How to build nanoblocks using DNA scaffolds

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Abstract – In recent years there have been a number of proposals to utilize the specificity of DNA-based interactions for potential applications in nanoscience. One interesting direction is the self-assembly of micro- and nanoparticle clusters using DNA scaffolds. In this letter we consider a DNA scaffold method to self-assemble clusters of "colored" particles. Stable clusters of identical microspheres have recently been produced by an entirely different method. Our DNA-based approach self-assembles clusters with additional degrees of freedom associated with particle permutation. We demonstrate that in the non-equilibrium regime of irreversible binding the self-assembly process is experimentally feasible. These color degrees of freedom may allow for more diverse intercluster interactions essential for hierarchical self-assembly of larger structures.

DNA has attracted significant attention for its potential applications in nanoscience [1–10]. One recent non–DNA-based advance is the self-assembly of stable clusters composed of identical microspheres [11]. In this letter we consider the self-assembly of micro- and nanoparticle clusters similar to those of [11], where DNA scaffolds govern the self-assembly process. The plan for the letter is the following. We first introduce the basic strategy of our self-assembly proposal. The goal is to maximize the yield for a particular type of cluster we call the star cluster. We analytically calculate the yield of the star cluster in the regime of irreversible binding. The analytical results are compared to the numerical results for the full aggregation equations. From an experimental perspective, the most important result is the determination of an optimal concentration ratio for experiments (see eq. (8)). To conclude we discuss the experimental feasibility of the self-assembly proposal.

The basic idea behind the procedure is as follows (see fig. 1). Particles are functionalized with single-stranded DNA (ssDNA) markers which determine the particle color. There may be many DNA attached to each particle, but on any given particle the marker sequence is identical. One then introduces DNA scaffolds to the system. The scaffold is a structure with f ssDNA markers, each marker
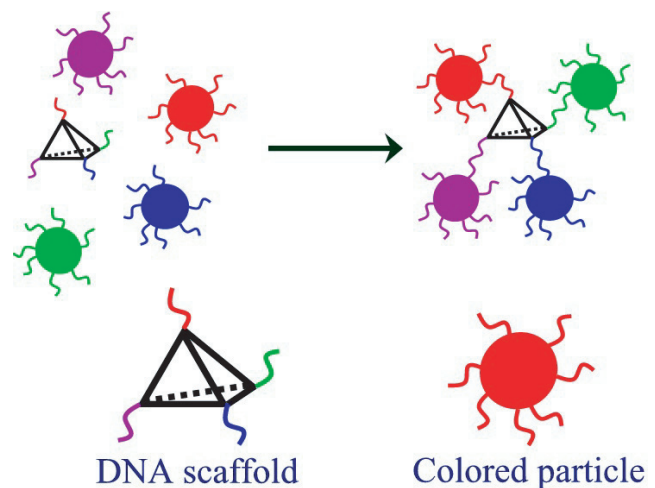

Fig. 1: (Colour on-line) DNA scaffolding. A graphical depiction of the scheme for self-assembling star clusters using DNA scaffolds. In the diagram (not drawn to scale) the scaffold funcionality $f = 4$.

complementary to one of the particle colors. Hybridization of the ssDNA markers on the particles to those on the scaffold results in the formation of colored particle clusters. Because there are many DNA attached to each particle, clusters can form which contain more than one scaffold. The essential goal of the procedure is to maximize

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the concentration of a particular type of cluster which we denote the star cluster. The star cluster contains one and only one scaffold to which f particles are attached, each particle having a distinct color.

We should note that the role of the scaffold could also be played by a patchy particle [12–14]. For example, these patches are regions on the particle surface where one can graft ssDNA markers. In this case there may be several DNA connections between a patch and colored particle. Our conclusions will still be valid, provided the patch size is chosen so that a patch interacts with at most one particle.

