

[*Electronic Supplementary Information (ESI)*]

**Diffraction grating of hydrogel functionalized with glucose oxidase
for glucose detection**

*Gang Ye, Xinyang Li, Xiaogong Wang**

Department of Chemical Engineering, Laboratory for Advanced Materials

Tsinghua University, Beijing, 100084, P. R. China

Materials. The PDMS prepolymer (Sylgard 184) and the silane-coupling agent 3-methacryloxy-propyltrimethoxysilane (Z603) were purchased from Dow Corning. The photoinitiator Esacure KIPEM@ was purchased from Satomer Company. Glucose Oxidase (EC 1.1.3.4 from *Aspergillus niger*) with a specific activity of 291.0 U/mg of lyophilized solid was purchased from CALBIOCHEM. Anhydrous β -D(+)-glucose was purchased from Fluka. Horseradish peroxidase (HRP) (EC 1.11.1.7, Type II, 220 purpurogallin units/mg solid) was purchased from Sigma. The *o*-dianisidine was purchased from Alfa Aesar. Deionized water (resistivity > 18 M Ω) was obtained from a Millipore water purification system. Other chemicals and solvents were commercially purchased and used without further purification. A phosphate buffer solution of pH 7.0 was prepared by mixing 5 mmol/L NaH₂PO₄ (aq.) and 5 mmol/L sodium citrate. The glass slides were ultrasonically treated in deionized water for 10 min, rinsed with acetone and blown dry with air before use.

Fabrication of masters and stamps. The grating preparation is characterized by using surface-patterned azo-polymer films as masters and replicated elastomeric stamps as major processing tools. The masters were prepared by inscribing surface-relief-gratings (SRGs) on films of an epoxy-based azo polymer (BP-AZ-CA) through interfering Ar⁺ laser beams irradiation (488 nm, 80 mW/cm²) (Y. N. He

* Corresponding author. e-mail: wxg-dce@mail.tsinghua.edu.cn

et al., *Polymer*, 2002, **43**, 7325-7333.). The writing beam was split by a mirror such that a half of the beam reflected onto the film surface was coincident with the other half beam to form an interference pattern. The grating depths were controlled by the irradiation time. The profile spacing (d) of the gratings (grating period) was adjusted according to the following equation

$$d = \lambda / (2 \sin \theta) \quad (1)$$

Where λ is the wavelength of the writing beam, and θ is the angle between the beam propagation axis and the mirror plane. The PDMS stamps with replicated SRGs on the surfaces were prepared by replica molding against the masters. The liquid prepolymer of PDMS was prepared by mixing the elastomer base and curing agent (Sylgard 184, Dow Corning) in a proper ratio (10:1, wt/wt). The cast molds containing the prepolymer were cured in a 60 °C oven for 4 h.

Fabrication of hydrogel gratings. Fabricating hydrogel gratings on the hydrogel surfaces is a process combining the photo-initiated free radical polymerization and soft-lithographic approach. The glass substrates were firstly spin-coated with a layer of silane-coupling agent 3-methacryloxypropyl-trimethoxysilane (Z603) and reaction was carried out at 40 °C for 10 h (A. G. Mayes et al., *Anal. Chem.* 1999, 71, 3390-3396.). The pre-gel solution was prepared by dissolving 2.0 g acrylic acid, 0.04 g *N, N'*-methlenebisacrylamide, and 0.02 g Esacure KIPEM@ in 10.0 g deionized water. Ultrasonic treatment was performed to make sure the precursor solution uniformly mixed. The solution was used as “ink” and dropped on a piece of the substrates. The stamp was pressed on the “inked” substrate in a conformal contact manner. After illumination under high-pressure mercury lamp (50 mW/cm²) for 15 min, the stamp was carefully peeled off and the hydrogel grating was obtained.

Immobilization of glucose oxidase (GOx). Hyperbranched diazonium salt (HB-DAS) was synthesized through the azo-coupling reaction of *N, N*-bis[2-(4-aminobenzoyloxy)ethyl]aniline in dimethylformamide (DMF) at 0 °C (P. C. Che et al., *Chem. Lett.*, 2004, **33**, 22-23.). Before use, 100 μL HB-DAS solution was diluted with 10 mL buffered solution, followed by dialysis to exclude the acid

molecules introduced during the preparation of the HB-DAS. The fabricated hydrogel gratings were dipped into the solution for 10 minutes to adsorb a HB-DAS layer. After washed with deionized water for several times and dried with air stream, the HB-DAS modified hydrogel gratings were then immersed into the GOx solution (prepared by dissolving 2.5 mg GOx into 5 mL buffered solution). After 10 minutes, the hydrogel gratings were washed repeatedly with neutral phosphate buffer solution, and then kept in the solution at 4 °C in a refrigerator before using.

