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Self-assembly of DNA-coded nanoclusters

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We present a theoretical discussion of a self-assembly scheme which makes it possible to use DNA to uniquely encode the composition and structure of microparticle and nanoparticle clusters. These anisotropic DNA-decorated clusters can be further used as building blocks for hierarchical self-assembly of larger structures. We address several important aspects of possible experimental implementation of the proposed scheme: the competition between different types of clusters in a solution, possible jamming in an unwanted configuration, and the degeneracy due to symmetry with respect to particle permutations.

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Over the past decade, a number of proposals have identified potential applications of DNA for self-assembly of microstructures and nanostructures [1–5]. Among these proposals, one common theme is finding a way to utilize the high degree of selectivity present in DNA-mediated interactions. An exciting and potentially promising application of these ideas is to use DNA-mediated interactions to program self-assembled nanoparticle structures [6–9]. Generically, these schemes utilize colloidal particles functionalized with specially designed ssDNA (markers), whose sequence defines the particle type (see Figs. 1 and 2). Selective, type-dependent interactions can then be introduced either by making the markers complementary to each other or by using linker-DNA chains whose ends are complementary to particular maker sequences. Independent of these studies, there are numerous proposals to make sophisticated nanoblocks which can be used for hierarchical self-assembly. One recent advance in the self-assembly of anisotropic clusters is the work of Manoharan *et al.* [10] They devised a scheme to produce stable clusters of n polystyrene microspheres. The clusters were assembled in a colloidal system consisting of evaporating oil droplets suspended in water, with the microspheres attached to the droplet interface. The resulting clusters, unique for each n, are optimal in the sense that they minimize the second moment of the mass distribution $M_2=\Sigma_{i=1}^{n}(\mathbf{r}_i-\mathbf{r}_{cm})^2$.

In this paper, we present a theoretical discussion of a method which essentially merges the two approaches. We propose to utilize DNA to self-assemble colloidal clusters, somewhat similar to those in Ref. [10]. An important aspect of the scheme is that the clusters are "decorated:" each particle in the resulting cluster is distinguished by a unique DNA marker sequence. As a result, the clusters have additional degrees of freedom associated with particle permutation and potentially may have more selective and sophisticated inter-cluster interactions essential for hierarchic self-assembly. In addition, the formation of such clusters would be an important step towards the programmable self-assembly of microstructures and nanostructures of an arbitrary shape, as suggested in Ref. [11].

We begin with octopuslike particles functionalized with dsDNA, with each strand terminated by a short ssDNA marker sequence. We assume that each particle i has a unique code—i.e., the maker sequence s_i of ssDNA attached to it. We then introduce anchor DNA to the system, ssDNA with sequence $\bar{s}_A\bar{s}_B\cdots\bar{s}_n$, with $\bar{s}_i$ the sequence complementary to the marker sequence s_i. The anchor is designed to hybridize with one particle of each type. Consider a cluster of n particles attached to a single anchor.

If we treat the DNA which link the particles to the anchor as Gaussian chains, there is an entropic contribution to the cluster free energy which can be expressed in terms of the particle configuration $\{\mathbf{r}_1,\ldots,\mathbf{r}_n\}$ as follows. Here R_g is the radius of gyration of the octopuslike DNA arms:

$$F=\frac{3k_BT}{2R_g^2}\sum_{i=1}^{n}(\mathbf{r}_i-\mathbf{r}_{anchor})^2. \tag{1}$$

This approximation of the DNA arms as Gaussian chains is acceptable provided their length L exceeds the persistence length $l_p\simeq 50$ nm and the probability of self-crossing is small [12]. The physical mechanism which determines the final particle configuration in our system is quite different from the capillary forces of Manoharan *et al.* However, because the functional form of the free energy is equivalent

