

Supporting Information for:

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

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Materials. Benzoyl peroxide (BPO), poly(vinylalcohol) (PVA), sodium dodecylbenzene sulfonate (DBS), 80% divinylbenzene (DVB), and all the monomers were purchased from Aldrich. The co-monomers were distilled under reduced pressure to remove the stabilizers and then stored at +4°C. Wang linker, 4-(hydroxymethyl)phenol, was crystallized from distilled water and dried under high vacuum before use. Anhydrous N,N-dimethylacetamide (DMA), sodium methoxide and all the other reagents were used as received from Aldrich. The other solvents: N,N-dimethylformamide (DMF), dichloromethane (DCM), 1,4-dioxane, toluene, ethanol, tetrahydrofuran (THF), and dimethylsulfoxide (DMSO) were distilled prior to use. An IKA-RW20 mechanical motor was used to maintain and control the stirring speed of the suspension polymerization reaction. The reaction vessels and impellers were designed according to the literature.¹ In a few cases a standard Morton flask (ChemGlass) was used as a reactor. FTIR spectra were recorded on a Perkin Elmer 2000 FTIR spectrometer. The beads and KBr were thoroughly mixed and the mixture was pressed to form a pellet, then the spectra were recorded. Single bead Raman spectra were recorded on a home-made Raman micro-imaging system.²

Suspension polymerizations. The micro-spherical beads were prepared by suspension co-polymerization following reported procedures.^{1,3} Typically, deionized water (200 mL), 10% (w/w) PVA/H₂O (4 g) were placed in the reaction vessel equipped with a mechanical stirrer, condenser and N₂ inlet. The reaction was kept under N₂ atmosphere throughout the entire polymerization process. An organic solution composed of co-monomers (for amounts see Table 1 of main text), DVB (0.125 g), chloromethylstyrene (0.50 g), BPO (0.15 g) was added to the reaction vessel. The mixture was stirred at a fixed speed (330 rpm) to produce the desired bead size distribution. The reactor was then immersed in a preheated oil bath maintained at 80°C. After 24 h, the motor was stopped and the beads formed were filtered and washed with deionized water and extracted with water and ethanol using Soxhlet extractors (24 h each). The beads were then sieved and dried under vacuum and characterized by FTIR and Raman spectroscopies, scanning electron microscopy and atomic force microscopy. Figure S1 summarizes the bead size distribution of the 24 barcoded resins synthesized. Under the above suspension polymerization conditions the main fraction

is in the range 70-140 mesh. Figure S2 shows that the IR and Raman spectra of the co-polymers is the sum of the spectra of the corresponding homopolymers. Figure S3 shows a typical scanning electron micrograph of the barcoded beads and an atomic force microscopy image of their surface morphology. The average surface roughness of the beads was determined to be 1.5 nm. Besides these surface fluctuations the AFM reveals randomly distributed holes with a depth of about 15 nm and a diameter in the range of 100 nm.

Effect of the dispersing agent (stabilizer) and stirring speed. The synthesis yield and size distribution of the barcoded resins depend on several empirically determined parameters including the reactor design, the ratio of organic to aqueous phase, the rate of mixing (stirring speed), the viscosity of both phases, and on the concentration and chemical nature of the dispersing agent (stabilizer). The latter two parameters have the greatest impact on the suspension polymerization outcome and were, as a result, optimized first. During the suspension polymerization process the microdroplets formed are directly converted into microbeads, which may coagulate as their viscosity increases. To prevent this undesired aggregation, a stabilizer, usually a water-soluble polymer, is added. Some of the key results obtained with PVA in the absence and presence of DBS are presented in Figure S4. The effect of stirring speed on the bead size distribution and overall yield of beaded material (after sieving) is summarized in Figure S5. Note that outside of the range 300-600 rpm the yield of beaded material drops steeply, and within this stirring range the distribution varies with the stirring rate. Based on these results the bead size distribution can be readily tuned to the desired size range.

