Topological Interaction by Entangled DNA Loops

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Topological Interaction by Entangled DNA loops

Lang Feng,[1] Ruojie Sha,[2] Nadrian.C.Seeman,[2] and Paul.M.Chaikin[1]

[1] *Center for Soft Matter Research, New York University, New York, New York, 10003, USA*
[2] *Chemistry Department of New York University, New York, New York, 10003, USA*

We have discovered a new type of interaction between micro/nanoscale particles that results from the entanglement of strands attached to their surfaces. Self-complementary DNA single strands on a particle can hybridize to form loops. A similar proximal particle can have its loops catenate with those of the first. Unlike conventional thermodynamic interparticle interactions , the catenation interaction is strongly history and protocol dependent, allowing for nonequilibrium particle assembly. The interactions can be controlled by an interesting combination of forces, temperature, light sensitive crosslinking and enzymatic unwinding of the topological links. This novel topological interaction may lead to new materials and phenomena such as particles strung on necklaces, confined motions on designed contours and surfaces and colloidal Olympic gels.

PACS numbers:

There is growing interest in the use of DNA for controlling interparticle interactions on the micro and nanoscale, because complementary DNA single strands lead to highly specific thermo-reversible binding [1-9]. DNA functionalized colloids and tiles have been used for temperature dependent aggregation, self-assembly, self-replication and crystallization [1-6]. Previously studied DNA-mediated interactions between particles [7-9] are topologically simple. Here we introduce a new type of DNA interaction which is topological, non-specific, nonequilibrium, highly irreversible and history dependent. It arises from entanglement and catenation of DNA loops on adjacent particles. The loops are from palindromic DNA. This entanglement interaction is different from any interaction derivable from a free energy and more like the interaction associated with "velcro". It can be controlled with temperature and the use of unlinking topoisomerase enzymes [10-13]. In our experiment, we observe and measure this topological entanglement interaction quantitatively. The interaction is most relevant for particles in contact and thus allows for new flexibility and process control in directed self-assembly.

DNA functionalized colloids with "Watson-Crick" interactions are highly attractive for their ability to recognize and bind only to particles with complementary coatings [3-5,7-8,14]. Recent studies on "self-protected" DNA interactions [15] show that loops and hairpins can weaken and help control these interactions. Here we demonstrate a model system in which the formation of loops and potentially hairpins also greatly enhances interparticle binding by the formation of entanglements. Our system is also topologically interesting; rather than being bound by hydrogen bonds exclusively , our DNA strands are linked and the link must be cut for joined particles to separate. The biological implications of such topological interaction [12,13] is also of significant importance. In our system as in Fig.1(a), two different palindromic DNA strands P1 and P2 are prepared [16]. P1 and P2 are placed on separate particles. P1 strands are self-complementary and can hybridize with strands on other P1 particles, or they can form loops by hybridizing with strands on the same particle. Hybridization takes place below a melting temperature, T_m. If P1 coated particles and P2 coated particles are held near one another when their temperature is lowered through T_m then some strands will entangle and some catenated loops will form. The particles are now linked and cannot be separated until the loops are opened by heating or by the use of an enzyme that catalyzes strand passage events. Note that thermodynamically the particles are bound metastabily and would lower their free energy if they separated.

