Diffraction Gratings

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Rapid Detection of *S*-Adenosyl Homocysteine Using Self-Assembled Optical Diffraction Gratings**

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Quantitative characterization of biomolecules is critical for molecular diagnostics and drug development.[1,2] Several assays based on spectrophotometry,[3,4] fluorometry,[5] chemiluminescence,[6] and electrochemical immunoassays have been reported for biomolecular detection.[7–9] These methods are often slow owing to multiple sample pretreatment steps that increase analysis time and cost. Immunoassays that combine high sensitivity with fast, robust, and inexpensive methods for biomolecular detection are of growing importance.[10] Herein the development of a self-assembled optical diffraction biosensor is described which is devoid of microfabrication[11] or enzymatic amplification[12] for the rapid detection of *S*-adenosyl homocysteine (SAH), a potential diagnostic marker for cardiovascular disease,[13] with a sensitivity limit of 24.5 pg mL^{-1}.

SAH is a low-molecular-mass analyte (384 Da) consisting of the nucleoside adenine joined to the amino acid homocysteine (Hcy) by a 5′ thioether linkage. Our method relies on a sandwich-binding approach, wherein SAH is bound by an antibody through the Hcy moiety while an adenine-specific RNA aptamer binds to the adenine moiety (Figure 1).

We designed a strategy using antibody-coupled (Ab) beads to capture SAH from solution in addition to aptamer-functionalized micropatterns specific for adenine which are stamped on a gold-coated glass slide (gold chip). Based on these specific interactions, SAH bound to the Ab beads produces a self-assembled optical diffraction grating upon exposure to the aptamer-functionalized micropatterns (Figure 2). The high-affinity adenosine-specific aptamer containing the 39-mer sequence CGG AUG AGA CGC UUG GCG UGU GCU GUG GAG AGU CAU CCG was chosen as the capture ligand for the SAH adenine moiety (binding constant: 5×10^{-8} mol L^{-1})[14] and was immobilized by coupling biotin to the 5′ end, and exposure onto streptavidin-saturated micropatterns. Since it is known that magnetic beads enable rapid concentration and isolation of the target molecules,[15] they were used in this study as target capture and grating-forming agents.

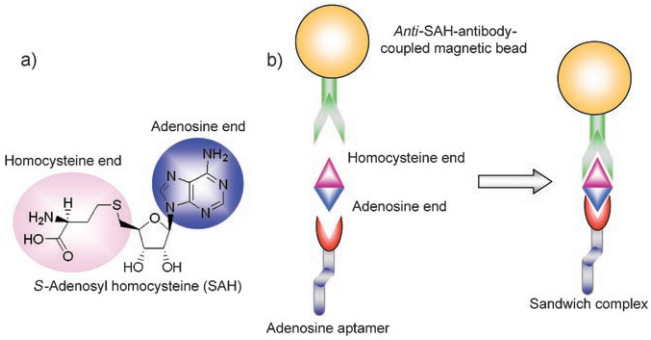

Figure 1. Sandwich complex formation: a) Structure of *S*-adenosyl homocysteine (SAH); b) Binding of *anti*-SAH-antibody-coupled magnetic bead to the homocysteine end of SAH produces a sandwich complex after binding of the adenosine-specific aptamer to the adenosine end of SAH.

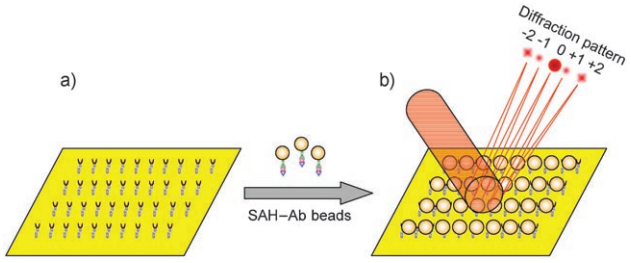

Figure 2. Detection strategy: a) Patterns of aptamer microcontact printed on gold chip. b) SAH-bound magnetic beads self-assemble onto the aptamer micropatterns to form diffraction gratings that, upon illumination with a laser, give a characteristic diffraction pattern.

SAH molecules dissolved in HEPES buffer (HEPES = 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid) were isolated by Ab beads. The beads were prepared by treating an SAH-specific antibody solution with magnetic beads activated with *N*-hydroxysuccinimide (see the Supporting Information). These Ab beads were then used to isolate

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SAH dissolved in buffer solutions with concentrations ranging from 40 nmol L^{-1} to 64 pmol L^{-1}.

