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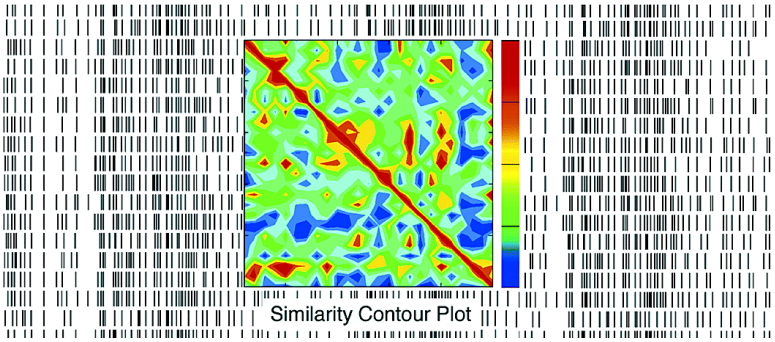

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Preparation, Physical Properties, On-Bead Binding Assay and Spectroscopic Reliability of 25 Barcoded Polystyrene−Poly(ethylene glycol) Graft Copolymers

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Abstract: Here we describe the preparation of 25 beaded polystyrene−poly(ethylene glycol) graft copolymers from six spectroscopically active styrene monomers: styrene, 2,5-dimethylstyrene, 4-methylstyrene, 2,4-dimethylstyrene, 4-*tert*-butylstyrene, and 3-methylstyrene. These polymers were thoroughly characterized by Raman, infrared, and $^{1}H/^{13}C$ NMR spectroscopies, and differential scanning calorimetry. Determination of the swelling properties, peptide synthesis, and on-bead streptavidin−alkaline phosphatase (SAP) binding assay further established that their physical and chemical properties where not significantly altered by the diversity of their encoded polystyrene core. Each of the 25 resins displayed a unique Raman and infrared vibrational fingerprint, which was converted into a "spectroscopic barcode". The position of each bar matches the peak wavenumber in the corresponding spectrum but is independent of its intensity. From this simplified representation similarity maps comparing 35 000 resin pairs were generated to establish the spectroscopic barcoding as a reliable encoding methodology. In effect, in 99% of the cases, the highest similarity coefficients were obtained for resin pairs prepared from the same styrene derivatives even after SAP binding assay. We have also shown that a small but unique combination of a resin's vibrations (30−40%) is sufficient for its identification. However, in rare cases where a resin's vibrational signature has been severely compromised, both the Raman and infrared barcodes were synergistically and reliably utilized to unequivocally identify its chemical make up.

Introduction

Combinatorial chemistry[1] concepts are changing the way in which academic research in many disciplines is conducted. The essence of this field is the rational and informed selection of diversity elements followed by their combinatorial association within a predefined framework to generate a chemical library. Two schools of thought emerged over the past few years regarding library design and synthesis: the first favors parallel synthesis and screening of relatively small target-oriented libraries. Conceptually, this strategy relies on retrosynthetic analysis and conventional organic synthesis[2] to generate a chemical diversity space that targets a specific biological function or biochemical pathway, a target-oriented synthesis (TOS) approach.[3] The second relies on the process of split-pool synthesis[4] to generate small or large, encoded or nonencoded, spatially resolved (resin-supported) chemical libraries. The most recent application of this strategy is the diversity-oriented synthesis (DOS) approach developed specifically for the search and validation of novel chemical and therapeutic targets.[5] Both TOS and DOS rely on similar theoretical and experimental descriptors to effectively explore the diversity space, structural complexity, and lead-like nature of the targeted library.[6]

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(1) See for instance: (a) Fenniri, H., Ed. *Combinatorial Chemistry: A Practical Approach*, Oxford University Press: Oxford, 2000. (b) Meldal, M.; Pirrung, M., Eds. *Curr. Opin. Chem. Biol.* **2002**, *6*, special issue on combinatorial chemistry. (c) Joyce, G. F.; Still, W. C.; Chapman, K. T. *Curr. Opin. Chem. Biol.* **1997**, *1*, special issue on combinatorial chemistry. (d) Drews, J. *Science* **2000**, *287*, 1960−1964. (e) Szostak, J. W., Ed. *Chem. Rev.* **1997**, *97*, 347−510, special issue on combinatorial chemistry.

(2) (a) Corey, E. J.; Cheng, X.-M. *The Logic of Chemical Synthesis*; John Wiley & Sons: New York, 1989. (b) Nicolaou, K. C.; Sorensen, E. J. *Classics in Total Synthesis*; VCH: New York, 1996.

(3) (a) Nicolaou, K. C.; Pfefferkorn, J. A.; Mitchell, H. J.; Roeker, A. J.; Barluenga, S.; Affleck, R. L.; Lillig, J. E. *J. Am. Chem. Soc.* **2000**, *122*, 9954−9967. (b) Nicolaou, K. C.; Winssinger, N.; Vourloumis, D.; Ohshima, T.; Kim, S.; Pfefferkorn, J.; Xu, J.-Y.; Li, T. *J. Am. Chem. Soc.* **1998**, *120*, 10814−10826. (c) Nicolaou, K. C.; Pfefferkorn, J. A.; Roeker, A. J.; Cao, G.-Q.; Barluenga, S.; Mitchell, H. J. *J. Am. Chem. Soc.* **2000**, *122*, 9939−9953. (d) Nicolaou, K. C.; Pfefferkorn, J. A.; Barluenga, S.; Mitchell, H. J.; Roeker, A. J.; Cao, G.-Q. *J. Am. Chem. Soc.* **2000**, *122*, 9968−9976. (e) Nicolaou, K. C.; Pfefferkorn, J. A.; Schuler, F.; Roecker, A. J.; Cao, G.-Q.; Casida, J. E. *Chem. Biol.* **2000**, *7*, 979−992. (f) Nicolaou, K. C.; Winssinger, N.; Pastor, J.; Ninkovic, S.; Sarabla, F.; He, Y.; Vourloumis, D.; Yang, Z.; Li, T.; Giannakakou, P.; Hamel, E. *Nature* **1997**, *387*, 268−272. (g) Wess, G.; Urmann, M.; Sickenberger, B. *Angew. Chem., Int. Ed.* **2001**, *40*, 3341−3350. (h) Burke, M. D.; Lalic, G. *Chem. Biol.* **2002**, *9*, 535−541. (i) Hanessian, S. *NATO ASI Ser., Ser. E* **1996**, *320 (Chemical Synthesis)*, 61−90. (j) Hijikuro, I.; Doi, T.; Takahashi, T. *J. Am. Chem. Soc.* **2001**, *123*, 3716−3722. (k) Lee, A.; Huang, L.; Ellman, J. A. *J. Am. Chem. Soc.* **1999**, *121*, 9907−9914.

(4) (a) Furka, Á.; Hamaker, L. K.; Peterson, M. L. In *Combinatorial Chemistry: A Practical Approach*; Fenniri, H., Ed.; Oxford University Press: Oxford, 2000. (b) Lam, K. S.; Salmon, S. E.; Hersh, E. M.; Hruby, V. J.; Kazmierski, W. M.; Knapp, R. J. *Nature* **1991**, *354*, 82−84. (c) Houghten, R. A.; Pinilla, C.; Blondelle, S. E.; Appel, J. R.; Dooley, C. T.; Cuervo, J. H. *Nature* **1991**, *354*, 84−87. (d) Lam, K. S.; Lebl, M.; Krchnak, V. *Chem. Rev.* **1997**, *97*, 411−448. (e) Furka, A.; Sebestyen, F.; Asgedom, M.; Dibo, G. *Int. J. Protein Res.* **1991**, *37*, 487−493.

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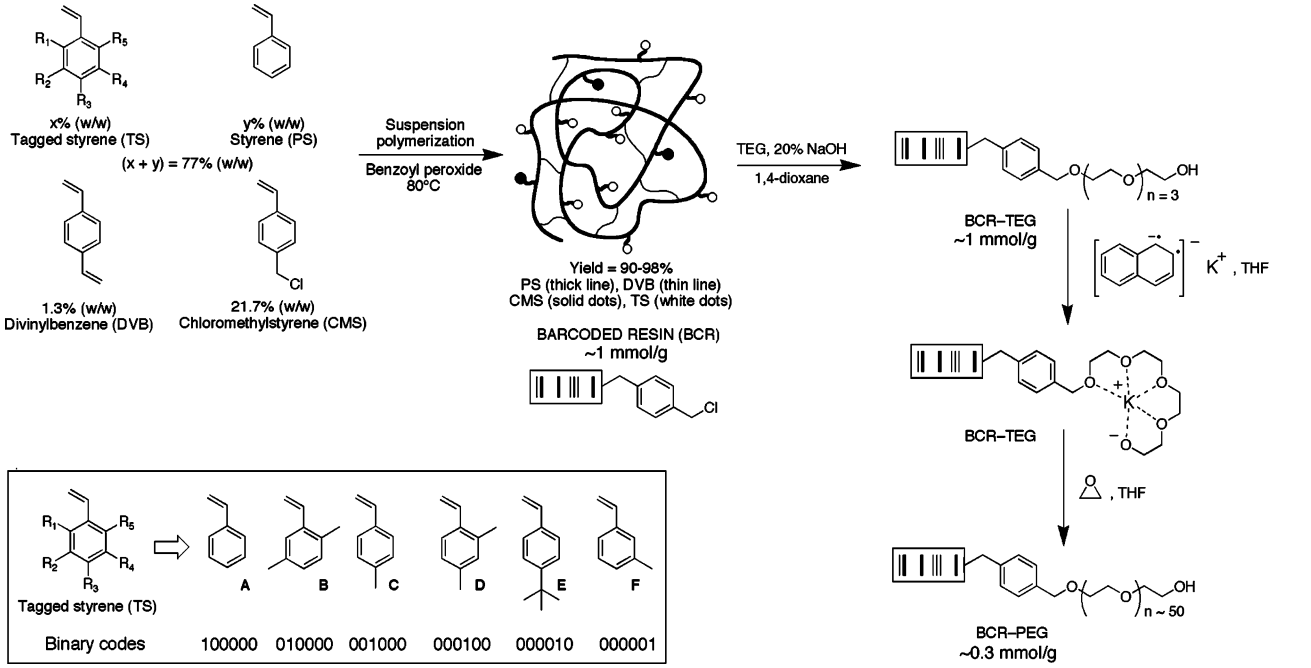


Figure 1. General synthetic strategy for the preparation of PS−PEG graft copolymers from spectroscopically distinguishable styrene monomers (inset).

While TOS offers the advantage of screening individual, well-characterized compounds obtained in multimilligram quantities, it is somewhat limited by the size of the library that can be produced and processed in a given period of time and is confined to a limited diversity space due to its target-oriented nature. DOS on the other hand takes advantage of split-pool synthesis on a polymeric support to dramatically reduce the synthetic effort and give rise to small or large libraries in which each compound is present in a relatively small quantity (<1 μmol/compound using macrobeads[5a−m,7]). This approach must rely on firmly established chemistry because the synthetic intermediates can be numerous, structurally complex, in small quantity, and not amenable to routine purification and characterization techniques. Thus, a cost-effective method that produces predetermined and sufficient quantities of well-characterized compounds, in a minimal number of synthetic steps, with a high level of diversity, and that eliminates the need for chemical encoding, would be an ideal compromise. In this contribution we describe a key ingredient of such strategy.

We have recently reported on a new class of resins prepared with built-in infrared and Raman spectroscopic barcodes (Figure 1).[8] This approach introduces a new paradigm in combinatorial chemistry, as the beads are no longer just carriers for solid-phase synthesis but are in addition the repository of the synthetic scheme to which they were subjected. In conjunction with a directed sorting strategy[3a,b,9] *at the single-bead level*, automated TOS and DOS of libraries in which each compound is assigned a unique barcode at the outset of a split-pool synthesis is now achievable. Because the loading, size, and the number of beads representing a given compound-barcode could be varied almost at will, the quantity of each synthetic intermediate and library member could, as a result, be tuned to carry out routine spectroscopic characterizations at any stage of the library synthesis and on-bead or solution-phase biological evaluation.

(5) (a) Schreiber, S. L. *Science* **2000**, *287*, 1964−1969. (b) Mayer, T. U.; Kapoor, T. M.; Haggarty, S. J.; King, R. W.; Schreiber, S. L.; Mitchison, T. J. *Science* **1999**, *286*, 971−974. (c) Kuruvilla, F. G.; Shamji, A. F.; Sternson, S. M.; Hergenrother, P. J.; Schreiber, S. L. *Nature* **2002**, *416*, 653−657. (d) Kubota, H.; Lim, J.; Depew, K. M.; Schreiber, S. L. *Chem. Biol.* **2002**, *9*, 265−276. (e) Spring, D. R.; Krishnan, S.; Blackwell, H. E.; Schreiber, S. L. *J. Am. Chem. Soc.* **2002**, *124*, 1354−1363. (f) Micalizio, G. C.; Schreiber, S. L. *Angew. Chem., Int. Ed.* **2002**, *41*, 152−154. (g) Blackwell, H. E.; Perez, L.; Stavenger, R. A.; Tallarico, J. A.; Eatough, E. C.; Foley, M. A.; Schreiber, S. L. *Chem. Biol.* **2001**, *8*, 1167−1182. (h) Clemons, P. A.; Koehler, A. N.; Wagner, B. K.; Sprigings, T. G.; Spring, D. R.; King, R. W.; Schreiber, S. L.; Foley, M. A. *Chem. Biol.* **2001**, *8*, 1183−1195. (i) Stavenger, R. A.; Schreiber, S. L. *Angew. Chem., Int. Ed.* **2001**, *40*, 3417−3421. (j) Blackwell, H. E.; Perez, L.; Schreiber, S. L. *Angew. Chem., Int. Ed.* **2001**, *40*, 3421−3425. (k) Sternson, S. M.; Louca, J. B.; Wong, J. C.; Schreiber, S. L. *J. Am. Chem. Soc.* **2001**, *123*, 1740−1747. (l) Tallarico, J. A.; Depew, K. M.; Pelish, H. E.; Westwood, N. J.; Lindslay, C. W.; Shair, M. D.; Schreiber, S. L.; Foley, M. A. *J. Comb. Chem.* **2001**, *3*, 312−318. (m) Spring, D.; Krishnan, S.; Schreiber, S. L. *J. Am. Chem. Soc.* **2000**, *122*, 5656−5657. (n) Komarov, P. G.; Komarova, E. A.; Kondratov, R. V.; Christov-Tselkov, K.; Coon, J. S.; Chernov, M. V.; Gudkov, A. V. *Science* **1999**, *285*, 1733−1737. (o) Chibale, K. *IUBMB Life* **2002**, *53*, 249−252. (p) Arya, P.; Joseph, R.; Chou, D. T. H. *Chem. Biol.* **2002**, *9*, 145−156. (q) Itami, K.; Nokami, T.; Ishimura, Y.; Mitsudo, K.; Kamei, T.; Yoshida, J. *J. Am. Chem. Soc.* **2001**, *123*, 11577−11585. (r) Pelish, H. E.; Westwood, N. J.; Feng, Y.; Kirchhausen, T.; Shair, M. D. *J. Am. Chem. Soc.* **2001**, *123*, 6740−6741.

(6) (a) Martin, E. J.; Critchlow, R. E. *J. Comb. Chem.* **1999**, *1*, 32−45. (b) Ghose, A. K.; Viswanadhan, V. N.; Wendoloski, J. J. *J. Comb. Chem.* **1999**, *1*, 55−68. (c) Blaney, J. M.; Martin, E. J. *Curr. Opin. Chem. Biol.* **1997**, *1*, 54−59. (d) Oprea, T. I. *J. Comput.-Aided Mol. Des.* **2000**, *14*, 251−264. (e) Fecik, R. A.; Frank, K. E.; Gentry, E. J.; Menon, S. R.; Mitcher, L. A.; Telikepalli, H. *Med. Res. Rev.* **1998**, *18*, 149−185. (f) Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Adv. Drug. Delivery Rev.* **1997**, *23*, 3−25. (g) Teague, S. J.; Davis, A. M.; Leeson, P. D.; Oprea, T. *Angew. Chem., Int. Ed.* **1999**, *38*, 3743−3748. (h) Hann, M. M.; Leach, A. R.; Harper, G. *J. Chem. Inf. Comput. Sci.* **2001**, *41*, 856−864. (i) Oprea, T. I.; Davis, A. M.; Teague, S. J.; Leeson, P. D. *J. Chem. Inf. Comput. Sci.* **2001**, *41*, 1308−1315. (j) Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Adv. Drug Delivery Rev.* **1997**, *23*, 3−25. (k) Sheridan, R. P.; Kearsley, R. K. *J. Chem. Inf. Comput. Sci.* **1995**, *35*, 310−320. (l) Cramer, R. D.; Clark, R. D.; Patterson, D. E.; Ferguson, A. M. *J. Med. Chem.* **1996**, *39*, 3060−3069. (m) Henkel, T.; Brunne, R. M.; Müller, H.; Reichel, F. *Angew. Chem., Int. Ed.* **1999**, *38*, 643−647. (n) Sadowski, J.; Wagener, M.; Gasteiger, J. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 2674−2677. (o) Patterson, D. E.; Cramer, R. D.; Ferguson, A. M.; Clark, R. D.; Weinberger, L. E. *J. Med. Chem.* **1996**, *39*, 3049−3059. (p) Ghose, A. K.; Viswanadhan, V. N.; Wendoloski, J. J. *J. Phys. Chem. A.* **1998**, *102*, 3762−3772. (q) Oprea, T. I.; Gottfries, J. *J. Comb. Chem.* **2001**, *3*, 157−166. (r) Oprea, T. I. *Curr. Opin. Chem. Biol.* **2002**, *6*, 384−389.

