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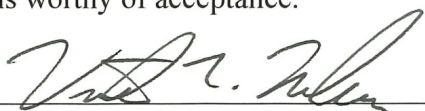
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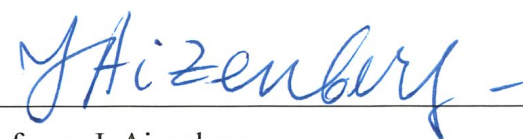
“Responsive and Dynamic Systems from DNA-Mediated Colloidal Interactions”

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August 10, 2018

Responsive and Dynamic Systems from DNA-Mediated Colloidal Interactions

A DISSERTATION PRESENTED
BY
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TO
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Responsive and Dynamic Systems from DNA-Mediated Colloidal Interactions

ABSTRACT

Grafting DNA oligonucleotides to colloidal particles leads to specific, reversible interactions between those particles. However, these interactions have been used almost exclusively to create non-responsive systems through equilibrium pathways. In this thesis, I explore different ways to precisely control the response of these particles to changes in their environment and demonstrate how this new control can be used to make systems with complex, dynamic behaviors.

DNA-mediated colloidal interactions are limited by the steep and monotonic dependence of the interaction strength on temperature, which hinders their use in self-assembly applications and limits the complexity of the systems that can be realized. I show how to modify the dependence on temperature in a controlled way by incorporating DNA strand-displacement reactions. This method allows us to make multicomponent systems that self-assemble over a wide range of temperatures, melt upon cooling, transition between structures with different compositions, or have multiple melting transitions. I create this wide range of behaviors simply by adding a small number of DNA strands to the solution, demonstrating that the approach is modular and straightforward to implement.

These strand-displacement reactions enable systems to dynamically rearrange in time. We demonstrate a system where, by thermal ratcheting, a single particle (the dancer) can be driven to move through a programmed sequence of steps along a one-dimensional track composed of other particles. We lay out the requirements for a system to exhibit controlled motion on the mesoscale, and we demonstrate how these conditions can be realized experimentally. Specifically, we show how the non-monotonic phase behavior enabled by strand-displacement reactions allows us to turn on and

off interactions between different pairs of particles, and thereby drive the motion of the dancer. We discuss the capabilities and limitations of using these interactions for applications in dynamic systems.

One such limitation is that a single variable—the temperature of the system—simultaneously controls interactions between several species of particles. We therefore aim to independently modulate interactions between many species. To this end, we present a new approach to dynamically control interactions between DNA-coated colloids using light. We infiltrate particles with dyes so that when we illuminate them with the appropriate wavelength, they heat. As a result, by uniformly illuminating samples with unfocused light, we can reversibly turn on and off the attractive interactions between particles. Although the light is incident on the entire sample, the heating is local to the particles. Therefore, by using multiple dyes, we can independently address the interactions between different sets of particles by using different wavelengths of light. This method of heating produces a short-range temperature gradient that builds up and dissipates on a time scale of milliseconds. Thus, the particles can be heated much more efficiently than by external heating, and the propensity of the entire system to aggregate can be modulated in less than 50 milliseconds. This rapid modulation opens the door to many applications, including non-equilibrium self-assembly.

Contents

1	INTRODUCTION	1
1.1	Colloidal self-assembly	1
1.2	DNA-mediated colloidal interactions	3
1.3	Overview	4
2	USING DNA STRAND DISPLACEMENT TO CONTROL INTERACTIONS IN DNA-GRAFTED COLLOIDS	6
2.1	Introduction	7
2.2	Fundamentals	11
2.3	Displacement-free binding	14
2.4	Single strand-displacement reaction	18
2.5	Two strand-displacement reactions	24
2.6	Combining displacement-free and displacement-mediated interactions	29
2.7	Conclusions and practical considerations	35
2.8	Methods and materials	38
3	PROGRAMMING DIRECTED MOTION ON THE MICROMETER SCALE BY THERMAL RATCHETING	46
3.1	Introduction	46
3.2	Design considerations	47
3.3	Results and discussion	52
3.4	Conclusions	61
3.5	Methods	62
4	MODULATING AND ADDRESSING COLLOIDAL INTERACTIONS USING LIGHT	65
4.1	Introduction	65
4.2	Results and discussion	66
4.3	Conclusions	74
4.4	Methods and materials	76
5	CONCLUSIONS AND FUTURE WORK	89
5.1	Addressable interactions for sequential assembly	90
5.2	Triggered release of free DNA strands	90
5.3	Non-equilibrium self-assembly	90
5.4	Broad vision	95

APPENDIX A	PROTOCOL FOR FUNCTIONALIZING POLYSTYRENE BEADS WITH DNA USING F108	97
A.1	Day 1 - Activate F108 with NPCF	97
A.2	Day 2 - React activated F108 with aminated-DNA, adsorb DNA-F108 to particles, and swell	100
A.3	Day 3 - Deswell and wash	103
A.4	Things needed for this protocol	104
APPENDIX B	PROTOCOL FOR FUNCTIONALIZING POLYSTYRENE BEADS WITH DNA USING PS-PEO	107
B.1	Chlorinate the PS-PEO	108
B.2	Azidize the Chlorinated PS-PEO	110
B.3	Functionalizing PS beads with PS-PEO-N ₃	111
B.4	Adding DNA	112
B.5	Things needed for this protocol	113
REFERENCES		122

Listing of figures

1.1	Cartoon of self-assembly	2
1.2	Specificity of DNA-mediated colloidal interactions	3
1.3	Temperature dependence of DNA-mediated interactions	4
2.1	Introduction to DNA-mediated interactions and toehold displacement reactions .	7
2.2	Colloidal phase behavior with different displacement schemes	10
2.3	Temperature-dependent behavior of particles grafted with complementary DNA .	16
2.4	Experimental and theoretical singlet fraction with one displacement reaction . . .	23
2.5	Experimental and theoretical singlet fraction with two displacement reactions . . .	28
2.6	Demonstration of a system that shows three phase transitions	30
2.7	Demonstration of a system that has a compositional transition	34
2.8	Summary of the phase behaviors that can be achieved with no displacement reactions, a single strand-displacement reaction, and two strand-displacement reactions	36
3.1	Introduction to dancer and explanation of interactions needed	48
3.2	Expected number of steps of a dancer	52
3.3	DNA sequences for dancer	53
3.4	Experimental demonstration of interactions between the dancer and track particles	55
3.5	Constructing a track using optical tweezers	57
3.6	Motion of a dancer on a track	61
4.1	Overview of light-driven modulation.	67
4.2	Fast and repeatable melting with light	69
4.3	Background temperature needed to melt clusters of different sizes	70
4.4	Theoretical temperature profile and melting curve	73
4.5	Probability of dimer separation after light pulses of varying duration	74
4.6	Addressable melting of different particle colors	75
4.7	Micrograph of dimer melting experiment	82
4.8	Dimer separation probabilities for different cutoff distances	83
4.9	Determining TrackPy cutoff value	84
4.10	Dimers present before each light pulse over the course of an experiment	84
5.1	Response of mixed systems of red and blue particles to light	91
5.2	Intuitive argument for timescales for pulsed interactions	93
5.3	Steady state behavior of a system under periodic light exposure	93
5.4	System behavior under light pulses of varying period and duty cycle	94
5.5	Encapsulating particles in droplets and wells	95

Prior Publications

Parts of this thesis are primarily based on the following previously published work:

- E. W. Gehrels, W. B. Rogers and V. N. Manoharan. Using DNA strand displacement to control interactions in DNA-grafted colloids. *Soft Matter* 14, 969–984 (2018).

Other publications relating to the work discussed in this thesis are in preparation.

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1

Introduction

1.1 COLLOIDAL SELF-ASSEMBLY

Self-assembly is the process by which individual components combine in solution and—owing to their random (Brownian) motion and their interactions with one another—form an ordered structure (see Figure 1.1). The Brownian motion of a particle can be understood as a random walk, where the probability density of its location begins as a delta function centered at its initial position and becomes a normal distribution as time goes on. The width of the normal distribution, or equivalently the mean-squared displacement $\langle x^2 \rangle$ of the particle, is described by the following set of equations:^{1,2}

$$\begin{aligned}\langle x^2 \rangle &= 2dDt \\ D(T) &= (k_B T) / (2d\pi\eta a) \\ \eta(T) &= 2.414 \times 10^{-5} \text{ Pa} \cdot \text{s} \, 10^{247.8 \text{ K} / (T - 140 \text{ K})},\end{aligned}\tag{1.1}$$

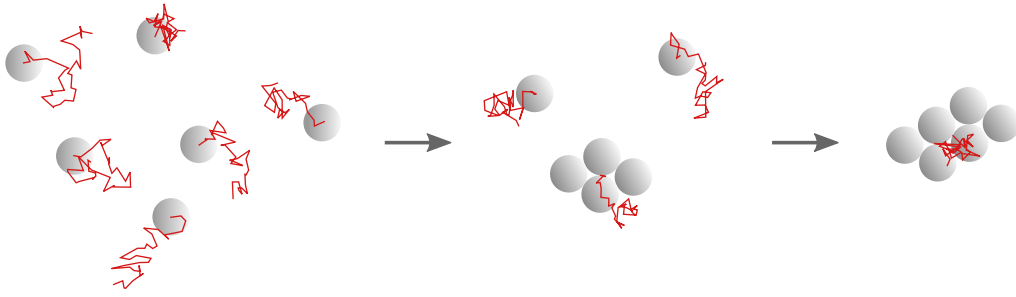


Figure 1.1: Six particles diffuse randomly until they collide. The red lines are simulated tracks representing typical diffusive motion of the center of mass of each particle (or group of particles) in time. When particles come into contact they attract one another.

where d is the number of dimensions in which the particle is diffusing, D is the diffusion coefficient, $\eta(T)$ is the viscosity of water as a function of temperature, a is the particle radius, k_B is the Boltzmann constant, and T is the temperature. Micrometer-sized colloidal particles are ideal for self-assembly applications because they are small enough to diffuse quickly (approximately $1 \mu\text{m}^2/\text{s}$ for a $1 \mu\text{m}$ particle) and large enough to visualize using optical microscopy.

Although interactions are a requirement for self-assembly, they must be carefully tuned for the desired structure.³ In equilibrium self-assembly, particles must be able to roll past one another, and the interaction must be weak enough to allow for neighbor exchange. At the same time, the bonds must be strong enough to allow structures to form and grow. This can be a fine balance to reach unless the bond strength can be dynamically tuned. Depletion forces are often used to drive self-assembly.⁴ However, in these systems, there is little to no control over which particles bind to each other, and, with a few exceptions,^{5,6} the interaction strength cannot be tuned without changing the composition of the solution.

The ideal colloidal self-assembly system will therefore have both specific and tunable interactions. DNA is a polymer that naturally confers these traits. Within the past twenty years, scientists have devised methods to coat colloidal particles with single stranded DNA (ssDNA) (see inset of Fig-

10 μm

Figure 1.2: Micrograph of a system containing two species of particles (one dyed red and the other dyed blue). The two species are coated with DNA that is complementary only to particles of the same color. Because of the specificity of the interactions, red particles bind only to other red particles and blue particles bind only to other blue particles.

ure 1.3).^{7,8,9}

1.2 DNA-MEDIATED COLLOIDAL INTERACTIONS

The usefulness of DNA-mediated colloidal interactions stems from their specificity and reversibility.^{7,8,9,10} Two particles grafted with DNA oligonucleotides bind if—and, in the absence of non-specific interactions, only if—the sequences are complementary (see Figure 1.2), and the temperature is in a range where the ssDNA on the two particles can hybridize to form a duplex. The duplexes bridge the particles together, binding them to one another. When the temperature is increased, the bridges melt, and the particles dissociate (see Figure 1.3). Lowering the temperature has the opposite effect.

With this combination of specificity and reversibility, it is possible to design many single-component and two-component systems that assemble into equilibrium crystals and clusters,^{3,9,11,12,13,14,15,16,17,18,19,20} with many more proposed in theory and simulation.^{21,22} However, com-

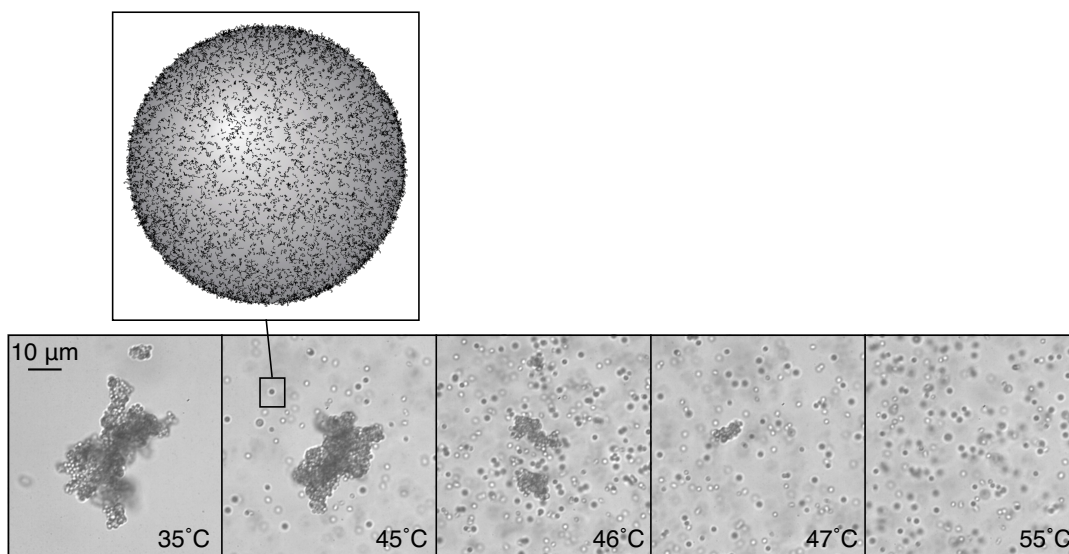


Figure 1.3: Particles coated in DNA aggregate at low temperatures and melt as the temperature is raised through the melting point. This behavior is shown in the brightfield micrographs at the bottom. Each dot in the micrographs is a single DNA-coated particle, as shown in the cartoon inset at the top, where the position and configuration of each DNA strand is simulated. This inset was created by W. Benjamin Rogers.

pared to biological system, the systems created using DNA-mediated colloidal interactions fall behind in one major way: almost all of the systems that have been realized to date remain in the same state once assembled. They cannot dynamically rearrange or respond to external cues.

1.3 OVERVIEW

In this thesis, I demonstrate how to make responsive mesoscale systems that can be controlled through external changes to their environment. I first explain how strand-displacement reactions, first developed for DNA nanotechnology, can modify the temperature dependence of DNA-mediated colloidal interactions (Chapter 2). I then demonstrate how this modified temperature dependence can be used to power a micrometer-scale “dancer” (Chapter 3). Next, I show that colloidal interactions can be modified quickly and specifically by using light to locally heat dyed particles (Chapter 4). Finally, I conclude with ideas for future directions, including using this fast control over binding for non-

equilibrium self-organization.

2

Using DNA strand displacement to control interactions in DNA-grafted colloids

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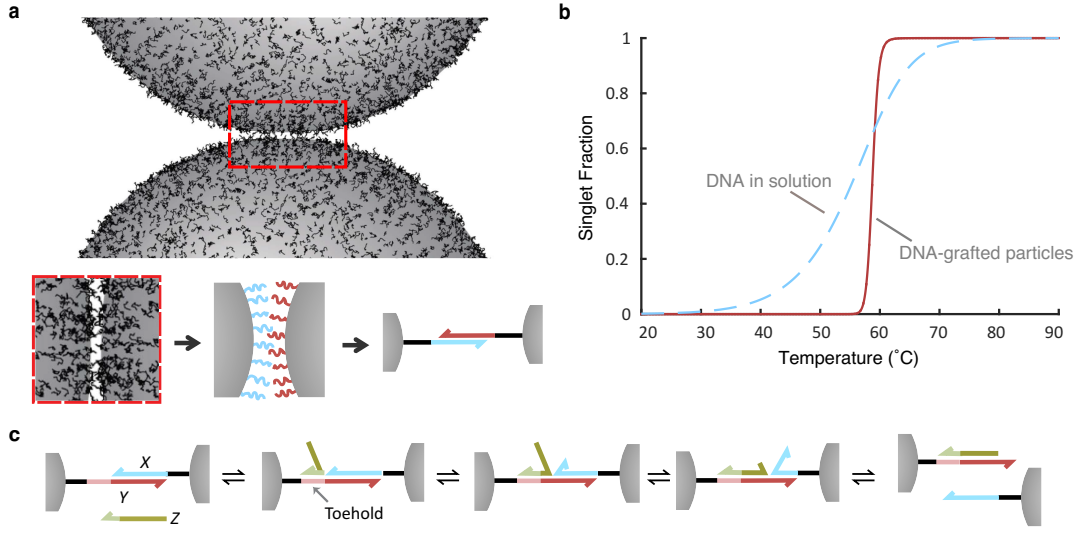


Figure 2.1: a) A scale rendering, generated by Monte Carlo simulation of polymer chain configurations, of DNA-grafted 1-μm polystyrene particles. The length of the DNA strands is 65 bases, such that the radius of gyration L is approximately 11 nm. The bottom images show how we schematically depict the bridges that bind the particles together. In these schematics, a zoomed-in portion of each particle is shown in gray, and the strands are not drawn to scale. b) Theoretical singlet fraction as a function of temperature for DNA in solution (blue, dashed) and for particles with grafted strands (red, solid), calculated using Equations 2.2, 2.4, and 2.5. c) Schematic of toehold-mediated strand displacement. At left, two particles are bridged by complementary ssDNA (X and Y). A displacing strand (Z), which is also complementary to Y , can bind to an exposed region of Y (the “toehold,” shown in pink) and displace X through a stochastic exchange of bases between the duplexes XY and ZY . This process is reversible if there remains an exposed region of Y where X can rebind after the displacement reaction.

2.1 INTRODUCTION

In this chapter, we demonstrate how the interactions and phase behavior of DNA-grafted colloidal particles can be altered by adding free DNA strands that can hybridize with the grafted strands. Rogers and Manoharan originally demonstrated this method in a short report in 2015.²³ The present chapter is a more comprehensive study that extends on this previous work, offers new experimental results, describes the theory behind them, and discusses some practical considerations for using the method.

The benefits of DNA-mediated colloidal interactions are laid out in the previous chapter. How-

ever, these interactions are limited in one significant way: the binding strength decreases steeply and monotonically with increasing temperature.^{24,25} Over a window of 1–2 °C, the binding strength typically decreases from many times the thermal energy $k_B T$ to negligible. As a result, the fraction of unbound particles (the “singlet fraction”) in a suspension decreases rapidly with decreasing temperature—much more rapidly than the singlet fraction of DNA strands that are identical to the ones on the particles, but are free in solution (Figure 2.1b).

This sharp transition makes it difficult for systems of particles to equilibrate. Equilibration happens only at temperatures where the DNA binding energies are strong enough to cause the particles to attract, but not so strong that the DNA bridges between the particles have long lifetimes.^{9,25,26} In this temperature regime, the single-stranded DNA molecules causing the attraction bind and unbind on timescales short compared to the particle motion, allowing the particles to explore different configurations without becoming trapped or falling apart. Because the binding strength varies so steeply with temperature, equilibration can occur only within a narrow window of temperatures. Thus, the steep dependence of the interactions on temperature limits the benefits of DNA-mediated interactions: while it is possible to design a system with a particular equilibrium structure, it is challenging to get that structure to form.

The problem becomes more acute for systems with several species of particles—where “species” means a set of particles grafted with a particular combination of oligonucleotides. In such systems, one can use the nearest-neighbor model of DNA thermodynamics²⁷ to predict how the melting temperatures should change with the DNA sequences. The model, in conjunction with additional theory that we describe later, allows one to design sequences such that a single species has a given melting temperature. But, owing to the uncertainty inherent to the model, it is difficult to design a set of sequences for a multi-species system such that all of the melting temperatures are the same. SantaLucia and Turner²⁸ quote a 6% uncertainty on the nearest-neighbor values of the DNA thermodynamic parameters, ΔH and ΔS . This uncertainty translates to roughly a 3 °C uncertainty in the melting

temperature under standard experimental conditions, which is larger than the width (2°C) of the entire melting transition. If different pairs of particles within a multi-species system must be annealed at different temperatures, it is impossible to equilibrate the system as a whole.

Here we show that these problems can be overcome by adding free strands of ssDNA that can hybridize to the grafted strands, inhibiting bridge formation. These competing DNA reactions, known as strand-displacement reactions (Figure 2.1c), have been widely used in the field of DNA nanotechnology to create dynamic and responsive systems.^{29,30,31,32} However, in the field of DNA-grafted colloids, strand displacement has, until recently,²³ been employed primarily to melt or change the lattice constants of systems of DNA-grafted nanoparticles.^{14,19,33,34}

We will show that the effect of strand displacement is to modify the free energy of hybridization of the grafted DNA strands, which in turn modifies the phase behavior of the particles. The phase behavior of the usual system of particles with complementary grafted strands consists of a single, steep melting transition. Adding a single displacement reaction leads to a broadened melting transition and solid and fluid phases that coexist over a wide range of temperatures. Adding a second displacement reaction leads to two melting transitions: a fluid-to-solid phase transition at low temperature and a solid-to-fluid transition at higher temperature. Confocal microscopy images of systems that show these phase behaviors are shown in Figure 2.2.

In what follows, we describe the origin of the sharp phase transition for particles grafted with complementary DNA (Section 2.2) and present a model for predicting this phase behavior for arbitrary sequences and surface concentrations of DNA (Section 2.3). We then extend this model to show how to overcome the steepness of this transition by adding a single displacement reaction, and we demonstrate the resulting phase behavior in an experimental system (Section 2.4). We go on to demonstrate further control of the phase behavior through the addition of a second displacement reaction, which yields a second melting transition upon cooling (Section 2.5). Finally, we show more complex phase behavior such as three transitions (Section 2.6.1) and transitions involving multiple species of particles

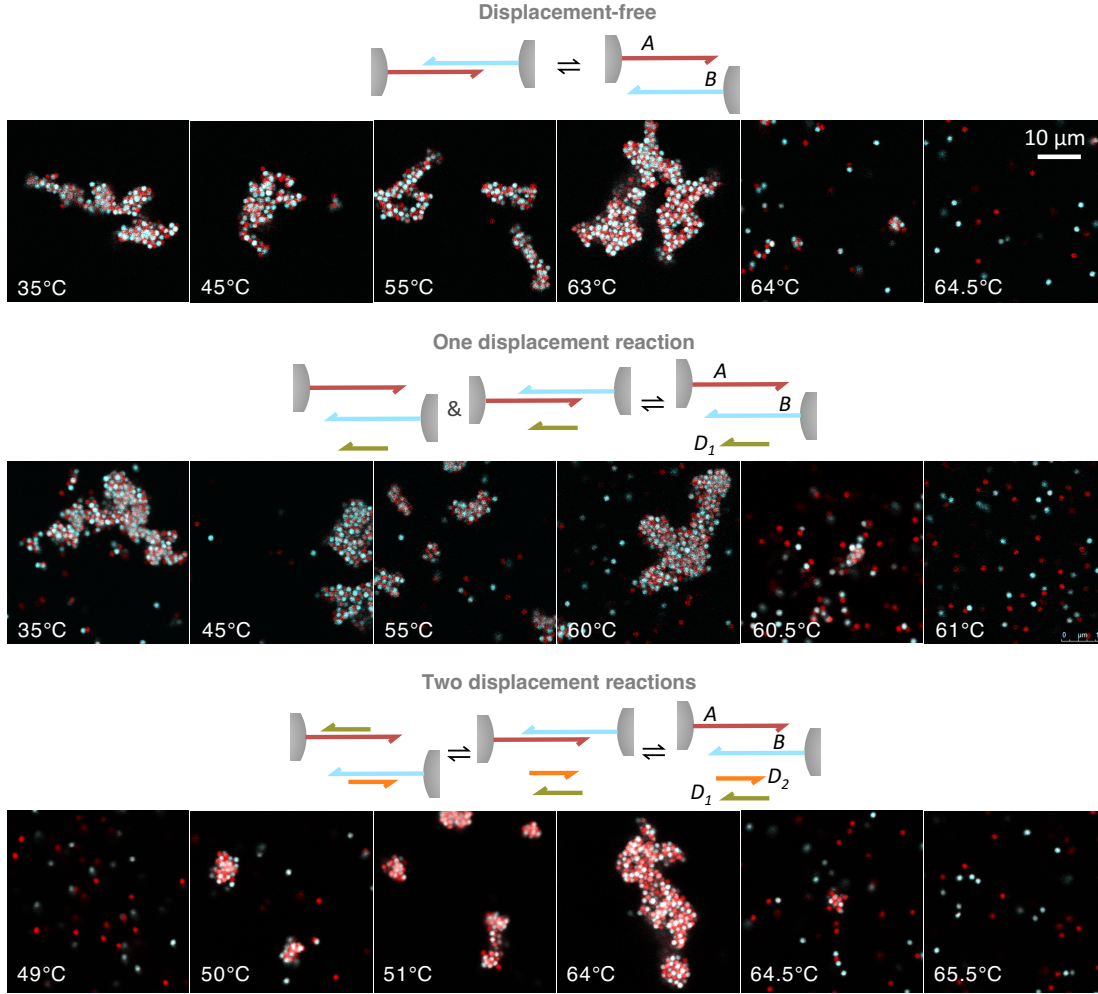


Figure 2.2: Fluorescence confocal microscopy images of a 3D system containing two complementary species (A , shown in red, and B , shown in blue) of 1- μm particles at different temperatures and under different displacement-reaction schemes. These experiments were performed with particles grafted with ssDNA with sequences from Table 2.3, with $C_{D_1} = 1.2 \mu\text{M}$ for the one-displacement-reaction scheme, and with $C_{D_1} = C_{D_2} = 100 \mu\text{M}$ for the two-displacement-reaction scheme. Top: The basic complementary system with no displacing strands. A single steep melting transition occurs in this system. Middle: Adding a single type of displacing strand (D_1 , green) introduces a new unbridged state that coexists with the bridged state. As a result, a stable coexistence exists between the solid and fluid phases over a wide range of temperatures. The coexistence is evidenced by the free particles in solution, which are present even at 35 °C. Bottom: Adding another displacing strand (D_2 , orange) creates three distinct binding configurations. As a result, a second phase transition emerges. The solid phase is now no longer stable below 50 °C, and it melts upon cooling.

(Section 2.6.2). We conclude with a discussion of future directions and practical considerations.

2.2 FUNDAMENTALS

Before describing how to use strand displacement reactions to modify the interactions between DNA-grafted particles, we first explain the mechanism of DNA-mediated interactions and why they have such a steep temperature dependence. Our argument follows that of Rogers and Crocker¹⁰ and Rogers, Shih, and Manoharan¹¹.

To understand the origin of the interparticle interaction in our system, we consider two particles grafted with ssDNA. They attract one another when the ssDNA on one hybridizes with that on the other to form a duplex—or a “bridge”—that links the two particles together

This attraction is mediated by many DNA strands. If the system is not too far from the duplex melting temperature, each of these strands fluctuates between bound and unbound states on timescales short compared to the diffusion time of the particles. Therefore we can treat the attraction as a time-averaged effective interaction, modeled by a free energy $F(r, T)$. $F(r, T)$ depends on the distance r between the particles and the temperature T , as well as on the sequences and concentrations of the DNA strands mediating the binding.^{10,11,24}

To illustrate the effect of DNA bridging on the free energy of the system, we consider two identical particles separated by a distance r . We calculate the difference in free energy between a state in which the particles are grafted with complementary DNA and a reference state in which they are grafted with non-complementary DNA. Then,

$$\Delta F(r, T) = F_{\text{comp}} - F_{\text{non-comp}} = -kT \ln \left(\frac{Z_{\text{comp}}(r)}{Z_{\text{non-comp}}(r)} \right), \quad (2.1)$$

where kT is the thermal energy and $Z(r)$ is the partition function. For the purposes of illustration, we assume that the configurational-conformational entropy of the unbound strands is the same in

both the complementary and the non-complementary cases.[†] Therefore, Z_{comp} and $Z_{\text{non-comp}}$ count only the ways in which the DNA bridges can form, along with their Boltzmann weights. Because we ignore the entropic contribution, there is only one possible combination of bridges for the non-complementary particles: no bridges exist ($Z_{\text{non-comp}}(r) = 1$). Hence, to solve Equation 2.1 we need only solve for $Z_{\text{comp}}(r)$.