Adenosine aptamer micropatterns were deposited on gold chips by microcontact printing (μCP).[16,17] A PDMS (poly(dimethylsiloxane)) stamp with 15-μm-wide alternating linear patterns was used for μCP (see the Supporting Information). As BSA (bovine serum albumin) binds to gold surfaces with high affinity, solid micropatterns with a high density of biotin were produced by microcontact printing biotinylated BSA (B-BSA).[18] The B-BSA micropatterned chip was then dipped in streptavidin solution to saturate the biotinylated sites followed by a treatment with biotin-coupled adenosine aptamer solution in RNA buffer, and a subsequent buffer wash.

The gold chip bearing the adenosine aptamer micropatterns was exposed to a suspension of SAH–Ab beads to produce adenosine aptamer–SAH–Ab bead grating patterns arising from specific binding of the SAH analyte. The gold chip was then rinsed with HEPES buffer, deionized water, dried, and examined under an optical microscope (Figure 3).

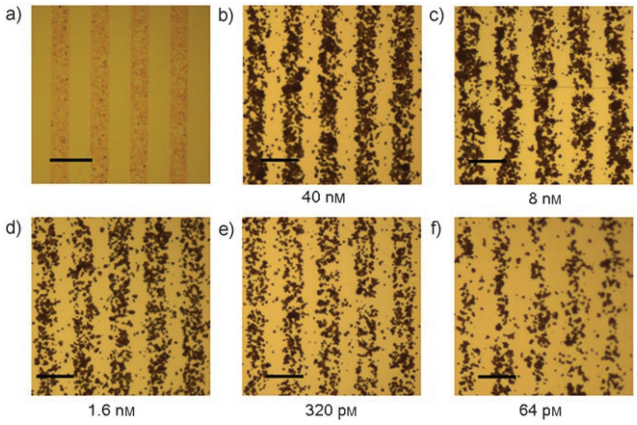

Figure 3. Self-assembled diffraction gratings. a) Neat aptamer micropatterns. b)–f) Diffraction gratings formed by the self-assembly of SAH–Ab beads on aptamer micropatterns. Scale bars = 30 μm.

Diffraction gratings formed by the SAH–Ab beads binding to the adenosine aptamer-modified surface produced a diffraction pattern upon illumination with laser light. The diffractometry setup is explained in the Supporting Information. The incident laser beam was adjusted to illuminate five grating periods, each being 15 μm wide and 150 μm long. The SAH concentrations were quantified by comparing the intensities of the diffracted light as a ratio of first mode (I_1) to zeroth mode (I_0).[19] The number of SAH–Ab beads bound to the micropatterns on the gold chip decreased with decreasing SAH concentration (Figure 3). As a consequence, the normalized diffraction mode intensities also decreased, from 0.045 at 40 nmol L^{-1} to 0.020 at 64 pmol L^{-1} SAH concentrations (Figure 4). The low-end concentration limit was 64 pmol L^{-1}, beyond which the intensity of the diffraction modes was very weak and their intensities could not be reliably detected.

To validate the mechanism of diffraction grating self-assembly and the specificity of SAH detection, several control

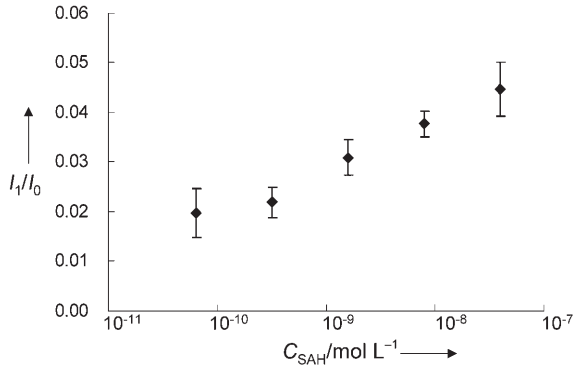

Figure 4. Dependence of normalized diffraction mode (I_1/I_0) intensities on SAH concentration (C_{SAH}) between 64 pmol L^{-1} to 40 nmol L^{-1}.

experiments were performed. In the absence of SAH, Ab beads did not self-assemble significantly on the aptamer micropatterns. SAH–Ab beads also did not bind to streptavidin micropatterns in appreciable amounts in the absence of the adenosine aptamer. To examine the requirement of an adenine moiety in SAH, a gold chip containing aptamer micropatterns was incubated with homocysteine (Hcy) bound magnetic beads (Hcy–Ab beads) instead of SAH–Ab beads. The Hcy–Ab beads did not bind in significant amounts on the aptamer micropatterns owing to the absence of an adenine moiety to enable binding. Similar observations were made when *S*-adenosyl methionine (SAM) was incubated with Ab beads before exposure to an adenosine-aptamer-micropatterned gold chip arising from the low binding affinity of the *anti*-SAH antibody for SAM (see the Supporting Information).

