Using Magnetic Levitation to Distinguish Atomic-Level Differences in Chemical Composition of Polymers, and to Monitor Chemical Reactions on Solid Supports

SUPPORTING INFORMATION

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General Methods. All chemicals and reagents were purchased from Sigma-Aldrich (Atlanta, GA) or EMD Biosciences (San Diego, CA) and used without further purification, unless noted otherwise. A Varian Inova spectrometer operating at 500 or 600 MHz ($^1$H) was used for NMR experiments. The magnets were purchased from K&J Magnetics (www.kjmagnetics.com); comparable magnets can also be obtained from Applied Magnets (www.magnet4less.com) at a lower price.

Derivation of Expression for $z_0$. Polymer beads levitate in the environment depicted in Fig. 1 when the gravitational ($\vec{F}_g$) and magnetic forces ($\vec{F}_{mag}$) acting on the beads balance one another (Eqn. S1). In this equation, $\rho_m$ is the density of the paramagnetic medium (kg·m$^{-3}$), $\rho_s$ is the density of the suspended particle (kg·m$^{-3}$), $V$ is the volume of the particle (m$^3$), $\vec{g}$ is the acceleration due to gravity (m·s$^{-2}$), $\chi_m$ and $\chi_s$ are the magnetic susceptibilities (unitless) of the paramagnetic medium and the suspended particle respectively, $\mu_0$ is the magnetic permeability of free space (T·m·A$^{-1}$), and $\vec{B}$ is applied magnetic field (T).

$$\vec{F}_g + \vec{F}_{mag} = (\rho_s - \rho_m) \vec{g} + \frac{(\chi_m - \chi_s)}{\mu_0} V \vec{B} \cdot \vec{V} \vec{B} = 0$$

(S1)

In a 3D Cartesian coordinate system in which the $z$-axis is aligned with the direction of gravity, the gravitational force balances the magnetic force only along the $z$-axis (Eqn. S2).

$$\left( \rho_s - \rho_m \right) g + \frac{(\chi_m - \chi_s)}{\mu_0} \left( B_x \frac{\partial B_z}{\partial x} + B_y \frac{\partial B_z}{\partial y} + B_z \frac{\partial B_z}{\partial z} \right) = 0$$

(S2)

A computer simulation (COMSOL Multiphysics) of the magnetic field between two identical permanent magnets in the anti-Helmholtz configuration showed that (i) the term
\[
\left( B_x \frac{\partial B_z}{\partial x} + B_y \frac{\partial B_z}{\partial y} \right) \text{ in Eqn. (S2) is much smaller than the term } B_z \frac{\partial B_z}{\partial z} \text{ along the centerline between the magnets and, therefore, can be safely neglected, and (ii) } B_z \text{ varies approximately linearly with the distance from the surface of the bottom magnet, } z \text{ (m) (Eqn. S3).}
\]

\[
B_z = \frac{2B_{0z}}{h} z - B_0 \tag{S3}
\]

By neglecting the \( B_x \frac{\partial B_z}{\partial x} + B_y \frac{\partial B_z}{\partial y} \) term in Eqn. (S2) and by substituting Eqn. (S3), we solve Eqn. (S2) to find the dependence of the levitation height, \( z_0 \) (m)—the equilibrium point between the two magnets where the force of gravity and the magnetic force acting on a particle balance each other—on the density of the particle, \( \rho \) (Eqn. S4).

