# Simple Quantitative Model for the Reversible Association of DNA Coated Colloids

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We investigate the reversible association of micrometer-sized colloids coated with complementary single-stranded DNA "sticky ends" as a function of the temperature and the sticky end coverage. We find that even a qualitative description of the dissociation transition curves requires the inclusion of an entropic cost. We develop a simple general model for this cost in terms of the configurational entropy loss due to binding and confinement of the tethered DNA between neighboring particles. With this easy-to-use model, we demonstrate for different kinds of DNA constructs quantitative control over the dissociation temperature and the sharpness of the dissociation curve, both essential properties for complex self-assembly processes.

Self-assembly of nano- to microscale particles is a powerful way to obtain new materials, provided that one has good control over the particle interactions. One approach is to coat particles with complementary, single-stranded DNA "sticky ends," which give rise to highly selective, thermoreversible attractions [1,2]. With this method, there recently has been a great deal of progress in forming ordered crystalline structures using intuition and phenomenology [3–5]. However, despite considerable experimental and theoretical work, e.g., Refs. [2,5–11], a clear quantitative description of the essential interactions and thermodynamics of the association behavior, together with a detailed comparison with experiments, is still lacking. In this Letter we present a general, easy-to-use quantitative model for the dissociation transition of particles coated with complementary DNA strands, based on readily measurable properties of the elementary constituents: the melting curves of sticky ends in solution, the length of the DNA construct, and the surface DNA coverage.

Jin *et al.* were the first to investigate systematically the dissociation transition of DNA-linked nanoparticles [12], which in general occurs at a higher temperature and is much sharper than the dehybridization of the same DNA in solution. For systems with DNA linkers suspended freely in solution, Lukatsky *et al.* showed that "entropic cooperativity" of the DNA-particle network can lead to a sharp phase transition [13], while Gibbs-Davis *et al.* demonstrated that close proximity of DNA duplexes ($\lesssim 5$ nm) may cause "cooperative melting" [14]. On the other hand, there are also models for DNA-mediated particle association in which these effects do not play a role [8,9], but in all cases a convincing comparison with experimental data is lacking.

To perform a quantitative test, we set up a series of experiments using micrometer-sized particles that interact directly through complementary sticky ends, and we systematically changed the sticky end coverage by dilution with "nonsticky" DNA. In most models the dissociation temperature and the sharpness of the transition are intimately related, whereas we find very sharp transitions at surprisingly low temperatures. We develop a simple quantitative model that in addition to hybridization-mediated attractions also includes an entropy cost due to the reduced configurational freedom of tethered DNA.

Our DNA construct consisted of a 61-nucleotide long oligomer (IDT, Coralville, IA), attached via a short poly (ethylene glycol) spacer to a $5'$ biotin group, and hybridized from its $5'$ end to a 49-nucleotide complementary strand (CS). The hybridization was done at an overall concentration of 15 $\mu$M in 50 mM phosphate/50 mM NaCl hybridization buffer ($p$H 7.5) by slowly cooling down from 90 to 22 °C. The result was a rigid $\sim$15 nm long double-stranded "rod" with a flexible single-stranded end of 11 bases designed with minimal sequence symmetry. We used three types of ends, of which two were complementary "sticky" sequences ($S/S'$); the other was a nonsticky thymine-only sequence ($N$), Fig. 1. We coated 1.05 $\mu$m diameter polystyrene Dynabeads (MyOne
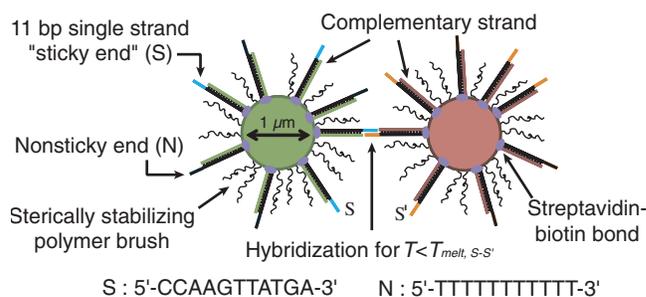


FIG. 1 (color). Schematic representation of the experimental system. The $\sim$15-nm-thick DNA coating is not drawn to scale and in reality many bonds form between the particles.

