INTRODUCTION
Phase separation of biomolecules is emerging as a fundamental principle of intracellular organization (1, 2). For the past few years, numerous works have shown that cells use phase separation as a mechanism to form diverse condensates that concentrate specific sets of biomolecules and facilitate cellular reactions required for their survival and proliferation (3, 4). Examples of intracellular condensates include not only canonical membrane-less organelles such as nucleoli (5), nuclear speckles (6), and cytoplasmic stress granules (7) but also recently described structures such as transcriptional, synaptic, and signaling clusters (8–10). Each condensate performs distinct cellular functions, mainly by controlling their specific compositions (11). Although much remains to be studied, a network of transient multivalent intermolecular interactions appears to determine the composition and organization of condensates (12). A complete understanding of how nanoscale intermolecular interactions can give rise to rich multiphase behaviors of micrometer-scale condensates is often hampered by the intrinsic complexity associated with protein-protein interactions.

As a building block, DNA provides a unique advantage over any known materials in that the thermodynamic stability and specificity of intermolecular interactions can be precisely defined from its linear sequence (13). This remarkable programmability has been widely demonstrated in numerous examples where the mixtures of DNA strands self-assemble into complex three-dimensional structures (14–16). The sequence programmability is initially used to build static assemblies (17–19) but quickly applied to construct dynamic ones as well (20–23). In particular, the demonstration of DNA strand displacement reactions has opened new avenues for a wide range of applications involving the dynamic exchange of individual DNA strands (24–26), which include molecular computation (27–29), controllable hydrogels (30, 31), and autonomous nanomachines (32–35). Notably, in most examples, the assembled DNA condensates function as isolated units either in the dilute solution or on the solid substrate surface (36, 37). In intracellular condensates, however, component macromolecules are loosely held together through transient interactions, causing dynamic rearrangements in their relative configuration with others (2). This dynamic internal environment facilitates various biochemical reactions such as ribosome biogenesis, RNA transcription, and DNA repair (1).

The biomolecular phase separation based on the Watson-Crick base pairing has been implicated in the assembly of intracellular condensates (38) and also demonstrated with DNA nanostructures of limited valency, called DNA nanostars (39, 40). The self-associating interactions in the DNA nanostars are solely mediated by the sticky ends of each arm, a feature highly useful to study the relation between intermolecular interactions and macroscale thermodynamic and rheological properties (41–44). DNA nanostar systems exhibit characteristic phase behaviors with the low-valence associated shrinkage of the coexistence region and accompanied emergence of an equilibrium gel (39, 45). Several recent works began to exploit the sequence programmability of DNA to build multiphase DNA liquid droplets (46, 47) and to study DNA partitioning into these structures (48). However, a systematic implementation of functional DNA-based condensates mimicking intracellular counterparts has not yet been achieved.

Here, we demonstrate a DNA-based synthetic condensate platform where the composition and function of condensates can be rationally designed from their individual sequences. Inspired by the way intracellular condensates are organized and function (Fig. 1A), our DNA condensates are composed of scaffolds that drive phase separation and clients that confer specific functions (Fig. 1B). We demonstrate that the condensation and dissolution of condensates can be controlled by the presence of specific signals that act on scaffolds. In addition, we characterize the kinetics and thermodynamics of...
the selective partitioning of client strands into specific condensates (Fig. 1C). We further show that the DNA condensates drastically accelerate molecular computation involving DNA strand displacement reactions in a highly selective manner, which can potentially be used to encode complex phase behaviors coupled with reactions. Our work set a stage for designing and building synthetic condensates that would be critical to construct more life-like artificial machines.

RESULTS

Design of the DNA-based synthetic condensates

Our DNA-based synthetic condensate system is inspired by the way biomolecular condensates organize the content of living cells (1, 2). Biomolecular condensates typically exhibit a unique set of internal components (Fig. 1A), which can be largely classified into two categories: scaffolds and clients (49). Scaffolds are drivers for phase separation, exhibiting self-association properties. They are required for the formation of condensates, and the strength of intermolecular interactions between scaffolds is often a target of biological regulation to modulate phase separation processes (50). In contrast, clients cannot induce phase separation by themselves but can contribute to define condensate functions by altering their compositions. Specific molecular interactions between clients and scaffolds drive the partitioning of clients into the condensate.

We adopt this organization of intracellular condensates into our DNA-based synthetic condensate platform. Taking advantage of the tunability of intermolecular interactions between DNA strands, we design DNA-based scaffolds and clients (Fig. 1, B and C; a list of the full sequences of strands is provided in the Supplementary Materials). Our scaffolds have properties of self-association and client binding. To implement self-association, we build our scaffolds from well-characterized Y-shaped DNA nanostars (hereafter referred to as a Y-scaffold) (39, 40, 46). Y-scaffolds have three arms with the sticky palindromic sequence (5′-GAGCTC-3′) of a single-stranded DNA (ssDNA) located at each end. Previous works showed that similar Y-shaped DNA nanostructures undergo self-associative phase separation (39, 41). For client binding, we then modify the Y-scaffold to append a short ssDNA overhang, which we call a toehold, in the middle of each scaffold arm. In addition to serving as a binding site for clients, the toehold in our system also functions as a handle for condensation control. Notably, both the toehold and the sticky end are part of a single oligonucleotide, termed assembler (Fig. 1A and fig. S1A). Thus, using toehold-mediated DNA strand displacement reactions, it is possible to release the assembler from the Y-core, leading to condensate dissolution. This feature enables a signal-dependent control over condensation and dissolution of the DNA-based synthetic condensates. Clients in our system can be any DNA elements displaying an ssDNA region complementary to the toehold of the Y-scaffolds. In this study, we mainly use ssDNAs or double-stranded DNAs (dsDNAs) up to 50 nucleotides (nt) in length as clients. We note that in our design of the DNA-based condensates, the sequences of sticky ends and toeholds can be chosen independently, thus enabling the orthogonal control of phase separation behaviors of the condensates and their composition.

