

A simple method to reprogram the binding specificity of DNA-coated colloids that crystallize

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Abstract

DNA-coated colloids can crystallize into a multitude of lattices, ranging from face-centered cubic to diamond and thereby contribute to our understanding of crystallization and open avenues to producing structures with useful photonic properties. Despite the broad potential design space of DNA-coated colloids, the design cycle for synthesizing DNA-coated particles is slow: preparing a particle with a new type of DNA sequence takes more than one day and requires custom-made and chemically modified DNA that typically takes the supplier over a month to synthesize. Here, we introduce a method to generate particles with custom sequences from a single feed stock in under an hour at ambient conditions. Our method appends new DNA domains onto the DNA grafted to colloidal particles based on a template that takes the supplier less than a week to produce. The resultant particles crystallize as readily and at the same temperature as those produced via direct chemical synthesis. Moreover, we show that particles coated with a single sequence can be converted into a variety of building blocks with differing specificities by appending different DNA sequences to them. This approach to DNA-coated particle preparation will make it practical to identify optimal and complex particle sequence designs and to expand the use of DNA-coated colloids to a much broader range of investigators and commercial entities.

1 Introduction

Due to the specificity of DNA hybridization, orthogonal interactions can be prescribed between microscopic objects by coating the objects with orthogonal, complementary pairs of single-stranded DNA[1, 2, 3]; building blocks with complementary sequences have short-ranged

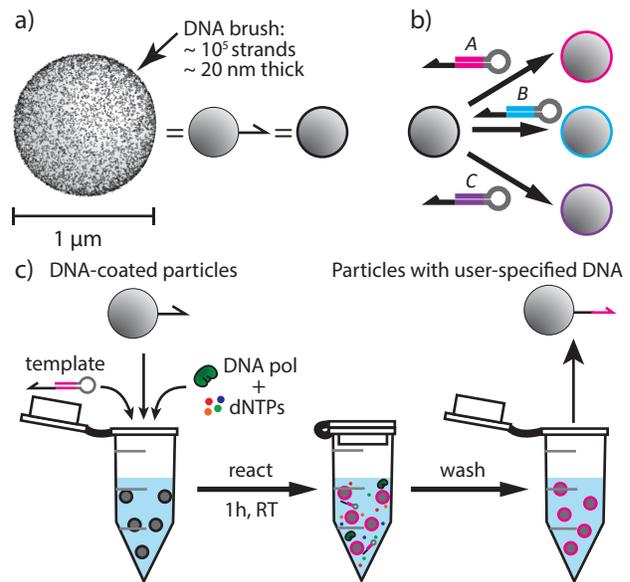


Figure 1: a) Schematic of DNA-coated colloids. Cartoon of DNA brush on colloid is copied from Ref [1] b) The primer exchange reaction enables the production of a range of DNA-coated colloids with distinct binding specificities from a single particle feed stock. c) Overview of the primer exchange reaction (PER) that extends the DNA on DNA-coated particles. DNA polymerase, a deoxynucleotide triphosphate (dNTP) mixture, and a DNA sequence template are mixed in an Eppendorf tube and left at room temperature. Then the particles are separated from the reaction mixture by centrifugation, at which point they are ready to be used in self-assembly experiments.

attractive interactions resulting from the hybridization of the DNA on their surfaces[4]. This use of DNA is an established strategy for producing building blocks that can assemble into a wide variety of microscopic structures, including stick figures [5], crystal lattices [6, 7, 8, 9], flexible bead-chains [10, 11], chiral clusters [12], and even cell aggregates [13]. Because DNA-coated microparticles (Fig. 1a) have sizes comparable to the wavelength of visible light, they are particularly promising building blocks for the self-assembly of photonic bandgap materials [14, 15, 16, 29], with applications in optical wave guides, lasers, and various light-harvesting technologies. DNA-coated microparticles are also useful as model systems for self-assembly, both in [17] and out of equilibrium [18, 19].

