMP in Tris–HCl buffer at pH 7.4. (Reproduced in part with permission from ref. 89. Copyright American Chemical Society.)

with lipid membranes[73,74] and the development of entirely artificial systems such as ionophore–polymer hybrids.[75]

With regard to structure and function, ion channels and ion pumps are usually built up of proteins, forming the pore. The pore as such thus has a certain diameter and inner polarity, determined by the chemical residues facing into the void. Access to such a pore is not generally restricted, but transport is controlled by size, polarity and charge. However, depending on the function of the channel, the terminal parts of the channel proteins which protrude on both sides of the membrane can behave as a control station that regulates the transport through the pores, *i.e.*, these pores are gated with the state of the gate often being triggered by a certain stimulus.[76,77] Both types of channels have inspired chemists to create artificial analogues.

3.1 Free access

The simplest biomimetic sensory approach to an ion-channel is the so-called ion-channel sensor (ICS). ICSs are usually electrochemical sensors for which the electrodes are modified with certain receptors.[78,79] The sensing paradigm relies on the binding of analytes—commonly macromolecules—to the receptors at the electrode surface, thus controlling the access of small redox-active probes to the electrode surface, which results in a modulation of the reduction or oxidation intensity.[80,81] These sensing systems are readily prepared through simple formation of

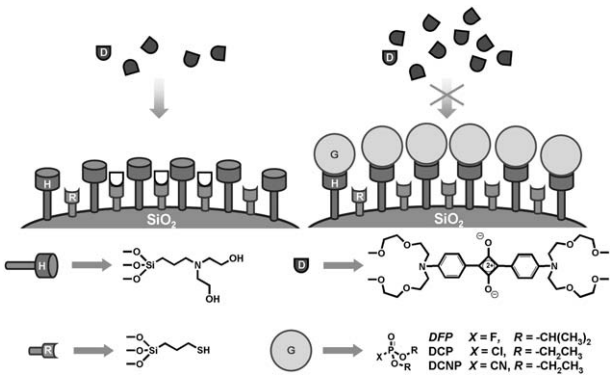


Fig. 15 Analyte-mediated suppression of indicator decolouration.

self-assembled monolayers (SAMs) on gold electrodes using the well-known spontaneous addition of organosulfur derivatives to Au surfaces to form the corresponding SAMs. For instance, in a representative example, gold electrodes were modified with SAMs of thioctic acid for the detection of protamine using the redox-active species $[Ru(NH_3)_6]^{3+}$ and $[Fe(CN)_6]^{3-}$ as probes.[82] In the absence of protamine, $[Fe(CN)_6]^{3-}$ is repelled by the SAM. Upon binding of the polycation protamine, the negative excess charge on the monolayer is reduced, the $[Fe(CN)_6]^{3-}$ probe can better access the electrode surface and reduction is enhanced (Fig. 14a). When exchanging a negatively for a positively charged probe, the detection scheme is reversed. In this case, $[Ru(NH_3)_6]^{3+}$ has free access to the electrode in the absence of the analyte and protamine addition reduces the current.

Since protamine is routinely used to neutralize heparin in blood, the detection of heparin is also possible with the previous ICS when protamine is attached to the SAM-covered electrodes.[83] In analogy to the described examples, many sensors were developed for different analytes from simple metal ions[84–86] *via* anions[87] to complex guests.[88,89] With regard to the latter, for instance, Fig. 14b illustrates the signal changes upon addition of cyclic AMP (cAMP) to a solution containing a gold electrode modified with a specifically designed cAMP-recognizing peptide and $[Fe(CN)_6]^{3-}$ as the redox probe.[89] Binding of the negatively charged cAMP to the peptide layer reduces the activity of the negatively charged redox probe due to electrostatic repulsion.

Recently, optical ion-channel-like sensors were also reported. Here, instead of gold, silica surfaces are decorated with two different moieties, a reactive group (R) and a receptor or host (H). The role of R is to react with a certain dye (D) inducing a change in colour or fluorescence. The sensing protocol relies on the inhibition of the reaction between R and D when a suitable guest (G) is bound to H, resulting in a modulation of the signal change (Fig. 15). For this optical approach, the host is able to control dye transport from the solution to the silica surface through binding to a targeted guest. In this manner, colorimetric assays for pyrophosphate,[90] heparin[91] and nerve agent mimics[92] were recently described. The coated silica nanoparticles used in this sensing protocol are commonly prepared from the corresponding trialkoxysilyl derivatives of R and H according to simple procedures that involve the heating of the silica nanoparticles in a mixture of water : ethanol : acetic acid (1 : 2 : 1) in the presence of the coating subunits.

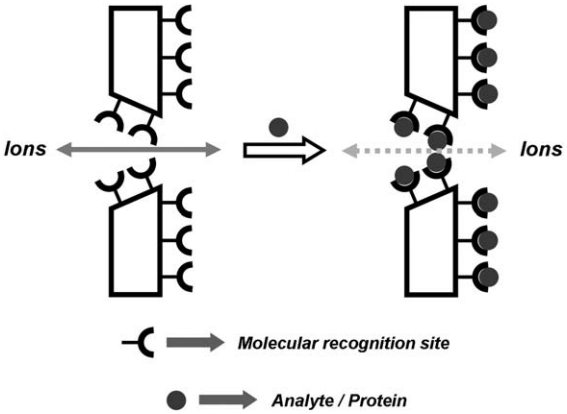


Fig. 16 Architecture of a hybrid material containing a single nanopore and mode of operation by inhibition of ion flux.

In a representative example, silica nanoparticles were functionalised with thiol groups as R and aliphatic alcohols as H. The role of the thiol groups is to react with a SQ dye as D, inducing bleaching.[93] In the sensing protocol, phosphorylation of the alcohol groups with a certain nerve gas agent (as G) inhibits the reaction between R and D, preventing decolouration. The degree of bleaching correlates with analyte concentration and low detection limits in the ppt range were achieved for the nerve gas stimulant diisopropylfluorophosphate. The material was also employed for the detection of nerve agents in the vapour phase,[92] stressing the versatility and simplicity of the approach toward promising sensing hybrids.

Besides these classic ICS mimics, another label-free electrochemical method has become popular in recent years, sensing with stochastic nanopore sensors. These sensors are able to detect analytes at the single-molecule level using an ion channel analogue that is included in a lipid bilayer or that can be fabricated in a solid-state membrane. These nanopore sensors detect substances based on the modulation of an ionic current that occurs when the target molecules is bound at the pore opening (Fig. 16). Typical single-channel stochastic sensors[94] were also created by introduction of one protein such as α-hemolysin into a lipid bilayer. As lipid bilayers are rather fragile objects, Martin and co-workers developed a different approach and embedded a single gold nanotube into a polymer layer and attached biochemical recognition sites at the pore openings (examples of supported bilayer membrane hybrids are discussed in Section 4).[95] For this purpose, the pores were decorated with a thin gold layer to facilitate further biochemical functionalization with the designated receptor sites, finally yielding a pore with openings of 4–5 nm.[96] Different pairs of analytes/recognition elements such as biotin/streptavidin, protein G/immunoglobulin G (IgG) and ricin/anti-ricin antibody were tested. The studies revealed that a protein that does not bind to the receptors does not change the flux and a protein that binds, reduces flux. The authors showed that electrochemical single-molecule detection is possible.[97]

