7th Functional DNA Nanotechnology Workshop

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Book of Abstracts

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Invited Lectures

Invited Lecture / 1

DNA-encoded soft matter with life-like properties: from origami ultrafast assembly with living cells to genetically encoded liquid transport

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Self-assembly is both an advantageously spontaneous process to organize molecular or colloidal entities into functional superstructures and a key-feature of how life builds its components. However, compared to their living counterparts, synthetic materials made by self-assembly may lack some of the key features of living systems such as their capability to grow, adapt, transform, evolve and/or move under rather fixed physiological conditions (temperature, ionic strength). In this talk, I will show how synthetic DNA can be used as a versatile code to program some of these life-like properties, all explored examples being done at strictly constant temperature. First, using DNA as a structural code, I will describe isothermal self- assembly methods where a few to hundreds of DNA strands can spontaneously assemble into userdefined structures, such as DNA origamis or DNA nanotubes, with capability to grow, evolve, dynamically reorganize and even transform at room or physiological temperature [1,2]. Strikingly, under physiological conditions, both 2D and 3D DNA origami self-assembly can be faithfully achieved in a few minutes only and directly in the presence of living cells, including complex structures such as human brain organoids [3]. I will also present a DNA base pairing-orthogonal approach to generate, nearly spontaneously, highly organized yet reversible superstructures, including rings, bundles and vast networks, out of preformed DNA nanotubes [4]. In a second part of this talk, I will show that synthetic DNA can be more than a structural building block and be advantageously used as a versatile designer “genome”to program synthetic soft matter properties, ranging from liquid interface functionalization and cellular recognition [5] to probably the first examples of genetically-encoded surface tension and fluid transport [6].

References:

[1] C. Rossi-Gendron, F. El Fakih, L. Bourdon, K. Nakazawa, J. Finkel, N. Triomphe, L. Chocron, M. Endo, H.
Sugiyama, G. Bellot, M. Morel, S. Rudiuk, D. Baigl. Isothermal self-assembly of multicomponent and evolutive DNA nanostructures. Nat. Nanotechnol. 2023, 18, 1311–1318
[2] Nanotubes Growth by Self-Assembly of DNA Strands at Room Temperature
L. Bourdon, S. P. Afrose, S. Agarwal, D. Das, R. Singh, A. Di Cicco, D. Lévy, A. Yamada, D. Baigl, E. Franco
ACS Nano 2025, 19, 18203
[3] L. Bourdon, G. D. Wilkens, S. Dehissi, S. Hotte, S. Rudiuk, M. Morel, A. Yamada, G. Bellot, D. Baigl.
Minutes-timescale in situ assembly of DNA origamis with living cells. Under review.
(available on ChemRxiv: https://doi.org/10.26434/chemrxiv-2025-mh3k7)
[4] L. Bourdon, X. Z. Xu, L. J. Michot, M. Morel, S. Rudiuk, A. Yamada, D. Baigl. DNA condensation-inspired assembly of DNA nanotubes into reversible superstructures: a base pairing-orthogonal way to create rings, bundles or vast networks. J. Am. Chem. Soc. 2025, in press
(available on ChemRxiv: https://doi.org/10.26434/chemrxiv-2024-3bggv-v2)
[5] C. Courrégelongue, S. Saha, Y. Abboud, S. Rudiuk, A. Yamada, M. Morel, C. Wunder, D. Baigl.
Manuscript in preparation
[6] S. Saha, C. Courrégelongue, A. Yamada, S. Rudiuk, M. Morel, C. Monteux, D. Baigl.
Manuscript in preparation

Invited Lecture / 2

Input-responsive CRISPR Systems Controlled by DNA Molecular Transducers for Sensing Applications

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CRISPR-Cas systems hold promise for molecular diagnostics,[1] yet achieving precise non nucleic acid-responsive control of their enzymatic activity remains challenging.[2] Dynamic DNA nanotechnology offers unique opportunities to address this challenge by programming input responsiveness directly into nucleic acid structures.

In this talk, I will present our work on DNA-based molecular transducers that provide external control over CRISPR-Cas12a activity in response to antibody and enzymes. We first designed antibody- responsive DNA toehold switches [3] that regulate PAM accessibility to Cas12a. These conformational transducers enable Cas12a activation only upon strand displacement triggered by specific antibodies, thereby coupling recognition events to robust signal amplification through collateral cleavage. This system achieves rapid, one-pot detection of IgG antibodies and small molecules with high sensitivity and specificity, even in complex samples.

Then, we report on synthetic transducers that can directly translate DNA repair enzyme activity into a CRISPR-readable output.[4] By converting base excision repair events into programmable DNA triggers, we link glycosylase activity (e.g., UDG, hOGG1) to Cas12a-mediated signal generation. This one-step assay operates in cell lysates, providing sensitive readouts of enzymatic activity and enabling throughput inhibitor screening.

Together, these strategies show how DNA molecular transducers can endow CRISPR systems with programmable input responsiveness, opening new avenues for molecular diagnostics, synthetic biology, and dynamic gene circuit design.

1. M.M. Kaminski, O.O. Abudayyeh, J.S. Gootenberg, F. Zhang, J.J. Collins. Nature Biomedical Engineering, 2021, 5, 643-656.
2. S. Del Giovane, N. Bagheri, A.C. Di Pede, A. Chamorro, S. Ranallo, D. Migliorelli, L. Burr, S. Paoletti, H. Altug, A. Porchetta TrAC Trends in Analytical Chemistry, 2024, 117594.
3. N. Bagheri, A. Chamorro, A. Idili, A. Porchetta Angewandte Chemie International Edition, 2024, 22, 63, e202319677.
4. N. Bagheri, A. Bertucci, R. Merlo, A. Porchetta bioRxiv, 2025, doi: https://doi.org/10.1101/2025.07.11.664310

Invited Lecture / 3

Modular sensing and programmable signal transduction with DNA nanotechnology

Author: Rebecca Schulman[1]

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Molecular biosensing—the ability to detect and quantify analytes and use this information to guide downstream behavior—remains a central challenge in bioengineering and nanotechnology. Existing methods depend on bulky instruments and manual intervention, whereas biological cells achieve sensitive, multiplexed sensing and autonomous response in sub-nanoscale volumes.

I will describe how DNA nanotechnology can enable similar capabilities. Using aptamer-regulated transcription (ART), binding events are transduced into catalytic activity, providing a modular and programmable mechanism for sensing. ART outputs can be linked to circuits that filter noise, tune dynamic range, or amplify weak signals, creating robust sensing modules that operate independently of context. This approach could make it possible to build modular, programmable, multiplexed sensors with uses ranging from diagnostics and biomanufacturing to autonomous devices such as feedback-controlled drug delivery systems and self-monitoring DNA assemblies.

Invited Lecture / 4

DNA-based active plasmonics

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DNA has emerged as one of the most versatile materials for bottom-up fabrication of plasmonic systems. Furthermore, incorporating various stimuli responses in DNA-based metal nanostructures is often more straightforward compared to structures created through top-down fabrication methods. In this talk I will present our recent advances in utilizing DNA for fabrication of plasmonic switches and dynamic plasmonic surfaces, with a particular emphasis on electrically driven systems.

Invited Lecture / 5

Programming stochastic self-assembly: what can be designed and how do we do it?

Author: Carl Goodrich[1]

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DNA provides unprecedented capacity for designing, synthesizing, and controlling microscopic interactions between self-assembling particles. However, our ability to take full-advantage of this vast design space to assemble nanomaterials with complex structure and function is hindered by the lack of inverse-design frameworks that connect the particle-level design attributes to the system-level assembly outcomes, like the yield of a user-specified target structure. I will show that stochastic binding rules –where particles can bind in multiple ways – present a powerful and scalable regime for complex assembly despite the presence of numerous competing structures. This regime is governed by an innate mathematical logic that robustly predicts which structures can be assembled at high yield and provides a tool for navigating the design space composed of binding energies and particle concentrations. Furthermore, this mathematical logic leads to nontrivial predictions that we verify experimentally using self-assembling DNA origami particles. I will also discuss assembly kinetics, and show how to incorporate equilibration times into the design process.

Invited Lecture / 6

DNA origami: nanomechanics and super-assemblies

Author: Friedrich Simmel

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The presentation will cover two distinct topics. The first part introduces a bistable nanomechanical switch constructed from DNA origami that can be actuated between its two conformational states using electric fields. Remarkably, some of these switches exhibit exceptional mechanical endurance, remaining fully functional over more than 200,000 switching cycles.
The second part focuses on the assembly of membrane-like superstructures from DNA origami barrels that emulate key aspects of lipid interactions. Using this approach, large compartments can be realized that reach the size of bacterial cells and, when templated on giant unilamellar vesicles, even attain dimensions comparable to eukaryotic cells.

Oral contributions

Oral contributions / 1

Brownian DNA computing

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DNA-based sensors have come a long way and are now capable of detecting ions, molecules, proteins, and nucleic acids, generating an output response. However, processing different inputs typically requires fuel for strand displacement reactions or purification steps, which makes DNA computing relatively slow.

In this talk, a novel approach to Brownian DNA computing is presented. It utilizes molecular balances on DNA origami structures as basic computational units that operate at the Brownian limit and does not bear on strand displacement reactions. Through Brownian motion, the system explores all possible states within the circuit. Computation is driven by input DNA strands that reduce the number of possible states.

By coupling multiple molecular balances, it becomes possible to construct circuits of greater complexity capable of performing all Boolean logic operations. These computations are read out using single-molecule fluorescence spectroscopy.

Beyond traditional DNA strand inputs, the molecular processing unit (MPU) can also respond to antibodies and protein–aptamer inputs. Additionally, the DNA-based system supports cooperative input design, enhancing sensitivity to small concentration changes—surpassing the typical linear input–response behavior.

The readout is not limited to binary true-or-false states but can also be designed as a decoder. Overall, Brownian DNA computing has the potential to serve as a foundational computational unit for molecular computing, sensing, and soft robotics.

Oral contributions / 2

Molecular encryption for DNA data storage

Authors Bas Bögels[1]; **Robin Vermathen[2]**

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Inspired by its natural function as an information carrying molecule and motivated by its promising properties such as high stability [1] and high information density [2], DNA has emerged as an alternative medium for the long-term storage of digital information. As with any data storage medium, protection of the encoded information is desired for DNA data storage. Previous work has focused on hiding DNA in objects [3], drowning out the signal of a message with a high background signal [4], or permanently deleting all data upon heating [5]. However, these methods either greatly decrease the information density of DNA or lead to loss of the stored data. Hence, we present a method of physical encryption in the form of a molecular locker. The locker consists of a DNA strand that is complementary to part of the data encoding strands of the file and contains a 3'-inverted T base. Due to sequence overlap, the locker competes with primers during an attempted amplification of the DNA file, greatly reducing the presence of the locked strands in the amplification product. This artificially creates drop-out when attempting to read the file using next-generation sequencing, preventing the data from being decoded. The locker can be removed by adding a password strand that is complementary to the locker. This allows for the repeated locking and unlocking of a DNA file without the loss of data.

References:
[1] Organick et al. 2021, An Empirical Comparison of Preservation Methods for Synthetic DNA Data Storage. Small Methods. 5.
[2] Organick et al. 2020. Probing the physical limits of reliable DNA data retrieval. Nature Communications. 11.
[3] Koch et al. 2020. A DNA-of-things storage architecture to create materials with embedded memory. Nature Biotechnology. 38:39-43.
[4] Clelland et al. 1999. Hiding messages in DNA microdots. Nature. 399:533-534.
[5] Kim et al. 2020. Metastable hybridization-based DNA information storage to allow rapid and permanent erasure. Nature Communications. 11.

Oral contributions / 3

Detection and quantification of counterion-mediated homologous recognition in double-stranded DNA

Author Andrew Stannard[1]

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In homologous recombination, DNA segments are exchanged between chromosomes, a process critical to establishing genetic variation and healing DNA damage. To avoid detrimental effects, cells must ensure that homologous, not heterologous, segments are exchanged. Prior to exchange, however, pairing must occur; in the chaos of the cell nucleus, how do homologous segments find each other?[1]. The potential for homologous pairing to occur in protein-free environments has been previously demonstrated[2,3]; here, by measuring dsDNA pairing in synthetic constructs, we test the hypothesis that homologous pairing is electrostatically-driven[4,5] and elucidate the physiological factors that facilitate homologous recognition.

Since the electrostatic-pairing theory predicts homologous recognition to be weak, here we tether duplexes together to entropically-bias their pairwise interactions, creating so-called DNA 'scissors'; FRET markers integrated into these constructs report on duplex-duplex separation. The physiologically- relevant, divalent cations of magnesium and calcium are known to specifically adsorb on anionic dsDNA, the resulting charge compensation reduces dsDNA-dsDNA electrostatic repulsion. Accordingly, for our DNA scissors, coaligned duplexes are observed for sufficient concentrations of these cations, an effect that disappears when duplex tethering is absent. By varying the nucleotide sequences of the duplexes-of-interest, the magnitude of divalent-cation-induced interactions between homologous and heterologous duplexes can be compared and the effect of electrostatically-driven homologous recognition can be quantified.

1 Barzel & Kupiec, *Nat. Rev. Genet.* **9**, 27 (2008)
2 Baldwin et al., *J. Phys. Chem. B* **112**, 1060 (2008)
3 Danilowicz et al., *PNAS* **106**, 19824 (2009)
4 Kornyshev & Leikin, *Phys. Rev. Lett.* **16**, 3666 (2001)
5 Haimov, Hedley, & Kornyshev, *J. Phys.: Condens. Matter* **36**, 40LT01 (2024)

Oral contributions / 4

Structure-switching biosensors: a bio-inspired strategy to accelerate sensor kinetics

Authors Alexis Vallée-Bélisle[1]; Carl Prévost-Tremblay[1]; **Dominic Lauzon[1]**

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Continuous Monitoring Biosensors (CMB) are at the forefront of the digital healthcare transformation, offering real-time measurement and novel metrics to improve disease management. While continuous glucose monitoring has set the gold standard, the development of CMB for other clinically relevant biomarkers remains challenging due to the absence of alternative enzymatic catalysts. To develop CMB, researchers have turned their attention towards other methods, with affinity-based structure-switching biosensing showing high potential. For example, aptamer-based biosensor has shown great results to monitor biomarkers in the nM to µM range. However, many clinically relevant biomarkers are found in the pM range and thus require stronger binders (e.g., antibody) to reach such low limit of detection. Stronger binders also rime with slower dissociation kinetics that would impact sensor regeneration and introduce lag time in the sensor response. Therefore, novel sensing architecture are required to improve sensor kinetics without affecting affinity. To address this specific challenge, we have investigated how Nature process chemical information using structure- switching receptors. Two mechanisms were identified: induced-fit and conformational selection. Each mechanism allows to activate or deactivate the switch, but proceed via two different pathways, enabling the programming of the switch kinetics (see graphic abstract). I will present how we have recreated these mechanisms using a DNA-based model, with an emphasis on their design principle, their programmability and their kinetic differences. I will finish my presentation with my future postdoc project (MSCA Postdoctoral Fellowships at TU/e, Netherlands) where we aim to implement these strategies into immunosensors and protein-based switches. Overall, this project shifts our focus from the thermodynamic to the kinetic optimization of structure-switching biosensors. It establishes a novel framework for CMB development, providing a scalable and adaptable strategy for *in vivo* continuous biomarker monitoring.

Oral contributions / 5

DNA Origami Enables Sub-Picomolar Sensitivity of Rapid Tests

Author Heini Ijäs[1]

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Lateral flow assays (LFAs) are paper-based rapid test devices with a wide range of applications enabled by their high speed, affordability, and ease of use. Most conventional LFAs operate on a sandwich immunoassay format, using antibodies to capture target analytes. A visual signal that indicates a positive test result is generated by nanoparticles, typically colloidal gold, conjugated to detection antibodies. In point-of-care diagnostics, LFAs are employed for *e.g.* detecting infectious diseases and monitoring health conditions, but they still lack the sensitivity required for many critical applications.

We demonstrate that DNA origami nanostructures can be integrated into standard LFA formats to tackle this sensitivity limitation. DNA origami enables the spatial organization of external molecules –such as proteins, fluorophores, and nanoparticles –on a single nanostructure with Ångström-level precision. We employ DNA origami nanostructures as molecular adaptors between the detection antibodies and labels in LFAs and demonstrate drastically improved detection sensitivity for a broad range of analytes. In particular, we highlight enhanced performance in detecting cardiac troponin I –a key biomarker for heart attacks –and viral proteins relevant to respiratory infections. Using spiked buffer and serum samples, we detect sub-picomolar concentrations of analytes. Further, we show superior performance compared to commercial LFAs in clinical settings.

With the demonstrated sensitivity enhancement and with material costs increased by less than one cent per test, DNA origami represents a practical and scalable strategy for upgrading existing LFAs, and a crucial step toward the integration of DNA nanotechnology into commercial diagnostic devices.

Oral contributions / 6

Development of a DNA origami nanosensing platform for diagnostic analysis of ADAMTS-13 proteolytic activity

Authors Gereon Brüggenthies[2]; Jasmin Arnold[1]

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Current advances in DNA nanotechnology have enabled the design and synthesis of complex and functional nanostructures, including artificial DNA motors, DNA crystals, and DNA nanopores[1]. A notable development is a DNA origami-based biosensor in which the biorecognition and signal transduction elements are completely decoupled[2]. This makes it a valuable tool for detecting single molecules of nucleic acids, single-nucleotide polymorphisms, as well as various protein targets. Proteases are an important class of biomolecules that require improved strategies for activity detection. In this work, we focus on a clinically relevant protease, A Disintegrin and Metalloprotease with ThromboSpondin repeats 13 (ADAMTS-13), which plays a key role in coagulation disorders[3]. We present a DNA origami-based biosensor capable of directly reporting ADAMTS-13 activity in blood samples. This approach enables rapid, near-patient analysis, offering a simple, cost-effective, and easy-to-use point-of-care testing (POCT) solution. Such a tool could significantly aid clinical decision- making in emergency settings by facilitating timely differentiation between coagulation-related conditions.

