Specificity, flexibility and valence of DNA bonds guide emulsion architecture†

Lang Feng,‡a Lea-Laetitia Pontani,‡a Rémi Dreyfus,b Paul Chaikina and Jasna Brujic*a

The specificity and thermal reversibility of DNA interactions have enabled the self-assembly of crystal structures, self-replicating materials and colloidal molecules. Grafting DNA onto liquid interfaces of emulsions leads to exciting new architectural possibilities due to the mobility of the DNA ligands and the patches they form between bound droplets. Here we show that the size and number of these adhesion patches (valency) can be controlled. Valence 2 leads to flexible polymers of emulsion droplets, while valence above 4 leads to rigid droplet networks. A simple thermodynamic model quantitatively describes the increase in the patch size with droplet radii, DNA concentration and the stiffness of the tether to the sticky-end. The patches are formed between droplets with complementary DNA strands or alternatively with complementary colloidal nanoparticles to mediate DNA binding between droplets. This emulsion system opens the route to directed self-assembly of more complex structures through distinct DNA bonds with varying strengths and controlled valence and flexibility.

1 Introduction

Self-assembly of particles is of great interest for the design of complex particulate architectures to create smart nano-materials with tunable optical,1 mechanical2 or electronic properties.3,4 The specific and programmable interaction between complementary DNA strands5 is a good candidate for self-assembly.6–9 Combining short DNA sequences with soft matter has led to diverse assemblies.10–17 Understanding the interactions of DNA coated colloids is important to control the assembly process,18–20 and there has been significant progress in experiments,20–25 theory and simulations.18–20,26–35 Most recently, DNA binding was used to assemble colloidal molecules with specific symmetries imposed by the positions of the grafted DNA.36,37 More degrees of freedom in the assembly process can be achieved by enabling the DNA to diffuse freely on the surface of phospholipid-stabilized emulsion droplets,38–40 and vesicles.41,42 Mixing two emulsions with complementary DNA strands leads to their specific binding through strong yet reversible adhesion patches. By making the emulsion droplets thermally activated, here we allow the system to explore all available configurations through the rearrangement of bound droplet structures. Moreover, we build a thermodynamic model that relates the size of adhesion patches to the binding free energy and reveals the important role of entropy loss upon binding. This model then allows us to control the droplet binding strength through the characteristics of the DNA, including the sequence-specific melting temperature, surface density and the stiffness of the tether to the sticky end. In addition, we control the valency of the self-assembled structures by tuning the amount of binders on the surface. We thus design and build linear droplet chains (i.e. emulsion polymers) or those that fold into compact structures with a given valency. Alternatively, on the macroscopic scale the emulsions assemble into 3D floppy networks as examples of novel amorphous materials. Consequently, this system represents a powerful tool for self-assembly applications.24–28,41

2 Materials and Methods

2.1 Lipid-stabilized streptavidin coated emulsion

We first functionalize the emulsion droplets with biotins as previously described in ref. 39. Here, the oil droplets are stabilized with egg y-2-phosphatidylcholine (EPC) lipids and the DSPE-PEG (2000) biotinylated lipids from Avanti Polar Lipids at a molar ratio of 92 : 8, respectively, and a total mass of 1.9 g L−1. The solvent containing the lipids is evaporated under nitrogen before 10 mL of silicone oil (viscosity = 350 cSt) is added to the dried lipids. The mixture is then sonicated for 30 min at room temperature and heated at 50 °C for 3 h. After cooling the sample to room temperature the lipid containing oil (10 mL) is first coarsely emulsified in 22 mL of buffer (5 mM SDS, wt = 18% dextran). This crude emulsion is then injected into a narrow gap Couette mixer with a gap size of 100 μm, and sheared at 22 rpm. The resulting emulsion is washed three times in an aqueous solution of 1 mM SDS and the average droplets size is ~8 μm. Smaller droplet sizes (4 μm average) can

† These authors contribute equally to this work.
be obtained with a more viscous buffer (5 mM SDS and wt = 4.5% alginate) and a faster shear rate of 30 rpm. These emulsions are stable during several weeks at 4 °C.

