Reaction Microarrays: A Method for Rapidly Determining the Enantiomeric Excess of Thousands of Samples

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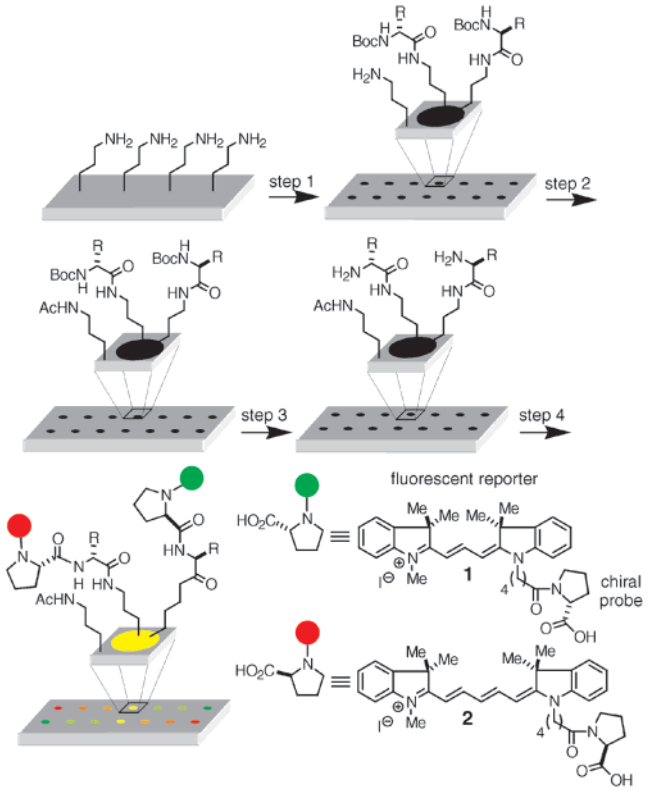
High-throughput experiments enhance our understanding of complex systems. For example, DNA microarrays are used to determine relative gene expression levels on a genome-wide basis, providing global views of cell biology.[1] This approach provides insights that are not possible by analyzing single gene expression experiments.[2] Related approaches in chemistry involve the use of combinatorial techniques that have been successfully applied to medicinal chemistry,[3] materials science,[4] and catalysis.[5] Recently, it has been demonstrated that the use of combinatorial methods can lead to the discovery of enantioselective catalysts and reactions.[6] However, these studies were limited to the evaluation of relatively small numbers of experiments due primarily to the lack of methods for high-throughput analysis.[7] Realization of the full potential of a combinatorial, screen-based approach in enantioselective synthesis requires a method for determining the enantiomeric excess (ee) of thousands of reaction products.[8] This contribution describes a new method that we have named reaction microarrays, in which DNA microarray technology has been adapted to measure the ee of tens of thousands of samples rapidly and *en masse*.

To evaluate the reaction microarray method, α-amino acids were used because of their fundamental chemical and biological importance, the availability of structural diversity, and their bifunctional structure. Samples of *N*-Boc-protected α-amino acids were arrayed and covalently attached to amine-functionalized glass slides. Automated contact printing of nanoliter volumes from 2 mM solutions chemoselectively attached $<10^{-11}$ moles of amino acid to each spot on a glass slide in a spatially arrayed manner (Scheme 1, step 1). Uncoupled surface amines were acetylated (step 2) and *en masse* Boc-deprotection yielded the free amine of the amino acids (step 3). In analogy to DNA microarrays, where relative gene expression levels are measured by a ratio of fluorescent reporters, reaction microarrays utilize two fluorescent probes to measure a ratio of enantiomers (step 4).

Scheme 1[a]



[a] Reagents and conditions: step 1) BocHNCH(R)CO_2H, PyAOP, ^{i}Pr$_2$NEt, DMF; step 2) Ac_2O, pyridine; step 3) 10% CF_3CO_2H and 10% Et_3SiH in CH_2Cl_2, then 3% Et_3N in CH_2Cl_2; step 4) pentafluorophenyl diphenylphosphinate, ^{i}Pr$_2$NEt, 1:1 mixture of **1** and **2**, DMF, −20 °C.

