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DNA origami nanorobot fiber optic genosensor to TMV

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ABSTRACT

In the quest of greater sensitivity and specificity of diagnostic systems, one continually searches for alternative DNA hybridization methods, enabling greater versatility and where possible field-enabled detection of target analytes. We present, herein, a hybrid molecular self-assembled scaffolded DNA origami entity, intimately immobilized via capture probes linked to aminopropyltriethoxysilane, onto a glass optical fiber end-face transducer, thus producing a novel biosensor. Immobilized DNA nanorobots with a switchable flap can then be actuated by a specific target DNA present in a sample, by exposing a hemin/G-quadruplex DNAzyme, which then catalyzes the generation of chemiluminescence, once the specific fiber probes are immersed in a luminol-based solution. Integrating organic nanorobots to inorganic fiber optics creates a hybrid system that we demonstrate as a proof-of-principle can be utilized in specific DNA sequence detection. This system has potential applications in a wide range of fields, including point-of-care diagnostics or cellular in vivo biosensing when using ultrathin fiber optic probes for research purposes.

1. Introduction

DNA origami technology enables increased control in self-assembly of 2D (Endo et al., 2011; Kozyra et al., 2017; Marini et al., 2011; Piantanida et al., 2015; Rothemund, 2006) and 3D nanoscale complex structures (Andersen et al., 2009; Douglas et al., 2009, 2012; Schüller et al., 2011; Torelli et al., 2014; Zadegan et al., 2012) of purpose designed shaped nanomaterials exhibiting high precision and specificity (Tørring et al., 2011). The method uses hundreds of short oligonucleotide 'staple' strands to direct the folding of a long single 'scaffold' strand of DNA into a pre-programmed arrangement (Rothemund, 2006).

To date, self-assembled DNA nanostructures have demonstrated a great potential in a wide range of innovative applications due to its programmable control of shape and size, precise spatial addressability, easy and high-yield preparation, and biocompatibility (Pandian and Sugiyama, 2016; Wang et al., 2013). Previous applications with DNA origami were found in photonics, therapeutics, nanofabrication and nanomechanics (Angell et al., 2016; Chandrasekaran et al., 2016; Chao et al., 2014; Peng et al., 2016; Kearney et al., 2016; Kuzuya and Ohya, 2014; Yang et al., 2015a; Yonamine et al., 2016). DNA nanostructures have been used previously for sensing applications. Self-assembled nucleic acid probe tiles were created for label-free detection of RNA: a ssDNA overhang can act as hybridization probes for specific RNA targets in solution and the ability to detect single molecule hybridization was explored (Ke et al., 2008). Also Koirala et al. (2014) used 2D and 3D DNA origami nanoassembly for the detection of multiple DNA synthetic targets anticipating a new sensing strategy. In a different approach, a switchable 2D DNA origami was used to detect the presence of synthetic DNA target (Marini et al., 2011).

Many applications are not only dependent on the structural design of the origami, but also on its immobilization to solid phase interphases or surfaces (Pillers et al., 2015). The immobilization of DNA nanostructures on a glass surface is a key step for the integration of DNA nanorobot onto optical fiber tips for sensing applications. Some immobilization on glass methodologies of 3D purpose DNA are known (Gietl et al., 2012), including with nanoantennas (Puchkova et al., 2015), nanopores (Bell et al., 2013; Hernández-Ainsa et al., 2013) and wafer pattern (Linko et al., 2015). DNA nanostructures can be selectively guided and anchored on glass capillaries by trapping them

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once a positive voltage is applied to a reservoir (Bell et al., 2013; Hernández-Ainsa et al., 2013). Recently, it has been shown that DNA origami can be uniformly deposited by a spray-coating technique without using pre-treatment of the substrate (Linko et al., 2015).

Nevertheless, the aforementioned methods could anchor the nanostructures on the glass interface in a way that would inhibit the actuation mechanism of the immobilized 3D DNA nanorobots, including changing its conformation from a closed state to an open one or making it unresponsive. Herein we utilized a DNA origami immobilization strategy that allows their immobilization to glass optical fibers, thereby enabling the use of a DNA origami, acting as a functional smart molecular device without losing its switchable feature, despite its solid-phase immobilization.

The motivation to combine the undisputed potential of DNA origami (i.e. addressability, versatility and loading capabilities) to the unique advantages of optical fiber transducers has been the key factor at the base of our new organic-inorganic hybrid system. We are the first, to the best of our knowledge to conjugate 3D DNA origami to an optical fiber transducer thereby producing a DNA origami genosensor. This new system could find use in many applications, such as diagnostics or water monitoring for pathogens.

2. Material and methods

2.1. Materials and reagents

Hemin was purchased from Porphyrin Products (Logan, UT) and used without other purification. A hemin stock solution (5 mM) was prepared in DMSO and stored in the dark at − 20 °C. Oligonucleotides without any modification and with Alexa 488 label at the 5' end were purchased from Integrated DNA Technologies Pte Ltd. (Singapore) and were resuspended in sterile MilliQ water to give stock solutions of 100 μM. Capture sequence, anchor sequence and labeled nucleotide sequences are shown in Supplementary information, Table S1. In the 3D DNA origami used in this work, 6 previously published sequences (Torelli et al., 2014) were labeled with Alexa 488 and one sequence (anchor sequence) was elongated in order to permit the attachment of the complementary capture sequence. 3D DNA nanorobot design was produced in caDNAno software from Douglas et al. (2009). M13mp18 was purchased from Bayou Biolabs, LA, USA. *Xmn*I enzyme and bovine serum albumin molecular biology grade were purchased from New England Biolabs, Singapore. All other chemicals were purchased from Sigma-Aldrich Pte Ltd (Singapore).

