Enzyme-Based Test Strips for Visual or Photographic Detection and Quantitation of Gaseous Sulfur Mustard

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ABSTRACT: Sulfur mustard is a chemical agent of high military and terroristic significance. No effective antidote exists, and sulfur mustard can be fairly easily produced in large quantity. Rapid field testing of sulfur mustard is highly desirable. Existing analytical devices for its detection are available but can suffer from low selectivity, laborious sample preparation, and/or the need for complex instrumentation. We describe a new kind of test strip for rapid detection of gaseous sulfur mustard that is based on its degradation by the enzyme haloalkane dehalogenase that is accompanied by a change of local pH. This change can be detected using pH indicators contained in the strips whose color changes from blue-green to yellow within 10 min. In addition to visual read-out, we also demonstrate quantitative reflectometric readout by using a conventional digital camera based on red-green-blue data acquisition. Organic haloalkanes, such as 1,2-dichloroethane, have a negligible interfering effect. The visual limit of detection is 20 μg/L, and the one for red-green-blue read-out is as low as 3 μg/L. The assays have good reproducibility ±6% and ±2% for interday assays and intraday assays, respectively. The strips can be stored for at least 6 months without loss of function. They are disposable and can be produced fairly rapidly and at low costs. Hence, they represent a promising tool for in-field detection of sulfur mustard.

Sulfur mustard [SM; bis(2-chloroethyl) sulfide] is a member of the vesicant class of chemical warfare agents. SM causes blistering of the skin and mucous membranes and, in high doses, is lethal.1,2 SM was first synthesized long ago, but its harmful properties became widely known only much later, mainly during World War I. It is banned by international conventions beginning with the Iraq–Iran war in the 1980s. Conceivably, SM may be abused by terrorists.3,4 Large quantities of SM are stockpiled, and others are sea-dumped and can be found as abandoned chemical ammunition.5 Its harmful properties, the absence of antidotes or specific treatments, and the ease of synthesis render SM a serious threat.

Methods for rapid detection of SM also find applications in alert procedures, selection of adequate protection, mapping of contamination areas, and during decontamination.6 Currently known tests include, for example, a colorimetric on-the-spot analytical test based on chemically doped papers and tubes.7 A simulant of SM was detected, for example, by a competitive chromogenic reaction between a dithiol and simulant with a squaraine dye to provide a blue coloration. The assay responds to the SM simulant, but not to the O-analogue of the mustard simulant and to other electrophilic agents. Detection is moderately sensitive with a limit of detection (LOD) of 1.25 μg/L.7 A fluorescence-based chemodosimeter was demonstrated for detection of SM in solution and the gas phase. It is based on S-alkylation followed by a desulfurization reaction of rhodamine-thioamide with SM. The assay is sensitive enough to visually detect 0.76 mg/L of SM.8 An optically transparent sol–gel-based sensor encapsulating Cu(II) acetate was fabricated for detection of analogues of SM via a charge-transfer mechanism. The LOD is said to be 0.03 μL of analogue per 1.5 mL of sensor volume.9 A rapid test was described for a related species (paraoxon) that can be fluorometrically determined, for example in human serum, by using a gold nanoparticle-immobilized organophosphorus hydrolase and coumarin 1 as a competitive inhibitor.10 These colorimetric tests are inex-
pensive, portable, and easy to use. However, they are not highly sensitive, are nonspecific, and are prone to false positive response. Hence, results have to be verified by other methods. More selective and sensitive detection is accomplished with more expensive instrumentation such as ion mobility spectrometry, flame photometry, infrared spectroscopy, or surface acoustic wave detection. An overview of these analytical techniques is given in Table S-1 in the Supporting Information. Even with these methods, false positive alarms have been reported. The most confirmatory results can be obtained using vehicle-mounted gas chromatographs coupled to mass-selective detectors (GC-MS). They can detect and quantify very low concentrations of SM, but assays are time-consuming and instrumentation is expensive (Table S-1, Supporting Information). While most reliable, GC-MS can hardly be applied to on-site analysis and/or at remote sites which the mobile lab cannot approach easily. Hence, a method for a rapid, selective, and inexpensive on-site monitoring of SM is needed.

