All-atom molecular dynamics of film supported flat-shaped DNA origami in water

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Abstract

Here we present a novel technique that utilizes a supporting inorganic film for MD simulations of flat-shaped DNA origami structures in explicit solvent. The number of atoms is typically over 16 million including water molecules. By utilizing a GPU capable simulation engine, we have addressed conformational changes of a DNA origami structure under normal ionic strength and deionized water conditions up to the order of one nanosecond simulation time. Our results demonstrate that DNA origami configuration undergoes a continual growth in the absence of cations, while it is not the case for normal ionic strength. Statistical analysis of helix forms for these DNA origami structures reveals that not only cations but also water permittivity contributed to the maintenance of B-DNA helix form during the stretching motion. These results will provide key features in designing molecular robots as assembly of DNA origami structural components such as scaffolds, connectors and channels.

Key Words: Molecular Robotics, DNA Origami, NAMD, Interface MD, TSUBAME3.0

Area of Interest: Emerging new technology
1. Introduction

DNA origami technology has been increasingly expanding its application fields such as nano-engineering, medical science and drug delivery, nano-chemistry, and robotics [1–5]. Among them, bio-mimetics and molecular robotics are cutting edge topics for researchers these days [3–5]. Construction of a DNA origami object uses a process of molecular self-assembly through DNA hybridization [6]. A long single-stranded DNA (scaffold), mostly M13 phage genomic DNA (7.2 kilo base long), is folded into prescribed objects by hundreds of short synthetic DNA single strands that are typically 20–60bps long. Those short DNA strands, named staples, are designed to be complementary to distinct positions of the scaffold DNA. The staples crosslink spatially separated segments in the scaffold together.

Thus the structure of a DNA origami object is essentially dependent on the sequence design of staples. This is one of the key benefits of the DNA origami method that allows nano-structure design without considering atomic details. Nevertheless, atomic-level DNA origami structure modeling is also important to assess structural properties of crossover motifs such as double and triple crossovers [7] which are frequently used in DNA origami designs. Moreover, in terms of comparison with experiments, assessing the physicochemical properties of their structure using molecular dynamics (MD) simulations with atomic detail has become increasingly important recently [8–11].

There have been known facts about shape deformation of DNA origami objects under low ionic strength, cations in particular. Chen et al. argued that if the exposition time of DNA origami “triangle” to low concentration of Mg$^{2+}$ is less than 10 minutes, it restores the original shape after addition of high concentration of Mg$^{2+}$ in reversible manner [8]. Ramakrishnan et al. showed that in the presence of a denaturing agent, urea in particular, Mg$^{2+}$ cations are ten times more efficient in stabilizing DNA origami triangles than monovalent Na$^+$ [9].

In addition, Kim et al. placed DNA origami triangles on silicon surface, immersing them into deionized water before obtaining their AFM (atomic-force microscopy) images. They observed a rapid decrease in the density of the DNA nanostructures. The shape of the remaining DNA nanostructure showed significant variation among experiments, and some of them are significantly damaged [10]. And very recently, Kielar et al. showed that EDTA, a cation chelating agent, may facilitate DNA origami denaturation [11]. They also found that phosphate ions, known as metal complexing agent, can also have an analogous effect.

In this study, we focus on these dynamics of DNA origami structures using an all-atom MD simulation approach. The hallmark of our approach is to incorporate an inorganic supporting film, mica film in this study, as a substrate for a DNA origami. To this end we developed a workflow to produce a combined system with a DNA origami and an inorganic film. This workflow consists roughly of 5 parts: (1) designing a DNA origami using caDNAno [12], (2) construction of an atomic model of the DNA origami using ENRG [13], (3) construction of an atomic model for the inorganic film using Interface MD [14], (4) the ion placement using CIONIZE [15], and finally (5) solvation using SOLVATE [16] which is followed by minimization with NAMD [17]. In addition, the latest refined force field, AMBER PARMBSC1 [18], is first utilized with the non-metallic species, Muscovite mica, in Interface MD.

We then observed continual stretching of DNA origami in MD runs under deionized water conditions. This result is reminiscent of the experimental DNA origami observations under deionized water [10] or in a solution with EDTA [11]. We also show that when realistic water permittivity (dielectric constant $\varepsilon >> 1$) is taken into account, DNA strands may withstand denaturation and exhibits a regular pattern similar to argyle lattice. By taking histograms of $Z_p'$ (a
measure of duplex forms) we analyze these characteristic features more quantitatively.