Tunable phase separation behaviors and material properties of the DNA-based condensates

We first characterize phase separation behaviors of the Y-scaffold. To assemble the scaffold, we mix equimolar concentrations of DNA
strands comprising the Y-scaffold and incubate them at 95°C followed by cooling at a rate of −1°C/min down to 25°C using a thermal cycler. Polyacrylamide gel electrophoresis (PAGE) analysis shows that the scaffolds are successfully assembled as designed (fig. S1B). We then image this sample at different temperatures using a laser scanning confocal microscope equipped with a temperature control stage. Because self-association between the Y-scaffold is mediated by hybridization of the sticky palindromic sequence, we expect that temperature strongly influences scaffold condensation (39, 46). We observe that condensate formation highly depends on temperature: At 60°C, no visible structures are present, but at 30°C micrometer scale, condensates with the concentration of Y-scaffolds much higher than the surrounding space are observed (Fig. 2A). To test the concentration dependence of condensate formation, we image samples of Y-scaffolds at different scaffold concentrations. At low scaffold concentration around 300 nM, no visible condensates are observed. As the Y-scaffold concentration increases, the volume fraction of condensates gradually increases (Fig. 2B), while both the dilute and condensed phase concentrations stay similar (figs. S2 and S3). This is consistent with binary phase separation behaviors that are widely observed in the solution of purified proteins acting as a scaffold in intracellular condensates (2, 51, 52). When the Y-scaffold concentration is high enough, a large amount of the condensed phase forms and settles down to the bottom of a microcentrifuge tube, which is visible even to the naked eye (Fig. 2B).

We next probe the material properties of the condensate using fluorescence recovery after photobleaching (FRAP) assay (Fig. 2C). At 40°C, FRAP experiments reveal a marked recovery, indicating that the condensates have liquid-like properties with highly mobile Y-scaffolds. However, when we lower the temperature to 4°C, the recovery is substantially suppressed. Thus, temperature is an important parameter for tuning the physical properties of the condensate, and lowering temperature leads to the formation of a gel state with reduced molecular dynamics. These data are consistent with previously known rheological behaviors of the DNA nanostar solution, where, for a concentration range above the coexistence region,
a gradual transition from a fluid-like to a solid-like state occurs as temperature drops, via a percolation transition (42–45, 53). The phase separation accompanying the percolation transition in the dense phase is a general characteristic of associative polymers where the time scales of intermolecular interactions govern the viscoelastic properties of the dense phase (54).

Because the hybridization strength between DNA strands is also highly sensitive to salt concentration (41), we examine the phase behaviors of Y-scaffolds at different salt concentrations. Consistent with previous works (40, 55), we find that the phase diagram, i.e., binodals, of Y-scaffolds is substantially affected by changes in salt concentration (Fig. 2D): Increasing NaCl concentrations leads to a marked expansion of the coexistence region. Varying the fraction of dye-labeled Y-scaffolds does not alter the measured values for condensed phase concentrations (Fig. S4), indicating that fluorescence labeling does not affect our phase diagram characterization.

At 350 mM NaCl, a condition mostly used in this study, we obtain the scaffold concentration of the condensed phase to be 194 ± 30 μM, equivalently 12.7 ± 2.0 mg/ml. In contrast, in vitro protein condensates typically exhibit much higher biomolecular concentrations, around 100 to 400 mg/ml (56, 57). The low-density nature of the trivalent or tetravalent DNA nanostructures was shown to be an inherent characteristic of systems with limited valency (39, 58).

The observed salt-dependent changes in scaffold phase diagram can be attributed primarily to the effect of salt concentrations on bond probability between the sticky ends of the Y-scaffold. For example, to reach a bond probability of 0.5, i.e., a condition for the half of sticky ends engaged in binding between scaffolds, or equivalently a condition for the percolation of trivalent particles in mean-field (53, 59), required scaffold concentrations are estimated to vary from ~230 to ~80 μM for 150 and 500 mM NaCl, respectively (fig. S5). Consistent with this notion, we find that changing salt concentrations heavily influences the material properties of condensates as well. In FRAP assay, the rates of fluorescence recovery progressively slow down as salt concentration increases (Fig. 2E), which translate into apparent diffusion coefficients of $D \approx 0.013, 0.004, and 0.002 \mu m^2/s$ for 200, 350, and 500 mM NaCl, respectively. The reduced molecular mobility of Y-scaffolds also affects the rate of shape relaxation of DNA condensates during fusion events. Upon contact with one another, surface tension tends to drive the relaxation of biomolecular condensate morphology back to the spherical one (2). At low salt concentration (200 mM NaCl), we observe progressive relaxation of the condensate shape with an inverse capillary velocity of $\sim 0.36 \text{min}/\mu m$ (Fig. 2, F and G), similar to previously reported values (40). Consistent with an increase in the binding stability between scaffolds, raising the salt concentration slows down the relaxation process (fig. S6). Together, our data demonstrate that the phase behaviors of Y-scaffolds are largely governed by the sticky end sequence, which can be tuned with environmental parameters such as temperature and salt concentration.

The control of condensation and dissolution of the DNA condensates

We then probe the controllability over the condensation and dissolution of DNA-based condensates (Fig. 3A). The molecular connectivity between multivalent macromolecules is a key parameter influencing the stability of liquid condensates (1, 12, 60). Consistent with this idea, we find that a molar ratio of the Y-core to the assembler strand containing the sticky end is critical for condensate formation (fig. S7): No condensation is observed for an equimolar mixture of the Y-core and the assembler, while strong condensation occurs in the mixture of threefold excess assemblers. Samples with twofold excess assemblers occasionally exhibit tiny puncta (fig. S7). This likely reflects the outcome of a hybridization equilibrium between the Y-core and the assembler, leading to small amounts of trivalent Y-scaffolds. When condensate is dynamically induced by flowing assemblers into a sample containing Y-cores, we observe that DNA condensates enriched with both components progressively settle down to the bottom of an imaging chamber, again in a stoichiometry-dependent manner (Fig. 3, B and C).