Chloromethylstyrene incorporation was quantified by potentiometric titration of the resins' chloride content⁴ (Table S1). The resin (0.05-0.1 g) and pyridine (1 mL) were sealed in a 10 mL glass vial and heated to 100°C for 2 hr. the solution was then transferred to a 100 mL beaker and 50% HNO₃ (5 mL) was added. The potentiometric titration was carried out on an Orion 720A potentiometer equipped with an Orion IonPlus selective chloride ion electrode (CIE) and using a standard solution of AgNO₃ (0.0025725 M). The resins' chloride content was derived from the following relationship:

$$\text{Chlorides (mMol / g)} = \frac{V \times M}{W}$$

V = volume of AgNO₃ added to reach the equivalence point

M = concentration of AgNO₃

W = weight of dry beads

Attachment of the Wang linker to the barcoded beads.⁵ 4-(hydroxymethyl)phenol (134 mg), DMA (8 mL) and sodium methoxide (58 mg) were magnetically stirred under nitrogen in a 50 mL 2-necked round-bottom flask. After complete dissolution of the sodium methoxide (~15 min), 800 mg of the resin were added and the mixture was heated to 50°C in an oil bath for 8 hr. The beads were then washed sequentially with 1,4-dioxane (3×30 mL), distilled water/1,4-dioxane (1/1) (3×30 mL), 1,4-dioxane (3×30 mL), and methanol (3×30 mL). The beads were then dried under high vacuum. The chloride content of the resin was measured as described above and when necessary a second coupling with the Wang linker was performed to cover the unreacted sites (Table S1).

(1) Arshady, R.; Ledwith, A. *React. Polym.* **1983**, 159-174.
(2) Fenniri, H.; Hedderich, H. G.; Haber, K. S.; Achkar, J.; Taylor, B.; Ben-Amotz, D. *Angew. Chem. Int. Ed.* **2000**, 39, 4483-4485.

(3) (a) Arshady, R. *J. Macromol. Struct. Rev. Macromol. Chem. Phys.* **1992**, C32, 101-132. (b) Arshady, R. *J. Chromatography*, **1991**, 586, 181-197. (c) Arshady, R. *J. Chromatography*, **1991**, 586, 199-219. (d) Arshady, R.; Kenner, G. W.; Ledwith, A. *J. Polym. Sci.* **1974**, 12, 2017-2025. (e) Arshady, R. *Makromol. Chem.* **1988**, 189, 1295-1303. (f) Borhan, B.; Wilson, J. A.; Gasch, M. J.; Ko, Y.; Kurth, D. M.; Kurth, M. *J. J. Org. Chem.* **1995**, 60, 7375-7378. (g) Lewandowski, K.; Svec, F.; Fréchet, J. M. J. *J. Appl. Polym. Sci.* **1998**, 67, 597-607.

(4) Ma, T.; Rittner, R. C. *Modern Organic Elemental Analysis*, Marcel Dekker Inc., New York, 1979, p. 164.

(5) (a) Wang, S. S. *J. Am. Chem. Soc.* **1973**, 95, 1328-1333. (b) Lu, G.-S.; Mojsos, S.; Tam, J. P.; Merrifield, R. B. *J. Org. Chem.* **1981**, 46, 3433-3436.

