

## Supplementary Information

for “Kinetics and non-exponential binding of DNA-grafted polymer colloids” by  
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### I. VERIFICATION OF THE HIDDEN MARKOV MODEL

To verify that the Viterbi algorithm does not significantly bias the most likely two-state trajectory or bound and unbound lifetime distributions, we analyzed simulated separation trajectories [Fig. S1(a)] with known lifetime distributions. The emission distributions in the bound and unbound state were chosen to match our experimental distributions. The unbound lifetime distribution was a single exponential [Fig. S1(b)]; the bound lifetime distribution was a power law multiplied by an exponential [Eq. (2)] [Fig. S1(c)]. Analyzing simulated separation trajectories with the approach described in the text, we found that the Viterbi algorithm reliably returned the imposed bound and unbound lifetime distributions, even for the case of highly non-exponential distributions ( $\alpha \approx 1$ ).

### II. CALCULATION OF FORWARD BINDING RATE

In general, the binding kinetics of molecules tethered to a surface will be different from the kinetics of the same molecules in solution, due to steric hinderance of other molecules, mutual alignment (which may be favorable or unfavorable to binding), or entropic forces accelerating unbinding. In a recent analysis of the energetics of binding between DNA-coated microspheres, we found that the hybridization in our experimental system was ‘ideal’, in the sense that the formation free energy of a DNA bridge could be accurately approximated by the hybridization free energy of the same DNA strands in solution at similar concentrations [1]. This was an intentional design feature of our DNA architecture, and is a consequence of our use of long flexible spacers at intermediate areal density.

Under such ‘ideal’ conditions, we might suppose that the kinetics of bridge formation, and not just the thermodynamics, would also resemble the solution case. The microscopic hybridization rate constant that prevails for complementary DNA in solution has been shown to depend only weakly on temperature, strand length and sequence [2–4]. For the ideal case, the collective rate of bridge formation  $k_f^*$  between two microspheres can be related

to a molecular forward reaction rate constant  $k_f$  by an integral over the spatially varying concentrations of reactive strands  $A$  and  $B$  in the gap between the spheres,

$$k_f^* = N_{Av} k_f \int d\mathbf{r} C_A^0(\mathbf{r}) C_B^0(\mathbf{r}), \quad (\text{S1})$$

where  $C_i^0(\mathbf{r})$  is the concentration of each sticky end species  $i$  and  $N_{Av}$  is Avogadro's number. The integral in Eq. (S1) corresponds to one we previously evaluated to quantitatively model measured interaction potentials [1], using numerically generated models for  $C_i^0(\mathbf{r})$ . The rate constant implied by our  $k_f^*$  data and Eq. (S1),  $k_f = (6 \pm 2) 10^5 \text{ s}^{-1} \text{ M}^{-1}$ , is indeed comparable to reported hybridization rate constants for short ssDNA strands at similar ionic strength [2, 3, 5]. This quantitative agreement demonstrates that DNA hybridization in the gap between our particles is also 'ideal' in the sense of not being significantly hindered by steric nor lubrication effects.

### III. INTERPRETATION OF EARLIER WORK ON BINDING KINETICS

We reported an earlier experimental effort to understand the non-exponential binding of DNA-grafted microspheres in 2008 [6]. That study was similar in some ways to the current one: DNA-grafted microspheres were observed in line optical tweezers under conditions of dynamic binding, the particles' bound lifetime distribution was computed from the trajectory, and the distribution was highly non-exponential. The notable difference was that the earlier experiment was designed to form predominantly single molecular bridges in an attempt to isolate the molecular contributions to the non-exponential behavior from those due to multiple bridge formation. For this reason, the amount of DNA on one particle was reduced to the level that the expected number of bridge-forming DNAs in the particle contact zone was only  $\langle N \rangle \approx 0.1$  (i.e. during 90% of collisions no bridges could form, due to lack of a binding partner). A very high concentration of DNA was used on the second particle to ensure that bridge formation would occur.