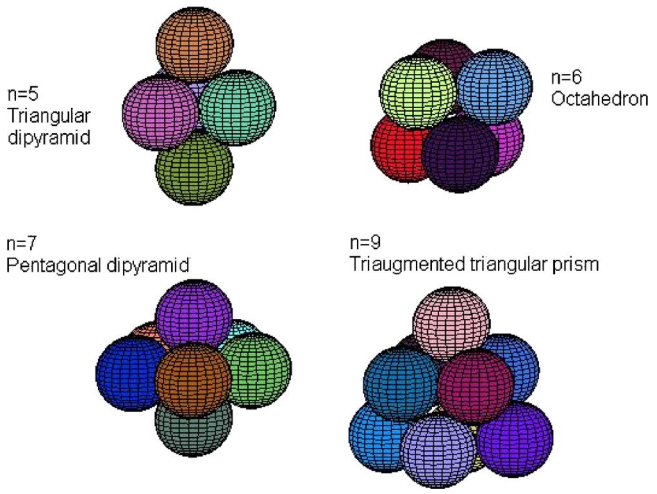

FIG. 1. (Color online) The minimal second moment clusters for $n=5$, 6, 7, and 9. Pictures of all the clusters from $n=4$ to 15 are available in Ref. [10].

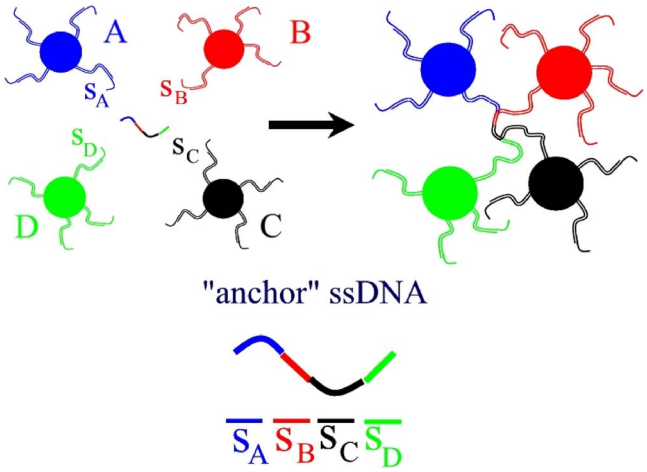

FIG. 2. (Color online) Schematic representation of the method for constructing decorated colloidal clusters using ssDNA "anchors."

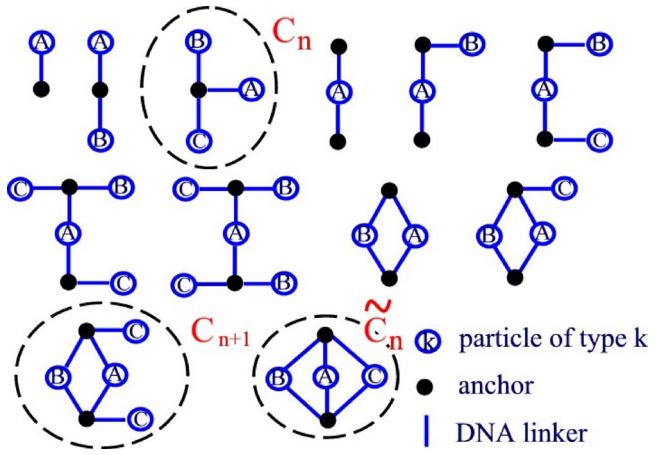

FIG. 3. (Color online) The topologically distinct one- and two-anchor structures for an anchor ssDNA with sequence $\bar{s}_A\bar{s}_B\bar{s}_C$. Different structure varieties may be obtained by relabeling the particle indices subject to the constraint that no more than one particle of each type is attached to a given anchor.

to the second moment of the mass distribution, the ground state of the cluster should correspond to the same optimal configuration.

Consider a system with n-particle species and an anchor of type $\bar{s}_A\bar{s}_B\cdots\bar{s}_n$. The clusters we would like to build contain n distinct particles (each particle in the cluster carries a different DNA marker sequence) attached to a single anchor. Let C_n denote the molar concentration of the desired one-anchor cluster. Because there are many DNAs attached to each particle, multiple-anchor structures can also form. The question is whether the experiment can be performed in a regime where the desired one-anchor structure dominates, avoiding gelation.

We consider the stability of type C_n with respect to alternative two anchor structures. To do so we determine the concentration C_{n+1} of $(n+1)$-particle structures which are maximally connected, but do not have a 1:1:...:1 composition. In particular, these structures contain more than one particle of each type, which could cause problems in our self-assembly scheme [11]. There are also n-particle structures $\tilde{C}_n$ with the correct composition, but which contain two anchors. We would like to avoid the formation of these structures as well, as their presence decreases the overall yield of type C_n. Figure 3 enumerates the various structures for an $n=3$ species system. If the experiment can be performed as hoped, we will find a regime where the ratios $\frac{C_{n+1}}{C_n}$ and $\frac{\tilde{C}_n}{C_n}$ are small. To this end, the equilibrium concentrations C_n, C_{n+1}, and $\tilde{C}_n$ are determined by equilibrating the chemical potential of the clusters with their constituents.