2.2 DNA coated emulsion droplets

To functionalize the droplets with DNA, the emulsion is first coated with two different fluorescent streptavidins: Alexa Fluor 488® (Invitrogen) and Alexa Fluor 633® streptavidin (Invitrogen). 100 μL of creamed emulsion is mixed with 10 μL of 1 mg mL⁻¹ streptavidin and 300 μL of buffer containing 2 mM Tris pH = 7 and 1 mM SDS. The solution is incubated for 1 hour at 4 °C and then washed twice with the same buffer, before a final wash in the magnesium buffer (1 mM SDS, 5 mM PBS, 4 mM MgCl₂, pH = 7). For each wash, we use either an Eppendorf centrifuge 5810R at 800 rcf for 4 minutes, or an Eppendorf mini-spin at 1400 rpm for 5 minutes.

Biotinylated DNA strands are purchased from IDT (Integrated DNA technology) and prepared with either a single-strand backbone or a pre-anneled double-strand backbone. Typical functional DNA strands in our studies consist of two major parts: an inert section and a sticky end. The longest sections of functional strands are inert sequences, which could hybridize with the complementary strand (CS) to form a double-stranded duplex if a relatively rigid backbone is desired. All sequences used in our studies can be found in the ESI.† and the most important sequences are:

S sequence with the sticky end italicized: 5′-GGA TGA AGA TGA GCA TTA CTT GCC CGG AGA GAC GTA A/G/ACG CTT CCC ATC GCT A/BiotinTEG/-3′

S′ sequence with the sticky end italicized: 5′-CAT CTT CAT CCA GCA TTA CTT GCC CGG AGA GAC GTA A/G/ACG CTT CCC ATC GCT A/BiotinTEG/-3′

Complementary strand (CS) 5′-TAG CGA TGG GAA GCGTGT CAGTTA GGT CTCTCG GGA CGG AAA GTA ATG C-3′.

The biotinylated DNA constructs are then added to the streptavidin coated emulsion. To achieve maximum coating of streptavidin sites on the droplets, 10 μL of 50 μM biotinylated DNA is added to the solution and incubated for 1 hour at room temperature (25 °C). The remaining unbound DNA is finally washed three times in the magnesium buffer. The magnesium buffer is used for DNA coated emulsions to increase the DNA binding energy at a relatively low ionic strength so as to ensure the stability of emulsion droplets. In all our experiments, one type of fluorescent streptavidin is specifically associated with one DNA strand in order to distinguish them by confocal microscopy.

2.3 Microscopy

Bright field Leica DMRXA microscope and Leica DM6000 CS confocal microscope (TCS SP5 II) are used to take bright field and fluorescent images of our samples, respectively.

The Leica DMRXA microscope is equipped with a built-in temperature controlled stage to provide fast in situ temperature control. On the temperature controlled stage, an ITO (indium tin oxide) glass was placed on a copper plate of thickness 3 mm, two ends of which were connected to Peltiers (2.5 cm × 2.5 cm) and then to an aluminum heat sink. The aluminum heat sink has an interior water circulation in which the temperature is kept constant by an external water bath. We were able to control and detect the temperature with <0.5 °C relative error using a LakeShore DRC 93C temperature controller and a LakeShore PT111 temperature sensor. The grey scale images are taken by a Qimaging Retiga 1300 camera on the microscope.

We use the confocal microscope to take all fluorescent images in our experiments. Excitations with 488 nm and 631 nm were used for imaging the samples labeled with streptavidin with Alexa® Fluor 488 and streptavidin with Alexa® Fluor 633. A 63× glycerol immersion objective (Leica) was used for all fluorescent imaging. The lateral resolution is about 150 nm for the immersion objective with 488 nm laser excitation and NA = 1.3, used in most of the experiments.

3 Results and discussion

3.1 Specificity, thermoreversibility and enrichment bonding of complementary DNA coated emulsion droplets

The model emulsion system in Fig. 1A is stabilized with a mixture of two phospholipids, egg-phosphatidylcholine (EPC) and PEG-biotinylated lipids, and a small amount of SDS surfactant. The biotinylated lipids are saturated with fluorescent streptavidin, which in turn binds to a second biotin from the functional DNA strand. The green emulsion (Alexa Fluor 488® streptavidin) is coated with the S sequence, while the red emulsion (Alexa Fluor 633® streptavidin) is coated with the S′ sequence (see Materials and methods). In addition to the complementary sticky ends, the binders have an identical backbone of either single stranded or double stranded DNA that serves as a tether to the binder. Alternatively, we study a hybrid system of interacting colloidal nanoparticles and emulsions, as illustrated in Fig. 1B. In this system the emulsion is only decorated with the S′ DNA sequence (red), which interacts with colloids coated with the complementary S sequence (green). As a result, a colloidal particle binds droplets together.

