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# Recent advances in self-assembled DNA nanosensors

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## To cite this article:

Karina M. M. Carneiro, Andrea A. Greschner. Recent Advances in Self-Assembled DNA Nanosensors. *American Journal of Nano Research and Application*. Special Issue: Nanomaterials and Nanosensors for Chemical and Biological Detection. Vol. 3, No. 1-1, 2015, pp. 1-7.

doi: 10.11648/j.nano.s.2015030101.11

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**Abstract:** Over the past 30 years DNA has been assembled into a plethora of structures by design, based on its reliable base pairing properties. As a result, many applications of DNA nanotechnology are emerging. Here, we review recent advances in the use of self-assembled DNA nanostructures as sensors. In particular, we focus on how defined nanostructures, such as rigid DNA tetrahedra, provide an advantage over traditional nanosensors consisting of arrays of single-stranded DNA. We also explore advances in DNA origami that have resulted in consistent detection of single molecules.

**Keywords:** Self-Assembly, DNA, Nanosensors, Tetrahedron, DNA Origami

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## 1. Introduction

The simple four-letter alphabet and predictable hydrogen bonding assembly patterns associated with the genetic code – adenine (A) binds to thymine (T) and cytosine (C) binds to guanine (G) – make DNA a reliable material for the construction of both nanostructures and nanomaterials.[1] This same high specificity also makes nucleic acids ideal candidates for use as sensors, and for binding and catalysis applications, coining the term ‘functional nucleic acids’.[2, 3] Being on the same length scale as many biological molecules – such as proteins, enzymes, and antibodies – rationally controlling the organization of molecules in the nanometer regime is particularly important in medical diagnostics.

Sensors detect the presence of specific analytes, ideally with high affinity and specificity, a fast response time, a long shelf life and reusability. In broad terms, a sensor consists of two parts: target recognition and signaling moieties. Nucleic acid aptamers (single-stranded nucleic acid molecules that have a well-defined three-dimensional structure and a high affinity to their target molecule) have been extensively used for target recognition within sensors due to their sequence-specific properties. A noteworthy combinatorial method called systematic evolution of ligands by exponential enrichment (SELEX) can be used to identify nucleic acid sequences that bind to a desired target with high affinity.[4] This step-wise protocol selects for the best aptamer sequences from a large pool of DNA strands, thereby streamlining aptamer development substantially. It has been reported that aptamers

can rival the binding performance of antibodies in certain aspects, and are thus valid target recognition alternatives to antibodies, the development of which presents its own challenges and limitations.[5] Hundreds of aptamers have been designed by scientists, and the widespread applicability of these aptamers has even motivated the creation of a searchable online database.[6] The range of analytes that have been targeted by aptamers is exceptionally wide; two noteworthy model aptamer targets are thrombin and adenosine triphosphate (ATP), although the full range also encompasses metal ions, organic small molecules, macromolecular examples of both natural and synthetic origin, and live organisms such as bacteria and eukaryotic cells. For an extensive review on functional nucleic acid sensors, please refer to Lu *et al.*[2] A second major class of nucleic acid sensors is referred to as molecular beacons (single-stranded DNA hairpins functionalized with a FRET pair). These functional nucleic acid sensors are typically employed to probe for the presence of a complementary target such as DNA or RNA, which may provide useful genetic or diagnostic information.[7] They have also been used to probe for the presence of the enzymatic target ligase, showcasing their potential beyond the area of nucleic acid sequence detection. The sensitivity of molecular beacons can be increased, for example, through conjugation with superquenchers and conjugated polymers. For more information on molecular beacons, please refer to Tan *et al.*[3]

Nucleic acids are already playing a large role in sensing applications. While the traditional sensors employ relatively simple or locally structured nucleic acid moieties, multiple

new developments in the hierarchical self-assembly of DNA have introduced DNA nanostructures to the field of molecular sensing. Here, we will present the most recent advances in using self-assembled DNA nanostructures to construct nanosensors. We will explore how the rational design of individual DNA nanostructures can increase sensitivity, amplify a signal, and allow detection of a variety of targets. In particular, we will review developments favoring the use of a DNA tetrahedron over the more traditional single duplex and hairpin sensors, as well as both static and dynamic DNA origami sensors.