Coupling with Fmoc-Gly and Fmoc-Phe. In a peptide synthesis vessel, the resin beads (~100 mg, 0.05 mMol hydroxyl groups based on potentiometric titration with a CIE) were soaked in DMF overnight then the solvent was drained and the resin washed with fresh DMF. A premixed solution of Fmoc-aminoacid (200 μ L of 0.5 M Fmoc-Gly in 1/1 DMF/DCM, or 400 μ L of 0.25 M Fmoc-Phe in 1/1 DMF/DCM, 2 equiv.), DCC (400 μ L of 0.5 M in 1/1 DMF/DCM, 4 equiv), and DMAP (200 μ L, 0.05 M in 1/1 DMF/DCM, 0.2 equiv) were successively added to the resin. The volume was adjusted to 1 mL with DMF/DCM (1/1) and the slurry was shaken for 30 min. The solvent was then drained and the procedure repeated twice. The solvent was removed and the resin washed thoroughly with DMF/DCM (1/1), DMF, DCM, and MeOH in this order (3 $\times$ 10 mL each). The resin was then dried under high vacuum and the level of aminoacid incorporation was determined by quantifying the fulvene-piperidine adduct ($\epsilon^{301\text{ nm}} = 7,800\text{ M}^{-1}\text{cm}^{-1}$) formed upon treatment with 20% piperidine/DMF (see Table S1).⁶ This method yielded a somewhat higher average loading in comparison with the CIE titration method (0.48 ± 0.06 mMol/g versus 0.40 ± 0.07 mMol/g). This apparent discrepancy was attributed to the intrinsic nature of the CIE titration method, which involves the release of the chlorides from the resin via nucleophilic displacement with pyridine. A reaction of this nature may not take place in some of the hydrophobic pockets within the bead, thereby resulting in an apparently lower loading.

Swellability. The swelling properties of the beads prior to the attachment of the Wang linker were investigated in DMSO, THF, toluene, ethanol, DMF, DMA, DCM, and 1,4-dioxane (Table S2). The weighed beads were immersed in the solvent until swelling equilibrium was achieved (24 hr). Excess solvent was removed and the solvated beads were weighed. The swelling degree was then derived from the following equation:

$$\text{Swellability (mL / g)} = \frac{(W_{\text{sol}} - W_{\text{d}}) / d_{\text{s}}}{W_{\text{d}}}$$

W_{sol} = solvated weight of the beads

W_{d} = dry weight of the beads

d_{s} = density of the solvent

Effect of the Wang Linker and aminoacids on the resins' Raman and IR barcodes. Figures S6 and S7 show that the linker and aminoacids have no effect on the main features of the IR and Raman spectra of the barcoded beads. (1) Bead #10241; (2) bead #10241 + Wang linker; (3) bead #10241 + Wang linker + Fmoc-Gly; (4) bead #10241 + Wang linker + Fmoc-Phe.

(6) (a) Fields, G. B.; Noble, R. L. *Int. J. Peptide Protein Res.* **1990**, *35*, 161-214. (b) Atherton, A.; Sheppard, R. C., *Solid Phase Peptide Synthesis: A Practical Approach*; Oxford University Press: Oxford, 1989.

Table S1. Loading of the resins in mMol/g measured with the CIE method before and after attaching Wang linker, and after aminoacid coupling to the Wang linker using the fulvene-piperidine adduct method.

Ref No.	Chloride content (mMol/g)			Amino acid loading (mMol/g)		Yield before (and after) sieving
	Prior to coupling with Wang linker	After 1 st coupling with Wang linker	After 2 nd coupling with Wang linker	after coupling with Fmoc-Gly	after coupling with Fmoc-Phe	
10262	0.456	0	–	0.481	0.492	98 (84)
10312	0.445	0	–	0.534*	0.504*	94 (79)
11012	0.411	0	–	0.483*	0.486*	96 (81)
11031	0.352	0	–	0.451*	0.475*	94 (81)
11032	0.373	0	–	0.453*	0.473*	95 (81)
11022	0.400	0	–	0.471	0.531	96 (82)
10311	0.430	0.041	0	0.509	0.414	95 (81)
11214	0.466	0.064	0	0.466	0.477	96 (80)
11011	0.427	0	–	0.529	0.494	95 (82)
10241	0.405	0	–	0.491	0.512	90 (81)
10191	0.392	0.062	0	0.485	0.492	92 (85)
10242	0.340	0	–	0.419*	0.416*	93 (83)
10131	0.406	0	–	0.527	0.505	92 (82)
10142	0.455	0	–	0.483	0.456	95 (82)
10232	0.367	0.048	0	0.484	0.457	93 (82)
10181	0.428	0	–	0.515	0.470	93 (82)
11021	0.377	0	–	0.480*	0.452*	94 (83)
10132	0.357	0.083	0	0.484	0.448	92 (83)
10172	0.338	0	–	0.427	0.436	92 (79)
10202	0.366	0	–	0.467	0.455	92 (80)
10192	0.417	0	–	0.536	0.515	94 (81)
10122	0.412	0	–	0.437	0.427	93 (79)
10212	0.393	0	–	0.485	0.464	93 (80)
10141	0.396	0.057	0	0.531	0.494	94 (81)
Average	0.40 ± 0.07	-	-	0.48 ± 0.06	0.47 ± 0.06	94 ± 4 (82 ± 3)