SFS400/440B Superguide G UV–vis silica fibers (Fiberguide Industries, Stirling, USA) were used for all experiments. The fibers had an original numerical aperture (NA) of 0.22, a core diameter of 400 μm (refractive index of 1.457 at 633 nm) and a surrounding silica cladding with a width of 40 μm (refractive index of 1.44 at 633 nm), in addition to a 150-μm-thick silicon buffer and a 210-μm-thick black Tefzel® jacket. The length of a single fiber used in the experiments was 20 cm. The black Tefzel® jacket and silicon buffer were mechanically stripped away using a fiber stripping tool (Micro-Strip®, from Micro-Electronics Inc., USA) to expose a 2 mm naked optical fiber core tip.

2.2. 3D DNA origami

The square-lattice version of the caDNAno software from Douglas et al. (2009) was used and 3D DNA origami was synthesized and purified as previously described (Torelli et al., 2014). Image J was used for gel image analysis to estimate the yield of purification (Abramoff et al., 2004).

2.3. Probe and target design

Sequences were previously drawn using the program BioEdit v.7.0.9 (Marini et al., 2011; Torelli et al., 2014). The probe was designed to target the TMV nucleocapsid gene whose gene sequence was retrieved from the NCBI database (accession number LN651239.1, KM407602.1, KJ406323.1, GQ370524.1, AJ239099.1). The specificity was confirmed *in silico* by aligning the probe sequences using BLASTn, available at the NCBI GenBank database.

2.4. 3D DNA origami gel electrophoresis

Folded and purified DNA-origami were run on a 0.7% (w/v) agarose gel (SeaKem Agarose, Lonza): gels were previously added with 0.5× Gel Red™ nucleic acid stain (Biotium, Hayward, CA) and run in 1× TAE (for 200 ml of a 50x buffer solution: 400 mM Tris-HCl, 11.42 ml acetic acid, 20 ml EDTA 0.5 M, pH 8.0) at 75 V for 45 min and then visualized and photographed under UV light (Molecular Imager Gel Doc™ XR+ Imaging System, Biorad). The 1 kb DNA ladder (Sigma-Aldrich, Singapore) was used as a molecular weight marker.

2.5. 3D DNA origami and Transmission Electron Microscopy (TEM) imaging

For TEM imaging 10 μl of the samples were adsorbed for 3 min on carbon-coated copper grids and imaged with a JEOL JEM 1011 transmission electron microscope (Tokyo, Japan) operating at 100 kV. The samples were stained for 2 min with a 2% (w/v) uranyl acetate solution and washed in milliQ water for three times.

2.6. 3D DNA origami and Atomic Force Microscopy (AFM) imaging

Samples were dispersed on the negative mica surface using 10× TAEM buffer and let deposit for 5 min. AFM images were obtained as previously described (Torelli et al., 2014).

2.7. Silanization of glass slides, capture probe binding and DNA origami attachment

The initial solid phase support used were microscope cover glasses, then silanized (Manzano et al., 2015; Marcello et al., 2013) with some modifications. The cover glasses were treated with 10% (w/v) NaOH at room temperature for 1 h, rinsed with deionized water and treated with 0.1 M HCl for 15 min. After a washing step with deionized water, the glass slides were rinsed in acetone and dried at 50 °C for a few minutes, and immersed in a 0.5% (v/v) APTES solution in deionized water for 30 min at room temperature. Slides were then rinsed three times in deionized water, dried at 160 °C for 1 h and cooled at room temperature for 30 min. 10 μl drops of the capture probe at 100 ng/μl were deposited in triplicate on each glass slide and incubated at 4 °C overnight to conjugate the microscope cover glass surface.

The glass slides with the bound capture probe were washed twice in deionized water prior to utilization. Then, 1 μl or 10 μl of the 3D DNA origami samples was spotted on each slide. The microscope cover glasses were incubated at room temperature for 2 h in the dark and they were washed twice in sterile deionized water to eliminate the unbound DNA nanostructures.

2.8. Silanization of the optical fiber tips and capture probe binding

In preparation for the silanisation procedure, fibers were soaked in a 1:1 methanol/37% (v/v) HCl solution for 10 min to purify the newly exposed fiber core from micro-contaminants. After sonication for 10 min, the fiber tips were dried under N_2 and dipped in Piranha solution (35% H_2O_2: 96% H_2SO_4 in the ratio 3:7) for 15 min at 90 °C to enhance the exposure of the hydroxyl groups on the silica surface. After a washing step with deionized water, the glass tips were rinsed in acetone and dried at 40 °C for a few minutes, and immersed in a 0.5% APTES solution in sterile deionized water for 30 min at room temperature. In the presence of water, the hydrolysis of ethoxy groups of