Enzymatic strips can provide the desired properties which have not been available in detection systems for SM yet. The strip sensing platform is anticipated for use as a disposable biosensor with a user-friendly format also enabling on-site assays by untrained personnel. Such qualitative tests are performed by visually observing the color intensity. Quantitative data can be obtained rather easily by recording the color of a sensing zone with either a digital camera or a smartphone camera. This kind of quantitation has not been reported for SM so far. However, the development of biosensor test strips for the specific detection of SM is confronted with the lack of biological recognition elements as a main challenge.

In this study, a biosensor test strip enabling simple and rapid detection of SM is demonstrated. This novel method utilizing enzyme as a biorecognition element is based on the recently discovered catalytic activity of enzyme haloalkane dehalogenases toward SM. The enzyme is immobilized on a commercially available paper strip that contains a pH color indicator. Enzymatic hydrolysis leads to the production of protons and, hence, a drop in local pH value which causes the pH indicator to change color. The strips were first examined using bis(2-chloroethyl) ether (BCEE), a nonblistering analogue of SM, as a much less toxic model compound (Table S-2, Supporting Information), and the optimized biosensor strips were finally applied to the detection of SM. The developed test strips are simple, disposable, and cheap. The strips are based on user-friendly format enabling also on-site assays by untrained personnel, without need for external power supply. Such tests could be particularly useful in less developed countries with higher risk of abuse of SM.

**Experimental Section**

**Chemicals and Materials.** Recombinant histidine-tagged haloalkane dehalogenase LinB from *Sphingobium japonicum* UT26 was expressed and purified as described previously. pH indicator strips covering the pH range from 2 to 9 were purchased from Merck (product no. 109543001; Darmstadt; Germany; [www.merck.de](http://www.merck.de)). Ampicillin sodium salt and isopropyl-β-D-thiogalactopyranoside were obtained from Duchefa (Haarlem; Netherlands; [www.duchefa-biochem.com](http://www.duchefa-biochem.com)). Nitric acid was purchased from Lach-Ner (Neratovice; Czech Republic; [www.lach-ner.com](http://www.lach-ner.com)). Ethanol, methanol, acetone, and acetonitrile were purchased from Chromservis (Prague; Czech Republic; [www.chromservis.eu](http://www.chromservis.eu)). HydroMed D4 hydrogel as a 5% (w/w) solution in ethanol/water (90/10, v/v) was obtained from AdvanSource Biomaterials (Wilmington, MA; [www.advbionmaterials.com](http://www.advbionmaterials.com)). SM was synthesized by the Military Repair Manufractory Zemianske Kostalany (Slovakia; [www.ulz.mil.sk](http://www.ulz.mil.sk)) and purified in the Military Research Institute Brno to final content of 96% of the active component. All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO; [www.sigmaaldrich.com](http://www.sigmaaldrich.com)). All reagents were of analytical grade and used without purification. Solutions were prepared with deionized water with a resistivity of 18.2 MΩ·cm using a Millipore Milli-Q water purification system (EMD Millipore; Billerica, MA; [www.merckmillipore.com](http://www.merckmillipore.com)).

**Preparation of Test Strips.** The enzyme was immobilized on the pH indicator strips as described in detail in the Supporting Information. In short, a solution of the hydrogel was spread onto the neutral and the alkaline pH-sensitive panels of a strip (50 µL per strip) using a homemade knife-coating device at a wet thickness of 125 µm. The hydrogel was allowed to dry in air for 1 h at 23 °C. The enzyme (lyophilized in 50 mM phosphate buffer of pH 7.5, 50 mg) and bovine serum albumin (BSA, 100 mg) were dissolved in 800 µL of water. The solution (40 µL per strip) was deposited on the hydrogel layer stepwise (in increments of 10 µL). Then the strips were exposed to glutaraldehyde vapor for 30 min. The resulting strips were stored in a dark, cool, and dry place before use.

**Analytical Procedure and Image Analysis.** For detection of BCEE and other potentially interfering chemicals, the strips were incubated in 10 mL of 100 mM glycine buffer of pH 8.6. For detection of SM, the strips were conditioned for 20 min at 23 °C with the same buffer but containing 20% dioxane. Hydrated strips were exposed to the gaseous analyte. A detailed protocol for preparation of vapors is given in the Supporting Information. Following an incubation time of typically 10 min, the response of the strips to the various chemicals was evaluated visually by comparison with a nonresponsive (blank) strip and a reference scale. Instrumental quantification was performed by using the red-green-blue readout (RGB) of a digital camera (Canon, type EOS 1100D; Canon, Japan; www.canon.com) equipped with a standard 18–125 mm objective (F 3.8–5.6 DC OS) and fixed at a distance of 30 cm above the strips. The strips were illuminated with a halogen lamp (50 W, 12 V). The camera was operated in manual mode with parameters set as follows: ISO sensitivity: 100; shutter speed: 1/500 s; focal length: 125 mm; exposure: 0 EV; white balance: custom. Images were stored in RAW-format. Data evaluation and processing was accomplished as described previously.