With regard to high priority analytes, stochastic nanopore sensors were recently successfully employed for the detection of several agents frequently used by terrorists.[98] For instance, an engineered α-hemolysin that contained a β-cyclodextrin host molecule was used as recognition element, the interaction of which with the hydrolysis products of cyclosarin and soman, *i.e.*, cyclohexyl methylphosphonic acid (CMPA) and pinacolyl methylphosphonate (PMPA), resulted in the blocking of the channel and a decrease in the current. The system showed lower detection limits than those required by the US Army, additionally displaying a remarkable selectivity.[99] Similar stochastic sensors were also reported for the sensing of certain liquid explosives.[100]

Although playing a less important role in the field of ICSs, porous silica supports such as MCM-41 were also employed in some cases. For instance, biotinylated MCM-41 was incorporated into lipid bilayer membranes and ion transport through the membrane was then controlled by avidin as the analyte.[101] Although some of the disadvantages of lipid bilayers were experienced, a large amount of the pores (*ca.* 80%) was blocked by avidin. In contrast, bovine serum albumin (BSA) as a negative control did not modulate the ion flux.

3.2 Controlled access

In contrast to free access as the underlying principle of ion channel sensors, most of the gated hybrid nanomaterials which were inspired by receptor-gated channels that require the binding of a co-factor or effector at the gatekeeper protein to activate the system and allow for transport were developed for cargo delivery.[102] Only few works demonstrated that gate-like scaffoldings can be employed in sensing protocols. Besides their bio-inspired architecture, the sensing performance of these devices is also reminiscent of biological systems. Signalling in cells is generally connected to the presence or a threshold concentration (effector concentration) of a certain substance that induces the release or uptake of another species.[103,104] Translated to the world of the analytical chemist this means that a porous support material is equipped with specific binding sites at the pore openings that can control the delivery of a loaded indicator. Like a ligand-gated channel protein that regulates ion trafficking across a cell membrane through binding of a co-factor, the analyte controls indicator release from the hybrid ensemble by opening or closing the pores, *i.e.*, the sensing process is based on the idea that a certain "key" analyte "opens" or "closes" the gate. In the absence of analyte, ideally no indicator delivery occurs whereas in the presence of the analyte, indicator molecules are released from the pores. If one further considers that a mesoporous 3D skeleton that is functionalised with specific receptors at the pore openings can contain a large number of indicator molecules in its pores, opening of these pores by one or few analytes can lead to the massive release of indicators, intrinsically equipping these systems with features of signal amplification.

Most of the reported systems use mesoporous silica materials of the MCM-41 type as suitable inorganic supports. The MCM-41 phase can be prepared using TEOS as hydrolytic inorganic precursor and the surfactant CTAB as porogen. Calcination of the meso-structured phase yields the MCM-41 solid. For the preparation of the sensing device, the mesoporous solid is first loaded with a suitable dye and then reacted, *i.e.*, grafted with the respective compounds necessary for the gating mechanism, resulting in a solid which contains the dye in the interior of the mesopores and the gating ensemble attached on the outside of the pores. In the first system designed for the chromo-fluorogenic

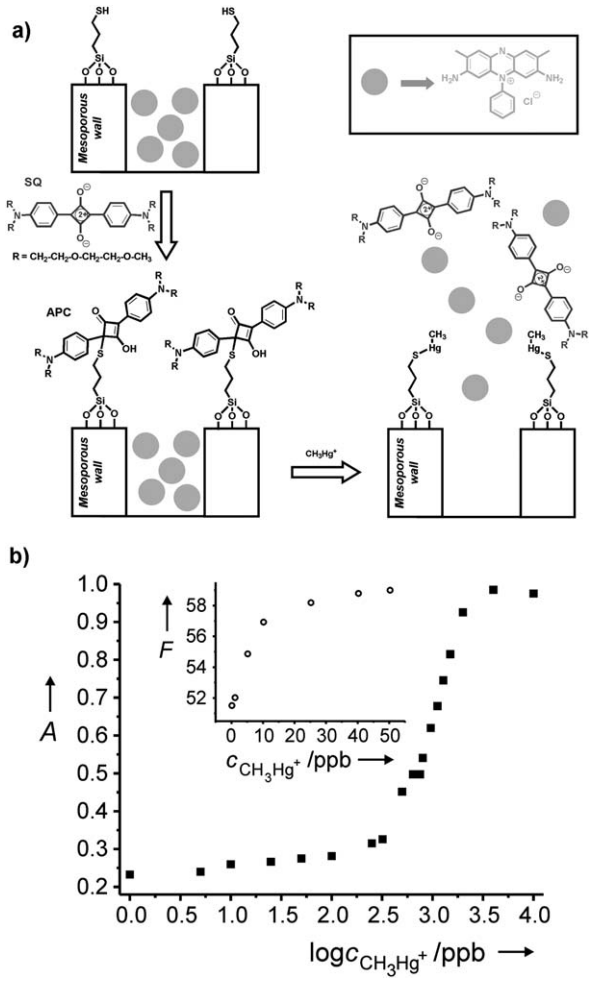


Fig. 17 a) Signal amplification by mesopore capping and target-induced uncapping and dye release; (b) spectrophotometric titration curves for safranine release monitored at 523 nm in acetonitrile/toluene 4 : 1 v/v in the presence of increasing amounts of CH_3Hg^+. Inset: corresponding titration curve obtained from the emission intensities at 610 nm. (Reproduced with permission from ref. 105. Copyright Wiley-VCH Verlag GmbH & Co. KGaA.)

detection of methylmercury, the pores of a mesoporous solid were initially loaded with a dye (safranine O) and then capped with APC groups (*vide supra*). The capping molecules were anchored on the surface through a reaction of previously attached mercaptopropyl groups with a squaraine derivative (*vide supra*). The uncapping protocol is based on the reaction of the thiophilic CH_3Hg^+ with the APC moieties, resulting in the coordination of the cation to the thiol groups and liberation of the SQ chromophore. Employing a straightforward protocol, this method could successfully be used to determine CH_3Hg^+ in fish samples.[105] An inherent feature of such systems is its previously mentioned nature of signal amplification. For instance, in this particular system reaction of one equivalent of CH_3Hg^+ with the APC groups leads to the delivery of an average of 200 safranine O molecules (Fig. 17). Since the gating APC groups are also converted to a dye (SQ) with complementary spectroscopic properties, the response is ratiometric and also internally referenced.