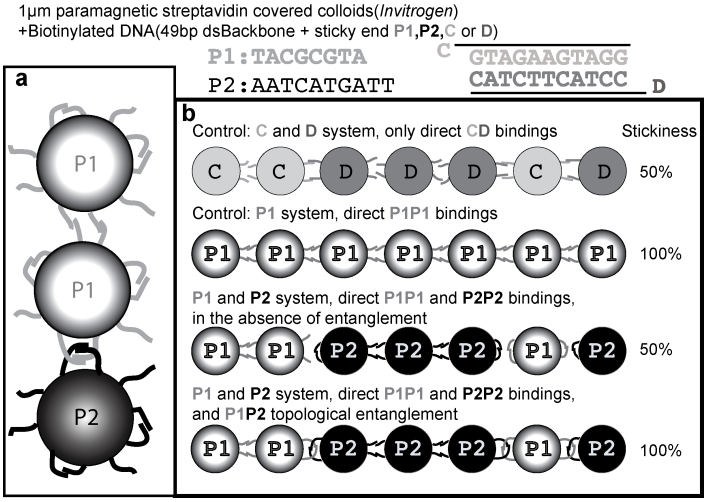

FIG. 1: The model system with direct binding and entanglement binding. 4 different sequences of DNA sticky ends P1, P2, C, D are shown on top. **(a)** Direct binding P1-P1 and topological entanglement binding P1-P2 schemes. **(b)** Random mixture of Watson-Crick chain system (C-D) gives binding fraction $P = 50\%$ **from CD direct bindings**; P1 chain system gives $P = 100\%$ **from P1P1 direct bindings**; Random mixture of P1 and P2, if P1P1 and P2P2 will bond but not P1P2 or P2P1 then $P = 50\%$; if there are entanglements for P1P2 and P2P1, P will increase to 100%

The topological interaction is fundamentally different from a potential interaction as illustrated by considering a Watson Crick bond (C-D in Fig.1) as contrasted with a P1-P2 link. Particles C and D will aggregate in suspension because they gain more free energy from hybridization when together than their entropy gain when apart. On the other hand, P1 and P2 are completely hybridized when they are either together or apart. They simply lose entropy when brought together. There is an entropic repulsion between them. Their linking depends on protocol and manipulation rather than thermodynamics. This should allow for novel directed self assembly schemes. The particles can be made "velcro-like" by adjusting the temperature. Near T_m, loops are constantly being opened and closed. Pushing two particles together will allow loops to be made and the particles to be linked, at least temporarily. Pulling them apart greatly speeds the unlinking.

To demonstrate and study topological interactions, we made the system shown in Fig.1. Our particles are 1 μm polystyrene spheres with a magnetic core and a streptavidin coating. Our DNA constructs consist of a biotin - polymer spacer - 49 base pair double strand as a backbone and a base paired hinge connecting the backbone to a DNA single stranded sticky end. The biotin connects the construct to the streptavinated particle. We make control particles in the form of Watson Crick complementary pairs, C and D, with sticky end sequences C: GTAGAAGTAGG, D: CCTACTTCTAC, and self complementary palindromic P1 and P2 particles with sticky end sequences P1:TACGCGTA and P2:AATCATGATT that are designed to avoid crosstalk. P1-P2 will not hybridize with each other above 0 ^oC. The basic idea of our experiment is shown in Fig.1(b). If we form a random chain of particles C and D and cool below their T_m then CD and DC bonds will form while CC and DD will not. Half of the contacts in the chain will bond, binding fraction P=50%. For a chain with just P1, all contacts P1P1 will form bonds, P=100%. For a random mixture of P1 and P2, P1P1 and P2P2 will bond but not P1P2 or P2P1 for P=50%. But if there are entanglements then P1P2 and P2P1will link for another 50%.

The experiment is shown in Fig.2. The DNA coated magnetic particles are mixed then made to form chains in a magnetic field $\sim 2mT$ at 52^oC. With the field on for about $20s$, over 95% of the chains are completed and stable, then we start a quench from 52^oC to T_{quench}(35^oC to 47^oC). The magnetic field is turned off after stabilizing at T_{quench}. The temperature is maintained at T_{quench} and **the number of objects (=chains+single particles)** on screen is monitored as the chain partially dissociates. Since our system has only single particles or chains of particles, we get the binding fraction as:

$$P = \frac{N_{initial} - N_{relax}}{N_{initial} - N_{mag}} \qquad (1)$$

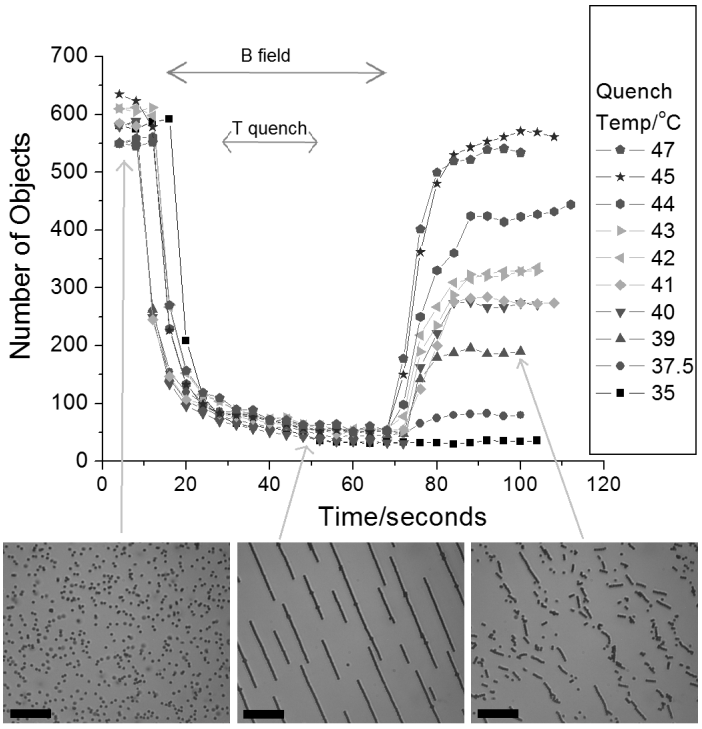

FIG. 2: Typical measurements of "number of objects" in the system. We use an evenly mixed P1 and P2 particle system. Initial soup (52^oC): particles are chained up with a magnetic field $\sim 2mT$, followed by a temperature quench to T_{quench}(refer to different shapes in the right column), and then released from the magnetic field. Bottom 3 pictures show the microscope images at different stages of the protocol. Scale bar is $20\mu m$ in all images.

where $N_{initial}$ is the number of objects in the initial particle soup without any field on; N_{mag} is the number of objects just before the magnetic field is turned off; N_{relax} is the average number of objects about $20s$ after turning off the field. Geometrically $N_{initial} - N_{mag}$ is the total number of particle contacts and $N_{initial} - N_{relax}$ is the number of bonds plus links from DNA hybridization. The ratio of the above 2 quantities gives the binding fraction P.

The entanglement can be explored when we plot the binding fraction, P, with respect to the quench temperature in Fig.3(a). Aside from the 1:1 P1:P2 particle system, 3 control experiments were done: pure P1 particles(triangles in Fig.3(a)), pure P2 particles (rounds in Fig.3(a)) and 1:1 C:D system (flipped triangles in Fig.3(a)). As expected the C D system saturated at $T_{quench} < T_m$ to $P = 50\%$ and the pure P1 and pure P2 particles to $P = 100\%$. Surprisingly, when T_{quench} is lowered from 47^oC, the P1 and P2 mixed system (square in Fig.3(a)) first undergoes a transition to a transient plateau around 50% at about $42 - 43^oC$, consistent with either P1 or P2 direct binding(round and triangles in Fig.3(a)) , however a second transition takes place to

$P \sim 100\%$ when the temperature is further lowered to 35^oC. The mid-point of the second transition is about 6^oC lower than direct binding of either P1 or P2. From this measurement we argue that entanglement binding could play an important role whenever two palindromic sequences are used.