This paper is organized as follows. In section 2, we show our design of a DNA origami and its atomic structure. Then we describe our workflow of building an atomic structure of mica film. Next we show our preparation strategy for ion placement and solvation. In section 3, we show the results of MD simulations carried out for the atomic model described in section 2. Then we describe a statistical analysis performed for the MD results under a normal ionic strength, deionized, and various dielectric constant conditions. In section 4, we discuss time complexity issues of system preparation, our ongoing studies on DNA origami MD simulations with other materials for supporting films and effects of self-ionization of water on DNA origami conformational dynamics. The last section is devoted to the conclusion of this study.

Figure 1. Atomic structure model of DNA origami “angry”
(a) The structure model shown in ball and stick (CPK color) representation using VMD [19]. Scale bar = 10 nm. (b) A zoom up view of the center part in (a).

2. Methods

2.1 Preparation of a DNA origami model

Figure 1 shows the atomic structure of the caDNAno designed DNA origami viewed in VMD [19]. We have used caDNAno [12], an interactive tool, for designing a DNA origami skeletal structure. This DNA origami, called “angry” hereafter, is an array of 33 double helices, each of which has 8 base pairs per helical phasing turn. A snapshot image from caDNAno for this DNA origami is shown in Appendix A.1. The number of staples in this model is 221. Among them the shortest ones have 10 nucleobases, while the longest ones have 48 nucleobases. The most frequently used ones have 32 nucleobases. They are responsible for making double crossovers among neighboring three lanes. The m13mp18 bacteria genome sequence (7.2 kilo base length) is used for the scaffold.

The atomic structure has been obtained through the ENRG server which automatically converts caDNAno data (JSON format file) to atom coordinates (PDB format file) [13]. Here we note that the atomic structure is made of a canonical B-DNA alone, and the total number of atoms in it is
approximately $4.5 \times 10^5$. The longer diameter, shorter diameter, and thickness of the DNA origami object are 105.6 nm, 78 nm and 21 nm, respectively. The ENRG server also generates topology data (PSF format file) necessary for starting MD simulations with CHARMM force field [20–23]. These coordinate and topology data are subsequently used as inputs for an MD simulation with NAMD2 [17].

2.2 Preparation of mica film structure

Muscovite mica (formula $\text{[Si}_2\text{O}_4\text{][Al}_2\text{O(OH)}_4]$) is the most commonly used inorganic material as substrate for a DNA origami object in solutions under AFM image observation. Its cleaved surface is known to give an ideal flat surface. As it will be described in Appendix A.2, we have developed a workflow to construct a mica film atomic MD model by utilizing the structure database, utility tools, and force field in Interface MD [14]. Then we have incorporated the mica film atomic structure into our MD model.

Figure 2. Atomic structure model for a mica double layer
(a) Unit cell structure in a ball-and-stick representation. Cell parameters: $a=5.19\text{Å}$, $b=9.02\text{Å}$, $c=20.0\text{Å}$, $\alpha=90.0^\circ$, $\beta=95.7^\circ$, $\gamma=90.0^\circ$. (Gray) silicon, (pink) aluminum, (red) oxygen, (purple) potassium, (white) hydrogen. (b) Structure of a 5x3x1 supercell. Cell parameters: $a=26.0\text{Å}$, $b=27.05\text{Å}$, $c=50.0\text{Å}$, $\alpha=90.0^\circ$, $\beta=90.0^\circ$, $\gamma=90.0^\circ$.

Figure 2(a) and (b) show the unit cell and a 3x5x1 supercell structures obtained from the clay minerals model database in Interface MD. The unit cell has 16 silicon, 8 aluminum, 48 oxygen, 8 aluminum, 4 potassium, and 8 hydrogen atoms. Aluminum, silicon, and potassium atoms bear positive charges (1.45/0.80, 1.10, and 1.00 respectively), while oxygen atoms bear negative charges (-0.78 to -0.55). We have used the 3x5x1 supercell as prototype for larger cells since the unit cell structure has an arbitrariness in localization of charge defects whereas that of the supercell has
already been optimized.

Figures 3(a) and (b) represent structural coordinates and topologies of a film constructed from the supercell, respectively. The longer side lengths of planer DNA origami objects are typically around 100nm. By taking account of this, we constructed a 130x135nm$^2$ film out of 50x50 copies of the supercell using a 4 step procedure consisting of 2 rounds of replication processes each of which is followed by a boundary bond reconnection process. Further details are explained in Appendix A.2.

