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Uncovering the Self-Assembly of DNA Nanostructures by Thermodynamics and Kinetics

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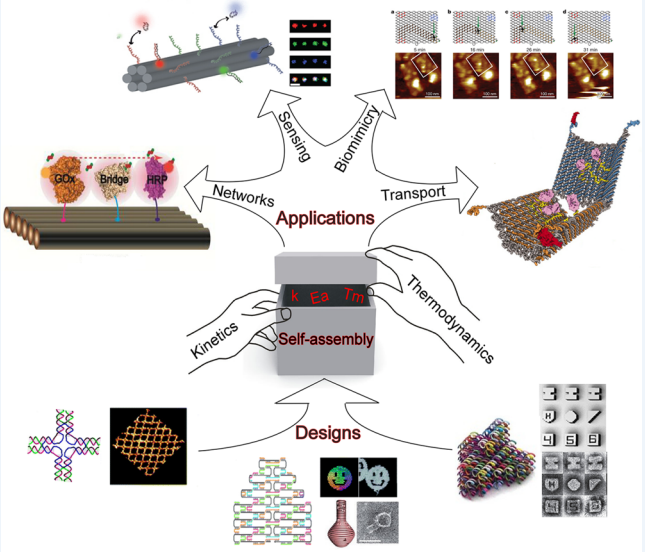
CONSPECTUS: DNA nanotechnology is one of the most flourishing interdisciplinary research fields. DNA nanostructures can be designed to self-assemble into a variety of periodic or aperiodic patterns of different shapes and length scales. They can be used as scaffolds for organizing other nanoparticles, proteins, and chemical groups, leveraging their functions for creating complex bioinspired materials that may serve as smart drug delivery systems, *in vitro* or *in vivo* biomolecular computing platforms, and diagnostic devices. Achieving optimal structural features, efficient assembly protocols, and precise functional group positioning and modification requires a thorough understanding of the thermodynamics and kinetics of the DNA nanostructure self-assembly process. The most common real-time measurement strategies include monitoring changes in UV absorbance based on the hyperchromic effect of DNA, and the emission signal changes of DNA intercalating dyes or covalently conjugated fluorescent dyes/pairs that accompany temperature dependent structural changes.



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Thermodynamic studies of a variety of DNA nanostructures have been performed, from simple double stranded DNA formation to more complex origami assembly. The key parameters that have been evaluated in terms of stability and cooperativity include the overall dimensions, the folding path of the scaffold, crossover and nick point arrangement, length and sequence of single strands, and salt and ion concentrations. DNA tile−tile interactions through sticky end hybridization have also been analyzed, and the steric inhibition and rigidity of tiles turn out to be important factors. Many kinetic studies have also been reported, and most are based on double stranded DNA formation. A two-state assumption and the hypothesis of several intermediate states have been applied to determine the rate constant and activation energy of the DNA hybridization process. A few simulated models were proposed to represent the structural, mechanical, and kinetic properties of DNA hybridization. The kinetics of strand displacement reactions has also been studied as a special case of DNA hybridization.

The thermodynamic and kinetic characteristics of DNA nanostructures have been exploited to develop rapid and isothermal annealing protocols. It is conceivable that a more thorough understanding of the DNA assembly process could be used to guide the structural design process and optimize the conditions for assembly, manipulation, and functionalization, thus benefiting both upstream design and downstream applications.

INTRODUCTION

DNA nanotechnology is a field in which artificial nucleic acid nanostructures are designed and constructed for a variety of technological purposes.[1−8] With accurate helical dimensions and predictable Watson−Crick hydrogen bond interactions, double helical DNA motifs have been widely utilized as programmable nanometer scale building blocks. DNA exhibits the combined rigidity and flexibility necessary to construct complex, higher order one-, two- and three- dimensional (1D, 2D, 3D) structures.[6−13] The growth and development of DNA nanotechnology has culminated in a variety of interesting structures and applications: from organizing nanoparticles, proteins, and nucleic acids, to serving as platforms for the assembly of complex biochemical machinery.[2−4] Most of these applications rely only on controlling the initial design parameters and observing the corresponding outcome, without much concern for the thermal features or mechanisms of nanostructure assembly.

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One major challenge in structural DNA nanotechnology is to increase the size and complexity of DNA assemblies while simultaneously controlling the error rate. The purity and relative stoichiometry of the participating single stranded DNA (ssDNA), and parameters such as structural constraint, DNA concentration, annealing profile, salt/ion concentration, and pH, should be optimized to reduce errors and improve the final assembly yield. Researchers have to perform tedious and iterative experimental analyses to identify the optimal assembly conditions for a particular design, often based solely on their own previous experience and intuition, due to a lack of understanding of the underlying mechanisms of assembly and the availability of pertinent thermodynamic and kinetic data.

In this Account, we will describe what is known about the thermodynamic and kinetic properties of complex DNA systems, two crucial parameters that shed light on the process of DNA nanostructure assembly. DNA nanotechnology represents a unique opportunity to gain insight about the dynamic changes and transition states of polyvalent binding events that accompany the association of DNA strands.[14,15] A few mechanistic studies of the formation of DNA nanostructures have already revealed various physical and chemical aspects of assembly, not only providing valuable predictive power that promotes upstream design efficiency, but also informing the construction of complex systems for downstream applications.

GENERAL OVERVIEW OF THE THERMODYNAMICS AND KINETICS OF DNA NANOSTRUCTURES

The thermodynamics of DNA structures explains the overall energy changes and transitions between single and double stranded states, reflecting the stability, cooperativity, and intrinsic flexibility of the assembled structures. When ssDNAs with rationally designed sequences are mixed together, heated to a high temperature to disrupt unwanted base pairing, and then gradually cooled, the DNA strands associate with their complementary sequences and they self-assemble into the designed shapes and patterns. In contrast, the assembled structures dissociate (melt) into the individual ssDNAs in response to increasing temperature. For cases in which the rate of temperature change is sufficiently low, dynamic equilibrium at each temperature is achieved, and the association/dissociation processes display overlapping traces that indicate reversibility. We can extract the melting temperature (T_m), which is the midpoint of the transition where half of the structures are associated and half are dissociated (Figure 1A), and the width of the transition, reflecting the cooperativity of assembly/disassembly, from these thermal melting curves. Other thermodynamic parameters that can be extracted from van't Hoff analyses include the free energy change (ΔG), enthalpy change (ΔH), and entropy change (ΔS), which reflect the overall thermal stability, contribution from intermolecular interactions, and internal rigidity/flexibility of the nanostructures, respectively.

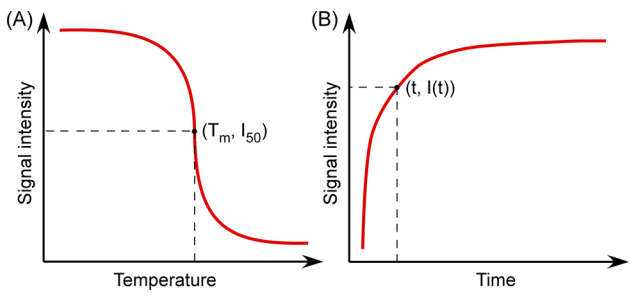

Figure 1. Data profiles of thermodynamic and kinetic measurements. (A) A representative thermal curve. The melting temperature (T_m) is the temperature at which 50% of the reaction is complete. (B) A representative kinetic curve.

