

Hyperuniform Phase-separated DNA Droplets

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Many recent studies of liquid-liquid phase separation in biology focus on phase separation as a dynamic control mechanism for cellular function, but it can also result in complex mesoscopic structures. We investigate a model system consisting of DNA nanostars: finite-valence, self-assembled particles that form micron-scale liquid droplets via a binodal phase transition. We demonstrate that, upon phase separation, nanostar droplets spontaneously form hyperuniform structures, a type of disordered material organization that combines the long-range order of crystals with the short-range isotropy of liquids. The hyperuniformity of the DNA droplets is modulated by the interplay of phase separation dynamics and droplet Brownian motion. We engineer a two-species system of immiscible DNA droplets and find two distinctly hyperuniform structures in the same sample, but with random cross-species droplet correlations. This rules out explanations that rely on droplet-droplet hydrodynamic interactions. The hyperuniformity exhibited by DNA droplets provides insight into the interaction of dynamics and structures in varied phase-separating systems.

I. INTRODUCTION

Liquid-liquid phase transitions represent an integral and pervasive mechanism underlying cellular organization [1, 2]. Most recent work on such phase-separated condensates has focused on their local biomolecular organization (droplet material properties, composition and phase behavior [3]), or on the regulatory effects they have on the cell [4]. In contrast, only a few studies have investigated long length-scale organization in phase-separating systems, including a theoretical work quantifying the long-range order in phase separation simulations [5], and an experiment that posited that phase separation forms the material structures that create non-iridescent structural colors of bird feathers [6].

The description of long-range material structure frequently focuses on the limits of perfect (i.e. crystalline) order, or complete disorder (e.g. as in an ideal gas). Others exist between those limits, notably ‘disordered hyperuniform’ structures [7], which are isotropic configurations that are locally disordered (i.e. non-crystalline), but nonetheless display more long-range regularity than expected for an ideal gas. The characterization of disordered hyperuniform structures have been important to understanding organizational mechanisms for various systems, including the packing of spheres [8], active matter [9], the structure of the early universe [10], and a universality class of dynamical phase transitions [11]. Hyperuniformity has also been observed in multicomponent systems, like the arrangement of the five types of cones in the chicken eye retina [12], in which each cone type is individually hyperuniform, and, simultaneously, all cone types together are hyperuniform. The concurrent hyperuniformity suggests that each component interacts with itself as well as with the other components, though the microscopic mechanism for this is still unclear.

As with other types of long-range order, hyperuniformity is analyzed through the spatial correlations in material density. Fluctuations in such density correlations are notably suppressed in hyperuniform materials relative to completely random structures. This can be quantified through the scattering function, $\chi(q)$ (which is the Fourier transform of the density-density correlations). Random, ideal-gas configurations show constant values of the scattering function ($\chi(q) \sim q^0$), while hyperuniform structures instead display a low $q$ (long-range) power-law scaling, $\chi(q) \sim q^{\alpha}$ with $\alpha > 0$. Hyperuniform scaling has been observed in a few experimental phase-separating systems, including decomposing polymer mixtures [13], with $\alpha = 2$, and solid-state dewetting in semiconductors [14], $\alpha = 4 - 6$.

To investigate the long-range structures formed by biomolecular phase separation, we perform experiments on DNA nanostars, a model system that phase-separates to form liquid DNA droplets. Nanostars are roughly 10 nm, finite-valence, self-assembled DNA particles consisting of four double-stranded “arms”, each decorated with a single-stranded palindromic sticky-end [15] (Fig. 1a). The sticky-ends enable two nanostars to bind through sequence-specific DNA hybridization, with a strength and specificity that is well known and easily modulated by sequence design and the ionic strength of the solvent [16]. Phase separation takes place when nanostar binding is energetically favorable, creating a percolated-network condensate with liquid-like features due to the transient nature of the nanostar-nanostar bonds (Fig. 1a). The phase diagram controlling condensation displays features similar to binodal demixing, notably including a coexistence regime where dense liquid droplets are in equilibrium with a dilute nanostar solution [15]. In addition, the sequence specificity of the nanostars provides an avenue to design separate particles with orthogonal attractive interactions that drive multi-component phase separation [17–19].

We show that liquid-liquid phase separation of DNA nanostars leads to long-range hyperuniform order of the
condensate with $\alpha = 2$. As the condensate matures into individual droplets, we find that the hyperuniformity is modulated and eventually removed by the Brownian motion of the droplets. To rule out the role of sedimentation, we investigate the long-range order of multi-phase nanostar solutions, and find that the hyperuniformity of each species is preserved while the hyperuniformity of the entire assembly is destroyed. Overall, this work implies a quite general mechanism for the formation of hyperuniform structures, while also showing how such structures are modified by diffusive fluctuations.

### II. NANOSTAR DROPLET HYPERUNIFORMITY MECHANISM

The experimental system consists of tetravalent DNA nanostars, which are formed by mixing together equal amounts of 4 DNA oligos (sequences given in Supplement) that assemble into star-shaped particles with four 20 bp ($\approx 8$ nm) arms tipped with single-stranded palindromic sticky ends (Fig. 1a). 10% of one strand is modified with a fluorescent dye, Cy5, for imaging. Salt (Potassium Acetate, KAce), is added to a fixed concentration of pre-annealed nanostars, to facilitate sticky-end binding.