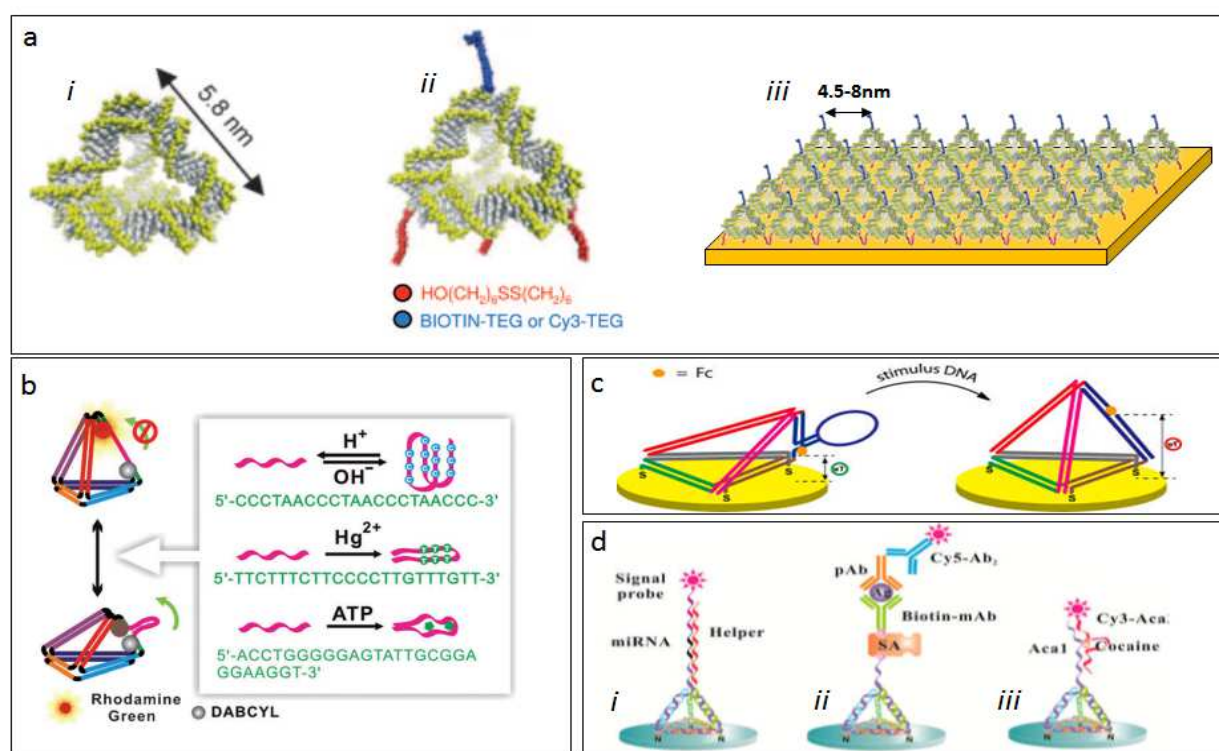
## 2. 3D DNA Nanostructures as Nanosensors

### 2.1. The Tetrahedron

In 2004, the emergence of a simple and reliable assembly strategy for small DNA nanostructures[8] led to the development of a new class of tetrahedron detectors. The tetrahedral shape of this molecule, obtained through the one-pot assembly of four DNA strands, had several characteristics that were ideal for nanosensing applications. Initial studies determined that the shape, size, and double-stranded nature of the tetrahedron makes it resistant to many types of enzyme digestion,[9] an important feature in developing nanosensors that may be exposed to serum

samples during routine testing (Scheme 1a*i*). In addition, the four-strand assembly pattern, with each strand terminating at a vertex of the tetrahedron, made it amenable to functionalization. Howorka *et al.*, were the first to take advantage of this feature. Through chemical modification of the 5' end of each DNA strand, they were able to append a disulfide molecule on each of three vertices, with either a biotin or Cy3 dye on the fourth (Scheme 1a *ii*). The three disulfide tags permitted strong adhesion to gold surfaces (Scheme 1a *iii*). A kinetic dissociation experiment determined that only 5% of triple-tagged tetrahedra were displaced after 2 hours, whereas 50% of doubly-tagged and 90% of singly-tagged tetrahedra were lost. AFM experiments with the biotin functionalized molecule confirmed that the fourth vertex was vertically oriented and remained accessible for complexation with streptavidin.[10, 11].

In sensor applications, the spacing of nanostructures on surfaces plays a large role in determining sensitivity. Common problems with single-stranded (ss) or hairpin-based nanosensors include molecular crowding at high concentration or long strand length, and probe strands adhering along the surface at low concentrations and/or short strand length. In both cases, access of the target to the sensing strand is diminished.[12, 13] In contrast, the three-dimensional shape and 3-point, 4-5.8 nm footprint[8, 14] of a tetrahedron ensures consistent spacing and proper orientation of the sensor, allowing easy access for targets (figure 1a *iii*).