The requirement of minimum trivalency for condensate formation suggests that removing just a single sticky end from each Y-scaffold would substantially reduce its capacity to interact with other scaffolds and can ultimately lead to condensate dissolution. To test this idea, we examine condensate stability in the presence of a dissolver strand of which sequence is fully complementary to the assembler. Dissolvers can thus bind to the toehold region of the Y-scaffold and then undergo branch migration to eventually displace the assembler from the Y-scaffold (Fig. 3A). The addition of dissolvers into the chamber containing preassembled Y-scaffold condensates leads to gradual dissolution of individual condensates (Fig. 3D). Compared to 2.5 μM dissolvers, condensates dissolve faster at higher dissolver concentration of 10 μM. At 20 μM dissolvers, condensates completely dissolve in less than 15 min with frequent formation of vacuoles (fig. S8); similar vacuole-forming behaviors were also observed for other biomolecular liquids (61, 62). To reliably quantify the dissolution process, we focus on the dissolver concentration range up to 10 μM. Notably, we observe that the effective radius of condensates, $R_{\text{eff}}$, defined by the condensate volume, $V$, as $V = (4/3)\pi R_{\text{eff}}^3$, decreases linearly over time (Fig. 3E). The condensate dissolution rate, i.e., the rate of radius changes of condensates, is independent of its radius (fig. S9), indicating that for experimental conditions used in this work, dissolver-mediated condensate dissolution is a surface area–limited process (62, 63).

To further probe the dissolution mechanism of condensates, we measure the dissolution rates of condensates as we systematically vary the concentrations of scaffolds and dissolvers (Fig. 3, F and G and fig. S9). We find that varying scaffold concentrations shows minimal effects on condensate dissolution, but the dissolver concentration is a key determinant of dissolution rates. This behavior can be attributed to binary phase separation of Y-scaffolds into a scaffold-rich dense phase and a scaffold-poor dilute phase. When the dissolver is added into this two-phase system, it would rapidly act on scaffolds in the dilute phase that are floating around freely in solution. Depending on the rate of interpenetration and strand displacement reaction, scaffolds located within the dense-condensate phase would experience different levels of sticky end loss. We thus first characterize the speed of dissolver interpenetration into DNA condensates during dissolution reactions. Immediately after adding dissolvers (<1 min), no difference in the fluorescence intensity of dissolvers is observed between the dense and the dilute phase (fig. S10), suggesting that dissolver interpenetration is a relatively fast process compared to the time scale of condensate dissolution (typically tens of minutes in our experimental conditions).

We then measure the rate of the strand displacement reaction mediated by dissolvers. For this purpose, we use the Y-scaffold composed of a dye-labeled Y-core and a quencher-labeled assembler strand (fig. S11A). The strand displacement reaction mediated by
dissolvers leads to a removal of the assembler from the Y-core, causing fluorescence unquenching. Using 300 to 600 nM dissolvers and 300 nM Y-scaffolds, we quantify kinetics of sticky end removal from scaffolds (fig. S11B). We note that at this Y-scaffold concentration, no condensation is induced (Fig. 2D). The dissolver-mediated strand displacement reaction can be well described by a simple bimolecular reaction with the rate constant of $k = 3.7 \pm 0.2 \times 10^4 \text{ M}^{-1} \text{s}^{-1}$.

Fig. 3. The control of condensation and dissolution of the DNA condensate. (A) Schematic of dynamic condensation and dissolution of the Y-scaffold. Binding of the assembler to the Y-core leads to the formation of the trivalent Y-scaffold, triggering phase separation. Upon addition of the dissolver, the toehold-mediated strand displacement reaction strips off the assembler from the Y-core, resulting in condensate dissolution. (B) Time-lapse images of dynamic condensation experiments with different concentrations of the assembler. Y-core (10 μM) is present in the chamber before the addition of 30 μM assembler. (C) Temporal changes of the SDs of the assembler channel images in (B). Identical quantification on the 10 μM assembler data is shown as well. (D) Time-lapse images of the side view of condensate dissolution obtained with different dissolver concentrations. Before the addition of dissolvers, condensates assembled at 5 μM Y-scaffolds are present in the sample. (E) Temporal changes of the effective radius of condensates measured with different concentrations of scaffolds and dissolvers. For each condition, the average and the SD of five different condensates are plotted. All data were acquired at 30°C and 350 mM NaCl. (G) Condensate dissolution rates obtained with different concentrations of scaffolds and dissolvers. Dissolver concentrations used are 2.5, 5, 7.5, and 10 μM. Data points are shifted slightly in the x axis for clarity. Scale bars, 20 μm (B) and 5 μm (D).

Collectively, these lines of evidence suggest that the depletion of sticky end containing Y-scaffolds in the dilute phase is a major driving mechanism for dissolver-mediated dissolution processes. In the case of coexisting phases at equilibrium, the solute concentration in the dilute phase is maintained at a fixed level but would decrease over time. A strong correlation observed between the condensate dissolution rates and the reduction of sticky end containing Y-scaffolds in the dilute-phase supports this view (fig. S13).

The composition control of the DNA condensates

Intracellular condensates play diverse physiological roles by facilitating biochemical reactions and sequestering key regulatory components, both of which are, in essence, driven by enrichment of...
specific molecules into the condensates (1, 2). In our DNA condensates, the Y-scaffold harbors the toehold, having bifunctional roles of controlling condensate assembly and composition, to which clients with complementary sequences can bind (Figs. 1, B and C, and 4A). We refer to DNAs without a region of sequence complementary to the toehold as spectators. Thus, the Y-scaffolds are designed to selectively enrich only clients, from the mixture of clients and spectators, into the condensate.