The alkaline pH-sensitive panel of the strip was used for calculation of the average gray value. The strips were photographed after 10 min of enzymatic reaction at 23 °C. Following acquisition, the images were saved as a 16-bit color TIF-file using the software Bibble 5 Pro (Bibble Laboratories; Austin, TX; [www.bibblelabs.com](http://www.bibblelabs.com)). This file was then split into the red, green, and blue channel information via ImageJ software (National Institute of Mental Health; Bethesda, MD; [www.nimh.nih.gov](http://www.nimh.nih.gov)). The green channel did not contain any useful information and was discarded. Next, the intensity data of the red channel image were divided by the data of the blue channel image to give the so-called red/blue (R/B) signal. These data are plotted on the graphs as a strip response.

**Statistics.** All major experiments were performed minimally in triplicate (n ≥ 3). The means and standard deviations of the data were calculated. The outliers were excluded on the basis of Dixon’s Q test. The statistical significance of the differences
between strip responses was determined using Student’s t test with a p value ≤0.05 considered to be statistically significant. The data were analyzed using GraphPad Prism 6 Software (GraphPad Software Inc.; San Diego, CA; http://graphpad.com).

Safety Statement. SM, BCEE, 1,2-dichloroethane, and ethyl bromoacetate are extremely toxic chemicals and therefore must be handled with extreme care and under appropriate safety precautions. A fume hood with a high volume flow must be used for preparation of gaseous analytes and measurements with the test strips under laboratory conditions. The material should be handled using appropriate personal safety equipment, including laboratory coats, chemical goggles, and protective gloves (made from butyl rubber in the case of SM and from nitrile rubber in the case of BCEE, 1,2-dichloroethane, and ethyl bromoacetate). The experiments with SM were performed at the specialized facilities of the Military Research Institute, Brno, Czech Republic. All materials exposed to SM have to be decontaminated later on with solutions of aqueous sodium hypochlorite.

RESULTS AND DISCUSSION

Design, Preparation, and Optimization of the Strips.
The selection of materials including the biological recognition element is a key step in the development of the sensing system because it governs selectivity and sensitivity. Haloalkane dehalogenases (EC 3.8.1.5) belong to one of a few classes of enzymes exhibiting catalytic activity toward SM. Among them, the haloalkane dehalogenase LinB from Sphingobium japonicum UT26 exhibits the highest conversion rate. This enzyme was therefore selected for the preparation of the strips for sensing SM.

The enzyme was immobilized in a highly inert polyurethane hydrogel of proven performance in sensors for pH values. This gel is biocompatible, soluble in a 9:1 ethanol/water mixture, and very permeable to protons. It forms transparent films on the support and increases the mechanical stability of the immobilized enzyme and the strip. However, other inert polymers such as certain thermogelling medical polycrylamides may also be applied. Rather than designing and fabricating one more optical pH sensor film, a commercially available cellulose-based pH indicator strip was used as a support for the hydrogel incorporating the immobilized enzyme. Changes of 0.3 pH units (or more) derived from the resolution of these paper strips can be detected visually.

When selecting a proper method for immobilizing the enzyme, previous experiments revealed that cross-linking with glutaraldehyde is appropriate. Detailed information on the immobilization procedures is given in the Supporting Information. Early attempts based on coimmobilization with BSA via cross-linking with glutaraldehyde and mixing it with hydrogel, or formation of water-insoluble particles such as cross-linked enzyme aggregates (CLEAs), did not result in adequate enzyme activity (maximal retention of 10%; Figure 1).

