ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/7097029

Digital Readout of Target Binding with Attomole Detection Limits via Enzyme Amplification in Femtoliter Arrays

ARTICLE *in* JOURNAL OF THE AMERICAN CHEMICAL SOCIETY · JUNE 2006

Impact Factor: 12.11 · DOI: 10.1021/ja058425e · Source: PubMed

CITATIONS
37

READS
46

2 AUTHORS, INCLUDING:

David M Rissin
Quanterix, Inc.
22 PUBLICATIONS **773** CITATIONS

SEE PROFILE

Available from: David M Rissin
Retrieved on: 17 January 2016

J|A|C|S
COMMUNICATIONS

Published on Web 04/20/2006

Digital Readout of Target Binding with Attomole Detection Limits via Enzyme Amplification in Femtoliter Arrays

David M. Rissin and David R. Walt*

Department of Chemistry, Tufts University, 62 Talbot Avenue, Medford, Massachusetts 02155

Received December 12, 2005; E-mail: david.walt@tufts.edu

Improving the detection limit and sensitivity of methods for capturing and accurately quantifying the concentration of a specific target is an ongoing challenge. In this communication, we demonstrate the capture and detection of single molecules of β-galactosidase on a 1 mm femtoliter array using biotin−streptavidin binding. The array contains 24 000 individual femtoliter reaction chambers, enabling digital concentration readout of the bulk target concentration by counting the number of reaction vessels that successfully capture a target molecule.

Methods that implement high-sensitivity and low-level analyte detection in conjunction with rapid and reproducible experimental protocols are the cornerstone of modern analytical chemistry. Currently, most techniques for quantifying low levels of target analyte in a sample matrix use amplification procedures to increase the number of reporter molecules and thereby provide a measurable signal. These processes include enzyme-linked immunosorbent assays (ELISA) for amplifying the signal in antibody-based assays, as well as the polymerase chain reaction (PCR) for amplifying target DNA strands in DNA-based assays. A more sensitive but indirect protein target amplification technique, called immuno-PCR,[1] makes use of oligonucleotide markers, which can subsequently be amplified using PCR and detected using a DNA assay.[2−4] While the immuno-PCR method permits ultra-low-level protein detection, it is a complex assay procedure and can be prone to false-positive signal generation.[5]

In this paper, we describe a proof-of-concept binding assay using enzymatic signal amplification in an array of femtoliter-sized reaction vessels. This method relies on enzymatic amplification and a small reactor size for signal generation, circumventing additional time-consuming and complex steps required in the immuno-PCR amplification technique. The current limit of detection (LOD) for binding streptavidin-β-galactosidase (SβG) to a biotinylated femtoliter array is 2.6 amol (150 μL of 17 fM solution) using a target binding time of 1 h.

Micro- and nanotechnologies have enabled the generation of small arrayed structures. These structures allow for small volume isolation,[6,7] enabling high-throughput and single molecule PCR amplification,[8,9] as well as single molecule interrogation.[7,10,11] Here, we employ an etched fiber optic array to create femtoliter-sized reaction vessels, each specifically functionalized and capable of capturing enzyme-labeled target molecules. Single enzyme molecules are confined to individual reaction vessels and catalyze the production of a sufficient number of fluorescent product molecules to generate a positive signal. At low target molecule concentrations, only a percentage of the capture sites bind a target molecule, enabling a binary readout of target concentration from the high-density array.[12]

The reactor vessel arrays are generated using an acid etch of the distal face of a polished 1 mm fiber optic array, consisting of 24 000 individual 4.5 μm optical fibers.[13] The core fiber material is silica, and the cladding around each fiber is germania-doped silica, which etches at a slower rate. The 4.5 μm fibers are etched to a depth of 2.9 μm, creating an array of reactor vessels, each with a 46 fL volume (Figure 1a). A major advantage of the optical fiber bundle is that the individual fibers in contact with each well can be used to carry both excitation and emission light to and from the wells, enabling remote interrogation of the well contents.[14,15] An array of optical fibers provides the capability for simultaneous excitation of molecules in adjacent vessels, without signal "cross-talk" between fibers.

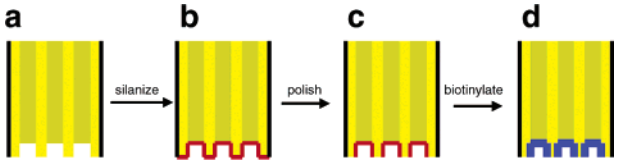

Figure 1. A side-view cross-section schematic representing the etched bundle modifications. (a) An etched fiber with the core represented by the gray columns and the cladding represented by the bright yellow columns. (b) Modification with an amino-functionalized silane (red). (c) Removal of the amine modification from the cladding material via polishing. (d) Functionalization of the fiber bundle with biotin succinimidyl ester (blue).