**Scheme 1.** a) The DNA tetrahedron is constructed by combining four oligonucleotide strands and allowing them to self assemble (*i*). Adding a functional group to the end of each strand allows for specific placement at each vertex after assembly (*ii*). In this case, three thiol modifications and one sensor group were attached. The three thiols anchor the tetrahedra to the gold substrate, resulting in a 4.5-8 nm separation between sensor groups. b) Dynamic structural changes can be achieved by introducing specific single-stranded DNA sequences to one side of the tetrahedron. Upon analyte recognition the sequence folds, quenching the fluorescence. c) Electrochemical sensing of a target strand fully complementary to the built-in hairpin. d) By functionalizing with amines in the place of thiols, glass substrates can be used. Three targets were tested: *i*) miRNA, *ii*) prostate-specific antigen, and *iii*) cocaine. Adapted from references 10, 15, 16, and 19 with permission from Wiley-VCH Verlag and the American Chemical Society.

## 2.2. Signaling Schemes

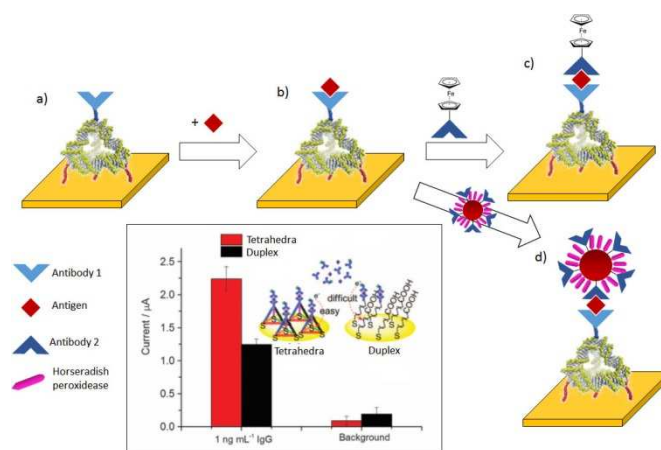
The signaling action of nanosensors can take many forms and has been a forum for recent advances. Similar to the classical hairpin probes, tetrahedral probes can signal a change in conformation. An elegant example of this is a system designed by Fan *et al.* In their study, they modified the traditional tetrahedron such that one side remained single-stranded. A dabcyl quencher and rhodamine green fluorophore were added to either end of the ssDNA portion. The ssDNA sequence was specifically chosen to respond to the presence of certain molecules or conditions by folding, thus bringing the FRET pair together and creating a detectable quench in fluorescence. By varying only a single strand, they were able to sense changes in pH (using an i-motif), the presence of mercury (T-rich mercury-specific oligonucleotide), and ATP (anti-ATP aptamer) (Scheme 1b). In the case of the pH sensor, the process was completely reversible. Sensor capabilities were expanded by creating two ssDNA sides with orthogonal targets. Sensitivity varied depending on the target, with detection limits of 2  $\mu\text{M}$  ( $\sim 1 \mu\text{g/mL}$ ) for ATP and 20 nM ( $\sim 4 \text{ ng/mL}$ ) for mercury. Regardless, selectivity was high, with similar molecules such as CTP, GTP, and UTP and other metal ions producing very little change in signal compared to the real targets. [15]

Electrochemical assays are gaining in popularity due to their low detection limits compared to fluorescence-based assays. The gold substrate common to many tetrahedron-based assays is appealing, as it can act as an electrode. While maintaining the substrate and tetrahedral design, many other adjustments have been pursued to obtain the lowest possible detection limits. Dynamic, surface-tethered DNA structures were combined with ferrocene to produce a sensor that modulates energy transfer based on surface-to-ferrocene distance. The ferrocene label was introduced above the hairpin near the peak vertex of the tetrahedron. A fully complementary target binding to the hairpin causes the tetrahedron to open, increasing the distance between ferrocene and the electrode and decreasing the signal substantially. The rigidity lent by the tetrahedral shape ensures that the ferrocene remains at the intended distance from the surface (Scheme 1c). [16]

Leong *et al.*, used an electrochemical antibody sandwich technique to detect IgG antigens (Scheme 2a-c). The first IgG antibody was coupled to the free vertex of the tetrahedron. Carbodiimide coupling was used to attach the second antibody to a ferrocene. When exposed to target, the IgG antigen binds to the antibody on the tethered tetrahedron and is sandwiched by the antibody-labeled ferrocene. The resulting electrochemical signal had a detection limit of 2.8 pg/mL. [17]