Immobilization was more successful, however, when applying the hydrogel to the support prior to the deposition of enzyme. In first attempts, mixtures of enzyme and BSA in varying weight ratios were applied to the hydrogel and cross-linked with glutaraldehyde. In the best cases, this procedure resulted in the retention of approximately 30% of enzyme activity (Figure 1). The highest activities were obtained by placing the enzyme with BSA onto the finished hydrogel (coating called upper layer in Figure 2). The optimal enzymatic activity is provided by a test strip with a concentration of 2.9 mg of enzyme per cm² (Figure 2). Its activity is still as high as 3.4 ± 0.6 nmol·s⁻¹·mg⁻¹.

Figure 1. Effect of immobilization procedure on activities of haloalkane dehalogenase LinB. Activities were measured with bis(2-chloroethyl) ether (1.2 g/L) at 21 °C after 0.5 h exposure of immobilized enzyme to a glycine buffer for washing out remaining free enzyme. The activity of 100% corresponds to 3.2 nmol·s⁻¹·mg⁻¹ of lyophilized enzyme. The standard deviations were calculated from three independent measurements (n = 3). Abbreviations: CLEA, cross-linked enzyme aggregates; D4, D4 hydrogel; GA, glutaraldehyde.

Figure 2. Effect of different coatings on the activities of immobilized haloalkane dehalogenase LinB: upper layer (placing LinB with BSA onto the hydrogel layer), sandwich (placing LinB with BSA between hydrogel layers), bottom layer (placing LinB with BSA below hydrogel layer). The following enzyme amounts were applied: 0.9 mg (white column), 2.0 mg (dotted column), 2.9 mg (gray column), and 3.8 mg of enzyme per cm² (black column). (A) Activities were measured with bis(2-chloroethyl) ether (1.2 g/L) at 21 °C after 0.5 h exposure of immobilized enzyme to a glycine buffer for washing out remaining free enzyme. The activity of 100% corresponds to 5.0 nmol·s⁻¹. The standard deviations were calculated from three independent measurements (n = 3). (B) Photographs of enzymatic and nonenzymatic pH strips after 30 min reaction with bis(2-chloroethyl) ether (1.2 g/L) at 21 °C.
relatively high concentration of enzyme was selected as a result of the following considerations: (i) The need for the conversion of sulfur mustard by the enzyme to occur earlier than abiotic (nonenzymatic) hydrolysis; (ii) a relatively low activity of haloalkane dehalogenase (0.0195 μmol·s⁻¹/mg); (iii) the stipulation of a short detection time.

The optimized strips were then used in further experiments as a compromise between activity and costs. They undergo pH changes in the order of 1.0 to 1.5 pH units after a 10 min exposure time to 1.2 g/L of BCEE, and this allows for an easy visual detection and semiquantitation.

Next, the strips were tested for their response to gaseous analyte. Haloalkane dehalogenases belong to the family of hydrolases, and these require the presence of water for the conversion of their substrate. Therefore, the strips were hydrated in buffer before exposure to vaporized analyte (Figure S-2). Response depends on the type of buffer used. Borate, Tris, and bicarbonate buffer (all of pH 8.5) provided negligible response to BCEE. Strips hydrated in a 100 mM glycine buffer of pH 8.6 exhibited significant concentration-dependent response; therefore, this buffer was used for hydration of the strips. A 10 min exposure time was found to be the best compromise between the requirement of a rapid assay and achievement of a sufficiently large signal change.

Analytical Performance of the Strips. The response of the test strips to gaseous BCEE at various concentrations was examined first to explore their performance (Figure 3). The calibration plot becomes flat at BCEE concentrations of 2 mg/L or higher. The overall shape of the calibration plot is sigmoidal and obeys the mass action law that governs the acid–base equilibrium of the pH indicator in the strips. The LOD was determined by linearizing the sigmoidal plot by using a log–logit function to give a LOD of 70 μg/L for gaseous BCEE (for n = 4 and a signal three times the standard deviation of the noise related to the intercept of the linear function). A control with test strips without immobilized enzyme showed no response to BCEE, neither visually nor using the RGB readout.

The selectivity of the strips was investigated in the presence of 1,2-dichloroethane and ethyl bromoacetate as potential interfering agents (Figure 4). The strips did not respond to 1,2-dichloroethane in a concentration as high as 6.3 mg/L. The response to mixtures of 1,2-dichloroethane and BCEE was the same as the response to pure BCEE. On the other hand, ethyl bromoacetate in relatively high concentration (6.2 mg/L) interfered. Both enzyme-loaded and nonloaded strips underwent color changes after exposure to ethyl bromoacetate with no statistical difference in the responses (p value = 0.49, Table S-4, Supporting Information), probably a result of chemical hydrolysis of this ester to form hydrobromic acid. This observation underpins the importance of using strips without an enzyme as a control to prevent false positive results. The response of the strips to mixtures of ethyl bromoacetate and BCEE was clearly distinguishable, representing minimally 80% of the response to pure BCEE.

