Toward Larger Chemical Libraries: Encoding with Fluorescent Colloids in Combinatorial Chemistry

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Received October 11, 1999

Chemical library technology plays a central role in research areas such as drug discovery and gene screening.[1] The most powerful combinatorial library synthesis method is the iterative "split and mix" synthesis on insoluble microscopic beads.[2] This technique is an efficient method for accessing all combinations of chosen monomers such as nucleic acids, amino acids, or sugars in a small number of reactions. Compound identification from such large pools of compounds, be it bound to resin (one compound per bead) or in solution, is achieved through covalent attachment of molecular tags to the beads[3] or through iterative deconvolution technologies.[4] Herein we introduce an encoding method that involves physically attaching fluorescent colloidal particles ("reporters") to the surface of solid support beads during split and mix syntheses, to produce an information-rich, colored barcode that can be easily, rapidly, and inexpensively decoded using fluorescence microscopy. This "colloidal barcoding" technique eliminates the need for compatible tagging chemistry in conventional molecular tagging[3] or optical encoding[5] procedures and permits unambiguous identification of compounds in libraries of any size and type.

In a split and mix synthesis, a large number of solid support beads is partitioned into several vessels, a different monomer is reacted with each portion, and then the beads are recombined to complete the cycle. The split and mix process is repeated for a chosen number of cycles, resulting in a chemical library, ideally consisting of all monomer combinations. The colloidal barcoding method involves the attachment of reporters to each solid support bead (Figure 1) during every reaction in the library synthesis. One type of reporter is used to code for each different reaction and every solid support bead receives multiple copies (50−400 reporters) at each reaction. Each type of reporter is distinguished by its specific combination of one or more fluorescent dyes. The power of the colloidal barcoding method lies in (a) the efficient use of relatively few dyes to record an enormous amount of information and (b) the ease with which the information can be retrieved using standard fluorescence microscopy techniques (described later). For example, with just 6 fluorescent dyes (carefully selected to prevent difficulties with spectral imposition), 64 types of reporter can be synthesized, each reporter containing a unique combination of dyes. This permits barcoding of a library of 8-residue peptides in every combination of 8 chosen amino acids. This library, of over 16 million peptides, could be completely and uniquely encoded via this method.

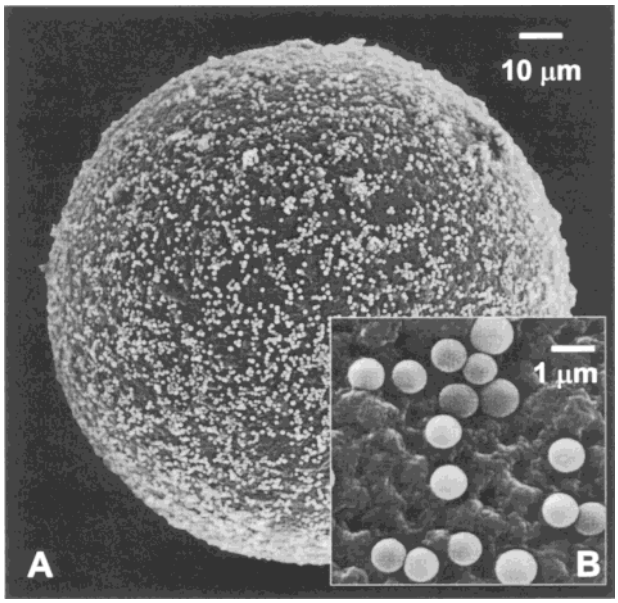

Figure 1. (a and b) Scanning electron microscope images of a polystyrene solid support bead with numerous silica particles 1 μm in diameter physically attached to the surface. The silica particles contain combinations of fluorescent dye that act as a barcode to identify the structure of the compound synthesized on the large bead.

To illustrate the concept, colloidal barcoding of a 100-member tripeptide library involving 20 different amino acids over 2 cycles was performed using standard Fmoc chemistry. Twenty different types of silica reporters were used, each reporter containing 1, 2, or 3 fluorescent dyes (see Supporting Information). The reporters were attached in the deprotection solution by simply adding piperidine/DMF (1:1) containing 1 mg of reporters to each portion of beads. Each of the 10 portions of beads in the first cycle was encoded with one type of reporter containing a unique combination of dyes. After mixing and splitting the beads into equal portions for cycle 2, each of the 10 portions received a different type of reporter from the remaining 10 reporter types not used in the first cycle. Consequently, the combination of reporters on any selected bead created a fluorescent barcode that permitted identification of the structure of the tripeptide on that bead.

A fluorescence microscope equipped with a number of optical filters provides an effective method of distinguishing reporter types and subsequent barcode reading of a chosen bead. The optical filters permit particular fluorescent dyes to absorb and emit while other dyes are prevented from doing so. Imaging under the different filters reveals the combination of dyes in each reporter. Figure 2 shows the colloidal barcode on a solid support bead, imaged by fluorescence microscopy under three different filters. In this example, the bead had been subjected to 3 cycles of a solid phase peptide synthesis using standard Fmoc procedures

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10.1021/ja993634i CCC: $19.00
Published on Web 02/17/2000

