FOLDING-UPON-BINDING AND SIGNAL-ON ELECTROCHEMICAL DNA SENSOR WITH HIGH AFFINITY AND SPECIFICITY

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Biomolecular receptors such as proteins or nucleic acids that shift between two or more conformations upon binding to a specific target can be used to build robust, sensitive and specific sensors. Since signal transduction is linked to the conformational change that occurs only upon binding, these receptors allow for detection of a specific target even within the incredibly complex media that exist within biological system. To create robust, rapid sensors that similarly link specificity and sensitivity, a number of structure-switching optical and electrochemical sensors have been reported in recent years for applications in the areas of diagnostics and imaging.

Among the various structure-switching strategies employed by naturally occurring receptors, the use of a clamp-like mechanism where the receptor comprises two recognition elements that both bind and recognize the target, remains one of the most effective. Inspired by this mechanism, we have recently explored the thermodynamics by which a DNA clamp-like molecular receptor, that recognizes a specific complementary oligonucleotide target through both Watson-Crick and triplex-forming Hoogsteen interactions, can improve both the affinity and specificity of recognition.

In the present work we fully realize and exploit the advantages of such molecular “double-check” mechanism, by adapting this clamp-like sensing strategy to a DNA-based electrochemical biosensor (hereafter named E-DNA). Specifically, here we have used a clamp-switch probe to develop a signal-on E-DNA sensor, a type of structure-switching DNA probe that enables the single-step detection of specific oligonucleotides in a reagentless fashion (i.e. without the need of adding exogenous reagents). We also demonstrate that this strategy results in significantly improved affinity and specificity relative to previously published E-DNA sensors.

We demonstrate that this original target-binding mechanism can improve both the affinity and specificity of recognition as opposed to classic probes solely based on Watson-Crick recognition. By using electrochemical signaling to report the conformational change, we demonstrate a signal-on E-DNA sensor with up to 400% signal gain upon target binding. Moreover, we were able to detect with nanomolar affinity a perfectly-matched target as short as 10 bases (KD = 0.39 nM). Finally, thanks to the molecular “double-check” provided by the concomitant Watson-Crick and Hoogsteen base pairings involved in target recognition, our sensor provides excellent discrimination efficiency towards a single-base mismatched target.

Enzyme Confinement and Regulation in a Self-Assembled, Nucleic Acid Matrix

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Programmable nanostructures obtained by nucleic acid self-assembly and enzymatic manipulation hold a central role towards developing miniaturized devices for innovative applications, ranging from biosensing to next-generation information technology. Very little is known, however, of the effect of confinement on the functional behaviour of biomolecules.

In our experimental work we form laterally-confined self-assembled monolayers of short nucleic acids on ultra-flat gold surfaces by self-assembly, and using an atomic force microscopy (AFM)-based nanolithography method termed nanografting. We have used these matrices to study the mechanism by which specific endonucleases, including ribonuclease III (RNase III) and an inactive, dsRNA-binding mutant (E110A) function inside the surface-bound matrices. We monitored these reactions from the decrease of the matrix height with respect to the surrounding surface, as measured by AFM. We addressed the effect of confinement by varying duplex density, and unequivocally show that the confinement has a quantifiable effect on the processes of enzyme diffusion and catalysis. Specifically we found that (i) the accessibility of a dsDNA matrix toward restriction endonucleases is sterically regulated. Also, endonucleases diffuse inside the matrix in a two-dimensionally manner, and gain access to the matrix
exclusively from the sides (that are generated by nanografting), rather than from the larger, topmost area; (ii) within matrices of a ds[DNA:DNA] chimera, the reactivity of the BamHI cleavage site, which is located in the middle of the 12 bp dsDNA segment proximal to the gold surface, is dependent upon the prior action of Ribonuclease III at its site, which is located in the middle of the upper dsRNA segment. Furthermore, in combination with BamHI, the RNase III and its catalytically inactive E110A mutant, as different inputs, effectively act as steric regulators of BamHI action. The AFM-measured height change of the matrix (output) is a step (i.e. digital) function of the dsRNA-specific input. These findings demonstrate that, in crowded nano-systems, enzymes function in a qualitatively different manner than in bulk. In general our results reveal novel properties of protein-nucleic acid interactions within a high-density array environment, which opens the door to novel applications of DNA nano-assemblies in combination with nucleic acid recognition and processing. Finally, our finding extends the versatility of AFM imaging as a bioanalytical tool for studying highly crowded biomolecules at the nanoscale.

