SYNSENSO

Cell-free synthetic biology for combinatorial biosensor design

Project no. 101072980

Deliverable D1.5
DNA-origami Scaffolds for Higher Translational Efficiency

Version 1

WP 1 – Building Cell-Free Computing Circuits

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Revision history

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Contents

Abbreviations

Abbreviations	Details
AFM	Atomic Force Microscopy
CAD	Computer-aided design

Partner Short Names

Abbreviations	Details
TUDa	Technische Universität Darmstadt
TUE	Eindhoven University of Technology
IIT	Istituto Italiano di Technologia
TVU	Tor Vergata Universita degli Studi di Roma
DBS	Dynamic Biosensors GmbH
UBI	Ulisse Biomed S.p.A.
ICL	Imperial College London
ABV	Abvance Biotech S.L.
ACC	accelopment Schweiz AG
LEB	LenioBio GmbH
BRA	BRAIN Biotech AG
NUC	Nuclera Nucleics Ltd.
BME	Biomerieux SA
UNA	University of Naples Federico II

Executive Summary

This document reports on deliverable D1.5, “DNA-origami scaffolds for higher translational efficiency”. This document outlines the scientific progress in WP1, i.e. design, production and characterization of DNA origami for usage in cell-free gene expression.

This Deliverable is a plan on WP1, Objective 3.

Objectives of the Deliverable

This deliverable describes the design, production and characterization of DNA origami for use in cell-free gene expression. This work was conducted by DC3 and DC4 and is part of WP1 of SYNSENSO.
The design, production and characterization of DNA origami allows to:

- Realize co-localization of two-component phosphorylation cascades.
- Realize co-localization of riboregulators.

Outcomes

We succeeded in preparing DNA origami at the TU/e as evidenced by gel electrophoresis, atomic force microscopy and fluorescence microscopy.

Next steps

In the next few months, we will combine DNA origami with use in cell-free gene expression, specifically to target co-localization of expressed proteins and riboregulators.

1 Production of DNA Origami

In this section, we describe the design and production of DNA origami at the TU/e.

1.1 Design of DNA Origami

DNA origami is a revolutionary approach in nanoscale engineering that leverages the unique self-assembling properties of DNA molecules. This innovative technique, first introduced by Paul Rothemund in 2006 [1], allows scientists to fold and shape DNA strands into precise and predetermined structures, much like origami paper folding.

The design process of DNA origami begins with a long single-stranded DNA scaffold derived from viral DNA, which is typically several thousand nucleotides in length. This scaffold serves as the backbone for the origami structure and is chosen based on its sequence and compatibility with the desired folding pattern. Complementary short DNA strands, known as staple strands, are then designed to bind to specific regions along the scaffold, guiding its folding into the desired shape. Computer-aided design (CAD) software plays a crucial role in this process, enabling simulation and visualization of the 3D structure of the intended origami. The software helps in determining the precise placement of staple strands to ensure the correct folding and formation of the final structure.

The success of DNA origami lies in the predictable nature of DNA base pairing: adenine (A) always pairs with thymine (T), and guanine (G) pairs with cytosine (C). By exploiting these base-pairing rules, researchers can meticulously program the interactions between the scaffold and staple strands, allowing the DNA to fold into intricate and specific shapes, such as triangles, squares, or even more complex nanoscale objects.

Here, a nanorod was folded using the p7560 scaffold, while a rectangle and nanoball were folded from the M13 scaffold. These scaffolds were obtained by bacterial infection (E. coli) with the phage (M13 bacteriophage) followed by single stranded DNA (ssDNA) extraction. The staple strands for the nanorod and rectangle designs were generated by the caDNAno v0.2 software [2] and are based on the structures initially designed by Rothemund [1]. To create the ball shaped origami, the vHelix plug-in for Autodesk Maya was used, as previously reported by Benson [3]. As an example, the rectangle origami design (as presented by caDNAno) is shown in figure 1. The resulting unique staples were ordered via the IDT website.

