Calculation of $\pi$ and Classification of Self-avoiding Lattices via DNA Configuration

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Numerical simulation (e.g., Monte Carlo simulation) is an efficient computational algorithm establishing an integral part in science to understand complex physical and biological phenomena related with stochastic problems. Aside from the typical numerical simulation applications, studies calculating numerical constants in mathematics, and estimation of growth behavior via a non-conventional self-assembly in connection with DNA nanotechnology, open a novel perspective to DNA related to computational physics. Here, a method to calculate the numerical value of $\pi$, and way to evaluate possible paths of self-avoiding walk with the aid of Monte Carlo simulation, are addressed. Additionally, experimentally obtained variation of the $\pi$ as functions of DNA concentration and the total number of trials, and the behaviour of self-avoiding random DNA lattice growth evaluated through number of growth steps, are discussed. From observing experimental calculations of $\pi$ ($\pi_{\exp}$) obtained by double crossover DNA lattices and DNA rings, fluctuation of $\pi_{\exp}$ tends to decrease as either DNA concentration or the number of trials increases. Based upon experimental data of self-avoiding random lattices grown by the three-point star DNA motifs, various lattice configurations are examined and analyzed. This new kind of study inculcates a novel perspective for DNA nanostructures related to computational physics and provides clues to solve analytically intractable problems.

A multitude of analytically intractable problems in various disciplines are addressed by performing numerical simulations that employ a computational model of a system to describe its complex behaviour over a time period by incorporating given variables. One such commonly used model is Monte Carlo (MC) simulation that refers to an effective computational algorithm adopted to perform an underlying stochastic and random sampling experiment on a computer to calculate various outcomes. MC simulation is used in science and engineering to understand complex physical phenomena, generate useful mathematical functions, and predict complicated algorithmic processes. Interestingly, the MC method has also been effectively used to understand complex biological process mechanisms such as the biological self-assembly behaviour, biomolecule dynamics, and the interaction between biomolecules and nanomaterials.

Among typical MC simulation applications, there are two interesting ones: calculating $\pi$ (one of most important mathematical constants defined as the ratio of a circle's circumference to its diameter), and interpreting a self-avoiding walk (an abstract model describing the behaviour of chain like entities where no two points can occupy the same place). Several approaches have been adapted to calculate $\pi$, among which the famously used one is Buffon's needle approach. The MC method is also used to enumerate the characteristics of the self-avoiding walk, to interpret the possibility to estimate proper paths.

The fabrication of various dimensional DNA nanostructures is well established due to the programmability of DNA base sequences and the stability of DNA molecules. Although these artificially designed DNA nanostructures find various applications as physical, chemical, or biomedical devices and sensors, calculating mathematical constants and incorporating abstract modeling via DNA nanostructures are rarely discussed.

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Here, we develop ways to calculate $\pi$ and evaluate the applicable number of self-avoiding walk paths with the aid of the computational simulation. In addition, we experimentally demonstrate the calculation of $\pi$ and evaluate applicable self-avoiding walk paths with two different DNA nanostructures (double crossover DNA lattices and DNA rings) and self-avoiding random DNA lattices (constructed by a three-point star DNA motif having a blunt-end), respectively. Finally, we analyze the trend of numerical $\pi$ variations controlled by DNA concentration, the total number of trials, and the characteristic growth behaviour of self-avoiding random DNA lattices evaluated through the total number of growth steps for the self-avoiding walk path.

**Results**

**Calculation of $\pi$ value.** The representative schematics for $\pi$ calculation with a different number of dots in a square having a quadrant of a circle are shown in Fig. 1a. For acquiring an calculated numerical value of $\pi$ ($= \pi_{est}$, where est stands for estimation), a random event needs to be considered which can be defined as drawing uniformly distributed dots (like throwing darts randomly at a board) over a square bounding box within the region.

![Figure 1](image-url)