To find the partition function for the complementary case, we begin by writing the probability of finding the pair of complementary DNA-grafted particles in an unbridged state. Because there is only one way to be in an unbridged state, $P_{\text{unbridged}} = 1/Z_{\text{comp}}(r)$, where $Z_{\text{comp}}(r)$ counts all bridged and unbridged configurations. Therefore we can replace $Z_{\text{comp}}(r)$ in Equation 2.1 by $1/P_{\text{unbridged}}$, yielding

$$\frac{\Delta F}{kT} = \ln P_{\text{unbridged}} .$$

Again for the purposes of illustration, we make another approximation: $P_{\text{unbridged}} \approx (1 - \chi)^N$, where χ is the probability of any two complementary strands forming a bridge, and N is the total number of grafted strands that can bind. The assumption behind this approximation is that bridges form independently of one another. Additionally, we assume that χ and N are constant with r up to some cutoff distance set by the radius of gyration of the ssDNA (see below). With these approximations we find that

$$\frac{\Delta F}{kT} = \ln(1 - \chi)^N . \quad (2.2)$$

The value of N is related to the surface concentration of the DNA on the particles C_{i0} and the volume V_{overlap} in which the binding portions of the two complementary DNA brushes overlap: $N = C_{i0} V_{\text{overlap}}$.[‡] The exponent N in Equation 2.2 is responsible for the steep transition from a

[†]If we were to consider this contribution, we would have $Z_{\text{configurational}} = \Omega_u$, where Ω_u is the number of configurational-conformational microstates in which the grafted strands do not overlap with each other or with the particles.

[‡]When the surface concentration of DNA on the two particles is not equal, the smaller of the two concentrations is used to calculate N .

bridged to an unbridged regime as the temperature increases. Because of this exponent, even a small increase in χ , caused by a small change in temperature, leads to a large decrease in the probability that two particles are unbridged.

In this paper, we will use this simplified expression for ΔF , herein referred to as the “simple model,” because it captures the essential features of the behavior of the system. One can derive a more accurate expression for the binding energy by accounting for how the density of the binding domains of the DNA strands varies with distance from the particle surface.¹⁰ To do so, we start by assuming that chemical equilibrium between grafted strands is established locally. We then integrate the density, corrected for dependence on distance from the particle surface, over the space between the particles:

$$\frac{\Delta F}{kT} \approx \int C_{i0}(r) \ln[1 - \chi(r, T)] dr, \quad (2.3)$$

where $C_{i0}(r)$ is the concentration of the binding domains of the grafted DNA strands, r is the spatial coordinate, and T is the temperature. In this more accurate model of the interaction, herein referred to as the “full model,” we also consider the repulsive contribution to the interparticle interactions, which arises from the compression of the DNA strands when the particle surfaces come close to one another. This entropic contribution and other details of this model are discussed in reference 10. In neither the simple nor full models do we assume that the number of bridges is Poisson-distributed, an approximation that has been discussed previously in the literature.^{10,35,36} However, we do assume independent binding, which is valid when N is large and binding is weak—conditions met in our experimental system.

With an expression for ΔF , from either Equation 2.2 or Equation 2.3, we can predict the singlet fraction f —the fraction of particles not bound to any others—as a function of temperature using the

equation derived by Dreyfus and coworkers²⁴:

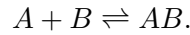
$$f = \frac{1 + 2KC_p - \sqrt{1 + 4KC_p}}{2(KC_p)^2}, \quad (2.4)$$

where $K = (l^2/4) \exp(-z\Delta F(T)/2kT)$, C_p is the concentration of particles per square micrometer, z is the average coordination of each particle (for a quasi-2D suspension, $z \approx 3$), and l is the range of the interaction. In our system, we assume $l = 15$ nm, which is a little bit larger than the radius of gyration of the grafted strands. While previously we made no assumptions about the geometry of our system, this expression for the singlet fraction assumes a quasi-two-dimensional (quasi-2D) suspension of particles. In the remainder of this paper, unless otherwise noted, all discussion and experimental measurements of the singlet fraction pertain to quasi-2D samples. We note, however, that the control over phase behavior achieved using strand-displacement reactions generalizes to three dimensions (3D), as demonstrated in Figure 2.2.

The expressions given in this subsection are general. They hold for systems containing only grafted strands and for systems containing both grafted and displacing strands. In the next three sections, we examine systems with zero, one, and two displacing strands. In each case, we derive expressions for the probability of hybridization $\chi(T)$ between the DNA strands grafted to the particles and combine them with Equations 2.2 and 2.4 to predict the temperature-dependent singlet fraction.

2.3 DISPLACEMENT-FREE BINDING

To calculate $\chi(T)$ for a pair of particles grafted with complementary DNA in the absence of any strand-displacement reactions, we model the hybridization of complementary DNA sequences A and B as a bimolecular reaction³⁷:



The equilibrium hybridization yield $\chi = C_{AB}/(C_A + C_{AB}) = C_{AB}/C_{A0}$ can be evaluated from the equilibrium constant of the reaction and from mass conservation:

$$\begin{aligned} K_{\text{eq}}(T) &= \frac{C_{AB}C^\circ}{C_A C_B} \\ C_{A0} &= C_A + C_{AB} \\ C_{B0} &= bC_{A0} = C_B + C_{AB}, \end{aligned}$$

where $C^\circ = 1 \text{ M}$ is a reference concentration, $K_{\text{eq}}(T) = \exp[-\Delta G_{AB}(T)/kT]$ is the equilibrium constant, and $\Delta G_{AB} = \Delta H_{AB} - T\Delta S_{AB}$ is the standard free energy difference between double-stranded AB and single-stranded A and B at the reference concentration C° . Here $C_{B0} = bC_{A0}$, where $b \geq 1$ and C_{A0} represents the local concentration if A is a grafted strand. We obtain the following equation for the hybridization yield:

$$\begin{aligned} \chi &= \frac{C_{AB}}{C_{A0}} \\ &= \frac{(b+1)K_{\text{eq}} \frac{C_{A0}}{C^\circ} + 1 - \sqrt{(b-1)^2(K_{\text{eq}} \frac{C_{A0}}{C^\circ})^2 + 2(b+1)K_{\text{eq}} \frac{C_{A0}}{C^\circ} + 1}}{2K_{\text{eq}} \frac{C_{A0}}{C^\circ}}, \end{aligned} \quad (2.5)$$

where χ depends only on b and the concentration-adjusted equilibrium constant $K'_{\text{eq}} = K_{\text{eq}}C_{A0}/C^\circ$.

To calculate the equilibrium constant, we use the nearest-neighbor model²⁷, which gives the concentration-adjusted standard free energy of binding $\Delta G'$:

$$-\ln K'_{\text{eq}} = \frac{\Delta G'}{RT} = \frac{\Delta H_{AB}}{RT} - \left[\frac{\Delta S_{AB}}{R} + \ln \left(\frac{C_{A0}}{C^\circ} \right) \right], \quad (2.6)$$

where ΔH_{AB} and ΔS_{AB} are calculated from the nearest-neighbor model using the actual se-

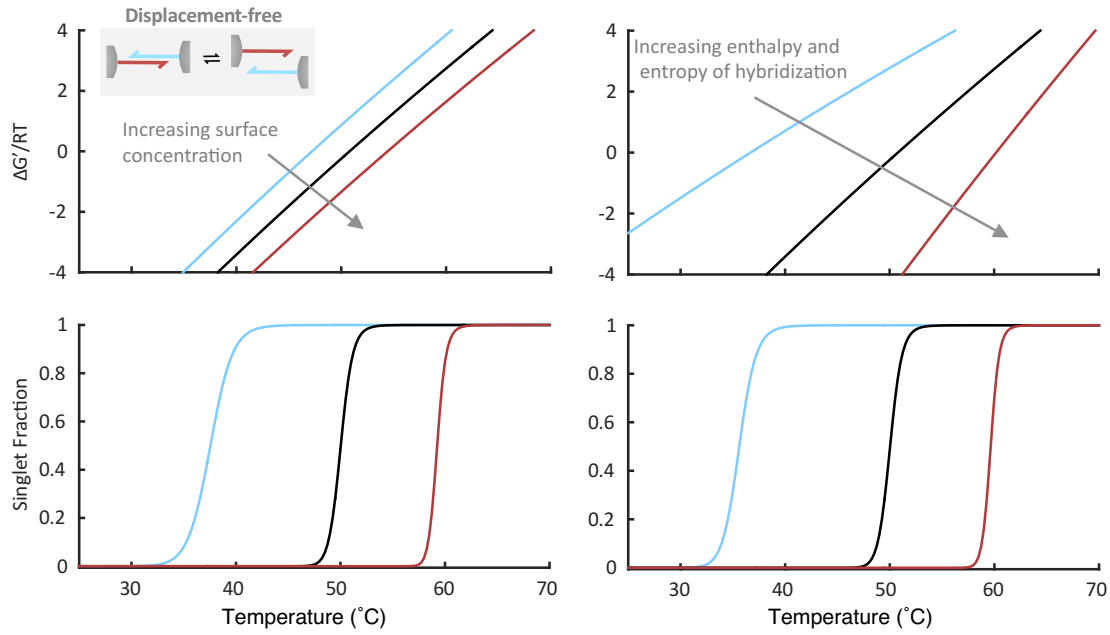


Figure 2.3: Temperature-dependent behavior of particles grafted with complementary DNA (see cartoon inset). Left: Plots of $\Delta G'$ (top) and singlet fraction (bottom) as a function of temperature and DNA surface concentration, calculated using Equations 2.2, 2.4, 2.5, and 2.6. The calculations are performed for DNA surface concentrations $C_{A0} = 76.7 \mu\text{M}$ (blue curves), $230 \mu\text{M}$ (black), and $690 \mu\text{M}$ (red). For all three curves the hybridization enthalpy and entropy of the DNA are $\Delta H_{AB} = -63.8 \text{ kcal/mol}$ and $\Delta S_{AB} = -180.3 \text{ cal/mol/K}$. Right: Plots of the same values, but now for constant surface concentration ($C_{A0} = 230 \mu\text{M}$) and varying enthalpy and entropy of DNA hybridization: $\Delta H_{AB} = -44.66 \text{ kcal/mol}$ and $\Delta S_{AB} = -126.21 \text{ cal/mol/K}$ (blue curves), $\Delta H_{AB} = -63.8 \text{ kcal/mol}$ and $\Delta S_{AB} = -180.3 \text{ cal/mol/K}$ (black), and $\Delta H_{AB} = -95.7 \text{ kcal/mol}$ and $\Delta S_{AB} = -270.45 \text{ cal/mol/K}$ (red). Note that changing either surface concentration or the enthalpy and entropy of hybridization shifts the melting transition, but does not significantly change its steepness. This calculation shows that sequence design and grafting density offer limited control over the shape of the melting transition.

quences of the strands.[§] The additional entropic term, $\ln(C_{A0}/C^\circ)$, results from the fact that two single-stranded reactants are consumed upon formation of one double-stranded product. Because ΔH_{AB} and ΔS_{AB} are assumed to be constant with temperature²⁷, the temperature dependence of $\Delta G'/RT$, and thus of Equations 2.2, 2.3, and 2.4, is determined entirely by the enthalpic term, the slope of which can be tuned by changing ΔH_{AB} .

[§]Here we switch to the Gibbs free energy instead of the Helmholtz free energy; while before we considered two particles held a fixed distance apart, now we consider a system of many components free to explore space.

With this expression for χ , we can use Equations 2.2, 2.4, 2.5, and 2.6 to predict $\Delta G'/RT$ and the singlet fraction as a function of temperature, given the sequences (which control ΔH_{AB} and ΔS_{AB}) and the surface concentrations of the grafted strands. By choosing values of these parameters that are typical for our experimental systems (described in Sections 2.4.2, 2.5.2, and 2.6) we can use our model to explore the phase behavior over a wide range of conditions. In Figure 2.3, we see that changing the surface concentration of DNA shifts $\Delta G'$ such that particles with higher surface concentrations melt at higher temperatures. This result has been validated experimentally in previous work.^{24,38} Modifying ΔH_{AB} and ΔS_{AB} changes the slope of $\Delta G'$, which also shifts the melting transition but does not significantly change its steepness. This effect—shifting the curve without changing the steepness—can be seen in the melting curves at zero displacing-strand concentration in Figures 2.4 and 2.5 and the blue curve in Figure 2.7. In each of these systems, the grafted strands have different sequences and therefore different ΔH_{AB} and ΔS_{AB} .

The plots in Figure 2.3 show that it is difficult to control the steepness of the melting transition in a displacement-free system. One can adjust the sequences of the grafted strands and their surface concentrations, but both of these parameters affect the singlet-fraction curves in a similar way: they shift the melting transition, but neither changes the steepness significantly. Although it is possible to reduce the steepness by using very low DNA surface concentrations, the DNA strands would need to bind strongly to keep the melting transition at a similar temperature. In this regime, the strands no longer fluctuate between bridged and unbridged configurations on timescales short compared to particle motion, and non-equilibrium behavior can result.^{38,39}

Fundamentally, what limits control over the shape of the melting transition is the linear dependence of $\Delta G'(T)$ on temperature. Changing the sequence or surface concentration can only change its slope. To control the shape of the melting transition we need to make $\Delta G'(T)$ a nonlinear function of temperature. In the following sections, we show how to do this by adding free strands that displace the grafted strands in a controlled way.

2.4 SINGLE STRAND-DISPLACEMENT REACTION

To increase the range of temperatures over which the system can equilibrate, we must significantly decrease the slope of $\Delta G'(T)$ near the melting transition. As shown in the previous section, this cannot be accomplished simply by modifying the grafted sequences or their surface concentrations. Instead, we introduce a competing interaction that effectively decreases the attraction between the two particles as the temperature decreases.

We create this competing interaction by introducing a freely dissolved displacing strand D_1 that can bind to one of the grafted strands (A). By binding A , these free strands can displace the hybridized AB pairs, causing the particles to unbind, while maintaining the same overall number of DNA duplexes (see diagram in the middle panel of Figure 2.2). As a result, the melting transition broadens such that coexistence between solid and fluid phases persists over a wide range of temperatures, as shown in the confocal micrographs in the middle panel of Figure 2.2.

2.4.1 MODELING THE SINGLE STRAND-DISPLACEMENT REACTION

To predict the singlet fraction of this modified system as a function of temperature, we again derive an expression for $\chi(T)$. We take into account the effect of a single displacement reaction on the probability that DNA bridges will form between the particles.

As in Section 2.3, we begin by writing down the equilibrium reactions for our system, now considering both the particle bridging and the displacement processes:



We derive the hybridization yield needed to complete Equation 2.2 from the equilibrium constant

and conservation of mass, assuming that the free strand D_1 is in large excess:^{||}

$$\begin{aligned} K_{eq}^{(1)}(T) &= \frac{C_{AB}C^\circ}{C_A C_B} \\ K_{eq}^{(2)}(T) &= \frac{C_{AD_1}C_B}{C_{AB}C_{D_1}} \\ C_{A0} &= C_A + C_{AB} + C_{AD_1} \\ C_{B0} &= bC_{A0} = C_B + C_{AB} \\ C_{D_10} &\approx C_{D_1}, \end{aligned}$$

where $K_{eq}^{(1)}$ is the equilibrium constant for the reaction shown in Equation 2.7 and $K_{eq}^{(2)}$ for Equation 2.8, and C_{D_1} is the free concentration of D_1 in the buffer in which the particles are suspended. The hybridization yield $\chi = C_{AB}/(C_A + C_{AB} + C_{AD_1}) = C_{AB}/C_{A0}$ can then be expressed in the same form as Equation 2.5,

$$\chi = \frac{(b+1)K'_{eq} + 1 - \sqrt{(b-1)^2(K'_{eq})^2 + 2(b+1)K'_{eq} + 1}}{2K'_{eq}}, \quad (2.9)$$

by redefining K'_{eq} in the following way:

$$-\ln K'_{eq} = \frac{\Delta G'}{RT} = -\ln \left[\frac{K_{eq}^{(1)} C_{A0}/C^\circ}{1 + K_{eq}^{(1)} K_{eq}^{(2)} C_{D_10}/C^\circ} \right]. \quad (2.10)$$

Thus we arrive at the same model as the one without displacing strands (Section 2.3), but with a modified expression for $\Delta G'(T)$.

We can understand how the displacement reaction modifies the temperature dependence of the

^{||}Because the grafted DNA strands are confined roughly within a spherical shell with volume $V_{\text{shell}} \approx 4\pi a^2 L$, this condition is satisfied so long as $C_{D_10}/C_{A0} \gg \phi/(1-\phi)V_{\text{shell}}/V_{\text{particle}} = 3\phi/(1-\phi)L/a$, where ϕ is the colloid volume fraction, a is the particle radius, and $L \approx 11$ nm is the thickness of the DNA layer, which is taken to be the same as the radius of gyration of the DNA. For our experimental conditions, $3\phi/(1-\phi)L/a \approx 0.005$.

interaction by examining limiting cases. In the high-temperature limit, where $K_{eq}^{(1)} K_{eq}^{(2)} C_{D_1 0} / C^\circ \ll 1$, $\Delta G'$ reduces to Equation 2.6. Thus, at high temperatures the displacement reaction has no effect on bridge formation—or, in fact, on the system at all. It can therefore be ignored.

In the opposite, low-temperature limit we find

$$\frac{\Delta G'}{RT} \xrightarrow{\text{low } T} \frac{(\Delta H_{AB} - \Delta H_{AD_1})}{RT} - \left[\frac{(\Delta S_{AB} - \Delta S_{AD_1})}{R} + \ln \left(\frac{C_{D_1 0}}{C^\circ} \right) \right].$$

In this limit we see that the displacement reaction simply lowers the free energy difference between the bridging and non-bridging configurations. As a result, if the grafted and displacing strands are designed to have the same enthalpy change upon hybridization (that is, $\Delta H_{AB} = \Delta H_{AD_1}$),

$$\frac{\Delta G'}{RT} \xrightarrow{\text{low } T} - \left[\frac{(\Delta S_{AB} - \Delta S_{AD_1})}{R} + \ln \left(\frac{C_{D_1 0}}{C^\circ} \right) \right]. \quad (2.11)$$

When $\Delta H_{AB} = \Delta H_{AD_1}$, we expect the melting transition to be broadened, since Equation 2.11 tells us that the free energy must become independent of temperature at low temperature. Therefore, adding the displacing strand makes the free energy of binding $\Delta G'(T)$ a nonlinear function of temperature.

The result of this nonlinearity in $\Delta G'(T)$ is a melting transition that can be tuned from steep to flat. To tune the transition, one can adjust either the sequences of the strands, which control ΔH_{AB} , ΔH_{AD_1} , ΔS_{AB} , and ΔS_{AD_1} , or the displacing-strand concentration C_{D_1} . Theoretical predictions of this behavior, obtained by inserting Equations 2.9 and 2.10 into our free-energy and singlet-fraction equations from Section 2.2, are shown by the curves in Figure 2.4. Experimental results are shown in the following subsection.

2.4.2 EXPERIMENTS USING A SINGLE STRAND-DISPLACEMENT REACTION

The wide region of coexistence between the solid and fluid phases that results from introducing a single displacing strand can be seen in the middle panel of Figure 2.2, which shows confocal micrographs of a 3D suspension of two species of fluorescently dyed particles grafted with sequences A (blue) and B (red) and mixed with displacing strand D_1 . The percent of unbound particles in this system remains non-zero and constant from 35 to 60 °C. Although in these experiments we have not waited long enough for nucleation of crystals to occur, our previous study²³ showed that, at longer times, crystals can form over a wide range of temperatures.

To generate DNA sequences that result in this wide range of coexistence, we follow a set of design rules intended to minimize the melting temperatures of secondary structures and the crosstalk between sequences (see Section 2.8.1). We then select sequences with melting temperatures between 40 and 65 °C and for which ΔH_{AB} and ΔH_{AD_1} fall within 5% of each other, so that Equation 2.11 applies. We then use Equations 2.2, 2.4, 2.9, and 2.10 to predict the 2D phase behavior and verify that the sequences produce the desired effect. The resulting sequences are shown in Table 2.1, and their thermodynamic parameters are given in Table 2.2.

Table 2.1: DNA sequences used for single strand-displacement experiments

Strand name	Base Sequence
A	5'-(T ₅₁)- GAGTTGCGGTAGAC -3'
B	5'-(T ₅₁)- AATGCCTGTCTACC -3'
D_1	5'-ACCGCAA-3'

Table 2.2: Thermodynamic parameters for single-displacement sequences used. The enthalpy and entropy of hybridization are calculated using the NUPACK software⁴⁰ for 50 mM NaCl and 10 mM MgCl₂.

Duplex name	ΔH (kcal/mol)	ΔS (cal/mol/K)
AB	-56.6	-163.0
AD_1	-53.6	-149.1

As predicted by our model, the location and steepness of the melting transition and degree of coexistence can be tuned by changing the concentration C_{D_1} of the displacing strand in solution (Figure 2.4). According to Equation 2.11, increasing the concentration of displacing strand should favor unbridged configurations over bridged configurations at low temperature. Our experiments on quasi-2D samples (described in detail in Section 2.8.4) verify this prediction. With no displacing strands, the system behaves as a simple complementary reaction: the singlet fraction rapidly increases to unity as the temperature increases. As the concentration of displacing strands is increased to 6 μM , a 10 $^{\circ}\text{C}$ -wide region of coexistence emerges between a solid phase containing about 25% of particles and a fluid phase containing the remainder. At higher concentrations of displacing strands we observe a fluid at all temperatures. Thus, the low-temperature equilibrium singlet fraction can be tuned between zero and one through a small change in the concentration of displacing strand, and the width of the melting transition can be varied from 2 to at least 10 $^{\circ}\text{C}$.

As shown in Figure 2.4, both the simplified model (shown as solid lines on the figure) and the full model (shown as dashed lines) detailed in Sections 2.2 and 2.4.1 capture the observed behavior of the system at different displacing-strand concentrations. To check the validity of our models, we fit them to the data using four free parameters (ΔH_{AB} , ΔS_{AB} , ΔH_{AD_1} , and ΔS_{AD_1}), as described in Section 2.8.7. We fit the four parameters for all displacing-strand concentrations simultaneously. For the simplified model, the best-fit parameters are all within 8% of those predicted by NUPACK. For the full model the parameters are all within 3% of the NUPACK predictions. The agreement shows that both models can be used, in conjunction with theoretical calculations of the thermodynamic parameters, to predict the phase behavior in the presence of displacing strands. Although the full model fits the experimental data much better than does the simplified model, the simplified model is, nonetheless, a useful tool for designing DNA strands that will produce a particular behavior and is considerably easier to implement.

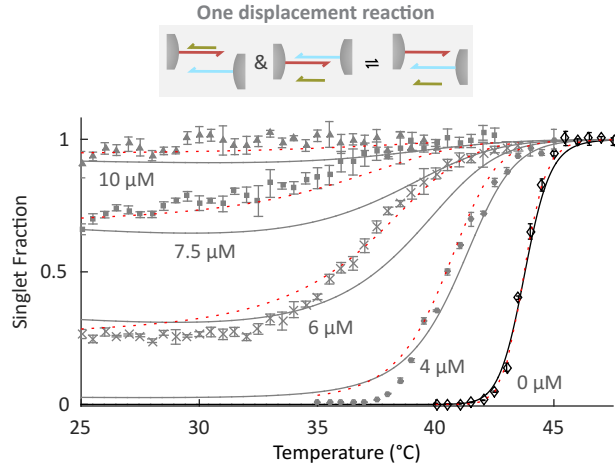


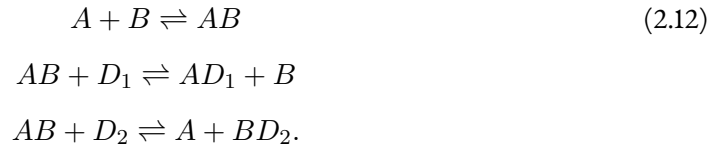
Figure 2.4: In an experimental system with one displacement reaction, changing the concentration of displacing strands can alter the width of the melting transition and the low-temperature singlet fraction. The plot shows the experimental singlet fraction (symbols) for different displacing-strand concentrations (from right to left, $C_{D_1} = 0, 4, 6, 7.5$, and $10 \mu\text{M}$). Fits of the model from Section 2.4.1 calculated using Equations 2.2, 2.4, 2.9, and 2.10 are shown as solid lines. For comparison, fits of the full model, calculated using Equation 2.3 instead of Equation 2.2 and including the interparticle repulsion, are shown as red dashed lines. Both the experiments and theory correspond to 6500 DNA strands per particle. The error bars correspond to the standard deviation from three measurements.

2.5 TWO STRAND-DISPLACEMENT REACTIONS

By adding a second displacing strand to the same solution we can make the free energy of binding $\Delta G'(T)$ not only nonlinear, but non-monotonic as well. This effect occurs if the second displacing strand D_2 can bind to the grafted strand B (see the diagram in the bottom panel of Figure 2.2). The result of this competition for binding between the various DNA strands is to invert the dependence on temperature, such that the interparticle attraction strengthens with increasing temperature over a range of temperatures before falling again at high temperatures. The effects of this inverted temperature dependence can be seen in the confocal micrographs in the bottom panel of Figure 2.2.

2.5.1 MODELING TWO STRAND-DISPLACEMENT REACTIONS

To model the effects of two strand-displacement reactions, we begin with the set of reactions



We assume that both D_1 and D_2 are in large excess, such that

$$\begin{aligned}
K_{eq}^{(1)}(T) &= \frac{C_{AB}C^\circ}{C_A C_B} \\
K_{eq}^{(2)}(T) &= \frac{C_{AD_1}C_B}{C_{AB}C_{D_1}} \\
K_{eq}^{(3)}(T) &= \frac{C_{BD_2}C_A}{C_{AB}C_{D_2}} \\
C_{A0} &= C_A + C_{AB} + C_{AD_1} \\
C_{B0} &= bC_{A0} = C_B + C_{AB} + C_{BD_2} \\
C_{D_1 0} &\approx C_{D_1} \\
C_{D_2 0} &\approx C_{D_2}.
\end{aligned}$$

To calculate the hybridization yield of bridge formation between the particles, $\chi = C_{AB}/(C_A + C_{AB} + C_{AD_1}) = C_{AB}/C_{A0}$, we first need an expression for the concentration-adjusted free energy. For the reaction network given by Equation 2.12,

$$\frac{\Delta G'}{RT} = -\ln \left[\frac{K_{eq}^{(1)} C_{A0}/C^\circ}{\left(1 + K_{eq}^{(1)} K_{eq}^{(2)} C_{D_1 0}/C^\circ\right) \left(1 + K_{eq}^{(1)} K_{eq}^{(3)} C_{D_2 0}/C^\circ\right)} \right]. \quad (2.13)$$

We can then calculate the concentration-adjusted equilibrium constant using $\ln K'_{eq} = -\Delta G'/RT$ and substitute into Equation 2.9 to find χ .

In the high-temperature limit, $\Delta G'$ again reduces to Equation 2.6 when the concentration of the two displacing strands are equal ($C_{D_1 0} = C_{D_2 0} = C_{D0}$) and when the hybridization free energies are the same for the bridging and displacing strands ($\Delta G_{AB} = \Delta G_{AD_1} = \Delta G_{BD_2}$). In the opposite, low-temperature, limit we find

$$\frac{\Delta G'}{RT} \xrightarrow{\text{low } T} -\frac{\Delta H_{AB}}{RT} + \left[\frac{\Delta S_{AB}}{R} + \ln \left(\frac{C_{A0}}{C^\circ} \right) + 2 \ln \left(\frac{C_{D0}}{C_{A0}} \right) \right]. \quad (2.14)$$

Note that the sign of the first term, $\Delta H_{AB}/RT$, is inverted from that in Equation 2.6. In other words, the free energy difference between bridging and non-bridging configurations actually becomes larger as the temperature decreases, making bridge formation less favorable.