We first probe time scales at which clients diffuse into the Y-scaffold condensate. When a mixture of both clients and spectators is flowed together into the chamber with preassembled condensates, we observe that over time, clients become enriched into the condensate while spectators are slightly excluded (Fig. 4, B to D). Initially, a concentration gradient of clients appears within the condensate, high along the periphery and low at the center, which decays over time to yield a uniform distribution of clients (Fig. 4B). After ~15 min, client concentration in the condensate reaches the steady-state value (Fig. 4C).

Having established the toehold-mediated recruitment of clients into condensates, we next sought to systematically vary toehold sequences and quantify the enrichment of dsDNA clients into the condensate. To characterize the degree of enrichment, we compute partition coefficients (PCs), which we define as the ratio of either client or scaffold concentration in the condensate versus that in the dilute phase. We find that client partitioning is heavily affected by the specific sequence used for toeholds (Fig. 4E). The partitioning tends to increase as either the length of the toehold or the fraction of G/C nucleotides increases. To further gain insights for client partitioning, we compute free energy changes associated with client hybridization using the web-based analysis tool DINAMelt (table S1) (64). We find that the free energy change of hybridization is a strong determinant for client partitioning (Fig. 4F); At |ΔG| ~ 6 kcal/mol, we begin to observe an increase in client partitioning, which ultimately saturates at ~10 kcal/mol. Overall, similar partitioning behaviors are observed for ssDNA clients as well, albeit with higher levels of partitioning at saturation (max PC was ~55 and ~100 for ds- and ssDNA clients, respectively; Fig. S14A).

We model the behaviors of client partitioning based on equilibrium binding between clients and scaffolds (11, 65). The model describes our DNA system as two compartments sharing free clients while allowing equilibrium binding between molecular species in each compartment (Fig. 4G; see Materials and Methods for details). The model successfully captures several key features of client partitioning data (Fig. 4F): (i) a sharp increase in partitioning as the hybridization becomes stronger, (ii) the saturation of partitioning above high enough hybridization strengths, and (iii) a difference in the saturation level of partitioning between ds- and ssDNA clients (Fig. 4F and fig. S14A). We note that only a single free parameter, a ratio of the association constant in the dense phase to that in the dilute phase, is used to fit the experimental data (K_{den}/K_{dil} = 0.32 ± 0.01 and 0.53 ± 0.01 for ds- and ssDNA clients, respectively; K is the association constant of the client to the scaffold either in the dilute or the dense phase). The difference in the association constants between the dilute and the dense phases can be attributed to the extra energetic cost of placing DNA clients in the network of scaffolds in the dense phase, which likely constrains the motion and configuration of DNA strands. This idea is also consistent with the observation that ssDNA clients, being more flexible than those of dsDNA, exhibit higher partitioning at saturation (Fig. 4F and fig. S14A).

On the basis of the polyphasic linkage formalism (66), the difference in the association constants of the client to the scaffold in two coexisting phases manifests as a change in the saturation concentration of the scaffold (65). Assuming the first-order binding polynomial, the saturation concentration of the scaffold in the presence of clients, S_{dil}^C, is expressed as S_{dil}^C = S_{dil} \left( \frac{1 + K_{dil} [C]}{1 + K_{den} [C]} \right) (65, 66), where S_{dil} and [C] are the saturation concentration of the scaffold in the absence of any clients and free client concentration, respectively. For the concentration used in the client partitioning experiments (100 nM), this client-mediated change in the scaffold saturation concentration is expected to be minimal (fig. S15). However, at higher client concentrations, the client can behave like a modulatory ligand altering thermodynamic phase behaviors of scaffolds (65). We observe the modulatory effect of clients on the saturation concentration of the scaffold in our DNA condensate system, with stronger effects observed for either higher client concentration or stronger binding affinity between the client and the scaffold (fig. S15). These results are consistent with our model prediction and a previous theoretical study (65), further confirming that condensate composition can be rationally designed using our DNA-based system.

The strong impact of binding strengths on partitioning indicates that the diffusive mobility of clients within DNA condensates would also highly depend on the nature of toeholds used. To probe the mobility of clients in the condensate, we perform a series of FRAP measurements on different clients. We again find a strong correlation between the rate of client diffusion and the free energy of hybridization (Fig. 4H). Over the hybridization free energy changes from ~−6.2 to ~−15.1 kcal/mol, a more than 100-fold change in the diffusion coefficients of clients is observed. Thus, the toehold displayed by the scaffold together with its cognate binding sequence in the client dictates condensate composition and internal client dynamics.

Our DNA condensate system is designed for the orthogonal control over condensation and composition by using separate sequence motifs. Clients used in previous studies typically shared with other scaffolds a binding site to the scaffold (46, 48). In this case, a direct competition between clients and scaffolds is expected, which can adversely affect the stability of condensates (12, 65). When the client harboring the same sticky end as the scaffold is added at a concentration comparable to the scaffold, we observe that condensates undergo a marked level of dissolution, in contrast to the orthogonal clients used in this study (Fig. 4, I to J). Together, we demonstrate that the composition of our DNA condensates can be precisely defined using toehold-mediated client partitioning while preserving the overall integrity of condensates.