The sample is loaded into a microcapillary tube coated with polyacrylamide [20] to prevent droplet adhesion, then visualized on a fluorescent microscope with a temperature control stage. The sample is heated above the melting temperature ($T_m \approx 45^\circ$C), such that the solution is a homogeneous, single-phase fluid, and quenched into the coexistence regime. Liquid DNA droplets form throughout the capillary, then, since the condensate is denser than water [21], sediment to the bottom of the capillary where they are imaged (Fig. 1c-d).

We investigate the structure of the nanostar droplets by calculating the density correlations of the fluorescent images. In Fourier space, density correlations are revealed by the scattering function, which is defined as $\chi(q) = \hat{I}(q)\hat{I}(-q)$, where $\hat{I}(q)$ is the discrete Fourier transform of the image $I(r)$ at wavevector $q$. We do not observe anisotropy in the droplet structures (see supplemental material Figure S1), so the scattering function is angularly averaged $\chi(q) = \chi(|q|) \equiv \chi(q)$. The low numerical aperture of the objective captures all of the fluorescence of the nanostar droplets (depth of focus~100 μm) so the two-dimensional images are equivalent to a projection along the optical axis.

For a sample of 30 μM nanostars and 500 mM KAce, the calculated scattering function shows that the nanostar droplet assemblies are hyperuniform, with a clear reduction of $\chi(q)$ at low $q$. Particularly, we find a power-law scaling $\chi(q \rightarrow 0) \sim q^\alpha$ with $\alpha = 2.0 \pm 0.1$ on long length scales (Fig. 1d).

The hyperuniformity exponent indicates the organizational principles of the system [22], so investigating which mechanisms produce $\alpha = 2$ scaling will provide insight into how phase separating droplet assemblies organize. We consider three effects that could modulate the hyperuniform scaling measured in the experiments: sedimentation, phase separation, and Brownian motion.

As nanostar droplets form, they sediment to the bottom surface of the sample chamber so it is possible that droplet-droplet hydrodynamic interactions during sedimentation are responsible for the observed hyperuniform scaling. Indeed, others have observed $\alpha = 2$ hyperuniform scaling in hydrodynamic calculations of sedimenting colloidal systems, though the suppression of density fluctuations required non-spherically shaped particles [23]. To test the role of sedimentation independent of phase separation, we perform experiments with polystyrene spheres of similar size to the droplets (10 μm diameter) suspended in a 10% glycerol-water mixture, and at a concentration that, after sedimentation, leads to a 20% area coverage (similar to the nanostar droplets). In these conditions, the sedimentation velocity of the colloids roughly matches that of a 10 μm diameter nanostar droplet [21], without altering the solvent viscosity significantly. We find that the $\chi(q)$ for sedimenting colloids does not display hyperuniformity (Fig. 1e). Instead, the structure looks random ($\chi \sim q^0$). At intermediate $q$-values, $\chi$ decreases slightly as expected from excluded volume interactions. As $q \rightarrow 0$, $\chi$ increases again representing a clustering effect as seen in Rayleigh-Taylor instabilities [24]. We also varied other parameters expected to affect sedimentation, including sample chamber height and volume fraction (see the supplemental material), but found that none produce hyperuniform structure.

Next we investigated if the long-range order could be generated by the phase separation process. To model this, we perform two-dimensional numerical simulations of the Cahn-Hilliard equation, which was formulated to describe spinodal decomposition [25], and has been applied to biomolecular systems [26–28]. The equation represents a free energy expansion of an inhomogeneous liquid with spatial- and time-dependent concentration $c(x,t)$, and includes the sum of the free energy of a homogeneous liquid with a term that penalizes concentration gradients. Simulations start from an initial condition where the concentration field is uniformly random $c(x,0) = [-0.5 - 10^{-4},-0.5 + 10^{-4}]$ which produces droplets of $c = +1$ in a background of $c = -1$ that occupy 25% area fraction.

Our simulations show that the structures formed by a Cahn-Hilliard simulation (Fig. 1d magenta) are hyperuniform with characteristic exponent $\alpha = 4$. This exponent is consistent with previous calculations of equal-ratio mixtures undergoing Cahn-Hilliard dynamics [5], which don’t form droplets but rather percolated patterns. To test if dimensionality modifies the characteristic exponent, we carried out Cahn-Hilliard simulations in 2D and 3D, finding $\alpha = 4$ in both cases (see the Supplemental Material).

While the Cahn-Hilliard simulation produces droplets with hyperuniform scaling, the structures generated do not scale with the same exponent as the experiments:
**FIG. 1.** Phase-Separating DNA nanostar droplets form hyperuniform structures.  

**a,** A schematic of the multiscale assembly of nanostar droplets. Nanostars are ≈10 nm, self-assembled DNA structures that bind, with strength $\Delta G$, through sticky-end interactions, condensing into dynamic, mesh-like networks that form liquid droplets on the micron scale. Bottom: Section of a typical fluorescent image, showing roughly 5,000 condensed nanostar droplets in a 1.2 mm by 0.7 mm area; full experimental images are 1.2 mm by 1.2 mm.  