References

1 Pengfei Zhan et al. 2023. Recent Advances in DNA Origami-Engineered Nanomaterials and Applications. Chemical Reviews. 123:3976-4050
2 Lennart Grabenhorst et al. 2025. Engineering modular and tunable single-molecule sensors by decoupling sensing from signal output. Nat. Nanotechnol. 20, 303–310
3 HM Tsai. 1996 Physiologic cleavage of von Willebrand factor by a plasma protease is dependent on its conformation and requires calcium ion. Blood. 87 (10): 4235–4244

Oral contributions / 7

Towards sequence-defined polymers with DNA-based catalytic templating

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Autonomous synthesis of sequence-defined polymers from a pool of monomers is considered to be one of the most important and difficult challenges in modern Chemistry and Synthetic Biology. In contrast, biological systems execute this task with astonishing efficiency while synthesizing nucleic acids and proteins from the available pools of NTPs or aminoacyl-tRNAs. Such accurate biopolymer syntheses are directed by information-bearing templates. In this work, we discuss the design of a DNA strand displacement network in which a catalytic DNA template assembles non-covalent DNA assemblies of length longer than two without the use of biological tools or external physical stimuli.

Forming a longer polymer via any template-assisted method has its own challenges. Firstly, a product consisting of multiple building blocks will have multiple recognition sites to stick to the template. This makes the autonomous detachment of template from the product harder due to increased cooperativity. Second, generating a product more than two units long always has a chance of producing smaller or truncated copies instead of the correct product. Third, the template has to be specific to be able to form the product with correct sequence of the building blocks.

Here we demonstrate the catalytic formation of products of length three to five units using single-stranded DNA templates under non-enzymatic isothermal conditions. We build this system by combining toehold- and handhold-mediated strand displacement reactions that was recently developed in our group along with the insights from a coarse-grained model of the templating mechanism. First we show that we can make trimeric products using DNA templates that works catalytically with high specificity. We noticed very low production of truncated products in this process. We followed the elementary steps of the network by fluorescence measurements, and validated the product formation by polyacrylamide gel electrophoresis (PAGE) of the aliquots from the reaction mixtures alongside pre-annealed controls. Then we further refined our design to produce tetramers which also showed turnover of the template. We finally extended the system to make pentameric assemblies. Our latest results shows definite signs of pentamer formation in presence of the template.

Oral contributions / 8

DNA tendrils for programmable membrane fusion in artificial cell design

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Membrane fusion is a ubiquitous phenomenon involved in a myriad of biological processes. It represents a crucial step in viral infection, liposome-based drug delivery, and the construction of fully biomimetic synthetic cells. While fusion between cellular membranes is tightly regulated by specialized proteins, reconstituting and controlling the behaviour of these proteins in synthetic systems remains a significant challenge. To address this, DNA-based nanodevices that mimic the activity of fusogenic proteins have been proposed.[1,2]

Here, we introduce minimal DNA structures –dubbed tendrils[3] –which can be easily modified to trigger fusion in response to external stimuli and to modulate fusion kinetics. Importantly, our design effectively decouples the more expensive, fusogenic cholesterol-modified strands from the control circuitry, enabling a plug-and-play strategy for designing DNA fusogens. Leveraging this modular design, we demonstrate control over membrane fusion kinetics (ranging from minutes to hours), logic-gated fusion responses (e.g., NOT, AND, and OR gates), and temperature-triggered fusion.

To demonstrate the applicability of our system in synthetic cell engineering, we encapsulated small unilamellar vesicles (SUVs) within micron-sized giant unilamellar vesicles (GUVs) and decorated both membranes with temperature-sensitive tendrils. Upon heating, fusion exposed the lipids from the encapsulated SUVs to the external solution, effectively mimicking the biological process of antigen presentation.

Altogether, our results highlight the potential of fusogenic DNA nanodevices as programmable tools for reshaping lipid assemblies and directing the behavior of compartmentalized systems, a key requirement in the development of advanced drug delivery systems and biomimetic synthetic cells. The modularity of DNA tendrils will facilitate the integration of other sensing moieties (e.g. aptamers), paving the way for the development of synthetic membrane-trafficking pathways.

1 Peruzzi et al. Angew. Chemie 2019
2 Stengel at al. J. Am. Chem. Soc. 2007
3 Paez-Perez et al. Soft Matter 2022

Oral contributions / 9

Do DNA Barcode networks benefit from promiscuity

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In sequencing-based microscopy, spatial information is encoded through proximity networks, where edges represent molecular interactions. While intuitively, increased connectivity, termed here as network promiscuity, might be expected to degrade spatial reconstructions due to the presence of false edges, this study systematically examines whether promiscuity can, conversely, enhance reconstruction accuracy. By using synthetic random geometric graphs (RGGs) and experimental DNA microscopy datasets, we demonstrate that moderate network promiscuity substantially improves spatial reconstruction quality, even in the presence of noise. Our findings show that adding edges, contrary to conventional expectations, can increase true spatial signals sufficiently to offset the negative impact of false edges.

However, this benefit exists only up to critical connectivity thresholds (edge densities around 0.75 to 0.8), beyond which accuracy sharply declines due to excessive edge density limiting spatial resolution. Comparative analysis of reconstruction algorithms reveals distinct performance regimes: random-walk-based methods (STRND) do well in sparse, moderate-noise conditions, whereas shortestpath-based methods (MDS) outperform in densely connected, noisy environments. Additionally, experiments on real sequencing-based microscopy data confirm that controlled promiscuity enhances reconstruction robustness, aligning closely with synthetic predictions. These findings suggest a fundamental behavior governed by edge density, highlighting the practical value of intentionally designing promiscuous networks to achieve high-quality spatial reconstructions in DNA barcode network imaging.

Oral contributions / 10

LASSO: versatile and selective biomolecule pulldown with combinatorial DNA-crosslinked polymers

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The ability to isolate molecular targets from complex biological samples is crucial for many areas such as diagnostics, genetic analysis, and bionanotechnology. A variety of commercial methods exist for this purpose; however, they are often inflexible with restrictive sample requirements and high cost. Here we introduce LASSO, a simple, cost-effective, and generalized method for the capture of diverse biomolecules on DNA-functionalized smart polymers. LASSO programmably binds targets through the addition of sequence-specific catcher strands. Gentle target pulldown is achieved by triggering polymer agglomeration with combinatorial DNA crosslinker libraries. We demonstrate selective capture of highly relevant biomolecular targets, including DNA oligonucleotides, SARS- CoV-2 RNA, human ribosomal RNA, and human thrombin, with >80% efficiency. Depletion of ribosomal RNA on LASSO enhanced the quality of RNA-seq libraries while exhibiting reduced off-target bias as compared to commercially available methods. Pulldown and release of thrombin take place under native conditions, preserving the protein's structure and enzymatic function. We anticipate that LASSO will simplify preparative workflows in broad applications including biosensing and transcriptomics.

Oral contributions / 11

Tackling new challenges in molecular interaction analysis: from small molecules to cells

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Oral contributions / 12

Immune-induced DNA-Antibody Hybrid Condensates

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DNA condensates have garnered increasing interest as synthetic analogs of living cells or membraneless compartments, offering potential applications in various fields. While membraneless organelles in cells typically form through complex protein–protein or protein–nucleic acid interactions, synthetic DNA condensates are generally formed by specific DNA–DNA interactions or non-specific hydrophobic interactions. To mimic the diversity of biomolecular interactions involved in the cellular microenvironment, we present here the combined use of specific DNA-DNA and antibody-antigen interactions to induce the formation of DNA-antibody hybrid condensates. To do this, we modified a four-arm DNA nanostar by replacing one sticky-end with one antigen. Only in the presence of the specific bivalent antibody, DNA-antigen nanostars phase-separate to form hybrid condensates. To demonstrate the versatility of the system, we employed multiple antigen-conjugated nanostars and their respective antibodies, achieving orthogonal and specific molecular recognition. This system could be an approach for applications in sensing and drug delivery.

Oral contributions / 13

Cation-controlled structure, activity, and organisation of biomimetic DNA receptors in synthetic cell membranes

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Biological membranes tightly regulate the spatio-temporal organisation of their machinery to facilitate key functionalities, such as signal transduction, molecular trafficking and cellular motion. Bottom-up synthetic biology aims to replicate in synthetic cells behaviours typically observed in living matter, allowing us to dissect biological phenomena as well as to unlock promised technological applications in biomedicine, biosynthesis, and bioremediation [1].

My work applies the tools of bionanotechnology, from self-assembly to chemical nanotechnology, to engineer functionality in otherwise inert lipid bilayer membranes [2]. Here, I will discuss the design and application of DNA nano-devices with controlled structure, activity, and membrane distribution. By interrogating biophysical principles governing structural and functional membrane- hosted responses, our DNA-based toolkit allows us to regulate biomimetic functionalities in syn thetic cellular membranes, including programmable transport of cargoes [3], control over two- and three-dimensional membrane re-structuring events [4], and cation-responsive, localised, catalytic action [5]. Besides fundamental contributions to our understanding of the organisation and activity of biological membranes, our biomimetic platforms pave the way for engineering sophisticated pathways for sensing, communication, motion, and division in synthetic cellular agents.

Key References:

1 B. C. Buddingh'& J. C. M. van Hest. 2017. Artificial Cells: Synthetic Compartments with Life-like Functionality and Adaptivity. Acc. Chem. Res. 50(4), 769–777.

2 R. Rubio-Sánchez*, G. Fabrini*, P. Cicuta, and L. Di Michele. 2021. Amphiphilic DNA nanostructures for bottom-up synthetic biology. Chem. Commun., 57, 12725.

3 R. Rubio-Sánchez, S. Eizagirre Barker, M. Walczak, P. Cicuta, and L. Di Michele. 2021. A Modular, Dynamic, DNA-Based Platform for Regulating Cargo Distribution and Transport Between Lipid Domains. Nano Lett., 21, 2800–2808.

4 R. Rubio-Sánchez•, B. M. Mognetti, P. Cicuta, and L. Di Michele•. 2023. DNA-Origami Line-Actants Control Domain Organization and Fission in Synthetic Membranes. J. Am. Chem. Soc., 145, 11265–11275.

5 E. Peters, D. A. Tanase, L. Di Michele•, and R. Rubio-Sánchez•. 2025. Cation-controlled assembly, activity, and organisation of biomimetic DNA receptors in synthetic cell membranes. bioRxiv, 10.1101.2024.04.27.650532

Oral contributions / 14

mRNA–DNA Origami Nanostructures for Cancer Vaccines

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mRNA vaccines rely on carriers to protect the payload and deliver it into cells. Lipid nanoparticles achieve this through encapsulation but leave the mRNA's structure untouched. Yet structure matters: the shape of a molecule can affect its uptake, translation, and immunogenicity.

We present a strategy where mRNA acts as both message and material. By hybridizing antigen- encoding mRNA with short complementary DNA strands, we fold it into defined mRNA–DNA origami. These assemblies mimic viral geometry, protect the mRNA backbone, and create a compact, programmable format. To further improve delivery, we coated the folded mRNA with Cowpea Chlorotic Mottle Virus (CCMV) capsid proteins. These proteins self-assemble around nucleic acids, forming virus-like shells. The coating enhanced resistance to nucleases.

We tested these constructs in antigen-presenting cells, comparing them to linear mRNA. Dendritic cells translated the folded mRNA and presented the encoded epitope on MHC I. Folding required careful design to keep the 5′ cap and coding region accessible for translation.

By engineering shape as well as sequence, this platform creates new possibilities for precise and modular cancer vaccines, designed from the molecule up.

Oral contributions / 15

UV-Triggered Assembly and Protein-Assisted Positioning of DNA-Based Cytoskeletal Filaments in Synthetic Cells

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Controlling the spatial and temporal dynamics of cytoskeletal components is a central challenge in the construction of synthetic cells. Here, we present a system in which synthetic cytoskeletal filaments composed of DNA tiles are assembled in situ via a UV-trigger and subsequently positioned through interaction with the Min protein system from Escherichia coli.

Specifically, we designed DNA tiles that remain inactive until exposed to a UV stimulus, enabling controllable self-assembly into micrometer-scale filaments. These DNA filaments are formed directly at the membrane, and their spatial distribution is influenced by the oscillatory behavior of the Min protein system, reconstituted on supported lipid bilayers (SLBs) and in giant unilamellar vesicles (GUVs). By tuning UV exposure times and Min protein concentrations, we demonstrate precise control over both filament nucleation timing and spatial localization, mimicking key aspects of cytoskeletal patterning in living systems. Using confocal microscopy and atomic force microscopy, we investigate filament formation, properties and behavior on SLBs and in GUVs.

Our system represents a modular platform for integrating photo-responsive DNA nanotechnology with protein-based reaction–diffusion systems. Beyond demonstrating compatibility between orthogonal synthetic and biological modules, our results suggest a path toward minimal cytoskeletal systems capable of adaptive organization and cooperative behavior. The coupling of external stimuli (UV light) with internal positioning mechanisms (Min proteins) provides a flexible framework for studying emergent properties in bottom-up synthetic biology and could be extended to orchestrate more complex tasks such as compartmentalization, membrane deformation, or active transport.

Our work highlights how rationally designed DNA architectures can function as responsive cytoskeletal elements, operating in unison with native protein systems, and opens new avenues for programmable morphogenesis in synthetic cellular environments.

Oral contributions / 16

From Lab to Product: A Case Study of Electrically Actuated Origami-Levers for Biosensing Applications

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DNA origami nanotechnology has seen widespread use in research, yet its translation into commercial products remains limited.
In the context of enabling technologies for drug discovery, we developed and commercialized DNA origami nanolevers as functional biosensing elements for analyzing binding-induced conformational changes in proteins.
These proteins are tethered to a chip surface via DNA origami levers. When subjected to alternating electric fields, the levers oscillate, moving the attached proteins through solution. The nanolever dynamics are influenced by the hydrodynamic friction of the tethered proteins, enabling detection of changes in their 3D conformation through measurements of switching speed. This assay is well-suited for high-throughput screening of drug candidates based on their ability to induce conformational changes in target proteins.
We describe the rationale behind using DNA origami to create distinct advantages for end users, and discuss practical considerations including manufacturability, product quality, and the establishment of a global supply chain for ready-to-use DNA origami kits. Application examples illustrate the analysis of protein conformational changes in workflows compatible with high-throughput screening.

Oral contributions / 17

Programmable Nanoscale Motion via Molecular Patterning on DNA Origami

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In the field of micro- and nanomotors, uncovering the principles that govern their motion is essential not only for developing active materials for diverse applications, but for deepening our understanding of the fundamental mechanisms underlying motion at the nanoscale. Active motion arises from the conversion of energy into mechanical work; however, effective propulsion requires a degree of structural anisotropy. In enzymatically powered systems, this asymmetry creates an out-of-equilibrium state where localized product gradients induce entropically driven fluid flows that propel the particle forward. Asymmetry can be introduced via particle shape or the anisotropic placement of catalytic units, as seen in functionalized DNA fibers or stochastically enzyme-coated spheres. While these systems have revealed key aspects of active motion, their inherent heterogeneity in enzyme number, spatial distribution, and particle geometry, limits the ability to dissect the individual contributions of each parameter to motile behaviour.

To overcome these limitations, we employ DNA origami as a programmable platform for nanomotor design, enabling precise control over enzyme number and spatial arrangement. This approach allows systematic investigation of how catalytic loading and structural anisotropy together dictate nanoscale motion. Specifically, we designed DNA nanorods (18-helix bundles) with increasing numbers of radially distributed urease enzymes (Fig. 1). Following assembly, urease-oligo conjugates were hybridized to the origami scaffolds, and structural and functional validation was performed via gel electrophoresis, atomic force microscopy (AFM), and enzyme kinetic assays. The motion behaviour of fluorescently labelled nanomotors was analysed using single-particle tracking (SPT). Trajectory analysis yielded key motility parameters, including mean-squared displacement (MSD), speed and diffusion coefficients. Strikingly, enhanced motility was observed only at intermediate enzyme densities, revealing a non-linear relationship between catalytic loading and propulsion efficiency. These findings highlight the existence of a critical balance between catalytic activity and structural asymmetry in driving effective motion.

Oral contributions / 18

Thousand-fold acceleration of single photon emission in DNA origamis with gold nanocube dimers

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The coherent interaction of light with a single quantum emitter is at the heart of several quantum technologies, such as communications and computing [1]. However, this process is not currently achievable at room temperature in an efficient manner because of ultrafast phonon-induced dephasing. To accelerate light-emitter interactions in order to try and beat dephasing, while maintaining high brightness, we optimize the coupling between a single fluorescent molecule and a plasmonic resonator using DNA origamis. This allows us to exploit the large field enhancements and Purcell factors available between the tips of gold nanocubes (Fig. 1-a) [2], reaching a thousand-fold acceleration of bright single photon emission.

In practice, we design tower-like DNA origami structures in order to position a single ATTO647N molecule specifically within the plasmonic hotspots achieved between two 40 nm gold nanocubes or two 60 nm gold nanospheres (Figures 1-b-e). These hybrid structures are assembled using plasmonic nanoparticles coated with DNA strands, which are complementary to strands located near the emitter on the DNA origamis. To ensure a precise tip-to-tip configuration and accurate molecular positioning, we develop a hollowed DNA origami structure, which directs the orientation of the nanocubes and confines the fluorophore within the plasmonic hotspot.