**The condensate-mediated acceleration of strand displacement reactions**

We next sought to test whether DNA condensates can perform designed functions based on the enrichment of specific components. DNA strand displacement reactions have been widely used to build reaction networks that perform diverse tasks such as molecular computation, autonomous stepping, and mRNA labeling (15, 24). Because our DNA condensates can concentrate specific clients, we reason that DNA condensates can facilitate DNA strand displacement reactions by recruiting a set of defined clients (67). We design a double-cascade DNA strand displacement reaction where a single-stranded output from the first cascade acts as an input for a
Fig. 4. Composition control of the DNA condensates through selective partitioning of clients. (A) Schematic of toehold-mediated composition control. Only clients with a region complementary to the toehold of the scaffold partition into the condensate. (B) Time-lapse images of the Y-scaffold condensate during client partitioning. The Y-scaffold with a single toehold of 8-nt GC 25% is used. (C) Temporal changes of the fluorescence intensities of clients within the condensate in (B). Intensities are normalized by corresponding values outside the condensate. (D) The fluorescence intensity of each component across the condensate at 10 min after the addition of clients. A two-step moving averaging of 5 pixels (0.8 μm) and 5 frames (40 s) is performed to reduce noise. (E) Merged fluorescence images of scaffolds (magenta) and clients (green). For each sample, 100 nM clients are incubated with a corresponding Y-scaffold (3 μM) for 12 hours. (F) The measured client PCs are plotted against the free-energy changes of hybridization of clients calculated from the DINAmeit (See Materials and Methods). Error bars are SDs (n = 30). A dashed curve denotes a result of the model fit based on the equilibrium binding model (See Materials and Methods). (G) The conceptual scheme of the equilibrium binding model. The scaffolds and clients in each phase undergo equilibrium binding while allowing the exchange of free clients between phases. (H) FRAP recovery curves of clients with different binding sites to the Y-scaffold. Error bars are SDs (n = 3). (I) Schematic of the partitioning into condensates for two different types of clients. (J) Time-lapse images of the condensates upon the addition of two different clients shown in (I). Clients (15 μM) are added to the solution of 3 μM scaffolds. Scale bars, 50 μm (E) and 20 μm (B and J).
downstream reaction (Fig. 5A). Each cascade component harbors a binding sequence to the toehold of the Y-scaffold, and the reaction progress can be monitored via fluorescence, which is produced when a quencher-labeled strand becomes displaced from a reporter strand at the end of cascade.

We first confirm that in the absence of scaffolds, the designed reaction cascade functions in an input-dependent manner (fig. S16). When the identical reaction is repeated in the presence of condensates, we observe that the reaction is greatly accelerated (Fig. 5B). In terms of the initial reaction rate over 1 min, ~7-fold enhancement in the reaction rate is observed for the condensate-mediated reaction cascade. Control experiments show that the reaction acceleration occurs only in the presence of client partitioning into condensates; removing either the sticky end or the cognate toehold from the scaffold abrogates the acceleration effect (Fig. 5B). To further probe the reaction progress within two coexisting phases, we monitor the identical cascade reaction using confocal microscopy. Upon addition of the input strand, a rapid increase in the reporter fluorescence is observed within the dense condensate phase, while no fluorescence increase is observed in the control sample with an incorrect toehold sequence (Fig. 5C and fig. S17). For condensates ranging in radius from 15 to 21 μm, the measured reaction speed within condensates is ~26 or 49 times faster, compared to the reaction in the dilute phase or scaffold-free condition, respectively (Fig. 5D), confirming that DNA condensates can dramatically accelerate strand displacement reactions by enriching clients with specific toehold binding sequences.

We find that even in the dilute phase, the initial reaction rate is 1.9-fold higher than the scaffold-free sample (Fig. 5E). This result appears contradictory to the law of mass action: The concentrations of reaction components in the dilute phase are lower than the scaffold-free condition, due to the partitioning into condensates, as reflected in the lower final reaction product (Fig. 5E). To probe this effect in detail, we first measure the degree of partitioning for each reaction component (table S2) and then quantify the reaction rates in the absence of scaffolds at the concentrations of reaction components equivalent to either dense condensate or dilute phase (Fig. 5F). For the condensate-equivalent condition, we observe a similar degree of reaction acceleration as condensates, indicating that the mass action, i.e., an increase in the concentrations of reaction components, alone can sufficiently explain the acceleration effect (Fig. 5G). In contrast, when the reaction rates in the dilute phase of the DNA condensate system are compared to those produced by the same concentrations of reaction components in the absence of scaffolds, the former condition exhibits 2.4- to 5.2-fold faster rates depending on the total client concentration used (Fig. 5G). Thus, the reaction in the dilute phase proceeds at a rate higher than expected by the mass action. This behavior may be due to a population of small solute clusters that tend to be present even in the subsaturated solutions (60, 68), which can affect the binding between reaction components through scaffolding. Similar behaviors and mechanisms are observed for SUMOylation reactions in the condensates of multivalent proteins (69).

We then sought to examine the effect of binding strengths between scaffolds and clients on the condensate-mediated reaction rates. We first confirm that in the absence of scaffolds, altering scaffold binding regions causes minimal impacts on the strand displacement reactions (fig. S18). As we characterized in this study, increasing the binding strength can cause two different effects on the condensate system: an increase in the concentration of reaction components (Fig. 4, E and F) and a decrease in their mobility within condensates (Fig. 4H). These features would affect the reaction rate in an opposite manner; increasing client partitioning can enhance reaction rates, while slow molecular dynamics would adversely affect the reaction. To probe relative contributions of these two behaviors, we measure the reaction rates of condensate systems made of five different types of toeholds and their binding pairs (Fig. 5H). Consistent with our expectation, we find that there is an optimal binding strength between clients and scaffolds, ΔG ≈ −9.2 kcal/mol, at which the reaction runs fastest: With the strongest binding pair, 12-nt GC 50%, the reaction rate drops to the half of the maximum rate. Direct imaging of the reaction progress in DNA condensates reveals the formation of a steep, persistent concentration gradient in the reporter fluorescence signal in the sample of a strong binding pair (Fig. 5I, 10-nt GC 50%), unlike the uniform distribution observed in the weak binding pair (Fig. 5C, 8-nt GC 25%). These findings confirm that strong binding between reaction components and scaffolds can indeed inhibit molecular diffusion and slow down the kinetics of overall reactions.

