Developing DNA tiles for oligonucleotide hybridization assay with higher accuracy and efficiency†

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We demonstrate the versatility of a DNA tile system for oligonucleotide hybridization assay and explored the detection limit of the probe tiles for DNA targets of varied lengths.

Monitoring gene expression through fast and accurate detection of DNA or RNA sequences is becoming increasingly important for a wide variety of biomedical applications.[1,2] Recently, the use of self-assembled water-soluble nucleic acid probe tiles as a molecular analog to gene chips was reported.[3] The nucleic acid probe tiles have been used to study position-dependent hybridization at the nanoscale and for label-free detection of RNA targets. These tiles[4–6] allowed target/probe strands hybridization in solution, circumventing the problems associated with slow liquid–solid interface kinetics.[7,8] A remarkable aspect of the system includes the ability to position probes on tiles with nanometre-scale precision, and to study the effects of probe placement with molecular resolution.[9,10] The system holds promise as a viable detection platform for nucleic acid targets, and therefore requires further studies on the positional effect to optimize its performance. It is also desirable to explore the use of the probe tile system for the detection of short oligonucleotides of different lengths.

It has been reported that the probe tiles display a strong position-dependent hybridization effect.[3] The original design of the probe tiles used rectangular-shaped DNA origami,[3,11] bearing identical probes at three distinct positions (Fig. 1a). Each line of capture probes consists of 12 selected pairs of neighboring helper strands that have been modified so that each contains a single stranded fragment of 11 nucleotides protruding out of the tile surface (Fig. 1b). Each dangling single strand in the capture pair serves as a half-probe for a single RNA target of 22 nucleotides (nt) in length. Upon hybridization of the target to a corresponding pair of half-probes, the strands form a stiff V-shaped structure that is readily sensed by AFM. Target hybridization had been shown to be most efficient when probes were located on the edge (position 3) of a tile, rather than in the middle (position 1).[3] The reported approach to overcome this positional limitation involved the use of bar-coded tile designs, in which different tiles, each carrying a unique bar-code and uniquely sequenced pairs of probes at the same edge positions, were designed to detect different targets with consistent hybridization efficiency.[3] However, it would be desirable to develop a system in which multiple targets could be detected on a single tile.[12] The combination of this development in conjunction with the bar-code system may expand the future applications.

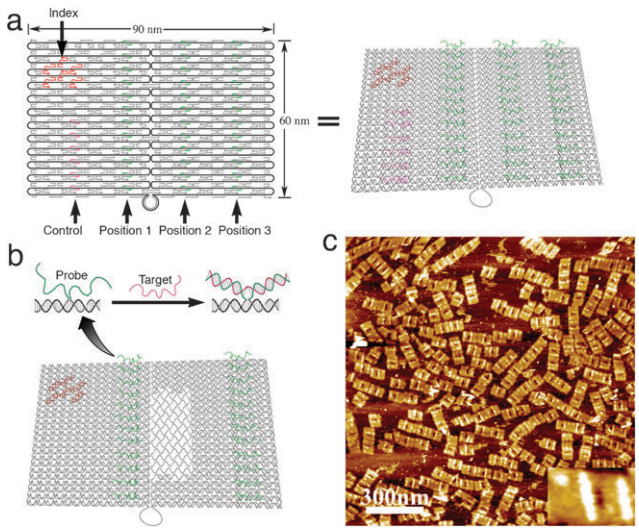

Fig. 1 (a) (Left) 90 × 60 nm DNA origami tile bearing an index, six control probes in a line, and three lines of identical probes (12 each) marked by position 1 to 3. (Right) The 3D view of the tile. (b) The 3D view of a tile bearing an index, and two lines of identical probes at positions 1 and 3 (same as shown in a). 21 helper strands were removed and left a hole in the middle of the tile. Upon target hybridization with the probes, DNA duplex forms V-shaped junction and can be detected with AFM. (c) AFM images showing high efficiency for the DNA target hybridization with probes at both position 1 and 3. Insert shows a zoom-in image with the index and both probe lines clearly visible.