DNA can be grafted onto colloidal particles in various ways, but not all methods produce particles that are compatible with equilibrium assembly of colloidal crystals [20]. When biotin-streptavidin chemistry is used to attach single-stranded DNA to particles, the particles tend to hit-and-stick and become kinetically trapped in fractal-like aggregates, even at temperatures at which the DNA-mediated interactions are reversible [21, 22]. Attaching DNA to particles using strategies based on strain-promoted click chemistry [24, 25, 26] produce DNA-coated colloids that crystallize [27, 28, 29]. However, these click-chemistry-based methods are time-consuming and require specialized knowledge of synthetic chemistry, which stands in the way of the widespread use of DNA-coated colloids. Moreover, these methods require dibenzocyclooctyne (DBCO)-functionalized DNA, which currently takes roughly a month to be commercially synthesized[30], so that—even if one has the expertise necessary for this synthesis—the time between the initial idea and the experiment is over a month. Last, once the particles are synthesized, their DNA sequences and thus their specificities for other particles are fixed. New particles must therefore be synthesized for each experiment or application that requires a unique DNA sequence.

Here we introduce a simple method to synthesize DNA-coated particles with user-prescribed sequences from a single particle feed stock (Fig. 1b). Our method decouples the expensive and time-consuming step of attaching DNA to colloidal particles from the step of tailoring the DNA sequence for its particular purpose, enabling one to convert the sequence on a batch of DNA-coated particles into another sequence for each new experiment, rather than redoing the DNA-coating procedure. Our method uses the Primer Exchange Reaction (PER), introduced by Kishi *et al.* in 2019 [31], to append a user-specified domain to the end of an input DNA sequence coating the particles. We show that this reaction can completely convert the particles’ DNA sequence within 1 hour (Fig. 1c) and that particles synthesized using this method—from now on referred to as PER-edited

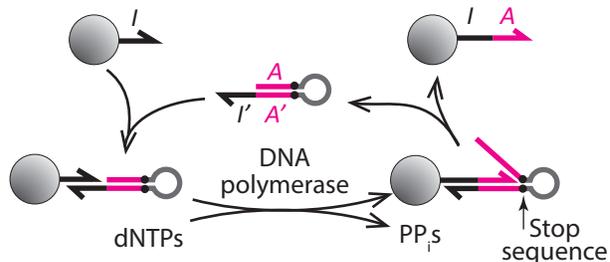


Figure 2: Schematic of the primer exchange reaction. dNTPs are DNA nucleotides and $PP_{i,s}$ are inorganic pyrophosphates. The input sequence, I , is 9 nucleotides, and the output domain, A , is 11 nucleotides. Sequences are in the Supplementary Information (SI section 1.6). To stop the DNA polymerase from copying more DNA after it has copied the output domain, an artificial stop sequence is incorporated in the template. This stop sequence consists of two nucleotides of which the complement cannot be incorporated because the corresponding NTP is not present in the reaction mixture. For example, in a solution that lacks GTP, the incorporation of a G stops the polymerase. More details on designing stop sequences are provided in the SI (section 2).

particles—assemble as readily and have the same melting temperature as particles produced directly *via* click chemistry—from now on referred to as reference particles. We also show that a single type of DNA-coated particle can be converted into a variety of particles with different DNA sequences and binding specificities (Fig. 1b), thereby overcoming some of the key technical bottlenecks to the synthesis of DNA-coated colloids and facilitating their widespread use.

2 Results

2.1 Conversion

We first ask whether the primer exchange reaction can be used to append new sequence domains onto DNA-coated colloids. The primer exchange reaction (schematically depicted in Figure 2) involves the reversible hybridization of a single-stranded input, I , to the input-binding domain, I' , of a catalytic hairpin. When I and I' are bound, DNA polymerase produces the complement to the hairpin’s template sequence, A' , appending the A domain onto the input strand, resulting in a longer single-stranded output, IA (Fig. 2a). The catalytic hairpin strand, which is only weakly bound to the output strand, is eventually released and can bind another input strand.