The condensate-based logic gate operation
The DNA strand displacement reaction has been widely used as an elementary reaction to build diverse reaction networks for applications such as molecular computation (24, 25). Having characterized the performance and mechanism of the condensate-mediated reactions, we then test whether our condensate platform can be applied to accelerate the DNA-based logic gate. For this purpose, we first use a two-layer OR gate comprising total 12 DNA reaction components (Fig. 6A) (28). When coupled to the condensate, we find that circuit computation is drastically accelerated: Depending on input types, the reaction rate is increased by 12- to 22-fold (Fig. 6, B to D). The demonstrated capacity of condensates in reaction acceleration can be highly useful for diverse DNA circuits; by simply appending scaffold binding sequences to the desired reaction components, selective reaction acceleration can be achieved.

To further demonstrate the applicability of our DNA condensate system toward molecular computation, we design a gating mechanism based on the condensate-mediated modulation of reaction kinetics (Fig. 6E). In our kinetic gating mechanism, the output of circuit operation depends on a selector strand that determines the specific reaction pathway to be coupled with condensates. The reaction acceleration in the chosen pathway can then be fed into other reaction elements to achieve the gating effect. As a proof of concept for the kinetic gating, we use parallel reaction cascades sharing a gate strand that functions exclusively for whichever cascade comes first. Thus, the coupling of the one cascade to condensates can lead to the depletion of the shared gate before the other cascade can act on, enabling the kinetics-based gating operation. We test the performance of the kinetic gating scheme using three parallel reaction cascades (fig. S19). Among them, two cascades participate in the kinetic gating by sharing an intermediate gate, while the remaining cascade is used as a bystander. Using this system, we indeed observe that the kinetic gating successfully operates as designed, displaying clear differences in the circuit output depending on the type of selector strands used (Fig. 6, F to H). Upon the selector-mediated coupling of the reaction cascade to condensates, the corresponding reaction is drastically accelerated with a concomitant inhibition of the other cascade engaged in the kinetic gating. Conversely, the
Fig. 5. Quantitative analysis of condensate mediated strand displacement reactions. (A) Schematics of a double-cascade DNA strand displacement reaction used in this figure. (B) Left: Kinetics of the reactions shown in (A) with either different inputs or scaffolds, measured with a plate reader. "T" and "F" indicate true and false sequences, respectively. (C) Fluorescence images of the condensate-mediated strand displacement reactions. (D) Kinetics of the reactions in the dilute and the dense phase, obtained from imaging data as in (C). (E) Kinetics of strand displacements in the dilute phase of the two-phase reaction and the single-phase one in the absence of scaffolds. (F) Schematic of the experiments to probe the contribution of the mass action in the condensate-mediated reaction. "SC." and "CL." indicate scaffolds and clients, respectively. (G) The results of experiments designed in (F) at indicated client concentrations. All data are normalized with the initial reaction rate in the absence of scaffolds at the client concentrations of 100 nM. Error bars are SDs (n = 3 independent experiments). (H) The reaction kinetics conducted with client-scaffold pairs of different binding strengths. The inset shows the normalized reaction rates during the initial 1 min plotted against the hybridization free energy between scaffolds and clients. Error bars are SDs (n = 3 independent experiments). (I) Time-lapse images of the condensate-mediated reactions conducted with scaffold-client pairs of different binding strengths. Scale bars, 20 μm (C and I). In this figure, unless otherwise specified, all reaction components are at 100 nM and have a scaffold binding site of 8-nt GC 25%. All the relative initial rates in the insets of this figure show the fluorescence increase over initial 1 min normalized with the corresponding value in the absence of scaffolds.
**Fig. 6. The condensate-mediated molecular computation.** (A) Schematic of a two-layer OR gate and the truth table for different inputs. (B) The fluorescence kinetics data of the two-layer OR gate for different inputs in the absence of scaffolds. (C) The fluorescence kinetics data of the two-layer OR gate for different inputs in the presence of condensates. All conditions are identical to (B) except for 1.5 μM Y-scaffolds added for condensate assembly. (D) Comparison of the half-time to completion for each logic gate operation in (B) and (C). The fold-changes between the half-times of the presence and absence of condensates are indicated. (E) Schematic of the kinetic gating mechanism. The selector strand defines which reaction cascade to be coupled to condensates for reaction acceleration. In our circuit of kinetic gating, the condensate-mediated acceleration of the chosen cascade leads to the depletion of an intermediate gate shared with the other reaction cascade. Because of the depletion, the latter cascade becomes strongly inhibited. (F to H) The fluorescence kinetics data of the kinetic gating for four different conditions specified in (H). Each selector strand couples the reaction components in the corresponding cascade to the condensate. The fluorescence signals are normalized with the average of maximal five data points of the reaction without selectors. All experiments were repeated three times, and error bars indicate SDs.
bystander reaction cascade stays unperturbed, regardless of the presence of either scaffolds or selectors.

Last, to expand the functionality of condensates, we take advantage of the immiscibility of DNA droplets (46, 47). Combining the orthogonality present in our system between condensation and composition control, multiple immiscible condensates with different compositions can be assembled. Thus, our DNA condensate system can be used to organize a multiphase reaction system in a way similar to diverse functional biomolecular condensates that coexist in living cells (1, 2). To demonstrate this feature in the simplest format, we prepare two different sets of scaffolds and clients so that each condensate recruits only one set of reaction components. The addition of input strands results in the fluorescence increase from reaction components cognate to each condensate, indicating that the specificity of reactions can be also conferred to the condensate (fig. S20). Thus, our DNA condensates represent a programmable immiscible phase that selectively recruits specific components and facilitates chemical reactions between them.