Let c_i denote the molar concentration of species i and c_a the molar anchor concentration. We consider the symmetrical case $\Delta G_i=\Delta G$ and equal initial particle concentrations $c_i^{(o)}=c^{(o)}$ for all species i. In this case we have $c_A=c_B=\cdots=c_n\equiv c$:

$$C_n = c_a\left(Nvc\exp\left[-\frac{\Delta G}{k_BT}\right]\right)^n. \tag{2}$$

The binding free energy of the cluster has a contribution from the hybridization free energy ΔG associated with attaching a particle to the anchor and an entropic contribution from the number of ways to construct the cluster (since each particle has N hybridizable DNA arms). In addition we must take into account the entropy for the internal degrees of freedom in the structure stemming from the flexibility of the DNA attachments to the anchor. In the Gaussian approximation, neglecting the excluded volume between particles, this localization volume $v=(2\pi/3)^{3/2}R_g^3$ can be calculated exactly by integrating over the various particle configurations weighted by the Boltzmann factor: $v^n=\int d^3\mathbf{r}_1\cdots d^3\mathbf{r}_n\exp[-F/k_BT]$.

We now consider the competing two-anchor structures C_{n+1} and $\tilde{C}_n$. The localization volumes $v_2=n^{-3/(n+2)}2^{-3(n-1)/2(n+2)}v$ and $v_3=n^{-3/2(n+1)}2^{-3n/2(n+1)}v$ can be calculated in a similar fashion to v. Since there are many DNA attached to each particle, in what follows we omit factors of $\frac{N-1}{N}$:

$$C_{n+1}\simeq\frac{v_2^{n+2}}{v^{2n}}\frac{C_n^2}{c^{n-1}}, \tag{3}$$

$$\tilde{C}_n\simeq\frac{v_3^{n+1}}{v^{2n}}\frac{C_n^2}{c^n}. \tag{4}$$

The concentration of free anchors, c_a, can be determined from the equation for anchor conservation:

$$c_a^{(o)}=c_a+C_n+2\tilde{C}_n+2nC_{n+1}. \tag{5}$$

We are interested in the low-temperature regime where there are no free anchors in solution. We determine the saturation values for the ratios of interest by noting that the Boltzmann factor $\delta\equiv\exp\left[-\frac{\Delta G}{k_BT}\right]\gg 1$ in this regime:

$$\frac{C_{n+1}}{C_n}\simeq\frac{n^{-3}2^{-3n+9/2}}{(R_g^3c^{(o)})^{n-2}}\frac{c_a^{(o)}}{c^{(o)}}+O\left(\frac{1}{\delta^n}\right), \tag{6}$$

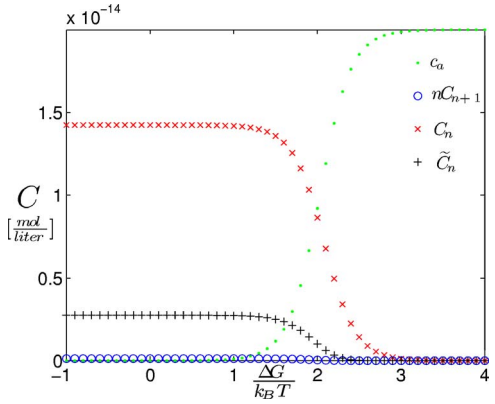

FIG. 4. (Color online) The molar concentrations c_a, C_n, nC_{n+1}, and $\tilde{C}_n$ in the symmetrical case for a system with $n=5$ particle species. The total particle volume fraction $n\phi \approx 0.25$ and $\frac{c_a^{(o)}}{c^{(o)}} = 10^{-3}$.

$$\frac{\tilde{C}_n}{C_n} \simeq \frac{n^{-3/2}2^{-3n+3/2}}{(R_g^3 c^{(o)})^{n-1}} \frac{c_a^{(o)}}{c^{(o)}} + O\left(\frac{1}{\delta^n}\right). \tag{7}$$