![Figure 3. Atomic structure model for a mica film](image)

(a) Ball-and-stick representation of a final mica film structure. (b) Bond representation of the same film structure. The x-y area of this film is 130x135nm$^2$. It is made from 50x50 copies of the supercell in Figure 2(b).

2.3 Alignment, ion placement and solvation

The next step is to align the DNA origami structure with the mica film structure prepared in the previous sections. The DNA origami structure is by default oriented perpendicularly to the x-y plane, and collides with the mica film. By rotating the DNA origami object $-90^\circ$ and $90^\circ$ about the y and x axes, respectively, it becomes in parallel with the x-y plane and also its internal axes aligned to global ones. Then we move the DNA origami object to centralize it in the area within the surface of mica film. This is accomplished by minimizing the differences of x- and y-margins. The margin sizes after this centralization are 12nm and 28.6nm for x- and y-margins, respectively. Finally, z-axis position of it is determined so that z-coordinate difference between atoms at the bottom of the DNA origami and at the top of the mica film is 1.6nm.

Ions and water molecules play key roles in a combined model with DNA origami and mica film in terms of comparison with experiments. Ion placement is primarily a measure for cancelling the local and global charge unbalances caused by solutes (here DNA-origami object) by placing counter ions in their nearby volumes. This is accomplished by calculating columbic potential map in a mesh grid of 4Å$^3$ resolution and then placing ions at points of minimum energy.
**Figure 4.** Atomic structure model of a combined system
Window shots taken after ion placement. (a) overview from the front face, (b) zoom up view in the center part of (a), and (b) a side view. Van der Waals (VDW) representations for (yellow) Na⁺, (green) Mg²⁺, (cyan) Cl⁻. The number of magnesium ions is smaller than that of the other two species, and thus they may not clearly be seen in (a) and (c).

**Figure 5.** Solvation of the combined system
(a) Side view of the system from a y-z cross section after solvation. DNA strands are placed in the middle of the z-axis boundaries while the mica film is placed their underneath. Scale bar = 100Å. (b) A closer view of the solvated system from an x-y cross section. Scale bar = 10 Å. Licorice: DNA, water, and mica film, VDW: (yellow) Na⁺, (cyan) Cl⁻. Population of Mg²⁺ is relatively small and thus are not seen this view.
We have used Na\(^+\), Mg\(^{2+}\), and Cl\(^-\) as ion species. In experiments, NaCl and MgCl salts are commonly used as cation sources for AFM imaging in a solution of DNA origami. Their concentrations are typically \(~150\text{mM}\) and \(15\text{~30mM}\) for NaCl and MgCl, respectively. In our MD model, the combined system with DNA origami and the mica film bear a net charge \(13897e^-\) before ion placement. In the present study, we have used \(14996x\text{Na}^+\), \(2999x\text{Mg}^{2+}\), and \(7097x\text{Cl}^-\) atoms for ion placement, for which the net charge negativity is cancelled while satisfying \([\text{Na}^+]\)=\(150\text{mM}\) and \([\text{Mg}^{2+}]\)=\(30\text{mM}\). Figures 4 shows overview (a), zoomed up view at the center (b), and side view of the structure after the ion placement.

Solvation of the system is then carried out by filling a rectangular parallelepiped box \((132x137.2x98\text{nm}^3)\) wrapping the DNA origami and the film with TIP3 waters [24] using the solvate plugin in VMD [16]. Approximately \(4.2x10^6\) TIP3 waters (\(12.6x10^6\) atoms) are added to the space within the water box. This procedure takes approximately 75 minutes by using only CPU (Xeon E5-2680v4, 14 cores, 2.4 GHz) and DRAM (256 Giga Bytes) resources. In Figure 5(a) and (b), we show a side view and a zoom up view from the front face of the atomic structure of the solvated system, respectively.

Figure 6 shows a flowchart summarizing our system preparation procedure described above. The alignment is processed automatically after structure files have been loaded. Before rendering the system to a solvation, we first passed it to ion placement since it requires greater computational cost after solvation than is needed in the absence of waters. The CUDA version of CIONIZE [15], turned out to be useful for this purpose since placement of approximately \(2.5x10^4\) ions took only \(~4\text{min}\), which is nearly 120 times faster than that for completing an ion placement after solvation using the default ionize plugin in VMD.