Pseudoenantiomeric fluorescent probes **1** and **2** combine a fluorescent reporter with a chiral probe and are synthesized by coupling Cy3 and Cy5 fluorophores to D- and L-enantiomers of proline, respectively. These chiral fluorescent probes are covalently attached to the immobilized amino acid samples by exposing the glass slide to an excess of an equimolar mixture of **1** and **2** under amide coupling conditions. Parallel kinetic resolution during the amide coupling reaction converts the ee information of the sample into a ratio of fluorophores that, upon excitation by an automated laser scanner, is observed as a ratio of fluorescent intensities. Fluorescence emission is measured following excitation of Cy3 at 532 nm and excitation of Cy5 at 635 nm. The resulting image is false-colored with Cy3 fluorescence represented as green and Cy5 fluorescence represented as red. Equivalent fluorescent emission intensity of Cy3 and Cy5 is yellow.

$$\%\ \mathrm{ee} = \left[\frac{(x-1)(s+1)}{(x+1)(s-1)}\right] \times 100\%; \quad \text{where } x = \frac{I_{\mathrm{fluor,Cy5}}}{I_{\mathrm{fluor,Cy3}}} \times \frac{1}{z} \quad \text{and } s = \frac{k_{\mathrm{fast}}}{k_{\mathrm{slow}}} \quad (1)$$

An extension of quantitative studies on the kinetic resolution of secondary alcohols[8] provides a relationship (eq 1) between ee and the measured fluorescent intensity ratio at each spot. The ratio of fluorescent intensities of **1** and **2** is divided by a

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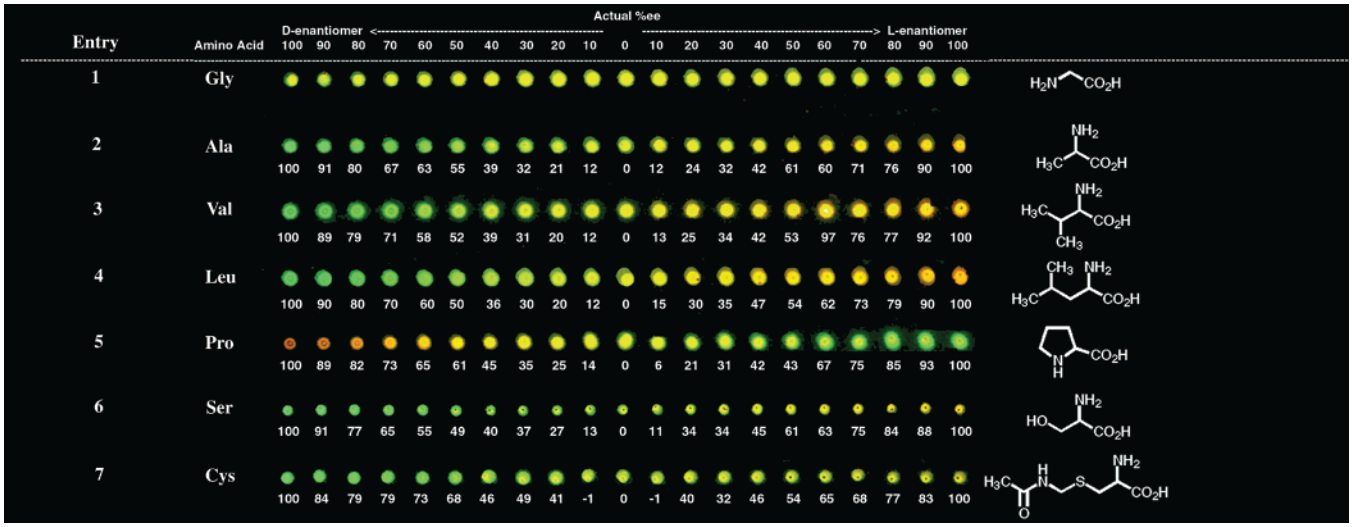