(7) Rapp, W. E. In *Combinatorial Chemistry*; Wilson, S. R., Czarnik, A. W., Eds.; Wiley: New York, 1997; pp 65−93.

(8) Fenniri, H.; Ding, L.; Ribbe, A. E.; Zyrianov, Y. *J. Am. Chem. Soc.* **2001**, *123*, 8151−8152.

Such a strategy engenders two challenges: (a) The repertoire of usable barcoded resins must be expanded, and (b) a rapid barcode-reading and bead-sorting device must be designed. This contribution addresses a key aspect of the latter problem. To sort the barcoded beads we opted to take advantage of flow cytometery.[10] Standard flow cytometry, however, was optimized for particles flowing in a stream of water or buffered solution and would, as a result, require BCRs (BarCoded Resins) that are compatible with such media. To address the water-compatibility issue and at the same time offer the opportunity for on-bead[1] and in-flow biological assays,[11] here we: (a) report the preparation of 25 spectroscopically encoded PS−PEG graft copolymers, (b) discuss key physical properties of these resins, (c) validate their utility by the synthesis of a test hexapapetide and its on-bead binding assay to streptavidin−alkaline phosphatase (SAP) conjugate, (d) establish that the barcodes are not altered by the loaded material (band shift/overlap) even after SAP binding assay, (e) show that potential differences in the physical and chemical properties of the BCRs generated from a range of styrene monomers do not adversely affect the SAP binding assay, and (f) demonstrate that despite the low amount of encoding material (in comparison with the previous generation of BCRs)[8] the barcodes remain reliable.

Results

Preparation of BCR, BCR−TEG, and BCR−PEG. PEG-based resins are well-documented in the literature,[12−19] and most of them are amenable to spectroscopic barcoding because of their polystyrene content.[12−17] Direct attachment of dihydroxyl PEG to chloromethylated Merrifield resin via Williamson coupling is possible,[14a,b,20] but it is inefficient with long-chain PEGs (>800 D); moreover, the PEG reacts partially at two sites, resulting in further cross-linking and decrease of the resin loading. Alternatively, PEG chains can be attached via amide or urethane bonds to aminomethyl copoly(styrene-1% divinylbenzene).[19a,21] A "PEG environment"[21j,k] can also be generated by partially functionalizing the aminomethyl groups with monomethoxypoly(ethylene glycol) chains. Although the resulting resins are suitable for solid-phase peptide synthesis, the amide (or urethane) linkage connecting the PEG to the PS matrix is relatively labile under strongly acidic/basic conditions utilized in solid-phase organic synthesis.

Among the reported methods for the preparation of PEGylated resins,[12−21] we have chosen Bayer and Rapp's original approach because it minimizes resin processing.[14] Thus, styrene, 2,5-

(9) (a) Nicolaou, K. C.; Xiao, X.-Y.; Parandoosh, Z.; Senyei, A.; Nova, M. P. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 2289−2291. (b) Nicolaou, K. C.; Vourloumis, D.; Li, T.; Pastor, J.; Winssinger, N.; He, Y.; Ninkovic, S.; Sarabia, F.; Vallberg, H.; Roschangar, F.; King, N. P.; Finlay, M. R. V.; Giannakakou, P.; Verdier-Pinard, P.; Hamel, E. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 2097−2103. (c) Xiao, X.-Y.; Li, R.; Zhuang, H.; Ewing, B.; Karunaratne, K.; Lillig, J.; Brown, R.; Nicolaou, K. C. *Biotechnol. Bioeng. (Combinatorial Chemistry)* **2000**, *71*, 44−50. (d) Moran, E. J.; Sarshar, S.; Cargill, J. F.; Shahbaz, M. J. M.; Lio, A.; Mjalli, A. M.; Armstrong, R. W. *J. Am. Chem. Soc.* **1995**, *117*, 10787−10788. (e) Herpin, T. F.; Van Kirk, K. G.; Salvino, J. M.; Yu, S. T.; Labaudiniere, R. F. *J. Comb. Chem.* **2000**, *2*, 513−521. (f) Shi, S.; Xiao, X.-Y.; Czarnik, A. W. *Biotechnol. Bioeng.* **1998**, *61*, 7−12. (g) Service, R. F. *Science* **1995**, *270*, 577. (h) Czarnik, T.; Nova, M. *Chem. Br.* **1997**, *33*, 39−41. (i) Miller, K. *Scientist* **2002**, *16*, 52. (j) Xiao, X.-Y.; Nova, M. P. In *Combinatorial Chemistry*; Wilson, S. R., Czarnik, A. W., Eds.; Wiley: New York, 1997; pp 135−152. (k) Andres, C. J.; Swann, R. T.; Severino, J.; Granat-Young, K.; Edinger, K.; Mongillo, J.; Deshpande, M. S. *Biotechnol. Bioeng.* **1998**, *61*, 93−94.

(10) (a) Trau, M.; Battersby, B. J. *Adv. Mater.* **2001**, *13*, 975−979. (b) Battersby, B. J.; Lawrie, G. A.; Trau, M. *Drug Discovery Today* **2001**, *6 (HTS Suppl.)*, 19−26.

(11) (a) Ormerod, M. G., Ed. *Flow Cytometry: A Practical Approach*; Oxford University Press: New York, 2000. (b) Keren, D. F., McCoy, J. P., Jr., Carey, J. L., Eds. *Flow Cytometry in Clinical Diagnosis*; ASCP Press: Chicago, 2001. (c) Rudbruch, A., Ed. *Flow Cytometry and Cell Sorting*; Springer: New York, 2000.

(12) (a) Miranda, L. P.; Lubell, W. D.; Halkes, K. M.; Groth, T.; Grøtli, M.; Rademann, G.; Gotfredsen, C. H.; Meldal, M. *J. Comb. Chem.* **2002**, *4*, 523−529. (b) Rademann, J.; Grøtli, M.; Meldal, M.; Bock, K. *J. Am. Chem. Soc.* **1999**, *121*, 5459−5466. (c) Renil, M.; Meldal, M. *Tetrahedron Lett.* **1996**, *37*, 6185−6188. (d) Buchardt, J.; Meldal, M. *Tetrahedron Lett.* **1998**, *39*, 8695−8698. (e) Grøtli, M.; Rademann, J.; Groth, T.; Lubell, W. D.; Miranda, L. P.; Meldal, M. *J. Comb. Chem.* **2001**, 3, 28−33. (f) Grøtli, M.; Gotfredsen, C. H.; Rademann, J.; Buchardt, J.; Clark, A. J.; Duus, J. Ø.; Meldal, M. *J. Comb. Chem.* **2000**, *2*, 108−119.

(13) (a) Renil, M.; Pillai, V. N. R. *Tetrahedron Lett.* **1994**, *35*, 3809−3812. (b) Renil, M.; Nagaraj, R.; Pillai, V. N. R. *Tetrahedron* **1994**, *50*, 6681−6688. (c) Roice, M.; Kumar, K. S.; Pillai, V. N. R. *Macromolecules* **1999**, *32*, 8807−8815. (d) Varkey, J. T.; Pillai, V. N. R. *J. Pept. Sci.* **1999**, *5*, 577−581. (e) Varkey, J. T.; Pillai, V. N. R. *J. Appl. Polym. Sci.* **1999**, *71*, 1933−1939. (f) Varkey, J. T.; Pillai, V. N. R. *J. Pept. Res.* **1998**, *51*, 49−54.

(14) (a) Bayer, E.; Hemmasi, B.; Albert, K.; Rapp, W.; Dengler, M. In *Peptides: Structure and Function*; Hruby, V. J., Rich, D. H., Eds.; Pierce Chemical Company: Rockford, Illinois, 1983; p 87−90. (b) Bayer, E.; Rapp, W. In *Chemistry of Peptides and Proteins*; Voelter, W., Bayer, E., Ovchinnikov, Y. A., Ivanov, V. I., Eds.; Walter de Gruyter: Berlin, 1986; Vol. 3, pp 3−8. (c) Rapp, W.; Zhang, L.; Bayer, E. In *Solid-Phase Synthesis*; Epton R., Ed.; SPCC (U.K.) Ltd.: Birmingham, U.K., 1990; p 205. (d) Rapp, W. In *Combinatorial Peptide and Non-Peptide Libraries: A Handbook*; Jung, G., Ed.; John Wiley & Sons: New York, 1998; pp 425−464. (e) Bayer, E.; Rapp, W. In *Poly(Ethylene Glycol) Chemistry: Biotechnical and Biomedical Applications*; Harris, J. M., Ed.; Plenum Press: New York, 1992; pp 325−345. (f) Bayer, E.; Dengler, M.; Hemmasi, B. *Int. J. Pept. Protein Res.* **1985**, *25*, 178−186. (g) Bayer, E.; Hellstern, H.; Eckstein, H. *Z. Naturforsch.* **1987**, *42*, 455−460. (h) Rapp, W.; Zhang, L.; Häbich, R.; Bayer, E. In *Peptides 1988*; Jung, G., Bayer, E., Eds; Walter de Gruyter: Berlin, 1989; p 199. (i) Bayer, E. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 113−129.

(15) (a) Itsuno, S.; Moue, I.; Ito, K. *Polym. Bull.* **1989**, *21*, 365−370. (b) Kamahori, K.; Ito, K.; Itsuno, S. *J. Org. Chem.* **1996**, *61*, 8321−8324. (c) Hird, N.; Hughes, I.; Hunter, D.; Morrison, M. G. J. T.; Sherrington, D. C.; Stevenson, L. *Tetrahedron* **1999**, *55*, 9575−9584. (d) Wilson, M. E.; Paech, K.; Zhou, W.-J.; Kurth, M. J. *J. Org. Chem.* **1998**, *63*, 5094−5099.

(16) (a) Moss, J. A.; Dickerson, T. J.; Janda, K. D. *Tetrahedron Lett.* **2001**, *43*, 37−40. (b) Toy, P. H.; Reger, T. S.; Garibay, P.; Garno, J. C.; Malikayil, J. A.; Liu, G.-Y.; Janda, K. D. *J. Comb. Chem.* **2001**, *3*, 117−124. (c) Chimomura, O.; Clapham, B.; Spanka, C.; Mahajan, S.; Janda, K. D. *J. Comb. Chem.* **2002**, *4*, 436−441.

(17) (a) Porco, J. A., Jr.; Deegan, T. L.; Devonport, W.; Gooding, O. W.; Heisler, K.; Labadie, J. W.; Newcomb, B.; Nguyen, C.; van Eikeren, P.; Wong, J.; Wright, P. *Mol. Diversity* **1996**, *2*, 197−206. (b) Gooding, O. W.; Baudart, S.; Deegan, T. L.; Heisler, K.; Labadies, J. W.; Newcomb, W. S.; Porco, J. A., Jr.; van Eikeren, P. *J. Comb. Chem.* **1999**, *1*, 113−122. (c) Gooding, O. W.; Hoeprich, P. D., Jr.; Labadie, J. W.; Porco, J. A., Jr.; van Eikeren, P.; Wright, P. In *Molecular Diversity and Combinatorial Chemistry. Library and Drug Discovery*; Chaiken, I. M., Janda, K. D., Eds.; American Chemical Society: Washington, DC, 1996; pp 199−206. (d) Deegan, T. L.; Gooding, O. W.; Baudart, S.; Porco, J. A., Jr. *Tetrahedron Lett.* **1997**, *38*, 4973−4976. (e) Labadie, J. W.; Deegan, T. L.; Gooding, O. W.; Heisler, K.; Newcomb, W. S.; Porco, J. A., Jr.; Tran, T. H.; van Eikeren, P. *Proc. Am. Chem. Soc.* **1996**, *75*, 389−390. (f) Swayze, E. *Tetrahedron Lett.* **1997**, *38*, 8465−8468.

(18) (a) Renil, M.; Ferreras, M.; Delaisse, J. M.; Foged, N. T.; Meldal, M. *J. Pept. Sci.* **1998**, *4*, 195−210. (b) Meldal, M. *Tetrahedron Lett.* **1992**, *33*, 3077−3080. (c) Auzanneau, F.-I.; Meldal, M.; Bock, K. *J. Pept. Sci.* **1995**, *1*, 31−44. (d) Meldal, M.; Auzanneau, F.-I.; Hindsgaul, O.; Palcic, M. M. *Chem. Commun.* **1994**, 1849−1850. (e) Renil, M.; Meldal, M. *Tetrahedron Lett.* **1995**, *36*, 4647−4650.

(19) (a) Barany, G.; Albericio, F.; Kates, S. A.; Kempe, M. In *Poly(Ethylene Glycol): Chemistry and Biological Applications*; Harris, J. M., Zalipsky, S., Eds.; American Chemical Society: Washington, DC, 1997; pp 239−264. (b) Kempe, M.; Barany, G. *J. Am. Chem. Soc.* **1996**, *118*, 7083−7093. (c) Kempe, M.; Barany, G. In *Peptides: Chemistry, Structure and Biology, Proceedings of the Fourteenth American Peptide Symposium*; Kaumaya, P. T. P., Hodges, R. S., Eds.; Mayflower Scientific Ltd.: Kingswinford, England, 1996; pp 865−866. (d) Kempe, M.; Barany, G. In *Innovations and Perspectives in Solid-Phase Synthesis and Combinatorial Chemical Libraries*; Epton, R., Ed.; Mayflower Worldwide Ltd.: Birmingham, England, 1995; pp 191−194.

(20) (a) Inman, J. K.; Du Bois, G. C.; Appella, E. In *Solid-Phase Methods in Protein Sequence Analysis, Proceeding of the Second International Conference on Solid-Phase Methods and Protein Sequence Analysis*; Previero, A., Coletti-Previero, M. A., Eds.; North-Holland Publishing Company: Amsterdam, The Netherlands, 1977; pp 81−94. (b) Regen, S. L.; Dulak, L. *J. Am. Chem. Soc.* **1977**, *99*, 623−625.(c) Warshawski, A.; Kalir, R.; Deshe, A.; Berkovitz, H.; Patchornik, A. *J. Am. Chem. Soc.* **1979**, *101*, 4249−4258. (d) Heffernan, J. G.; MacKenzie, W. M.; Sherrington, D. C. *J. Chem. Soc., Perkin Trans.* **1981**, *2*, 514−517. (e) Becker, H.; Lucas, H.-W.; Maul, J.; Pillai, V. N. R.; Anzinger, H.; Mutter, M. *Makromol. Chem., Rapid Commun.* **1982**, *3*, 217−223. (f) Hellerman, H.; Lucas, H.-W.; Maul, J.; Pillai, V. N. R.; Mutter, M. *Makromol. Chem.* **1983**, *184*, 2603−2617. (g) Tsuchida, E.; Nishide, H.; Shimidazu, M.; Yamada, A.; Keneko, M. *Makromol. Chem., Rapid Commun.* **1981**, *2*, 621−626.

dimethylsytrene, 4-methylstyrene, 2,4-dimethylstyrene, 4-*tert*-butylstyrene, and 3-methylstyrene were used to prepare lightly cross-linked (1%) Merrifield-like BCRs following a standard suspension polymerization process (Figure 1).[8,22] These monomers were selected on the basis of their relative chemical inertness, commercial availability and cost, unique IR and Raman spectral features, and their amenability to react in high yield (>95%) in a standard suspension polymerization reaction. This choice was also motivated by the necessity to maintain the same chemical robustness as Merrifield resins and yet incorporate subtle changes that significantly alter the spectroscopic properties of the resulting (co-)polymers. 1,4-Divinylbenzene (1.3%) was used as a cross-linking agent, and 4-chloromethylstyrene was introduced as an attachment point for the PEG graft.

The resulting barcoded polystyrene beads were then treated with excess tetraethylene glycol under basic conditions to yield the corresponding hydroxylated BCR−TEG.[14a,b] Residual chloride titration ruled out the presence of unreacted chloromethyl groups.[23] Anionic polymerization of ethylene oxide on BCR−TEG was then carried out to produce the PEGylated BCRs (BCR−PEG). Anionic polymerization proceeds at higher rate when the propagating anionic species exist as free ions in solution. Therefore, a polar nonprotic solvent (THF) and a weakly coordinating counterion (K^+) were chosen to reduce the ion-pairing effect. Minimal ion-pairing leads to facile proton exchange between initiation and polymer growth sites. This equilibrium-controlled polymerization leads to nearly equivalent chain growth from every initiation site.[20e,f,24] To activate the BCR−TEG resins toward anionic polymerization and improve the reaction rate between the base and the buried hydroxyl sites we have chosen a relatively hydrophobic base (potassium naphthalide) instead of the previously reported metallic potassium[14] and tBuOK.[17] Under these conditions the propagating center, potassium alkoxide, was rapidly generated, and the polymerization proceeded in 2 h instead of the 24−48 h reported with metallic potassium[14] and tBuOK.[17] Furthermore, linear PEG, a side product of anionic polymerization of ethylene oxide in the presence of tBuOK, elimination reactions, and potential degradation of the PEG chain were minimized.[20e,f,24] Although the benzyl ether bond connecting the PEG to the PS backbone is relatively labile under strongly acidic conditions, a phenetyl ether[25] or aliphatic[12d,17] linkage could be introduced to overcome this drawback.