As a result, the singlet fraction shows two transitions: a freezing transition from fluid to solid that occurs upon heating from low temperature, and a melting transition from solid to fluid upon further heating. These transitions can be understood from the molecular-scale interactions shown in the diagram at the top of Figure 2.5. At low temperatures, enthalpy favors two displacing strands binding to two grafted strands, which has more base pairs than two grafted strands binding together. As a result, the grafted strands are unavailable to form bridges, the particles do not attract one another, and the system remains in a fluid phase. On heating, the displacing strands can detach from the grafted strands. Although the enthalpy of this state is higher than that of the low-temperature state since there are fewer base pairs, this enthalpic increase is compensated by the increase in entropy caused by the liberation of the two displacing strands. As a result, the grafted strands are free to form bridges, and the particles can form a solid phase. The solid phase is therefore entropically stabilized, and the range of temperatures over which it is stable can be tuned by changing the concentration of the displacing strands, as predicted by the model. Finally, at high temperature the grafted strands dissociate, and the solid phase melts.

As in the single displacement-reaction case, we can theoretically predict the behavior of the system by solving for χ as described above and then using Equation 2.2 to calculate ΔF . Finally, we substitute this value of ΔF into Equation 2.4 to solve for the singlet fraction. The resulting theoretical singlet fraction curves are shown by the solid lines in Figure 2.5. The experimental realization of this system is described in the following subsection.

2.5.2 EXPERIMENTS USING TWO STRAND-DISPLACEMENT REACTIONS

The appearance of a second phase transition in the two-displacement-reaction system is shown experimentally in the bottom panel of Figure 2.2. This figure shows confocal images from a 3D system containing two species of particles (A and B) and two displacing strands (D_1 and D_2). The concentrations of the two displacing strands are equal. Our experiment shows that at 65.5 °C the entire system is in a fluid state that, on cooling, transitions to a solid at 64 °C. This solid phase persists to 51 °C, at which point the system melts on further cooling, so that at 49 °C it is once again fully in the fluid state.

Table 2.3: DNA sequences used for experiments with two strand-displacement reactions

Strand name	Base Sequence
A	5'-(T ₅₄)-CTAACTGCGGT-3'
B	5'-(T ₅₄)-CTTACCGCAGT-3'
D_1	GCAGTTAG
D_2	GCGGTAAG

Table 2.4: Thermodynamic parameters for sequences used for two displacement reactions, calculated using the NUPACK software for 250 mM NaCl.

Duplex name	ΔH (kcal/mol)	ΔS (cal/mol/K)
AB	-63.6	-173.7
AD_1	-64.4	-186.1
BD_2	-69.4	-195.7

We design our sequences in the same way as for the single displacement scheme, but with the constraint that the free energies of hybridization are all equal ($\Delta G_{AB} = \Delta G_{AD_1} = \Delta G_{BD_2}$) to within 5%. The sequences are shown in Table 2.3 and their thermodynamic parameters in Table 2.4. With this constraint, we can control the width of the solid region in the phase diagram by changing the concentration of the two displacing strands jointly ($C_{D_1} = C_{D_2}$), as predicted by Equation 2.14 and shown in the quasi-2D measurements in Figure 2.5. Increasing the concentration of displacing

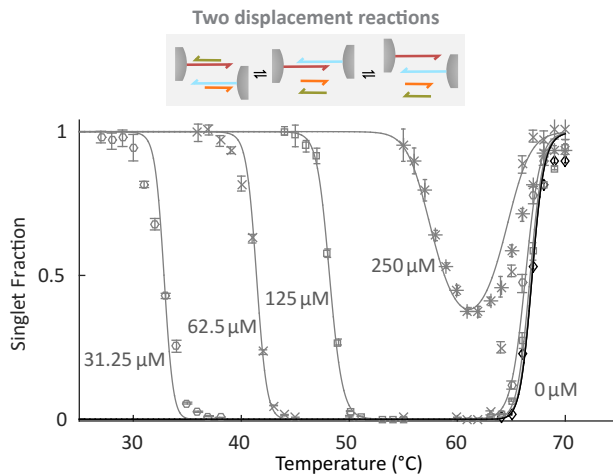


Figure 2.5: In an experimental system with two displacement reactions, changing the concentration of displacing strands alters the width (in temperature) of the regime where the solid phase is stable. The experimentally measured singlet fractions are shown by the symbols, where each type of symbol represents a different displacing-strand concentration. The solid lines represent fits of the model from Section 2.5.1 using Equations 2.2, 2.4, 2.9, and 2.13. In both the experiments and the fits, there are 6500 DNA strands per particle. Error bars denote the standard deviation from three measurements.

strands favors the fluid phase, where more displacing strands are bound, over the solid phase.

This tunability is shown experimentally in Figure 2.5. At low concentration of displacing strand (31 μM) there is a 30 $^{\circ}\text{C}$ -wide solid regime between two fluid phases. At higher concentration of displacing strand, the width of this regime decreases, so that at 125 μM it is approximately 13 $^{\circ}\text{C}$ wide. At 250 μM of displacing strands there is no temperature at which the system is completely solid. Thus, the minimum width of a regime in which the sample is fully solid is about 10 $^{\circ}\text{C}$.

Our simplified model, shown as solid lines in the figure, again captures the behavior of our experimental measurements. We fit the model to the data, allowing ΔH_{AB} , ΔH_{AD_1} , and ΔH_{BD_2} to vary while holding ΔS_{AB} , ΔS_{AD_1} , and ΔS_{BD_2} at the values calculated with NUPACK. The values returned by each of the fits are within 3.3% of their NUPACK values, within the uncertainty of calculations based on the nearest-neighbor model (which NUPACK uses). Again, the agreement validates the use of the model as a tool for designing DNA sequences to yield prescribed phase behaviors.

2.6 COMBINING DISPLACEMENT-FREE AND DISPLACEMENT-MEDIATED INTERACTIONS

Having shown that our model explains and predicts the experimentally observed phase behavior in both the one- and two-displacement-reaction schemes, we now show how to combine these schemes to design more complex behavior. We show that with careful design of the DNA sequences, so as to remove crosstalk between non-complementary sequences, the different strand-displacement reactions can operate independently in the same solution. Thus, multiple such reactions can be combined to create multiple transitions at different temperatures between the same pair of particles, or to create independent phase transitions between multiple species of particles in the same solution.

2.6.1 THREE PHASE TRANSITIONS

The different interactions described in the previous sections can be combined to create more complex interactions by grafting multiple strands of DNA to each particle. Here we demonstrate a system in which there are three transitions between fluid and solid phases. We create this behavior by combining a transition at low temperature, which arises from the reaction of grafted strands only, with freezing and melting transitions at higher temperatures, which arise from a two-displacement-reaction scheme. Our system consists of two particle species, depicted as gray and red in Figure 2.6a.

Realizing this set of transitions requires four grafted DNA strands ($5A$ and $5C$ on red and $5B$ and $5E$ on gray) and two displacing strands ($5D_1$ and $5D_2$), as shown in Figure 2.6a and Table 2.5. The thermodynamic parameters are given in Table 2.6. We design the sequences for $5A$, $5B$, $5D_1$, and $5D_2$ following the protocol described in Section 2.5.2, so as to place the high-temperature melting transition near 55°C . We make strands $5C$ and $5E$ short compared to $5A$ and $5B$ so that the melting transition caused by this interaction is at a low enough temperature (30°C) to leave room for a third transition between the two. To allow the two sets of transitions (displacement-free and two-displacement) to occur independently of one another, we design the DNA sequences so that they do

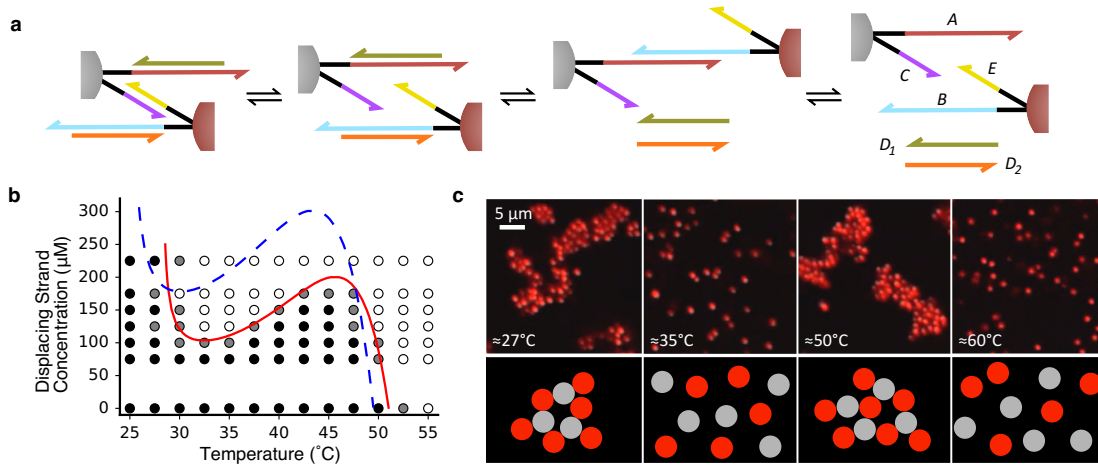


Figure 2.6: Demonstration of a system that shows three phase transitions (solid to fluid to solid to fluid). There are two types of particles, one identified by red fluorescent dye and the other undyed (shown in gray on diagrams). a) Schematic of the DNA and particles. The combination of a low-melting-temperature interaction between complementary grafted strands and a two-displacing-strand-mediated interaction creates the three phase transitions. b) Phase diagram showing the state of aggregation of a 3D suspension of the particles as a function of temperature and displacing-strand concentration $C_{D1} = C_{D2}$. The black symbols indicate an aggregated state (less than approximately 15% of particles unbound), the white symbols indicate a fluid state (more than approximately 75% of particles unbound), and the grey symbols indicate that the system is between these two bounds. The red line shows the best fit of a model for the phase boundary, which represents the displacing-strand concentration at which the singlet fraction is 50% for each temperature. The dashed blue line shows this same phase boundary using the nearest-neighbor values for the thermodynamic parameters instead of the best-fit values. c) Fluorescence confocal images of this system with displacing-strand concentration $C_{D1} = C_{D2} = 125 \mu\text{M}$. As shown in the schematic below the micrographs, the aggregated states occur when the dyed and undyed particles interact with one another, and the fluid states occur when there are no interactions. The temperatures given for the micrographs are approximate.

not contain any complementary domains across the two sets of reactions, as discussed in Section 2.8.1. Here, one set involves the sequences 5*A*, 5*B*, 5*D*₁, and 5*D*₂, and the other involves 5*C* and 5*E*. Finally, we tune the location of the third transition (a freezing transition) by changing the concentration of displacing strands in the solution.

The phase behavior of the resulting experimental system agrees with the predictions of a model for the phase boundary, as shown in Figure 2.6b. Here we measure the experimental phase behavior for a 3D sample as a function of displacing-strand concentration and temperature, and we compare to the predictions of a model that combines the elements of Sections 2.3 and 2.5.1.

To model the behavior of this combined system, we solve for the hybridization probability of *A* and *B*, $\chi_{AB} = C_{AB}/C_{A0}$, using Equations 2.9, 2.13, and the relation $\ln K'_{eq} = -\Delta G'/RT$. We solve for the hybridization probability of *C* and *E*, $\chi_{CE} = C_{CE}/C_{E0}$, using Equations 2.5 and 2.6. We then calculate the singlet fraction using Equation 2.4, where the binding strength is given by

$$\frac{\Delta F}{kT} = \ln(1 - \chi_{AB})^{N_A} + \ln(1 - \chi_{CE})^{N_E} , \quad (2.15)$$

where N_i is related to the surface concentration of DNA of type *i*, as discussed in Section 2.2.

We use our simple 2D model to fit a theoretical phase boundary to the data using three parameters, f_1 , f_2 , and f_3 , which are coefficients for the nearest-neighbor thermodynamic parameters: $f_1\Delta H_{AB}$, $f_1\Delta S_{AB}$, $f_2\Delta H_{AD_1}$, $f_2\Delta S_{AD_1}$, $f_2\Delta H_{BD_2}$, $f_2\Delta S_{BD_2}$, $f_3\Delta H_{CE}$, $f_3\Delta S_{CE}$. The f_i therefore characterize the deviation from nearest-neighbor predictions. Section 2.8.7 provides a detailed description of the fitting method. Given the best-fit coefficients, we then calculate the theoretical phase boundary by calculating the displacing-strand concentration at which the singlet fraction is 0.5 for a range of temperatures from 25 to 55 °C. This phase boundary is shown as the red curve in Figure 2.6b.

The fits show that the actual thermodynamic parameters are within 11% of the nearest-neighbor values, which are given in Table 2.6. The agreement is good, considering that the the measurements

were performed on a 3D sample and compared to a singlet fraction model developed for quasi-2D systems. By comparison, the phase boundary calculated using our model and the nearest-neighbor values (instead of the best-fit values) has the same shape but is shifted slightly toward decreasing temperature and higher displacing-strand concentration (blue curve in Figure 2.6b). Therefore, the 11% uncertainty in the predictions for the thermodynamic parameters translates to an uncertainty of about a factor of two in the displacing-strand concentration required to achieve a given phase behavior.

Nonetheless, the simple model (Equation 2.15) with the nearest-neighbor predictions of the thermodynamic parameters still proves useful in design. To demonstrate, we use the model to design a system that shows solid-fluid-solid-fluid phase behavior, where the transitions are spaced equally as a function of temperature. Using the nearest-neighbor parameters in our model, we predict that the displacing-strand concentration should be less than 250 μM to satisfy these constraints. We then vary the concentration of displacing strands until we realize the desired behavior. In the example shown in Figure 2.6c, we find that the experimental system at 125 μM shows an interior fluid-solid transition that occurs about 10 $^{\circ}\text{C}$ above the low-temperature solid-fluid transition and about 15 $^{\circ}\text{C}$ below the high-temperature solid-fluid transition, close to our design goals. Although the design process requires some experimental tuning, the model provides a good starting point. Also, the tuning does not require varying the sequences, only the displacing-strand concentrations—which are easy to vary, as we discuss in Section 2.7.

We estimate that as many as nine phase transitions can be incorporated into a single system, assuming that crosstalk between the DNA strands can be avoided and that each of the grafted strands is in high enough surface concentration to allow the melting transition to occur at the desired temperature (see Figure 2.3). The limits are set by the regime in which water is liquid (0–100 $^{\circ}\text{C}$) and by the minimum width of the regime where the solid phase is stable—approximately 10 $^{\circ}\text{C}$, as discussed in Section 2.5.2.

Table 2.5: DNA sequences used for a system showing three phase transitions with temperature (solid to fluid to solid to fluid). The concentrations refer to surface concentration in the case of the grafted strands and solution concentrations in the case of the displacing strands. Surface concentrations are estimated from the expected number of DNA strands of each type, assuming a total of 6500 DNA strands per particle confined to a shell the width of the radius of gyration of the DNA (11 nm).

Particle	Strand name	Base Sequence	Concentration
red	5 <i>A</i>	5'-(T ₅₄)-CA ATG GAG GCT-3'	150 μ M
red	5 <i>C</i>	5'-(T ₅₉)-CAG GTG-3'	150 μ M
undyed	5 <i>B</i>	5'-(T ₅₄)-TA TAG CCT CCA-3'	112.5 μ M
undyed	5 <i>E</i>	5'-(T ₅₉)-CAC CTG-3'	75 μ M
—	5 <i>D</i> ₁	5'-CT CCA TTG-3'	125 μ M
—	5 <i>D</i> ₂	5'-AG GCT ATA-3'	125 μ M

Table 2.6: Thermodynamic parameters for sequences used for the system with three phase transitions, calculated using the nearest-neighbor model at 250 mM NaCl.

Duplex name	ΔH (kcal/mol)	ΔS (cal/mol/K)
5 <i>A</i> 5 <i>B</i>	-53.5	-145.6
5 <i>A</i> 5 <i>D</i> ₁	-55.6	-157.9
5 <i>B</i> 5 <i>D</i> ₂	-50.4	-143.8
5 <i>C</i> 5 <i>E</i>	-41.0	-115.9

2.6.2 COMPOSITIONAL TRANSITIONS

All of the examples that we have shown thus far involve two species of particles. We now show that the same displacement-mediated interactions can be used in systems with multiple species. We demonstrate an experimental system with a controlled transition between binary crystals with different compositions (Figure 2.7).

This system contains three different particle species (shown as red, green, and blue in the figure). Similarly to the system discussed in Section 2.6.1, the green species is grafted with two different sequences of DNA that allow it to bind to both the red and the blue species. The sequences are designed such that at low temperature, the blue and green particles are bound while the red particles remain in a fluid state. At high temperature, the red and green particles are bound while the blue particles are in a fluid state. These two regimes are separated by a 10 °C window in which the entire system is a fluid

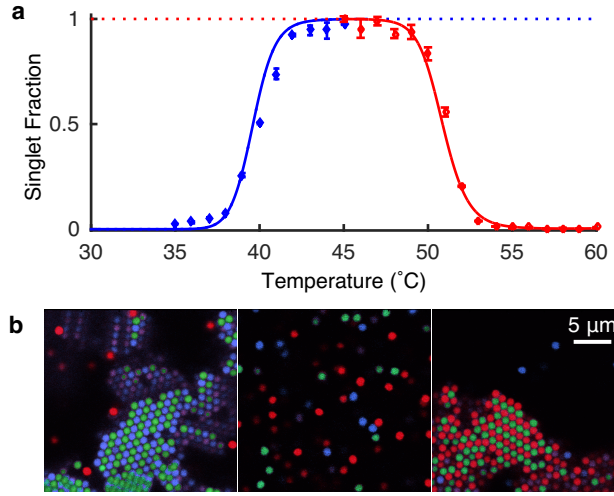


Figure 2.7: a) Experimental singlet fraction measurements for blue/green interactions (blue symbols) and red/green interactions (red symbols) with a fit to the model overlaid (lines). b) Confocal fluorescence micrographs showing the transition from a blue/green crystal phase at low temperature to a red/green crystal phase at high temperature through an intermediate fluid phase. The intermediate fluid phase is used to anneal the crystal into the two phases on cooling or heating. The displacing-strand concentration used is $C_{D_1} = C_{D_2} = 62.5 \mu\text{M}$.

(Figure 2.7b).

Table 2.7: DNA sequences used for compositional transitions. Concentrations are defined as in Table 2.5.

Particle	Strand name	Base Sequence	Concentration
red	<i>A</i>	5'-(T ₅₁)-CT AAC TGC GGT-3'	300 μM
green	<i>B</i>	5'-(T ₅₁)-CT TAC CGC AGT-3'	150 μM
green	<i>E</i>	5'-(T ₅₉)-CAG GTG-3'	150 μM
blue	<i>F</i>	5'-(T ₅₉)-CAC CTG-3'	300 μM
—	<i>D</i> ₁	5'-GC AGT TAG-3'	62.5 μM
—	<i>D</i> ₂	5'-GC GGT AAG-3'	62.5 μM

This phase behavior is designed through the combination of a displacement-free interaction between the green and blue particles and a two-displacement interaction between the red and green particles (Tables 2.7 and 2.8). The blue and green particles, which are grafted with complementary DNA, bind together at low temperature and unbind above 40 °C. The green particles are grafted with a

Table 2.8: Thermodynamic parameters for sequences used for compositional transitions, calculated using the NUPACK software for 500 mM NaCl.

Duplex name	ΔH (kcal/mol)	ΔS (cal/mol/K)
<i>AB</i>	-63.6	-171.5
<i>AD</i> ₁	-64.6	-184.8
<i>BD</i> ₂	-69.6	-194.1
<i>EF</i>	-49.0	-138.0

second strand of DNA that allows them to interact with the red particles through a two-displacing-strand interaction, such that they are dispersed at low temperatures and aggregated at temperatures above 51 °C (and eventually redisperse at temperatures higher than those reached in the experiment). Therefore, the same system can be driven to assemble into two crystals of different composition. Both the equilibrium structure of the phases *and* the transitions between these phases are encoded in the DNA sequences.

2.7 CONCLUSIONS AND PRACTICAL CONSIDERATIONS

We have shown, through theory and experiment, that strand-displacement reactions are a powerful way to alter—in fundamental ways—the temperature dependence of DNA-mediated interactions between colloidal particles. Using a small number of experimental building blocks, one can create broadened melting transitions, temperature-independent coexistence between fluid and solid phases, and inverted transitions where particles bind with increasing strength as temperature increases. A summary of the phase behaviors that can be achieved with no displacing strands, a single strand-displacement reaction, and two strand-displacement reactions is shown in Figure 2.8. The diversity of the melting curves highlights the versatility of the strand-displacement schemes. We have also shown that these schemes can be combined with multiple strands of DNA on a single species of particle to realize more complicated phase transitions or with multiple species of particles to realize compositional transitions.

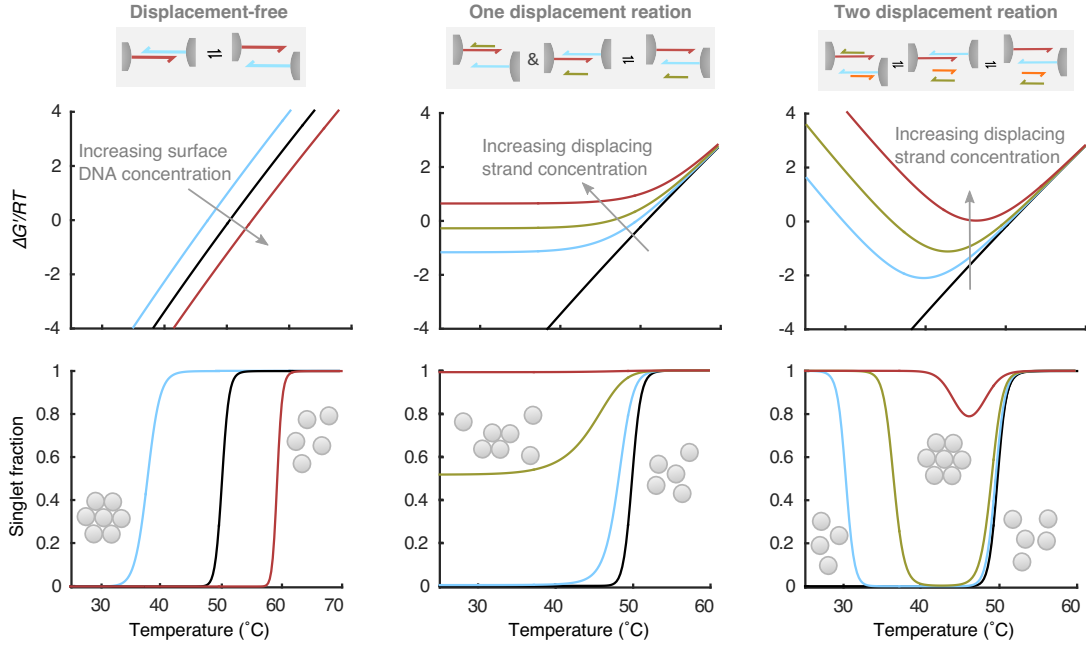


Figure 2.8: A summary of the basic phase behaviors that can be achieved with no displacement reactions (left), a single strand-displacement reaction (middle), and two strand-displacement reactions (right). Plots of $\Delta G'$ (top row) and singlet fraction (bottom row) as a function of temperature calculated using Equations 2.6, 2.10, and 2.13 for $\Delta G'$ and Equations 2.2, 2.4, 2.5, and 2.9 for singlet fraction. The calculations for the black curve on each plot are performed for typical experimental values of the concentrations and thermodynamic parameters: the DNA surface concentration is $C_{A0} = 230 \mu\text{M}$, and the hybridization enthalpy and entropy of the DNA are $\Delta H_{AB} = \Delta H_{AD_1} = \Delta H_{BD_2} = -63.8 \text{ kcal/mol}$ and $\Delta S_{AB} = \Delta S_{AD_1} = \Delta S_{BD_2} = -180.3 \text{ cal/mol/K}$. The surface concentrations used in the displacement-free case are 76.6 (blue) and $690 \mu\text{M}$ (red). The displacing-strand concentrations used in the one-displacement-reaction case are $C_{D_1} = 76$ (blue), 184 (green), and 460 (red) μM . The displacing-strand concentrations used in the two-displacement-reaction case are $C_{D_1} = C_{D_2} = 6.9$ (blue), 18.4 (green), and 57.5 (red) μM . For one displacement reaction, the concentration-adjusted free energy of the system flattens, leading to a wide coexistence region between fluid and solid. The width of the transition and the degree of coexistence can be tuned with the displacing-strand concentration. For two displacement reactions, the free energy reverses slope at low temperature, leading to a second melting transition. The position of this second, low temperature transition is controlled by the concentration of displacing strands.

The model that we present provides a theoretical basis for the change in phase behavior resulting from changes in the concentrations of the displacing strands in solution. It also allows us to design the DNA sequences required to realize these systems experimentally.

This strand-displacement toolkit may make it easier to assemble DNA-grafted colloidal particles. For example, broadening the melting transition could allow for easier annealing of structures. This effect is especially important in systems containing multiple species of particles. In such systems the melting temperatures of different pairs of species must be matched so that they can all anneal in the same temperature range. It is much easier to match these temperatures by controlling the concentration of displacing strand in solution than by trying to control the grafted strand concentrations, which requires modifying the synthesis of the particles. Also, the ability to use several species of particles in the same system, where each pair can exhibit phase behavior with multiple transitions, opens the door to systems that can dynamically reconfigure between multiple structures as the temperature is tuned (see Chapter 3).

Incorporating displacement reactions into a DNA-grafted system is straightforward—so much so, in fact, that we now design all of our DNA-grafted particles so that there are “toeholds” on each strand for displacement reactions to occur, as shown in Figure 2.1c. By adding toeholds, we always have the choice to use displacement reactions. If we choose to use them, we can simply add the displacing strands to the solution, and we can easily control the shape of the melting transition(s) by varying the concentration of free strands. The displacing strands, being a few bases long, are inexpensive and do not require purification.

If we choose not to include displacement reactions, there is no harm in including the toeholds, so long as we are careful to avoid crosstalk between the toeholds and the binding domains. The toeholds are designed such that the free energy of hybridization between the grafted and displacing strands is the same as that between the grafted strands, as discussed above. Practically, we find that it is easiest to meet this constraint and avoid crosstalk through a few guidelines. First, we avoid sequences with

more than two C or G bases in a row. These repeats lead to unintended interactions and hairpins, owing to the high stability of C-G bonds. Second, we avoid using *any* base three or more times in a row. Third, we design our toeholds to be three bases long, so that they bind the displacing strands strongly enough to ensure that displacement occurs on short timescales, but weakly enough so that the displacing strands do not remain bound to the grafted strand through the toehold alone.^{31,32} Further details of our DNA sequence design process are discussed in Section 2.8.1.

Finally, we note that while the experiments here rely on the method shown by Kim, Manoharan, and Crocker⁴¹ to graft DNA onto the particles, the control over the temperature dependence afforded by displacement reactions should work for particles with much higher surface coverage, including those demonstrated recently by Pine and collaborators.^{42,43,44} We also foresee no obstacles to integrating strand-displacement reactions into the emulsion systems with the mobile DNA strands demonstrated by Brujic and coworkers.^{45,46}

2.8 METHODS AND MATERIALS

2.8.1 SEQUENCE DESIGN

All grafted oligonucleotides are 65 bases long, single stranded, and consist of an inert poly-T spacer and a binding domain on the 3' end. The poly-T spacer sets the range of interaction, and the binding-domain sequence sets the strength and specificity of binding.

To design the base sequence of a binding domain, we generate many random sequences of bases of the appropriate length from the complete set of three-base codons (where codon refers to a unique three-base segment of DNA), each used only once to minimize unintended crosstalk and hairpins. We then use either the nearest-neighbor model²⁷ or the online software NUPACK⁴⁰, which uses the nearest-neighbor parameters along with other corrections, to calculate the thermodynamic parameters (enthalpy and entropy contribution to binding energy) of each sequence. We calculate

these parameters at salt concentrations matching the conditions of the corresponding experimental measurements. We then filter the sequences such that they meet the specifications for the single-displacement scheme of Section 2.4 ($\Delta H_{AB} = \Delta H_{AD_1}$) or the two-displacement scheme of Section 2.5 ($\Delta G_{AB} = \Delta G_{AD_1} = \Delta G_{BD_2}$). We match these thermodynamic parameters within 5%.