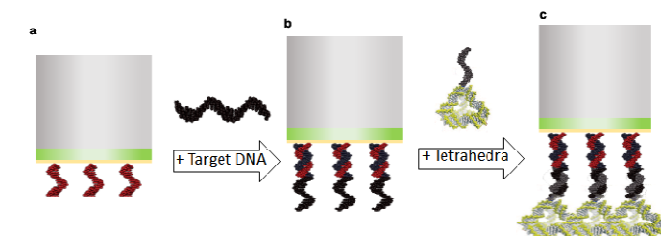
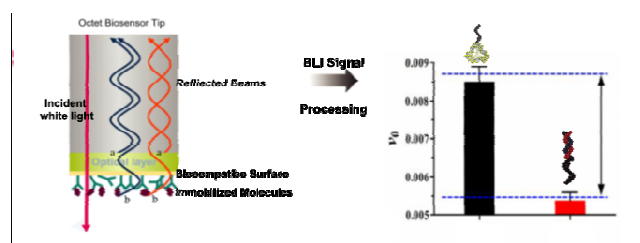
The sandwich motif can also be used for signal amplification. Zuo *et al.* constructed a sandwich-based tetrahedral nanosensor to detect prostate-specific antigen. They modified the detection method by using a gold nanoparticle multiply-labeled with antibody and horseradish

peroxidase (HRP) instead of ferrocene. Because multiple HRP's were associated with each antibody binding action, the resulting signal was greatly augmented. The combination of ideal spacing (provided by the tetrahedron) and signal amplification (via the gold nanoparticle) resulted in a detection limit of 1 pg/mL, a full order of magnitude greater than singly-labeled sensors (Scheme 2d). [18]



**Scheme 2.** a) A gold-tethered tetrahedron functionalized with an antibody. b) When a suitable antigen is added to the solution, it binds to the antibody. c) Addition of a second, ferrocene-labeled antibody creates a sandwich and results in electron transfer. d) Alternatively the sandwich can be completed with a gold nanoparticle multiply labeled with antibodies and HRP's. The many HRP's result in signal amplification. Inset: Compared to single-duplex sensors, tetrahedra provide twice the signal. Adapted from references 17 and 18 with permission from Nature Publishing Group and the American Chemical Society.

## 2.3. Accessibility



**Scheme 3.** Inset: General BLI sensor consists of a fibre optic (grey), with an optical biolayer (green) and a biocompatible surface (yellow). Light shines through the fibre optic and reflects off both a reference layer and biolayer. The signal from the biolayer depends on thickness and density, and is greatly increased when a pendant tetrahedron is used. a) Fibre optic functionalized with half an aptamer (red). b) Target molecule (black) is partially recognized by the immobilized half-aptamer. c) A tetrahedron, conjugated to the remaining half of the aptamer, also recognizes the target, amplifying the signal. Adapted from references 22, 23 with the permission of Elsevier and the American Chemical Society.

While the above methods have excellent detection limits, their cost can be prohibitive, with both gold substrate and chemical labelling required. Several steps are being taken towards making these techniques more accessible. One approach is to use a different substrate. Fan *et al.* modified the DNA tetrahedron by replacing the thiol functional groups used previously with amines, allowing for immobilization on glass substrates. The group surveyed several possible targets using a variety of sandwich assays. Even though unamplified fluorescence detection methods were used in lieu of electrochemical methods, they were able to achieve respectable detection limits of 40 pg/mL for prostate-specific antigen and 100 nM (~30 ng/mL) for cocaine and demonstrate good correlation with clinical tests, while using a readily available, inexpensive substrate (Scheme 1d).[19]

Still others are moving towards label-free analyses. These nanosensors are easier to work with, less expensive, and often quicker to prepare, although they lack somewhat in sensitivity.[20, 21] One label-free method is biolayer interferometry (BLI). In general, the tip of a fibre optic sensor is coated with a layer of molecules for trapping the designated targets. White light is shone through the fibre optic, and partially reflected by the two layers at the tip (the reference layer and biolayer). Reflected light is captured by a spectrometer. When the target is bound to the biolayer, it alters the reflectance and can be detected (Scheme 3, inset).[22] Ye *et al.* used tetrahedra to enhance this signal by splitting the sequence of an aptamer. Half of the sequence was immobilized on the fibre optic tip (Scheme 3a), the other half was pendant on a vertex of a tetrahedron. Dipping the fibre optic into a solution containing target allowed for initial binding to the sensor (Scheme 3b). When tetrahedra are added, the remaining half of the aptamer was also recognized by the target, creating a tetrahedron-target-sensor sandwich (Scheme 3c). The added bulk of the tetrahedra created a thicker and denser biolayer, enhancing the signal by two orders of magnitude and decreasing the detection limit to 200 pM (~1.4 ng/mL).[23]

#### 2.4. Summary

Moving from ssDNA and hairpin sensors to discrete self-assembled DNA nanostructures has many benefits. Easily addressed vertices allow for a variety of functionalities, including surface tethers (thiol for gold substrates, and amines for glass substrates), fluorophores, and aptamers. The size and shape of the tetrahedron provides ideal spacing between sensors, and ensures that they are oriented in an upright position for easy target access. The programmability of the DNA alphabet allows for target-triggered dynamic movement of the tetrahedron itself, or it can be used as a scaffold for electrochemical, fluorescence, sandwich, and label-free assays.

