Electronic Supplementary Information (ESI)

Aptamer-functionalized Hydrogel Diffraction Grating for the Human Thrombin Detection

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1. Materials

The epoxy-based azo polymer (BP-AZ-CA) was synthesized in this laboratory. The preparation and characterization details of BP-AZ-CA have been given in our previous paper.[1] The PDMS prepolymer (Sylgard 184) and the curing agent were purchased from Dow Corning. Acrylamide (electrophoresis grade, 99%) was purchased from Acros Organics, *N*, *N*-methylene-bisacrylamide (MBAA), 3-(methacryloyloxy)propyltrimethoxysilane (97%) were purchased from Alfa Aesar. 2-Hydroxy-4'-(2-hydroxyethoxy)-2-methylpropiophenone was purchased from Energy Chemical. *N*-succinimidylacrylate (NSA) was synthesized in this laboratory. The preparation and characterization details of NSA have been given in our previous paper.[2] Deionized water (resistivity > 18 MΩ·cm^{-1}) was obtained from a Millipore water purification system. The aptamer (A: NH_2-$(CH_2)_6$-5'-AC'-TGT'GGT'TGG' TGT'GGT'TGG-3') and its complementary sequence (B: 3'-TG'ACA'CCA' ACC'A-5'-$(CH_2)_6$-NH_2) were obtained from Invtrogen Company with 5'-Aminolinker-C6 modified. The human thrombin was purchased from HTI Company. EDTA-2Na, $MgCl_2$, Tris and other chemicals and solvents were commercially purchased and used without further purification.

2. Preparation of masters and stamps

The masters and stamps were the soft-lithographic processing tools used for preparing the hydrogel gratings (Fig. S1). The masters were prepared by inscribing surface-relief-gratings (SRGs) on films of the epoxy-based azo polymer (BP-AZ-CA) through irradiation with interfering Ar^+ laser beams. In the process, the BP-AZ-CA films were obtained by spin-coating the polymer solution in DMF (10 wt%) on glass slides and dried under the vacuum condition for 48 h. SRGs were inscribed by exposing the films to interfering Ar^+ laser beams (488 nm, 80 mW/cm^2). A typical AFM image of SRG is given in Fig. S2. The periods and trough depths of the gratings were controlled by adjusting the intersection angle and irradiation time (5–20 min). PDMS prepolymer was prepared by mixing the elastomer base and curing agent (Sylgard 184, Dow Corning) in a ratio of 10:1 (weight/weight). PDMS stamps were

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obtained by casting the prepolymer against the masters and curing in the molds at 60 °C for 4 h.

3. Preparation of the human thrombin-responsive Gratings

The gratings were fabricated through microcontact printing by using the PDMS stamps and the precursor solution of the hydrogel as "ink" (Fig. S1). To obtain the precursor solution, the aptamer and its complementary sequence were chemically modified by reaction with N-succinimidylacrylate (NSA) in a TE (10 mM Tris-HCl 1 mM EDTA-2Na, pH = 8.0) solution. After purification, the resulted vinyl-aptamer (A) and vinyl-complementary sequence (B) were used as monomers to be copolymerized with acrylamide. The pre-gel solution was prepared by adding monomer acrylamide (500 mg, 14 M), cross-linker *N*, *N*-methylenebisacrylamide (10 mg, 0.13 M), photoinitiator 2-hydroxy-4'-(2-hydroxyethoxy)-2-methylpropiophenone (5 mg, 0.04 M) into modified aptamer (A) and complementary sequence (B) solution containing A 15.5 μM and B 24.8 μM in TE (500 μL). After mixing, the pre-gel solution used as the "ink" was dropped on the glass slides. The PDMS stamps were pressed on the "inked" glass slide surfaces in a conformal contact manner with a moderate pressure (such as 50 kPa). The curing was carried out under UV lamp (20 mW/cm^2) irradiation. After carefully peeling off the stamps, the gratings of the hydrogel with good optical transparency were obtained. The gratings were kept in the TE solution at the room temperature before the measurements.

The concentrations of aptamers and complementary sequences in hydrogel mentioned above can be adjusted for different analyte concentration ranges. Decreasing the detection minimum limit of the analyte is an important goal in the hydrogel preparation rationale. Moreover, a proper density of crosslinking points in the hydrogel network can show the most sensitive response to analyte in solution. As the oligonucleotides mainly perform the recognition function, the concentration should be adjusted according to the analyte concentration. When keeping the same crosslinking density, decreasing the concentration of the aptamers and complementary sequences in hydrogel preparation did not show effect to significantly decrease the minimum detection limit. To further decrease the detection limit for the analyte, both adjusting the cross-linking density and optimizing the grating parameter should be required. On the other hand, a higher concentration of aptamers and complementary sequences in the hydrogel is not always necessary, as the typical detection concentration range is below several μM magnitudes.