Lifetime measurements conducted in a high-refractive-index glycerol-based aqueous solution demonstrate an enhancement of the emission decay rate by approximately 1000-fold (Figure 1-g), combined with emission intensities enhanced by ~100. These sub-10 ps lifetimes confirm the potential of such hybrid DNA-origami plasmonic nanostructures to achieve coherent light-matter interactions in an efficient manner at room temperature, such as indistinguishable single photon emission or strong coupling [3].

1 C. Toninelli et al., Nat. Mater., 20, 1615 (2021)
2 J. Heintz et al., J. Phys. Chem. Lett., 13, 11996 (2022)
3 J. Heintz et al., ACS Nano, 15, 14732 (2021)

Oral contributions / 19

DNA Origami Clathrate Crystals Exhibit Structural Color in the Visible

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The bottom-up self-assembly of photonic crystals with complex symmetries, such as diamond-type structures, requires molecular building blocks with programmable geometry and directional binding, which is challenging in conventional colloidal systems.[1] The DNA origami technique [2], which allows rational design of complex structures, is a powerful tool for this task. A direct rod-connected cubic diamond structure photonic crystal can be achieved by assembling tetrapod DNA origami structure using single-stranded DNA "sticky ends" stretched out from the four tips of the structure [3]. By carefully designing the pattern of the sticky ends, one can control the rotation between neighboring monomers. A 60° rotation between monomer arms, for example, yields the cubic diamond structures while a rotation by 0° at one of the four arms can lead to hexagonal diamond structures. We designed a system of competing sticky ends in which the neighboring monomers have both the possibility to bind with 60° and 0° rotation. By further tuning parameters such as temperature and salt concentrations during crystallization, we were able to observe stacking of cubic/hexagonal diamond structures (Fig. 1a and b6), as well as the emergence of clathrate-type lattices which has exclusively 0° rotation between all monomers (Fig. 1c).

Our clathrate lattice has a lattice constant of 440 nm with 136 monomers forming the unit cell. This spacing leads to the occurrence of structural color in the visible range after stabilizing the DNA origami lattice with an SiO2 coating through a sol-gel process [4]. Upon drying, the resulting structures exhibit blue or green reflections under dark-field illumination, depending on the orientation of the individual crystals and the angle of the incoming light [5].

References:
1 He, Mingxin, et al. "Colloidal diamond." Nature 585.7826 (2020): 524-529.
2 Rothemund, Paul WK. "Folding DNA to create nanoscale shapes and patterns." Nature 440.7082 (2006): 297-302.
3 Posnjak, Gregor, et al. "Diamond-lattice photonic crystals assembled from DNA origami." Science 384.6697 (2024): 781-785.
4 Nguyen, Linh, et al. "DNA-origami-templated silica growth by sol–gel chemistry." Angewandte Chemie International Edition 58.3 (2019): 912-916.
5 Yin, X, et al. in preparation.

Oral contributions / 20

Differentiable Programming of Indexed Chemical Reaction Networks and Reaction-Diffusion Systems

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Many molecular systems are best understood in terms of prototypical species and reactions. The central dogma and related biochemistry are rife with examples: gene i is transcribed into RNA i, which is translated into protein i; kinase n phosphorylates substrate m; protein p dimerizes with protein q. Engineered nucleic acid systems also often have this form: oligonucleotide i hybridizes to complementary oligonucleotide j; signal strand n displaces the output of seesaw gate m; hairpin p triggers the opening of target q. When there are many variants of a small number of prototypes, it can be conceptually cleaner and computationally more efficient to represent the full system in terms of indexed species (eg for dimerization, M_p, D_pq) and indexed reactions (M_p+ M_q→ D_pq). Here, we formalize the Indexed Chemical Reaction Network (ICRN) model and describe a Python software package designed to simulate such
systems in the well-mixed and reaction-diffusion settings, using a differentiable programming framework originally developed for large-scale neural network models, taking advantage of GPU acceleration when available. Notably, this framework makes it straightforward to train the models' initial conditions and rate constants to optimize a target behavior, such as matching experimental data, performing a computation, or exhibiting spatial pattern formation. The natural map of indexed chemical reaction networks onto neural network formalisms provides a tangible yet general perspective for translating concepts and techniques from the theory and practice of neural computation into the design of biomolecular systems.

Oral contributions / 21

Programmable DNA-based nanosystems for spatiotemporal signal processing and actuation

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The goal of this project is to develop a synthetic system that can sense, process and actuate molecular information in a programmable manner. For this purpose, we developed a DNA based reaction cascade that undergoes two steps, generating two temporally resolved output signals. The two reaction centers, A and B, are localized few nanometers apart inside a DNA origami compartment. Upon addition, the trigger DNA strand interacts with center A liberating an intermediate species that sequentially reacts with center B. Recording the first and second step of the reaction cascade by FRET, we investigate whether and how the spatial arrangement of the input species, different types and concentrations of crowding agents, as well as the attachment of different cargo sizes to the trigger strand, affect the kinetic signature of the process; both at the ensemble and single-molecule levels. We also show that the output signal can be coupled to a chain growth event, leading to the formation of filament-like appendices on one side of the origami cage. Such a large-scale morphological change is hypothesized to alter the overall hydrodynamic properties of the nano-object and affect its random diffusion behavior in solution. Hence, our model system enables not only monitoring and analyzing signal transmission within a nanosized environment but permits also to couple an input signal to a physical observable. We envision that the programmability of the DNA origami object, both inside and outside the cavity, will offer a versatile tool for studying reaction kinetics at the nanoscale and creating more sophisticated nano-sized objects that can self-propel in response to certain stimuli.

Oral contributions / 22

Inverse thermodynamics for programmable self-assembly thrives with DNA-based systems

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Self-assembly refers to the spontaneous organisation of components into ordered structures without external guidance. This phenomenon underpins many natural processes and is increasingly harnessed in materials science to build complex nano-structures. Inverse self-assembly seeks to leverage the tunability of colloidal interactions to design building blocks that drive the system toward a target structure, while avoiding kinetic traps and competing phases. In this work, we extend the inverse design paradigm beyond structure alone to include selected thermodynamic properties. We present a framework for inverse thermodynamics, i.e. the design of interaction potentials that realise specific thermodynamic behaviours [1]. By precisely controlling both bonding topology and bond energetics, we demonstrate how to program complex phase behaviour in multi-component systems. Our focus is on the design principles for azeotropic demixing, given the critical role of azeotropic points in self-assembly [2,3]. DNA nanotechnology offers an ideal experimental platform for realising these inverse-designs: DNA origami and DNA functionalised colloids can be decorated by complementary DNA strands [4]. This versatile control makes DNA-based systems uniquely suited to implement the highly specific, short-range and anisotropic bonding required for inverse thermodynamics.

1 Beneduce, C., Mastriani, G., Šulc, P. et al. (2025). Inverse Thermodynamics: Designing Interactions for Targeted Phase Behavior. arXiv preprint arXiv:2506.11856.

2 Beneduce, C., EP Pinto, D., Šulc, P. et al. (2023). Two-step nucleation in a binary mixture of patchy particles. The Journal of Chemical Physics, 158(15).

3 Beneduce, C., Sciortino, F., Sulc, P. et al. (2023) Engineering azeotropy to optimize the self-assembly of colloidal mixtures. ACS nano, 17(24), 24841-24853.

4 Liu, H., Matthies, M., Russo, J. et al. (2024). Inverse design of a pyrochlore lattice of DNA origami through model-driven experiments. Science, 384(6697), 776-781.

Oral contributions / 23

Binding code between DNA oligomers and long single-stranded chains: multiplicity of partial pairings vs accessibility due to secondary structures

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The design of optimal nucleic acid oligomers capable of rapid and specific isothermal binding to long single-stranded DNA or RNA sequences often exceeding a thousand bases in length (e.g., genomes, mRNA, or lncRNA), remains a significant challenge. This endeavor holds substantial implications not only for DNA nanotechnology but also for understanding fundamental biological processes and developing novel analytical systems or therapies. We addressed this challenge by studying the binding of long sequence models, such as M13mp18, to a large panel (> 50) of DNA oligomers immobilized on a label-free biosensor microarray. Our observations revealed that fewer than 10% of the oligomers complementary to the long sequence exhibited substantial binding capability. These findings unveil an unexpected hierarchy of factors influencing binding: the primary parameters are the multiplicity of partial pairing with above-average strength and the polymorphic binding of degenerate sequences, while the secondary structure of the strands plays a relevant, but secondary, role [1]. To further validate this concept, we selected a new panel of oligomer probes designed to provide specific response patterns in the presence of different genome strands. Biosensor experiments confirmed the expected behavior. Computational selection, based on both the multiplicity of binding and the strength of transient secondary structures, enabled the identification of a small fraction of optimal probes (less than 1% of complementary probes) that provided excellent discrimination capabilities. This fraction of optimal probes is further reduced when detection oligomer probes providing an amplification signal (e.g., single-nanoparticle detection) are used in addition to surface-immobilized capture oligomers. We found that, in such cases, cross-interactions between probes, mediated by polymorphic binding, must also be considered. Overall, the results of this study provide a key to cracking the code for optimal sequences in capturing and detecting low concentrations of various genomic sequences, down to single-molecule counting.

1 Nava, G., Carzaniga, T., Casiraghi et al. (2024). Nucleic Acids Research Vol. 52, Issue 15, pp. 8661–8674.

Oral contributions / 24

Magnetic DNA origami nanorotors: programmable torques on the nanoscale

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Magneto-responsive actuators offer minimally-invasive and deep-tissue perturbation of cellular processes, features not available in methods using optical implants and visible light as stimulus. Despite progress, the magnetic manipulation of cells at the single receptor level is still highly challenging; magnetic nanoparticles (MNPs) as nanoactuators can only exert ~ femtonewton forces and torques. To achieve piconewton forces, a biologically relevant regime, it is necessary to integrate MNPs together in a controllable manner. This integration has not yet been achieved utilizing soft-synthetic templates, where control over the number, patterning, and orientation of MNPs remains a challenge. In contrast to soft templates, DNA origami holds promise in overcoming these limitations, specifically so for its capacity to arrange nanoparticles in a site-specific manner at high spatial resolution. Here, we demonstrate programmable assembly of custom magnetic nanocubes at pre-defined position and number on 6 helix-bundle (HB) DNA origami and show the controlled magnetic rotation of hundreds of magnetic DNA bundles on glass surface under circulating magnetic fields of 8 mT. Nano-rotation assays with two different designs at various magnetic field amplitudes and frequencies will be presented. By varying the quantity and position of magnetic nanocubes, torque values spanning the order of 10 to 100 pN·nm are reachable, using magnetic field amplitudes of ~ 8 mT. These magnetic frameworks therefore offer a foundation for advanced biocompatible nanorobotics and magnetic force and torques tweezers.

Oral contributions / 25

Optimizing Large-Scale DNA Origami Lattices on Silicon

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Recently, periodic nanostructures made of DNA origami tiles have become a viable option for applications in fields like diagnostics, electronics, sensing and optics, especially in challenging applications like the creating metasurfaces with unique optical properties [1]. Main benefit with origami is its superior self-assembly properties and vast variety of functionalization schemes [2], and in particular the possibility to assembly them into larger nanostructured constructions with precision comparable to e-beam lithography while avoiding the slow and expensive patterning. Self-assembled DNA origami lattices with large surface coverage and high order, have been achieved mostly on very smooth substrates, like mica or lipid layers [3], which usually are not compatible with any traditional nanofabrication process. To overcome this limitation and allow further processing, e.g., by DNA-assisted lithography [4], we have demonstrated large-scale assembly of 2D fishnet-type lattices on a silicon substrate using cross-shaped DNA origami, so-called Seeman tile, as the building block [5].

Here, we further investigate how different parameters, like tile-tile and tile-surface interaction strengths, temperature, concentrations and time, affect the assembly, concentrating especially on how the size of the single crystalline domains within the formed polycrystalline type of layer can be increased towards completely single crystalline lattice. Results are analysed for precision of formation, surface coverage of well-formed lattice and achieved domain size. For this, systematic statistical counting as well as FFT analysis for periodicity and correlation lengths were made from AFM data. These origami-lattices on silicon substrate can be further used for any traditional fabrication processes like etching or metallization, to turn them for example into metamaterial with novel optical properties.

1. L. Lermusiaux *et al*. Nano Ex. **3**:021003 (2022).
2. F. Hong *et al*. **117**:12584 (2017).
3. J.M. Parikka *et al*. Molecules **26**:1502 (2021).
4. B. Shen *et al*. Science Adv. **4**:eaap8978 (2018).
5. K. Tapio *et al*. Chem. Mater. **35**:1961 (2023).

Oral contributions / 26

Structure-Based Prediction of Context-Dependent Ribozyme Activity for Functional RNA Design

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The growing interest in RNA therapeutics and dynamic nucleic acid systems has drawn increasing attention to ribozymes as postor cotranscriptional editors of RNA oligonucleotides. However, both the ribozyme and the remaining transcript consist of ribonucleotides that can potentially interact in undesirable ways, disrupting the ribozyme structure and reducing catalytic activity. While the prediction of ribozyme functionality from sequence using machine learning or physics-based models is a very active field of research, predicting the influence of the surrounding upand downstream ribonucleotides on ribozyme functionality has received less attention.

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Self-Multimerising Single-Layer 3D Curved DNA origami with ENSnano

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DNA origami is a highly effective technique for constructing DNA nanostructures with high yield (Rothemund, 2006). However, the achievable size is typically limited to ~ 40–100 nm by the length of the DNA scaffold strand. To overcome this constraint, several strategies have been developed to assemble multiple DNA origami units into larger architectures (Dietz et al., 2011; Gerling et al., 2015; Wagenbauer et al., 2017; Monferrer et al., 2023). These approaches rely on Watson–Crick base pairing through specifically designed staples, helix stacking (optionally combined with barcoding to ensure proper alignment), or complementary docking sites. The latter strategy is particularly powerful but typically requires thick, multi-layer designs, which increase the number of components and thicken the design. More broadly, ensuring the stability of each independent component has remained a challenge. ENSnano has recently demonstrated that single-layer, three-dimensional curved DNA origami can be assembled stably and with high yield (Levy, Finkel et al., in preparation). Here, we present a new set of ENSnano design tools that facilitate the precise alignment of DNA helices in multicomponent DNA origami. These tools minimize strain along each helix while ensuring exact helix alignment between components, continuously varying arc lengths, and strict matching of prescribed scaffold lengths. We demonstrate their versatility by assembling, in a single-pot annealing reaction, toroidal nanostructures composed of either 3 or 11 identical copies of the same single-layer DNA origami using the 8064-nt scaffold. Each origami unit self-assembles with other copies either through 3-bp connecting staple strands or purely via helix stacking. Alignment is enforced by barcoding (Woo et al., 2011), i.e., by shifting pairs of helices so that any misregistration disrupts at least 50 % of the stacks. Preliminary AFM imaging of unpurified samples confirms that individual origami fold correctly and self-assemble with very high yield into the expected multi-component structures: (A) 3- component torii with circular cross-sections of 32 helices, inner diameter 51 nm and outer diameter 109 nm; and (B) 10–11-component torii with 62 helices, inner diameter 97 nm and outer diameter 206 nm—both in agreement with AFM measurements. Experimental conditions have not yet been optimized. These tools and algorithms will be fully integrated into ENSnano in the near future to support the streamlined design of complex, self-assembling DNA nanostructures.

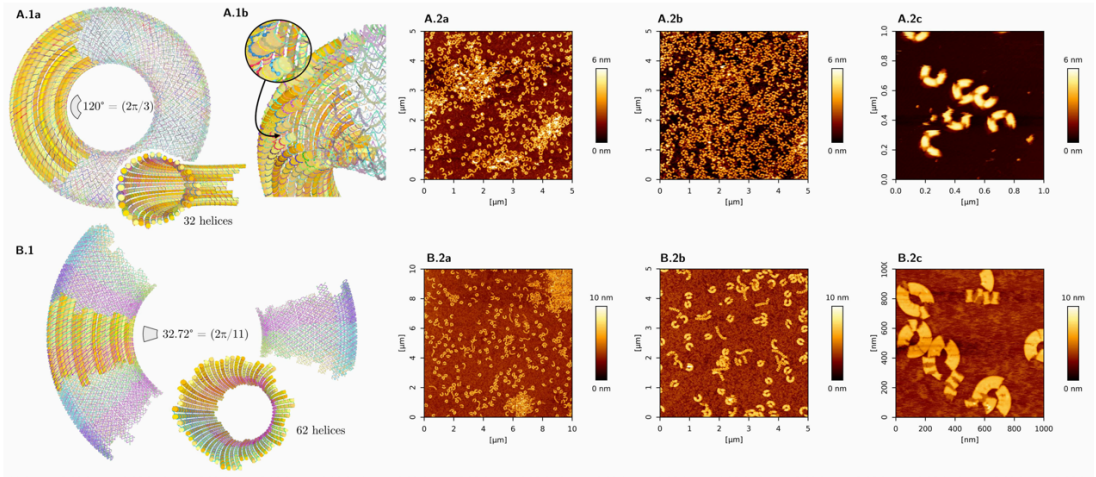

Figure 1: Toroidal nanostructures assembled from 3 (A) and 11 (B) identical single-layer curved origami units. (A) 120° segment design with 32 helices in a circular cross-section (A.1a) and automatic helix adjustment to ensure perfect alignment with other copies (A.1b). AFM images of unpurified samples assembled using 3-bp connecting staples (A.2a) or pure helix stacking (A.2b,c). (B) 32.72° segment design with 62 helices in a circular cross-section (B.1a). AFM images of unpurified samples assembled via pure helix stacking (B.2a–c)

Oral contributions / 28

Integrating Nucleic Acid Enzymes with DNA Origami for Advanced Digital Biosensing

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In digital biosensing, sensitive detection is enabled through isolation of individual biomarkers, followed by localized signal generation. While this approach has been widely adopted, its broader applicability can be enhanced by implementing nucleic acid (NA)-based components, offering advantages such as simple synthesis and high thermal stability. Additionally, alternative target confinement strategies could replace conventional (and challenging) methods, like microwells or droplet microfluidics.