The particles whose DNA we set out to “edit” using PER are 600-nm and 1- μ m-diameter polystyrene colloids with single-stranded DNA grafted onto their surface *via* the click chemistry method developed by Oh *et al.* [25] (detailed methods in SI). The grafted sequence

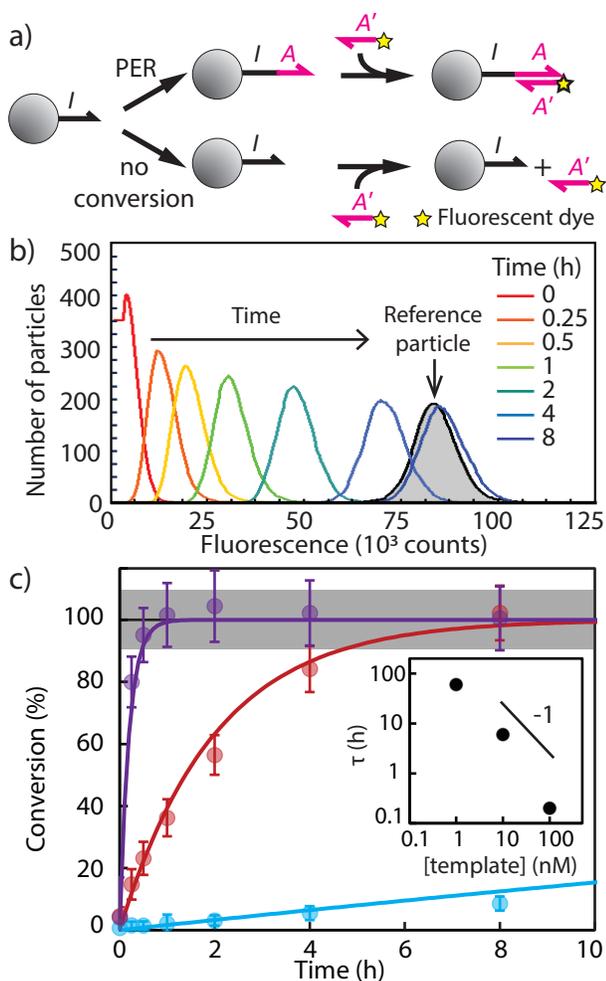


Figure 3: a) Schematic of the labeling reaction used to quantify the DNA conversion on particles. The fluorescently labeled strand with sequence A' is complementary to the DNA on PER-edited particles, but not to the input sequence so that the amount of fluorescence indicates the degree of conversion. b) Distribution of the single-particle fluorescence of DNA-coated particles after increasing reaction times, as measured using flow cytometry. The average fluorescence is a measure of conversion. The gray shaded region indicates the fluorescence of reference particles to which the sequence A' was attached through click chemistry. Details of flow cytometry experiments are provided in the Supporting Information (SI section 1.4). Each histogram represents ten thousand particles. The template concentration was 10 nM. c) PER conversion as function of time for 1 nM (blue), 10 nM (red), 100 nM (purple) template. Higher template concentrations lead to faster conversion. Error bars represent the standard deviations of the fluorescence distributions. The particles are 600 nm in diameter. The inset shows the typical reaction time, τ , as a function of template concentration. The inset shows that the typical reaction time τ scales linearly with the inverse template concentration.

consists of a 40-nucleotide poly-T spacer followed by a 9-nucleotide input domain, I . We measured a grafting density of $3.6 \pm 0.2 \times 10^4$ strands/ μm^2 on these particles (Fig. S4).

To test whether PER could append a new domain onto the DNA on the 500 nm particles, we mixed the particles at 0.1% (v/v) with 1 – 100 nM hairpin strand, 100 μM of each nucleotide triphosphate, and 0.13 U/ μL DNA polymerase, and let the reaction proceed at room temperature for 1 hour. After the reaction, we washed the particles by centrifugation and resuspension. See SI for details of the synthesis and DNA sequences.

To quantify the PER conversion of DNA on the DNA-coated colloids, we added fluorescently labeled strands to the particles after the reaction and measured the fluorescent signal of ten thousand individual particles using flow cytometry (detailed methods in SI). The fluorescently labeled DNA strands had sequence A' and thus could only bind to PER-edited particles. Therefore, the fluorescent signal of each particle is a measure of the fraction of its DNA that has been converted (Fig. 3a). We determined the percent yield of the reaction by comparing the fluorescence intensity of PER-edited particles to that of reference particles to which the sequence IA (the target sequence of the PER reaction) was attached directly *via* click chemistry (gray shaded curve in Fig. 3b). When 10 nM hairpin was used in the PER reaction, conversion of the DNA on the particles was complete after 8 hours (Fig. 3b).

