All-atom molecular dynamics simulations of membrane spanning

DNA origami nanopores

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Abstract:

Building on the recent technological advances, all atom molecular dynamics (MD) simulations have become an indispensable tool to study the molecular behavior at nanoscale. Molecular simulations have been used to characterize the structure, dynamics, mechanical and electrical properties of DNA origami nanostructures. In this chapter we describe a method to build all-atom model of lipid spanning DNA origami nanopores and perform molecular dynamics simulations in explicit electrolyte solutions.

1. Introduction

State-of-art DNA nanotechnology has enabled an efficient route to construct and control synthetic nanoscale systems which perform specific functions[1]. Introduced in 2006, DNA origami method[2] has filled fresh aspirations to the field of DNA nanotechnology. Computer-aided design of staple strands has simplified the protocols for synthesizing DNA origami constructs[3]. In 2012, Simmel lab at the Technical University of Munich demonstrated the insertion of a cholesterol-anchored DNA origami barrel in a lipid vesicle[4]. Simultaneously, the Howorka group at the university college of London also characterized a series of lipid spanning DNA origami nanopores using various hydrophobic modifications including ethyl-phosphorothioate[5], streptavidin, porphyrin[6] and added functionalities[7]. Membrane spanning DNA nanostructures have opened new avenues in the area of synthetic membrane channels for various applications[8] such as transmembrane molecular transport[9], DNA translocation[10], mimicking the natural enzymes[11] etc. The highly programmable functionalities of the DNA backbone provide DNA nanopores an advantage over the biological protein nanopores. One of important feature of the DNA origami nanopore is that their diameter can be controlled externally by sequence design. Keysers group at the Cambridge university has synthesized DNA channels is variable shape and size DNA channel[12-14].

With the recent advancement in computer architecture and numerical algorithms, computational methods, in particular coarse-grained and all-atom molecular dynamics (MD) simulations, can now provide accurate microscopic account of the structure and dynamics of self-assembled DNA nanosystems.[15, 16] Previously all-atom MD simulation have been successfully used to revealed the fine details about the structural[17-19], mechanical[20, 21] and electrical[22] properties of DNA nanostructures. MD simulation of DNA nanostructures using coarse-grained oxDNA model[23] successfully reproduced the experimental observations such as cry-TEM structures[24]. Due to their contrasting interaction, the assembly of DNA and lipid is not very common in nature. Molecular simulations can be particularly helpful in understanding the molecular mechanism and interaction governing the self-assembly of DNA nanopore in lipid bilayer membranes[25].

Our group has pursued the application oriented computational exploration of membrane tethered DNA nanosystems using all-atom and coarse-grained molecular dynamics simulations. In 2015, in the first of its own kind of study of membrane embedded DNA origami nanopores using all-atom MD simulations, we characterized the mechanism of ionic conductance, mechanical gating and electro-osmotic transport of ATP molecules across the membrane[26]. In the subsequent studies, based on the results of all-atom MD simulation trajectories, we hypothesized a novel mechanism of toroidal pore formation in lipid bilayer membrane by different kind of DNA nanopores[12, 13, 27]. In one of the recent studies, all-atom and coarse-grained MD simulation revealed that DNA origami nanopore in lipid bilayer membrane catalyzes the spontaneous transport of lipid from one leaflet to other with a rate which is three order of magnitude higher than their biological archetype[11]. The fluorescence microscopy experiments confirmed the predication of microscopic MD simulations. In this chapter, we summarize the general protocols used to perform all-atom MD simulations of membrane embedded DNA origami nanopore in lipid bilayer membranes.

2. Materials.

Software and online servers

1. **caDNAno**. Developed by Douglas et al, caDNAnano[3] is most widely used computer program to design the DNA origami nanostructures. The latest version of caDNAno can be downloaded from http://cadnano.org. There are several other interactive and command-based interfaces available to design the DNA nanostructure including Nanoengineer-1, DAEDALUS[28], NAB[29], Tiamat[30] which can also be useful to create DNA nanopore depending on the design the nanopore. More details about this can be found in our previous tutorial[31].

2. **caDNAno to pdb convertor**. The output "json" file of caDNAno design can be uploaded into the ENRG-MD server http://bionano.physics.illinois.edu/origami-structure and converted to the PDB file format representing all atoms in the structure. Alternatively, one can also use mrDNA package for json to all-atom PDB conversion, please refer to the website for the details about mrDNA https://gitlab.engr.illinois.edu/tbgl/tools/mrdna

3. **MarvinSketch.** Developed by ChemAxon, MarvinSketch is a useful and open source software to draw the chemical structure of non-standard residues and export their 3D all-atom model. It can be downloaded from https://chemaxon.com/products/marvin

4. **VMD.** VMD[32] is a molecular visualization program to display and animate atomistic structures. It can be download from http://www.ks.uiuc.edu/Research/vmd. VMD is supported by majority of operating systems (Windows, UNIX, Mac-OS) and also provides a tcl-based programming interface which is very helpful for the analysis of the simulation trajectories. For more instruction, please refer to VMD use guide[33] and VMD tutorials[34].

