

## Supporting Information

### Combining Step-Gradients and Linear Gradients in Density

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**Contents:** The Supporting Information contains a detailed Materials and Methods section. Characterizations of the aqueous multiphase systems and the magnetic susceptibility are provided. A description of the methods used to measure the height of levitating beads and to estimate uncertainty in calculated densities is also provided.

## ***Materials and Methods.***

**Chemicals.** We purchased the following chemicals and reagents for experiments in this paper: manganese chloride tetrahydrate (Alfa Aesar), manganese sulfate monohydrate (Ward's Science), polyethylene glycol (PEG) (MW= 20 kDa) (Fluka Analytical), polyethylene glycol (MW = 1.5 kDa) (Fluka Analytical), and dextran (MW = 500 kDa) (Spectrum).

**Materials.** Glass density standard beads with a reported accuracy of 0.0002 g/cm<sup>3</sup> were purchased from American Density Standards. MagLev devices were custom made with machined aluminum and permanent magnets purchased from K&J Magnetics. Solutions in experiments were held in standard, square plastic cuvettes (VWR) used for spectroscopy.

**Preparation of AMPS.** We prepared the PEG-dextran AMPS by adding 5 g of polyethylene glycol (MW = 20 kDa) and 10 g of dextran (MW = 500 kDa) into a 100-mL volumetric flask. We then added 20 mL of a solution of MnCl<sub>2</sub> with a concentration of 1,575 mM. Finally, we added deionized water (MilliQ) and dissolved all the components until a final volume of 100 mL was attained. We prepared the PEG-MnSO<sub>4</sub> AMPS by mixing 5 mL of a stock aqueous solution of 40% (wt/vol) PEG (MW = 1.5 kDa)—that is, 40 g of PEG in 100 mL of total solution—with 7 mL of an aqueous solution of 1,500 mM MnSO<sub>4</sub>. Both systems were mixed vigorously with a vortexer. The PEG-dextran system was centrifuged for 15 minutes at 2,000 g to attain phase separation. The PEG-MnSO<sub>4</sub> system separated on the bench top without centrifugation in under 5 minutes.

**Analysis of Phase Composition in AMPS.** We used gel permeation chromatography (GPC; Agilent PL-GPC 50) to estimate the concentration of polymer in each phase of an AMPS. We used an aqueous mobile phase and two PL aquagel-OH MIXED-M columns arranged in series. We constructed separate standard curves for each polymer present in an AMPS by plotting known concentrations of polymer in % (wt/vol) against peak area. By

noting the elution times of each polymer and comparing the peak areas from the chromatograms of each phase to the corresponding polymer standard curves, we were able to identify in which phase a polymer was either enriched or depleted.

**Density Measurements.** To measure the density of the phases of an AMPS, we first separated the phases of the AMPS. Using 15-mL conical tubes, we centrifuged ~4 mL of AMPS solutions at 2000 *g* for 30 minutes. We then used a blunted pipette tip and a pipettor to remove 1 mL of the top phase without disturbing the interface. A syringe allowed us to puncture the bottom of the tube and drain out 1 mL of the bottom phase without contamination from the top phase. We then used a U-tube densitometer (DMA 35, Anton Paar) to measure the density of each phase with an accuracy of  $\pm 0.001$  g/cm<sup>3</sup>.

**Estimation of Levitation Heights.** We took digital pictures of the levitating glass beads in the MagLev device after beads came to rest. Each picture contained a ruler by the side of the cuvette containing the beads. Using ImageJ, we measured, in pixels, the locations of the upper and lower surfaces of the magnets, the solid/liquid interface between the bottom phase and the container, the liquid/liquid interface of the AMPS, and the air/liquid interface at the meniscus of the top phase. Using the ruler as a reference in the image, we converted pixels to millimeters. As described in the manuscript, we measured the centroid of each bead to use as a levitation height. We traced the outer edge of the bead and calculated the centroid numerically in ImageJ. The resolution of the camera allowed us to determine levitation heights with a precision of ~1% of the height ( $\pm 0.1$  mm).

**Characterization of the Magnetic Field of MagLev Device.** We measured the strength to the magnetic field at the surface of the magnets of the MagLev device using a DC magnetometer (AlphaLab Inc.). The probe measured the maximum field at the center of the magnetic face reproducibly to three significant figures.

**Determination of the Concentration of Manganese in the Phases.** We used flame atomic absorption (FAA) spectroscopy to measure the concentration of manganese in each phase of an AMPS. We constructed a standard curve by plotting known concentrations of manganese in ppm against absorption intensity. Using the equation of the line generated from the plot, we were able to calculate the concentration of manganese in an AMPS.