Streptavidin C1, Molecular Probes, 3% polydispersity)
with $N$ and $S(S')$ DNA in the ratio $\chi = n_S/(n_S + n_N)$,
where $n_S$ and $n_N$ are, respectively, the number of $S(S')$
and $N$ DNA strands per bead. We did this by combining
5 $\mu$l of bead suspension with 10 $\mu$l of a DNA solution and
60 $\mu$l of suspension buffer (10 mM phosphate/50 mM
NaCl and 0.5% w/w Pluronic surfactant, $p$H 7.5), and
allowing this mixture to incubate for 30 min at room
temperature. To remove excess and nonspecifically ad-
sorbed DNA we centrifuged and resuspended the particles
3 times in 100 $\mu$l suspension buffer; we repeated this
washing procedure twice, heating in between for 30 min
at 55 °C.

For radioactive determination of the DNA coverage we
labeled part of the CS-DNA with a $^{32}$P isotope and mixed
this with unlabeled CS-DNA in a known number ratio
($\sim$1:1000), before hybridization with $S$-DNA; $N$-DNA
was hybridized with unlabeled CS-DNA only. After incu-
bation and washing we determined the number of decay
events with an Intertechnique SL30 scintillation counter
and related this to the number of DNA strands per particle.
As expected, the number of $S(S')$ sticky ends that attached
to the particles depended linearly on the mixing ratio $\chi$.
Furthermore, our washing procedure indeed eliminated
initial nonspecific adsorption, with the coverage reaching
a constant value of $\sim$2.2 × 10$^4$ strands/particle after two
washing cycles.

We placed the sample on a temperature gradient stage on
the light microscope. After 1 h equilibration time, we
imaged the suspension, going from a fully aggregated state
at the "cold" end to a fully dissociated state at the "hot"
end. For each point we measured the fraction of nonag-
gregated particles, or "singlet fraction," by videomicro-
scopy [15]. Because of sedimentation, the system was
essentially two dimensional with a concentration $C_p =$
0.1 particles/$\mu$m$^2$. The temperature gradient was constant
at 0.7 °C/cm and the measured uncertainty was $\pm$0.2 °C at
each point. The dissociation curves were found to be
independent of the equilibration time and the steepness
of the temperature gradient.

The solid blue curve in Fig. 2 shows a typical "melting
curve" that we measured for our sticky ends in solution
(UV-260 absorption). While earlier studies relied on pre-
dictions from nearest-neighbor thermodynamics [16], we
determined the enthalpic, $\Delta H^0_{\text{DNA}} = -322$ kJ/mol, and
entropic, $\Delta S^0_{\text{DNA}} = -936$ J/mol K, contributions to the
hybridization free energy, $\Delta F^0_{\text{DNA}} = \Delta H^0_{\text{DNA}} - T\Delta S^0_{\text{DNA}}$,
from the concentration dependence of such curves [17] ($T$
is the absolute temperature). In Fig. 2, $\Delta F^0_{\text{DNA}}$ is shown as
the solid red line. At $T_0 = \frac{\Delta H^0_{\text{DNA}}}{\Delta S^0_{\text{DNA}}}$ the interaction between
the DNA strands becomes attractive. The fraction of un-
hybridized strands ("singlets") decreases as the tempera-
ture is lowered below $T_0$ and hybridized pairs are formed
substantially when the hybridization free energy exceeds
the translational entropy of singlets in solution. At the so-
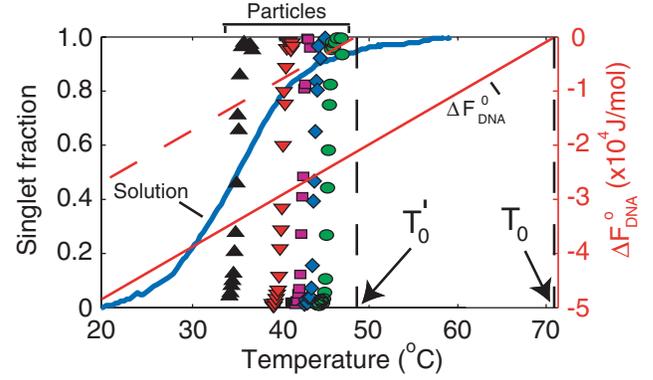called melting temperature $T_m$, 50% is hybridized and