5. **CGenFF.** CGenFF[35] webserver can be accessed at https://cgenff.umaryland.edu/. It is a utility to create the CHARMM compatible topology and parameters of small organic molecules to perform all-atom MD simulations.

6. **CHARMM-GUI** CHARMM-GUI[36] is a webserver to interactively build the membrane systems. The preferred lipid type membrane system can be assembled and downloaded from http://www.charmm-gui.org.

7. **NAMD.** NAMD is a highly parallel molecular dynamics code which also supports CUDA-based acceleration. NAMD is compatible with Linux/UNIX, Mac OS X, or Windows operating systems and runs on laptops as well. However, for performing the all-atom MD simulation of membrane spanning DNA nanopore, it is recommended to use supercomputers with parallel programming environment. For more details on NAMD, please refer to the NAMD user guide[37] and NAMD tutorials[38].

8. **CHARMM topology and parameter files**. CHARMM topology files are required to create the all-atom structures. Also, we use CHARMM force field parameters[39] with latest CUFIX[40] to describe the inter and intramolecular interaction in the system. CHARMM topology and parameters can be downloaded from https://www.charmm.org/charmm/. The latest non-bonded (CUFIX) corrections to ion-DNA and ion-lipid interaction parameters can be download from http://bionano.physics.illinois.edu/CUFIX .

9. **PERL.** Perl is a general-purpose scripting language available across all the major operating systems. In this chapter, we will use a file created from perl script to enforce hexahydrate water structure around Mg2+ ions.

2.2 Required files:

1. **DNPinMembraneTutorial package**. The files used in this chapter (scripts and other support files) at
http://bionano.physics.illinois.edu/sites/default/files/DNPinMembraneTutorial.tar.gz

2. **origamiTutorial package**. Some files used in our guide to simulate DNA origami nanostructure as described in our previous chapter[41], will also also be used in this chapter. These files can be found on our website at
http://bionano.physics.illinois.edu/sites/default/files/origamitutorial.tar.gz

3. Methods In this section, we describe the steps involved in the building an all-atom model of DNA origami nanopore in lipid bilayer membranes, performing MD simulations in explicit electrolyte solution and calculating the ionic current in MD simulation. The whole section is arranged in the following manner; in section §3.1 we will describe how to build and assemble atomistic models of various components of the system including DNA origami nanopore, hydrophobic lipid anchor, lipid bilayer membrane, water and ions; in section §3.2 we will describe the methodology to run equilibrium MD simulations; in section §3.3 we will describe the method of applying the electric field in MD simulation using NAMD. Finally, in section §3.3 will be discuss how we calculate ionic currents from the all-atom MD simulation trajectories.

3.1 Assembling all-atom model of DNA origami nanopore in lipid bilayer membrane.

Step 1: The first step in building the system is to create the caDNAnano design of DNA origami nanopore. We chose a honeycomb lattice and draw the design of a four-helix DNA nanopore in caDNAno as per the design used in experiments[11], figure 1. We saved the output "json" file of the caDNAnao design used in this chapter which can be find in the step1 subdirectory of the DNPinMembraneTutorial folder.

Step 2: Next**,** we uploaded the json file, figure 2a, into the ENRG-MD webserver http://bionano.physics.illinois.edu/origami-structure and followed the instruction provided over the webpage. We downloaded and unzipped the output file from the ENRG-MD webserver, in the subdirectory step2 of the DNPinMembraneTutorial folder. The output folder contains the necessary files to run NAMD simulations. These files are the CHARMM format structure file (.psf), coordinate (.pdb), the extrabonds file to implement the restraints of elastic network in the origami structure (.exb) and NAMD configuration file (.namd). The downloads will also contain a folder with CHARMM parameter files. A detailed description on how to assemble and simulate DNA origami is provided in our practical guild for DNA origami similations[41]. Figure 2b shows the all-atom pdb structure of DNA origami nanopore.

Inserting a negatively charged barrel of DNA into lipid bilayer membrane, can disrupt the structure of membrane. In order to overcome this instability in membrane embedded DNA nanostructues, several modifications to the DNA backbone using hydrophobic moieties such as, cholesterol[4, 7, 11-14], ethyl groups[5], porphyrins[6, 42] and biotin-streptavidin[9], etc. have been proposed and realized experimentally. Using the coarse-grained MD simulations, we have shown that cholesterol

anchors can account for the free energy of inserting a DNA barrel in lipid bilayer membrane[12]. After obtaining the all-atom topology and coordinate of DNA origami nanopore in step 2, the next step is to covalently connect the hydrophobic lipid anchors (cholesterol group with tetraethylene glycol (chol-TEG) linker in our case) to the O3' atom of the DNA backbone as schematically shown in figure 1a. Before connecting the lipid anchors, we equilibrated the DNA origami nanopore in vacuum using ENRG-MD restraints as discussed in our chapter on DNA origami simulation protocols[41].