Once all of these requirements are met, we ensure that the melting transitions fall in the desired range using a Matlab program (available at <https://github.com/manoharan-lab/DNA-colloid-design>). This program calculates the singlet fraction as a function of temperature for any given combination of sequences using the model described in Sections 2.2, 2.3, 2.4.1, and 2.5.1. We then check for stable secondary structures and unintended crosstalk between non-interacting sequences using NUPACK and Mfold⁴⁷. We reject sequences with secondary structures that are stable above 20 °C. We also reject if unintended crosstalk is predicted to occur more than 1% as often as the desired structure within the working temperature range for the experiment.

We then order single-stranded DNA oligonucleotides, with the sequences we have designed, from Integrated DNA Technologies, Inc. with an amino-C6-modifier added to the 5' end of the surface-grafted strands. Surface-grafted strands are purified by high-performance liquid chromatography, and short, soluble strands are purified by standard desalting. These purification steps are performed by the supplier.

2.8.2 FUNCTIONALIZING PARTICLES

We functionalize our colloidal particles with DNA by chemically bonding our DNA strands to a triblock copolymer, which we then physically graft to the surface of the particles. Our protocol is modified from that described in reference 41 (see Appendix A).

We begin by activating the hydroxyl end groups of a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) triblock copolymer (Pluronic F108, BASF) by dissolving 500 mg of Pluronic F108 in 2 mL of dichloromethane (anhydrous, $\geq 99.8\%$, Sigma-Aldrich) and 30 μ L of triethylamine

(Sigma-Aldrich). We add 100 mg of *p*-nitrophenyl chloroformate (Sigma-Aldrich), cool the solution to 0 °C and stir for at least 4 hours to allow the reaction to complete.

After the reaction, we wash the activated Pluronic once in 3% v/v hydrochloric acid (38% ACS grade, EMD) in ethanol (200 proof, Koptec) and three times in 1% v/v hydrochloric acid in ethanol. After the final wash, we pour off the supernatant and allow the pellet to dry in a vacuum desiccator for at least 7 hours.

We then react the activated Pluronic with 5'-amino-C6-modified oligonucleotides (IDT) by first dissolving 15 mg of the activated Pluronic in 1 mL of 10 mM pH 4.0 citric acid buffer (1.1 mM Anhydrous Citric Acid, EMS 99.5%, 8.9 mM Sodium Citrate, Spectrum, 99.0-100.5%). We then combine 15 μ L of DNA (1 mM in molecular-biology-grade water) with 1 μ L of 1 M pH 9.5 carbonate buffer (0.85 M Sodium Bicarbonate, EMD 99.7-100.3%, 0.15 M Sodium Carbonate, EMD $\geq$ 99.5%) and 4 μ L of the activated Pluronic in citric acid buffer. We allow this solution to react on a room temperature vortexer for at least 4 hours to allow the DNA to bind to the activated end groups of the Pluronic.

Once the reaction has completed, we physically graft the DNA-functionalized Pluronic to the surface of 1- μ m polystyrene sulfate particles (Invitrogen). To graft, we first wash the particles in aqueous buffer containing 10 mM Tris and 1 mM EDTA (diluted to 1xTE from Serva TE Buffer (100x), pH 8), finishing at a total volume fraction of 10%. If the particles do not appear well dispersed, we sonicate until there is no aggregation. After the particles are washed, we combine 340 μ L of citric acid buffer with 40 μ L of the DNA-functionalized Pluronic solution and 40 μ L of the 10% particle solution. We then add 4 μ L of toluene (anhydrous, Sigma) being careful to not strongly agitate the solution through vortexing, sonication, or centrifugation. We allow the solution to mix gently on a room temperature rotator for at least 7 hours to allow the central block of the Pluronic to infiltrate into the swollen matrix of polystyrene. At the end of this time we heat an open container of the solution to 90 °C in a vented oven for 12 min to allow the toluene to evaporate, thus deswelling the particles. Finally we wash the particles at least five times in 1xTE buffer to remove any excess Pluronic.

We estimate our total DNA density to be 6,500 DNA strands per particle by flow cytometry and melting-curve measurements, and we use this value in all calculations. We are easily able to incorporate hydrophobic dyes, such as BODIPY 650 (Life Technologies) or BODIPY 488 (Life Technologies), or a mixture of the two, into the polystyrene core by dissolving them in the toluene used for swelling.

2.8.3 SAMPLE PREPARATION

Functionalized colloidal particles are stored separately in a 4 °C refrigerator at 1% in 1xTE (Tris-EDTA pH 8.0) buffer. When we need to prepare a sample, we mix the particles with displacing strands at appropriate concentrations in a 1xTE solution with a final concentration of 250 mM NaCl. Exceptions are the singlet-fraction measurements for the single-displacement-reaction case, where the samples were prepared with 50 mM NaCl and 10 mM MgCl₂, and the singlet-fraction measurements for the system with three species of particles, where the samples were prepared at 500 mM NaCl. This solution is then loaded into a sample chamber prepared as described in the following three subsections.

2.8.4 PERFORMING SINGLET-FRACTION EXPERIMENTS

Melting-curve measurements are performed in 2D sample chambers to allow for comparison to the theoretical singlet-fraction equations discussed in Section 2.2.

Samples are made of a 1:1 mixture of the two particles species at a total volume fraction of 4%. This solution is sealed in a 2D sample chamber made from two coverslips (No. 1; VWR) that we plasma-clean for approximately 1 min to prevent nonspecific binding of the DNA-grafted microspheres to the glass surfaces. 1.8- μ m-diameter silica microspheres are deposited to provide the correct spacing between the coverslips for 2D confinement. Once the sample is added, the coverslips are bonded together and sealed by UV-curable optical adhesive (Norland 63).

The sample is imaged on an inverted optical microscope (Nikon TE2000-E) under 100 $\times$ magnifi-

cation. The sample temperature is controlled by a resistive heater (Bioscience Tools) wrapped around the objective, which is in contact with the sample through immersion oil. This heater is driven by a low-noise temperature controller (Bioscience Tools). The sample is also heated using a thermoelectric cooler (TE Technology, Inc.) bonded directly to the sample by silicone vacuum grease. The thermoelectric cooler is driven by a separate high-performance digital temperature controller designed to drive thermoelectric cooler elements (Thorlabs). The sample is equilibrated at each temperature point for about 15 min before data is acquired. Each data point represents the average of three frames at a given temperature. Each frame is approximately $10^4 \mu\text{m}^2$ and contains about 1000 particles.

The singlet fraction is determined using traditional image analysis routines.⁴⁸ We account for the systematic bias arising from the presence of particles that are in close proximity but unbound by comparing our measured singlet fraction to a simple Monte Carlo simulation of hard disks at the same concentration as our experiments.

2.8.5 PERFORMING PHASE BEHAVIOR EXPERIMENTS

Phase behavior experiments such as those used to produce Figure 2.6b are performed on an inverted optical microscope (Nikon TE2000-E) under $100\times$ magnification.

Samples are prepared by making a 1:1 mixture of particles at a total volume fraction of 0.5%. The sample is sealed between two plasma-cleaned coverslips using silicone vacuum grease (Dow Corning). The space between the coverslips in the resulting sample chambers is about $30 \mu\text{m}$. Thus the particles can move about in a 3D volume.

The sample is heated as described in Section 2.8.4.

The phase of the sample is determined by visual inspection of micrographs and videos of the sample. In cases where the fraction of unbound particles does not clearly fall into one of the three categories described in Section 2.6.1, the number of unbound particles in a single field of view is manually counted and compared to the number of unbound particles for the same sample far above the

melting temperature.

2.8.6 PERFORMING CONFOCAL EXPERIMENTS

Confocal experiments are performed on a Leica SP5 confocal microscope. For general confocal experiments (for example, Figures 2.2 and 2.6c), samples are prepared as described in Section 2.8.5.

For confocal crystallization experiments (for example, Figure 2.7), samples are prepared by making a 1:1 mixture of particles at a total volume fraction of 5% and density matching the particles to the solvent by adding 6% w/w sucrose to the buffer. The sample is sealed and heated as in the general experiments.

All two and three-color images from confocal fluorescence microscopy experiments are produced from two-color images, which are measured when two detectors simultaneously collect emitted photons in different wavelength bands. The signals from these detectors become red (r') and blue (b') channels. Red (r), green (g), and blue (b) channels of the three-color images in Figure 2.7 are computed according to:

$$\begin{aligned} r &= r' - \sqrt{r'b'} \\ g &= \sqrt{r'b'} \\ b &= b' - \sqrt{r'b'}. \end{aligned}$$

2.8.7 FITTING THE MODELS TO EXPERIMENTAL DATA

In Figures 2.4, 2.5, 2.6, and 2.7 we fit the model from Sections 2.2, 2.3, 2.4.1, 2.5.1, and 2.6.1 to experimentally measured singlet-fraction curves using non-linear least squares fitting.

For the single-displacement case in Figure 2.4, we use two different methods to perform the fits. In the first method (black and gray lines), we fit the simplest version of the model, defined by Equations 2.2, 2.4, 2.9, and 2.10, where ΔH_{AB} , ΔH_{AD_1} , ΔS_{AB} , and ΔS_{AD_1} are the fit parameters. This

simplified model is simultaneously fit to all of the data for the five different displacing-strand concentrations. The fitted values returned are $\Delta H_{AB} = -57.28$ kcal/mol, $\Delta S_{AB} = -165.06$ cal/(mol K), $\Delta H_{AD_1} = -58.05$ kcal/mol, and $\Delta S_{AD_1} = -159.66$ cal/(mol K).

In the second method (shown in red), we fit the full model defined by Equation 2.3, including the interparticle repulsive discussed in reference 10. The fit parameters are the same four thermodynamic parameters as above. This fitting method is described in reference 23. The fitted values returned are $\Delta H_{AB} = -55.46$ kcal/mol, $\Delta S_{AB} = -159.07$ cal/(mol K), $\Delta H_{AD_1} = -55.21$ kcal/mol, and $\Delta S_{AD_1} = -150.51$ cal/(mol K).

For the two-displacement case in Figures 2.5 and 2.7, we fit only the simplified model based on Equations 2.2, 2.4, 2.9, and 2.13. Here we fit each curve separately. First we fit the measurements for the system with no displacing strands for ΔH_{AB} , holding ΔS_{AB} fixed at the value predicted using NUPACK (Table 2.4). For each of the remaining curves, we hold ΔH_{AB} and ΔS_{AB} constant and use a single fit parameter f , which serves as a common multiplicative factor to the enthalpic changes on hybridization of the two displacing strands: $f\Delta H_{AD_1}$ and $f\Delta H_{BD_2}$. We fix ΔS_{AD_1} and ΔS_{BD_2} to the values predicted from the nearest-neighbor model using NUPACK. The final fitted values are: $\Delta H_{AB} = -64.40$ kcal/mol, $f = 1.016$ (for $C_D = 31.25$ μ M), $f = 1.024$ (for $C_D = 62.5$ μ M), $f = 1.028$ (for $C_D = 125$ μ M), and $f = 1.033$ (for $C_D = 250$ μ M).

For the three-species system shown in Section 2.6.2 and Figure 2.7, we perform two separate fits for the interactions between the two different pairs of particles. For the displacement-free interaction between the blue and green particles, we fit the model given in Sections 2.2 and 2.3 for ΔH_{AB} , holding ΔS_{AB} fixed at the value predicted by NUPACK (Table 2.8). This fit returns $\Delta H_{AB} = 48.59$ kcal/mol.

For the two-displacement-strand reaction between the red and green particles, we simultaneous fit for ΔH_{AB} and for a common multiplicative factor f to the enthalpic changes on hybridization of the two displacing strands: $f\Delta H_{AD_1}$ and $f\Delta H_{BD_2}$. We hold the entropic changes on hybridization

fixed at the value predicted by NUPACK (Table 2.8). The final fitted values are $\Delta H_{AB} = -64.2314$ kcal/mol and $f = 1.0147$.

In the system with three transitions (Section 2.6.1), we again consider the simplified model, now given by Equation 2.15. Although our measurements are now performed in 3D, we calculate the singlet fraction using the 2D model given by Equation 2.4. For simplicity, we keep the particle coordination number and areal particle concentration the same as for the 2D measurements. The model depends on ΔH_{AB} , ΔH_{AD_1} , ΔH_{BD_2} , ΔH_{CE} , and the corresponding entropic changes on hybridization for those four duplexes. We set all of these quantities to the values predicted by the nearest-neighbor model (Table 2.6) and then fit for a multiplicative factor for each of them. To simplify the fit, we constrain the enthalpic and entropic changes to vary together for each set of sequences, and we further constrain the enthalpic and entropic changes for both of the displacement reactions to vary by a common factor. The result of these constraints is a set of three fitting parameters, f_1 , f_2 , and f_3 , where our thermodynamic changes on hybridization become $f_1\Delta H_{AB}$, $f_1\Delta S_{AB}$, $f_2\Delta H_{AD_1}$, $f_2\Delta S_{AD_1}$, $f_2\Delta H_{BD_2}$, $f_2\Delta S_{BD_2}$, $f_3\Delta H_{CE}$, $f_3\Delta S_{CE}$.

We fit these three factors separately by first considering the phase-behavior data for the case with no displacing strands. We manually vary f_1 until the melting transition for the model occurs in the location indicated by the experimental data (around 52.5 °C). We then consider the case with high concentration of displacing strands ($C_{D1} = C_{D2} = 225$ μ M), where we manually vary f_3 so that the melting transition of the model matches the observed phase transition at 30 °C. Finally, we hold f_1 and f_3 constant at the values found using this procedure, and we manually vary f_2 until the model for the singlet fraction simultaneously fits the observed transitions for the remaining 5 experimental data sets: $C_{D1} = C_{D2} = 75, 100, 125, 150, 175$ μ M. The fitted values found for these multiplicative factors are $f_1 = 1.04$, $f_2 = 1.11$, and $f_3 = 1.07$.

3

Programming directed motion on the micrometer scale by thermal ratcheting

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3.1 INTRODUCTION

Though DNA-mediated colloid interactions have been proposed for use in the assembly of many complex structures,^{21,22,49,50,51,52,53} the systems developed to date have, with a few exceptions,^{14,18,54,55} remained in a constant, unresponsive state once their assembly is complete. With the introduction

of strand-displacement reactions (as described in Chapter 2) we can control not only the structures that form, but also the response of these systems to changes in temperature. Here we show that this temperature control can be leveraged to make dynamic, programmable systems on the micrometer scale.

We tackle the problem of controlled transport over micrometer-scale distances. In biological systems, motor proteins such as kinesin can travel long distances (micrometers) inside cells by taking discrete steps along microtubules, powered by the consumption of ATP.⁵⁶ This stepping behavior has been mimicked in synthetic systems composed entirely of DNA that can take steps along patterned tracks.^{57,58,59,60}

Our goal is to create a synthetic system that behaves not as a simple motor that moves autonomously in a single direction, but instead as a “dancer” that takes a precisely controlled sequence of steps in response to external triggers. By working with colloidal particles, we aim to direct motion on the micrometer scale.

3.2 DESIGN CONSIDERATIONS

We aim to create a system consisting of a single colloidal particle (the dancer) that moves through a controlled sequence of steps along a one-dimensional (1D) track in response to external cues. To control the behavior of the dancer, we must carefully design the track on which it will move. The dancer must always be able to distinguish a track particle in front of it from one behind. Thus, the track must be asymmetric. We therefore design a track composed of a repeated sequence of three different species of particles, where different species refer to particles that have different interactions with the dancer. We refer to these three species of track particles as *red*, *green*, and *blue*.

We must be able to dynamically and reversibly vary the strength of these interactions to actuate the dancer. This type of specific, tunable interaction can be achieved using DNA-mediated colloidal

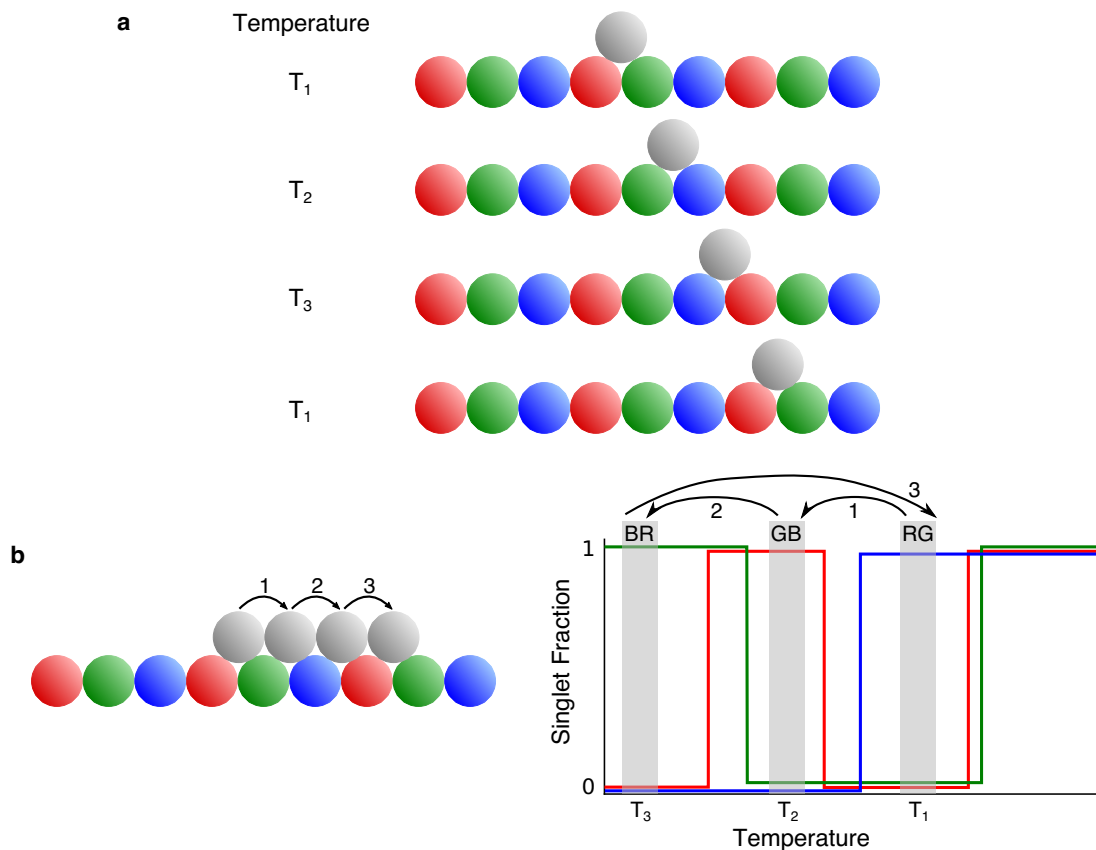


Figure 3.1: a) Schematic of dancer system. A single undyed DNA-coated particle (the dancer, shown in gray) is placed on a track composed of three other types of DNA-coated particles. The colors denote surface coverage with different sequences of DNA. At different temperatures, the dancer binds to different pairs of track particles, allowing the dancer to move in response to changes in temperature. b) Schematic of the temperature dependence of the interactions between each track particle and the dancer required to achieve the motion shown in (a). At low temperature the dancer binds to *blue* and *red*, at intermediate temperature to *blue* and *green*, and at high temperature to *red* and *green*. By slowly decreasing the temperature through these three states and then rapidly raising the temperature, we can move the dancer progressively in one direction. Reversing this cycle moves the dancer in the opposite direction.

interactions, as discussed in Section 1.2. We therefore construct both the dancer and the track from DNA-functionalized colloidal particles and change the interaction strengths by varying the temperature of the solution.

For the dancer to remain bound to the track at all times, it must move from one position on the track to the next by rolling across a track particle. When it is not moving, it is bound to two species (see Figure 3.1a). This scheme requires three interaction (and therefore temperature) regimes in which the dancer binds to the three different pairs of track particle species (T_1 : *red/green*, T_2 : *green/blue*, and T_3 : *blue/red*). At the temperatures between these regimes, we require the dancer to remain bound to at least one track particle.

The simplest set of interparticle interactions needed to realize these conditions is shown in Figure 3.1b. The dancer can be controlled by changing the temperature from T_1 to T_2 , T_2 to T_3 , and ratcheting (increasing the temperature rapidly) from T_3 to T_1 . If the time required to heat from T_3 to T_1 is short compared to the time that it takes the dancer to diffuse backwards one step, the dancer should be able to take another step forward. By varying the sequences of temperature changes, keeping track of which changes in temperature must be fast and which can be slow, we should be able to precisely control the timing and direction of each step (“choreographing the dance”).

The interaction curves in Figure 3.1b show multiple and non-monotonic phase transitions, similar to those resulting from the use of strand-displacement reactions as discussed in Chapter 2. We can therefore use the principles laid out in that chapter to design the DNA necessary to realize these interactions. The details of the DNA design process are described in detail in Sections 3.3 and 3.5.1.

We must also consider how the binding strength affects the rolling rate of the dancer and the probability of it unbinding from the track. We would like the dancer to remain bound to the track for the entire course of an experiment (1–2 hours). We minimize the probability that the dancer falls off of the track by making the track two particles wide instead of one (see Figure 3.6a). This way, the dancer binds to four track particles when it is stationary and to two track particles when it is moving

between sites. The additional bonds should increase the average time the dancer stays bound to the track.

To understand how the detachment probability—the probability of the dancer falling off of the track within a step time t_s —depends on the interaction strength, we derive the probability of breaking two independent bonds. We start with the expression for the probability of a single bond breaking at time t , given a rate constant k :⁶¹

$$p(t_b = t) = ke^{-kt}.$$

The probability of a bond breaking anytime before t is then

$$p(t_b < t) = \int_0^t ke^{-kt'} dt' = 1 - e^{-kt},$$

and the probability of two bonds independently breaking before t is

$$p(t_{b12} < t) = p(t_{b1} < t, t_{b2} < t) = p(t_{b1} < t) p(t_{b2} < t) = \left(1 - e^{-kt}\right)^2,$$

where we have assumed that the rate constant is the same for both bonds. Therefore, the probability of two bonds breaking before the first step completes is:

$$p_b = p(t_{b12} < t_s) = \left(1 - e^{-kt_s}\right)^2.$$

The probability that the dancer stays on the track for n steps and falls off on step $n + 1$ is:

$$p_n = p_b (1 - p_b)^n = \left(1 - e^{-kt_s}\right)^2 \left[1 - \left(1 - e^{-kt_s}\right)^2\right]^n,$$

which allows us to find the expected value of the number of steps before detachment:

$$\begin{aligned} \langle n \rangle &= \sum_{n=0}^{\infty} np_n \\ &= \sum_{n=0}^{\infty} n \left(1 - e^{-kt_s}\right)^2 \left[1 - \left(1 - e^{-kt_s}\right)^2\right]^n \\ &= \left(1 - e^{-kt_s}\right)^2 \sum_{n=0}^{\infty} n \left[1 - \left(1 - e^{-kt_s}\right)^2\right]^n. \end{aligned}$$

We can sum the infinite series using the expression

$$A \sum_{i=0}^{\infty} ix^i = A \frac{x}{(x-1)^2},$$

where $x = 1 - (1 - e^{-kt_s})^2$. Thus,

$$\langle n \rangle = \left(1 - e^{-kt_s}\right)^2 \frac{1 - (1 - e^{-kt_s})^2}{(1 - e^{-kt_s})^4} = \left(1 - e^{-kt_s}\right)^{-2} - 1.$$

We can estimate the rate constant using the Arrhenius equation:⁶²

$$k = k_d e^{(-\Delta G/RT)},$$

where ΔG is the free energy of attraction per mole, RT is the thermal energy, and k_d is the rate at which the particles diffuse out of the range of their mutual interaction. Rogers, Sinno, and Crocker³⁹ measured $k_d = 100 \text{ s}^{-1}$ for micrometer-scale particles similar to ours. By plotting the expected number of steps as a function of the bond strength ΔG (see Figure 3.2), we find that the expected number of steps is a very strong function of the binding energy. This result would seem to imply that we should set the binding energy as high as possible.

However, the derivation above does not account for the time it takes for the dancer to take one step, which also depends on the binding energy; DNA-functionalized particles can roll only when the binding energy is low, since rolling requires DNA bridges to actively break and re-form quickly.⁴² In the following section, we describe how we experimentally tune ΔG for each interaction to maximize the rolling rate while preventing unbinding over the course of the experiment.

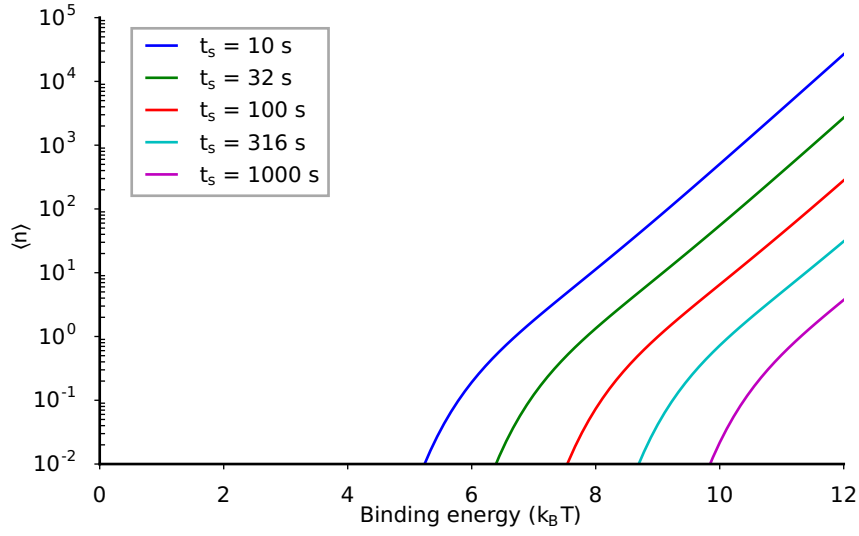


Figure 3.2: Expected number of steps of a dancer as a function of the time it takes to roll one step and its binding energy with the track particles.

3.3 RESULTS AND DISCUSSION

3.3.1 DNA SEQUENCES AND STRAND-DISPLACEMENT REACTIONS

To experimentally realize the design discussed in Section 3.2, we first choose DNA sequences that yield a response like that shown in Figure 3.1b. Each of the interactions between the dancer and the track falls into one of the categories described in Chapter 2. The interaction between the *green* and dancer particles must be weak at low and high temperatures and strong in between. This non-monotonic interaction strength is achieved using a two strand-displacement reaction, as described in Section 2.5. The interaction between the *red* and dancer particles must be strong at low and high temperatures, and weak at intermediate temperatures, as described in Section 2.6.1. Finally, the interaction between the *blue* and dancer particles behaves like a standard complementary reaction (strong at low temperatures and weak at high temperatures). However, we add a single strand-displacement reaction (as in Section 2.4) to allow the dancer to roll across *blue* track particles even at temperatures

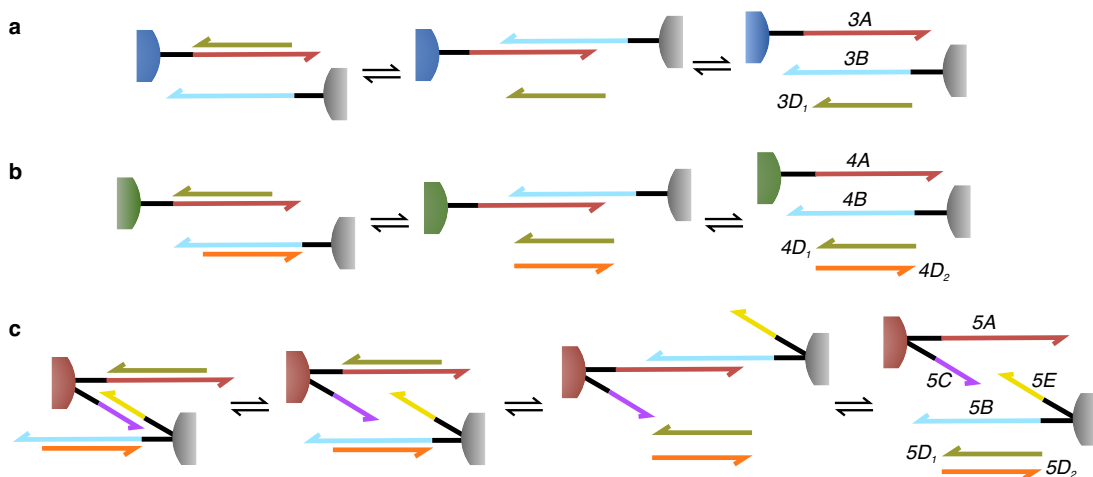


Figure 3.3: The dancer system requires four different species of particles: the dancer (shown in gray), and the three species of track particles (shown in blue, green, and red). To illustrate the interactions between these particles we zoom in on a small area of each track particle and show a single DNA strand and the ways that it can bind to a single DNA strand on the dancer. a) The interaction between the *blue* particle and the dancer requires one sequence of DNA grafted to each (3A and 3B) and a single displacing strand (3D₁). b) The interaction between the *green* particle and the dancer requires one sequence of DNA grafted to each (4A and 4B) and two displacing strands (4D₁ and 4D₂). c) The most complex interaction is that between the *red* particle and the dancer, which requires two sequences of DNA grafted to each species (5A and 5C on *red* and 5B and 5E on the dancer) and two displacing strands (5D₁ and 5D₂).

far from the melting transition. All in all, this set of interactions requires eight surface-bound DNA strands (one on *blue*, one on *green*, two on *red*, and four on the dancer) and five displacing strands free in solution (see Figure 3.3).