Figure 1. Calculation of $\pi$ using Monte Carlo simulation. (a) The representative schematics for $\pi$ calculation with a different number of dots in a square. Calculated numerical value of $\pi$ ($= \pi_{est}$, where est stands for estimation) is defined as $(N_{D,in}/N_D) \times 4$, where $N_{D,in}$ and $N_D$ stand for the number of dots inside a quadrant of a circle (with a radius of $R$) and the total number of dots in a square (with a length of $R$). By definition, $\pi_{est}$ with four different $N_{D,in}$ i.e. 10, 50, 100, and 1000 are calculated to be 2.40 ($= 6/10 \times 4$), 2.72, 2.88 and 3.09 respectively, showing that roughly larger $N_{D}$ gives a more accurate known value of $\pi$ ($\pi_{known} \approx 3.14$). (b) A flow chart depicting algorithmic steps to obtain $\pi_{est}$ with various $N_D$ and total number of trials ($n_T$). (c) $\pi_{est}$ as a function of $n_T$ at a given $N_D$ (e.g. 10, 50, 100, or 1000). In general, $\pi_{est}$ approaches to $\pi_{known}$ with the increasing $n_T$ at relatively larger $N_D$ values, as expected. (d) $\pi_{est}$ as a function of $N_D$ at a fixed $n_T$ (e.g. 1, 5, 10, 50, or 100 marked as a dotted line in (c). From observation, $\pi_{est}$ approaches to $\pi_{known}$ with the increasing $N_D$ at relatively smaller $n_T$ but $\pi_{est}$ is roughly independent with $N_D$ at relatively larger $n_T$. (e) A representative graph of $\pi_{est}$ as a function of $N_D$. As $N_D$ is increased, fluctuation of $\pi_{est}$ from $\pi_{known}$ tends to decrease. Insets show tendencies of fluctuation of $\pi_{est}$ in the two different ranges of $N_D$. 

At this point, the text ends with the conclusion that $\pi$ can be effectively calculated through the Monte Carlo simulation method, with accuracy increasing for larger $N_D$ and $n_T$. The experimental demonstration involves two DNA nanostructures and random DNA lattices, each with distinct growth behaviours that are explored through numerical analysis.

**Conclusions**

The development of methods for calculating $\pi$ and evaluating self-avoiding walk paths has significant implications for computational simulations in biological and physical sciences. The use of DNA nanostructures as platforms for these calculations offers new avenues for research into self-avoiding walks and their applications in areas such as molecular computing and nanotechnology. Further exploration into the characteristics of different DNA structures and their interactions with self-avoiding walks could lead to novel insights and practical applications.
whose area is to be determined. By considering a quadrant of a circle with a radius R bounded by a square with a length R, the ratio of the quadrant area to the square area is approximately equal to the ratio of the total number of dots falling inside the quadrant (N_{D-in}, marked as blue) to the total number of dots inside the square (N_D) due to the uniformly distributed dots within the square. Therefore, \( \pi_{est} \) can be defined as \( (N_{D-in}/N_D) \times 4 \). By definition, representative \( \pi_{est} \) with four different \( N_D \) (i.e. 10, 50, 100, and 1000) are calculated to be 2.40 (\( = (6/10) \times 4 \)), 2.72, 2.88 and 3.09 respectively. This shows that a roughly larger \( N_D \) gives a relatively more accurate known value of \( \pi \) (\( \approx 3.14 \)). Consequently, the magnitude (i.e. 0.060 = [3.2–3.14], 0.020, 0.019, and 0.001) of the deviation of \( \pi_{est} \) from \( \pi_{known} \) (\( \Delta \pi_{est} = |\pi_{est} - \pi_{known}| \)) will be smaller as \( N_D \) (10, 50, 100, and 1000) increases at a given optimum \( N_{D-in} \) (i.e. 8, 39, 79, and 785, which provides the most accurate \( \pi_{est} \) compared to \( \pi_{known} \) at a given \( N_D \)).

Figure 1b shows a flowchart representing algorithmic steps in order to obtain \( \pi_{est} \) as a function of either \( N_D \) or the total number of trials (\( n_T \)). By assigning an initial input of \( N_D \) with the unit-step increment of \( i \), dots are randomly sampled in a square. Then, \( N_{D-in} \) are counted until \( i \) reaches \( N_D \) followed by evaluation of \( \pi_{est} \) calculated as \( (N_{D-in}/N_D) \times 4 \). Similarly, when the unit-step increment of \( i \) reaches an initial input of \( n_T \), summed \( \pi_{est} \) is divided by \( n_T \) to get the average \( \pi_{est} \).