The intra-assay reproducibility of the biosensor test strips was found to be 1.6% (for n = 15) by assaying strips prepared in one batch at three different days (Table S-5, Supporting Information). Similarly, the interassay reproducibility was determined to be 5.7% (again for n = 15) by assaying strips prepared from three different batches of enzyme and immobilized independently (Table S-5). This indicates an excellent reproducibility of the strips. Storage stability was investigated with strips prepared in a single batch and stored in a dried and lyophilized state at different temperatures (Figure S-3). The optimized strips were then used in further experiments as a compromise between activity and costs. They undergo pH changes in the order of 1.0 to 1.5 pH units after a 10 min exposure time to 1.2 g/L of BCEE, and this allows for an easy visual detection and semiquantitation.

Figure 3. Calibration plot for gaseous bis(2-chloroethyl) ether with linear fit of data (in inset). (A) The response of enzymatic pH strips after 10 min at 23 °C was evaluated using the RGB readout. The signal of the nonenzymatic strip (control) was subtracted from the signal of the enzymatic strip. The standard deviations were calculated from four independent measurements (n = 4). (B) Photographs of enzymatic and nonenzymatic pH strips after 10 min exposure to bis(2-chloroethyl) ether at 23 °C.

Figure 4. Effect of interferents on the response of the enzymatic pH strips. The activities were determined at 23 °C: (1) Bis(2-chloroethyl) ether (7.1 mg/L, control); (2) 1,2-dichloroethane (6.3 mg/L); (3) 1,2-dichloroethane (6.9 mg/L) and bis(2-chloroethyl) ether (6.8 mg/L); (4) 1,2-dichloroethane (3.4 mg/L) and bis(2-chloroethyl) ether (7.4 mg/L); (5) ethyl bromoacetate (6.2 mg/L); (6) ethyl bromoacetate (6.7 mg/L) and bis(2-chloroethyl) ether (9.4 mg/L); (7) ethyl bromoacetate (2.8 mg/L) and bis(2-chloroethyl) ether (9.8 mg/L). The response of the strips was evaluated using the RGB readout. The signal of the nonenzymatic strip (control) was subtracted from the signal of the enzymatic strip. The standard deviations were calculated from three independent measurements (n = 3).
S-3, Supporting Information). The dried strips were stored at 23 °C under dry conditions in the dark for 6 months but retained more than 95% of their initial response (with relative standard deviation of 3.2% after 6 months, n = 3; Table S-6, Supporting Information). The same retention of response was observed for strips stored in dried form at 4 °C or in lyophilized form for 9.5 months (with relative standard deviations of 3.2 and 6.3%, respectively, after 9.5 months, n = 3; Table S-6). Finally, the response of strips exposed to repeated cycles of freezing and thawing remained uncompromised compared to the initial response (p value = 0.40 after six cycles, Figure S-3, Table S-7, Supporting Information).

The effect of temperature on the response of strips was tested (Figure S-4, Supporting Information). The strips are applicable at both 23 °C and 37 °C without significant difference in their responses (p value ≤ 0.8, Table S-8, Supporting Information). The loss of enzymatic activity is expected after exposure of strips to higher temperatures because the haloalkane dehalogenase LinB possesses a melting temperature of 48 °C. On the other hand, temperatures lower than 20 °C usually cause a fast decrease of enzymatic activity of haloalkane dehalogenases.23

Application of Strips to the Detection of SM. The optimized strips were tested for response to SM. Fractions of 10% of acetone and acetonitrile and of up to 20% of dioxane were added in order to increase the solubility of SM in the strip and to suppress its spontaneous hydrolysis. Subsequently, the moist enzymatic and nonenzymatic strips were exposed to vapors of SM simultaneously (Figure 5A). The response of the strips was evaluated visually at first. The most significant color change was observed for enzyme-loaded test strips hydrated with buffer containing 20% of dioxane. The blue-green color in the alkaline panel of the strips changed to yellow after a 10 min exposure to air saturated with SM vapor. The nonloaded strip, however, did not give a visually detectable change. Again, a more precise evaluation was performed using the RGB readout method. The most significant difference between the response of enzymatic and nonenzymatic strips was observed, in agreement with the visual evaluation, for strips hydrated with buffer containing 20% of dioxane, which, therefore, was used for hydrating the strips used for calibration.