We now want to develop a simple quantitative model to predict the binding fraction in Fig.3(a). Since our system "equilibrates" metastabily about $10s$ after the field is turned off, we argue that a temperature dependent probability : $P(T) = \frac{\exp[-\beta\Delta F(T)]}{\exp[-\beta\Delta F(T)]+1/(CA_w)} = 1/(1+\exp[\beta\Delta F(T) - \ln[C_0 A_w]])$ **describes the binding fraction, when the particles are held together as in the experiments.** C_0 is 2-D concentration of colloids and A_w is the wiggling area when bound [7], $\Delta F(T)$ and $\ln[C_0 A_w]$ are the free energy difference and entropy difference between bound states and unbound states. For direct binding, we could simply take the form $\Delta F_{direct}(T) = -RT\ln[(1+k\exp(-\frac{\Delta F_{DNA}}{kT}))^N - 1]$ [7]. For entanglement binding, the partition function is $Z_{entangle} = Z_{total} \times < g_{ij}(T) >$ which gives

$$F_{entangle}(T) = -RT\ln(\frac{Z_{entangle}}{Z_{total} - Z_{entangle}}) = -RT\ln(\frac{< g_{ij}(T) >}{1 - < g_{ij}(T) >}) \qquad (2)$$

where $Z_{total} = (1 + k_{l1}\exp(-\frac{\Delta F_{DNA1}}{kT}))^{N_{l1}}(1 + k_{l2}\exp(-\frac{\Delta F_{DNA2}}{kT}))^{N_{l2}}$ is the total partition function for loops on both surfaces, subscripts 1 and 2 label the 2 different DNA surfaces, index i and j refer to the number of loops on different particles, P1 and P2 in our case. $\Delta F_{DNA} = \Delta H_{DNA} - T\Delta S_{DNA} - T\Delta S_p$ are free energies for DNA sticky ends, including the configurational entropy ΔS_p [7]. $k_l \sim 18$ is the number of DNA one can reach on the same surface. $N_l \sim 70$ is the maximum number of loops that can be formed in the contact region. $< g_{ij}(T) >$ is the ensemble averaged probability of having at least one entanglement bond and it is temperature dependent. It is derived from a Poisson distribution considering the area of the contact region $S \sim 24000nm^2$, and a reduced entanglement area $A \sim 100nm^2$ which counts the number of ways two loops can entangle. [See SM for more detail] With $\Delta F_{entangle}$ and ΔF_{direct}, we can calculate the binding fraction using $P(T)$ (Fig.3(b)). For our $P1$:$P2$ mixed system, direct binding and entanglement occur over different temperature ranges, (Fig.3(c)), **where we plot** $\mathbf{P(T) = P_{entangle}(T)/2 + P_{direct}(T)/2}$ **with** the estimated entangled area A ranging from $50nm^2$ to $130nm^2$.

Topoisomerase I (E.Coli)(from *New England Biolabs*) is an enzyme which unentangles single stranded or nicked DNA by splitting one strand, passing another strand through the gap and then re-ligating the split. To further test our catenation scenario we took our P1:P2 system as in Fig.4(a) at $T_{quench} = 36^oC$