Because the melting transitions have a characteristic width of a few degrees Centigrade, we must separate the temperature regimes to allow each of the transitions to complete between them. We therefore set $T_3 \approx 25^\circ\text{C}$, $T_2 \approx 35^\circ\text{C}$, and $T_1 \approx 45^\circ\text{C}$. These values drive the design of the DNA sequences, and their concentrations. The sequences we choose are shown in Table 3.1 (see Section 3.5.1 for details of the sequence design procedure).

We then combine all of the components in solution, observe the composition of any aggregates as a function of temperature, and adjust the DNA concentrations to tune the temperature at which each transition occurs. The positions of the high-temperature melting transitions and the low-temperature

Table 3.1: DNA sequences grafted to the dancer and track particles and present in solution as displacing strands.

Particle	Strand name	Base Sequence
<i>blue</i>	3A	5'-(T ₅₄)-GA TCG TCA GAA-3'
dancer	3B	5'-(T ₅₉)-CTG ACG-3'
—	3D ₁	5'-TG ACG ATC-3'
<i>green</i>	4A	5'-(T ₅₄)-CT AAC TGC GGT-3'
dancer	4B	5'-(T ₅₇)-AC CGC AGT-3'
—	4D ₁	5'-GC AGT TAG-3'
—	4D ₂	5'-GC GGT AAG-3'
<i>red</i>	5A	5'-(T ₅₄)-CA ATG GAG GCT-3'
<i>red</i>	5C	5'-(T ₅₉)-CAG GTG-3'
dancer	5B	5'-(T ₅₄)-TA TAG CCT CCA-3'
dancer	5E	5'-(T ₅₉)-CAC CTG-3'
—	5D ₁	5'-CT CCA TTG-3'
—	5D ₂	5'-AG GCT ATA-3'

melting transition for the *red* particles are controlled by the surface concentration of DNA on the particle. The positions of the low-temperature transition for the *green* particle and the intermediate transition for the *red* particle are controlled by the concentration of displacing strands in solution. This procedure results in the DNA concentrations given in Table 3.2.

Bulk confocal experiments show that these concentrations lead to the desired temperature responses (Figure 3.4). Since the track particles cannot bind to one another and the dancer particles are not dyed, the observation of an aggregate containing a specific color of particles in the confocal images indicates an attractive interaction between that track particle and the dancer. The data show that these attractive interactions have the desired responses: at 24 °C *red* and *blue* track particles bind to the dancer while *green* remains free, at 36 °C only *green* and *blue* bind to the dancer, at 46 °C only *red* and *green* bind to the dancer, and at 60 °C and higher, there are no attractions, as expected. This is the first demonstration of an experimental system where this many interactions have been coordinated in the same solution, and the result is a precisely controlled phase space.

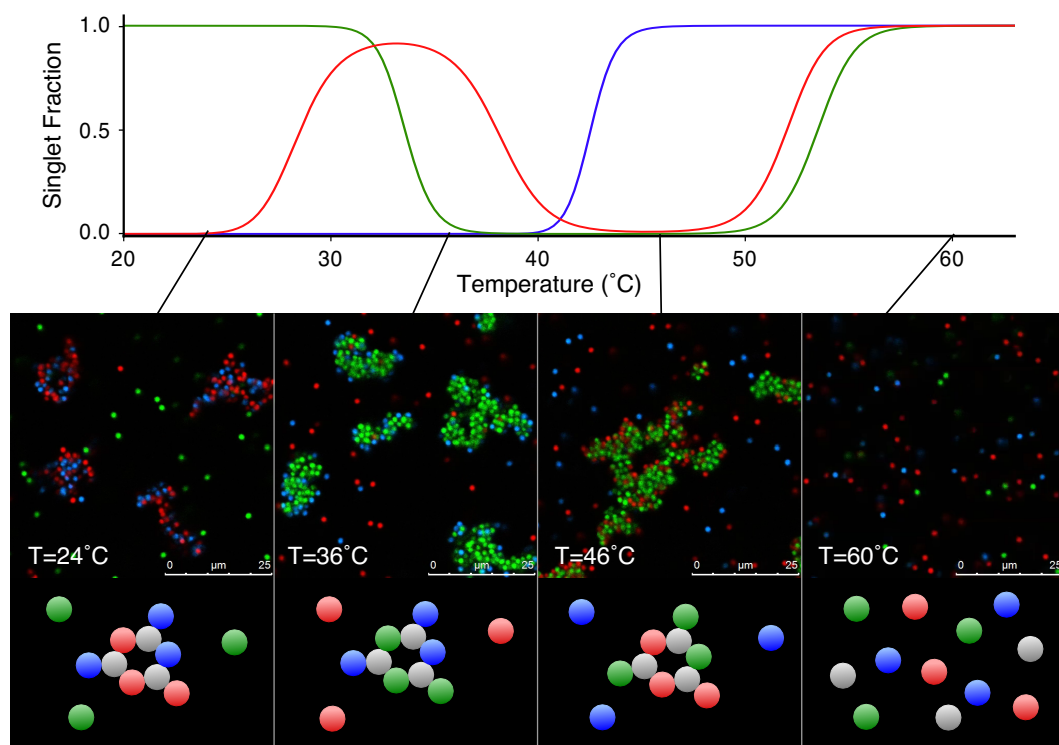


Figure 3.4: Three species of track particles, identified by fluorescent dyes (red, blue, green), each have different interactions with the undyed dancer particle. At the top is a plot of the calculated singlet fractions showing how each color of track particle interacts with the dancer particle as a function of temperature. The middle panel shows fluorescence confocal images of this system with schematic representation of the particles in the images on the bottom.

Table 3.2: DNA concentrations used for the experiments shown in Figure 3.4. The concentrations refer to surface concentration in the case of the grafted strands and solution concentrations in the case of the displacing strands. Surface concentrations are estimated from the expected number of DNA strands of each type, assuming a total of 6500 DNA strands per particle confined to a shell the width of the radius of gyration of the DNA (11 nm).

Particle	Strand name	Concentration (μM)
<i>blue</i>	3A	300
dancer	3B	75
—	3D ₁	0.087
<i>green</i>	4A	300
dancer	4B	37.5
—	4D ₁	7
—	4D ₂	7
<i>red</i>	5A	150
<i>red</i>	5C	150
dancer	5B	112.5
dancer	5E	75
—	5D ₁	175
—	5D ₂	175

3.3.2 CONSTRUCTING TRACKS

Making the dancer dance requires more than just the appropriate interactions; it also requires tracks. To construct the track, we first fluorescently dye the track particles so that we can distinguish them when they are mixed together (Section 3.5.2). We can then use optical tweezers to manipulate individual particles and place them in the appropriate sequence (see Figure 3.5b). To keep the track particles in their locations, we must immobilize them once they touch the surface. Our immobilization scheme relies on non-specific binding between DNA-grafted particles and cover-slip glass. If untreated, glass cover slips have a non-specific, irreversible attraction to DNA-coated particles, perhaps owing to van der Waals attractions at the high salt concentrations required for systems with DNA-mediated interactions. Creating a sample chamber with such “sticky” glass quickly depletes the number of particles free in solution. Therefore, we create a sample chamber that is constructed with cover glass that is half sticky and half passivated (Figure 3.5a and Section 3.5.5). We use the passivated region

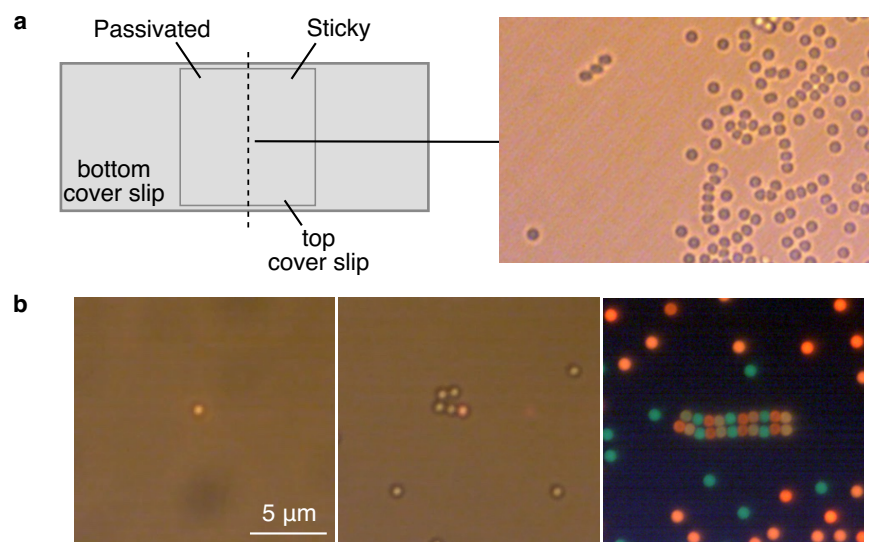


Figure 3.5: Process of assembling a track using optical tweezers. a) Cartoon of a sample chamber that is half “sticky” and half passivated. To the right is an optical micrograph of the boundary between the two regions. b) Optical micrographs showing the process of using optical tweezers to construct a track. We first grab a particle with the tweezers and identify its color using fluorescence as shown in the first micrograph. We then deposit the particle into the correct position in the track as shown in the second micrograph. By repeating this process many times, we build a full track as shown in fluorescence in the third micrograph.

of the sample chamber as a reservoir of particles, which we then use to construct a track in the sticky region. With these techniques, we can reliably construct long three-color tracks (see Figure 3.5b).

3.3.3 INTERACTIONS BETWEEN THE DANCER AND TRACK

Using the method described in the previous section, we can construct a track, place a dancer on it in a given position, and observe the dancer’s behavior as a function of temperature. Because the dancer can bind to only a few track particles at a time, the response to temperature is different from that observed in bulk, where each dancer binds to many track particles. We must therefore once again adjust the concentrations of DNA in the sample. For this purpose, we construct “tracks” consisting of squares containing four identical track particles, and we place a single dancer on top of each. We can then measure—and consequently tune—the transition temperatures of each interaction. Because

we can directly observe the behavior of single dancers, we also use these experiments to measure and adjust the temperature range in which each dancer can roll.

Because rolling relies on weak binding, we adjust the transition temperatures so that, when the dancer is moving across a track particle, it does so at a temperature no more than 5 °C from the melting temperature for that species of particle.³ To allow the dancer to roll across the *red* and *green* track particles, we place the high-temperature *red* melting transition near T_1 and low-temperature *green* melting transition near T_2 . However, the step across the *blue* particle occurs between T_2 and T_3 , and, unlike in the other two cases, it is not possible to place a *blue* melting transition near this range of temperatures. Thus, we must ensure rolling between the dancer and *blue* track particles over a wide range of temperatures. As mentioned in Section 3.3.1, we add a displacing strand to solution that competes with the *blue*/dancer interaction, thereby keeping the interaction from strengthening as the temperature is lowered. The amount of this displacing strand must be tuned to maximize the rolling rate and maintain a low detachment probability.

We then modify the concentrations of the DNA strands to ensure that T_1 , T_2 , and T_3 are as well separated as possible and that the particles roll between these temperatures. The resulting concentrations are shown in Table 3.3. The measurements of the rolling ranges and melting temperatures are shown in Table 3.4. For these conditions we choose $T_1 = 42$ °C, $T_2 = 32$ °C, and $T_3 = 23$ °C.

3.3.4 CONTROL OVER THE DANCER

To explore the response of the dancer to thermal ratcheting, we heat the sample as described in Section 2.8.4. To keep the dancer from moving in the wrong direction during the step from *blue/red* position to the *red/green* position, we must increase the temperature from 25 °C to 33 °C before the dancer rolls away from the *red* position on the track. Because the maximum rate at which we can heat over this temperature range is 0.22 °C/s, heating takes approximately 36 s. Observations of the motion of dancers on the 2D square tracks reveal that it takes a dancer approximately 1 min to diffuse

Table 3.3: Optimized DNA concentrations for dancing on a track. The concentrations are defined as in Table 3.2.

Particle	Strand name	Concentration (μM)
<i>blue</i>	3 <i>A</i>	300
dancer	3 <i>B</i>	99
—	3 <i>D</i> ₁	0.1
<i>green</i>	4 <i>A</i>	300
dancer	4 <i>B</i>	30
—	4 <i>D</i> ₁	3.5
—	4 <i>D</i> ₂	3.5
<i>red</i>	5 <i>A</i>	150
<i>red</i>	5 <i>C</i>	150
dancer	5 <i>B</i>	105
dancer	5 <i>E</i>	66
—	5 <i>D</i> ₁	70
—	5 <i>D</i> ₂	70

across a single step. Therefore, the heating rate should be sufficient for the ratcheting step.

As a proof of concept, we build a full three-color track, set the temperature to 40 °C, and place a dancer particle between *red* and *green* track particles, as shown in Figures 3.6b and c. When the temperature is lowered to 32 °C, the dancer releases from the *red* particles, diffuses back and forth across the *green* particles, and comes to rest between the *green* and *blue* particles. When the temperature is further lowered to 30 °C, the dancer moves to the *blue-red* position. This step occurs at a higher temperature than the expected 23 °C, likely because the *green* bond breaks prematurely. Because we direct each step while observing the system, we adjust for the broken bond by rapidly increasing the temperature to strengthen the *red* bond. When we increase the temperature to 42 °C, the dancer moves forward another step to the next *red-green* position. To demonstrate external control, we then send the dancer backward and forward by rapidly lowering the temperature to 32 °C and then raising it back to 42 °C.

In repeated experiments it is often possible to drive the dancer a step or two, but consistent motion without the dancer getting stuck at a certain location or prematurely falling off of the track is

Table 3.4: Experimentally measured rolling ranges and melting temperatures for the interactions between each track particle species and the dancer. The experiments are performed at the DNA concentrations given in Table 3.3 with the exception of the low-temperature *green*/dancer measurements, which are performed at 3 μM displacing strand and are therefore shifted to slightly lower temperatures than in the dancing experiments, and the high-temperature *green*/dancer measurements, which are performed with 6.6 μM displacing strand, but should not be significantly shifted.

Interaction	Rolling temperatures ($^{\circ}\text{C}$)	Melting temperature ($^{\circ}\text{C}$)
<i>blue</i> /dancer	26–39	39
<i>green</i> /dancer	25–30	25
	42–50	50
<i>red</i> /dancer	22–25	25
	33–44	33
	33–44	44

rare. These failure modes arise from uncertainty and variability in the melting transitions. While we can measure the melting transitions from experiments conducted on 2D squares of track particles, the measurements vary from square to square by a few degrees Centigrade, even in the same suspension. Also, the response of a dancer on the full three-color track is not always consistent with the measurements from the single-color square experiments. This discrepancy could arise either from the sensitivity of the melting transitions to variations in the DNA concentrations in solution and from particle to particle (as discussed in Chapter 2), or from interactions between strands of DNA on one color of particle with other particles’ displacing strands, leading to a change in the effective displacing-strand concentrations when all species are mixed.

A dancer system that performs as well as that shown in Figure 3.6 requires repeated experiments on both single-color squares and three-color tracks, in which we tune the displacing-strand concentrations iteratively. For instance, if the dancer gets stuck at a track location, it indicates that the system did not yet cross the melting transition required for the dancer to unbind from the previous track position. If the dancer unbinds entirely from the track instead of taking a step, it indicates that the system prematurely crossed the melting temperature for the particles in front of the dancer.

The number of steps that can be taken by the dancer is fundamentally limited by the trade-off

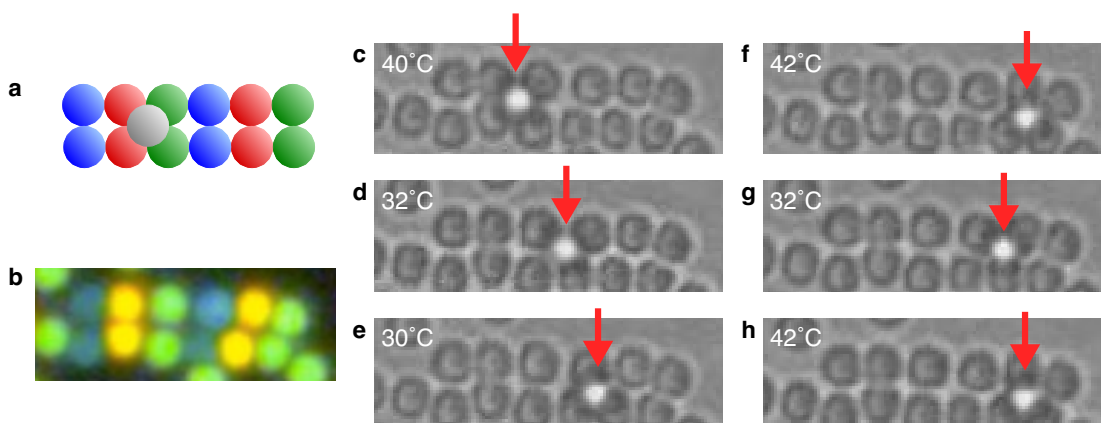


Figure 3.6: a) A schematic of the track with dancer particle on top (in grey). b) Fluorescence image of the track. c-h) Brightfield optical micrographs of dancer motion after a dancer is added to the track. As the temperature of the system is lowered, the dancer (indicated with the red arrow) processes towards the right end of the track. The temperature is then quickly raised to allow the dancer to take one more step forward. Quickly lowering the temperature then allows the dancer to move in reverse for one step. Finally the temperature is quickly raised, allowing the dancer to take a final step to the right.

between the rolling rate and detachment probability (as discussed in Section 3.2). Because we experimentally minimized the detachment probability for a given rolling rate, we believe that our experiments demonstrate nearly optimal behavior of the dancer for the given system. It may be possible to decrease the detachment probability and maintain rolling by using new methods for functionalizing the particles^{42,43}. Nonetheless, our experiments show an important proof of concept for the directed motion scheme.

3.4 CONCLUSIONS

We have shown that strand-displacement reactions can be used to construct complex interactions between four species of particle in the same solutions and that these interactions can be used to realize directed micrometer-scale motion in a system of isotropic particles. This system provides a basis for understanding what is required to create directed motion. We need a substrate with a repeated pattern that breaks symmetry, a motile species that is able to interact with different locations on the

substrate independently under different conditions, a way to ratchet the interactions to drive the motion forward across the substrate pattern, and a way to reduce the probability of detachment without sacrificing high rates of diffusion. These principles are not limited to DNA-functionalized colloids, or even to 1D transport; they are applicable in a wide range of contexts. For example, these design strategies could be extended to realize controlled motion on a 2D substrate or assembly-line-type guided construction of a desired structure.

More broadly, this experimental system, which enables observation of rates of motion under different thermal ratcheting conditions, could be used to study how to extract work from a finite-size system dominated by thermal fluctuations. While the extraction of mechanical work from thermodynamic cycling has been studied for close to a century,⁶³ it is only recently that Blickle and Bechinger⁶⁴ extended this field to systems with energy scales of a few $k_B T$. Their system, however, focuses on work arising from the fluctuation of a single particle within an optical trap. They did not use their microscopic heat engine to drive transport.

While the experimental realization of the dancer is currently limited, these limitations could be mitigated if the behavior of each of the interactions did not depend on the same signal, the temperature of the solution. In the following chapter, I discuss a system in which we can independently tune the interactions between different species of particles using light.

3.5 METHODS

3.5.1 DNA DESIGN

When designing the DNA to mediate the interactions between the dancer and each of the track particles, we follow the criteria in Sections 2.7 and 2.8.1. Because some of the particle species are coated in multiple sequences of DNA, the overall surface-concentration of each strand is up to 75% lower than the maximum grafting density. This decreased surface-coverage must be taken into account dur-

ing DNA design. In addition, all DNA must be designed to eliminate unintended cross-talk and secondary structure, as discussed in Section 2.8.1. Finally, we ensure that each DNA sequence has a toehold to maximize the kinetics of the system and allow the particles to roll.

3.5.2 FUNCTIONALIZING PARTICLES WITH DNA AND DYEING THEM FLUORESCENTLY

The method for functionalizing particles with DNA is given in detail in Appendix A.

For confocal microscopy, we label the red particles with BODIPY 650 (Life Technologies), the blue particles with BODIPY 488 (Life Technologies), and the green particles with a 50/50 mixture of the two.

In the dancing experiments, the particles must remain stable under repeated exposure to fluorescence illumination. However, when fluorescently-dyed, DNA-coated particles are excited, and the melting temperature drops irreversibly within seconds. The change in the melting temperature is related to the time the particles are exposed to light, and can be tens of degrees Centigrade. This irreversible change is thought to occur because free oxygen radicals that damage DNA are generated near the surface of the particle when the dye is excited.⁶⁵ To circumvent this problem, we carefully select fluorescent dyes so that the particles are stable under exposure to light for as long as possible. We use Coumarin 545 (Exciton) for the red particles, BODIPY 558 (Life Technologies) for the blue particles, and a 50/50 mixture of these two dyes for the green particles.

3.5.3 SAMPLE PREPARATION AND IMAGING

Samples used for confocal experiments contain a 1:1:1:1 mixture of particles at a total volume fraction of 0.66% at 250 mM NaCl. The sample is sealed between two plasma-cleaned coverslips using silicone vacuum grease (Dow Corning). The methods for confocal imaging are described in Section 2.8.6. Samples used for dancing experiments contain a 1:1:1:1 mixture of particles at a total volume fraction

of 0.25% at 250 mM NaCl. The sample chamber is prepared as described in Section 3.5.5.

3.5.4 OPTICAL TWEEZERS

The optical tweezer setup is constructed on a Nikon TE2000-E microscope. A 671 nm, 40 mW laser beam (Shanghai Dream Lasers Technology Co., Ltd.) is collimated and expanded so that it is approximately 1 inch in diameter. We then send the beam into the back of a 100 $\times$ oil immersion objective (NA 1.4, Nikon) by sending it through a 45 $^\circ$ 670 nm dichroic mirror (Thorlabs) placed directly below the objective. Two mirrors in the beam path allow us to adjust the beam's position and orientation as it enters the objective, allowing us to center and align the beam.

3.5.5 TRACK CONSTRUCTION

We construct our tracks using 24 $\times$ 60 and 22 $\times$ 22 No. 1 cover glass (VWR). The cover slips can be passivated by plasma cleaning to functionalize the surface with OH $^-$ groups. We passivate the entire surface of the large cover slip, but passivate only half of the small cover slip to create a sticky region in which we can construct tracks. To passivate only half of the chamber, we offset two slides from one another and hold them together with binder clips such that half of each slide is covered by the other. We then assemble the sample chamber with these slides using the method described in Section 2.8.5. To construct the track, we work near the interface between the sticky and passivated regions. Because the track is two particles wide, the chance that the dancer will diffuse off of the edge of the track and stick to the glass is small.

4

Modulating and addressing colloidal interactions using light

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4.1 INTRODUCTION

As discussed in the previous two chapters, the reversibility of DNA hybridization can be used to create novel, temperature-responsive materials. However, there remain two major limitations with using temperature to control DNA-mediated colloidal interactions. First, changing the temperature

is slow. One must heat the entire sample, and it can take minutes for a sample to equilibrate. This is a problem because many proposed applications of programmed interactions require rapid modulation of the interparticle interactions.^{66,67,68,69} Second, all particles in a sample respond simultaneously to changes in temperature. It is not possible, for example, to independently control the interactions between one pair of particles without affecting those between another pair. In other words, the particles cannot be addressed independently with temperature.

Here we show that light can be used to reversibly and independently control interactions between different particle species on sub-second time scales. To achieve this control, we add light-absorbing, non-fluorescent dyes to DNA-grafted colloids. We infiltrate 1 μm polystyrene particles with the dyes by swelling the particles with a dye solution while functionalizing them with ssDNA (Figure 4.1a). As we shall show, the dyes absorb certain wavelengths of light and convert that energy to heat, resulting in a rapid change in temperature—and, consequently, interaction strength.

In contrast to photochemical methods for controlling interactions such as azobenzene modifications,^{70,71} our method is easy to implement and requires no chemical modification. Furthermore, as we show, it allows us to independently address interactions among different sets of particles by using different wavelengths of light.

4.2 RESULTS AND DISCUSSION

To demonstrate the effect of light on dye-infiltrated particles, we first place ssDNA-functionalized particles into a sample chamber and allow them to aggregate by holding the entire sample at a temperature below the melting transition. The particles are dyed with Oil Red O dye (Sigma-Aldrich), which has an absorption peak at 520 nm. When we illuminate the particles with a wide-field beam at 560 nm, which is close to the absorption peak (see Figure 4.6b), we find that the aggregates melt (Figure 4.1c). The melting happens only when the dye is present: aggregates of undyed particles grafted with identi-

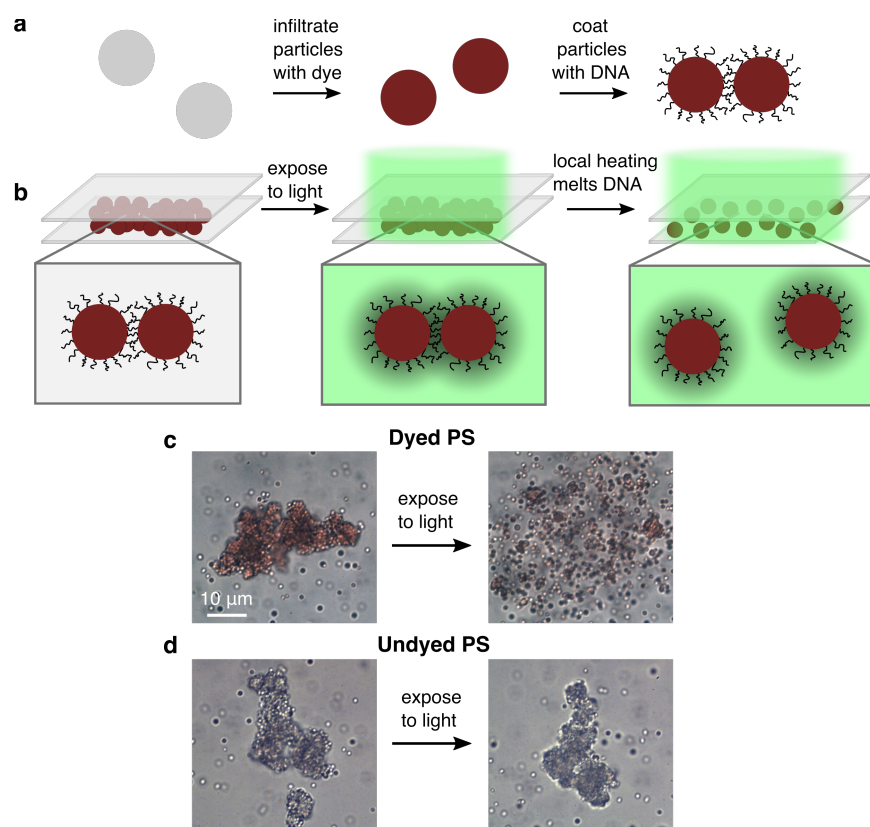


Figure 4.1: Overview of light-driven modulation. a) We begin with undyed 1 μm polystyrene particles (shown schematically in gray), which we infiltrate with a large concentration of non-fluorescent dye (here shown as red). b) When coated with complementary strands of ssDNA, the particles aggregate at temperatures below their melting temperature. When exposed to light (here shown in green) the particles heat up (as illustrated by the dark gray corona around the particles) and the aggregates fall apart. c, d) Optical micrographs of dyed and undyed particles coated in identical sequences of DNA, held 1 $^{\circ}\text{C}$ below their melting temperatures, and exposed to green light. The undyed particles remain aggregated while the dyed particles melt.

cal ssDNA do not melt under the same illumination (Figure 4.1d). Furthermore, when we illuminate aggregates of dyed particles with a wavelength far removed from the absorption peak, 648 nm, they do not melt. We can therefore conclude that the melting is caused by absorption of light by the dye.

