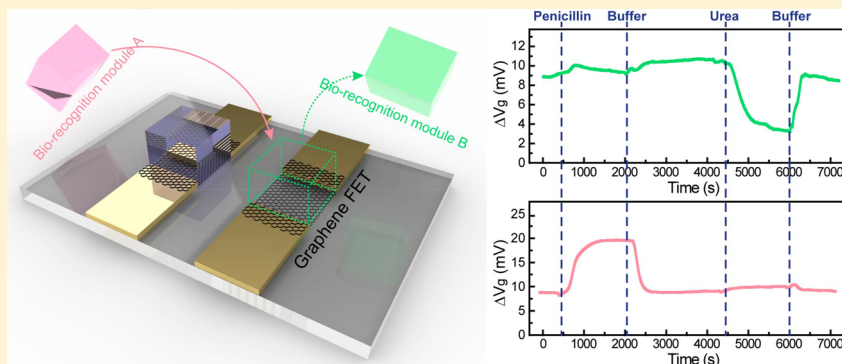


Modularized Field-Effect Transistor Biosensors

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S Supporting Information



ABSTRACT: Field-effect transistors (FETs), when functionalized with proper biorecognition elements (such as antibodies or enzymes), represent a unique platform for real-time, specific, label-free transduction of biochemical signals. However, direct immobilization of biorecognition molecules on FETs imposes limitations on reprogrammability, sensor regeneration, and robust device handling. Here we demonstrate a modularized design of FET biosensors with separate biorecognition and transducer modules, which are capable of reversible assembly and disassembly. In particular, hydrogel “stamps” immobilizing bioreceptors have been chosen to build biorecognition modules to reliably interface with FET transducers structurally and functionally. Successful detection of penicillin down to 0.25 mM has been achieved with a penicillinase-encoded hydrogel module, demonstrating effective signal transduction across the hybrid interface. Moreover, sequential integration of urease- and penicillinase-encoded modules on the same FET device allows us to reprogram the sensing modality without cross-contamination. In addition to independent bioreceptor encoding, the modular design also fosters sophisticated control of sensing kinetics by modulating the physiochemical microenvironment in the biorecognition modules. Specifically, the distinction in hydrogel porosity between polyethylene glycol and gelatin enables controlled access and detection of larger molecules, such as poly-L-lysine (MW 150–300 kDa), only through the gelatin module. Biorecognition modules with standardized interface designs have also been exploited to comply with additive mass fabrication by 3D printing, demonstrating potential for low cost, ease of storage, multiplexing, and great customizability for personalized biosensor production. This generic concept presents a unique integration strategy for modularized bioelectronics and could broadly impact hybrid device development.

KEYWORDS: Bioelectronics, hydrogel-gate, modular design, programmable, customizable, mass production

Rapid progress in biosensor development has led to many technological advances in healthcare research and has impacted important clinical applications.^{1,2} Field-effect-transistor (FET)-based biosensors represent a unique class of analytical tools for label-free specific detection of bioanalytes with unprecedented sensitivity,^{3–6} fast response time,^{7,8} potential for miniaturization and multiplexing,^{3,9} and facile integration with developed semiconductor electronics technologies.^{4,10} Specific sensing with an FET biosensor involves structural and functional integration of biorecognition molecules, such as enzymes, antibodies, aptamers, and so on, with FETs, where the selective interaction between bioanalytes and bioreceptors could result in biophysical or biochemical changes that can be further transduced and amplified via a field effect toward external signal readouts.^{3,6,10,11} For example, enzyme-functionalized FET biosensors can react with specific

substrates, where the enzymatic transformation leads to modulation of electric potential at the FET channel/electrolyte interface and induces a change of the charge carrier density and the conductance of FET devices as readouts in real time.^{10,12} Similarly, an antibody-functionalized FET biosensor may specifically bind to the corresponding antigen molecules. Because most biomolecules carry electrostatic charges (or can carry electrostatic charges at suitable pH), such binding events will also lead to local electric potential change at the surface of FET channels.^{3,6,11,13}

