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DYNAMIC INTERACTIONS BETWEEN LIPID-TETHERED DNA AND PHOSPHOLIPID MEMBRANES

Allocation: Blue Waters Professor/240 Knh
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EXECUTIVE SUMMARY

Lipid-anchored DNA can attach functional cargo to bilayer membranes; this has applications in DNA nanotechnology, synthetic biology, and cell biology research. An understanding of DNA membrane-binding strength and structural dynamics at the nanoscale is required to optimize the DNA anchoring for these applications. Using simulations performed on the Blue Waters supercomputer, the research team elucidated how membrane binding of cholesterol-modified DNA depends on electrostatic and steric factors involving the size and charge of the lipid headgroup, duplexed or single-stranded DNA, and the buffer composition. Atomistic molecular dynamics (MD) simulations explain the experimental findings and elucidate the dynamic nature of anchored DNA, such as the mushroom-like conformation of single-stranded DNA hovering over the bilayer surface in contrast to a straight-up conformation of double-stranded DNA. The information from this study is expected to facilitate the development of biomimetic DNA versions of natural nanopores and cytoskeletons for research and nanobiotechnology.

RESEARCH CHALLENGE

A lipid molecule attached to the end of a DNA strand can anchor the strand to a lipid bilayer membrane. This simple principle has been applied to design lipid-spanning DNA nanopores [1,2] for applications in the fields of nanobiotechnology [3], biosensing [4], and synthetic biology [5]. Controlling the interactions between anchored DNA and a bilayer membrane is critical to attaining the desired performance of such lipid-spanning DNA nanopores. There are numerous unanswered questions about the nature of such interactions; in particular, about the affinity and the conformation of the tethered DNA strands to and near the bilayer membrane and how those are affected by the charge and size of the lipid headgroup.

METHODS & CODES

The research team performed explicit-solvent all-atom MD simulations of several lipid-conjugated DNA systems using the latest version of NAMD2 [6]; the CHARMM36 [7] forcefield to describe the bonded and nonbonded interactions among DNA, lipid bilayer membranes, water, and ions; and the team's custom NBFIX corrections for nonbonded interactions [8]. The analysis and postprocessing of the simulation trajectories were performed using VMD and CPPTRAJ [9,10].

RESULTS & IMPACT

Complementing the gel-shift experiments carried out by the collaborators in the Howorka group at the University College London, the researchers at the University of Illinois at Urbana–Champaign built and simulated several all-atom models of DNA–lipid systems that differed from one another by the composition of the lipid membrane, the type of DNA molecules, and the buffer conditions. The lipids were chosen on the basis of their wide use in research and to cover a representative set of headgroup charges and sizes.

This study has shown that the composition of a lipid membrane, the buffer electrolyte, and the type and length of DNA constructs can considerably affect the ability of a cholesterol-terminated DNA molecule to insert into a lipid membrane. Thus, negatively charged lipids were found to weaken the binding of DNA to the membrane. Surprisingly, the study found the type of monovalent cations in the buffer solution also affected DNA binding, which the MD simulations explained by stronger specific interactions of smaller-sized cations with the lipid headgroups. In addition to electrostatics, this study elucidated the role of steric interactions, predicting the maximum coverage of lipid membranes by various DNA constructs. It also found that, while being tethered to lipid bilayers, the DNA molecules can diffuse along the membrane surface and that such diffusion is limited by the properties of the lipid bilayer.

By synergistically combining the experiment and all-atom MD simulations, the research team examined how cholesterol-modified DNA strands interact with lipid bilayer membranes. Based on the titration results from the gel-shift assay, this study for the first time quantified the binding affinity of cholesterol-conjugated DNA to lipid membranes in terms of their equilibrium dissociation constant. The quantitative insights into the binding affinity and molecular accessibility of DNA will facilitate rational design of membrane-spanning DNA nanopores and broaden the usage of cholesterol-conjugated DNA for sculpting and assembling lipid bilayer membranes into functional biomimetic systems.