By using the algorithm for \( \pi_{est} \), numerical values of the \( \pi_{est} \) as functions of \( N_D \) and \( n_T \) can be obtained and analyzed. \( \pi_{est} \) as a function of \( n_T \) at four different \( N_D \) values (i.e. 10, 50, 100, and 1000) are obtained, which approaches \( \pi_{known} \) with the increasing \( n_T \) at any given \( N_D \) values, as expected (Fig. 1c). The \( \pi_{est} \) with varying \( N_D \) at a fixed \( n_T \) (e.g. 1, 5, 10, 50, or 100 marked as a dotted line in Fig. 1c) are extracted in order to evaluate the trend of \( \pi_{est} \) as a function of \( N_D \), which shows that \( \pi_{est} \) heavily relies on \( N_D \) at relatively smaller \( n_T \) but it is roughly independent of \( N_D \) at larger \( n_T \) (Fig. 1d). A representative graph of \( \pi_{est} \) as a function of \( N_D \) is shown in Fig. 1e. As \( N_D \) is increased, the fluctuation of \( \pi_{est} \) from \( \pi_{known} \) tends to decrease. Insets show the fluctuation tendency of \( \pi_{est} \) in the two different ranges of \( N_D \) (i.e. between 0–20 and 980–1000), which clearly shows that fluctuation of \( \pi_{est} \) from \( \pi_{known} \) tends to decrease with the increase in \( N_D \) as expected. In addition, the differentiation of \( \pi_{est} \) per unit number of dots (\( -\Delta \pi_{est}/\Delta N_D \)) as a function of \( N_D \) is shown in Supplementary Fig. 1. Differences in the \( \pi_{est} \) per unit number of dots tend to decrease with the increase in \( N_D \) because \( \pi_{est} \) at a relatively larger \( N_D \) has a greater chance to give an accurate value of \( \pi \).

**Experimental observation of \( \pi \) using DNA nanostructures.** Experimental observation of \( \pi \) (\( \pi_{exp} \)) is demonstrated by constructing two types of DNA nanostructures, i.e. double crossover (DX) DNA lattices33,34 and DNA rings35-37 (Fig. 2). Two sets of DX DNA motifs (i.e. PR and PS) are designed for construction of DX DNA lattices. Here, P stands for PR (\( \pi \)) and R/S indicate opposite helical directionalities of the duplexes within the motifs (See Supplementary Fig. 2, Supplementary Tables 1 and 2). Each set has two DX motifs, without and with hairpins marked as PR(S)0 and PR(S)1, respectively (Fig. 2a). A DX motif having hairpins ~3.5 nm long protruding up and down is called DXH (i.e. PR1 and PS1). DX and DXH motifs, having identical sets of sticky ends in each set with the equal probability of binding (two exemplified binding sites are indicated by question marks in Fig. 2b), can hybridize to form a DX lattice with the aid of complementary colour-coded and shape-coded sticky ends. In addition, DNA rings comprised of T motifs (non-crossover based DNA motifs having three double-stranded domains connected through single strands) are fabricated in order to obtain \( \pi_{exp} \). A ring with inner and outer diameters of 13 nm and 29 nm is constituted through the complementary base-pairs of the sticky ends in T motifs (Fig. 2d).

Representative structural configurations of DX DNA lattices and DNA rings are shown in Fig. 2e,h, respectively. Atomic force microscopy (AFM) images of DX lattices with different concentrations of DXH (0, 25, 50, 100, 150, and 200 nM) are shown in Fig. 2e (Supplementary Fig. 3, and Supplementary Tables 3 and 4) are fabricated in order to obtain \( \pi_{exp} \). A ring with inner and outer diameters of 13 nm and 29 nm is constituted through the complementary base-pairs of the sticky ends in T motifs (Fig. 2d).