However, the biorecognition elements are commonly immobilized directly in close proximity on the FET channel/

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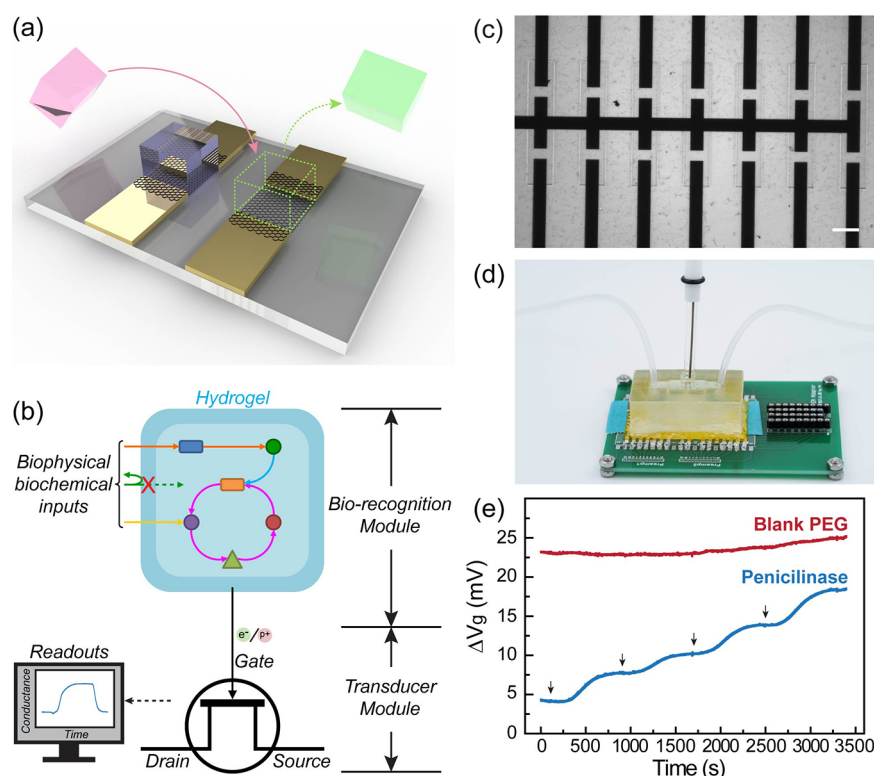


Figure 1. Modularized FET biosensor. (a) Schematic representation of modularized graphene FET biosensor with reversibly assembled and disassembled biorecognition modules (pink, green and blue cubes). Gold, electrode interconnects for the graphene FET devices. Black, graphene channels. (b) Diagram showing stitched two-step signal transduction in the biorecognition module and the FET transducer module, respectively, enabled by modularized FET biosensors. Squares, triangles, circles, and colored arrows conceptually denote biologically relevant processes undergoing in hydrogel biorecognition module. (c) Optical image of graphene FET arrays protected with S1813 photoresist, which will be removed with acetone before being used for sensing. Dark lines are evaporated Cr/Au interconnects. Scale bar, 200 μm . (d) Photograph of the device chip. Cr/Au interconnects are connected to printed circuit board with silver paste and then wired to the data acquisition systems through receptacle connector arrays. A 3D-printed fluidic channel is aligned and attached onto the center of the device chip. Silicone inlet and outlet tubing are inserted. Ag/AgCl electrode was placed into the channel as reference electrode. (e) Real-time penicillin sensing. Blue and red traces represent recording from FET biosensors integrated with penicillinase-encoded receptor module and blank hydrogel module, respectively. Arrows depict the time points chronologically of solution switching to phosphate buffer with 0.25, 0.5, 1.0, and 2.0 mM penicillin.