Since $\frac{C_{n+1}}{\tilde{C}_n} \ll 1$, Eq. (7) provides the experimental constraint for suppressing the two-anchor structures. Taking the radius of the hard spheres, $R \sim R_g$, it can be interpreted as a criterion for choosing the initial anchor concentration $c_a^{(o)}$ for an n species system with $\phi = \frac{4\pi}{3} R_g^3 c^{(o)}$ the particle volume fraction for an individual species:

$$\frac{c_a^{(o)}}{c^{(o)}} \lesssim n^{3/2}(2\phi)^{n-1}. \tag{8}$$

The condition gives the maximum anchor concentration for the two-anchor structures to be suppressed. Since $\phi \leqslant \frac{1}{n}$, the theoretical limits are $\frac{c_a^{(o)}}{c^{(o)}} \lesssim 1$, 0.29, 0.06, and 0.01 for $n=4$, 5, 6, and 7, respectively. In Fig. 4 we plot the solution for the concentrations. There is a large temperature regime $\left(\frac{\Delta G}{k_B T} \lesssim 2\right)$ where the two-anchor structures are suppressed in favor of the desired one-anchor structures.

The experimental criterion for suppression of the two-anchor structures [Eq. (8)] provides a fairly strict bound on the anchor concentration and, hence, the cluster yield for $n \geqslant 5$. However, the previous discussion considers only the equilibrium concentrations. Below the melting temperature (see Fig. 4), the connections are nearly irreversible. In the irreversible regime where the DNA binding is very strong, the probability that a cluster of type C_n becomes attached to a second anchor is $p \sim n \frac{c_a^{(o)}}{c^{(o)}}$. The factor of n counts the possible attachment sites to the cluster. In terms of the fraction $f_n \sim \frac{c_a^{(o)}}{c^{(o)}}$ of particles in clusters of type C_n, the fraction of particles, f_2, in the two-anchor structures will be $f_2 \sim \frac{p}{2} f_n = \frac{n}{2}\left(\frac{c_a^{(o)}}{c^{(o)}}\right)^2$. Here the factor of $\frac{1}{2}$ is necessary since the initial attachment can be to either of the two anchors. As indicated in Fig. 5, provided the anchor concentration is

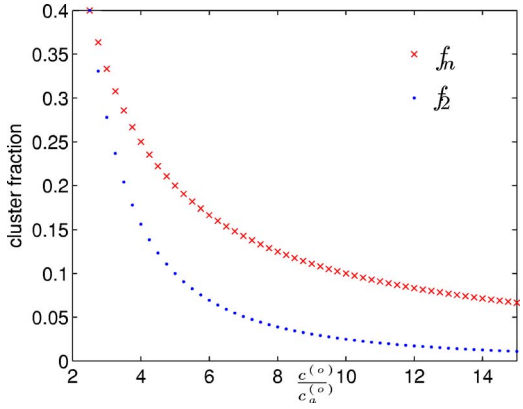

FIG. 5. (Color online) Cluster fractions as a function of the concentration ratio $\frac{c^{(o)}}{c_a^{(o)}}$ for an $n=5$ species system in the irreversible binding regime. f_n is the fraction of the desired one-anchor structures and f_2 is the fraction of two-anchor structures.

dilute enough, $\frac{c^{(o)}}{c_a^{(o)}} \gtrsim 4$, the formation of the two-anchor structures is suppressed.

We now present a brief discussion of the role that jamming plays in preventing the one-anchor structures from assuming the minimal second-moment configuration. We performed simulations of the assembly of optimal colloidal clusters up to $n=9$ particles by numerically integrating the particles' Langevin equations:

$$b^{-1}\frac{d\mathbf{r}_i}{dt} = -\nabla_i H + \eta_i. \tag{9}$$

Here b is the particle mobility and the thermal noise has been artificially suppressed (i.e., $\eta=0$). The model Hamiltonian H used in these simulations has been discussed in detail elsewhere [11]. As indicated in Fig. 6, the hard-sphere system gets trapped in a configuration with a larger M_2 than the

