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Barcoded Resins: A New Concept for Polymer-Supported Combinatorial Library Self-Deconvolution

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Deconvolution strategies can be grouped into five families, dual-defined scanning,[1] positional scanning,[1] indexed libraries,[2] recursive deconvolutions,[1,3] and deletion synthesis deconvolution[4] methods. Encoded strategies evolved from DNA-[5] to chemically-[6] and radio frequency-encoded[7] combinatorial libraries. Recently, dual recursive deconvolution (DRED) was introduced as a hybrid strategy,[8] as it adds a new (encoding) dimension to the classical deconvolution strategies. DRED operates through the iterative identification of the first and last randomized positions of active members of combinatorial libraries generated through split synthesis. The last position can be readily obtained from pool screening[1−4] after the last step of the split synthesis,[9] while the first position can be “encoded” by the unique vibrational fingerprint of the resin beads used. Once the first and last positions are identified, the second and second-to-last positions are subjected to the same deconvolution process. Remarkably, the synthesis *and* DRED of a 64 million hexapetide library from the 20 natural amino acids would barely double the number of steps required for the split synthesis (246 versus 120) and would require only 20 spectroscopically distinguishable beads.

While this method has much merit, it is limited by the chemical and physical heterogeneity of commercial resins used as encoding elements. Furthermore, the requirement for a hyperspectral imaging system limits its accessibility to the vast majority of academic and industrial laboratories. To address these issues, here we introduce a novel and simple concept whereby new resins are synthesized with built-in infrared (IR) and Raman *spectroscopic barcodes*, readily identifiable visually or with standard data processing programs.[10] This approach introduces a new paradigm in combinatorial chemistry, as the beads are no longer just carriers for solid-phase synthesis, but are rather the repository of the synthetic steps to which they were subjected.

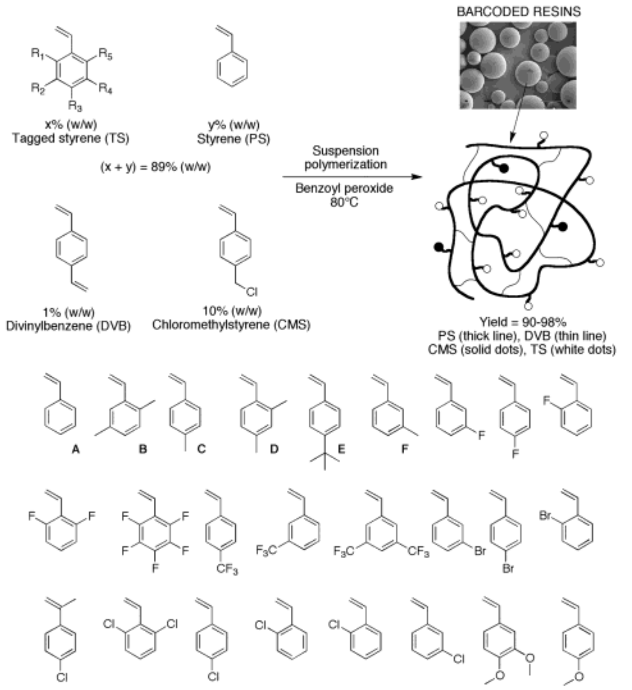

Figure 1. Suspension polymerization components for the preparation of the barcoded resins (top) and styrene derivatives investigated as encoding elements (bottom).

Since the vast majority of solid-phase reactions are performed on standard Merrifield resins (1−2% divinylbenzene/polystyrene),[11] the first generation of barcoded resins was based on the same chemistry with the fundamental difference, however, that each of the beads generated displayed unique IR and Raman spectral features.

A selection of 25 comonomers investigated is shown in Figure 1. These compounds were selected on the basis of their commercial availability, unique IR and Raman spectral features, relative chemical inertness, and amenability to polymerization in a standard suspension polymerization reaction.[12] IR and Raman spectroscopic studies of the corresponding homo- and copolymers led to the following two conclusions: (a) A comonomer added to the suspension polymerization[12] mixture below ∼10% (w/w) threshold does not display strong and unique vibrations; the vibrational spectrum is dominated by the main comonomer(s). (b) Copolymers composed of two or more comonomers exhibit IR and Raman spectral features that are the sum of the spectral features of the corresponding homopolymers. For instance, the Raman (or IR) spectrum of poly(styrene-*co*-4-methylstyrene) is the sum of the Raman (or IR) spectrum of poly(styrene) and poly(4-methylstyrene). This additivity was instrumental in streamlining comonomer selection and, ultimately, the resulting DRED beads' composition and properties.

To maintain the same chemical inertness and physical properties of a Merrifield resin, yet incorporate subtle changes that significantly alter their spectroscopic properties we selected alkyl-substituted styrenes A−F (Figure 1). Table 1 summarizes the formulations used for the preparation of 24 Merrifield-like

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(10) The barcodes can be decoded using standard laser barcode readers or with commercial data analysis and pattern recognition software packages.

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(12) See Supporting Information.