Table 1. TEG and PEG Vibration Modes in BCR−TEG and BCR−PEG Resins (±3 cm^{-1}), Respectively

IR	d	Raman	d	vibration mode[e]
~3500[a]	*s*			ν(OH)
~2900[a,b]	*s*			ν(CH)
2742[b]	*w*			ν(CH)
2696[c]	*w*			ν(CH)
1965[b]	*w*			summation band
		1480[b]	*s*	$\delta_s(CH_2)$, ν(CC)
1466[b]	*m*	1468[c]	*w*	$\delta_s(CH_2)$, ν(CC)
1461	*w*			$\delta_a(CH_2)$
1454[a]	*w*			$\delta_a(CH_2)$
1448[a]	*w*	1448	*w*	$\delta_a(CH_2)$
1411[a]	*w*			$\omega_s(CH_2)$
1360[a]	*w*			$\omega_a(CH_2)$
1342[a]	*w*			$\omega_a(CH_2)$
1278[b]	*m*	1279[b]	s	$t_s(CH_2)$, $t_a(CH_2)$
1240[b]	*m*			$t_a(CH_2)$
		1233[a]	*m*	$t_s(CH_2)$, $\omega_a(CH_2)$, $t_a(CH_2)$, $t_s(CH_2)$
1147[b]	*s*	1140[b]	*s*	$r_s(CH_2)$, ν_a(COC)
		1126[b]	*m*	$r_s(CH_2)$, ν_s(COC)
1109[a,b]	*s*			ν_s(COC), ν_a(COC), ν_a(COC), $r_a(CH_2)$
		1062[b]	*m*	ν(CC), ν_s(COC), ν(CC), $r_s(CH_2)$
~950[b]	*s*			$r_a(CH_2)$, ν_a(COC), ν(CC), ν_a(COC)
		858[c]	*s*	ν_s(COC), $r_s(CH_2)$
844[b]	*m*	843[a]	*s*	$r_a(CH_2)$, ν_a(COC)
		580	*w*	
~530	*w*	537	*m*	δ_a(CCO), δ(COC), δ_a(CCO), $r_a(CH_2)$
509	*w*			δ_a(CCO), $r_a(CH_2)$
		360[b]	*s*	δ(COC), δ_s(CCO)
		275	*s*	δ_s(CCO), δ(COC)

[a] These vibrations were recorded for BCR−TEG and BCR−PEG. [b] The underlined vibrations are intensified in BCR−PEG.[27] [c] Although these vibrations are known for PEG, they were not observed in BCR−TEG and BCR−PEG spectra. Furthermore, several vibrations arising from the TEG and PEG grafts are not listed here because they do not occur regularly (see underlined values in Tables 5 and 6 in the Supporting Information section). [d] *s*, strong; *m*, medium; *w*, weak. [e] δ_a, deformation; ν, stretch; ω, wagging; t, twisting; r, rocking. Subscripts "a" and "s" refer to asymmetric and symmetric modes, respectively.

Except for the disappearance of the chloromethyl vibration at 1265 cm^{-1}, no significant changes were recorded by Raman upon attachment of the TEG spacer. The IR spectra of BCR−TEG showed the disappearance of the 700 cm^{-1} CCl streching and the 1266 cm^{-1} CH_2Cl wagging, and the appearance of new (or stronger) bands at ~3460 (OH stretch), ~2900, 1342, and 1109 cm^{-1} due to TEG spacer (see Table 1 for assignments). The vibration at 1721 cm^{-1} ascribed to carbonyl groups in BCR disappeared upon grafting the TEG spacer. This functional group

(21) (a) Zalipsky, S.; Chang, J. L.; Albericio, F.; Barany, G. *React. Polym.* **1994**, *22*, 243−258. (b) Zalipsky, S.; Albericio, F.; Barany, G. In *Peptides, Structure and Function*; Hruby, V. J., Rich, D. H., Eds.; Pierce Chemical Company: Rockford, IL, 1983; pp 257−260. (c) Zalipsky, S.; Albericio, F.; Barany, G. In *Peptides, Structure and Function*; Deber, C. M., Hruby, V. J., Kopple, K. D., Eds.; Pierce Chemical Company: Rockford, IL, 1985; pp 257−260. (d) Barany, G.; Albericio, F.; Solé, N. A.; Griffin, G. W.; Kates, S. A.; Hudson, D. In *Peptides*; Schneider, C. H., Eberle, A. N., Eds.; Escom Science Publishers: Leiden, The Netherlands, 1992; pp 267−268. (e) Kates, S. A.; McGuinness, B. F.; Blackburn, C.; Griffin, G. W.; Solé, N. A.; Barany, G.; Albericio, F. *Biopolymers (Peptide Science)* **1998**, *47*, 365−380. (f) McGuiness, B. F.; Kates, S. A.; Griffin, G. W.; Herman, L. W.; Solé, N. A.; Vágner, J.; Albericio, F.; Barany, G. In *Peptide Chemistry, Structure and Biology: Proceedings of the Fourteenth American Peptide Symposium*; Kaumaya, P. T. P., Hodges, R. S., Eds.; Mayflower Scientific Ltd.: Kingswinford, England, 1996; pp 125−126. (g) Barany, G.; Albericio, F.; Biancalana, S.; Bontems, S. L.; Chang, J. L.; Eritja, R., Ferrer, M.; Fields, C. G.; Fields, G. B. In *Peptide Chemistry and Biology: Proceedings of the Twelfth American Peptide Symposium*; Smith, J. A., Rivier, J. E., Eds.; Escom Science Publishers: Leiden, The Netherlands, 1992; pp 603−604. (h) Albericio, F.; Bacardit, J.; Barany, G.; Coull, J. M.; Egholm, M.; Giralt, E.; Griffin, G. W.; Kates, S. A.; Nicolás, E.; Solé, N. A. In *Peptides 1994, Proceedings of the Twenty-Third European Peptide Symposium*; Maia, H. L. S., Ed., ESCOM Science Publishers: Leiden, The Netherlands, 1995; pp 271−272. (i) Ferrer, M.; Woodward, C.; Barany, G. *Int. J. Pept. Protein Res.* **1992**, *40*, 194−207. (j) Barany, G.; Solé, N. A.; Van Abel, R. J.; Albericio, F.; Selsted, M. E. In *Innovation and Perspectives in Solid-Phase Synthesis*; Epton, R., Ed.; Intercept Limited: Andover, UK, 1992; pp 29−38. (k) Adams, J. H.; Cook, R. M.; Hudson, D.; Jammalamadaka, V.; Lyttle, M. H.; Songster, M. F. *J. Org. Chem.* **1998**, *63*, 3706−3716.

(22) (a) Arshady, R.; Ledwith, A. *React. Polym.* **1983**, *1*, 159−174. (b) Arshady, R., *Makromol. Chem.* **1989**, *189*, 1295−1303. (c) Yu, Z.; Bradley, M. *Curr. Opin. Chem. Biol.* **2002**, *6*, 347−352.

(23) See Supporting Information for experimental details.

(24) (a) Malkemus, J. D. *J. Am. Oil Chem. Soc.* **1956**, *33*, 571. (b) Inman, J. K.; Du Bois, G. C.; Apella, E. In *Solid-Phase Methods in Protein Sequence Analysis*; Proceedings of the Second Annual Conference; North-Holland Publishing: Amsterdam, 1977; pp 81−91. (c) Harris, J. M.; Sedaghat-Herati, M. R.; Sather, P. J.; Brooks, D. E.; Fyles, T. M. Synthesis of Poly-(Ethylene Glycol) Derivatives. In *Poly(Ethylene Glycol) Chemistry: Biotechnical and Biomedical Applications*; Harris, J. M., Ed.; Plenum Press: New York, 1992; pp 371−381. (d) Price, C. C.; Carmelite, D. D. *J. Am. Chem. Soc.* **1966**, *88*, 4039−4044. (e) Rapp, W.; Bayer, E. In *Innovation and Perspectives in Solid-Phase Synthesis and Related Technologies: Peptides, Polypeptides and Oligonucleotides*; Epton, R., Ed.; Intercept: Andover, 1992; pp 259−266. (f) Gee, G.; Higginson, W. C. E.; Livesley, P.; Taylor, K. J. *J. Chem. Soc.* **1959**, 1338−1344. (g) Gee, G.; Higginson, W. C. E.; Livesley, P.; Taylor, K. J. *J. Chem. Soc.* **1959**, 1345−1352.

(25) Park, B. D.; Lee, H. I.; Ryoo, S. J.; Lee, Y. S. *Tetrahedron Lett.* **1997**, *38*, 591−594.

Table 2. Binary Codes, Composition, Loading, PEG Content, and Peptide Synthesis Yield

		resin loading (mmol/g)		PEG graft			AFGHPQ
binary code	composition[a]	[–Cl][b]	[–OH][c]	wt[d]	L1[e]	L2[f]	yield (%)[g]
100000	A	0.89	0.25	72	67	60	96.0
110001	A/B/F	1.00	0.33	67	47	44	111
100011	A/E/F	0.90	0.42	53	29	32	98.1
010011	B/E/F	0.99	0.45	54	28	26	87.5
110010	A/B/E	0.87	0.31	64	50	47	97.9
011001	B/C/F	0.90	0.26	71	63	60	101
010001	B/F	0.91	0.23	74	73	81	92.2
100010	A/E	1.03	0.32	68	49	54	98.9
101001	A/C/F	1.03	0.36	65	42	42	105
001000	C	0.91	0.31	66	50	51	108
001001	C/F	0.99	0.38	62	38	39	82.4
001010	C/E	0.88	0.31	62	44	23	125
101000	A/C	1.01	0.38	62	38	33	97.8
011000	B/C	0.93	0.35	63	42	38	109
011010	B/C/E	0.92	0.37	60	37	30	81
100001	A/F	1.00	0.35	65	44	41	101
001011	C/E/F	0.92	0.28	70	58	50	82.4
100100	A/D	1.14	0.31	73	55	50	100
010010	B/E	0.86	0.40	53	31	27	98.3
101010	A/C/E	1.00	0.34	65	49	50	84.6
000011	E/F	0.89	0.26	71	64	62	99.0
010000	B	0.96	0.25	74	68	72	96.2
111000	A/B/C	0.99	0.37	62	39	37	111
110000	A/B	1.07	0.36	64	42	39	94.2
000001	F	1.06	0.42	61	33	29	106
average:		0.96	0.34	65	47	47	98.5

[a] A = styrene, B = 2,5 dimethylstyrene, C = 4-methylstyrene, D = 2,4-dimethylstyrene, E = 4-*tert*-butylstyrene, F = 3-methylstyrene, CMS = 4-chloromethylstyrene, DVB = 1,4-divinylbenzene. [b] Resin loading determined by titration of the chloride content prior to TEG/PEG grafting.[23] [c] Loading obtained by attaching Fmoc-Phe to BCR−PEG followed by titration of the fulvene−piperidine adduct released upon treatment with 20% piperidine/DMF.[23] The same titration experiment was carried out with Fmoc-Gly, and the results were within ±5% (data not shown). [d] PEG content in % weight after epoxide polymerization, determined from the ratio of the loading (mmol/g) before and after PEG grafting or gravimetrically from the weight gain after PEG grafting (final hydroxyl loading = initial loading × [initial weight/final weight]). [e] Length of the PEG graft in number of ethylene oxide units obtained from the resin loading and PEG content. [f] Length of the PEG graft in number of ethylene oxide units derived from ^{1}H NMR data. [g] Yield of hexapeptide synthesis obtained from the fulvene−piperidine adduct method.[23]

results most likely from the polymerization initiator, benzoyl peroxide.[26] Upon grafting of the PEG chain several new IR and Raman vibrations were recorded, while others were enhanced (Table 1).

Loading and PEG Content. The chloromethyl styrene contents of the parent BCRs were adjusted to ∼1 mmol/g so that upon grafting of the PEG chain the weight gain would reduce the resin loading to ∼0.3 mmol/g. Chloromethylstyrene incorporation (hence the loading of the BCRs) was titrated using a chloride ion-selective electrode.[23] The hydroxyl content of BCR−PEG was determined after coupling with Fmoc-Phe and Fmoc-Gly followed by spectrophotometric titration of the fulvene−piperidine adduct generated upon treatment of the resin with 20% piperidine/DMF.[23] On the basis of the loading before and after PEGylation the average PEG content and its length in the BCR−PEG resins were determined to be ∼65% w/w and ∼47 ethylene oxide units, respectively (Table 2).[23]

Swelling Properties. The swelling properties of BCR, BCR−TEG, and BCR−PEG were determined by using the syringe method.[23] BCR and BCR−TEG displayed swelling properties similar to those of Merrifield resins, except in DMF where the swelling properties of BCR−TEG were significantly enhanced. BCR−PEG displayed enhanced and broad swelling properties including those in methanol and water (Figure 2).

Magic Angle Spinning[28] ^{1}H NMR and Gel Phase[14f,i,29] ^{13}C NMR Experiments. All the ^{1}H NMR spectra were recorded at 298.15 K (uncorrected) with a spin rate of 3 kHz at the magic angle on a Varian Infinity CMX 400 solid-state spectrometer equipped with a 5 mm ^{1}H/*X*/*Y* triple resonance MAS probe. From peak integrals, the PEG content was determined (Table 2). Because of the smaller line widths and better chemical shift dispersion of ^{13}C lines, gel phase ^{13}C NMR spectroscopy in a standard liquids probe was sufficient to assess the composition of the polymers synthesized. Thus, all the proton-decoupled ^{13}C NMR spectra were recorded at 298.15 K (uncorrected) with a spin rate of 20 Hz using WALTZ decoupling with an effective field of 3125 Hz on a Bruker DRX 500 MHz or Varian Unity

(26) (a) Moad, G.; Solomon, D. H.; Willing, R. H. *Macromolecules* **1988**, *21*, 855−857. (b) Moad, G.; Solomon, D. H.; Johns, S. R.; Willing, R. H. *Macromolecules* **1982**, *15*, 1188−1191.

(27) (a) Yokoyama, M.; Ochi, H.; Tadokoro, H.; Price, C. C. *Macromolecules* **1972**, *5*, 690−698. (b) Rosenberg, M.; Loewenschuss, A.; Marcus, Y. *Spectrochim. Acta Pt.* A **1998**, 54, 1819−1826.

(28) (a) Maas, W. E.; Laukien, F. H.; Ory, D. G. *J. Am. Chem. Soc.* **1996**, *118*, 13085−13086. (b) Chin, J.; Fell, B.; Shapiro, M. J.; Tomesch, J.; Wareing, J. R.; Bray, A. M. *J. Org. Chem.* **1997**, *62*, 538−539. (c) Dhalluin, C.; Boutillon, C.; Tartar, A.; Lippens, G. *J. Am. Chem. Soc.* **1997**, *119*, 10494−10500. (d) Riedl, R.; Tappe, R.; Berkessel, A. *J. Am. Chem. Soc.* **1998**, *120*, 8994−9000. (e) Warrass, R.; Wieruszeski, J.-M.; Lippens, G. *J. Am. Chem. Soc.* **1999**, *121*, 3787−3788. (f) Chin, J.; Fell, B.; Pochapsky, S.; Shapiro, M. J.; Wareing, J. R. *J. Org. Chem.* **1998**, *63*, 1309−1311. (g) Klein, J.; Meinecke, R.; Mayer, M.; Meyer, B. *J. Am. Chem. Soc.* **1999**, *121*, 5336−5337. (h) Sarkar, S. K.; Garigipati, S.; Adams, J. L.; Keifer, P. A. *J. Am. Chem. Soc.* **1996**, *118*, 2305−2306. (i) Meissner, A.; Bloch, P.; Humpfer, E.; Spraul, M.; Sørensen, O. W. *J. Am. Chem. Soc.* **1997**, *119*, 1787−1788. (j) Pursch, M.; Schlotterbeck, G.; Tseng, L.-H.; Albert, K.; Rapp, W. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2867−2869. (k) Anderson, R. C.; Jarema, M. A.; Shapiro, M. J.; Stockes, J. P.; Ziliox, M. *J. Org. Chem.* **1995**, *60*, 2650−2651. (l) Garigipati, R. S.; Adams, B.; Adams, J. L.; Sarkar, S. K. *J. Org. Chem.* **1996**, *61*, 2911−2914. (m) Lippens, G.; Bourdonneau, M.; Dhalluin, C.; Warrass, R.; Richert, T.; Seetharaman, C.; Boutillon, C.; Piotto, M. *Curr. Org. Chem.* **1999**, *3*, 147−169. (n) Keifer, P. A. *Drug Discovery Today* **1997**, *2*, 468−478. (o) Duus, J. O.; St. Hillaire, P. M.; Meldal, M.; Bock, K. *Pure Appl. Chem.* **1999**, *71*, 755−765. (p) Schneider, B.; Doskocilová, D.; Dubal, J. *Polymer* **1985**, *26*, 253−259. (q) Anderson, R. C.; Jarema, M. A.; Shapiro, M. J.; Stokes, J. P.; Ziliox, M. *J. Org. Chem.* **1995**, *60*, 2560−2561. (r) Stöver, H. D. H.; Fréchet, J. M. J. *Macromolecules* **1991**, *24*, 883−888. (s) Jelinek, R.; Valente, A. P.; Valentine, K. G.; Opella, S. J. *J. Magn. Res.* **1997**, *125*, 185−187. (t) Gibb, S. J.; Johnson, S. J. *J. Magn. Res.* **1991**, *93*, 395−402. (u) Dingley, A. J.; Mackay, J. P.; Chapman, B. E.; Morris, M. B.; Kuchel, P. W.; Hambly, B. D.; King, G. F. *J. Biomol. NMR* **1995**, *6*, 321−328. (v) Altieri, A. S.; Hinton, D. P.; Byrd, R. A. *J. Am. Chem. Soc.* **1995**, *117*, 7566−7567. (w) Stranix, B. R.; Gao, J. P.; Barghi, R.; Salha, J.; Darling, G. D. *J. Org. Chem.* **1997**, *62*, 8987−8993. (x) Ruhland, T.; Andersen, K.; Pedersen, H. *J. Org. Chem.* **1998**, *63*, 9204−9211.