**b,** Schematic of the nanostar phase diagram. Below a critical temperature, nanostars phase separate into droplets with concentration $c_{\text{dense}}$ that coexist with unbound, dilute nanostars at concentration, $c_{\text{dilute}}$.  

**c,** Droplet formation dynamics from experiments on nanostars (top), and from Cahn-Hilliard simulations (bottom). Nanostar solutions are prepared in the melted state $T > T_m$ and quenched at $t = 0$. For both the experiment and simulation, droplets form quickly and grow over time, but with characteristically different dynamics.  

**d,** The scattering function, $\chi(q)$ of the droplet intensity as a function of wavenumber, $q$, shows hyperuniform scaling $\chi(q \to 0) \sim q^\alpha$ in the experiment (green), with exponent $\alpha = 2$, as well as the Cahn-Hilliard simulation (magenta), with exponent $\alpha = 4$. We recover the experimental structure by randomly displacing Cahn-Hilliard droplets with a displacement magnitude of one droplet spacing (orange). Plotted nanostar droplet experiment $\chi$ is the average of six different quenches and two different sample preparations at a 30 minute quench time. Error bars correspond to standard deviation of different quenches and samples. Values of $\chi$ are normalized for comparison, $\tilde{\chi} = \chi/\chi_{\text{peak}}$ and $\tilde{q} = q/q_{\text{peak}}$. 
they have much stronger suppression of density fluctu-
ations, $\alpha = 4$, as opposed to the experimental value,
$\alpha = 2$. We posit that the discrepancy is due to the
Brownian motion of the droplets, which occurs in ex-
periment, but is not captured by the simulation. The
difference can be seen by comparing movies of the exper-
iment and the Cahn-Hilliard droplet formation process
in the Supplemental Material. Cahn-Hilliard droplets do
not move; they either grow or shrink via Ostwald ripen-
ing, so coalescence events only occur when droplets grow
large enough to merge. In contrast, the Brownian motion
of the nanoastar droplets allows for droplet coalescence
without growth.

Diffusion has also been shown to modulate the hy-
peruniform scaling in several non-equilibrium numerical
models, including absorbing-state models in the active
phase [29], chiral active particle simulations [30], model
soft polymers [31] and driven hard-sphere fluids [32], all
producing $\alpha = 2$. To explore this, we add an approxi-
mation of Brownian motion to the simulation. Partic-
ularly, we locate and size the droplets [33], then dis-
place them in a random direction with a magnitude, $\delta$,
proportional to each drop’s Stokes-Einstein mobility, i.e.
$\delta \sim 1/R$, where $R$ is droplet radius. We find that ran-
donmly displaced Cahn-Hilliard droplets recover the $\alpha = 2$
scaling from the experiments (Fig. 1d). We see qualita-
tive agreement of $\chi(q \to 0)$ between the experiment and the randomly displaced Cahn-Hilliard drops if we
choose a displacement magnitude equal to one average
particle spacing (see Supplemental Material Figure S8).
For small displacements, the Cahn-Hilliard hyperuniform
scaling $\alpha = 4$ is retained. As the displacement magnitude
approaches the mean droplet size $\chi(q)$ starts to exhibit
$\alpha = 2$ at the lowest $q$-values. For displacements larger
than an average droplet size, the structure looks random
$\chi(q) \sim q^0$ for length scales smaller than the displace-
ment size while $\chi$ is hyperuniform with $\alpha = 2$ for larger length
scales. This suggests that the nanoastar droplets form via
a Cahn-Hilliard phase separation, but then diffuse one
average particle spacing before being imaged. In the ex-
pertiment, we never observe the $\alpha = 4$ scaling predicted
by Cahn-Hilliard, even at the earliest times, indicating
that the lateral diffusion occurs concurrently with sedi-
mentation.

III. DYNAMICAL ASSEMBLY OF DROPLET
STRUCTURES

The success of the “Cahn-Hilliard plus diffusion” pic-
ture (Fig. 1d) assumes that the timescale for droplet
growth is much smaller than the timescale for Brownian
motion. Therefore, it is compelling to explore the den-
sity fluctuations in the opposite regime, where Brownian
motion occurs on shorter timescales relative to droplet
growth. We found that a sample with a large nanoastar
concentration ($30\mu$M), and at high ionic strength ($K\text{Ac} = 500\text{mM}$), formed droplets much more quickly than a
sample with a lesser concentration ($10\mu$M), and lower
ionic strength ($K\text{Ac} = 100\text{mM}$) (Fig. 2). In both cases,
the nanoastars are prepared in the melted state, then the
temperature is quenched to 38°C and held for the length
of the experiment.

The vastly different phase separation dynamics pro-
duce qualitatively different droplet structures. For both
cases, when the temperature is above the melting point,
unbound nanoastars form a homogeneous fluid, and we
measure a constant random structure, $\chi(q) \approx 10^{-4}$
(Fig. 2b). As the system is quenched below melting, we
observe the emergence of hyperuniform scaling $\chi(q \to 0) \sim q^\alpha$ with $\alpha = 2.0 \pm 0.1$ in the case where the droplets
form quickly. The $\alpha = 2$ scaling emerges almost immedi-
ately after droplets form, at quench times of about 6 min-
utes. We don’t observe evidence of the $\alpha = 4$ scaling seen
in the Cahn-Hilliard simulations. In contrast, the ran-
donm structure of the homogeneous fluid, $\chi(q \to 0) \sim q^0$,
is preserved after quenching the slow system, even just after
droplets appear. This observation supports the hy-
pothesis that droplet growth kinetics relative to Brownian
motion controls the long-range structures.