In a second example, the mesoporous support was functionalised with a certain hapten at the pore outlets able to interact with an antibody that can act as a nanoscopic cap. In this case, a MCM-41-type solid was selected and loaded with the dye $[Ru(bipy)_3]Cl_2$. Then, a suitable derivative of the hapten 4-(4-amino-benzenesulfonylamino)-benzoic acid was anchored on the outer surface of the mesoporous support. Finally, the pores were capped with a polyclonal antibody for sulfathiazole. The delivery of the dye in the presence of a family of sulfonamides was studied in aqueous PBS 1× (pH 7.5). A selective uncapping of the pores and dye delivery was observed for sulfathiazole.[106] Other gated systems that can selectively be opened or closed in the presence of target analytes were developed for the chromogenic signalling of ATP,[107] long-chain carboxylates,[108] anionic surfactants[109] and borate.[110]

4 Stabilizing vesicles

Mimicking ion channels is not the only strategy chemists follow to utilize features of natural membrane systems, but the modelling of simplified cell membranes and biomimetic membrane platforms as such is another highly topical issue. Imitating cell membranes at the molecular level of course is a complex endeavour. Moreover, several attempts toward synthetic lipid bilayers as model systems were in fact made to better understand the complexity of native cell membranes. Regarding applications and systems within the scope of this article, one has to consider that spherical bilayer membranes (s-BLMs) or liposomes into which amphiphilic lipids such as phosphatidylcholines, phosphatidyl ethanolamines and phosphatidyl glycerols, that are composed of a hydrophilic polar phosphoric head group and hydrophobic hydrocarbon tails, self-assemble in water are rather fragile objects, limiting their use in many practical applications. Chemists thus strived to stabilize membrane systems and developed spherical supported lipid bilayer membranes (ss-BLMs) which are stable compositions still providing many characteristics of natural systems such as lateral fluidity and semi-permeability for ionic species and molecules (Fig. 18). Additionally, the combination of particles and lipid bilayers to biomimetic hybrid materials allows the insertion of hydrophobic molecules such as integral membrane proteins into the bilayer's hydrocarbon chain region as well as the covalent attachment of biomolecules and the assembly of hydrophilic compounds such as specific receptors at the hydrophilic outer surface of the supported membrane. Like hollow vesicles, assemblies supported by an inorganic carrier such as porous or non-porous silica particles

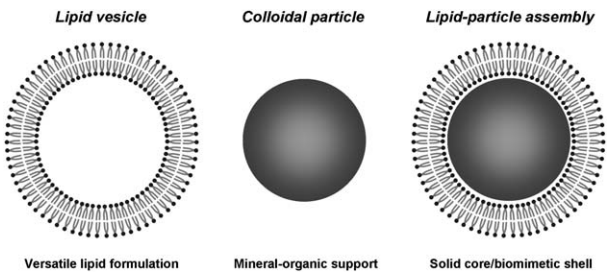


Fig. 18 Schematic representation of a lipid vesicle, a colloidal particle and a lipid/particle assembly or ss-BLM.

and coated with lipid bilayers can thus display a wide range of the desired properties of native cell membranes. ss-BLMs can be formed by spontaneous adsorption and fusion of liposomes as unilaminar vesicles with the hydrophilic solid substrate or by Langmuir–Blodgett techniques, resulting in a single bilayer membrane coating on the substrate. Because silica particles have hydrophilic surfaces, depending on the composition and temperature, membranes are typically separated from the substrate by a thin layer of water and, like natural membranes, possess macroscopic long-range fluidity with mobile components of both leaflets freely diffusing over the entire surface of the particle support. In such systems, particle–lipid interactions can be assessed by determining adsorption isotherms of lipids on particles and ζ potentials derived from dynamic light scattering measurements. Colloidal stability can be measured by kinetic turbimetry and particle sedimentation over time. With regard to sensory applications, silica particles possess many favourable features such as chemical stability, superior optical properties, good biocompatibility and a high surface-to-volume ratio which also significantly increase their performance in ss-BLM hybrids. Based on the facile preparation chemistry of silica particles, it is possible to create ss-BLMs with defined shapes and sizes in the range of *ca.* 50 nm to *ca.* 80 μm, comparable with the size range of vesicles in biological systems.

Although the sketch in Fig. 18 looks rather straightforward, the functional reconstruction of complex transmembrane proteins for chemical and biosensing is still a challenge. The introduction of membrane proteins during ss-BLM formation and the resulting effect on protein performance was recently evaluated and improved by Brozik and co-workers.[111] Issues to be considered are the protrusion of the protein from the liposomal surface, hampered diffusion and the inhibition of protein functions due to protein–substrate interactions. They investigated the effects of pore structure and substrate curvature of nanoporous silica microspheres on the stability and functionality of membrane proteins, focusing on the incorporation efficiency of liposomal and detergent-mediated introduction of a serotonin-gated ion channel (5HT3 receptor) and the proton pump bacteriorhodopsin into the ss-BLM and the resulting protein function. To determine the bilayer unilamellarity, fluorescent and non-fluorescent liposomes and proteoliposomes were used for the preparation of the nanoporous microbead-supported bilayers. Bilayer stability, detergent solubilization and protein functionality were probed with a Ca^{2+}-sensitive electrode by loading the beads with 10 mM $CaCl_2$, followed by the daily recording of the external Ca^{2+} concentration. The authors found that the larger the diameter of the particles the higher the capacity to retain the compartmentalized fluorescent dyes and Ca^{2+} ions and the better the resistance against solubilization through detergents. Moreover, the membrane surface area increases when the pore size is larger than the bilayer thickness, because the membrane invaginates into the pores. The proteins were successfully incorporated into and reconstituted in the membrane and provided a basis for applications in ionic and fluorescent dye-based assays.

Lopez, Whitten and co-workers established a fluorescence quenching assay for biointeractions of molecules with supported bilayers to assess the catalytic activity of human serum-derived phospholipase (PLA2), elaborating their earlier findings on membrane-mediated superquenching.[112,113] The lipoprotein-associated phospholipase A2 (Lp-PLA2) can be applied as potential biomarker for atherosclerotic diseases and is found in the core of atherosclerotic plaques which induce the degenerative disease atherosclerosis. PLA2 enzymes specifically recognize the acyl bond of phospholipids and release arachidonic acid and lysophospholipids by catalytic hydrolysis, resulting in membrane disruption. In this study, fluorescent cationic conjugated polyelectrolytes (CPEs), poly(*p*-phenylene-ethynylene) (PPE) derivatives, were physically adsorbed or surface-grafted onto oppositely charged non-porous 5 μm silica microspheres (MS), yielding MSPPE hybrids, which were subsequently coated with an anionic phospholipid bilayer of (1,2-dimyristoyl-*sn*-*glycero*-3 [phosphor-*rac*-(1-glycerol)] (DMPG). An external fluorescence quencher (AQS) was used to detect the activity of the enzyme which disrupts the lipid bilayer (Fig. 19). In this assembly, the lipid bilayer simultaneously acts as a substrate for PLA2 and as a barrier between the fluorescent polymer and the quencher. Quenching of the CPE fluorescence is hindered by the presence of the phospholipid coating, but revived when the coating is digested by the enzyme PLA2. The sensitivity of the assay can be improved by modifying the structures of the CPEs. In addition,