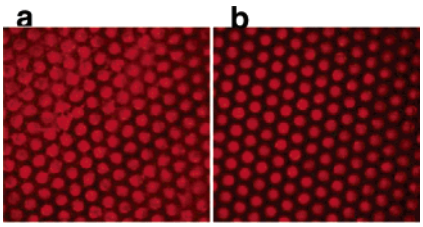

Figure 2. Streptavidin Alexa Fluor 568 binding to (a) an unpolished biotin-modified fiber optic array, and (b) a polished biotin-modified fiber optic array. As seen in image (a), streptavidin binding occurred on all surfaces, in comparison to image (b), where binding occurred only on the surfaces of the microwell reactors (Pseudocolor added using IPlab software).

The fibers were first modified with an aminopropyl silane bound to both the core and cladding surfaces (Figure 1b). To avoid biotin attachment to the cladding, the amino-silanized fibers were polished for 10 s with 0.3 μm lapping film to remove the amino-silanized layer from the cladding (Figure 1c). After polishing, NHS−biotin was attached to the amino groups on the well surfaces (Figure 1d). To test the effectiveness of this surface modification procedure, streptavidin Alexa Fluor 568 was attached directly to the biotin groups on the surfaces of both a polished and an unpolished fiber, followed by image acquisition of the modified surface (Figure 2). As seen in the figure, the unpolished fiber shows dye over the entire array, including the cladding surface. In contrast, the polished fiber shows dye localized only on the well surfaces.

After array modification, the biotinylated fiber arrays were incubated for 1 h at room temperature in 150 μL of PBS buffer containing varying amounts of SβG. The SβG concentration was chosen so that, during the incubation, statistically either one molecule or no molecules would bind to each well. The arrays were then washed repeatedly in PBS buffer, to ensure that unbound target was removed.

10.1021/ja058425e CCC: $33.50 © 2006 American Chemical Society

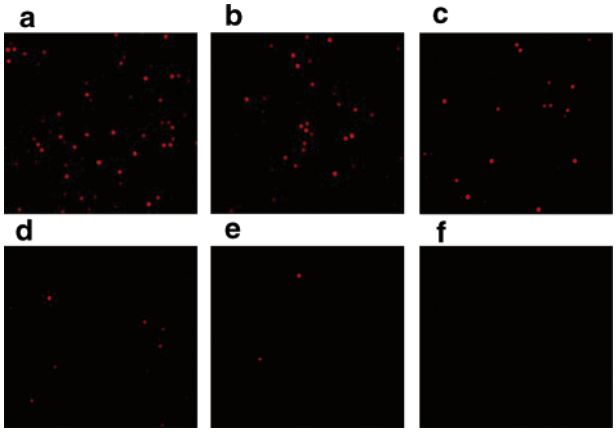

Figure 3. A portion of the fiber array for each SβG binding experiment containing (a) 128 amol, (b) 51 amol, (c) 25 amol, (d) 7.5 amol, (e) 2.6 amol, and (f) one of the two controls, showing a representative number of active versus inactive reaction chambers (Pseudocolor added using IPlab software).

For a binary readout of SβG binding, the fiber array was loaded and secured on an upright microscope system equipped with a mechanical platform. A solution of β-galactosidase substrate, resorufin-β-D-galactopyranoside (RDG), was introduced to the distal end of the fiber containing the reaction vessels and subsequently sealed. The substrate was sealed using a 0.01 in. thick silicone elastomer gasket sandwiched between a microscope slide and the fiber array by means of a mechanical platform located beneath the microscope stage. This platform applied a uniform pressure to the gasket material, across the entire bundle, sealing off and isolating each reaction chamber and enabling well to well interrogation of enzyme activity.[12] β-Galactosidase hydrolyzes RDG to form resorufin, which builds up to a locally high concentration in each sealed reaction vessel, generating a detectable fluorescent signal (Figure 3). Analysis of over 5000 reaction vessels for each experiment allowed for a correlation between the percentage of reaction vessels that captured an enzyme molecule and the amount of enzyme present in the interrogated sample. The variation seen in the intensity differences from active well to active well is most likely a result of molecule-to-molecule variation in catalytic activity,[16,17] combined with surface effects that may modulate the relative activities of different enzyme molecules based on their orientation on the reaction chamber surface. Two control experiments were conducted to ensure that the binding of enzyme to the surface of the reactors was based exclusively on the biotin−streptavidin interaction and not on nonspecific binding to the glass surface. One control experiment consisted of an etched, unmodified fiber incubated with the most concentrated SβG target solution (128 amol in 150 μL). The second control experiment was performed using the modified fiber incubated in a solution of β-galactosidase lacking streptavidin (128 amol in 150 μL). Both control experiments generated a negligible active well percentage (less than 0.06% versus 0.2% for the 2.6 amol experiment discussed below).