**Analysis of \( \pi \) using experimental observation.** The analysis of \( \pi_{exp} \) controlled by [DX] and \( n_T \) are conducted and results are displayed in Fig. 3. The histogram in Fig. 3a shows an average of \( \pi_{exp} (\pi_{exp}) \) obtained from more than four data sets at a given [DXH] as a function of [DXH] (the concentration sum of DXH is in each set of motif, \( [\text{DXH}]_1 + [\text{DXH}]_2 \)) i.e. 25, 50, 75, 100, 150, and 200 nM at a fixed [DXH] and \([\text{DXH}]_2 \) of 100 nM. For example, 150 nM of [DXH] indicates 150 nM of [DXH]_1 + [DXH]_2 with 50 nM of [DXH]_2 + [DXH]_3. Although the standard deviation of an error bar generally decreases as [DXH] increases, the magnitude of the deviation of \( \pi_{exp} \) from \( \pi_{known} \) (\( \Delta \pi_{exp} (\pi_{exp} - \pi_{known}) \)) is almost constant above 50 nM of [DXH]. A plot of \( \pi_{exp} \) as a function of [DX] (\( = [\text{DXH}]_1 + [\text{DXH}]_2 \)) is shown in Fig. 3b. By observation, 100 nM of [DX] gives a more accurate \( \pi_{exp} \) (\( \pi_{exp} \)) of -0.009 than 50 (\( \approx 0.062 \)) or 200 nM (\( \approx 0.049 \)) of [DX]. Figure 3c displays \( \Delta \pi_{exp} \) as a function of [DX] (ranging between 2.75 and 3.20 measured by \( (N_{D-in}/N_D) \)) for \( n_T \) and captures more number of rings inside a quadrant and total number of rings in an image) are shown in the images. Lastly, a plot of \( n_T \) as a function of [T] (roughly sigmoidal) analyzed by AFM images is shown in Fig. 2i.
Figure 2. Experimental observation of π using DNA nanostructure configuration. (a,b) Cartoon representations of two sets – PR and PS – of DNA double-crossover (DX) motifs and corresponding DX lattice formed by complementary colour-coded sticky ends. Each set has two DX motifs, without and with hairpins marked as PR(S)0 and PR(S)1, respectively. Hairpins with a length of 3.5 nm protruding up and down on a DX motif called as a DXH. DX and DXH motifs having identical sets of sticky ends in each set can hybridize to form a DX lattice (two exemplified binding sites are indicated by question marks) with the equal probability of binding. (c,d) Schematics of unit building block (called as a T motif) and a DNA ring made of T motifs. The complementary-counterparts are colour-coded with the same colours. (e,f) AFM images of DX lattices with different concentrations of DXH (0, 25, 50, 100, 150 and 200 nM represented as DXH0, DXH0.25, DXH0.5, DXH1.0, DXH1.5 and DXH2.0, respectively) annealed in free solution. An arc (shown in blue) in each image is drawn representing first quadrant in a circle. Experimental observation of π through images (πexp) can be obtained by (NH-in/NH)×4, where NH-in and NH represent the number of hairpins inside a quadrant and total number of hairpins in an image. A scan size of all images in (e,f) is 100×100 nm2 (200×200 nm2). (g) A graph of concentration of DXH ([DXH])-dependent NH analyzed by AFM images with scan size of 100×100 nm2. Theoretical and experimental NH are plotted as red-dotted and black-solid lines, respectively. (h) AFM images of DNA rings with different concentrations of a T motif (2, 5, 8, 10 nM with the scan size of 3×3 μm2, 1 and 20 nM with 2×2 μm2, and 20 nM with 600×600 nm2 indicated as R2, R5, R8, R10, R1, R20 and R20, respectively) annealed through a mica-assisted growth method. Arcs are drawn in third quadrants and corresponding πexp (measured by (NR-in/NR)×4, where NR-in and NR represent number of rings inside a quadrant and the total number of rings in an image) are shown in images. (i) A plot of NR as a function of [T] analyzed by AFM images with scan size of 1×1 μm2.
Figure 3. The analysis of experimentally obtained $\pi$ ($\pi_{\text{exp}}$) controlled by DNA concentrations ([$\text{DNA}$]) and the number of trials ($n_T$). (a) A histogram plot of $\pi_{\text{exp}}$ as a function of concentrations of a DX motif with the hairpin ([DXH]) at a fixed concentration of each motif set ([DXPR] = [DXPS] = 100 nM). Here, [DXH] is defined as the concentration sum of DXHs in each set of motif ($=[\text{DXPR}]+[\text{DXPS}]$). For instance, 50 nM of [DXH] means 50 nM of [DXPR] + [DXPS] with 150 nM of [DXPR] and [DXPS]. Average $\pi_{\text{exp}}$ is obtained from more than four data sets at a given [DXH]. The magnitude of the deviation of $\pi_{\text{exp}}$ from $\pi_{\text{known}}$ ($\Delta \pi_{\text{exp}} = |\pi_{\text{exp}} - \pi_{\text{known}}|$) is almost constant above 50 nM of [DXH]. (b) A plot of $\pi_{\text{exp}}$ as a function of [DX] ($=[\text{DXPR}]$ or [DXPS] with the condition of [DXPR] = [DXPS]) with the equal amount of DX motifs without and with hairpins ([DXPR] = [DXPS] and [DXPS] = [DXPR]). As an example, 100 nM of [DX] indicates [DXPR] = [DXPS] = 100 nM having 50 nM of each [DXPS] and [DXPR] as well as 50 nM each of [DXPR] and [DXPS]. By observation, 100 nM of [DX] gives more accurate $\pi_{\text{exp}}$ (3.15) than 50 (3.08) or 200 nM (3.19) of [DX]. (c) Plots of the deviation of $\pi_{\text{exp}}$ ($\Delta \pi_{\text{exp}}$) (arranged in a descending order) and average $\pi_{\text{exp}}$ ($\langle \pi_{\text{exp}} \rangle = \sum \pi_{\text{exp}}^{\text{nd}} / n^\text{nd}$) as a function of $n_T$. Here, 100 nM of [DX] ($=[\text{DXPR}]$) with 50 nM of each [DXPS] and [DXPR] are used. (d) A histogram plot of $\pi_{\text{exp}}$ as a function of [T]. Accidentally, $\Delta \pi_{\text{exp}}$ are roughly independent of [T]. (e) Plots of $\Delta \pi_{\text{exp}}$ arranged in a descending order as a function of $n_T$ at 2, 5, and 20 nM of [T]. Although 20 nM of [T] shows slightly less $\Delta \pi_{\text{exp}}$ than other [T], roughly ($\pi_{\text{exp}}$) are independent with [T] which is in agreement with (d). (f) A graph of $\pi_{\text{exp}}$ against normalized [DXH] ($[\text{DXH}]_{\text{Norm}} = [\text{DXH}] / [\text{DXH}]_{200}$) and normalized [T] ($[\text{T}]_{\text{Norm}} = [\text{T}] / [\text{T}]_{20}$). It shows comparison of $\pi_{\text{exp}}$ with the two different DNA nanostructure configurations (i.e. lattices and rings).