The thermal reversibility of DNA interactions have been previously studied on the colloidal scale. In our emulsion system it allows us to switch the adhesion between droplets on and off by cycling the temperature above and below the DNA melting temperature of Tm = 50 °C. At room temperature, complementary emulsions are mixed together and diffuse to form adhesive clusters (Fig. 1C left panel) that dissociate upon heating above Tm (Fig. 1C right panel and ESI Movie†). Similarly, the emulsion-colloid system below Tm reveals multivalent particle-emulsion structures (Fig. 1D left panel) that are separated upon heating (Fig. 1D right panel).

When complementary droplets meet, their DNA strands hybridize to form double-stranded (ds) DNA. Therefore, the presence of green and red streptavidin, associated with each DNA strand, lead to yellow adhesion patches in regions of hybridization, as shown in Fig. 2A (dsb) and Fig. 2B (ssb). Interestingly, the patches in emulsions with double stranded backbone (dsb) tethers are significantly larger than those with single stranded backbone (ssb) tethers. In both cases, the plot of the patch size as a function of the reduced radius of each pair of contacting droplets in Fig. 2C reveals a linear dependence. Thus, the measured ratio of dsb to ssb patch sizes of ~1.6 is
independent of droplet size. This result implies that the strength of binding between droplets can be modulated in situ by adding the complementary strand to ssb functionalized emulsions. Indeed, we observe that the patch size transitions from the average size expected for ssb DNA interactions to the ~1.6 times larger adhesions corresponding to dsb DNA in less than one hour, as shown in Fig. 2D.

Another way to increase the binding strength, as well as the number of patches per droplet, is to increase the DNA coverage on the droplets, $C_{\text{max}}$, as shown in the 3D images in Fig. 3A. We can vary the relative DNA coverage $d = N_{\text{DNA}}/N_{\text{max}}$ by changing the amount of biotinylated DNA construct introduced in the system $N_{\text{DNA}} \sim 1 \text{ pmol}, 2 \text{ pmol}, 4 \text{ pmol}, 8 \text{ pmol} \text{ or } 20 \text{ pmol}$ to achieve the relative DNA coverage of $d \sim 8.3\%, 17\%, 34\%, 67\%$ and 100\%. (Here $N_{\text{max}}$ is calibrated as 12 pmol per emulsion sample of 30 μL, see ESI.) The patch size ($\theta$) and the relative intensity, defined as the ratio of the intensity of fluorescence in the patch and that of the droplet surface, increase as a function of DNA coverage in Fig. 3B and C. This capability allows one to tune the reversibility of droplet interactions and the temperature at which the structures melt.

3.2 Modeling the experimental observations with a statistical mechanical model

DNA coated solid colloidal particles have been extensively studied in theories\textsuperscript{18,20,24–27,30} and simulations.\textsuperscript{26–28,30,32–35} The understanding of complementary DNA interaction between colloidal particles built the framework of an efficient toolbox for the design of colloidal crystallization\textsuperscript{26,28,30,32,33} on both the nano and micron-scale. The thermoreversibility and programability of DNA interactions led to interesting phase behavior\textsuperscript{23,33} and sophisticated ways to assemble mesoscopic colloidal architecture.\textsuperscript{27,35} However, most theories and simulations dealt with solid particles in which the surface anchored DNA strands are not allowed to freely diffuse on the spherical surface. Here we develop a mean-field statistical model to understand the binding mechanism between soft particles with mobile DNA strands on the surface.