Unlike with BCEE, the calibration with SM had to be performed at lower vapor concentrations, because vapors of SM are difficult to prepare due to its low volatility and its hazardous properties (Table S-2, Figure 5B). The data for vapors of SM also show larger standard deviations. This is likely due to some condensation of SM vapors and the possible formation of aerosols. Simultaneous exposure of enzymatic and nonenzymatic strips to SM enabled comparison of their responses. While both tested strips did not change color on exposure to SM in concentrations between 0 and 20 μg/L, a statistically significant difference in their response was observed (p value ≤ 0.005, Table S-9, Supporting Information) when the RGB technique was utilized. This approach enabled detection of SM in concentrations down to 3 μg/L. According to Wattana and Bey,7 the acute emergency guideline level 3 for a 10 min exposure is 3.9 μg/L. Above this level, exposures may become life-threatening or result in long-term complications. Such an increase in LODs when using RGB readout was also found in other cases.24 This low LOD is virtually the same as that of other colorimetric papers and tubes. It is better by factors of 10 and 1000 compared with that of surface acoustic wave sensing and infrared spectroscopy, respectively. The LODs are higher than those of ion mobility spectrometers and GC-MS with their LODs in the pg/L if not fg/L range.25 On the other hand, the strips described here are much more simple, smaller, easy to handle, and affordable in terms of production. Potentially, they can be used in-field and by less skilled personnel (Table S-1).

Read-out is based on a photographic technique which most potential users are familiar with. Conceivably, digital cameras may be replaced by smartphones with fully integrated read-out software.26–28 Furthermore, we are working on the application of protein engineering for improvement of catalytic properties of haloalkane dehalogenases with SM, in parallel with screening of known haloalkane dehalogenases to find the enzymes with better catalytic efficiency with SM. Application of these enzymes enables decreasing the LOD of the test strips.

![Figure 5](image-url)

Figure 5. (A) Response of the enzymatic pH strips hydrated in organic cosolvents to saturated sulfur mustard vapors. The enzymatic strips (black column) and hydrated nonenzymatic strips (white column) were exposed to sulfur mustard (0.6 mg/L) at 23 °C. (B) Calibration plot for gaseous sulfur mustard as determined using the enzymatic pH strips hydrated in 20% dioxane at 24 °C. The response in both tests was evaluated after 10 min using the RGB readout. The signal of the nonenzymatic strip (control) was subtracted from the signal of the enzymatic strip for the particular concentration in the calibration plot. The standard deviations were calculated from three independent measurements (n = 3).

**CONCLUSIONS**

The test strips described in this article allow for a fast and fairly simple detection and quantitation of SM. The activity of haloalkane dehalogenase is well retained after immobilization. The pH indicator strips used as a solid support are readily available and represent a viable transducer for detecting local changes in pH value that occur as a result of enzymatic action. The test strips enable rapid detection of SM, with LODs of 20 μg/L for visible detection, and 3 μg/L for RGB read-out. The strips display long-term storage stability and a high reproducibility in terms of detection and production. Conceivably, the method may be extended to fiber optic remote sensing, as previously shown for nerve agents acting as acetylcholine esterase inhibitors,29 which can be performed over large distances and reduces the risk of exposure to SM by persons. The method also is likely to be applicable to other detection schemes for organic toxicants if enzymes can be found that cause the formation or consumption of protons during their action.
There are no analytical techniques for detection of SM, properties of detected analytes, experimental details and results of optimization, reproducibility, and storage stability of enzymatic pH strips (Tables S-1 to S-9 and Figures S-1 to S-4) (PDF).

**REFERENCES**


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**Notes**

The authors declare the following competing financial interest(s): Two authors declare the following competing financial interests. Jiri Damborsky and Zbynek Prokop are founders of Enantis, spol. s r.o., a biotechnology spin-off company of Masaryk University.

**ASSOCIATED CONTENT**

**Supporting Information**

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.anal-chem.6b01272.