Previously we performed an equilibrium calculation to determine the yield of the star cluster [15]. The results of that study indicated that the concentration of scaffolds must be kept very small to prevent the aggregation of larger clusters. From an experimental perspective this result is somewhat disappointing, since the overall yield of the star cluster is proportional to the scaffold concentration. The situation is considerably improved in the regime of irreversible binding of particles to scaffolds. In what follows we present a calculation for the yield of the star cluster far from equilibrium.

The remainder of the paper is divided into two major sections. In the first part, we introduce a set of simplifying assumptions which permit an analytical calculation of the star cluster yield. The major assumption is that the aggregation process between scaffolds and particles is diffusion limited. Motivated by the disparity in size between the particles and scaffolds, we discuss how the aggregation is effectively a two stage process. In the second section we remove these simplifying assumptions. In general the rate constants will depend on both the clusters' diffusion coefficients, as well as the finite chemical rates for cluster-cluster aggregation. The cost associated with making these changes is that we must resort to a numerical solution of the aggregation equations. The results of the analytical calculation can then be checked against the numerical results.

To understand the basic physics behind the aggregation process we consider the mobility mismatch between the particles and the scaffolds. In solution, a particle with radius $R \simeq 1\,\mu$m has a diffusion coefficient given by the Stokes-Einstein relation $D = k_B T/6\pi\eta R$. On the other hand, the size of the scaffold $a \simeq 10\,$nm. As a result the scaffolds diffuse $R/a \simeq 100$ times faster than the particles. To first approximation the resulting aggregation is a two-stage process. In the first stage the particles recruit different numbers of scaffolds via the fast scaffold diffusion and subsequent DNA hybridization. Since we consider the regime of strong binding where these bonds are irreversible, the result is a Poisson distribution over the concentration of particles with m scaffolds attached. Let C_i denote the concentration of particles with color i, and c denote the total concentration of scaffolds. The total particle concentration $C_{tot} = \sum_{i=1}^{f} C_i$. The concentration

$C_i^{(m)}$ of particles of color i with m scaffolds attached is

$$C_i^{(m)} = C_i \frac{p^m \exp(-p)}{m!}, \qquad (1)$$

$$p = \frac{c}{C_{tot}}. \qquad (2)$$

In the second stage there are no free scaffolds left in solution, and these particles decorated with scaffolds aggregate to form the final clusters. The seed to build a star cluster is a particle of any color with exactly one scaffold attached. This seed must aggregate with $f - 1$ particles of different colors, each of which has no scaffolds. We now calculate the concentration of the star cluster C_*. The yield of the desired star cluster is quantified in terms of the star mass fraction $M_* = (fC_*)/C_{tot}$.

$$M_* = \frac{f}{C_{tot}} \sum_{i=1}^{f} C_i^{(1)} \prod_{\substack{j=1 \\ j \neq i}}^{f} \frac{C_j^{(0)}}{C_j} = x \exp(-x). \qquad (3)$$

Here $x = fp$ is the scaffold functionality f multiplied by the concentration ratio p. By choosing $p = 1/f$ the mass fraction attains a maximum of $\exp(-1) \simeq 0.37$! This result indicates that by selecting the appropriate scaffold concentration, in the non-equilibrium regime up to 37% of the particles will aggregate to form star clusters. This is a significant improvement over the situation in the equilibrium regime.

This treatment of the problem captures the physics of star cluster formation, but it does not account for the loss of star clusters due to aggregation. In particular, as long as there are scaffolds with markers available for hybridization, when these scaffolds encounter a star cluster they can aggregate to form a larger cluster. We now estimate how this aggregation effects the final concentration of star clusters.