**Enzyme Reactivity of DNA Origami**
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The application of self-assembled DNA nanoparticles in nanomedicine, such as for carrying and delivering drugs, greatly depends on their stability and/or capability to interact with complex biological systems such as the cellular environment. Recent studies suggest that in cell lysate, or in living cells, or in animal model DNA origami are more stable than the unfolded ss- and dsDNA filaments (Q. Mei, Nanolett., 2011), while previous work of our group demonstrated that the activity of restriction enzymes within surface-supported self-assembled monolayer (SAM) of dsDNA molecules depends on steric hindrance, and is inhibited when the DNA density is higher than a certain critical threshold. Collectively, these results suggest that the enhanced stability of self-assembled DNA nanostructures compared to other naturally available DNA forms, is due to the inherent high level of packing. In the attempt of establishing a quantitative approach to control the interactions between self-assembled DNA nanoparticles and nucleases, we experimentally studied the effect of 14 different enzymes on two, different DNA Origami originated from the same ssDNA scaffold (M13mp18). We found that some of these enzymes produce shape-dependent fragmentation patterns. Our hypothesis is the enzymatic activity is steric regulated by the spatial arrangement of restriction sites within the DNA nanostructure. By using the open-access software Cadnano we designed a three layers parallelepiped of 7x24x34 nm3 in size, as formed by 30 segments of 112 bp-long dsDNA, which are arranged within a honeycomb lattice. In particular, structural rigidity highly depends on the pattern of crossovers between adjacent DNA duplexes: the higher is their number, the tighter is the structure. We designed 5 versions of our DNA parallelepiped, by gradually removing crossovers in a specific area. The mechanical properties of each version were qualitatively examined with the open-source program CanDo, which approximates DNA double helices with elastic rods, and predicts shapes deformations and thermal fluctuations based on input, structural parameters, including crossovers positions, among others. CanDo-related results show that in the area where crossovers are altered, the rigidity of the parallelepiped decreases as a function of the amount of deletions. Next step will be inserting enzyme-specific restriction sites within this area for studying the influence of packing and rigidity on enzymatic accessibility and kinetics.

**Motility of DNA-functionalized Janus particles**
**Günther, Katrin**
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Motility of DNA-functionalized Janus particles
Katrin Günther1, Andreas Bregulla2, Frank Cichos2, and Michael Mertig1,3
Directed transport of cargo on a microscopic scale requires control of rotational diffusion. DNA and DNA-based artificial structures were coupled to micron-sized Janus particles in order to favor translational motion. The influence of length and stiffness of the stabilizing DNA elements on the behavior of the optically heated Janus particles is investigated.

**Conjugation of magnetic nanoparticles to chiral DNA-assembled plasmonic structures**

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We intend to create dynamic plasmonic nanostructures exhibiting switchable circular dichroism. Our group recently demonstrated surface-bound DNA-assembled gold particle helices that were aligned and switched between orientations in a drying - resolving process.1,2,3 With the goal to observe switching in solution, we now designed another approach based on the attachment of magnetic iron oxide nanoparticles to the ends of the DNA structures that also bear the gold nanoparticles.

The DNA skeleton of the plasmonic nanohelix structures used in our experiments has a length of 100 nm, a diameter of 16 nm and consists of a bundle of 24 double helices with nine gold nanoparticle attachment sites.2 DNA bound magnetic nanoparticles (Fe_3O_4) with a diameter of 30 nm are attached to the ends of the DNA skeleton of the plasmonic nanohelix. We observed that almost all magnetic nanoparticle attachment sites on the DNA structures were occupied by exactly one magnetic nanoparticle. However, strong clustering of the helix constructs was observed possibly due to magnetic interactions of the magnetic particles. In some cases two DNA structures attached to the same magnetic particle resulting in a chainlike structure of up to four segments. We found, that the magnetic nanoparticles can be added before or after the gold nanoparticle attachment to the DNA structure or even simultaneously.

In conclusion, we successfully bound magnetic iron oxide nanoparticles to the plasmonic helices, though clustering and purification issues still have to be solved. In upcoming experiments we will measure the circular dichroism spectrum of the nanostructures in a switching magnetic field.

**References:**  

**Gold nanolenses for surface-enhanced Raman scattering on DNA origami substrates**

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The scattering signal detected in Raman spectroscopy can be amplified by many orders of magnitude if the analyte is positioned in the hot spot formed between metal nanostructures. Surface-enhanced Raman spectroscopy exploits this effect, enabling characterization of analytes at the single-molecule level. The nanometer-scale spatial arrangement of the individual components has a crucial effect on the observed signal enhancements and represents one of the greatest challenges in the technique.

In our group DNA origami is used to create scaffolds for the precise positioning of gold nanoparticles and respective analyte molecules. Highest sensitivities are to be expected in the gold nanolenses we assembled, employing rows of 3 differently-sized gold nanoparticles. In calculations with the FDTD method we estimate the attainable electromagnetic field enhancements in the synthesized systems. Ultimately we aim to develop a versatile platform for investigations on biological samples, providing structural information –e.g. of binding events- at the single-molecule level.
Nano scale metallization of DNA nanostructures

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Tuning the optical, electromagnetic and mechanical properties of a material requires simultaneous control over its composition and shape. Noble metal nanostructures are very promising candidates for applications in sensing, light harvesting, spectroscopy, and catalysis. In particular gold and silver nanoparticles feature extraordinary optical properties which are due to their strong localized plasmon resonances. Depending on the material, the size of the particles and especially on the shape of the nanostructures these resonances can be tuned from the ultra violet over the visible to the near infrared region of the electromagnetic spectrum.

While noble metal particles with different shapes can be fabricated using specialized protocols, so far no method exists, that allows a programmable fabrication of arbitrarily shaped particles. This maybe however realized using the tool box of DNA nanotechnology. In particular DNA origami emerged as a convenient technique to build nanostructures with many different shapes in a programmable manner and with very high precision and yield.

DNA has already previously been used as a template for metal deposition, e.g. to produce wire-like structures with linear DNA molecules or more complex structures with DNA origami. However, the homogeneity and continuity of the resulting structures remained problematic due to the use of many nucleation centers distributed over the whole template.