Here, we present a DNA nanosensor concept, where DNA origami disks (~100 nm diameter) are functionalized with a multicomponent NA enzyme (MNAzyme) and its fluorophore-quencher labeled substrates. Upon detection of a single-stranded DNA (ssDNA) target, the partzymes assemble into an active MNAzyme. This leads to substrate cleavage, releasing the quenchers and producing a localized fluorescent signal ("ON"state). Without target, the MNAzyme does not assemble and no signal is generated ("OFF"state). After design and characterization (gel electrophoresis, AFM) of the origami, we studied the system using single-molecule TIRF microscopy. DNA-PAINT confirmed the ring-shaped arrangement of substrate linkers on the origami. Then, substrate cleavage upon target detection was measured at single-nanosensor level. Based on signals from nanosensors incubated with 0 and 100 nM ssDNA target, a partial least squares discriminant analysis (PLS-DA) model was developed to distinguish "ON"and "OFF"states. This model was then applied to nanosensors exposed to target concentrations between 0 and 100 nM, demonstrating a concentration-dependent response. Finally, to enable precisely controlled DNA nanosensor immobilization, we optimized a nanosphere lithography protocol to produce DNA nanoarrays. Our modified protocol decouples the resulting binding site size and interspacing, allowing individual nanosensor immobilization at user-defined distances and ensuring single-nanosensor signal evaluation.

Future efforts will focus on enhancing sensitivity, expanding to different biomarker types, and integrating the system into a self-powered microfluidic device. As such, our approach paves the way towards a versatile digital biosensing platform.

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Self-assembled DNA nanostructure applied on immunomodulation of microglia in neuroinflammation

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Alzheimer's disease is one of the most prevalent cognitive disorders, affecting approximately 35 million people worldwide. This condition is partly driven by chronic neuroinflammation, which leads to progressive neuronal degradation. A key factor in this inflammatory response is the uncontrolled release of cytokines, signaling proteins, extracellular DNA, and notably, microRNAs (miRNAs) which activates microglia, shifting them from a resting phenotype to a pro-inflammatory phenotype.

In drug delivery, due to poor bioavailability or limited stability, certain advanced therapies often require a delivery system. Among them, framework nucleic acids (FNA) have emerged as precise, programmable, biocompatible, biodegradable, self-assembling, and non-immunogenic systems that are spontaneously taken up by cells. DNAzymes, which are catalytic DNA sequences capable of hydrolyzing RNA, can be engineered to selectively bind and cleave target RNA sequences. In this thesis project, we aim to develop a tetrahedron-shaped DNA nanostructure (TET) incorporating a DNAzyme targeting a pro-inflammatory miRNA implicated in neuroinflammation.

Size and shape are the most important factors regarding the internalization of the FNA. Endocytic pathways include mainly clathrin-mediated, caveolin-mediated and phagocytosis, the latter being restricted to macrophages. As our model is phagocytosis-competent cell (microglia, N9), we are also interested to compare the internalization of the TET with the internalization of a bigger nanostructure made by origami. Moreover, we functionalize TET with aptamer allowing to specifically targeting activated microglia, we are interested to compare the difference of uptake by the resting and activated macrophages.

Both by fluorescence microscopy and polymerase chain reaction (PCR), we observed an uptake of TET into the cells around 1000 structures each. Additionally, PCR and western blot analysis revealed a decrease in the level of miR-34a in activated microglia treated with TET and a decrease of the abundance of inflammation markers (iNOS) respectively. These promising results suggest that TET could enhance therapies against neurodegenerative diseases.

Poster contributions

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Light-induced 3D-printing of DNA-based Responsive Hydrogels

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Nucleic acids are versatile biomolecules containing specific sequences of bases which define their properties and reactivity. The use of nucleic acids, and in particular DNA, is a promising strategy in the designing of advanced materials when selective responsivity is required. In this context, the fabrication of DNA-responsive hydrogels has been recently reported, exploiting standard photolithography to fabricate DNA-activated soft robots.
Here, a step forward in DNA-based technologies is proposed, employing Digital Light Processing (DLP) 3D printing to fabricate 3D hybrid hydrogels, and adding acrydite-modified DNA as comonomer in the photocurable formulations. The 3D printing process does not damage those groups and, additionally, their presence enables the triggering of the macromolecular structure in response to complementary DNA strands, leading to a controlled volumetric expansion through the hybridization chain reaction (HCR) process. Furthermore, by optimizing 3D printing parameters, well-defined complex objects can be fabricated employing minimum amounts of photocurable formulations (less than 100 µm). The possibility to fabricate multi-material structures is also demonstrated, in which modified hydrogels maintained their selective responsivity towards precise DNA sequences. This work opens new perspective in the realization of advanced DNA based devices, in which materials characteristics, 3D design, and advanced fabrication play synergistically.

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Cost-efficient folding of functionalized DNA origami nanostructures via staple recycling

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DNA origami nanostructures (DONs) offer a highly programmable platform for applications ranging from biosensing to drug delivery, and increasingly, for molecular data storage [1]. However, high production costs, especially for modified staples, limit their broader use. Standard assembly protocols require a large staple excess [2], much of which is discarded after folding.
In this work [3], we present a cost-efficient and straightforward strategy to recover and recycle unmodified, biotinylated and ATTO488-labeled staples. A rectangular DON and six-helix bundle served as model systems and molecular weight cut-off filtration enabled effective separation of folded structures from excess staples. The recovered staples retained folding efficiency, structural integrity and functionality over at least ten folding cycles verified by agarose gel electrophoresis and atomic force microscopy.
Recycling reduces staple costs by 33% after five cycles, with savings up to 41% for extended cycles of high-density modifications. This approach improves resource efficiency and is particularly relevant for DNA-based data storage applications requiring extensive staple functionalization.

References:
[1] Doricchi, A., et al., ACS Nano 16, 17552-17571, 2022
[2] Rothemund, P. W. K., Nature 440, 297-302, 2006
[3] Tomm, E., et al., Nanoscale 17, 17265-17273, 2025

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Targeting of insulin nanoclusters for tailored insulin therapies in zebrafish

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Insulin receptors (IRs) organize into nanoclusters on the cell membrane, as demonstrated by our group and others using super-resolution microscopy. The nanocluster arrangements of IRs provide the ideal environment for multivalent interactions with insulin. We designed an insulin-DNA nanostructure that offers precise control over the number and spatial distribution of insulin molecules within each nanostructure. Our group found that certain multivalent insulin configurations significantly increased IR activation, extended receptor engagement, and improved glucose regulation in a zebrafish model of diabetes. These findings highlight the potential of modulating insulin valency and spatial organization within nanoclusters as a strategy to enhance insulin-based therapies.

Our ongoing work investigates the biodistribution and functional activity of insulin-DNA origami nanostructures in wild-type and diabetes zebrafish models. Future work aims to expand this work in wild-type and diabetes mouse models. Understanding tissue-specific IR patterns may enable the development of new types of insulin nanocluster-based therapies that can selectively target key tissues to fine-tune insulin efficacy at the cellular level.

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RECENT ADVANCES IN STUDYING THE INTERPLAY OF DNA TOPOLOGY AND DNA-BINDING PROTEINS: A KEY TO PROTEIN- DNA DYNAMICS

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DNA topology is an intrinsic property of DNA molecules that influences fundamental cellular processes such as chromatin architecture and gene expression. Genomic DNA is organised into topological domains, with supercoiling levels regulated by DNA topoisomerases. These enzymes modify the DNA supercoiling of covalently closed DNA molecules by either removing or introducing supercoils. Each enzyme has its specificity, leading to the production of a range of differently supercoiled molecules [1], a capability exploitable in both fundamental research and biotechnology. Understanding how DNA topology modulates protein–DNA interactions offers crucial insights into genome architecture and function, especially in terms of binding affinity, specificity, and the conformational flexibility of DNA-binding proteins. In our research, we combine computational modelling and biochemical assays to investigate how DNA supercoiling affects protein structure, and how, in turn, protein binding can reshape DNA conformation. From a biotechnological perspective, modulating protein conformational dynamics through DNA topology offers an innovative strategy for regulating the in vivo activity of proteins involved in pathological pathways, with potential implications for the development of novel therapeutic tools [2].

REFERENCES

1 Bettotti P, Visone V, Lunelli L, et al. (2018). Sci Rep, 8(1): 61-63. doi: 10.1038/s41598-018-24499-5
2 Li S, Vemuri C, Chen C. (2024). Curr Opin Struct Biol, 87:102868. doi: 10.1016/j.sbi.2024.102868.

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Enzyme-mediated dissipative HCR for programmable DNA assembly and disassembly

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Dynamic behavior is a key feature of biological supramolecular assemblies that is essential for environmental adaptability and functional regulation. The reproduction of such non-equilibrium dynamics in synthetic materials is promising for the creation of adaptive systems with life-like properties. Dissipative DNA nanotechnology has recently gained attention as a powerful approach to develop responsive biomaterials. It exploits the programmability of DNA-DNA interactions and the use of nucleic acids as chemical fuels to control non-equilibrium processes. In this work, we present a new method to implement dissipative control over the hybridization chain reaction (HCR), a well-established technique based on the polymerization of two metastable DNA hairpins. By implementing a "tailed HCR" strategy, we introduce dissipative behavior through enzyme-mediated fuel consumption. We have redesigned a fuel that employs strand displacement to disassemble HCR polymers into small oligomers. The DNA nanostructure can then reform in a cyclic, dissipative process when an enzyme specifically recognizes the fuel involved in the oligomer structure and degrades it. In particular, we have developed two orthogonal strategies using two different enzyme-fuel pairs: RNA fuel/RNase H and phosphorylated DNA fuel/lambda exonuclease. These systems enable programmable assembly and disassembly of the DNA nanostructure and demonstrate the potential of dissipative DNA nanotechnology for time-controlled, responsive biomaterials.

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Toward Multivalent Aptamers

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Aptamers are short, single-stranded DNA or RNA oligonucleotides that exhibit high specificity and affinity for target molecules. Their potential applications range from medical diagnostics to cancer therapy. Aptamers are synthetically generated through a selection process in which large libraries of random oligonucleotide sequences are screened to identify those with the strongest binding affinity to a given target. This process is known as SELEX (Systematic Evolution of Ligands by Exponential Enrichment) [1]. Usually, SELEX processes result in strands exhibiting a single binding domain. The evolution of multivalent binders, in contrast, has been difficult to achieve.
Here, we demonstrate that a single DNA origami structure can serve both as a production platform for short RNA oligonucleotides and as a site for hosting multiple copies of the same RNA aptamer. Crucially, due to their local proximity, RNA oligonucleotides produced on one origami primarily bind to DNA handles presented on the same origami platform. This feature in principle allows for the simultaneous screening of sequence libraries within a single reaction vessel potentially leading to multivalent aptamers. We illustrate this approach using two DNA Origamis with two distinct RNA species detected by fluorescence microscopy.

1) Tuerk C, Gold L (August 1990). “Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase”. Science. 249 (4968): 505–10. Bibcode:1990Sci…249..505T. doi:10.1126/science.2200121. PMID 2200121.

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Bio-inspired tools for improved dynamic data operability and random access in DNA-based digital data storage

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Starting several decades ago, our digital revolution has resulted in a continuous and ongoing exponential increase in digital data being produced, with estimates indicating that this data will soon outpace the storage capacity of current technologies [1,2]. This impending data storage crisis has led researchers to investigate alternative storage methods. DNA has been proposed as a promising candidate due to its high data density, long term stability and low maintenance costs, and important advances in the field of DNA-based digital data storage have been observed in recent years [1,2]. However, DNA-based systems still suffer from several limitations in terms of cost and speed, with limited dynamic data operability and random access capabilities. Random access of information in particular is a key aspect to consider, with the ability to selectively retrieve the specific data of interest without having to read out the entire pool of information becoming increasingly important as the amount of data stored increases [3]. In order to address these needs, we have developed a system that utilizes nucleic acid-guided enzymatic reactions to improve the capacity for dynamic data operation and random access in DNA-based data storage solutions. For one such data operation, the reading operation, we implemented an in vitro transcription-based data read out approach with primer-based selectivity for improved random access, circumventing several of the limitations of previous approaches. This system builds on the utilization of single-stranded data encoding strands carrying a unique primer binding site acting as data ID, allowing the targets of interest to be selectively converted to double-stranded form enabling their read out through T7 RNA polymerase-based transcription.

References:
1 Wang, S. et al. Advanced Materials, 36(6), 2307499, 2024
2 Doricchi, A. et al. ACS Nano, 16(11), 17552–17571, 2022
3 Zhou, Y. et al. ACS Applied Materials & Interfaces, 16(33), 43102–43113, 2024

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Antigen-triggered Activation of a DNA Origami Nanopore

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Antigen-triggered Activation of a DNA Origami Nanopore

Fabricating nanostructures capable of reacting to physiological stimuli by dynamic reconfiguration is of great interest when considering new diagnostics and therapeutics. We envision designing nanopores made of DNA, transitioning between an inactive and active state in response to the presence of specific antigens. To this end, we use programmable triangular building blocks constructed from DNA, assembling with high yield into shell objects composed of 20 individual triangles [1]. The triangles are extended by a potential pore-forming moiety and display membrane anchoring units. When assembled into the DNA origami shell, the pore protrusions point into the shell's interior and are, therefore, hidden, meaning that the nanopores are inactive. IgG antibodies are used as structural elements to kinetically trap the DNA origami shell in a metastable state to control the nanopore's activity and trigger the shell system's disassembly once the corresponding antigen is present [2]. The soluble antigens displace the IgGs from the shells and cause disassembly into the individual monomers, thereby transferring the nanopores into an active state. We have designed and produced triangular building blocks equipped with a potential pore-forming moiety and characterized the structures using negative-stain transmission electron microscopy and cryo-electron microscopy. We demonstrate the disassembly of DNA origami shells only in the presence of the corresponding antigen, which would activate the individual nanopores. We aim to investigate the pore-forming capability of the individual triangles in dye influx/efflux studies on vesicles.

References:

1. C. Sigl, E. M. Willner, W. Engelen, et al., Programmable icosahedral shell system for virus trapping, Nat. Mater., 2021, 20, 1281-1289.
2. W. Engelen, C. Sigl, K. Kadletz, E. M. Willner, H. Dietz, Antigen-Triggered Logic-Gating of DNA Nanodevices, J. Am. Chem. Soc., 2021, 143, 21630-21636.

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Chemically Fuelled DNA Origami Rotational Motors

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Molecular motors are biological machines that convert chemical energy into mechanical work. Constructing synthetic molecular machinery is essential for understanding their natural counterparts and advancing the development of artificial biological systems. Although significant progress has been made in the realm of synthetic motors, the development of a chemically fueled, rotational motor remains elusive. Here, we conceptualize two synthetic rotational molecular motors powered by the principles of the molecular Brownian ratchet. The first proposed motor uses the enzyme adenylate kinase to induce conformational changes in the DNA origami-based rotor structure1, modulating the internal potential energy landscape leading to unidirectional motion. The second motor functions via a replenishing burnt bridge Brownian ratchet, inspired in part by the motility of influenza viruses on cell surfaces. In this system, a DNA origami rotor forms transient tethers via a chemical fuel with substrates on the wall of the stator. These tethers (bridges) are subsequently enzymatically cleaved (burnt), creating a directional bias for unvisited substrate, resulting in continuous rotational motion. These two chemically fuelled molecular motors provide a foundation for future synthetic nano-machinery, expanding the possibilities for autonomous nanoscale motion and controlled energy transduction.

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In Vitro DNA Cryptography

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With digital data increasing exponentially, available space and longevity of energy demanding data centres are reaching their limit. With regards to storage density and durability, archiving data in DNA provides a sustainable solution (1). Rapidly evolving DNA synthesis and sequencing technologies have permitted such concepts to be achieved. Accordingly, raising questions around security and confidentiality is crucial to store information securely, particularly by regularly re-encrypting this massive amount of molecular data. An efficient strategy would be to perform these operations at the molecular level, without the use of a digital intermediary (2). In this work, we investigate an experimental in vitro DNA cryptography operation. Combining concepts of DNA origami and cryptography, a design involving a message containing DNA strand, written using a designed character to codon basis, can be encrypted by the formation of a ciphered DNA sequence.

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Controlling The Orientation Of Chiral Double-L Dna Origami On Different Substrates

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The ability to precisely control DNA origami orientation holds immense potential for a wide range of applications.1 This includes the development of advanced metamaterials, highly sensitive chiral sensing platforms, high-density data storage devices, and sophisticated drug delivery systems. Any method to achieve DNA origami orientation control is therefore attractive for both fundamental research and technological innovation.

This presentation explains the simple yet powerful approach to control the orientation of DNA origami nanostructures upon deposition on different substrates. By varying the Mg^{2+} concentra tion of the buffer solution, we demonstrate the ability to control the orientation of a chiral 2D DNA origami shape on the mica surface (Figure 1). A chiral double-L (**CDL**) DNA origami structure was used that can adopt either an **S** or **Z** orientation upon adsorption. **CDL** adsorption on mica was probed by atomic force microscopy (AFM), both for dried samples as well as at the liquid-solid interface. Distributions of **S** and **Z** orientations are shown to depend dramatically on the Mg2+ concentration, ranging from randomly oriented CDLs to exclusive **S**. The results are explained by considering Mg^{2+} induced conformational transitions in the 3D shape of the 2D **CDL** DNA origami. In the second part of the presentation, the influence of different substrates such as silica, graphite, and graphene on **S** or **Z** orientation will be discussed.