Consider the beginning of the second stage in our aggregation process. There are no longer any free scaffolds in solution, but a scaffold can have up to $f - 1$ DNA markers still available for hybridization. We would like to determine how the star cluster mass fraction $M_*(y)$ changes as a function of the fraction of saturated scaffolds y. Here a saturated scaffold has particles hybridized to all f of its DNA markers, and is therefore unreactive. If s is the expectation that a slot on the scaffold is filled, then the fraction of saturated scaffolds is $y = s^{f-1}$. The average number of open slots on a scaffold is $(f-1)(1-s)$. Consider filling an open slot on the scaffold. The probability that the particle which filled the slot was part of a star cluster is $M_*(y)$. The average rate $r(y)$ at which star clusters are lost to aggregation is then

$$r(y) = -M_*(y)\frac{\mathrm{d}}{\mathrm{d}y}\left[(f-1)(1-s)\right] = M_*(y)y^{-\alpha}. \qquad (4)$$

Here the exponent $\alpha = (f-2)/(f-1)$. We can then construct a differential equation for M_* taking into account this loss due to aggregation.

$$\frac{dM_*}{dy} = \frac{dM_*^{(o)}}{dy} - xr(y). \tag{5}$$

In the absence of this loss term the result of the calculation should recover our previous result eq. (3). This zeroth-order approximation is just $M_*^{(o)}(y) = xy\exp(-xy)$ which gives the correct star cluster concentration once all of the scaffolds are saturated ($y=1$). To simplify the analysis a bit we take $\alpha = 1$ which is an excellent approximation in the limit of large scaffold functionality f. This is an inhomogeneous first-order differential equation which can be solved by introducing an integrating factor $u(y) = y^x$. The initial condition which must be satisfied is $M_*(0) = 0$. We are interested in the final star mass fraction M_*, which is $M_*(y=1)$. The result is

$$M_* = x\sum_{k=0}^{\infty} \frac{(-x)^k}{k!}\left[\frac{1}{x+k+1} - \frac{x}{x+k+2}\right]$$
$$= x\exp(-x) + x^2 E_{-x}(x) - x^{1-x}\Gamma(1+x). \tag{6}$$

Here $\Gamma(x)$ is the gamma function and $E_\nu(x) = \int_1^\infty t^{-\nu}\exp(-xt)dt$ is the exponential integral of order ν.

We can perform a similar type of analysis in the case when there is only one particle color. In this case the f ssDNA markers on the scaffold all have identical sequences complementary to this color. It turns out that the result for the mass fraction is the same. Because the mass fraction is the same in both cases, we can gain insight into the behavior of the system with many colors by analyzing the much simpler one-color system.

With the analytic solution at hand, we now turn to the second part of the paper. To study the aggregation process in further detail, we numerically solved a system of differential equations which models the irreversible aggregation between particles (one color) and scaffolds.

$$\frac{dC_{IJ}}{dt} = \frac{1}{2}\sum_{\substack{i+i'=I \\ j+j'=J}} K_{iji'j'}C_{ij}C_{i'j'} - C_{IJ}\sum_{i,j} K_{ijIJ}C_{ij}. \tag{7}$$

This equation is the Smoluchowski coagulation equation [16] adapted to our system. C_{ij} is the concentration of the cluster with i scaffolds and j particles. $K_{iji'j'}$ is the rate constant for the irreversible reaction $C_{ij} + C_{i'j'} \rightarrow C_{i+i'j+j'}$. There are two contributions to the rate constant [17,18]. The first is the Smoluchowski rate $K_{smol} = 4\pi(R_{ij} + R_{i'j'})(D_{ij} + D_{i'j'})$. Here the hydrodynamic radius for the cluster $R_{ij} \sim j^{1/3}$ and $D_{ij} = k_B T/6\pi\eta R_{ij}$ is the diffusion constant for the cluster. The second contribution is the finite chemical rate for cluster formation. In analogy to the Flory-Stockmayer model for gelation in branched polymers [19,20], by counting the number of ways to connect the two clusters, we
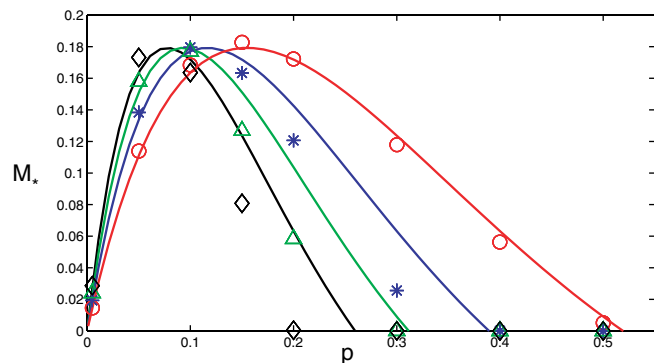

