Self-Assembly Science Research Section

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1. Introduction

Nucleic acids and their nanostructures, such as scaffolded DNA origami,[1] have been used for organizing enzyme cascades and shown to enhance the efficiency and rate of sequential reactions.[2] Besides some proof-of-concept experiments, the use of DNA origami for templating the biomass-related enzymes is hampered by their unsatisfactory stability to withstand the folded structure under application-specific conditions. For example, the origami structures are only stable below the melting temperature of around 50 °C.[3] Also, the origami materials poorly withstand the mechanical forces and break even under the mild forces applied during the structural analysis by force-based methods such as atomic force microscopy (AFM).[4] Further, it requires a significantly high ionic strength of typically 5-20 mM of $MgCl_2$.[5] Ionic strengths in physiological conditions where enzymes are often handled are much lower than needed to ensure origami stability. The typical Mg^{2+} concentration in cell culture media is 0.04 to 0.8 mM, and that of Na^+ and K^+ are about 150 and 5.5 mM, respectively, making these environments significantly destabilizing toward DNA origami materials.[6] Moreover, the solutions containing enzymes often undergo desalting and buffer exchanging processes and may also be stored in pure water to adapt the optimum conditions suitable for the enzymatic reactions. However, in pure water or low ionic strength buffers, the DNA origami immediately unfolds, and it is not very convenient to store the templated enzymes under these conditions and carry out multienzyme reactions. Biomass often undergoes chemical pretreatments using strong acids or bases to break down the lignin. Also, the biomass product contains several carboxylic acids with a pH of 2 to 2.5. Though the DNA origami materials were shown to be stable up to a pH of 11, the low pH values below 4 were found to denature the DNA origami.[5] Thus, it is necessary to develop methods to stabilize the DNA origami nanomaterials for various applications, particularly for handling the enzymes related to biomass energy conversion.

2. Methods to improve the stability of DNA origami

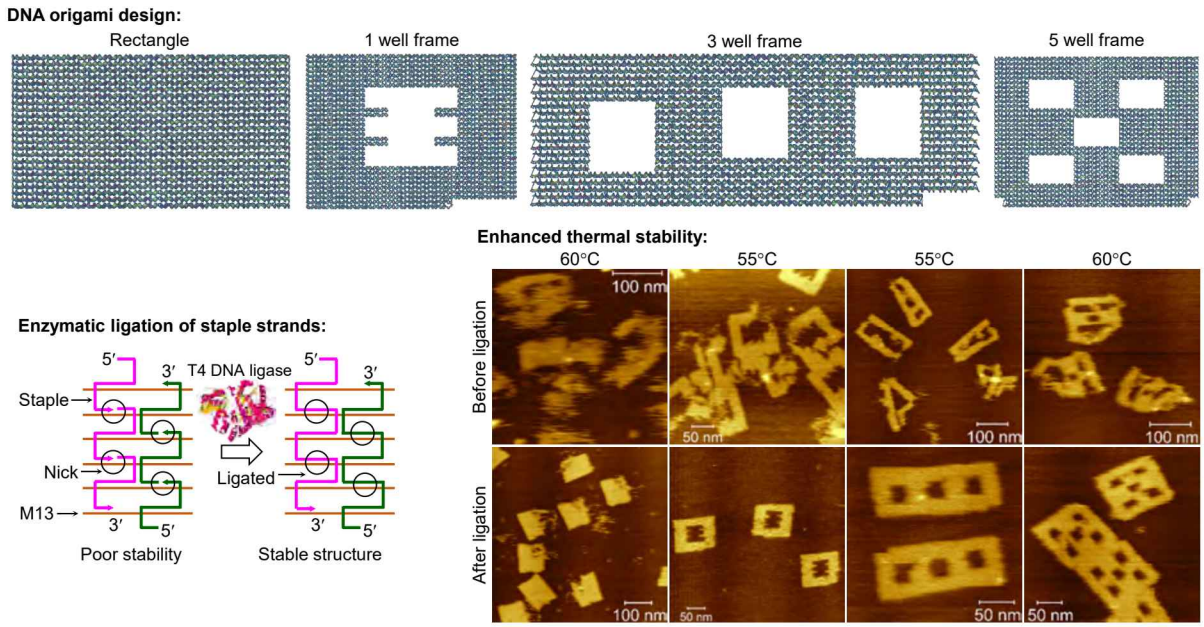

Figure 1. Top: Structures of the 2D DNA origami used in this study. Bottom left: Schematic illustration of enzymatic ligation of the nicks in DNA origami. Bottom right: AFM images evidencing the enhanced thermal stability of origami after enzymatic ligation.