That we can control the hyperuniformity of the droplet
structures by the relative timescales of droplet growth
and diffusion implies there is a hyperuniformity length
scale, $\xi$. For length scales shorter than $\xi$ the density
fluctuations are random, $\chi \sim q^0$, while for length scales
longer than $\xi$ density fluctuations are hyperuniform, with
$\chi \sim q^2$ scaling. Due to the mechanisms for hyperuniform
organization we have identified, $\xi$ should evolve with time
and the only other mesoscopic length scale in the system,
the droplet size. To assess evolution of $\xi$ quantitatively,
we fit $\chi(q)$ to a functional form,

$$\chi(q) = V_c^2 \frac{1 - e^{-(q\xi)^2}}{1 + (q\langle R \rangle)^\beta}$$

with three fitting parameters: $V_c$, the condensate quan-
tity; the hyperuniformity length scale, $\xi$; and the mean
particle size, $\langle R \rangle$. Eq. 1, while phenomenological, is jus-
tified by decomposing the scattering function $\chi(q)$ into a
structural component $S(q)$ that incorporates long-range
structure and a form factor $F(q)$ that accounts for the
particle size and profile. This is done by assuming that
the scattering function is separable, such that the small-$q$
behavior is dominated by $S(q)$ and goes to 1 at large-$q$,
and vice-versa for $F(q)$. To replicate the hyperuniformity
we measure in Fig. 1, we chose the form $S(q) = 1 - e^{-(q\xi)^2}$
where $S(q) \sim q^2$ for length scales larger than $\xi$. This
exponential form is the solution to the diffusion equation
in Fourier space where $\xi = \sqrt{D\beta}$ [34–36]. The choice of
form factor, $F(q) = 1/(1 + q\langle R \rangle)^3$, with $\beta = 5$, isphe-
nomenological, but related to the form factors expected
for polydisperse droplets (See Supplement).

For all cases of fast and slow droplet growth, the func-
tional form, Eq. 1, fits well over the whole range of $q$-
values and times after the quench (Fig. 2a-b). The fit pa-
parameter $V_c(t)$ reflects the qualitative differences in nanos-
FIG. 2. Dynamics of hyperuniform structure formation. 

a, Images of nanostar droplet formation for a fast condensation process (30µM nanostars, 500mM ionic strength) and a slow condensation process (10µM nanostars, 100mM ionic strength).

b, Scattering functions, χ(q), for the fast- and slow-condensing systems, for different times after quench. The scattering function in both cases displays a large-q decay indicative of the particle size as expressed by Eq. 1. At small-q, the fast condensation process produces hyperuniform structures, χ ∼ q², as droplets form, while structures are not hyperuniform for slow condensation. Each χ(q,t) is fit to Eq. 1 (dashed lines).

c, Fit parameters of Eq. 1 to χ(q) data show the volume of condensate, Vc, increases quickly and plateaus for the fast process, while slow condensation only produces droplets after a significant delay (~100 min). This same dynamical behavior is reflected in the mean droplet size, ⟨R⟩ (circles). For fast condensation, the hyperuniformity length scale, ξD, (triangles) increases with time, after a brief decline at early times when droplets are still forming. At later times (>10 min), ξD displays scaling consistent with diffusive equilibration of the droplet structure, ξD ∼ √t. Plotted χ values are the average of three different quenches and two different sample preparations.

tar droplet growth dynamics; the fast condensation process produces droplets quickly, <5 minutes, and reaches a plateau value at 30 minutes, while Vc(t) of slow condensation doesn’t show evidence of droplet formation until ~100 minutes and does not a plateau even after 300 minutes (Fig. 2c). The mean droplet size ⟨R⟩(t) reflects the dynamics of the condensate volume Vc(t), quickly plateauing for fast condensation while a significant delay plus slow droplet growth is characteristic of the slow condensation process (Fig. 2c).

For the slow condensation, we cannot resolve a hyperuniformity length scale ξ. ξ for the fast condensation process displays a non-monotonic behavior as droplets form, at first decreasing then increasing again at longer times. The early time decrease in ξ could be due to the rapid droplet growth in this phase of the quench, where small, quickly diffusing droplets slow down dramatically as they grow. In the late phase of droplet growth, ξ is consistent with a diffusive scaling, where modes of a length scale ξ relax back to equilibrium with ξ ∼ t¹/². The diffusive behavior of ξ doesn’t only agree in the plateau region of Vc(t) but at even earlier times as well.