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Monitoring the dynamics of a DNA-encaged unfoldase at the single-molecule level

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We have recently constructed a modular chimera that mimics the functional architecture of the 26S proteasome, coupling protein unfolding and proteolysis [1]. The nanomachine consists of two sequentially aligned compartments, A(p97) and B(aCt), made by scaffolded DNA origami cages. The first compartment contains p97, an ATP-dependent unfoldase, that mechanically unfolds the substrate. This then translocates into the second compartment, where it is proteolytically degraded by alphachymotrypsin. We observed that the substrate of p97, the protein I3, is unfolded by A(p97) three times faster than by non-compartmentalized p97, and proteolysis by B(aCt) is five times faster than by non-compartmentalized alpha-chymotrypsin. These findings confirm the widely observed enhanced performance of DNA-scaffolded enzymes relative to their freely diffusing counterparts.[2, 3] A key finding from our previous study, although not yet fully understood, is the uniform spatial orientation of p97, whereby its N-terminal domains consistently align toward one of the cage's apertures. In this work, we propose a strategy for studying the dynamics of p97 in different settings while monitoring motor's operation and relative orientation at the single-molecule level. For this purpose, we will engineer an encaged p97 that functions like a molecular pulley, actively drawing the substrate protein toward itself for unfolding. We envision that the obtained results will contribute to a deeper understanding of p97's operation and will help to shed light onto the mechanisms underlying the enhanced reactivity and preferred orientation of natural and artificial compartmentalized biological nanomachines.

1. Huang J, et al. 2024. Nat Nanotechnol 19: 1521-31
2. Huang J, et al. 2023. Small 19: e2202253
3. Lin P, et al. 2025. Chempluschem 90: e202400483

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Cancelled poster.

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Novel Combinatorial Strand Displacement Mechanisms for DNA Nanostructure-Based Data Storage Devices

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As global data storage demands surge, conventional technologies like magnetic tape, SSD and hard drive face critical limitations in scalability and sustainability. DNA offers a promising alternative due to its exceptional density and durability. Current sequence-based DNA storage methods, however, are hindered by the high cost of DNA synthesis, which requires unique sequences for each bit of data.
This work introduces a novel approach that encodes data using the geometry of DNA nanostructures, much like a compact disc, but at the nanoscale. We've developed two new combinatorial strand-displacement reactions—4-way/6-way strand displacement and associative handhold-mediated strand displacement (AHMSD)—that use two separate domains on different strands of DNA to generate unique N2 orthogonal species from just 2N strands. This dramatically reduces the synthesis cost per addressable location within the nanostructures.
Our preliminary results demonstrate successful, reversible data writing and deletion. In solution, the 4-way mechanism achieved ~60% completion in 3 minutes, while AHMSD reached ≈50% yield within 2 hours. Deletion using 6-way displacement was completed at ≈50% in 15 minutes, with AHMSD taking 2 hours. We further showed position-specific writing on a DNA nanostructure, visualized by AFM imaging as a ~4 nm bump with DNA dumbbell at a targeted location. To identify the set of orthogonal species that we can use, we have designed tested 9 orthogonal toehold pairs and 10 distinct handhold sequences. Both mechanisms showed variable kinetics and about 20% of the combinations showed leakage. Hidden energy drive has been incorporated into the reaction by mismatch repair scheme.
In conclusion, our research establishes fundamental design principles for a cost-effective generation of orthogonal DNA species with applications in geometry-based DNA storage system. These novel strand displacement mechanisms could be generalised in building a large set of DNA species using a small set of DNA strands.

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Controlled 3D Shape Switching in DNA Origami Using Strand Displacement and Thermodynamic Design

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DNA nanostructures that can change shape in response to external inputs are useful for applications in areas like biosensing [1], medicine [2], and adaptive materials [3]. DNA origami allows for building these structures with precise control over geometry and dynamic behavior [4].

In this project, we designed a reconfigurable DNA origami made of triangular units that can fold into two different 3D shapes: an octahedron and a boat. The system is designed to switch between these shapes based on two types of input: (1) strand displacement and (2) temperature changes. To enable higher-throughput screening of experimental conditions, we developed a fluorophore-quencher assay that distinguishes between the octahedron and boat configurations based on their structural differences. Initial validation includes TEM imaging and fluorophore-quencher assays for the strand displacement system which offers programmable, sequence-specific control and could be used for applications such as targeted molecular release triggered by microRNAs.

For the temperature-based switching, oxDNA free energy simulations combined with entropy estimates were used to guide sequence design. The goal is to engineer thermodynamically balanced states that favor one shape over the other depending on ambient temperature. This kind of thermal control could enable applications in autonomous sensing or responsive nanodevices that adapt passively to environmental changes. oxDNA was also used to simulate structural stability, and to identify potential design failures before experimental work.

Overall, this work demonstrates how integrating computational modeling with experimental design can support the development of dynamic, programmable DNA origami devices. By combining multiple design strategies, this approach contributes to a broader toolkit for building shape- changing nanostructures with responsive behavior.

1 Cabane, E. et al., Biointerphases 7, 1-4, 2012
2 Ramesh, M. et al., Biosensors 13, 1 40, 2022
3 Tanjeem, N. et al., Advanced materials 34, 3, 2022
4 Rothemund, P., Nature 440, 297–302, 2006

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Reusable plasmonic microarrays for optical sensing of oligonucleotides

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Sensing and quantifying nucleic acids is of fundamental importance for diagnostics, development of new drugs and the application of personalized therapies. Nanotechnology allows the creation of biosensors that are cost-effective, fast, sensitive and easy to use.
We have realized a reusable multi-readout biosensor that is able to detect hybridization between two complementary sequences by absorption spectroscopy, photoluminescence, fluorescent imaging, and photothermal assessment. The biosensor exploits the optical and morphological properties of gold nanorods (AuNRs) immobilized on a substrate via layer-by-layer electrostatic assembly and functionalized with DNA oligonucleotides. DNA is not modified for the functionalization and the whole technique is water-based. SEM and AFM characterizations revealed that the AuNRs–DNA microarray has suitable morphological properties to promote DNA hybridization. The photothermal properties of the plasmonic microarray are employed to monitor oligonucleotide hybridization under near-infrared laser irradiation and allow its reusability [1].
This biosensor offers promising potential for bioanalytical applications, particularly diagnostics.

1 M. L. Sforza, E. De Marinis, A. Masi, C. Nervi, L. De Sio, F. Petronella. “Multi-readout thermoplasmonic biosensor for rapid detection of oligonucleotides”. VIEW, 20240123, 2025.

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Cisplatin-Cross-Linked DNA Nanoblocks for Combined ChemoRadiotherapy

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DNA nanostructures are promising multifunctional carriers for therapeutic applications, but their use in biological environments is challenged by their limited stability [1]. We address this by cross- linking DNA nanostructures with cisplatin [2], exploiting its DNA-binding, cytotoxic, and radiosensitizing properties to create more robust structures for combined chemo- and radiotherapy. Cisplatin loading of DNA nanoblocks was quantified by inductively coupled plasma mass spectrometry (ICP-MS) identifying an optimal loading of ~350 cisplatin molecules per nanostructure. Structural integrity was assessed using atomic force microscopy (AFM) and transmission electron microscopy (TEM).

Cytotoxicity was evaluated in FaDu and MCF7 cancer cell lines using the MTT assay, showing significant effects at nanomolar concentrations of cisplatin-cross-linked nanoblocks. Cellular uptake of cisplatin in cells was quantified by ICP-MS and shown to persist for up to 96 hours consistent with the observed uptake of Cy3-labeled cisplatin-cross-linked nanoblocks in both cell lines in fluorescence microscopy images. Clonogenic assays following gamma irradiation revealed a radiosensitizing effect in FaDu cells at higher cisplatin-cross-linked nanoblock concentrations, but not in MCF7 cells, despite similar uptake levels—suggesting that cell-line-specific biological responses may play a role in the radiation response. Preliminary in vivo imaging data will also be presented highlighting the biodistribution of intravenously injected IRDye800-labeled DNA nanoblocks, supporting their potential for systemic delivery.

Our results demonstrate the potential of cisplatin-cross-linked DNA nanostructures as stable carriers for chemotherapeutic and radiosensitizing agents, as well as fluorescent probes for imaging. The platform shows promise for multimodal cancer therapy, with identified areas for further optimization to enhance biomedical applicability.

We acknowledge the support from the Czech Science Foundation grant no. 24-11503S.

1 Keller, A., and Linko, V., Angew. Chem. Int. Ed. 2020, 59,15818–15833.
2 Sala, L., et al., ACS Appl. Nano Mater. 2022, 5, 13267–13275.

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Manipulating communication between DNA origami compartments

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Communication is one of the fundamental properties of living systems. Cells constantly exchange information using small molecules; however, understanding the molecular pathways remains difficult due to the overall complexity of living organisms. One of the ways to address this problem is a bottom-up approach, which uses simplified model systems to study individual biological processes. The aim of this project is to create a system of communicating nanosized compartments to study how spatial organization and segregation affect the kinetics of the signal transfer between different populations. This can be achieved by combining the spatial addressability of structural DNA nanotechnology with the reaction programmability of dynamic DNA nanotechnology. As a model reaction cascade, we implement an enzyme-free AND logic gate based on the toehold-mediated strand displacement (TMSD) principle, with its steps segregated into two populations of DNA origami compartments, referred to as 'sender'and 'receiver'. The cascade starts with an input of single-stranded

DNA, which is decoded in the sender compartment and transformed into an output signal. This molecular information is then transferred from the sender to the receiver via passive diffusion. Once in the receiver, the signal will be re-encoded generating a feedback response that will be sent back to the sender, closing the cycle. Our objective is to examine how reaction kinetics vary based on the stoichiometry of each component within the cascade and their spatial segregation in physically distinct compartments, as opposed to freely diffusing species in solution. In the long term, we aim to immobilize DNA origami on surfaces and connect them into more ordered structures, creating a spatially organized system that mimics communication between populations of protocells positioned at distances comparable to their size. This approach will enable us to study paracrine-like signaling and spatial pattern formation in a simplified manner.

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Engineering colloidal DNA-magnetic nanoparticles for programmable biosensing

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Colloidal DNA-labelled magnetic nanoparticles (MNPs) enable enzyme-free biosensing and molecular recognition of nucleic acids and proteins, with their inherent magnetic properties being minimally interfered by background signals [1]. Upon a successful molecular recognition between capture ssDNA strands on MNPs and target strands in solution, the particle hydrodynamic size increases, thus slowing down the Brownian relaxation dynamics of MNPs that can be detected using magnetic particle spectroscopy (MPS). However, the interaction between DNA strands on MNPs and how their grafting density influences their molecular recognition towards targets remain poorly understood1. Here, we encapsulate single-core custom MNPs inside a thin polymeric shell, enabling their assembling with ssDNA strands of different lengths at various grafting densities through copper-free click chemistry conjugation (Fig. 1a). To investigate the interaction between ssDNA strands on MNPs, we conducted titration assays at four distinct DNA grafting densities by adding complementary strands to DNA-labelled MNPs over a broad concentration range (Fig. 1b). We then measured changes in magnetic relaxation dynamics by recording MPS harmonics spectra. As a complementary technique, we applied dynamic light scattering (DLS) to measure changes in particle hydrodynamic size upon binding to target and established a correlation between MPS and DLS results.

To characterize the titration curves, we fitted a Hill-like function to the data (Fig. 1b and c) and extracted two characteristic parameters $K_{1/2}$ and n, which represent the target concentration where half of the capture strands are hybridized, and the extent of cooperativity, respectively. The titration experiments indicate that low DNA grafting density yields a Hill coefficient $n < 1$, indicating minimal cooperativity between ssDNA strands, thereby resulting in a shallower response to target DNA and benefiting a broader dynamic range for detection. While at high DNA grafting densities, the dense packing of ssDNA strands promotes their cooperativity on the MNPs, as indicated by $n > 1$, resulting in a sharper response to target binding but a narrower response window. We therefore propose that DNA strands are coiled on MNPs at low grafting densities, whereas at high grafting densities they adopt a brush-like conformation (Fig. 1b, inset). Our atomic force microscopy studies reveal a transition of ssDNA from being coiled to forming brushes as their grafting density increases. We will further discuss what is the length of the brushed shell and how this influences the multiplexed target detection.

References:
1 A. Lak, Y. Wang, et al. Cooperative dynamics of DNA-grafted magnetic nanoparticles optimize magnetic biosensing and coupling to DNA origami. Nanoscale 16, (2024): 7678-7689.

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ATP-responsive membrane-less compartments in synthetic cells

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Adenosine triphosphate (ATP) plays a crucial role in a wide range of cellular processes. It provides cells with a source of chemical energy to perform essential functions necessary to sustain life, from the molecular transport across cell membranes to the biosynthesis of macromolecules [1].

One of the main goals of synthetic biology is to design artificial systems with life-like structures and behaviour, often referred to as synthetic cells [2]. Owing to its interdisciplinary nature, DNA nanotechnology has become an established approach for building both structure and function in synthetic cellular systems [3]. Given the fundamental importance of ATP and the ubiquity of membrane-less compartments in cells, it would be highly desirable to engineer pathways in synthetic cells that allow them to store and respond to ATP.

Here, we introduce ATP-responsive membrane-less compartments assembled from DNA nanostructures. We achieve this by rationally embedding split ATP aptamers into a tetravalent DNA nanostructure. In the presence of ATP, the split ATP aptamers bind together, triggering the assembly of the DNA-based condensates, thus functioning as ATP storage units. Through systematic design, we can tune the melting temperatures of these condensates, providing control over the amount of stored ATP. Finally, when assembled inside synthetic cells, our ATP-responsive membrane-less organelles can be coupled to biological processes that depend on ATP, leading to controlled organelle disassembly and subsequent release of ATP.

Our strategy expands the toolkit for engineering responsive behaviours in synthetic cellular systems by enabling ATP-dependent compartmentalisation and controlled release, thereby regulating energy availability within synthetic cells.

1 Dunn, Jacob; Grider, Michael H. “Physiology, Adenosine Triphosphate”. StatPearls. StatPearls Publishing 2023
2 Benner, S.; Sismour, A. Synthetic biology. Nat. Rev. Genet. 2005, 6, 533–543.
3 Jungmann, R.; Renner, S.; Simmel, F. C. From DNA nanotechnology to synthetic biology. HFSP J. 2008, 2, 99–109.

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Engineering Active Biomimetic Nanomotor in Lipid Membranes

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The development of artificial nanomachines that mimic the functionality of biological membrane motors represents a frontier in synthetic biology and nanotechnology. In this work, we present the design and construction of a dual-component DNA-based transmembrane nanomotor—engineered through scaffolded DNA origami—inspired by the rotary mechanism of the FoF_1-ATP synthase. This nanomotor features a DNA nanopore as the stator and a twisted DNA structure as the rotor. The nanopore stator is designed for membrane insertion, enabling the nanomotor to operate in biocompatible membrane systems. The rotor couples to the hydrodynamic flow inside the DNA nanopore, converting the transmembrane potential gradient into mechanical torque. Salinity gradients or transmembrane voltage will power the assembled dual-component nanomotor, and the nanomotor's performance will be characterised by tracking unidirectional rotation using high-speed single-molecule fluorescence microscopy.

This work establishes a new class of active, energy-consuming membrane DNA nanomachines. It will open avenues for the development of synthetic nanoscale systems with potential applications in targeted drug delivery and the construction of autonomous synthetic cells. By bridging the gap between passive sensing and active mechanical function, our DNA turbine represents a significant step toward programmable, membrane-integrated nanorobotics.

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A Universal Approach for DNA Origami Folding and Functionalization Using a Reactive Polyamine

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DNA origami is a powerful bottom-up technique that leverages specific Watson-Crick-Franklin base pairing to build complex and reproducible nanostructures with precise size and shape control. These biocompatible nanostructures offer diverse applications in biomedicine and nanobiotechnology, such as therapeutic delivery, biosensing, and biomimetics. However, many of these applications do normally require origamis to be chemically equipped with functional moieties.

Here, we present a versatile strategy to fold and chemically modify DNA origami exploiting the reactivity of the polycation spermine-azide (SpAz). SpAz facilitates DNA origami folding and enables its subsequent conjugation via Strain-Promoted Alkyne-Azide Cycloaddition (SPAAC) with three dibenzocyclooctyne (DBCO)-functionalized molecules: a fluorophore (Cy5), polyethylene glycol (PEG), and a phosphatidylethanolamine (PE) tag. Successful SpAz-mediated folding of DNA origami was verified by gel electrophoresis (GE), dynamic light scattering (DLS), atomic force microscopy (AFM), and transmission electron microscopy. Cy5 conjugation resulted in a high functionalization degree, confirmed by UV-Vis spectroscopy, GE, and confocal microscopy. PEG modification increased the hydrodynamic diameter, height, and volume of the origamis, as shown by DLS and AFM. Interestingly, the incorporation of PEG altered the nanomechanical properties of the origamis, leading to a decrease in Young's modulus, as verified by nanoindentation measurements.

Additionally, SPAAC functionalization was exploited to mediate the attachment of SpAz-folded origamis to giant unilamellar vesicles (GUVs), which serve as biomimetic models for cell membranes. Confocal microscopy confirmed successful binding to PE-DBCO-containing GUVs. No attachment was observed in the absence of PE-DBCO, confirming that the process was SPAAC-mediated. Heterovalent functionalization with both Cy5 and PE was also demonstrated, with a remarkable influence of the stoichiometric ratio between Cy5 and SpAz.

Our study introduces a facile strategy for folding and functionalizing DNA nanostructures by leveraging the reactive polyamine SpAz. This approach enables the efficient conjugation of diverse functional tags while preserving origami structural integrity, making it adaptable for various applications in bioimaging, therapeutic delivery, and biomimetics. By providing a straightforward and versatile method, this work expands the potential of DNA origami and DNA nanotechnology for the development of functional nucleic acid-based materials.

