

Cooperative Melting in Caged Dimers with Only Two DNA Duplexes

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Abstract: Small molecule–DNA hybrids with *only* two parallel DNA duplexes (rSMDH₂) displayed sharper melting profiles compared to unmodified DNA duplexes, consistent with predictions from neighboring-duplex theory. Using adjusted thermodynamic parameters obtained from a coarse-grain dynamic simulation, the experimental data fit well to an analytical model.

DNA-hybrid materials have been explored in a wide range of research areas such as sensors and actuators,^{1,2} molecular electronics,³ template synthesis,^{4,5} and computing.^{6–11} In diagnostics, DNA-linked gold nanoparticle (GNP)¹² and comb-like polymer¹³ aggregates possess a unique switch-like melting property, which has been utilized to distinguish single-nucleotide polymorphisms (SNPs). This switch-like melting phenomenon can be explained by a “neighboring-duplex/effective-concentration” (ND/EC) model,¹⁴ which predicts an enhanced melting temperature (T_m) and sharp melting due to two effects: (1) entropic constraints associated with the melting of structures in which the tethered DNAs have a higher effective concentration than when they are free in solution, and (2) an increase in the effective ion concentration between neighboring duplexes that leads to the higher overall stability of fully hybridized structures and cooperative thermal melting.¹⁵ This model, which has been verified using coarse-grain molecular dynamics (CGMD) simulations,¹⁴ predicts that stabilization can be observed with as few as 2–3 parallel DNA duplexes, giving rise to sharper melting transitions in DNA-linked nanoparticle¹² and comb-like polymer¹³ aggregates than that observed for the double-stranded DNA duplex.^{16,17}

We recently reported the synthesis and cooperative melting behavior of a small molecule–DNA hybrid containing only three DNA duplexes (SMDH₃) around a rigid (r) small-molecule core (C).¹⁸ Herein, we report the cooperative melting of SMDH₂, possessing only *two* closely associated double-stranded DNAs, with or without a rigid core. Using adjusted thermodynamic parameters obtained from coarse-grain molecular dynamics simulations, the experimental data fit well to an analytical model where the melting of these dimeric duplexes is considered as two distinct steps where most of the entropy is released in the second step.

rSMDH₂ **3** and **3'** (Figure 1, **3** = 3'-A-5'-T₃CT₃-3'-B-5', **3'** = 3'-A'-5'-T₃CT₃-3'-B'-5') containing two asymmetric strands (one of the strands is the reverse sequence of the other) were designed to facilitate only the formation of cage-like dimers and avoid face-to-face bulge-containing DNA structures (Figure S3 in the Supporting Information (SI)). Synthesis of asymmetric rSMDH₂'s was achieved by adding the phosphoramidite core **1**¹⁹ to the initial DNA arm grown from the surface of a controlled porosity glass bead (CPG), followed by synthesis of the second arm via 3'-phosphora-

midite chemistry (Figure 1). As an improvement from our previous synthesis,¹⁸ we added an extra ethylene glycol (CH₂CH₂O) linker between the core and the DNA strand; this makes the products more stable during the DMT-on reversed-phase HPLC purification. To evaluate the effect of the core **C** on melting behavior, we also prepared analogous SMDH₂'s where the arms are simply linked together via a 6-base pair ss-DNA TTTT sequence (T₆ spacer; i.e., 3'-A-5'-T₆-3'-B-5' or A-T₆-B for short).