It is tempting to assign the slow dynamics to a nucleation process, and the fast to spinodal decomposition [37, 38]. For sufficiently deep quenches, one expects the phase separation is spinodal, defined by dynamics that lack a time delay or an energy barrier; i.e. the mixture spontaneously demixes once the system is quenched below a certain temperature. In contrast, shallow quenches typically undergo nucleation and growth...
The role diffusion plays in the long-range structure is illuminated by varying the droplet size. We vary the mean droplet size, from \((R) \approx 10\,\mu\text{m} \) to \(40\,\mu\text{m}\), by adjusting the nanostar concentration (Fig. 3a), then measure the scattering function a fixed time after quench. We find a separation of the length scales, \(\xi\) and \(\langle R \rangle\), as the droplets get smaller, resulting in a plateau in \(\chi(q)\) at intermediate \(q\)-values (Fig. 3b). This occurs because, as droplets get smaller, they diffuse further in a given time; thus, as \(1/(\langle R \rangle)\) moves to larger \(q\), \(1/\xi\) moves to smaller \(q\). We confirm this quantitatively by fitting the \(\chi(q)\) to Eq. 1, extracting best-fit \(\xi\) and \(\langle R \rangle\), and finding \(\xi \sim 1/\sqrt{\langle R \rangle}\) (Fig. 3b Inset), which is consistent with the Stokes-Einstein prediction, \(D \sim 1/R\), given the expectation that \(\xi \sim \sqrt{Dt}\). The emergence of the plateau, along with the relation of the length scales, confirms that the hyperuniform structure is erased by diffusion.

**V. DISTINGUISHABLE HYPERUNIFORMITY OF MULTICOMPONENT PHASE SEPARATION**

The ability to precisely control nanostar sequences allows us to test the phase-separation driven hyperuniformity hypothesis through multicomponent condensation. We design two orthogonal nanostar species such that each species’ sticky ends only bind to other particles of the same species [17, 39]. Particularly, we create nanostars with two binding sequences, GGAATTC and CTAGC-TAG, which are palindromic sequences with similar binding strength (ensuring strong same-species binding, and similar melting temperatures), but that are chosen to minimize the cross-species hybridization energy. Accordingly, when quenching solutions of the two nanostar species, we observe two separated droplet species, without adhesion (Fig. 4a). We find the structures of each droplet species, individually, i.e. \(\chi_{1,1}\) and \(\chi_{2,2}\), are hyperuniform (Fig. 4b) with the same \(\chi(q \to 0) \sim q^2\) scaling that we measure in the single-species case (Fig. 1d). However, when both species are analyzed together, the total scattering function \(\chi_{\text{tot}}\) is not hyperuniform (Fig. 4b). Instead, the structure is consistent with that expected for a hard disk fluid occupying area fraction \(\phi = 0.29\) (see Methods for more details), which is consistent with a significant role played by excluded volume interactions between the two orthogonal droplets. There is a small deviation between the hard-disk prediction and the measured \(\chi_{\text{tot}}\) near the peak (Fig. 4b); this likely occurs because the hard disk solution assumes a monodisperse population of particles, whereas the droplets are polydisperse. The lack of hyperuniformity in the summed (two-species) structure is opposite to that found for multi-species cones in the chicken eye retina [12]. While the mechanism at play in the chicken retina is unclear, here we attribute the lack of multi-species hyperuniformity to the independence of the phase-separation processes in each species. We have shown that the spatial structure associated with Cahn-Hilliard spinodal decomposition dominates the nanostar system, leading to hyperuniformity within each species individually. However, the two phase separation processes are made independent by the molecular design, leading to random cross-species droplet placements, and a non-hyperuniform total structure \((\chi_{\text{tot}}(q \to 0) \not\sim q^2)\). This result also suggests, very clearly, that sedimentation is not the mechanism for
Hyperuniformity of multicomponent nanostar droplets. a, Subsection of a two-color fluorescent image of a two-component mixture of nanostars with orthogonal sticky-ends GGAATTCC (yellow), and CTAGCTAG (pink). b, The scattering function shows that each component ($\chi_1, \chi_2$) retains $\alpha = 2$ hyperuniform scaling (dashed line) even in the presence of another orthogonal nanostar species. However, the scattering function of both droplet species ($\chi_{\text{tot}}$) is not hyperuniform, but rather consistent with the scattering function of a 29% area fraction hard disk fluid (dotted line) due to excluded-volume interactions between unlike droplets. Plotted $\chi$ values are the average of five different quenches.

VI. CONCLUSIONS

We observe assemblies of condensates that exhibit suppressed, hyperuniform density fluctuations that result from the interplay of a spinodal decomposition phase separation process and Brownian motion. The structure is controlled by a diffusive length scale $\xi$ which produces $\chi \sim q^2$ hyperuniformity for length scales larger than $\xi \sim \sqrt{t/(R)}$, for quench time $t$ and mean droplet size $\langle R \rangle$. The bottom-up design of orthogonal droplet species preserves the long-range hyperuniform order of individual components, while the hyperuniformity of the entire assembly is destroyed. This observation further supports that phase separation, as driven by specific biochemical interactions, results in the measured long-range order, rather than any non-specific physical mechanism, such as droplet-droplet hydrodynamic interactions.