Our model is based on the assumption that binders are recruited into the contact area until the binding energy balances the energy cost upon droplet deformation and the entropy penalty due to the immobilization of the DNA tether in the patch. Let us consider two complementary emulsion droplets with the same radius $R_p$ and DNA surface density $N_b/(4\pi R_p^2)$, where $N_b$ is the total number of DNA strands on the droplet. The two droplets interact to form an adhesive patch of radius $r_p$ and deformation angle $\theta = r_p/R$, as shown in Fig. 1A. We denote as $N_b$ the number of DNA binders inside the patch between two complementary droplets, and $N_a$ the amount of free DNA strands remaining on the droplet surface. The global free energy change from the unbound to the bound state is then:

$$\Delta F = \Delta E_{\text{DNA}}, - 2T(S_b + S_a) + E_{\text{deform}} - \Delta F_{\text{unbound}} $$  \hspace{1cm} (1) 

where $\Delta E_{\text{DNA}}$ is the binding energy, $T$ is the temperature, $S$ is the entropy of binding and $E_{\text{deform}}$ is the energy cost to deform the
interface. Subscripts $\beta$ and $\alpha$ indicate the binding patch region and the unbound surface, respectively. In the dilute case, $S_{\alpha,\beta} = -kN_{\alpha,\beta} \ln[C_{\alpha,\beta}/C_0]$ where $C_{\alpha,\beta} = N_{\alpha,\beta}/\pi r_p^2$ is the surface density of DNA in the unbound surface or in the adhesive patch, and $C_0$ is the reference concentration, which cancels out in the calculation.

The binding free energy for the mobile DNA patch is estimated in mean field:\cite{24,25}

$$\Delta E_{\text{DNA},\beta} = -k_B T \ln\left( 1 + \exp\left[ -\frac{\Delta G_{\text{DNA}} - T \Delta S_r - k_B T \ln[C_{\beta}A_w]}{k_B T} \right] \right)^{\frac{1}{N_s}} - 1$$

where $\Delta G_{\text{DNA}}$ is the free energy of hybridization of free DNA in solution calculated from the Nearest Neighbor model,\cite{45,46} $\Delta S_r$ is the entropy loss due to rotational constraints of hybridized DNA strands,\cite{24} $A_w$ is the area in which two DNA strands can move relative to each other when hybridized and $\ln[A_wC_0]$ is the translational entropy penalty for two DNA strands bound in the patch. In the strong binding regime,\cite{25} at low temperature compared to $T_m$, the binding energy from mobile DNA can be estimated as

$$\Delta E_{\text{DNA},\beta} = N_{\beta}(\Delta G_{\text{DNA}} - T \Delta S_r - k_B T \ln[C_{\beta}A_w])$$

The deformation energy is given by $E_{\text{deform}} = \sigma \pi r_p^2 \theta^4/2$, where $\sigma$ is the surface tension.\cite{26} If the contacting emulsions are of different sizes, $R_1$ and $R_2$, the deformation energy is estimated to be $E_{\text{deform}} = \frac{1}{4} \sigma \pi \frac{r_p}{R_1^3} + \frac{1}{4} \sigma \pi \frac{r_p}{R_2^3}$. The reduced radius $\langle R \rangle = \sqrt{\frac{2R_1^2R_2^2}{R_1^2 + R_2^2}}$ is used to simplify the deformation energy $E_{\text{deform}} = \frac{1}{2} \sigma \pi \frac{r_p}{\langle R \rangle^3}$ and it is used in both Fig. 2 and 3. Since
there are only two independent parameters in the problem, \( C_b \) and \( r_p \), we minimize the global energy to obtain the profile of \( C_b(A_s, A_w) \) and \( \theta(A_s, A_w) \):

\[
\theta(A_s, A_w) = \frac{r_p}{(R)} = \sqrt{k_B T (-2 \ln(1 - C_b A_{step}) - C_b A_{step})} A_{step}
\]

(2)

assuming that the binding free energy \( \Delta G_{DNA} \) the surface tension \( \sigma \), the streptavidin size \( A_{step} \) and the temperature \( T \) are kept constant (see ESI for details).

Since the double stranded tether is much longer and can reach as far as \( \sim 26 \) nm, further than that of the single stranded tether of \( \sim 4.5 \) nm, the area of relative motion of bound DNA strands, \( A_w \), is also much larger, estimated to be \( \sim 2000 \) nm\(^2\) compared to only \( \sim 60 \) nm\(^2\). Therefore, the dsb case loses less entropy upon binding (see ref. 24 and 25 and ESI†), which quantitatively explains the \( \sim 1.6 \) fold larger average patch size, as shown in Fig. 2C. Moreover, we capture the adhesion strength dependence on the DNA surface coverage with only 2 fitting parameters: the rotational entropy loss \( \Delta S_r = -16 R \) (where \( R \) is the gas constant) and the maximum DNA binding capability, \( N_{max} = 12 \) pmol, for an emulsion sample of 30 \( \mu \)L. These fitting parameters are consistent with previously reported values²⁴,²⁵,²⁹ (see ESI†). The agreement of the model with the experimental data for the three trends shown in Fig. 2C and 3B and C gives validity to our theoretical framework.