(29) (a) Bayer, E.; Albert, K.; Willisch, H.; Rapp, W.; Hemmasi, B. *Macromolecules* **1990**, *23*, 1937−1940. (b) Ford, W. T.; Mohanraj, S.; Periyasamy, M. *Br. Polym. J.* **1984**, *16*, 179−187. (c) Look, G. C.; Holmes, C. P.; Chinn, J. P.; Gallop, M. A. *J. Org. Chem.* **1994**, *59*, 7588−7590. (d) Brown, J. M.; Ramsden, J. A. *Chem. Commun.* **1996**, 2117−2118. (e) Svensson, A.; Fex, T.; Kihlberg, J. *Terahedron Lett.* **1996**, *37*, 7649−7652. (f) Keifer, P. A.; Baltusis, L.; Rice, D. M.; Tymiak, A. A.; Shoolery, J. N. *J. Magn. Reson. A* **1996**, *119*, 65−75. (g) Frechet, J. M. J. *Tetrahedron* **1981**, *37*, 663−683. (h) Barany, G.; Kneib-Cordonier, N.; Mullen, D. G. *Int. J. Pept. Protein Res.* **1987**, *30*, 705−739. (i) Epton, R.; Goddard, P.; Irvin, K. J. *Polymer* **1980**, *21*, 1367−1371. (j) Giralt, E.; Rizo, J.; Pedroso, E. *Tetrahedron* **1984**, *40*, 4141−4152. (k) Feliz, M.; Goralt, E.; Ribó, J. M.; Trull, F. R. *Makromol Chem.* **1988**, *189*, 1551−1559. (l) Giralt, E.; Albericio, F.; Bardella, F.; Eritja, R.; Feliz, M.; Pedroso, E.; Pons, M.; Riso, J. In *Innovation and Perspectives in Solid-Phase Synthesis*; Epton, R., Ed.; SPCC Ltd: Birmingham, UK, 1990; p 111. (m) Sterlicht, H.; Kenyon, G. L.; Packer, E. L.; Sinclair, J. *J. Am. Chem. Soc.* **1971**, *93*, 199−208. (n) Liebfritz, D.; Mayr, W.; Oekonomopulos, R.; Jung, J. *Tetrahedron* **1978** *34*, 2045−2050. (o) Manatt, S. L.; Amsden, S. F.; Bettison, C. A.; Frazer, W. T.; Gudman, J. T.; Lenk, B. E.; Lubetich, J. F.; McNelly, E. A.; Smith, S. C.; Templeton, D. J.; Pinnell, R. P. *Tetrahedron Lett.* **1980**, *21*, 1397−1400. (p) Jones, A. J.; Leznoff, C. C.; Svirskaya, P. I. *Org. Magn. Reson.* **1982**, *18*, 236−240. (q) Blossey, E. C.; Cannon, R. G.; Ford, W. T.; Periyasamy, M.; Mohanraj, S. *J. Org. Chem.* **1990**, *55*, 4664−4668. (r) Keifer, P. A. *Curr. Opin. Biotechnol.* **1999**, *10*, 34−41. (s) Shapiro, M. J.; Wareing, J. R. *Curr. Opin. Chem. Biol.* **1998**, *2*, 372−375. (t) Kanemitsu, T.; Kanie, O.; Wong, C.-H. *Angew. Chem., Int. Ed.* **1998**, *38*, 3415−3418.

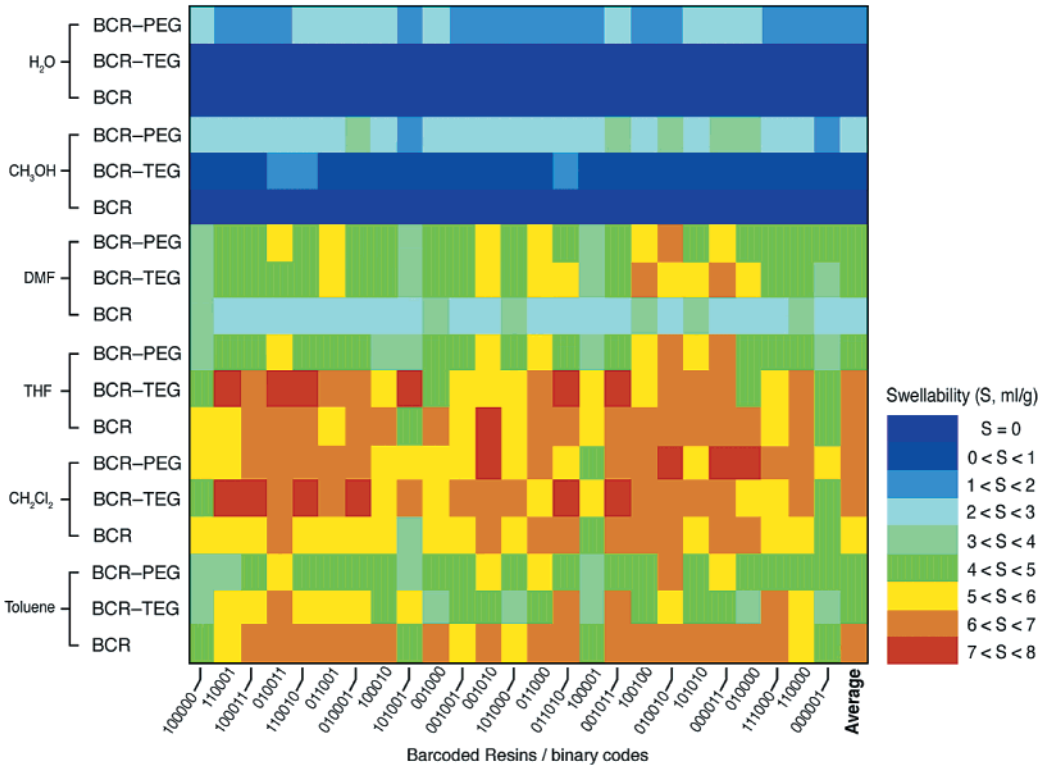


Figure 2. Swellability of BCR, BCR−TEG, and BCR−PEG (mL/g) in toluene, dichloromethane (CH_2Cl_2), tetrahydrofuran (THF), *N*,*N*-dimethylformamide (DMF), methanol (CH_3OH), and deionized water (H_2O).

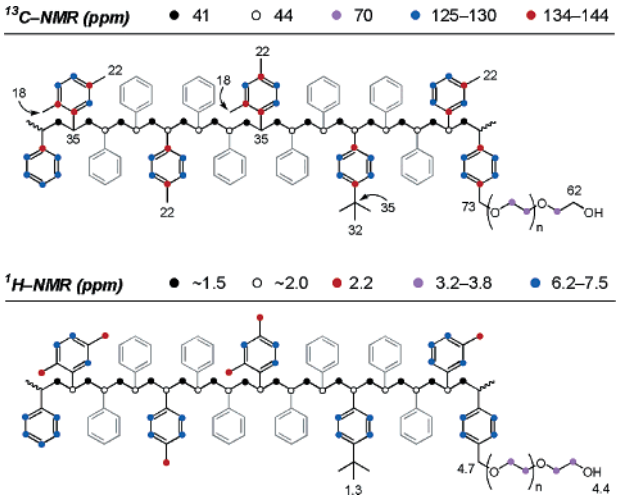


Figure 3. Summary of ^{13}C (top) and ^{1}H (bottom) NMR resonances and assignments of the 25 BCR−PEG graft copolymers synthesized. Divinylbenzene bridges were not included because they represent only 1.3% of the polymers' weight and were as a result undetectable.

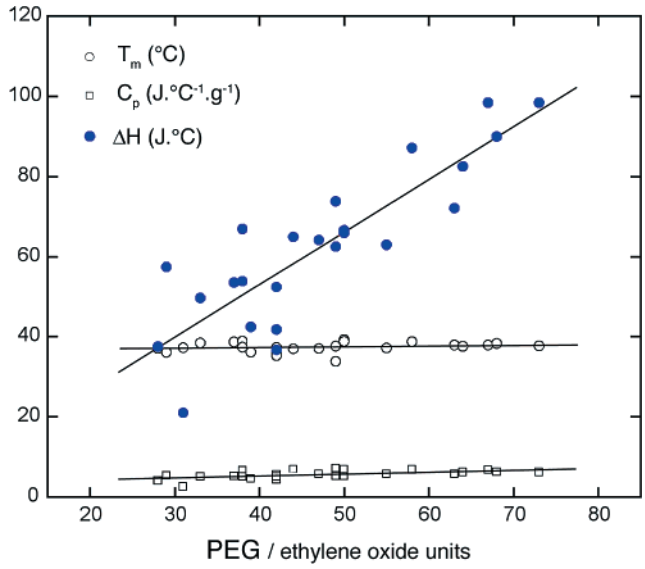


Figure 4. Melting temperature (T_m), heat capacity (C_p) and melting enthalpies (ΔH) of the BCR−PEG resins prepared as a function of PEG length (28−73 ethylene oxide units).

Plus 600 MHz spectrometers. All the polymers synthesized displayed ^{1}H and ^{13}C resonances characteristic of their parent monomers. Figure 3 summarizes the ^{1}H and ^{13}C NMR assignments.

Differential Scanning Calorimetry. DSC thermograms were obtained at a heating rate of 10 °C/min over the temperature range of 20−150 °C on a TA 2920 differential scanning calorimeter equipped with a computer-analyzer system. Figure 4 and Table 4 (Supporting Information) summarize the thermodynamic parameters recorded. T_m and C_p where found to be insensitive to PEG length between 28 and 73 ethylene oxide units, whereas ΔH increased quasi-linearly with a correlation coefficient $r = 0.86$ according to the following relationship: $\Delta H = 0.358 + 1.32 \times L$, where L is the PEG graft length in number of ethylene oxide units.

Streptavidin−Alkaline Phosphatase (SAP) Binding Assay. A hexapeptide (H_2N-QPHGFA-OH) was synthesized on BCR−PEG in high yield (>98.5%) using Atherton and Sheppard[23,30] Fmoc/tBu peptide synthesis protocol. The QPH tripeptide sequence at its N terminus was previously identified by Østergaard[31a] and Lam[4b,31b,c] as a ligand for streptavidin. Thus,

(30) Atherton, A.; Sheppard, R. C. *Solid-Phase Peptide Synthesis: A Practical Approach*; Oxford University Press: Oxford, 1989.

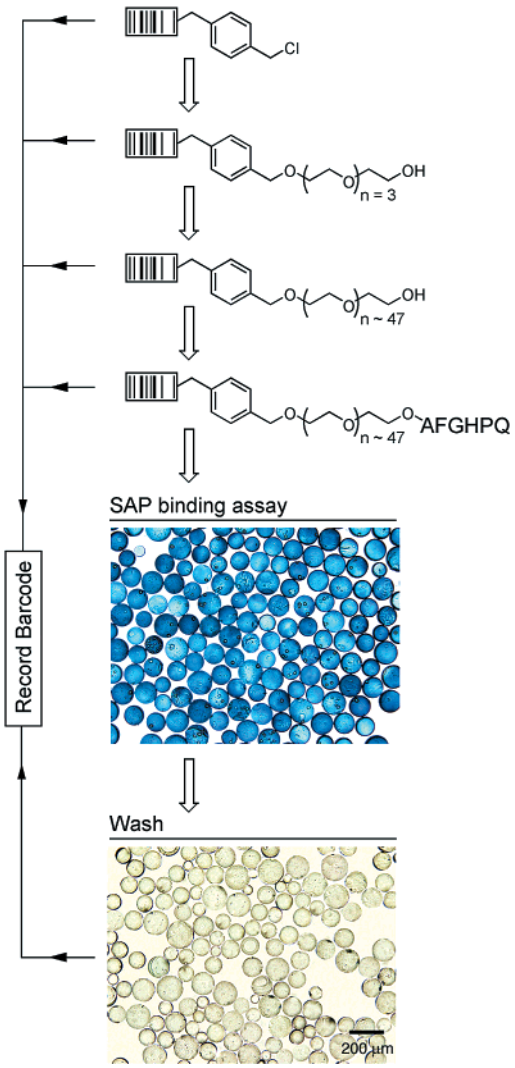


Figure 5. Sequence of steps leading to BCR−PEG-AFGHPQ-NH_2 for the SAP binding assay. The blue beads show resin-bound streptavidin−alkaline phosphatase, whereas resins without peptide remained colorless (not shown). The barcodes generated for BCR and for BCR−PEG-AFGHPQ-NH_2 after the SAP binding assay were found to be essentially identical.

upon addition of BCIP (5-bromo-4-chloro-3-indolyl phosphate, alkaline phosphatase substrate) and SAP, the beads turned blue turquoise from the local hydrolysis of BCIP, revealing the beads bound to streptavidin. This experiment was implemented to further establish the reliability of the barcoding strategy as a means to unequivocally identify the BCRs, even after a binding assay, and to demonstrate that the heterogeneity of the PS backbone does not alter the binding assay. Indeed, all the BCR−PEG synthesized turned blue (Figure 5), and their Raman and IR barcodes prior and after the SAP assay were found to be essentially identical.

Binary Code and Spectroscopic Barcode Generation. We have arbitrarily assigned a basic binary code for each of the six monomers (Figure 1). The presence (1) or absence (0) of a particular styrene monomer within a given polymer determines the binary code assigned to each resin. Thus, a six-digit binary code was assigned for each resin obtained from the six parent styrene monomers. The Raman[32] and IR[33] spectra of the BCR,

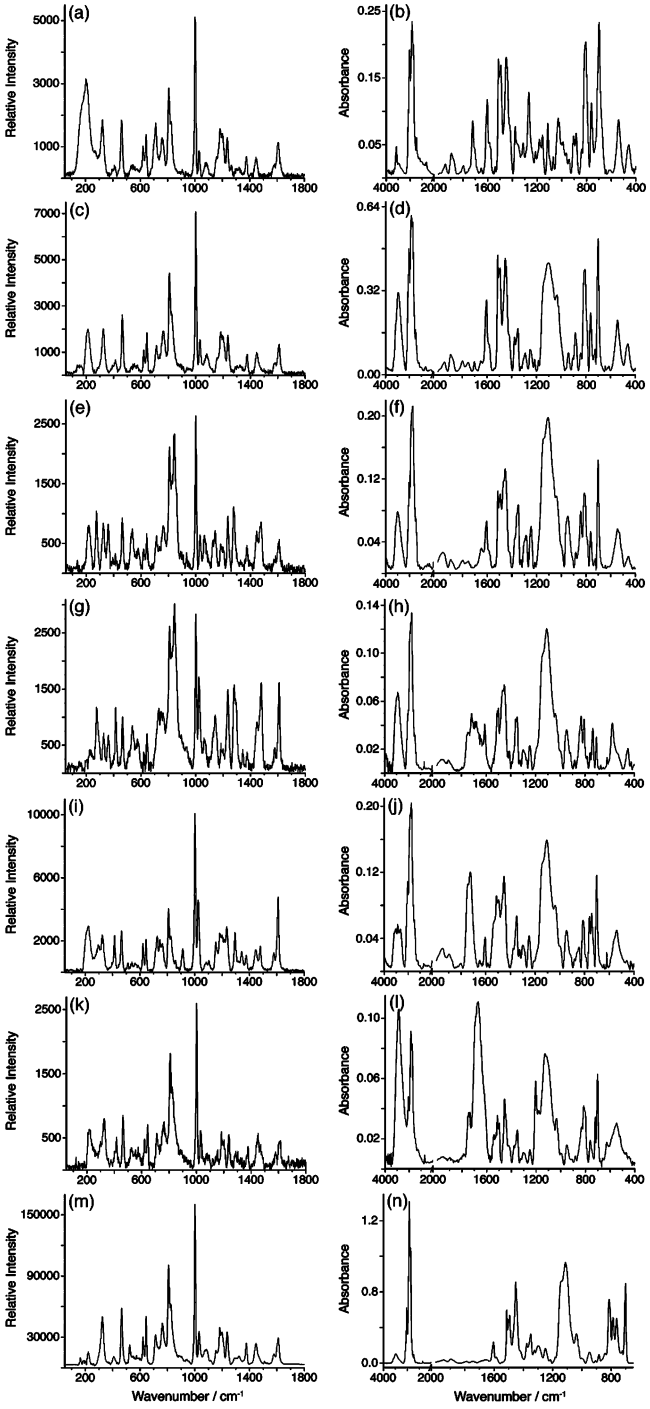


Figure 6. Raman (left) and infrared (right) spectra of resin 111000: BCR (a,b), BCR−TEG (c,d), BCR−PEG (e,f), BCR−PEG-FmocGly (g,h), BCR−PEG-FmocPhe (i,j), BCR−PEG-AFGHPQ before SAP assay (k,l), and BCR−PEG-AFGHPQ after SAP assay (m,n). These spectra show that most of the vibrations of the parent BCR appear in subsequent resins, particularly in BCR−PEG-AFGHPQ after SAP assay.