Step 3: We created a pdb (*cholTEG.pdb*) of chol-TEG using the MarvinSketch and saved it in the ste3 subdirectory of the DNPinMembraneTutorial folder. For the purpose of providing additional flexibility to the anchor, we added an unpaired DNA nucleotide (adenine) to the chol-TEG molecule. We uploaded the pdb structure of chol-TEG into the CGenFF webserver[35] and obtained the topology and parameter file (*cholteg.str*) for the cholesterol anchors. Using VMD, we strategically connected the chol-TEG with O3' atom of DNA such that chol-TEG extends diagonally opposite directions away from the DNA nanopore, figure 2c. We created the necessary "patches" to make covalent connections between DNA and chol-TEG using psfgen plugin of VMD. A complete tcl script, *gen_psf.tcl*, to create the topology of chol-TEG conjugated DNA origami pore can be found in subdirectory step3 of the DNPinMembraneTutorial folder.

Step 4: Next step is to insert the chol-TEG conjugated DNA origami nanopore into the diphytanoyl phosphatidylethanolamine (DPhPE) lipid bilayer membrane. The equilibrated patch of the DPhPE lipid bilayer membrane all-atom structure was obtained from the CHARMM-GUI website. The dimensions of the membrane patch should be large enough to avoid the undesirable interaction between the periodic images of the DNA origami nanopores. If only a smaller patch of equilibrated lipid is available, "writemol" commands from the psfgen plugin of VMD can be utilized to create a larger patch by adding multiple periodic images of the original patch. We used tcl commands in VMD to embed the atomistic structure of DNA origami nanopore in the middle of the square patch (12.6 nm x 12.6 nm) of pre-equilibrated lipid bilayer membrane. After embedding the DNA nanostructure into the lipid membrane, lipid molecules located either within 3 Å of the nanostructure or inside the nanostructure were removed, figure 2d. The psf and pdb files of the membrane embedded DNA nanopore are kept in the step4 subfolder of the DNPinMembraneTutorial folder.

Step 5 and 6: Mg^{2+} ions are crucial for the stability of the DNA origami nanostructures. Previously, we have shown that instead of bare Mg^{2+} ions, a magnesium hexahydrate (MGHH2+) complex model reproduces the simulated DNA–DNA forces in better agreement with experiments[43-45]. Using a perl script available in sub-folder step5_6, we placed 151 of MGHH2+ ions to compensate its electrical charge of DNA. Following that, we solvated the structure in box of water using Solvate plugin of VMD, figure 2e. Finally, we added K^+ and Cl^- ions into the system using Autoionize plugins of VMD to acquire 1M KCl electrolyte concentration. A script, *solvate_ionize.tcl* used to solvate and ionize the system is given in the sub-folder step5_6 of the DNPinMembraneTutorial folder. Thus, obtained topology (psf) and coordinate (pdb) files will be used to run the simulations. The final assembled system measured 12.5 x 12.5 x 17 nm_3 and contained 235, 646 atoms. It is advisable to load the structure and coordinate files into VMD and visualize the individual components carefully. Figure 2f shows the fully assembled all-atom model of the system.

3.2 Equilibrating the structure of DNA origami nanopores in lipid bilayer membrane.

After creating the psf and pdb files of solvated structure of DNA origami nanopore embedded in lipid bilayer membrane, we prepared the NAMD configuration files to run the equilibrium MD

simulations. To remove the possible clashes between the DNA, lipid and solvent in the system, we first minimize the system for 1200 steps using the conjugate gradient method. Subsequently to achieve the correct density of the simulation box, we equilibrated the system using constant number of atoms (N), pressure (P = 1 atms) and temperature (T = 295 K), i.e. NPT ensemble. To maintain the pressure and temperature in the system, we use Nosé-Hoover Langevin piston[46, 47] and Langevin thermostat[48, 49], respectively. We used anisotropic pressure coupling in these simulations, the ratio of system's dimensions in membrane plane (x-y plane) were kept constant using the "useConstantRatio" keyword in NAMD program. The system's dimension to the bilayer normal (Z axis) was allowed adjust independently.