FIG. 2 (color). Particle singlet fraction as a function of tem-
perature for sticky end fraction $\chi = 0.2$, $\chi = 0.4$, $\chi = 0.6$,
$\chi = 0.8$, and $\chi = 1.0$ (symbols from left to right). The blue
line is the melting curve for the same sticky end DNA in so-
lution. The red line shows the hybridization free energy of DNA
in solution ($\Delta F^0_{\text{DNA}}$) as a function of temperature. The dashed
red line shows the effect of an entropy correction on $\Delta F^0_{\text{DNA}}$.

$\Delta F^0_{\text{DNA}}(T_m) = RT_m \ln(C/4)$ ($R$ is the gas constant and $C$
is the overall DNA concentration).

Let $N_b$ be the number of bonds that can form between
two DNA coated particles. In our case of high coverage,
simple geometry gives [2]:

$$N_b = 2\pi\chi\rho R_p^2 \frac{L - h/2}{R_p + L} \approx 154\chi. \qquad (1)$$

Here, $\rho$ is the total DNA coverage ($6.4 \times 10^3$ strands/$\mu$m$^2$),
$R_p$ the particle radius (525 nm), $L$ the length of the DNA
construct ($\sim$15 nm), and $h \approx L$ the distance between the
particle surfaces, as determined by strand-surface inter-
actions (see [18]). Intuitively one might expect that

$$\Delta F_{\text{bead}} \approx N_b \Delta F^0_{\text{DNA}} = N_b(\Delta H^0_{\text{DNA}} - T\Delta S^0_{\text{DNA}}). \qquad (2)$$

Particle dissociation then occurs when the binding free
energy equals the entropy change between unbound,
$R\ln(C_u)$, and bound, $R\ln(1/v)$, particles. Here, $v$ is the
interaction volume corresponding to the range of the
DNA-mediated attraction. The dissociation temperature
$T_d$ is then given by $\Delta F_{\text{bead}}(T_d) \approx RT_d \ln(vC_p/4)$ and the
dissociation transition is complete when the entropy
change per singlet is about twice the binding energy
$R(T_d + \delta T)\ln(vC_p/2) \approx 2\Delta F_{\text{bead}}(T_d + \delta T)$. The disso-
ciation temperature and width of the transition are then

$$T_d \approx \frac{\Delta H^0_{\text{DNA}}}{(\Delta S^0_{\text{DNA}} + \frac{R\ln(vC_p/4)}{N_b})}, \quad 2\delta T \approx -\frac{RT_d\ln(vC_p/2)}{N_b\Delta S^0_{\text{DNA}}}.$$
$$(3)$$

In this scenario, the temperature below which particle
interaction is attractive, $T_0 = \frac{N_b\Delta H^0_{\text{DNA}}}{N_b\Delta S^0_{\text{DNA}}}$, remains the same
as for the free DNA in solution. However, from the equa-
tions it can be seen that when the number of simulta-
neously formed DNA bonds increases, the slope of

$\Delta F(T)$ increases with a factor $N_b$, which should push the dissociation temperature up toward $T_0$ and which should make the transition sharper by a factor $1/N_b$. Thus, the dissociation temperature and the sharpness of the transition are expected to be intimately related.

In Fig. 2 we show the experimentally obtained dissociation curves for our particles with sticky end fractions in the range $\chi = 0.2$–$1.0$. These dissociation curves indeed are much sharper ($2\delta T \approx 1.0\,^\circ\text{C}$) than the melting curve of the same DNA in solution ($2\delta T \approx 20.0\,^\circ\text{C}$), but surprisingly they are not shifted up in temperature toward $T_0$ (see also note [19]). Rather, it appears that for increasing $\chi$ (and thus $N_b$) the dissociation curves approach a lower $T_0'$. This would be associated with a $\Delta F_{\text{DNA}}^{0'}$ which is shifted down by a certain energy cost that occurs when tethered DNA hybridizes and not when it is free in solution.