Kinetic analyses describe reaction rates in nonequilibrium states and provide instructional information about transition states and the time required to reach equilibrium under certain conditions. In particular, kinetic studies of DNA nanostructures that focus on the rate of structural formation and underlying mechanisms such as the activation energy (E_a), reveal details not accessible through thermodynamics studies. Temperature dependent rate constants (k) can be determined from kinetic curves (Figure 1B). The E_a of a reaction reflects the energy barrier required to facilitate a given reaction pathway and can be obtained from temperature dependent kinetic measurements (Arrhenius plots).

Understanding DNA nanostructure assembly involves evaluating the single-stranded DNA interactions that form structural motifs, and the overall structural stability and flexibility of the final assembly. The main factors that determine the thermodynamic and kinetic behavior of the structural motifs are the length and sequence of the participating ssDNAs and their binding domains; other internal factors include the overall dimensions of the nanostructure (i.e., its translational and rotational diffusion dynamics), the locations of binding domains that may be sterically hindered, the rigidity of the structures before and after assembly, the folding path of the ssDNAs, and the distances between crossover points. There are external factors that also affect the assembly process, including metal ion concentration. Mg^{2+} (12−22 mM) is regularly used to stabilize the negatively charged backbone in DNA structures, with increasing concentration as the complexity of the structure increases.[19,29] In addition, excess staple strands are necessary to effectively direct folding of the scaffold strand, but eventually the presence of excess strands does not further improve the yield or rate of assembly.[23]

MEASUREMENT STRATEGIES

To date, a few methods have been reported for observing the dynamic assembly of DNA nanostructures, including optical spectroscopy, atomic force microscopy,[16] and microcalorimetry.[17] The latter two are less commonly used due to the slow (delayed) read out, large sample volume requirements, and potential interference from environmental factors.

In optical spectroscopy methods, researchers commonly utilize the increase in UV absorbance at 260 nm that occurs when DNA double helices change from ordered (native) to disordered (denatured) structures, referred to as the hyperchromic effect. The primary drawbacks of this method are the relatively small signal change, especially for DNA origami samples in the presence of a large excess of staple strands, and the potential structural damage to DNA caused by prolonged UV exposure.

DNA intercalating dyes such as SYBR Green SYBR Gold, and YOYO dyes preferentially bind to double rather than single stranded DNA, exhibiting a concurrent increase in fluorescence quantum yield when inserted between the DNA base pairs. They have been used for studying the DNA self-assembly process in real time by monitoring the change in fluorescence intensity with temperature or time.[18] The ratio of dye molecules to DNA base pairs must be carefully controlled to produce a usable signal

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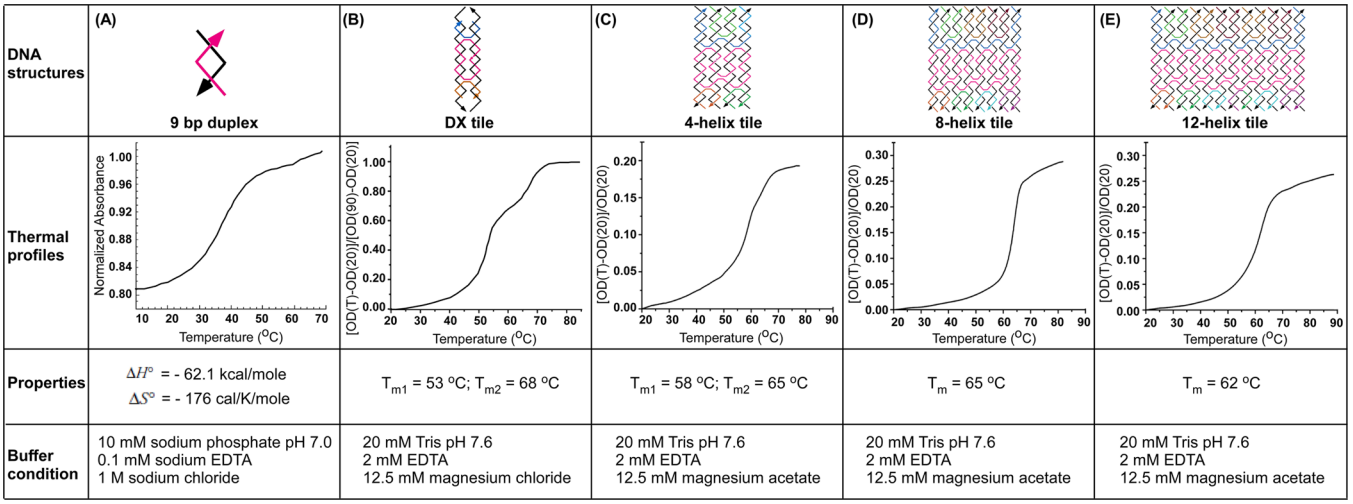

Figure 2. Examples of thermodynamic analyses of DNA tile nanostructures: (A) 9 bp duplex[28] (Adapted with permission from ref 28. Copyright 2000 American Chemical Society); (B) DX tile (Adapted with permission from ref 33); (C) 4-helix tile (Adapted with permission from ref 10); (D) 8-helix tile (Adapted with permission from ref 10); (E) 12-helix tile (Adapted with permission from ref 10). A representative thermodynamic profile, values of T_m (or ΔH and ΔS if reported), and buffer conditions are listed.

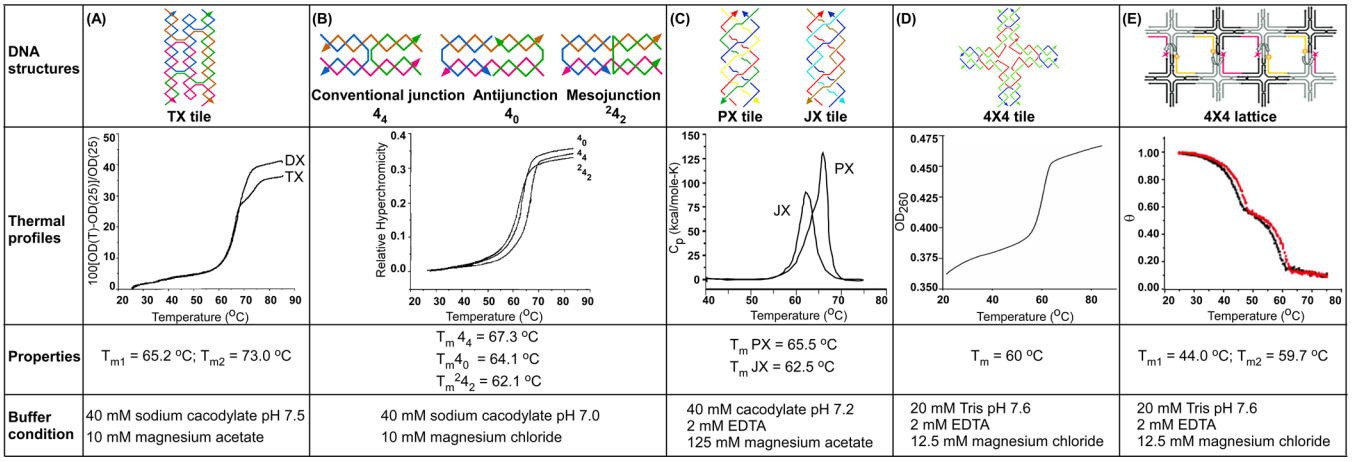

Figure 3. Examples of thermodynamic analyses of complex DNA tiles and tile array formation: (A) TX tile (Adapted with permission from ref 34. Copyright 2000 American Chemical Society.); (B) Holliday junction, antijunction, and mesojunction tiles (Adapted with permission from ref 36. Copyright 1992 American Chemical Society.); (C) PX and JX tiles (Adapted from ref 35 with permission from Elsevier.); (D) 4 × 4 tile (From ref 7. Adapted with permission from AAAS.); (E) 4 × 4 lattice (Adapted from ref 21). The reported T_m values and buffer conditions are listed.