Initially, the system was equilibrated for 205 ns having all non-hydrogen atoms of the DNA nanostructure harmonically restrained to their initial coordinates using a spring constant of 1 kcal mol$_{-1}$ Å$_{-2}$ which allowed the lipid and water to adopt equilibrium configurations around DNA nanopore. Following that, the spring constants of the restraints were decreased to 0.5 and then to 0.1 kcal mol$_{-1}$ Å$_{-2}$, the system was equilibrated at each spring constant value for 4.8 ns. Next, spatial restraints were replaced by a network of harmonic restraints that maintained distances between atomic pairs at their initial values; such elastic restraints excluded hydrogen atoms, phosphate groups, atoms in the same nucleotide and pairs separated by more than 8 Å. These types of the restrains are implemented using the "extraBonds" utility of NAMD program, the required extrabonds file for the origami structure is obtained from the [ENRG-MD server]. The system was simulated under such network of elastic restraints for 14.4 ns; the spring constants of the restraints were decreased from 0.5 to 0.1 and then to 0.01 1 kcal mol$_{-1}$ Å$_{-2}$ in 4.8 ns steps.

All MD simulations were performed using the program NAMD[50] using periodic boundary conditions, the CHARMM36 parameter set for water[51], ions[52], nucleic acids[53], lipid bilayer[54]. We used the latest CUFIX parameters for of ion-DNA, ion-ion and DNA–lipid interactions[40, 55]. We invoked a 2–2–6 fs multiple time-stepping method in NAMD to integrate the equation of motion. We employed SETTLE algorithm[56] to keep water molecules rigid and RATTLE algorithm[57] to keep all other covalent bonds involving hydrogen atoms rigid. A 8-10-12 Å cutoff scheme was adopted to compute the van der Waals and short-range electrostatic forces. Long-range electrostatic interactions were computed using the particle mesh Ewald (PME) method[58] over a 1.2-Å resolution grid[59]. We saved the coordinates of the system after every 2.4 picosecond.

Finally, we performed the 2.2 μs production simulation of DNA origami nanopore embedded in lipid bilayer membrane. The system's coordinates were recorded every 240 ps. Production simulation of the system was performed on Anton 2 using simulation parameters equivalent to those described above for, except that temperature and pressure were maintained using the Nosé-Hoover thermostat[60, 61] and the Martyna-Tobias-Klein barostat.[46] Figure 3a and 3b show the instantaneous snapshot of the system at the beginning 0 μs and after and after 2.2 μs long production MD simulations respectively. The DNA origami nanostructure overall maintained its structure during the course of the prolong MD simulations. Also, we observed significant scrambling of the lipids from one leaflet to other[11].

3.3 Electric field simulations

In order to calculate the ionic current through the DNA origami nanopores, we performed MD simulations under transmembrane potential differences of ± 30 mV, ± 0.100 V, ± 0.150 V and ± 0.250 V along the bilayer normal. The voltage values are similar to those used in typical experiments to measure the ionic conductance across the membrane spanning DNA origami nanopore[4, 6, 7]. Figure 4a shows a cut away view of the system set up to measure the ionic currents in all-atom MD simulations. We used the conformation obtained at the end production simulation

and applied constant electric field along the bilayer normal (z axis). All simulations were performed under using constant number of atoms (N), volume (average system size) and temperature (T = 295 K) ensemble, i.e. NVT ensemble. The dimensions of the system in all three directions were kept constant to the average system dimensions from last 5 ns of production MD simulation. The desired voltage difference across the system was maintained using an externally applied electric field, E given by $E=-V/L$, where L is the length of the simulation box along the direction of applied field (z-axis). In order to obtain the desired value of electric field in the unit of kcal/mol/Å/e which is used in NAMD configuration file, a factor of 23.0609 needs to be multiplied in above expression, (given that the voltage and length are provided in volts and Å). We ran simulation of each bias for approximately 100 ns and saved the saved the coordinate of the system at every 2.4 ps.

3.4 Calculation of ionic current and lipid scrambling.

The transmembrane ionic current (I) can be measure by counting the number of ions translocated through the DNA origami nanopore as follows,

$$I = \frac{Nq}{t}$$

where the N is the number of ions permeated across the membrane and q is charge of ion and t is the simulation time.

In a steady state, the current in the system must be same in direction of the applied electric field. We centered all the frames of the simulation trajectory around DNA origami nanopore. To reduce the thermal noise originating from the stochastic displacements of ions in bulk solution, we computed the ionic current in the region within $L/2 \leq z \leq L/2$ using the following equation as explained in our previous work[62].

$$I\left(t + \frac{\Delta t}{2}\right) = \frac{1}{\Delta t\, L} \sum_{i}^{N} q_i[(\zeta_i(t + \Delta t) - \zeta_i(t)],$$