### 3. DNA Origami in Nanosensing

The above-presented techniques are excellent for detecting target analytes. However, the signal obtained in response to

target detection is inevitably an average of all the sensor-target interactions.

DNA origami is a system for making self-assembled nanoscale DNA structures. Each shape is pre-designed and based on a single long DNA strand folded by a series of added staple strands.[24] The resulting structures can be static or dynamic,[25, 26] and are characterized via atomic force microscopy (AFM), precluding the need for fluorescent tags or electrochemical sensing techniques. DNA origami offers an opportunity to isolate and identify specific interactions. These structures can be functionalized in a variety of ways, based on their shape and design.

#### 3.1. Static DNA Origami Sensors

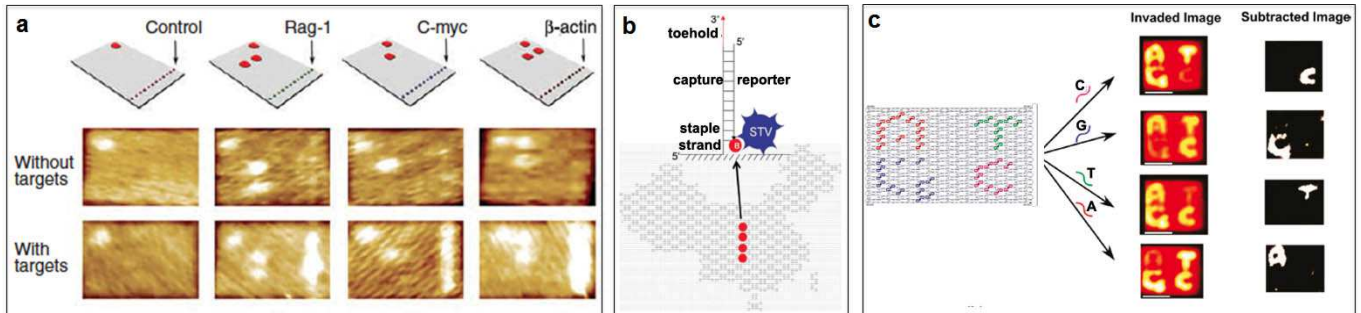
Static origami nanosensors rely on a change of surface characteristics for detection. Yan and co-workers designed a DNA origami tile (60 x 90 nm) with distinct bar codes or labels (specifically positioned DNA dumbbell structures on the surface of the tiles) to differentiate between tiles targeting individual RNA sequences.[27] Each tile was functionalized with adjacent 20-nucleotide single-stranded regions for hybridization with a 40-nucleotide RNA target (namely *Rag-1*, *c-myc* and  $\beta$ -*actin*) as seen in Scheme 4a. Once the target hybridized to the complementary sequences on the tile, a V-shaped structure formed, visible via AFM. The authors found that the edge of the tile provided the best position for target hybridization, presumably due to steric interactions and charge repulsion being greater at the center of the tile. These tiles could be used at a 5:1 ratio with the target, with 30 minutes incubation time, no stirring and at room temperature, for visualization by AFM. The targets were also able to successfully detect an RNA sequence in the presence of a large excess of cellular RNA, with no cross-hybridization events reported. This system is a proof-of-concept for nucleic acid sensing, but is limited by tile concentration (minimum reported 200 pM) and AFM as a characterization technique.

An asymmetric DNA origami (with the shape of the map of China) was also used for the detection of specific DNA strands in solution.[28] This structure differs from the one proposed by Yan *et al.*[27] due to its asymmetric nature, and therefore relinquishes the need of a label within the assembly. It is also different with regards to the DNA probe used: linear versus V-shaped. The group used this asymmetric tile to detect DNA strands with a specific sequence in solution through a sandwich approach, whereby a short overhang strand hybridized to half of the target strand, and the other half of the strand hybridized to a biotin-functionalized DNA strand which is subsequently conjugated to a streptavidin. The increased height due to the presence of streptavidin on the surface of the map can be visualized by AFM. A similar streptavidin-based approach was used for the detection of DNA strands containing a single nucleotide polymorphism (SNP). In this case, the tile was labelled with a toehold strand pre-labelled with a streptavidin. The term 'toehold' indicates that the strand is slightly longer than its complement, allowing for displacement of the shorter (streptavidin-labelled) strand if the fully complementary strand is introduced. The group was

able to use the toehold-displacement strategy to distinguish between fully complementary targets and those with a single mismatch (Scheme 4b).[29]

Seeman and co-workers designed a DNA origami tile decorated with hairpins in the shape of the DNA base letters: A, C, T and G, which were visualized by AFM.[30] These hairpins had a toehold, such that they could be displaced in the presence of a DNA strand fully complementary to the hairpin strand (via the strand displacement method). In the presence of