To overcome these problems we propose a method that uses only a single nucleation center. In particular we use single gold nanoparticles attached to origami structures that serve as nucleation centers for a subsequent gold deposition through a seeded growth procedure. The origami template is then expected to influence the shape of the resulting metal structure.

Smart DNA origami nanopores

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DNA origami[1] has emerged as a unique and versatile method to fabricate tailored nanostructures with exciting applications in numerous scientific fields. Accurate control at the nanoscale level can be easily performed thanks to the programmability of DNA base-pairs interactions. This salient property is extremely important in the construction of artificial systems in which nanometre control of shape and dimensions is required. Synthetic nanopores used for biomolecules sensing represent an important example[2].

Using the DNA origami method, we combined DNA origami structures with glass nanocapillaries to yield hybrid nanopores with controlled surface chemistry and precise diameters [3,4]. We were able to reversibly and repeatedly form these DNA origami nanopores by voltage-driven trapping of the DNA origami structures onto the glass nanocapillary. Using single molecule fluorescence imaging with simultaneous ionic current measurements, we presented the first direct visualisation of the trapping of DNA origami structures. We also showed the possibility to control the folding of the translocating \(\lambda\)-DNA molecules by tuning the size of the aperture in the DNA origami structure [4].

We are now creating smart DNA origami nanopores by incorporating stimuli-responsive domains in the DNA origami structure. We have recently demonstrated the possibility to exert a control in the passage frequency of \(\lambda\)-DNA molecules by mechanically distorting the DNA origami nanopore as a function of voltage[5]. Thus DNA origami is a promising artificial platform to mimic biological nanopores.

4. S. Hernández-Ainsa et al. ACS Nano 2013, 7, 6024
Superresolution fluorescence imaging of nanopatterned DNA-origamis with semisynthetic fluorescent protein-DNA conjugates.

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DNA-origamis[1] provide unique possibilities in fabrication of nanostructures which can be functionalized with nanometer-scale accuracy via base pairing. The positioning of single organic dye molecules via labeled oligonucleotides has led to the development of a diverse set of 2D or 3D and single- or multicolor molecular probes for fluorescence and superresolution microscopy standards (e.g. dSTORM and STED) [2,3]. However, for biological applications fluorescent proteins (FPs) became a unique tool of noninvasive superresolution imaging in living cells, since they are fully genetically encoded fluorescent labels. In order to compare the features of different FPs for superresolution microscopy techniques or the influence of different surrounding conditions, cellular structures are not appropriate due to e.g. structural variability. Thus, it is crucial to develop superresolution standards based on FPs. In this work we synthesized FP-DNA conjugates[4], immobilized them in specific patterns on DNA origamis and characterized them by localization based microscopy.


Probing electron-induced biodamage at the single-molecule level using DNA origami templates

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The ability of low-energy electrons (LEEs) to induce various chemical reactions has been identified as a phenomenon of great importance in many areas of fundamental and applied research including atmospheric and astrochemistry, radiation biology, and nanotechnology. In these particular fields, especially the role of LEE-induced bond cleavage due to dissociative electron attachment (DEA) has been highlighted as it, e.g., is considered a major source of DNA damage induced by ionizing radiation. Here we present a novel approach[1,2] that allows for the first time to visualize the electron-induced dissociation of single chemical bonds within complex, but well-defined DNA nanostructures. This technique is based on the detailed atomic force microscopy analysis of designed, synthetic DNA targets attached to DNA origami templates and can be used to study the dissociation of selected chemical bonds and the complex cleavage of DNA strands of predefined sequence, and topology. We apply this technique
to quantify the reactivity of a single disulfide bond, a thymine dimer, and the essential vitamin biotin toward LEEs. Furthermore, we demonstrate that this novel method enables the fast and parallel quantitative investigation of multiple target species in a single irradiation experiment.


EGNAS: a software for exhaustive DNA sticky end design

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The molecular recognition of DNA is based on complementary base pairing. This allows the formation of a double helix by nucleobase sequence selective hybridization of single-stranded DNA. A correct pairing of specific DNA strands is essential for applications in the fields of DNA microarrays, DNA nanotechnology, DNA origami, DNA computing and genetics. A proper DNA sequence design is especially required for the use of DNA strands as unique identifiers. These identifiers represent molecular bar codes on DNA microarrays or facilitate the self-assembly of DNA tiles to form nanoscale structures. These bar codes are applied as so called "sticky ends" or molecular tags. Furthermore, such sticky ends can be used to attach molecular constructs to DNA origami in a site-specific way.

The goal of sticky end sequence design is to generate the largest set of sequences that provides efficient and selective hybridization. We present an exhaustive DNA sequence design algorithm which is realized in the software tool EGNAS. Sets with maximum possible number of sequences are generated with EGNAS within a reasonable computing time of a few second to some minutes on a commercially available computer. Secondary structures (self-complementarity and hairpin structures) as well as possible cross hybridizations can be avoided. User defined sequences can be included to have minimum interactions with the generated sequences. Additionally, arbitrary subsequences can be forbidden and the guanine-cytosine content can be defined.

In summary, EGNAS is a software tool for designing sticky ends used for self-assembly of DNA tiles, for selective attachment of molecular constructs to DNA origami and for the development of molecular bar codes on DNA microarrays.