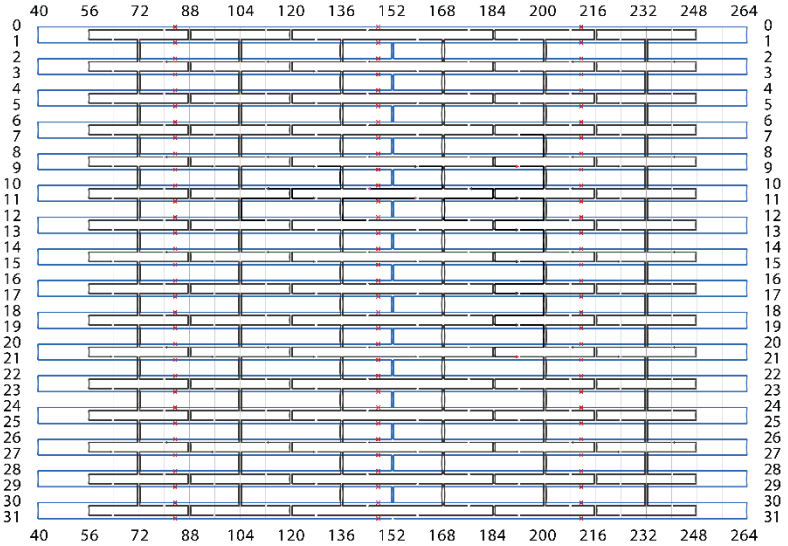

Figure 1: Schematic overview of the DNA origami rectangle in caDNAno. The scaffold strand is shown in blue and the staple strands in black. The arrows indicate the directionality of the DNA (5'-> 3'). Numbers on the top and bottom designate the nucleotide positions, while the numbers on the right and left indicate helix numbers.

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1.2 Self-assembly of DNA Origami

In order to assemble the desired nanostructures, the staples strands were added to the ssDNA scaffold (10x molar excess) in a folding buffer containing NaCl, Tris, EDTA and $MgCl_2$. This mixture was heated to 95°C for 15 min and then slowly cooled to 20°C. During this process, the staples will sequentially anneal to the scaffold, thereby self-assembling the desired structure. After the reaction, the origamis are purified from the excess staples. For the rod shaped origami, purification was performed via polyethyleneglycol (PEG) precipitation whereas for the nanoball and rectangle, spin filtration was used (Amicon 100kDa)[4]. The reconstituted origamis can be used for further characterization.