**Figure 6.** Flowchart of the system preparation procedure after film building

Green: structure data of the DNA origami and the mica film prepared by the procedures explained in sections 2.1 and 2.2, respectively. Light blue: alignment, combine, ion placement, and solvation processes. Yellow: an initial structure model for an MD simulation with NAMD2 [17].

### 2.4 Incorporation of PARMBSBC1

According to a recent benchmark test for long time simulations of duplex B-form DNA, the accuracy of the latest AMBER MD force field, PARMBSBC1 [18], is shown to have the best quality among other previously developed ones in terms of agreement with NMR experimental data [25].
We initially tested DNA origami MD simulations with the CHARMM force field [20–23] since Interface MD only provides CHARMM force field parameters for non-metallic species including mica. Then it turned out that individual double helix in a DNA origami undergo noticeable configurational change during a simulation due to the electrostatic repulsions among minus charged phosphates in their backbone. By taking account of this fact, we focused on incorporation of PARMBSC1 into our CHARMM NAMD simulations. As a result, we have successfully developed a working protocol for this task and made it utilized in our simulations. Further details of this method will be described in Appendix A.3.

3. Results

3.1 Configuration dynamics

We used the conjugate gradient method for energy minimization of the system up to $12 \times 10^3$ steps, and it is followed by a 40 pico seconds of NVT MD run for early time equilibration. During these processes harmonic restraints are assumed for nucleic acids' backbone. In the equilibration process, we have rendered the system a temperature increase protocol through Langevin dynamics temperature control mechanism. After that, a production MD run has been carried out for 1–1.6ns with using 1 node in TSUBAME3.0 (Intel Xeon E5-2680, 14cores/28threads 2.4GHz * 2CPU, 256 Giga Bytes Main memories, NVIDIA TESLA P100 for NVlink * 4).

In Figure 7, we show panels of snapshot images obtained for DNA origami angry during a production MD run. Those images were taken at every 0.2 ns. After sufficient time (>0.4 ns), we could observe a distinctive pattern that associate well with the chicken-wire pattern found in a DNA origami object “pointer” [26]. Phosphates in the backbone of DNA bear minus sign charge, i.e. $e^{-1}$ per one nucleotide. Counter ions, here cations, sodium and magnesium ions, have shown to reside continually in the neighborhood of DNA (data not shown). These cations play the role of neutralize those negative charges and reduce the electrostatic repulsion between neighboring DNA strands. This mechanism helps maintain the DNA origami shape as designed by preventing it from becoming untied [27]. In fact, it agrees with what was analyzed in a previous MD study on DNA condensates in the presence of multivalent cations [28].

Here we demonstrate the effect of cations in restraining DNA origami shape by a comparative experiment with the lowest ionic strength. Figure 8 shows panels of images generated from snapshots, taken at every 0.2 ns, of a MD run in the absence of ion charges. In this result, DNA origami continues to grow and will not seem to stop until it touches the boundaries of the simulation box. Apparently the electrostatic repulsion mechanism due to the negatively charged phosphates in backbones of DNA dominates the configuration dynamics of DNA origami. The apertures between neighboring strands now form a pattern associates with argyle lattice. Each strand in this argyle pattern are elongated and seems to undergo a strain coming from a stress force. Figures 9(a) and (b) show profiles of x-directional width, y-directional height, and z-directional thickness of DNA origami obtained from Figures 7 and 8, respectively. The time courses of width and height of DNA origami in Figure 9(a) remain constant. On the other hand, those for Figure 9(b) continually grow and tend to converge at later time. This result clearly demonstrates the effectiveness of condensation mechanism in the presence of cations in restraining DNA origami shape.
**Figure 7.** Snapshots of DNA origami angry taken from an MD run under a typical ionic strength. Here $[\text{Na}^+] = 150\text{mM}$ and $[\text{Mg}^{2+}] = 30\text{mM}$. VDW representation. Movie is in Supplements S.1.

**Figure 8.** Snapshots of DNA origami angry taken from a MD run under deionized water. Movie file is in Supplements S.2.
Figure 9. Time courses of x-, y-, and z-directional sizes
(a) These sizes are measured for the DNA origami in the MD run in Figure 7. Top panel: absolute size, bottom: relative size. (b) They are measured from the MD run in Figure 8.