Where L = 30 Å (the region where DNA nanopore is inside the membrane) Δt is saving frequency in the simulation trajectory, N is the number of ions present in the system, q_i is the charge of the respective ion, and ζ_i is z coordinate of the respective ion at that time instant

$$if\, abs(\zeta_i) \leq L/2; \zeta_i = z_i(t)$$

$$if\, abs(\zeta_i) > L/2; \zeta_i = L/2$$

$$if\, abs(\zeta_i) < -L/2; \zeta_i = -L/2$$

A tcl script (*currentTraj.tcl*) available under the analysis subfolder of the DNPinMembraneTutorial folder, utilizes this equation to calculate the ionic current. The ionic current values were sampled every 2.4 ps, and a running average over 1000 windows is shown in figure 4b for different biases. Integrating the instantaneous ionic current values over whole the interval of simulation time, we obtained the total charge permeated across the membrane in the direction of applied field, figure 4c. In MD simulation, we can count the number of ions translocated across the membrane which must be similar to the integrated current values as plotted in figure 4c. Comparing the number of ions translocated to the integrated ionic current, one can cross-check if the script used to calculate the ionic currents is working correctly. Finally, we averaged the ionic current for whole 100 ns MD

simulations using the blocks of 70 ps, figure 4d. The I-V response of the DNA nanopore appears to be non-linear at the higher voltage biases (>100 mV).

4. Notes.

During the initial equilibration, DNA origami simulation can crash due various reasons. It is recommended to monitor the energy, temperature, pressure, density etc. in the log file during the course of the simulation. In UNIX based operating system (Linux, Ubuntu, Fedora, Redhut etc), *awk*, *sed*, *grep* etc. commands can be useful to print and plot these quantities from NAMD simulation log files. For example, following terminal command will extract simulation time step, kinetic energy, potential energy, temperature and pressure from the NAMD log file to a new file 'output.dat'.