BCR−TEG, BCR−PEG, BCR−PEG-Fmoc-Gly, BCR−PEG-Fmoc-Phe, BCR−PEG-AFGHPQ before and after SAP assay for each type of parent polymer were recorded and baseline-corrected.[23] Most of the vibrations present in the BCR series were consistently identified in subsequent derivatives of the same parent polymers (BCR−TEG and BCR−PEG). Figure 6 illustrates the changes in Raman and infrared spectra observed upon grafting TEG, PEG, PEG-FmocGly, PEG-FmocPhe, and PEG-AFGHPQ (before and after SAP assay) to BCR 111000.

(31) (a) Østergaard, S.; Hansen, P. H.; Olsen, M.; Holm, A. *FEBS Lett.* **1995**, *362*, 306−308. (b) Lam, K. S.; Lebl, M. *Immunomethods* **1992**, *1*, 11−15.

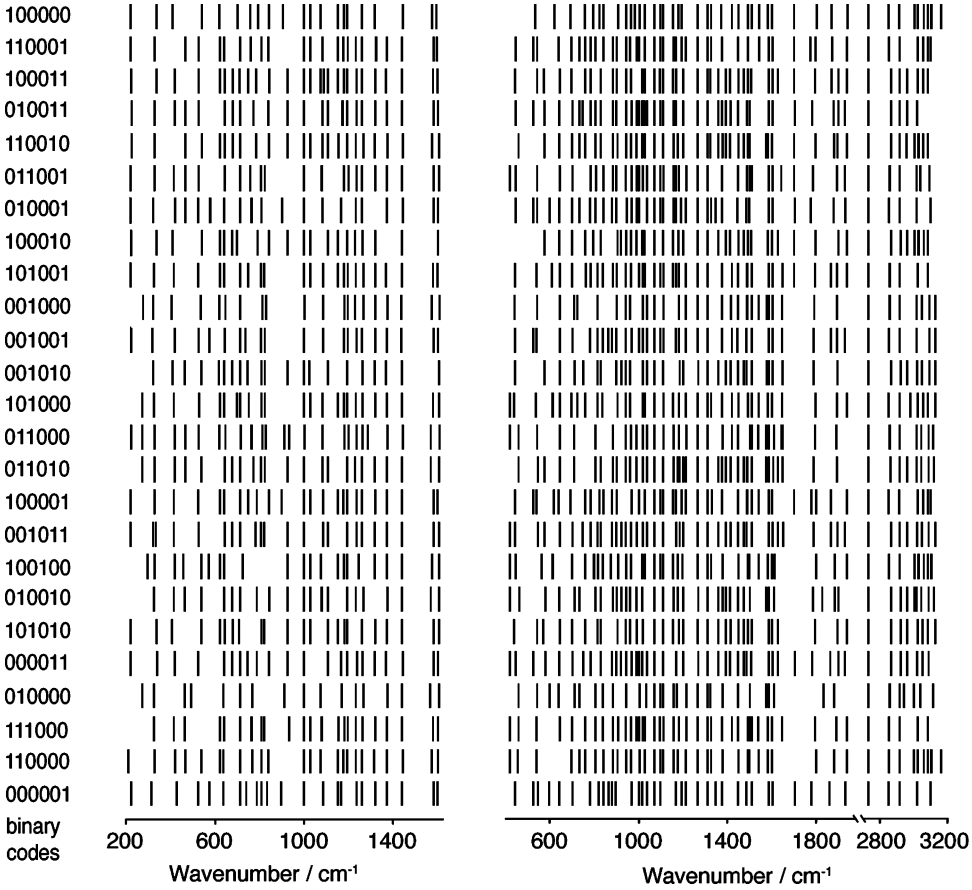


Figure 7. Raman (left) and infrared (right) spectroscopic barcodes of the parent BCRs used in the preparation of BCR−TEG, BCR−PEG, BCR−PEG-FmocGly, BCR−PEG-FmocPhe, and BCR−PEG-AFGHPQ.

These vibrations were converted into barcodes in which the position of each bar matches the peak wavenumber in the corresponding spectrum (Figure 7).

Conservation of the Vibrational Fingerprint. To determine the level of similarity between the parent BCRs and their derivatives we processed the Raman and IR spectral data as follows:

Method 1. The Raman (IR) vibrations for each series of 25 resins were combined, and their occurrences and standard deviations within each series were determined (Tables 5 and 6, Supporting Information). For instance, the Raman vibration at 1445 cm^{-1} appears in the spectra of 25 BCR, BCR−TEG, BCR−PEG, BCR−PEG-FmocGly, BCR−PEG-FmocPhe, and BCR−PEG-AFGHPQ (before and after SAP assay) with a standard deviation of 1.6, 1.7, 0.4, 1.4, 1.8, 1.7, and 1.8 cm^{-1}, respectively. The IR vibration at 3023 cm^{-1} appears in the spectra of 21 BCR, BCR−TEG and BCR−PEG, 20 BCR−PEG-FmocGly, 17 BCR−PEG-FmocPhe, and 21 BCR−PEG-AFGHPQ (before and after SAP assay) with a standard deviation of 2.7, 2.7, 2.9, 2.4, 2.5, 2.5, and 2.9 cm^{-1}, respectively. This comparative analysis allowed us to rapidly probe the overall persistence of the parent BCR's vibrational fingerprint.

Method 2. A similarity coefficient (S)[34] between each resin pair was calculated according to the following equation:

$$S = \frac{\sum_i (v_{iA} \cdot v_{iB})}{\sum_i v_{iA} + \sum_i v_{iB} - \sum_i (v_{iA} \cdot v_{iB})}$$

where V_{iA} is 1 when vibration i is present in resin A and 0 when it is absent. Similarly, V_{iB} is 1 when vibration i is present

(32) (a) Gift, A. D.; Ma, J.; Haber, K. S.; McClain, B. L.; Ben-Amotz, D. *J. Raman Spectrosc.* **1999**, *30*, 757−765. (b) Hashimoto, M.; Araki, T.; Kawata, S. *Opt. Lett.* **2000**, *25*, 1768−1770. (c) Ma, J.; Ben-Amotz, D. *Appl. Spectrosc.* **1997**, *51*, 1845−1848. (d) Fenniri, H.; Hedderich, H. G.; Haber, K. S.; Achkar, J.; Taylor, B.; Ben-Amotz, D. *Angew. Chem., Int. Ed.* **2000**, *39*, 4483−4485. (e) Fenniri, H.; McFadden, R. M. Multispectral Imaging for the High-Throughput Evaluation of Solid Phase-Supported Chemical Libraries. In *Optimization of Solid-Phase Combinatorial Synthesis*; Yan, B., Czarnik, A. W., Eds.; Marcel Dekker: New York, 2001; pp 287−304.

(33) (a) Yan, B.; Yan, H. *J. Comb. Chem.* **2001**, *3*, 78−84. (b) Yan, B.; Chu, Y.-H.; Shapiro, M.; Richmond, R.; Chin, J.; Liu, L.; Yu, Z. in *Combinatorial Chemistry: A Practical Approach*, Fenniri, H., Ed.; Oxford University Press: Oxford, 2000; pp 263−286. (c) Yan, B. *Curr. Opin. Chem. Biol.* **2002**, *6*, 328−332. (d) Yan, B.; Kumaravel, G.; Anjaria, H.; Wu, A.; Petter, R. C.; Jewel, F. C., Jr.; Wareing, J. R. *J. Org. Chem.* **1995**, *60*, 5736−5738. (e) Gremlich, H.-U.; Berets, S. L. *Appl. Spectrosc.* **1996**, *50*, 532−536. (f) Yan, B.; Fell, J. B.; Kumaravel, G. *J. Org. Chem.* **1996**, *61*, 7467−7472. (g) Gosselin, F.; De Renzo, M.; Ellis, T. H.; Lubell, W. D. *J. Org. Chem.* **1996**, *61*, 7980−7981. (h) Yan, B.; Sun, Q.; Wareing, J. R.; Jewell, C. F. *J. Org. Chem.* **1996**, *61*, 8765−8770. (i) Russell, K.; Cole, D. C.; McLaren, F. M.; Pivonka, D. E. *J. Am. Chem. Soc.* **1996**, *118*, 7941−7945. (j) Li, W.; Yan, B. *J. Org. Chem.* **1998**, *63*, 4092−4097. (k) Yan, B. *Acc. Chem. Res.* **1998**, *31*, 621−630. (l) Shapiro, M. J.; Lin, M.; Yan, B. On-Resin Analysis in Combinatorial Chemistry. In *A Practical Guide to Combinatorial Chemistry*; Czarnik, A. W., DeWitt, S. H., Eds.; American Chemical Society: Washington, DC, 1997; pp 123−151. (m) Yan, B.; Gstash, H. *Tetrahedron Lett.* **1996**, *37*, 8325−8328. (n) Luo, Y.; Ouyang, S.; Armstrong, R. W.; Murphy, M. M. *J. Org. Chem.* **1998**, *63*, 8719−8722. (o) Yan, B.; Gremlich, H.-U.; Moss, S.; Coppola, G. M.; Sun, Q.; Liu, L. *J. Comb. Chem.* **1999**, *1*, 46−54. (p) Chen, C.; Ahlberg Randell, L. A.; Miller, R. B.; Jones, A. D.; Kurth, M. J. *J. Am. Chem. Soc.* **1994**, *116*, 2661−2662. (q) Frechet, J. M.; Schuerch, C. *J. Am. Chem. Soc.* **1971**, *93*, 492−496.

in resin B and 0 when it is not. Thus, the numerator corresponds to the intersection of the family of vibrations present in A with the family of vibrations present in B (i.e. number of vibrations common to both resin A and resin B). The denominator corresponds to the union of families A and B (i.e. sum of the vibrations unique to resin A, the vibrations unique to resin B, and the vibrations common to both resins). The result is a similarity table for each pair of resin series (Tables 7−62, Supporting Information). To simplify the comparison of the Raman and IR data, the *S* values were normalized along each column by considering that the diagonal values (for homologous resins) are the highest, which is indeed the case in 99% of the 35 000 calculated similarity coefficients. The resulting normalized tables were then presented as contour plots in which the highest similarity intersections appear in red and the lowest appear in blue (Figure 9 and Figure 1 in Supporting Information section).

Discussion

BCR−PEG Graft Copolymers. Grafting PEG on PS was carried out in the 1970s and early 1980s by Inman,[20a] Regen,[20b] Warshawski and Patchornik,[20c] Sherrington,[20d] and Mutter,[20e,f] but it was not until Bayer and Rapp introduced TentaGel[14] in the mid 1980s that this formulation became frequently used in solid-phase organic synthesis[12−19,21,35] and on-bead biological assays.[21k,36] This study was further motivated by their ease of preparation and the need to generate encoded resins amenable to directed sorting using flow cytometry.[10,11]

The defining characteristic of the BCR−PEG is their preparation from spectroscopically active styrene monomers displaying unique IR and Raman vibrational fingerprints. The styrene monomers' substitution pattern is the source of spectral diversity of the resulting polymers. The frequencies used to generate the barcodes are mainly due to aromatic ring skeletal bending and C−H stretching modes. Tables 63−68 (Supporting Information) summarize the IR and Raman vibrations of the homopolymers generated from the parent monomers.[33,37] The vibrational spectra (and barcodes) of the corresponding copolymers are, in all cases studied, a linear combination of the parent homopolymers' frequencies,[23] in addition to new vibrations unique to the copolymers as illustrated in Figure 6.[23] This result was instrumental in streamlining the choice of styrene derivatives used in the suspension polymerization and predicting the resins' spectroscopic barcodes, composition, and properties.

NMR Studies. Solvent motion in the polymer network leads to chain expansion observable on a macroscopic scale as a swelling of the bead (Figure 2) and is accompanied by an enhanced local mobility of the PEG chains at the microscopic level. As a consequence of the enhanced molecular mobility, the various interactions that govern the line width of the NMR signal will be motionally averaged, including the homonuclear dipolar interaction that can broaden proton signals up to 20 kHz in a static solid-state sample. This motional averaging may be incomplete due to the anisotropic environment of the solvent molecules in the polymer. The resulting residual dipolar broadening can be further reduced by spinning the sample at the magic angle (54.7° from the *z*-axis) at a rate higher than the nonspinning residual line width. This last condition on the spinning rate can be advantageously exploited to suppress the lines of the more rigid supporting cross-linked polystyrene backbone while obtaining a liquidlike spectrum for the more mobile PEG graft. Magic angle spinning (MAS) eliminates also the magnetic-susceptibility broadening caused by the difference in (electronic) magnetic susceptibility between the polymer and the pure solvent, combined with the irregular shape of the solvent−polymer interface. Thus, MAS NMR at a spinning rate of 2−5 kHz eliminates to a large extent both mechanisms of line broadening, provided the NMR solvent is chosen to maximize resin swelling.

Although from the reactions' high yield we were able to infer the incorporation of all the styrene monomers used as well as the level of PEG grafting, this result was further established by NMR. All the polymers showed the characteristic ^{13}C PEG resonances at 62 ppm ($\underline{C}H_2OH$), 70 ppm ($\underline{C}H_2O\underline{C}H_2$), and 73 ppm ($Ar\underline{C}H_2O$). The aromatic $\underline{C}H$ (125−130 ppm) and $\underline{C}$ (134−146 ppm) resonances were generally weakly resolved and broad as a result of their limited mobility.[14f,i,28,29] The $\underline{C}H_2$ and $\underline{C}H$ resonances of the polymer backbone gave two characteristic peaks at 41 ppm (sharp) and 44 ppm (broad), respectively. The benzene ring substituants gave the following diagnostic ^{13}C resonances that allowed us to confirm the composition of the polymers and the incorporation of all the styrene monomers: (a) resins prepared from 4-*tert*-butylstyrene featured resonances at 32 ppm ($C\underline{C}H_3$) and 35 ppm ($\underline{C}CH_3$) ppm, (b) resins with 2,5-dimethylstyrene or 2,4-dimethylstyrene gave resonances at 18 ppm (2-methyl), 22 ppm (4-methyl or 5-methyl), and 35 ppm ($\underline{C}H$), (c) resins containing 3-methylstyrene or 4 methylstyrene gave a unique resonance at 22 ppm (3-methyl or 4-methyl). These data are summarized in Figure 2. From ^{1}H NMR peak integration we were also able to confirm the PEG content, which was found to be similar to that determined gravimetrically (∼47 ethylene oxide units on average, Table 2).

Differential Scanning Calorimetry (DSC) Studies. Standard gel-type copoly(styrene-1% divinylbenzene) resins are glassy materials with a glass transition temperature (T_g) ∼110 °C.[38]

(34) Woodruff, H. B.; Lowry, S. R.; Ritter, G. L.; Isenhour, T. L. *Anal. Chem.* **1975**, *47*, 2027−2030.
(35) (a) Swali, V.; Wells, N. J.; Langley, G. J.; Bradley, M. J. *J. Org. Chem.* **1997**, *62*, 4902−4903. (b) Winther, L.; Almdal, K.; Batsberg Pedersen, W.; Kops, J.; Berg, R. H. In *Peptides: Chemistry, Structure and Biology. Proceedings of the Thirteenth American Peptide Symposium*; Hodges, R. S., Smith, J. A., Eds.; Escom Science Publishers: Leiden, 1994; pp 872−873. (c) Haralambidis, J.; Duncan, L.; Tregear, G. W. *Tetrahedron Lett.* **1987**, *28*, 5199−5202. (d) de la Torre, B. G.; Avino, A.; Tarrason, G.; Piulats, J.; Albericio, F.; Eritja, R. *Tetrahedron Lett.* **1994**, *35*, 2733−2736. (e) Fenniri, H.; Janda, K. D.; Lerner, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 2278−2282. (f) Gao, H.; Gaffney, B. L.; Jones, R. A. *Tetrahedron Lett.* **1991**, *32*, 5477−5480. (g) Wright, P.; Lloyd, D.; Audrus, A.; Rapp, W. *Tetrahedron Lett.* **1993**, *34*, 3373−3376.
(36) (a) Quarell, R.; Claridge, D. W.; Weaver, G. W.; Lowe, G. *Mol. Diversity* **1995**, *1*, 223−232. (b) Pillai, V. N. R.; Mutter, M. *Acc. Chem. Res.* **1981**, *14*, 122−130. (c) Smith, H. K.; Bradley, M. *J. Comb. Chem.* **1999**, *1*, 326−332. (d) Burger, M. T.; Bartlett, P. A. *J. Am. Chem. Soc.* **1997**, *119*, 12697−12698. (e) Leon, S.; Quarrell, R.; Lowe, G. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2997−3002.
(37) The assignments are based in part on: (a) Raynolds, N. M.; Hsu, S. L. *Macromolecules* **1990**, *23*, 3463−3472. (b) Painter, P. C.; Koenig, J. L. *J. Polym. Sci., Polym. Phys. Ed.* **1977**, *15*, 1885−1903. (c) Snyder, R. W.; Painter, P. C. *Polymer* **1981**, *22*, 1633−1641. (d) Sears, W. M.; Hunt, J. L.; Stevens, J. R. *J. Chem. Phys.* **1981**, *75*, 1589−1598. (e) Sears, W. M.; Hunt, J. L.; Stevens, J. R. *J. Chem. Phys.* **1981**, *75*, 1599−1602. (f) Yan, B.; Sun, Q. *J. Org. Chem.* **1998**, *63*, 55−58. (g) Altava, B.; Burguete, M. I.; Garcia-Verdugo, E.; Lius, S. V.; Vicent, M. J. *Tetrahedron* **2001**, *57*, 8675−8683. (h) Colthup, N. B.; Daly, L. H.; Wiberley, S. E. *Introduction to Infrared and Raman Spectroscopy*; Academic Press: New York, 1975. (i) Varsanyi, G. *Assignments for Vibrational Spectra of Seven Hundred Benzene Derivatives*; Wiley: New York, 1974. (j) Painter, P. C.; Howe, S. E.; Coleman, M. M. *Appl. Spectrosc.* **1984**, *38*, 184−190.
(38) The T_g is ∼110 °C for polystyrene with an average molecular weight above 36 000: (a) Boyer, R. F. *J. Appl. Polym. Sci.* **1987**, *33*, 955−969. (b) Akiba, I.; Ohba, Y.; Akiyama, S. *Macromolecules* **1999**, *32*, 1175−1179.

