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Programming Directed Motion with DNA-Grafted Particles

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Cite This: *ACS Nano* 2022, 16, 9195–9202

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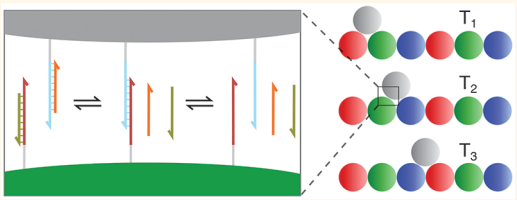
ABSTRACT: **Colloidal particles can be programmed to interact in complex ways by functionalizing them with DNA oligonucleotides. Adding DNA strand-displacement reactions to the system allows these interparticle interactions to respond to specific changes in temperature. We present the requirements for thermally driven directed motion of colloidal particles, and we explore how these conditions can be realized experimentally using strand-displacement reactions. To evaluate the concept, we build and test a colloidal "dancer": a single particle that can be driven to move through a programmed sequence of steps along a one-dimensional track composed of other particles. The results of these tests reveal the capabilities and limitations of using DNA-mediated interactions for applications in dynamic systems. Specifically, we discuss how to design the substrate to limit complexity while permitting full control of the motile component, how to ratchet the interactions to move over many substrate positions with a limited regime of control parameters, and how to use technological developments to reduce the probability of detachment without sacrificing speed.**



KEYWORDS: *colloids, DNA, self-assembly, thermal ratchet, directed motion*

Though DNA-mediated interactions can be used to program the self-assembly of colloidal particles into many different complex structures,[1−7] it remains a challenge to make colloidal assemblies that can respond in a programmed manner to external cues. Recent progress toward this goal has focused on colloidal structures that can be reconfigured upon the introduction of DNA strands or a change in solution conditions.[8−13] We examine another kind of programmable response: directed motion.

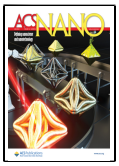
Directed motion at the nano- and microscale can be achieved in several ways. In Brownian motors and ratchets, the diffusion of particles is biased through the periodic application of asymmetrical potentials.[14] This principle has been used to transport and sort colloids, DNA, and other microscopic species.[15−18] Motor proteins such as kinesin can travel long distances (micrometers) inside cells by taking discrete steps along microtubules, powered by the consumption of ATP.[19] This stepping behavior has been mimicked in synthetic systems composed entirely of DNA that can take steps along patterned tracks.[20−23]

Here we address the challenge of adapting programmable colloidal interactions to make colloidal systems that display directed microscale motion. We describe a system in which changes in temperature are used to steer a colloidal particle along a track. Our system is a type of Brownian ratchet, in that it relies on thermal fluctuations to move the particle, but it can also be viewed as a heat engine, since the motion is achieved by changing the thermal bath that is coupled to the system. The changes in temperature lead to changes in interactions between the moving particle and other fixed particles that make up a one-dimensional track.

We design these temperature-tunable interactions using colloidal particles grafted with a high density of complementary DNA strands. The interaction strength between these particles varies steeply with temperature, ranging from many times the thermal energy k_BT to negligible over a 1 °C range. We use DNA strand-displacement reactions to modify this thermal response.[12,24] Adding a single type of free DNA displacing strands to solution can make the temperature dependence less steep. Adding two types of displacing strands can create interparticle interactions that are nonmonotonic with temperature. We show that by optimizing several design parameters related to these strand-displacement reactions, we can cause a DNA-grafted particle to move in a chosen direction by applying a particular temperature sequence.