change while simultaneously minimizing the background. However, the intercalating dyes may induce a change in the helical twist of the DNA, leading to conformational distortions of the structures.[19] Moreover, the switches between single and double stranded states induce a new equilibrium between the dyes, which may result in a delayed detection of the signal change.[19,20] Finally, strong interactions between the intercalating dyes and the bases may cause changes in the thermal stability of the DNA structures. Nevertheless, the sensitivity and convenience of this method have been utilized in many thermal studies.[18,20]

An alternative optical spectroscopy method involves covalent incorporation of fluorescent dyes, either pairs of Förster resonance energy transfer (FRET) dyes or fluorescent dye–quencher pairs. FRET is a well-established measurement technique commonly used to study distance-dependent molecular events. It is well suited for studying dynamic DNA nanostructure assembly/disassembly due to the predictable energy transfer that occurs on the nanometer scale. The FRET or quenching efficiency reflects the assembly yield of the DNA structures accurately, sensitively, and instantly, which makes fluorescence spectroscopy a popular method in DNA thermodynamic and kinetic studies.[21,22] Fluorescently labeled ssDNA is commercially available for a variety of dye choices with unique excitation/emission wavelengths. Since only one or two labeled strands are required for experiments, there is minimal background interference. For multimolecular ($n > 2$) reactions, a FRET pair can simplify data analysis by enabling the use of a two-component model to describe the assembly process. Moreover, a recent study demonstrated that the sensitivity of some reporter dyes to the local environment can be used not only to probe global structure but also to distinguish fine structural changes within a larger structure.[23] Some fluorophores display significant signal changes upon hybridization to ssDNA, possibly due to changes in their interaction with neighboring nucleotides (accompanied by a change in quantum yield), which makes it possible to use a single fluorophore to indicate structural changes.[24]

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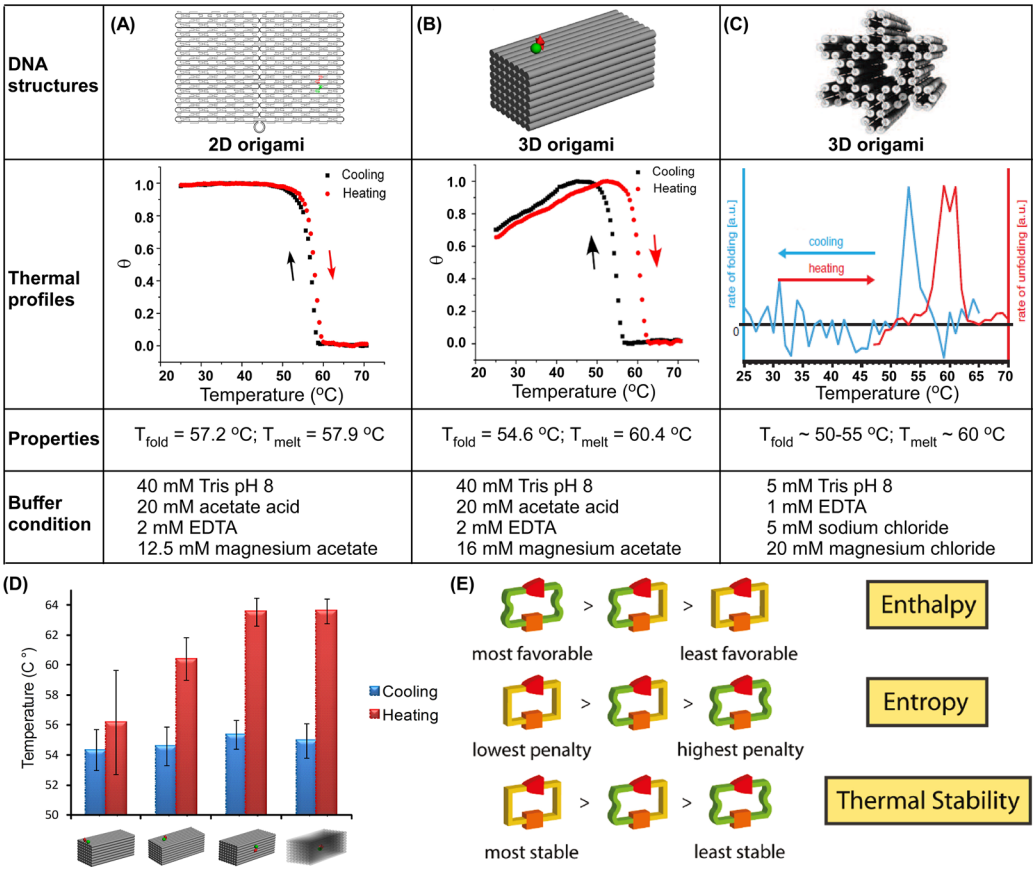

Figure 4. Examples of thermodynamic analyses of DNA origami and tile−tile interactions. (A) 2D[23] and (B,[23] C[18]) various 3D origami with the corresponding thermal profiles and buffer conditions reported. (Adapted from refs 23 and 18, respectively.) (D) Heterogeneity during the assembly/disassembly of different parts of a 3D cuboid origami structure. (From ref 23. Reprinted with permission from American Chemical Society.) (E) Illustration of the thermodynamic stabilities of dimers formed from DX tiles with varying flexibility (Adapted from ref 15).