3.3 Helix form analysis

The helical nature of DNA could be related to onsets of characteristic pattern observed in the bulk and peripheries of DNA origami, such as chicken-wire and argyle lattice patterns. We have conducted a statistical analysis of helix forms in DNA origami objects undergoing the normal and deionized water conditions. The measure $Z_p'$ adopted in the present study is based on the one introduced in a previous MD simulation study on DNA in water [29]. Figure 10 schematically illustrates the calculation method of $Z_p'$. We first calculate a standard vector between paired nucleobases using relative coordinates from C1' atom in the sense strand to that in the anti-sense one. This is carried out for a consecutive 8 residues (orange pointers in Figure 10). Then we obtain orthogonal vectors as cross products of neighboring standard vectors (black arrows in Figure 10). After that, summation of those orthogonal vectors are computed (cyan arrow in Figure 10). Then a vector of interest is calculated as relative coordinates from the fourth phosphor atom in the sense strand to that in anti-sense strand (magenta arrow in Figure 10). Finally, $Z_p'$ is computed as projection of this vector onto the sum of orthogonal vectors (red arrow in Figure 10).

Figure 11 shows a profile of normalized histograms of $Z_p'$ obtained from a MD run. The distribution shows a strong peak initially at early time domain ~0.1 pico second of simulation time, which reflects the fact that DNA origami is prepared using a canonical B-DNA atomic model. The correlation to the initial condition becomes almost negligible after 0.6 nano second of simulation time. Then the peak shape is stabilized in a delta form with a width 15~20Å over 1 nano second. The expected value of the peak is 0.53±5.77 which is close to 0.78 estimated for a canonical B-DNA atomic model. Although this result strongly suggests that B-DNA form is still the most stable duplex form in DNA origami during the stationary phase, the wide peak width tells us that there will be contributions from other helix types including A-DNA form. In Figures 11(a) and 11(b), histograms of $Z_p'$ obtained from atomic structure models of canonical B- and A-DNA are also included to show this fact.
**Figure 10.** Calculation model of the measure $Zp'$
(Yellow orange) standard vectors for a consecutive 8 base pairs, (black) normal vectors calculated from the standard vectors, (cyan) summation of the normal vectors, (magenta) vector of interest between 4th phosphor atoms, and (red) $Zp'$ as the projection onto the cyan vector.

**Figure 11.** Time series of histograms obtained for $Zp'$
These histograms are estimated from a MD run in Figure 8 calculated at every 100 ps. Vertical cyan line: histogram of $Zp'$ calculated for a canonical B-DNA. Vertical orange line: histogram of $Zp'$ calculated for a canonical A-DNA. Atomic structure models of the canonical forms are obtained by submitting a 352 base length sequence to the 3D-DART server [30].

We found that the statistical nature of B-DNA form in DNA origami is quite robust and almost ubiquitous when we use standard ionic strength and dielectric constants that are typically stronger.
than 20. Figure 12 shows the normalized histograms of Zp' obtained for DNA origami objects in MD simulations under different ionic strength and dielectric constants. The simulation time chosen for taking these histograms are approximately 1 nano second. As it can clearly be seen that in the presence of cations, the histogram exhibits the sharpest peak, of which the maximum is centered at a peak estimated from a canonical structure model of B-DNA. Interestingly, in the absence of cations, the sharpness is degraded and the expected value is slightly shifted to minus. Most notably, if we assume $\varepsilon=1$, where $\varepsilon$ is the dielectric constant for electrostatic potentials, the histogram exhibits a broader shape with multiple humps. The mechanism underlying this form shift can be attributed to the strain in response to the stress force applied on each strand, as we mentioned in section 3.1. Nevertheless, if we assume $\varepsilon>>1$, such an effect is shown to be less significant. In fact, we found that although histogram shape for $\varepsilon=20$ still has stronger contribution from the strain than others, those for $\varepsilon=40$–100 equally exhibit similar delta shaped peaks whose maxima are centered at the peak of canonical B-DNA. These results suggest a qualitative difference between mechanisms of stretching motion under $\varepsilon>>1$ and $\varepsilon\sim1$.