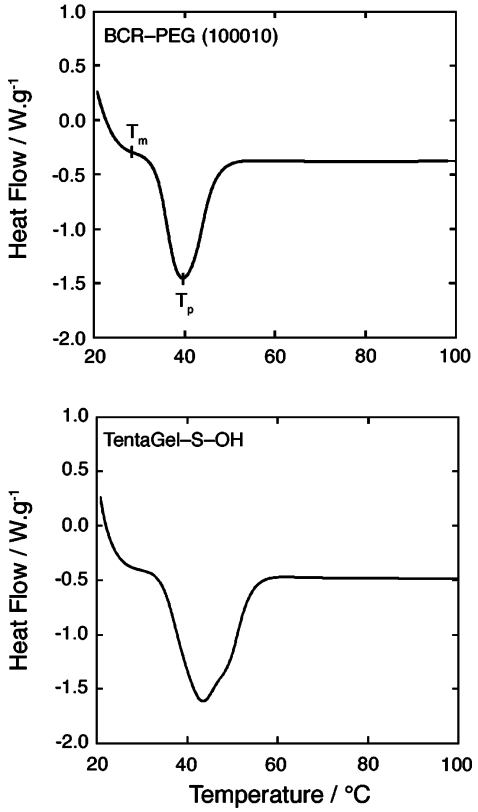


Figure 8. Differential scanning calorimetry (DSC) endotherms of BCR−PEG (100010) (top) and of 130 μm TentaGel-S-OH (bottom). The latter is a commercially available PS−PEG graft copolymer with an average PEG length of ~68 ethylene oxide units. These profiles document the uniformity of the chain graft length in BCR−PEG resins (see Supporting Information for DSC endotherms of other BCR−PEG resin prepared).

The thermal behavior of PS−PEG graft copolymers, on the other hand, depends on the PEG content and the length of the grafts. For illustration, the DSC endotherms of BCR−PEG (100010) and of TentaGel-S-OH are shown in Figure 8. The endotherm is associated with a phase transition in which the PEG graft changes from the solid (crystalline) to the fluid state. The melting temperatures (T_m's) ranged from 34 to 39 °C (average T_m = 37.4 °C), which is an indication of the uniformity of the PEG graft, and compares well with that of commercial TentaGel-S-OH (T_m = 34.5 °C). These values are also comparable with those reported for unbound linear PEG of corresponding length.[39] Furthermore, while T_m and C_p were independent of PEG length, ΔH varied quasi-linearly in the range 23−71 ethylene oxide units, in agreement with reported literature on linear unbound PEG.[39] As previously observed for TentaGel and ArgoGel,[17] no T_g's associated with glassy PEG or polystyrene domains were observed in the range of 20−120 °C, confirming that the crystalline PEG in BCR−PEG resins dominates the solid-state properties of the copolymer.

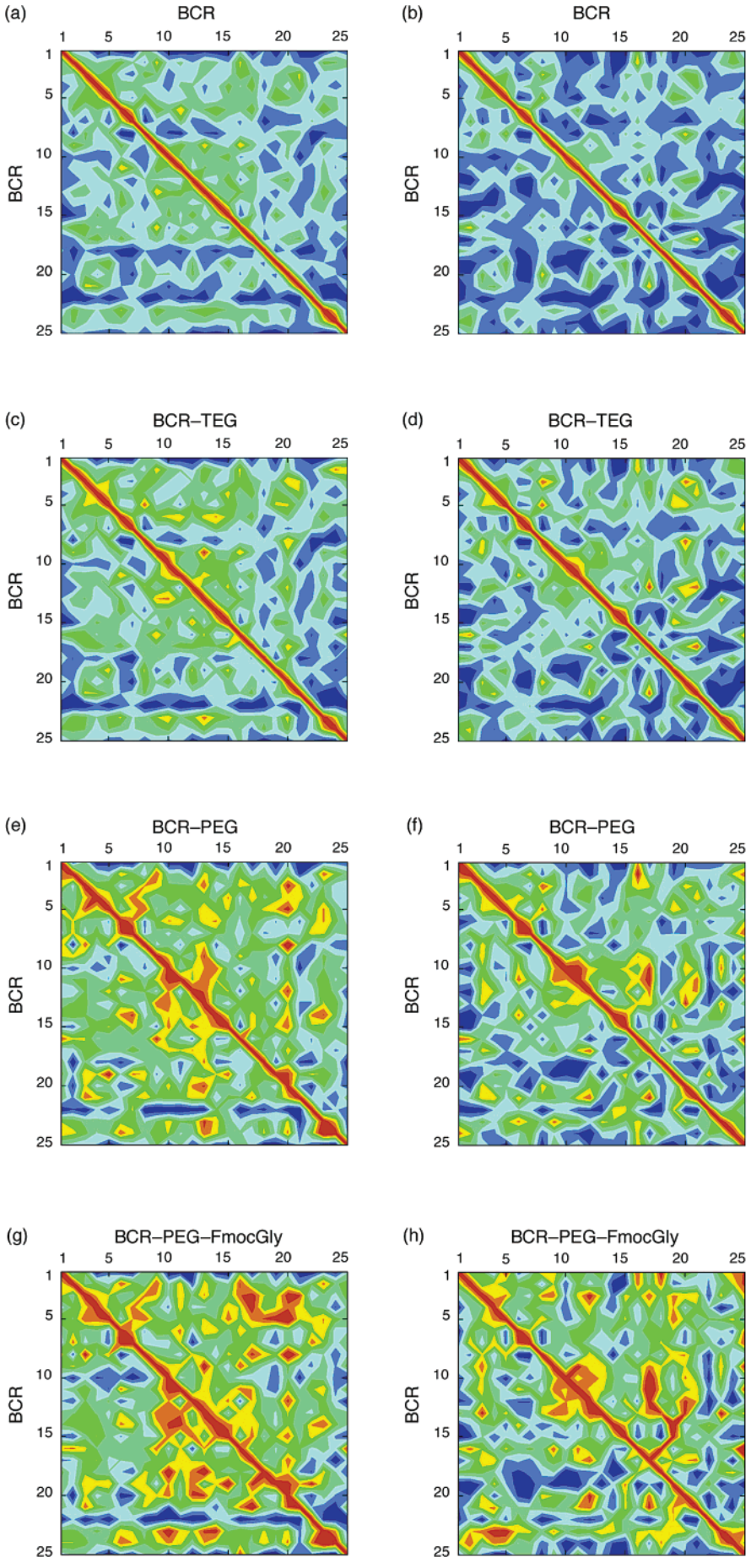
SAP Binding Assay. Central to the success of this methodology in combinatorial screening is the conservation of the vibrational fingerprint of BCRs not only upon PEG grafting but also after undergoing multistep syntheses and on-bead binding assays. To this end we synthesized a hexapeptide, known to bind to streptavidin, on all 25 resins and subjected them to the SAP binding assay. All the beads turned blue in the presence of BCIP (alkaline phosphatase substrate) demonstrating that streptavidin recognized its peptide substrate regardless of the diversity of the resin's BCR core. The barcodes were recorded before and after the SAP binding assay and compared to those of BCR, BCR−TEG, and BCR−PEG (next section).

Reliability of the Spectroscopic Barcoding. Two methods were used to determine the reliability of the spectroscopic barcoding. The first consisted in generating a repertoire of vibrations appearing in the BCR series and determining the occurrence of each vibration in subsequent resins. Of the 54 BCR Raman vibrations in the 1800−200 cm^{-1} spectral range (two chloromethyl vibrations omitted), 100% appeared in BCR−TEG, 83% in BCR−PEG, 80% in BCR−PEG-FmocGly and BCR−PEG-FmocPhe, 93% in BCR−PEG-AFGHPQ (before SAP assay), and 87% in BCR−PEG-AFGHPQ (after SAP assay) (Table 5, Supporting Information). Similarly, of the 84 BCR infrared vibrations in the 4000−650 cm^{-1} spectral range (two chloromethyl vibrations omitted), 91% appeared in BCR−TEG, 75% in BCR−PEG, 68% in BCR−PEG-FmocGly, 67% in BCR−PEG-FmocPhe, 69% in BCR−PEG-AFGHPQ (before SAP assay), and 76% in BCR−PEG-AFGHPQ (after SAP assay) (Table 6, Supporting Information). This simple comparison shows that neither PEG, nor PEG-amino acid or PEG-AFGHPQ which make up ~65, 68, and 71% of the respective resins' weight, did significantly alter the vibrational modes of the BCR core. It is thus anticipated that a small-molecule library with an average molecular weight of 650 g/mol or less would have a marginal effect on the vibrational fingerprint of BCR−PEG and an undetectable effect on that of BCR resins.

To further challenge the robustness of the barcoding strategy we calculated the similarity between all the resins synthesized according to method 2 described above. For instance, the Raman and IR barcodes of 25 BCR resins were cross-checked against themselves as well as against 25 BCR−TEG, 25 BCR−PEG, 25 BCR−PEG-FmocGly, 25 BCR−PEG-FmocPhe, 25 BCR−PEG-AFGHPQ (before SAP assay), and 25 BCR−PEG-AFGHPQ (after SAP assay). The resulting database of 35 000 similarity coefficients showed that in 99% of the cases, the highest S values were obtained for resins with identical binary codes (diagonal values in Tables 7−62, Supporting Information). To simplify the comparison of the IR and Raman similarity tables, the S values were normalized and presented as contour plots as shown in Figure 9 (see also Figure 1, Supporting Information).

Several important conclusions were drawn from this analysis. First, all the contour plots show high similarity along the diagonal, demonstrating that resins with identical binary codes maintain the highest level of similarity despite the attachment of TEG, PEG, PEG-amino acid, or PEG-AFGHPQ to the BCR core. Second, neither the hexapeptide AFGHPQ nor the SAP binding assay altered the reliability of the spectroscopic barcoding. Third, the Raman and infrared contours are significantly different, therefore allowing us to use them synergistically for the unequivocal identification of a barcoded resin. To illustrate this synergy, consider the extreme case where BCR−PEG-FmocPhe 10 (binary code 001000, Figure 9i) showed high Raman similarity with BCR 10 (001000), BCR 13 (101000), BCR 14 (011000), BCR 18 (100100), BCR 23 (111000), and BCR 24 (110000). The IR similarity contour (Figure 9j), however, ruled out every possibility except the correct one, BCR

(39) Ginés, J. M.; Arias, M. J.; Rabasco, A. M.; Novák, C.; Ruiz-Conde, A.; Sánchez-Soto, P. J. *J. Therm. Anal.* **1996**, *46*, 291−304.


(a)
BCR
(b)
BCR
(c)
BCR–TEG
(d)
BCR–TEG
(e)
BCR–PEG
(f)
BCR–PEG
(g)
BCR–PEG–FmocGly
(h)
BCR–PEG–FmocGly
1 5 10 15 20 25
BCR

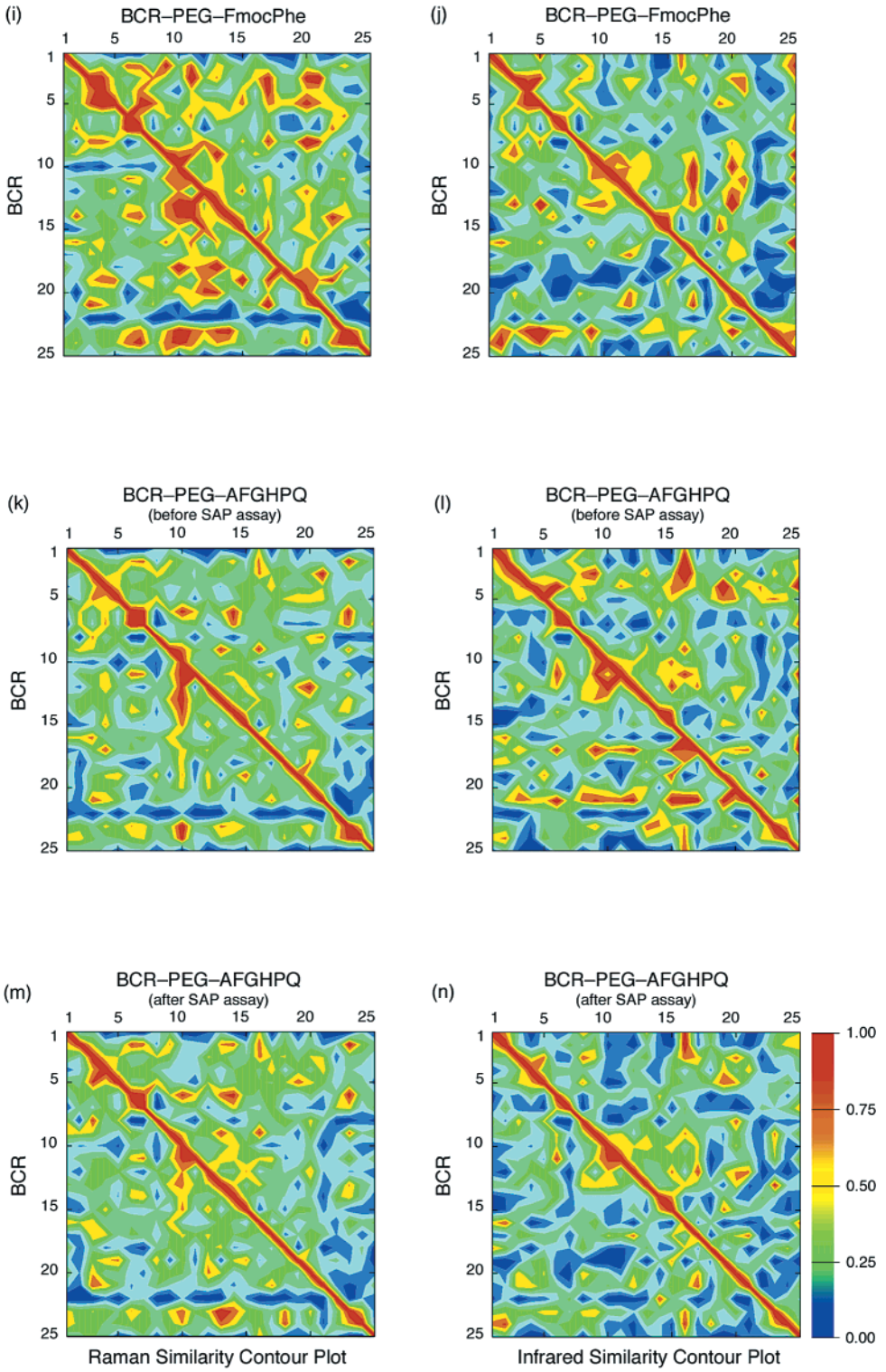


Figure 9. Raman (left) and infrared (right) similarity contour plots of (a,b) BCR versus BCR, (c,d) BCR versus BCR−TEG, (e,f) BCR versus BCR−PEG, (g,h) BCR versus BCR−PEG-FmocGly, (i,j) BCR versus BCR−PEG-FmocPhe, (k,l) BCR versus BCR−PEG-AFGHPQ (before SAP assay), and (m,n) BCR versus BCR−PEG-AFGHPQ (after SAP assay). The highest similarity intersections are depicted in red and the lowest in blue.