**Characterization of the Magnetic Susceptibilities by the Evans Method.** We estimated the mass magnetic susceptibilities for the manganese ions in the levitating media by NMR using the Evans method.<sup>1,2</sup> The NMR spectroscopy was performed on a Varian Mercury 400 MHz spectrometer in standard (5 mm diameter, 8" length) NMR tubes (Wilmad LabGlass).

We added the aqueous solution of interest (to which we previously added 2% v/v of dioxane) to a NMR tube containing a capillary insert filled with D<sub>2</sub>O containing 2% v/v of dioxane. We obtained two resonance peaks for dioxane: the solvent in the insert showed the standard values of chemical shift for this compound, while the chemical shift of the solvent in the rest of the tube is affected by the paramagnetic ions in solution. We calculated the effective mass magnetic susceptibility of the manganese ions in the studied solutions using the following equation (Equation S1):

$$\chi_m = \frac{3\delta_\nu}{4\pi\nu_0 m} + \chi_0 + \frac{\chi_0(\rho_0 - \rho_a)}{m} \quad (\text{Equation S1})$$

Where  $\nu_0$  is the frequency of the spectrometer,  $\delta_\nu$  is the observed difference in chemical shift,  $m$  is the mass concentration of the paramagnetic species (in g/cm<sup>3</sup>),  $\chi_0$  is the magnetic susceptibility of the solvent,  $\rho_0$  is the density of the solvent and  $\rho_a$  is the density of the analyte solution. The second and third term correct for the diamagnetism of the solvent and the difference in diamagnetism between the solvent and the analyte solution.

Using the mass magnetic susceptibility and the molecular weight of manganese, we calculated the molar magnetic susceptibility. Combining this with the molar concentration of  $\text{Mn}^{2+}$  measured by FAA provided the volume magnetic susceptibility that we could then use in equation 1 to calculate the density of an object based on its levitation height. The measured magnetic susceptibilities of each phase differed slightly from that of manganese ions in a purely aqueous solution. Using the magnetic susceptibilities calculated by Evans method generally provided better estimates of density when using density-standard glass beads (**Table S-1**).

**Calculation of Density of Levitated Beads.** Solving equation 1 for the density of the levitating object, we find:

$$\rho_s = \rho_m + \left(h - \frac{d}{2}\right) \times \left(\frac{4B_0^2(\chi_s - \chi_m)}{g\mu_0 d^2}\right) \quad (\text{Equation S2})$$

By using diamagnetic objects in paramagnetic media, we assume  $\chi_m \gg \chi_s$  and, therefore, that  $\chi_s - \chi_m \approx -\chi_m$ . We calculate the unitless volume susceptibility as  $\chi_m = \chi_M C$ , where  $\chi_M$  is the molar magnetic susceptibility of the paramagnetic component of the solution as measured by the Evans method,  $C$  is the concentration of  $\text{Mn}^{2+}$  measured by FAA, and  $\chi_w$  is the volume susceptibility of the diamagnetic component of the solution. We make the simplifying assumption that the dominant diamagnetic component of the solution is water, and we use  $9.024 \times 10^{-6}$  as the SI value of  $\chi_w$  based on standard values for water at 20 °C. We assume that the phases of AMPS are predominantly water. Even with this slight overestimation, the change to the calculated density from accounting for diamagnetism is smaller than the overall uncertainty of the estimate.<sup>3</sup> Substituting in only measured and constant variables into equation S1, we have:

$$\rho_s = \rho_m + \left(h - \frac{d}{2}\right) \times \left(\frac{4B_0^2(\chi_w - \chi_M C)}{g\mu_0 d^2}\right) \quad (\text{Equation S3})$$

All numbers are calculated using SI units, but final densities are reported in units of  $\text{g}/\text{cm}^3$ .

**Table S-1.** Comparison of measurements of density by MagLev when calculated using the assumption that the molar magnetic susceptibility of a phase is the same as in aqueous solution versus the measured magnetic susceptibility.

System	Phase	$\chi_M$ ( $10^{-6} \text{ cm}^3/\text{mol}$ )		Density of Beads ( $\text{g}/\text{cm}^3$ )		
		In water	In AMPS	Uncorrected <sup>[a]</sup>	Corrected <sup>[b]</sup>	Claimed <sup>[c]</sup>
PEG-dextran	top	14,786	13,981	$1.040 \pm 0.001$	$1.040 \pm 0.001$	1.040
	bottom	14,786	14,535	$1.120 \pm 0.001$	$1.120 \pm 0.001$	1.120
PEG-MnSO <sub>4</sub>	top	14,786	14,246	$1.078 \pm 0.001$	$1.079 \pm 0.002$	1.081
	bottom	14,786	15,417	$1.248 \pm 0.002$	$1.249 \pm 0.002$	1.250

[a] Densities calculated using the  $\chi_M$  of MnCl<sub>2</sub> in an aqueous solution.