With this approach, we can melt particle aggregates using a wide-field configuration; that is, we do not need to focus the beam onto any particular set of particles. The area of illumination in Figure 4.1c is $100 \times 100 \mu\text{m}$. Aggregates outside the area of illumination are not affected.

Next, we demonstrate that the light-driven melting is reversible. We hold a sample of particles dyed with Oil Red O dye at 45°C , 3°C below their melting transition, and turn the illumination on and off multiple times (Figure 4.2a). The aggregates melt each time the light is on and re-form when the light is off. The reversibility requires an oxygen scavenging system: we add glucose oxidase and catalase to the sample, as discussed in Section 4.4.3. Without the scavenging system, aggregates do not re-form after a few cycles. The irreversibility in the absence of an oxygen scavenger likely occurs because free oxygen radicals that damage the DNA are generated near the surface of the particle when the dye is excited.⁶⁵ With the oxygen scavenging system, we find that we can modulate the system for at least 1 hour, and possibly much longer.

The light causes only melting; it does not affect the particles after they unbind, as evidenced in Figure 4.2b. Within the first second of illumination, aggregates begin to expand and then spread outward by approximately $5 \mu\text{m}$ over the next 5 s. The $5 \mu\text{m}$ displacement is consistent with the expected mean-squared displacement for non-interacting particles,¹ which we calculate to be $4.8 \mu\text{m}$ over 5 s. When we turn the light off after 5 s, the particles immediately start to bind to one another, and within 25 s they re-form large aggregates. We estimate that the time to form aggregates of this size for a diffusion-limited process is approximately 21 s (see Section 4.4.6), in good agreement with the measurements.

Having shown that the light-driven melting is caused by the dye and is both rapid and reversible, we are left with two possible explanations for its origin: either the light triggers a photochemical change

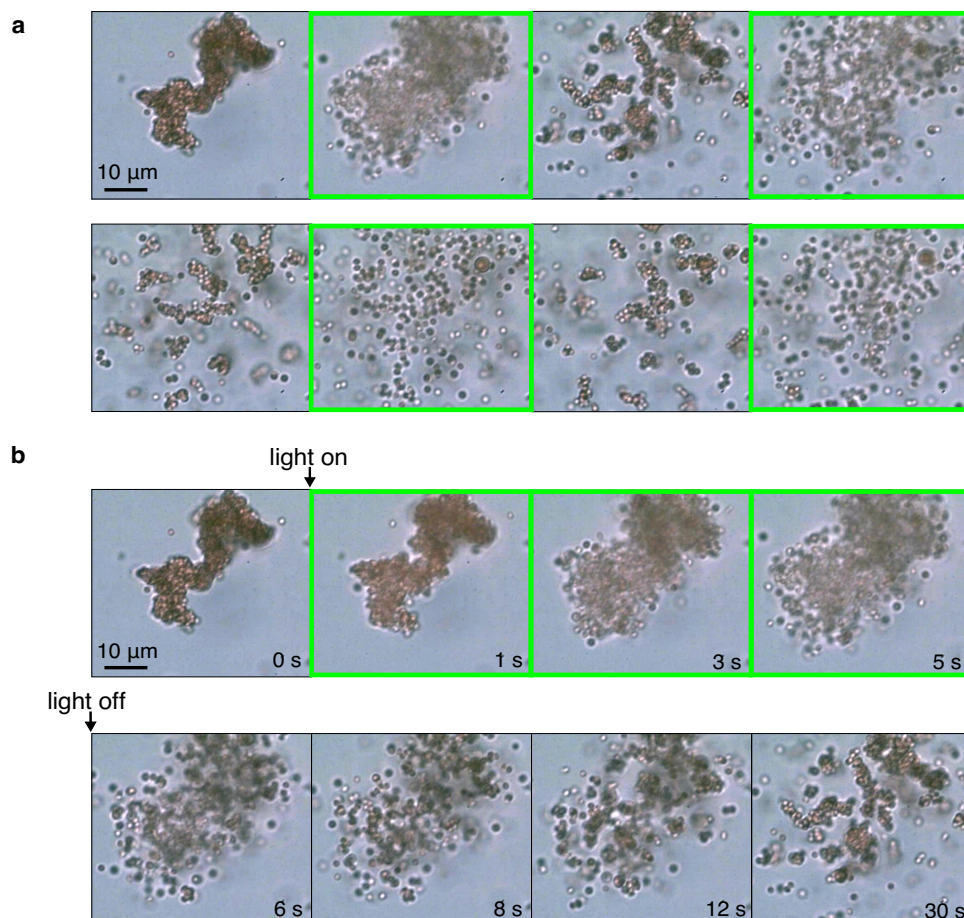


Figure 4.2: Optical micrographs of light-driven melting. When a stable aggregate (first micrograph) is exposed to light (denoted by a green frame) the aggregate melts. When the light is turned off the aggregate re-forms. a) This melting-reaggregation process can be repeated many times. b) This light-induced aggregate melting is shown in the top row as a function of time for which the cluster has been exposed to green light. When the light is subsequently turned off, the particles reaggregate (shown as a function of time in the bottom row).

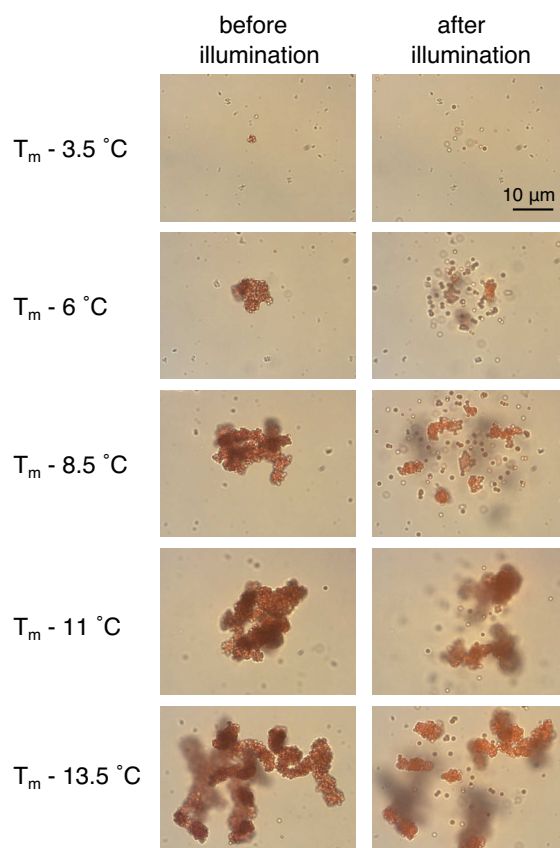


Figure 4.3: Optical micrographs showing melting of clusters of different sizes under illumination. For each temperature, we show the smallest cluster that begins to melt. Any larger clusters melt entirely, while smaller clusters remain stably aggregated.

in the DNA that is thermally reversible when the light is off, or the light is converted to heat, which modulates the interactions between particles. To determine which effect occurs, we examine the melting of aggregates of different sizes. We find that the larger the aggregate, the lower the temperature at which it melts (Figure 4.3), under constant illumination conditions. We are not aware of any photochemical process that would account for such a relation between aggregate size and melting temperature. However, our observations *are* consistent with the hypothesis that the light heats the dyed particles, leading to melting of the DNA bonds bridging the particles together.

If the heating hypothesis is correct, we expect the temperature change to depend on four factors:

the rate of heat dissipation into the medium, the illumination time, the illumination intensity, and the rate of heat generation—which is a function of the illumination time and intensity, and of the absorption of the light by the dye. We model the effects of these factors by solving the time-dependent thermal diffusion equation for a sphere—which represents either a single dyed particle or an aggregate of particles—exposed to light in an infinite medium of water. We solve the following set of equations⁷² for the change in temperature above the background, T :

$$\begin{aligned} \frac{1}{k_s} \frac{\partial T_s}{\partial t} &= \frac{1}{r^2} \left(r^2 \frac{\partial T_s}{\partial r} \right) + \frac{A}{K_s} \quad \text{for } 0 \leq r \leq a \\ \frac{1}{k_w} \frac{\partial T_w}{\partial t} &= \frac{1}{r^2} \left(r^2 \frac{\partial T_w}{\partial r} \right) \quad \text{for } r > a, \end{aligned} \tag{4.1}$$

where A is the (constant) rate of heat produced by the sphere per unit volume per unit time, k is the thermal conductivity, K is the thermal diffusivity, a is the radius of the sphere, t is the time since the start of illumination, and the subscripts s and w refer to the sphere and to the water. The boundary conditions are

$$\begin{aligned} T_s &= T_w = 0 \quad \text{at } t = 0 \\ T_s &= T_w \quad \text{at } r = a \\ K_s \frac{\partial T_s}{\partial r} &= K_w \frac{\partial T_w}{\partial r} \quad \text{at } r = a. \end{aligned} \tag{4.2}$$

T_s must remain finite as $r \rightarrow 0$, and T_w must remain finite as $r \rightarrow \infty$.

We analytically solve Equation (4.1) subject to the boundary conditions in Equation (4.2) to obtain both the steady-state and time-dependent temperature profiles. At steady state, the temperature falls off quadratically with distance from the particle center within the particle, and as $1/r$ outside the particle (Figure 4.4a). From the time-dependent solution, we find that the relaxation time of the temperature gradient is only 5 ms (Section 4.4.5). Because the timescale for the temperature gradient to develop is smaller than the timescale for particles to diffuse a distance equal to their diameter (approx-

imately 1 s), we can view heating and diffusion as separate, sequential processes: once the light heats the particles, the ssDNA strands melt, and the particles then diffuse. Hence the calculations explain why the melting and re-aggregation processes are diffusion-limited.

The model also explains why, at constant illumination conditions, larger aggregates melt at lower temperatures. For a single particle, the temperature gradient outside the particle decays by a fraction of a degree Centigrade over a distance comparable to the ssDNA length (Figure 4.4a). Because the interaction strength is a steep function of temperature, as shown by the theoretical singlet fraction in Figure 4.4b, even this small change in temperature can cause a small aggregate to melt, if it is close to the melting temperature. However, for a 10- μm , spherical aggregate of particles, the temperature can increase by nearly 11 $^{\circ}\text{C}$ at the center and 6 $^{\circ}\text{C}$ near the edge. Such an aggregate can melt even when the solution temperature is many degrees below the melting temperature.

Finally, the model predicts that heating should be rapid—on the order of a few milliseconds, as discussed above. To test this prediction, we measure how quickly the interaction changes. We use optical tweezers to construct particle dimers composed of 2- μm particles infiltrated with Oil Blue N dye and expose them to pulses of 560 nm light with a controlled duration. We then track the positions of the particles for the next second to see whether the dimer separates. We repeat this experiment many times and for pulses of different durations. We then infer the probability of the dimer breaking apart during or after the pulse as a function of the pulse duration (see Section 4.4.7). We plot this probability $\hat{P}_b$ and its uncertainty in Figure 4.5. We then estimate the timescale over which the interaction changes from the duration at which $\hat{P}_b$ is significantly higher than when there is no illumination. This timescale is between 20 and 50 ms, compared to the hundreds of seconds required to heat the sample on a heating stage. Thus, light-driven heating decreases the time to change the interaction potential by over four orders of magnitude.

Having shown that light causes rapid and reversible changes in interaction potential between DNA-grafted particles, we now show that it can be used to address different species of particles. We

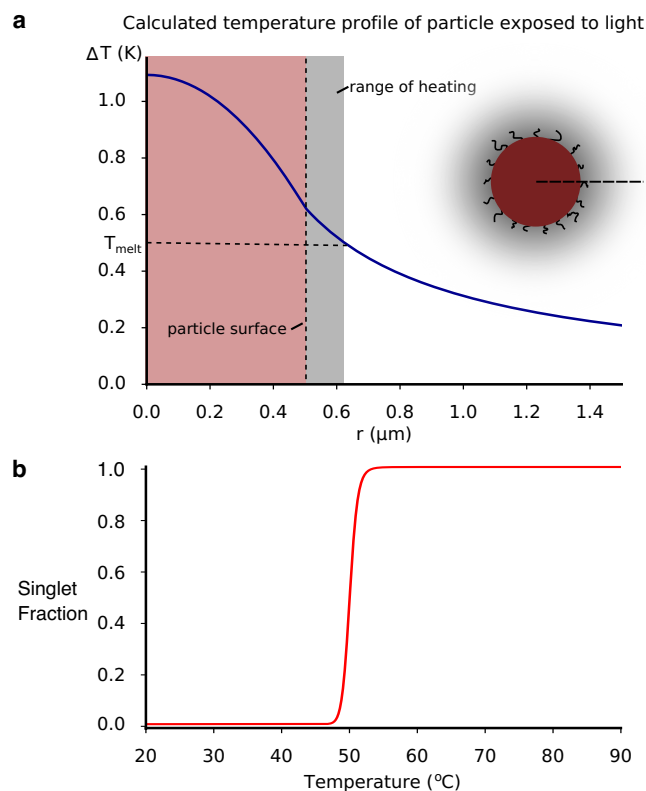


Figure 4.4: a) Theoretical calculation of the steady-state temperature profile of a dyed 1 μm polystyrene particle in an infinite medium of water under exposure to light that the dye absorbs. The center of the particle continuously heats in response to light absorption, and the heat is continuously dissipated into the bath. The steady state is reached within approximately 5 ms. The amount of heating depends on the illumination power and the absorptivity of the dye in the wavelength used. b) Typical theoretical singlet fraction curve. At low temperatures the particles are aggregated. Above the melting temperature the system melts over a range of about 2 $^{\circ}\text{C}$.

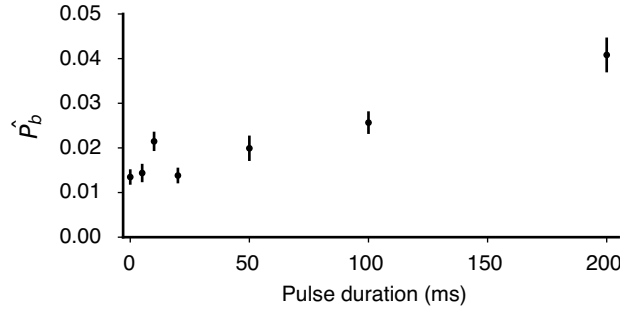


Figure 4.5: Mean probability that a dimer separates within 1 s of the beginning of a light pulse, as a function of the duration of the pulse. Error bars correspond to a 1σ credible interval as described in Section 4.4.7. We use a cutoff distance of $2.21\text{ }\mu\text{m}$ to determine when two particles are no longer bound to one another. See Figure 4.8 for results at different cutoff distances.

infiltrate two batches of particles with different dyes: Oil Red O, which absorbs in the blue, and Oil Blue N, which absorbs in the red (see absorption spectra in Figure 4.6). We then coat these particles, as well as undyed particles in a control batch, with identical ssDNA. We bring a sample of each species to 1°C below the melting temperature and expose each sample to 485-nm and 648-nm light. The undyed particles remain aggregated under exposure to both wavelengths of light, indicating that the light does not heat the solution or the polystyrene itself in the absence of dye. The Oil Red O-dyed particles remain aggregated under exposure to 648-nm light, where the dye does not absorb, and the aggregates melt under exposure to 485-nm light, where the dye absorbs strongly. Conversely, the Oil Blue N-dyed particles remain aggregated under exposure to 485-nm light, and the aggregates melt under exposure to 648-nm light. Hence, light-driven heating can be used to address interactions between different species of particles with independent triggers.

4.3 CONCLUSIONS

In conclusion, we have demonstrated a simple method to rapidly modulate and address interactions between colloidal particles. Compared to existing protocols, our method decreases the timescale

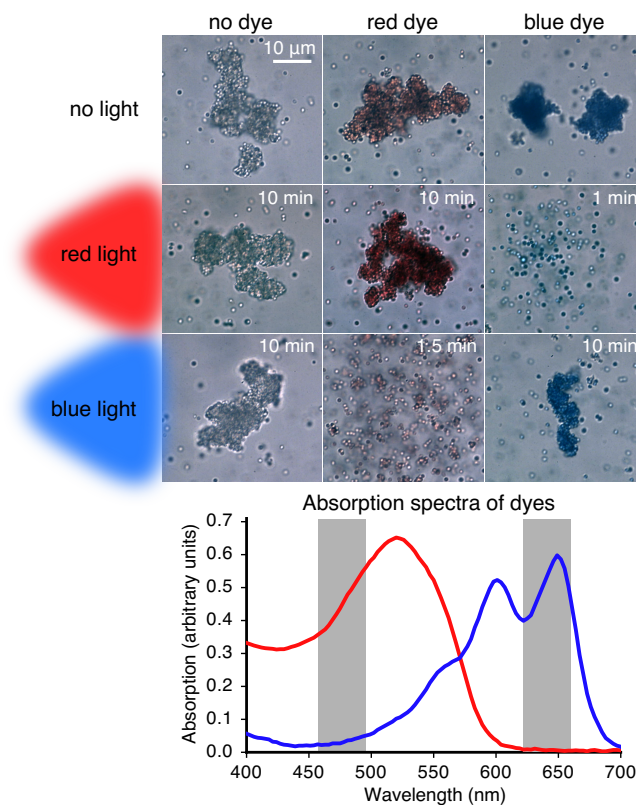


Figure 4.6: Aggregates of three different species of particles (undyed, red, and blue) each coated in the same sequence of self-complementary DNA and held 1 °C below their melting temperature. The response of these aggregates to exposure of different wavelengths of light is shown (the amount of time that they were exposed is noted in the top right corner of each micrograph). The absorption spectra of the dyes are shown below with the regions of illumination shaded in grey. While the undyed particles remain aggregated under exposure to all colors of light, the dyed particles only remain aggregated when exposed to wavelengths of light in which they do not absorb. When exposed to wavelengths of light where they do absorb (red light for the blue particles and blue light for the red particles) the particles disperse.

for changing the temperature, and therefore the interparticle interaction strength, by an estimated four orders of magnitude. In addition, our method provides a way to independently change the interactions between multiple species in the same solution, which is not possible with direct heating. Furthermore, our method is simple to implement: it requires only adding dyes to the particles and an oxygen scavenging system to the solution. No chemical modification of the DNA is necessary.

There are many potential applications for this method. First, it can enable sequential self-assembly schemes, since the interactions between different species can be modified independently. Second, it can be used to controllably trigger release of DNA into solution upon exposure to light. Finally, it can be used to explore non-equilibrium self-assembly, because the interparticle interactions can be modulated on timescales shorter than the characteristic diffusion time. The non-equilibrium behavior of the system could enable us to study the effects of breaking detailed balance on reaction rates and steady-state structures. Multiple theoretical and computational studies have shown that rapidly modulating interactions can lead to phenomena that do not occur in equilibrium, such as rapid annealing of large crystal domains, stable particle chain assemblies, and non-equilibrium steady states.^{66,67,68,69,73} The experimental developments needed to study these effects are discussed in Section 5.3.

4.4 METHODS AND MATERIALS

4.4.1 DNA SEQUENCES

DNA oligos are ordered from IDT with a 5' Dibenzocyclooctyne (DBCO) modification and with HPLC purification. The specific sequence used for the experiments presented in this article is self-complementary (5' - /5DBCOTEG/ - (T₁₆) - CGCG - 3').

4.4.2 FUNCTIONALIZING PARTICLES WITH DNA

Our protocol is modified from that of ref. 43 and described in detail in Appendix B. We modify PS-*b*-PEO with an azide group as described in the reference. We then mix 240 μL of 417 μM PS-*b*-PEO- N_3 in deionized (DI) water with 120 μL Tetrahydrofuran (THF, Sigma) and 3 μL toluene (anhydrous, Sigma-Aldrich) saturated with dye (Oil Blue N, 96%, Sigma-Aldrich or Oil Red O, BSC certified, Sigma-Aldrich). Finally we add 40 μL of 1 μm sulfate-modified polystyrene particles (Molecular Probes) that have been washed into 1x TE buffer, suspended to 10% v/v, and sonicated. The suspension is shaken at room temperature for 30 min. After shaking, an excess of DI water is added to the suspension to decrease the fraction of THF below 10% followed by heating at 70 $^{\circ}\text{C}$ for 1 h to evaporate the THF. The azide functionalized particles (PS-PS-*b*-PEO- N_3) are then washed several times in DI water.

Any dye that is soluble in toluene and polystyrene, but insoluble in water can be used to dye particles in this way.

To attach the ssDNA to the PS-PS-*b*-PEO- N_3 we mix 10 μL of 70 μM DBCO-modified ssDNA (IDT), 40 μL of PS-PS-*b*-PEO- N_3 (1% v/v), and 150 μL aqueous buffer containing 10 mM Tris and 1 mM EDTA (diluted to 1x TE from Serva TE Buffer (100x), pH 8) as well as 0.05% Pluronic F 127 (BASF) and 1 M NaCl. The solution is shaken for 24 h after which the mixture is washed with 1x TE several times.

4.4.3 SAMPLE PREPARATION

To prevent oxidative damage to the DNA on the particles during illumination, we add a Glucose Oxidase / Catalase enzyme system that scavenges oxygen from the solution following the procedure in ref. 74 (Section 3.2.3, pg 17). We mix 8 μL 1x TE with 625 mM NaCl, 1 μL Glucose Oxidase / Catalase Stock (125 mM NaCl, 20 mg/mL Glucose Oxidase (from *Aspergillus niger*, Type VII, lyophilized

powder, Sigma) and 3.5 mg/mL Catalase (Lyophilized, Spectrum) in 1x TE), and 0.1 μ L 340 nm carboxylate modified latex particles (Opti-Link, Thermo) and leave to react for 15 min. We separately prepare a 30 μ L suspension of 0.1% v/v PS-PS-*b*-PEO-DNA particles at 500 mM NaCl in 1x TE. We then add 1 μ L 450 mg/mL D(+) Glucose (99.5%, Sigma) in DI water to our solution containing Glucose Oxidase / Catalase. We add 0.6 μ L of the resulting solution to the particle suspension.

We sprinkle 10–30 μ m glass spacer beads (Polysciences) onto a 24×60 No. 1 coverslip and then plasma clean that and a 22×22 No. 1 coverslip for 45 s. We place 20 μ L of the final solution onto the 24×60 No. 1 coverslip and gently cover with the 22×22 No. 1 coverslip, taking care to avoid trapping bubbles. We wick away the excess solution with a Kimwipe and seal the edges with UV-curable optical adhesive (Norland 63). Finally we cure the sample under UV for 5 min keeping the center of the sample covered with aluminum foil.

4.4.4 IMAGING

The methods used to heat and image are described in refs. 23 and 75. Wide-field, wavelength-dependent illumination is provided by a Lumencor Spectra-X light engine. The illumination intensities at the sample are 95 mW for green light (560/32 nm), 21.7 mW for red light (648 nm), and 32 mW for blue light (485/25 nm) over an area of approximately $(100 \mu\text{m})^2$ as measured with a power meter at the back aperture of the objective. For experiments comparing the response of samples illuminated with both red and blue light (see Figure 4.6), we make the illumination intensities the same by decreasing the power of the blue light to match that of the red light source.

4.4.5 CALCULATING HEAT PROFILES

Temperature profiles for a heated polystyrene bead are calculated following the method in ref. 72. We assume a single bead in an infinite medium of water that perfectly absorbs 3 MW/m² (which

corresponds to the approximate true illumination intensity) of light, which it then perfectly radiates as heat.

The time dependent results for the change in temperature above the background V are

$$V = \frac{a^2 A}{K_s} \left(\frac{1}{3} \frac{K_s}{K_w} + \frac{1}{6} \left(1 - \frac{r^2}{a^2} \right) - \frac{2ab}{r\pi} \int_0^\infty \frac{\exp(-y^2 t / \gamma_s)}{y^2} \frac{(\sin y - y \cos y) \sin(ry/a)}{[(c \sin y - y \cos y)^2 + b^2 y^2 \sin^2 y]} dy \right)$$

if $r \leq a$, and

$$V = \frac{a^3 A}{r K_s} \left(\frac{1}{3} \frac{K_s}{K_w} - \frac{2}{\pi} \int_0^\infty \frac{\exp(-y^2 t / \gamma_s)}{y^3} \frac{(\sin y - y \cos y) [by \sin y - y \cos \sigma y - (c \sin y - y \cos y) \sin \sigma y]}{[(c \sin y - y \cos y)^2 + b^2 y^2 \sin^2 y]} dy \right)$$

if $r \geq a$. Here a is the particle radius (0.5 μm), K_s is the thermal conductivity of polystyrene (0.4 W/m/K), K_w is the thermal conductivity of water (0.6 W/m/K), ρ_s is the density of polystyrene (1055 kg/m³), ρ_w is the density of water (1000 kg/m³), c_s^v is the heat capacity of polystyrene (1300 J/kg/K),⁷⁶ c_w^v is the heat capacity of water (4185.5 J/kg/K), k_s is the thermal diffusivity of polystyrene ($k_s = K_s / (\rho_s c_s^v)$), k_w is the thermal diffusivity of water ($k_w = K_w / (\rho_w c_w^v)$), A is the heat generated in the particle per unit volume per unit time (4.5 TW/m³ assuming all light incident on the 1 μm particle is absorbed and perfectly re-radiated as heat) and

$$\begin{aligned} b &= \frac{K_w}{K_s} \sqrt{\left(\frac{k_s}{k_w} \right)} \\ c &= 1 - \frac{K_w}{K_s} \\ \gamma_s &= \frac{a^2}{k_s} \\ \sigma &= \left(\frac{r}{a} - 1 \right) \sqrt{\frac{k_s}{k_w}}. \end{aligned}$$

The steady-state results are

$$V = \frac{a}{6K_s} \left(a^2 - r^2 + 2a^2 \frac{K_s}{K_w} \right)$$

if $r \leq a$, and

$$V = \frac{Aa^3}{3K_w r}$$

if $r \geq a$. The time-dependent equations predict a value within 10% of the steady-state result within 5 ms for the conditions given above.

4.4.6 AGGREGATION AND DIFFUSION TIME

In Figure 4.2 we show the expansion of a cluster of particles exposed to light for 5 s. We then compare the observed expansion with the estimated expansion from a diffusion model:^{1,77}

$$\begin{aligned} \langle x^2 \rangle &= 6Dt \\ D(T) &= \frac{k_B T}{6\pi\eta a} \\ \eta(T) &= 2.414 \times 10^{-5} \text{ Pa} \cdot \text{s} 10^{247.8 \text{ K}/(T-140 \text{ K})}, \end{aligned} \tag{4.3}$$

where η is the viscosity of water, D is the diffusion coefficient, a is the particle radius (0.5 μm), k is the Boltzman constant ($1.38 \times 10^{-23} \text{ m}^2 \text{ kg}/(\text{s}^2 \text{ K})$), and T is the temperature (318 K).

To calculate the expected time for a cluster of particles to aggregate, as shown in the bottom panel of Figure 4.2b, we assume that the limiting time scale is that for the particles on the outer edge of the cluster to diffuse inward and bind to those in the center. We estimate this distance to be 10 μm by examining the first and last frames of the bottom row of Figure 4.2b. We then use Equations 4.3 to calculate the average time required to diffuse this distance.

4.4.7 DIMER MELTING EXPERIMENTS AND ANALYSIS

For the dimer melting experiments, we make a sample as described in Section 4.4.3, except that we use 2 μm particles to increase the amount of heating per particle, we work at lower volume fraction, and we make the sample quasi-two-dimensional. To do this, we forgo the use of spacer beads, place only 10 μL of sample on the slide, and squeeze the two slides together using binder clips while curing the epoxy.

To make the sample, we mix 38 μL 1x TE with 263 mM NaCl and 0.1% F127, 1 μL Glucose Oxidase / Catalase Stock, and 0.1 μL 340 nm carboxyl-modified latex particles and wait 15 min. We separately prepare an 18 μL suspension of 0.05% v/v PS-PS-*b*-PEO-DNA particles at 250 mM NaCl in 1x TE and place the suspension in a 55 $^{\circ}\text{C}$ water bath to keep the particles from aggregating. We then add 1 μL glucose stock to the Glucose Oxidase / Catalase solution and 2 μL of the resulting mixture to the particle suspension.

