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Templated xerogels as platforms for biomolecule-less biomolecule sensors

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Abstract

We report on a new sensor strategy that we have termed protein imprinted xerogels with integrated emission sites (PIXIES). The PIXIES platform is completely self-contained, and it achieves analyte recognition without a biorecognition element (e.g., antibody). The PIXIES relies upon sol–gel-derived xerogels, molecular imprinting, and the selective installation of a luminescent reporter molecule directly within the molecularly imprint site. In operation the templated xerogel selectively recognizes the target analyte, the analyte binds to the template site, and binding causes a change in the physicochemical properties within the template site that are sensed and reported by the luminescent probe molecule. We report the PIXIES analytical figures of merit for and compare these results to a standard ELISA. For human interleukin-1 the PIXIES-based sensor elements exhibited the following analytical figures of merit: (i) ∼2 pg/mL detection limits; (ii) <2 min response times; (iii) >85 selectivity; (iv) <6% R.S.D. long term drift over 16 weeks of ambient storage; (v) >95% reversibility after more than 25 cycles; and (vi) >85% recoveries on spiked samples.
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1. Introduction

The goal to simultaneously measure "everything" within a sample is common in many disciplines [1]. This desire has helped drive the development of a wide variety of sensor array strategies [2–8], including artificial "noses" and "tongues" [9]. Interestingly, current array-based sensor platforms have much in common with biochips, DNA microarrays, and protein microarrays [10–12]. For example, each technology requires that one identify an appropriate recognition element (e.g., antibody, cell or phage lysate, recombinant protein or peptide, oligonucleotide/oligonucleoside) that can selectively recognize the target analyte (e.g., a protein, DNA fragment), one must implement a suitable detection/transduction method, and one must "immobilize" the recognition element [13–15]. In an ideal platform the recognition element must also remain stable over time, the target analyte must have access to the recognition element, and the target analyte-recognition element association/interaction should be, for a sensor or any other device designed for real-time monitoring, "reversible" or at least easily dissociated/reset following each measurement.

Several research teams have developed biosensor arrays [16–20] for simultaneous multi-analyte detection. Representative examples include the Tan group's molecular beacon-based DNA biosensors [16], the electrostatically complexed monolayers deposited on photolithographically patterned gold microelectrodes described by Revzin et al. [17], the photopatternable enzyme membranes reported by Moser et al. [18], the Walt group's high density fiber optic arrays that are based on intact bacteria and yeast cells [19], and McDevitt's "electronic taste chips" based on immunochemistry [20]. A more recent development has been work from our laboratories coupling pin printing, protein-doped xerogels, and light emitting diodes to form novel (bio)sensor arrays [21].

Despite the progress on biosensor and protein microarrays based on proteinaceous biorecognition elements [2–20], there are well-documented limitations associated with this strategy [22–25]. For example, according to Swanson and co-workers

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[22], "... immuno-based assays are difficult to implement ... owing to poor stabilities of antibodies and the need for unstable reagents." Thus, although biologically based recognition elements are clearly being used to detect proteins, there are compelling reasons for developing inexpensive, robust, and reusable alternatives for these expensive and labile biorecognition elements. What if one could develop "biomolecule-less" sensors for the detection of biomolecules?

Over the past decade, the creation of specific binding domains within synthetic polymers by template-directed cross-linking of functional monomers has attracted considerable attention [23,24]. Molecular imprinting involves arranging polymerizable functional monomers around a suitable template molecule (e.g., the actual target analyte or a surrogate) followed by polymerization and template removal. Arrangement is typically achieved by non-covalent interactions (e.g., H-bonds, ion pairing) or reversible covalent interactions. After template removal, these molecularly imprinted polymers can recognize and bind the template (i.e., the target analyte). The advantages of molecularly imprinted polymer-based materials include [23,24]: (i) specificity comparable to a biomolecule (nM dissociation constants have been reported); (ii) robustness and stability under extreme chemical and physical conditions; and (iii) an ability to design recognition sites even for analytes that lack suitable biorecognition elements *or* for unknown analytes.