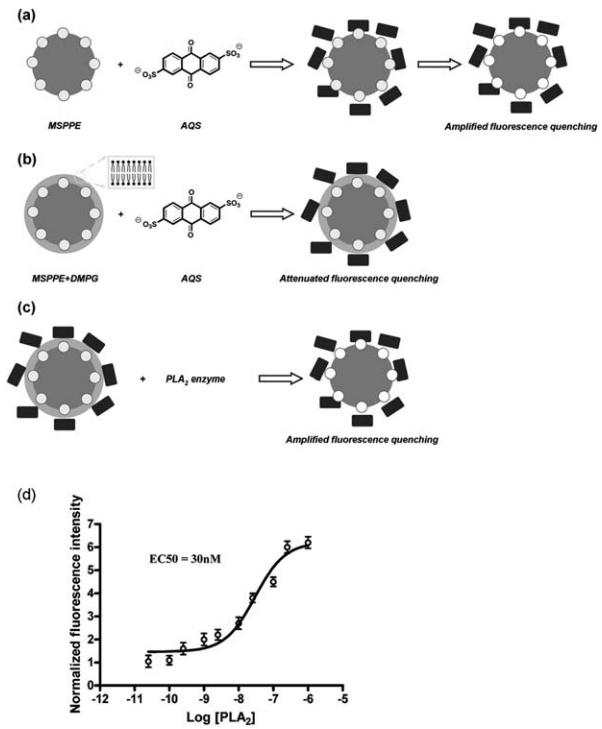


Fig. 19 Polymer-supported bead-based fluorescence "turn off" assay: (a) 5 μm silica microspheres coated with PPE (MSPPE) are quenched in the presence of AQS; (b) fluorescence quenching is attenuated when a lipid bilayer is formed around the polymer coated beads, preventing attack of PPE by AQS; (c) upon introduction of an enzyme, lipid bilayer digestion and amplified fluorescence quenching similar to that in (a) occurs. (d) MSPPE + DMPG hybrids incubated with different concentrations of PLA_2 for 50 min in PBS. Flow cytometric examination was performed immediately after the addition of AQS. (Reproduced with permission from ref. 112. Copyright American Chemical Society.)

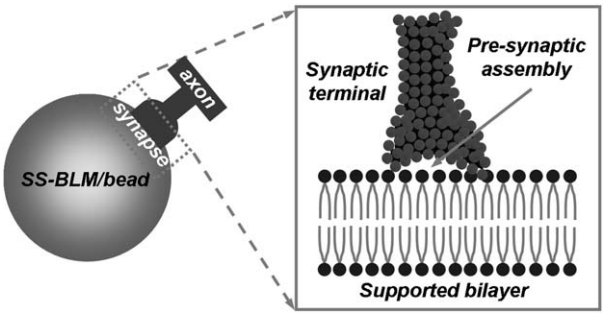


Fig. 20 Pre-synaptic spherical supported bilayer membrane assembly.

the system can be modified so that other members of the phospholipase family can be targeted.

Synthetic lipid bilayer membranes are also interesting candidates for neuroengineering applications which offer the possibility of replacing damaged neurons or coaxing neuronal circuits due to their close structural similarity with pre- and postsynaptic membranes, allowing for the simulation of axon surfaces. Until recently, it has been very difficult to repair damages associated with chronic neurodegenerative diseases or acute brain trauma as a result of concussive or penetrating injury of neuronal pathways because of the high degree of complexity of the central nervous system. Being less sensory in nature from a molecular recognition or analytical chemistry point of view, the mimicking of neuronal activities might harbour potential for the development of chemical transduction circuits, *i.e.*, a chemical analogy to artificial neural networks successfully used in sensor arrays for several years.[114,115]

An important step toward the artificial organization of functional synapses is the search for a suitable supporting platform. In this regard, Gopalakrishnan *et al.* recently developed a lipid bilayer membrane for triggering the presynaptic assembly of vesicles for the *in vitro* contacting of synapses with live axons (Fig. 20).[116] For this purpose, ss-BLMs on silica were generated from different mixtures of lipids that were co-cultured with hippocampal neurons. The ss-BLMs were prepared according to a procedure that includes a biotin–avidin tethering protocol. A mixture of zwitterionic phospholipids and cationic lipids forms the lipid bilayer membrane, which promotes the adhesion of the beads to the neurons. For quantification, synaptophysin immunostaining was used which showed that the accumulation of the pre-synaptic vesicles occurs at the contact point between the axons and the ss-BLM-covered beads. To detect and locate other important synaptic proteins at the bead–axon contact points in a single culture the authors performed a triple immunostaining experiment. With this arrangement they could show that in addition to synaptophysin, actin filaments, which are important cytoskeletal components involved in many synaptic processes including signal transduction and synaptic vesicle trafficking, and bassoon, an important scaffolding protein present at the presynaptic active zones, are also present at the bead–axon contacts. The results indicated that the nature of the lipid phase plays a crucial role in the assembly of presynaptic vesicles at the neuron–ss-BLM contacts as do the chemical and electrostatic interactions between the neurons and the ss-BLM.

The last approach to a biomimetic hybrid material in this section is also less sensory in nature, yet in our view harbours interesting aspects for immunoanalytical chemistry. Lincopan *et al.* investigated silica-supported cationic bilayers of dioctadecyldimethylammonium bromide (DODAB), which were established as effective immunoadjuvants able to stimulate dendritic cells, with regard to cellular response for vaccine design in comparison to alum, a commonly used inorganic aluminium salt. In general, the antigen is responsible for the specific immunoresponse and the adjuvant amplifies this response through a secondary signalling pathway such as local tissue irritation. Because the adjuvant also binds the antigen and releases its cargo slowly in the cell, antibody production is often enhanced. The authors took advantage of the biocompatibility of silica to produce DODAB BLM-coated particles for the immobilization and antigen presentation of the two different model antigens BSA and recombinant 18 kDa heat-shock-protein (hsp).[117] The latter is an important target for the immunoresponse to mycobacteria. The authors showed that the hybrid system can effectively adsorb antigens with less amount of immunoadjuvant to provoke a superior immunoresponse *in vivo* compared to the conventionally used alums. In addition, the DODAB–silica hybrids were taken up much better than DODAB vesicles or silica beads alone by the dendritic immune cells. Such enhanced antibody production is potentially attractive for developers of immunoassays.

These examples showed that spherically supported lipid bilayer membranes are significantly more stable than their unsupported counterparts. Nevertheless, it is still a challenge to incorporate special types of transmembrane proteins into ss-BLMs while preserving their natural function, especially with regard to their specific location, *i.e.*, when their activity is connected to a certain protrusion from the membrane surface. Returning briefly to the ICSs of Section 3, it should be mentioned that supported bilayer membranes were also realized on planar porous inorganic substrates such as alumina,[118] silicon nitride[119] and gold[120] as well as disordered network-like carbon nanotube,[121] silicon nanowire-type[122] or deposited metal droplet[123] supports, operating as artificial analogues of membrane pores and harbouring exciting prospects for chemical and biosensing.

5 Adapting colour

Stimuli-triggered colour changes are not only a vital part of many analytical detection protocols, but also play an important role in the life of many amphibians, reptiles and fishes when they adapt their body colour to a certain light intensity or background colour.[124] In these organisms, chromatophores as specific pigment cells are responsible for the collective change of colour, which is orchestrated by a complex interplay of hormones, cyclic AMP (cAMP) and other key effectors of the responsible parts of the nervous and endocrine systems, the mechanisms of which can differ with animal and pigment cell.[125] Melanophores for instance contain black and brown granules of melanin and erythrophores incorporate carotenoid vesicles and pteridine granules. The major mechanisms involved in colour changes are the translocation and dispersion or aggregation of granules or microplatelets in pigment cells.[126] Light and background colour are the main causes of chromatic responses. Nonetheless, other

stimuli such as Ca^{2+} concentration[127] or temperature[128,129] were also frequently reported.