We call this system a "dancer" rather than a "motor" because the particle takes a controlled sequence of steps in response to external cues (see Movie 1). The dancer system is distinct from existing systems of dynamic colloids such as "swim-

Received: February 11, 2022
Accepted: June 3, 2022
Published: June 10, 2022



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ACS Nano 2022, 16, 9195−9202

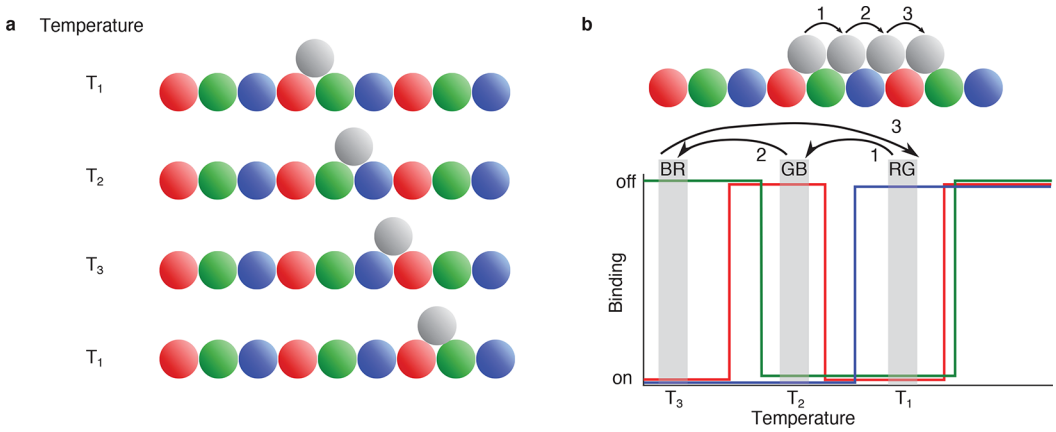

Figure 1. Introduction to dancer system and an explanation of interactions needed. (a) Schematic of the dancer system, showing desired behavior. A side view of a single DNA-coated particle (the dancer, shown in gray) placed on a track composed of three other types of DNA-coated particles. The colors denote surface coverage with different sequences of DNA. At different temperatures, the dancer must bind to different pairs of track particles, allowing it 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, it binds to *blue* and *green*. At high temperature, it binds to *red* and *green*. By slowly decreasing the temperature through these three states and then rapidly raising it, we aim to move the dancer progressively in one direction. Reversing this cycle should move the dancer in the opposite direction.

mers"[25] and "monowheels"[26] because each step of the motion is controlled and reversible.

DESIGN CONSIDERATIONS

We first describe the design criteria that must be met to make a dancer and provide an overview of our approaches to satisfying these criteria experimentally. Our overall goal is 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 design the track on which it will move. The track must be composed of as few unique elements as possible to simplify the design, while at the same time ensuring that forward and backward motions are differentiable. We therefore design an asymmetric track composed of a repeated sequence of three different species of particles, each of which has a specific, temperature-dependent interaction with the dancer, as illustrated in Figure 1a. We refer to these three species of track particles as *red*, *green*, and *blue*.

To begin, the dancer is bound to two adjacent species of track particles (see Figure 1a). To actuate the dancer, we turn on and off its interactions with the different track particles in time so that it unbinds from a track particle on one side, rolls across the middle track particle, and binds to the track particle on the other side, thereby reaching the next fixed position along the track.

This scheme requires three regimes of interaction that are controlled by variation in temperature, as illustrated schematically in Figure 1. The temperatures are T_1, where the dancer is bound to the *red* and *green* track particles, T_2, where the dancer is bound to the *green* and *blue* track particles, and T_3, where the dancer is bound to the *blue* and *red* track particles. At temperatures between these regimes, we require the dancer to remain bound to at least one track particle. The simplest set of temperature-dependent interparticle interactions required to realize these conditions is shown in Figure 1b.

Moving the dancer using these interactions requires ratcheting the temperature of the system by first slowly lowering the temperature from T_1 to T_2, then from T_2 to T_3, and then rapidly increasing the temperature 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 backward one step, then the dancer should be able to take another step forward. But if the heating time is long compared to the time that it takes the dancer to diffuse backward one step, then the dancer will reverse the first two steps instead of progressing forward. By varying the sequences of temperature changes, keeping track of which changes in temperature must be fast and which can be slow, we aim to choreograph the motion of the dancer, that is, control the timing and direction of each step.

The design principles laid out above represent all that is required in an idealized system. However, we must also consider the physical challenges to realizing such behavior in a real experimental system. These challenges include experimentally creating the temperature-dependent interactions described above, adapting the track design to realistic conditions, and sustaining the motion over a long distance and at a reasonable speed.