*Triplicate measurements

Table S2. Swelling properties of the barcoded resins in DMSO, THF, Toluene, DMF, ethanol, DMA, DCM, and 1,4-dioxane.

Ref No.	Swellability (mL/g)							
	DMSO	THF	Toluene	Ethanol	DMF	DMA	DCM	1,4-Dioxane
10262	0.8	7.9	7.1	1.8	4.9	6.0	7.4	6.9
10312	0.9	6.6	6.2	2.4	3.1	4.3	5.2	4.3
11012	0.9	4.2	4.7	2.3	1.5	3.3	4.7	4.4
11031	1.0	5.6	5.4	3.2	1.3	2.9	5.0	4.3
11032	1.1	3.6	4.3	2.7	1.4	2.9	4.9	3.9
11022	0.8	3.7	4.7	2.2	1.5	3.1	4.4	3.6
10311	1.0	4.9	4.8	2.6	1.6	3.3	4.5	3.8
11214	0.8	4.3	4.2	2.5	1.6	2.7	4.2	3.9
11011	1.1	5.6	5.7	3.4	1.5	3.9	5.0	4.2
10241	0.8	6.5	6.1	3.5	2.0	4.7	5.7	5.1
10191	1.1	4.8	4.9	2.2	1.7	3.4	4.7	4.3
10242	0.8	5.0	5.2	2.3	1.5	2.9	4.4	4.0
10131	1.0	5.4	5.4	2.5	1.4	4.3	5.1	4.7
10142	0.8	5.4	5.3	2.7	1.9	3.2	4.9	4.3
10232	1.0	4.6	4.8	2.3	1.4	2.6	4.3	4.0
10181	1.1	5.5	5.4	2.6	1.6	3.6	4.9	4.5
11021	0.9	5.5	5.3	2.7	1.6	3.2	4.9	4.6
10132	0.9	5.7	5.3	2.7	1.6	3.5	5.1	4.7
10172	1.2	5.2	5.1	2.4	1.5	2.6	4.6	4.1
10202	0.9	5.8	5.7	2.9	1.6	3.6	5.0	4.4
10192	0.9	5.1	5.3	2.3	1.4	3.0	4.8	4.3
10122	1.0	4.8	5.0	4.4	1.3	3.0	4.7	4.2
10212	0.9	4.8	4.9	2.3	1.3	2.8	4.3	3.8
10141	0.9	5.4	5.2	2.4	1.6	3.7	5.1	4.8