Leaving the aggregation-induced colour change of receptor-functionalised gold nanoparticles (AuNPs) in the presence of (bio)chemical target analytes aside,[130] the majority of hybrid systems that respond to a stimulus with a change in colour were developed for temperature. For instance, Suzuki and Kawaguchi incorporated AuNPs or Au/AgNPs in a polymer microgel and observed colour changes connected to the swelling and shrinking of the hybrid particles as a function of temperature.[131] When the polymer network shrinks, the AuNPs come in closer contact and can efficiently couple, which leads to a shift of the surface plasmon absorption band and hence colour, reminiscent of the aggregation of granules in chromatophores. Like in the natural systems, the colour changes are fully reversible in the hybrid. Turning to bi-particulate-polymer systems, sophisticated design allows for the development of a reversible nanothermometer for precise temperature measurement at nanoscale dimensions.[132,133] System design involves the connection of two different NPs, an AuNP and a CdTe QD, through a poly(ethylene glycol) (PEG) polymer as a "molecular spring" in such a way that resonance conditions between AuNP plasmon and CdTe exciton lead to luminescence enhancement (Fig. 21). Temperature changes now have an effect on the PEG linker, leading to changes in the AuNP–QD distance and hence the degree of coupling which is visible through luminescence intensity changes.

Whereas such a particle–polymer–particle architecture was also used for pH sensing with a pH-responsive polymer linker,[134] the combination of molecular probes and nanoparticles has recently led to another elegant temperature sensing hybrid, completing the examples discussed in this article.[135] Palacio, Carlos and co-workers designed a composite system that contains 100–400 nm nanoclusters of magnetic maghemite (γ-Fe_2O_3) nanoparticles of *ca.* 21 nm diameter in a shell of APTES/TEOS (final size of 5 μm), which is additionally doped by a Eu^{3+} and a Tb^{3+} β-diketonate chelate. Whereas the maghemite core allows the system to be moved magnetically, the organic–inorganic silica layer is part of the photophysical sensing mechanism. Excitation of the Tb^{3+} chelate at 357 nm leads to a thermally controlled energy transfer to a slightly higher lying excited state of the hybrid silica bulk with subsequent non-radiative depopulation. The higher the temperature is, the more efficient is this internal quenching pathway and reduces the intensity of the Tb^{3+} emission lines at *ca.* 495 and 550 nm. On the other hand, the Eu^{3+} levels are much lower lying in energy and thermally driven deactivation into the bulk is impossible. If system design further takes into account a sufficiently large distance between the single Tb^{3+} and Eu^{3+} centres, avoiding energy transfer between the two lanthanides, the typical Eu^{3+} emission intensity at *ca.* 615 and 700 nm remains virtually insensitive to temperature changes. The system is thus internally referenced. The authors could show that, once calibrated, absolute temperature measurements with an emission intensity change of 4.9% K^{-1} are possible at nanoscopic dimensions. Moreover, although the inflection point of the APTES/TEOS sensor lies at *ca.* 140 K, it was possible to shift the working range of the system by >100 K to higher temperatures when using a different hybrid silica precursor, a bifunctional di-ureapropyltriethoxysilane cross-linker, that forms a network with a slightly higher lying excited state than the APTES/TEOS mixture.

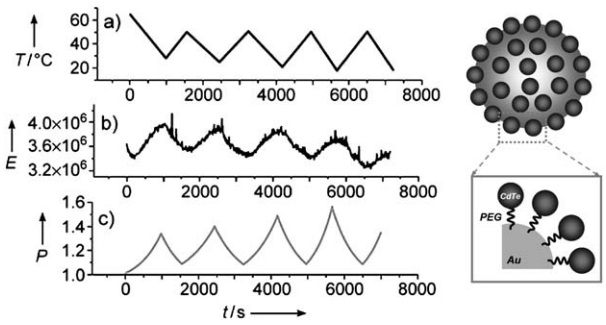


Fig. 21 Left: Variation of the photoluminescence intensity E (b) of the PEG-functionalized Au and CdTe nanoparticles depending on the temperature (a); (c) shows the calculated photon-field enhancement factor P of the CdTe nanoparticles as a function of time. Right: schematic representation of a dynamic nanothermometer based on a nanoparticle superstructure. This superstructure consists of two types of nanoparticles (gold and CdTe) connected by polymeric PEG spacers. Left: reproduced with permission from ref. 133. Copyright Wiley-VCH Verlag GmbH & Co. KGaA.

6 Conclusions

During the last two decades, inspiration from several stimuli-driven recognition, transportation and translocation processes perfected by nature over billions of years of evolution led to the development of a wealth of hybrid sensory ensembles with sophisticated functions and unprecedented performance. Enhanced performance and function generally arise from synergistic effects upon combination of an inorganic support with the suitable (bio)organic functionalization and bio- or supramolecular recognition chemistry. As presented in sections 2 and 3.1, considerable progress was already achieved in the field of artificial binding pockets and flux-controlled channel-like sensors. Nonetheless, whether mesoporous silicas, imprinted sol–gel networks or modified gold electrodes are concerned, the step toward mass production and industrial application for most of these formats still lies ahead. Equipping functional chemical ensembles with elements of control is a particularly fascinating area of research, whether optical, electrochemical, chemical, thermal, or magnetic means are concerned. It is thus not surprising that, despite the sophistication realized for some of the examples discussed in section 3.2, much of the enormous inherent potential of gated signalling systems remains yet to be explored. Especially the broad palette of tools in the hand of a chemist suggests that an integration of magnetic or optical stimuli, which do not play a major role for many recognition-based processes at the cellular level in an organism such as an arbitrary cell in the body, with biomimetic recognition chemistry will presumably generate unique sensing systems in future. In a similar way, combining (parts of) evolutionary optimized entities with the stability and versatility of almost limitlessly configurable artificial supports holds a great promise for new complex hybrid devices. Extrapolating the examples in section 4 into the future, artificial neural networks with chemical communication or

transduction pathways might become an alternative in sensor networks. Finally, the aggregation-induced colour changes in chromatophores sketched in section 5 show that processes in nature which are not intrinsically related to (bio)molecular recognition can also be reflected by the design of artificial sensing systems. In summary, the major aspects of the chemistry presented here are the synergisms of bio- and supramolecular concepts and nanoscale inorganic materials which have created a variety of enhanced functions and properties that are difficult to achieve in the single areas alone.