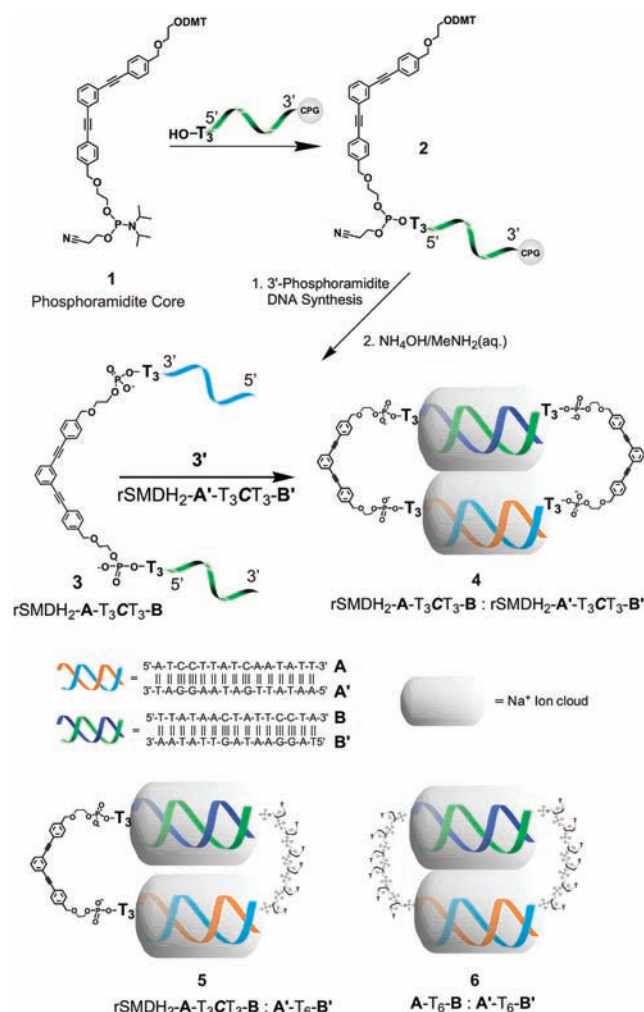


Figure 1. Synthesis of rSMDH₂-A-T₃CT₃-B and its dimeric structures **4–6** (dimeric cages **5** and **6** are formed using rSMDH₂, an unmodified DNA with T₆ linker).

Hybridization of rSMDH₂'s was performed by combining equimolar amounts of two complementarily functionalized rSMDH₂'s in phosphate buffer at 25 °C, annealing the hybridized mixture at 85 °C for 10 min, and allowing it to cool to room temperature over 4 h. The melting profile of the hybridized mixture

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was obtained by heating the samples from 20 to 70 °C at a rate of 1 °C per minute while monitoring the increase in UV-vis absorbance at 260 nm at 0.1 °C intervals. For comparison, we also formed cage-like structures **5** and **6**, from rSMDH₂ **3** with the complementary A'-T₆-B', and from A-T₆-B:A'-T₆-B', respectively (Figure 1).

Similar to the previously reported rSMDH₃ experiment,¹⁸ non-denaturing PAGE-gel analyses of the hybridized 0.38–2 μM solutions containing caged dimers **4** and **6** (Figure 2; see also Figures S8 and S9 in the SI) displayed sharp bands. However, at higher concentrations (4–16 μM), solutions of these caged dimers show new bands and smearing, corresponding to higher cyclic and oligomeric structures, respectively.

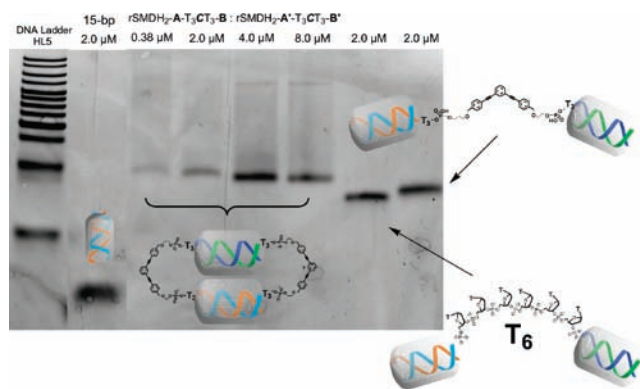


Figure 2. Nondenaturing PAGE-gel image (10%) of DNA duplexes and hybrids. From left to right: lane 1 = HL5 DNA ladder, lane 2 = A:A' (2 μM), lanes 3–6 = rSMDH₂-A-T₃CT₃-B:rSMDH₂-A'-T₃CT₃-B' (0.38 to 8 μM), lane 7 = A:A'-T₆-B'B (2.0 μM), lane 8 = A:rSMDH₂-A'-T₃CT₃-B'B (2.0 μM).

The neighboring-duplex/effective-concentration model¹⁵ predicts that when an aggregate possesses two or more parallel DNA duplexes in close proximity (25–40 Å),¹⁷ its melting point (T_m) increases and its melting profile sharpens. Consistent with this conjecture, the rSMDH₂ dimer **4** (**3:3'**) displayed a sharper melting profile (full width at half-maximum (fwhm) = 6.3 ± 0.2 °C) and a higher melting temperature (T_m = 43.5 ± 0.4 °C) compared to the A:A' unmodified 15 base-pair DNA duplex (fwhm = 10.6 ± 0.1 °C, T_m = 41.3 ± 0.1 °C) (Table 1). Surprisingly, dimers **5** and **6** (Figure 3), where either one or both partners do not have a rigid core linking the two DNA arms together, also show similarly sharp melting profiles and higher T_m values (Table 1, entries 11–16) as in the case of **4**. This observation suggests that as long as the linkers are short enough to keep the DNA arms in close proximity, cooperative melting can occur readily in the absence of a rigid linker.