3.3 Diffusion of the bound lipids on the surface

The fluidity of the droplet surface enables rearrangements in bound structures and allows for the self-assembly of programmable geometries. Adhesion patches are free to diffuse despite the high binding energy of \( \sim 20,000 \) DNA connections in an average-sized patch with a 1.6 \( \mu \)m diameter. Fig. 4A shows the diffusion of droplets that are bound through DNA interactions, but free to rotate with respect to each other and thus explore available configurations. We use hybrid systems of particles and emulsions to quantify the diffusion of adhesion patches (Fig. 4B).

The beads serve as reporters for the lipid motion on the monolayer surface. To measure relative motion, two colloidal particles coated with the S DNA sequence are attached onto the surface of a S functionalized droplet through at least 200 DNA bonds.

The mean square displacement of one bead with respect to the other (graph in Fig. 4B) yields a diffusion constant of \( D \sim 0.012 \) \( \mu \)m\(^2\) s\(^{-1}\). This value is significantly smaller than both the diffusion of a single lipid of size \( \sim 1 \) nm in a fluid model membrane with \( D \sim 1-10 \) \( \mu \)m\(^2\) s\(^{-1}\) (ref. 49-52) and that of a 1 \( \mu \)m colloidal particle with \( D_{particle} \sim 0.5 \) \( \mu \)m\(^2\) s\(^{-1}\). This slow diffusion of the particle is due to the strong hydrodynamic drag of an adhesive lipid patch of radius \( \sim 100 \) nm, which is expected to be two orders of magnitude lower than that of a single lipid.

Allowing the emulsions to cream to the surface assembles floopy networks of bound droplets that are organized by the specificity of the DNA bonds, as shown in Fig. 4C and ESI†. Once the maximum droplet connectivity is achieved, no further rearrangements in the structure are observed. Nevertheless, the bonds continue to be mobile owing to the liquid interfaces. While the coordination number distribution of such networks can be tuned by the concentration of binders on the surface, their structure remains amorphous.

3.4 Building structure of controlled valency with emulsions and colloids

This system also allows us to control the valency of self-assembled emulsion droplets. Indeed our thermodynamic model suggests that, for large enough binding energies \( \Delta G_{DNA} \) of \( \sim -40 \) kT, the enrichment patch will reach a saturated DNA density \( (C_b)_{sat} \sim 1.5 \times 10^4 \) per \( \mu \)m\(^2\), limited by the size of streptavidin.
Experimentally the DNA hybridization energy is increased by using longer sticky ends on the surface of the droplets (see ESI†). Limiting the amount of available binders on the surface therefore fixes the number of patches a droplet can engage with its complementary binding partners, since the maximum patch angle $\theta \sim 0.15$ is determined by the dramatic deformation energy cost ($E_{\text{deform}} \propto \theta^4$) and higher order terms. According to the predictions of our model, to achieve monovalent ($n = 1$) and divalent ($n = 2$) self-assembly structure, DNA surface densities $C_a$ of $\sim 84$ per $\mu m^2$ and $\sim 168$ per $\mu m^2$ (corresponding to 6% and 12% relative DNA coverage) should be used, respectively. Indeed, experimentally we find that the relative DNA coverages of 7.5%, 15% and 30% on the emulsion droplets result in monovalent complementary dimers with $\sim 80\%$ yield (Fig. 5A left), divalent linear chains with $\sim 60\%$ yield (Fig. 5A middle, see also ESI movie†) and multivalent clusters (Fig. 5A right) with over 90% yield, respectively. These results are in good agreement with the concentrations predicted by the model (see ESI†) and thus present a leap in the design and control of self-assembled architectures.