[b] Densities calculated using the  $\chi_M$  measured by Evans method for each solution.

[c] Densities claimed by the manufacturer to a precision of  $0.0002 \text{ g}/\text{cm}^3$ .

**Estimation of Uncertainty in Calculations of Density.** Assuming the variables in equation S2 are independent, we estimate the uncertainty using the standard approximation for the propagation of error:

$$\sigma_{\rho_s} = \sqrt{\left(\sigma_{\rho_m}^2 + \left(\frac{\partial \rho_s}{\partial h}\right)^2 \sigma_h^2 + \left(\frac{\partial \rho_s}{\partial d}\right)^2 \sigma_d^2 + \left(\frac{\partial \rho_s}{\partial B_0}\right)^2 \sigma_{B_0}^2 + \left(\frac{\partial \rho_s}{\partial \chi_M}\right)^2 \sigma_{\chi_M}^2 + \left(\frac{\partial \rho_s}{\partial C}\right)^2 \sigma_C^2\right)}$$

(Equation S4)

Uncertainty for each variable is based on the standard deviation of replicate measurements for  $\chi_M$  ( $n = 7$ ) and  $C$  ( $n = 8$ ), and by measurement uncertainty for  $h$ ,  $d$ ,  $B_0$ , and  $\rho_M$ .

### ***Experimental Details***

**Details of Characterization of each AMPS.** We characterized the composition, density, concentration of manganese ions, and molar magnetic susceptibility for each phase of the AMPS we investigated (**Table S-2**).

**Characterization of Poly(ethylene glycol)–dextran Two-phase System.** We prepared an AMPS from a mixture with a final concentration of 5% (wt/vol) poly(ethylene glycol), 10% (wt/vol) dextran, and 315 mM  $\text{MnCl}_2$ . From our GPC results, we determined that the top phase was enriched for poly(ethylene glycol) and the bottom phase was enriched for dextran. From flame atomic absorption spectroscopy (FAA), we determined that the concentration of  $\text{Mn}^{2+}$  was  $323.0 \pm 3.1$  mM in the poly(ethylene glycol)-rich top phase of the AMPS and  $304.6 \pm 2.5$  mM in the dextran-rich bottom phase of the AMPS. These results confirmed that neither polymer interacts preferentially with  $\text{Mn}^{2+}$ , which establishes a nearly homogenous distribution of paramagnetic ions throughout both phases of the AMPS. We determined the molar magnetic susceptibility of the manganese ions in the two phases of the AMPS by Evans method, and found a value of  $\chi_M = 13,981 \times 10^{-6}$  emu•mol<sup>-1</sup> for the poly(ethylene glycol)-rich top phase of and a value of  $\chi_M = 14,535 \times 10^{-6}$  emu•mol<sup>-1</sup> for the

**Table S-2.** Final compositions and physical properties of AMPS.

<b>System</b>	<b>Phase</b>	<b>Composition</b>	<b>Density (g/cm<sup>3</sup>)</b>	<b>[Mn<sup>2+</sup>] (mM)</b>	<b><math>\chi_M</math> (10<sup>-6</sup> cm<sup>3</sup>/mol)</b>
PEG-dextran	Top	PEG-rich	1.048	323	13,981
	Bottom	dextran-rich	1.104	305	14,535
PEG-MnSO <sub>4</sub>	Top	PEG-rich	1.090	327	14,246
	Bottom	MnSO <sub>4</sub> -rich	1.210	1,496	15,265
Mn <sup>2+</sup> (aq) <sup>[a]</sup>	<i>NA</i>	<i>NA</i>	<i>NA</i>	<i>NA</i>	14,786

[a] measurement based on solutions of MnCl<sub>2</sub> in water

dextran-rich bottom phase. Both values deviate slightly, from the literature value of  $14,786 \times 10^{-6} \text{ emu} \cdot \text{mol}^{-1}$  for aqueous manganese(II) ions.

When one phase of an AMPS is separated and used alone as a paramagnetic solution, the range of densities that can be levitated in a standard cuvette is a function of the density and the magnetic susceptibility of the phase. The range of densities available for both the PEG-rich top phase and the dextran-rich bottom phase are described in **Table S-1**. In an AMPS, each phase occupies a specific region of the cuvette and the range of densities that can be levitated in each phase is limited to the range of densities at the levitation heights occupied by the specific phase (**Table S-3**).