Molecular barcoding for SPR-based DNA microarrays

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DNA microarrays are used for the sequence selective detection of nucleic acids. They are supposed to enable a fast and highly parallel detection with a low sample volume requirement. Usually, fluorescent markers are used to detect the hybridization between a probe and, e.g., a PCR product with a single-stranded overhang as target DNA. However, novel marker-free techniques are of growing interest.

We present the development of DNA microarrays utilizing surface plasmon resonance (SPR). No fluorescent markers are necessary, because the detection is based on refractive index changes due to hybridization events occurring at the sensor surface. This surface is a 50 nm thick gold film. On this film, DNA probes are immobilized in a microarray. These probes are single DNA strands with so called anti-
tag sequences. The complementary tag sequences are included in the primer sequences that are the single-stranded parts of PCR products. This tag/anti-tag system can be considered as molecular barcoding identifying PCR products on a microarray with unique tag/anti-tag sequences. We show that secondary structures of probes and primers resulting from the base sequence have significant influence on the kinetics and efficiency of the hybridization on the sensor surface. Influence of the immobilization, the hybridization conditions and the PCR product length on the sensor performance is studied.

**Microinjected DNA Nanotubes as Intracellular Delivery Vehicles in vivo**

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DNA-based nanoconstructs are promising materials for biological and biomedical applications due to their ability to present a variety of (bio)chemical moieties with nanometer precision while providing full control over stoichiometry. Moreover, DNA nanostructures are highly biocompatible and stable in cell media, blood serum, and cultured cells for elongated periods of time. Unmethylated CpG sequences are a hallmark of microbial DNA and they have been used as adjuvants for immunostimulation. These CpG sequences are recognized by Toll-like receptor 9 (TLR9), present on lymphocytes and antigen-presenting cells, including macrophages, and thus initiate an immune response. In this study, we investigated the use of DNA-based nanotubes as carrier systems for CpG delivery and their effect on immune cells in vivo and in real time. DNA nanotubes were designed using the single-stranded tile assembly method where each strand has 10-11 base domains complementary to four neighboring strands. For this purpose, 48 unique DNA strands were mixed to form 8-helix nanotubes which have a length of ~40 nm and a diameter of ~8 nm. The strands without CpG sequences were enzymatically labeled with a fluorescence dye for the visualization of nanotubes in vivo.

CpG-decorated DNA nanotubes, DNA nanotubes, and CpG oligonucleotides (500 nM, 300 nl) were microinjected into the cremaster muscle of anesthetized mice. As visualized by in vivo fluorescence microscopy, all three types of DNA were rapidly internalized by F4/80 positive cells (incl. macrophages and mast cells) and colocalized with lysotracker dye in these cells. Only microinjection of CpG-decorated DNA nanotubes but not of DNA nanotubes or CpG oligonucleotides induced a significant increase in leukocyte adhesion (30 min after injection) and transmigration (60 min after injection) in postcapillary venules of the cremaster muscle as observed by in vivo microscopy. Interestingly, CpG-decorated DNA nanotube-elicited leukocyte recruitment was almost completely blocked in animals treated with an inhibitor of mast cell degranulation.

Confocal microscopy of immunostained muscle tissue revealed that only after treatment of CpG-decorated DNA nanotubes, nuclei of cells surrounding the microinjection site were positive for phosphorylated p65, indicating TLR-9 mediated activation of the NF-κB pathway. Taken together, these in vivo findings suggest that DNA nanotubes are promising delivery vehicles capable of targeting tissue macrophages and mast cells. The immunogenic potential apparently depends on the decoration of DNA tubes with CpG-oligonucleotides.

**Using DNA origami as templates for precise assembly of inorganic nanoparticles**

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DNA origami offers the unique possibility to tailor desired template structures, which become especially attractive for nanoscaled electronic and/or photonic devices because of their versatility and high precision. To prepare multifunctional devices made of DNA origami structures and nanoparticles, a targeted functionalization of well-defined metal and semiconductor nanoparticles (NPs) needs to be achieved. Therefore, both a synthesis that follows colloidal wet chemical routes and the control of a defined surface chemistry of the NPs with respect to the DNA functionality is needed. The deposition of
NPs onto pre-functionalized contact areas on the DNA origami structures will then allow the preparation of precisely controllable hybrid structures. The present work will show a promising approach towards DNA functionalized NPs and their attachment to DNA origami templates including various characterization techniques.

**Self-assembled fluorescent Ag:DNA emitters on DNA tubes**

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Fluorescent silver clusters stabilized by single-stranded DNA, can self-assemble into arrays of emitters, for example on DNA nanotubes. The properties of the emitters, created on the tubes, are different from the properties of their free counterparts. Namely, the emission intensity reduces by a factor of 5 and the position of the emission peak blue shifts by approximately 10nm.

We measured absorbance and emission properties of the emitters before and after detaching from the tubes, and concluded that the difference has its origin in the structural change of the cluster-DNA system, depending on whether they are attached or not. This remarkable observation implies that Ag:DNAs can be used not only as a bright optical markers but also as a witness of dynamical changes in the DNA.

**Strategies to tune extend and narrow the dynamic range of DNA-based nanoswitches**

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The high specificity and affinity binding of proteins and nucleic acids have inspired decades of research aimed at employing biomolecular recognition in novel diagnostic tools. Despite this, however, biological recognition elements often exhibit a potentially significant limitation: the single-site binding characteristic of the majority of such receptors produces a hyperbolic dose-response curve with a fixed dynamic range. This can limit the utility of biomolecular receptors in applications which require the measurement of large changes in target concentration or that require a strong sensitivity response (a steeper variation of output signal with small amount of target concentration).

