# Self-Assembly Science Research Section

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## 1. Introduction

Structural DNA nanotechnology[1] has attracted much attention during the past one and a half decades due to the addition of the scaffolded DNA origami method[2] to the field. This method has enabled the synthesis of DNA nanomaterials with a dimension of ~100 nm in diameter, whereas the initially prepared non-scaffolded structures were ~10 to 20 nm in size. Since the invention of scaffolded origami, various two- (2D) and three-dimensional (3D) DNA materials have been synthesized and self-assembled further to create even larger materials in the dimension of micrometers. Due to their self-assembly nature and addressability, these materials have been used as novel scaffolds for nanopatterning of various nanoparticles and quantum dots, attachment of carbon nanotubes, immobilization of biomolecules such as proteins[3] and viral capsids, carriers of drugs, a platform for the analysis of single molecular reactions and processes,[4] and so on. The specific interest to us is the application of these materials as scaffolds for organizing enzyme cascades related to biomass energy conversion.[3]

Besides the application of origami materials, one major issue with these nano-biomaterials is their unsatisfactory thermal stability, which prevents them from being used in various conditions to withstand thermal, mechanical, and chemical modifications.[5] For instance, the DNA origami structures that use most of the staples of length 32 bases melt below 50 °C. Similar to the non-scaffolded DNA tube,[6] the 3D DNA origami structures such as cuboid may also break open when deposited on mica or scanned by force-based methods such as atomic force microscopy (AFM), and disintegrate in deionized water. The biomass product contains several carboxylic acids with a pH of 2 to 2.5. However, origami materials are stable only between pH 4.5-10 but denature at a lower pH.[7] The reason for the stability issues of the origami materials is the presence of breaks in the phosphate backbone, the so-called nicks, in the staple strands. Increasing the staple length would improve the thermal stability, but at the same time would 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.

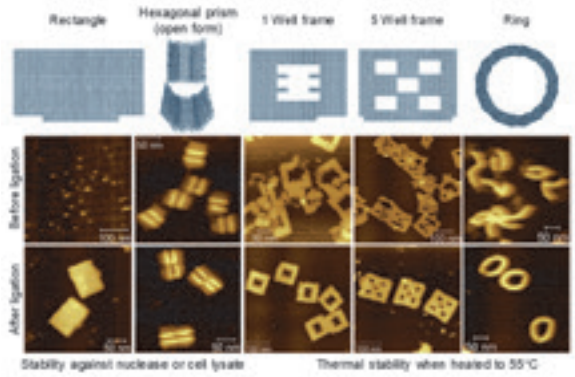
## 2. Currently available methods

There have been few reports on stabilizing DNA origami structures, including photo-cross-linking using 8-methoxypsoralen. Another method is the UV light-induced cyclobutane pyrimidine dimer formation by placing thymidines nearby within DNA origami.[9] However, these methods are unsuitable 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,[10] which is routinely used in molecular biology. Enzymatic ligation was also applied for the tiny DNA nanostructures such as the DNA nanotubes with the size of ~10 nm in diameter that contain only five nicks or only two sticky ends, DNA triangles containing double crossover molecules, four-arm DNA junctions, and the DNA triple crossover complexes. Among these structures, the short DNA nanotube was ligated with an average efficiency of 68 to 77%, while no ligation yield was reported for other structures. Also, enzymatic ligation is not widely applied to scaffolded DNA origami. DNA origami structure is a bundle of tightly packed anti-parallel duplexes. Thus, the enzymatic ligation on that is expected to differ from that of a simple duplex DNA or non-scaffolded DNA nanostructures consisting of relatively few ssDNAs. Crystal structures of ligases suggest that the enzymes completely encircle the nicked DNA to ligate the nick. The tightly packed DNA origami may restrict access to the ligase; thus, optimization of the conditions and well characterization of the ligation is necessary. Further, it is essential to develop methods to stabilize DNA origami nanomaterials for diverse applications, especially when dealing with enzymes involved in biomass energy conversion.