10. Finally, the mean similarity coefficient for each pair of resin series was calculated by averaging *S* values along the diagonal of each similarity table. As reflected by the increased red/orange contours on going from (a,b) to (i,j) in Figure 9, the mean *S* values decreased with the weight % of the BCR core. For instance, the mean similarity of BCR's Raman and infrared vibrational fingerprints versus those of BCR, BCR−TEG, BCR−PEG, and BCR−PEG-amino acid decreased from 100% to under 40% (Table 3). Despite this decrease in overall similarity, the reliability of the barcoding strategy remained uncompromised. This analysis shows also that not all the vibrations need to be present for a barcode to be identified and assigned to a bead, a unique combination of 30−40% of the bands appears to be sufficient. For the same reason, the

Table 3. Average *S* Values along the Diagonals of Tables 7−62 (Supporting Information), Reflecting the Similarity Level between the Raman and Infrared Vibrational Spectra of Various Resin Series[a]

	BCR	BCR–TEG	BCR–PEG	BCR–PEG -FmocGly	BCR–PEG -FmocPhe	BCR–PEG -peptide before SAP	BCR–PEG -peptide after SAP
BCR	1.00 ± 0.00 (1.00 ± 0.00)	0.88 ± 0.05 (0.70 ± 0.04)	0.54 ± 0.07 (0.44 ± 0.05)	0.48 ± 0.06 (0.34 ± 0.04)	0.48 ± 0.06 (0.38 ± 0.05)	0.65 ± 0.08 (0.38 ±0.04)	0.66 ± 0.10 (0.54 ± 0.06)
BCR−TEG	−	1.00 ± 0.00 (1.00 ± 0.00)	0.57 ± 0.07 (0.52 ±0.08)	0.50 ± 0.06 (0.40 ± 0.07)	0.50 ± 0.06 (0.44 ± 0.07)	0.68 ± 0.09 (0.44 ± 0.05)	0.69 ± 0.11 (0.64 ± 0.09)
BCR−PEG	−	−	1.00 ± 0.00 (1.00 ± 0.00)	0.73 ±0.14 (0.60 ± 0.08)	0.70 ± 0.16 (0.66 ± 0.06)	0.60 ± 0.12 (0.48 ± 0.07)	0.55 ± 0.07 (0.55 ±0.10)
BCR−PEG -FmocGly	−	−	−	1.00 ± 0.00 (1.00 ± 0.00)	0.82 ± 0.15 (0.77 ± 0.11)	0.57 ± 0.10 (0.44 ± 0.05)	0.51 ±0.06 (0.47 ± 011)
BCR−PEG -FmocPhe	−	−	−	−	1.00 ± 0.00 (1.00 ± 0.00)	0.58 ± 0.09 (0.47 ± 0.06)	0.51 ± 0.07 (0.54 ± 0.11)
BCR−PEG-peptide before SAP	−	−	−	−	−	1.00 ± 0.00 (1.00 ± 0.00)	0.76 ± 0.10 (0.58 ± 0.09)
BCR−PEG -peptide after SAP	−	−	−	−	−	−	1.00 ± 0.00 (1.00 ± 0.00)

[a] Values in parentheses were derived from the infrared similarity tables.[23]

appearance of vibrations unique to the material loaded on the resin is not an issue. In extreme cases, however, where the resin's vibrational fingerprint may be severely altered, the release of the support-bound compound or even degradation of the PEG chain under strongly acidic conditions would unveil the underlying barcode.

On the basis of the similarity trend between BCR and BCR−TEG, BCR−PEG, and BCR−PEG-amino acid, one may anticipate that hexapeptide synthesis would induce further decrease in overall similarity with the parent BCR. Counterintuitively, an apparent improvement of the average similarity between BCR and BCR−PEG-AFGHPQ was observed (first row in Table 3). It is known that long PEG chains feature strong Raman and infrared vibrations as a result of their ordered helical structure, whereas short or amorphous PEG chains display broad and weaker bands.[40] We propose, therefore, that the enhancement observed is due to an attenuation of PEG's vibrations in BCR−PEG-AFGHPQ which could be the result of an induced disorder attributed to the hexapeptide. Alternatively or concurrently, BCR−PEG-AFGHPQ may have a higher propensity to retain solvent molecules and salts (from the buffer), thereby destabilizing PEG's secondary structure and resulting in broadening/attenuation of the PEG vibrations.

Conclusions

Encoded combinatorial chemistry[1] emerged over the past decade as a strategy for tracking the chemical identity of individual compounds in a chemical library, the main goal being that large numbers of compounds can be tested simultaneously and only those with the desired properties would be decoded. There are two main approaches to accomplish this. The first relies on spatial segregation on a 2D matrix, wherein each library member is identified by its (*x*,*y*) coordinates. The second relies on microcarriers bearing each a unique compound along with its encoding element. While the first approach reached the market rapidly, its scope is limited to a few classes of compounds and chemistries, namely DNA,[41] protein and peptides,[42] presynthesized small molecules,[43] and inorganic/organic materials microarrays.[44] The second approach benefits from the multitude of microcarriers available, their amenability to split-pool synthesis,[4] and their compatibility with a broad spectrum of encoding/code readout strategies.[14f,i,28,29,31,32,45−57] The microcarriers can be encoded during library synthesis by adding a detectable chemical tag at each synthesis cycle that encodes for that particular step (parallel encoding approach). Alterna-

(40) (a) Koenig, J. L.; Angood, A. C. *J. Polym. Sci.* **1970**, *8*, 1787−1961. (b) Maxfield, J.; Shepherd, I. W. *Polymer* **1975**, *16*, 505−509. (c) Kuptsov, A. H.; Zhizhin, G. N. *Handbook of Fourier Transform Raman and Infrared Spectra of Polymers*; Elsevier: New York, 1998; pp 116−120.

(41) (a) Stoll, D.; Templin, M. F.; Schrenk, M.; Traub, P. C.; Vohringer, C. F.; Joos, T. O. *Front. Biosci.* **2002**, *7*, C13−C32. (b) Lockhart, D. J.; Winzeler, E. A. *Nature* **2000**, *405*, 825−836. (c) Brown, P. O.; Botstein, D. *Nat. Genet.* **1999**, *21 (Suppl. 1)*, 33−37. (d) Case-Green, S. C.; Mir, K. U.; Pritchard, C. E.; Southern, E. M. *Curr. Opin. Chem. Biol.* **1998**, *2*, 404−410. (e) Chee, M.; Yang, R.; Hubbell, E.; Berno, A.; Huang, X. C.; Stern, D.; Winkler, J.; Lokhart, D. J.; Morris, M. S.; Fodor, S. P. A. *Science* **1996**, *274*, 610−614. (f) Southern, E. M. *TIG* **1996**, *12*, 110−115. (g) Fodor, S. P. A.; Rava, R. P.; Huang, X. C.; Pease, A. C.; Holmes, C. P.; Adams, C. L. *Nature* **1993**, *364*, 555−556. (h) Pirrung, M. C. *Chem. Rev.* **1997**, *97*, 473−488. (i) Pease, A. C.; Solas, D.; Sullivan, E. J.; Cronin, M. T.; Holmes, C. P.; Fodor, S. P. A. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 5022−5026. (j) McGall, G. H.; Barone, A. D.; Diggelmann, M.; Fodor, S. P. A.; Gentalen, E.; Ngo, N. *J. Am. Chem. Soc.* **1997**, *119*, 5081−5090. (k) Pirrung, M. C.; Bradley, J.-C. *J. Org. Chem.* **1995**, *60*, 6270−6276. (l) Fodor, S. P. A.; Read, J. L.; Pirrung, M. C.; Stryer, L.; Lu, A. T.; Solas, D. *Science* **1991**, *251*, 767−773. (m) Winzeler, E. A.; Richards, D. R.; Conway, A. R.; Goldstein, A. L.; Kalman, S.; McCullough, M. J.; McCusker, J. H.; Stevens, D. A.; Wodicka, L.; Lockhart, D. J.; Davis, R. W. *Science* **1998**, *281*, 1194−1197.

(42) (a) Wilson, D. S.; Nock, S. *Angew. Chem., Int. Ed.* **2003**, *42*, 494−500. (b) Sundberg, S. A.; Barrett, R. W.; Pirrung, M.; Lu, A. L.; Kiangsoontra, B.; Holmes, C. P. *J. Am. Chem. Soc.* **1995**, *117*, 12050−12057. (c) Holmes, C. P.; Adams, C. L.; Kochersperger, L. M.; Mortensen, R. B.; Aldwin, L. A. *Biopolymers* **1995**, *37*, 199−211. (d) Adams, C. L.; Kochersperger, L. M.; Mortensen, R. B.; Aldwin, L. A.; Holmes, C. P. *Tech. Protein Chem.* **1994**, *5*, 525−532. (e) Kramer, A.; Volkmer-Engert, R.; Malin, R.; Reineke, U.; Schneider-Mergener J. *Pept. Res.* **1993**, *6*, 314−319. (f) Tegge, W.; Frank, R.; Hofmann, F.; Dostmann, W. R. G. *Biochemistry* **1995**, *34*, 10569−10577. (g) Kramer, A.; Shuster, A.; Reineke, U.; Malin, R.; Volkmer-Engert, R.; Landgraf, C.; Schneider-Mergener, J. *Methods (Methods Enzymol.)* **1994**, *6*, 388−395. (h) Jacobs, J. W.; Fodor, S. P. A. *Trends Biotechnol.* **1994**, *12*, 19−26. (i) Malin, R.; Steinbrecher, R.; Jannsen, J.; Semmler, W.; Noll, B.; Johannsen, B.; Frömmel, C.; Höhne, W.; Schneider-Mergener, J. *J. Am. Chem. Soc.* **1995**, *117*, 11821−11822. (j) Frank, R. *J. Biotechnol.* **1995**, *41*, 259−272. (k) Molina, F.; Laune, D.; Pau, B.; Granier, C. *Pept. Res.* **1996**, *9*, 151−155. (l) Pirrung, M. C.; Huang, C.-Y. *Bioconjugate Chem.* **1996**, *7*, 317−321. (m) Brockman, J. M.; Frutos, A. G.; Corn, R. M. *J. Am. Chem.* **1999**, *121*, 8044−8051. (n) Stanková, M.; Wade, S.; Lam, K. S.; Lebl, M. *Pept. Res.* **1994**, *7*, 292−298.

(43) MacBeath, G.; Koehler, A. N.; Schreiber, S. L. *J. Am. Chem. Soc.* **1999**, *121*, 7967−7968.

(44) (a) Danielson, E.; Devenney, M.; Giaquinta, D. M.; Golden, J. H.; Haushalter, R. C.; McFarland, E. W.; Poojary, D. M.; Reaves, C. M.; Weinberg, W. H.; Wu, X. D. *Science* **1998**, *279*, 837−839. (b) Cremer, P. S.; Yang, T. *J. Am. Chem. Soc.* **1999**, *121*, 8130−8231. (c) Baker, B. E.; Kline, N. J.; Treado, P. K.; Natan, M. J. *J. Am. Chem. Soc.* **1996**, *118*, 8721−8722. (d) Wang, J.; Yoo, Y.; Gao, C.; Takeuchi, I.; Sun, X.; Chang, H.; Xiang, X.-D.; Schultz, P. G., *Science* **1998**, *279*, 1712−1714. (e) Briceño, G.; Chang, H.; Sun, X.; Schultz, P. G.; Xiang, X.-D. *Science* **1995**, *270*, 273−275. (f) Xiang, X.-D.; Sun, X.; Briceño, G.; Lou, Y.; Wang, K.-A.; Chang, H.; Wallace-Freedman, W. G.; Chen, S.-W.; Schultz, P. G. *Science* **1995**, *268*, 1738−1740.

tively, the microcarriers can be encoded before the synthesis (pre-encoding approach),[58−64] in which case they must be decoded at each synthetic cycle to keep track of their chemical history (directed sorting strategy).[3a,b,9]

Parallel encoding requires the physical separation of the tags from the microcarrier followed by their analysis to uncover the chemical identity of the encoded material. Common molecular tags include oligonucleotide,[45] halo-aryls,[46] trityls,[47] secondary amines,[48] fluorescent dyes,[49] or peptides.[4d,46c,50] While the detection methods for the elucidation of the codes are generally difficult to automate,[51] they include a broad spectrum of techniques including mass spectrometry,[47,52] high-resolution magic angle spinning[28] and gel phase[14f,i,29] ^{1}H, ^{13}C, and ^{19}F NMR spectroscopies,[53] energy-dispersive X-ray spectroscopy,[54] X-ray photoelectron spectroscopy,[55] infrared and Raman spectroscopies,[31,32,56] and fluorescence spectroscopy.[57] Pre-encoding requires simply matching the microcarrier's preset code with the corresponding library member. The encoding methods in this case include optical,[49,58] colloidal,[10,59] organic[60] and inorganic[61] dye, radio frequency,[3a,b,9] graphical,[62] size,[63] and shape[64] encoding.

Spectroscopic barcoding[8] was recently introduced as a new pre-encoding strategy wherein the resin beads are not just carriers for solid-phase synthesis but are in addition the repository of the synthetic scheme to which they were subjected. The goal of the present study is to establish the potential of this methodology in diversity-oriented library synthesis and

(45) (a) Needles, M. C.; Jones, D. G.; Tate, E. H.; Heinkel, G. L.; Kochersperger, L. M.; Dower, J. W.; Barrett, R. W.; Gallop, M. A. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 10700−10704. (b) Nielsen, J.; Brenner, S.; Janda, K. D. *J. Am. Chem. Soc.* **1993**, *115*, 9812−9813. (c) Brenner, S.; Lerner, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 5381−5383.

(46) (a) Ohlmeyer, M. H. J.; Swanson, R. N.; Dillard, L. W.; Reader, J. C.; Asouline, G.; Kobayashi, R.; Wigler, M.; Still, W. C. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 10922−10926. (b) Dolle, R. E.; Guo, J.; O'Brien, L.; Jin, Y.; Piznik, M.; Bowman, K. J.; Li, W.; Egan, W. J.; Cavallaro, C. L.; Roughton, A. L.; Zhao, Q.; Reader, J. C.; Orlowski, M.; Jacob-Samuel, B.; DiIanni Carroll, C. *J. Comb. Chem.* **2000**, *2*, 716−731. (c) Tan, D. S.; Burbaum, J. J. *Curr. Opin. Drug Discovery Dev.* **2000**, *3*, 439−453. (d) Hwang, S.; Tamilarasu, N.; Ryan, K.; Huq, I.; Richter, S.; Still, W. C.; Rana, T. M. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 12997−13002. (e) Still, W. C. *Acc. Chem. Res.* **1996**, *29*, 155−163. (f) Nestler, H. P.; Bartlett, P. A.; Still, W. C. *J. Org. Chem.* **1994**, *59*, 4723−4724. (g) Baldwin, J. J.; Burbaum, J. J.; Henderson, I.; Ohlmeyer, M. H. J. *J. Am. Chem. Soc.* **1995**, *117*, 5588−5589. (h) Burbaum, J. J.; Ohlmeyer, M. H. J.; Reader, J. C.; Henderson, I.; Dillard, L. W.; Li, G.; Randle, T. L.; Sigal, N. H.; Chelsky, D.; Baldwin, J. J. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 6027−6031. (i) Chabala, J. C. *Curr. Opin. Biotechnol.* **1995**, *6*, 632−639. (j) Chabala, J. C.; Baldwin, J. J.; Burbaum, J. J.; Chelsky, D.; Dillard, R. W.; Henderson, I.; Li, G.; Ohlmeyer, M. H. J.; Randle, T. L.; Reader, J. C.; Rokosz, L.; Sigal, N. H. *Perspect. Drug Discovery Des.* **1994**, *2*, 305−318.

(47) Shchepinov, M. S.; Chalk, R.; Southern, E. M. *Tetrahedron* **2000**, *56*, 2713−2724.

(48) (a) Ni, Z.-J.; Maclean, D.; Holmes, C. P.; Murphy, M. M.; Ruhland, B.; Jacobs, J. W.; Gordon, E. M.; Gallop, M. A. *J. Med. Chem.* **1996**, *39*, 1601−1608. (b) Liu, X.; Takahashi, L. H.; Fitch, W. L.; Rozing, G.; Bayle, C.; Couderc, F. *J. Chromatogr., A.* **2001**, *924*, 323−329. (c) Fitch, W. L.; Baer, T. A.; Chen, W.; Holden, F.; Holmes, C. P.; Maclean, D.; Shah, N.; Sullivan, E.; Tang, M.; Waybourn, P. *J. Comb. Chem.* **1999**, *1*, 188−194. (d) Maclean, D.; Schullek, J. R.; Murphy, M. M.; Ni, Z.-J.; Gordon, E. M.; Gallop, M. A. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 2805−2810. (e) Scott, R. H.; Barnes, C.; Gerhard, U.; Balasubramanian, S. *Chem. Commun.* **1999**, 1331−1332. (f) Atuegbu, A.; Maclean, D.; Nguyen, C.; Gordon, E. M.; Jacobs, J. W. *Bioorg. Med. Chem.* **1996**, *4*, 1097−1106. (g) St. Hilaire, P. M.; Lowary, T. L.; Meldal, M.; Bock, K. *J. Am. Chem. Soc.* **1998**, *120*, 13312−13320.

(49) Egner, B. J.; Rana, S.; Smith, H.; Bouloc, N.; Frey, J. G.; Brocklesby, W. S.; Bradley, M. *Chem. Commun.* **1997**, 735−736.

(50) (a) Nokolaiev, V.; Stierandova, A.; Krchnak, V.; Seligmann, B.; Lam, K. S.; Salmon, S. E.; Lebl, M. *Pept. Res.* **1993**, *6*, 161−170. (b) Kerr, J. M.; Banville, S. C.; Zuckermann, R. N. *J. Am. Chem. Soc.* **1993**, *115*, 2529−2531. (c) Cheung, Y. W.; Abell, C.; Balasubrmanian, S. *J. Am. Chem. Soc.* **1997**, *119*, 9568−9569. (d) Salmon, S. E.; Lam, K. S.; Lebl, M.; Kandola, A.; Khattri, P. S.; Wade, S.; Patek, M.; Kocis, P.; Krchnák, V.; Thorpe, D.; Felder, S. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 11708−11712. (e) Krchnák, V.; Weichsel, A. S.; Cabel, D.; Lebl, M. *Pept. Res.* **1995**, *4*, 198−205. (f) Liu, R.; Marik, J.; Lam, K. S. *J. Am. Chem. Soc.* **2002**, *124*, 7678−7680. (g) Vagner, J.; Barany, G.; Lam, K. S.; Krchnák, V.; Sepetov, N. F.; Ostrem, J. A.; Strop, P.; Lebl, M. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 8194−8199. (h) Lebl, M.; Krchnák, V.; Sepetov, N. F.; Seligmann, B.; Strop, P.; Felder, S.; Lam, K. S. *Biopolymers (Pept. Sci.)* **1995**, *37*, 177−198.