\[
z_0 = \left[ \frac{g \mu_{0} h^2}{(\chi_s - \chi_m)4B_0^2} \right] \rho + \left[ \frac{h}{2} - \frac{\rho \mu_{0} g h^2}{(\chi_s - \chi_m)4B_0^2} \right] \tag{S4}
\]
**Figure S1.** Photograph of the apparatus used for monitoring solid-supported reactions. The device consists of two 5 cm × 5 cm × 2.5 cm NdFeB magnets oriented with like poles towards each other. The magnets are positioned 4.5 cm apart and held in place within aluminum blocks that have cavities for hosting the magnets. The clusters of beads in the cuvette correspond to the resin-bound starting material (top cluster) and product (bottom cluster) of the condensation reaction shown in Fig. 3. The beads are levitated in a 4 mL quartz cuvette filled with 650 mM GdCl$_3$ in DMF.
**General Procedure for Levitating Polymeric Beads within the Device.** Beads levitate within the device depicted in Fig. S1 when the magnetic and gravitational forces acting on the beads are balanced. The beads levitate below the vertical midpoint between the magnets when \( \rho_s > \rho_m \), and above the midpoint when \( \rho_s < \rho_m \). Beads that sink to the bottom or float to the surface of the liquid at the top of the container have densities of \( \rho_s \gg \rho_m \) or \( \rho_s \ll \rho_m \), respectively. We used these general observations as a guide for selecting optimum conditions for levitation. The density of the beads used throughout this study was within the range of 1.05 – 1.25 g/cm\(^3\), and we found that concentrations of 500 – 800 mM GdCl\(_3\) in H\(_2\)O or DMF were able to levitate most of the beads. For monitoring the progress of the condensation reaction depicted in Fig. 3, we first chose the concentration of GdCl\(_3\) empirically, such that the density of the paramagnetic medium approximately matched the density of the beads. We then fine-tuned the concentration of GdCl\(_3\) to obtain an optimized dynamic range for monitoring this reaction, such that the starting material levitated above and the product below the midpoint of the device.

**General Procedure for Preparing Derivatives (a-j) of 4-Benzylxybenzaldehyde Polystyrene by Reductive Amination.** Into a Poly-Prep chromatography column (Bio-Rad catalog # 731-1550) was added 4-benzylxybenzaldehyde polystyrene resin (0.150 g, 3.5 mmol –CHO/g resin, 35–75 µm), NaCNBH\(_3\) (0.330 g, 5.2 mmol), N,N-dimethylformamide (DMF) (6 mL), acetic acid (0.3 mL, 5.2 mmol), and the corresponding amine or amine hydrochloride (5.2 mmol). The resulting reaction mixture was agitated using a wrist-action shaker (Burrell) at 25 °C for 24 hours. The resin was washed with DMF (3 × 6 mL), CH\(_2\)Cl\(_2\) (3 × 6 mL), MeOH (2 × 6 mL), and H\(_2\)O (2 × 6 mL) and dried under vacuum for at least 30 minutes. An aliquot of each sample of resin tested negative for the presence of aldehydes using 2,4-dinitrophenylhydrazine.\(^2\)
General Procedure for Measuring Densities of Derivatives (a-j) of 4-
Benzyloxybenzaldehyde Polystyrene by Sink-Float Technique. The resin (~1 mg) was
suspended in 0.5 mL of aqueous CaCl$_2$ solution and centrifuged for 2 minutes at 9100 g. The
beads either floated or sedimented in the CaCl$_2$ solution when the density of the beads did not
match the density of solution; the beads, however, remained dispersed evenly throughout the
solution when the density of the beads matched the density of the solution. The centrifugation
procedure was repeated with different sets of beads at different concentrations of CaCl$_2$ until the
concentration of CaCl$_2$ was found that would allow the beads to remain dispersed evenly
throughout the solution. The density of CaCl$_2$ at that point was taken as the density of the resin.
The concentration-density dependence of CaCl$_2$ was obtained from the CRC Handbook of
Chemistry and Physics.

Characterization of Derivatives (a-j) of 4-Benzylxybenzaldehyde Polystyrene by
Elemental Analysis. The percent composition of Carbon, Hydrogen, Nitrogen for each resin
was determined by QTI (Whitehouse, NJ) using a Perkin-Elmer 2400 Elemental Analyzer. To
compare relative amounts of Nitrogen for each resin we normalized the values by dividing the
Nitrogen content by the Carbon content and multiplying by 100%. The similarity between the
N/C ratio for resins functionalized with different small molecules indicates similar loading of
small molecule per bead.
Table S1. Elemental analysis of derivatives (a-j) of 4-benzyloxybenzaldehyde polystyrene resin.