## 3. Cosolvent-assisted enhanced enzymatic ligation

The previous investigations of the effect of organic solvents, such as DMSO, on the ligation indicated that it increased the extent of the ligation reaction, or showed no effect, or increased the specificity while decreasing the efficiency. This motivated us to test the effect of DMSO and other solvents on the

Figure 1. Top panel: DNA origami nanomaterials used in this study. Middle panel: AFM images indicating the




unsatisfactory stability of the origami materials. Bottom panel: AFM images of the ligated origami materials indicate improved stability against thermal treatment, nuclease digestion, and cell lysate.

ligation of DNA origami. The initial ligation experiments were carried out with four different 2D DNA origami structures, namely rectangle (Rec)[2] and frame-shaped origami containing 1 (1WF),[11] 3 (3WF),[12] and 5 wells (5WF, Figure 1, top panel).[13] Several organic solvents were screened to determine the best-performing cosolvent on the enzymatic ligation of origami using 1WF. All the staple stands were purified to eliminate the kinase inhibitors such as ammonium and phosphate ions, 5′-phosphorylated with ~100% yield, and utilized to fold M13mp18. After folding, the excess staples were removed by spin column filtration, and the origami was ligated by T4 DNA ligase in the absence or presence of cosolvent. To our surprise, the ligation proceeded much better and resulted in near-quantitative ligation in the presence of DMSO when compared to ligase alone. Additionally, we confirmed that only DMSO successfully enhanced the enzymatic ligation of origami, and other organic solvents failed to do so. As we anticipated, the ligated origami resulted in much better stability of the origami materials against various treatments (Figure 1, middle and bottom panels).

## 4. Chemical method to stability the DNA origami

CNBr was used to ligate the origami staples chemically. Agarose gel electrophoresis (AGE) was used to characterize the ligation reaction. The chemically ligated origami migrated faster than the native and enzymatically ligated origami. This indicated that the chemical ligation method is more efficient than other methods. Moreover, the chemical ligation reaction was completed within 5 min, highlighting the advantage over enzymatic ligation, which typically requires overnight reaction time for saturation. Reaction conditions were carefully adjusted to optimize the ligation yield. As a result, the highest possible ligation efficiency, exceeding 90%, was achieved under the optimized conditions. This ligation method improves the structural stability up to 30ºC, enhances stability during electrophoresis and subsequent extraction, and provides resistance against nuclease and cell lysate. In addition to its superior efficiency, this method is straightforward and non-tedious, with a cost advantage over other methods, making it an attractive option.[14]

## 5. Conclusions

Our study demonstrates the use of organic solvent on enzymatic ligation and chemical ligation as efficient methods for nick ligation in DNA origami nanomaterials. We could ligate staple nicks in origami with near-quantitative yields by optimizing the reaction conditions. These methods were successfully tested on four different 2D DNA origami structures. The advantages of using CNBr-mediated ligation include a faster reaction time of just 5 min, along with quantitative reaction yields and native phosphate ligation. Additionally, the ligation of origami using these methods enhances their stability against thermal treatments, during electrophoresis and purification, and provides resistance against nuclease and cell lysate. Overall, our findings suggest two highly effective methods for achieving efficient and stable nick ligation in DNA origami, with potential applications in biomass-related enzymes in particular and in nanotechnology, biophysics, and synthetic biology in general.

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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 and processes

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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Rajendran Arivazhagan, Scientific Research (C), Retroviral integration into topologically-interlocked DNAs to probe the role of DNA structure and screen viral inhibitors, FY2021-FY2023

## 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.

## Presentations

A. Rajendran, K. Krishnamurthy, E. Nakata, T. Morii, Development of methods for the efficient ligation of staple nicks in DNA origami, The 103rd Annual Meeting of the Chemical Society of Japan, Tokyo University of Science, Noda Campus, Chiba, Japan, 2023.3.22-25

A. Rajendran, DNA nanomaterials: Design, synthesis and applications, International Webinar on Recent Advances in Material and Chemical Sciences (RAMCS-2023), (Online) Guru Nanak College, Chennai, India, 2023.2.2

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