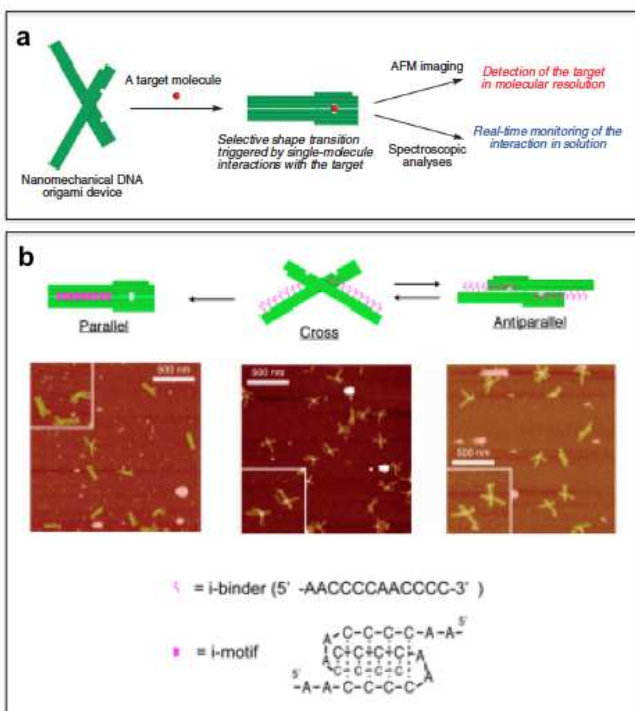
an analyte fully complementary to the strand, the hairpin structure is destabilized and removed as a duplex from the surface of the origami tile, therefore erasing the letter from the surface of the tile. This method provides a visual readout of the SNP type by AFM. The authors also created a computer program that calculates an average of 25 images and subtracts the background for a direct readout of the mismatched base (Scheme 4c).



**Scheme 4.** a) Rectangular DNA origami tile functionalized for label-free detection of specific RNA targets. The 'barcode' of each tile is visible as dots on the main tile surface, whereas the strip along the right edge indicates target detection. b) DNA origami in the shape of the map of China for the detection of single nucleotide polymorphisms (SNPs). c) Rectangular DNA origami tile used for a visual readout of SNPs based on AFM images. Reproduced and adapted with permission from references 27, 29, 30 with permission from the American Association for the Advancement of Science, John Wiley & Sons, and the American Chemical Society.

### 3.2. Dynamic Origami Sensors

Dynamic origami nanosensors are those that change their overall shape in response to the presence of an analyte. The changes are distinctly visible through AFM.



**Scheme 5.** a) Dynamic DNA origami assembly changes shape in the presence of target molecules. b) pH responsive shape change of a nanomechanical DNA origami sensor. Adapted from references 31 and 32 with permission from Nature Publishing Group and MDPI.

Komiyama *et al.*, designed what they referred to as DNA origami 'pliers' and 'forceps'. These designs consisted of two origami arms (170 x 20 nm) connected by a type of four-way junction referred to as a Holliday junction. The natural form of the Holliday junction is x-shaped. As such, when the entire origami structure settles on mica prior to imaging, the preferred orientation of the arms is a cross shape. In order to detect individual molecules, ligands were placed inside a small notch on each arm. When the target is recognized by the ligands, the two notches are aligned, resulting in a parallel arm orientation (Scheme 5a). This switch from cross to parallel is easily visible via AFM, and indicates that a single molecule of analyte has been detected and immobilized. The proof-of-concept was performed using biotin/streptavidin and FAM/antifluorescein IgG as ligand/target pairs.[31]

The same team also investigated 'zipper' sensors. The tweezer shape and cross versus parallel detection method remained the same, but instead of ligands, they attached pendant DNA strands to each lever. The pendant strands were chosen for their ability to self-assemble under target conditions. For example, by using a 12-base telomeric excerpt, the pendant arms will join together to form a G-quadruplex in the presence of  $K^+$  or  $Na^+$  (depending on the sequence).[31] The system has since been adapted as a pH sensor, using the acid-sensitive i-motif sequence as pendant strands (Scheme 5b).[32]

Using origami assemblies as sensors for nucleic acids and RNA allows for aqueous quantification and very small sample amounts are required. However, the sensor results can only be interpreted by AFM imaging. This would cause a technical challenge in clinical settings, as personnel would need to be trained on the instrument, and imaging can be time-consuming

and challenging. This difficulty may be overcome by the continuing efforts in simplifying AFM characterization, and with fast scan AFM.