We demonstrate here different strategies to tune, extend and narrow the dynamic range of classic DNA-based probes. Following our first demonstration using classic molecular beacons we have demonstrated multiple, complementary approaches by which we can tune, extend and narrow the dynamic range of a model aptamer-based cocaine sensor. Specifically, using a mutational approach we have generated sets of cocaine aptamers varying in their affinity for the target. Using various combinations of these receptors we were able to both narrow and broaden the dynamic range of biochemical receptors. In a second approach we have used a model cocaine aptamer and have changed its affinity using allosteric effectors. Compared to the mutational approach, this method provides a more rational, more efficient, and more cost-effective approach by which we can tune the affinity of an oligonucleotide-based receptor.

Finally, we demonstrated the rational design of allosterically controllable, metal-ion-triggered molecular switches. Specifically, we engineered DNA-nanoswitches which contains mismatches sites serving as specific recognition sites for mercury(II) or silver(I) ions. Both switches contain multiple metal binding sites and thus exhibit homotropic allosteric (cooperative) responses. As heterotropic allosteric effectors we employ single-stranded DNA sequences that either stabilize or destabilize the non-binding state, enabling dynamic range tuning over several orders of magnitude.

The ability to rationally introduce these effects into target-responsive switches could be of value in improving the fine activation of DNA-based nanomachines.
DNA Origami Substrates for Highly Sensitive Surface-Enhanced Raman Scattering


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Surface-enhanced Raman scattering (SERS) is a very promising method for the investigation of analyte molecules at trace levels. One of the major challenges for reliable SERS experiments, that is, the optimization of the enhancement effect from the SERS hot spot, can be overcome using DNA origami substrates which enable the precise attachment of metal nanoparticles with well-defined interparticle distances.

In current experiments we created dimers of gold nanoparticles (AuNPs) arranged on DNA origami substrates to achieve an extremely high field enhancement for Raman scattering. By using carboxytetramethylrhodamine (TAMRA) as a Raman reporter molecule and optimizing the gap size between the AuNPs SERS experiments down to a single-molecule level could be performed. [1] In subsequent studies the SERS hot spots shall be further optimized by exploiting the increased field enhancement of Au-Ag core-shell nanoparticles within the visible range of the electromagnetic spectrum. Furthermore, the field of application shall be extended by using the origami-AuNPs assemblies as biosensors, e.g. for the detection of DNA.


Standards for Superresolution Fluorescence Microscopy based on DNA Origami

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The improvement of superresolution microscopy methods is an important topic for many scientists around the world. Due to the fact that the results of such improvements have to be compared to those of existing methods, there is a need for test-structures that have an expectation-value for distances and ensure reproducible results. Since conventional test-structures like actin filaments give only single and random distances on a SR-image, we developed standards based on the DNA-origami-structures because they allow a very reliable positioning of dye-molecules with a precision of ~1 nm. DNA origami nanorulers have marks with known distances in the desired range from 6 to 400 nm. These distances can be found hundreds of times on every SR-image which allows statistical analysis. DNA origami nanorulers were specifically designed for a variety of methods including dSTORM, STED, SIM and also for three dimensional superresolution microscopy [1-3]. With our new software CAEOBS (Computer Aided Evaluation of Origami Based Standards) thousands of DNA origami stuctures can be analyzed automatically in only a few minutes [3]. This makes DNA origami standards an easily manageable tool for checking the performance of a fluorescence microscope or a new fluorescence microscopy method.


Simulations of DNA brick self-assembly
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In 2012, Ke and co-workers [Science 338, 1177 (2012)] found that large numbers of distinct DNA strands can be designed to assemble into complex 3D target structures. This finding represented something of a conundrum for the self-assembly community, as no other colloidal system had previously achieved the same degree of complexity. Ke and co-workers suggested that in their system, seeding is slower than the subsequent growth of the desired structure, thereby minimising the tendency for incorrect assembly, but that this should be the case is far from obvious, and is furthermore difficult to prove experimentally. We use simulations to address the question of why DNA can succeed where colloids fail. In particular, we have simulated a system comprising ∼1000 types of DNA strand modelled using a very simple potential and have found that the designed structure can reproducibly self-assemble within a narrow temperature window. Using rare event simulation methods, we have also calculated the free-energy barrier to cluster growth, confirming the experimentalists' hypothesis that the process is nucleation-initiated. The presence of a free-energy barrier allows target structures to form with high yield because incomplete structures are unlikely to meet and stick together. Finally, given the highly simplified nature of our DNA model, our work implies that other systems, such as colloids, could also be designed to assemble into truly complex structures.

Transfer of a protein pattern from self-assembled DNA origami to a functionalized gold surface
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Two patterns of the protein streptavidin from assembled DNA origami are transferred onto a functionalized gold surface. The streptavidin molecules are linked to DNA via a biot-streptavidin interaction and a disulfide spacer and these conjugates self-organise into a predetermined pattern on DNA origami. The structures are first immobilized on a biotin-functionalized Au surface and after disulfide linker cleavage the origami is separated from the streptavidins, leaving the protein pattern at the surface.