4. Diffraction efficiency measurement

In the experiments, the responsive gratings were immersed in the test solutions with different concentrations in a sample cell. The medium used for test solutions was a TE (10 mM Tris-HCl 1 mM EDTA-2Na, pH = 8.0) solution, which was the same as those used for preparation and storage of hydrogels. A beam from He–Ne laser

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(LDM635, 635 nm, 4.5 mW Red Laser Module) was employed as the incident light and two silicon photodiodes (Thorlabs, DET100A, High-Speed Si Detector, 400–1 100 nm, 45 ns Rise Time) were worked as the light intensity detectors. The photodiodes were fixed on the optical stages for fine adjustment of their positions. The generated current was converted into voltage by the resistors connected to the photodiodes, and then fed into Preamp Module (NT57-988). The amplified signals were finally transmitted to a computer and processed with Labview Workstation (NT56-825, Data Acquisition System Package). The signals were detected in a real-time manner under an air-ambient condition. The diffraction efficiency was obtained from the intensity ratios of the first-order and zero-order diffraction beams.

5. Relationship between the grating parameters and diffraction efficiency

The diffraction gratings can be divided into the thin gratings and thick gratings which depending on the ratio of depth to period. The gratings used in the experiments are sinusoidal thin gratings. The diffraction efficiency can be approximately estimated by using the formula below:

$$\eta(\lambda) = \exp[-\frac{2.3D(\lambda)}{\cos\theta}](\frac{\pi d}{\lambda\cos\theta})^2\{[\Delta k(\lambda)]^2 + [\Delta n(\lambda)]^2\}$$

where $D(\lambda) = 4\pi dk/2.3\lambda$ is optical density, d is the trough depth of the grating, Δk and Δn are k and n (imaginary and real parts of the refractive index) of gratings relative to surrounding media, θ is the Bragg angle.[3] In the above experiments, the parameters of Δk, Δn, and θ were approximated constants at the same wavelength λ. Therefore, the diffraction should increase with the trough depth (d) increase.

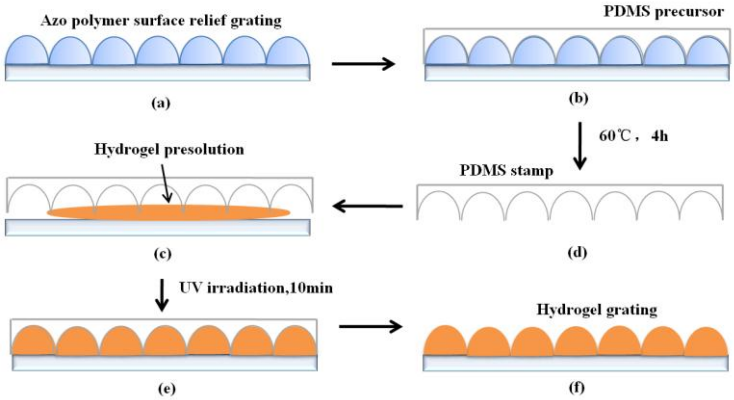

Fig. S1 The schematic diagram of the preparation of the hydrogel gratings.

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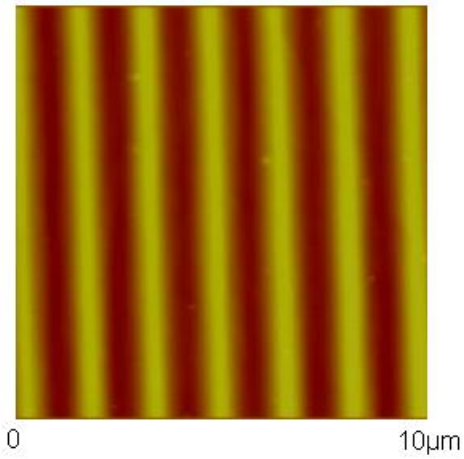

Fig. S2 AFM image of the azo polymer surface-relief-grating (10 μm × 10 μm).

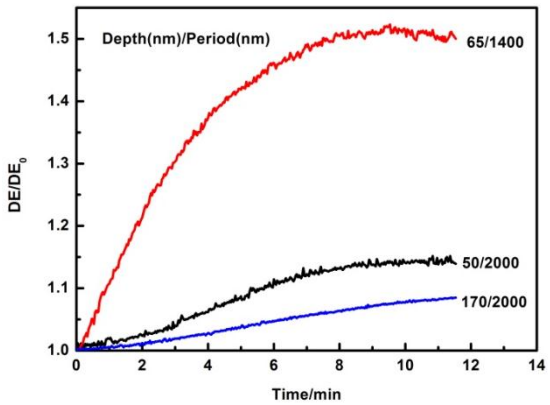

Fig. S3 The DE/DE_0 responses of the SRG hydrogel gratings with different trough depths and periods. The figure shows that when the periods are the same (2000 nm), the grating with the trough depth of 50 shows the higher DE/DE_0. Compared with the grating with the period of 2000 nm, the grating with the period of 1400 μm shows a significantly higher DE/DE_0. The analyte concentrations were 0.2 mg/mL.

Reference

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