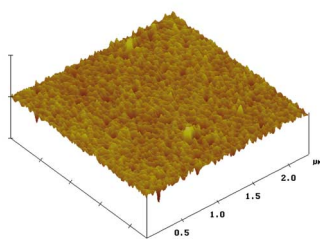
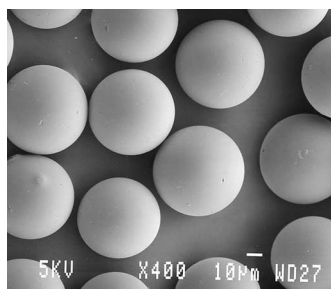
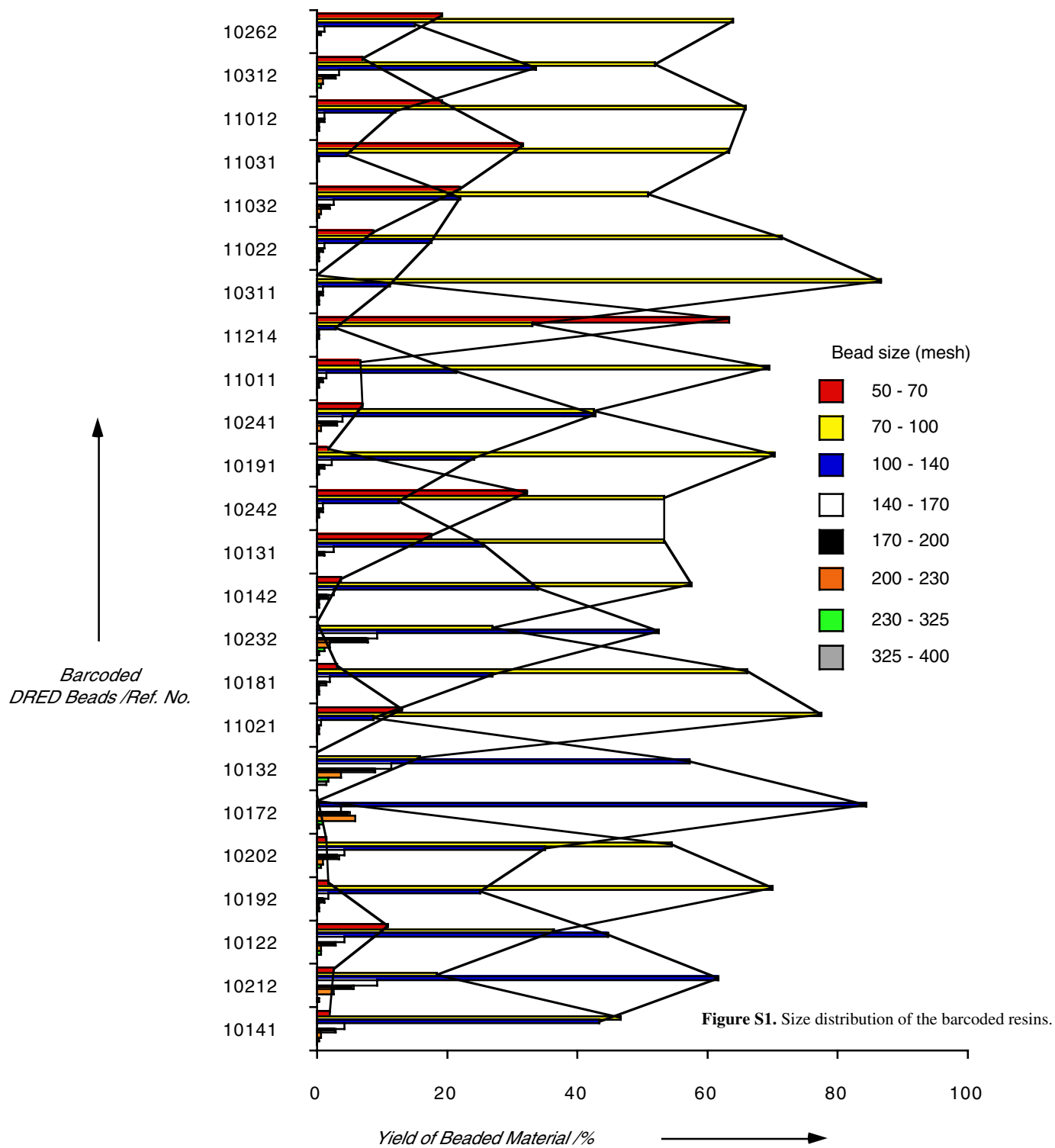


Figure S2. (Left) Representative SEM micrograph of the barcoded beads showing their spherical, smooth and monodisperse nature (scale bar = 10 μm). (Right) Representative tapping-mode atomic force microscopy image of a $2.5 \times 2.5 \mu\text{m}^2$ area on the surface of a barcoded bead. (Height data scale = 50 nm).