In order to understand this mechanism responsible for the qualitative difference between the result for $\varepsilon=1$ and others, we carried out MD simulations for a simpler DNA origami structure, called rectangle, a rectangular shaped DNA origami. It has 20 rows of duplex DNA having 352 base pair length. Neighboring three lanes are bound by a 32 nucleotide base length staple forming a double crossover, and they are placed at every 32 bases interval. Figure 13 and 14 show the panels of snapshot images taken at every 0.2 ns during a production MD run for rectangle under $\varepsilon=1$ and 60, respectively. Noticeably, the DNA origami under $\varepsilon=1$ exhibits damaged structures in top and bottom halves. Scaffolds in those damaged part have lost their counterpart staple strands and have changed their form into arched conformations that face nucleobases to center of the DNA origami. On the other hand, the DNA origami under $\varepsilon=60$ remains undamaged while it keeps growing. As a result, a regular pattern associated with argyle lattice develops in all part of the DNA origami.
Figure 13. Snapshots from an MD run for the DNA origami rectangle in deionized water under $\varepsilon=1$
VDW representation. Movie file is in Supplements S.3. Scale bar = 100Å.

Figure 14. Snapshots of an MD run for the rectangle motif in deionized water under $\varepsilon=60$
Movie file is in Supplements S.4.
4. Discussion

Nowadays turn-around time has not been a major issue of MD simulation studies since the emergence of GPU capable MD engines, such as NAMD, AMBER, DESMOND [31], and others. In terms of molecular robotics, a more crucial issue is how and to what extent we can reduce the time needed for preparation of large scale MD models with more than 10 million number of atoms. We have addressed this issue by leveraging GPU capable plugins in VMD, CIONIZE in particular, by developing the supporting inorganic film insertion technique for reduction of the simulation box, and by incorporating script based automating tool of preparation workflow. These tools help us to reduce the time lag occurred when a MD model is revised. For instance, in the case that we change the DNA origami structure from angry to rectangle, or change the inorganic martial from clay minerals to silica. In fact, the introduction of the supporting film reduced the solvation time from 6 hours to 75 minutes (single Tesla P100 GPU), the use of CUDA capable CIONIZE decreased the processing time of ion placement down to 4 minutes, and the automating tool have gotten rid of human interaction with these processes through VMD GUI.

We have introduced the supporting film for our MD simulation study on flat-shaped DNA origami conformation dynamics. The model inorganic material for the film in the present study is only mica and its atom coordinates and force fields have been obtained from Interface MD. Although it has not been mentioned in our results, we have also been carrying out MD studies for DNA origami structures on other material models, i.e. quartz alpha and crystal silicon films. There have been observed some interesting features in their dynamics that are different from what has been shown in the present study. For instance, we could observe adsorption of DNA origami on the surface of quartz alpha film in presence of cations. Such result is reminiscent of DNA adsorption on silica surface in previously published experiments [32, 33]. Also we observed faster movement of DNA origami on silicon surface than that observed for other films. This implies mica also adds moderately attractive force to DNA origami due to the positively charged cations, i.e. potassium ions. These results will be presented elsewhere in the near future.

Our analysis on helix forms in DNA origami using MD simulations (section 3.2) will be helpful not only for improving precision and accuracy of DNA origami design but also for obtaining new insights in the mechanism of how DNA origami shapes are robustly controlled under various ionic strength. The result of helix type analysis tells us that not only ionic strength but dielectric constant may be relevant for the maintenance of DNA origami shape. Water permittivity, or dielectric constant, depend on ionic strength [34] and possibly on pKw value of water (pKw~14 at room temperature). Self-ionization of water [35] determines this value and has been studied in terms of mechanism underlying the water permittivity [36], but has not been explicitly taken into account in any conventional DNA origami MD simulations. Thus we are now conducting a study on how such property of water has an impact on DNA origami shapes. Molecular weight of a single proton is ~1, and this fact implies that inclusion of this atom species in a MD simulation will significantly slow down the computation speed below that is expected for typical simulations with TIP3 waters, since smaller time step value is necessary for numerical integration of their trajectories. We have successfully overcome this issue with NAMD, by using only a half smaller time step value from the default value, 1fs. Interestingly, we found that once if protons and hydroxide ions are incorporated, the elongation dynamics of DNA origami becomes less sensitive to the variation of [Na⁺] and [Mg²⁺] than those observed for simulations with conventional TIP3 water solvents. We will show those results elsewhere in the near future.
5. Conclusions

By introducing a supporting film technology for MD simulations of flat-shaped DNA origami atomic structures in explicit solvent, we have successfully found a novel feature in dynamics, that is, the stretching motion. It can be attributed to negatively charged phosphates in backbones of DNA strands. We hope that future technological development in experimental devices, such as HAFM (high-speed AFM), will allow for direct/indirect observation of such feature. Our results suggest that although the efficiency of shape fixing is the highest for the addition of cations, increasing solvents' dielectric constant also has a secondary contribution to this, in the sense that B-DNA form is maintained during the stretching motion. This in turn suggests that conventional water models underestimate some factors necessary for realizing higher dielectric constant and need further sophistication.

