Molecularly Imprinted Xerogels as Platforms for Sensing

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ABSTRACT

Detection and quantification of analytes in clinical settings (e.g., routine blood testing), at home (e.g., glucose monitoring), in the field (e.g., environmental monitoring, war fighter protection, homeland security), and in the factory (e.g., worker health, beverage and food safety) is exceedingly challenging. Chemical sensors and biosensors have attracted considerable attention because of their perceived ability to meet these challenges. Chemical sensors exploit a recognition element in concert with a transduction strategy. When the recognition element is biological (e.g., antibody, aptamer, enzyme), the sensor is termed a biosensor. There is substantial literature on biosensing; however, there are compelling reasons for developing inexpensive, robust, and reusable alternatives for the expensive or unstable biorecognition elements. This Account summarizes recent research on designing and producing analyte-responsive materials based on molecularly imprinted xerogels.

Introduction

Sol–gel processing[1] offers an attractive pathway to produce high-quality optical materials for photonic applications.[2–4] In contrast to conventional glass production methods from molten inorganic oxides, sol–gel processing allows one to prepare a wide variety of glasses under ambient conditions. Xerogels (Figure 1) are produced when the sol is allowed to gel and then dry under ambient conditions. Xerogels have been investigated widely for optosensor development because the processing conditions are mild, one can easily dope them with a plethora of active agents, they exhibit good stability, and they can be made optically transparent.[1,5–8] Xerogels also provide a convenient means to produce materials with tailored physicochemical properties.[8–10]

Figure 2 illustrates our research group's overall approach for producing tailored optical sensors from xerogels. By choosing the precursor chemistry (i.e., R′); molar ratios of precursor, water, catalyst, and cosolvent; and the processing conditions (Figure 2A) that one uses to prepare the colloidal sol, one can design and create xerogels with specific chemical (Figure 2A), nanoscale (Figure 2B), and micrometer scale (Figure 2C) features that can be integrated into complete sensing systems (Figure 2D).

In this Account, we review strategies for producing analyte-responsive materials based on molecularly imprinted xerogels.

Xerogels Doped with Active Agents

Active agent immobilization is important in numerous chemistry subdisciplines (e.g., catalysis, extractions, sensing, separation sciences).[11–13] Immobilization falls into three main categories: (1) physisorption, (2) covalent attachment, and (3) entrapment. Physisorption techniques are the simplest; however, the approach has several disadvantages. Random orientation of the active agent at the surface can lead to its inaccessibility to the target molecule in question (e.g., analyte, substrate), and because this method lacks covalent chemical bonds, the "immobilized" agent sometimes leaches or desorbs from the surface. Covalent attachment schemes generally form more stable interfaces in comparison to physisorption, and active agent leaching from the surface is lessened. However, orientation can remain an issue, the immobilization chemistry is more elaborate, and attachment can be challenging, costly, and time intensive. Active agent sequestration within an intrinsically porous three-dimensional network is an attractive alternative strategy.

In the late 1980s and early 1990s, we[14–16] and others[17,18] were developing ways to create oriented, surface-immobilized biorecognition elements (e.g., antibodies) for sensor and separation applications. In our hands, antibody orientation proved very challenging and frustrating. In late 1991, a UB colleague, Paras N. Prasad, came to one of the authors (F.V.B.) for help in addressing a problem associated with dopant behavior with xerogels. At the time, Prasad's group had been developing xerogels for fascinating nonlinear optical applications. In early 1992, Prasad and Bright were able to convince a new graduate student, Upvan Narang, to work with them on this problem. At that time, xerogels were totally unknown to the Bright group, but it did not take long for us to become intrigued, especially after reading excellent books and papers authored by, for example, Avnir,[19] Brinker,[1,20] Dunn, Valentine and Zink,[21] and Reisfield.[22] To say our interest was "peaked" is a mild understatement!

Our laboratory's early xerogel research focused on the behavior of organic luminophores sequestered within simple xerogels (i.e., ones composed of tetramethylorthosilane (TMOS) or teteraethylorthosilane (TEOS)).[23,24]

Ellen L. Holthoff (nee Shughart) earned her B.S. in chemistry from Lebanon Valley College in 2002 and a chemistry Ph.D. from the University at Buffalo, The State University of New York, in 2007 studying with Frank Bright. Her scientific research centers on developing molecularly imprinted xerogels for small molecules and site-selectively templated and tagged xerogels as sensor platforms.

Frank V. Bright earned his B.S. in chemistry from the University of Redlands in 1982 and a chemistry Ph.D. from Oklahoma State University in 1985 studying with Linda McGown. He was a postdoctoral fellow at Indiana University working with Gary Hieftje and then joined the faculty at the University at Buffalo, The State University of New York, in 1987. He is currently UB Distinguished Professor, A. Conger Goodyear Chair, and Department Chair. His scientific research, appearing in more than 230 publications, centers on determining and quantifying key molecular-level events to develop new and improved devices, materials, and methods. Current research foci include (i) tailored materials for antifouling applications and sensors, (ii) supercritical fluids and ionic liquids as environmentally benign solvents, (iii) multiphoton excited luminescence for chemical analysis of things as they are, and (iv) biodegradable constructs to accelerate and sustain wound repair.

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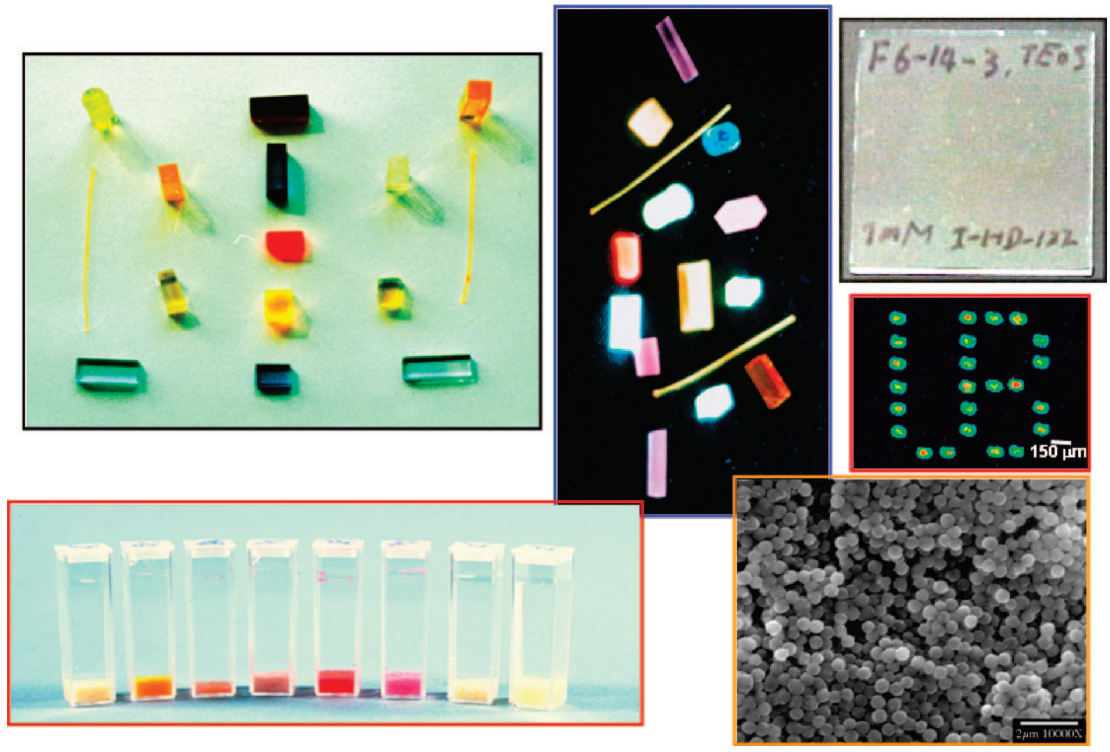

FIGURE 1. Examples of xerogel-based monoliths, films, arrays, and nanoparticles.