## 4. Conclusion

While DNA aptamers, hairpins, and molecular beacons have become quite common in sensor applications, the use of self-assembled DNA nanostructures remains fairly new. Replacing molecular beacons and single-stranded sensors with rigid, three-dimensional tetrahedra promotes proper probe spacing, thereby reducing steric hindrance and improving target access. The stiffness of the structure also keeps the probes properly oriented. Because of the innate programmability of DNA, dynamic sensors can be designed to utilize changes in tetrahedron side lengths as signaling devices. A shift has also been seen towards more accessible DNA nanosensors, with alternative substrates and label-free detection methods being explored.

Functionalization of DNA origami has also led to advances in molecular-level sensing. Based on AFM characterization, target recognition can be detected either through changes in the surface of a DNA origami tile, or through dynamic shape changes in the overall origami structure. Clever design techniques have enabled static DNA origami to be programmed to not only indicate the presence of a target, but to also identify the target. Dynamic DNA origami, on the other hand, is capable of signaling the detection of a single analyte molecule.

Overall, research into self-assembled DNA nanosensors is yielding a variety of benefits. Work towards accessible and affordable substrates, label-free sensors, and fast-scan microscopy techniques will continue to drive this field.

## References

- [1] K.M. Carneiro, N. Avakyan, H.F. Sleiman, "Long-range assembly of DNA into nanofibers and highly ordered networks", Wiley interdisciplinary reviews. Nanomedicine and nanobiotechnology, 2013, pp.
- [2] J. Liu, Z. Cao, Y. Lu, "Functional nucleic acid sensors", Chemical Reviews, 109, 2009, pp. 1948-1998.
- [3] K. Wang, Z. Tang, C.J. Yang, Y. Kim, X. Fang, W. Li, Y. Wu, C.D. Medley, Z. Cao, J. Li, P. Colon, H. Lin, W. Tan, "Molecular engineering of DNA: Molecular beacons", Angewandte Chemie International Edition, 48, 2009, pp. 856-870.
- [4] C. Tuerk, L. Gold, "Systematic evolution of ligands by exponential enrichment: Rna ligands to bacteriophage t4 DNA polymerase", Science, 249, 1990, pp. 505-510.
- [5] S.D. Jayasena, "Aptamers: An emerging class of molecules that rival antibodies in diagnostics", Clinical Chemistry, 45, 1999, pp. 1628-1650.
- [6] K. Robison, A.M. McGuire, G.M. Church, "A comprehensive library of DNA-binding site matrices for 55 proteins applied to the complete escherichia coli k-12 genome", Journal of Molecular Biology, 284, 1998, pp. 241-254.
- [7] S. Tyagi, F.R. Kramer, "Molecular beacons: Probes that fluoresce upon hybridization", Nat Biotech, 14, 1996, pp. 303-308.
- [8] R.P. Goodman, R.M. Berry, A.J. Turberfield, "The single-step synthesis of a DNA tetrahedron", Chemical Communications, 2004, pp. 1372-1373.
- [9] J.-W. Keum, H. Bermudez, "Enhanced resistance of dnananostructures to enzymatic digestion", Chemical Communications, 2009, pp. 7036-7038.
- [10] N. Mitchell, R. Schlapak, M. Kastner, D. Armitage, W. Chrzanowski, J. Riener, P. Hinterdorfer, A. Ebner, S. Howorka, "A DNA nanostructure for the functional assembly of chemical groups with tunable stoichiometry and defined nanoscale geometry", Angewandte Chemie International Edition, 48, 2009, pp. 525-527.
- [11] M. Leitner, N. Mitchell, M. Kastner, R. Schlapak, H.J. Gruber, P. Hinterdorfer, S. Howorka, A. Ebner, "Single-molecule afm characterization of individual chemically tagged DNA tetrahedra", ACS Nano, 5, 2011, pp. 7048-7054.
- [12] D.Y. Petrovykh, V. Pérez-Dieste, A. Opdahl, H. Kimura-Suda, J.M. Sullivan, M.J. Tarlov, F.J. Himpsel, L.J. Whitman, "Nucleobase orientation and ordering in films of single-stranded DNA on gold", Journal of the American Chemical Society, 128, 2005, pp. 2-3.
- [13] H. Pei, X. Zuo, D. Pan, J. Shi, Q. Huang, C. Fan, "Scaffolded biosensors with designed DNA nanostructures", NPG Asia Mater, 5, 2013, pp. e51.
- [14] H. Pei, N. Lu, Y. Wen, S. Song, Y. Liu, H. Yan, C. Fan, "A DNA nanostructure-based biomolecular probe carrier platform for electrochemical biosensing", Advanced Materials, 22, 2010, pp. 4754-4758.
- [15] H. Pei, L. Liang, G. Yao, J. Li, Q. Huang, C. Fan, "Reconfigurable three-dimensional DNA nanostructures for the construction of intracellular logic sensors", Angewandte Chemie International Edition, 51, 2012, pp. 9020-9024.
- [16] A. Abi, M. Lin, H. Pei, C. Fan, E.E. Ferapontova, X. Zuo, "Electrochemical switching with 3d DNA tetrahedral nanostructures self-assembled at gold electrodes", ACS Applied Materials & Interfaces, 6, 2014, pp. 8928-8931.
- [17] L. Yuan, M. Giovanni, J. Xie, C. Fan, D.T. Leong, "Ultrasensitive igg quantification using DNA nano-pyramids", NPG Asia Mater, 6, 2014, pp. e112.
- [18] X. Chen, G. Zhou, P. Song, J. Wang, J. Gao, J. Lu, C. Fan, X. Zuo, "Ultrasensitive electrochemical detection of prostate-specific antigen by using antibodies anchored on a DNA nanostructural scaffold", Analytical Chemistry, 86, 2014, pp. 7337-7342.
- [19] Z. Li, B. Zhao, D. Wang, Y. Wen, G. Liu, H. Dong, S. Song, C. Fan, "DNA nanostructure-based universal microarray platform for high-efficiency multiplex bioanalysis in biofluids", ACS Applied Materials & Interfaces, 6, 2014, pp. 17944-17953.
- [20] M. Zhang, B.-C. Ye, "Label-free fluorescent detection of copper (ii) using DNA-templated highly luminescent silver nanoclusters", Analyst, 136, 2011, pp. 5139-5142.
- [21] M. Zhang, S.-M. Guo, Y.-R. Li, P. Zuo, B.-C. Ye, "A label-free fluorescent molecular beacon based on DNA-templated silver nanoclusters for detection of adenosine and adenosine deaminase", Chemical Communications, 48, 2012, pp. 5488-5490.