The source of the additional energy cost becomes clear from the schematic drawing in Fig. 3, which shows how the available states for a pair of hybridized DNA tethers is strongly restricted, as compared to the unhybridized case. Because of the PEG spacer at its $5'$ end, our DNA construct is like a freely pivoting rigid rod. Unhybridized, the sticky end of each strand independently explores the surface of a half sphere, but in a hybridized pair the complementary sticky ends can only move together. This gives rise to a "configurational entropy cost," $\Delta S_p$, per tethered DNA bond:

$$\Delta F_{\text{tether}} = \Delta F_{\text{DNA}}^0 - T\Delta S_p. \tag{4}$$

From geometry, it can be estimated that $\Delta S_p \approx -R\ln(4\pi L^3 C_0) \approx -10R$ [9] ($C_0 = 1$ mol/l) and as the red lines in Fig. 2 demonstrate, this configurational entropy cost is the dominant cause of the shift of the particle dissociation temperature towards $T_0'$.

We calculate the binding free energy of the particles, $\Delta F_{\text{bead}}$, where we allow the particles to form any number of bonds up to $N_b$. Geometrically, each sticky end can bind to $k$ different complementary strands on the opposing particle surface, in our case: $k = 3\pi L^2 \rho \chi \approx 13\chi$. The partition function for two interacting, DNA coated bead surfaces becomes

$$Z_s = (1 + ke^{-\beta\Delta F_{\text{tether}}})^{N_b} \tag{5}$$

and the particle binding free energy is $\Delta F_{\text{bead}} = -RT\ln Z_s$. In the experiments, the particles do not just pair up, but form extensive fractal-like aggregates with coordination number $z \approx 3$, as estimated by videomicroscopy. Accord-
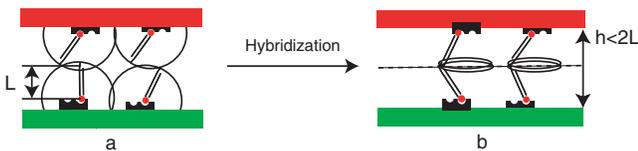
ing to the cell model of Ref. [20], the chemical potential $\mu$ of a particle with coordination number $z$ inside a cluster is $\mu = \frac{z}{2}\Delta F_{\text{bead}} - RT\ln(A_p)$. Here, $A_p$ is the area over which a particle can move without loosing the attractive interaction with its neighbors. In a square well model, $A_p \approx (\frac{\delta}{2})^2$, where $\delta$ is the range of the interaction. We take $\delta = l$, with $l \approx 3.6$ nm the length of the sticky end when hybridized. The chemical potential $\mu_i$ of a gas of clusters of $i$ particles is $\mu_i = i\mu + RT\log(C_i)$, where $C_i$ is the concentration of clusters of size $i$. The aggregation is modeled by the following series of equilibria: $C_i + C_1 \rightleftharpoons C_{i+1}$. In equilibrium, $\mu_i + \mu_1 = \mu_{i+1}$ and the equilibrium constant is $K = \frac{[C_{i+1}]}{[C_i][C_1]} = (\frac{l}{2})^2 e^{-\beta\frac{z}{2}\Delta F_{\text{bead}}}$. Taking the equilibrium conditions for all sizes of clusters, the singlet fraction is

$$f = \frac{1 + 2KC_p - \sqrt{1 + 4KC_p}}{2(KC_p)^2}. \tag{6}$$

In Fig. 4, we compare the predictions of this model with the experimentally observed dissociation curves. As the inset in Fig. 4(a) shows (for sticky end fraction $\chi = 1.0$), the dissociation temperature indeed is strongly overestimated if the configurational entropy correction is not included in the hybridization free energy of the tethered DNA. Figure 4(a) shows a comparison of the melting temperatures obtained from the model and the experiments by varying the ratio $\chi$. The solid black line results from the model by taking the number of strands on the particles to be $N_{\text{DNA}} = 22\,000$. The two dashed lines are the predictions for $N_{\text{DNA}} = 22\,000 \pm 2200$ (10% relative uncertainty due to the estimated pipetting error and coverage polydispersity). Therefore, once a single entropy correction $\Delta S_p = -14.6R$ is set, our model predicts the melting temperatures measured for different coverage with excellent agreement. Finally, Fig. 4(b) (black curves) shows that by adjusting $N_{\text{DNA}}$ and $\chi$ within the experimental error, both the dissociation temperature and the width of the transition match perfectly. The configurational entropy cost that we find from fitting the dissociation curves contains several terms (including about a 5% contribution from steric repulsion between unhybridized strands) but is dominated by the $-10R$ estimated above from simple geometrical considerations: the shift in $\Delta F_{\text{tether}}$ is therefore mostly accounted for by the entropy loss of the tethered rods.