Inspired by the Jerusalem, New Mexico, and UCLA researchers, we took the leap in 1993 and showed that intact IgG antibodies could function when sequestered within xerogels.[25] These results were eye opening. They meant no more struggling to create Fab fragments and dealing with surface loadings and tedious covalent bond formation protocols. One could directly sequester an intact antibody within a xerogel, and the antibody "functioned". In several public forums, Bright often referred to the entire process of antibody sequestration within xerogels as "full professor chemistry". In retrospect, this was not a particularly smart statement given that Bright himself had yet to attain the rank at that particular point in time! In 2000, our laboratory finally drummed up the courage to quantify how well an antibody functioned within a xerogel in comparison to aqueous solution.[26] Ultimately we discovered that there was *less than a 10-fold decrease* in the antibody binding affinity when it was sequestered within the xerogel, and the antibodies were remarkably stable when sequestered within the xerogel. Stability was *not* a hallmark of our earlier biosensor platforms.

Figure 3 illustrates the fabrication of an active agent-doped xerogel wherein one or more of a wide variety of artificial receptors,[27] antibodies,[25,26,28,29] cells,[30–33] enzymes,[34–38] or luminophores[39–42] can be introduced into the sol and sequestered within the xerogel network. In a sensor scheme, the target analyte partitions into the intrinsically porous xerogel and interacts with the active agent (recognition element) to produce an optical, mass, thermal, or electrochemical response that is related to the analyte concentration within the sample.[8–10] This concept has, of course, been parallelized by using xerogel-based sensor arrays[43–48] for simultaneous multianalyte detection.

Between our initial xerogel foray in 1993 and the early 2000s, the primary focus of our xerogel research was to determine how more complex active dopants were behaving within xerogels and how the processing conditions and precursor chemical structures influenced the dopant's behavior and photophysics. However, the sensor aspects of our research relied on active agents, on being able to sequester these agents within the xerogel, and then on being able to exploit the entrapped agent for sensor applications. Our focus began to shift in late 2002 after Bright made a visit to The College of Wooster. At Wooster there was a new faculty member on staff named Paul L. Edmiston. Edmiston was a graduate student studying for his Ph.D. with S. Scott Saavedra at the University of Arizona when Bright visited there in 1996. Bright and Edmiston had known one another for several years; Edmiston and one of Bright's former graduate students (Emily D. Niemeyer) were also ACS Analytical Division Fellows in the late 1990s. During Bright's 2002 Wooster visit and meetings with Edmiston, he became intrigued by Edmiston's efforts (*vide infra*) to create small molecule responsive chemical sensors based on xerogels and molecular imprinting. It was obvious that this basic concept had serious potential and might eliminate the need for labile biorecognition elements like antibodies and enzymes, but there was much to overcome.

Molecularly Imprinted Materials

Biorecognition elements, although selective, have their limitations.[49–52] For example, according to Swanson and

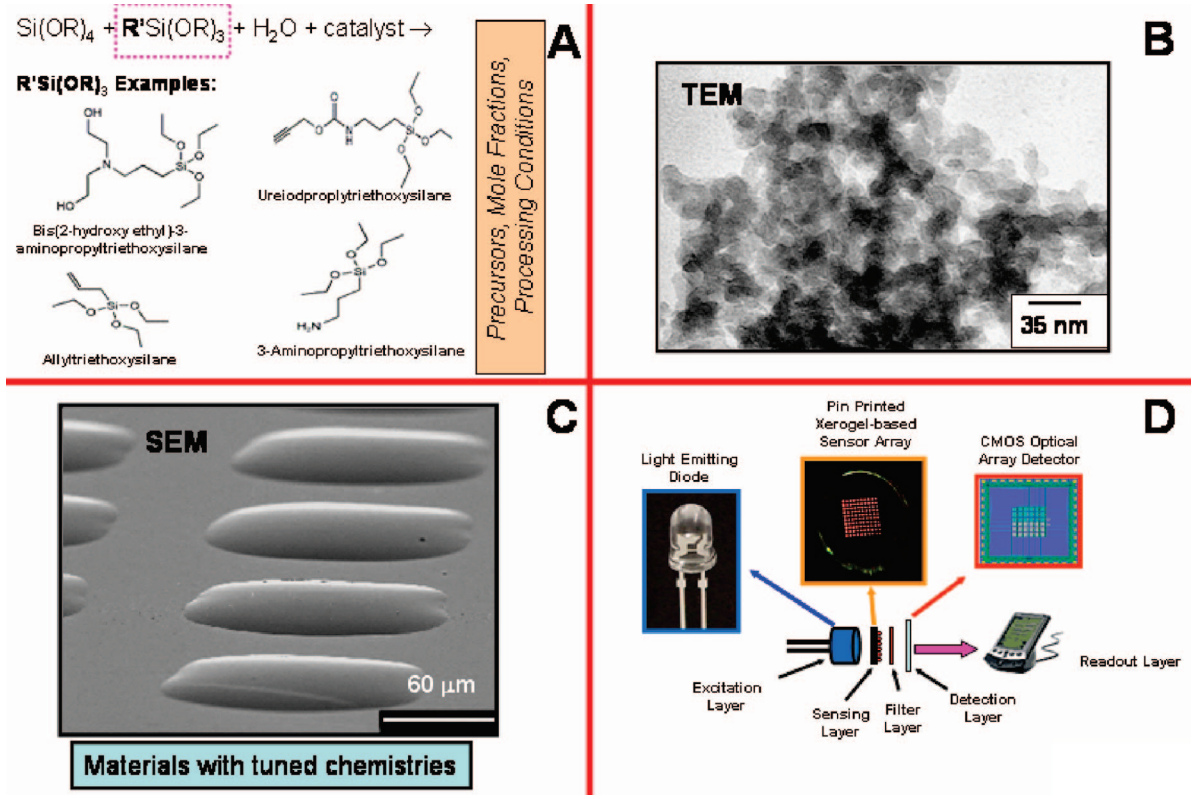

FIGURE 2. Overall philosophy depicting the use of reactive silanes (A) to form tailored colloidal sols and xerogels (B), micrometer-sized xerogel-based sensor elements (C), and xerogel-based sensor devices (D).

