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Liquid-to-Gel Transition for Visual and Tactile Detection of Biological Analytes

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Abstract

So far all visual instrument-free methods are based on the color change. However, colorimetric assays cannot be used by blind or color blind people. Here we introduce a liquid-to-gel transition as a general output platform. The signal output (a piece of gel) can be unambiguously distinguished from liquid both visually and by touch. The approach promises to contribute to the development of accessible environment for visually impaired persons.

Among the variety of analytic methods, those that enable direct visual output are particularly attractive due to the possibility of instrument-free signal readout.¹ Indeed, such analytical formats as pH test strips, home pregnancy test, enzyme-linked immunosorbent assay (ELISA) or gold-nanoparticle-based DNA analysis² can be said to be ultimate in terms of the easiness of output readout. Here we challenge this statement by introducing an alternative signal readout for molecular sensors, which can be detected not only visually but also by touch. The tactile signal readout can be used by blind and color blind people or under the conditions when visualization is not possible (e.g. in the dark).

The method is based on the radical-mediated enzymatic polymerization of acrylamide into polyacrylamide by horseradish peroxidase (HRP)/acetylacetone/H₂O₂ ternary system.³ In this system acetylacetone is oxidized by HRP to the acetylacetone-radical, which results in radical-initiated polymerization of acrylamide accompanied by a gel-to-liquid transition. However, HRP itself cannot be transformed easily into a sensor capable of detecting biological analytes. On the other hand, a number of DNA and RNA sequences were reported to have HRP-like activity in the presence of hemin.⁴ The peroxidase-like DNAzymes (PxD) contain guanine-rich DNA sequences that fold into a stable G-quadruplex structure, which can bind hemin and catalyze H₂O₂-dependent oxidation of colorless substances and convert

Electronic Supplementary Information (ESI) available: Oligonucleotide sequences, detailed experimental procedure and limit of detection for SSA.

^{††}The DF was calculated according to the formula: $DF = (F_m - F_o) / (F_{mm} - F_o)$, where F_m and F_{mm} are average fluorescence of samples in the presence of matched and mismatched analytes, respectively; F_o is a background fluorescence in the absence of analytes.

them into the colored products. Furthermore, a great variety of colorimetric biosensors have been developed based on PxD.⁵ Here, we aimed at achieving an analyte-dependent activation of PxD followed by polymerization of acrylamide solution rather than color change. If polymerization is completed, the gel fragments would be observed at the tips of the inversed tubes, or by touch if the contents of the tubes are released on the filter paper (Figure 1). In this proof of concept study, we report on the detection of biologically important analytes: ATP and two DNA sequences by a liquid-to-gel transition method.

We first turned our attention to the detection of ATP, an important biological analyte. The sequence for ATP aptamer was isolated by Huizenga and Szostak.^{6a} The aptamer is known to bind two ATP molecules and was used in a number of assays for ATP detection.⁶ In our design, the ATP aptamer sequence was split into two fragments, **PxD1** and **PxD2** (Figure 1A). Each half was attached to a part of G-rich PxD sequence. Binding of ATP to the aptamer stabilized the G-quadruplex structure, which catalyzed the polymerization reaction in the presence of hemin. To prevent nonspecific association of the sensor strands in the absence of the analyte, **PxD2** strand had additional nucleotides, allowing for the formation of a stable stem-loop structure within one of the sensor strands (Figure S1). Five mM ATP was detected in 1 hr of incubation at room temperature (Figure 1B, sample 3). This concentration is within the physiological range of ATP level.⁷ Addition of 10 mM ATP resulted in polymerization within ~30 min (Figure 1B, sample 4). No polymerization was observed in the presence of just two sensor strands (Figure 1B, sample 2). Gel fragments were characterized under microscope (Figure S2).

The dependence of polymerization rates on the analyte concentration promised to become a basis for a quantitative detection, in which the analyte concentration is determined based on the time required for polymerization. We performed a series of experiments aiming at determining a correlation between ATP concentration and polymerization time. However, no clear dependence was determined in this study. Higher concentrations of the analyte triggered polymerization of acrylamide at various times within one hour and varied from experiment to experiment. This can be explained by the presence of various amounts of residual oxygen in the samples, which is known to inhibit the polymerization reaction.⁸ This observation correlates with irreproducible inhibition period for the HRP-mediated polymerization of acrylamide due to the presence of residual oxygen reported by Lalot et al.⁸ Due to the experimental setup, the presence of residual oxygen is inevitable and varies in each experiment from tube to tube, thus making it practically difficult to correlate concentration of the analyte and polymerization time. Based on this observation, we would primarily recommend the presented method for qualitative analyte detection, when the presence or absence of an analyte (e.g. pathogen) in a sample needs to be determined. However, we do not exclude that an improved oxygen-free setup will eventually enable applications of this method for quantitative analysis.

To demonstrate a general applicability of the approach, we used similar strategy for a different biologically significant analyte, DNA. As a targeted sequence, we chose DNA fragments of **AMEL_X** and **AMEL_Y** genes, which encode for amelogenin – protein involved in the development of tooth enamel. Different amelogenin-encoding sequences are located in X and Y chromosomes, which is used for sex determination in forensic and

anthropological applications.⁹ The split DNA sensor was designed as reported earlier for colorimetric DNA detection (Figure 2A).¹⁰ Two strands **PxD3** and **PxD4** hybridized to the abutting positions of **AMEL_X** analyte and formed a **PxD** G-quadruplex structure, which triggered the polymerization reaction. In the absence of the analyte, no polymerization occurred (Figure 2B, sample 2), whereas in the presence of 10 μ M **AMEL_X** analyte liquid-to-gel transition was observed within 1 hr (Figure 2B, sample 3). Importantly, no polymerization took place in the presence of a single-base mismatched analyte (Figure 2B, sample 4), which strongly suggests that the method can be used for detection of point mutations and single nucleotide polymorphisms in DNA.

Even though conventional hybridization probes can detect nucleic acid down to $\sim$ 1-100 nM,¹¹ modern amplification technologies (e.g. LAMP, NASBA) produce highly concentrated nucleic acid amplicons. Furthermore, complete elimination of the contact between assay and O₂ can potentially further reduce the LOD of this assay. Therefore, the utility of the reported method in clinical DNA analysis has to be further validated.

Some analytical applications could benefit if the presence of an analyte maintained liquid phase, while polymerization into gel occurred in the absence of the analyte. Therefore, we designed a sensor that detects a DNA analyte in the inversed ('NOT' logic) format, e.g. presence of an analyte inhibits gel formation. We used **AMEL_Y** DNA analyte in this design to demonstrate more general applicability of the assay for the DNA detection. In this design, the catalytic DNA sequence was again split in two parts: **PxD5** and **PxD6** strands in Figure 3A. The two sensor strands associated and formed G-quadruplex, which triggered gel formation in the absence of the analyte (Figure 3B, sample 2). On the contrary, addition of the analyte disrupted G-quadruplex formation due to preferential binding of **PxD5** strand to the **AMEL_Y** analyte. Consistent with the design, polymerization of acrylamide was observed only in the presence of both sensor strands, but not the analyte (Figure 3B).

Methods for user-friendly detection of biological analytes are under continuous development. For example, Macdonald and colleagues created a 7-segment display to vividly display an output of a molecular automaton that analyzed DNA sequence of Ebola and Marburg viruses.¹² Furthermore, Gormley et al. developed an analytical output to form polymers that can associate with multiple gold nanoparticles into aggregates, resulting in a visible color change.¹³ Ikeda et al. reported peptide-based hydrogels that have the ability to encapsulate enzymes and detect small molecules including glucose, lactose and sarcosine.¹⁴ However, these and other approaches^{15,16} all relied only on visual monitoring of the output signals. On the other hand, a great variety of elcrosensors including smartphone-based, RGB-Depth, electromagnetic and others, have been developed for visually impaired people.¹⁷

Here we report a first method that can be used for a tactile detection of biological analytes and therefore can be used by blind and color blind persons without the need for any electronic device. Using this method, we were able to unambiguously detect ATP and two different DNA sequences (**AMEL_X** and **AMEL_Y**). Importantly, the DNA sequences can be detected with high selectivity even at room temperature, which is important in practice for the detection of drug-resistant bacteria and single nucleotide polymorphisms in human

genomes. Importantly, PxD-based sensors are known for the detection of a broad variety of analytes including metal ions, small molecules and proteins.⁵ This method, therefore, has potentially a broad spectrum of bioanalytical applications.

The disadvantage of the reported assay is its sensitivity to atmospheric oxygen, which inhibits polymerization and complicates quantitative analysis. We do not exclude that future converting the technology in an oxygen-free format may solve this problem. The toxicity problem of unpolymerized acrylamide can be easily solved by wearing gloves or by covering the gel pieces by a plastic film. Alternatively, acrylamide can be replaced with less toxic monomers, e.g. hydroxyethyl methacrylate (HEA).¹⁸ Analysis of RNA or DNA sequences is not compatible with whole blood samples and, as for other nucleic acid detection technologies, requires isolation of DNA prior analysis. However, we do not exclude that the method might be adoptable for some analytes (e.g. K⁺, drugs etc.) in whole blood, which would represent another major advance of the liquid-to-gel transition approach. We also believe that the new output signal reported in this study has a great potential beyond a tactile-sensing platform. For example, the liquid-to-gel transition can be used for analyte-dependent mechanical blocking of tubes or channels in (micro)fluidic devices thus regulating liquid flow in analyte-depending manner.

Conclusions

We demonstrated an assay for analyte-dependent liquid-to-gel transition that can be sensed not only visually but also by touch. We hope that this method will make accessible home testing systems for blind and color blind persons, as well as inspire future developments of chemical products for physically-challenged people.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Polymerization-based visual and tactile detection of ATP. A) Sensor design: DNA strands **PxD1** and **PxD2** bind two ATP molecules,⁶ which stabilize a G-quadruplex structure. The G-quadruplex binds hemin (brown oval) and catalyzes H_2O_2 -dependent oxidation of acetylacetone to acetylacetone radical, which then triggers polymerization of acrylamide and bisacrylamide. Dotted lines represent triethylene glycol linkers. B) Visual signal output can be detected by turning a reaction test-tube upside down. All samples contained reaction buffer: 50 mM HEPES pH 6.6, 50 mM MgCl_2 , 20 mM K^+ , 120 mM NaCl, 1 % DMSO v/v, 0.03 % w/v Triton $\times$ 100, 38 % w/v acrylamide, 2% w/v bisacrylamide, 127 mM acetylacetone, 5 μM hemin and 5.2 mM H_2O_2 . Polymerization was observed only when either 5 or 10 mM of ATP were present (samples 3 and 4, respectively). No gel fragments are observed at the tip of the tube in the absence of ATP (sample 2). Sample 1 (negative control) contained only the buffer components; sample 5 (positive control) contained full **PxD** 5'-GGG TAG GGC GGG TT GGG; Low panel: tactile readout is possible if the samples are placed on a piece of filter paper. Liquid samples 1 and 2 are absorbed by the paper, while gel shapes from samples 3, 4 and 5 can be sensed by touch. All samples were colored cyan by addition of 0.1 % w/v xylene cyanol for easy visualization.

Fig. 2.

Polymerization-based visual and tactile detection of **AMEL_X** DNA analyte. A) Sensor design: two DNA strands **PxD3** and **PxD4** hybridize to a DNA analyte (**AMEL_X**) and form a G-quadruplex structure, which binds hemin and catalyzes polymerization of acrylamide solution (see Figure 1 legend and SI for experimental details). Dotted lines represent triethylene glycol linkers. B) Visual signal output. Polyacrylamide gel is observed in the presence of fully matched **AMEL_X**. (3), but not in its absence (2) or presence of a mismatched analyte (4). Sample 5 did not contain DNA strands (negative control); Sample 1 contained full **PxD** (positive control). Low panel: the gel fragments can be detected by touch after transfer on a filter paper.

Fig. 3.

Detection of **AMEL_Y** DNA analyte in a NOT logic mode. A) The predicted secondary structure of the **AMEL_Y** sensor in the absence of **AMEL_Y** analyte. Green line indicates a fragment complementary to **AMEL_Y** analyte. The sensor acts in a 'NOT' format: it triggers polymerization in the absence of **AMEL_Y** analyte. B) Polymerization was observed in the absence of **AMEL_Y** analyte (sample 2), but not in its presence (sample 3). Sample 1 contained full **PxD** (positive control); sample 4, contained all, but DNA components (negative control). Lower panel: samples after release on the filter paper; only samples 1 and 2 can be detected by touch.