The analysis of the data in the earlier study was fundamentally flawed, based on an untested assumption that the binding kinetics was reaction-limited. As a result, the particle bound lifetime distribution was incorrectly taken to describe the lifetime distribution of single DNA bridges. In other words, the effects of rebinding were not considered. Based on our current understanding of the microscopic rate constants, we estimate that even when

there is only a single reactive DNA molecule present in the gap between the particles, the particle sticking probability is high,  $P_{stick} > 0.85$ , due to the very high concentration of DNA on the second particle. Rather than being negligible, rebinding will typically occur more than six times before the particles are able to diffuse apart. Given the diffusion-limited conditions of the earlier experiment and that experiments uncertainty in grafted DNA density, it is not practical to separate the effects of DNA density fluctuations [case (ii) in the main text] from molecular effects [case (iii)] in a meaningful way.

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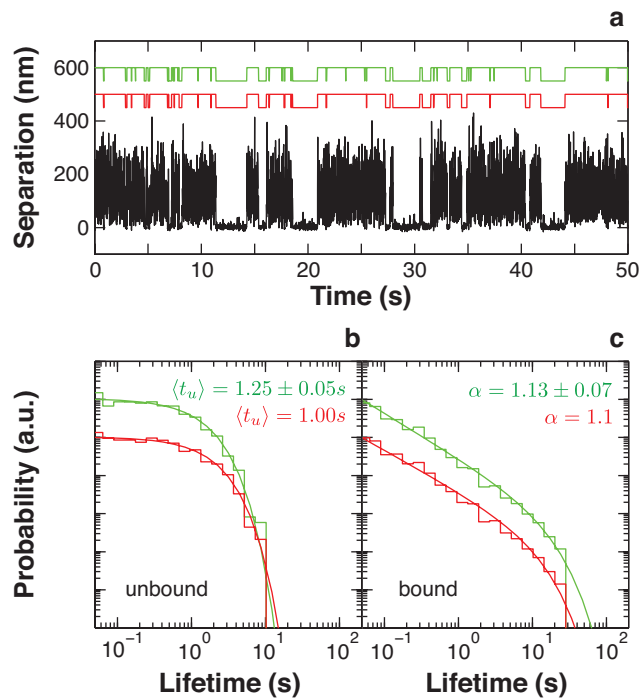


FIG. S1. (a) Simulated separation (black) and two-state (red) trajectories with known bound and unbound lifetime distributions, truncated power law and exponential, respectively. The Viterbi algorithm predicts a two-state trajectory (green) that resembles closely the simulated two-state trajectory and does not bias the functional form of the unbound (b) or bound (c) lifetime distributions, simulated (red) and predicted (green). We do observe a slight systematic bias in the mean unbound lifetime, a result of missed short-lived bound events.

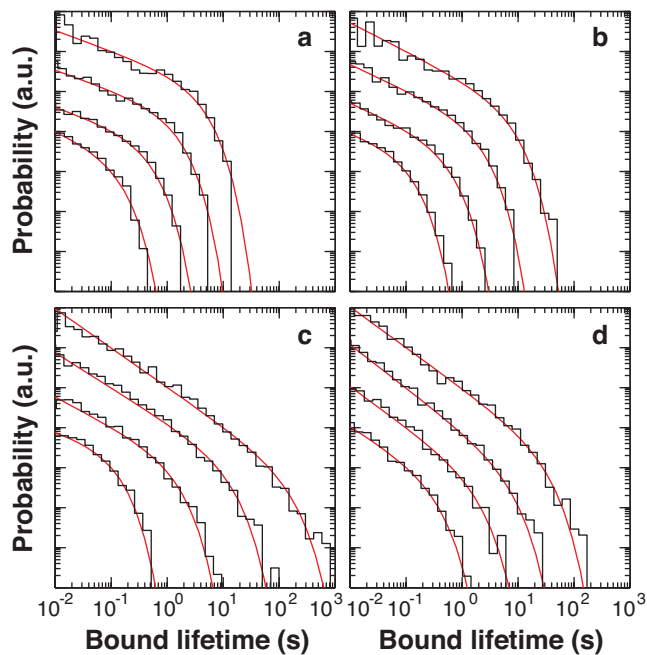


FIG. S2. The simulated bound lifetime distributions can be fitted well by a power law multiplied by an exponential [Eq. (2)]. The four panels (a-d) correspond to the lifetime distributions for Fig. 4(b-e) at  $P_{stick} = 0$ . Within each panel the curves show the distribution for  $EB = [1, 2, 3, 4]k_B T$  moving from the bottom curve to the top curve. The distributions have been offset for clarity.