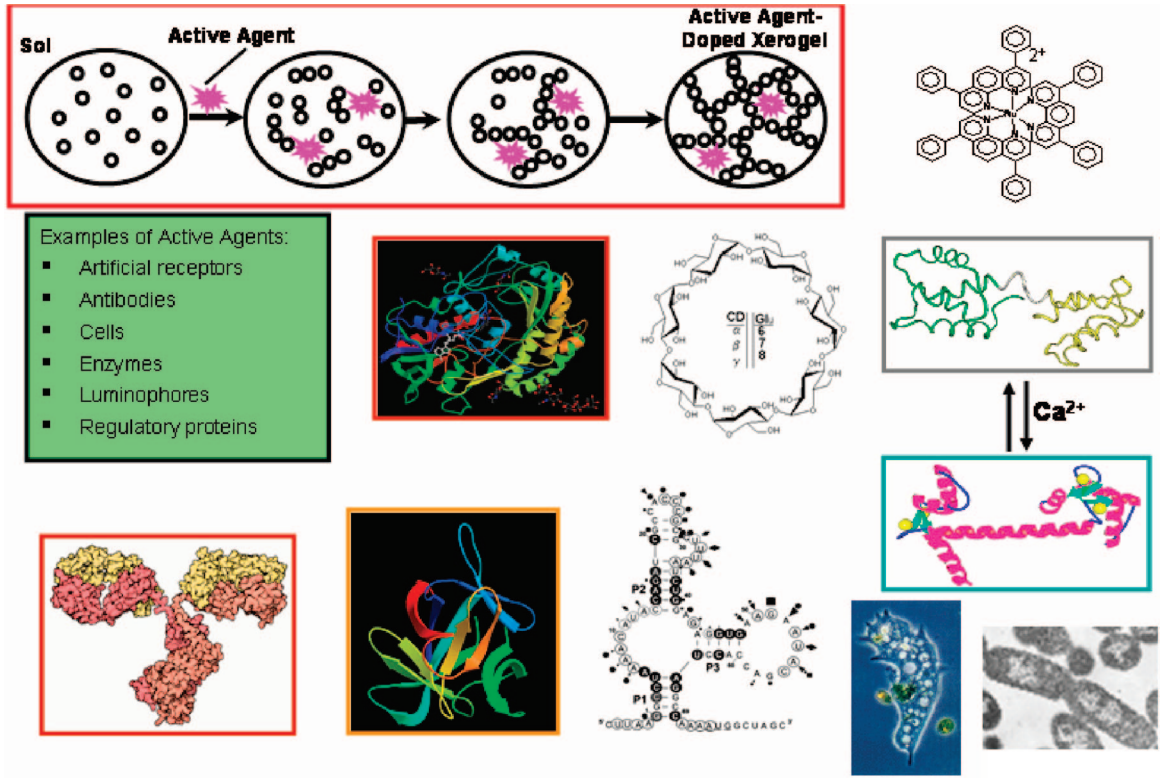

FIGURE 3. Formation of an active agent doped xerogel. Representative examples of active agents are shown.

coworkers,[53] "...immuno-based assays [ones that use antibodies for recognition] are difficult to implement... owing to poor stabilities of antibodies and the need for unstable reagents." Aptamers[54] can certainly address several of these issues, but aptamers are not yet available for a wide range of analytes, and they can be costly.

The introduction of specific binding domains within synthetic polymers (organic and inorganic) by template-

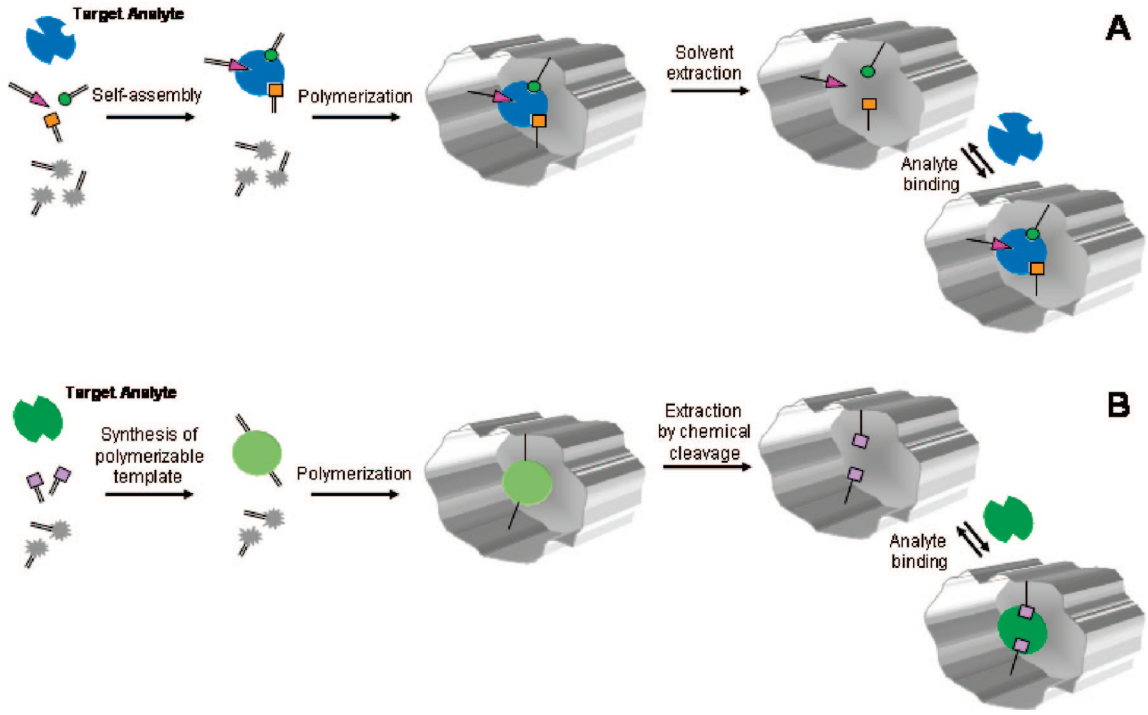

FIGURE 4. Schematic of the two most common MIP fabrication strategies: (A) noncovalent imprinting; (B) covalent imprinting.

directed cross-linking of functional monomers (molecular imprinting) has attracted considerable attention.[49–52,55–60] Interestingly, the molecular imprinting concept can be traced back to Dickey's seminal work on imprinted silica.[61] Molecular imprinting involves arranging polymerizable functional monomers around a template followed by polymerization and template removal (Figure 4). Arrangement is typically achieved by noncovalent interactions (e.g., hydrogen bonds, ion pair interactions, Van der Waals forces, dipole–dipole bonds) or reversible covalent interactions. A properly designed molecularly imprinted polymer (MIP) can then bind the template or structurally similar analytes. The attraction of molecularly imprinted materials include (1) binding affinities comparable to a biological recognition element (nanomolar dissociation constants have been reported), (2) robustness and stability under a range of chemical and physical conditions, and (3) the potential to design recognition sites for analytes that lack suitable biorecognition elements.

The three-dimensional arrangement of functional residues around the template is generally achieved by (1) noncovalent or (2) reversible covalent interactions. Mosbach[62,63] has described the most commonly practiced imprinting strategy (Figure 4A), which is based on noncovalent interactions between specific functional groups on polymerizable monomers and the template to position the monomers in a particular orientation with respect to the template molecule prior to polymerization. Following polymerization and template removal, the three-dimensional arrangement of functional groups within the imprinted polymeric matrix can subsequently recognize and bind the target using the same noncovalent interactions. Arrangement using noncovalent interactions requires the template and target to form a sufficient number of noncovalent intermolecular interactions to produce the three-dimensional binding pocket during polymerization. As a result, noncovalent imprinting has not been particularly successful for templates or target molecules that do not possess functional groups (i.e., strong noncovalent interactions between the functional monomers and template are a key requirement).[64] Despite this limitation, noncovalent molecular imprinting is very flexible in terms of the functional monomers, the possible target molecules, and the use of the imprinted materials.[65]

Reversible covalent interactions can be used to overcome many of the limitations associated with noncovalent imprinting (Figure 4B). Wulff and coworkers[66–68] introduced a covalent molecular imprinting method that exploits reversible covalent bonds between binding site monomers and a template molecule. During imprinting, the strong (covalent) interactions aid in controlling the functional groups within/inside the templated three-dimensional binding cavity. The covalent bonds are then cleaved to release the template molecule, but they are later renewed or derivatized so the template site selectively binds the target molecule or transduces its presence upon binding. This type of imprinting creates strong interactions as a result of covalent bond restoration between the three-dimensional site within the matrix and the target; however, it is limited by the rather small number of useful reversible covalent interactions that can be used.[64] Binding site monomers with boronic acid, diol, aldehyde, or amine functional groups have been successfully used for covalent molecular imprinting applications.