electrolyte interface via physical absorption or chemical conjugation for efficient signal coupling.^{3,13–15} This is especially critical for detection in physiologically relevant environments where the chemical “outputs” from enzymatic reactions will be quickly diluted and/or neutralized, while the electric field generated by charged analytes will also diminish at distance of nanometer scale as a result of enhanced electrostatic screening in high ionic strength solutions, known as Debye screening.^{15,16} Such demanded structural intimacy between biorecognition and FET elements in traditional FET biosensors has largely limited the design versatility and translational applications. For example, reprogramming the sensing specificity to detect different target molecules is often difficult or requires complicated and time-consuming steps after the initial functionalization. Additionally, after sensing tests are performed, it lacks versatile methods to regenerate the sensing capability of the receptors without compromising the functionality of the bioreceptor¹⁷ especially ones that irreversibly bind to target with a high affinity coefficient. Moreover, the bioreceptor functionalization process must be compatible with postfabricated FETs and the lack of in-vitro stability of bioreceptors limits the lifetime of the hybrid device.¹⁸ A modular design of FET biosensors, consisting of separate electronic and biorecognition elements capable of reversible assembly and disassembly, has the

potential to overcome these limitations (Figure 1a), yet challenges remain to formulate an intermodule interface that can simultaneously allow effective signal coupling when integrated and the absence of cross-contamination once disassembled/reassembled.

To this end, the design and construction of biorecognition module needs to fulfill the following criteria: (1) the biorecognition module has to structurally support the robust immobilization and handling of bioreceptors; (2) the biorecognition module should allow open access of biochemical “inputs” (i.e., the analyte molecules); (3) the “outputs” from biorecognition modules need to be effectively coupled to and reliably transduced by the FET module. A functional hydrogel represents the ideal backbone material for the biorecognition modules to enable seamless structural and functional integration with FET transducers. By creating a confined microenvironment with tunable diffusion barriers, it offers controlled access to biochemical “inputs” and highly localized enrichment/amplification of biochemical “outputs” before they are diluted/neutralized by the buffer (Figure 1a). It has also been demonstrated to modulate the local dielectrics to mitigate Debye screening, thus enabling charged-based sensing in high-ionic-strength solutions.¹² These amplified biochemical and/or charge signals are critically important to the reliable signal transduction across the modular interface (Figure 1b). It

is worth noting that the biorecognition module can grant the possibility for modulating selective accessibility or controlled diffusion dynamics of specific “input” through porosity engineering as well as rationally designed matrices properties. Additionally, the excellent structural integrity and biocompatibility of hydrogel matrices allow the biorecognition modules to be independently designed, manufactured, and preserved with significantly improved scalability and shelf life.^{12,18} The unitary design of the biorecognition module may allow for more complex biorecognition modules with spatial organization and integration over multiple biological processes, such as enzymatic cascades for intricate biosensing. Through reversible assembly and disassembly of diverse bioactive hydrogel modules with FET modules, the two-step tandem signal transduction can be easily connected and disconnected on demand to achieve reprogrammable sensing capabilities.

For proof-of-concept demonstration, graphene was chosen as the channel material for fabricating the FET modules. Specifically, single-layer graphene synthesized on the copper foil catalyst via chemical vapor deposition was transferred onto photolithographically patterned gold electrodes and configured as FET channels (Figure 1c).¹² Bioactive enzymes, such as penicillinase and urease, are PEGylated and copolymerized with polyethylene glycol diacrylate (PEGDA)¹⁹ to create hydrogel “stamps” as biorecognition modules. After mounting the FET sensing chip onto the printed circuit board with receptacle connectors, a 3D-printed chamber with configured fluidic channel was aligned and mounted on the FET sensing chip (Figure 1d).²⁰ Inlet/outlet tubing and Ag/AgCl reference electrode were then connected onto the fluidic channel for delivery of analyte solutions and buffers as well as applying the electrolyte gate, respectively (Figure 1d).²⁰ The encoded hydrogels “stamps” (biorecognition module) were placed in direct contact with the graphene channels (transducer module) to complete the assembly of FET biosensors with the respective sensing modalities. Following the connection of the FET sensing chip to the data acquisition system, real-time electrical characterization of FET biosensor and the sensing experiments were performed by continuously recording the conductance of individual FET devices while introducing analyte solutions or buffers in desirable sequence via the inset/outlet tubes. Figure S1 shows a representative conductance variation as a function of electrolyte-gate voltage for graphene FET, with clear bipolar transconductance on each side from Dirac point.^{13,21,22}