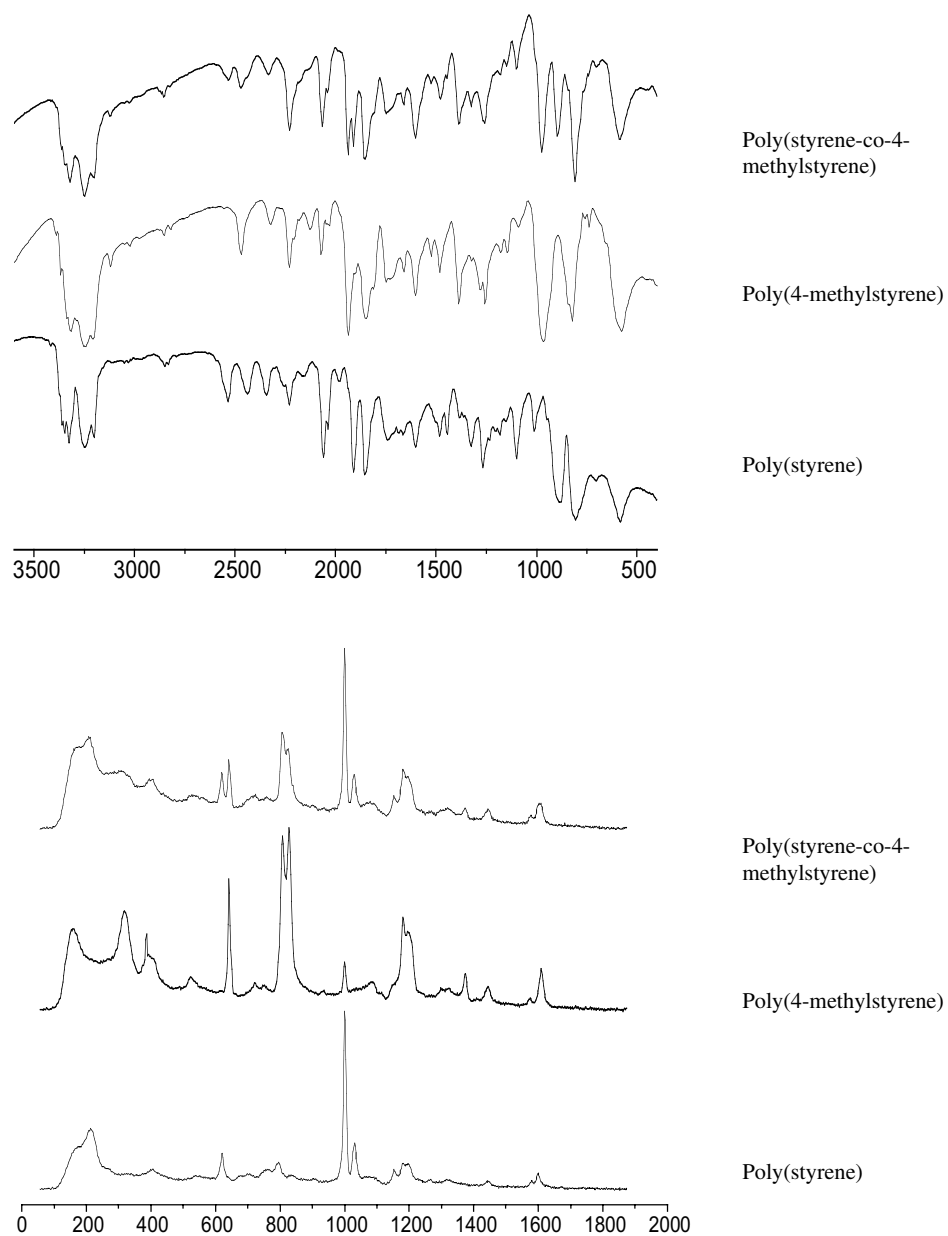


Figure S3. IR (top) and Raman (bottom) spectra of polystyrene (bead #10262), poly(4-methylstyrene) (bead #10241), and poly(styrene-co-4-methylstyrene) (bead #10131) demonstrating the additive nature of the spectra. All the barcoded beads behave similarly.