Self-avoiding random lattice growth. A self-avoiding random walk path (called a lattice configuration) constructed by a unit building block is demonstrated via MC simulation in order to understand the feasibility to predict proper paths. A self-avoiding random lattice has a growth path on a lattice configuration that does not visit the same place more than once. Schematics of various lattice configurations constructed by a three-point star motif having single blunt-end (3PSB) are represented in Fig. 4a. A blunt-end in a 3PSB, which is introduced to generate asymmetric self-avoiding random lattices, is marked with a black (serves as a seed), a red (grown to the left), or a green dot (grown to the right). Formation of a self-avoiding random lattice starts from a seed 3PSB ($N_0 = 0$, where $N_k$ indicates a step number) through the arrow facing of the incoming 3PSB from the next step. Lattice configurations are named as (a step number, NS)-(configuration number from the previous step)-(configuration number at the present step). For examples, 2-3-1 and 3-34-2 indicate 1st configuration of 2nd step obtained from 3rd configuration in 1st step for 2-3-1, and 2nd configuration of 3rd step obtained from 3rd configuration in

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given substrate. Curves of $\Delta \pi_{\text{exp}}$ arranged in a descending order as a function of $n_T$ at 2, 5, and 20 nM of [T] are displayed in Fig. 3e. Although 20 nM of [T] shows slightly less $\Delta \pi_{\text{exp}}$ than other [T], roughly ($\pi_{\text{exp}}$) are independent from [T] which is in good agreement with Fig. 3d. $\pi_{\text{exp}}$ against normalized [DXH] ($[\text{DXH}]_{\text{Norm}} = [\text{DXH}] / [\text{DXH}]_{200}$) and normalized [T] ($[\text{T}]_{\text{Norm}} = [\text{T}] / [\text{T}]_{20}$) are shown in Fig. 3f in order to compare $\pi_{\text{exp}}$ with respect to either largest [DXH] or [T], as well as to understand comparison of $\pi_{\text{exp}}$ with the two different DNA nanostructure configurations, i.e. DNA lattices and DNA rings.
1st step and 4th configuration in 2nd step for 3-34-2. All possible lattice configurations up to NS = 3 are shown in Supplementary Fig. 4. In order to predict applicable numbers of self-avoiding lattices, available lattice configurations at a given NS are analyzed. There are two types of available lattice configurations, i.e. open and blocked (half- and fully-filled circles, respectively) lattice configurations. (b) A pedigree lattice configuration chart of self-avoiding random growth. 32 blocked lattice configurations — 10 (2) half-blocked happened on the left (right) side of the lattices, and 20 full-blocked configurations — out of 256 available configurations (Ω = 4^NS) after 4th step (NS = 4) of lattice growth are shown. Total number of the 3PSB (excluding a seed 3PSB) participated in that configuration is indicated by magenta. (c) A flow chart depicting algorithmic steps to obtain the total numbers of open (ΩO) and blocked (ΩB) lattice configurations at a given NS.
lattice configurations are easily determined by counting available numbers of arrows (binding sites for the next step) in a lattice (i.e., 2, 1, and 0 arrows in the lattices indicate open, half-, and full-blocked lattice configurations, respectively).