Figure 1. Amino acids were printed on glass slides as mixtures of enantiomers ranging from 100% ee D- to 100% ee of the L-enantiomer in steps of 10% ee. The number under each spot is the mean measured ee (%) from 12 experiments.

normalization factor, *z*, to account for nonequivalent equimolar fluorescent intensities of Cy3 and Cy5. For any substrate, the value of *z* is defined as the fluorescent intensity ratio of the racemate. The value of *s* quantifies the kinetic resolution and is obtained from *z* and the measured value of *x* for a sample of known ee. For 100% ee, eq 1 simplifies to $s = x$. Previous work[9,10] has shown that perfect recognition is not necessary and that small *s* values are sufficient for accurate determination of ee.

The accuracy of this method was tested on structurally diverse α-amino acids of varying ee (Figure 1). The glass slide was arrayed with Gly, Ala, Val, Leu, Pro, Ser, and Cys and treated as described in Scheme 1. The six chiral amino acids were printed as mixtures of enantiomers ranging from 100% ee D to 100% ee L in steps of 10% ee. The achiral α-amino acid Gly produced yellow spots (equivalent incorporation of red and green fluorophores), indicating no preference for either chiral probe. The six chiral amino acids exhibited a noticeable change of spot color in the progression from one enantiomer to the other, and more importantly, measured ee values correlated well with actual ee values. The red or green color of each spot can be used to assign the absolute configuration of the predominant enantiomer in each sample when correlated to the color of a sample of known absolute configuration. Observed *s* values ranged from 1.2 to 3.6, with higher values generally corresponding to more accurate ee measurements. Standard error of the mean was <8% ee in all but one sample, and the average measured ee values of 116 out of 126 samples (92%) were within ±10% ee of the actual value.

A high-throughput analysis of ee was performed by arraying 15 552 samples of proline onto a glass slide (Figure 2) and manipulating the slides as indicated in Scheme 1. Of these samples, 15 550 were printed from 20 standard mixtures that were between 0 and 20% ee of either enantiomer. A single sample was >99% ee of the D-enantiomer and the remaining sample was >99% ee of the L-enantiomer. Pronounced differences in color enabled both rapid identification and determination of absolute configuration of the two samples known to be >99% ee in less than 48 h. In this experiment, spot diameters were ≤140 μm, which would allow approximately 75 000 samples to be arrayed onto a 25 mm × 75 mm glass slide and analyzed in a comparable amount of time.

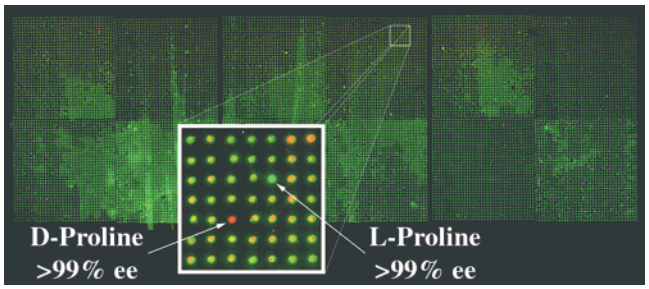

Figure 2. Identification of two >99% ee samples of proline in a collection of 15552 samples.

In conclusion, DNA microarray technology has been adapted for the high-throughput determination of ee. While several novel high-throughput screens for catalysis have been developed to determine reactivity,[4,5b,f] reaction microarrays provide information regarding enantioselectivity. Improvements in this method will parallel improvements in the efficiency of DNA microarray technology and the development of improved methods for the attachment of molecules to a glass surface.[11] These developments and the discovery that a single set of probes can be used to determine the ee of six structurally diverse α-amino acids together provide a foundation for reaction microarrays as a general method for high-throughput ee analysis.

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Supporting Information Available: Syntheses of **1** and **2**, all microarray protocols, and data analysis (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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