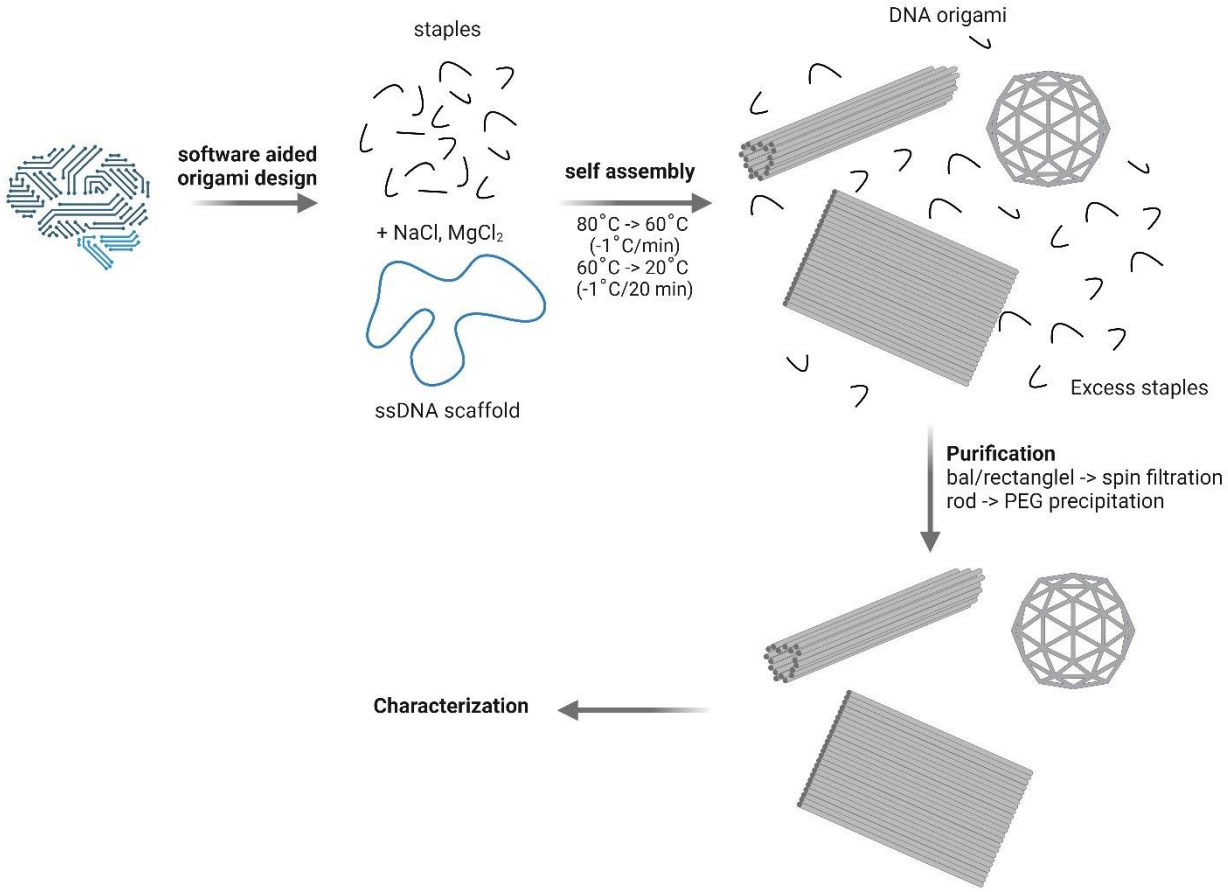

Figure 2: Schematic overview of DNA origami workflow. First, origamis are designed using caDNAno software and the required staples are ordered. In a one-pot thermal annealing reaction (carried out in a thermocycler), the staples hybridize to the scaffold, giving rise to the desired DNA origami. Removal of excess staple strands was achieved by PEG precipitation for the nanorod and rectangle and by spin filtration (amicon filter 100 kDa) for the nanoball.

2 Characterization of DNA Origami

In this section, we describe the characterization of DNA origami at the TU/e, including gel electrophoresis, atomic force microscopy (AFM) and fluorescence microscopy.

2.1 Gel electrophoresis

Gel electrophoresis serves as a rapid and effective quality control step, ensuring the success of the folding process and identifying any issues during assembly.

First of all, gel electrophoresis allows us to verify the size and integrity of DNA origami structures. By running the origami samples through an agarose gel, the different folded origamis can be separated based on their molecular weight or structural properties, providing a visual confirmation of the expected sizes. In addition, gel electrophoresis helps to assess the purity of the final product by revealing the presence of excess staples, incomplete assemblies, contaminants or other by-products.
Figure 3 shows an agarose gel of the rectangle, ball and rod origami before and after purification.
For the ball and rectangle, the M13 scaffold is used as the reference, since those origamis have been reported to run slightly higher compared to their scaffold, thereby confirming successful folding [5]. The rod, on the other hand, is known to run further towards the positive pole compared to its scaffold (p7560) [6].

