Supporting Information

Precursor preparation

The micellar solution was prepared by adding appropriate amount of Tween-80 and ML in deionized (DI) water and mixing at room temperature for 24 h, then leaving the solution at rest for 2 days to ensure equilibrium before experiments. Enzyme stock solutions were prepared in 5 mL of sodium acetate buffer (0.05 M, pH 6.5) in DI-water. Glucose solutions (1–30 mM) were prepared from a pure substrate, dissolved in distilled water and equilibrated at room temperature before the experiments for mutarotation. We prepared the precursor solution by mixing Tween-80, ML, DI-water with GOx in the buffer solution (sodium acetate) for 2 hours at room temperature. The final concentration of GOx in the precursor solution was 1 U/μL.

Microdevice design

The micro-devices were fabricated using standard soft lithography techniques [1]. The synthesis of the nanogel encapsulated with GOx (nanogel-GOx) requires a continuous flow of the precursor pumped through the microchannel. Figure 1 below illustrates detailed design of the microchannel. In the microfluidic device, we pump 2 counter streams of precursor solutions from the left/right sides to the center of the microchannel. After the precursor each passes through the micropost array, the nanogel-GOx forms due to the spatial confinement and high strain rates and high strain introduced by the microfluidic flow (see video submitted as the supporting information). Meanwhile, a stagnation point in the center of the device can facilitate the maximum amount of nanogel-GOx collection and is connected with an outlet tubing. The nanogel-GOx can then be easily collected and stored in DI-water at room temperature for further experiments. This procedure avoids jamming and blockage in the microchannel. The microfluidic device can be used multiple times (up to 20 times). If the microchannel gets jammed due to multiple usage or the accidental debris accumulations during the experiment, new devices will be used.

Sample collection for material characterizations

Since the conversion rate from the precursor to the nanogel-GOx is not 100% from the microfluidic process, we collected the sample downstream containing nanogel-GOx, liquid precursor, and un-encapsulated GOx and

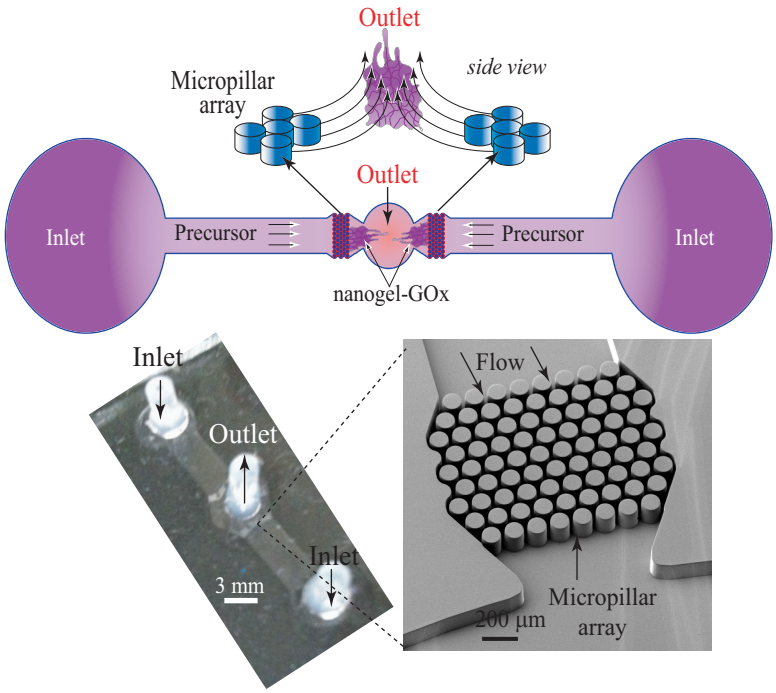

Figure 1: Schematics of the flow configuration to form nanogel-GOx material. Two counter streams of precursor solutions would each pass a set of micropost array and form nanogel-GOx. Meanwhile a stagnation point in the center of the device can facilitate the maximum amount of nanogel-GOx collection and is connected with an outlet tubing. The nanogel-GOx material is then collected and stored (at room temperature) in DI-water for further experiments.

added 20 mL of DI-water. We then centrifuged this diluted solution at 1500 RPM for 30 minutes and left the centrifuged solution at rest for 2 days, until a thin layer containing the concentrated nanogel-GOx scaffold was formed at the top of the stored solution.

Cryo-EM sample preparation

The plunging-freeze method was used for the cryo-sample preparation. Cryo-EM images were collected at liquid nitrogen temperature on a Tecnai G2 F20 transmission electron microscope (FEI Co., Hillsboro, OR) operated at 200 kV and equipped with a field emission gun.

Experimental setup to for chronoamperometry response

After collecting the sample containing nanogel-GOx scaffolds, we loaded 5 μL of the sample onto a home-made gold electrode with 5 μm in gap size (see Figure 2 below).

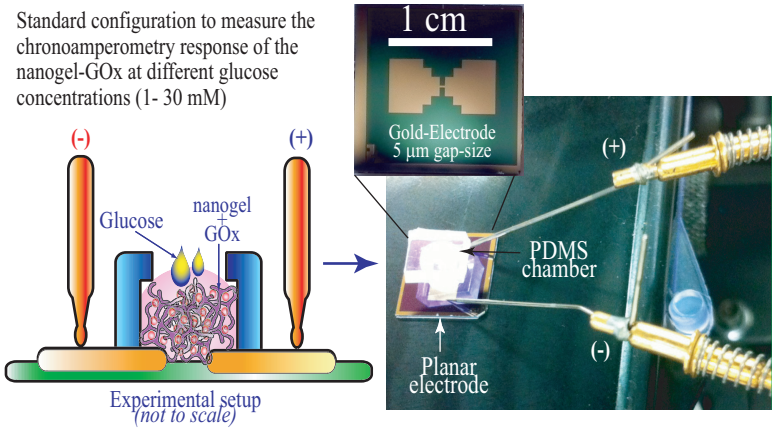

Figure 2: Experimental setup to measure the chronoamperometry response of the nanogel-GOx material at different glucose concentrations (1–30 mM). The nanogel-GOx material is loaded onto a planar gold electrode, then glucose (at different concentrations) is loaded onto the nanogel-GOx material to measure the chornoamerometry behavior of the nanogel-GOx for glucose sensing purposes. The experiments were conducted at room temperature.

Rational of glucose concentration range

We chose 1–30 mM glucose concentration range because of its relevance to biomedical and industrial applications. For example, metabolic disorders resulting from insulin deficiency and hyperglycemia exhibit higher blood glucose concentrations. The normal range of blood glucose concentration lie in 3.9 mM–6.2 mM (empty stomach), and 3.9 mM–7.8 mM (2 hours after food) [2–4]. The detection of glucose in industrial applications (e.g., food processing) can range from 15 mM to 50 mM.

References

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