Experimental details for the synthesis of **1a**, **1g**, **3b**–**h**, **6**, **9j**, **9i**, and **9k** are found in the Supporting Information.

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A New Screen for Combinatorial Catalysis; On-Bead Testing in Agarose Gel**

Michael Müller, Trevor W. Mathers, and Anthony P. Davis*

Catalyst discovery through combinatorial chemistry is under study in many laboratories.[1] Much of this work involves small- to medium-sized libraries, synthesized in parallel and tested in compartmentalized apparatus.[2] There are still rather few methods for screening the very large, bead-bound libraries accessible through "split-and-mix" synthesis.[3] Realistically, such libraries must be screened visually under conditions that allow picking of active beads. Visual screening for catalysis is nontrivial, given that starting materials and products can diffuse freely between beads. Published solutions rely on a) thermographic screening,[4] b) test reactions that give insoluble, colored products,[5] c) bead-bound indicators,[6] or d) screening for reactivity, as an indicator of potential catalytic properties.[7] A very recent method developed by Miller et al. employs a gel, covalently bonded to a fluorescent indicator, formed by polymerization around the library beads.[8]

Herein we report an alternative gel-based protocol for screening for bead-bound catalytic activity in aqueous media.

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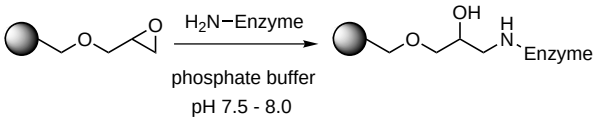
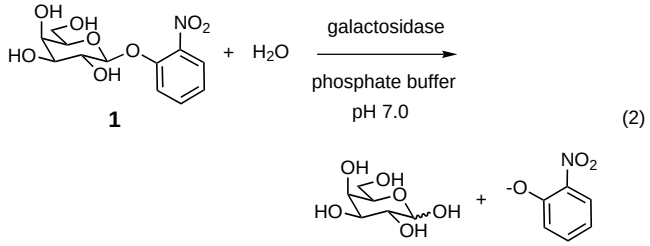
Like the method of Miller et al., it relies on the gel to limit diffusion of reaction products, allowing a visual indication of reaction (e.g. a color change) to be associated with a particular bead. However, the present method requires no preparation of specialist materials, and no special equipment other than a low-power optical microscope.

The new screen employs "low-melting agarose", a gel which is widely used in electrophoresis.[9] The gel phase can be prepared simply by dissolving the powdered agarose in hot water, cooling below a critical temperature, and waiting for a few minutes. The gel can be remelted and recast as necessary. To test its potential as a medium for catalysis screening, we used enzymes immobilized by reaction with oxirane acrylic beads [Eq. (1)].[10] Inactive control beads were produced by



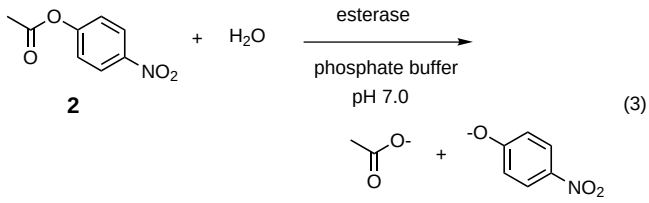
(1)

reaction of the oxirane beads with benzylamine. Early experiments employed β-D-galactosidase with the standard test substrate *o*-nitrophenyl-β-D-galactopyranoside (**1**) [Eq. (2)], the reaction being signalled by the yellow color of



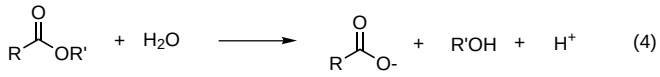
(2)

o-nitrophenolate. Samples of the agarose gel were placed in Petri dishes and remelted. Small quantities of **1** were dissolved in the molten gel, which was then allowed to cool and set. Mixtures of immobilized enzyme beads (3–5%) and inactive control beads (97–95%) were spread on the gel surfaces. Active beads were clearly identifiable, acquiring a yellow color within a few minutes (Figure 1a). Control experiments established that the gel was indeed necessary; in the absence of agarose, the nitrophenolate products diffused rapidly throughout the mixture (Figure 1b). Similar results were obtained with pig liver esterase, for which *p*-nitrophenyl acetate **2** was employed as substrate [Eq. (3); Figure 1c].