The first challenge, creating the specific and temperature-dependent interactions, can be achieved using DNA-mediated colloidal interactions.[27−30] DNA-mediated interactions are well-suited for this application for two main reasons. First, the interactions can be made nonmonotonic with temperature using DNA strand-displacement reactions.[12,24] Second, each pair of particles can be bound together by tens to hundreds of individual DNA strands. The dynamic breaking and reforming of individual DNA bonds allows the particles to roll with respect to each other while remaining bound.[31−33] We therefore aim to construct both the dancer and the track from DNA-functionalized colloidal particles and to change the interaction strengths by varying the temperature of the solution. The details of the DNA design process are described in the "Results and Discussion" and "Methods" sections.

To address the second challenge, adapting the track to realistic conditions, we consider that the dancer must remain bound to the track for the entire course of an experiment (1−2 h). If it detaches from the track at any point, then it will diffuse

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ACS Nano 2022, 16, 9195−9202

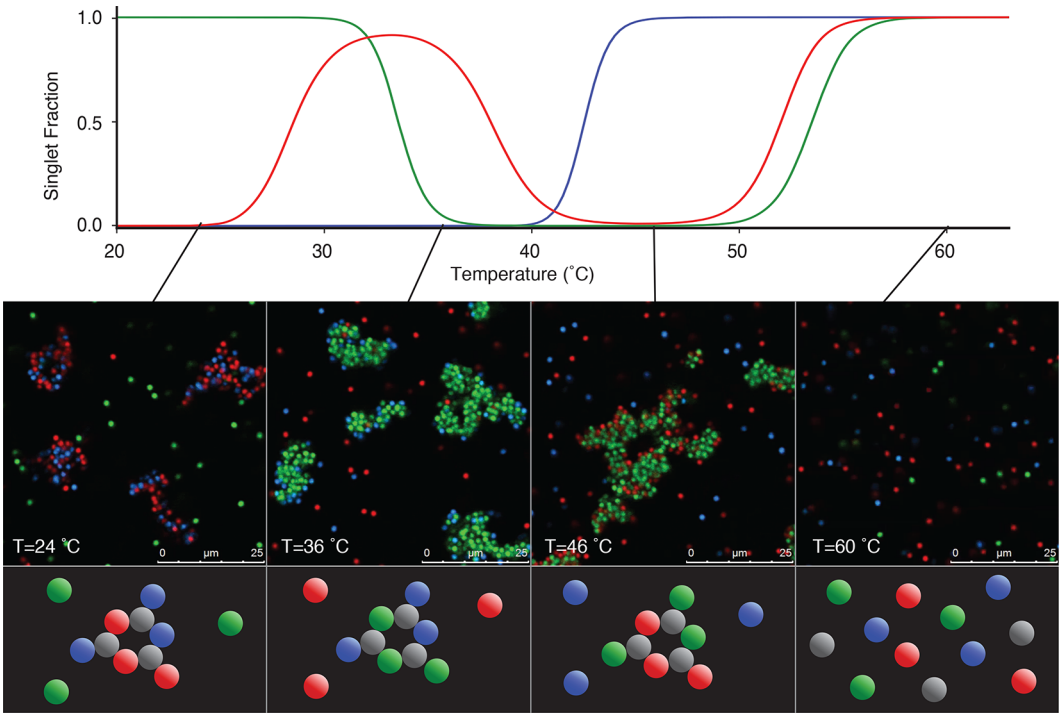

Figure 2. Experiments showing that interactions between the dancer and track particles follow the desired temperature dependencies. Three species of track particles, identified by fluorescent dyes (red, blue, and green), each have different interactions with the undyed dancer particle. Top: plot of the fraction of track particles that are not bound to a dancer particle (singlet fraction), calculated using the model of ref 12. The plot shows how each color of track particle interacts with the dancer particle as a function of temperature. Middle: fluorescence confocal images of this system. The dancer particles do not appear in the confocal images because they are not dyed. Because there are no interactions between the dyed particles in the absence of the dancer, any aggregate of dyed particles must contain dancer particles. Bottom: schematic representation of the aggregates in the images, showing binding between the dancer particles and dyed particles.

away into the bulk. We can reduce the probability of the dancer detaching by making the track two particles wide instead of one (see Figure 3a). 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 increase the average time the dancer stays bound to the track.