Immobilized enzyme assay. Activity study of the immobilized GOx was performed using horseradish peroxidase (HRP) coupled assay by UV-visible spectroscopy (H. U. Bergmeyer et al., *Methods of Enzymatic Analysis*, Academic Press Inc., New York, 1974, pp. 457–458.). The following reagents were used for the conduct of the enzyme assay: (A) 0.05 M phosphate buffer at 25 °C, pH = 7.0 adjusted with 0.05 mol/L sodium citrate; (B) 0.27 mmol/L *o*-dianisidine in reagent A; (C) HRP solution containing 60 purpurogallin units/mL in cold deionized water; (D) 0.1 g/mL β -D-glucose in deionized water. The reactions were carried out by mixing 0.5 mL of freshly prepared D, 0.1 mL C, and 2.4 mL B in a colorimetric ware, maintaining the mixture at 25 °C for 10 min. Then a piece of the hydrogel gratings immobilized with GOx was dipped in the mixture solution, followed by recording the increase of the absorbance at 436 nm in every 1 minute over a period of 4 minutes. One enzyme unit is defined as the amount of the enzyme required to increase 0.001 of the absorbance at 436 nm per minute on 1 cm² of the hydrogel grating. Based on such definition, about 65 units of the GOx were immobilized onto the surface of the hydrogel grating.

Optical setup for diffraction efficiency measurement. The optical setup to monitor the diffraction efficiency is illustrated in Figure S1. A He-Ne laser beam (LDM635, 635 nm, 4.5 mW Red Laser Module) was passed through an aperture which made the beam diameter adjustable. The beam was perpendicularly irradiated on the hydrogel grating immersed in the sample cell. The diffracted lights then transmitted out of the cell and were collected by two silicon photodiodes (Thorlabs, DET100A,

High-Speed Si Detector, 400-1100 nm, 45 ns Rise Time). The photodiodes, affixed with band-pass filters, were fixed on translational stages for fine adjustment. The output currents were converted into voltages by the resistors connected to the photodiodes, and then fed into low-pass amplifiers (NT57-988, PREAMP MODULE). The amplified signals finally were transmitted to a computer and processed with Labview Workstation (NT56-825, DATA ACQUISITION SYSTEM PACKAGE). So, when there are chemical stimulations happened on the hydrogel gratings in the cell, the real-time responses can be detected by monitoring the first-order diffraction efficiency (DE).

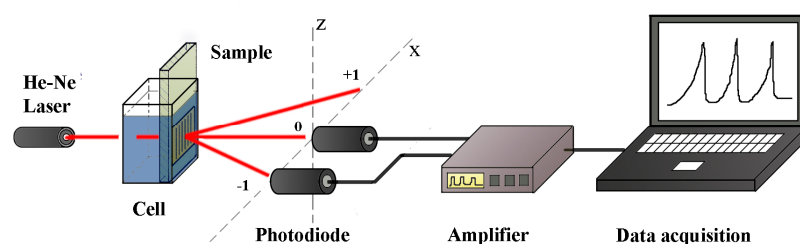
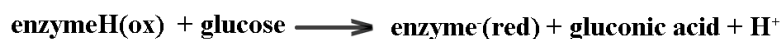


Figure S1 Illustration of the setup for the diffraction efficiency measurement

Mechanism. The diffraction grating biosensors work on basis of the enzymatic reaction of GOx. For the hydrogel gratings, the immobilized GOxs by the HB-DAS act as the direct receptors of the glucose molecules in the solution. Glucose oxidase converts β -D-glucose to gluconic acid in a two-step process. In the first step, glucose is converted to gluconic acid, and the enzyme is reduced. Then, the enzyme is reconverted to its oxidized form by oxygen in the solution, producing H_2O_2 as a byproduct:



The gluconic acid and the ionic species generated during the enzymatic reaction then affect on the hydrogel gratings. With ionizable weak acidic side-groups, poly(acrylic acid) hydrogel shows the volume shrinking when exposed to the solutions with low pH or higher ionic concentrations. The shrinking process does not stop until that the elastic force inside the hydrogel is in equilibrium with the

osmotic force difference between the gel and the solution. The volume shrinking of the hydrogel will cause the decrease of trough depth of the grating and afford a measurable modulation of the diffraction efficiency (DE) for the glucose sensing. Because of the mechanism, the glucose detection needs to be carried out in the dilute PBS (such as 5 mmol/L). Increasing the electrolyte amount in PBS showed the effect to decrease the sensitivity and the responsive rate (Figure S2). The osmotic pressure resulted by the enzymatic reaction is diminished in the presence of the substantial amount of electrolyte.

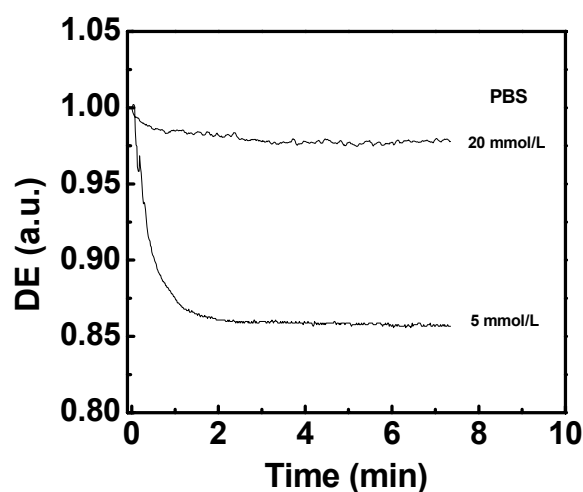


Figure S2 The diffraction efficiency variations of the hydrogel gratings in 0.75 mmol/L glucose PBS solutions with different electrolyte concentrations