Notes and references

1 M. Ma, *Crit. Rev. Biochem. Mol. Biol.*, 2007, **42**, 463.
2 T. Leinders-Zufall, A. P. Lane, A. C. Puche, W. Ma, M. V. Novotny, M. T. Shipley and F. Zufall, *Nature*, 2000, **405**, 792.
3 C. H. Serezani, M. N. Ballinger, D. M. Aronoff and M. Peters-Golden, *Am. J. Respir. Cell Mol. Biol.*, 2008, **39**, 127.
4 T. Shimada, *Drug Metab. Pharmacokinet.*, 2006, **21**, 257.
5 M. C. Duncan, D. G. Ho, J. Huang, M. E. Jung and G. S. Payne, *Proc. Natl. Acad. Sci. U. S. A.*, 2007, **104**, 6235.
6 E. J. M. Helmreich, *Biophys. Chem.*, 2003, **100**, 519.
7 E. V. Anslyn, *J. Org. Chem.*, 2007, **72**, 687.
8 K. Rurack and R. Martínez-Máñez, in *Encyclopedia of Inorganic Chemistry*, ed. R. H. Crabtree, John Wiley, Chichester, 15 March 2009, DOI: 10.1002/0470862106.ia420.
9 A. B. Descalzo, R. Martínez-Máñez, F. Sancenón, K. Hoffmann and K. Rurack, *Angew. Chem., Int. Ed.*, 2006, **45**, 5924.
10 R. Martínez-Máñez, F. Sancenón, M. Hecht, M. Biyikal and K. Rurack, *Anal. Bioanal. Chem.*, 2011, **399**, 55.
11 *The Supramolecular Chemistry of Organic–Inorganic Hybrid Materials*, ed. K. Rurack and R. Martínez-Máñez, J. Wiley & Sons, Inc., Hoboken, 2010.
12 D. E. Koshland, *Proc. Natl. Acad. Sci. U. S. A.*, 1958, **44**, 98.
13 G. G. Hammes, *Biochemistry*, 2002, **41**, 8221.
14 V. S.-Y. Lin, C.-Y. Lai, J. Huang, S.-A. Song and S. Xu, *J. Am. Chem. Soc.*, 2001, **123**, 11510.
15 D. R. Radu, C.-Y. Lai, J. W. Wiench, M. Pruski and V. S.-Y. Lin, *J. Am. Chem. Soc.*, 2004, **126**, 1640.
16 A. B. Descalzo, K. Rurack, H. Weisshoff, R. Martínez-Máñez, M. D. Marcos, P. Amorós, K. Hoffmann and J. Soto, *J. Am. Chem. Soc.*, 2005, **127**, 184.
17 M. Comes, M. D. Marcos, R. Martínez-Máñez, F. Sancenón, J. Soto, L. A. Villaescusa, P. Amorós and D. Beltrán, *Adv. Mater.*, 2004, **16**, 1783.
18 S. Basurto, T. Torroba, M. Comes, R. Martínez-Máñez, F. Sancenón, L. Villaescusa and P. Amorós, *Org. Lett.*, 2005, **7**, 5469.
19 B. García-Acosta, M. Comes, J. L. Bricks, M. A. Kudinova, V. V. Kurdyukov, A. I. Tolmachev, A. B. Descalzo, M. D. Marcos, R. Martínez-Máñez, A. Moreno, F. Sancenón, J. Soto, L. A. Villaescusa, K. Rurack, J. M. Barat, I. Escriche and P. Amorós, *Chem. Commun.*, 2006, 2239.
20 M. Comes, M. D. Marcos, R. Martínez-Máñez, M. C. Millán, J. V. Ros-Lis, F. Sancenón, J. Soto and L. A. Villaescusa, *Chem.–Eur. J.*, 2006, **12**, 2162.
21 M. Comes, G. Rodríguez-López, M. D. Marcos, R. Martínez-Máñez, F. Sancenón, J. Soto, L. A. Villaescusa, P. Amorós and D. Beltrán, *Angew. Chem., Int. Ed.*, 2005, **44**, 2918.
22 M. Comes, M. D. Marcos, R. Martínez-Máñez, F. Sancenón, J. Soto, L. A. Villaescusa and P. Amorós, *Chem. Commun.*, 2008, 3639.
23 M. Comes, E. Aznar, M. Moragues, M. D. Marcos, R. Martínez-Máñez, F. Sancenón, J. Soto, L. A. Villaescusa, L. Gil and P. Amorós, *Chem.–Eur. J.*, 2009, **15**, 9024.
24 M. Vasak, *J. Trace Elem. Med. Biol.*, 2005, **19**, 13.
25 J. M. Slocik and D. W. Wright, *Biomacromolecules*, 2003, **4**, 1135.
26 J.-W. Lee and J. D. Helmann, *BioMetals*, 2007, **20**, 485.
27 M. H. Lee, S. J. Lee, J. H. Jung, H. Lim and J. S. Kim, *Tetrahedron*, 2007, **63**, 12087.
28 C. Song, X. Zhang, C. Jia, P. Zhou, X. Quan and C. Duan, *Talanta*, 2010, **81**, 643.
29 R. Métivier, I. Leray, B. Lebeau and B. Valeur, *J. Mater. Chem.*, 2005, **15**, 2965.
30 S. J. Lee, J.-E. Lee, J. Seo, I. Y. Jeong, S. S. Lee and J. H. Jung, *Adv. Funct. Mater.*, 2007, **17**, 3441.
31 E. Palomares, R. Vilar and J. R. Durrant, *Chem. Commun.*, 2004, 362.