(51) Czarnik, A. W. *Curr. Opin. Chem. Biol.* **1997**, *1*, 60−66.

(52) (a) Brummel, C. L.; Lee, I. N.; Zhou, Y.; Benkovic, S. J.; Winograd, N. *Science* **1994**, *264*, 399−402. (b) Zambias, R. A.; Boulton, D. A.; Griffin, P. R. *Tetrahedron Lett.* **1994**, *35*, 4283−4286. (c) Youngquist , R. S.; Fuentes, G. R.; Lacey, M. P.; Keough, T. *J. Am. Chem. Soc.* **1995**, *117*, 3900−3906. (d) Brummel, C. L.; Vickerman, J. C.; Carr, S. A.; Hemling, M. A.; Roberts, J. D.; Johnson, W.; Weinstock, J.; Gaitanopoulos, D.; Benkovic, S. J.; Winograd, N. *Anal. Chem.* **1996**, *68*, 237−242. (e) Egner, B. J.; Cardno, M.; Bradley, M. *Chem. Commun.* **1995**, *21*, 2163-2164. (f) Egner, B. J.; Langley, G. J.; Bradley, M. *J. Org. Chem.* **1995**, *60*, 2652−2653. (g) Geysen, H. M.; Wagner, C. D.; Bodnar, W. M.; Markworth, C. J.; Parke, G. J.; Schoenen, F. J.; Wagner, D. S.; Kinder, D. S. *Chem. Biol.* **1996**, *3*, 679−688. (h) Lane, S. J.; Pipe, A. *Rapid Commun. Mass Spectrom.* **2000**, *14*, 782−793. (i) Walk, T. B.; Trautwein, A. W.; Richter, H.; Jung, G. *Angew. Chem., Int. Ed.* **1999**, *38*, 1763−1765. (j) Chu, Y.-H.; Kirby, D. P.; Karger, B. L. *J. Am. Chem. Soc.* **1995**, *117*, 5419−5420. (k) Pomerantz, S. C.; McCloskey, J. A.; Tarasow, T. M.; Eaton, B. E. *J. Am. Chem. Soc.* **1997**, *119*, 3861−3867. (l) Winograd, N.; Braun, R. N. *Spectroscopy* **2001**, *16*, 14−27. (m) Enjalbal, C.; Maux, D.; Combarieux, R.; Martinez, J.; Aubagnac, J.-L. *J. Comb. Chem.* **2003**, *5*, 102−109.

(53) (a) Hochlowski, J. A.; Whittern, D. N.; Sowin, T. J. *J. Comb. Chem.* **1999**, *1*, 291−293. (b) Pirrung, M. C.; Park, K. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2115−2118. (c) Stones, D.; Miller, D. J.; Beaton, M. W.; Rutherford, T. J.; Gani, D. *Tetrahedron Lett.* **1998**, *39*, 4875−4878. (d) Svensson, A.; Bergquist, K.-E.; Fex, T.; Kihlberg, J. *Tetrahedron Lett.* **1998**, *39*, 7193−7196.

(54) Neilly, J. P.; Hochlowski, J. E. *Appl. Spectrosc.* **1999**, *53*, 74−81.

(55) Yoo, S. E.; Gong, Y.-D.; Seo, J.-S.; Sung, M. M.; Lee, S. S.; Kim, Y. *J. Comb. Chem.* **1999**, *3*, 177−180.

(56) (a) Rahman, S. S.; Busby, D. J.; Lee, D. C. *J. Org. Chem.* **1998**, *63*, 6196−6199. (b) Hochlowski, J.; Whittern, D.; Pan, J.; Swenson, R. *Drugs Future* **1999**, *24*, 539−554. (c) Pivonka, D. E. *J. Comb. Chem.* **2000**, *2*, 33−38. (d) Deben, I. E. A.; van Wijk, T. H. M.; van Doornum, E. M.; Hermkens, P. H. H.; Kellenbach, E. R. *Mikrochim. Acta* **1997**, *14*, 245−246. (e) Chan, T. Y.; Chen, R.; Sofia, M. J.; Smith, B. C.; Glennon, D. *Tetrahedron Lett.* **1997**, *38*, 2821−2824.

(57) (a) Scott, R. H.; Balasubramanian, S. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1567−1572. (b) Yan, B.; Martin, P. C.; Lee, J. *J. Comb. Chem.* **1999**, *1*, 78−81.

(58) Sebestyen, F.; Szendrei, G.; Mark, M.; Doda, M.; Illiyes, E.; Szokan, G.; Kindla, K.; Rapp, W.; Szego, P.; Campian, E.; Furka, A. *J. Pept. Sci.* **1998**, *4*, 294−299.

(59) (a) Trau, M.; Battersby, B. J. *Adv. Mater.* **2001**, *13*, 975−979. (b) Battersby, B. J.; Lawrie, G. A.; Trau, M. *Drug Discovery Today* **2001**, *6 (HTS Suppl.)*, 19−26. (c) Battersby, B. J.; Bryant, D.; Meutermans, W.; Matthews, D.; Smythe, M. L.; Trau, M. *J. Am. Chem. Soc.* **2000**, *122*, 2138−2139. (d) Battersby, B. J.; Lawrie, G. A.; Johnston, A. P. R.; Trau, M. *Chem. Commun.* **2002**, 1435−1441. (e) Grøndahl, L.; Battersby, B. J.; Bryant, D.; Trau, M. *Langmuir* **2000**, *16*, 9709−9715.

(60) (a) Fulton, R. J.; McDade, R. L.; Smith, P. L.; Kienker, L. J.; Kettman, K. R., Jr. *Clin. Chem.* **1997**, *43*, 1749−1756. (b) Kettman, J. R.; Davies, T.; Chandler, T.; Oliver, K. G.; Fulton, R. J *Cytometry* **1998**, *33*, 234−243. (c) Prabhakar, U.; Eirikis, E.; Davis, H. M. *J. Immunol. Methods* **2002**, *260*, 207−218. (d) Martins, T. B. *Clin. Diagn. Lab. Immun.* **2002**, *9*, 41−45. (e) Ye, F.; Li, M. S.; Taylor, J. D.; Nguyen, Q.; Colton, H. M.; Casey, W. M.; Wagner, M.; Weiner, M. P.; Chen, J. *Hum. Mutat.* **2001**, *17*, 305−316. (f) Taylor, J. D.; Briley, D.; Nguyen, Q.; Long, K.; Iannone, M. A.; Li, M. S.; Ye, F.; Afshari, A.; Lai, E.; Wagner, M.; Chen, J.; Weiner, M. P. *Biotechniques* **2001**, *30*, 661−669. (g) Yang, L.; Tran, D. K.; Wang, X. *Genome Res.* **2001**, *11*, 1888−1898. (h) Dunbar, S. A.; Jacobson, J. W. *Clin. Chem.* **2000**, *46*, 1498−1500. (i) Vignali, D. A. A. *J. Immunol. Methods* **1999**, *227*, 41−52. (j) Carson, R. T.; Vignali, D. A. A. *J. Immunol. Methods* **2000**, *243*, 243−255. (k) Gordon, R. F.; McDade, R. L. *Clin. Chem.* **1997**, *43*, 1799−1801. (l) WalkerPeach, C. R.; Smith, P. L.; DuBois, D. B.; Fulton, R. J. *Clin. Chem.* **1997**, *43*, 21. (m) Smith, P. L.; WalkerPeach, C. R.; Fulton, R. J.; DuBois, D. B. *Clin. Chem.* **1998**, *44*, 2054−2056. (n) Oliver, K. G.; Kettman, J. R.; Fulton, R. J. *Clin. Chem.* **1998**, *44*, 2057−2060. (o) Bellisario, R.; Colinas, R. J.; Pass, K. A. *Clin. Chem.* **2000**, *46*, 1422−1424. (p) Pantano, P.; Walt, D. R. *Chem. Mater.* **1996**, *8*, 2832−2835. (q) Walt, D. R. *Science* **2000**, *287*, 451−452. (r) Michael, K. L.; Ferguson, J. A.; Healey, B. G.; Panova, A. A.; Pantano, P.; Walt, D. R. In *Polymers in Sensors*; ACS Symposium Series, 690; American Chemical Society: Washington, DC, 1998, pp 273−289. (s) Michael, K. L.; Taylor, L. C.; Schultz, S. L.; Walt, D. R. *Anal. Chem.* **1998**, *70*, 1242−1248. (t) Steemers, F. J.; Ferguson, J. A.; Walt, D. R. *Nat. Biotechnol.* **2000**, *18*, 91−94. (u) Ferguson, J. A.; Steemers, F. J.; Walt, D. R. *Anal. Chem.* **2000**, *72*, 5618−5624. (v) Ferguson, J. A.; Boles, T. A.; Adams, C. P.; Walt, D. R. *Nat. Biotechnol.* **1996**, *14*, 1681−1684. (w) Healy, B. G.; Matson, R. S.; Walt, D. R. *Anal. Biochem.* **1997**, *251*, 270−279. (x) Szurdoki, F.; Michael, K. L.; Walt, D. R. *Anal. Biochem.* **2001**, *291*, 219−228. (y) Keij, J. F.; Steinkamp, J. A. *Cytometry* **1998**, *33*, 318−323. (z) Kürner, J. M.; Klimant, I.; Krause, C.; Pringsheim, E.; Wolfbeis, O. S. *Anal. Biochem.* **2001**, *297*, 32−41.

(61) (a) Bruchez, M.; Maronne, M.; Gin, P.; Weiss, S.; Alivisatos, A. P. *Science* **1998**, *281*, 2013−2016. (b) Han, M.; Gao, X. H.; Su, J. Z.; Nie, S. *Nat. Biotechnol.* **2001**, *19*, 631−635. (c) Alivisatos, A. P. *Sci. Am.* **2001**, *285*, 66−73.

screening, biomedical diagnosis and genomics. Thus, high loading, chemically "inert" polystyrene-*co*-divinylbenzene resins were prepared from six spectroscopically active styrene monomers and subjected to graft copolymerization with ethylene oxide to generate 25 new polystyrene−poly(ethylene glycol) graft copolymers (BCR−PEG) each possessing a unique intrinsic vibrational fingerprint that serves as a spectroscopic barcode for their rapid identification. These resins were characterized by infrared, Raman, and $^{1}H/^{13}C$ NMR spectroscopies and DSC. Their compatibility with biological assays was tested by determining their swelling properties in solvents ranging in polarity from toluene to water, and by subjecting them to peptide synthesis and SAP binding assay. The key result is that their chemical and physical properties were not significantly altered by the chemical diversity of the polystyrene core. Central to the success of this methodology is to reliably read and assign the resins' vibrational fingerprint, particularly after library synthesis and screening. To simulate these conditions we used two methods to investigate several derivatives of the BCRs, (BCR−TEG, BCR−PEG, BCR−PEG-FmocGly, BCR−PEG-FmocPhe, and BCR−PEG-AFGHPQ before and after SAP assay). The first method consisted in enumerating the vibrations of the parent BCRs that appeared in subsequent derivatives of this polymer. This comparison shows that neither PEG, PEG-amino acid or PEG-AFGHPQ which make up ∼65, 68, and 71% of the respective resins' weight, did alter the vibrational fingerprint of the BCR core. We anticipate, therefore that a small-molecule library prepared on BCR, BCR−TEG, or BCR−PEG would have a marginal effect on the barcoding strategy. The second method consisted in calculating the level of similarity between resin pairs. The 35 000 similarity coefficient database generated showed that any of the resins prepared could be unequivocally identified even after SAP binding assay.

Because of the amenability of the BCRs to directed sorting strategies,[3a,b,9] the synthetic steps common to all the beads or any subset thereof can be combined, thereby reducing the overall synthetic effort (e.g. by using split-mix synthesis[4]). As a result, each individual compound or group of compounds could be synthesized on a bead characterized by a unique barcode. Directed sorting, bead loading (up to 1 mmol/g), and size (100−1000 μm)[65] offer the possibility to control the amount of each synthetic intermediate and library member. As a result, routine spectroscopic characterizations at any stage of the library synthesis as well as on-bead or solution-phase biological evaluations could be carried out. Read-out of the barcodes can be done using single-bead microspectroscopy[23,32,33] or can be dramatically speeded up by using hyperspectral imaging of dozens of beads simultaneously.[32d,e] Bead synthesis can be automated at the laboratory scale to produce at least 50 BCRs per day in 25 g batches,[22c] and more in a production setting. The beads' pore size can be readily controlled between 5 and 500 nm[66] to improve on-bead biological assays.[18d,21k,36]

A particularly attractive aspect of these resins is their potential use in "semi-dynamic" combinatorial chemistry.[67] For instance, a dynamic library with two diversity inputs can be readily deconvoluted if each of the diversity positions is immobilized on BCRs and subjected separately to the dynamic selection in the presence of the complementary diversity inputs. Identification of the most active elements at each position, via barcode read-out of the active beads, would result in a subset of optimal building blocks. Combinatorial association of these elements, using solution-phase parallel synthesis or directed synthesis on BCRs, would then result in a focused library of hits from which the most active compounds can be rapidly identified. Work to establish this concept is underway in our laboratories.

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Supporting Information Available: Procedures for the preparation of BCR, BCR−TEG, BCR−PEG, BCR−PEG-FmocGly, BCR−PEG-FmocPhe, BCR−PEG-AFGHPQ. Procedures for swellability, $^{1}H/^{13}C$ NMR, DSC, SAP binding assay. Raman and IR peak assignment tables. Similarity tables. Raman and IR similarity contour plots. Raman and infrared spectra of all the resins synthesized. $^{1}H/^{13}C$ NMR and DSC endotherms of all the BCR−PEG resins (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(62) (a) Houghten, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 5131−5135. (b) Xiao, X.; Zhao, C.; Potash, H.; Nova, M. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 780−782. (c) Zhou, H. H.; Roy, S.; Schulman, H.; Natan, M. J. *Trends Biotechnol.* **2001**, *19 (Suppl.)*, 34−39. (d) Martin, B. R.; Dermody, D. J.; Reiss, B. D.; Fang, M.; Lyon, L. A.; Natan, M. J.; Mallouk, T. E. *Adv. Mater*. **1999**, *11*, 1021−1025. (e) Nicewarner-Peña, S. R.; Freeman, R. G.; Reiss, B. D.; He, L.; Peña, D. J.; Walton, I. D.; Cromer, R.; Keating, K. D.; Natan, M. J. *Science* **2001**, *294*, 137−141. (f) Wedekind, P.; Kubitscheck, U.; Heinrich, O.; Peters, R. *Biophys. J.* **1996**, *71*, 1621−1632.

(63) (a) McHugh, T. M.; Miner, R. C.; Logan, L. H.; Stites, D. P. *J. Clin. Microbiol.* **1988**, *26*, 1957−1961. (b) Scillian, J. J.; McHugh, T. M.; Busch, M. P.; Tam, M.; Fulwyler, M. J.; Chien, D. Y.; Vyas, G. N. *Blood* **1989**, *73*, 2041−2048.

(64) Vaino, A. R.; Janda, K. D. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 7692−7696.

(65) (a) Ahmed, S. M. *J. Dispersion Sci. Technol.* **1984**, *5*, 421−432. (b) Kangwansupamonkon, W.; Damronglerd, S.; Kiatkamjornwong, S. *J. Appl. Polym. Sci.* **2002**, *85*, 654−669.

(66) (a) Labadie, J. W. *Curr. Opin. Chem. Biol.* **1998**, *2*, 346−352. (b) Arshady, R. *J. Chromatogr.* **1991**, *586*, 199−219. (c) Galia, M.; Svec, F.; Frechet, J. M. J. *J. Polym. Sci., Part A: Polym. Chem.* **1994**, *32*, 2169−2175. (d) Liang, Y.-C.; Svec, F.; Frechet, J. M. J. *J. Polym. Sci., Part A: Polym. Chem.* **1997**, *35*, 2631−2643. (e) Wang, Q. C.; Svec, F.; Frechet, J. M. J. *J. Polym. Sci., Part A: Polym. Chem.* **1994**, *32*, 2577−2588. (f) Lewandowski, K.; Svec, F.; Frechet, J. M. J. *J. Appl. Polym. Sci.* **1998**, *67*, 597−607.

(67) (a) Ramstrom, O.; Lehn, J.-M. *Nat. Rev. Drug Discovery* **2002**, *1*, 26−36. (b) Lehn, J.-M.; Eliseev, A. V. *Science* **2001**, *291*, 231−232. (c) Karan, C.; Miller, B. L. *J. Am. Chem. Soc.* **2001**, *123*, 7455−7456. (d) Hof, F.; Nuckolls, C.; Rebek, J., Jr. *J. Am. Chem. Soc.* **2000**, *122*, 4251−4252. (e) Rowan, S. J.; Cantrill, S. J.; Cousins, G. R. L.; Sanders, J. K. M.; Stoddart, J. F. *Angew. Chem., Int. Ed.* **2002**, *41*, 899−952. (f) Calama, M. C.; Timmerman, P.; Reinhoudt, D. N. *Angew. Chem., Int. Ed.* **2000**, *39*, 755−758. (g) Linton, B.; Hamilton, A. D. *Curr. Opin. Chem. Biol.* **1999**, *3*, 307−312.