The SAH detection approach presented herein is simple, devoid of special labeling or signal amplification steps, and possesses significant detection sensitivity. For example, the fluorescence-based coupled enzyme assay detection limit is reported to be 1 μmol L^{-1},[20] whereas the SAH detection sensitivity of LC/MS based method is 50 nmol L^{-1}.[21] Capillary electrophoresis and stable-isotope dilution LC/ESI-MS methods are capable of detecting SAH concentrations of 7.2 nmol L^{-1} and 1 nmol L^{-1}, respectively.[22,23] The most sensitive method known, HPLC-coulometric electrochemical detection, has an ultimate SAH detection sensitivity of 40 fmol L^{-1};[24] however, the analysis time and instrumentation cost are much greater than the method we report.

From an analytical perspective, there are several advantages provided by this detection strategy. The analyte concentrates on the surface of the antibody coupled bead, where it can bind strongly to the micropatterned capture surface by multivalent interactions with the aptamer layer. This produces a rugged optical diffraction grating mediated by antibody–analyte–aptamer self-assembly. Naive aptamer micropatterns did not show a diffraction pattern. Distinct and measurable diffraction patterns were obtained only when SAH–Ab beads were exposed to adenosine aptamer micropatterns. In summary, this rapid and simple method offers competitive limits of detection with significantly lower cost and simpler instrumentation. Our efforts are now focused on

lowering the limits of detection even further and extending the method to the analysis of methyltransferase reactions.

Experimental Section

Microcontact printing of B-BSA: The surface of the PDMS stamp was exposed to a solution of B-BSA (1 mg mL^{-1} of phosphate-buffered saline (PBS)) with a cotton swab for 2 min followed by drying the stamp with nitrogen gas. The B-BSA-inked stamp was pressed against the gold-coated slide (gold chip) for 2 min to obtain a well-defined pattern. The stamp was then removed, the gold-coated slide was rinsed with PBS and dried.

Saturation of micropatterns with streptavidin: The gold chip, microcontact-printed with B-BSA, was dipped into streptavidin solution (1 mg mL^{-1} PBS) for 15 min, then rinsed with PBS and dryed under a gentle stream of nitrogen. This step saturated all the biotin groups present on the micropatterns with streptavidin. The gold chips with streptavidin-saturated micropatterns were used for immobilizing biotin-coupled aptamer.

Saturation of micropatterns with biotin-coupled aptamer: SAH–aptamer solution (50 μL, 1 mg mL^{-1} in RNA buffer: NaCl (300 mM), 2-amino-2-hydroxymethyl-1,3-propanediol (Tris, 25 mM), pH 7.6, and $MgCl_2$ (5 mM)) was transferred with a micropipet onto the gold chip containing streptavidin-saturated micropatterns, and incubated at room temperature for 15 min. At the end of this period, the chips were rinsed with buffer to remove any unbound SAH–aptamer sticking to the gold surface.

Bead-based capture of SAH: From a stock solution, a series of concentrations of SAH solutions (40 nM to 64 pM) were prepared in HEPES buffer (1M, pH 7.3). An aliquot of Ab beads (1.8×10^8 beads suspended in 10 μL of PBS) was transferred into a 1 mL Eppendorf tube in a magnetic separator and the liquid was removed. SAH (50 μL) in HEPES was added to the Ab beads (1.8×10^8) and gently stirred for 30 min, followed by rinsing with HEPES to remove unbound SAH. The beads were then resuspended in 50 μL HEPES.

Self-assembly of SAH–Ab beads on aptamer micropatterns: The SAH–Ab bead suspension (1.8×10^8 beads in 50 μL of HEPES) was spotted with a micropipet onto gold chips containing aptamer micropatterns. After 10 min, the gold chips were rinsed with HEPES and nanopure water, and then dried with nitrogen gas. The chips were examined under an optical microscope for micropatterns containing magnetic beads.

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