**Characterization of Poly(ethylene glycol)–MnSO<sub>4</sub> Two-phase System.** We prepared a mixture with a final concentration of 16.7% (wt/vol) PEG and 875 mM MnSO<sub>4</sub>. From our GPC results, we determined that top phase was enriched for PEG and the bottom phase was predominantly salt. From flame atomic absorption (FAA) spectroscopy, we determined that the concentration of Mn<sup>2+</sup> was  $326.7 \pm 2.0 \text{ mM}$  in the PEG-rich top phase of the ATPS and  $1.496 \text{ M} \pm 0.012 \text{ M}$  in the bottom phase of the ATPS. These results support a large difference in the concentration of the paramagnetic ion in the two phases to create a large step in the magnetic susceptibility. We applied Evan's method to measure the molar magnetic susceptibility of the manganese ions in both phases, and found  $\chi_M = 14,246 \times 10^{-6} \text{ emu} \cdot \text{mol}^{-1}$  for the poly(ethylene glycol)-rich top phase of and a value of  $\chi_M = 15,265 \times 10^{-6} \text{ emu} \cdot \text{mol}^{-1}$  for the manganese sulfate rich bottom phase.

The concentration of Mn<sup>2+</sup> in the bottom phase of the AMPS is more than four times that of the top phase; correspondingly, the range of densities available in the bottom phase is more than four times the range of densities available in the top phase (**Table S-4**).

**Table S-3.** Values of buoyant densities accessible by magnetic levitation (MagLev) using a poly(ethylene glycol)–dextran aqueous multiphase polymer system (AMPS) containing MnCl<sub>2</sub> compared to the densities accessible by each phase alone. The concentration of Mn<sup>2+</sup> in the top and bottom phases is 323 mM and 305 mM, respectively. The interface of the AMPS marks this step in density and is located at a vertical position of 23 mm within the MagLev device.

paramagnetic medium	phase density (g/cm <sup>3</sup> )	buoyant density (g/cm <sup>3</sup> ) at levitation height		
		45 mm	23 mm / interface	0 mm
top phase only	1.048	1.027	1.048	1.071
bottom phase only	1.104	1.081	1.104	1.129
AMPS		1.027	1.048–1.104	1.129

**Table S-4.** Values of buoyant densities accessible by magnetic levitation (MagLev) using a poly(ethylene glycol)–MnSO<sub>4</sub> aqueous multiphase polymer system (AMPS) compared to the densities accessible by each phase alone. The concentration of Mn<sup>2+</sup> in the top and bottom phases is 327 mM and 1,496 mM, respectively. The interface of the AMPS marks this step in density and is located at a vertical position of 21 mm within the MagLev device.

paramagnetic medium	phase density (g/cm <sup>3</sup> )	buoyant density (g/cm <sup>3</sup> ) at levitation height		
		45 mm	21 mm / interface	0 mm
top phase only	1.090	1.063	1.092	1.118
bottom phase only	1.210	1.077	1.222	1.350
AMPS		1.063	1.092–1.222	1.350

**Tuning the Step in Density of a Paramagnetic AMPS.** To demonstrate how changing the position of the interface of a paramagnetic AMPS in a magnetic field gradient could be used to tune the step in density at the interface, we used a two-phase AMPS of 4.5% (wt/vol) PEG (MW = 20 kDa) and 9% (wt/vol) dextran (MW = 500 kDa) with 584 mM  $\text{MnCl}_2$ . After dispensing the AMPS into a short (~19 mm) plastic cuvette, we covered the system and allowed phase separation to occur overnight. We added two polystyrene and Nylon beads into the system and allowed the AMPS to degas under vacuum for 30 minutes. We used a more powerful MagLev device (5.08 cm by 10.2 cm surface with a thickness of 5.08 cm, a separation distance between magnets of 47 mm, and a surface field of 0.40 T) to provide a smaller slope in the effective density gradient in each phase; reducing the slope leads to a larger shift in density for an interface moving between two heights.

In order to position the AMPS just below the top magnet, we placed the cuvette on a stack of 27 glass slides above the bottom magnet. We then allowed the system to equilibrate for two minutes. In this configuration (with the interface near the top of the MagLev device), the interface bins the lower density objects like polystyrene while higher density objects like Nylon rest at the bottom of the container (**Figure 5**). We then removed one glass slide at a time, allowed the system to equilibrate, and took a photo of the beads. Each glass slide had a thickness of 1 mm and, thus, we captured images of the cuvette at a height above the bottom magnet ranging from 0 to 27 mm.

From the original AMPS solution, we also dispensed 14 mL into a conical tube and centrifuged it for 20 minutes at 2,500 g. After centrifugation, phases were fully separated. Using a pipette, we removed ~6 mL of the top phase. We then punctured the bottom of the conical tube with a 16 gauge needle and dripped ~6 mL of the bottom phase into a separate container. We put each of these fractions into standard, square plastic cuvettes and used density standard glass beads to measure the slope of the effective gradient in density of each

phase. We also used these systems to levitate the individual Nylon and polystyrene beads to determine their density. The slopes and density values were used to construct the plots in

**Figure 5.**

## References

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