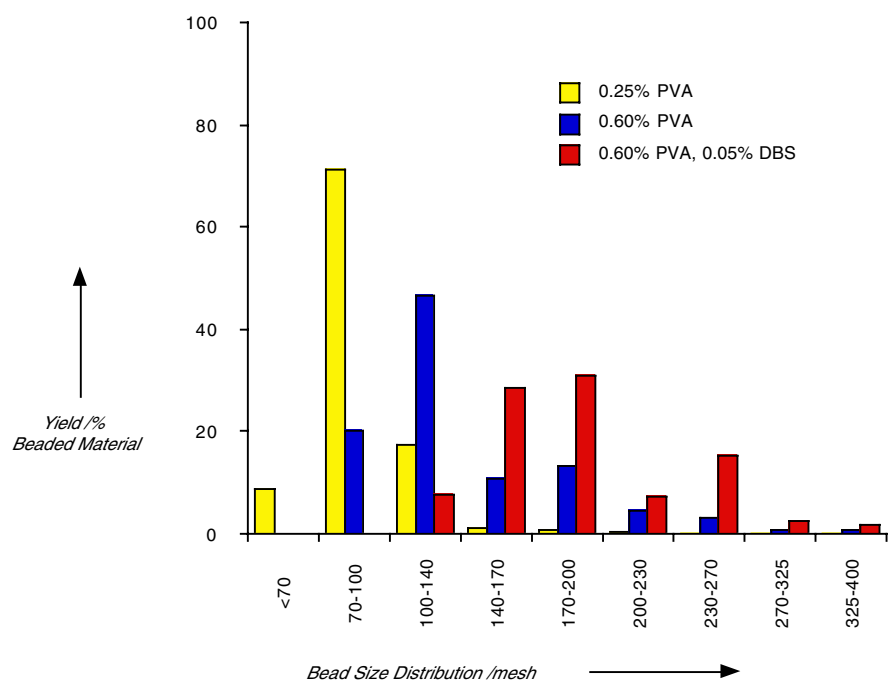


Figure S4. Barcoded beads' size distribution as a function of dispersing agent.

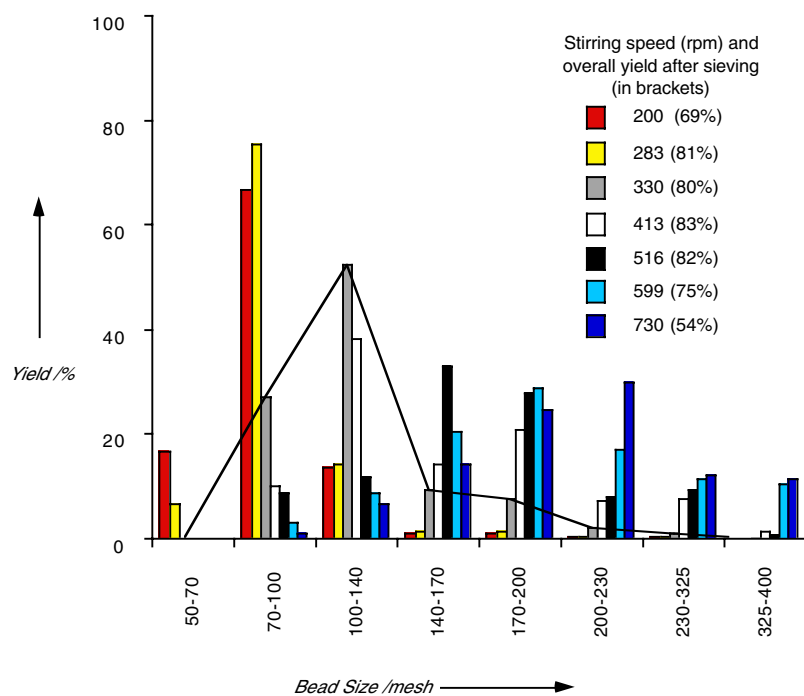


Figure S5. Effect of the stirring speed on the barcoded beads' size distribution and overall yield of beaded material (after sieving).