THERMODYNAMICS OF DNA NANOSTRUCTURES

As early as 1987, Breslauer and co-workers studied the thermal behavior of DNA junction motifs by UV absorbance and other calorimetry methods.[25] With the development and application of improved measurement approaches, more accurate and thorough analyses of the thermodynamic properties of DNA nanostructures have since been achieved. Estimating the thermal parameters of Watson−Crick base pairing based on the nearest neighbor model when the salt conditions and sequences are provided is commonly calculated by software such as Mfold.[26,27] The formation of a 9 bp duplex was shown to have $\Delta H = -62.1$ kcal mol^{-1} and $\Delta S = -176$ cal K^{-1} mol^{-1} by Howard (Figure 2A).[28] Duplexes with bulges or mismatches have lower T_m's, which can be remitted using higher Na^+ or Mg^{2+} concentrations.[29] The thermodynamic properties of 8 bp DNA/DNA, RNA/RNA, and DNA/RNA hybrid duplexes indicated that RNA/RNA duplexes are the most stable, with T_m's in the 60−66 °C range and ΔG_{37} of −13 kcal mol^{-1}, compared with DNA/DNA or hybrid duplexes, both with T_m's between 42 and 54 °C and $\Delta G_{37} \approx -9$ kcal mol^{-1}.[30]

Double crossover (DX) motifs are composed of two duplexes linked side by side at two double crossover points (as in a Holliday junction) and have been used to construct periodic 1D and 2D arrays via sticky end associations.[21,31] The thermal behavior of individual DX tiles show multiple transitions between 45 and 70 °C,[13,32,33] where the folding of long undisrupted duplexes and duplexes with a nick point are distinguishable by two transitions (Figure 2B). Four-helix tiles are composed of two DX tiles linked side by side, and they display similar thermal transitions as DX tiles (Figure 2C), while more complex 8- and 12-helix tiles exhibit a single thermal transition indicating the existence of more cooperative assembly processes in larger systems (Figure 2D,E).[10]

More complicated junction tiles, including triple crossover (TX) and parallel crossover (PX) tiles, were investigated by the Seeman group.[9,34−36] When they compared DX and TX tiles of the same length and similar GC content, they found that TX tiles also displayed two transitions and concluded that the overall stability of TX and DX tiles is comparable (Figure 3A).[34] Beyond that, they also examined the thermal stability of conventional Holliday junctions, which have T_m's higher than antijunctions and mesojunctions (involving one or two nick points in the backbone), indicating that more flexible stacking domains in the latter junctions destabilize the base pairing interactions that flank the junction point (Figure 3B).[36] Another study from their group investigated PX tiles and demonstrated the thermally preferred formation of PX tiles over juxtaposed parallel (JX_1) tiles (Figure 3C).[35] Compared with simple duplexes, both PX and JX_1 tiles have comparable enthalpic gains but higher entropic penalties due to the formation of more compact crossovers, resulting in a kilocalorie per mole of base pair penalty in free energy.

To develop more complex and larger structures, researchers covalently linked four Holliday junctions together and created four-arm tiles (4 × 4) that were subsequently used to assemble 2D arrays (Figure 3D).[7] An accurate thermal study of 4 × 4 tiles and the corresponding arrays was performed by the Niemeyer

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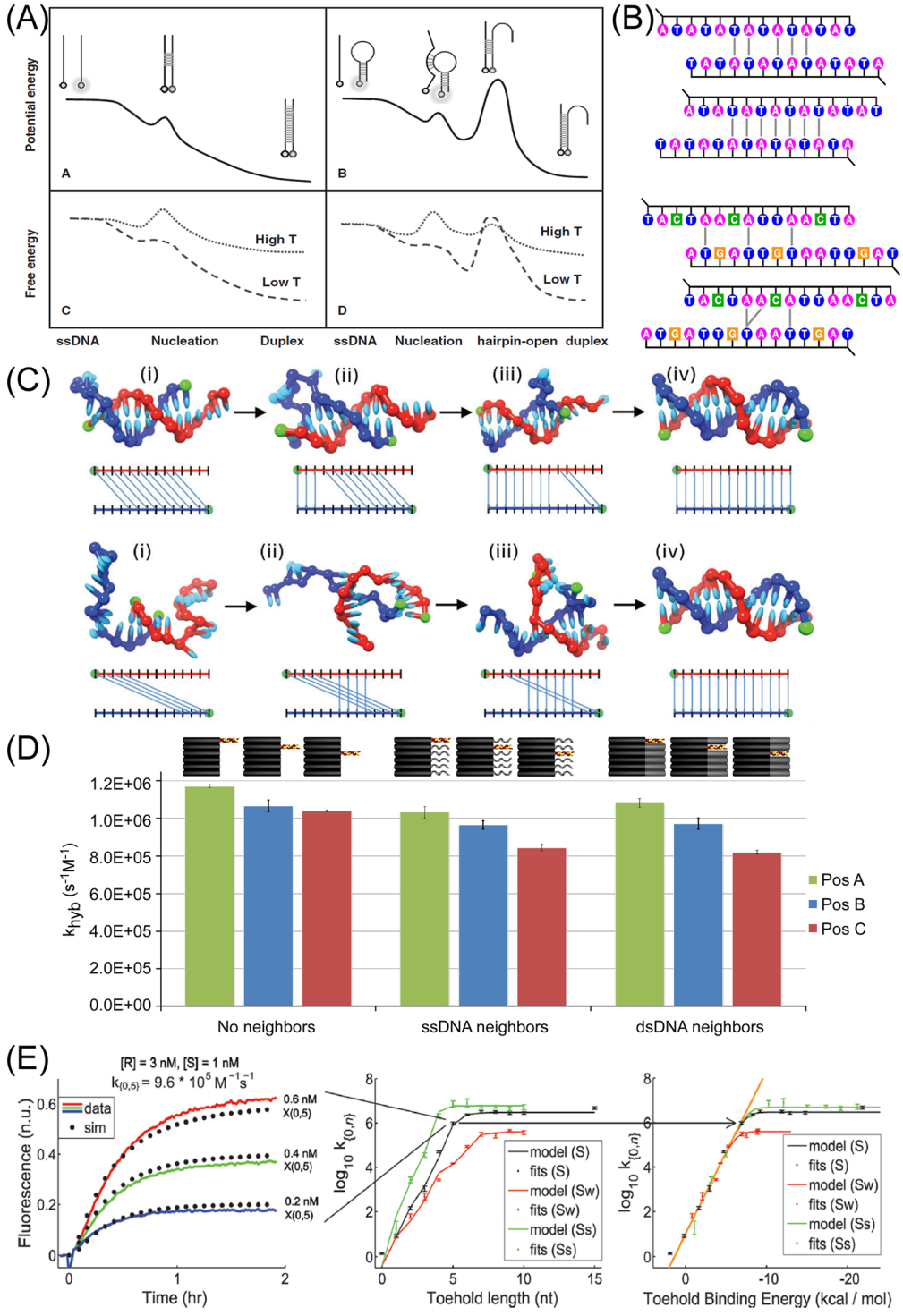

Figure 5. Examples of kinetic studies of DNA nanostructures. (A) Schematic representation of various energy profiles with and without intrastrand hairpin structures at high and low temperature. (Adapted with permission from ref 43. Copyright 2007 Oxford University Press.) (B) Illustration of two mechanisms of duplex formation from complementary ssDNAs, slithering of a repeated sequence (top) and required contacts of a random sequence (bottom). (Adapted with permission from ref 44.) (C) Coarse-grained model to explain the internal displacement mechanism of hybridization of complementary, repetitive sequence ssDNAs: the inchworm mechanism (top) with a bulge loop from alternative binding passing along the helix, and pseudoknot mechanism (bottom) with correct long ssDNA tail binding initiated by short misalignment. (Adapted with permission from ref 45. Copyright 2013 Oxford University Press.) (D) Hybridization rate constants decrease with increasing steric crowding and reducing binding site accessibility. (Adapted from ref 24.) (E) Kinetic profiles of strand displacement reactions corresponding to different toehold lengths. Models S, Sw, and Ss refer to typical toehold, weak toehold, and strong toehold, which contain equal, less, and more GC content, respectively. (Reprinted with permission from ref 37. Copyright 2009 American Chemical Society.)

group[21] using fluorescence spectroscopy and a FRET pair labeled close to the sticky ends. Compared with previous studies using UV absorbance measurements, they observed two distinguishable transitions during periodic lattice formation: the higher

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temperature transition is attributed to preformation of dispersed tiles, and the lower one reflects the cooperative formation of 2D arrays from the individual tiles through sticky end associations (Figure 3E). This study demonstrated the accuracy of applying FRET pairs to provide full thermodynamic characterization of tile assembly and array growth.

