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Unraveling the Self-Assembly Nature and Properties of DNA Tesseract Designs

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1. Introduction

DNA origami nanostructures, achieved through precise folding of DNA molecules, provide exceptional control over nanoscale architecture.

This research focuses on the development of a DNA origami structure known as "Tesseract", formed through DNA base pairing. The Tesseract consists of a large cube enclosing a smaller cube, demonstrating excellent resistance to nuclease degradation and thermal stability, and thus having potential application in drug delivery.

Verification of successful Tesseract formation was achieved using Cryo-EM and gel electrophoresis. Cryo-EM provided high-resolution visualization, confirming the structure's integrity, while gel electrophoresis supported successful assembly, FRET assays, utilizing Cy3 and Cy5 labels, were employed to investigate Tesseract dynamics. FRET signals demonstrated conformational changes of the structure, offering valuable insights.

2. Figures & Results

Figure 2.1: Four Building Blocks of Tesseract

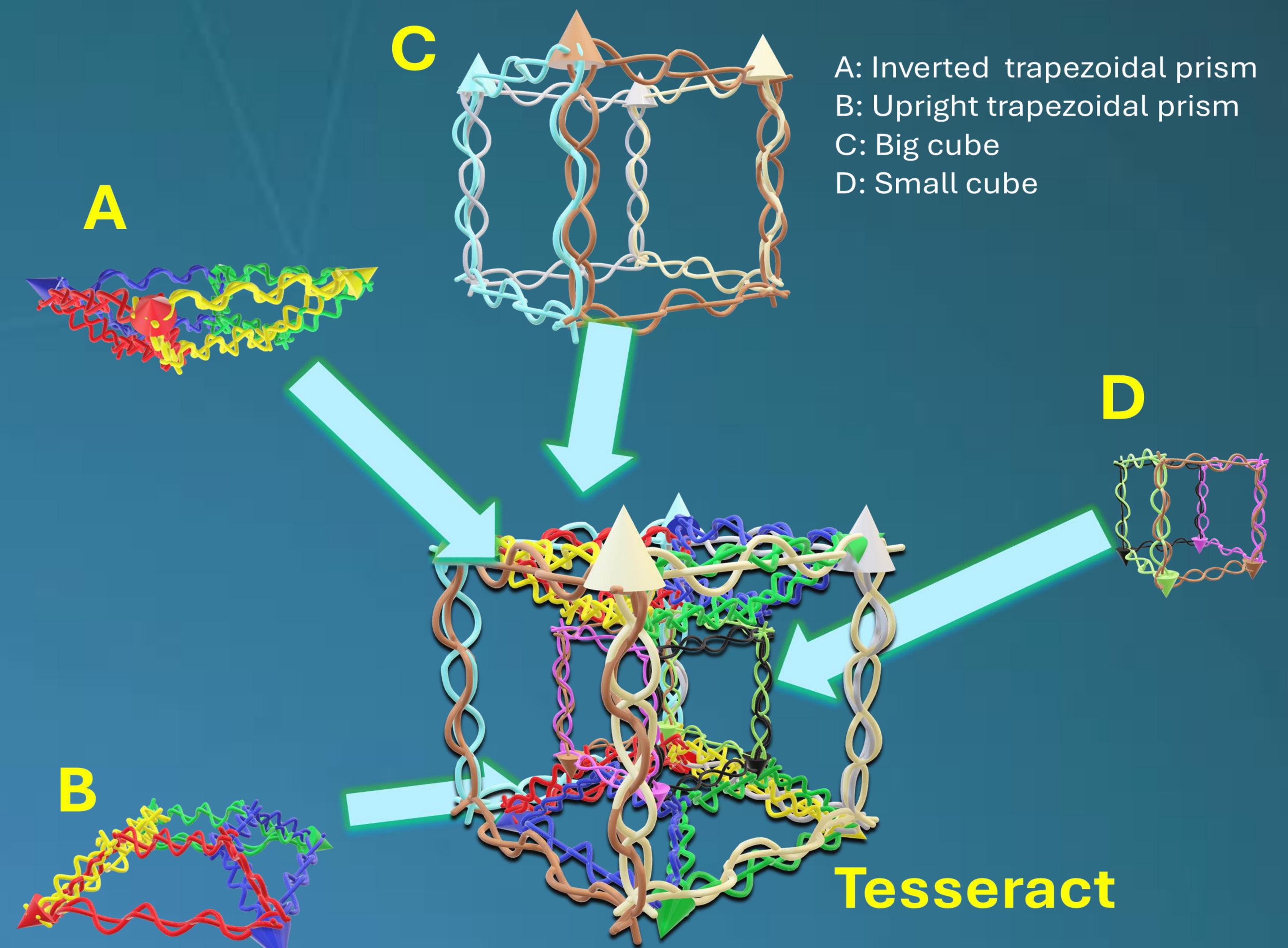


Figure 2.2:

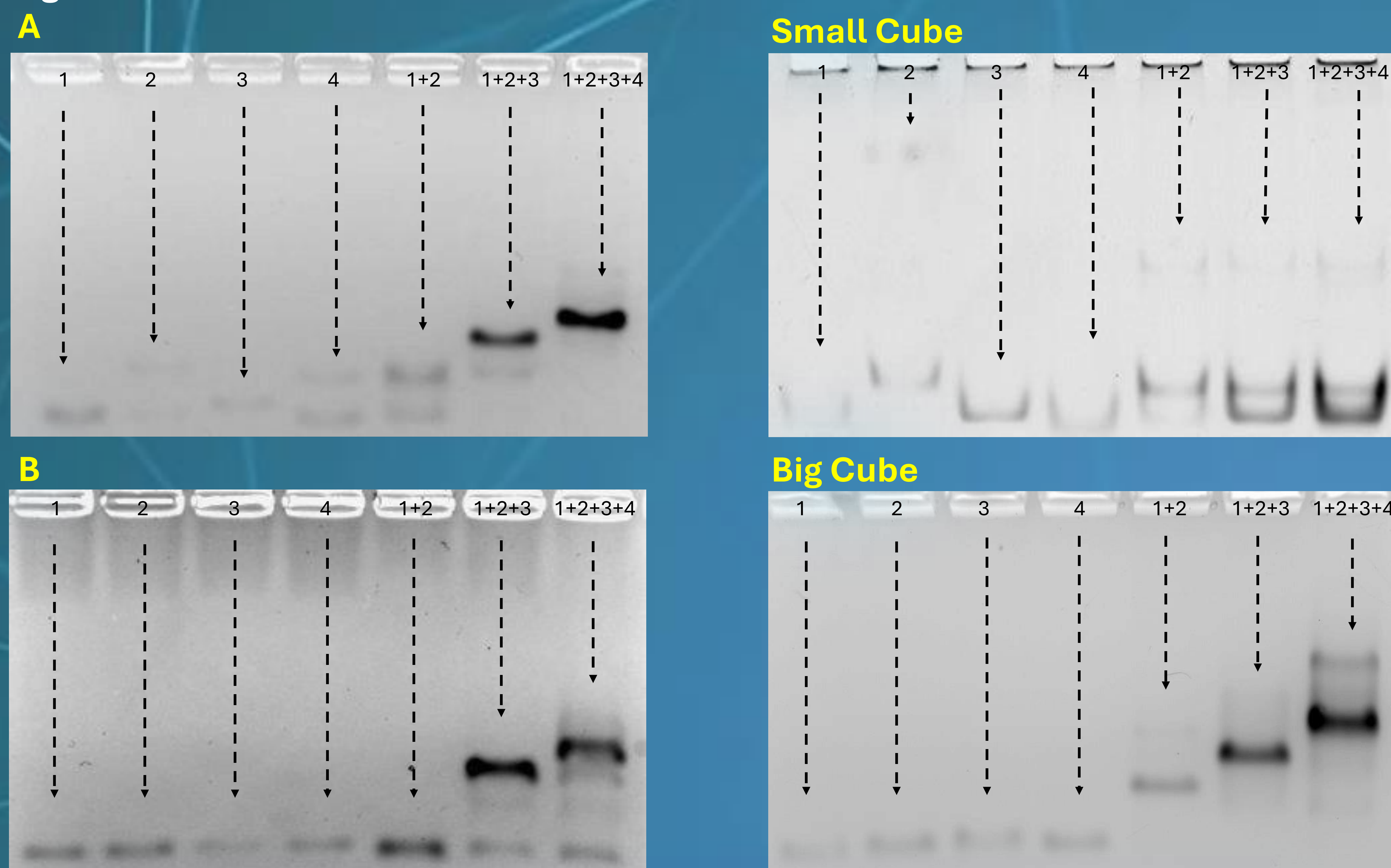


Fig. 2.2 | Gel Electrophoretic Analysis of the Four Constituent Components of the Tesseract
This figure depicts the gel electrophoresis of the four distinct components that constitute the Tesseract structure. The four components are labeled as A, B, Small Cube, and Big Cube. The 1, 2, 3, and 4 labels indicate the DNA strands used to build the respective components, with each component having its own distinct DNA strand, for a total of four DNA strands. A and B are structurally similar, resulting in comparable gel migration patterns. The Small Cube component was run on a PAGE (polyacrylamide gel electrophoresis) due to its smaller size, while the remaining parts were run on a 2.5% agarose gel. Importantly, all components except the Small Cube can be obtained individually.

Figure 2.3:

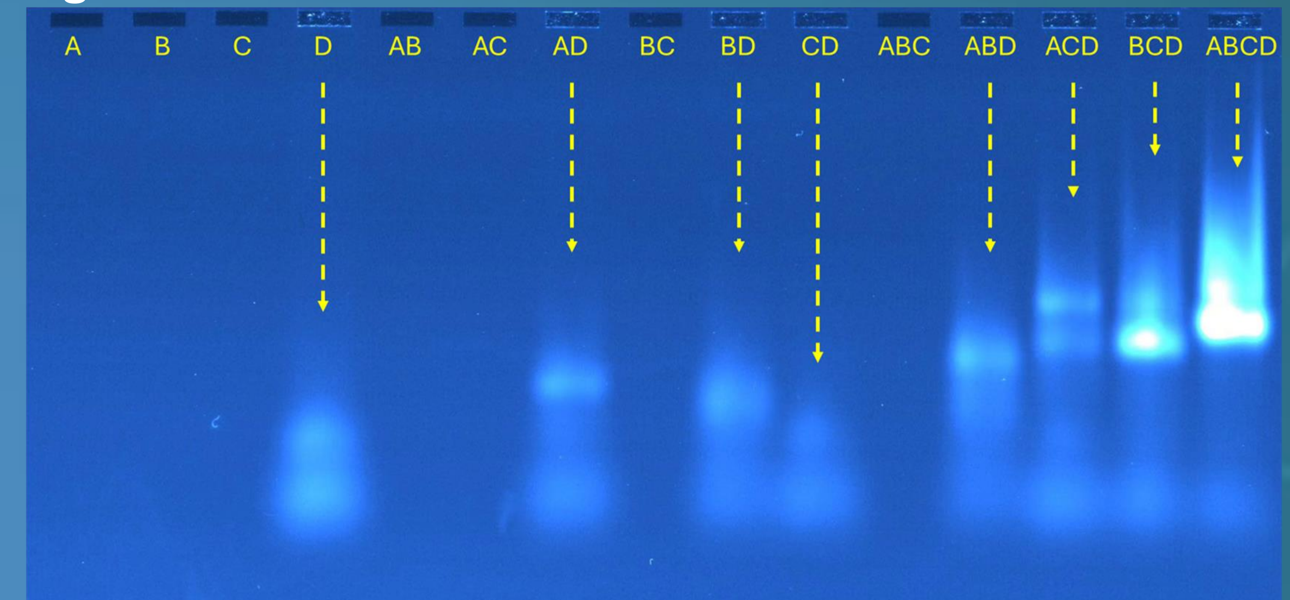
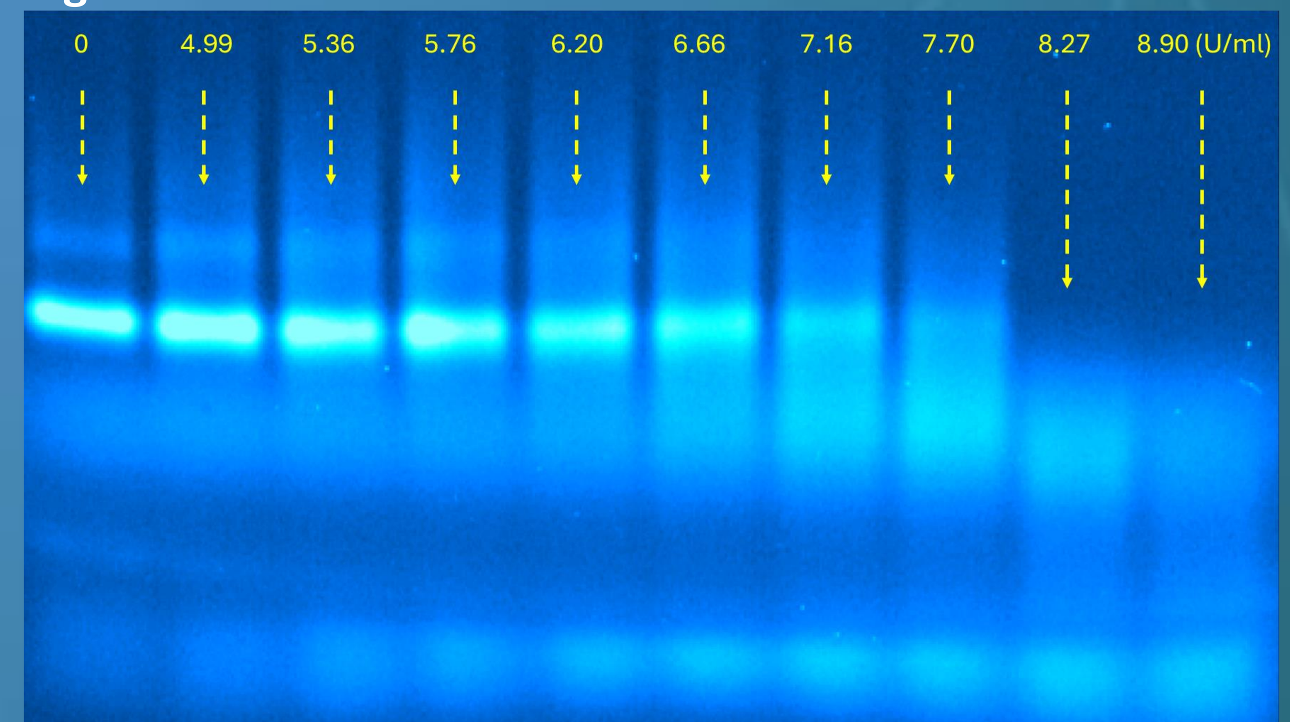