Measurements of particle fluorescence after increasing reaction times indicated that the average conversion per particle increases monotonically until complete conversion is reached. Notably, when the conversion is partial, similar fractions of DNA on each particle are converted. In other words, no two sub-populations exist of entirely unconverted and entirely converted particles (Fig. 3b). This observation suggests that by tuning the reaction time, a controllable fraction of the DNA on DNA-coated colloids can be edited with PER.

To test whether we could tune the conversion rate, we varied the concentration of catalytic hairpin used in the PER reaction. Figure 3c shows the conversion as a function of the reaction time for hairpin concentrations of 1 nM, 10 nM, and 100 nM. We found that the two highest concentrations reach complete conversion with a rate that increases approximately linearly with hairpin concentration. The time to complete conversion was 1 hour with 100 nM hairpin and 8 hours with 10 nM hairpin (Fig. 3c). With 1 nM hairpin, the reaction did not go to completion within 7 days (Fig. S1). Fitting the measured conversion as a function of time to a single exponential yielded estimates of the typical reaction time where $1/e$ of the reactant was converted (inset Fig. 3c). We measured values that are a factor of 3 larger than predictions based on the rates of the PER reaction in solution (Fig. S1) [34]. The decreased PER rate when the substrate

DNA is grafted onto colloidal surfaces compared to DNA free in solution is likely due to the steric hindrance of the DNA polymerase in the dense DNA coating.

To check whether any unintended side products formed, we also performed PER on particles coated with streptavidin to which biotinylated DNA was attached (Fig. S2). The streptavidin-biotin bond can be broken by heat denaturing streptavidin at 95°C in 50% formamide solution [35]. Using this method, we removed the DNA from the surfaces of the particles after the primer exchange reaction and analyzed the product sequences using gel electrophoresis. From this experiment we learned that only DNA strands with lengths that correspond to the lengths of the reactant and product sequences were present on the particles after the reaction. No significant concentrations of unintended side products were formed (Fig. S2).

2.2 Crystallization

Our PER-editing method is intended to produce self-assembly building blocks, so we next asked whether PER-edited particles have similar assembly properties to the reference particles (to which the full sequence is synthesized and then grafted to the particles). Typically, when a binary system of colloids coated with complementary single-stranded DNA sequences is combined, the particles form aggregates below a certain transition temperature, called the melting temperature. Above the melting temperature the colloids are unaggregated and form a stable dispersion[1]. The melting temperature increases with the hybridization free energy of the DNA strands involved in the inter-particle binding and with their grafting density[36], so if the PER-edited particles and the reference particles have the same grafting density and sequence, as we expect based on the flow cytometry data, their melting temperature should also be the same.

To determine the melting temperature we prepared samples containing either the reference particles or the PER-edited particles, and particles to which DNA containing a 7-nucleotide complementary domain was attached, which we call co-assemblers. We placed these samples on a custom-built heating element (SI section 1.3) on a microscope and found that both samples had aggregated. We then heated the sample slowly to the melting temperature, *i.e.* the temperature at which approximately half the particles were part of aggregates and half were dispersed. Measurements of the fraction of single particles as function of the temperature are shown in the Supplementary Information (Fig. S6). While observing the behavior of particles during heating with a 60x oil-immersion lens, the thermistor on our heating stage showed that aggregates of PER-edited particles and their co-assemblers melted at 63°C and aggregates of the reference particles and their co-assemblers at 65°C (Fig. 4). These measurements indicate that both particles have similar binding free energies. The 60x oil im-

mersion objective changes the thermal load of the sample, so that the actual temperature of the sample may be lower than that reported by the temperature controller. Indeed, with a 40x air objective we found melting temperatures around 52°C for reference particles and 50°C for PER-edited particles.

Below the melting temperature, the structure that corresponds to a global minimum in the free energy landscape for a binary mixture of DNA-coated colloids is a crystal lattice, isostructural to cesium chloride[27], that maximizes the number of contacts between the complementary particles. However, this equilibrium structure is kinetically difficult to reach, and only accessible if the particles can roll on the surface of their neighbours after binding, which requires both a high density and a homogeneous distribution of grafted DNA[20, 29]. Both reference particles and input particles for PER are produced in a way that results in a DNA coating that facilitates crystallization, so we asked whether the ability to crystallize is maintained after PER.

We found that both the reference particles and the PER-edited particles crystallized readily when kept near the melting temperature (Fig. 4), indicating that the PER-edited particles are suitable building blocks for equilibrium self-assembly.