M1.3 – A Small Scaffold for DNA Origami
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The 'origami method' allows for the assembly of designed DNA nanostructures of a wide range of geometries and sizes.1 Origami structures form when a long, usually single stranded scaffold strand anneals to a mixture of short, synthetic staple strands in aqueous buffer. One scaffold strand is dominating the field: M13mp18, a bacteriophage-derived vector 7249 nucleotides in length, which is typically induced to fold in the designed structure by using ≥200 staple oligonucleotides. The number of staples and the size of the final structures make it difficult to generate chemically modified structures routinely. We have recently reported the convenient preparation of a 704 nt fragment dubbed "M1.3" as linear or cyclic scaffold and the assembly of small origami structures with just 15-24 staple strands.2 Small origami with M1.3 as scaffold show similar thermal stability as larger M13 origami structures.3,4 The much smaller structures are more readily prepared from chemically reinforced strands, prepared individually using the power of synthetic organic chemistry.

References
Elucidating the assembly of brick-based DNA nanostructures

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Brick-based DNA nanostructures are a powerful approach for designing and creating DNA nanostructures at high speed [Ke et al., 2012]. As its name implies, the structures are constructed from a collection of several hundred synthetic single-stranded DNA oligonucleotide “bricks”, designed to link to neighboring bricks through complementary base pairing. From a predefined pool made up of several thousand DNA strands which collectively form a 3D canvas, nearly any arbitrary structure can be created in a matter of days. Due to its modularity and ability to be integrated with computer-assisted automated design and production, this technique is promising for potential industrial-scale applications. However, this necessitates an intimate understanding of the molecular assembly process so that factors such as production yield, speed of assembly and basic design can be fully optimized.

Studies on DNA origami have shown that scaffolded nanostructures can assemble within a relatively narrow temperature range [Sobczak et al., 2012; Wei et al., 2013], possibly due to cooperative effects arising from the long underlying scaffold strand. While brick-based DNA nanostructures are lacking this underlying scaffold, the highly regular pattern of hybridization linking together neighboring bricks could also give clues into optimization strategies for the assembly process. First estimations on brick-based DNA nanostructures indicated that during the thermal-based assembly, initial outer “shell” sections could be formed at higher temperatures followed by the hybridization of the inner portions of the structures at much lower temperatures. More exact nearest-neighbor thermodynamic calculations of melting temperatures for hybridized sections of neighboring bricks pointed out two broad temperature ranges. The higher of these temperature ranges may form outer-shell sections which could act as nuclei for successive assembling. Real-time fluorometric assembling experiments will display the relevance of this theory. Preliminary experimental data show some similarity with previously described calculations of melting temperatures. Future experiments are planned to determine the influence of outer-shell sections in the entire assembling process.

Characterizing the bending and flexibility induced by bulges in DNA duplexes

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Advances in DNA nanotechnology have grown considerably in the last few years, which has stimulated the search for simple structures that can be used to control certain properties of a variety of self-assembled nano-scale objects. We use a coarse-grained model of DNA, oxDNA, to examine the properties of structures assembled from star-tiles, including chiral DNA triangular prisms created by Chendge Mao's group, tetrahedrons, as well as 2D arrays. We also examine the Z-tile, which can be used to grow 1D nanostructures. All of these structures have the following feature in common: a bulged duplex, that is, two helical segments that connect at a bulge loop. This motif has been used extensively in experiments involving various tiles. The bulged duplex can occupy two main populations, one where the stacking at the center of the system is preserved where the system is straight with the bulge on one side, and the other where the stacking at the center is broken, which allows the system to act as a hinge to allow flexibility. For small loop sizes, often some of the bases are inserted into the duplex whereas with increasing bulge length the range of bend angles available becomes wider and the relative twist angles between the two helical sections are less correlated.

The properties of this basic structural motif clearly correlate with the structural behavior of certain nano-scale objects, and the enhanced flexibility associated with larger bulges has indeed been used to tune the
self-assembly product as well as structure of DNA nano-structures. For example, in the case of the chiral triangular prisms, the bulge size can be adjusted so that the prism prefers to twist in one direction over another. Our results from simulations of the different prisms mainly reproduce the experimental measurements of the twist, as well as suggesting that the chiral cages are relatively pliable and have a tendency to shear somewhat. Current investigations include understanding the tendency of certain structures, for example rings made of three star-tiles, having a particular curvature that causes a particular isoform of the prisms to form over other possible isoforms. This curvature tendency has consistently been seen in the simulations, and has been investigated experimentally in Mao's group. Additionally, we are currently exploring the effect of bulges on the dynamics of ring closure, a key assembly process in the self-assembly of chiral cages.

**Dielectrophoretic trapping and electrical measurement of 3D DNA origami**

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DNA origami as a self-assembled, non-periodic structure has great potential in nanoelectronics and nanophotoics. To utilize DNA origami in such areas, it is important to understand their electrical properties. Our group has studied the conductance of individual rectangular origami by dielectrophoretic (DEP) trapping and impedance spectroscopy (IS) and found that the conductance of DNA origami is very low in dry, but become higher in high humidity. Data analysis based on the IS also suggest that the water molecules play an big role in DNA origami conductance, which means the newly developed 3D DNA origami may have an improved conductance since their compact structure can hold more water molecules. Therefore, we trapped and measured 3D DNA origami with the shapes of brick and "L". The results proved that the individual 3D DNA origami can also be trapped by DEP, which is a nice tool for manipulating 3D DNA origami. But there is no evidence show significant improvement in conductance yet. In addition, incidences of localized destruction of the substrate after trapping were observed, the cause of this is still under investigation.

