Electronic Supplementary Material (ESI) for Soft Matter.

Supporting Information

Salt-dependent properties of a coacervate-like, self-assembled DNA liquid

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DNA concentration in NS-liquid phase

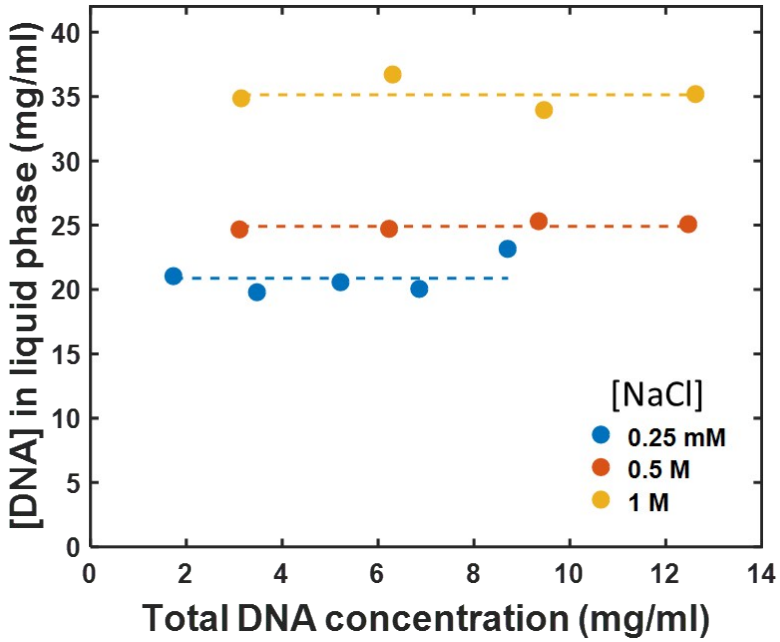

Figure S1: The concentration of DNA in the NS liquid phase is independent of the total DNA concentration of the NS solutions at all three salt conditions explored.

A small amount of YOYO-1 dye was added to NS solutions (NS:YOYO-1=100:1) for clear visualization of the two phases, i.e. a DNA-dilute NS 'gas' phase and a DNA-dense NS 'liquid' phase, upon bulk phase separation in 200 uL PCR tubes. Samples from each phase were carefully isolated and measured using UV/vis spectroscopy. We confirm that the effect of YOYO-1 dye on DNA NS phase separation is negligible. With YOYO-1, at 0.5 M NaCl, the [DNA] in the dense NS-liquid is measured to be 25 ± 0.3 mg/mL; without YOYO-1, at the same 0.5 M NaCl, [DNA] is measured to be 25 ± 0.4 mg/mL. We also confirm that [DNA] in the NS-liquid phase is independent of the overall [DNA] in the NS solution (Fig. S1) for all three explored NaCl concentrations.

Sedimentation of NS-liquid droplets

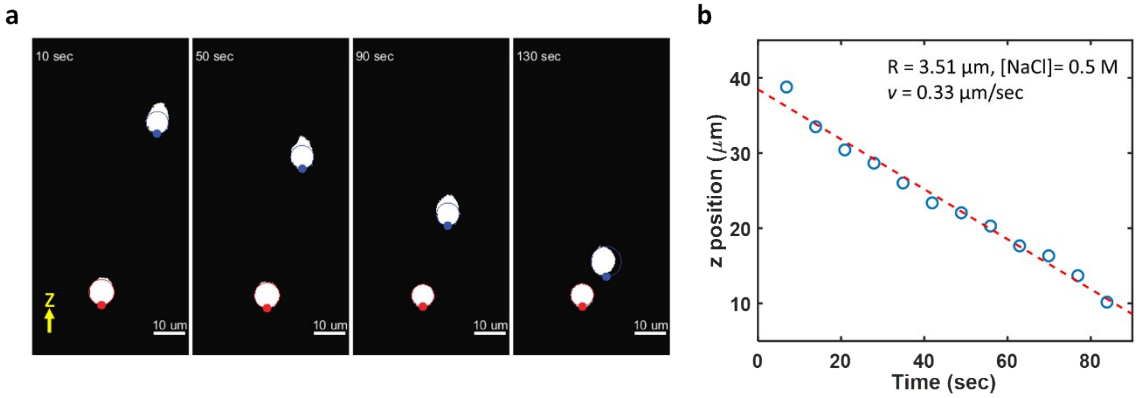

Figure S2: (a) Binarized images of projections onto xz-plane of a sedimenting droplet. The droplet encircled in red is settled on the surface while the droplet encircled in blue is sedimenting. Panels correspond to 10, 50, 90, and 130 sec respectively. (b) A plot of z position of a sedimenting droplet vs. time at [NaCl]=0.5 M.