The flow cytometry data in Figure 3 show that a specific fraction of DNA on each particle can be converted by choosing an appropriate template concentration and reaction time. To check if such partially converted particles are also suitable for self-assembly we asked how the melting temperature scales with the percentage of DNA on particles that is converted and what conversion is required for the particles to crystallize. To this end, we prepared PER-edited particles with conversion percentages ranging from 0.1% to 100% by varying the reaction time and template concentration. We mixed these partially converted particles with co-assemblers, similar to the experiment in Fig 4, and measured the melting temperatures.

As the conversion increases from 0%, the melting temperature also increases until the melting temperature of the reference particles, 52°C, is reached at approximately 40% conversion. Above 40% conversion the melting temperature plateaued (Fig. 5). The observed increase of the melting temperature is consistent with the increase in melting temperature as a function of grafting density observed in earlier work[36, 37], but the apparent plateau at 40% is surprising. A notable difference with previous studies that could explain this observation is that we only varied the conversion of one of the two binding partners, and kept the grafting density of the other binding partner constant, so that the average grafting density of DNA containing sticky ends involved in the two-particle interaction goes from 50% to 100% as the conversion goes from 0% to 100%. We also found that only particles with conversions over 30% could crystallize

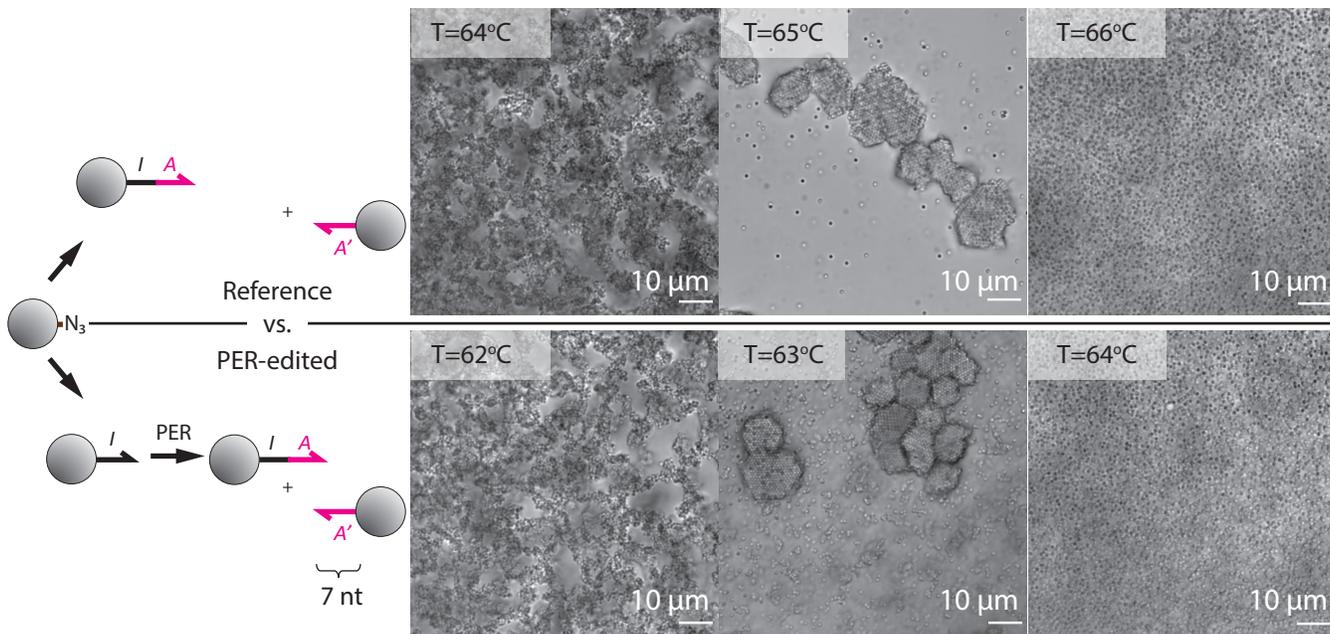


Figure 4: The self-assembly of PER-edited particles (bottom row) is compared to that of reference particles (top row). When mixed with particles coated with the complementary DNA sequence, both the reference and PER-edited particles formed random aggregates below the melting temperature, crystallized when held near the melting temperature, and were unassembled above the melting temperature. The complementary sequence, A' , contains 7 consecutive bases complementary with sequence A . All particles are 600 nm in diameter. The images were recorded with an oil-immersion objective which increases thermal contact with the sample so the actual sample temperature is lower than the reported temperature.

with their binding partners. Below that conversion random aggregates formed even at the melting temperature consistent with the notion that crystallization requires a threshold grafting density[20].