To address the third challenge, sustaining the motion at a reasonable speed, we must also consider that the probability of the dancer detaching depends strongly on the binding strength: The stronger the binding, the lower the detachment rate. This result would seem to imply that we should set the binding strength as high as possible. However, the time it takes for the dancer to take one step also depends on the binding strength; DNA-functionalized particles can roll only when the multiple DNA bridges can actively break and reform quickly.[31] A derivation of the expected number of steps given the binding strength and rolling rate is shown in Supporting Information section S1. We describe how to experimentally tune binding strength for each interaction to maximize the rolling rate while preventing unbinding over the course of the experiment in the "Results and Discussion" section.

RESULTS AND DISCUSSION

DNA Sequences and Strand-Displacement Reactions. To experimentally realize the dancer system, we design sequences for both the particle-bound and displacing DNA strands. Each of the interactions between the dancer and the track requires different types of strand-displacement reactions previously described by Gehrels, Rogers, and Manoharan.[12,24] A detailed description of these interactions is given in Supporting Information section S2 and Figure S2. In short, a single type of displacing strand is added to solution to weaken the low-temperature interaction between the surface-bound DNA on the dancer and the *blue* particle species. We weaken the bonds so that they can continually break and reform, thereby allowing the particles to roll with respect to one another over a large range of temperatures. Two types of displacing strands compete with bonds between the surface-bound DNA on the dancer and the *green* particle species to create the two-transition interactions (see the green curves in Figures 1b and 2). Finally, two types of displacing strands and two types of particle-bound DNA are needed for the three-transition interactions between the dancer and the *red* particle species (see the red curves in Figures 1b and 2). All in all, the interactions between the dancer and the three track particle species require eight types of particle-bound DNA strands (one each on *blue* and *green*, two on *red*, and four on the dancer) and five types of displacing strands that are free in solution (see Figure S2).

Because each melting transition has a characteristic width of a few degrees Centigrade (as shown in the top panel of Figure 2), we must separate the temperature regimes to allow each of the transitions to complete. We therefore set $T_3 \approx 25$ °C, $T_2 \approx 35$ °C, and $T_1 \approx 45$ °C. These values drive the design of the DNA sequences as well as their concentrations. The sequences we use are given in Table S1 (see the "Methods" section for details of the sequence design procedure).

By combining all of the components in solution, we can observe the composition of any solid structures that form as a function of temperature, and we can adjust the DNA

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ACS Nano 2022, 16, 9195−9202