Overall self-avoiding random lattice configurations are represented by a pedigree chart in Fig. 4b. Although all blocked lattice configurations (up to $N_S = 4$) are fully displayed, some open configurations are skipped (indicated by dots) for clarity. Total numbers of open ($\Omega_O$) and blocked (full- and half-blocked) ($\Omega_B$) lattice configurations at $N_S = 3$ are 60 and 4 (2 and 2) among 64 available configurations ($\Omega = 4^N$). Similarly, there are 224 open and 32 blocked lattice configurations (20 full-blocked and 12 half-blocked configurations (10 happened on the left side of the lattice and 2 on the right)) out of 256 ($\Omega = 4^N$) at the 4th step of lattice growth. The total number of 3PSB (excluding a seed 3PSB) that participated in specific lattice configurations varied with (and even within) $N_S$, which are indicated by magenta in the pedigree chart. Figure 4c shows a flowchart with algorithmic steps for acquiring $\Omega_O$ and $\Omega_B$ as a function of $N_S$. By initially assigning the total number of trials ($n_T$) and $N_S$ with $i$ and $j$ for the unit-step increments of the trial and the step respectively, $\Omega_O = 4^n_T$, $\Omega_O$, and $\Omega_B$ at a given $N_S$ as well as from analytical evaluation of open lattice configuration ($\Omega_A$) are depicted. The intersection between $\ln \Omega_O$ and $\ln \Omega_B$ (occurred at 9.12 of $N_S$) and the ratio of $\Omega_B$ and $\Omega_A$ are shown in the bottom and top insets, respectively. $\Omega_A$ is larger and smaller than $\Omega_B$ at below and above regions of the thin dotted line (marked at $\Omega_B/\Omega_O = 1$ in the graph of $\Omega_B/\Omega_O$), respectively.

Analysis of self-avoiding random lattice configurations. Physical configurations of self-avoiding random lattices with the symbolic representations of configurations grown up to $N_S$ of 20 (50 and 100) generated by the self-avoiding walk algorithm are shown in Fig. 5a–d (Supplementary Figs 5 and 6). Two-dimensional
self-avoiding random lattices are self-assembled through the subsequent 3PSB bindings to a seed tile of 3PSB, which has two binding sites, left and right leading the paths of the red and green, respectively. Here, open, half-blocked (growth blocked on either the left (a red path) or right (a green) side of the lattice), and full-blocked configurations are symbolized by a hollow, half-filled and fully-filled circle, respectively.

Figure 5e and f show logarithmic numbers of lattice configurations (ln Ω = S/k, where S is entropy and k is a constant) and its difference for open and blocked self-avoiding random lattice configurations as a function of NS. ln ΩN, ln ΩO, ln ΩH, and ln ΩB are easily obtained from the total number of available, open, and blocked (including half-blocked and full-blocked) lattice configurations (i.e. ΩN = 4S, ΩO, and ΩB) respectively at a given NS. In addition, the total number of open lattice configurations ΩA for a 2-dimensional hexagonal lattice model can be analytically extracted

\[ \Omega_A = 0.415 \cdot (\sqrt{2} + \sqrt{2})^{3S+1} \cdot (2N_5 + 1)^{11/2} \]

as shown in Fig. 5e.14 Although ln ΩO and ln ΩB differ by ~3% relatively at smaller NS, they tend to overlap completely with the difference percentage ratio (100 × ln ΩA − ln ΩO)/ln ΩA of ~10⁻² to 6% at larger NS. The intersection between ln ΩA and ln ΩB (occurred at 9.12 of NS) and the ratio of ΩA and ΩB are shown in the bottom and top insets, respectively. ΩB is larger and smaller than ΩO at below and above regions of the thin dotted line (marked at ΩB/ΩO = 1 in the graph of ΩB/ΩO), respectively. In order to compare occurrences of open and blocked lattice configurations, difference (D) of ln ΩO and ln ΩB as a function of NS are discussed (Fig. 5f). As mentioned, D becomes 0 at NS of 9.12 and magnitude of D increases with increasing or decreasing NS from the cross point at NS = 9.12.

Experimental observation of self-avoiding random lattices. Three different DNA nanostructures (a honeycomb lattice, a hexagonal ring, and a three-point star dimer) are constructed by slightly modified three-point star DNA motifs in order to test their applicability in the growth of self-avoiding random lattices (See Fig. 6, Supplementary Fig. 7, and Supplementary Table 5). Figure 6a shows a schematic of a three-point star DNA motifs in order to test their applicability in the growth of self-avoiding random lattices (a honeycomb lattice, a hexagonal ring, and a three-point star dimer) are constructed by slightly modified palindromic self-complementary sticky-end sequences (indicated as S1, S2, and S3) located at the end of each DNA motif (3PSHL) for construction of a honeycomb lattice (a simplified one shown at a right bottom) and its representative AFM images of a honeycomb lattice. A 3PSHL is comprised of 7 strands (marked as #1–#7) with palindromic self-complementary sticky-end sequences in #7 are replaced from S3 to S1. A blunt-end in a simplified 3PS shown in the right bottom of Fig. 6d is marked with either a black (served as a seed), a red (grown to the left), or a green dot (grown to the right) in order to easily evaluate the lattice configurations. Representative AFM images with the lattice configurations (either an open, a half-blocked or a full-blocked configuration at a given step number) of self-avoiding random lattices comprised of 3PSB are displayed in Fig. 6e–p. Simplified 3PSB motifs are overlaid on AFM images to enhance the visibility of lattice configurations. Representative AFM images of a honeycomb lattice, hexagonal rings, and 3PS dimers are well formed in agreement with the design schemes with relatively higher production yields than cross-tile lattices made of four-point star motifs.13