The collapse of the measured melting temperatures as a function of fractional PER conversion for different hairpin concentrations onto a single curve shows that the fraction of DNA that has been converted is the only factor that determines the melting temperature of the PER-edited particles and how that conversion is reached does not affect the outcome. These findings show that particles with controllable conversion and—by extension—controllable melting temperature can be prepared by tuning the reaction time and catalyst concentration.

The propensity of PER-edited particles to crystallize also depended on the PER conditions. Letting the reaction go for longer than necessary to reach 100% conversion, or using more than 0.13 U/ μ L Bst DNA polymerase resulted in particles that displayed non-specific aggregation even well above the melting temperature and did not crystallize (Fig. S3). The non-specific aggregation could be due to slow primer-independent or template-independent polymerization reactions [32, 33].

2.3 Reprogramming binding specificity

The key advance enabled by our synthesis method is that a single feed stock of DNA-coated colloids can be converted into multiple types of self-assembly building blocks with distinct binding specificities. To demonstrate this capability, we show that three different sequences can be appended onto the DNA on one type of “input” particle and that the resultant PER-edited particles have differing binding specificities (Fig. 6).

To this end we converted precursor particles with sequence I into assembly building blocks with sequences IA , IB , and IC using 100 nm of three different templates. We then mixed the three PER-edited particles with a set of three co-assemblers—DNA-coated particles that contain DNA complementary to each of the PER-edited particles: A' , B' , and C' . The co-assembler particles were fluorescently labeled with a magenta dye, a cyan dye, or both the magenta and the cyan dyes (shown as purple), respectively. The PER-edited particles were not fluorescently labeled and are not visible in the images. We annealed a suspension of all four particle types at the melting temperature and imaged the resultant crystals using confocal microscopy. If the single feedstock of DNA-coated particles has successfully been converted into three distinct types of building blocks with differing binding specificities, each of the PER-edited build-

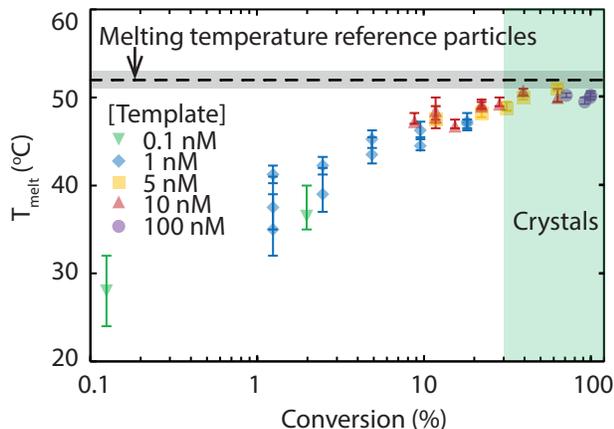


Figure 5: The melting temperature as a function of approximate conversion. The approximate conversions are calculated using the fits in Fig. 3. The melting temperature increased with conversion and plateaued near the melting temperature of the reference particles (dashed black line). The green area indicates conversions for which we observed that the particles could crystallize. Below 30% conversion, particles did not crystallize even if they were kept near the melting temperature. The melting temperature of unedited particles with the complementary particles is approximately 23°C , due to partial sequence complementarity between I and A' (no more than 2 sequential bases).

ing blocks should bind only to their target co-assembler and leave their off-target co-assembler particles free in solution.

Figure 6 shows that in each of the three samples, the PER-edited particles indeed co-crystallized only with their intended co-assembler; each of the crystals are either fully magenta, cyan, or purple. Notably, the co-assembler particles for the purple aggregates were also produced from the initial feed stock using the primer exchange reaction, showing that crystals form even if both types of DNA-coated particles in a binary system were produced using PER.