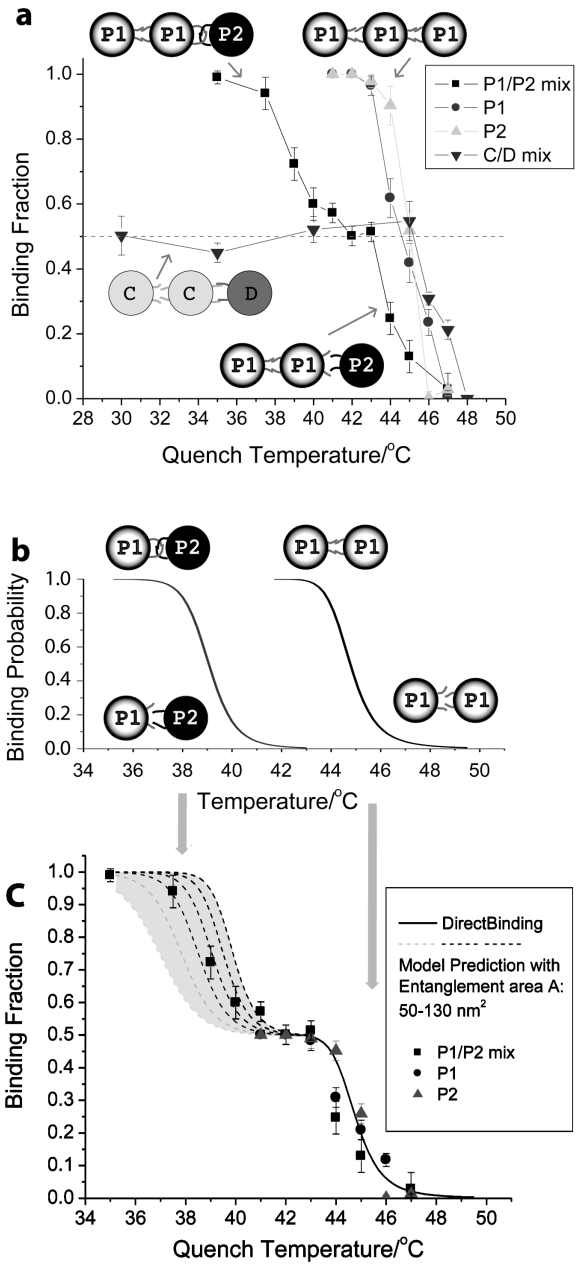

FIG. 3: Experimental data compared with model prediction. **(a)** Experimental data: binding fraction vs. temperature in 4 different experiments. The 2 transitions on the square curve show direct binding(higher T) and entanglement binding(lower T) in the P1 and P2 mixed system. Triangle or round transition curves show direct binding of P1 or P2. The flipped triangle transition curve shows direct binding of the C and D mixed system, there is no entanglement possibility in this system, the binding fraction remains at 50% at low temperature. **(b)** Model prediction for entanglement binding transition and direct binding transition with effective entangled area $A = 100nm^2$. **(c)** Direct P1 and P2 binding curves are rescaled to 50% for easy comparison to square curve model system. **After using a fitting parameter ΔS_p set by the pure P1 or P2 melting transition[7], we can predict the entanglement melting transition by** $P(T) = P_{entangle}(T)/2 + P_{direct}(T)/2$**.** Dashed lines and grey zone show prediction with uncertainty in A from $50nm^2$ to $130nm^2$. **The error bars result from statistics on several experimental runs and an estimate of error in analyzing the number of independent objects from each image.**

and added Topoisomerase I (to a final 1x concentration for NE and BSA buffer [17], $1unit/25\mu L$ for topoisomerase). We indeed observe significant breaking of chains in Fig.4(b), a decrease in P to about 56%. In contrast, either adding only NE and BSA buffer to the mixed system or adding topoisomerase to the pure P1 system does not destroy the chains.

To test that the breaks occur specifically at the catenation links between P1 and P2 particles we used non-magnetic fluorescent Neutravidin covered polystyrene particles(*Invitrogen*) , red for P1, green for P2. The particles have a gravitational height of $20\mu m$ and a dissociation temperature around $42 - 43^oC$. Single color aggregates form below 40^oC as in Fig.4(c). As the temperature is lowered to 37^oC, we see differently colored clusters connecting with each other because of dynamical entanglement binding(Fig.4(d)). In this temperature range, there is a small probability that on particle loops will open, and as clusters occasional closely approach, they can entangle and catenate. When topoisomerase I is added, the two differently colored clusters start to diffuse away from each other (as shown in the three small images in Fig.4(f), see SM movies for more detail), until differently colored clusters are separate (Fig.4(e)). The average connecting number between differently colored clusters decreases from 11 ± 3 down to 0.6 ± 0.5 on each screen. This corresponds to more than 95% efficient breaking of the topological links.