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AutoMod: Automated Modification Placement for DNA Origami Design

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Several software have been developed for the design of curved DNA origami structures, including DNAxiS [1] and MagicDNA 2.0 [2]. However, these software have limitations that result in some structures being either completely outside their design space or only realizable with low design accuracy.

In this work, we present a general solution for finding the length differences between helices of a curved design and translating them to a pattern of insertions and deletions, as well as a set of optimal crossover positions, in a caDNAno [3] file. This solution is formulated in terms of arc length differences between parametric curves that represent the desired locations of helical axes. We introduce novel software, called AutoMod, that implements the aforementioned solution. AutoMod aims to complement the previous design software by achieving high-accuracy curvature in the cases where previous software either cannot be applied or produce structures whose equilibrium conformation notably deviates from the desired target shape.

We demonstrate the functionality of AutoMod by designing several curved DNA origami structures and showcase significant improvements in design accuracy by comparing oxDNA [4] simulation results for structures designed with AutoMod and other methods.

[1] D. Fu et al., "Automated design of 3D DNA origami with non-rasterized 2D curvature," *Science Advances*, 8(51), eade4455, 2022. doi: 10.1126/sciadv.ade4455

[2] W. G. Pfeifer et al., "Versatile computer-aided design of free-form DNA nanostructures and assemblies," *Science Advances*, 9(30), eadi0697, 2023. doi: 10.1126/sciadv.adi0697

[3] S. M. Douglas et al., "Rapid prototyping of 3D DNA-origami shapes with caDNAno," *Nucleic Acids Research*, 37(15), pp. 5001-5006, 2009. doi: 10.1093/nar/gkp436

[4] E. Poppleton et al., "oxDNA: coarse-grained simulations of nucleic acids made simple," *Journal of Open Source Software*, 8(81), 4693, 2023. doi: 10.21105/joss.04693

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Nanoelectrode arrays (NEAs) as electrochemical sensor platforms for CRISPR-Cas based diagnostics

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The applicability of CRISPR-Cas has gone beyond genome editing and has found expansive use in molecular diagnostics. While there have been many fluorescent sensors, electrochemical sensors for CRISPR-Cas are now rapidly gaining attention as they reduce the need for expensive instruments, are low-cost, and can be used in low-resource settings, satisfying the requirements for an ideal point- of-care testing (POCT) device [1].
Conventional electrochemical sensors focus on a signal-off strategy, which limits detection range and sensitivity. In this study, we present the initial development of a novel electrochemical sensing platform based on nanoelectrode arrays (NEAs) [2] coupled with a signal-on strategy.
NEAs are fabricated by forming arrays of nanoholes in a thin polycarbonate (PC) film deposited on a conductive substrate through Thermal Nanoimprinting Lithography (TNIL). The presence of carboxylic acid groups on PC enables covalent functionalization with oligonucleotide probes, forming the basis of a biosensing interface [3]. Surface activation methods were evaluated, with NaOH treatment demonstrating superior performance compared to UV/Ozone (UVO). Conditions for covalent DNA immobilization were optimized, with improved functionalization observed at basic pH 8 and optimal DNA concentration of 0.5 µM [4]. Initial CRISPR-Cas assay results on PC substrates are also presented. Future work will explore the use of NEAs in electrochemical sensing and the development of microfluidic devices for single and multiplexed CRISPR-Cas assays. The combination of NEAs and CRISPR-Cas holds strong potential to advance molecular diagnostics for clinical and environmental applications.

References

[1] Y. Dai, R. A Somoza, L. Wang, J. F. Welter, Y. Li, A. I Caplan, C. C. Liu, *Angew. Chem. Int. Ed*. 2019, 58, 17399.
[2] D.W.M Arrigan, *Analyst*. 2004, 129, 1157-1165
[3] A. Zanut, A. Cian, N. Cefarin, A. Pozzato, M. Tormen, *Biosensors*. 2020, 10(8), 90
[4] S. Fortunati, I. Vasini, M.Giannetto, M. Mattarozzi, A.Porchetta, A.Bertucci, M. Careri, *Anal. Chem*. 2022, 94, 5075-5083

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A Graph-Theoretical Approach to the Optimal Self-Assembly of Polyhedral Capsids from DNA Origami Building Blocks

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Controlling the self-assembly of complex nanostructures is one of the central goals in DNA nanotechnology, where DNA origami provides a versatile platform for designing programmable interactions. While polyhedral structures like the icosahedron have been successfully assembled both computationally and experimentally, more complex targets such as the snub cube pose additional challenges due to kinetic traps and competing configurations.

In this work, we focus on designing a self-assembling system based on DNA origami building blocks to form snub cubes. Using patchy particles as a coarse grained model for DNA origami monomers, we simulate the assembly of finite-sized polyhedral structures. The patchy interactions encode directional binding rules that reflect the selective hybridization behavior of DNA strands.

To design optimal interaction patterns, we use the SAT-assembly framework, which reformulates the self-assembly problem into a Boolean satisfiability (SAT) problem. This approach allows us to efficiently explore a vast design space, identifying conditions under which the desired structures form while avoiding undesired or kinetically trapped configurations.

While the icosahedron can be reliably assembled using this method and has been validated experimentally, the snub cube presents additional complexity. Among the SAT derived solutions, only one initially led to successful in silico assembly of the snub cube. A graph-theoretical analysis of this solution revealed a key degenerate connected component shared across all species. Using this insight as a design principle, we incorporated constraints on this component within the SAT formulation, which enabled us to generate additional valid solutions and markedly improved the yield, reaching up to 90%.

Overall, our approach combines algorithmic design and graph-theoretical analysis within a DNA origami driven framework, offering a novel and robust strategy for programming the self-assembly of complex nanostructures with high precision.

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Following the kinetics of single molecular events on DNA origamis with gold nanosphere dimers

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We develop a colorimetric sensing platform compatible with single-molecule detection by assembling gold-nanosphere dimers on a Y-shaped DNA origami that acts as a nanoscale actuator (Fig.1-a). DNA origamis are a highly programmable and flexible platform that can be precisely engineered to undergo controlled conformational changes in response to specific molecular targets, such as DNA/RNA strands, proteins, or specific cations [1]. To translate such conformational changes in colorimetric information, we exploit the nanoscale dependence of plasmon coupling between two gold nanospheres [2,3].

The scaffold of our DNA origami features an active site with a conformation that can be tuned by hybridizing specific DNA single strands (Fig. 1-b). One-step digital colorimetric sensing of DNA single strands is achieved using a strand displacement reaction. These measurements are carried out both by performing single-nanostructure scattering spectroscopy (Fig.1-c) and by analyzing the hue of single dimers in dark-field images (Fig.1-d), obtaining similar statistical responses. The kinetics of these single DNA sensing events are monitored as a function of the concentration of the target strands (Fig. 1-e) and by introducing one or two base mismatches in the DNA sequence to characterize the sensitivity and specificity of these hybrid nanosensors. These results highlight the versatility of DNA origamis for colorimetric sensing, in particular to monitor single DNA or RNA strands on a simple color camera.

1 Y. Ke et al., Nat. Commun, 7:10935, 2016

2 L. Lermusiaux et al., ACS Nano, 9:978, 2015

3 L. Lermusiaux et al., Langmuir, 34:14946, 2018

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DNA origami nanocompartments to model confined nanospaces

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Metabolic compartmentalization enables the separation of pathways and components in the cell and is a key feature found throughout biology [1]. Compartments offer two primary advantages: isolation and condensation [2]. While the former shields the reaction from the environment, the latter typically increases the local concentration of reactants. In this study, we use a DNA origami compartment to model spatial confinement. The programmability of DNA opens unique opportunities to explore the effect of stoichiometry, segregation and spatial distribution on the internalized reaction [3]. Our compartment contains two DNA species, a sender and a receiver. Addition of a trigger strand initiates a DNA strand displacement (DSD) reaction that releases a transmitter from the sender complex. The transmitter then binds to the receiver complex, terminating the cascade. Since the DSD species are modified with fluorophores, the entire cascade is monitored by fluorescence spectroscopy. We hypothesize that the kinetics of the reaction and diffusion of the transmitter within the compartment or across its boundaries will be affected by the degree of permeability of the DNA walls and by the effective concentration of the species, which is determined by the spatial constraints. These aspects essentially represent the isolation and condensation features typically observed in natural compartments. To systematically examine these phenomena, we aim to modulate the permeability and internal dimensions of the DNA compartments through various strategies, including the addition of an miniscaffold-based inner layer of DNA helices [4], and to complement ensemble measurements with single-molecule FRET to investigate the process in detail.

1. Bar-Peled, L., Kory, N. Nat Metab 4, 1232-1244 (2022).
2. Ichihashi, N., Yomo, T. Curr Opin Chem Biol 22, 12-17 (2014).
3. Huang, J., Gambietz, S., Sacca, B. Small 19, e2202253 (2023).
4. Ke, Y. et al. Chem Sci 3, 2587-2597 (2012).

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pH-sensitive DNA Zippers for enhanced nanopipette biosensors

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Dynamic DNA nanostructures (DONs) can be used as triggerable switches in novel chemical and biotechnological applications, including electrochemical sensing. One such example is a so-called Zipper DON [1] that can adopt either a closed or open conformation based on the formation of pH-sensitive DNA triplexes. This property makes the Zippers excellent sensor components for pH-dependent sensing devices. Nanopipette as an electrochemical biosensor is an emerging class of low-cost, rapid sensing devices that measure changes to the surface charge of its internal walls. The surface charge creates an electrical double layer overlap at the tip, resulting in a non-ohmic electrical response known as ion current rectification. Here, in combination with the Zippers, the device allows for a highly sensitive and tunable pH-relevant biosensor as the DON modulates the ion current rectification based on its pH-dependent conformation.[2]

[1] P. Williamson, H. Ijäs, B. Shen, D. K. Corrigan, and V. Linko, “Probing the Conformational States of a pH-Sensitive DNA Origami Zipper via Label-Free Electrochemical Methods,”Langmuir, vol. 37, no. 25, pp. 7801–7809, Jun. 2021, doi: 10.1021/acs.langmuir.1c01110.

[2] R. Davis, O. Reinsalu, V. Linko and R. Johnson, article in preparation, 2025

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Modular Virus Capsid Coatings for Biocatalytic DNA Origami Nanoreactors

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Micro- and nanoscale compartments are essential in living organisms. They offer a unique environment promoting chemical reactions with high specificity and efficiency due to high enzyme and substrate concentrations while protecting possible intermediates and reducing competing cross- reactions. [1] Protein cages and custom DNA origami structures have emerged as artificial nanocontainers to mimic compartmentalization of naturally occurring biocatalytic reactions. While the highly ordered protein capsids excel in gating interactions between enzyme and substrate, [2,3] stoichiometric control over the cargo remains challenging. [4] The high addressability of DNA origami, on the other hand, allows for a high degree of control over the enzyme type, amount and precise location along the surface of the nanostructure. [5] Nevertheless, both approaches would benefit from overcoming challenges related to controlled enzyme loading and enzyme-substrate interactions.

In this work, we cleverly merge virus capsid-based protein cages and DNA origami to exploit their synergistic properties for biocatalysis and to gain control over enzyme-substrate interactions while regulating the cargo load. We demonstrate size selectivity towards the substrate molecule depending on the amount and type of capsid protein used for the encapsulation. The capsid cage additionally protects the biocatalytic unit from nuclease degradation, and functionalizing the nanoreactor's surface with antigen-targeting moieties allows targeting for delivery purposes. Thus, we believe our approach provides an attractive platform not only for biomedical applications but also, because of its modularity, for rapid investigation of the physicochemical properties of capsid proteins. [6]

References

[1] A. Küchler et al. 2016. Enzymatic Reactions in Confined Environments. Nat. Nanotechnol. 11:409– 420

[2] E. Selivanovitch et al. 2021. Molecular Exclusion Limits for Diffusion Across a Porous Capsid. Nat. Commun., 12:2903

[3] J. Glasgow et al. 2015. Influence of Electrostatics on Small Molecule Flux Through a Protein Nanoreactor. ACS Synth. Biol., 4:1011-1019.

[4] D. McNeale et al. 2023. Protein Cargo Encapsulation by Virus-Like Particles: Strategies and Applications. Wiley Interdiscip. Rev.: Nanomed. Nanobiotechnol., 5:e1869

[5] J. Huang et al. 2023. Self-Assembled Artificial DNA Nanocompartments and Their Bioapplications. Small. 19:2202253

[6] I. Seitz et al. 2025. submitted

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Controlling in-vitro transcription through covalent DNA-dimerization netwoks

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Living systems are an inspiring example of how fundamental functions can emerge from networks of interacting molecules. Competitive dimerization networks, for example, are composed by families of proteins that bind to each other to form a combinatorial library of dimers that play a crucial role in the downstream activation of specific signalling pathways. In recent years, synthetic DNA has emerged as an extraordinary nanomaterial to build synthetic molecular networks that exhibit complex input-output behaviour. Inspired by naturally-occurring systems, we demonstrate here a strategy to rationally program in-vitro transcription using synthetic DNA-based dimerization networks. The approach we propose is based on the use of a DNA-based competitive dimerization network consisting of DNA monomers modified with reactive groups that can covalently bond to each other and create a library of DNA dimer outputs. In the presence of specific DNA input strands that sequester DNA monomers, we can trigger the formation of a specific DNA dimer output able to activate an in-vitro transcription system. The strategy is highly versatile and we demonstrate the possibility to finely modulate the in-vitro transcription process using different network sizes and input sets. The programmability of these DNA-based dimerization networks also enables the orthogonal transcription of different fluorogenic aptamers. Finally, the DNA networks proposed here allow to perform complex input-output computations in a highly predictable and programmable manner.

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Interaction of DNA Nanomotors with Lipidic Membranes for Cell-Inspired Ultra-Soft Microrobots

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Most state-of-the-art nanomedicine protocols rely on passive diffusion of nanoparticles (1), which results in limited tissue targeting and penetration efficiency. Active nanomotors can overcome this by achieving active and directed movement by sourcing energy from the environment (2). Herein, we propose a hybrid micro-nanoscale system comprised of an ultra-soft, microscale body, coupled with DNA-origami nanomotors. Inspired by amoeboid locomotion of immune cells, this vesicle- based body has proven capable of infiltrating interstices smaller than its diameter thanks to passive, large body deformations (3, 4). The embedding of nanomotors aims to induce active and controllable deformations that can be harnessed to transmit forces to the environment and achieve displacement (5).

We fabricated nanomotors by decorating self-assembled DNA nanotubes with enzymes (6). To achieve a microscale effect of the nanomotor activity, we performed an in-depth study on the design parameters modulating their interactions with the vesicle membrane, including aspect ratio, number and localisation of enzymes, number and length of membrane links. We analysed the localisation of the motors and the effect of the membrane-nanomotor coupling on the membrane dynamics and on the nanomotor motility. We studied the interaction by real time confocal microscopy and single particle tracking.

This hybrid cell-inspired microrobot design opens the path for ultra-soft medical devices, whose body compartment can be loaded with chemically complex and customisable cargo. Moreover, it potentially offers a platform to study the minimal elements of cellular locomotion.

1. C.-Y. Hsu et al., South African Journal of Chemical Engineering. 46, 233–270 (2023).
2. S. Chen et al., (2022), doi:10.1039/D2TB00556E.
3. E. De Remigis et al., IEEE Transactions on Medical Robotics and Bionics. 7, 123–129 (2024).
4. E. De Remigis, O. Tricinci, M. Ibrahimi, V. Iacovacci, S. Palagi, (Delft, The Netherlands, 2024).
5. G. Charras, E. Paluch, Nature reviews. Molecular cell biology. 9, 730–736 (2008).
6. T. Patiño Padial et al., J. Am. Chem. Soc. 146, 12664–12671 (2024).

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DNA-based MicroRNA-sensing Artificial Cells for Prostate Cancer Diagnosis

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MicroRNAs (miRs) control protein expression in cells and some have been found to be deregulated in cells and bodily fluids of cancer patients compared to healthy individuals. Despite their great potential as minimally invasive biomarkers, there is currently no cancer diagnostic test based on circulating miR detection, mainly due to their low endogenous concentration. Prostate cancer (PCa) is the most prevalent cancer in men and novel early-detection strategies could potentially reduce deaths from PCa through earlier diagnosis and more timely intervention. We are using DNA nanotechnology to build membraneless artificial cells, using cholesterol-functionalised DNA nanostars (C-Stars), for multiplexed detection of PCa-associated miRs directly from blood plasma, forgoing the need for cumbersome extraction and PCR amplification methods[1]. Target miRs can diffuse and interact with specific binding sites within the artificial cell, leading to characteristic reaction-diffusion patterns, which can then be recorded and modelled for quantitative analysis[2].

Initial proof-of-concept studies were successfully conducted using DNA equivalent versions of miR-141-3p, which was found to be upregulated in PCa patients. The C-Star artificial cells were introduced into wells containing miR solutions at different concentrations and Z-stack images were taken over time using confocal microscopy. A catalytic hairpin assembly mechanism was also introduced for signal amplification, allowing a detectable signal to be generated from target concentrations as low as 10 nM.

Future work will include integration of a multiplexed design to simultaneously detect miR-141-3p and miR-375-3p, as well as further limit of detection improvement. The adjustable miR binding site sequence on the artificial cells also allows this platform to be tailored for any type of cancer that shows an overexpression of specific miRs. This work highlights the potential of DNA nanotechnology to improve accurate diagnosis and monitoring through public screening.