Molecularly imprinted polymers have been developed for amino acid derivatives, nucleotide bases, pesticides, pharmaceuticals, polycyclic aromatic hydrocarbons, proteins, sugars and their derivatives, and vitamins. However, according to Lam and co-workers [25], "One of the major issues in the development of molecularly imprinted polymer-based biomimetic sensors is signal transduction." There is literature on the use of amperometry/voltammetry [26], acoustic waves [27], conductometry [28], colorimetry/fluorimetry [29], and radio-labeled assays [30] to convert a molecularly imprinted polymer into a sensor; however, reports are scarce on luminescence-based molecularly imprinted polymer sensors for detecting non-luminescent analytes [25,31,32].

Sol–gel processing is an attractive method to sequester and exploit chemically active species [33,34]. Sol–gel-derived xerogels are also attractive because one can tailor a xerogel's physicochemical properties by using different precursor(s) or changing the processing protocol [33,34]. Xerogel-based sensor platform can also exhibit remarkable stability over time [35]. Further, as demonstrated by, for example, Lam and co-workers [25] and Edmiston and co-workers [31] one can exploit molecularly imprint xerogels to design luminescence-based chemical sensors for the detection of small molecules. In related research, the Chambers group [36] has used molecular imprinting to prepare molecularly imprinted xerogels that can recognize the protein Ricin. However, although Chambers and co-workers used the intrinsic fluorescence from the tryptophan residues within Ricin to investigate the Ricin–xerogel interactions, these authors did *not* actually report a sensing strategy.

High-throughput screening (HTS) methods are common in combinatorial chemistry and drug discovery [37–40]. For example, HTS methods have been used to identify promising H_2-producing catalytic materials [41], ceramics [42], flame retardants [43], dielectric thin films [44], and other materials [45–47]. We became attracted to HTS as a way to more efficiently prepare and screen biodegradable polymer and sol–gel-derived xerogel formulations for bioengineering and general sensor applications, respectively [48,49].

Despite the obvious attraction of luminescence-based detection, xerogels, and molecular imprinting, researchers have *not* developed a protein detection strategy that exploits the power of luminescence, the tunability of xerogels, and molecular imprinting. In this paper we report on a new strategy for fabricating protein-responsive chemical sensor elements based on sol–gel-derived molecularly imprinted xerogels. We term these new xerogel-based sensor elements protein imprinted xerogels with integrated emission sites (PIXIES). We outline the PIXIES production protocol, we describe a methodology for rapidly producing and screening a wide variety of sol–gel-derived xerogel-based formulations, and we compare the analytical figures of merit for our PIXIES to standard antibody-based assays.

2. Experimental

2.1. Reagents and supplies

The following reagents were used: human serum albumin, ovalbumin, tetramethoxysilane (TMOS) and urea (Sigma–Aldrich); human interleukin-1 alpha and beta (R&D Systems); tetraethoxysilane (TEOS) and methyltrimethoxysilane (C1-TMOS) (United Chemical Technologies); ethyltrimethoxysilane (C2-TMOS), *n*-propyltrimethoxysilane (C3-TMOS), *n*-butyltrimethoxysilane (C4-TMOS), *n*-hexyltrimethoxysilane (C6-TMOS), *n*-octyltrimethoxysilane (C8-TMOS), *n*-decyltriethoxysilane (C10-TEOS), bis(2-hydroxy-ethyl)aminopropyltriethoxysilane (HAPTS), 3-aminopropyltriethoxysilane (APTES), ureidopropyltriethoxysilane (U-TEOS), and 3,3,3-trifluoropropyltrimethoxysilane (TFP-TMOS) (Gelest, Inc.); succinimidyl ester of BODIPY FL (BODIPY-FL,SE) and 4-azido-2,3,5,6-tetrafluorobenzyl amine, hydrochloride (ATFA) (Invitrogen); HCl (Fisher Scientific); and EtOH (Quantum Chemical).

Aqueous solutions were prepared from deionized water that was treated with a Barnstead NANOpure II system to a specific resistivity of $>18\ \mathrm{M\Omega\ cm}$.

Glass microscope slides (Fisher Scientific Co.) were used as the substrates. The slides were cleaned by an EtOH rinse, 1–2 h soak in 1 M NaOH, 1–2 h soak in 1 M HCl, deionized water wash, and oven drying at 80 °C.

2.2. Formulation development and preliminary PIXIES screening

We set out to prepare, screen, and identify xerogel formulations that yielded PIXIES with good analytical figures of merit. Toward this end, we used a statistical design strategy [50] and prepared a series of sols based on several of the precursors that were listed in the previous section. As an example, a representative series of sols was prepared form TEOS, APTES,

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