Once the sample is prepared we place it on the heating stage and image it as described above. We bring the sample to just above the melting temperature and then use optical tweezers (785 nm) to drag approximately 20 single particles to an empty space in the sample. We then lower the temperature to approximately 10 $^{\circ}\text{C}$ below the melting temperature and assemble the single particles into dimers in a 3 by 3 grid with as much space between the dimers as possible within the field of view (see Figure 4.7). We then raise the temperature to 3 $^{\circ}\text{C}$ below the temperature at which most dimers break apart within a few seconds, even in the absence of illumination.

We use a CMOS Photon Focus camera with the frame rate set to 50 frame/s with an exposure time of 13.336 ms. We trigger the Lumencor light source with a square pulse of a given length (5 ms to 200 ms) with a period of 1 s. We record for 3000 frames (60 pulses). The light source and camera are not synchronized.

We repeat the experiment 9 times at each pulse length on the same set of 9 dimers. We rebuild the

A 3x3 grid of dimers used to measure the response to different length pulses of light. A scale bar indicates 10 μm.

Figure 4.7: Three by three grid of dimers used to measure the response to different length pulses of light.

dimers using the optical tweezers between each experiment.

We post-process the micrographs to locate dimers and determine how many dimers break after a given light pulse using TrackPy⁷⁸, an open-source software package that is based on the Crocker-Grier centroiding algorithm⁴⁸. To set the TrackPy parameters, we identify particles in micrographs of dimers well below the melting temperature. We plot the particle positions from TrackPy on top of the micrographs and verify their locations by eye. By iterating over a range of TrackPy parameters, we optimize the particle positions while eliminating points that TrackPy falsely identifies as particles. We then use TrackPy to locate particles in space and time for the duration of the movie. TrackPy assigns each particle a unique identification number that allows us to associate particles between frames.

To identify which particles are bound together, we locate the particles and then classify particles as bound or unbound using a cutoff distance d_b . Particles that are less than d_b apart are said to be bound. We run the analysis for several different cutoff distances (see Figure 4.8).

We use a cutoff distance of $d_b = 20.75$ pixels ($2.21 \mu\text{m}$) for the rest of our analysis. This value is chosen by tracking dimers that are well below their melting temperature and measuring the distri-

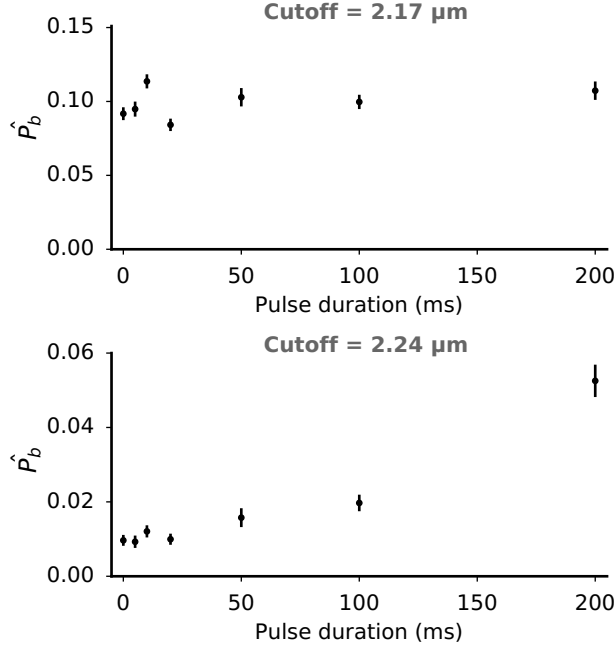


Figure 4.8: Analysis of dimer breaking probabilities at different particle separation cutoffs. See also Figure 4.5.

bution of interparticle separations at each of the over 3000 frames (see Figure 4.9). The 3σ value is 20.75 pixels or, equivalently, 2.21 μm .

For each movie, we identify the frame before the i th light pulse ($f_{\text{init},i}$) by eye. We calculate the separation between all pairs of particles in this frame and identify which particles are bound to only one other particle. This pair of particles is classified as a dimer; it is uniquely identified by the constituent particles' identification numbers.

To determine whether these dimers break, we calculate their interparticle distances for all frames in the movie after the i th pulse and before the $(i + 1)$ th pulse. If the particle separation ever exceeds the cutoff distance d_b , we count the dimer as unbound. We repeat this procedure for every light pulse i in the movie.

We discard the frames when the light pulse is visible by eye because the images become saturated and the particle center tracking is unreliable. Thus, the total time we watch the dimers varies with the

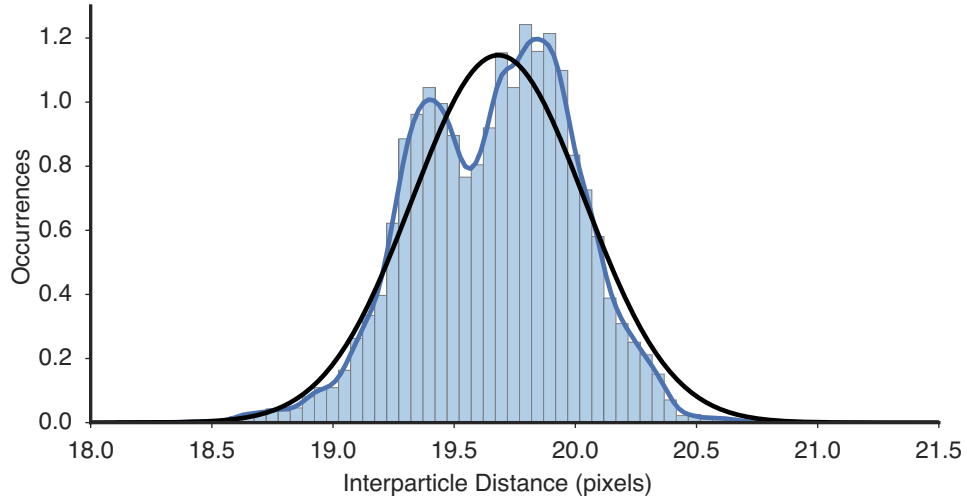


Figure 4.9: Distance between particles that are bound in dimers. Occurrences are counted over 3000 frames. The histogram is overlaid with a kernel density estimation (blue line), and fit to a Gaussian with the form $a \exp(x - x_o)^2 / (2w)^2$ (black line). The fit values are $a = 1.1466$, $x_o = 19.6825$, $w = 0.3560$.

pulse length, with longer pulse lengths being analyzed for fewer frames. As a result, we may underestimate the number of breaking events at longer pulse lengths.

To show that the system is not heating over the course of each experiment, we plot the number of dimers present in the system before each light pulse for a movie containing 60 light pulses (see Figure 4.10). We see dimers re-forming after some of the pulses, and there is little evidence for long-term drift.

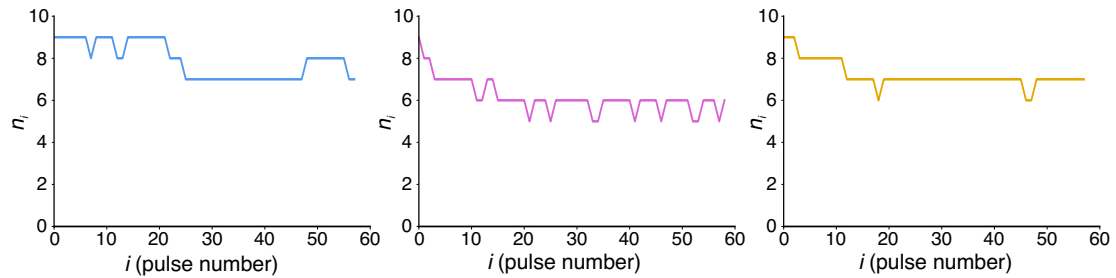


Figure 4.10: Number of dimers present immediately preceding each pulse of a movie plotted for 3 different movies, recorded for 100 ms pulses with a cutoff distance of $2.21 \mu\text{m}$.

We want to infer the probability (plus uncertainty) of a dimer breaking anytime after the pulse and before the next. Call this probability P_b . By plotting P_b as a function of pulse width, we can determine what pulse widths have an effect on the melting of the dimers. We need the uncertainty on P_b because we need to determine whether the effect is statistically significant or not. We also need to determine the effect of the threshold d_b .

We therefore seek to determine the posterior probability distribution

$$p(P_b \mid \mathbf{D}, d_b),$$

where $\mathbf{D} = \{(b_1, n_1), (b_2, n_2), \dots, (b_l, n_l)\}$, n_i is the number of dimers present immediately before light pulse i , b_i is the number of dimers that break between pulse i and $i + 1$, and l is the total number of pulses in all the movies for a particular pulse width. The posterior probability distribution gives us all of the information that the data contain about P_b , given our assumptions.

From Bayes's rule, the posterior probability is related to the product of the prior probability $p(P_b)$ and the likelihood function $p(\mathbf{D} \mid P_b, d_b)$:

$$p(P_b \mid \mathbf{D}, d_b) = \frac{p(P_b) p(\mathbf{D} \mid P_b, d_b)}{p(\mathbf{D} \mid d_b)},$$

where the denominator is a normalization factor that we can ignore for now. We retain the d_b on the right side of the “given” symbol ($\mid$) to remind us that all of our data and calculations are conditioned on the threshold we choose. We then need to set a prior and calculate the likelihood function.

We use a uniform prior for P_b ; that is,

$$p(P_b) = \begin{cases} 1 & 0 < P_b < 1 \\ 0 & \text{otherwise} . \end{cases}$$

This prior represents complete ignorance of P_b . It is a reasonable prior to choose, because we don't have a good estimate of P_b before doing the experiment: even with no light pulses, P_b depends on many variables that are not precisely measured, such as the volume fraction of dimers in the system and the interaction between the particles at the temperature of the experiment.

We assume that all the measurements are independent. That is, each dimer breaks (or does not break) independently of the others in a given pulse. Also, we assume no correlation between the measured fraction of broken dimers at pulse i and that at pulse j . We also assume that each pair of particles has the same interaction potential. In the actual experiment, there may be some heterogeneity in the interaction potential, such that the dimers that don't melt on a given pulse are those with a stronger interaction. We do not account for such heterogeneity in this simple estimation procedure.

With these assumptions, we can write down a likelihood function for the number of broken dimers after one pulse. The likelihood is a binomial distribution, since there are two possible outcomes for each dimer (broken or not broken), and each dimer is independent:

$$p(b_i, n_i \mid P_b, d_b) = \frac{n_i!}{b_i! (n_i - b_i)!} P_b^{b_i} (1 - P_b)^{n_i - b_i}.$$

Since each measurement at each pulse is independent, the likelihood of the entire data set (all the pulses in all the movies at a given pulse width) is the product of likelihood functions for a single pulse:

$$p(\mathbf{D} \mid P_b, d_b) = \prod_{i=1}^l p(b_i, n_i \mid P_b, d_b).$$

Because we have a uniform prior, Bayes's rule (above) tells us that the posterior probability distribution is proportional to the likelihood function:

$$p(P_b \mid \mathbf{D}, d_b) \propto p(\mathbf{D} \mid P_b, d_b).$$

Substituting our expression for the likelihood function into the above equation, we obtain

$$p(P_b | \mathbf{D}, d_b) \propto \prod_{i=1}^l \frac{n_i!}{b_i! (n_i - b_i)!} P_b^{b_i} (1 - P_b)^{n_i - b_i}.$$

We can then group terms in the product:

$$p(P_b | \mathbf{D}, d_b) \propto \left[\prod_{i=1}^l \frac{n_i!}{b_i! (n_i - b_i)!} \right] P_b^{\sum_i b_i} (1 - P_b)^{\sum_i n_i - b_i}.$$

The term in brackets is a constant that depends only on the data. We can ignore it because it will become part of the normalization factor. If we then define

$$S_b = \sum_{i=1}^l b_i$$

and

$$S_{nb} = \sum_{i=1}^l (n_i - b_i),$$

the posterior probability becomes

$$p(P_b | \mathbf{D}, d_b) \propto P_b^{S_b} (1 - P_b)^{S_{nb}}.$$

The posterior probability distribution is a beta distribution. The beta distribution of x , $0 < x < 1$, has two parameters, α and β , where $\alpha, \beta > 0$:

$$p(x | \alpha, \beta) = \frac{\Gamma(\alpha + \beta)}{\Gamma(\alpha)\Gamma(\beta)} x^{\alpha-1} (1 - x)^{\beta-1}.$$

Here Γ is the gamma function, $x = P_b$, $\alpha = S_b + 1$, and $\beta = S_{nb} + 1$.⁷⁹

We can analytically calculate the best estimate for P_b , $\hat{P}_b$, and the uncertainty on that estimate

from the known properties of the beta distribution. There are several possible choices for our estimator: we might use the mean of the distribution, its maximum (or mode), or its median. We choose the mean for our estimate and the standard deviation to characterize the uncertainty because there are analytical formulas for both. First, though, we note that the mean can be different from the maximum value of the distribution (the maximum *a posteriori* value or MAP, or mode) which is:⁸⁰

$$\text{MAP} = \frac{\alpha - 1}{\alpha + \beta - 2} = \frac{\sum_{i=1}^l b_i}{\sum_{i=1}^l n_i} = \frac{S_b}{S_n},$$

where

$$S_n = \sum_{i=1}^l n_i.$$

The MAP value makes sense as an estimate of P_b : it is the total number of dimers that break divided by the total number of dimers. But because the beta distribution can be skewed, the mean value need not correspond to the MAP. The mean value, which we will use as our estimate $\hat{P}_b$, has the form⁷⁹

$$\hat{P}_b = \frac{\alpha}{\alpha + \beta} = \frac{1 + \sum_{i=1}^l b_i}{2 + \sum_{i=1}^l n_i} = \frac{1 + S_b}{2 + S_n}.$$

There will not be much difference between the MAP and the mean if the sums are large, though. The advantage of using the mean (versus the MAP) is that we can use an analytical formula for the variance to estimate the uncertainty about the mean. The variance is:⁸⁰

$$\sigma_{P_b}^2 = \frac{\alpha\beta}{(\alpha + \beta)^2(\alpha + \beta + 1)} = \frac{(1 + S_b)(1 + S_{nb})}{(2 + S_n)^2(3 + S_n)}.$$

Thus, we can estimate the uncertainty from the limits of a 1σ credible interval. The interval is

$$(\hat{P}_b - \sigma_{P_b}) < \hat{P}_b < (\hat{P}_b + \sigma_{P_b}).$$

5

Conclusions and future work

I have shown several ways in which DNA-mediated colloidal interactions can be used to create systems with controlled responses to changes in their environment. Strand-displacement reactions enable controlled response to temperature: melting on cooling, multiple and non-monotonic phase transitions, temperature-independent interactions, and compositional phase transitions. We used these responses to create a dynamic system that shows directed motion on the micrometer scale in response to temperature cycling. Finally, by coupling light absorption to heat production, we demonstrated a new, species-specific method for modulating the interactions between particles. This method is more than four orders of magnitude faster than direct heating. These new capabilities enable many new applications and fundamental studies of non-equilibrium self-organization, as discussed in the following sections.

5.1 ADDRESSABLE INTERACTIONS FOR SEQUENTIAL ASSEMBLY

The ability to independently modulate interactions between different species of particles (Section 4.2) enables experiments on sequential assembly. By sequentially exposing systems containing multiple colors of particles to different wavelengths of light, we might direct the self-assembly of different structures from the same initial solution. A first step towards such an experimental system is shown in Figure 5.1. We coat red and blue particles with identical, self-complementary sequences of ssDNA and allow them to aggregate into a random red/blue network. Exposing the system to either red or blue light causes the large aggregates to break apart and smaller aggregates to form. The small aggregates contain primarily the particle color that did not absorb the light.

5.2 TRIGGERED RELEASE OF FREE DNA STRANDS

Addressable heating of DNA-coated particles can also be used to trigger the release of free DNA oligonucleotides into solution. Instead of coating different particles with complementary sequences of DNA, we make the DNA complementary to part or all of the sequence of a free oligonucleotide and then anneal the free strands to the surface of the particles. When the particles are illuminated and, as a result, heat up, the free oligonucleotides are released into solution. Each type of colored particle can sequester and release a different sequence under different illumination conditions. This specific, triggered release might be used to set off cascading reactions or to create spatially controlled gradients of signaling molecules.

5.3 NON-EQUILIBRIUM SELF-ASSEMBLY

Now that we can control interparticle interactions on sub-second timescales (Section 4.2), we can explore non-equilibrium self-assembly driven by rapid and periodic modulation of the interactions.

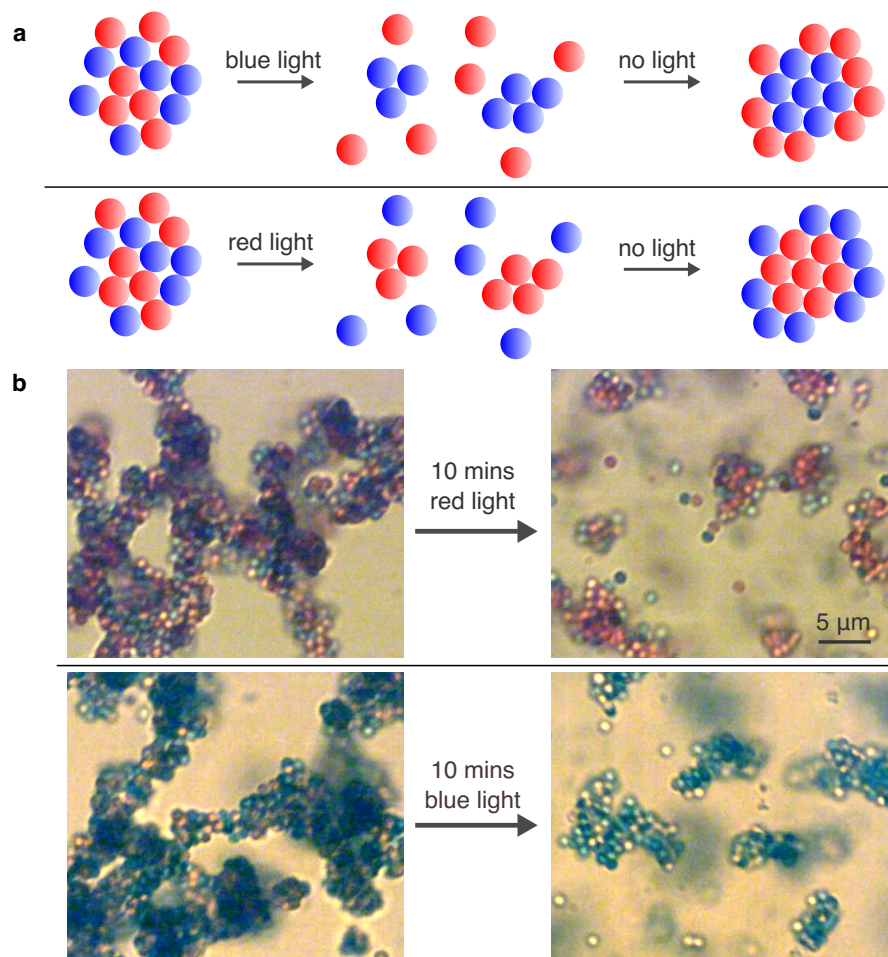


Figure 5.1: a) Cartoon of how different structures can be driven to assemble from the same initial suspension containing red and blue particles. Shining blue light on a mixed aggregate causes red clusters to break because red/red and red/blue bonds melt. After annealing, the light is removed and a structure with a blue core and a red shell forms. The inverse core-shell structure forms when red light is used instead of blue. b) Optical micrographs of a mixed system of red and blue particles exposed to either red or blue light. Before exposure, the particles are randomly mixed in aggregates. During exposure, small aggregates form that contain a majority of particles of the same color as the light to which the sample was exposed.

To understand the timescales needed to achieve these non-equilibrium phenomena, we consider a random collection of particles that initially attract one another. The interactions are turned off for a specified amount of time and then turned back on again (see Figure 5.2). There are three regimes of response. If the interactions were left off for a short time, the particles would not diffuse away from their initial configuration before the interactions were turned back on. In this case, the aggregated particles would not rearrange and the system would be kinetically trapped. If the interactions were left off for a long time, the particles would diffuse far enough apart to lose any memory of their original positions and would form a different structure when the light was turned on. Finally, if the interactions were off for a time comparable to that for a particle to diffuse its own diameter, the particles could rearrange without losing all memory of their nearest neighbors. Over many pulses, the system might anneal. The ideal off time can be approximated by

$$t_{\text{off}} = \frac{\delta^2}{D},$$

where δ is the interaction length (approximately 10 nm) and D is the diffusion coefficient (approximately $1 \mu\text{m}^2/\text{s}$ for a $1 \mu\text{m}$ particle). This calculation yields timescales on the order of milliseconds to seconds, which are accessible using light-induced heating.

Preliminary experiments, in which we shine light pulses onto densely packed clusters of particles, show that the steady state of the system depends on the pulse duration (see Figure 5.3). For very short pulses, the system remains in its initial aggregated state at long times. For very long pulses, the particles diffuse far enough apart that they do not re-form bonds when the interactions are on. For intermediate pulse durations, small clusters form in coexistence with singlets.

We can then test whether this behavior is merely an effect of averaging the strength of the interaction in time. We hold the duty cycle constant and vary the period of the pulsing. The results are shown in Figure 5.4. If the effect of pulsing were merely to average the interactions, we would expect

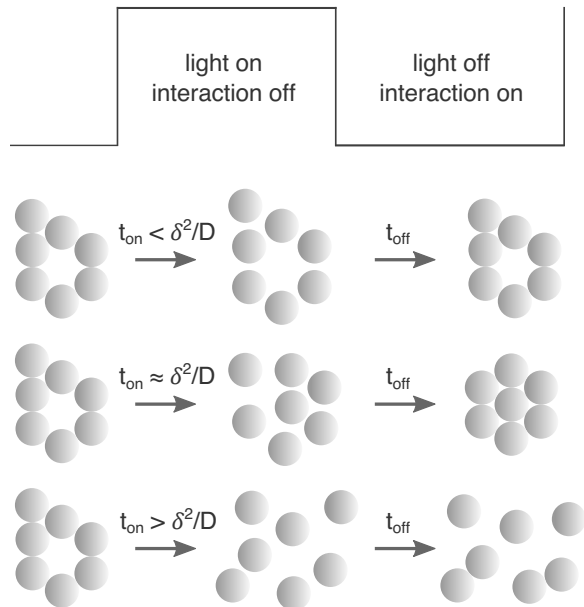


Figure 5.2: Diagram showing three possible responses of an aggregate to light that is turned on for a fixed amount of time, t_{on} , and then turned back off again. Adapted with permission from Zachary M. Sherman and James W. Swan. Dynamic, directed self-assembly of nanoparticles via toggled interactions. *ACS Nano*, 10(5):5260–5271, May 2016.⁷³ Copyright 2016 American Chemical Society.

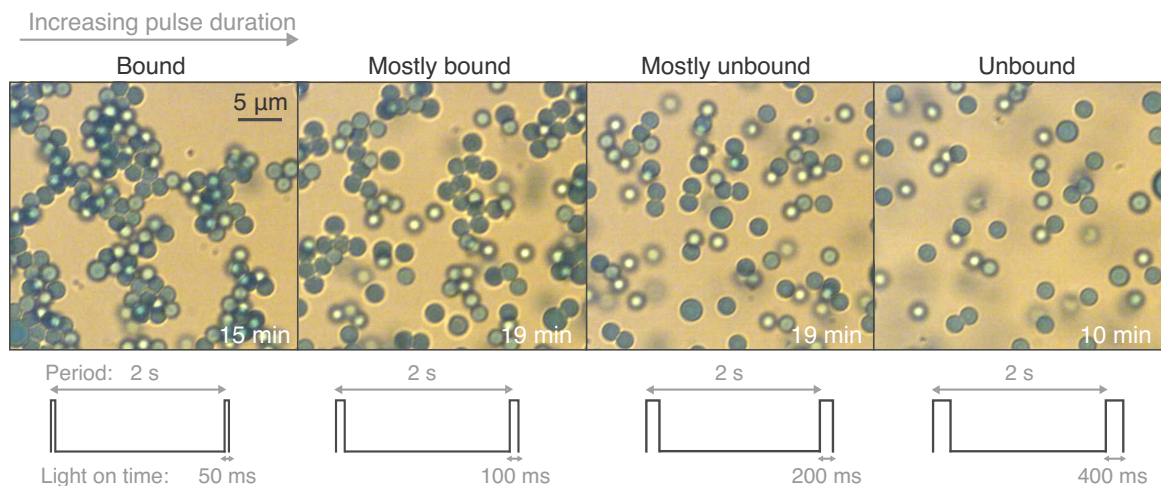


Figure 5.3: Optical micrographs of a system containing 2 μm particles dyed with Oil Blue N and coated with self-complementary DNA. The system is held 10 $^{\circ}\text{C}$ below the particles' melting temperature and exposed to pulses of light of varying duration with a period of 2 s. Micrographs show the steady-state behavior of the system after 10–19 min (as indicated on the images) of pulsing.

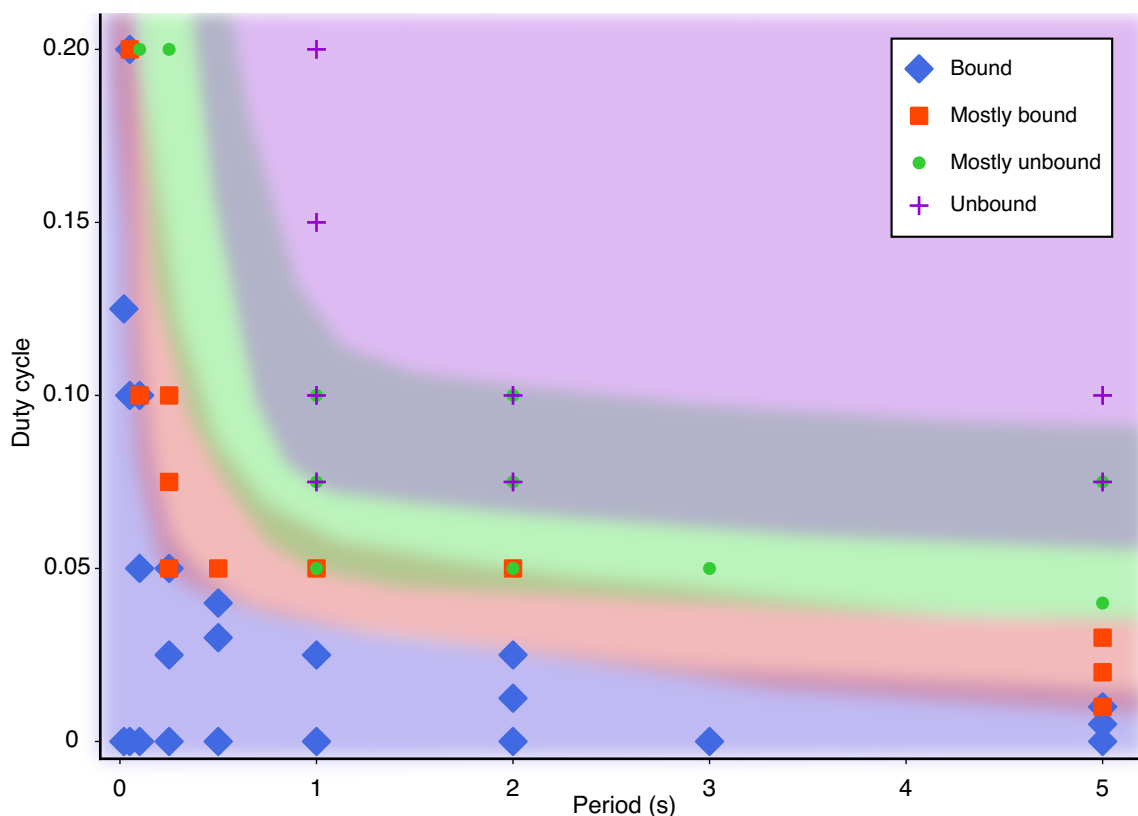


Figure 5.4: Plot of the aggregation state of a system exposed to light pulses as a function of the period and duty cycle. Each data point shows the observed degree of aggregation (as shown in Figure 5.3) in a single experiment where light is pulsed continuously for 10 min. The background colors serve as a guide for the eye.

the behavior to depend only on the duty cycle and not the period. Instead we see that the steady-state behavior of the system depends on the actual interaction on and off times.