Fig. 2: (Colour on-line) Star cluster mass fraction. The mass fraction M_* as a function of p for scaffolds with functionality $f=3$ (red circles), 4 (blue asteriks), 5 (green triangles), and 6 (black diamonds). The results determined numerically from the full solution of the Smoluchowski coagulation equation (markers) can be compared to results of the anlaytical calculation (lines) eq. (6). In the plot the parameter k_0 is chosen so that in the $f=3$ system $\langle K_{smol}\rangle = \langle K_{chem}\rangle$.

have $K_{chem} = k_0[(1 + (f-1)i - j)j' + (1 + (f-1)i' - j')j]$. Together these two rates determine the overall aggregation rate by $\frac{1}{K_{iji'j'}} = \frac{1}{K_{smol}} + \frac{1}{K_{chem}}$. To simply matters we only consider tree like structures, i.e. we do not consider the formation of clusters with internal loops. We have truncated the set of equations by considering clusters with a maximum of 10 scaffolds.

By solving these equations we can determine the concentration of stars $C_* = C_{1f}$ in this notation and test the validity of our two stage ansatz. As indicated in fig. 2, the results of our analytical calculation are in good agreement with the results of the full numerical calculation, even upon introduction of a finite chemical rate comparable to the Smoluchowski rate. Several points are in order.

The optimal concentration ratio p for experiments is easily determined from $\frac{dM_*}{dx} = 0$. The result is $x_{max} \simeq 0.47$. For scaffolds of functionality f the concentration ratio should be chosen as

$$p = \frac{0.47}{f}. \tag{8}$$

Note that the maximum attainable star cluster yield $M_*(x_{max}) \simeq 20\%$ does not decrease with increasing f. Solving the aggregation equations becomes computationally expensive, but it can still be done by reducing the maximum number of scaffolds in a cluster. For example, considering clusters with up to 5 scaffolds for $f=10$ gives $M_*(x_{max}) \simeq 0.2$. These results are important from the perspective of experimental feasibility for the self-assembly method. This is to be contrasted with the earlier equilibrium treatment. There the condition to suppress the aggregation of larger clusters imposed a fairly strict constraint [15] on the concentration ratio $p \lesssim f^{1/2}\left(\frac{2}{f}\right)^{f-1}$. From the perspective of self-assembling stars with large

f this renders the regime of irreversible binding far more appealing than the equilibrium regime.

If an experiment is performed with the optimal concentration ratio, the clusters which self-assemble are easily separated by density gradient centrifugation [21]. In this regime most of the particles are monomers, in star clusters, or in saturated two scaffold clusters. These clusters contain, 1, f, and $2f - 1$ particles, respectively. The disparity in hydrodynamic radius and sedimentation velocity of these clusters makes the separation procedure experimentally feasible.

In this letter we considered a DNA scaffold method for self-assembling star clusters of f colored particles. By taking advantage of the mobility mismatch between particles and scaffolds, we were able to formulate a non-equilibrium calculation of the star mass fraction. The results of the calculation were compared to the numerical results of the full Smoluchowski coagulation equation for the system. Good agreement is established between the analytical calculation and the numerics. In the regime of irreversible binding the yield of the desired star cluster is drastically improved in comparison to earlier equilibrium estimates. In non-equilibrium we find an experimentally feasible regime for the self-assembly of star clusters with a maximum mass fraction $\simeq 20\%$. We determined the optimal concentration ratio for an experimental implementation of our proposal. The additional color degrees of freedom associated with particle permutation in these clusters makes them ideal candidates as building blocks in a future hierarchical self-assembly scheme. In addition, these clusters can serve as the starting point to self-assemble structures of arbitrary geometry [22]. The experimental realization of self-assembling star clusters using DNA scaffolds would constitute an important step towards realizing the full potential of DNA mediated interactions in nanoscience.

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