The mechanistic link between phase separation and the emergence of hyperuniformity can be understood by considering the Cahn-Hilliard model. Fourier analysis of the Cahn-Hilliard equation indicates the amplitude, $A_q(t)$, of a concentration mode with wavevector $q$, evolves, from a homogeneous initial state where $A_q(0) \sim$ constant, with the form $A_q(t) \sim e^{D(q^2-\gamma q^4)t}$ at early times [40]. $D$ is the particle (nanostar) diffusion coefficient, and $\gamma$ is related to the interfacial energy between the two phases. There is thus a fastest growing mode (with $q_{\text{max}} \approx 1/\sqrt{\gamma}$) that separates low $q$, diffusion-dominated, slow-growing modes, from high $q$, interface-dominated, exponentially-suppressed modes. $A_q$ grows with time for all $q < q_{\text{max}}$, indicating phase separation increases density fluctuations on all long length scales. However, at increasingly small $q$ the amplitude grows increasingly slowly (i.e. to lowest order, as $A_q(t) \approx Dq^2t$)–this is because diffusion is progressively slower over longer scales. Thus, at finite time the density fluctuations at low $q$ are much smaller than those at $q_{\text{max}}$, and it is this relative suppression that corresponds to the hyperuniform structure. Ultimately, then, the long length scale structure of the nanostar droplets arises from two diffusion mechanisms: the dynamics of diffusion of individual nanostars drives the hyperuniform Cahn-Hilliard mode structure, while, at long times, droplet diffusion destroys the hyperuniformity.

The phase-separation driven disordered hyperuniform structures that we observe imply that long-range ordering might be more universal than previously considered. The Cahn-Hilliard equation is pervasive in biology, physics, chemistry, ecology, image processing, and astronomy, implying that hyperuniformity could be broadly observed, from the pattern formation of mussels [41] to the structure of Saturn’s rings [42]. Similarly, the modulation of these structures by Brownian motion is not limited to the micro-scale, because random fluctuations exist at all length scales.

The hyperuniform structure of droplets demonstrated in this work could lend itself to a variety of applications. For example, we have shown that the loss of hyperuniformity can be quantitatively linked to thermal droplet diffusion driven by the environment; it is thus likely possible to use the long-range structure of droplets as probes of the dynamic processes in more complex, non-equilibrium environments (e.g. in active systems [43], or even liv-
ing matter [44]). Alternatively, droplet hyperuniformity might be exploited in a chemical reactor, where unique inter-droplet spatial couplings could affect, e.g., interdependent reactions occurring in separate droplets. Finally, given previously-established connections between spinodal decomposition and material properties [45], we posit that hyperuniform, phase-separated droplets could be used as a means to create patterned materials with exotic photonic or mechanical behaviors.

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VII. METHODS

A. Sample Preparation

The sample chamber consists of a borosilicate glass microcapillary tube (Vitrocom) with interior dimensions: 300 µm height, 3 mm width and 25 mm length. Capillaries are internally coated with polycrylamide [20] to keep droplets from sticking. We image the droplets with a Nikon Ti-2 fluorescent microscope with a 10x objective, typically finding approximately 10,000 droplets at a quench time of 43 minutes for the fast condensation process. The full size of the images is 1200 µm (see Supplementary Movies).

For imaging, 10% of nanostars are modified by labelling one strand with a fluorescent dye on the 3’ end. We use Cy5 dyed strands (NS1: GGAATTCC) for most experiments, and include a FAM label for the two-component mixtures in Fig 4. All samples are buffered with 10 mM Potassium Phosphate (pH ∼ 6.9). Salt, in this case Potassium Acetate, is added to concentrations of 100 mM and 500 mM.

B. Image Analysis

The scattering function is $$\chi(q) = \langle \hat{I}(q) \hat{I}(-q) \rangle$$, where $$\hat{I}(q) = FFT(I(r))$$ is the two dimensional discrete Fourier transform of the image $$I(r)$$. The images are normalized by dividing the melted, uniform nanostar image due to the spatial inhomogeneity of the incident LED light source. To account for the lack of periodicity in the experimental images, we apply a Hanning window to suppress edge effects, $$\hat{I}(q) = FFT(I(x,y)W(x,y))$$ where $$W(x,y) = \frac{1}{4}(1 - \cos(x/L))(1 - \cos(y/L))$$ and $$L$$ is the image size.

C. Cahn-Hilliard Simulations

The evolution of $$c(x,t)$$ is described by

$$\frac{\partial c}{\partial t} = D \nabla^2 \mu$$

where $$\mu = c^3 - c - \gamma \nabla^2 c$$ is the chemical potential, $$D$$ is the diffusion constant and $$\sqrt{\gamma}$$ is the length scale of transition region between the phases. We solve the Cahn-Hilliard equation numerically in two dimensions with discrete time step $$\Delta t = 1$$ on a 2000x2000 grid with periodic boundaries. All simulations are calculated with parameters $$D = 0.0125$$ and $$\gamma = 0.1$$ and numerically evaluated for $$10^5$$ time steps, when the dynamics are self-similar and the structures are fractal (the data collapses after scaling $$\chi$$ by its peak location $$q_{\text{peak}}$$ and value $$\chi(q_{\text{peak}})$$).

We start from a biased random initial condition $$c(x,t) = 0.5 - 10^{-4} X$$ where $$X$$ is a uniform random number between 0 and 1, which generate droplets of $$c = 1$$ in a background of $$c = -1$$ with an area fraction of 25%. The scattering function calculation is analogous to the experiments, where $$\chi(q) = \langle \hat{c}(q) \hat{c}(-q) \rangle$$. $$\hat{c}(q)$$ is the discrete Fourier transform of the concentration field. Here, we do not apply a window because the system is evaluated with periodic boundary conditions. We show data for the 2D Cahn-Hilliard equation here because we can resolve smaller $$q$$-values given a fixed number of grid points, which is imposed by memory constraints, though the hyperuniform scaling exponents are the same in two and three dimensions.

