Self-Assembly Science Research Section

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1. Introduction

In recent years, DNA origami has emerged as an innovative technique for constructing materials ranging from nano to micrometer scale, with sub-nanometer precision.[1] This method is widely used in various material science, chemical, and biomedical applications.[2] Despite this, the broad applicability of DNA origami is limited by stability issues. DNA origami structures typically melt below 60°C, break when deposited on mica or scanned by force-based methods such as atomic force microscopy (AFM),[3] disintegrate in deionized water and protein-denaturing conditions, and are susceptible to digestion by cellular enzymes.[4]

A few strategies have been employed to enhance the stability of DNA origami under specific conditions.[5] However, there is no reliable method for achieving the desired stability. Recently, we have developed near-quantitative and native nick ligation methods for DNA origami, which show promise for enhancing stability.[6] While these methods have been tested in vitro, the in-cell stability remains unexplored. In addition, the ability to anchor enzymes on DNA origami structures and control their spatial arrangement is crucial for enhancing enzymatic activity, particularly for biomass-related reactions. However, DNA origami naturally adopts a twisted conformation due to its design with 10.67 base pairs per helical turn, which introduces unwanted conformational distortion that disrupts the positioning of enzymes and hampers the efficiency of cascaded enzymatic reactions. This twist in DNA origami is expected to significantly affect enzyme kinetics, limiting its potential for catalysis applications.

This study focused on addressing these structural challenges by employing DNA intercalators to flatten the DNA origami structures, thus improving their performance in biomolecular applications. Also, this research aimed to understand how structural modifications, such as ligation and intercalation, influence enzyme activity and small molecule binding. Additionally, it aimed to develop strategies to stabilize DNA origami for cascaded enzymatic reactions, particularly in biomass-related processes. Furthermore, this study explored the potential of DNA origami as a drug carrier by examining its interactions with small molecules. The selective binding of specific drugs based on size and other properties is a compelling area of study. Most drug carriers rely on the covalent linkage of drugs to the carrier, requiring extensive chemical modifications and often resulting in low drug loading.[7] Therefore, developing a drug carrier based on non-covalent binding with high drug loading is highly desirable. Finally, it also aims to establish a size-dependent biomolecular sensing platform.

2. Stable DNA nanomaterials

The initial research focused on addressing the structural instability of DNA origami. Two ligation methods were explored: 1) Enhanced enzymatic ligation by dimethyl sulfoxide (DMSO): This method effectively improved the stability of 2D DNA origami, achieving over 90% ligation efficiency. 2) Chemical ligation using CNBr: This non-enzymatic approach was also successful, with ligation efficiencies of over 80% in both 2D and 3D DNA origami structures, offering a faster reaction time compared to the enzymatic method.[4,6] While these ligation methods addressed some stability issues, the inherent conformational twist of DNA origami remained a challenge. The next step in the research was to explore the flattening of these twisted structures using DNA intercalators, which are known to unwind duplex DNA.

3. Effect of intercalators on origami structure and function

DNA intercalator [Ex: ethidium bromide (EtBr)] was used to test for its ability to relax the twisted DNA origami structures. Intercalators bind to nucleic acids by inserting between the base pairs of DNA, unwinding the helix, and reducing the twist. Intercalators like ethidium bromide were found to effectively flatten the DNA origami, allowing for more uniform and planar structures. This flattening is critical for improving the relative orientation and inter-enzyme distance in cascaded enzymatic reactions. Steady-state fluorescence studies provided insights into the binding dynamics of the intercalator with DNA origami. Intercalation enhanced the fluorescence of the molecules, indicating successful binding to the DNA.

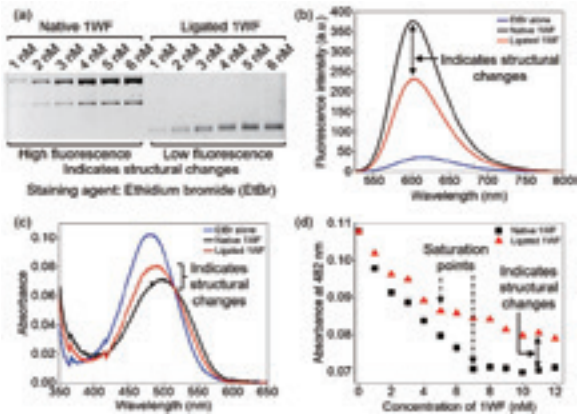

Figure 1. a) Agarose gel electrophoresis of native and ligated 1WF. Staining agent: EtBr. b) Fluorescence spectra of EtBr alone, EtBr with native and ligated 1WF. c) Absorption spectra of EtBr alone, EtBr with native and ligated 1WF. d) Plot of concentration of 1WF vs absorbance at 482 nm (λ_{max} of EtBr alone).