Electrostatic repulsions between the target and the underlying tile, as well as repulsions between probes, are stronger close to the center of the tile than close to the edge. These factors, in addition to the reduced steric hindrance at the tile's edge, presumably contribute to the observed positional effect. We propose that by altering the geometry of the tile to alleviate the electrostatic repulsions and steric hindrance close to the center of the tile, it may ultimately improve the target-probe hybridization efficiency. To test this hypothesis a modified version of the original tile design was employed. In this assembly, a series of helper strands located in the middle of the tile were intentionally omitted from the assembly mixture. By removing these helper strands we generated an origami tile

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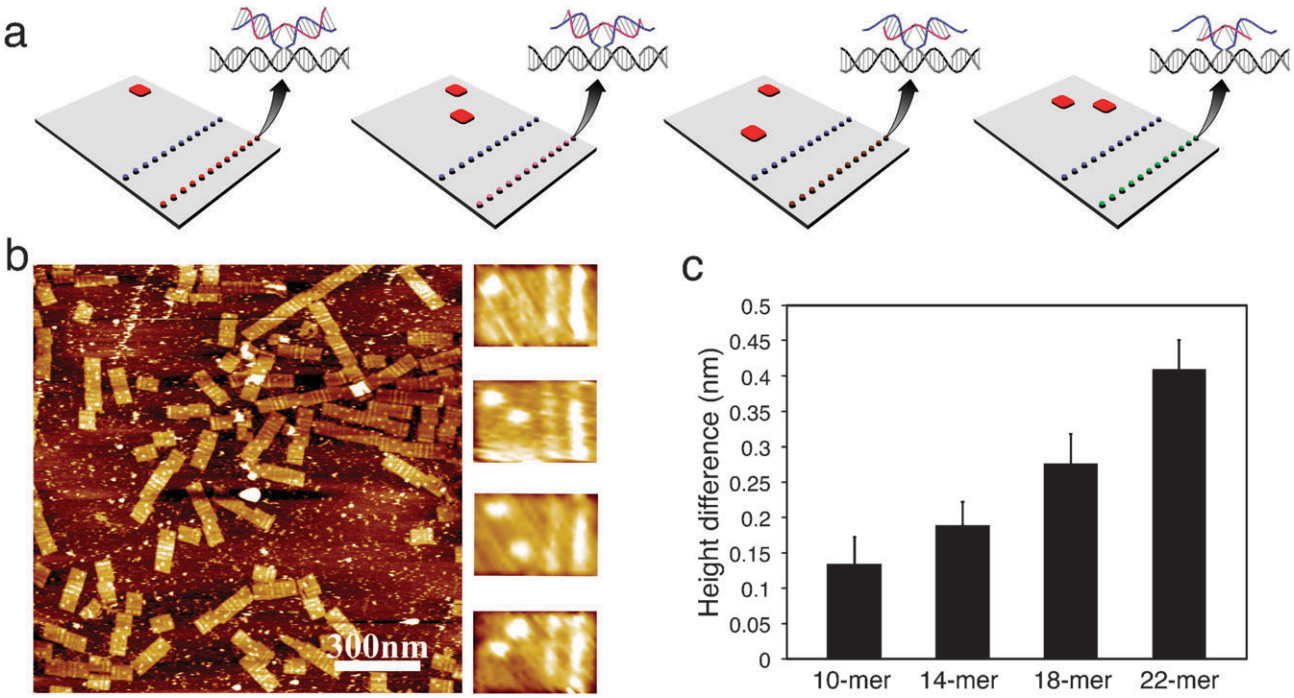

Fig. 2 (a) Topographic illustration of the bar-coded tile designs for short DNA detection. The top left corners of the tiles are modified with one or two groups of indexes forming distinctive bar-codes for each tile. Each tile carries a line of probes near the right edge of the tile for detecting DNA targets of different lengths (22, 18, 14 and 10 mer, from left to right). A line of control probes near the middle of the tile is used as a reference to measure the height difference by AFM. (b) AFM images of an equimolar mix of the bar-coded tiles with 4 different length DNA targets. Zoom-in images demonstrate the details of each of the 4 bar-coded tiles. (c) Height difference measurement chart showing the height difference between the probe lines (hybridized with DNA targets) and the control line. 50 tiles are arbitrarily chosen to calculate the average height difference for each bar-coded tile.

pattern with a roughly 35 × 15 nm rectangular shaped "hole" located at position 2 and close to position 1 (Fig. 1b). The "hole" is generated because that without the hybridization of complementary helper strands, the M13 scaffold in this region remains single stranded and is flexible enough to appear like a "hole" in the tile when imaged by AFM. Probe lines were placed at position 1 (close to the inner edge of the hole) and position 3 (close to the outer edge of the tile) (Fig. 1b).

The hybridization efficiency of target molecules to the probes located at the two positions in the new design were compared to that of the original design. The DNA tiles were first self-assembled in solution, and purified using centrifugal filtration to remove excess helper strands. We adjusted the tile concentration to 10 nM and added 240 nM DNA target (at 1 : 1 ratio of [probe]/[target]) into the solution. We arbitrarily chose 50 tiles from AFM images and analyzed the percentage of DNA target hybridization (Fig. 1c) by measuring the relative height changes from the AFM images. A detailed explanation of the data analysis procedure can be found in the supporting material available online (Fig. S1).† The results reveal that the hybridization percentage at position 1 increases dramatically from 50% to 80%, compared to that of the tile without the hole (Fig. S3).† Note that the hybridization percentage at position 3 also increases slightly from 71% to 80%. These results demonstrated that positioning probes close to both the outer and inner edges can help to improve the hybridization efficiency.