Figure 6d–s show the representative experimental results and analysis of self-avoiding random lattices grown by the 3PS DNA motifs (3PS). In 3PSB, a #6 strand from 3PSHL is removed and self-complementary sticky-end sequences in #7 are replaced from S3 to S1. A blunt-end in a simplified 3PSB shown in the right bottom of Fig. 6d is marked with either a black (served as a seed), a red (grown to the left), or a green dot (grown to the right) in order to easily evaluate the lattice configurations. Representative AFM images with the lattice configurations (either an open, a half-blocked or a full-blocked configuration at a given step number) of self-avoiding random lattices comprised of 3PSB are displayed in Fig. 6e–p. Simplified 3PSB motifs are overlaid on AFM images to enhance the visibility of lattice configurations. Figure 6r,s display percentages of the total number of open, and double blunt ends (3PSB, for formation of a 3PS dimer) are shown in Fig. 6b and c. A 3PSHL (a black dot in simplified 3PSHL indicates a blunt end arm as shown in Fig. 6b) and a 3PSD (two black dots in simplified 3PSB, represent the blunt end arms in Fig. 6c) need 6 strands (strand #7 removed from 3PSHL) with two sets (S1 and S2) of palindromic self-complementary sticky-end sequences, and 5 strands (#6 and #7 removed from 3PSB) with a single set (S1) of palindromic self-complementary sticky-end sequences, respectively. From the observation of the AFM images, honeycomb lattices, hexagonal rings, and 3PS dimers are well formed in agreement with the design schemes with relatively higher production yields than cross-tile lattices made of four-point star motifs.13

Discussion

We discuss methodologies to calculate the numerical value of π and to evaluate a possible number of self-avoiding walk paths with the aid of computational MC simulation. Additionally, we demonstrate the calculation of π and evaluation of applicable self-avoiding walk paths by distinct DNA nanostructures. Finally, we analyze the trend of numerical variations of π as functions of DNA concentration and the total number of trials for π calculation, and the behaviour of self-avoiding random DNA lattice growth evaluated through number of growth steps for the self-avoiding walk path. From observation of experimental calculations of π (πexp) demonstrated by constructing two different types of DNA nanostructures (i.e. double crossover DNA lattices and DNA rings), fluctuation of πexp from known π tends to decrease as either DNA concentration or the number of trials increases. Based upon experimental observation of self-avoiding random lattices grown by the three-point star DNA motifs, the percentage of lattice configurations is examined. Open (blocked) lattice configurations are dominant below (above) the step number of 9.12 (at this step number obtained by simulation, numbers of open and blocked configurations are the same). This in depth study of numerical calculation of mathematical constants and characteristic estimation of abstract models via DNA provides a novel perspective for the applicability of DNA in the field of science and engineering.
Figure 6. Experimental observation of self-avoiding random lattice growth with the three-point star DNA motif. (a) A schematic of a three-point star DNA motif (3PS₃H) for construction of a honeycomb lattice and its representative AFM image (scan size of 500 × 500 nm²) of a honeycomb lattice. Seven strands constituting 3PS₃H are numbered as #1~#7, where palindromic self-complementary sticky-end sequences located at the end of each arm are indicated as S1, S2, and S3. A simplified 3PS₃H and a magnified honeycomb lattice (100 × 100 nm²) are shown at the right bottom corners of them. (b) A schematic of a three-point star DNA motif with a single blunt end (3PS₃BH) for fabrication of a hexagonal ring and its AFM image. Six strands (strand #7 removed from 3PS₃H) and two sets (S1 and S2) of palindromic self-complementary sticky-end sequences are required. A black dot in simplified 3PS₃BH indicates a blunt end arm. Inset in AFM image is 3-dimensional visualization of a hexagonal ring. (c) A schematic of a three-point star DNA motif with double blunt ends (3PS₃D) for formation of a 3PS dimer and its AFM image. Five strands (#6 and #7 removed from 3PS₃H) and single set (S1) of palindromic self-complementary sticky-end sequences is required. Inset in AFM image is 3-dimensional visualization of 3PS dimers. (d) A schematic of a three-point star DNA motif with a blunt end (3PS₃B) for demonstration of a self-avoiding random lattice. Strand #6 is removed from 3PS₃H and self-complementary sticky-end sequences in #7 are modified. A blunt-end in a simplified 3PS₃B is marked with a black (served as a seed), a red (grown to the left), or a green dot (grown to the right) in order to easily analyze the lattice configurations. (e–q) Representative AFM images of self-avoiding random lattices comprised of 3PS₃B. Either an open, a half-blocked or a full-blocked lattice configuration at a given step number is indicated in each image. In order to clarify the growth visualization of lattice configurations, simplified 3PS₃B are overlaid on AFM images. (r) A plot of percentage of total number of 3PS₃B motifs (α) in that specific range, i.e. below 10, 11–20, 21–30, and above 30. (s) A bar graph of percentages of the total number of open, half-blocked, and a full-blocked lattice configurations (β).
Methods