1 Brady et al. 2017. Nano Lett. 17:3276.
2 Leathers et al. 2022. JACS. 144:17468.

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Expressing membrane-less RNA organelles in lipid-based synthetic cells

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Bottom-up synthetic biology aims to create biomimetic synthetic cells to perform out-of-equilibrium, 'life-like'functions such as growth and division [1]. Giant unilamellar vesicles (GUVs) are good cell mimics due to having sizes similar to living cells, and faithfully imitating the properties of their membranes [2]. Recently, Fabrini et al. have developed branched RNA constructs, or "nanostars", which can be transcribed from DNA templates via in vitro transcription. The nanostars fold and assemble co-transcriptionally via complementary kissing loop (KL) domains, creating synthetic biomolecular condensates with controlled number, size, composition, and the ability to selectively recruit pro-teins.[2] Here, we demonstrate the encapsulation of transcription machinery using the emulsion phase transfer technique to grow these RNA organelles in GUVs. These organelles can grow over time and can be engineered to bind to the GUV membrane allowing with different levels of adhesion.

1 Hirschi S et al. 2022. Synthetic Biology: Bottom-Up Assembly of Molecular Systems. Chemical Reviews. 122: 16294-16328.

2 Fabrini, G., Farag, N., Nuccio, S.P. et al. 2024 Co-transcriptional production of programmable RNA condensates and synthetic organelles. Nat. Nanotechnol. 19, 1665–1673

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Improving internalization of DNA origami and tetrahedron via cell penetrating peptides (CPPs) complexation for future RNA therapeutic delivery

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KEYWORDS: *RNA based therapeutics, DNA nanotechnology, nucleic acid nanostructures (NANs), cell penetrating peptides (CPPs), non-viral vectors, RNA therapies*

RNA-based therapeutics, including small interfering RNAs (siRNAs) and microRNAs (miRs), have emerged as promising tools to modulate regenerative pathways in ischemia-damaged cardiac tissue. However, their clinical application remains limited by the lack of safe and effective delivery systems.

DNA nanotechnology provides a versatile and biocompatible platform to overcome this limitation. By exploiting Watson–Crick–Franklin base pairing, DNA can be used as a programmable building block to construct nucleic acid nanostructures (NANs) with customizable properties (1). These structures can encapsulate or hybridize with different RNA cargos, through sequence complementarity, allowing for controlled loading and efficient release.

To improve the cellular uptake of these NANs, cell penetrating peptides (CPPs) are used as delivery enhancing agents. These short, cationic peptides facilitate translocation across negatively charged cellular membranes and interact electrostatically with DNA nanostructures (2). The CPP to NAN ratio (N/P) must be optimized to ensure the formation of stable complexes suitable for cellular delivery.

In this proof of concept study, NANs in the form of origami and tetrahedron were assembled and functionalized with two CPP families (PF14-AH and PF14-AK). Their uptake was evaluated in U87 Luc2 and HaCaT cells, showing a marked improvement in internalization without compromising cell viability.

These results provide a starting point for the development of non-viral delivery strategies in RNA therapies. Current efforts are focused on expanding this approach to alternative nanostructures, such as DNA nanohydrogels, with the ultimate goal of delivering miRs to hiPSC-derived cardiomyocytes as a proof of concept of their efficacy in a translational model.

REFERENCES

1. Kosara Sm Singh R, Bhatia D. Structural DNA nanotechnology at the nexus of next-generation bio applications: challenges and perspectives. Nanoscale Adv. 2023;6(2):386–401.

2. Huang YW, Lee HJ, Tolliver LM, Aronstam RS. Delivery of nucleic acids and nanomaterials by cell- penetrating peptides: Opportunities and challenges. Biomed Res Int. 2015;2015:11–3.

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Toward a Functional DNA Nanoturbine Built from a Single Double Helix

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We propose the design and ongoing development of a DNA-based transmembrane nano turbine—an innovative molecular machine that converts ionic gradients or electric potentials into unidirectional mechanical rotation. Constructed using DNA origami techniques, this nanoturbine integrates a single double-helix rotor within a robust, membrane-spanning nanopore. Inspired by biological rotary motors, the device aims to achieve controlled, nanoscale rotation, potentially establishing the world's smallest functional turbine. The project combines coarse-grained molecular dynamics simulations with experimental validation through Atomic Force Microscopy (AFM), Transmission Electron Microscopy (TEM), electrophysiology, and high-speed fluorescence imaging. This work not only addresses fundamental challenges in nanopore engineering but also opens new avenues for powering nanoscale devices and advancing synthetic molecular machinery.

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Rewiring DNA repair activity into CRISPR signal transduction via synthetic DNA Transducers

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Nature's enzymatic reaction networks rely on intricate communication pathways to coordinate complex biochemical functions. Translating such communication to synthetic systems remains challenging, particularly between unrelated enzymes. Here, we present DNA-based molecular transducers (DNA Transducer) that enable artificial communication between DNA repair enzymes and CRISPR- Cas12a (Figure 1). The Transducers are rationally designed hairpin DNA probes bearing specific lesions (e.g., uracil or 8-oxoguanine) that serve as substrates for mono- or bifunctional glycosylases (UDG and hOGG1, respectively). Enzymatic repair induces structural reconfiguration of the DNA Transducer, activating Cas12a-mediated collateral cleavage. This one-step cascade converts enzymatic activity into amplified fluorescence output, enabling the direct monitoring of repair activity in cell lysates without the need for auxiliary enzymes or multistep processing. Crucially, the system supports throughput screening of small-molecule inhibitors, as demonstrated by profiling 8-oxoguanine glycosylases-targeted compounds. By coupling DNA repair dynamics to a CRISPR output signal, this modular platform offers a scalable and generalizable strategy for real-time diagnostics, mechanistic studies, and drug discovery applications.

References

(1) Srivastava, D.; Bernhard, S. Enzyme–enzyme interactions and the regulation of metabolic reaction pathways. Current topics in cellular regulation 1986, 28, 1-68.

(2) Del Giovane, S.; Bagheri, N.; Di Pede, A. C.; Chamorro, A.; Ranallo, S.; Migliorelli, D.; Burr, L.; Paoletti, S.; Altug, H.; Porchetta, A. Challenges and perspectives of CRISPR-based technology for diagnostic applications. TrAC Trends in Analytical Chemistry 2024, 117594.

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Functional DNA Nanodevices for Programmable Enzyme-Triggered Activation and Deactivation of Transcription

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Here, we develop orthogonal enzyme-driven DNA transcriptional timers capable of rationally programming a tunable delay of in vitro transcription onset. These timers are based on blocker strands that, by binding to the promoter region of the DNA template, prevent transcription initiation. The blockers can subsequently be removed via specific enzymatic reactions. Once the blocker strand is removed, an input strand binds and completes the promoter, thus activating transcription. The kinetics of enzymatic blocker degradation control the timing of transcription onset. We designed three tunable timers using RNase H, Uracil-DNA Glycosylase (UDG), and DNA-formamidopyrimidine glycosylase (Fpg) with their respective blocker strands. By varying the concentrations of the blocker or the enzyme, we can modulate the onset of transcription. Moreover, following the same design strategy, we successfully programmed not only the transcriptional activation (turning on) but also the deactivation (turning off) of the reaction. This provides a versatile strategy for rationally programming both the onset and termination of in vitro transcription. Additionally, the orthogonality and high programmability of these timers allow us to control the timing of all three systems in a one-tube reaction. This technology can further be applied to modulate the collateral activity of gene-editing enzymes like Cas12.

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Advancing continuous biosensing through single particle plasmonic imaging of DNA nanoswitches

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Continuous biosensors rely on affinity-based nanoswitches that undergo target-induced conformational changes and as such enable real-time biomarker detection in healthcare and biotechnology applications1. However, developing sensitive and fully reversible affinity-based nanoswitches for continuous biosensing is hindered by limited understanding of their mechanism of action.

We recently developed a fiber optic surface plasmon resonance (FO-SPR) biosensor using affinity-based DNA nanoswitches with AuNPs for sensitive target detection via competitive DNA hybridization2. To deepen our understanding of nanoswitches' behavior, in this work we demonstrate a novel approach of imaging AuNPs tethered to the gold-coated surface of an optical fiber. By exciting localized surface plasmons on the AuNPs via propagating plasmons on the gold film, we resolved 40 nm AuNPs with high signal-to-noise ratio and low background scattering. This single particle (SP) method established a robust toolbox for analyzing nanoswitches' behavior by enabling simultaneous, nanoscale tracking of the distance of AuNPs from the surface through intensity changes, as well as their XY motion at the nanoscale over time.

Our SP tracking approach offered previously inaccessible insight into the nanoswitch heterogeneity, providing spatial and temporal information about their behavior. Additionally, it visually confirmed that the bulk signal originates from the spatial redistribution of AuNPs upon target binding.

In conclusion, we believe these SP measurements pave the way for advancing mechanistic understanding of nanoswitches' behavior, promoting the design and development of novel nanoswitches. This platform will provide quantitative insight into the influence of key parameters such as AuNP- film spacing and the affinities among different bioassay components (e.g., the capture probe, competitor, and target) on nanoswitch performance.

1 Dillen, Lammertyn, Analyst 2022, 147, 1006–1023.
2 Dillen et al., ACS Sensors 2023, 8 (2), 811-821.

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Nucleic Acid-based Plasmonic Materials for Nanodevice Applications

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In recent years, DNA ability to self-assemble into predetermined geometries, following Watson−Crick base-pairing rules, allowed the tailoring of functional supramolecular systems, which opened several opportunities in the fields of nanotechnology and material science. In this context, the DNA origami technique represents a general way to construct nano-objects with defined static 3D shapes, while machine-like dynamic assemblies, whose structure can be programmed to switch across states, showed the potential to exert precise motion control over matter at the nano-size level. In a recent study, large tubular assemblies of hybrid DNA-based plasmonic nanomaterials, with programmable chiral properties, were attained 1. Specifically, micrometer-long DNA templates were used to organize gold nanoparticles, providing an example of large chiroplasmonic nanostructures synthesized by a simple self-assembly process. Such DNA nanotube structures, detected with atomic force microscopy and electron microscopy, were nicely twisted in a “fusilli” fashion (i.e., a type of Italian pasta). These large structures could find application in the development of metamaterials for protein structure characterization and to manipulate circular dichroism responses.
DNA is also an exceptional building block for the fabrication of dynamic supramolecular systems with switchable geometries. In another recent study 2, a self-assembled and tunable plasmonic–fluorescent nanostructure was built using DNA as the scaffold. By controlling strand displacement reactions, we were able to obtain a precise sliding motion mechanism of two single-strand DNA rails which were connected by a DNA quasi-ring. This system resulted in a nano-mechanical structure, where six discrete configurations could be obtained. Depending on the configuration, specific distances were set between a DNA-tethered gold nanoparticle and a fluorophore, Sulfo-Cyanine3 (Cy3), which could be detected by fluorescence emission plasmonic enhancement/inhibition changes. Since the mechanism worked as a multi-state fluorescent reporter of sequence-specific nucleic acid strands, it could therefore find application as a biosensing platform and for in vivo imaging.

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Development of DNA aptamers as therapeutic tools against Pseudomonas aeruginosa

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Antibiotic resistance is posing a serious threat to human health. Therefore, innovative therapeutic tools are needed to efficiently treat resistant bacteria. Aptamers are small synthetic oligonucleotides which show high specificity and affinity for a designated target. They have multiple applications in the biotechnological field and can be considered as biological drugs to target resistant microorganisms. In this work we selected DNA aptamers to specifically target the hemophore of the heme assimilation system (HasA) from Pseudomonas aeruginosa. HasA was produced in house with a 6xHistidine-tag at the N-terminal side. The protein was also expressed with a deletion of the last 21 aa residues at the C-terminal side (HasA trunc), since the truncated version makes HasA a more efficient hemophore. Starting from a DNA library with a random region of 40 nucleotides, we used SELEX to select DNA aptamers with both HasA full length and HasA trunc as targets. Twelve rounds of SELEX were performed; in the first eight rounds the target was HasA full length, while the last four rounds the target was changed to HasA trunc. The selection was monitored by qPCR and melting curve analysis. From round 1 to 12, the main fluorescence signal peak at 70°C, typical of the library, gradually shifted to 84°C. Selected putative aptamers were sequenced by Next Generation Sequencing and, the most abundant and relevant candidates are currently being tested for their binding affinity and specificity. At the same time, our group is also working on the aptamer selection towards the receptor of the heme assimilation system (HasR). Since iron is an essential micronutrient for P. aeruginosa, as for many bacteria. Targeting and potentially blocking elements of the bacterium's iron acquisition mechanisms, such as HasR and HasA, can put the bacterium in significant metabolic disadvantage.

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Covalent Dynamic DNA Networks to Translate Multiple Inputs into Programmable Outputs

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Many biochemical signal-processing pathways rely on families of proteins that competitively dimerize in diverse combinations. Such competitive dimerization networks (CDNs) enable complex input-output computations and context-specific adaptability by varying component expression levels. Inspired by this biological occurring paradigm and introducing the predictability and sequence specificity of DNA hybridization, we propose the development of a fully synthetic DNA-based dimerization network capable of sophisticated computational ability.

Our system employs DNA oligonucleotide monomers functionalized with reactive groups that covalently bond to form dimer outputs in an all-to-all or many-to-many fashion. Inputs can selectively bind and sequester specific monomers, preventing them from participating in the dimerization process and thus controlling the network's outputs. This design enables highly programmable input- output computation, offering precise control over the synthesis of a selected dimer output. Furthermore, we demonstrate that the network's size and complexity can be readily scaled, significantly expanding its computational capacity. Notably, DNA-based dimerization networks can regulate the yield of functional dimers outputs to drive downstream reactions, such as the controlled assembly or disassembly of multiple synthetic DNA nanostructures.

Building on these capabilities, we are also exploring strategies to integrate enzymatic control into these networks. By designing DNA components that respond to specific enzymatic actions, we can selectively activate or deactivate parts of the network, adding new layers of regulation and adaptability. Furthermore, by finely tuning the enzyme-driven reactions it is possible to modulate the availability of reactive sites in a temporal fashion. By incorporating such enzyme-responsive modules, we aim to create DNA-based dimerization networks whose behavior can be externally regulated by enzymatic cues, further expanding the versatility and programmability of these synthetic computational systems.

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DNA nanostructures targeting activated platelets

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DNA nanostructures can be made by combining a long single-strand DNA scaffold with more than 200 short DNA oligonucleotides (staples) that will direct the folding of the scaffold to any predesigned shape[1]. DNA nanostructure products have numerous functions, such as studying nanoscale biological interactions and facilitating drug delivery. It has been shown that DNA nanostructures can bind platelet membranes without triggering activation[2], however, DNA nanostructures have not been explored for their potential to target activated platelets and deliver thrombolytics. In this project, we investigate the interaction of various DNA nanostructures with activated platelets, and we assess their potential for anticoagulant drug delivery. DNA nanostructures with different shapes (triangle, tetrahedron, 5-well-frame) were synthesized and visualized using atomic force microscopy (AFM) and transmission electron microscopy (TEM). Flow cytometry reveals that 1 nM folded DNA nanostructures (triangular, tetrahedral, and 5-well-frame structures) specifically bind to activated platelets with high affinity in 30 minutes of incubation time, without modifications. Confocal microscopy images suggest the internalization of these structures into activated platelets, where they gather in the cell center. Future work will focus on conjugating thrombolytic agents to DNA nanostructures and evaluating their effects on platelet function and clot dissolution. Peptides will also be attached onto the nanostructures for higher affinity and tuning of the binding. This study highlights the potential of DNA nanotechnology in developing targeted anticoagulant therapies for thrombotic diseases.

References
[1] Rothemund, P. W. K. Folding DNA to create nanoscale shapes and patterns. Nature 440, 297–302 (2006).
[2] Roka-Moiia, Y. et al. DNA Origami–Platelet Adducts: Nanoconstruct Binding without Platelet Activation. Bioconjugate Chem. 33, 1295–1310 (2022).

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Unveiling Spatial Rules of Co-Stimulation with Dual-Ligand DNA Origami Nanorods

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Immunotherapy has revolutionized the treatment of cancer and immune-related diseases by harnessing the body's own immune system. A key factor in modulating immune cell activation, particularly T-cells 1, is the precise spatial presentation of ligands [1, 2]. However, controlling this at the nanoscale remains a major challenge [3, 4]. In this study, we investigated how spatial patterning of activation signals affects T-cell activation using DNA origami.
We designed a library of DNA origami nanorods presenting anti-CD3 and anti-CD28 antibodies, to provide TCR targeting and co-stimulatory pathways, respectively, in distinct spatial arrangements: vertical, horizontal and random distribution across varying ligand densities.
Flow cytometry analysis of activation markers CD25 and CD69 revealed that nanorods with vertically aligned ligands significantly enhanced T cell activation compared to both horizontally patterned and randomly distributed configurations. This effect was consistent across multiple ligand densities and donor samples, highlighting the importance of spatial organization in modulating immune responses. These findings demonstrate that not only spatial arrangement but also the coordinated presentation of primary (anti-CD3) and co-stimulatory (anti-CD28) signals is critical for modulating T-cell activation. This work highlights the potential of DNA origami as a modular, programmable platform for the study of immune signaling and the design of antigen-presenting systems with fine-tuned control over both signal type and spatial organization.

1 A. C. Wauters et al., 'Artificial Antigen-Presenting Cell Topology Dictates T Cell Activation', ACS Nano, vol. 16, no. 9, pp. 15072–15085, Sep. 2022, doi: 10.1021/acsnano.2c06211
2 Y. Sun et al., 'DNA origami–based artificial antigen-presenting cells for adoptive T cell therapy', Sci Adv, vol. 8, no. 48, Dec. 2022, doi: 10.1126/SCIADV.ADD1106,.
3 P. Dobosz and T. Dzieciątkowski, 'The Intriguing History of Cancer Immunotherapy', Front Im munol, vol. 10, p. 2965, Dec. 2019, doi: 10.3389/FIMMU.2019.02965.
4 N. E. Papaioannou, O. V. Beniata, P. Vitsos, O. Tsitsilonis, and P. Samara, 'Harnessing the im-mune system to improve cancer therapy', Ann Transl Med, vol. 4, no. 14, p. 261, Jul. 2016, doi: 10.21037/ATM.2016.04.01.