One primary reason for the stability issues of the origami structures is the presence of breaks in the phosphate backbone, the so-called nicks of the staple strands. Increasing the staple length would improve the thermal stability but, at the same time, will lead to practical difficulties such as increased cost of synthesis, decreased product yield and purity, and limitations on the maximum length of synthetic oligo DNAs. We have previously reported a method to stabilize the DNA origami structures by photo-cross-linking of 8-methoxypsoralen.[3] Another method is the UV light-induced cyclobutane pyrimidine dimer formation by placing thymidines in close proximity within the DNA origami.[7] However, these methods are not suitable where native-like DNA is anticipated, as they introduce chemical cross-links to the DNA strands. Also, the irradiation of UV light for 1 to 2 h often leads to DNA damage. The alternative method is the enzymatic ligation of the nicks, which is routinely used in molecular biology. Enzymatic ligation was applied for a few small non-scaffolded and scaffolded DNA structures.[8-12] However, the conditions for enzymatic ligation of DNA origami were not optimized, and no evidence was provided to support the success of the ligation. Thus, to improve the stability of DNA origami, in this study, we aimed to perform the enzymatic ligation, cosolvent-assisted enhancement in enzymatic ligation, and chemical ligation of DNA origami. For this purpose, we have made a collaboration with Prof. Takashi Morii's group at the Biofunctional Chemistry Research Section of the Advanced Energy Utilization Division.

3. Enzymatic ligation of DNA origami nanomaterials

At first, we have carried out a detailed analysis and optimization of the conditions for the enzymatic ligation of the staple strands in four types of 2D square lattice DNA origami, namely rectangle (Rec),[1] 1 (1WF),[13] 3 (3WF),[14] and 5 well-frame (5WF,[15] Figure 1). Our results indicated that the ligation takes overnight, is efficient at 37 °C rather than the usual 16 °C or room temperature, and typically requires a much higher concentration of T4 DNA ligase.[16] Under the optimized conditions, up to 10 staples ligation with a maximum ligation efficiency of 55% was achieved. Also, the ligation is found to increase the thermal stability of the origami as low as 5 °C to as high as 20 °C, depending on the structure (Figure 1). Further, our studies indicated that the ligation of the staple strands influences the globular structure/planarity of the DNA origami, and the origami is more compact when the staples are ligated. The globular structure of the native and ligated origami was also found to be altered dynamically and progressively upon ethidium bromide intercalation in a concentration-dependent manner.[16] Moreover, our results shed light on the structural features and mechanistic insights on the DNA-ligase interaction and accessibility to the nick site in DNA origami.

4. Cosolvent-assisted enhanced enzymatic ligation of DNA origami

Besides our initial demonstration, due to the tightly-packed anti-parallelly oriented arrangement of multiple duplexes in the origami and the difficulties in the accessibility of the nicks by ligase, enzymatic ligation was only partly successful. To further enhance the enzymatic ligation of origami, we have carried out the effect of cosolvent and identified the best performing cosolvent. Our results indicated that the cosolvent enhances enzymatic ligation. Further, we have successfully carried out the chemical ligation of 2D and 3D DNA origami and enhanced the thermal stability of the DNA origami materials.

Overall, our results are useful to understand the optimized conditions for the enzymatic and chemical ligations of DNA origami structures, ligation-induced structural rigidity and compactness, the access of ligase enzyme in a tightly packed environment, and the nature of ethidium bromide binding and its influence on the conformational change in DNA origami materials.

5. References

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Collaboration Works

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

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

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

森井孝，中田栄司，Rajendran Arivazhagan，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

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