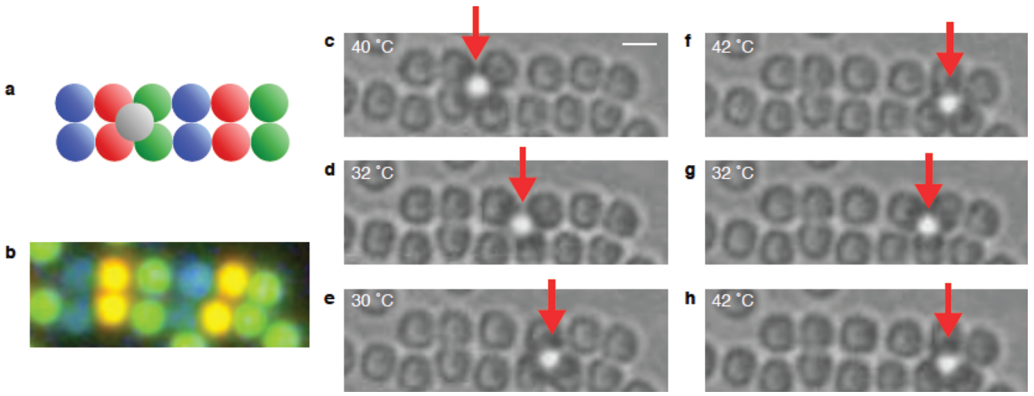

Figure 3. Particle dancing on a track. (a) Schematic of the track with dancer particle on top (in gray). (b) Fluorescence image of the track. (c–h) Optical micrographs showing dancer motion. After a dancer is added to the track (c), the temperature of the system is lowered. The dancer (indicated with the red arrow) takes two steps toward the right end of the track (d, e). The temperature is then quickly raised to allow the dancer to take one more step forward (f). Quickly lowering the temperature then allows the dancer to move in reverse for one step (g). Finally the temperature is quickly raised, allowing the dancer to take a final step to the right (h). (scale bar = 1 μm). See Movie 3 for a recording of the full experiment.

concentrations to tune the temperature at which each transition occurs. The positions of the high-temperature melting transitions and the low-temperature 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* particles and the intermediate transition for the *red* particles are controlled by the concentration of displacing strands in solution. This procedure results in the DNA concentrations given in Table S2.

Confocal Imaging of Resulting Phase Behavior. Bulk confocal experiments show that these concentrations lead to the desired temperature responses in the colloidal suspension (Figure 2). Since the track particles cannot bind to one another and the dancer particles are not dyed, the observation of a dense structure containing a specific color of particles in the confocal images indicates an attractive interaction between that track particle and the dancer. Our data show that these attractive interactions have the desired responses: At 24 °C, the *red* and *blue* track particles bind to the dancer particles, while the *green* particles remain free. At 36 °C, only the *green* and *blue* particles bind to the dancer. At 46 °C, only the *red* and *green* particles bind to the dancer. At 60 °C and higher, there are no attractions, as expected. By coordinating so many interactions in the same solution, we obtain precise control over the composition of structures formed over a wide range of temperatures.

Constructing Tracks. Making the dancer dance requires more than just the appropriate interactions; it also requires a track. To construct the track, we first fluorescently dye the track particles so that we can distinguish them when they are mixed together (see the "Methods" section). We then use optical tweezers to manipulate individual particles and place them in the appropriate sequence (see Figure S3b). To keep the track particles in their locations, we must immobilize them once they touch the surface. Our immobilization scheme relies on nonspecific binding between DNA-grafted particles and coverslip glass. We find that untreated glass coverslips have a nonspecific, irreversible attraction to DNA-coated particles, likely due 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 S3a and the "Methods" section). The passivated region of the sample chamber serves as a reservoir of particles, which we use to construct a track in the sticky region. With these techniques, we can reliably construct three-species tracks (see Figure S3b and Movie 2).

Interactions between the Dancer and Track. Using the method described in the previous section, we can construct a track as shown in Figure 3a,b, place a dancer on it, and observe the dancer's behavior as a function of temperature. We find that the response to temperature is different for the single dancer on the track than that observed for the bulk suspension of dancer and track particles. The differences might arise because the measurements of the melting transitions in suspension were averaged over a large number of dancer particles, each able to bind up to 12 track particles, while the 2D track measurements involved single dancers binding no more than four partners at once.

To align the temperature-dependence of the interactions with the design criteria, we adjust the concentrations of DNA in the sample. Increasing the concentration of particle-bound DNA increases the temperature of the high-temperature melting transitions for the interactions between the dancer and the *blue*, *green*, and *red* particles. It also increases the low-temperature melting transition between the dancer and *red* particles. Increasing the concentration of displacing strands increases the temperature of the low-temperature melting transition between the dancer and *green* particles and the moderate-temperature melting transition between the dancer and *red* particles (see Figure S2).