In both types of molecular imprinting (Figure 4), once the template is removed, three-dimensional binding cavities are exposed that correspond to the template in size, shape, and functionality. Essentially, one creates a three-

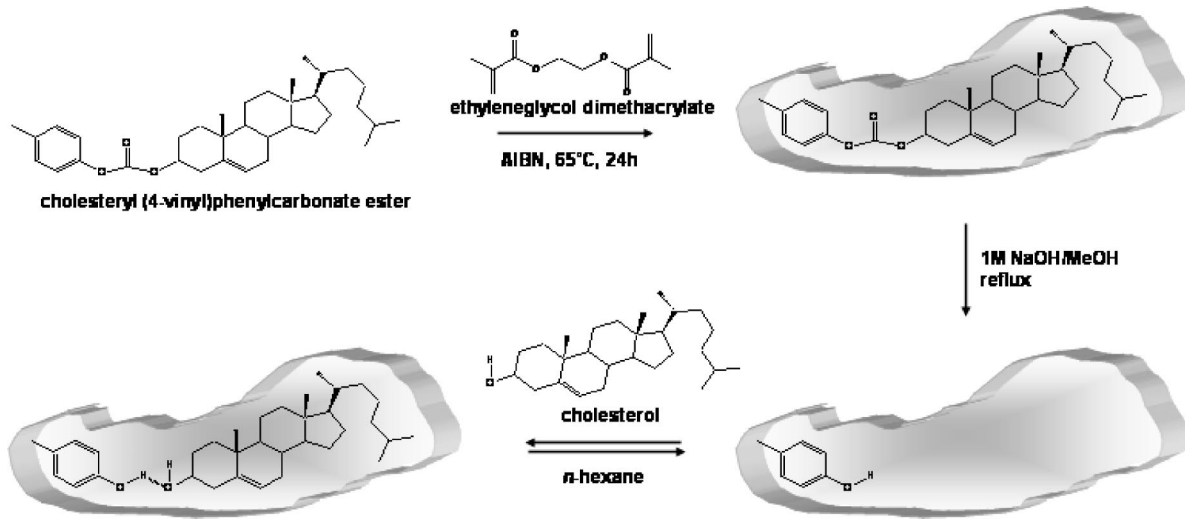

FIGURE 5. The sacrificial spacer approach to molecular imprinting. A cholesterol-responsive MIP is fabricated by using cholesteryl (4-vinyl)phenylcarbonate ester as the template. Adapted from ref 52.

dimensional "memory" of the template within an imprinted polymer matrix.[58]

To overcome the drawbacks associated with covalent and noncovalent approaches to molecular imprinting, Whitcombe et al.[52] described an alternative scheme. In the sacrificial spacer (SS) approach (Figure 5), first developed for cholesterol binding, the authors used cholesteryl (4-vinyl)phenylcarbonate ester as a functional monomer; it operated as the covalently bound template monomer, but it could be cleaved and removed from the matrix following templating. This combination resulted in the formation of three-dimensional noncovalent recognition sites within the final material *in addition to* a phenolic residue capable of interacting with the template (i.e., cholesterol) through hydrogen bonding. The Whitcombe group[69] has also reported on imprinting of small aromatic heterocycles in which MIPs are prepared by using two different SS methodologies (phenyldimethylsilylmethacrylate template) and evaluated by comparison with pyridine-imprinted polymers prepared by the noncovalent molecular imprinting method. The noncovalently imprinted polymers exhibited no size selectivity for the smaller pyridine template. In contrast, polymers prepared by using the SS method showed more selective binding, particularly at analyte concentrations below 1.0 mM.

The majority of molecular imprinting research has focused on the use of organic acrylic and vinylic precursors (among other functionalities) and a cross-linker (e.g., bis- or trisacrylates, vinylbenzenes, acrylamides, and piperazines) with an initiator like azobisisobutylonitrile (AIBN). The final polymers are not necessarily porous, and there are issues associated with solvent, temperature or both plasticizing the polymer.

Molecularly Imprinted Xerogels

Xerogels can easily form cross-linked materials. Xerogels are also intrinsically porous, their porosity can be tuned by the processing conditions (e.g., pH), and they are not as prone to plasticize in comparison to organically-based MIPs. Xerogels are also attractive hosts for labile biomolecule sequestration (*vide supra*). In 1949, Dickey[61] first imprinted silica to create materials that were 4–20 times more effective at binding the target molecules (methyl, ethyl, propyl, and butyl orange) in comparison to unimprinted silica controls. Much has transpired since Dickey's seminal work and sol–gel processing has allowed researchers to create a wide variety of molecularly imprinted xerogels (MIXs) and MIX-based sensors.

For example, Lin et al.[70] synthesized a molecularly imprinted organic–inorganic hybrid polymer to bind caffeine. In this work, random copolymers of poly(methacrylamide-*co*-(vinyl trimethoxysilane)) (MAAM–VTMOS) and poly((methacrylic acid)-*co*-(vinyl trimethoxysilane)) (MAA–VTMOS) were synthesized by a free radical polymerization reaction in benzoylperoxide. These polymers, along with TEOS and caffeine, then underwent an acid-catalyzed sol–gel process to create hybrid xerogel monoliths. Ionic and nonspecific adsorption, which are considered to be the main disadvantage of many MIP-based platforms, were minimized by end capping the surface silanol groups with a mixture of chlorotrimethylsilane and 1,1,1,3,3,3-hexamethyldisilazane. The end-capped hybrid MAAM-based MIX exhibited superior recognition properties for caffeine in comparison to a pure MAA-based MIPs. The MAAM-based MIX yielded an "imprint factor" of 9.50 in comparison to 6.80 for the pure MAA-based MIP. Both types of end-capped hybrid materials exhibited improved caffeine recognition in comparison to a polyacrylonitrile-based caffeine imprinted organic copolymer. The hybrid MIX selectivity for caffeine was also higher in comparison to the organic-only MIP. The hybrid MIX caffeine selectivity was assessed by using structural analogues, theobromine and theophylline. The selectivity factor, SF (defined as the response ratio from the MIX when it is challenged by the target analyte and an equivalent concentration of interferent) for a caffeine-responsive MIX were 17.5 for theobromine and 20 for

theophylline. The corresponding polyacrylonitrile-based caffeine MIP yielded a SF of only 1.4 for theophylline.

Marx and coworkers created interesting MIX-based thin films that bound parathion.[71] The authors combined TEOS, phenyltrimethoxysilane (PTriMOS), and aminopropyltriethoxysilane (APTES) in varying concentrations and mole ratios with parathion to prepare the MIXs. The MIXs exhibited high selectivity toward parathion (equilibrium binding of 0.26 ± 0.01 μg) in comparison to similar organophosphates, including paraoxon (0.0087 ± 0.006 μg), fenitrothion (0.007 ± 0.003 μg), diazinon (0.026 ± 0.013 μg), and quinalphos (0.012 ± 0.004 μg). These researchers also demonstrated that analyte binding depended on the functional monomer and the composition that one used to prepare the MIX.