Recently, researchers have begun to investigate the thermodynamic properties of more complex DNA nanostructures such as DNA origami, although a more thorough study of these structures has yet to be achieved and new approaches to deconvolute the energetics are needed. Dietz and co-workers[18] reported higher melting temperatures than folding temperatures for a series of 3D origami structures (Figure 4C), which is in agreement with a study of 3D origami from the Liu group[23] (Figure 4B). In the latter work, they also compared the thermal profiles of 2D (Figure 4A) and 3D origami. FRET probes were used to study the global and local environments and thermal behavior of several partially formed origami structures, and the nearly homogeneous assembly of 2D origami and overlap between the folding and melting curves were verified, indicating a highly cooperative and energetically favorable scaffold topology. In contrast, 3D origami displayed a 7−10 °C hysteresis with a much slower formation rate in the cooling phase, since the long scaffold strand, under the direction of hundreds of staple strands, must overcome a relatively high energy barrier to realize the complicated folding pathway (Figure 4B). They propose that disassembly likely occurs in steps, since the parallel double helices may dissociate from both ends toward the middle, until the final structure is completely dissolved at higher temperatures (Figure 4D). Thus, the diverse formation/dissociation behavior of 3D origami exhibits strong dependence on the scaffold path and staple arrangement.

The Liu group also performed systematic thermodynamic studies of tile−tile interactions.[14,15] Multivalent sticky-end association between two complementary multihelical DNA tiles was evaluated, and they found that increasing the number of intertile interactions enhanced dimer stability and changing the relative positions of the sticky ends resulted in unique superstructure T_m's and free energy changes. The formation of dimer structures from more flexible tiles was shown to proceed with favorable enthalpic gains due to reduced energetic strain but involved much higher entropic penalties because of the order induced on the tiles, resulting in an overall lower thermal stability (Figure 4E).

All these studies provided useful quantitative thermodynamic information describing discrete DNA motifs and periodic arrays. By carefully comparing thermal profiles, the less favorable conformational arrangements such as parallel crossovers and complex scaffold topologies were revealed. The existence of extended, undisrupted, double helical domains, higher GC content, greater numbers of longer sticky ends with favorable positions, and higher Mg^{2+} concentration were demonstrated to significantly improve the stability and formation of DNA nanostructures.

KINETICS OF DNA HYBRIDIZATION AND STRAND DISPLACEMENT

Compared to the thermodynamic studies of DNA nanostructures, less effort has been spent on investigating the kinetics and reaction pathways of structural formation due to the difficulty in capturing information about any intermediate states. The development of advanced techniques and suitable models will facilitate a deeper understanding of the dynamic aspects of DNA nanostructure assembly.

Porschke and co-workers studied the formation of DNA duplexes in the 1970s. The bimolecular rate constant of DNA duplex formation is demonstrated on the order of 10^6−10^7 M^{-1} s^{-1},[37−40] which can be accelerated by increasing Na^+ and Mg^{2+} concentration,[39,40] decreasing the possibility of complex secondary structures within ssDNA, or performing surface meditated reactions.[38] The activation energy (E_a) of DNA nanostructure formation is affected by sequence, intrastrand secondary structure, and reaction temperature. However, these factors may not function separately but are restricted by each other, for example, the E_a of DNA hybridization has been reported as negative for AT rich sequences and positive for GC rich sequences at temperatures around the T_m, although these results are complicated at other temperatures.[41] Researchers have suggested that the dissociation rate constant (k_{off}) of DNA nanostructures increases at higher temperatures,[22,41−43] while the association rate constant (k_{on}) may increase[22,41] or decrease[42] at higher temperatures. Zhao and co-workers claimed that there is a temperature dependent switching of the rate limiting step in the formation of DNA nanostructures, based on a change in the free energy profile with varying temperature.[43] By analyzing the formation of duplexes from ssDNA with/without intrastrand hairpin structures, they found that at low temperatures the enthalpic cost of disrupting the transient intramolecular secondary structure of ssDNA was the major limitation while at high temperatures the dominant limiting step of the reaction becomes the entropic cost of nucleation (Figure 5A). The Deckert group demonstrated that k_{off} contributes more to the stability of a duplex than k_{on}, where k_{off} is determined by the energetic penalty to break hydrogen bonds and base stacking interactions.[30]

The underlying mechanisms of duplex formation are still under discussion. Pablo and co-workers demonstrated that the initial contacts between ssDNAs during nucleation are necessary for the hybridization of complementary, random sequences, while they used a "slithering" mechanism to explain the higher hybridization rates of repeating sequences, that is, slithering of the strand and snapping into the correct duplex formation (Figure 5B).[44] This hypothesis is in agreement with another hybridization mechanism proposed by Louis and co-workers who studied sequence dependent duplex formation pathways.[45] They applied a coarse-grained model, oxDNA, to simulate the formation of flexible and stiff DNA duplexes and proposed that complementary, nonrepetitive sequences of ssDNAs prefer to detach after forming the initial contacts, which are not favorable for full duplex formation, but follow a zipping pathway upon the formation of the correct initial contacts. However, for the case of complementary, repetitive sequences of ssDNAs, metastable intermediates with mis-bonded base pairs can be rearranged by "pseudoknot" and "inchworm" internal displacement mechanisms (Figure 5C). These internal displacements can significantly accelerate the rate of hybridization reactions in repetitive and GC rich sequences.

Researchers are interested in understanding the kinetics of hybridization for other DNA nanostructures. The Liu group recently employed a series of 6 Helix DNA tiles with protruding ssDNA probes and studied the effect of local steric bulk on the hybridization rate of a ssDNA target.[24] Lower rate constants of binding were observed on probes displayed from the interior positions, where steric crowding from neighboring ssDNAs reduced the probability of effective nucleation (Figure 5D).