<table>
<thead>
<tr>
<th>Label</th>
<th>% C</th>
<th>% H</th>
<th>% N</th>
<th>(N/C)</th>
<th>N per small molecule on resin</th>
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**Attachment of Leucine to Wang Polystyrene Resin.** To a solution of N-(9-
Fluorenylmethoxycarbonyl)-L-leucine (3.89 g, 11.0 mmol) in anhydrous CH₂Cl₂ (50 mL) was added N,N’-diisopropylcarbodiimide (0.85 mL, 5.5 mmol) at 0 ºC. After stirring at 0 ºC for 15 minutes, the resulting suspension was concentrated under reduced pressure, and then transferred to a glass solid-phase reaction vessel containing Wang polystyrene (0.500 g, 2.2 mmol –OH/g resin, diameter = 75–150 µm), DMF (30 mL), N,N-diisopropylethylamine (0.96 mL, 5.5 mmol), and a catalytic amount of 4-(dimethylamino)pyridine (13 mg, 0.1 mmol). The resulting reaction mixture was agitated using a wrist-action shaker (Burrell) at 25 ºC for 45 minutes. The resin was washed with DMF (3 × 10 mL), CH₂Cl₂ (3 × 10 mL) and the coupling and washing procedure was repeated once more to ensure complete modification of the resin with leucine. The N-(9-
Fluorenylmethoxycarbonyl) protecting group was removed by suspending the resin in 20 % (v/v) piperidine in DMF and agitating the reaction mixture using a wrist-action shaker at 25 ºC for 30 minutes. After deprotection, leucine-functionalized resin was washed with DMF (3 × 10 mL),
CH₂Cl₂ (3 × 10 mL), CH₃OH (2 × 10 mL), and H₂O (2 × 10 mL) and dried under vacuum. The presence of amines was confirmed by staining an aliquot of the beads with ninhydrin⁵.

**Monitoring the Reaction of 2,5-Diiodobenzoic Acid with Leucine-Functionalized Wang Resin.** Leucine-functionalized Wang resin (0.200 g, 1.8 mmol –NH₂/g resin) was placed in a glass container, suspended in 50 mL of DMF, and cooled to 0 °C. To this container was added 2,5-diiodobenzoic acid (0.678 g, 1.8 mmol), O-(Benzotriazol-1-yl)-N,N,N′,N′-tetramethyluronium hexafluorophosphate (0.688 g, 1.8 mmol), and diethylisopropylamine (0.316 mL, 1.8 mmol). The entire mixture was agitated on an orbital shaker, while maintaining the temperature of the reaction at 0 °C. Aliquots (1.5 mL) were withdrawn from the reaction mixture over time, and the resin from each aliquot was washed with DMF (3 × 6 mL) and CH₂Cl₂ (3 × 6 mL), and dried under vacuum for 30 minutes. The beads (~100 beads) from each aliquot were suspended in a quartz cuvette containing 650 mM GdCl₃ in DMF at 25 ºC for 15 minutes to ensure complete swelling of the beads in DMF. The cuvette was placed along the central axis between the two NdFeB magnets for ~15 minutes and the levitation height of the beads was recorded using a ruler with mm-scale markings (the levitation height was measured at the vertical midpoint of each cluster of beads).

The remaining resin from each aliquot was placed into a glass vial and suspended in 0.5 mL of 20% (v/v) trifluoroacetic acid in CH₂Cl₂. This mixture was agitated for 1 hr using a VWR Vortex Analog Mixer at room temperature. The contents of each vial were concentrated under vacuum and dried under reduced pressure for 16 hours. Dichloromethane (2 mL) was added to each vial and the vials were agitated using a VWR Vortex Analog Mixer for 30 minutes to extract the cleaved compound from the resin. Each sample of resin was filtered through a 1 mL
glass Pasteur pipette equipped with a plug of glass wool and washed with CH$_2$Cl$_2$ (2 × 1 mL), H$_2$O (2 × 1 mL), CH$_2$Cl$_2$ (2 × 1 mL), and MeOH (2 × 1 mL). The eluent from each sample was concentrated under reduced pressure, dried under vacuum for 16 hours, and re-dissolved in a 1:1 mixture of CDCl$_3$ and DMSO-d$_6$. The ratio of product to starting material was measured using $^1$H NMR.