**DISCUSSION**

Cells use a mechanism of phase separation to organize their contents, via the formation of dynamic membraneless condensates (1, 2). Specific biomolecules recruited into the condensates interact with one another to perform diverse cellular reactions. In this study, we introduce DNA-based synthetic condensates mimicking the way intracellular condensates are organized and function. Using the remarkable features of DNA in programming well-defined intermolecular interactions, we design DNA strands of scaffolds and clients that together self-assemble into functional condensates. The low valency of our scaffolds enables the formation of low-density condensates (39, 58) that can accommodate short DNA clients on demand. Using a separate motif for scaffold-client interactions from scaffold-scaffold interactions, a full control over the identity of individual condensates, including composition and miscibility, is conferred. We demonstrate that selective partitioning and enrichment of clients in condensates can drastically facilitate DNA strand displacement reactions, a feature that we apply to molecular computation. Thus, building on previous works (46, 47), our DNA condensates represent a programmable platform where their composition and function can be designed in a predictable manner.

In our work, we primarily focus on the demonstration of DNA-based rational design of synthetic condensates, but our approach can be extended in several straightforward manners. Although we use the DNA scaffold of three arms in this study because of its ease of implementation (46) and high sensitivity to perturbation, scaffolds of higher valency (40, 47) can give rise to condensates with different physical properties. As the valency increases, the binodals for condensate assembly would expand with a concomitant change in viscoelastic properties (39, 42). This would allow for the operation of condensates at higher temperatures or a broader concentration range, with potentially different reaction rates. Moreover, we expect that the technology we laid out here can be also used to build multimodal condensates. Similar to the way natural condensates function (1, 2), synthetic DNA condensates may release signals that can, for example, act on other DNA circuits in solution or other condensates. It would be also feasible to build synthetic condensates that function based on the sequestration mechanism, by depleting the pool of specific factors.

We envision that the DNA-based condensate platform can provide a layer of complex high-order functions that cannot be achieved from biomolecules in the dilute state. We expect that the unique advantages of DNA, including the sequence programmability of intermolecular interactions, biocompatibility, and the ease of interfacing with other biomolecules or nanomaterials, will make it an important ingredient to build more life-like synthetic systems.

**MATERIALS AND METHODS**

**Sequence design and DNA stock preparation**

All sequences of DNA strands used in this article are available in the Supplementary Materials (tables S3 to S15). NUPACK software was used to design sequences. DNA oligonucleotides were purchased from Bionics and Bioneer Corporation. All fluorophore- or quencher-labeled oligos were purified with high-performance liquid chromatography, and the other oligos were purified with PAGE. Delivered lyophilized DNA oligonucleotides were diluted to 100 μM with Millipore ultrapure water. To confirm the accurate concentration of the strands, absorbance values of the samples at 260, 280, and 320 nm were measured by using a microplate spectrophotometer (Epoch, BioTek). Before using in experiments, 100 μM DNA stocks were stored at 4°C. For preparing a 50 μM Y-shaped scaffold sample, lyophilized DNA oligonucleotides were diluted to 500 μM with Millipore ultrapure water.

**The assembly of Y-scaffolds**

Before assembling scaffolds, DNA stock solutions are dissolved to the desired NaCl condition. Unless otherwise specified, all experiments were conducted at 30°C and 350 mM NaCl. Then, diluted DNA solutions constituting the scaffold were mixed and vortexed together. To assemble the Y-scaffold completely, mixed samples were denatured and annealed by using a thermocycler (Eppendorf). Samples were heated to 95°C for 5 min and cooled down −1°C/min to 25°C. Typically, 10% of the scaffold population contains a single DNA strand labeled with Cy5 dye.

**Condensate imaging and FRAP experiments**

All imaging experiments were performed using a Nikon confocal A1 microscope with four-laser units (405, 488, 561, and 640 nm). For experiments requiring a large degree of temperature control, samples were imaged in an imaging channel made of a glass slide sandwiched with a cover glass by double-sided sticky tape on Linkam PE100 stage heater. In all other experiments, samples were observed in 96-well plates placed in a microscope stage incubator that can be set at desirable temperatures up to 40°C. All FRAP experiments were performed using the ROI (region of interest) stimulation function in the Nikon A1 confocal microscope. For comparison, the size of the stimulation ROI was set to be the same. During recovery, images of Fig. 2 (C and E) were acquired every 20 s for an hour. Images of Fig. 4H and fig. S14B were acquired every 10 s for 10 min. To obtain recovery curves, the fluorescence intensities within the ROI were integrated and normalized with the fluorescence intensity after bleaching. The recovery curves were fitted to the 2D infinite model to obtain the characteristic diffusion time scales (70).

**Polyacrylamide gel electrophoresis**

PAGE analysis was performed to verify the formation of the DNA nanostructures. One-millimeter-thick polyacrylamide gel was
prepared by mixing 6 ml of 19:1 40% acrylamide/bis acrylamide solution, 400 μl of 50× tris-acetate-EDTA (TAE) buffer, 13.6 μl of Millipore ultrapure water, 120 μl of 10% (w/v) ammonium persulfate solution and 12 μl of tetramethylethylenediamine. Hardened PAGE gel was mounted on a Mini-PROTEAN Tetra Cell (Bio-Rad) filled with 1× TAE buffer. All test samples were mixed with gel loading dye purple (B7024S, New England Biolabs), and 1 μl of each sample was loaded to the gel. After running the gel at 10 V for 70 min, it was stained with SYBR Gold solution for 10 min. All PAGE gel images were visualized by Gel Doc XR+ gel documentation system (Bio-Rad).

Quantification of the phase diagram of the Y-scaffolds
To measure the phase diagram of the scaffold, samples of 5 μM Y-scaffolds are prepared for various buffer conditions of different NaCl concentrations. Ten percent of the scaffolds are labeled with Cy5. The fluorescent intensities of the dilute and condensed phase are measured from z-stack images. To prevent the potential under-estimation of fluorescence intensity associated with small-sized objects, we only include condensates that are several times larger than the diffusion-limited confocal volume. To convert fluorescence intensities into the concentrations of the scaffold, the calibration process is performed using the sample without scaffold condensation.