To confirm that the DNA concentration in NS-liquid droplets matches that of the bulk phase, we examined NS-liquid droplets sedimenting. Stokes' Law dictates the drag force, F_{drag}, exerted on hard spheres when moving through a viscous fluid with low Reynold's number[1] as $F_{drag} = 6\pi\eta_{fluid}Rv$, where η_{fluid} is the fluid viscosity, R is the radius of the spherical object, and v is the velocity. For a spherical particle sedimenting at terminal velocity, the drag force is equal to the force due to gravity, $F_g = \frac{4}{3}\pi R^3 \Delta\rho g$, where $\Delta\rho$ is the density difference between the particle and the surrounding fluid and g is gravitational acceleration (g=9.8 m/s^2), yielding:

$$\Delta\rho = \frac{9v\eta_{fluid}}{2\ gR^2} \qquad \text{(S1)}$$

We took confocal volumes, with a depth of 150 µm, of sedimenting NS-liquid droplets and extracted terminal sedimentation velocities at [NaCl] = 0.25, 0.5, and 1 M, as shown in Fig. S2, to calculate $\Delta\rho$ (Eq. S1). Assuming that NS-liquid droplets are composed only of DNA nanostars and the surrounding fluid, one can obtain the DNA volume fraction in the NS-droplets as:

$$\phi_{DNA} = \frac{\rho_{droplet} - \rho_{fluid}}{\rho_{DNA} - \rho_{fluid}} \qquad \text{(S2)}$$

where ρ_{DNA} is taken to be 1700 mg/mL and ρ_{fluid} is 1008, 1019, and 1040 mg/mL for 0.25, 0.5, and 1 M NaCl, respectively[2].

The concentration of DNA in the droplet phase increases from 12 (± 5) to 24.0 (± 9) mg/mL with an increase in [NaCl] from 0.25 to 1 M, consistent with the DNA concentrations measured from bulk phase separation. Slightly lower values from the sedimentation method could be attributed to the limited z-resolution in

tracking sedimenting NS droplets, our assumption that the surrounding solution lacks NS (whose presence would slightly alter η_{fluid} and ρ_{fluid}), and possible deformation of the sedimenting droplets.

