

CENTER FOR DNA NANOTECHNOLOGY 2016 - HIGHLIGHTS

About the center

Center for DNA Nanotechnology (CDNA) was established in March 2007 by Kjems, Besenbacher and Gothelf in collaboration with the American researchers Yan and LaBean. In 2012 the center was extended to 2017 by which four scientists, Ferapontova, Dong, Birkedal and Andersen at iNANO were included as senior members, and William Shih at Harvard University became associated with CDNA. The purpose of the research at CDNA is to explore fundamental aspects of DNA as a programmable tool for directing the assembly of molecules and materials into nanoarchitectures and functional structures. Since this is the final year of CDNA, and since many of the activities at the center will continue beyond the lifespan of the center, the highlight will focus on new results obtained in 2016 that have not yet been published.

Aptamer-based FRET on RNA origami devices for intracellular sensing

The Andersen lab has in collaboration with the Gothelf Lab demonstrated Förster Resonance Energy Transfer (FRET) between fluorescent aptamers and use RNA origami scaffolds to develop FRET-based conformational sensor devices that offers ratiometric outputs (Fig 1). *In vitro* detection of micro RNAs (miRNAs) was demonstrated as well as to the small molecule S-Adenosyl Methionine (SAM). Notably, the sensitivity against SAM to be an order of magnitude higher than existing riboswitch-based systems. The FRET constructs are expressed in *E. coli* cells where FRET outputs are recorded, and as proof-of-concept, SAM is also detected *in vivo*. It is anticipated that the design of expressible RNA FRET sensors for a diverse set of targets will allow real-time studies of fluxes of metabolic pathways inside cells.

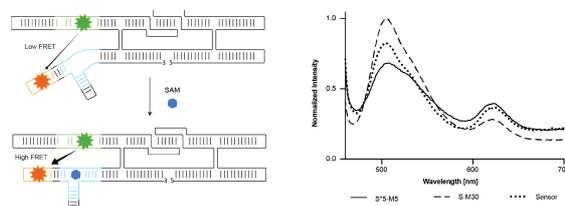


Figure 3. The RNA based aptasensor, where the presence of an analyte such as SAM can be detected through the change of FRET signal.

Multifunctional protein complexes assembled by DNA

Saporin is a ribosome inactivating protein (RIP) that acts by cleaving a single adenine from the large subunit of the ribosome leading to cell death. The relatively low toxicity of saporin compared to other RIPs is due to its poor ability to enter cells. The Gothelf lab has in collaboration with Kjems developed a method to couple the iron transporter protein transferrin (Tf) to saporin (Fig 2). By conjugating DNA strands to the two proteins they are assembled via hybridization to a common template.

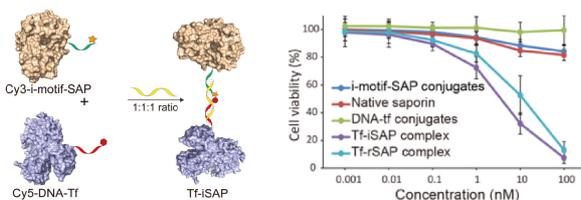


Figure 2 DNA templated assembly of the iron transporter protein transferrin (Tf) and the toxin saporin (SAP), leading to a protein complex with significantly higher cytotoxicity than the individual proteins.

In vitro studies demonstrates that the DNA-linked saporin-transferrin conjugates dramatically enhanced cell uptake and

cytotoxicity. Furthermore, conjugates that are linked by a DNA i-motif are more cytotoxic than conjugates linked by a regular DNA duplex. This study provides an effective strategy for the modular design of immunotoxins and for incorporation of three or more biomolecular components in the complex.

Control of enzyme reactions by a reconfigurable DNA nanovault.

The group of Andersen in collaboration with the Kjems group has introduced a DNA origami device that functions as a nanoscale vault: an enzyme is loaded in an isolated cavity and the access to free substrate molecules is controlled by a multi-lock mechanism (Fig. 2). The DNA vault is characterized for features such as reversible opening/closing, cargo loading and wall porosity, and is shown to control the enzymatic reaction catalysed by an encapsulated protease. Our general approach can be further developed to control a wide range of enzyme-substrate systems with applications in basic research, synthetic biology and medicine.

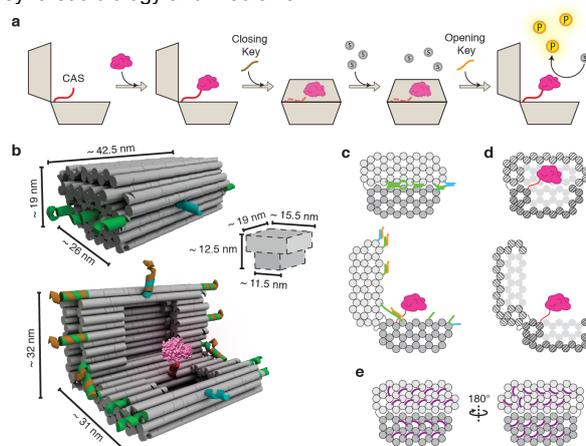


Figure 3 A DNA origami vault a) Schematic illustration of the function, b) design of the vault.

Inserting antibodies in DNA origami structures

A method for covalent and orientation-selective immobilization of antibodies in origami structures was developed. The immobilization was achieved through DNA-templated protein conjugation previously developed in the Gothelf lab. For this purpose 2D and 3D DNA origami structures were constructed. The structures contain a cavity that is designed to host the Fc domain of an antibody. On each side of the cavity are placed two DNA staple strands modified with ligand-metal complexes for binding to histidine clusters on the Fc domain. Subsequent covalent coupling to the antibody was achieved by coupling of surface lysines to reactive esters installed in the cavity of the DNA nanostructure. Atomic force microscopy (AFM) and transmission electron microscopy (TEM) validated efficient antibody immobilization in the origami structures (Fig. 4). The increased ability to control the orientation of antibodies in nanostructures and at surfaces has potential for directing the interactions of antibodies with targets and to provide more regular surface assemblies of antibodies.

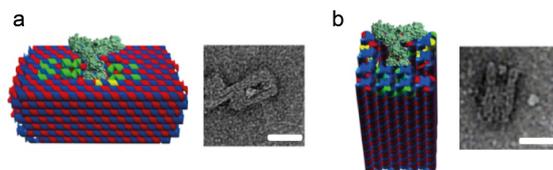


Figure 4 Three dimensional DNA origami structures for covalent immobilization of IgG antibodies. a) Illustration and TEM image of a structure where the cavity is placed orthogonal to the DNA helices, b) Illustration and TEM image of a structure where the cavity is in parallel with the DNA helices. Scale bar = 20 nm.