The final product of the reaction (N-(2,5-diiodobenzoyl)-L-leucine) was cleaved from the beads using the procedure described above and purified using flash chromatography on silica gel using 1:1 ethyl acetate/dichloromethane. $^1$H NMR (500 MHz, CDCl$_3$, referenced to CDCl$_3$ at 7.26 ppm): δ 7.69 (1H, s), 7.55 (1H, d, J = 8.5 Hz), 7.40 (1H, d, J = 8.0 Hz), 6.26 (1H, d, J = 7.0 Hz), 4.78 (1H, m), 1.84 (2H, m), 1.72 (1H, m), 1.03 (3H, d, J = 6.5 Hz), 1.00 (3H, d, J = 6.0 Hz). $^{13}$C NMR (125 MHz, CDCl$_3$, referenced to CDCl$_3$ at 77.0 ppm): δ 171.2, 167.7, 143.2, 141.4, 140.3, 137.0, 93.6, 91.6, 60.4, 41.1, 25.0, 22.9, 21.9. HRMS (ES) $m/z$ calculated for C$_{13}$H$_{15}$I$_2$NO$_3$ [M+H]$^+$: 487.9214, found: 487.9166.
Figure S2. Representative $^1$H NMR spectra of samples from the reaction of 2,5-diiodobenzoic acid with leucine-functionalized Wang Resin (in 1:1 CDCl$_3$/DMSO-d6).
Figure S3. $^1$H NMR spectrum of N-(2,5-diiodobenzoyl)-L-leucine in CDCl$_3$. 

![NMR spectrum image](image-url)
Measuring Densities of Samples from the Reaction of 2,5-Diiodobenzoic Acid with Leucine-Functionalized Wang Resin by Sink-FLOAT Technique. Densities of samples from this reaction were measured using a method analogous to the one described above. Briefly, the resin (~1 mg) was suspended in 0.5 mL of GdCl$_3$ solution in DMF and centrifuged for 2 minutes at 9100 g. The centrifugation procedure was repeated with different sets of beads at different concentrations of GdCl$_3$ until a concentration of GdCl$_3$ was found that allows the beads to remain dispersed evenly throughout the solution. The density of GdCl$_3$ was measured by weighing a fixed volume of the solution on an analytical balance.

Calculating the Rate of Consumption of Polymer-Bound Amine in the Reaction of 2,5-Diiodobenzoic Acid with Leucine-Functionalized Wang Resin. We calculated the initial concentration $[-\text{NH}_2]_{0\%\text{conversion}}$ per bead using equation S5. In this equation 1.8 mmol/g is the loading level of the resin and 1.06 g/cm$^3$ is the density of the beads as measured by the sink-float technique.

$$[-\text{NH}_2]_{0\%\text{conversion}} = \left(\frac{1.8\text{mmol}}{g}\right)\left(\frac{1\text{mol}}{1000\text{mmol}}\right)\left(\frac{1.06\text{g}}{1000\text{cm}^3}\right)\left(\frac{1\text{L}}{1\text{cm}^3}\right) = 1.9\text{mol/L} \quad (S5)$$

We calculated the value of $[-\text{NH}_2]$ for each time point during the course of the reaction using equation S6 for levitation, where $z$ is the “levitation height”, and equation S7 for NMR, where $\phi$ is the fraction of the starting material remaining on the beads obtained by integration of the $^1\text{H}$ of the NMR spectra.

**levitation:** $[-\text{NH}_2]_{\text{experimental}} = \left(\frac{z_{\text{experimental}} - z_{100\%\text{conversion}}}{z_{0\%\text{conversion}} - z_{100\%\text{conversion}}}\right)[-\text{NH}_2]_{100\%\text{conversion}} \quad (S6)$

**NMR:** $[-\text{NH}_2]_{\text{experimental}} = \phi[-\text{NH}_2]_{10\%\text{conversion}} \quad (S7)$

S12
References: