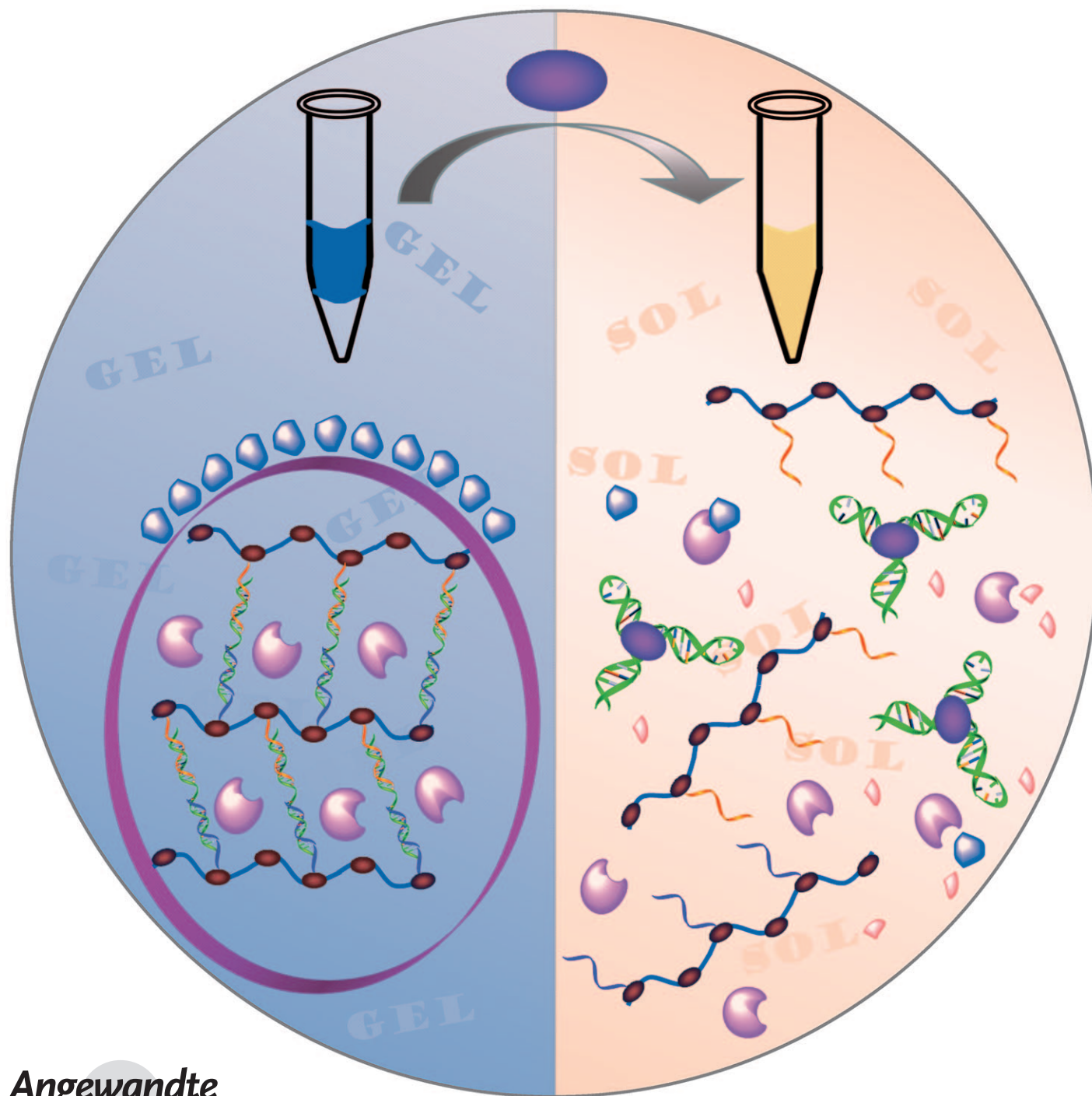


An Aptamer Cross-Linked Hydrogel as a Colorimetric Platform for Visual Detection**

Zhi Zhu, Cuichen Wu, Haipeng Liu, Yuan Zou, Xiaoling Zhang, Huaizhi Kang, Chaoyong James Yang,* and Weihong Tan*



Visual detection is an increasingly attractive method in many fields because both qualitative and semiquantitative assessment can be performed in real time without any advanced or complicated instrumentation. It is especially useful for rapid diagnostics in disaster situations, home healthcare settings, and in poorly equipped rural areas, where low cost, rapidity, and simplicity are essential. A variety of colorimetric reagents, such as visible dyes,^[1] polymers,^[2] enzymes,^[3] and gold nanoparticles (AuNPs),^[4–9] can be used for visual detection of specific targets. The color change of these reagents is based on diverse, yet selective, molecular interactions. Examples include stimuli-induced release or absorbance of dye molecules, polymers whose color changes are initiated by target binding, or enzymatic reactions triggered by molecular recognition. Meanwhile, “stimuli-responsive” or “smart” hydrogels have attracted particular attention in the development of biosensor devices that utilize a broad spectrum of triggers, including temperature, pH, ionic strength, and electric field. However, most biosensing devices operate on the basis of mechanical work performed by gel swelling and shrinking, or property changes of free-swelling gels, such as changes in optical transmission,^[10] refractive index,^[11] or resonance frequency,^[12] most of which must rely on time-consuming manipulation and sophisticated instruments. Herein, we propose a colorimetric agent-caging hydrogel as a novel visual detection platform that relies on DNA base-pair recognition and aptamer–target interactions for simple and rapid target detection with the naked eye.

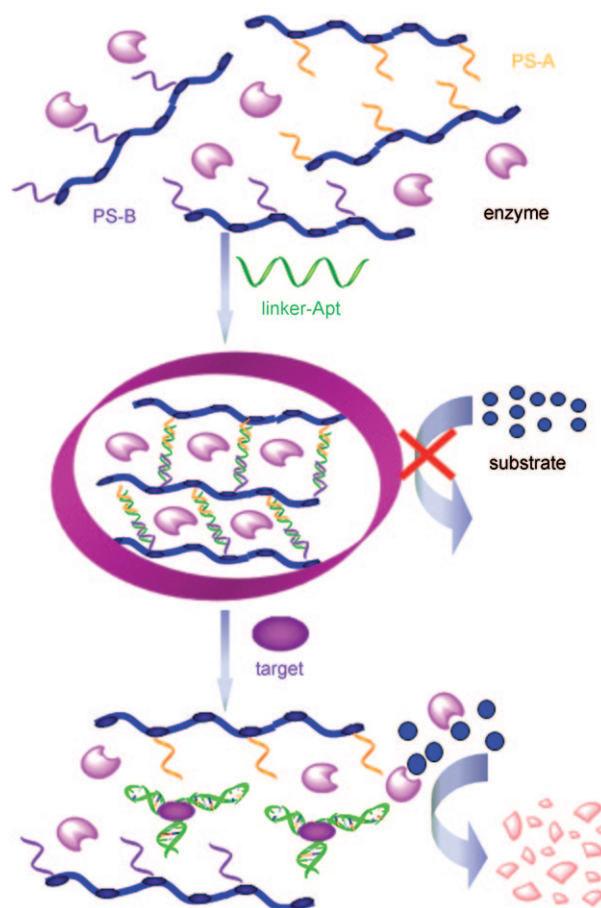


Figure 1. Working principle of DNA cross-linked hydrogel for signal amplification and visual detection.

Figure 1 illustrates the working principle of our visual detection method. Two pieces of DNA, strand A and strand B, are grafted onto linear polyacrylamide polymers to form polymer strands A and B (PS-A and PS-B), respectively. The sequences of DNA strands A and B are complementary to an adjacent area of a DNA aptamer sequence. When mixed in equal amounts, the polymers grafted with strand A and strand B are in transparent liquid form. The addition of aptamer linker-Apt initiates hybridization of strand A and strand B with the aptamer sequence, thus cross-linking the linear polyacrylamide polymers. As the hybridization proceeds, the cross-linking ratio of polyacrylamide increases, which results in the increase of viscosity of the polymer solution. The polymer will finally transform into a gel.^[13] Upon introduction of a target, the aptamer will bind with it, and the gel will be dissolved as a result of reducing the cross-linking density by competitive target–aptamer binding.^[14] If an enzyme is added prior to the addition of the aptamer, the enzyme will be trapped inside the 3D network of the hydrogel (represented as pink symbols in Figure 1). When target molecules are introduced to dissolve the gel, the enzyme is released and can take part in its catalytic role for signal amplification. A cascade of events is thus set in motion, whereby target binding triggers an enzymatic reaction, which, in turn, changes the substrate color, thus allowing visual

[*] C. Wu, Y. Zou, Prof. Dr. C. J. Yang
State Key Laboratory for Physical Chemistry of Solid Surfaces
The Key Laboratory for Chemical Biology of Fujian Province and
Department of Chemical Biology
College of Chemistry and Chemical Engineering
Xiamen University, Xiamen 361005 (China)
Fax: (+86) 592-218-9959
E-mail: cyyang@xmu.edu.cn

Z. Zhu, H. Liu, Prof. X. Zhang, H. Kang, Prof. Dr. W. Tan
Center For Research at Bio/nano Interface, Department of
Chemistry and Department of Physiology and Functional Genomics
Shands Cancer Center, UF Genetics Institute and
McKnight Brain Institute, University of Florida
Gainesville, FL 32611-7200 (USA)
Fax: (+1) 352-846-2410
E-mail: tan@chem.ufl.edu

Prof. Dr. W. Tan
Biomedical Engineering Center, State Key Laboratory of Chemo/
Biosensing and Chemometrics
College of Chemistry and Chemical Engineering
Hunan University, Changsha 410082 (China)

Prof. X. Zhang
Department of Chemistry, School of Science
Beijing Institute of Technology, Beijing 100081 (China)

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detection. Because our aptamer cross-linked hydrogel colorimetric platform can be targeted to any ligand for which there is a corresponding aptamer,^[15] we anticipate that it will find many visual detection applications in a wide variety of fields.

There can be no argument against the statement that drug misuse is a major challenge confronting public health and law enforcement. In this work, cocaine was used as the model target to test our new visual sensing method. A cocaine aptamer has previously been obtained by Landry's research group through an *in vitro* selection process,^[17] and has been already used for the design of several aptasensors.^[18] Our design of cocaine strands A and B and linker-Apt have been adopted from the recent report of the Lu group using gold nanoparticles and an aptamer for colorimetric cocaine sensors.^[18bc]

To systematically study the principles of the hydrogel platform and to optimize the system, we trapped gold nanoparticles (AuNPs) inside the hydrogel. AuNPs were adopted as indicating reagents or signal-amplifying agents based on their unique optical properties and chemical stability. Firstly, gold nanoparticles with diameters of only a few nanometers can be easily obtained. Such a diameter range is equivalent to that of most enzymes (3–15 nm); therefore, the behavior of hydrogel-trapped enzymes can be extrapolated by studying that of gold nanoparticles. Secondly, and more importantly, the remarkably large extinction coefficient of AuNPs at the visible wavelength (around 520 nm) makes them a sensitive indicating reagent for visual detection. Thus, either trapping or release of AuNPs by the aptamer cross-linked hydrogel through molecular recognition can be directly visualized by their characteristic red color.

In our experiment, 13 nm water-soluble AuNPs were prepared by following an established protocol,^[19] and modified with bovine serum albumin (BSA) to avoid aggregation caused by the high salt concentration. Before addition of linker-Apt, the modified AuNPs were added into the sol system, and were mixed thoroughly with PS-A and PS-B. After introduction of linker-Apt, a homogeneous red-colored hydrogel formed with evenly dispersed AuNPs trapped inside. After washing three times with buffer solution to remove surface-bound AuNPs, the gel was placed in a buffer solution and was found to remain in gel form. In buffer solution, the gel appeared red, while the upper buffer solution layer remained colorless (Figure 2a). Upon addition of the target, the gel dissolved and released AuNPs to the upper layer of the buffer solution. As a result, the buffer solution turned from colorless to intense red, a change that can be easily seen with the naked eye.

The greatest response sensitivity in such a sensing scheme relies on optimizing the hydrogel pore size to maximize the diffusion rate of target molecules into the gel for target recognition and rapid detection, while minimizing the non-specific leaking of cargoes to avoid false positive results. The pore size of the gel is determined by the cross-linking ratio of DNA. Accordingly, four hydrogels with different DNA cross-linking densities (0.1, 0.3, 0.5, 0.7 mM) were prepared, and the kinetics of target-triggered release of AuNPs from hydrogels was investigated by both the naked eye and UV/Vis spectrometry (Figure 2). The gel was prepared with AuNPs

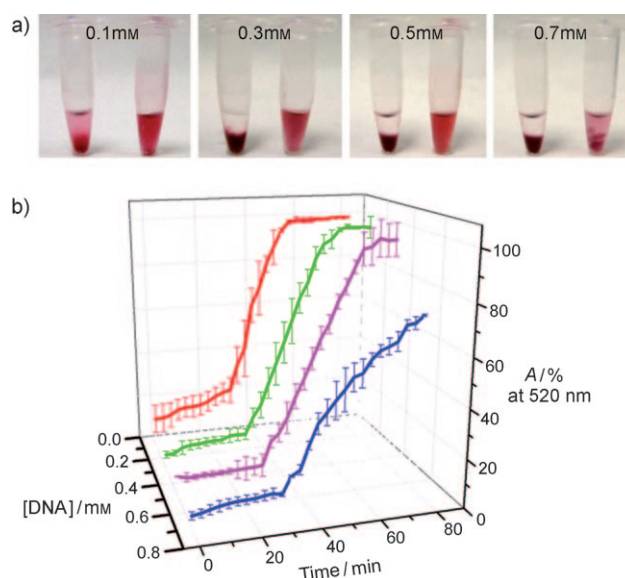


Figure 2. Release of AuNPs from the hydrogel upon introduction of cocaine. a) Photograph of the hydrogel before (left) and 30 min after (right) addition of cocaine. Four hydrogels with different DNA cross-linking densities (0.1–0.7 mM) were prepared to study cargo release kinetics. AuNPs were trapped in the DNA hydrogel with a cover layer of 10 mM tris(hydroxymethyl)aminomethane (Tris-HCl) buffer (pH 8.0, 200 mM NaCl). Upon introduction of 1 mM cocaine, AuNPs were released from the DNA hydrogel to form a uniform red solution. b) Release kinetics of AuNPs from four types of hydrogels upon introduction of cocaine. The encapsulating stability of each hydrogel was examined for 30 min before addition of 1 mM cocaine.

that were encapsulated and placed at the bottom of a quartz microcell with a buffer solution on top. The release of AuNPs to the buffer solution over time could be quantitatively monitored through the strong AuNP absorption at 520 nm. The absorption curves on the buffer solution from the four types of hydrogels during the release of AuNPs are shown in Figure 2b. The gels were monitored for 30 min before the introduction of 1 mM cocaine in order to check the encapsulating stability of the hydrogel. The 0.1 mM hydrogel showed the fastest response, but the lowest encapsulating stability. The 0.3 mM and 0.5 mM hydrogels gave a similar response; the 0.5 mM hydrogel had a lower background, as well as somewhat slower kinetics. As for the 0.7 mM hydrogel, the response was much slower and did not reach equilibrium during the monitoring period. The quantitative results indicate a 3.7 times signal-to-background difference for the 0.1 mM hydrogel, 8.1 times for the 0.3 mM hydrogel, 11 times for the 0.5 mM hydrogel, and 7.7 times for the 0.7 mM hydrogel. In particular, if the readable signal was set to be three times higher than the background signal, it took less than 10 min for all these four types of gel to reach their three-times signal-to-background difference, which indicated fast detection. Figure 2a shows photographs taken 30 min after introducing 1 mM cocaine, when the reactions were almost completed. The tubes on the left are the control experiments under the same working conditions without cocaine. By correlating with the spectrometric data, leaking is a problem for the 0.1 mM

hydrogel, and the 0.7 mm hydrogel has a slower reaction rate. In contrast, the 0.3 mm and 0.5 mm hydrogels gave the best results. This difference among four hydrogels clearly demonstrated the concentration-dependent encapsulation and release capability upon target binding. That is, low-concentration cross-linking hydrogels tend to dissolve much faster and easier than high-concentration cross-linking hydrogels, but have a stability problem. On the other hand, high-concentration cross-linking hydrogels might have slower kinetics for the gel-sol transition, and thus prolong the detection time. As a consequence, the optimal condition was determined empirically to be the 0.5 mm DNA cross-linker concentration, which was used in the next step. The AuNP model also suggested that nanoparticles or molecules with dimensions of approximately 10 nm can be doped inside the hydrogel and then released.

As a further step, we attempted to introduce an enzyme into the gel system. A common test for amylose is to mix it with a small amount of iodine solution. The amylose induces a color change from yellow to dark blue. On the other hand, amylase can break amylose down into sugar, which is colorless in the presence of iodine. Even though these two phenomena are well known, they have not, to the best of our knowledge, been combined into a colorimetric sensing platform. Therefore, we chose the amylose- I_2 -amylase system because of the specificity of its color change, the fact that no toxic reagents are involved, and the simplicity and cost-effectiveness of its operation. More importantly, both amylose and amylase are large polymers with high molecular weight. As a result, they can be separated physically by the hydrogel, with amylase trapped inside the gel and amylose outside the gel. Therefore, no amylose is digested by amylase unless the enzyme is released as a result of gel dissolution upon target recognition. However, once the target dissolves a certain area of the hydrogel and releases enough amylase, the color change would be sufficiently distinguishable to draw a clinically sound conclusion, even though the whole gel is not completely dissolved. Hence, the use of enzymes for signal amplification and colorimetric reaction delivers a method for visual detection with high sensitivity. Because the complex formed between amylose and I_2 might affect the enzyme function, I_2 solution was introduced 10 min later as the last step in order to evaluate the results of the reaction.

Similar to the trapping procedure for AuNPs, an amylase-caged hydrogel was prepared by adding linker-Apt into a well-mixed solution containing PS-A, PS-B, and amylase. The loading capacity of amylase for hydrogels was found to be as high as 2 μ g per 10 μ L gel. After introduction of linker-Apt, a homogeneous colorless hydrogel formed with evenly dispersed enzyme trapped inside. No change of catalytic activity of the enzyme was observed after trapping, thus suggesting that the trapping process is very mild.

The enzyme-hydrogel response to cocaine was investigated by visually observing the reaction in an Eppendorf tube (Figure 3a). Several tubes were prepared: In tubes 1 and 2, no amylase was trapped in the gel. Tube 1 had gel on the bottom and a blue solution of the amylose- I_2 complex on top. No color change or gel dissolution was observed. 1 mM cocaine was introduced into tube 2, and the gel was totally dissolved.

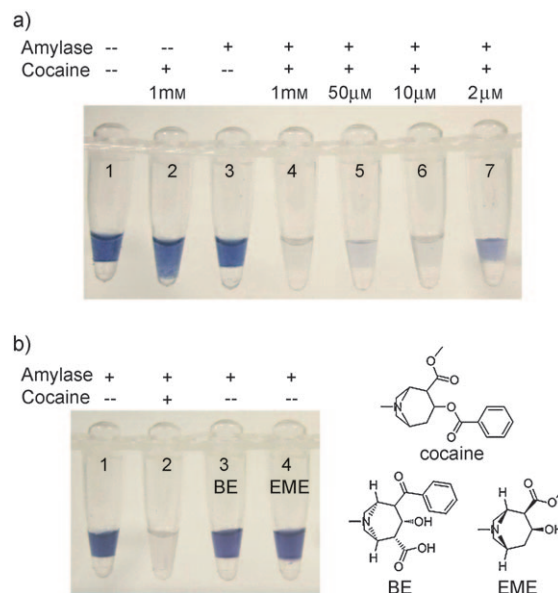


Figure 3. Photograph of gels with enzymatic reaction for visual detection of cocaine, I_2 solution was always introduced 10 min later as the last step to evaluate the results of the reaction. a) Gel response to different amounts of cocaine. b) Control tests for two cocaine analogues, benzoylecgonine (BE) and ecgonine methyl ester (EME).

Since no enzyme was trapped, only a homologous blue solution was obtained. The gel in tube 3 was preloaded with amylase. However, without the target, tube 3 behaved in a manner similar to tube 1, where amylase and amylose blue solutions were well separated by the gel. Then, different amounts of cocaine were introduced into the upper solution of tubes 4–7. In tube 4 with cocaine, the gel dissolved and the solution was colorless. In tubes 5 and 6, a much smaller amount of cocaine was added, which was not enough to completely dissolve the gel, and the solution was colorless after introduction of I_2 . This result occurred because the gel partially dissolved and released enough enzyme to hydrolyze the amylose. In this regard, even 10 μ M cocaine, which was only 100 ng in our experimental conditions, could be detected directly with the naked eye. We also tried to lower the cocaine concentration to 2 μ M in tube 7. Although the blue color did not fade completely, it could still be distinguished from tube 3. From the comparison of tubes 1–7, we demonstrated how the introduction of an enzyme reaction into this system amplifies the signal and enables the direct detection of lower amounts of target with the naked eye, thus improving the overall sensitivity of this visual detection method.

It has been reported that two cocaine metabolites, benzoylecgonine (BE) and ecgonine methyl ester (EME) have no affinity for the cocaine aptamer,^[17] and should therefore not cause hydrogel dissolution. We then used these two metabolites as negative controls. Our results indicated that even at a concentration of 1 mM, neither benzoylecgonine (BE) nor ecgonine methyl ester (EME) caused gel dissolution or color fading (Figure 3b), thus confirming that the gel-sol transition and enzymatic reaction were indeed triggered by cocaine-aptamer recognition. It should be noted that this aptamer sequence has been found to bind with

steroids^[20] and quinine.^[21] To use the sensor developed for cocaine detection, one should consider the potential false positive signal caused by these interferences. An aptamer with better selectivity is thus much desirable.

In conclusion, we have demonstrated the general design for a colorimetric visual detection platform based on an aptamer cross-linked hydrogel. Competitive binding of the target to the aptamer causes the reduction of cross-linking density and therefore induces gel dissolution. We were able to use this simple system to detect less than 20 ng of cocaine with the naked eye within 10 min. This result is comparable to the most sensitive methods^[18] reported to date, but can be achieved without the aid of sophisticated instrumentation. As no special features on the aptamers are required, our technique might be a generic approach that can be applied with different aptamer sequences for the detection of other molecules. Since the hydrogel is convenient for either micro- or nanopatterning, this colorimetric visual detection platform can be further developed into lab-on-a-chip devices for diversified applications, such as forensic analysis, medical diagnostics, and environmental monitoring.

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