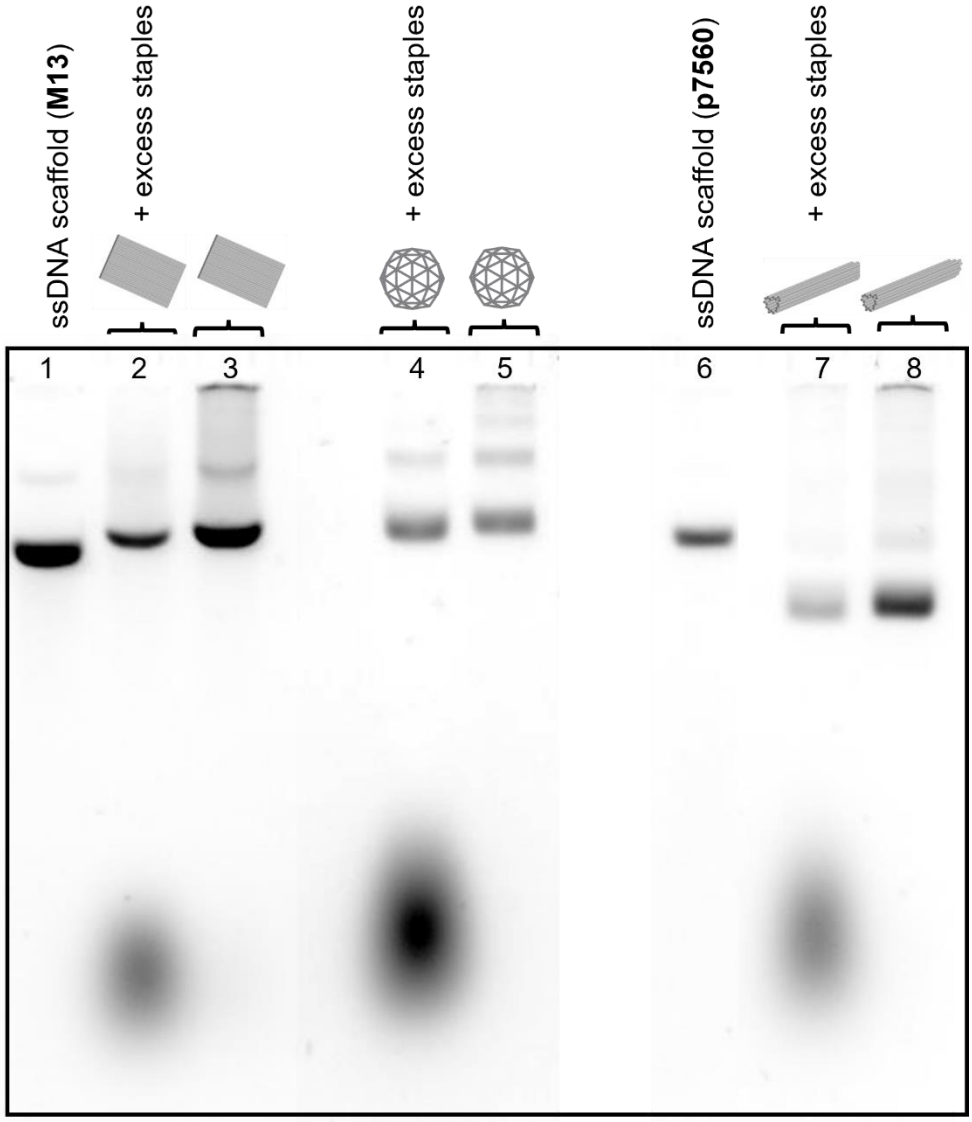

Figure 3: Image of an 1.5% agarose gel stained for DNA with SYBR safe of the rectangle, ball and rod origamis before purification (lane 2,4 and 7) and after purification (lane 3, 5 and 8). As a reference, the origamis are compared to the ssDNA scaffolds (M13, lane 1 and p7650, lane 6).

2.2 Atomic force microscopy

Atomic Force Microscopy (AFM) is an effective tool for characterizing DNA origami structures. Its high-resolution imaging provides detailed visuals at the nanoscale, allowing confirmation of design accuracy and overall quality assessment. AFM's unique ability to perform three-dimensional profiling captures

intricate structural details. Additionally, the technique's versatility in various environmental conditions, including liquid settings, enhances its applicability for studying DNA origami. For the origamis developed in this study, liquid phase AFM was used to characterize the structures. According to the AFM results in figure 4, origamis are correctly self-assembled and uniform.

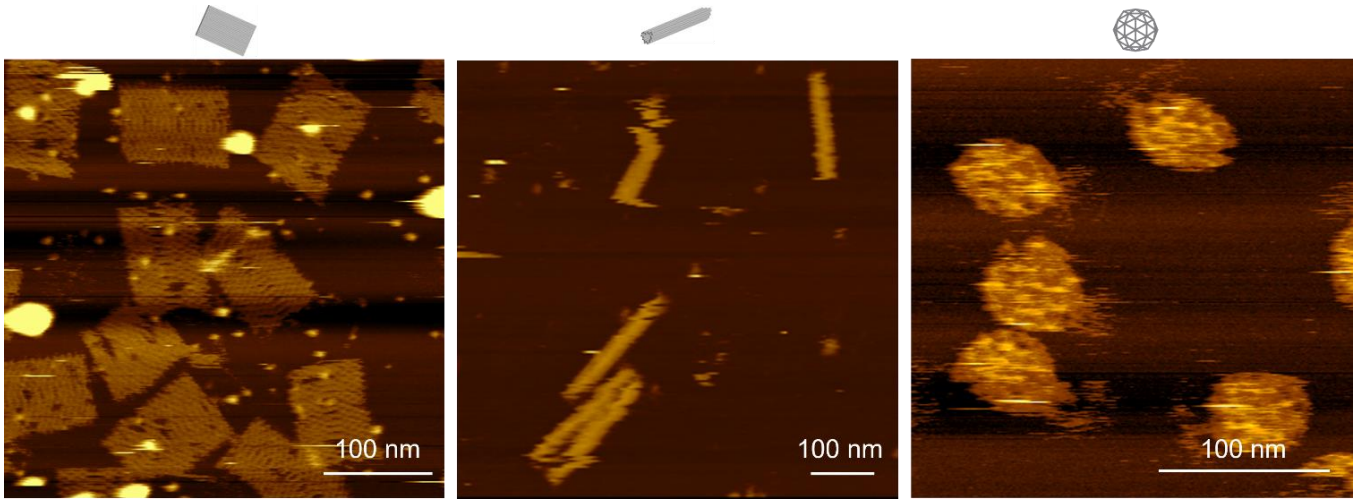

Figure 4. Liquid phase AFM images of rectangles, rods and ball origamis.