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Appendix

A.1 DNA origami “angry” designed using caDNAno

caDNAno allows intuitive editing of DNA origami by providing manual and automatic layout functions for the scaffold and staples. It’s based on mouse operations and without the need for in-depth knowledge about the helix structure of DNA. Figure A.1 shows a snapshot image taken from caDNAno GUI in which a 2D diagram of skeletal structure for a flat-shaped DNA origami “angry” is edited. By flat-shaped we mean DNA origami is constructed by only using a 2 dimensional fold of the scaffold.

Figure A.1. Screenshot taken from a window of caDNAno [12] before assignment of a nucleotide sequence
This DNA origami is designed as a sheet-like planer object and is named “angry” in this paper. The guidelines (gray rulers) are initially generated upon selection of 33 consecutive circles column-wise out of square grid of candidates from cross sectional view. The top row in each odd lane is for staples, while the bottom one is for scaffold, and vice versa for even lanes. The color lines represent traces of staples, starting from filled squares and ending at arrow heads. Scaffold is a single trace and colored blue. Crossovers are denoted by vertical line segments connecting neighboring horizontal lines.

A.2 Construction of mica film

In Figure A.2, the coordinate file (mica15_double_layer.car) and topology file (mica15_double_layer.mdf) are originally prepared as native Materials Studio MD input models.
The utility programs msi2lmp and car_lmp_TO_pdb_psf bundled with Interface MD are utilized to convert them into PDB and PSF formats. More specifically, the program msi2lmp has been used for translation of the coordinate and topology files into a single lammps format file [37], and thereby car_lmp_TO_pdb_psf has been used for converting it into PDB and PSF files (mica15_double_layer.pdb and mica_double_layer.psf). At this point these files are prepared to run a MD simulation using NAMD which refers charmm27_interface_v1_5.prm provided from Interface MD. These two files are then used as a prototype model for the subsequent replication procedure. A utility function replicatemol in TopoTools plugin [38] in VMD, with addition of customized codes for assignments of unique segment/residue indexing, has been utilized for this replication procedure.

Figure A.2. Flowchart of film building procedure

Figure A.3(a) shows the structure of a 2x2 replicated cell from the prototype model. In addition, we have developed an in-house tool, called RemoveLongBonds, for reconnection of boundary bonds in the replicated cells. Figure A.3(b) shows the structure of the cell after application of this reconnection procedure.

As it is described in section 2.2, we used a 4 step replication protocol for building a 130x135nm² size film out of an original 26x27nm² size supercell. We tried to optimize the replication numbers in those steps in terms of real time performances. We have evaluated processing times for two types of protocols: (1) initially construct a 10x10 replicated cell from original supercell then build another 5x5 replicated cell of it, (2) construct a 25x25 cell from the original supercell then make them replicated to be a 2x2 cell of it. As a result, protocol (1) was shown to require only ~70 minutes for processing, whereas ~7 hours of time is necessary for (2) (Xeon Gold 6148, 20 cores, 754 Giga Bytes). Figure 3(a) and (b) in section 2.2 show the final structure obtained from the protocol (1) above.

Figure A.3(a) shows the structure of a 2x2 replicated cell from the prototype model. In addition, we have developed an in-house tool, called RemoveLongBonds, for reconnection of boundary bonds in the replicated cells. Figure A.3(b) shows the structure of the cell after application of this reconnection procedure.

As it is described in section 2.2, we used a 4 step replication protocol for building a 130x135nm² size film out of an original 26x27nm² size supercell. We tried to optimize the replication numbers in those steps in terms of real time performances. We have evaluated processing times for two types of protocols: (1) initially construct a 10x10 replicated cell from original supercell then build another 5x5 replicated cell of it, (2) construct a 25x25 cell from the original supercell then make them replicated to be a 2x2 cell of it. As a result, protocol (1) was shown to require only ~70 minutes for processing, whereas ~7 hours of time is necessary for (2) (Xeon Gold 6148, 20 cores, 754 Giga Bytes). Figure 3(a) and (b) in section 2.2 show the final structure obtained from the protocol (1) above.
Figure A.3. Replication of mica supercell
(a) Line representation of bonds after 2x2 replication of the supercell in Figure 2 of section 2.2. (Yellow) silicon, (pink) aluminum, (red) oxygen, (cyan) potassium. (b) Line representation of bonds after bond reconnections.

A.3 Incorporation of PARMBSC1

To utilize PARMBSC1 as DNA force field in our simulation there is a hurdle that should be overcome. Although NAMD has already been capable of running a MD simulation with AMBER force field, it may not run a simulation under a mixed environment where CHARMM and AMBER force fields are coexisting. In fact, Interface MD only provides CHARMM style force field for non-metallic materials including mica. In order to solve this issue, we redefine PARMBSC1 as CHARMM force field, and convert the prepared structure files so that they can be MD inputs under the new force field.

More specifically, we have addressed this issue by incorporating a group of procedures illustrated in Figures A.4-6. They consist roughly of four parts: (1) cropping the DNA origami structure part (Figure A.4), (2) translation of PARMBSC1 into CHARMM format (Figure A.5), (3) conversion of structure file format of DNA origami using TLEAP [39] and PARMED [40] (Figure A.6), and (4) re-integration of DNA origami with other part (Figure A.4). In the following, (2) and (3) parts are explained in more detail.

Figure A.5 shows a flowchart of the translation process of PARMBSC1 into CHARMM style force field. PARMBSC1 only defines additional ones to GAFF, generalized AMBER force field. Hence we have used GAFF and PARMBSC1 as source data set. They are fed into AMB2CHM utility program available from AmberTools18 [41–43], and are then converted into a single CHARMM format force field file, “amb2chm.par”.

The definitions of atom types in amb2chm.par file are now redefined and the new force field parameter set is available in terms of these atom types. This means that the previously built structure files (“nucleic.pdb” and “nucleic.psf”) may no longer be used as MD inputs, since they are written based on CHARMM's atom type definitions. Therefore, other structure files, written in new atom types and compatible with amb2chm.par, need to be created.
Figure A.4. Flowchart of pre- and post-processes for AMBER's PARMSC1 force field incorporation
Yellow: extracted coordinate and topology files. Sky blue: converted files through procedure explained in Figure A.6. Red: a procedure explained in Figure A.6.

Figure A.5. Flowchart of AMBER's force field translation into CHARMM format

To deal with this issue, we developed a procedure which utilize TLEAP [39] and PARMED [40], as illustrated in Figure A.6. TLEAP is also a utility program in AmberTools, which provides a command-line/scripting interface for creation of AMBER MD input coordinate and topology dataset, and thus have the same role as PSFGEN for creation of NAMD MD inputs [44]. PARMED provides a generalized manipulation tools for coordinate and topology data in various MD simulation packages including AMBER, CHARMM, and others. In Figure A.6, the original structure file (nucleic.pdb) is pre-processed using pdb4amber so as to be read safely by TLEAP. After that TLEAP reads PARMSC1 and then loads the pre-processed structure file (nucleic_clean_ter.pdb). Then it exports AMBER format MD input files (“nucleic.inpcrd” and “nucleic.prmtop”). They are passed subsequently to a python script that translate them into PDB and PSF format files using PARMED.
**Figure A.6.** Flowchart of AMBER's force field translation into CHARMM format
Supplements

S.1 MD simulation result of DNA origami “angry” on mica film

[angry_e80.mp4]
A movie file created from trajectory data of a MD simulation for DNA origami “angry” under [Na+] = 150 mM, [Mg2+] = 30mM.

S.2 MD simulation result of “angry” under a deionized condition

[angry_deion_e80.mp4]
A movie file created for DNA origami “angry” under the deionized condition.

S.3 MD simulation result of “rectangle” under $\varepsilon$ = 1

[rectangle_deion_e1.mp4]
A movie file created for DNA origami “rectangle” under $\varepsilon$ = 1 and a deionized condition.

S.4 MD simulation result of “rectangle” under $\varepsilon$ = 60

[rectangle_deion_e60.mp4]
A movie file created for DNA origami “rectangle” under $\varepsilon$ = 60 and a deionized condition.