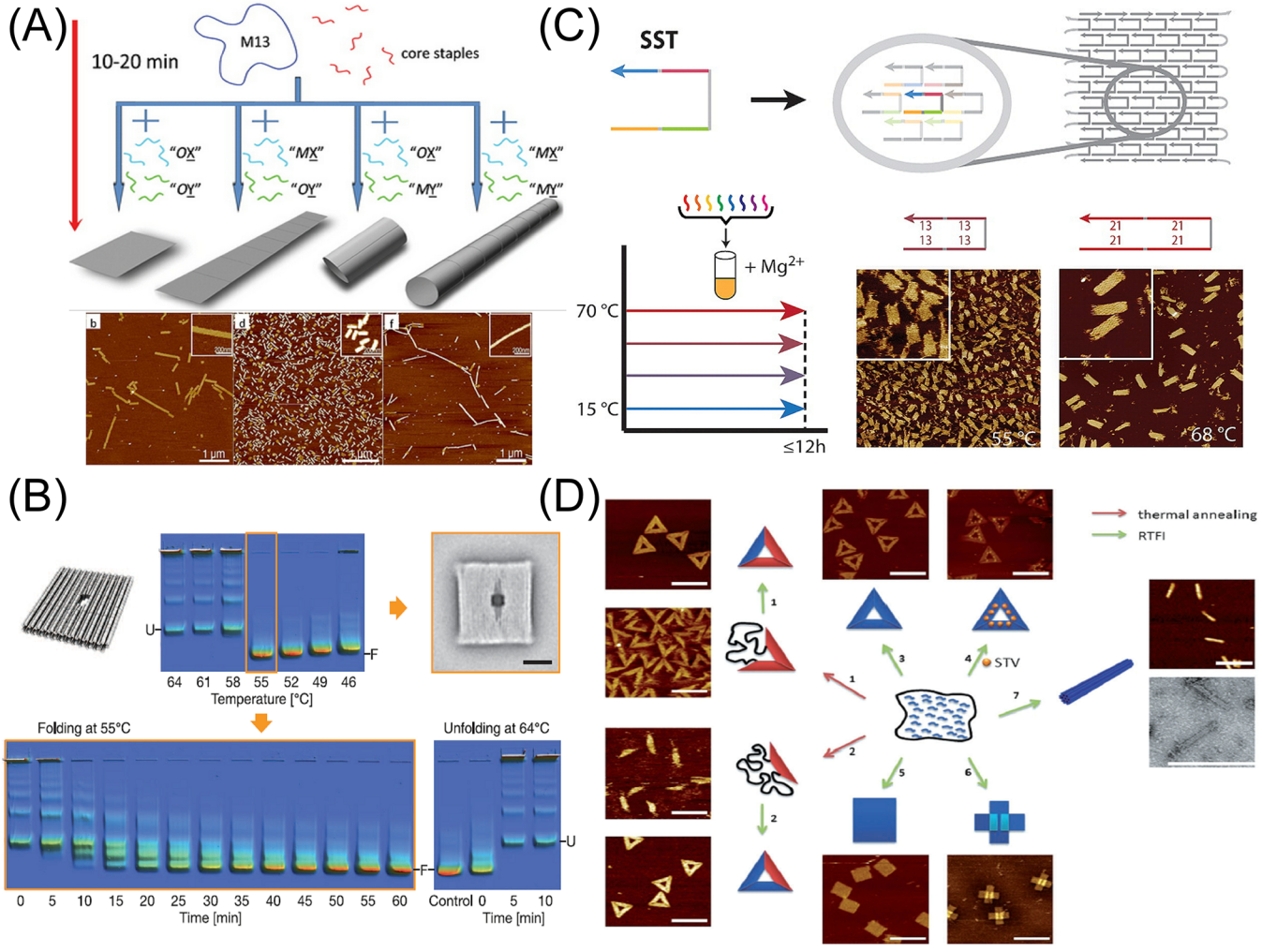

Figure 6. Examples of rapid and isothermal assembly of DNA nanostructures. (A) One pot annealing of combinatorial origami structures and the corresponding AFM images of the products. (Adapted with permission from ref 46. Copyright 2013 American Chemical Society.) (B) Time dependent folding and unfolding of 3D origami at constant temperature analyzed by native gel electrophoresis and the corresponding TEM image of the product. (Reprinted from ref 18 with permission from AAAS.) (C) Illustration of SST structures, the isothermal assembly protocol used, and the corresponding AFM images of the products. (Adapted with permission from ref 47. Copyright 2013 American Chemical Society.) (D) Schematics and AFM images of different origami assembly strategies, all achieved at room temperature with 30% or 40% formamide (pathways 1−6 or 7). (Adapted with permission from ref 48.)

Strand displacement reactions are frequently employed in dynamic DNA nanostructure systems. Winfree and co-workers performed a series of investigations on the kinetics of DNA strand displacement and concluded that the binding rate of the toehold region is critical to determine the final efficiency.[11,37] A three-step model was applied to explain that the rate constant of overall strand displacement reactions is approximately the same as that of the nucleation step (followed by a much faster zipping step). They found that longer toehold length, higher GC content, and higher concentration can increase the probability of nucleation and provide favorable binding energy, resulting in a higher rate constant of strand displacement (10^5−10^6 M^{-1} s^{-1}) (Figure 5E).[37]

RAPID AND ISOTHERMAL ASSEMBLY OF DNA NANOSTRUCTURES

Based on knowledge of the thermodynamic and kinetic characteristics of DNA assembly, researchers have begun to develop isothermal techniques that facilitate faster and milder assembly of DNA nanostructures. Fan and co-workers used rationally designed "edge" strands to construct DNA origami based nanoribbons and nanotubes in a single-pot, where the size of tubes was controllable and the assembly occurred within 10−20 minutes (Figure 6A).[46] This assembly strategy is much faster than standard protocols in which DNA is mixed and slowly cooled over 12 hours. The Dietz group demonstrated that DNA origami can be folded within a few minutes with high yield at a fixed temperature, typically at the low temperature boundary of the folding curve of the thermodynamic profile (Figure 6B).[18] Yin and co-workers examined assembly over a wide range of constant temperatures, from 15 to 70 °C, and successfully assembled scaffoldless single-stranded tile (SST) structures over 12 hours under various buffer conditions (Figure 6C).[47] Winfree and co-workers attempted to optimize strand displacement reactions using deprotector or catalyst strands and successfully demonstrated the isothermal assembly (at room temperature) of >10 μm long nanotubes from DX tiles.[11] It seems that rapid hybridization at a constant temperature right below the melting point ensures successful and efficient assembly. Rapid and isothermal assembly conditions have significantly shortened

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sample preparation times and can potentially facilitate the application of functional modifications with unique buffer and temperature restrictions.

Moreover, chemicals and detergents have been employed to achieve assembly of DNA structures at even lower temperatures. In 2008, Simmel and co-workers demonstrated isothermal assembly of DNA origami by slowly reducing the concentration of formamide.[49] Recently, the Gothelf group realized the construction of partial and full DNA origami and SSTs at room temperature with formamide[48] in relatively high yield (Figure 6D). The ability to assemble complex DNA nanostructures at room temperature is ideal for applications in which the DNA structures serve as scaffolds for other thermally sensitive functional molecules, such as proteins, since high temperature assembly is often detrimental to such molecules.

■ SUMMARY AND PERSPECTIVES

Researchers in DNA nanotechnology have put significant effort into designing more complicated and functional structures and are exploring a wider range of applications but have long been lacking vital information about the thermodynamics and kinetics of assembly to guide them. Uncovering the intricate details of assembly will allow us to thoroughly understand, expertly control, and efficiently optimize structural design and applications.

Although the current tools and techniques have already revealed much about the thermodynamic and kinetic properties of DNA nanostructures, there are many remaining challenges to unravel the complexities of assembly. For example, the rate of crossover formation during the assembly of DNA tile motifs has rarely been studied and is an important factor different from hybridization. In addition, the folding/disassociation behavior of DNA origami structures is still largely unknown due to the great number of interactions among many strands, and it is important for researchers to continue developing appropriate analytical tools, approaches, and models to explore the kinetic behavior of complex DNA nanostructures.