Experiments involved running native and CNBr-ligated 1WF origami at varying concentrations through an agarose gel. As shown in Figure 1a, native samples displayed relatively high fluorescence, whereas ligated samples exhibited markedly weaker fluorescence, indicating that ligation alters base stacking and intercalative binding. Steady-state fluorescence spectroscopy further supported these findings (Figure 1b). While the addition of native 1WF to EtBr significantly increased fluorescence, the same amount of ligated 1WF resulted in notably lower fluorescence enhancement, suggesting ligation-induced changes in the binding pocket. Upon intercalative binding, the absorbance of EtBr decreases with a significant bathochromic shift in the absorption maximum. This phenomenon was used to test the difference in the binding pocket of native and ligated origami (Figure 1c). Native origami showed a greater reduction in absorbance compared to ligated samples. The concentration-dependent absorbance at 482 nm also highlighted significant differences between native and ligated origami (Figure 1d). Similar results were obtained with DMSO-assisted near-quantitative ligation by ligase. While base stacking and intercalative binding were the focus, similar alterations are anticipated for inter-helical distances and groove binding. The study also examined the impact of DNA ligation on the intercalation process. Ligation-induced structural rigidity reduced the available space for intercalators to bind, suggesting that ligation and intercalation affect in a manner that could be harnessed for controlling molecular binding in drug delivery applications.

The time-resolved fluorescence and fluorescence anisotropy measurements will be carried out to get further insight on the binding affinity and structural changes in DNA origami upon intercalation. Additionally, small-angle X-ray scattering (SAXS) will be carried out to obtain direct structural information, so as to confirm that intercalation led to significant flattening of the DNA origami. The next phase of the research will focus on immobilizing enzymes relevant to biomass-related processes on the stabilized, planar DNA origami. By fixing enzymes in close proximity on the origami scaffold, this study aimed to facilitate cascaded enzymatic reactions, which are crucial for converting biomass into valuable products like bioethanol or biobutanol. In addition to enzyme immobilization, DNA origami will be explored as a potential drug delivery system.

4. Conclusions

This research represents a significant advancement in the field of DNA origami, addressing key challenges related to stability, functional diversity, and application in enzyme immobilization and drug delivery. By developing novel ligation and intercalation strategies, the study has successfully created stable, planar DNA origami structures that are ideal for precise enzyme positioning in cascaded enzymatic reactions. These advances have the potential to revolutionize the use of DNA origami in biotechnology, particularly in biomass conversion processes. Additionally, the research highlights the potential of DNA origami as a versatile drug delivery system. The ability to selectively bind and release small molecules opens new avenues for targeted therapies and diagnostic tools. The insights gained from this study contribute to our understanding of how DNA's structural dynamics influence its interactions with small molecules, which is valuable for designing more effective drug delivery systems and biosensors. Future research will focus on further refining ligation techniques, exploring alternative condensing agents for 3D DNA origami, and optimizing drug loading and release dynamics. The findings from this study have broader implications for the development of DNA-based nanostructures for applications in nanomedicine, materials science, and molecular diagnostics.

5. References

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Collaboration Works

Arivazhagan Rajendran, Amrita Vishwa Vidyapeetham （インド）, Metal oxide nanoparticles for biological applications

Arivazhagan Rajendran, Visvesvaraya Technological University（インド）, Stabilization of DNA nanomaterials by enzymatic and chemical methods

Arivazhagan Rajendran, National Institute of Technology, Calicut（インド）, DNA nanomaterials for the analysis of single molecular reactions and processes

Arivazhagan Rajendran, Vanderbilt University School of Medicine（アメリカ）, Topoisomerase 反応の可視化

Arivazhagan Rajendran, Ewha Womans University（大韓民国）, 小分子による酵素機構の解明

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Publications

K. Krishnamurthy, A. Rajendran, E. Nakata, T. Morii, Near quantitative ligation results in resistance of DNA origami against nuclease and cell lysate, *Small Methods*, **2024**, *8*, 2300999.

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Presentations

A. Rajendran, K. Krishnamurthy, E. Nakata, T. Morii, Chemical Ligation of Staple Nicks in DNA Origami, The 104th Annual Meeting of the Chemical Society of Japan, Nihon University College of Science and Technology, Funabashi Campus, Japan, **2024.03.18-21**.

Reports/News Paper Articles

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