*awk '/^ENERGY:/{print $2, $11, $14}' npt1.log >output.dat*

When the simulation crashes unexpectedly, the log file generally writes the reason of the crash. One of the common causes which can lead to the unstable simulation during the initial stages is the piercing of bonds through the aromatic rings in DNA or the linker molecule. The best way to avoid these errors is to altogether remove the clashes between DNA and lipids at the first place. Alternatively, we can load the molecule into VMD and move the coordinates of the atoms or residues interactively (by using keys 5 and 6) and remove the clashes.
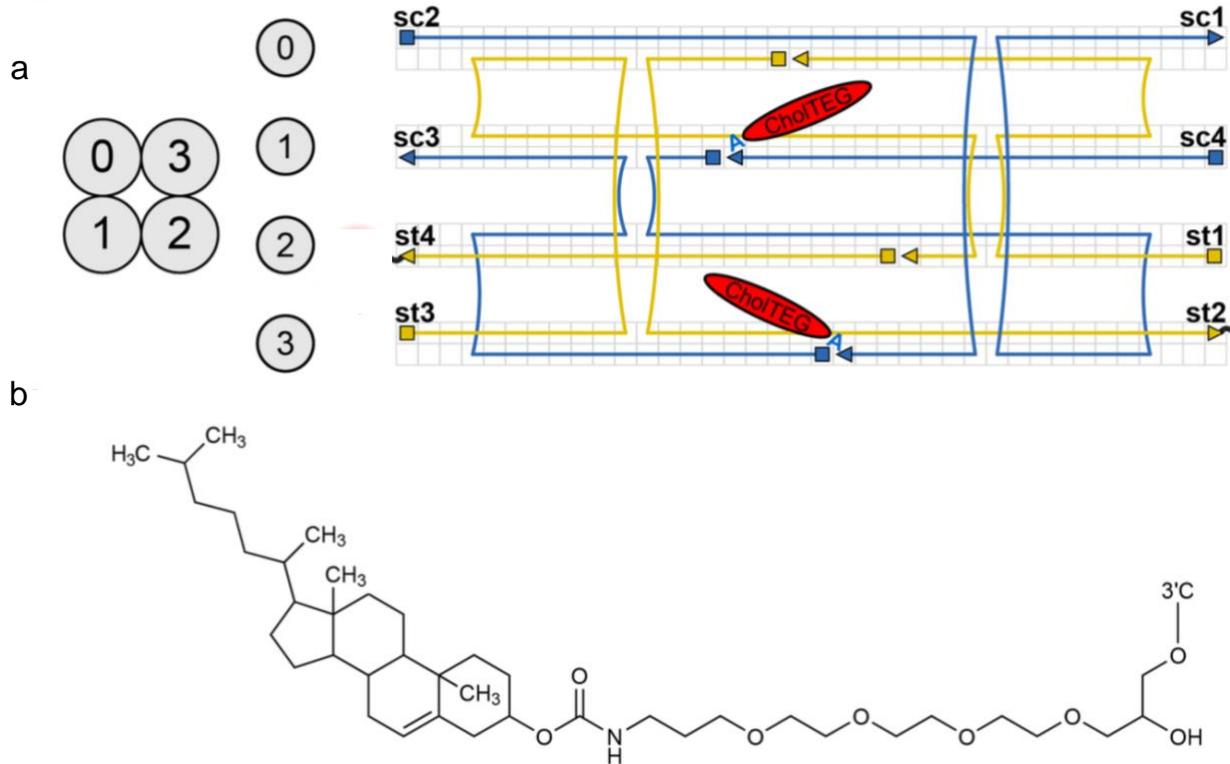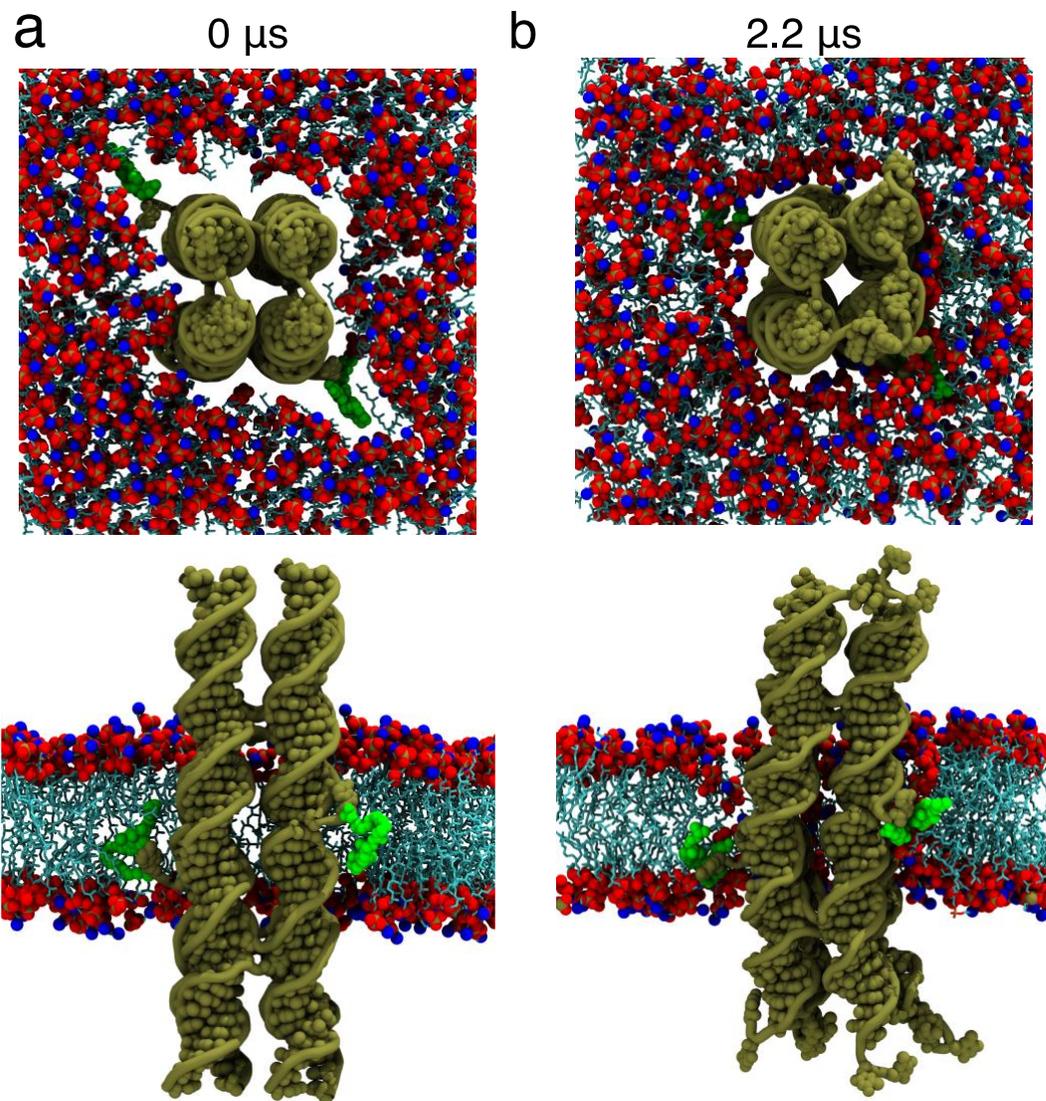
Figures

