A Polymeric and Fluorescent Gel for Combinatorial Screening of Catalysts

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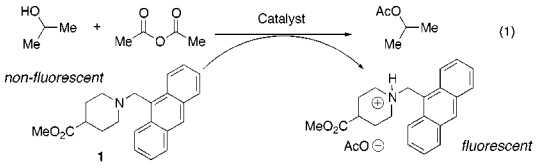
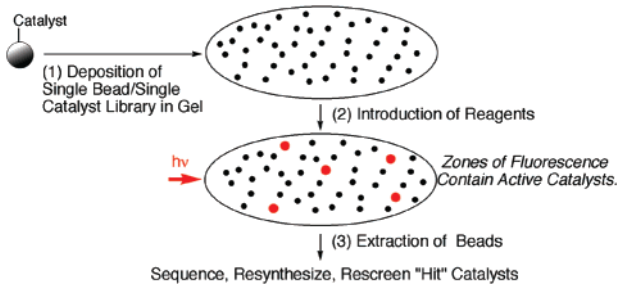
Methods of increasing the pace of materials and catalyst discovery have become a topic of intense interest in the field of chemical synthesis.[1] In particular, techniques that allow for the parallel preparation and simultaneous screening of numerous catalysts have gained particular attention as they promise to accelerate what may be the rate-determining step in the catalyst development process, the discovery of the catalyst itself.[2] One way to determine a catalyst's activity is to monitor reaction progress through the detection of a product with an appropriate chemical sensor.[3] In the present study, we report the design, synthesis, and initial implementation of a sensor-functionalized polymeric gel that allows for the pooled screening of certain types of catalyst libraries.

The method we describe involves deposition of catalysts that have been immobilized on conventional resin-beads within a polymeric matrix. The matrix is designed such that it possesses sufficient permeability for diffusion of reagents to the individually localized beads (Scheme 1).[4] Simultaneously, the polymer incorporates (by covalent attachment) a fluorescence-based sensor that signals the presence of an appropriate reaction product by an increase in fluorescence intensity.[5] The method relies on the slow diffusion of product out of the bead as it is formed into the matrix, which affords a fluorescent zone around the site of an active catalyst. Regions of the polymer surrounding beads that are inactive remain dark as none of the product is present.

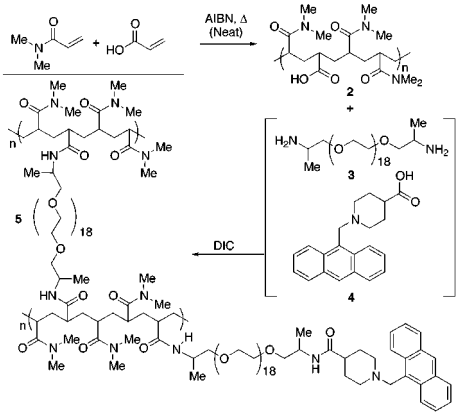
For an initial platform upon which to screen catalyst libraries, we chose to synthesize a gel-based matrix in which we could screen catalysts that mediate reactions that afford carboxylic acids as products. Specifically, we chose to study catalysts for acylation reactions of alcohols with acetic anhydride (eq 1). Work from our laboratory has established that aminomethylanthracene **1** is a suitable sensor for detection of the acidic products of this reaction[6] and is an excellent probe for in situ monitoring of catalytic activities.[7] Earlier efforts focused on simultaneous attachment of the fluorophore and catalyst to a unique bead; the present study demonstrates that polymer-bound catalysts and polymer-bound sensor may also be spatially segregated.



Scheme 1



Scheme 2



The matrix we set out to synthesize is based on the precedent of Meldal who synthesized poly(ethylene glycol) dimethylacrylamide (PEGA) gels as peptide synthesis supports.[8] These materials have the advantage of swelling in both organic and aqueous solvents. We speculated that the rate of diffusion of reagents through these gels would be adequate to allow efficient mass transport, but sufficiently slow such that real-time observation of analyte migration would be possible. Therefore, we proposed that sensor-functionalized gels, with a derivative of fluorophore **1** incorporated, would provide an ideal medium in which catalyst activities might be monitored.[9] The synthesis of such an aminomethylanthracene-functionalized gel is outlined in Scheme 2. A 3:1 molar ratio of *N*,*N*-dimethylacrylamide and acrylic acid is copolymerized with AIBN as a radical initiator to afford prepolymer **2**. Cross-linking is accomplished in a standard gel caster using a solution of poly(ethylene glycol)-bridged diamine **3** that contains a 0.1 M concentration of carboxylic acid-functionalized fluorophore **4**.[10] This procedure affords a gel-like polymer with the microstructure depicted by structure **5**.

As in the case of the polymers reported by Meldal, this material swells in a wide variety of solvents (e.g., DMF, *i*-PrOH, H_2O). The material is also reversibly fluorescent. Treatment of gel **5** with acetic acid (HOAc) induces the gel to fluoresce (excitation λ, 390 nm; emission λ, 420 nm); washing with base followed by rinsing with DMF results in a gel that is nonfluorescent. Of

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(10) For details on the synthesis of **5**, see the Supporting Information.

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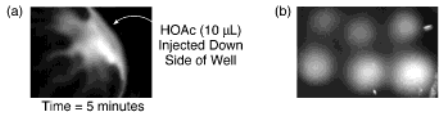

Figure 1. Gel reponse to HOAc. (a) Injection of 10 μL down the side of a well (diameter = 1 cm). (b) Spotting of six samples, v/v (HOAc/*i*-PrOH): (i) 30:70; (ii) 40:60; (iii) 50:50; (iv) 60:40; (v) 70:30; (vi) 80:20.