In conclusion we have introduced a new type of interaction between colloidal particles involving topological entanglement, catenation, of DNA loops formed on separate particles. **It is non specific in its ability to bind any flavor of DNA loops, but there is no such binding to unlooped DNA structures** . The interaction is intrinsically nonequilibrium. This topological entanglement is a history-dependent way of binding particles and introduces a new approach to colloidal dynamics and self-assembly, not predicted theoretically [18,19]. The interaction can be controlled by designed protocol, including the sequence of particle positions and temperatures and the use of disentangling enzymes. The loops can be made temperature insensitive by fixing the hybridized stands with a cross linker such as psoralen or cinnamate [20] (see also SM). The "velcro" - like interactions should lead to novel means of micro and nanoscale construction and nonequilibrium phenomena.

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Supplementary Material is linked to the online version of the paper

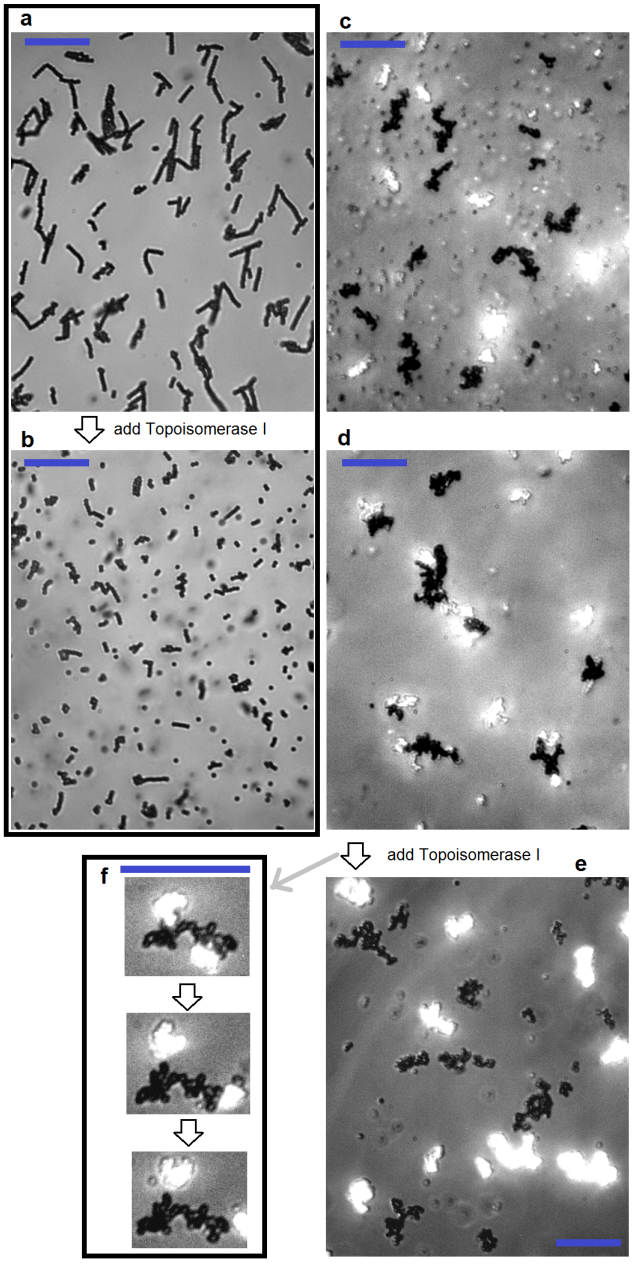

FIG. 4: Topoisomerase unlinking experiments.**(a)** a typical image for particles chained together: P1 and P2 mixed system with only 1x NE and BSA buffer, P1 system with topoisomerase I ($1unit/25\mu L$),1x NE and BSA buffer; **(b)** P1 and P2 mixed system with topoisomerase,1x NE and BSA buffer. Breaking of the chains proves our entanglement interaction is topological. **(c) P1(bright) and P2(dark)** mixed system at 40^oC forms separate P1 and P2 clusters. **(d)** at low temperature $\sim 37^oC$, P1 and P2 clusters are connected by entanglement and catenation. **(e)** and **(f)** P1 and P2 clusters separate after adding topoisomerase. The scale bar indicates $20\mu m$ in all images.

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