Zhang et al.[72] reported a piezoelectric sensor coated with a thin film MIX for L-histidine. The L-histidine imprinted MIX was derived from PTriMOS and methyltrimethoxysilane (MTriMOS). The L-histidine binding to the MIX was investigated by the piezoelectric microgravimetry and electrochemical impedance. This sensor yielded an L-histidine detection limit of 25 nM. Scatchard analysis showed that the MIX exhibited a binding site loading of 23.7 μmol/g. The SF for an L-histidine-responsive MIX were as follows: D-histidine (4.2), D,L-phenylalanine (10), L-arginine (12.5), L-tyrosine (10.4), imidazole-4-propanoic acid (4.8), and imidazole-4-ethanamine (5.3).

Lee and coworkers[73] developed a series of MIX films that were imprinted with the aromatic carboxylic acids 4-(4-propyloxyphenylazo)-benzoic acid (C_3AzoCO_2H), anthracene-9-carboxylic acid (9-$AnCO_2H$), and anthracene-2-caroxylic acid (2-$AnCO_2H$). MIX films were created by repeatedly immersing a gold-coated quartz crystal microbalance electrode or a quartz plate in a solution of titanium-*n*-butoxide ($Ti(O\text{-}n\text{-}Bu)_4$) mixed with a given carboxylic acid dissolved in toluene/ethanol. The template molecules were completely removed from the films upon treatment with 1% ammonia solution. The MIX films exhibited mass increases for guest binding in the presence of various carboxylic acids (e.g., C_3AzoCO_2H, $AzoCO_2H$, 9-$AnCO_2H$, 2-$AnCO_2H$, cinnamic acid, octanoic acid, and benzoic acid) and the isomeric structure of the two anthracene carboxylic acids was readily distinguished by the MIXs. The authors defined the imprinting efficiency (IE) as a molar ratio of bound guest and template molecules. The IE in the C_3AzoCO_2H-imprinted MIX is $AzoCO_2H$ (0.88), 2-$AnCO_2H$ (0.60), cinnamic acid (0.48), benzoic acid (0.36), 9-$AnCO_2H$ (0.33), and octanoic acid (0.31). The IE in the 9-$AnCO_2H$-imprinted MIX is $AzoCO_2H$ (0.76), 2-$AnCO_2H$ (0.88), cinnamic acid (0.75), C_3AzoCO_2H (0.67), benzoic acid (0.64), and octanoic acid (0.36). The IE in the 2-$AnCO_2H$-imprinted MIX is $AzoCO_2H$ (0.76), 9-$AnCO_2H$ (0.72), C_3AzoCO_2H (0.71), benzoic acid (0.61), cinnamic acid (0.54), and octanoic acid (0.39).

Li et al.[74] fabricated MIX-based materials for parathion by exploiting noncovalent π–π interactions with the functional monomer *p-tert*-butylcalix[6]-1,4-crown-4. TEOS, hydroxyterminated silicone oil, poly(methylhydrosiloxane), *p-tert*-butylcalix[6]-1,4-crown-4, and parathion were mixed together to form the MIX. Parathion binding was characterized electrochemically by cyclic voltammetry, linear sweep voltammetry, chronoamperometry, and alternating current impedance spectroscopy. The reported parathion detection limit was 1 nM. The authors evaluated the selectivity of this MIX-based sensor by challenging it with fenitrothion, methyl-parathion, α-hydroxy-4-nitrophenyl-dimethyl phosphonate, α-hydroxy-4-nitrophenyl-diethyl phosphonate, and hydrophobic compounds containing nitro groups (e.g., 2-nitroso-1-naphthol, *o*-, *m*-, and *p*-nitrophenol, *o*- and *m*-dinitrobenzene, and nitrobenzene). Remarkably, no distinguishable change in the peak current was reported in the presence of these interfering compounds.

Zhang and coworkers[75] reported a MIX-based thin film for cytidine. The MIX was produced from 3-(aminopropyl)trimethoxysilane (APTMS) and cytidine by electrodeposition on a gold electrode surface. The authors applied a sufficiently negative potential to the electrode surface to generate hydroxyl ions, which were used as the catalyst for the APTMS hydrolysis and condensation at the electrode surface. The binding capacity and selectivity of the MIX film were studied using piezoelectric quartz crystal impedance, electrochemical impedance, and capacitance. Dissociation constants ranging from 0.084 to 0.19 nM were observed, indicating a strong imprinted interaction between the electrodeposited MIX-based film and cytidine. The SF for a cytidine-responsive MIX is as follows: guanosine (4.8), thymidine (2.8), uridine (2.3), deoxythymidine (2.6), deoxyguanosine (5), adenosine (5.6), and adenosine monophosphate (7.7). The authors observed a 14% decrease in binding capacity over 4 weeks.

The Lam group[56] used a photoinduced electron transfer strategy to form a 2,4-dichlorophenoxyacetic acid (2,4-D)-responsive MIX by copolymerizing 3-[*N*,*N*-bis(9-anthrylmethyl)amino] propyltriethoxysilane (fluorophore) with TEOS and PTriMOS using 2,4-D as the template. The so-formed MIX exhibited a change in fluorescence with pH (apparent pK_a near 7.2), and it yielded a 15% decrease in fluorescence in the presence of 750 μM 2,4-D. Tests with benzoic acid and acetic acid revealed SF values of 5.0 and 1.1, respectively.

In 2002, Edmiston and coworkers[64] reported an elegant approach to fabricate a fluorescence-based MIX for the detection of 1,1-bis(4-chlorophenyl)2,2,2-trichloroethane (DDT). The approach exploited the SS scheme, introduced by Whitcombe[52,57] (*vide supra*), wherein the authors reacted 3-isocyanatopropyltriethoxysilane with 4,4′-ethylidenebisphenol to form the SS. They then prepared the fluorescent monomer by reacting APTES with the fluorophore 4-chloro-7-nitrobenzofurazan (NBD) (attaching the NBD to the APTES amine, NBD–APTES). The imprinted xerogel was then formed by mixing NBD–APTES, SS, and bis(trimethoxysilyl)benzene (BTB). Once the xerogel was formed, the authors cleaved the SS carbamate bond with $LiAlH_4$, forming amines within the template site and liberating the SS from the xerogel. In the presence of DDT, this MIX-based sensor exhibited up to a 3% change in