32 Y. Wang, B. Li, L. Zhang, L. Liu, Q. Zao and P. Li, *New J. Chem.*, 2010, **34**, 1946.
33 L.-L. Li, H. Sun, C.-J. Fang, J. Xu, J.-Y. Jin and C.-H. Yan, *J. Mater. Chem.*, 2007, **17**, 4492.
34 H. Zhang, P. Zhang, K. Ye, Y. Sun, S. Jiang, Y. Wang and W. Pang, *J. Lumin.*, 2006, **117**, 68.
35 L. Gao, J. Q. Wang, L. Huang, X. X. Fan, J. H. Zhu, Y. Wang and Z. G. Zou, *Inorg. Chem.*, 2007, **46**, 10287.
36 J.-Q. Wang, L. Huang, M. Xue, Y. Wang, L. Gao, J. H. Zhu and Z. Zou, *J. Phys. Chem. C*, 2008, **112**, 5014.
37 L. Gao, Y. Wang, J. Wang, L. Huang, L. Shi, X. Fan, Z. Zou, T. Yu, M. Zhu and Z. Li, *Inorg. Chem.*, 2006, **45**, 6844.
38 T. Balaji, M. Sasidharan and H. Matsunaga, *Anal. Bioanal. Chem.*, 2006, **384**, 488.
39 T. Balaji, S. A. El-Safty, H. Matsunaga, T. Hanaoka and F. Mizukami, *Angew. Chem., Int. Ed.*, 2006, **45**, 7202.
40 S. A. El-Safty, A. A. Ismail, H. Matsunaga and F. Mizukami, *Chem.–Eur. J.*, 2007, **13**, 9245.
41 S. A. El-Safty, A. A. Ismail, H. Matsunaga, T. Hanaoka and F. Mizukami, *Adv. Funct. Mater.*, 2008, **18**, 1485.
42 J. V. Ros-Lis, R. Casasús, M. Comes, C. Coll, M. D. Marcos, R. Martínez-Máñez, F. Sancenón, J. Soto, P. Amorós, J. El Haskouri, N. Garró and K. Rurack, *Chem.–Eur. J.*, 2008, **14**, 8267.
43 S. J. Lee, D. R. Bae, W. S. Han, S. S. Lee and J. H. Jung, *Eur. J. Inorg. Chem.*, 2008, 1559.
44 H. Y. Lee, D. R. Bae, J. C. Park, H. Song, W. S. Han and J. H. Jung, *Angew. Chem., Int. Ed.*, 2009, **48**, 1239.
45 K. Haupt and K. Mosbach, *Chem. Rev.*, 2000, **100**, 2495.
46 G. Wulff, *Chem. Rev.*, 2002, **102**, 1.
47 B. Sellergren, *Trends Anal. Chem.*, 1997, **16**, 310.
48 M. E. Diaz-Garcia and R. B. Laino, *Microchim. Acta*, 2005, **149**, 19.
49 A. Bossi, F. Bonini, A. P. F. Turner and S. A. Piletsky, *Biosens. Bioelectron.*, 2007, **22**, 1131.
50 I. A. Nicholls and J. P. Rosengren, *Bioseparation*, 2001, **10**, 301.
51 Y.-S. Chang, T.-H. Ko, T.-J. Hsu and M.-J. Syu, *Anal. Chem.*, 2009, **81**, 2098.
52 J. D. Bass and A. Katz, *Chem. Mater.*, 2003, **15**, 2757.
53 C. A. Carlson, J. A. Lloyd, S. L. Dean, N. R. Walker and P. L. Edmiston, *Anal. Chem.*, 2006, **78**, 3537.
54 E. L. Shughart, K. Ahsan, M. R. Detty and F. V. Bright, *Anal. Chem.*, 2006, **78**, 3165.
55 S. A. Trammell, M. Zeinali, B. J. Melde, P. T. Charles, F. L. Velez, M. A. Dinderman, A. Kusterbeck and M. A. Markowitz, *Anal. Chem.*, 2008, **80**, 4627.
56 R. Makote and M. M. Collinson, *Chem. Mater.*, 1998, **10**, 2440.
57 R. Makote and M. M. Collinson, *Chem. Commun.*, 1998, 425.
58 S. Fireman-Shoresh, D. Avnir and S. Marx, *Chem. Mater.*, 2003, **15**, 3607.
59 S. Marx, A. Zaltsman, I. Turyan and D. Mandler, *Anal. Chem.*, 2004, **76**, 120.
60 N. W. Turner, C. W. Jeans, K. R. Brain, C. J. Allender, V. Hlady and D. W. Britt, *Biotechnol. Prog.*, 2006, **22**, 1474.
61 H.-H. Yang, S.-Q. Zhang, W. Yang, X.-L. Chen, Z.-X. Zhuang, J.-G. Xu and X.-R. Wang, *J. Am. Chem. Soc.*, 2004, **126**, 4045.
62 C. Xie, B. Liu, Z. Wang, D. Gao, G. Guan and Z. Zhang, *Anal. Chem.*, 2008, **80**, 437.
63 J. Tan, H.-F. Wang and X.-P. Yan, *Anal. Chem.*, 2009, **81**, 5273.
64 H.-F. Wang, Y. He, T.-R. Ji and X.-P. Yan, *Anal. Chem.*, 2009, **81**, 1615.
65 T. J. Jentsch, V. Stein, F. Weinreich and A. A. Zdebik, *Physiol. Rev.*, 2002, **82**, 503.
66 S. Morbach and R. Krämer, *ChemBioChem*, 2002, **3**, 385.
67 J. A. Wemmie, M. P. Price and M. J. Welsh, *Trends Neurosci.*, 2006, **29**, 578.
68 H. Bayley and C. R. Martin, *Chem. Rev.*, 2000, **100**, 2575.
69 Y. Jung, H. Bayley and L. Movileanu, *J. Am. Chem. Soc.*, 2006, **128**, 15332.