We also designed a new rSMDH₂ **7** (3'-A'-5'-T₃CT₃-5'-B'-3') to hybridize with rSMDH₂ **3** (3'-A-5'-T₃CT₃-3'-B-5') and form a hairpin-like dimer **8** (**3:7**). The melting profile of this dimer **8** displays a broader (fwhm = 9.1 ± 0.3 °C, Figure 4) transition compared to rSMDH₂ dimer **4** (**3:3'**) (fwhm = 6.2 ± 0.1 °C). This again supports our hypothesis that ion-cloud sharing is crucial to cooperative melting; the parallel orientation of the DNA duplexes in dimer **4** leads to better overlap between the ion clouds in the two partner duplexes, and sharper melting profile, in comparison to that in dimer **8**.

As a control experiment, the melting profiles of the hybrids formed between T₆-linked A'-T₆-B' and its complementary sequences **A** and **B** (Table 1, entries 5–7) are broader and have lower T_m values compared to dimer **6** (Table 1, entries 11–17). As predicted by the neighboring-duplex/effective-concentration

Table 1. Melting Data for 15-bp DNA Duplexes (A:A'), A:A'-T₆-B':B', Caged- (**4**, **5**, **6**), and Hairpin-like (**8**) Dimers Formed by Unmodified DNA and rSMDH₂ Hybrids

entry	hybridization mixture	[NaCl] (mM) ^a	T_m (°C)	fwhm (°C)	N_c (±0.1)
1	A:A' ^b	75	35.6 ± 0.4	10.3 ± 0.4	–
2	A:A' ^b	150	40.9 ± 0.2	10.6 ± 0.1	–
3	A:A' ^b	300	45.3 ± 0.4	10.8 ± 0.3	–
4	A:A' ^{b,c}	300	45.8	10.5	–
5	A:A'-T ₆ -B':B' ^d	75	36.4 ± 0.1	10.2 ± 0.1	1.0
6	A:A'-T ₆ -B':B' ^d	150	41.0 ± 0.3	10.3 ± 0.1	1.0
7	A:A'-T ₆ -B':B' ^d	300	46.5 ± 0.2	10.3 ± 0.3	1.0
8	4 ^b	75	37.5 ± 0.2	6.3 ± 0.2	1.7
9	4 ^b	150	43.5 ± 0.4	5.9 ± 0.1	1.8
10	4 ^b	300	49.1 ± 0.3	7.5 ± 0.1	1.6
11	5 ^b	75	36.9 ± 0.1	5.9 ± 0.1	2.0
12	5 ^b	150	43.5 ± 0.1	5.8 ± 0.1	2.1
13	5 ^b	300	47.9 ± 0.1	6.2 ± 0.1	1.8
14	6 ^b	75	36.5 ± 0.1	6.0 ± 0.1	1.8
15	6 ^b	150	42.2 ± 0.1	6.1 ± 0.1	2.0
16	6 ^b	300	48.5 ± 0.1	5.2 ± 0.2	1.8
17	6 ^{b,c}	300	48.8	4.7	–
18	8 ^{b,e}	75	35.4 ± 0.2	8.9 ± 0.2	1.1
19	8 ^{b,e}	150	41.8 ± 0.1	9.1 ± 0.3	1.2
20	8 ^{b,e}	300	46.6 ± 0.1	8.9 ± 0.1	1.3

^a NaCl concentration in a 10 mM PBS buffer. ^b 0.76 μM total [DNA]. ^c Theoretical value. ^d 2.00 μM total [DNA]. ^e Hairpin-like dimer **8** (**3:7**).

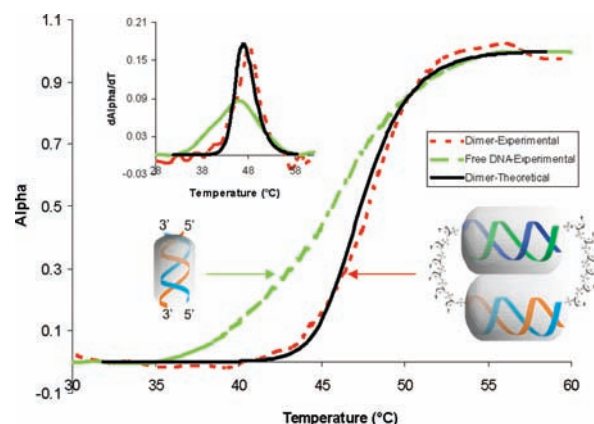


Figure 3. Experimental and theoretical melting curves for A-T₆-B:A'-T₆-B' (0.76 μM) and the experimental melting curve for A:A' (0.76 μM) in PBS buffer (10 mM, pH 7.0, 300 mM NaCl). Inset shows the first derivatives of the melting curves.

model,¹⁷ when the salt concentration increases, the T_m difference between the A:A' duplex and the dimer **4** also increases (Table 1, cf. entries 1–3 vs 8–10). Similar comparisons hold for dimers **5** and **6**.