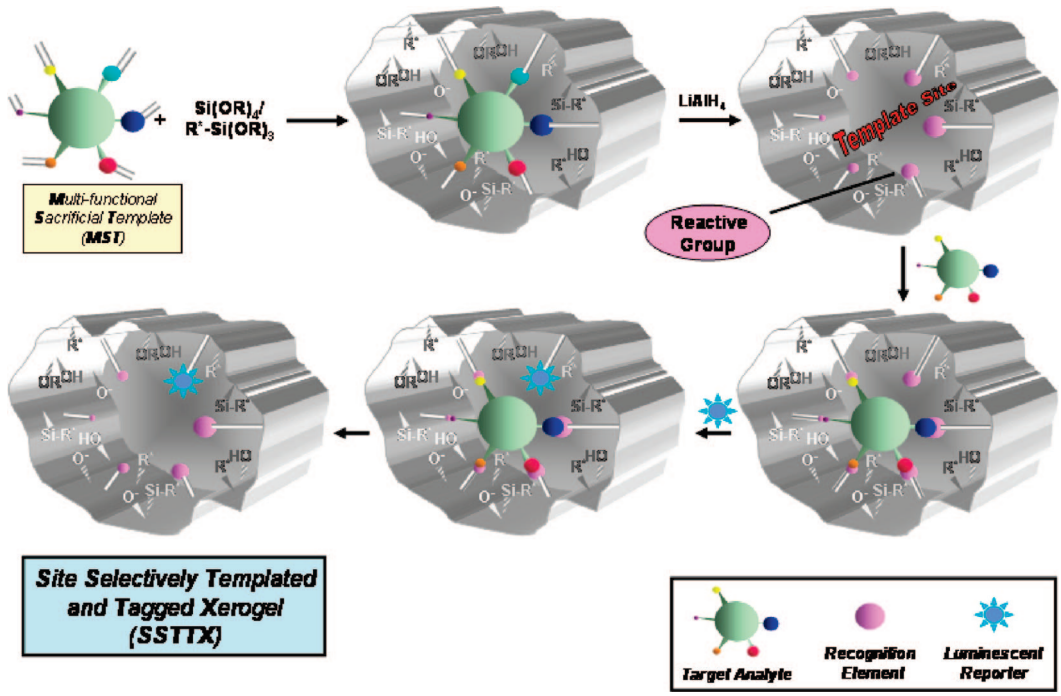

FIGURE 6. Generic schematic describing the SSTTX fabrication protocol. The view shown is from the perspective of a single pore within a xerogel.

NBD fluorescence. Single-digit parts per billion level detection limits were reported. The DDT-responsive MIX exhibited a SF between 1 and 5 for 2,2-bis(4-chlorophenyl)-1,1-dichloroethylene, 1-(2-chlorophenyl)-1-(4-chlorophenyl)-2,2-dichloroethane, 2,2-bis(4-chlorophenyl)-1,1-dichloroethane, diphenylmethane, 4,4′-dibromobiphenyl, and 4,4′-bis(chloromethyl)-1,1′-biphenyl. The primary limitation of this strategy was that the reporter molecules were randomly distributed within the MIX, and they were not necessarily close to the template site.

The aforementioned examples clearly show that sol–gel processing can be used to create materials with diverse functionalities (cf., Figure 2A), one could create xerogel-based sensor arrays,[29,43–46] xerogel-based sensors can be made that exhibit high stability,[47,48] and MIX-based sensors were possible. However, the issue of efficient transduction and universality was not solved.

In 2006, Shughart et al.[76] overcame the problem of randomly distributed reporter molecules within the MIX. In the site selectively templated and tagged xerogel (SSTTX) strategy (Figure 6), one forms a MIX by using a multifunctional sacrificial template (MST). The requirements for an effective MST are twofold: (1) a structural analog that resembles the target analyte in size, shape, and functionality and (2) strategically placed functional groups for forming easily altered bonds that can be cleaved to remove the MST. An important feature of the MST, in comparison to its target analyte, is that the template possesses one additional residue. This additional residue allows for the covalent attachment of a reporter molecule at the template site close to the target analyte's binding site. In operation, analyte binding to the SSTTX's template site causes changes in the physicochemical properties that surround the reporter molecule. If the reporter molecule is a properly selected luminophore, the local change in physicochemical properties results in an analyte concentration-dependent change in the reporter molecules emission intensity, spectra, lifetime, or anisotropy.

In the original offering, the SSTTX strategy was used to create a MIX for a model compound, 9-anthrol (Figure 7). Toward this end, 3-isocyanatopropyltriethoxysilane was reacted with 9,10-anthracenediol to form the bifunctional sacrificial template (BST; Figure 7A). The MIX was formed by using the BST and TMOS. Once the xerogel formed, the BST carbamate bonds were cleaved with $LiAlH_4$, forming amines within the three-dimensional template site and liberating the BST remnant from the xerogel. The xerogel was then challenged with 9-anthrol to fill the template sites. NBD-Cl (the fluorescent reporter) was then introduced, and it reacted with and attached to any free, accessible amine. 9-Anthrol binding was detected by a change in the NBD fluorescence intensity. This first-generation SSTTX (Figure 7B) exhibited a reversible response, provided 0.3 μM detection limits for 9-anthrol, and exhibited a response time of $<$45 s. (Note, control xerogels including ones with NBD randomly distributed within the xerogel were essentially nonresponsive to the target analyte.) As shown in Figure 7C our 9-anthrol-responsive SSTTX yielded a SF up to 520 for a wide variety of potential interferents.

More recently, we evaluated the ability to tune a MIX's selectivity by adjusting the precursors that were used to prepare the sol. Figure 8 presents results for a series of hybrid MIXs that were designed to recognize tetracycline (TC) when they are challenged by several TC analogs. These results demonstrate that the precursor chemistry