Today, dynamic and transformable structures are mostly achieved by strand displacement reactions, where the transformation of a preformed structure is triggered by external fuel strands.[12,50] In the future, it may be possible to realize structural transformations by subtly controlling temperature fluctuations (and thus energy input/release). Recently, advanced techniques like cryo-electron microscopy (cryo-EM)[51] and all-atom molecular dynamics (MD)[52] have been applied to reveal additional characteristics of dynamic DNA nanostructures. These methods may provide precise and perceptual macro- and microscopic analyses of these complex structures.

With a substantial understanding of the thermodynamic and kinetic properties of DNA nanostructures, including the stabilities and energy requirements to achieve certain designs, one can identify the ideal temperatures for assembly and predict the rates of nanostructure formation, which will lead to optimized design outcomes (higher yield, faster rate), efficient suppression of unwanted side-products and minimize the labor-intensive, trial-and-error approach. Moreover, software that takes these parameters into account can potentially predict the outcomes of any structural design and optimize strand sequences, lengths of sticky ends, positions of crossovers, etc. A database may be established for screening the most frequent and efficient motifs for practical, customized conditions. Researchers using both experimental and theoretical methods can benefit from it, and eventually much of the design process can be automated.

Based on more accurate control and stabilization, biocompatible DNA nanostructures can be utilized for many attractive applications. By more careful optimization of the capture sequences, interactions, and orientations, DNA structures can serve as ideal platforms to arrange and organize other materials. Employing milder assembly conditions will ensure more stable immobilization and minimize the damage to functional components. With controllable, predicable, and dynamically transformable DNA structures, it is possible to achieve *in vivo* diagnosis and therapy, for example, controlled drug delivery modules that sense, activate, and actuate.

We foresee that a thorough understanding of DNA assembly processes will guide structural designs, reduce accumulated errors during self-assembly, and optimize the conditions for preparation, manipulation, and functionalization of DNA nanostructures, ultimately benefiting both upstream design and downstream applications.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Lund, K.; Manzo, A. J.; Dabby, N.; Michelotti, N.; Johnson-Buck, A.; Nangreave, J.; Taylor, S.; Pei, R.; Stojanovic, M. N.; Walter, N. G.; Winfree, E.; Yan, H. Molecular robots guided by prescriptive landscapes. *Nature* **2010**, *465*, 206−210.

(2) Lin, C.; Jungmann, R.; Leifer, A. M.; Li, C.; Levner, D.; Church, G. M.; Shih, W. M.; Yin, P. Submicrometre geometrically encoded fluorescent barcodes self-assembled from DNA. *Nat. Chem.* **2012**, *4*, 832−839.