To find the correct values for each of these DNA concentrations, we construct miniature "tracks" consisting of squares containing four identical track particles, and we place a single dancer on top of each. Because we can directly observe the behavior of single dancers, we can see when the dancer changes position to roll around on the square, when it detaches from all four track particles to float away into solution, or when it rolls off of the edge of the track and sticks to the glass. We use these experiments to measure and adjust the temperature ranges for rolling and for detachment.

https://doi.org/10.1021/acsnano.2c01454
ACS Nano 2022, 16, 9195–9202

Because rolling relies on weak binding, we adjust the transition temperatures so that in the temperature range where the dancer is supposed to step across a track particle, it is no more than 5 °C from the melting temperature for that species of particle.[34] 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 the 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. Therefore, we must adjust the concentration of the displacing strand that competes with the *blue*/dancer interaction to maximize the rolling rate while maintaining a low detachment probability.

We then modify the concentrations of the DNA strands to ensure that T_1, T_2, and T_3 are separated as much as possible and that the particles roll between these temperatures. The resulting concentrations are shown in Table S3. The measurements of the rolling ranges and melting temperatures are shown in Table 1. For these conditions, we choose T_1 = 42 °C, T_2 = 32 °C, and T_3 = 23 °C.

Table 1. Experimentally Measured Rolling Ranges and Melting Temperatures for the Optimized Interactions between Each Track Particle Species and the Dancer[a]

interaction	rolling temps (°C)	melting temp (°C)
blue/dancer	26−39	39
green/dancer	25−30	25
	42−50	50
red/dancer	22−25	25
	33−44	33
	33−44	44

[a]The experiments are carried out at the DNA concentrations given in Table S3 with two exceptions: The low-temperature *green*/dancer measurements are carried out at 3 μM displacing strand and are therefore shifted to slightly lower temperatures than in the dancing experiments; the high-temperature *green*/dancer measurements are carried out at 6.6 μM displacing strand but are not significantly shifted.

Heating Rate. To explore the response of the dancer to thermal ratcheting, we heat the sample using a Peltier heater (see the "Methods" section). To keep the dancer from moving in the wrong direction during the step from the *blue*/*red* to the *red*/*green* position, we must increase the temperature from 25 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 across a single step. Therefore, the heating rate should be sufficient for preliminary tests of the ratcheting step, although a faster heating rate would reduce susceptibility to thermal fluctuations.

Evaluation of Concept. To evaluate the concept, we build a full three-color track, set the temperature to 40 °C, and use optical tweezers to place a dancer particle between *red* and *green* track particles (Figure 3b,c). When the temperature is lowered to 32 °C, the dancer is released from the *red* particles, rolls back and forth across the *green* particles, and comes to rest between the *green* and *blue* particles (Figure 3d). In this test, when the temperature is further lowered to 30 °C, the dancer moves to the *blue*−*red* position (Figure 3e). This step occurs at a higher temperature than the expected 23 °C, suggesting that the *green* bond broke prematurely. Because we observe the system in real time, 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 (Figure 3f). To test external control, we then send the dancer backward and forward by rapidly lowering the temperature to 32 °C (Figure 3g) and then raising it back to 42 °C (Figure 3h).

Failure Modes. In repeated experiments, we find that we can consistently move the dancer through a sequence of programmed steps, though the distance it travels varies from experiment to experiment. There are two common failure modes: The dancer can get stuck at a certain location or prematurely detach from the track. Sometimes these failures occur after only one or two steps. By diagnosing how they occur, we gain insight into how to achieve better control.

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 variability could arise from the sensitivity of the melting transitions to variations in the DNA concentrations in solution in different samples, from stochastic variation in the density of strands from particle to particle, or from slight variations of the positions and spacings of the track particles.

A dancer system that performs as well as that shown in Figure 3 requires repeated experiments on both single-color squares and three-color tracks in order to iteratively tune the displacing-strand concentrations. For instance, when 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. When 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.

Additional Discussion. Fundamentally, the number of steps that the dancer can take is limited by the trade-off between the rolling rate and detachment probability. As discussed in the "Design Considerations" section, as the interaction strength between the dancer and the track increases, both the detachment probability and the rolling rate decrease.[31−33] We choose our sequences and concentrations to optimize the rolling rate while maintaining a low detachment probability. Because this optimization is done experimentally, our result, a maximum of five sequential steps, is probably close to the best that can be obtained for this system.