Alternatively, the complementary colloid-emulsion hybrid system (see ESI†), shown in Fig. 1B, is a versatile tool for self and directed-assembly. Combining the dilute emulsion ($\sim 1000$ droplets per $mm^2$) with complementary nanoparticles at a low particle/droplet ratio of $\sim 5$ enables the formation of linear emulsion polymers of different lengths, as shown in Fig. 5B. The linear arrangement of the droplets is induced by constraining them in a 1D line at the edge of a tilted rectangular capillary (see ESI†). The binding colloids are recruited exclusively to the emulsion contacts after overnight incubation to form two patches per emulsion droplet and thus prevent branching. This leads to polymer chains with a valency of 2 that diffuse over time due to the mobility of the particulate joints between the droplets. They remain in a linear configuration because the particles are too small to bridge three droplets. On the other hand, at a higher particle/droplet ratio of $\sim 100$ we observe multivalency and folding of the linear chains into compact structures over time, as shown in Fig. 5C. In that case, the binding energy of the multivalent particles and their connectivity is sufficient to arrest the resulting structure. The geometry of the final compact structure depends on the valency, the number of droplets in the cluster, as well as the kinetic pathway, and ranges from triangular lattices to flowers, as shown in Fig. 5D. These structures lower their energy by maximizing the number of colloids that occupy emulsion contacts. Unlike droplet–droplet patches, shown in Fig. 2 and 5A, the solid polystyrene particles are large enough to form a binding ring surrounding the contact points of two emulsion droplets without reaching their contact point. This leads to the ring structure in the adhesion zone (see ESI Movie†) that maximizes the number of particles per emulsion contact and thereby minimizes the global free energy.

4 Conclusions

We have achieved the self-assembly of thermal emulsion polymer chains with programmable droplet interactions.

![Fig. 5](A) Monovalent doublets (left), divalent droplet chains (center) or higher valency clusters are self-assembled by controlling the binders concentration. The scale bars are 10 $\mu m$ here. (B) Chains of divalent emulsion droplets (red) are formed using complementary nanoparticles (green) with a nanoparticle/droplet ratio $\sim 5$. (C) A higher nanoparticle/droplet ratio leads to a trivalent 2D structure. (D) Large particle/droplet ratios $\sim 100$ produce multivalent droplets and lead to compact rigid structures in which the beads all assemble between the droplet contacts to minimize the system’s energy.
Controlling the number of binders and the length of the chain we obtain divalent, trivalent and multivalent structures. In addition, the mobility of adhesive patches within these structures allows them to evolve into geometries that are governed by the underlying free energy landscape. Furthermore, DNA interactions allow one to program the shape of the free energy landscape through the control of bond specificity, strength, flexibility and valency. Further engineering the bond specificity should allow one to design a divalent droplet chain which can fold into a designed shape with controllable chirality, similar to the chiral structures assembled by nanoparticles and DNA.\textsuperscript{35,36} More complex DNA designs have been recently exploited to realize information processing and Boolean logic on a molecular level,\textsuperscript{35,36} by constructing programmable DNA logic circuits in bulk solution\textsuperscript{36} or with nanoparticles.\textsuperscript{37} Applying these strategies\textsuperscript{35,36} to the mobile DNA strands on droplet surface may lead to novel DNA computing algorithms on a 2D substrate. Controlling the size and valency of each droplet enables one to mimic the behavior of atoms\textsuperscript{36} and construct structures with a low-coordination number. Alternatively, the thermally agitated networks of complementary droplets are an example of an active jammed system with rheological properties that are of much interest in the field of soft matter. The many control parameters confer a great versatility to this system which therefore represents a new step forward in the field of self-assembly, with the potential of building intelligently designed materials, such as colloidal crystals or artificial self-replicating materials, with no external inputs.

\section*{Acknowledgements}

We would like to thank Ruojie Sha and Nadrian Seeman for useful discussions and a careful reading of the manuscript. This work is supported partially by the MRSEC Program of the National Science Foundation under Grant no. DMR-0820341 for materials support, the National Science Foundation Career Grant no. 0955621 for characterizations of emulsions, NASA NNX08AK04G for microscopy, and L.F and P.C acknowledge support for thermal and optical data acquisition and analysis from DOE-BES-DE-SC0007991.

\section*{Notes and references}

50 W. L. Vaz, R. M. Clegg and D. Hallmann, Biochemistry, 1985, 24, 781–786.