**Figure 1: Design of DNA origami nanopore with cholesterol anchor for membrane tethering (a)** caDNAno design of DNA nanostructure. The sequence of the structure is given in the reference article.[11] The "CholTEG" tag indicates a cholesterol group chemically linked to a nucleotide. **(b)** Chemical structure of cholesterol group with TEG linker (CholTEG). The "3'C" tag indicates the 3' carbon of the modified nucleotide.
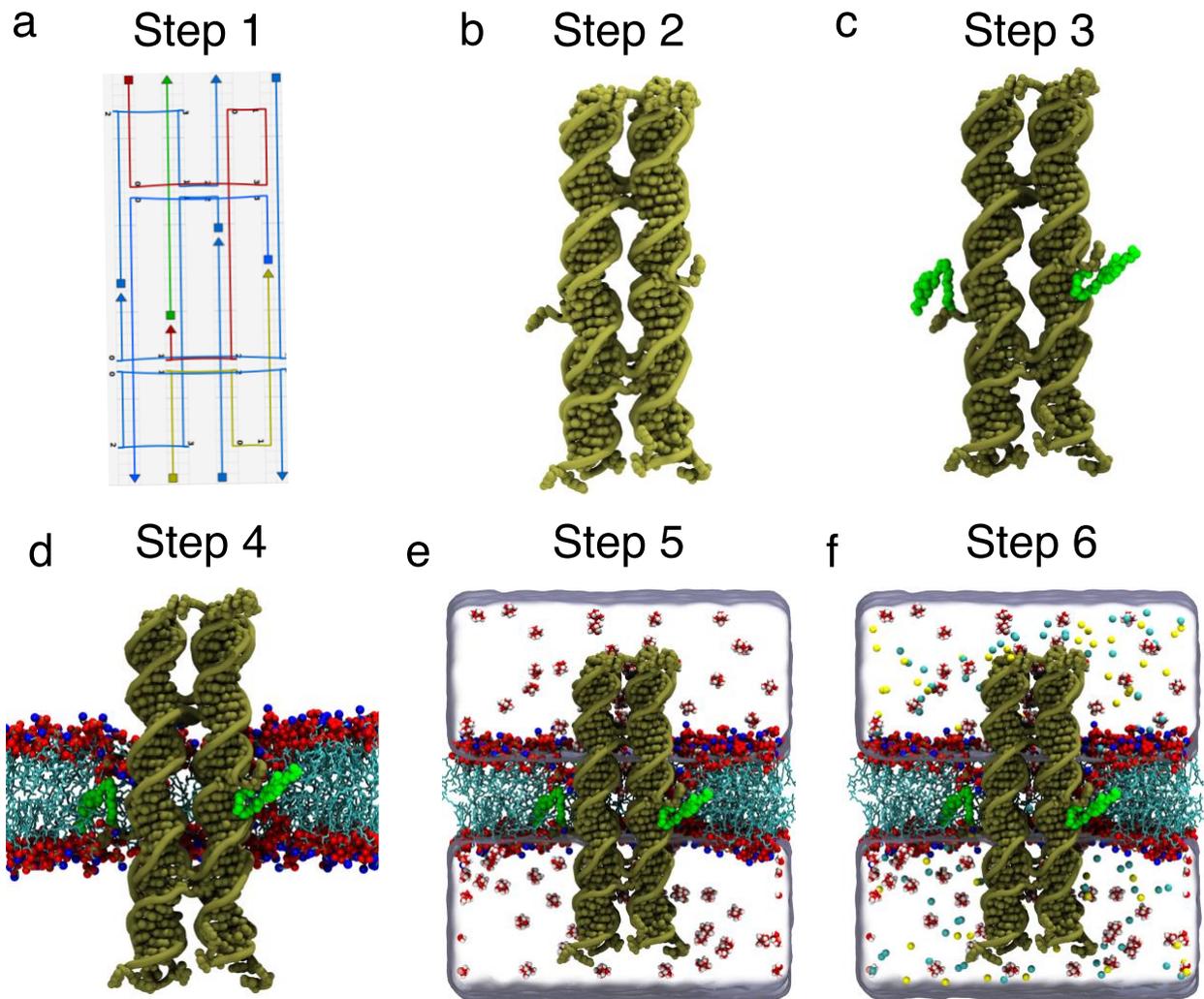
Figure 2: Steps involved in assembling the all-atom MD simulation system for membrane spanning DNA origami nanopore. (a), The design of DNA origami nanopore as visualized in caDNAnano. (b) An all-atom model of DNA origami by converting the json file to atomistic representation using ENRG-MD webserver, atoms of DNA are shown using tan spheres. (c), All-atom representation of covalently conjugated cholTEG (shown using green sphere) to DNA nanopore, the atoms of chol-TEG are shown using green spheres. (d) DNA nanopore embedded into a patch of the lipid bilayer membrane. Nitrogen, phosphorus and oxygen atoms of the lipid headgroups are highlighted in blue, tan and red spheres respectively and the rest of the lipid is shown in cyan licorice representation. (e) Membrane embedded DNA origami nanopore with added MGHH and solvated in a box of water. (f) Fully assembled DNA origami nanopore embedded in DPhPE lipid bilyar membrane solvated in specific concentration of electrolyte solution of K+ (yellow) and Cl- ions (cyan).