Finally, we tested whether multiple domains could be sequentially attached to a single particle. We tested the sequential attachment of up to three 11-nucleotide domains and found that the binding specificity of the particles successfully changed with each added DNA sequence (Fig. S5). However, particles to which more than one domain was added with PER were not able to crystallize with their complements.

3 Conclusions

We have introduced a method to rapidly and easily reprogram the binding specificity of DNA-coated colloids by appending new DNA domains onto the DNA grafted onto

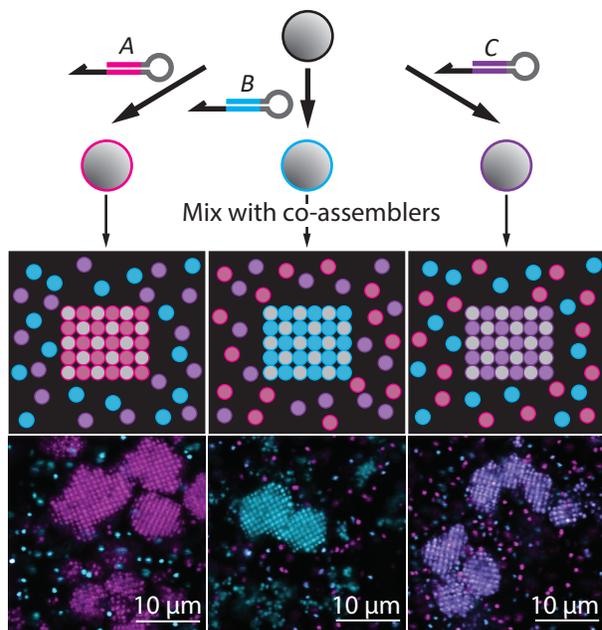


Figure 6: PER converts generic DNA-coated particle into three building blocks for self-assembly with differing binding specificities. The input particles (not fluorescently labeled) are converted into three batches of DNA-coated particles with differing sequence: A, B, and C. Each batch is mixed with three types of fluorescently labeled particles: magenta particles are coated with sequence A' , cyan particles are coated with B' , and purple particles are coated with C' . The samples are annealed at the melting temperature and imaged at the melting temperature under a confocal microscope. Each type of converted particle aggregated only with their respective complementary particle. Scale bars are $10\ \mu\text{m}$.

colloids. We showed that DNA-coated particles with differing binding specificities could be prepared from a single feed stock by appending new domains to the DNA on the colloids and that the particles maintained their ability to crystallize after the DNA extension procedure.

Colloid science and self-assembly have a range of open challenges that require access to building blocks with many orthogonal, tunable interactions to be addressed, such as the self-assembly of finite-sized structures of arbitrary shapes and sizes [18, 40, 41, 5] and understanding the nucleation and growth of multi-component crystals[42, 29]. DNA-coated particles are a logical choice of building block because their DNA-hybridization-mediated interactions enable on the order of 100 orthogonal interactions[43] with tunable strengths. However, the complexity and long duration of the synthesis—and importantly the optimization—of DNA-coated colloids has long been a barrier for their use. We think that our method will help quickly produce a wide variety of different DNA-coated colloids from a single feed-stock and optimize their designs so applications

and experiments requiring many different DNA-coated colloids come within reach. Our method may also help expand the use of DNA-coated particles beyond crystallization and self-assembly to sub-fields of colloid science such as gelation and rheology[44, 45, 46].

Our approach could potentially be extended beyond particles, to other systems where the grafting of DNA onto an object is expensive, time-consuming, or difficult, such as cells[13] and antibodies[39]. Our method could also be extended to change the binding specificity of single probes in DNA micro-arrays[47].

Beyond synthesis methods, this work also opens up the possibility of altering the binding properties of particles over time within one experiment, which could be a useful tool in dissipative self-assembly. Time-dependent interactions are increasingly sought after for their ability to create dynamic, reconfigurable, and adaptive structures, but currently few chemical strategies for achieving time-dependent interaction strengths are available [48, 49]. The primer exchange reaction could also be used to initiate sequential assembly stages and freeze objects into kinetically trapped structures by converting the DNA on particles at varying rates, controlled by the hairpin concentration.

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5 Author contributions

P.G.M., R.S., & W.B.R. conceived the experiments. P.G.M., T.E.V., & H.F. performed the experiments and analysis. All authors contributed to writing of the paper.

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