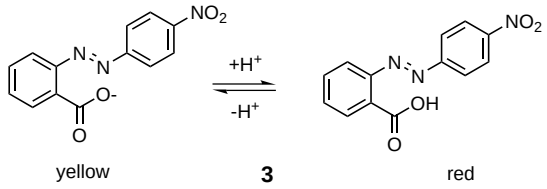


(3)

The reactions in Equations (2) and (3) involve specific substrates which may not be good models for compounds of interest. Screens with broader scope are clearly desirable. Ester hydrolysis may be monitored simply by the change in pH [Eq. (4)]. Color pH indicators may therefore provide a


(4)

general visual screen for esterase activity.[11] We looked particularly for indicators which would change color at moderate pH, and which showed the stronger color when protonated. Methyl Red (**3**), which is yellow at pH 6.2 and red at pH 4.2, seemed a good candidate. The esterase/inactive



bead mixture was tested as above with gels containing ethyl acetate and a small quantity of methyl red sodium salt. Within 5 minutes the few active beads acquired an intense red color, clearly visible against the yellow background (Figure 2a,b). Closer examination revealed that the red (protonated) indicator was insoluble, precipitating on the surface of the beads (Figure 2c). Presumably this assists the experiment, increasing the contrast between beads and background, and providing a further barrier to diffusion. The gel is still necessary, however. In control experiments omitting agarose, all the beads acquired the red color of protonated indicator (Figure 2d). We also investigated a second procedure, in which the beads are suspended in the molten gel just before it sets. In this case the inactive beads acquired a faint pink color,

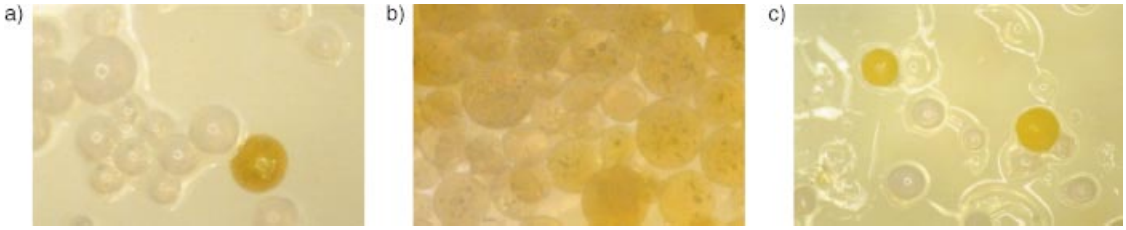


Figure 1. Micrographs from experiments employing nitrophenolate substrates [Eqs. (2), (3)]. a) Galactosidase bead + controls, on agarose + **1**, after 5 min. b) Galactosidase beads + controls (2:98) + **1** in aqueous solution, after 5 min. Nitrophenolate disperses through active and inactive beads. c) Esterase beads + controls, on agarose + **2**, after 5 min. Despite the uniform yellow background, due to spontaneous hydrolysis of **2**, the two active beads are still distinguishable.