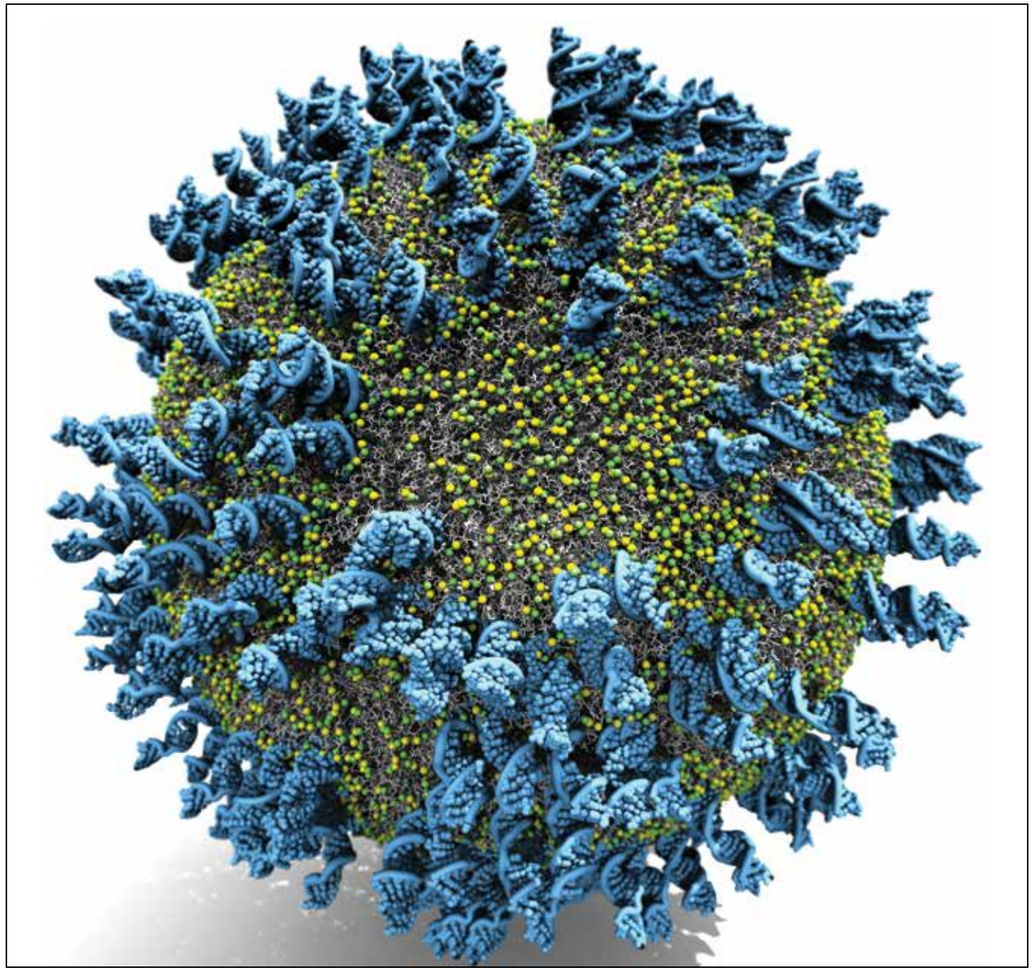

Figure 1: Molecular graphics representation of cholesterol-modified DNA fused into a phospholipid vesicle. The nitrogen and phosphorous atoms of lipid headgroups are shown in lime and yellow, lipid tails in white and gray, and DNA molecules in blue.

WHY BLUE WATERS

Explicit-solvent all-atom MD simulations were needed to examine the fine details of lipid-tethered DNA interactions with the bilayer membrane and to accurately characterize the effect of various factors such as the charge and the size of the lipid headgroups as well as the type of the DNA constructs (single- or double-stranded DNA). Because of the long timescale needed to decipher these details, such MD simulations are computationally demanding. The large number of XK nodes on Blue Waters with graphics processing unit accelerators connected by the fast Gemini interconnect makes it one of the best publicly available systems for performing simulations studying DNA–lipid interactions in atomistic detail. Over the past several years, the research team has used Blue Waters to carry out a set of landmark simulations in the area of nucleosome and DNA dynamics, bringing high-performance simulations to the forefront of this research field.

PUBLICATIONS & DATA SETS

P. M. Arnott, H. Joshi, A. Aksimentiev, and S. Howorka, "Dynamic interactions between lipid-tethered DNA and phospholipid membranes," *Langmuir*, vol. 34, no. 49, pp. 15084–15092, 2018, doi: 10.1021/acs.langmuir.8b02271.