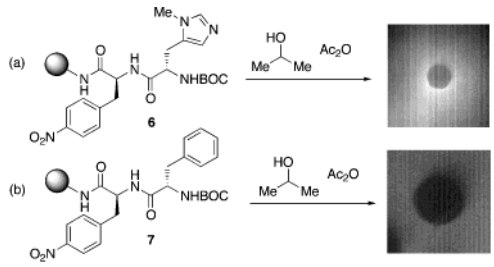

Figure 2. Fluorescence micrographs of catalyst-functionalized beads immobilized in gel **5**. (a) Micrograph of active bead (**6**) after introduction of reagents (t = 5 min). (b) Micrograph of catalytically inactive bead (**7**) after introduction of reagents (t = 5 min).

particular note is that the rate of diffusion of HOAc within the polymer is slow, a fact that suggests that localization of a fluorescence response in a spatially arrayed region of the polymer should be possible. In Figure 1a, we show a fluorescence micrograph of a portion of the polymer cast in a microwell (diameter = 3 mm). At t = 0 min, no fluorescence is evident in the gel. After one drop (~10 μL) of HOAc is injected down the side of the well, an increase in fluorescence intensity is apparent, but the rate of diffusion is greatly decreased relative to the rate in free solution. The fluorescence intensity is also proportional to the concentration of the HOAc that is present. Figure 1b shows a fluorescence micrograph of six spots placed upon the gel, increasingly more concentrated in HOAc.

To demonstrate that sensor-functionalized polymer **5** is a suitable medium in which to screen catalysts, we prepared samples of **5** in which gel cross-linking was accomplished around samples of resin beads that are functionalized with potential catalyst candidates (Figure 2). As controls, we prepared two sets of beads. In one case, we made beads that contained the dipeptide sequence *p*(NO_2)Phe−π(Me)His-BOC (**6**). This sequence contains the *N*-alkylimidazole heterocycle as part of the π(methyl)histidine (Pmh) residue; this moiety is known to be highly active for acyl-transfer reactions involving alcohols and anhydrides.[11,12] A second set of beads was made that contained the sequence *p*(NO_2)Phe−Phe-BOC (**7**), which we have shown is catalytically inactive under these acyl transfer conditions. Each set contained *p*(NO_2)Phe to ensure that all beads are uniformly dark throughout the experiment as a result of efficient fluorescence quenching.[13] When a sample of the gel containing the active beads was exposed to the reaction conditions for 5 min, an increase in fluorescence intensity was noted in zones of the gel where beads were localized (Figure 2a). In contrast, when the same experiment was performed with a gel sample containing the inactive beads (Figure 2b), the gel remained dark.

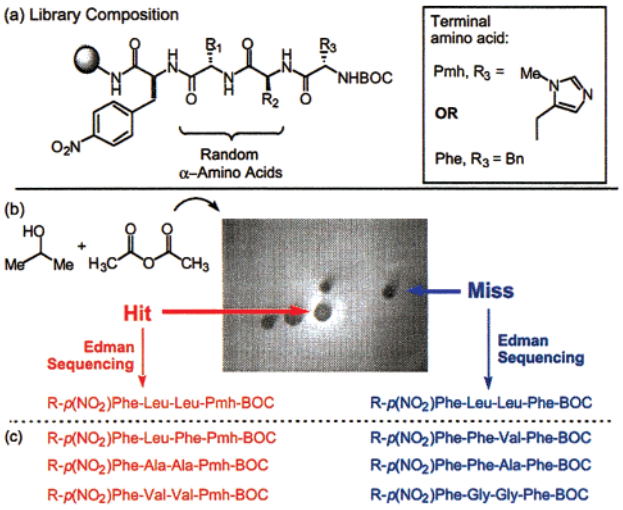

Figure 3. Screen of peptide library deposited in sensor-functionalized gel. (a) Library composition. Each member is terminated in either Pmh or Phe. (b) Excerpt of fluorescence micrograph of gel containing library upon exposure to reaction conditions. (c) Sequences of other "hit" and "miss" beads obtained by single-bead Edman degradation.

We then screened an actual library of catalyst candidates that had been deposited in sensor-functionalized gel **5** (Figure 3). We synthesized a 50-member tetrapeptide library using the split-and-pool method.[14] Each member of the library was initiated with the *p*(NO_2)Phe residue (Figure 3a). The second and third residues were composed of five different amino acids introduced in two "split" steps.[15] The final residue of the library was either the catalytically competent residue Pmh or the catalytically inactive residue Phe. Sensor-functionalized gel **5** was then cross-linked around the beads containing the library, and the gel was exposed to the reaction conditions. Figure 3b shows a fluorescence micrograph of a portion of the gel. Four beads surrounded by halos of fluorescence were extracted from the gel, washed, and subjected to single-bead Edman degradation[16] to determine the sequences of the peptides immobilized on the beads. In addition, four beads that produced no increase in fluorescence intensity were extracted and subjected to the same analysis. In each of the four "hits" selected, the peptides were found to contain the catalytically active Pmh residue. In each of the four "miss" cases, the peptides were found to be terminated with Phe (Figure 3c).

These results validate the concept of using sensor-functionalized gels as a screening tool for reactions that produce appropriate analytes. Future studies from our laboratory will address the incorporation of additional sensors for other products, and on applications.

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Supporting Information Available: Experimental details for the preparation of the gel, catalyst library and for screening (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(15) Residues two and three: Ala, Gly, Leu, Phe, and Val.
(16) Single-bead Edman degradation analyses were performed at Midwest Analytical, Inc., St Louis, MO 63123.