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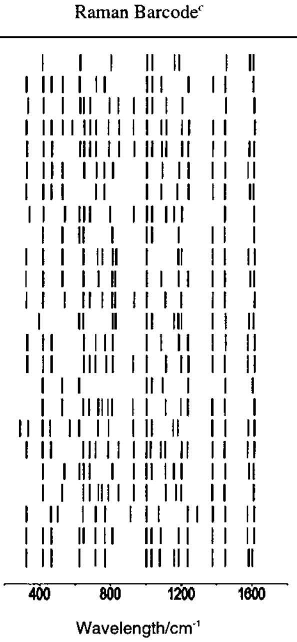
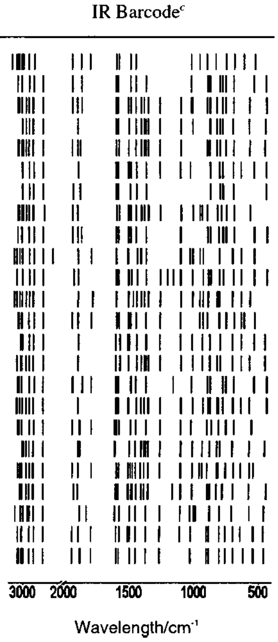
Table 1. Composition, Overall Yield and, Raman and IR Barcodes of the 24 New Resins Synthesized

Ref No.	Monomers (g)[a]			Yield/%[b]
10262	A, 4.50	—	—	98
10312	A, 1.50	B, 1.50	F, 1.50	94
11012	A, 1.50	E, 1.50	F, 1.50	96
11031	B, 1.50	E, 1.50	F, 1.50	94
11032	A, 1.50	B, 1.50	E, 1.50	95
11022	B, 1.50	C, 1.50	F, 1.50	96
10311	B, 2.25	F, 2.25	—	95
11214	A, 2.25	E, 2.25	—	96
11011	A, 1.50	C, 1.50	F, 1.50	95
10241	C, 4.50	—	—	90
10191	C, 2.25	F, 2.25	—	92
10242	C, 2.25	E, 2.25	—	93
10131	A, 2.25	C, 2.25	—	92
10142	B, 2.25	C, 2.25	—	95
10232	B, 1.50	C, 1.50	E, 1.50	93
10181	A, 2.25	F, 2.25	—	93
11021	C, 1.50	E, 1.50	F, 1.50	94
10132	A, 2.25	D, 2.25	—	92
10172	B, 2.25	E, 2.25	—	92
10202	A, 1.50	C, 1.50	E, 1.50	92
10192	E, 2.25	F, 2.25	—	94
10122	B, 4.50	—	—	93
10212	A, 1.50	B, 1.50	C, 1.50	93
10141	A, 2.25	B, 2.25	—	94





[a] A = styrene, B = 2,5-dimethylstyrene, C = 4-methylstyrene, D = 2,4-dimethylstyrene, E = 4-*tert*-butylstyrene, F = 3-methylstyrene. [b] The high overall chemical yields obtained for all of the barcoded beads before sieving indicates that the bulk composition of the produced beads is consistent with that of the corresponding comonomer mixture. Furthermore, scanning electron microscopy and atomic force microscopy studies of the beads' shape and morphology established their homogeneous texture.[12] The average yield of beaded material after sieving is ~82%. [c] The thicker bars denote the best-resolved vibrations. Single-bead Raman and FTIR spectra were recorded according to reported procedures.[12]

barcoded beads derived from this family using standard suspension polymerization techniques;[12] literally, hundreds of other compositions are accessible. Detailed analysis of the vibrational spectra of each bead in the range 200−1800 cm^{-1} for Raman and 400−3200 cm^{-1} for IR resulted in a set of unique peaks that were converted into absolute spectroscopic barcodes (Table 1). These barcodes could be readily modulated by modifying the styrene comonomer ratio and composition in the suspension polymerization reaction. Chloromethylstyrene was incorporated into the beads at a level proportional to its molar ratio in the polymerization mixture to serve as a handle for subsequent reactions on the beads. In this study, the level of incorporation was readily modulated between a final loading of 0 to 1.5 mmol/g of resin without affecting the barcodes. Chloromethyl group incorporation was titrated using a selective chloride ion electrode[12] and was subsequently confirmed using the fulvene−piperidine adduct method.[12]

Vital to the success of this approach in library deconvolution is the conservation of the resins' key spectral features (barcodes) regardless of the beads' cargo. Three experiments were performed to address this question: The barcodes were checked before and after (a) attachment of the Wang linker, (b) attachment of the Wang linker and Fmoc-Gly, and (c) attachment of the Wang linker and Fmoc-Phe. In all three cases not a single significant variation in all 24 spectroscopic barcodes was noted. Finally, key to a successful solid-phase organic synthetic scheme, are the swelling properties which were found to mirror those of Merrifield resins in a variety of solvents.[12] In contrast with this, resins derived from chlorinated and brominated resins swelled mostly in chlorinated solvents. Fluorinated resins exhibited the poorest swelling properties in common organic solvents, while resins derived from methoxy-containing monomers displayed the widest solvent compatibility (data not shown).

In conclusion, 24 barcoded resins with chemical and physical properties similar to those of Merrifield resins were prepared and characterized. On the basis of the spectroscopic and chemical behavior of these resins many more can be specifically tailored to the libraries to be deconvoluted. The series described here was designed to address a limitation associated with using multiple resins with different physical and chemical properties in the dual recursive deconvolution of resin-supported libraries and to streamline resin bead identification. A standard FTIR or Raman spectrometer equipped with microspectroscopic capabilities (for single-bead analysis)[12] is sufficient to implement the DRED methodology. The barcoded resins are also amenable to a directed sorting strategy wherein each barcode encodes a unique library member.[7a] The added value of this method is that it is a noninvasive screening technique, it is inexpensive, and it does not involve the development of any encoding chemistry since the barcoded beads are prepared in a one-step-large-scale suspension polymerization reaction from commercial starting materials.

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Supporting Information Available: Procedures for the preparation of the barcoded resins and for the coupling with the Wang linker, Fmoc-Gly, and Fmoc-Phe; bead size distribution; representative Raman and FTIR spectra; chloride and hydroxyl titrations; and swelling properties (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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