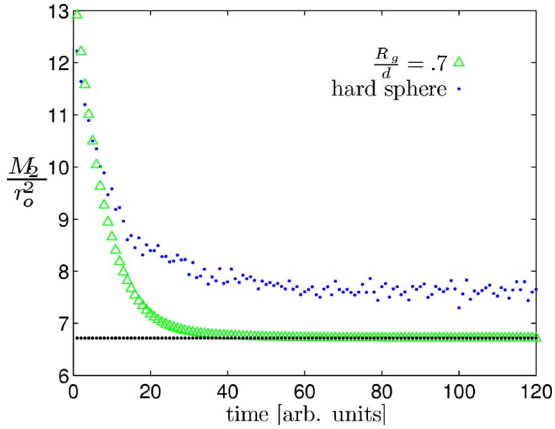

FIG. 6. (Color online) Plot of the dimensionless second moment $\frac{M_2}{r_0^2}$ as a function of time for $n=9$ particles. Results are shown for the case of hard spheres and also for a system with a soft-core repulsion with geometric parameter $\frac{R_g}{d}=0.7$. The dashed line is the theoretical moment for the triaugmented triangular prism, which is the minimal $n=9$ structure.

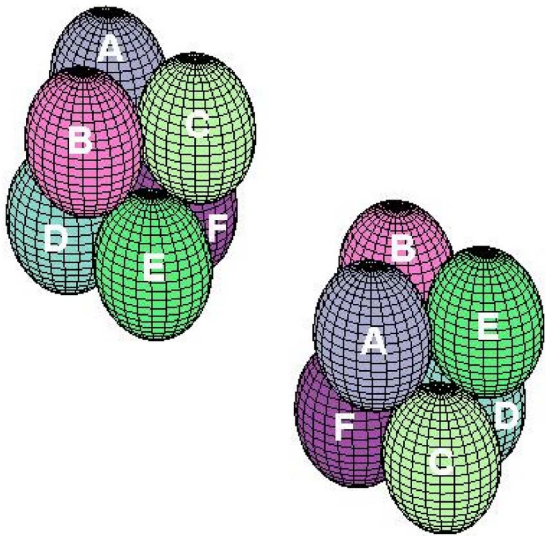

FIG. 7. (Color online) An illustration of degeneracy in DNA-coded nanoclusters. Two different $n=6$ isomers are pictured, both with the same minimal second-moment configuration, the octahedron.

optimal cluster, whereas the soft-core system is able to fully relax. The jamming behavior is largely determined by the single control parameter $\frac{R_g}{d}$, with d the diameter of the hard sphere. Beyond the critical value $\frac{R_g}{d} \gtrsim 0.5$ the jamming behavior is either completely eliminated or greatly reduced in the case of larger clusters.

Building these decorated colloidal clusters is the first major experimental step in a new self-assembly proposal. However, in order to utilize the resulting clusters as building blocks, an additional ordering is necessary. The problem is that the decoration introduces degeneracy in the ground-state configuration. This degeneracy was not present in Ref. [10] since all the polystyrene spheres were identical. Namely, in the colloidal clusters self-assembled by our method, permuting the particle labels in a cluster does not change the second moment of the mass distribution (see Fig. 7). We need a method to select a single "isomer" out of the many present after self-assembly. In the DNA-colloidal system considered here, this isomer selection can be facilitated by "linker" ssDNA. These are short ssDNA with sequence $\bar{s}_A \bar{s}_B$ to connect particles A and B. We first construct a list of nearest neighbors for the chosen isomer and introduce linker DNA for each nearest-neighbor pair. The octopuslike DNA arms of the given particles will hybridize to the linkers, resulting in a springlike attraction between the selected particle pairs. Note that the length L of the DNA arms must be on the order of the linear dimension of the original cluster. Otherwise the interparticle links cannot form upon the introduction of linker DNA to the system. It should be noted that although this method breaks the permutation degeneracy of a cluster, the right-left degeneracy will still be present.

In conclusion we discussed a method which uses DNA to self-assemble anisotropic colloidal building blocks. We found an experimentally accessible regime where the resulting clusters are minimal second-moment configurations. In addition, the clusters are decorated: each particle in the cluster is distinguished by a unique marker DNA sequence. The cluster formation process provides an interesting model system to study a type of jamming-unjamming transition in colloids. Constructing decorated colloidal clusters would represent a major step towards realizing the long-term potential of DNA-based self-assembly schemes.

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