DNA nanostructure fabrication. Synthetic oligonucleotides purified via high-performance liquid chromatography were purchased from Bioneer (Daejeon, Korea). Double-crossover (DX) DNA lattices were formed by the 2-step free solution annealing method. First, individual strands of either DX (without hairpins, PR0 and PS0) or DXH (with hairpins, PR1 and PS1) motif were mixed with equimolar concentration (800 nM) in 1× TAE/Mg2+ buffer solution (40 mM Tris, 20 mM Acetic acid, 1 mM EDTA (pH 8.0), and 12.5 mM magnesium acetate). These strand mixtures of each motif (i.e., PR0, PS0, PR1, and PS1) in the test tubes were then slowly cooled from 95 to 25 °C by placing them in a Styrofoam box containing 2 L of boiled water for about 2 days to facilitate hybridization. In succession, an appropriate amount of each motif was added into a new test tube to obtain DXH0, DNA lattices (final concentrations of individual motifs were [PR0] = [PS0] = 100 nM, and [PR1] = [PS1] = 0 nM). Similarly, sets of motif concentrations ([PR0], [PS0], [PR1], and [PS1] = 75, 100, 25, and 0 nM; 50, 100, 50, and 0 nM; 0, 50, 100, and 50 nM; 0, 0, 100, and 100 nM) were prepared to construct DXH0, DXH0.5, DXH0.75, DXH1.0, DXH1.5, and DXH2.0 DNA lattices, respectively. Second step annealing was performed by placing sample test tubes in a Styrofoam box containing 2 L of water (initial temperature, 40 °C) and cooling them from 40 °C to 25 °C for about 24 hours to obtain DX DNA lattices. (Fig. 2, Supplementary Fig. 2, Supplementary Tables 1 and 2)

DNA rings were formed by mixing a stoichiometric quantity of each strand in a buffer containing a mica substrate (size of 5 × 5 mm²). This strand mixture with mica was annealed in a test tube by slowly cooling from 95 to 25 °C in a Styrofoam box. Eventually, DNA rings formed on the mica surface with different coverages depending upon the concentration of a T motif. DNA rings with a five different T motif concentrations of 2, 5, 8, 10 and 20 nM were prepared and analyzed. (Fig. 2, Supplementary Fig. 3, Supplementary Tables 3 and 4)

Honeycomb lattices, hexagonal rings, 3PS dimers, and self-avoiding random lattices were constructed by specific three-point star motifs; 3PSH, 3PSL, 3PSR, and 3PSB motifs. They were formed by mixing stoichiometric quantities of each strand in the buffer by cooling from 95 °C to 25 °C in a Styrofoam box. Final concentrations of 3PS for all DNA nanostructure configurations were 200 nM. (Fig. 6, Supplementary Fig. 7, Supplementary Table 5)

AFM imaging. 5 μL of DNA nanostructures (i.e., DX lattices, honeycomb lattices, hexagonal rings, 3PS dimers, and self-avoiding random lattices) in buffer solution prepared via the free-solution annealing method were dropped on a freshly cleaved mica surface. A 30 μL of 1× TAE/Mg2+ buffer solution was then placed onto the mica, and another 20 μL was placed onto the silicon nitride AFM tip (NP-S10, Veeco Inc., CA, USA). To image DNA rings fabricated through the MAG method, a mica substrate with preformed DNA rings was taken from a test tube and placed on a metal puck. Then, 30 μL of buffer was pipetted onto the mica substrate, and another 20 μL was dispensed onto an AFM tip. Corresponding AFM images were then obtained using a Multimode Nanoscope (Veeco Inc., CA, USA) in the fluid-tapping mode (Figs 2 and 6).

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Author Contributions
A.T. and S.K. initiated and directed the project, designed experiments, performed the experiments, carried out the theoretical modelling and calculations, analysed data and wrote the first version of the paper. Y.S., H.C., S.B. and J.S. performed the experiments and revised the paper. T.H.H. and S.H.P. initiated and supervised the project.

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