  0044-8249/01/11320-3930 $ 17.50+.50/0

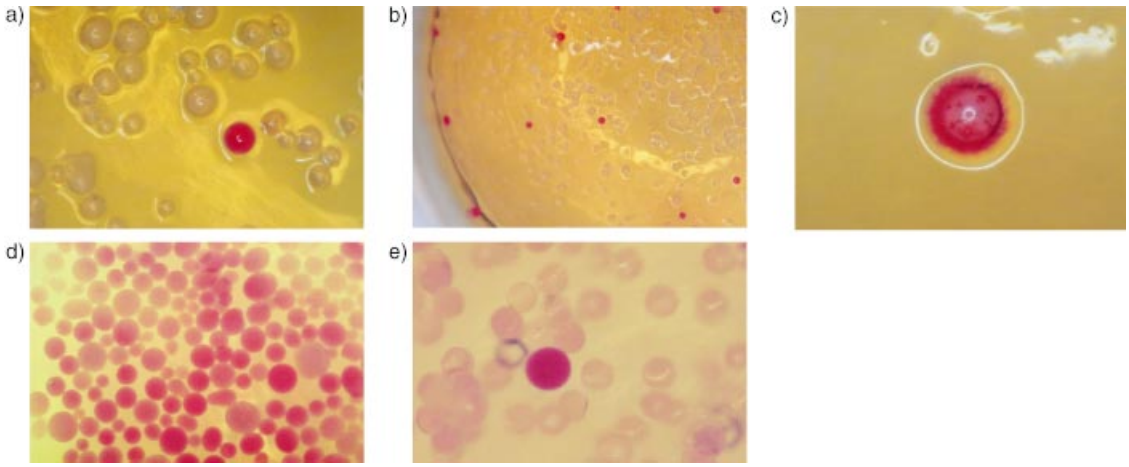


Figure 2. Micrographs from the hydrolysis of ethyl acetate in the presence of **3**. a) Esterase bead + controls, on agarose + EtOAc + **3**, after 4 min. b) As in a), at lower magnification. c) Esterase bead on agarose + EtOAc + **3**, after 15 min. Precipitated **3** forms a filamentous halo around the bead. d) Esterase beads + controls (5:95) + EtOAc + **3** in aqueous solution, after 4 min. e) Esterase bead + controls suspended in agarose + EtOAc + **3**, after 4 min.

but the contrast between active and inactive beads was still perfectly clear (Figure 2e).

In conclusion, we have used immobilized enzymes to demonstrate a protocol potentially usable for catalysis screening in "split-and-mix" combinatorial libraries.[12] Although it has been used just for two reaction types, both in aqueous media, it should be generalizable to other transformations and also to organic solvents (given an appropriate gelator). The examples include a pH-based screen for ester hydrolysis, which avoids the use of elaborate, specific substrates. In future work we hope to exploit this method to detect "synthetic esterase" activity in steroidal podand libraries.[7]

Experimental Section

Materials: Agarose gel was prepared by dissolving low-melting agarose (0.5 g: Life Technologies, GibcoBRL, 15517-014) in boiling water (50 mL), then allowing to cool and set. β-D-Galactosidase (Sigma, G 2513) and porcine liver esterase (Sigma, E 2884) were immobilized on oxirane acrylic beads (Sigma, O 7628) following the procedure of Laumen et al.[10]

Galactosidase screen (Figure 1a): Agarose gel (0.5 mL) was melted in a small Petri dish in a drying oven. Phosphate buffer (pH 7.0, 0.02 M, 2 drops) and *o*-nitrophenyl-β-D-galactopyranoside (5 mg) were stirred into the gel, which was then filtered through cotton wool to remove undissolved substrate and allowed to set in a second Petri dish. A mixture of galactosidase and control beads (ca. 5:95) was spread onto the surface of the gel. The active beads became perceptibly yellow after about one minute.

Esterase/Methyl Red screen (Figure 2a,b,e): Agarose gel (0.5 mL) was melted in a small Petri dish in a drying oven. An aqueous solution of Methyl Red, sodium salt (Acros 41484-0100, $\sim$1 mg mL^{-1}) was added dropwise to give the solution a moderate yellow color (final concentration between 0.1 and 0.2 mM). Ethyl acetate (two drops) was added with stirring and the gel was allowed to set. A mixture of esterase and control beads (ca. 2:98) was spread onto the surface of the gel. The active beads became perceptibly red within 1 min, and acquired a visible halo of precipitated indicator after about 20 min. Alternatively, the mixture of esterase and control beads was stirred into the cooling gel at just above the setting temperature (ca. 40 °C). Again, the active beads became red within 1 min.

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[12] There is potential concern that a screen developed by using true enzymes may not be capable of detecting the lower activities expected for artificial enzymes. However a rough estimate suggests that the indicator-based esterase screen, at least, should be usefully sensitive. Titration of aqueous AcOH into a solution of Methyl Red sodium salt in deionized water (1.4×10^{-4} M, as used in the screen) established that about five equivalents of the acid are required for complete conversion to the red precipitate. Therefore, a bead capable of generating $[AcOH]_{local} = 0.7$ mM should certainly be detected. A potential catalyst synthesized on Tentagel would be present at about 10 mM within the bead. While the effective concentration of the catalyst may be less, it seems likely that catalysts would be detectable after one turnover at most.

  0044-8249/01/11320-3931 $ 17.50+.50/0