(3) Douglas, S. M.; Bachelet, I.; Church, G. M. A logic-gated nanorobot for targeted transport of molecular payloads. *Science* **2012**, *335*, 831−834.
(4) Fu, J.; Liu, M.; Liu, Y.; Woodbury, N. W.; Yan, H. Interenzyme substrate diffusion for an enzyme cascade organized on spatially addressable DNA nanostructures. *J. Am. Chem. Soc.* **2012**, *134*, 5516−5519.
(5) Rothemund, P. W. Folding DNA to create nanoscale shapes and patterns. *Nature* **2006**, *440*, 297−302.
(6) Ke, Y.; Ong, L. L.; Shih, W. M.; Yin, P. Three-dimensional structures self-assembled from DNA bricks. *Science* **2012**, *338*, 1177−1183.
(7) Yan, H.; Park, S. H.; Finkelstein, G.; Reif, J. H.; LaBean, T. H. DNA-templated self-assembly of protein arrays and highly conductive nanowires. *Science* **2003**, *301*, 1882−1884.
(8) Han, D.; Pal, S.; Nangreave, J.; Deng, Z.; Liu, Y.; Yan, H. DNA origami with complex curvatures in three-dimensional space. *Science* **2011**, *332*, 342−346.
(9) Seeman, N. C.; Kallenbach, N. R. DNA branched junctions. *Annu. Rev. Biophys. Biomol. Struct.* **1994**, *23*, 53−86.
(10) Ke, Y.; Liu, Y.; Zhang, J.; Yan, H. A study of DNA tube formation mechanisms using 4-, 8-, and 12-helix DNA nanostructures. *J. Am. Chem. Soc.* **2006**, *128*, 4414−4421.
(11) Zhang, D. Y.; Hariadi, R. F.; Choi, H. M.; Winfree, E. Integrating DNA strand-displacement circuitry with DNA tile self-assembly. *Nat. Commun.* **2013**, *4*, 1965−1974.
(12) Han, D.; Pal, S.; Liu, Y.; Yan, H. Folding and cutting DNA into reconfigurable topological nanostructures. *Nat. Nanotechnol.* **2010**, *5*, 712−717.
(13) Rothemund, P. W.; Papadakis, N.; Winfree, E. Algorithmic self-assembly of DNA Sierpinski triangles. *PLoS Biol.* **2004**, *2*, 2041−2053.
(14) Nangreave, J.; Yan, H.; Liu, Y. Studies of thermal stability of multivalent DNA hybridization in a nanostructured system. *Biophys. J.* **2009**, *97*, 563−571.
(15) Nangreave, J.; Yan, H.; Liu, Y. DNA nanostructures as models for evaluating the role of enthalpy and entropy in polyvalent binding. *J. Am. Chem. Soc.* **2011**, *133*, 4490−4497.
(16) Song, J.; Arbona, J. M.; Zhang, Z.; Liu, L.; Xie, E.; Elezgaray, J.; Aime, J. P.; Gothelf, K. V.; Besenbacher, F.; Dong, M. Direct visualization of transient thermal response of a DNA origami. *J. Am. Chem. Soc.* **2012**, *134*, 9844−9847.
(17) SantaLucia, J., Jr.; Turner, D. H. Measuring the thermodynamics of RNA secondary structure formation. *Biopolymers* **1997**, *44*, 309−319.
(18) Sobczak, J. P.; Martin, T. G.; Gerling, T.; Dietz, H. Rapid folding of DNA into nanoscale shapes at constant temperature. *Science* **2012**, *338*, 1458−1461.
(19) Ke, Y.; Bellot, G.; Voigt, N. V.; Fradkov, E.; Shih, W. M. Two design strategies for enhancement of multilayer−DNA-origami folding: Underwinding for specific intercalator rescue and staple-break positioning. *Chem. Sci.* **2012**, *3*, 2587−2597.
(20) Reed, G. H.; Kent, J. O.; Wittwer, C. T. High-resolution DNA melting analysis for simple and efficient molecular diagnostics. *Pharmacogenomics* **2007**, *8*, 597−608.
(21) Sacca, B.; Meyer, R.; Feldkamp, U.; Schroeder, H.; Niemeyer, C. M. High-throughput, real-time monitoring of the self-assembly of DNA nanostructures by FRET spectroscopy. *Angew. Chem., Int. Ed.* **2008**, *47*, 2135−2137.
(22) Morrison, L. E.; Stols, L. M. Sensitive fluorescence-based thermodynamic and kinetic measurements of DNA hybridization in solution. *Biochemistry* **1993**, *32*, 3095−3104.
(23) Wei, X.; Nangreave, J.; Jiang, S.; Yan, H.; Liu, Y. Mapping the thermal behavior of DNA origami nanostructures. *J. Am. Chem. Soc.* **2013**, *135*, 6165−6176.
(24) Pinheiro, A. V.; Nangreave, J.; Jiang, S.; Yan, H.; Liu, Y. Steric crowding and the kinetics of DNA hybridization within a DNA nanostructure system. *ACS Nano* **2012**, *6*, 5521−5530.
(25) Marky, L. A.; Kallenbach, N. R.; McDonough, K. A.; Seeman, N. C.; Breslauer, K. J. The melting behavior of a DNA junction structure: A calorimetric and spectroscopic study. *Biopolymers* **1987**, *26*, 1621−1634.
(26) SantaLucia, J., Jr.; Hicks, D. The thermodynamics of DNA structural motifs. *Annu. Rev. Biophys. Biomol. Struct.* **2004**, *33*, 415−440.
(27) SantaLucia, J., Jr. A unified view of polymer, dumbbell, and oligonucleotide DNA nearest-neighbor thermodynamics. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 1460−1465.
(28) Howard, K. P. Thermodynamics of DNA Duplex Formation: A Biophysical Chemistry Laboratory Experiment. *J. Chem. Educ.* **2000**, *77*, 1469−1471.
(29) Hou, M. H.; Lin, S. B.; Yuann, J. M.; Lin, W. C.; Wang, A. H.; Kan Ls, L. Effects of polyamines on the thermal stability and formation kinetics of DNA duplexes with abnormal structure. *Nucleic Acids Res.* **2001**, *29*, 5121−5128.
(30) Rauzan, B.; McMichael, E.; Cave, R.; Sevcik, L. R.; Ostrosky, K.; Whitman, E.; Stegemann, R.; Sinclair, A. L.; Serra, M. J.; Deckert, A. A. Kinetics and thermodynamics of DNA, RNA, and hybrid duplex formation. *Biochemistry* **2013**, *52*, 765−772.
(31) Fu, T. J.; Seeman, N. C. DNA double-crossover molecules. *Biochemistry* **1993**, *32*, 3211−3220.
(32) Chen, H. L.; Schulman, R.; Goel, A.; Winfree, E. Reducing facet nucleation during algorithmic self-assembly. *Nano Lett.* **2007**, *7*, 2913−2919.
(33) Rinker, S.; Liu, Y.; Yan, H. Two-dimensional LNA/DNA arrays: estimating the helicity of LNA/DNA hybrid duplex. *Chem. Commun.* **2006**, 2675−2677.
(34) LaBean, T. H.; Yan, H.; Kopatsch, J.; Liu, F.; Winfree, E.; Reif, J. H.; Seeman, N. C. Construction, analysis, ligation, and self-assembly of DNA triple crossover complexes. *J. Am. Chem. Soc.* **2000**, *122*, 1848−1860.
(35) Spink, C. H.; Ding, L.; Yang, Q.; Sheardy, R. D.; Seeman, N. C. Thermodynamics of forming a parallel DNA crossover. *Biophys. J.* **2009**, *97*, 528−538.
(36) Du, S. M.; Zhang, S.; Seeman, N. C. DNA junctions, antijunctions, and mesojunctions. *Biochemistry* **1992**, *31*, 10955−10963.
(37) Zhang, D. Y.; Winfree, E. Control of DNA strand displacement kinetics using toehold exchange. *J. Am. Chem. Soc.* **2009**, *131*, 17303−17314.
(38) Gao, Y.; Wolf, L. K.; Georgiadis, R. M. Secondary structure effects on DNA hybridization kinetics: a solution versus surface comparison. *Nucleic Acids Res.* **2006**, *34*, 3370−3377.
(39) Carrillo-Nava, E.; Mejía-Radillo, Y.; Hinz, H.-J. Dodecamer DNA Duplex Formation Is Characterized by Second-Order Kinetics, Positive Activation Energies, and a Dependence on Sequence and Mg2+Ion Concentration†. *Biochemistry* **2008**, *47*, 13153−13157.
(40) Pörschke, D. Model calculations on the kinetics of oligonucleotide double helix coil transitions. Evidence for a fast chain sliding reaction. *Biophys. Chem.* **1974**, *2*, 83−96.
(41) Pörschke, D.; Uhlenbeck, O. C.; Martin, F. H. Thermodynamics and kinetics of the helix-coil transition of oligomers containing GC base pairs. *Biopolymers* **1973**, *12*, 1313−1335.
(42) Craig, M. E.; Crothers, D. M.; Doty, P. Relaxation kinetics of dimer formation by self complementary oligonucleotides. *J. Mol. Biol.* **1971**, *62*, 383−401.
(43) Chen, C.; Wang, W.; Wang, Z.; Wei, F.; Zhao, X. S. Influence of secondary structure on kinetics and reaction mechanism of DNA hybridization. *Nucleic Acids Res.* **2007**, *35*, 2875−2884.
(44) Sambriski, E. J.; Schwartz, D. C.; de Pablo, J. J. Uncovering pathways in DNA oligonucleotide hybridization via transition state analysis. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 18125−18130.
(45) Ouldridge, T. E.; Sulc, P.; Romano, F.; Doye, J. P.; Louis, A. A. DNA hybridization kinetics: zippering, internal displacement and sequence dependence. *Nucleic Acids Res.* **2013**, *41*, 8886−8895.
(46) Fu, Y.; Zeng, D.; Chao, J.; Jin, Y.; Zhang, Z.; Liu, H.; Li, D.; Ma, H.; Huang, Q.; Gothelf, K. V.; Fan, C. Single-step rapid assembly of DNA origami nanostructures for addressable nanoscale bioreactors. *J. Am. Chem. Soc.* **2013**, *135*, 696−702.
(47) Myhrvold, C.; Dai, M.; Silver, P. A.; Yin, P. Isothermal self-assembly of complex DNA structures under diverse and biocompatible conditions. *Nano Lett.* **2013**, *13*, 4242−4248.

(48) Zhang, Z.; Song, J.; Besenbacher, F.; Dong, M.; Gothelf, K. V. Self-assembly of DNA origami and single-stranded tile structures at room temperature. *Angew. Chem., Int. Ed.* **2013**, *52*, 9219−9223.
(49) Jungmann, R.; Liedl, T.; Sobey, T. L.; Shih, W.; Simmel, F. C. Isothermal Assembly of DNA Origami Structures Using Denaturing Agents. *J. Am. Chem. Soc.* **2008**, *130*, 10062−10063.
(50) Zhang, F.; Nangreave, J.; Liu, Y.; Yan, H. Reconfigurable DNA origami to generate quasifractal patterns. *Nano Lett.* **2012**, *12*, 3290−3295.
(51) Bai, X. c.; Martin, T. G.; Scheres, S. H. W.; Dietz, H. Cryo-EM structure of a 3D DNA-origami object. *Proc. Natl. Acad. Sci. U.S.A.* **2012**, *109*, 20012−20017.
(52) Yoo, J.; Aksimentiev, A. In situ structure and dynamics of DNA origami determined through molecular dynamics simulations. *Proc. Natl. Acad. Sci. U.S.A.* **2013**, *110*, 20099−20104.