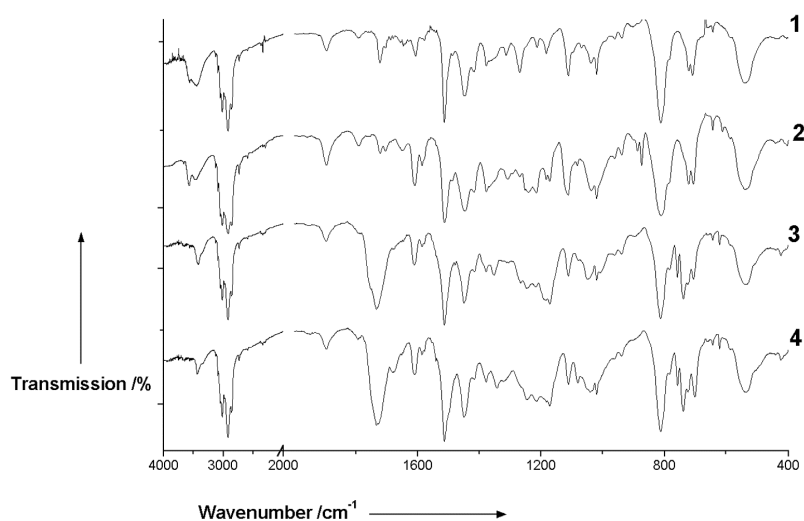


Figure S6. FTIR spectra of (1) bead #10241; (2) bead #10241 + linker; (3) bead #10241 + linker + Fmoc-Gly; (4) bead #10241 + linker + Fmoc-Phe.

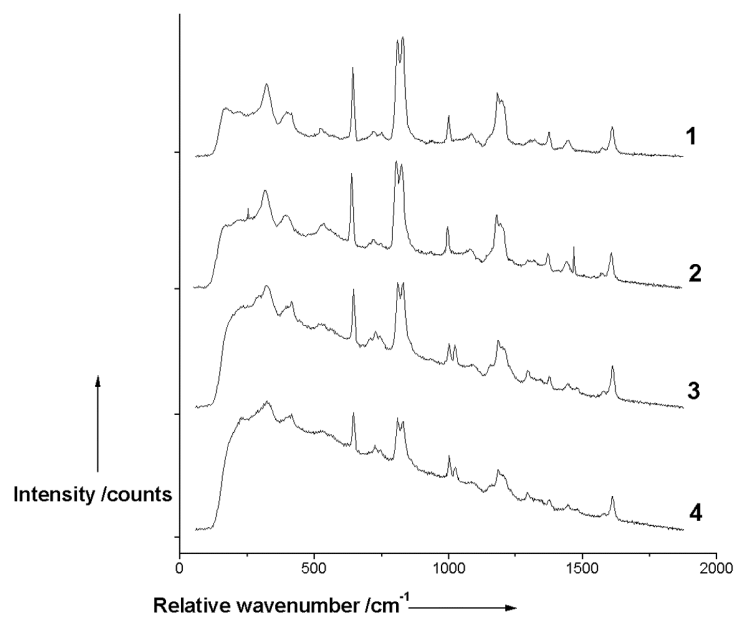


Figure S7. Raman spectra of (1) bead #10241; (2) bead #10241 + linker; (3) bead #10241 + linker + Fmoc-Gly; (4) bead #10241 + linker + Fmoc-Phe.