2.3 Fluorescence microscopy

By incorporating fluorescent labels into the DNA strands, the origamis can be visualized via fluorescence microscopy, thereby allowing use to study the interactions between the origamis and a particular environment or even their real-time motility. The incorporation of fluorescent dyes onto the origamis was done by modifying specific staples strand with an additional ssDNA tail, referred to as a handle. This handle is complementary to a ssDNA strand containing a fluorescent dye, for instance, Cy5. The ssDNA containing the fluorescent dye can be added directly into the origami folding reaction, generating fluorescently labeled origamis.
Figure 5a shows a confocal image of Cy5 -labeled nanorods that are deposited on a glass coverslip, showing successful origami labeling. In addition, the diffusion of the Cy5-labeled nanorods in water was quantified via single particle tracking (figure 5b).

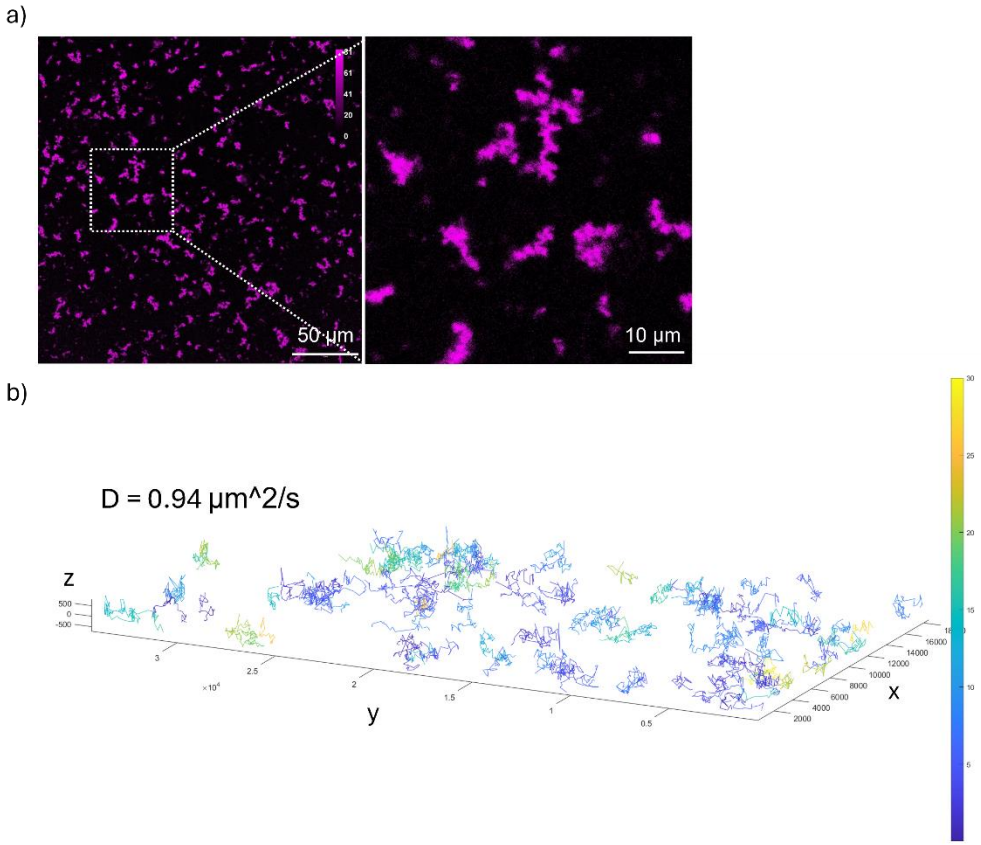

Figure 5. Characterization with fluorescence microscopy. a) Confocal fluorescence microscopy image of Cy-5 labeled nanorods deposited on glass. b) Three dimensional tracks of Cy-5 labeled nanorods obtained via single particle tracking on a multiplane wide field microscope. From the tracks, a diffusion coefficient of 0.94 µm^2/s could be determined for nanorods in water.

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