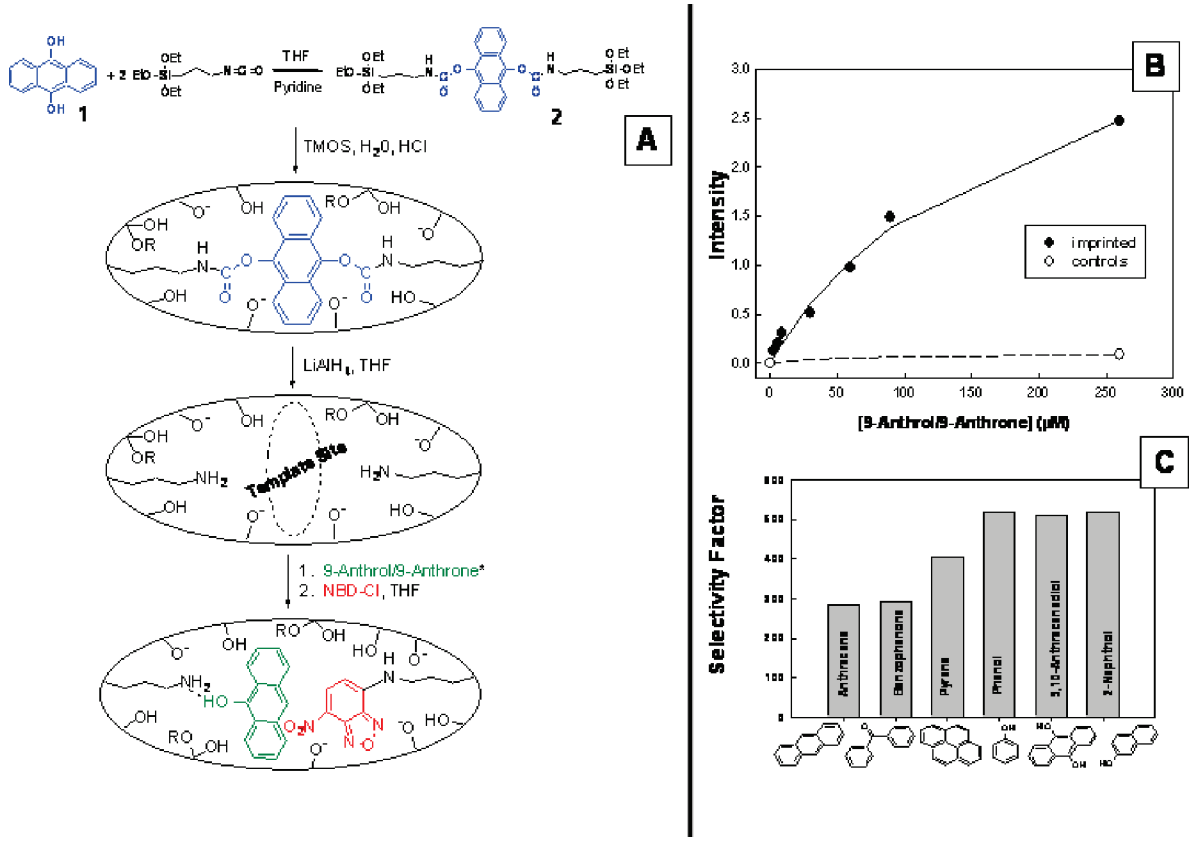

FIGURE 7. Results from an SSTTX sensor for the detection and quantification of 9-anthrol: (A) the reaction protocol used to produce the SSTTX; (B) 9-anthrol response profile for an SSTTX and series of xerogel controls; (C) selectivity of a 9-anthrol-responsive SSTTX when challenged by several interferents with structures similar to 9-anthrol. Adapted from ref 76.

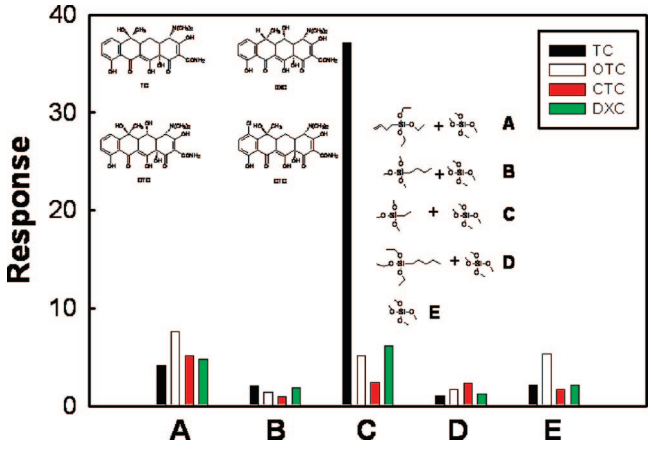

FIGURE 8. Effects of precursor chemistry (50/50 for all except E) on the selectivity of a tetracycline (TC)-responsive MIX.

and functionality and the precursor mole ratios can be used to tune the MIX selectivity.

The Edmiston group[77] recently reported on a second strategy for overcoming randomly distributed reporter molecules within the MIX. In this approach, the authors reacted 3-isocyanatopropyltriethoxysilane with *N*-(9-fluorenylmethoxycarbonyl-β-phenyl-D-phenylalaninol (FMCPPA) to form the FMCPPA–silane template. The MIX was formed by mixing BTB and the FMCPPA–silane template. Once the xerogel formed, the FMCPPA–silane template was chemically removed via cleavage of a carbamate linkage by iodotrimethylsilane. Following template removal, an amine group was left that provided an attachment site for a fluorescent reporter molecule, NBD-Cl. Fluorene binding was detected by a change in NBD fluorescence intensity. The sensor responded to fluorene (40% change in NBD fluorescence), yielded detection limits below 10 ppt for fluorene, and responded in <60 s. Unfortunately, the MIX fluorene response was irreversible. The reported SF ranged from 1.4 to 5 for fluorene in comparison to fluorene-2-carboxaldehyde, anthracene, fluoranthracene, and naphthalene.

The target analytes in all the previous examples were small to medium sized molecules (i.e., < 1000 g/mol). Could the MIX strategy be extended to develop selective sensors for protein detection? When our laboratory first thought about this challenge, we were encouraged by related research from the Chambers group[78] where they had prepared imprinted xerogels that recognized the proteinaceous biotoxin Ricin. However, although Chambers and coworkers used tryptophan fluorescence to investigate the Ricin–xerogel interactions, they did *not* report a sensing strategy.

In 2006, Tao et al.[79] reported on a new sensor strategy that they called protein-imprinted xerogels with integrated emission sites (PIXIES) (Figure 9). PIXIES are a completely self-contained protein-sensing platform, achieving analyte recognition without a biorecognition element (e.g., antibody). PIXIES rely on a MIX and the protein target itself

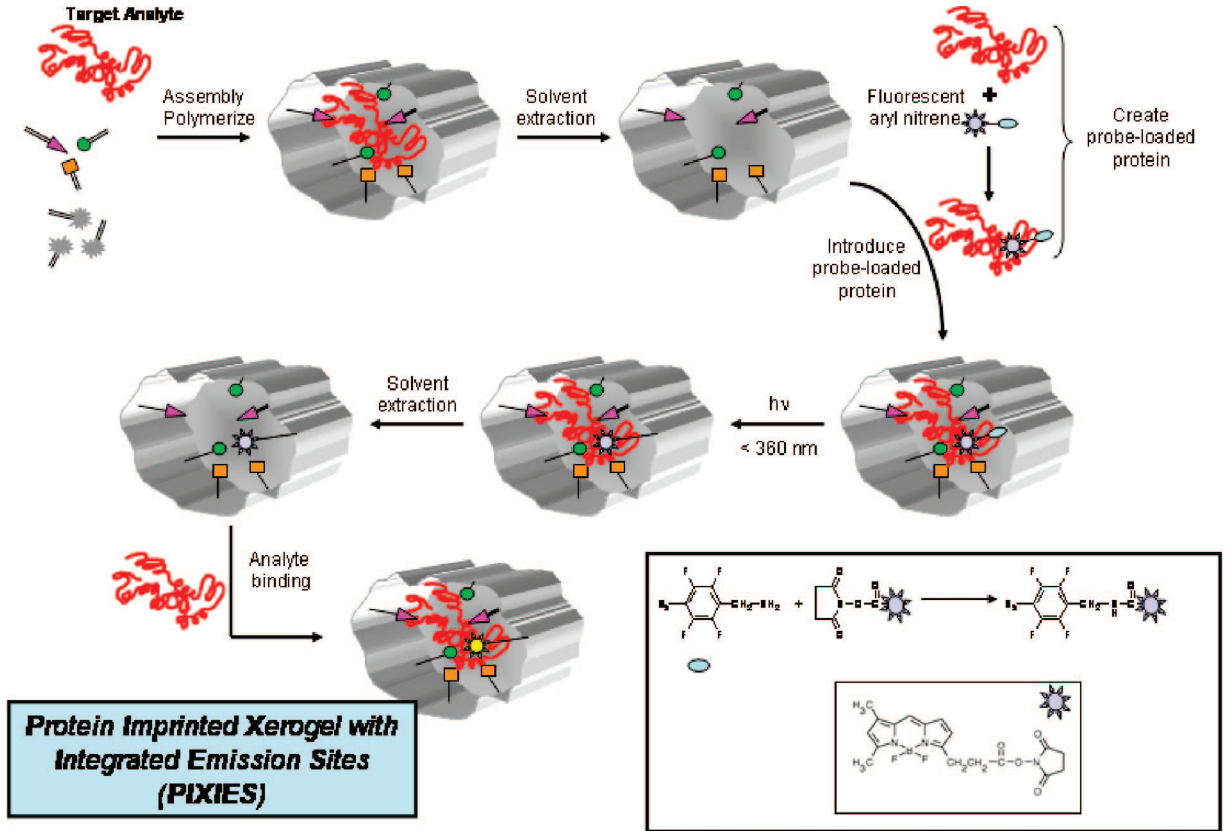

FIGURE 9. Outline of the generic PIXIES production protocol. Adapted from ref 79.

to simultaneously create the template site within the MIX and assist in the selective attachment of a luminescent reporter molecule directly within the three-dimensional molecularly imprinted cavity. Ovalbumin was selected as the initial protein target. The protein-imprinted xerogel was formed by preparing a range of sol solutions that contained different mole ratios of TEOS, APTES, *n*-octyltrimethoxysilane (C8-TriMOS), and bis(2-hydroxyethyl)aminopropyltriethoxysilane (HAPTS). These particular precursors were selected after we had used our high-throughput screening tools[80,81] to conduct preliminary materials screening experiments.