The detection sensitivity of nucleic acid tiles depends on the height change of the probe lines upon the hybridization sensed by AFM cantilever. In order to test if it is possible to detect oligonucleotides of different lengths and sequences simultaneously using the DNA tile system, we explored the relationship between the length of oligonucleotide targets and the observed height change. Four uniquely bar-coded probe tiles were used for the detection of four distinct DNA targets ranging from 10 to 22 nt in length (Fig. 2a). Each of the four bar-coded tiles contained a unique combination of index spots for identification. Similarly, each control and capture probe contained a single stranded fragment of 11 nucleotides that protruded out of the tile surface that functioned as a half-probe for a single DNA target. The control probes on each of the four tiles were identical and consist of a random 11 base long sequence, serving as both a control to evaluate non-specific binding of the target DNA and as a reference for height comparison with the probe/target complex. Upon hybridization of the target to a corresponding pair of half-probes, the strands formed a stiff V-shaped structure that was readily sensed by AFM. The size and stability of the rigid portion of the "V" structure depended on the length of the target, which would be reflected in the height change in AFM images. The four bar-coded tiles were self-assembled in solution, purified using centrifugal filtration and mixed together at 2.5 nM concentrations. Then 30 nM 10-, 14-, 18-, and 22-base long DNA targets were added into the mixed bar-coded tile solution and the hybridization was imaged by AFM after 30 min incubation (Fig. 2b). It was observed that the height difference between the control and capture probes was proportional to the length of the hybridized DNA

target (Fig. 2c). It is noted that the average height difference for 10 mer DNA target hybridization was $\sim$0.15 nm. This was a few-fold larger than the noise level of AFM, $\sim$0.02 nm, in the *z* direction on an atomic flat surface, like a freshly cleaved mica, in a well vibration-isolated environment. This indicated that a DNA target as short as 10 nt could be detected using this technique. The only limit was the thermal stability of the hybridization at room temperature when the number of perfectly matched base-pairs was reduced to smaller than 5.

In conclusion, we have modified the nucleic acid tile to study the positional effect of hybridization. The experimental results supported our hypothesis that placing the probes at positions more accessible to the targets in solution, *e.g.* at edges, can increase the hybridization efficiency of oligonucleotide targets on the tile probes. A possible result of creating more edges (or digging holes) in the tile surfaces could be the decrease of electrostatic repulsion and steric hindrance between the tile surface and targets. In addition, the increased flexibility of the center part of the tile close to the "hole" may also contribute to this improvement of the hybridization efficiency. The above studies, as well as the capability to detect oligonucleotides as short as 10 nt make it possible to design more complicated nucleic acid tiles for multi-target detection with high efficiency.

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Notes and references

1 I. Ghosh, C. I. Stains, A. T. Ooi and D. J. Segal, *Mol. BioSyst.*, 2006, **2**, 551–560.
2 R. T. Ranasinghe and T. Brown, *Chem. Commun.*, 2005, 5847–5502.
3 Y. Ke, S. Lindsay, Y. Chang, Y. Liu and H. Yan, *Science*, 2008, **319**, 180–183.
4 N. C. Seeman, *Nature*, 2003, **421**, 427–431.
5 E. Winfree, F. Liu, L. A. Wenzler and N. C. Seeman, *Nature*, 1998, **394**, 539–544.
6 H. Yan, S. H. Park, G. Finkelstein, L. H. Reif and T. H. LaBean, *Science*, 2003, **301**, 1882–1884.
7 A. W. Peterson, R. L. Heaton and R. M. Georgiadis, *Nucleic Acids Res.*, 2001, **29**, 5163–5168.
8 Y. Gao, L. K. Wolf and R. M. Georgiadis, *Nucleic Acids Res.*, 2006, **34**, 3370–3377.
9 K. Lund, Y. Liu, S. Lindsay and H. Yan, *J. Am. Chem. Soc.*, 2005, **127**, 17606–17607.
10 S. H. Park, C. Pistol, S. J. Ahn, J. H. Reif, A. R. Lebeck, C. Dwyer and T. H. LaBean, *Angew. Chem., Int. Ed.*, 2006, **45**, 735–739.
11 P. W. K. Rothemund, *Nature*, 2006, **440**, 297–302.
12 L. J. Kricka, *Clin. Chem. (Washington, DC, U.S.)*, 1999, **45:4**, 453–458.