But there are other choices of systems. Our method of functionalizing the particles is straightforward to implement, but it leads to a narrow parameter range in which the particles neither fall off the track nor get stuck. Other, more recent methods for functionalizing particles[31,35] result in a much higher density of DNA strands (more than 100 000 strands for a 1 μm particle, compared to the approximately 6500 strands using the present method). By functionalizing the particles with a higher density of strands, each of which interacts more weakly with its complement, it should be possible to break the trade-off between detachment probability and rolling rate:

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ACS Nano 2022, 16, 9195−9202

Owing to the weak interactions between the complementary strands, each DNA duplex would break more rapidly and reform, while owing to the high coverage, the probability of having no duplexes linking the particles at any given time would remain low. If the rolling rate can be increased to the diffusion limit (where particles diffuse as quickly as hydrodynamics allows), then the maximum number of steps would increase dramatically for the same interaction strength (see Figure S1). Furthermore, the concentrations and sequences would likely not need to be tuned so carefully to yield a dancer that can take a large number of steps without detaching from the track. A higher density of DNA strands would also reduce any particle-to-particle stochasticity in the number of interacting strands and thus the interaction strengths.

Another possible modification for our system would be to decouple the interactions of the different track particles entirely. This could be accomplished using a system where, instead of changing the bulk solution temperature, one modifies the temperature of each particle species independently through the absorption of different wavelengths of light.[36]

Nonetheless, the system we have tested provides an important demonstration of concept and, more generally, a basis for understanding how to program motion on the micrometer scale. The basic requirements include a substrate with a repeated pattern that is asymmetric, 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 speed.

CONCLUSIONS

We have shown that isotropic colloidal particles can be driven to undergo directed motion using a sequence of temperature changes by exploiting the versatility of DNA strand-displacement reactions. This programmability is essential to creating the thermal ratcheting behavior we have demonstrated.

Our thermally operated dancer sheds light on design principles and fabrication strategies that are not limited to a specific experimental system. These strategies could be extended to controlled motion on a two-dimensional substrate or within a three-dimensional network. Beyond transport, the ability to direct rearrangements of particles could be used to create structures with reconfigurable geometries. These programmable rearrangements could ultimately enable assembly line-type construction of desired structures that cannot be assembled through bulk equilibrium pathways.

More broadly, this type of 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 two centuries,[37] it is only recently that heat engines have been reduced to micrometer sizes and energy scales of a few k_BT.[38] Future variants of our system, which is also a heat engine, could be coupled to external loads so that the displacement and work done could be directly measured.

METHODS

DNA Design. When designing the DNA to mediate the interactions between the dancer and each of the track particles, we follow the criteria laid out in ref 12. Most importantly, this involves correctly designing the thermodynamic parameters (enthalpy and entropy contribution to binding energy) of the DNA sequences. For the single-strand-displacement case, we must satisfy the case $\Delta H_{3A,3B} = \Delta H_{3A,3D_1}$ so that the interaction is weakened at lower temperatures. For the two-displacement case, we must set $\Delta G_{4A,4B} = \Delta G_{4A,4D_1} = \Delta G_{4B,4D_2}$ to realize a low-temperature unbound state and an intermediate-temperature bound state. These last equalities must also hold for strands 5A, 5B, $5D_1$, and $5D_2$.

Beyond fulfilling these constraints on the thermodynamic parameters, the specific DNA sequences must be chosen to yield the correct melting temperatures. 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 during DNA design. In addition, all DNA must be designed to eliminate unintended cross-talk and secondary structure. Finally, we ensure that each DNA sequence has a toehold to maximize the kinetics of the system and allow the particles to roll. Each of these requirements is verified using the online software NUPACK,[39] MFold,[40] and our in-house software package (https://github.com/manoharan-lab/DNA-colloid-design).

Functionalizing Particles with DNA and Dyeing Them Fluorescently. The method for functionalizing particles with DNA is adapted from ref 41. A detailed protocol (including safety hazards) is given in Supporting Information section S6. In short, we chemically bonded our DNA oligomer (IDT) to a triblock copolymer. We swelled the particles in the presence of this DNA-functionalized polymer to allow the hydrophobic central block to infiltrate the particle's surface and then deswelled to physically entrap the triblock copolymer and therefore physically graft the DNA to the particle.