The linear relationship between the percentage of active reaction vessels and the moles of target in the log−log plot (Figure 4) suggests that this binary readout detection method can be used for the detection of targets, such as DNA and antigens. The method permits rapid analysis and accurate concentration information via a digital readout, while maintaining a straightforward assay procedure. The technique is limited by the number of individual reaction vessels that can be viewed with an acceptable resolution. Expanding the number of vessels that are interrogated by using higher density CCD chips will decrease the limit of detection as the lower limit is defined by the statistics of the small number of active wells that

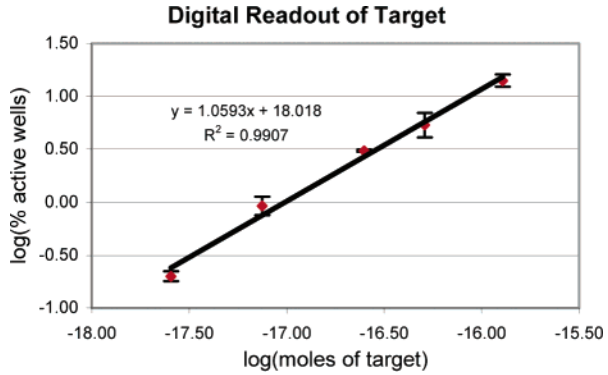

Figure 4. A log−log plot of the moles of target enzyme present in a sample with the resulting percentage of active reaction vessels. Experiments were performed in triplicate with standard deviation represented by error bars.

light up at the lower target concentrations. The upper limit of the dynamic range is controlled by the deviation from a binary readout. As target concentrations are increased, the binary readout is lost because the distribution function moves from Poissonian to Gaussian and multiple enzyme molecules begin to occupy the wells, leading to a nonlinear increase in the percentage of active wells.

DNA and protein target detection should be possible using this digital readout system by modifying the reactor wells with specific capture antibodies or DNA probes, followed by target capture and a final enzyme labeling of the captured target.

The use of an array of reaction vessels in combination with enzymatic signal generation facilitates the digital readout of target concentration in a sample. The ease of performing this assay, along with the simple binary readout, should facilitate further development of this technology for the detection of specific analytes of interest. This capture and readout approach should prove useful for DNA and antibody assays that utilize an enzyme label to catalyze the generation of a fluorescent signal.

Acknowledgment. We thank DARPA for their financial support of this work.

Supporting Information Available: Related instrumentation and experimental procedures (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Sano, T.; Smith, C. L.; Cantor, C. R. *Science* **1992**, *258*, 120−122.
(2) Nam, J. M.; Thaxton, C. S.; Mirkin, C. A. *Science* **2003**, *301*, 1884−1886.
(3) Niemeyer, C. M.; Adler, M.; Pignataro, B.; Lenhert, S.; Gao, S.; Chi, L. F.; Fuchs, H.; Blohm, D. *Nucleic Acids Res.* **1999**, *27*, 4553−4561.
(4) Zhou, H.; Fisher, R. J.; Papas, T. S. *Nucleic Acids Res.* **1993**, *21*, 6038−6039.
(5) Niemeyer, C. M.; Adler, M.; Wacker, R. *Trends Biotechnol.* **2005**, *23*, 208−216.
(6) Whitesides, G. M. *Nat. Biotechnol.* **2003**, *21*, 1161−1165.
(7) Rondelez, Y.; Tresset, G.; Tabata, K. V.; Arata, H.; Fujita, H.; Takeuchi, S.; Noji, H. *Nat. Biotechnol.* **2005**, *23*, 361−365.
(8) Nakano, M.; Komatsu, J.; Matsuura, S.; Takashima, K.; Katsura, S.; Mizuno, A. *J. Biotechnol.* **2003**, *102*, 117−124.
(9) Nagai, H.; Murakami, Y.; Yokoyama, K.; Tamiya, E. *Biosens. Bioelectron.* **2001**, *16*, 1015−1019.
(10) Lipman, A. E.; Shuler, B.; Bakajin, O.; Eaton, W. A. *Science* **2003**, *301*, 1233−1235.
(11) Chiu, D. T.; Wilson, C. F.; Ryttsen, F.; Stromberg, A.; Farre, C.; Karlsson, A.; Nordholm, S.; Gaggar, A.; Modi, B. P.; Moscho, A.; Garza-Lopez, R. A.; Orwar, O.; Zare, R. N. *Science* **1999**, *283*, 1892−1895.
(12) Rissin, D. M.; Walt, D. R. *Nano Lett.* **2006**, *6*, 520−523.
(13) Pantano, P.; Walt, D. R. *Chem. Mater.* **1996**, *8*, 2832−2835.
(14) Monk, D. J.; Ueberfeld, J.; Walt, D. R. *J. Mater. Chem.* **2005**, *15*, 4361−4366.
(15) Song, L. N.; Ahn, S.; Walt, D. R. *Emerging Infect. Dis.* **2005**, *11*, 1629−1632.
(16) Lee, J. Y.; Li, H. W.; Yeung, E. S. *J. Chromatogr. A* **2004**, *1053*, 173−179.
(17) Xue, Q. F.; Yeung, E. S. *Nature* **1995**, *373*, 681−683.

JA058425E