From the salt-concentration dependence of the melting temperature of the aggregates (Table 1, entries 5–17), the number of cooperative duplexes (N_c , defined in eq S2 in the SI) can be calculated for the various SMDH₂ hybridization mixtures (see SI).¹⁵ As expected for a cooperative dimer pair, N_c for the caged dimers averages between 1.6 and 2.1 ± 0.1. Interestingly, while the T_m 's for the control groups A:A'-T₆-B':B' and A:rSMDH₂-A'-T₃CT₃-B':B' do not differ significantly from that of A:A', their N_c 's still average in the range 0.9–1.0 ± 0.1, clearly indicating that there is no cooperativity between the duplexes.

The formation of DNA dimers such as **4–6** from the dissociated DNA partners ($[a]$ and $[b]$) can be viewed as a three-state event where the first arms come together initially to form $[ab_1]$ as a bimolecular reaction (Figure 5). Hybridization of the second arms to give $[ab_2]$ is nominally unimolecular but can be thought of as a

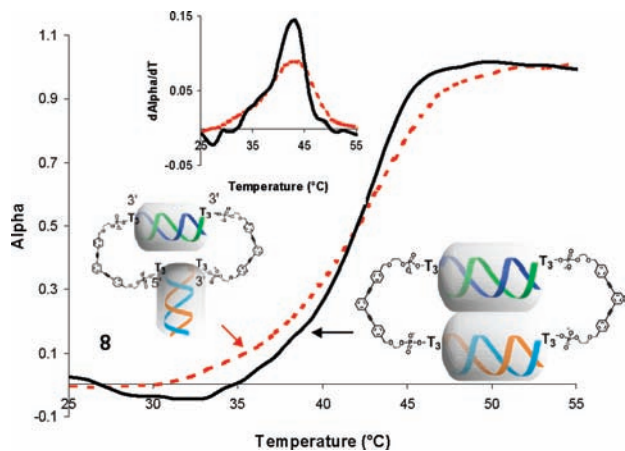


Figure 4. Melting curves for dimer **8** (3:7; 0.76 μM) and dimer **4** (3:3'; 0.76 μM) in PBS buffer (10 mM, pH 7.0, 150 mM NaCl). Inset shows the first derivatives of the melting curves.

bimolecular reaction in which the effective local concentration of the second arms (see SI) is greatly enhanced compared to the solution concentration. For the reverse (melting) process, the first step ($[ab_2] \rightarrow [ab_1]$) is therefore entropically disfavored, but the second step ($[ab_1] \rightarrow [a] + [b]$) is strongly favored. Additional factors that favor cooperative melting arise as a result of counterion clouds which are shared between the two duplexes in $[ab_2]$, but not in later stages, and can be readily modeled by CGMD simulations.¹⁴

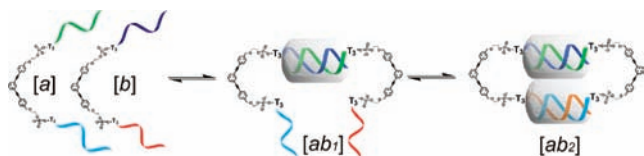


Figure 5. A schematic presentation of the hybridization of rSMDH₂ dimer showing three possible states.

Based on the aforementioned effective concentration model and adjusted thermodynamic parameters from CGMD simulations (see SI), analytical melting curves can be generated for dimer **6** (fwhm = 4.7 °C, Figure 3) that closely mimic the experimental melting profiles and T_m increases. This close agreement suggests that our entropic attribution for the melting of SMDH₂ materials is a reasonable one: while the first dehybridization is entropically disfavored in comparison to the melting of free DNA duplexes in solution, the second step, where most of the entropy is released is

strongly favored, is primarily responsible for the sharp melting behavior in a cooperative, cascade melting mechanism.

In conclusion, we have experimentally demonstrated for the first time that aggregates with two parallel DNA duplexes in close proximity can interact cooperatively, consistent with the prediction by the neighboring-duplex/effective-concentration model.^{14,15} With the help of coarse-grain molecular dynamics simulations, we can now begin to construct accurate analytical models that allow us to understand the thermodynamic parameters governing the cooperative interactions that occur when DNA duplexes aggregate. This knowledge will enable researchers to design better DNA-hybrid materials for a wide range of applications.

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Supporting Information Available: Synthetic procedures and characterization data for rigid small molecule–DNA hybrids; methods and data for hybridization experiments; theoretical equations for the analytical model. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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