Figure 3: **Instantaneous snapshots of the simulated system.** Microscopic configuration of the simulated system **(a)** at the beginning of the simulation and **(b)** at the end of the 2.2 μs equilibration MD simulation. Top panel shows the top view of the system whereas the bottom panel illustrates a cut-away view. DNA base-pairs are shown using tan spheres, whereas the backbone of DNA is shown using a tubular representation. Lipid bilayer membrane is shown in cyan licorice representation whereas the nitrogen, phosphorus and oxygen atoms of the lipid headgroups are highlighted in blue, tan and red spheres respectively. CholTEG is shown in green spheres, the electrolyte solution is not shown for clarity.
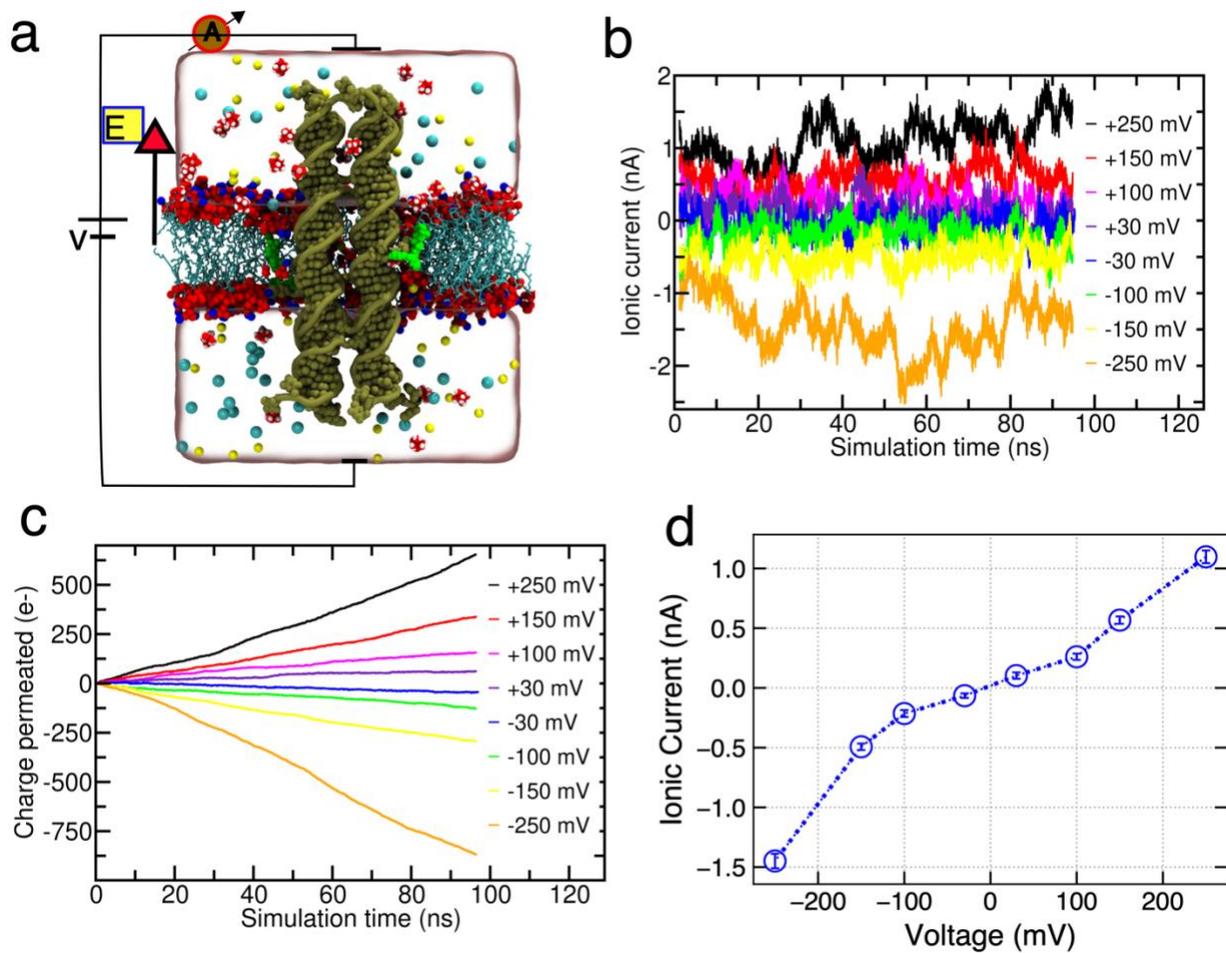
Figure 4: Simulated ionic current through DNA origami nanopore. (a) A cut-away view of the simulation system with applied voltage bias. (b) Total ionic current through the DNA origami nanopore at ±30 mV, ±100 mV ±150 mV and ±250 mV as a function of simulation time. (c) Total charge permeated across the membrane through the DNA origami nanopore at different applied voltage biases as a function of the simulation time. (d) The average ionic current obtained from the block average of instantaneous ionic current obtained from the simulation trajectories. The error bar shows the standard error in the ionic current.

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