Bulk rheology with NS-liquid

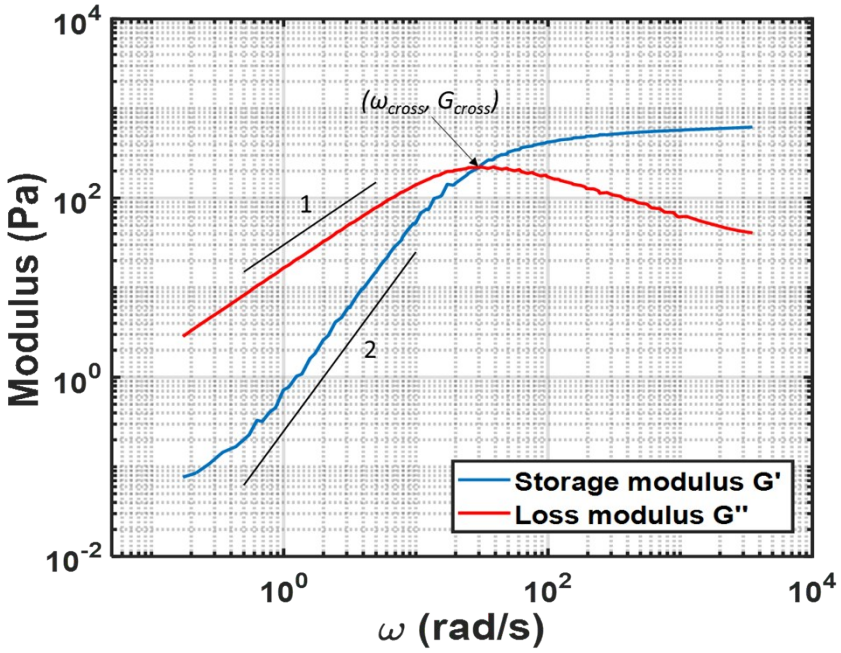

Figure S3: Storage and loss moduli (G' and G'') of the NS liquid phase at 0.5 M NaCl at 20 °C from bulk rheology. The viscosity η is calculated from the crossover modulus G_{cross} and frequency ω_{cross} as $\eta = 2\pi G_{cross}/\omega_{cross} \approx 45.86$ Pa·sec. The solid lines are guides for slopes of 1 and 2.

To validate the viscosity results from microrheology, we measured viscosity of the NS-liquid phase using a conventional rheometer (ARG2, TA instruments). NS-liquid (> 0.5 mL) was extracted from bulk scale phase separation at [NaCl] = 0.5 M and transferred to a rheometer with 20 mm diameter flat plates and a 0.2 mm gap. The storage and loss moduli were measured over a range of frequencies at 5% strain, which is confirmed to be in the linear regime. The viscosity was calculated using $\eta = \frac{2\pi G_{cross}}{\omega_{cross}}$, where G_{cross} and ω_{cross} are the modulus and frequency, respectively, of the point at which the storage and loss modulus curves intersect, as shown in Fig. S3. At 20 °C, the viscosity was calculated to be about 46 Pa·sec, very close to the viscosity measured from microrheology, which was about 45 Pa·sec at [NaCl] = 0.5 M.

Fluorescence recovery after photobleaching (FRAP)

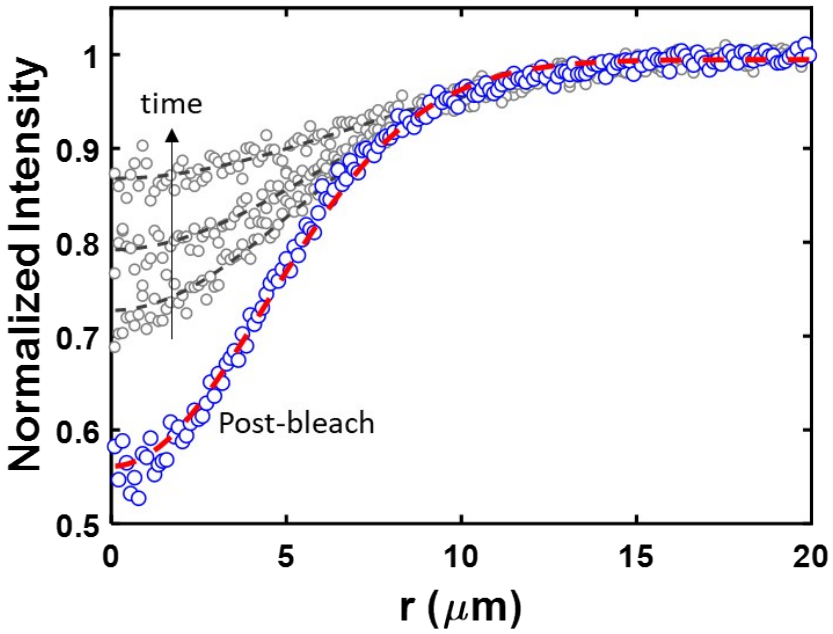

Figure S4: Normalized averaged intensities at radial distance r from the bleach spot at 0 (blue), 1, 2, and 5 min after bleach at [NaCl] = 0.25 M. At each time point, the data are fit to $I(t,r) = I_0(t) - A(t)exp(-r^2/2v(t)^2)$.

After point bleaching near the center of large NS droplets at different [NaCl], the radial averaged fluorescence intensity near the bleach spot was calculated from the FRAP images at each recovery time and fit to a Gaussian function $I(t,r) = I_0(t) - A(t)exp(-r^2/2v(t)^2)$, where I is the averaged intensity at radial distance r from the bleach spot and v^2 is the variance of the Gaussian function. The NS diffusion coefficients at different [NaCl] are obtained from plots of v^2 vs. time as shown in Fig. 2c in the main text.