For confocal microscopy, we labeled 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. To incorporate the dye into the particles, we added dye to the solvent that was used to swell the particles. The brightness of the signal can be adjusted by changing the concentration of the dye in the solvent.

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.[42] To circumvent this problem, we carefully selected fluorescent dyes so that the particles were stable under exposure to light for as long as possible. We used 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.

Sample Preparation and Imaging. Samples used for confocal experiments contained a 1:1:1:1 mixture of particles at a total volume fraction of 0.66% in 250 mM NaCl. The sample was sealed between two plasma-cleaned coverslips using silicone vacuum grease (Dow Corning). Confocal experiments were carried out on a Leica SP5 confocal microscope where the dyes are imaged simultaneously as three detectors collect emitted photons in different wavelength bands.

Samples for dancing experiments contained a 1:1:1:1 mixture of particles at a total volume fraction of 0.25% in 250 mM NaCl. The sample chamber was prepared as described in the "Track Construction" section below. Imaging was carried out using an inverted optical microscope (Nikon TE2000-E) using a 100× oil-immersion objective (NA 1.4, Nikon). Micrographs and videos were collected in brightfield illumination or under fluorescence imaging using a Lumencor Spectra-X light engine containing 7 bandpass filters (390/22, 438/29, 485/25, 513/22, 560/32, 585/29, and 648/20) in conjunction with a multiband filter set (Semrock) that allows for imaging of up to four fluorophores simultaneously (exciter: 387/485/559/649 nm quad-pass bandpass filter, emitter: 440/521/607/700

https://doi.org/10.1021/acsnano.2c01454
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nm quad-pass bandpass filter, dichroic: 410/504/582/669 nm quad-edge beam splitter).

The temperature of the sample was controlled concurrently by two heaters. Silicone vacuum grease was used to hold the sample in direct contact with a thermoelectric cooler (TE Technology, Inc.), which was driven by a high-performance digital temperature controller (Thorlabs). To minimize heat loss through the objective, we wrapped a resistive heater (Bioscience Tools) around the objective, which was in contact with the sample through immersion oil. This heater was driven by a low-noise temperature controller (Bioscience Tools). The two heaters were adjusted synchronously for each change in temperature to ensure the most homogeneous temperature possible. The actual temperature at the sample was measured and recorded in real time using a resistive temperature detector (Thorlabs) that was held between the thermoelectric cooler and the sample.

Track Construction. We constructed our tracks using 24 × 60 and 22 × 22 No. 1 cover glass (VWR). The coverslips can be passivated by plasma cleaning to functionalize the surface with OH^- groups. We passivated the entire surface of the large coverslip, but we passivated only half of the small coverslip to create a sticky region in which we could construct tracks. To passivate only half of the chamber, we offset two slides from one another and held them together with binder clips such that half of each slide was covered by the other. We then assembled the sample chamber with these slides using the method described above. To construct the track, we worked near the interface between the sticky and passivated regions. Because the track was two particles wide, the chance that the dancer would diffuse off of the edge of the track and stick to the glass was small.

Optical Tweezers. The optical tweezer setup was constructed on a Nikon TE2000-E microscope. A 671 nm, 40 mW laser beam (Shanghai Dream Lasers Technology Co., Ltd.) was collimated and expanded so that it had an approximately 1 in. diameter. We then sent the beam into the back of a 100× oil-immersion objective (NA 1.4, Nikon) by sending it through a 45°, 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. ***Caution:*** *Laser safety glasses should be worn when aligning lasers.*

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsnano.2c01454.

Calculations, and experimental details (PDF)

Movies 1−3 showing a simulation of dancer motion, the process of constructing the track, and the dancer moving on the track (MP4, MP4, MP4)

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was funded by the National Science Foundation through grant no. DMR-1435964, the Harvard MRSEC through grant no. DMR-1420570, and the Army Research Office through the MURI program under award no. W911NF-13-1-0383.

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https://doi.org/10.1021/acsnano.2c01454
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