Figure 2.4:



Remark: Cy3 and Cy5 are labeled on the same face of the small cube, activation distance is ~10nm

Figure 2.3 | FRET Signal of 15 Combinations
This figure presents the gel electrophoresis results, which display the FRET signal. The band representing the combination of components 'ABCD' exhibits a significantly higher fluorescence signal. This indicates a strong activation of the Cy3 fluorophore, implying that Cy3 and Cy5 are in close proximity, allowing for efficient FRET detection. Furthermore, the gel also suggests the assembly of a larger-sized structure, potentially a Tesseract, when all the individual parts come together.

Figure 2.4 | FRET Signal of Tesseract
This figure depicts the gel electrophoresis results for 15nM-Tesseract, 1X TAEM buffer under DNase I treatment for 1 hour. A distinctive band disappears when the concentration of DNAase I is between 6.6 and 7.16 U/ml.

Figure 2.5:

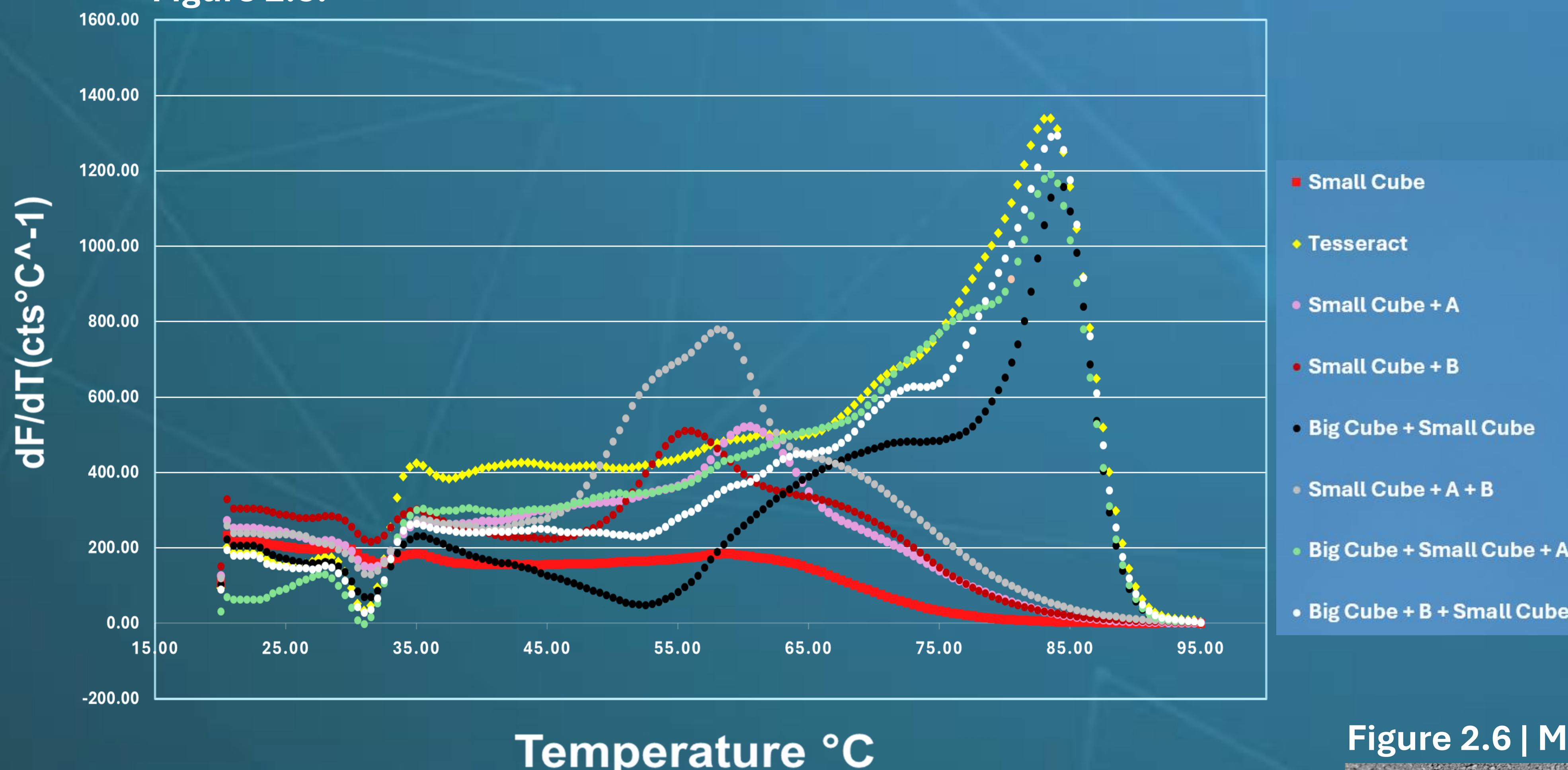


Figure 2.5 | qPCR of Combinations With Small Cube
This figure depicts the derivative of the qPCR results for all possible combinations involving the small cube. Tesseract exhibits the highest signal detection at its maximum point. Additionally, the melting point of the structure with the larger cube is higher compared to the scenario where the larger cube is absent, suggesting that the presence of the larger cube contributes to the increased melting point.

Figure 2.6 | Micrograph (Data Collection)

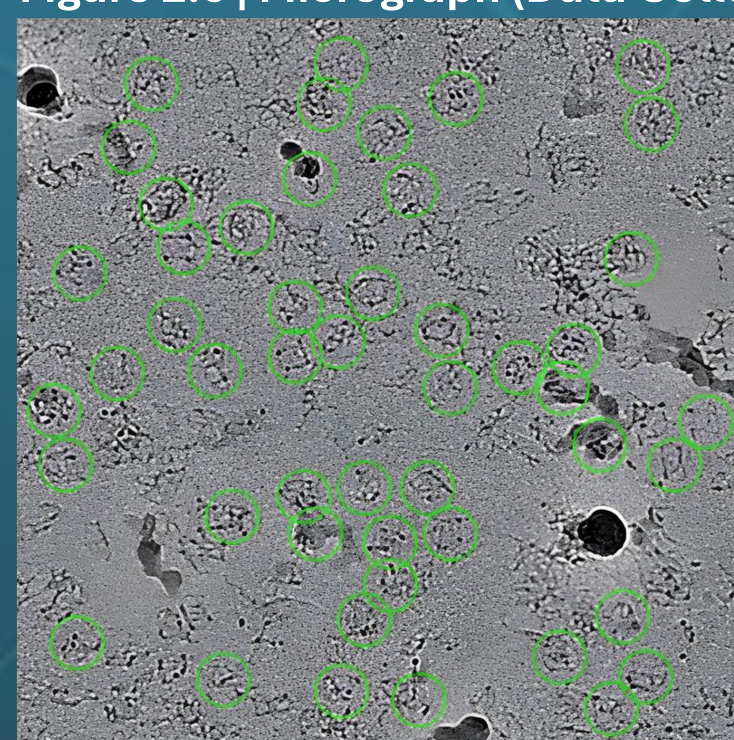


Figure 2.7 | Cryo-EM Reconstruction

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J.A. Tanner, S.C.C. Shiu, Facet-Based Nucleic Acid Platonic, Kepler-Poinsot Polyhedra and Four-Dimensional Tesseract for Therapeutic, Diagnostic and Analytical Applications. US Provisional Patent Application No.63/638,955 filed on 26 April 2024