We are currently limited by the difficulty of maintaining a constant volume fraction of particles for long times. Because the sample is illuminated only over the field of view (approximately $100\text{ }\mu\text{m}$ by $100\text{ }\mu\text{m}$), any particle that diffuses out of the field of view is no longer illuminated and can bind to other particles outside of the field of view. As a result, the volume fraction in the illuminated region decreases over time. Because singlets diffuse faster than aggregates, experiments with higher singlet fractions have much higher rates of particle loss. This issue could be remedied by constraining the

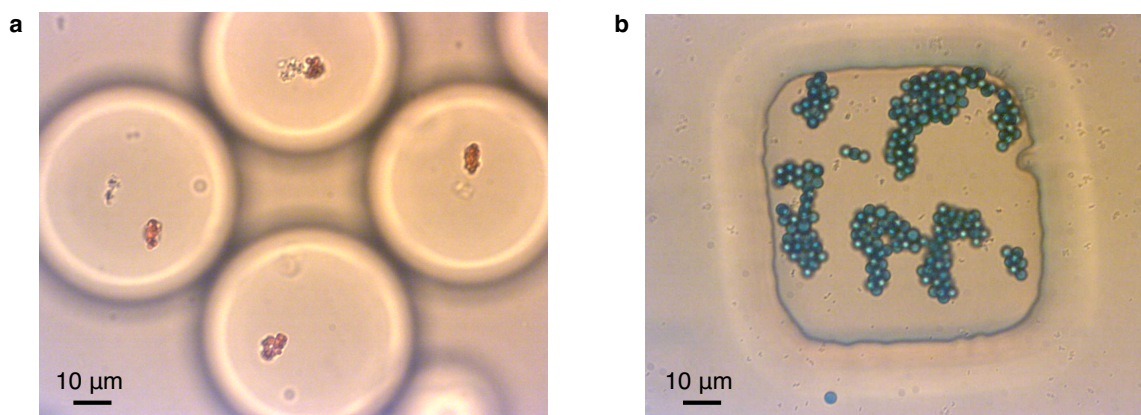


Figure 5.5: Optical micrographs demonstrating two methods of encapsulating particles in a finite volume. a) Water-in-oil emulsion droplets confining 1 μm red particles. b) 50 by 50 μm micro-patterned PDMS well confining 2 μm blue particles.

particles to a region contained within the field of view. Two possible systems for encapsulating particles are shown in Figure 5.5.

5.4 BROAD VISION

Billions of years of evolution have served to create biological systems that exhibit intricate, dynamic, intensely complicated, and yet unbelievably robust behaviors. A single fertilized sex cell, left in the right conditions, will reliably develop into a living, breathing animal.

Synthetic systems are incredibly simple by comparison. They often contain only a handful of components, with constant interactions, and they assemble in equilibrium, without the consumption of fuel. It has always been, and continues to be, my goal to understand the criteria necessary for designing more complex and dynamic systems.

My ultimate, grand aspiration is to, by studying biological systems, break self-organization down to its minimal necessary components. With this new understanding, we could make synthetic tools to realize these components, and thereby build a toolbox that could be used to create any system, regardless of complexity. The work in this thesis may seem laughably simple by comparison, but it is my

hope that the ideas developed here serve to point to a new way of designing systems that incorporates energy input, fuel consumption, and non-equilibrium effects, and in this way to enter a new era of responsive and dynamic systems.



Protocol for functionalizing polystyrene beads with DNA using F108

This protocol makes 400 μL of 1 μm polystyrene particles (sulfate or CML) at 1% volume fraction. Minor modifications are needed to make a different volume or a different size of particles. The protocol is adapted from ref. 41.

A.1 DAY 1 - ACTIVATE F108 WITH NPCF

1. Wipe down hotplate and bench with 70% ethanol
2. Turn on hotplate to 80 °C with stirring on
3. Wash two 3 mL glass vials, two small stir bars (VWR 976), two spatulas
 - (a) Remove the lining from the tops of the vials and clean off any leftover adhesive

- (b) Rinse three times with Alconox solution, Milli-Q water, acetone, Milli-Q water, ethanol, Milli-Q water
 - (c) Dry with nitrogen
 - (d) Dry vials (containing stir bars) and lids on a hot plate (takes approximately 45 mins)
4. While waiting for vials to dry, get out:
- (a) Pluronic F108
 - (b) Nitrophenylchloroformate (4NPCF)
 - i. Use either a new bottle or work from aliquots that are stored with desiccant
 - ii. Make sure the 4NPCF is at room temperature before opening
 - (c) Triethylamine (TEA)
 - (d) Dichloromethane (DCM)
 - (e) 3 mL syringe and 27 G 1 1/4 in needle
 - (f) 100 μ L pipette and tips
5. When the vials are dry, take one and gently blow the inside and the cap with nitrogen, and put the cap on.
6. Turn off the heating on the hotplate, but leave stirring on
7. Add 500 mg F108 to the vial using a clean spatula
- (a) Blow nitrogen over the spatula first
 - (b) Minimize the amount of time the F108 container is open and reseal with Parafilm when finished
8. Use the syringe and needle to measure out 2 mL DCM and add it to the vial. Either use a glass syringe or work quickly to keep the plastic from dissolving.
9. Use the pipette to add 30 μ L of TEA to the vial
10. Hold the vial over the hotplate to allow the stir bar to mix the solution and dissolve the F108 without the solution heating up. Do not let the solution touch the plastic cap.
11. Add 100 mg 4NPCF to the vial
- (a) Blow off the spatula with nitrogen first
 - (b) Try to minimize the amount of 4NPCF that ends up on the sides of the vial
12. Hold over the hotplate until the 4NPCF is dissolved

- (a) Do not let the solution touch the cap
 - (b) If the solution turns yellow at this point, something has gone wrong
- 13. Seal vial with Parafilm
- 14. Fill a glass dish with ice
- 15. Add some Milli-Q water to the ice
- 16. Place the vial in the ice on the hotplate so that the level of ice is below the level of the Parafilm
- 17. Leave stirring in ice for at least 4 hours
- 18. In the mean time, prepare 4 washing solutions:
 - (a) Get out:
 - i. 200 proof ethanol
 - ii. 25 mL serological pipette tip
 - iii. pipette bulb
 - iv. 1 mL serological pipette tip
 - v. 37% hydrochloric acid (HCl)
 - vi. four 50 mL falcon tubes
 - (b) Mark one tube with a star
 - (c) Add 14.9 mL of ethanol to the 3 unmarked tubes and 14.6 mL ethanol to the starred tube
 - (d) Add 0.1 mL HCl to the 3 unmarked tubes and 0.4 mL HCl to the starred tube. Work in fume hood using proper PPE.
 - (e) Vortex to mix and put in the freezer for at least 45 min
- 19. When the 4 hours are up, take the vial out of the ice, dry it off, and remove the Parafilm. The solution should not be yellow at this point or something has gone wrong.
- 20. Take the starred tube out of the freezer, dry off the outside, and warm the top with your hand to prevent condensation when it is opened
- 21. Pour the vial into the starred tube being careful not to pour in the stir bar
- 22. Vortex the mixture for a bit. It will most likely turn turbid and white.
- 23. Return the tube to the freezer for 1 hour
- 24. At least 15 mins before you perform step 25

- (a) Set the big centrifuge:
 - i. Max rate for ramping up and down
 - ii. 10 min run
 - iii. 4000 rpm
 - iv. 2 °C
 - (b) Let it run once to allow it to cool down
 - (c) Make a water counter balance
 - (d) Open vacuum desiccator and put 6 Eppendorf tubes in a rack inside
 - (e) Pump desiccator back down, checking to make sure that the seal is good
25. When the hour is up, remove starred tube from the freezer and vortex
 26. Place tube and counter balance in the centrifuge and run on previous setting
 27. Warm top of tube with hand and dry outside
 28. Pour off supernatant into an appropriate waste receptacle
 29. Take another tube from the freezer, warm top and dry outside
 30. Pour contents of new tube into the starred tube
 31. Vortex until redispersed
 32. Repeat steps 26–31 two more times
 33. After last wash, pour off the supernatant and gently heat (by holding in your hand) until the pellet is liquefied
 34. Open the vacuum desiccator and pour the liquid into 3 or 4 of the predried Eppendorf tubes
 35. Leave this solution to dry in the desiccator overnight

A.2 DAY 2 - REACT ACTIVATED F108 WITH AMINATED-DNA, ADSORB DNA-F108 TO PARTICLES, AND SWELL

1. Take DNA out of freezer
 - (a) If dried:
 - i. Briefly centrifuge
 - ii. Suspend to 1 mM in Molecular Biology Grade H₂O by adding as many µL of water as there are nmol of DNA

- iii. Vortex and briefly centrifuge
 - (b) If already suspended to 1mM, allow to thaw
2. Get out:
 - (a) Citrate buffer (see Section A.4.3)
 - (b) 0.5–10 μ L pipette and tips
 - (c) 10–100 μ L pipette and tips
 - (d) 100–1000 μ L pipette and tips
 - (e) Carbonate buffer (see Section A.4.3)
 - (f) A spatula
 - (g) Two Eppendorf tubes (or 1+ the number of different DNA sequences you want to prepare)
 3. Wash spatula using same method as on day 1
 4. Remove an Eppendorf tube of activated F108 from the vacuum desiccator and then pump it back down
 5. Measure out 15 mg activated F108 into an empty Eppendorf tube, first blowing nitrogen over the spatula
 6. Add 1 mL of citrate buffer
 7. Vortex until redispersed
 8. In a new, empty Eppendorf tube mix together:
 - (a) 15 μ L 1 mM DNA in water
 - (b) 1 μ L carbonate buffer
 - (c) 4 μ L activated F108 in citrate
 - (d) Pipette mix with each addition
 - (e) Vortex and briefly centrifuge after each addition
 9. Put Eppendorf tubes containing DNA/ F108 mixtures on a vortexer and turn it to a low setting so that solution remains in bottom of the Eppendorf tube
 10. Leave them this way for at least 4 hours
 - (a) Come back to check on them a few times, and centrifuge them down if the solution gets shaken up onto the walls

- (b) Solution should start to turn yellow after about 1 hour
- 11. In the mean time wash your particles:
 - (a) Get out:
 - i. 1 μm sulfate (or CML) particles
 - ii. Three Eppendorf tubes
 - iii. Appropriate volume pipette and tips for washing
- 12. Invert the bottle of particles several times
- 13. Squeeze out approximately the desired amount of particles into an Eppendorf tube
 - (a) You need 40 μL of particles at 10% for each different type of DNA coated particle you want to make
 - (b) You should make a larger volume (~ 60 μL each) in case you lose some during washing
 - (c) If the particles don't come suspended at 10% you'll need to start with a larger volume and concentrate them appropriately during washing
- 14. Measure the amount that you want to wash and split it equally into two Eppendorf tubes
- 15. Centrifuge them for 7 min at 4000 g
- 16. Remove as much supernatant as you can and replace with an equal volume of 1x TE (see Section A.4.3)
- 17. Vortex until recombined. This takes a good amount of work. Make sure that when you invert no particles remain in a pellet at the bottom. You may need to resort to sonicating.
- 18. Repeat steps 15–17 for a total of 5 washes (making sure to end up with particles suspended at 10% volume fraction)
- 19. After the last wash, dilute a small amount of particles to approximately 1% volume fraction and look at them under a microscope to make sure that there are no aggregates
 - (a) If there are aggregates (which is likely with the sulfate beads) sonicate them for a while
 - (b) Repeat checking and sonicating until there is fewer than one dimer per (100 $\times$) field of view
- 20. After 4 hours is up, get out:
 - (a) Citrate buffer
 - (b) Washed particles at 10% volume fraction

- (c) 1 low adhesion Eppendorf tube (or as many as you want to make different types of DNA coated particles)
- 21. Remove the F108/ DNA solutions from the vortexer and centrifuge briefly. The solution should now be bright yellow.
- 22. Measure 340 μL citrate buffer into the low adhesion Eppendorf tube
- 23. Add 20 μL F108/ DNA solution
- 24. Pipette mix, vortex, and centrifuge briefly
- 25. Add 40 μL of washed 1 μm particles at 10% volume fraction
- 26. Pipette mix, vortex, and centrifuge briefly
- 27. Let this mixture sit on your bench for approximately 15 mins
- 28. Get out:
 - (a) Toluene (either plain or mixed with dye)
 - (b) 0.5–10 μL pipette and tips
- 29. Add 4 μL of toluene to your particle/DNA solution
- 30. Pipette mix and gently invert a few times. DO NOT VORTEX OR CENTRIFUGE at this point.
- 31. Seal the Eppendorf tube with Parafilm. If you used dyed toluene, also wrap the tube in aluminum foil.
- 32. Place the tube on a room temperature rotator overnight (or for at least 7 hours)

A.3 DAY 3 - DESWELL AND WASH

- 1. Turn the oven to 90 °C
- 2. Get an Eppendorf tube rack
- 3. Remove Parafilm (and foil) from sample(s)
- 4. VERY briefly centrifuge the particles (about 1 second) to remove any droplets from the walls
- 5. Open sample to atmosphere and put in 90 °C oven for approximately 12 min (the amount of time depends slightly on the number of samples and the volume of each sample)
- 6. Remove samples from oven and let sit open on your bench for approximately 15 min

7. Pipette sample into a new Eppendorf tube. When pipetting draw the sample from the center of the tube and leave behind about 1-2 μL at the meniscus.
8. Wash the particles 5 times in 1x TE using the same method as on day 2

A.4 THINGS NEEDED FOR THIS PROTOCOL

A.4.1 EQUIPMENT

1. Hot plate
2. Vacuum desiccator (preferably with solvent trap)
3. Freezer
4. Vortexer
5. Refrigerated centrifuge
6. Oven

A.4.2 MATERIALS

1. Two 1 Dr black topped vials from VWR
2. Two stir bars # 976 from VWR
3. Two metal spatulas
4. Parafilm
5. Aluminum foil
6. One 3 mL syringe
7. One 27 G 1 1/4 in needle
8. One glass dish about 4" in diameter and 2.5" deep
9. Five 45–50 mL falcon tubes
10. Macro pipettor
11. 1 mL serological pipette tip
12. 25 mL serological pipette tip
13. A bunch of Eppendorf tubes

A.4.3 CHEMICALS

1. 1 μm sulfate or carboxyl modified polystyrene beads (Invitrogen)
2. 5' Amine modified DNA (IDT)
3. Pluronic F108 (BASF)
4. Milli-Q water
5. Molecular biology grade water
6. *p*-nitrophenyl chloroformate (4NPCF, Sigma-Aldrich)
7. 200 proof ethanol (Koptec)
8. 70% ethanol
9. 1% Alconox in Milli-Q water
10. Sodium chloride (NaCl)
11. Toluene (anhydrous, Sigma)
 - (a) Use in hood
 - (b) Health (2) Flammability (3)
12. Hydrochloric acid (HCl, 38% ACS grade, EMD)
 - (a) Health (3)
 - (b) Wear acid gloves
 - (c) Work in hood
13. Triethylamine (TEA, Sigma-Aldrich)
 - (a) Work in hood
 - (b) Health (2) Flammable (3)
14. Dichloromethane (DCM, anhydrous, $\geq 99.8\%$, Sigma-Aldrich)
 - (a) Penetrates through nitrile gloves in 1 min
 - (b) Always work in hood
 - (c) Health (2)
15. Citrate buffer (10 mM pH 4.0 citric acid buffer)

- (a) 1.1 mM Anhydrous Citric Acid (EMS 99.5%)
 - (b) 8.9 mM Sodium Citrate (Spectrum, 99.0–100.5%)
16. Carbonate buffer (1 M pH 9.5)
- (a) 0.85 M Sodium Bicarbonate (EMD 99.7–100.3%)
 - (b) 0.15 M Sodium Carbonate (EMD $\geq$ 99.5%)
 - (c) 3.57 g NaHCO_3 and 0.792 g Na_2CO_3 in 50 mL Milli-Q water, mixed thoroughly, then filtered
17. 1x TE (Tris-EDTA)
- (a) Diluted to 1x TE from Serva TE Buffer (100 $\times$), pH 8
18. Acetone (for washing, any purity is fine)

B

Protocol for functionalizing polystyrene beads with DNA using PS-PEO

This is a protocol to first add an azide group to PS-PEO diblock copolymers and then incorporate those polymers into PS particles by a swelling/deswelling procedure. The presence of the azide group then allows for simple click chemistry functionalization of particles with DNA. The protocol is expanded from that given in reference 43.

Note: Most of this procedure should be done in the fume hood

B.1 CHLORINATE THE PS-PEO

B.1.1 DAY 1

1. Wipe down hot plate and bench with 70% ethanol
2. Turn on hot plate to 80 °C and turn stirring on as well
3. Wash two 4 mL glass vials (1 Dram), two stir bars (VWR 976), two spatulas
 - (a) Rinse three times with Alconox solution, Milli-Q water, acetone, Milli-Q water, ethanol, Milli-Q water
 - (b) Dry with nitrogen
 - (c) Dry vials (containing stir bars) and lids on a hot plate (takes approximately 45 min)
 - (d) Blow dry vials and caps with nitrogen and close them (seal one with Parafilm and set aside for later)
 - (e) Turn off heating
4. Measure out 100 mg PS-PEO into one of the vials
 - (a) First blow off spatula with nitrogen
 - (b) After using, seal the PS-PEO with Parafilm and return to desiccator
5. Add 2 mL DCM using a 3 mL syringe with a 27 G 1 1/4 in needle
 - (a) Work in fume hood
 - (b) DCM penetrates through nitrile gloves so change gloves quickly if you get any on you
6. Add 42 µL TEA in the fume hood
7. Seal the vial with Parafilm
8. Hold over the stir plate until the PS-PEO is dissolved (this doesn't take long)
9. Fill a dish with ice and place it on the (now cool) stir plate
10. Put the vial in the ice so that the solution is submerged, but the bottom of the Parafilm is above the level of the water
11. Let stir for 15 min
12. Remove from ice, thoroughly dry and open. Add 23.5 µL of MsCl
 - (a) Note: MsCl is very toxic. Double glove and work in fume hood.

13. Parafilm again and return to ice bath for 2 hours (periodically check on the level of water/ice)
14. After 2 hours, remove from the ice bath, dry, and put back on stir plate at room temperature for 22 hours

B.1.2 DAY 2

1. After the reaction, transfer the solution to a Falcon tube and evaporate in the vacuum desiccator until dry (at least 6 hrs)
2. While it is drying, prepare two Falcon tubes containing 10 mL MeOH and 243 μ L of 37% HCl and put them in the freezer
 - (a) Pull 45 mL of anhydrous methanol out of bottle with a big syringe and needle and put in a separate falcon tube to work from
 - (b) Note: Methanol penetrates through nitrile in less than 1 min so be careful not to get any on you, and change gloves immediately if you do.
 - (c) Work in the hood.
3. When the PS-PEO-Ms is dry, remove one of the containers of MeOH/HCl from the freezer, warm and dry top of falcon tube to keep water from condensing inside when you open it, pour it into the Falcon tube from the desiccator, and vortex
4. Return to the freezer for 1 hr. The solution should turn white and turbid.
5. Centrifuge at 4500 rpm for 10 min at 2 °C
6. Pour off supernatant and pour the second Falcon tube from freezer onto the pellet
 - (a) Dispose of supernatant as hazardous waste
 - (b) Warm and dry top of Falcon tube from freezer before opening
7. Vortex to resuspend
 - (a) The solution should immediately begin to become cloudy
8. Return to freezer for 1 hr
9. Centrifuge again and pour off supernatant
10. Add approximately 3 mL MeOH and swirl to dissolve pellet (couple of minutes)
11. Pour in 40 mL of diethyl ether
12. Vortex until white and turbid

13. Return to freezer for 1 hr
14. Centrifuge and pour off supernatant
15. Repeat steps 10–14
16. Dry pellet in vacuum desiccator (in the falcon tube)

B.2 AZIDIZE THE CHLORINATED PS-PEO

B.2.1 DAY 3

1. Wash vials and stir bars and spatulas. You can use leftover washed vial and stir bars from day 1 (blow out with nitrogen)
2. Add 0.01 g of NaN_3 to the glass vial (in the hood using proper PPE)
 - (a) Note: NaN_3 (Sodium Azide) is VERY TOXIC and poses an explosion risk. Work in fume hood. Double glove. Dispose of anything that comes in contact as hazardous waste.
3. Add 2 mL of DMF using a 3 mL syringe with a 27G 1 1/4 in needle
4. Hold over stir plate until dissolved (approximately 30 min)
5. Transfer PS-PEO-Ms to the vial and dissolve by swirling in hand or with gentle stirring for approximately 10 min
6. Stir the solution at 1500 rpm in a 65 °C oil bath for 24 hr

B.2.2 DAY 4

1. Pour the solution into a Falcon tube
2. Add 40 mL diethyl ether
3. Vortex until white and turbid
4. Put in freezer for 1 hr
5. Centrifuge and pour off supernatant
6. Add ~3 mL MeOH and swirl to dissolve pellet
7. Pour in ~40 mL of diethyl ether
8. Vortex until white and turbid

9. Return to freezer for 1 hr
10. Repeat steps 5–9 two or three times
11. After centrifuging and pouring off the last supernatant, dry the PS-PEO-N₃ in vacuum desiccator

B.2.3 DAY 5

1. Wash a larger (~ 20 mL) vial, cap, stir bar, and spatula
 - (a) It is now less important for them to be super dry, but they need to be dry enough that the weight doesn't drift as water evaporates
2. Remove the PS-PEO-N₃ from the vacuum desiccator and weigh into the vial
3. Add Molecular Biology Grade water to suspend the PS-PEO-N₃ to 1 mM
 - (a) The molecular weight of the PS-PEO-N₃ is 10,342 g/mol
 - (b) The PS-PEO-N₃ should be white and dissolve into a translucent solution in water
 - (c) You should get approximately 4 mL of the 1 mM PS-PEO-N₃ solution. Since large volume pipettes are less accurate, add water in stages, weighing between.
4. Stir gently, trying not to tilt, so that you don't have chunks of PS-PEO on the walls

B.3 FUNCTIONALIZING PS BEADS WITH PS-PEO-N₃

1. Wash particles five times and suspend to 10% volume fraction
 - (a) Centrifuge at 4000 g for 7 min
 - (b) Replace supernatant with 1x TE (or Milli-Q H₂O)
 - (c) After washing look at a small amount diluted to 0.5% on the microscope to see if there are aggregates. If there is more than one doublet per field of view sonicate them and repeat.
2. Get out a low-adhesion Eppendorf tube (1.5 mL)
3. Put 100 μ L of PS-PEO-N₃ solution into the tube (for particles that are not 1 μ m multiply this volume by 1 μ m / diameter)
4. Without dye:
 - (a) Add 100 μ L Milli-Q H₂O (this number plus the PS-PEO volume should add to 200 μ L)

- (b) Add 160 μL THF, mix gently on the vortexer, then immediately
- (c) Add 40 μL of 1 μm PS particles at 10%
- 5. With dye:
 - (a) Add 140 μL Milli-Q H_2O (this number plus the PS-PEO volume should add to 240 μL)
 - (b) Add 120 μL THF
 - (c) Add 3 μL of dye (0.5 mg/mL) in toluene, mix gently on the vortexer, then immediately
 - (d) Add 40 μL of 1 μm PS particles at 10%
- 6. Parafilm shut and place on vortexer for 30 min
- 7. Centrifuge very briefly to get any liquid off of the cap
- 8. Fill Eppendorf tube up to 1.5 mL with Milli-Q H_2O , and flick tube a bit
- 9. Centrifuge very briefly to get any liquid off of the cap
- 10. Put tube into 70 $^\circ\text{C}$ oven with cap open for 1 hr (or until solvent smell is gone).
- 11. Shake tube every 10 min to mix up particles, and centrifuge down very briefly to get any liquid off of the cap.
- 12. Transfer to new eppendorfs
- 13. Wash three times (or more) into Milli-Q H_2O (NOT 1x TE!) ending up with 400 μL of suspension at 1%

B.4 ADDING DNA

- 1. Combine:
 - (a) 10 μL DBCO DNA 70uM (for particles that are not 1 μm multiply this volume by 1 μm / diameter)
 - (b) 40 μL azidized particles 1%
 - (c) 150 μL 1x TE, 1M NaCl, 0.05% (by weight) F127
- 2. At room temperature, shake for 24h (don't use stir-bar as this deforms particles)
- 3. Wash 5 $\times$ and suspend in whatever you'd like (eg. Milli-Q H_2O , 1x TE, PBS, ...)

B.5 THINGS NEEDED FOR THIS PROTOCOL

B.5.1 EQUIPMENT

1. Hot plate
2. Vacuum desiccator (preferably with solvent trap)
3. Freezer
4. Vortexer
5. Refrigerated centrifuge
6. Oven

B.5.2 MATERIALS

1. Two 1 Dr glass vials
2. One ~20 mL glass vial
3. Two small stir bars (# 976 from VWR)
4. One metal spatula
5. One disposable spatula (for using with NaN_3)
6. Parafilm
7. Two 3 mL syringe
8. Two 27 G 1 1/4 in needle
9. One 40+ mL syringe
10. One 16 G 1 1/2 in needle
11. One glass dish approximately 4" in diameter and 2.5" deep
12. Five 45–50 mL falcon tubes
13. Macro pipettor
14. 10 mL serological pipette tip
15. Several 50 mL serological pipette tips
16. A bunch of 1.5 mL Eppendorf tubes

B.5.3 CHEMICALS

1. PS-PEO from Polymer Source (special order)
 - (a) PS - 3800 MW
 - (b) PEO - 6500 MW
 - (c) Electronic grade
2. 70% ethanol
3. 1% Alconox in Milli-Q water
4. 1 μ m sulfate or carboxyl modified polystyrene beads (Invitrogen)
5. 5' DBCO-modified DNA (IDT)
6. 1x TE
 - (a) Diluted to 1x TE from Serva TE Buffer (100 $\times$), pH 8
7. Sodium Chloride (NaCl)
8. Pluronic F127 (BASF)
9. Dichloromethane (DCM, anhydrous, $\geq 99.8\%$, Sigma-Aldrich)
 - (a) Penetrates through nitrile gloves in 1 min
 - (b) Always work in hood
 - (c) Health (2)
10. Triethylamine (TEA, Sigma-Aldrich)
 - (a) Work in hood
 - (b) Health (2) Flammable (3)
11. Methanesulfonyl chloride (MsCl, $\geq 99.7\%$, Aldrich)
 - (a) VERY Toxic (4)
 - (b) Always work in hood
 - (c) Never inhale it
 - (d) Health (4)
12. Methanol (MeOH, 99.8% anhydrous)

- (a) Penetrates through nitrile in <1 min
 - (b) Always work in hood
 - (c) Health (2) Flammable (3)
13. Diethyl ether (reagent grade, $\geq 98\%$, Sigma-Aldrich)
- (a) Very flammable (4)
 - (b) Health (1) Flammable (4)
14. Hydrochloric acid (HCl, 38% ACS grade, EMD)
- (a) Health (3)
 - (b) Wear acid gloves
 - (c) Work in hood
15. N,N-Dimethyl formamide (DMF, anhydrous, 99.8%, Sigma-Aldrich)
- (a) Health (2) Flammable (2)
16. Sodium Azide (NaN_3 , ReagentPlus, $\geq 99.5\%$, Sigma-Aldrich)
- (a) VERY TOXIC
 - (b) EXPLOSION RISK
 - (c) Use in hood
 - (d) Double glove
 - (e) Dispose of anything that comes in contact (including gloves) as hazardous waste
 - (f) Health (4)
17. Tetrahydrofuran (THF, anhydrous, $\geq 99.9\%$, inhibitor-free, Sigma-Aldrich)
- (a) Health (1) Flammability (3)
 - (b) Peroxide former, dispose within 1 year if unopened or 6 months after opening
18. Toluene (anhydrous, Sigma)
- (a) Use in hood
 - (b) Health (2) Flammability (3)
19. Acetone (for washing, any purity is fine)
20. Ethanol (for washing, any purity is fine)

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