The protein-imprinted xerogel (PIX) was formed by mixing the sol with an aqueous buffer that contained ovalbumin. The xerogel was formed and ovalbumin was removed from the PIX by using either aqueous urea or dilute phosphoric acid. Next, we needed a way to selectively deliver and position a suitable reporter molecule into the template site and covalently attach it in or near the template site. We were aware that many fluorescent probe molecules tended to bind with modest affinity to proteins. Given this, we considered using the target protein itself as a sort of Trojan Horse to selectively deliver one or more reporter molecules into the template site. The question then was how to attach the reporter to the xerogel matrix?

Earlier in Bright's career, he was a committee member on a graduate student's synopsis defense. The student was reviewing for the committee how paclitaxol (an anticancer drug) interacts with microtubules and discussing how researchers had used photoreactive cross-linking reagents as a tool to determine the unique mode of drug action. Cross-linking chemistry seemed like a possible solution to our reporter molecule attachment problem. After researching the subject, it appeared as if fluorinated aryl azides might be a good choice for attaching a reporter molecule to the xerogel template site. A bit of additional research revealed that Molecular Probes (now Invitrogen) offered fluorinated aryl azides that could be coupled directly to a luminescent reporter molecule. As illustrated in the bottom right panel in Figure 9, reaction of 4-azido-2,3,5,6-tetrafluorobenzyl amine hydrochloride (ATFA) and the succinimidyl ester of dipyrromethane boron difluoride (BODIPY-FL, SE) in the dark yielded the desired luminophore-tagged aryl azide.

A 1:1 mixture of ovalbumin and the luminophore-tagged aryl azide was then prepared in the dark. The ovalbumin serves to deliver the luminophore-tagged aryl azide into the template site. Illumination of the ovalbumin/luminophore-tagged aryl azide doped PIX with UV light creates the aryl nitrene, which undergoes CH insertion into the xerogel. Washing removes the ovalbumin and any misreacted aryl nitrene, creating the PIXIES.

Figure 10 shows that an ovalbumin-templated PIXIES exhibits high selectivity for ovalbumin over human serum albumin (HSA) and the phenylsulfonamide of ovalbumin with a selectivity factor greater than 200. When an ovalbumin-templated PIXIES was challenged with a ternary protein mixture that contained ovalbumin, the phenylsulfonamide of ovalbumin, and HSA, the observed

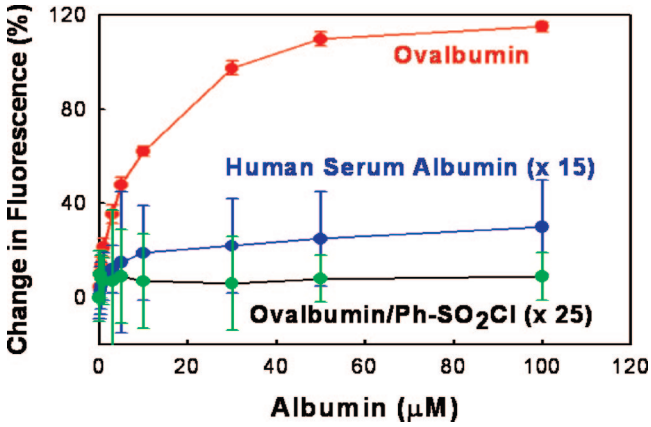

FIGURE 10. Response curves for an ovalbumin-responsive PIXIES when challenged with ovalbumin, human serum albumin, and ovalbumin treated with Ph-SO_2-Cl. Adapted from ref 79.

response was equivalent only to the sample's ovalbumin content (±5%). Control experiments with BODIPY FL randomly distributed within a PIX yielded no response to ovalbumin.

We explored the PIXIES ability to discriminate between two structurally similar proteins. Human interleukin 1-α (hIL-1α) and human interleukin 1-β (hIL-1β) were used as the targets. Spiked samples were prepared in human plasma. The PIXIES performance was compared with results from standard enzyme-linked immunosorbent assay (ELISA) kits for the same proteins. The PIXIES-based sensors exhibited detection limits of ~2 pg/mL and response times of <2 min. The PIXIES yielded a SF for these two proteins of at least 85. The PIXIES detection limits and SF are within a factor of three of the ELISAs and PIXIES response times are at least 100-fold faster in comparison to the corresponding ELISA.

Conclusions and Future Directions

The seminal work on molecularly imprinted silica appeared nearly 60 years ago. Molecular imprinting represents an exciting and promising technique that is being explored increasingly as a platform for creating a wide variety of responsive materials like chemical sensors. Molecularly imprinted materials appear to offer an inexpensive, robust, and reusable alternative to expensive and labile biorecognition elements. Molecularly imprinted materials can exhibit binding affinities that are comparable to antibody–antigen binding, yet they offer one the ability to design three-dimensional recognition sites for a plethora of analytes, including those for which biorecognition elements do not exist. Specificity for molecularly imprinted materials for their target analyte in comparison to structurally similar molecules is good, but they do not yet surpass existing biorecognition elements. Molecularly imprinted materials offer researchers an opportunity to design suites of materials and recognition elements for the same target analyte with unique binding characteristics and subsequent responses.

Recently, researchers have created sensors that are based on molecularly imprinted xerogels. Here, the state-of-the-art has progressed to the point where molecularly imprinted xerogels can now be designed and developed for sensing small molecules and proteins. Strategies now exist to attach one or more reporter molecules near the three-dimensional template site as a way to improve transduction and detection limits. Xerogel-based materials also exhibit good long term stability under ambient storage conditions.

Molecularly imprinted materials also have their issues, and many questions remain unanswered. For example, in an honest head-to-head comparison, what are the quantifiable differences in performance from molecularly imprinted materials based on organic and inorganic precursors? Organic luminophores are not ideal reporters; they tend to bleach or otherwise degrade over time or in the presence of light and heat. Solvatochromic, all inorganic luminophores (e.g., quantum dots) may prove useful, but how can they be effectively positioned close to the template site? The full potential of molecularly imprinted materials for chemical sensing also awaits their extension to the detection and quantification of a wider variety of analytes (e.g., DNA, RNA) and their use in the analysis of real samples. The issue of sensor fouling during long-term deployment remains largely unknown for molecularly imprinted materials. There is little fundamental information on the relationship between precursor chemistry, templating variables, and the final material properties. In short, we lack firm rules on what governs a molecularly imprinted material's performance. We have only rudimentary insights into the processes that actually occur within the template sites of a molecularly imprinted material upon analyte binding/dissociation. We do not know how selectivity arises in molecularly imprinted materials. Finally, monomer/precursory diversity (i.e., R′ in Figure 2A) needs to be expanded (e.g., amino acid analogs) to allow researchers to create materials with high affinity and selectivity.

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