**3D plasmonic chiral colloids**

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3D plasmonic chiral colloids are synthesized through deterministically grouping of two gold nanorod AuNRs on DNA origami. These nanorod crosses exhibit strong circular dichroism (CD) at optical frequencies which can be engineered through position tuning of the rods on the origami. Our experimental results agree qualitatively well with theoretical predictions.

**Studying DNA Origami Using a Coarse-Grained Model of DNA**

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DNA origami is a powerful and flexible method of DNA nanostructure fabrication through directed self-assembly, which enables the formation of large nanostructures made of thousands of DNA base pairs. It is a relatively new and fast-growing technique with many potential applications. However, many aspects of DNA origami are not well understood, such as the assembly mechanism, mechanical properties, and the precise structural form of the origami objects in solution. We attempt to understand the assembly and the structural properties of these objects by simulating a coarse-grained model of DNA, oxDNA, which reproduces the thermodynamic, mechanical and structural properties of single and double DNA strands by representing DNA on the single-nucleotide level. We use molecular dynamics run on GPUs to simulate large DNA origami structures with this relatively detailed model.

In origami assembly, we find evidence of kinetic traps caused by competition between staple strands, where identical strands partially bind to the same scaffold strand and prevent each other from fully...
binding, which may lead to misfolding. We also see quasi-topological problems associated with completing the hybridisation of a partially bound scaffold domain, a design-dependent effect which would result in lower rates of assembly than one might expect. Meanwhile, simulations of 2D origami structures in solution show that they are very flexible when not bound to a surface. In addition, these 2D structures exhibit the well-known weave pattern between DNA helices within the plane of the structure, as well as an out-of-plane pattern which we call corrugation; in 3D structures, these effects combine to cause each DNA helix to itself trace out a left-handed helix with a period dependent on the origami design.

Rational design of structured substrates for DNAzyme signaling

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I’ll describe DNAzyme signaling cascades developed in my lab and their application to multi-strain pathogen detection.

DNA Origami as Matrices for Enzyme imprinting

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We plan to use two-dimensional (2D) DNA Origami as a nanochips for detecting short nucleic acids such as micro (mi)RNA, as captured with a probe protruding from the nanochip surface. Following previous results on surface-bound nucleic acids monolayers (Redhu S.K. et al., Sci. Rep. 2013), we plan to use an ancillary, restriction enzyme for site-specifically imprinting the presence of captured miRNAs on the Origami scaffold, in solution. With such detection approach, the 2D DNA Origami serves as a matrix for imprinting and storing biological information that is subjected to alteration due to the rapid degradation of miRNA content. Recent studies already reported on the enhanced stability of DNA Origami of different shapes against different nucleases, in purified solutions and in complex biological mixtures (Castro et al., 2011; Lee et al., 2012; Qian, Yan, & Meldrum, 2011). There is, however, a lack of information on the structural factors that determine enzymatic reactions in such highly dense DNA nanostructures.

We studied the behavior of 14 different restriction enzymes towards two well-known, 2D DNA Origami, a triangle and a rectangle, both originating from the same single stranded (ss)DNA scaffold (M13mp18), by denaturing gel electrophoresis analysis of the reactions products. Whereas the DNA scaffold sequence naturally includes a number of restriction sites (regardless the 2D shape), our results suggest that most of the restriction enzymes tested differently access to their sites as a function of the 2D shape. The enzyme accessibility of these restriction sites must depend on the pattern of the staples-based canvas, which is shape-dependent. In addition, data analysis suggests that within such a highly dense, 2D DNA Origami, a restriction site is fully accessible only when it is exposed on the surface of the DNA Origami, and is protected by the presence of an adjacent double helical segment, which covers its surface.

These results are essential towards developing our enzyme imprinting-based sensor. In particular, we plan to modify two staples near one restriction site. In turn, two ssDNA overhangs will be bridged only upon target binding, thus leading to the formation of a double helix segment, which will cover the surface of the restriction site, thus protecting it from the action of the enzyme. Another (control) site will be located within an unstructured region of the DNA Origami, as to have maximum accessibility. Successful enzymatic reaction on both sites (i.e. no target present on the DNA Origami’s surface during the enzyme reaction) will lead, after melting the DNA self-assemblies, to the formation of two distinct fragments of the scaffold. On the contrarily, in the case that a copy of the target binds to the DNA Origami, the scaffold will be enzyme-cleaved in single site, and thus linearized. A miRNA target will be quantified by measuring the molar ratio between the linearized scaffold and its miRNA-specific fragments, by means of electrophoretic separation and/or southern blot analysis.

Topography-Controlled Alignment of DNA Origami Nanotubes on Nanopatterned
Surfaces
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Since its discovery, DNA origami technique [1] has attracted considerable attention from various research fields. By this technique, it has become possible to generate complex 2D and 3D DNA nanostructures with any desired shape. Quasi-one dimensional DNA nanostructures hold particularly great promise as scaffolds for nanoelectronic device fabrication. However, the fabrication of functional nanoelectronic devices from such DNA nanostructure templates requires their controlled arrangement and orientation on a conventional substrate. In the past, various techniques have been employed to control the alignment of immobilized DNA nanostructures on different surfaces [2], [3]. However, most techniques rely on lithographic pre-patterning and often also a chemical functionalization of the substrate.