- [22] T. Do, F. Ho, B. Heidecker, K. Witte, L. Chang, L. Lerner, "A rapid method for determining dynamic binding capacity of resins for the purification of proteins", *Protein Expression and Purification*, 60, 2008, pp. 147-150.
- [23] M. Zhang, X.-Q. Jiang, H.-N. Le, P. Wang, B.-C. Ye, "Dip-and-read method for label-free renewable sensing enhanced using complex DNA structures", *ACS Applied Materials & Interfaces*, 5, 2013, pp. 473-478.
- [24] P.W.K. Rothmund, "Folding DNA to create nanoscale shapes and patterns", *Nature*, 440, 2006, pp. 297-302.
- [25] E.S. Andersen, M. Dong, M.M. Nielsen, K. Jahn, A. Lind-Thomsen, W. Mamdoh, K.V. Gothelf, F. Besenbacher, J. Kjems, "DNA origami design of dolphin-shaped structures with flexible tails", *ACS nano*, 2, 2008, pp. 1213-1218.
- [26] E.S. Andersen, M. Dong, M.M. Nielsen, K. Jahn, R. Subramani, W. Mamdoh, M.M. Golas, B. Sander, H. Stark, C.L.P. Oliveira, J.S. Pedersen, V. Birkeedal, F. Besenbacher, K.V. Gothelf, J. Kjems, "Self-assembly of a nanoscale DNA box with a controllable lid", *Nature*, 459, 2009, pp. 73-U75.
- [27] Y. Ke, S. Lindsay, Y. Chang, Y. Liu, H. Yan, "Self-assembled water-soluble nucleic acid probe tiles for label-free rna hybridization assays", *Science*, 319, 2008, pp. 180-183.
- [28] Z. Zhang, Y. Wang, C. Fan, C. Li, Y. Li, L. Qian, Y. Fu, Y. Shi, J. Hu, L. He, "Asymmetric DNA origami for spatially addressable and index-free solution-phase DNA chips", *Advanced Materials*, 22, 2010, pp. 2672-2675.
- [29] Z. Zhang, D. Zeng, H. Ma, G. Feng, J. Hu, L. He, C. Li, C. Fan, "A DNA-origami chip platform for label-free snp genotyping using toehold-mediated strand displacement", *Small*, 6, 2010, pp. 1854-1858.
- [30] H.K.K. Subramanian, B. Chakraborty, R. Sha, N.C. Seeman, "The label-free unambiguous detection and symbolic display of single nucleotide polymorphisms on DNA origami", *Nano Letters*, 11, 2011, pp. 910-913.
- [31] A. Kuzuya, Y. Sakai, T. Yamazaki, Y. Xu, M. Komiyama, "Nanomechanical DNA origami 'single-molecule beacons' directly imaged by atomic force microscopy", *Nat Commun*, 2, 2011, pp. 449.
- [32] A. Kuzuya, R. Watanabe, Y. Yamanaka, T. Tamaki, M. Kaino, Y. Ohya, "Nanomechanical DNA origami ph sensors", *Sensors*, 14, 2014, pp. 19329-19335.