D. Percus-Yevick Scattering Function

We approximate the structure of the hard disk fluid by solving the Ornstein-Zernike equation with the Percus-Yevick closure relation [46–48]. Then, the numerical Taylor series expansion as $$q \to 0$$ results in $$S(q \to 0, \phi = 0.29) \approx 0.10 + 0.31 q^2 + O(q^4)$$. We retain the same form factor, $$F(q) = F_1(q) + F_2(q)$$, so the hard-sphere scattering function is $$\chi(q) \approx V^2(0.1 + 0.31 q^2)/(1 + (q/R)^2)^2$$.

Therefore, we fit the summed scattering function $$\chi_{\text{tot}}$$ with one fit parameter $$V_c$$ and find agreement at small $$q$$ even without any structural fit parameters. We determine the area fraction by locating and sizing the particles from images (such as in Fig. 4a).

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Supplemental Material: Hyperuniform Phase-separated DNA Droplets

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I. DNA SEQUENCES

| Nanostar 1       | Strand 1               | GGAATCCCTGTATCAGGCTCCGATGCGCTTCATACTCTTCTTAACAACC |
|------------------|------------------------|--------------------------------------------------|
|                  | Strand 2               | GGAATCCCTGGTTGTTAAGAAGAGTATGTCTTGCCGCGCCTCGAGTCCC |
|                  | Strand 3               | GGAATCCCTGGGACTCGAGGCGGCCAGTTCCGCATCCCTCCATTTTCG |
|                  | Strand 4               | GGAATCCCTCGAAATGAGGGATGCCGTTGCGCATCGGAGCTGATAC   |
|                  | Dyed Strand 1          | GGAATCCCTGTATCAGGCTCCGATGCGCTTCATACTCTTTAACAACC/3Cy5Sp/ |

| Nanostar 2       | Strand 1               | CTAGCTAGTGGAATGCTACTACGAGCGGTTGTGTTGGCGCTGCGAGGTGC |
|------------------|------------------------|-----------------------------------------------------|
|                  | Strand 2               | CTAGCTAGTGCCAATGCGACACCGCCACAGTTCCGACAGGCAGCAGAAC  |
|                  | Strand 3               | CTAGCTAGTGTTCCGCGTAATTGCGATGTCTTGACGGCGCGCCATG   |
|                  | Strand 4               | CTAGCTAGTGCAATGCTTGGGGGGATGGCTGCGCTGCGGTGATGAGTC  |
|                  | Dyed Strand 1          | CTAGCTAGTGGAATGCTACTACGAGCGGTTGTGTTGGCGCTGCGAGGTGC/36-FAM/ |

II. CONDENSATION MOVIES

**Movie S1: Fast Condensation.** Time series movie of the fast condensation process in Figure 2 of the main text. Frame Rate: 1/3 minutes. 30 μM nanostars, and 500 mM KAce.

**Movie S2: Slow Condensation.** Time series movie of the slow condensation process in Figure 2 of the main text. Frame Rate: 1/6 minutes. 10 μM nanostars, and 100 mM KAce.

**Movie S3: Cahn-Hilliard Simulation.** Time series movie of the Cahn-Hilliard simulation in Figure 1 of the main text. Frame Rate: 1/2000 timesteps. Droplet area fraction: 25%.

III. FORM FACTOR ESTIMATION

It is not trivial to model the form factor $F(q)$ for nanostar droplets because of their polydispersity, as well as the non-uniform intensity profile inside the droplet. For mono-disperse spheres of uniform intensity, the scattering profile is that of an Airy disk so $F(q)$
decays like \( F(q \rightarrow \infty) \sim q^{-4} \). Polydisperse spheres also decay as a power law but with a different power. For example, polydisperse Cahn-Hilliard droplets decay like \( F(q \rightarrow \infty) \sim q^{-5} \) (Fig. S3). Additionally, the droplet intensity is integrated along the optical axis by the low numerical aperture objective, so the intensity profile inside the droplet looks like \( I(r) \sim I_{\text{max}} - (r/R)^2 \) with droplet radius \( R \). We estimate \( F(q) \) of the form \( F(q) \sim \frac{1}{1+(q\langle R \rangle)^\beta} \) where we fit \( \beta = 5 \pm 1 \) to the final \( \chi(q) \) in each experimental condition and fix the value for all earlier times.

IV. SEDIMENTATION OF NANOSTAR DROPLETS

As the temperature of the sample decreases below \( T_m \), droplets start to form in the bulk of the sample and then sediment once they reach a size such that the buoyant force of the DNA droplets drives them to the bottom of the sample chamber. It has been shown that the volume fraction of DNA inside the droplet \( \phi_{DN} \) is approximately 1\% [1], so we estimate the buoyant density of the droplets is \( \Delta \rho_{\text{drop}} \approx 0.07 \text{g/cm}^3 \). The critical size for droplet sedimentation into a region where we can image them is roughly \( r = ((k_B T)/\Delta \rho(4\pi/3)gh)^{1/3} \approx 0.5 \mu m \), where the height \( h \approx (\lambda/NA^2) + C_{\text{pix}}/(M \times NA) \approx 100 \mu m \) is the depth of field the objective, for visible wavelengths \( \lambda \approx 0.55 \mu m \), numerical aperture \( NA = 0.3 \), camera pixel size \( C_{\text{pix}} = 7.3 \mu m \), and magnification \( M = 10 \).