In this work, we demonstrate a compelling alternative approach to generate ordered arrays of DNA nanotubes on topographically patterned surfaces. To this end, we combine two bottom-up techniques for nanostructure fabrication, i.e., DNA origami self-assembly and self-organized nanopattern formation on silicon surfaces during ion sputtering [4], thus avoiding the necessity of lithographic processing or chemical modifications. The self-alignment of the six-helix-bundle (6HB) DNA origami nanotubes is purely driven by electrostatic interactions with the nanorippled Si/SiO2 surface during adsorption. By tuning the pattern dimensions to match the dimensions of the DNA origami nanotubes, we obtain an alignment yield of 70% [5].

Reference

Optimization of self-assembled nanoantennas for diagnostics
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Self-assembled nanoantennas [1] are not only able to highly enhance fluorescence in a well-defined ultrasmall volume but also to improve the signal-to-noise ratio and enable detection of single molecules in an environment of substantial fluorescent background. This effect offers great potential for practical applicability in many fields of research such as molecular diagnostics.
Basically, the nanoantenna consists of two gold nanoparticles that are able to focus light between them. By placing a fluorescent molecule in this ultra-small hot-spot between the two gold nanoparticles the fluorescence of this molecule gets enhanced. The major advantage of the self-assembled nanoantenna over other nanophotonic structures is that it is based on well-defined DNA origami nanostructures [2]. These DNA nanostructures allow precise placement of the gold nanoparticle dimer and also the fluorescent dye. Still, it has some considerable drawbacks like the relative big gap between the gold nanoparticles and the arbitrary angular placement of the DNA origami on the surface which directly effect and reduce fluorescent enhancement. Within this study, we present a new DNA origami design that reduces the gap between the gold nanoparticles and assures an up-right placement to achieve both a higher fluorescence enhancement and also very accurate and consistent results. In addition, the synthesis of the nanoantenna is further improved and simplified. Due to these improvements practical applicability of the self-assembled nanoantenna for nucleic acid detection in connection with diagnostics is enabled.


**Designed DNA Folding Motifs that Store Energy-Rich Biomolecules**

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Most complex nanotechnological devices require an energy source. If based on biomacromolecules, functions such as motion or emission of light can most easily be driven by energy-rich small molecules. For the device to be switchable, a storage motif or 'molecular battery' is called for that can release energy-rich compounds in controlled fashion. We have initiated a research program that studies de novo designed DNA or RNA sequences capable of forming binding sites for small molecules. The sequences are distantly related to aptamers in that they bind biologically relevant molecules like ATP1, NADH2, FAD2 and cAMP3. Aptamers are evolved and not designed motifs, though, that often fold into non-canonical secondary structures.4 The designed binding motifs presented here are based on triple helices and bind their targets via Watson-Crick and Hoogsteen base pairing.5,6 We show that our de novo designed binding motifs are able to bind and release energy-rich, biologically important molecules in temperature-controlled fashion. Further, is is shown that it is possible to create macroscopic storage devices by immobilizing DNA motifs on a porous solid support.

References

**Perylene Bisimides in DNA and their potential usage as Photonic Wires**

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Optical properties, such as a high fluorescence quantum yield, molar absorptivity and photo stability make Perylene-3,4:9,10-tetra carboxylic acid bisimides (PBl)s very interesting building blocks in a DNA scaffold [1]. The supramolecular structure of DNA allows controlling the chemical environment of implemented dyes. Therefore a variety of different adjacencies, which characteristics considerably differ from those of the chromophore itself, can be studied [2].
Modifications in the so called bay area of PBIs allow changes in optical and electronic characteristics and therefore they appear to be ideal candidates for usage photonic wires. Groups in the bay area need to be small to allow good intercalation of the introduced base surrogates. Bulky groups are leading to a disorganization of the DNA structure and consequently disturb stacking of the nucleobases causing less efficient energy transfer.

In our poster contribution we will report on the synthesis, electrochemical and optical properties of differently substituted PBI dyes, their incorporation into oligonucleotides and spectroscopic behavior in various DNA duplexes.

References


A Highly Sensitive, Label-free Sensing Platform Based on DNA Origami and Graphene Oxide Nanosheets
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Here, we report a label-free sensing platform with high sensitivity based on DNA origami and graphene oxide nanosheets. The aromatic ring-rich graphene oxide nanosheets provide a selective adsorbing interface for single-stranded DNA molecules with higher affinity than duplex DNA. The adsorption is mediated via stacking interaction between the exposed nucleobases and the graphene nanosheets. We design a series of DNA origami structures bearing single-stranded probing staples that can hybridize with analyte DNA or RNA molecules. By only two simple experimental steps, the analyte-binding DNA origami structures can be isolated and the original amount of analyte present can be determined via fluorescence with the help of conventional DNA dyes such as Ethidium Bromide or SYBR Gold/Green. Our sensing strategy demonstrates a significantly higher fluorescence level than the single-dye labeled probing systems and a high sensitivity without the need of any enzymatic amplification treatments, and shows a simple and versatile platform for the detection of nucleic acids and other related analytes.