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Multiplexed Resolution Enhancement with Fluorescence Lifetime Imaging for DNA-Based Nanoscale Imaging

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Super-resolution microscopy has transformed biological imaging by enabling visualization of subcellular structures at nanometer scales beyond the diffraction limit, while also opening new possibilities for single-molecule sensing, nanomaterials characterization, and the study of molecular interactions with unprecedented spatial precision. Single-molecule localization microscopy (SMLM) techniques, such as DNA-PAINT, routinely achieve lateral resolution of approximately 10 nm. Sequential imaging approaches, such as Exchange-PAINT and Resolution Enhancement by Sequential Imaging (RESI), further improve spatial precision to the sub-nanometer scale by capturing targets over multiple imaging rounds. While these methods achieve remarkable accuracy, they come at the cost of long imaging cycles and reduced experimental practicality. Simultaneous multiplexed imaging presents a promising alternative, but current implementations are hindered by a limited fluorophore palette, spectral crosstalk, chromatic aberrations, and the need for elaborate alignment and calibration, typically restricting them to three colors. To overcome these limitations and push the boundaries of both spatial resolution and multiplexing, we introduce a novel wide-field SMLM technique that integrates fluorescence lifetime imaging microscopy (FLIM). By encoding different targets with distinct fluorescence lifetimes, our method enables simultaneous multi-target imaging in the same field of view. Benchmarked on DNA origami, our technique offers a scalable, high-resolution alternative for structural biology and cell imaging applications.

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Active Learning Guided Optimization of Cell-Free Protein Expression

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Cell-free platforms are multi-component systems comprising Transcription and Translation (TX- TL) machinery liberated from cellular metabolism [1,2]. These systems are composed of three main compartments, cell lysate, Energy Buffer, and DNA template. The nature of multiplicity in cell-free systems bring along a number of variety in terms of TX-TL yield. The underlying cause unfortunately cannot be attributed to a single compartment as the effects are collective and accumulative during TX-TL. However, it is evident in previous studies that cell-free systems are prone to variability [3,4] and therefore, need to be optimized every time one of the compartments is changed. Active learning approaches have been used so far to identify the optimal conditions for cell-free systems [5,6]. Our approach to investigate the best working conditions for high yield protein expression, i.e. high TX-TL yield, is based on investigating Energy Buffer components for specific cell-lysate and DNA template combinations. Our goal is to scan the experimental space of Energy Buffer combinations that will result in maximized TX-TL performance with the help of active learning algorithms. Our approach is based on Energy Buffer combination generation using active learning algorithms. These combinations are then tested for TX-TL yield using a single batch of cell lysate and a fluorescent protein encoding DNA template. The data obtained from each combination is provided to the algorithm allowing it to update its predictions to be tested. This feed-forward loop is conducted multiple times resulting in TX-TL performances, i.e. protein expression yields, almost 12-fold higher than the Energy Buffer combinations provided in the literature.

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Hybridization Chain Reaction (HCR) Amplification in Cell-Free Biosensors

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In recent decades, advances in synthetic biology have enabled the transition from cell-based to cell-free biosensors, significantly expanding their application range from environmental monitoring [1] to medical diagnostics [2]. Indeed, cell-free biosensors are emerging as cost-effective, user-friendly and field-deployable platforms for the detection of viral nucleic acids [3] and small molecules [4]. In the last years our group reported the first examples of cell-free biosensors for the detection of clinically- relevant antibodies [5], [6] that exploit the use of synthetic antigen-conjugated DNA strands. However, improving the sensitivity of such platforms remains a major challenge. Several strategies have been proposed to address this limitation, including enzymatic amplification by CRISPR-based systems [7], [8]. Here, we develop one-step cell-free biosensors for the detection of different biomarkers. Specifically, our approach by combining the molecular recognition of programmable antigen- conjugated synthetic nucleic acids and genetic circuits with the robust, enzyme-free amplification capabilities of Hybridization Chain Reaction (HCR) [9] enables the sensitive, specific and selective of different molecular biomarkers.

References

1 J. K. Jung et al., “Cell-free biosensors for rapid detection of water contaminants,”Nat. Biotechnol., vol. 38, no. 12, pp. 1451–1459, 2020, doi: 10.1038/s41587-020-0571-7.
2 X. Tan, J. H. Letendre, J. J. Collins, and W. W. Wong, “Synthetic biology in the clinic: engineering vaccines, diagnostics, and therapeutics,”Cell, vol. 184, no. 4, pp. 881–898, 2021, doi: https://doi.org/10.1016/j.cell.2021.0 3 K. Pardee et al., “Rapid, Low-Cost Detection of Zika Virus Using Programmable Biomolecular Components.,”Cell, vol. 165, no. 5, pp. 1255–1266, May 2016, doi: 10.1016/j.cell.2016.04.059.
4 A. D. Silverman, U. Akova, K. K. Alam, M. C. Jewett, and J. B. Lucks, “Design and Optimization of a Cell-Free Atrazine Biosensor,”ACS Synth. Biol., vol. 9, no. 3, pp. 671–677, Mar. 2020, doi: 10.1021/acssynbio.9b00388.
5 S. Bracaglia, S. Ranallo, and F. Ricci, “Electrochemical Cell-Free Biosensors for Antibody Detection,”Angew. Chemie Int. Ed., vol. 62, no. 8, p. e202216512, Feb. 2023, doi: https://doi.org/10.1002/anie.202216512.
6 A. Patino Diaz, S. Bracaglia, S. Ranallo, T. Patino, A. Porchetta, and F. Ricci, “Programmable CellFree Transcriptional Switches for Antibody Detection,”J. Am. Chem. Soc., vol. 144, no. 13, pp. 5820–5826, Apr. 2022, doi: 10.1021/jacs.1c11706.
7 F. Miceli, S. Bracaglia, D. Sorrentino, A. Porchetta, S. Ranallo, and F. Ricci, “MAIGRET: a CRISPRbased immunoassay that employs antibody-induced cell-free transcription of CRISPR guide RNA strands.,”Nucleic Acids Res., vol. 53, no. 6, Mar. 2025, doi: 10.1093/nar/gkaf238.
8 H. de Puig et al., “Minimally instrumented SHERLOCK (miSHERLOCK) for CRISPR-based point of-care diagnosis of SARS-CoV-2 and emerging variants.,”Sci. Adv., vol. 7, no. 32, Aug. 2021, doi: 10.1126/sciadv.abh2944.
9 R. M. Dirks and N. A. Pierce, “Triggered amplification by hybridization chain reaction.,”Proc. Natl. Acad. Sci. U. S. A., vol. 101, no. 43, pp. 15275–15278, Oct. 2004, doi: 10.1073/pnas.0407024101.

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Programmable Regulation of Enzyme-Inhibitor Complexes through synthetic gene networks

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Precise control of biomolecular interactions is central to synthetic biology, therapeutics, and biosensing [1,2]. Enzyme-inhibitor complexes, in particular, represent a powerful but underutilized axis for programmable regulation due to their inherent reversibility and specificity [3]. However, conventional approaches to modulate these systems often lack dynamic tunability and contextual responsiveness [4]. Here, we present a modular platform that harnesses the programmability of synthetic nucleic acids and the versatility of gene networks to dynamically regulate enzyme-inhibitor complexes. Specifically, synthetic gene circuits are engineered to respond to specific targets by transcribing functional RNA strands in a highly programmable and orthogonal manner. These RNA strands act as regulatory elements that modulate enzyme-inhibitor interactions. By encoding these logic-driven responses in transcriptional modules, we achieve repression or activation of enzymatic activity. We demonstrate this concept using different enzyme-inhibitor systems and validate how transcriptionally encoded RNA regulators can be used to achieve input-specific and dose-responsive control in a tunable and reversible way. This platform offers a new route to dynamic biochemical control, with broad implications for therapeutic enzyme regulation, biosensing and synthetic biology.

References

1) Silverman AD, Gollakota DV, & Jewett MC (2020). Cell-free gene expression: an expanded repertoire of applications. Nature Reviews Genetics, 21, 151-170. https://doi.org/10.1038/s41576-019-01863

2) Jung JK, Alam KK, Verosloff MS, Capdevila DA, Desmau M, Clauer PR, Lee JW, Nguyen PQ, Pastén PA, Matiasek SJ, Gaillard JF, Giedroc DP, Collins JJ, & Lucks JB (2022). Cell-free biosensors for rapid detection of water contaminants. Nature Biotechnology, 40, 682-692. https://doi.org/10.1038/s41587022-01208-7

3) Pandi A, Vasquez JJ, Ferrante T, Balasubramanian S, & Del Vecchio D (2023). Design of dynamic enzyme regulation using modular transcriptional inhibitors. Nature Communications, 14, 1853. https://doi.org/10.1038/s41467-023-37341-1

4) Xu H, Beisel CL, & Lu TK (2021). Synthetic biology strategies for programmable enzyme control. Trends in Biotechnology, 39, 472-485. https://doi.org/10.1016/j.tibtech.2020.10.012

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MAIGRET: an antibody-responsive cell-free transcription for CRISPR-based ultrasensitive immunoassay

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The programmability of CRISPR-based techniques, combined with the advantages of nucleic acid nanotechnology, has enabled the development of innovative diagnostic tools.
Here we present a two-step CRISPR-based immunoassay, named MAIGRET (Molecular Assay based on antibody-Induced Guide-RNA Enzymatic Transcription). This platform enables the sensitive and versatile detection of specific antibodies and other proteins by integrating the advantages of CRISPR-based sensing with cell-free transcription systems.
In the first step of the assay, the target antibody binds to a pair of antigen-conjugated DNA strands, inducing their co-localization and the formation of a bimolecular complex. This complex hybridizes to the single-stranded region of an inactive synthetic template that encodes the CRISPR RNA guide strand (crRNA) specific to the Cas12a enzyme. Only upon formation of this complex the synthetic template is activated, allowing the cell-free transcription of the crRNA strand. In the second step, an aliquot of the first reaction is transferred into a mixture containing Cas12a, its double-stranded DNA activator, and a DNA hairpin reporter labeled with a fluorophore-quencher pair. The crRNA produced in the first step activates Cas12a, triggering its collateral cleavage activity and resulting in the reporter cleavage and a measurable fluorescence signal.
MAIGRET achieves highly sensitive (detection limits in the low picomolar range), specific (no signal in the presence of non-target antibodies), and selective (effective even in complex samples such as 50% blood serum) detection of target proteins. Thanks to its programmable design, the platform can be tailored for different targets, either in direct or competitive assay formats, and supports multiplexed detection using orthogonal CRISPR enzymes (e.g., Cas13), each coupled with a distinct, antibody-responsive transcriptional template. Considering the versatility of MAIGRET, we are currently developing an adaptation with a colorimetric readout by employing the collateral cleavage of a horseradish peroxidase (HRP)-DNA strand anchored on the magnetic beads as reporter system.

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Nuclease-mediated cleavage of DNA origami nanostructures for controlled cutting in a time-dependent manner

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Self-assembled deoxyribonucleic acid (DNA) origami nanostructures (DONs) are highly versatile, possessing unique structural and mechanical properties that make them promising candidates for diverse applications such as biosensing, drug delivery, and advanced nanodevices. However, realizing their full potential often requires precise pre- or post-assembly processing/ modification for functionalization or dynamic applications. Nucleases offer a powerful toolkit for targeted and localized structural modification of DONs such as controlled cutting and joining; however, achieving this through traditional enzymatic processing remains challenging because of their topological complexity. Our research investigates the controlled action of specific and non-specific nucleases on both 2D and 3D DONs. Triangular 2D DONs (with trapezoidal domains bridged through single stranded linkers) were assembled and processed with various exonucleases in a time-lapse manner and then characterized using agarose gel electrophoresis (AGE) and atomic force microscopy (AFM). Our initial findings reveal distinct degradation patterns: non-specific nucleases (e.g., DNase I, Exonuclease III) lead to rapid, uncontrolled degradation of DONs, while Mung Bean Nuclease (MBN) demonstrated relatively selective and controlled cutting at exposed single stranded linkers and overhangs. These results highlight the potential of MBN and other specific exonucleases as powerful tools for controlled cleavage and modification of complex DNA origami nanostructures in a programmable manner. On-going work aims to further elucidate the dynamic interactions between various specific and non-specific nucleases (DNase I, Exonuclease III, MBN) and both 2D and 3D DONs for their utility in advanced DNA nanotechnology applications.

Keywords: Self-assembly; DNA origami; Enzyme reactions; Nucleases; Exonucleases; Electrophoresis; AFM.

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DNA Condensates as a Theranostic Platform

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Membraneless organelles, such as DNA/RNA condensates formed via liquid-liquid phase separation (LLPS), provide a flexible and dynamic platform for biosensing applications in therapeutic advancements.1, 2 The DNA condensates may offer a biomimetic environment for molecular recognition, enabling the detection of disease biomarkers with high sensitivity and specificity. By leveraging multivalent interactions, DNA condensates can be engineered to respond to specific nucleic acid sequences, proteins, or small molecules, making them ideal for sensing and targeted drug delivery. [1-3] The condensates also play a crucial role in regulating intracellular machinery and maintaining physiological homeostasis in biological system. [2,4] In the context of cancer, DNA condensate-based biosensors may facilitate real-time monitoring of disease progression and therapeutic response. Additionally, their tunable physicochemical properties may allow for controlled drug release, improving therapeutic efficacy while minimizing off-target effects. [2,5]
Here, we aim to develop nucleic acid condensate as a biosensing and delivery platform of therapeutic for cancer and other critical diseases. We employ circular dichroism (CD) spectroscopy as a label-free technique to detect DNA condensates and explore potential of condensates as delivery platform for therapeutic oligonucleotides. DNA Y-stem nanostars were used to generate DNA- based droplets, which could serve as delivery vehicles for therapeutic oligonucleotides such as anti- miRNA21. Time-dependent CD and fluorescence imaging confirmed formation and growth of condensate over time. The oligonucleotide anti-miRNA21 were observed to be compartmentalized in phase separated droplets via functionalization with Zipped Nucleic Acid (ZNA) as revealed using fluorescence imaging. As a next step, DNA droplets loaded with therapeutic are intended for delivery into cellular systems to evaluate their real-time cargo release potential on target sites, contributing to the development of advanced therapeutic strategies. This work could open a new avenue for targeted delivery of therapeutics in the new era of personalized medicine.

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DNA condensates containing client Silica Nanoparticles (SiNPs) for theranostic applications

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Biomolecular condensates−dynamic, biomolecule-enriched droplets which arise owing to Liquid-Liquid Phase Separation (LLPS)−have been recently described to be implicated in many key vital processes within cells [1,2]. Although intracellular LLPS is extremely complex, the use of nucleic acids'high programmability, governed by Watson-Crick-Franklin base-pairing, offers a straightforward mechanism to form predictable artificial condensates [2]. Specifically, DNA nanotechnology provides a powerful platform for the engineering of condensates with tuneable structures and functions by capitalizing on the vast toolbox available to the field [3]. Consequently, recent studies have demonstrated the formation of DNA condensates capable of selective-partitioning target molecules of interest, spatiotemporally controlled biochemical reactions and stimuli-responsive phase-separation and partitioning behaviours [4,5,6,7].

Herein, we report on the partitioning of mesoporous silica nanoparticles (SiNPs) into DNA-based condensates. Our strategy leverages DNA nanostars as programmable building blocks for condensate formation, which are rationally designed to include an anchoring single-stranded DNA (ssDNA) overhang for the recruitment and local concentration of SiNPs functionalized with complementary DNA sequences. To explore the parameters governing nanoparticle partitioning, we constructed a toolbox of SiNPs varying in size (from 90 to 700 nm) and surface chemistry (DBCO-modified and carboxylated), allowing us to investigate the combined effects of particle size, surface properties, and sequence-specific hybridization on partitioning behaviour within the condensate. Our findings indicate that there is no strict size threshold limiting SiNP partitioning into DNA condensates and provide insights into how biomolecular condensates mediate selective client loading and molecular interactions.

1. S. Brocca, R. Grandori, S. Longhi, V. Uversky. International Journal of Molecular Sciences, 2020, 21(23), 9045. Doi:10.3390/ijms21239045

2. J. M. Stewart, S. Li, A. A. Tang, M. A. Klocke, M. V. Gobry, G. Fabrini, L. Di Michele, P. W. K. Rothemund, E. Franco. Nature Communications, 2024, 15(1), 6244. Doi:10.1038/s41467-024-50003-x

3. S. Agarwal, D. Osmanovic, M. Dizani, M.A. Klocke, E. Franco. Nature Communications, 2024, 15(1), 1915. Doi:10.1038/s41467-024-46266-z

4. S. Xu, Y. Ouyang, Y. Qin, D. Chen, Z. Duan, D. Song, D. Harries, F. Xia, I. Willner, F. Huang. Nature communications, 2025, 16(1), 3352. Doi:10.1038/s41467-025-58650-4

5. M. Dizani, D. Sorrentino, S. Agarwal, J. M. Stewart, E. Franco. Journal of the American Chemical Society, 2024, 146(43), 29344–29354. Doi:10.1021/jacs.4c07555

6. W. Yu, K. Jin, D. Wang, N. Wang, Y. Li, Y. Liu, J. Li, G. Du, X. Lv, J. Chen, R. Ledesma-Amaro, L. Liu. Nature Communications, 2024, 15(1), 7989. Doi:10.1038/s41467-024-52411-5

7. S. Zhou, X. Ju, H. Chen, Y. Zhang, J. Zheng, K. Wang, X. Yang, J. Liu, Angewandte Chemie (International ed. in English), 2025, e202505316. Doi:10.1002/anie.202505316