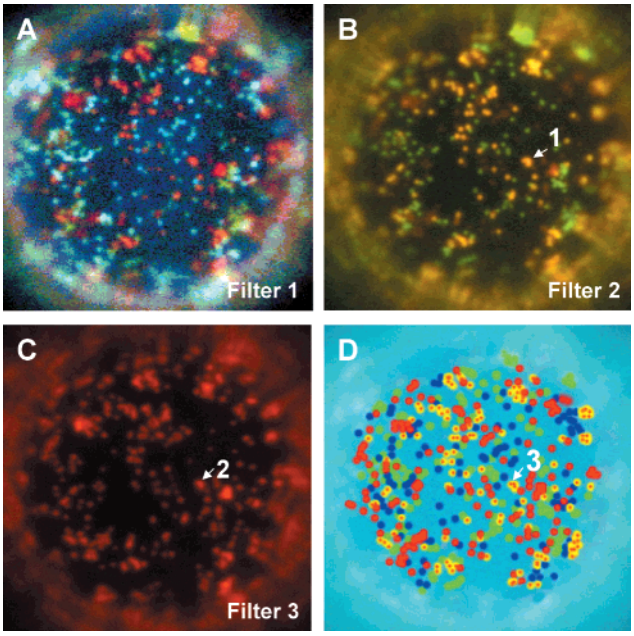

Figure 2. Fluorescence microscopy is used to read the colloidal barcode that identifies the chemical structure of the compound on a single bead in a library. (a−c) The bead is analyzed under three different filters and the individual dyes present within each reporter are identified. (d) A schematic representation showing the combinations of dyes present in each reporter. For example, the yellow dye within a particular reporter is detected under Filter 2 (Arrow 1), Filter 3 detects red dye within the same reporter (Arrow 2), and the red/yellow combination is revealed and depicted in the schematic (Arrow 3). The red, green, blue, and red/yellow reporters present on the solid support codes for the peptide, Fmoc-Ala-Lys-Gly-Glu-OH.

(see Supporting Information). The bead was barcoded with four different types of reporter (which included encoding with fluorescent green reporters for the amino acid, glutamic acid, present on the Fmoc-Glu-Wang support beads prior to peptide synthesis). Cycles 1 to 3, involving the amino acids glycine, lysine, and alanine, were encoded with red, blue, and red/yellow fluorescent reporters, respectively. Each type of reporter was present in sufficiently large numbers (∼100 reporters/bead) at the end of 3 synthesis steps to permit simple and rapid decoding. The green, red, blue, and combined red/yellow emissions of the attached reporters generated a colloidal barcode that determined the exact sequence of monomer couplings that formed the compound. Examining the colloidal barcode under different filters allows immediate identification of the chemical structure of the compound (Fmoc-Ala-Lys-Gly-Glu-resin). Mass spectrometry analysis of the compound after trifluoroacetic acid (TFA) cleavage from the bead documented good quality crude peptides, indicating that the barcoding did not interfere with compound synthesis.

To optimize reporter−bead adhesion and to prevent reporter dislodgment or exchange from interfering with barcode reading, manipulation of macroscopic colloidal forces[6] was performed. Prior to their use in a library synthesis, reporters were treated with charged polymers (polyelectrolytes). Multilayers of positively and negatively charged polyelectrolytes were built up on the reporter surface to enhance the electrostatic attraction between reporter and solid support bead. Once attached, the polyelectrolyte chains formed a permanent interlocking molecular web between the particles. Reporters used in this study were coated with a layer of positively charged polyelectrolyte, poly(diallyldimethylammonium chloride) (PDADMAC), followed by a layer of negatively charged polyelectrolyte, poly(acrylic acid) (PAA). Coating was performed by soaking the reporters in an aqueous solution of PDADMAC for 24 h and washing with water, and then soaking the PDADMAC-coated reporters in an aqueous solution of PAA followed by thorough washing (see Supporting Information).

Solid support beads involved in split and mix syntheses (e.g. three monomers and three cycles, see Supporting Information) display colloidal barcodes that are intact and easily distinguishable after the synthesis. The manipulation of colloidal forces via polyelectrolyte coating of reporters ensures that a large number of purposefully attached reporters remain on each bead.

The coating of reporters with polyelectrolyte multilayers greatly increases adhesion and provides a durable link that is able to survive a great variety of reagent and solvent conditions. Two portions of solid support beads, one portion barcoded with red reporters, the other barcoded with green reporters, were mixed together in various solvents and reagents (see Supporting Information). In all of the examined cases, the reporters remained attached to the beads (100−400 reporters per bead) and did not cross-contaminate to any significant extent (less than 5 but most often 0 contaminant reporters per bead). The colloidal barcodes remained intact and distinguishable. Colloidal barcoding was successful on several resins, including Wang, PEGA, TentaGel, aminomethyl PS, and PAM resins.

The colloidal barcode can be read before or after TFA cleavage of the compound from the solid support since the barcode remains intact under cleavage conditions. Furthermore, on-bead assays can be performed, with the colloidal barcode remaining intact, even at high ionic strengths.

The simplicity of colloidal barcoding, as compared to existing molecular tagging or optical encoding procedures, makes it an extremely attractive and economical route to production and screening of large chemical libraries. It is envisaged that this novel method will be generically suitable for many applications, including on-bead binding and functional assaying, and will offer significant benefits to drug lead identification.

Acknowledgment. This work was supported by the Australian Research Council (98/ARCL227). We acknowledge the assistance of Geoff Edwards with the scanning electron microscopy.

Supporting Information Available: Experimental details, a table giving the scheme for colloidal barcoding of a 100-member peptide library, and figures showing the colloidal barcoding of a 27-compound library prepared by the split and mix technique and the mass spectrum of compounds cleaved from the glutamic acid portion after the third cycle in the split and mix synthesis (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA993634I

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