In order to check the generality and robustness of the model, experiments were also performed on double-stranded constructs with a shorter (8 bases) sticky end. Using measured hybridization parameters and the same entropic penalty $\Delta S_p = -14.6R$, the model predicts the experimental results for two different coverages (red curves Fig. 4). Further, using the $S/S'$ sticky ends, the construct was changed from double to single stranded. In this case, we choose the surface separation equal to the equilibrium end-to-end distance of the DNA bridge ($h = 21.7$ nm for a



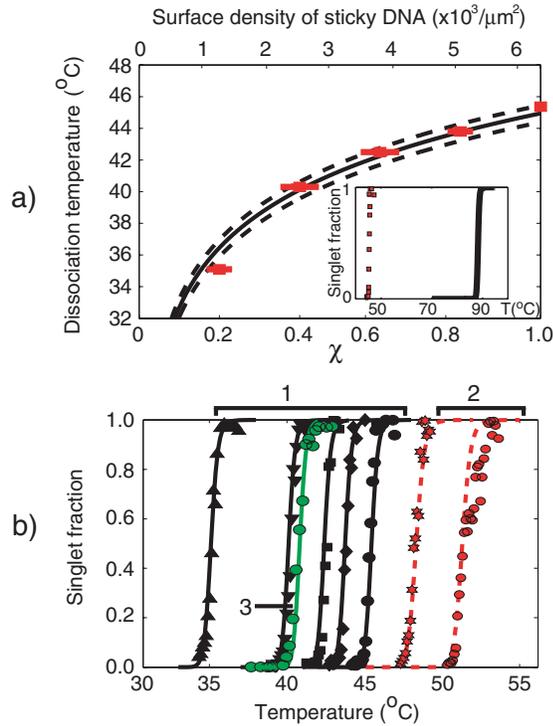FIG. 3 (color).   Configurational freedom of tethered DNA before hybridization (a) and after hybridization (b).

FIG. 4 (color).   (a) Experimental and theoretical dissociation temperature as a function of $\chi$, the fraction of sticky DNA. The red squares indicate the measured dissociation temperatures. The red error bars on $\chi$ reflect the estimated 10% uncertainty in the coverage. The solid line is the predicted dissociation temperature. The dashed lines show the effect of a $\pm 10\%$ error in the total number of tethered strands. Inset: experimental (squares) and theoretical dissociation curves (solid line) before the entropy correction. (b) Experimental dissociation curves and the fits from our model for different systems. Black (1): double-stranded construct and $S/S'$ sequences, ($\chi = 0.2$–$1.0$) as in Fig. 2, red and dashed lines (2): double-stranded construct and shorter sticky end ($\chi = 0.65$ and $\chi = 1$), green (3): single-stranded construct and $S/S'$ sequences ($\chi = 1$).

Kuhn length of 3.4 nm) [21]. The end-to-end distance of a single tethered DNA strand is $R_{ee} = 14.4$ nm. Using these geometrical parameters, the model agrees with the experimental data [Fig. 4(b), green curve] once the entropy correction is adjusted to $-14.8R$. This correction is due to the loss of entropy of one sticky end, $-R \ln(\frac{2\pi}{3} R_{ee}^3 C_0) \approx -8.2R$, and the confinement of the DNA between the particles $\approx -2.5R$, which gives a total of $-10.7R$, close to the fitted entropy cost.

In conclusion, we have developed a simple quantitative model for DNA-mediated particle association, which incorporates a significant entropic cost for each interparticle bond, due to the loss of configurational freedom when two tethered strands hybridize. Given $\Delta F_{\mathrm{DNA}}^0$ from calculation or solution measurement, a single constant shift, $\Delta S_p$, is sufficient to characterize the aggregation behavior of particles coated with that DNA. This $\Delta S_p$ can be obtained from one sample of coated colloids or nanoparticles and

subsequently used in designing the desired dissociation transitions for other samples.

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