The critical size is a measure of equilibrium structure, indicating the size above which droplets will equilibrate within the microscope focal depth; alternatively, we can estimate the kinetics of sedimentation, specifically what size droplets will sediment in the timescale of the experiment. With similar parameters, the droplets that are large enough to sediment the height of the sample chamber (300 \( \mu m \)) during the timescale of the experiment, \( \sim 45 \text{min} \), have radius \( r > (v\mu/(2/9)\Delta \rho g) \approx 1 \mu m \). Given these relevant scales, we are confident that all of the condensates larger than 1 \( \mu m \) are within the depth of focus of our imaging setup.

V. LOCATION AND SIZING OF DROPLETS

Droplets are located with a Crocker-Grier style algorithm [2] at times throughout the condensation process. For experiments: Droplets are sized assuming they are spherical and the objective evenly collects fluorescence throughout the droplet and is projected along z-
axis. The droplet profile is fit to the form $I(r) = I_0(1 - (r/R)^2)$ with fit parameters $I_0$ and $R$ (Droplet Radius), and $r = \sqrt{(x - x_{\text{center}})^2 + (y - y_{\text{center}})^2}$. For Cahn-Hilliard Simulation: Droplets are sized similarly by fitting to an error function $I(r) = \frac{I_0}{2}(erf(\frac{r-R}{\sqrt{\gamma}}) + 1)$. The resulting droplet distributions for the fast and slow condensation experiments as well as the Cahn-Hilliard simulation are shown in Fig. S2.

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FIG. S1. **Scattering Function Isotropy.** Image of scattering function $\chi(\vec{q})$ for the fast condensation process 60 minutes after quench. The droplet configurations are isotropic at all $q$-values, justifying an angular average of the scattering function. Yellow square near $q = 0$ is a result of the Hanning window.
FIG. S2. Droplet size distributions for Fast condensation, Slow condensation experiments and Cahn-Hilliard Simulations. The droplet distribution of the fast process shows quick accumulation of small droplets that coarsen into two populations of small and large droplets. Droplets coalescence is indicated by the decrease the total drop count. Over time, the number of small droplets decreases relative to large ones presumably because they diffuse faster and coalesce with another droplet. The slow condensation process, however, shows coarsening behavior, as opposed to coalescence. The droplet distribution slowly shifts toward larger droplet sizes and broadens. The droplet distribution for the Cahn-Hilliard simulation shows coarsening behavior as well, with a slow broadening of the distribution and increased mean droplet size.
FIG. S3. Cahn-Hilliard Scattering Function in two and three dimensions. Cahn-Hilliard droplets show clear power law scaling in both the small-\( q \) and large-\( q \) limits. At large-\( q \), \( \chi(q \to \infty) \sim q^{-5} \), indicative of the polydispersity of the the droplets (Monodisperse droplets: \( \chi(q \to \infty) \sim q^{-4} \)). At small-\( q \), the system is hyperuniform with \( S(q) \sim q^4 \) for both two and three dimensions.
Scattering function of sedimenting colloidal suspension samples do not show hyperuniform scaling for a range of sample chamber height, $h$, and area fraction $\phi$. Colloidal suspensions consist of 10µm diameter polystyrene spheres suspended in a 10% glycerol-water solution. Suspensions are prepared by mixing uniformly then loaded into the sample and allowed to sediment. Images are taken once all particles sediment to the bottom surface of the sample chamber and the scattering function is calculated. Oscillations in $\chi$ near the inverse particle diameter correspond to excluded volume interactions at larger area fractions. At 50% area fraction, small-$q$ increase in $\chi$ is due to some clustering, indicative of a Rayleigh-Taylor type instability.
FIG. S5. Random Displacement of Cahn-Hilliard Droplets produces $\alpha = 2$. The structure factor, $S(q)$, is plotted for Cahn-Hilliard configurations of droplets that have been randomly displaced. We locate and size the droplets then displace them in a random direction with a magnitude, $\delta$, proportional to each droplet’s Stokes-Einstein mobility, i.e. $\delta \sim 1/R$, where $R$ is droplet radius. The resulting structure of varied displacement magnitudes, $|\Delta r|$, relative to mean droplet diameter, $\langle d \rangle$, is plotted. For very small displacements, $|\Delta r| \ll \langle d \rangle$, the Cahn-Hilliard hyperuniform scaling $\alpha = 4$ is retained. As the displacement magnitude approaches the mean droplet size, $|\Delta r| \approx \langle d \rangle$, $S(q)$ starts to exhibit $\alpha = 2$ at the lowest $q$ values. For displacements larger than an average droplet size, the structure is random $S(q) \sim 1$ for $q > 1/|\Delta r|$ and is hyperuniform with $\alpha = 2$ for $q < 1/|\Delta r|$. The dashed lines correspond to $S(q) = 1 - e^{-(q|\Delta r|)^2}$ (no fit parameters).