NS overhang binding probabilities in NS-liquids

To estimate the effect of salt on NS interactions, we calculate the binding probabilities of overhangs. To do this, we use a mean-field picture in which we ignore connectivity of the overhangs within a NS. That is, individual overhangs are assumed to be freely translating at a concentration set by the measured c_{DNA} in the DNA liquid phase. Using the c_{DNA} data from Table 1, the NS overhang concentrations are 1.28, 1.66, and 2.34 mM for [NaCl]=0.25, 0.5, and 1 M, respectively. Then, given the SantaLucia parameter set[3], and T = 293 K, we find the binding probabilities for this NS overhang sequence (5'-CGATCGA-3') to be 98.7, 99.2, and 99.5% at [NaCl]=0.25, 0.5, and 1 M, respectively. We note that this mean-field picture will overestimate the binding probability: the actual connectivity (4 overhangs per NS) will lead to geometric constraints that will likely decrease the actual achieved binding probability.

Effect of NS overhang sequence

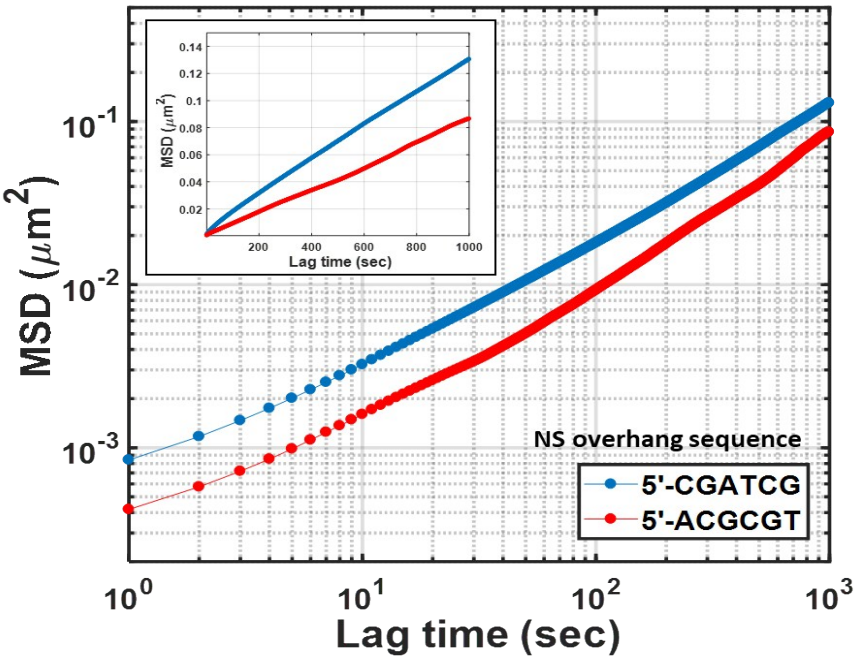

Figure S5: Averaged mean squared displacements (MSD) of 200 nm probe particles in liquid droplets of two different NSs, NS-CGATCG and NS-ACGCGT, as a function of lag time at 0.5 M NaCl. The lower MSD values for NS-ACGCGT lead to a higher viscosity, when calculated from $MSD = 4D_{probe}\tau^{\alpha}$.

We clearly show in the main text that the base-paring between NS overhangs dominantly controls the physical properties of the NS liquids. In addition to changing [salt] (see main text), we also tested whether the NS liquid properties can be controlled by varying the overhang sequence. Fig. S5 shows the microrheology result of a new NS liquid system (overhang sequence: ACGCGT) at [NaCl]=0.5 M compared to that of NS-CGATCG at the same [NaCl]. The averaged MSD of the 200 nm probe particles are seen to be significantly lower in the liquid droplet of the new NS (ACGCGT). We calculate the viscosity of NS-ACGCGT to be about 86 Pa·sec, which is higher than the 45 Pa·sec exhibited by NS-CGATCG at the same [salt]. This higher viscosity of the new NS-liquid is consistent with a lower ΔG for overhang base pairing, where, based on DNA nearest-neighbor thermodynamics[3], ΔG for NS-ACGCGT is estimated to be about -9.5 kcal/mol compared to ΔG for NS-CGATCG of about -9.1 kcal/mol, at [NaCl] = 0.5 M and 20 ^{o}C.

References

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