Fusion dynamics of the Y-scaffold condensates
To measure the fusion dynamics of the Y-scaffold condensate, 5 μM Y-scaffolds solutions were prepared in 200 and 500 mM NaCl buffer. The surface of the imaging cell in the glass-bottom 96-well plate is pretreated with 5 μl of fluorinated oil with 2% surfactant (008-FluoroSurfactant in HFE 7500, RAN Biotech) before adding 100 μl of the Y-scaffold solution in different salinity. The fusion dynamics of condensates is imaged every 15 s using a laser scanning confocal microscope. For each image, the condensate aspect ratio, AR(t) = \( \frac{l_{\text{long}}}{l_{\text{short}}} \), where \( l_{\text{long}} \) and \( l_{\text{short}} \) are the long and short axes of the condensate, is measured using the ImageJ. The shape relaxation time, \( \tau \), is obtained by fitting the following expression: \( \text{AR}(t) = 1 + (\text{AR}(0) - 1) \exp(-t/\tau) \). We define the characteristic length scale of the condensate time trace in this calculation. Close to the end of condensate dissolution, the contact angle of DNA condensates to the surface of the imaging chamber approaches below 90°, and the morphological difference between condensates and ideal spheres becomes consequential. Thus, the effective radius data after entering this regime were excluded from the calculation of the dissolution rates.

DNA strand displacement reactions
All sequences for strand displacement reactions are designed using a seesaw compiler (71). The reactions in the solution phase were measured using a hybrid multimode microplate reader (Synergy H1M, BioTek). For strand displacement reactions in the Y-scaffold condensates, all reaction components except an input strand were preincubated with the condensate solution typically for an hour before the addition of the input. Individual reaction components (100 nM) are used together with 1.5 μM scaffolds. The reactions were performed in 350 mM NaCl buffer at 30°C.

Normalizing of DNA strand displacement reactions
Unless otherwise specified, all raw data of DNA strand displacement reactions measured by the multiplate reader or the confocal microscope were normalized as follows: The raw fluorescence intensity data during reactions were subtracted from the average intensities of each sample before adding the input strands and then divided by the average intensity values of the maximal five data points in reactions under comparison.

Modeling of client partitioning
The partitioning of clients into DNA condensates was modeled as a result of equilibrium binding (11, 65). In the client partitioning experiments, the Y-scaffold with a single toehold binding site was used. In the equilibrium binding model, the total scaffold concentrations in each phase were assigned using the experimentally measured phase boundary. Considering the modulatory effect of clients in the scaffold saturation concentration as described in the polyphase linkage formalism (65, 66), the total scaffold concentration in the dilute phase, \( C_{\text{tot,dil}} \), is expressed as \( C_{\text{tot,dil}} = C_{\text{tot,dir}} \left( \frac{1 + K_{dil} [C]}{1 + K_{den} [C]} \right) \), where \( C_{\text{tot,dir}} \) and \( [C] \) are the saturation concentration of the scaffold in the absence of any clients and free client concentration, respectively. \( K_i \) represents the association constant of the client to the scaffold. For each phase, the binding equilibrium between clients, \( C \), and scaffolds, \( S \), is given by

\[
K_i = \frac{[S_i]C}{[S][C]}.
\]

The binding of clients to scaffolds is mediated by hybridization of DNA strand displacement reactions in the toehold region of the scaffold. Thus, for the dilute phase, the association constant is given by

\[
K_{\text{dil}} = e^{-\Delta G_{\text{hyd}}/k_B T}/c_{\text{ref}}
\]

where \( \Delta G \) is the free energy difference of hybridization and \( c_{\text{ref}} \) is a reference concentration (\( c_{\text{ref}} = 1 \) M) (41). The hybridization free energy for each client is estimated using the web-based software DINAfold (64). In the dense phase, we assume that there is a free energy cost associated with the presence of clients within the dense network of DNA scaffolds (48, 65). Because clients used in this study are similarly sized (20 to 50 nt), we used a single value to account for the sequence-independent free-energy cost for all clients (\( K_{\text{den}} = \alpha K_{\text{dil}} \)). The two phases are linked through their free client concentrations, equal in both phases. On the basis of the lever rule, the total scaffold concentration is related to the volume fraction of the dense phase, \( \phi \)

\[
S_{\text{tot}} = (1 - \phi) ([S_{\text{dil}}] + [S_{\text{dil}}][C]) + \phi ([S_{\text{den}}] + [S_{\text{den}}][C])
\]
A similar conservation law applies to the client. Once the affinity ratio, $\alpha$, is given, the system of equations above can be solved together to yield the concentrations of all molecular species. The PC of clients is then expressed as below

$$ PC = \left( \frac{[S]_{\text{den}}[C]}{[S]_{\text{dil}}[C]} \right) $$

Using MATLAB least-square fits, the experimental data are fitted to the model described above with the affinity ratio, $\alpha$, as the only free-fitting parameter.

**Estimation of bond probability between scaffolds**

The bond probability between Y-scaffolds was estimated following a procedure described elsewhere (41). Briefly, individual bindings between the sticky ends are driven by the free-energy difference, $\Delta G$, associated with the hybridization reaction. Thus, the bond probability, i.e., the fraction of formed bonds, is given by

$$ P_b = 1 - \frac{1 + \sqrt{1 + 4fc\Delta}}{2fc\Delta} $$

where, $f$ and $c$ are the valence and molar concentration of Y-scaffolds, respectively. $\Delta$ is associated with the free energy of hybridization reaction, $\Delta = \exp(-\Delta G/k_bT)$, where $k_b$ is the Boltzmann constant. The free-energy differences, $\Delta G$, for the sticky end sequence and salt concentration used in this study are obtained from the web-based software, DINAMelt (64).

**SUPPLEMENTARY MATERIALS**

Supplementary material for this article is available at https://science.org/doi/10.1126/sciadv.abj1771

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