70 A. T. A. Jenkins, N. Boden, R. J. Bushby, S. D. Evans, P. F. Knowles, R. E. Miles, S. D. Ogier, H. Schönherr and G. J. Vancso, *J. Am. Chem. Soc.*, 1999, **121**, 5274.
71 L. Rose and A. T. A. Jenkins, *Bioelectrochemistry*, 2007, **70**, 387.
72 H. Tsukube, K. Takagi, T. Higashiyama, T. Iwachido and N. Hayama, *Inorg. Chem.*, 1994, **33**, 2984.
73 O. Murillo, I. Suzuki, E. Abel, C. L. Murray, E. S. Meadows, T. Jin and G. W. Gokel, *J. Am. Chem. Soc.*, 1997, **119**, 5540.
74 N. Sakai, K. C. Brennan, L. A. Weiss and S. Matile, *J. Am. Chem. Soc.*, 1997, **119**, 8726.
75 M. F. M. Roks and R. J. M. Nolte, *Macromolecules*, 1992, **25**, 5398.
76 J. T. Finn, M. E. Grunwald and K. W. Yau, *Annu. Rev. Physiol.*, 1996, **58**, 395.
77 I. B. Levitan, *Nat. Neurosci.*, 2006, **9**, 305.
78 L. M. Goldenberg, M. R. Bryce and M. C. Petty, *J. Mater. Chem.*, 1999, **9**, 1957.
79 Y. Umezawa and H. Aoki, *Anal. Chem.*, 2004, **76**, 320A.
80 P. Bühlmann, H. Aoki, K. P. Xiao, S. Amemiya, K. Tohda and Y. Umezawa, *Electroanalysis*, 1998, **10**, 1149.
81 M. Sugawara, A. Hirano, P. Bühlmann and Y. Umezawa, *Bull. Chem. Soc. Jpn.*, 2002, **75**, 187.
82 V. P. Y. Gadzekpo, K. P. Xiao, H. Aoki, P. Bühlmann and Y. Umezawa, *Anal. Chem.*, 1999, **71**, 5109.
83 V. P. Y. Gadzekpo, P. Bühlmann, K. P. Xiao, H. Aoki and Y. Umezawa, *Anal. Chim. Acta*, 2000, **411**, 163.
84 K. Bandyopadhyay, H. Liu, S.-G. Liu and L. Echegoyen, *Chem. Commun.*, 2000, 141.
85 S. Flink, H. Schönherr, G. J. Vancso, F. A. J. Geurts, K. G. C. van Leerdam, F. C. J. M. van Veggel and D. N. Reinhoudt, *J. Chem. Soc., Perkin Trans. 2*, 2000, 2141.
86 H. Aoki, Y. Umezawa, A. Vertova and S. Rondinini, *Anal. Sci.*, 2006, **22**, 1581.
87 H. Aoki, K. Hasegawa, K. Tohda and Y. Umezawa, *Biosens. Bioelectron.*, 2003, **18**, 261.
88 H. Aoki and Y. Umezawa, *Analyst*, 2003, **128**, 681.
89 Y. Katayama, Y. Ohuchi, H. Higashi, Y. Kudo and M. Maeda, *Anal. Chem.*, 2000, **72**, 4671.
90 E. Climent, R. Casasús, M. D. Marcos, R. Martínez-Máñez, F. Sancenón and J. Soto, *Chem. Commun.*, 2008, 6531.
91 E. Climent, P. Calero, M. D. Marcos, R. Martínez-Máñez, F. Sancenón and J. Soto, *Chem.–Eur. J.*, 2009, **15**, 1816.
92 E. Climent, A. Martí, S. Royo, R. Martínez-Máñez, M. D. Marcos, F. Sancenón, J. Soto, A. M. Costero, S. Gil and M. Parra, *Angew. Chem., Int. Ed.*, 2010, **49**, 5945.
93 J. V. Ros-Lis, B. García, D. Jiménez, R. Martínez-Máñez, F. Sancenón, J. Soto, F. Gonzalvo and M. C. Valldecabres, *J. Am. Chem. Soc.*, 2004, **126**, 4064.
94 L. T. Sexton, L. P. Horne and C. R. Martin, *Mol. BioSyst.*, 2007, **3**, 667.
95 Z. Siwy, L. Trofin, P. Kohli, L. A. Baker, C. Trautmann and C. R. Martin, *J. Am. Chem. Soc.*, 2005, **127**, 5000.
96 Z. Siwy, E. Heins, C. C. Harrell, P. Kohli and C. R. Martin, *J. Am. Chem. Soc.*, 2004, **126**, 10850.
97 E. A. Heins, Z. S. Siwy, L. A. Baker and C. R. Martin, *Nano Lett.*, 2005, **5**, 1824.
98 A. Liu, Q. Zhao and X. Guan, *Anal. Chim. Acta*, 2010, **675**, 106.
99 D. Wang, Q. Zhao, R. S. S. Zoysa and X. Guan, *Sens. Actuators, B*, 2009, **139**, 440.
100 D. A. Jayawardhana, J. A. Crank, Q. Zhao, D. W. Armstrong and X. Guan, *Anal. Chem.*, 2009, **81**, 460.
101 K. Nozawa, C. Osono and M. Sugawara, *Sens. Actuators, B*, 2007, **126**, 632.
102 B. G. Trewyn, I. I. Slowing, S. Giri, H. T. Chen and V. S.-Y. Lin, *Acc. Chem. Res.*, 2007, **40**, 846.
103 T. C. Rich and J. W. Karpen, *Ann. Biomed. Eng.*, 2002, **30**, 1088.
104 M. Oh-hora and A. Rao, *Curr. Opin. Immunol.*, 2008, **20**, 250.
105 E. Climent, M. D. Marcos, R. Martínez-Máñez, F. Sancenón, J. Soto, K. Rurack and P. Amorós, *Angew. Chem., Int. Ed.*, 2009, **48**, 8519.
106 E. Climent, A. Bernardos, R. Martínez-Máñez, A. Maquieira, M. D. Marcos, N. Pastor-Navarro, R. Puchades, F. Sancenón, J. Soto and P. Amorós, *J. Am. Chem. Soc.*, 2009, **131**, 14075.
107 R. Casasús, E. Aznar, M. D. Marcos, R. Martínez-Máñez, F. Sancenón, J. Soto and P. Amorós, *Angew. Chem., Int. Ed.*, 2006, **45**, 6661.
108 C. Coll, R. Casasús, E. Aznar, M. D. Marcos, R. Martínez-Máñez, F. Sancenón, J. Soto and P. Amorós, *Chem. Commun.*, 2007, 1957.
109 C. Coll, E. Aznar, R. Martínez-Máñez, M. D. Marcos, F. Sancenón, J. Soto, P. Amorós, J. Cano and E. Ruiz, *Chem.–Eur. J.*, 2010, **16**, 10048.
110 E. Aznar, C. Coll, M. D. Marcos, R. Martínez-Máñez, F. Sancenón, J. Soto, P. Amorós, J. Cano and E. Ruiz, *Chem.–Eur. J.*, 2009, **15**, 6877.
111 R. W. Davis, A. Flores, T. A. Barrick, J. M. Cox, S. M. Brozik, G. P. Lopez and J. A. Brozik, *Langmuir*, 2007, **23**, 3864.
112 S. Chemburu, E. Ji, Y. Casana, Y. Wu, T. Buranda, K. S. Schanze, G. P. Lopez and D. G. Whitten, *J. Phys. Chem. B*, 2008, **112**, 14492.
113 R. Zeineldin, M. E. Piyasena, L. A. Sklar, D. Whitten and G. P. Lopez, *Langmuir*, 2008, **24**, 4125.
114 R.-I. Stefan, J. F. van Staden and H. Y. Aboul-Enein, *Crit. Rev. Anal. Chem.*, 1999, **29**, 133.
115 G. Orellana and D. Haigh, *Curr. Anal. Chem.*, 2008, **4**, 273.
116 G. Gopalakrishnan, P. Thostrup, I. Rouiller, A. L. Lucido, W. Belkaïd, D. R. Colman and R. B. Lennox, *ACS Chem. Neurosci.*, 2010, **1**, 86.
117 N. Lincopan, M. R. A. Santana, E. Faquim-Mauro, M. H. B. da Costa and A. M. Carmona-Ribeiro, *BMC Biotechnol.*, 2009, 9, Art.-No. 5.
118 C. Kepplinger, I. Höfer and C. Steinem, *Chem. Phys. Lipids*, 2009, **160**, 109.
119 A. Studer, X. Han, F. K. Winkler and L. X. Tiefenauer, *Colloids Surf., B*, 2009, **73**, 325.
120 G. Favero, L. Campanella, S. Cavallo, A. D'Annibale, M. Perrella, E. Mattei and T. Ferri, *J. Am. Chem. Soc.*, 2005, **127**, 8103.
121 Y. Huang, P. V. Palkar, L.-J. Li, H. Zhang and P. Chen, *Biosens. Bioelectron.*, 2010, **25**, 1834.
122 J. A. Martinez, N. Misra, Y. Wang, P. Stroeve, C. P. Grigoropoulos and A. Noy, *Nano Lett.*, 2009, **9**, 1121.
123 L. Becucci, M. D'Amico, S. Daniele, M. Olivotto, A. Pozzi and R. Guidelli, *Bioelectrochemistry*, 2010, **78**, 176.
124 L. E. M. Nery and A. M. L. Castrucci, *Comp. Biochem. Physiol., Part A: Mol. Integr. Physiol.*, 1997, **118**, 1135.
125 M. A. Visconti, G. C. Ramanzini, C. R. Camargo and A. M. L. Castrucci, *J. Exp. Zool.*, 1999, **284**, 485.
126 N. Oshima, *Pigm. Cell Res.*, 2001, **14**, 312.
127 J. C. McNamara and M. R. Ribeiro, *Biol. Bull.*, 2000, **198**, 357.
128 W. C. Sherbrooke, A. M. D. Castrucci and M. E. Hadley, *Physiol. Zool.*, 1994, **67**, 659.
129 R. B. King, S. Hauff and J. B. Phillips, *Copeia*, 1994, 422.
130 Z. Wang and L. Ma, *Coord. Chem. Rev.*, 2009, **253**, 1607.
131 D. Suzuki and H. Kawaguchi, *Langmuir*, 2006, **22**, 3818.
132 J. Lee and N. A. Kotov, *Nano Today*, 2007, **2**, 48.
133 J. Lee, A. O. Govorov and N. A. Kotov, *Angew. Chem., Int. Ed.*, 2005, **44**, 7439.
134 R. Lupitskyy, M. Motornov and S. Minko, *Langmuir*, 2008, **24**, 8976.
135 C. D. S. Brites, P. P. Lima, N. J. O. Silva, A. Millán, V. S. Amaral, F. Palacio and L. D. Carlos, *Adv. Mater.*, 2010, **22**, 4499.

