ACS central science

FIRST REACTIONS

# Attack on the Cell Membrane: The Pointy Ends of DNA Nanostructures Lead the Way

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**A combination of molecular simulations and experiments helps explain how DNA nanostructures interact with cell membranes to initiate cell entry.**

Nanoparticles capable of entering cells have been widely utilized for biomedical applications including intracellular sensing and drug delivery. Such nanoparticles are often designed to be cationic in order to engage with negatively charged cell membranes. Nevertheless, some anionic nanoparticles, including DNA nanostructures, can be readily engulfed by cells. In spite of their high potential in biomedical applications, very limited work has been done to investigate the cell entry mechanism of such negatively charged DNA nanostructures. In this issue of *ACS Central Science*, Fan and colleagues combined molecular simulations and experiments to examine the like-charge, attraction-mediated interactions at the interface between DNA nanostructures and the cell membrane.[1]

Short DNA molecules (e.g., plasmids, antisense DNAs) typically lack the capability to enter cells, while DNA nanostructures (e.g., spherical nucleic acids) can be actively internalized by cells with the aid of receptors residing in the cell membrane.[2] Self-assembled DNA nanostructures have drawn increasing attention owing to their unique characteristics, including precisely programmable physical (i.e., size, shape, charge) and chemical (i.e., surface functionality) properties and excellent biocompatibility.[3] Recently, researchers started looking into critical mechanistic questions regarding the cellular uptake of DNA nanostructures, such as the internalization pathway[4] and how their properties mediate

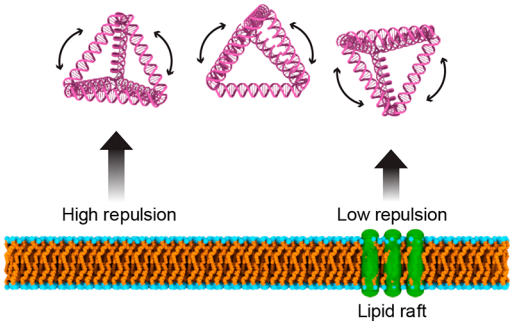


**Figure 1.** Molecular simulation of designer DNA nanostructures at the cell membrane interface. Anionic tetrahedral DNA nanostructures (TDN) take on a "corner-attack mode" to approach hot spots on the negatively charged cell membrane to minimize repulsive forces.

internalization,[5,6] solely based on experimental observations. The simulation-experiment combination strategy in Fan's work delivered a much-needed new tactic for gaining a deeper understanding of the mechanism of the cellular uptake process.

The authors assembled a classic tetrahedral DNA nanostructure with the edge length of 20 base pairs (TDN-20).[4] Experiments revealed that a lipid raft/protein-mediated transport pathway is closely associated with the uptake of TDNs into cancer cells. A model cell membrane filled with negatively charged and neutral lipids, along with lipid raft proteins, was constructed to mimic the like-charge attraction and lipid raft-mediated transport. In this model, anionic lipids induce a repulsive force, while membrane proteins imitate short-range attraction to TDNs. Dissipative particle dynamics simulations revealed that many of TDN-20s reached the membrane within 15 $\mu$s when initially placed 10 nm above the membrane, while none reached the

  

DOI: 10.1021/acscentsci.8b00600
*ACS Cent. Sci.* XXXX, XXX, XXX−XXX

membrane if the attraction force by membrane protein was omitted as a parameter. The majority of TDN-20s rotate prior to landing onto the membrane, thus realizing a "corner-attack orientation", presumably, to minimize repulsive force from the anionic lipids (Figure 1). Besides the orientational preference, it was found that TDN-20s are "picky" over the landing spots as well. Simulations showed that TDN-20s have a higher tendency of landing on hot spots of the membrane where lipid raft proteins reside and create a minimal negative charge. Since cell membranes have a semifluid nature, the approach of TDN-20s to the membrane surface induces charge redistribution in the membrane by pushing away negatively charged lipids and attracting positively charged lipids. This phenomenon was indirectly verified by experiments on the uptake of TDN-20s in cells with varied membrane fluidity.

The range of sizes and shapes of the DNA nanostructures was further expanded to make shorter (TDN-13) and longer (TDN-32) nanostructures, as well as TDN-20 dimers (TDN-20d) and 6-helix tubular nanostructures. Similar to TDN-20, molecular simulation revealed that all structures adapted the "corner-attack mode" regardless of differences in their size and shape. Three tetrahedral structures exhibited similar cellular uptake efficiencies, while TDN-20d and 6-helix structures had a reduced uptake, which was independently verified by simulations and flow cytometric experiments. Authors suggest that within the size and shape range studied in this work, the cellular uptake of DNA nanostructures is highly shape-dependent, while likely size-independent. According to Fan et al., such shape-dependence may be attributed to the slower rotational dynamics of TDN-20d and 6-helix tube when initiating the corner-attack orientation. Both simulation and experiments revealed a slower rotational diffusion rate and slower cellular uptake rate for TDN-20d as compared to TDN-20.

This work offers critical insights into the rational structural design of anionic nanoparticles by dissecting the dynamic behaviors of designer DNA nanostructures at the cell membrane interface.

This work offers critical insights into the rational structural design of anionic nanoparticles by dissecting the dynamic behaviors of designer DNA nanostructures at the cell membrane interface, although many other aspects might be explored further. For instance, the cell membrane is substantially more crowded than the model used in this study. The cell model could be improved by taking into account more interactive entities, which demands more complex models, higher computing power, and a better understanding of the cell membrane. Moreover, the size and shape range of DNA nanostructures could be expanded further as the observations might vary drastically.[5,6] At last, in order to complete the picture, the critical role of membrane receptors in the active transportation of different DNA nanostructures across the membrane could be carefully investigated at the molecular level. The current computational model includes the interaction between TDNs and a membrane protein receptor by using a simplified coarse-grained model without molecular details. Therefore, it may not be considered an accurate interpretation of complex receptor-mediated transport. Nevertheless, these limitations should not dampen the enthusiasm for the excellent work reported here as it represents the first example of utilizing computational modeling to probe the cellular uptake of DNA nanostructures and paves the way for future studies.


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**Notes**

The authors declare no competing financial interest.

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DOI: 10.1021/acscentsci.8b00600
*ACS Cent. Sci.* XXXX, XXX, XXX−XXX