To investigate the effectiveness of signal coupling and transduction across the separate modules, we have demonstrated the detection of penicillin G using penicillinase-encoded hydrogel as the biorecognition module. Penicillinase catalyzes the hydrolysis of penicillin into penicilloic acid, releases protons, and therein leads to a decrease of the local pH, which is then transduced and detected as a decrease in conductance via field effect.¹⁰ On the basis of the electrolyte-gate measurement of the graphene FET (Figure S1), the sensing signal amplitude was calculated by dividing the conductance change with the transconductance value at V_g of the sensing tests. Figure 1e shows the real-time recording of signal amplitude while sequentially switching the 5 mM phosphate buffer with various concentration of penicillin G from 0.25 mM to 0.5 mM, 1.0 mM and 2.0 mM,²³ demonstrating the concentration-dependent detection (Figure 1e, blue curve). The detection amplitude and limit of detection (Figure S2) are consistent with the hydrogel-gate FET

biosensor where the hydrogel is directly photopolymerized on top of graphene FETs, presenting the uncompromised signal transduction across two modules.¹² In clear comparison, stable conductance was recorded from another FET biosensor assembled with a blank PEG hydrogel module as a control experiment (Figure 1e, red curve), demonstrating the signal coupling is well-confined within the hydrogel biorecognition modules which is critical for potential multiplexing biosensors.

The modularization of FET biosensor also allows us to freely connect and disconnect the signal coupling between the biorecognition and transducer modules and, as a result, switch the sensing modalities of the same FET transducer. As a proof of the concept, we choose urease-encoded biorecognition module in addition to penicillinase to demonstrate the capability of reprogramming the modularized FET biosensors. Urease catalyzes the hydrolysis of urea to generate ammonium, which will lead to an increase of the local pH and an increase in conductance of the measured device.²⁴ First, the urease-encoded biorecognition module was integrated onto the FET transducer (Figure 2a, I). When 5 mM phosphate buffers

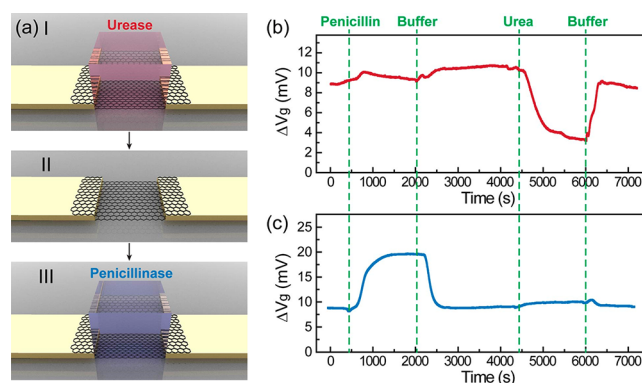


Figure 2. Switchable module. (a) Schematic of switching biorecognition modules from urease-encoded (red) to penicillinase-encoded (blue) PEG. (b,c) Real-time recording from the FET biosensors integrated with urease-encoded (red trace) and penicillinase-encoded biorecognition module (blue trace), respectively. Green dashed lines indicate the time points chronologically of solution switching to phosphate buffer with 1.0 mM penicillin, pure phosphate buffer, phosphate buffer with 1.0 mM urea, and pure phosphate buffer.

containing 1 mM penicillin, no analyte, 1 mM urea and no analyte²³ were sequentially introduced, a highly specific signal corresponding to urea injection was recorded with a minimal nonspecific signal from penicillin (Figure 2b). Following the sensing test, the urease-encoded biorecognition module was detached from the FET devices, and a similar penicillinase-encoded biorecognition module was then assembled onto the same FET device (Figure 2a, II and III). The sensing experiment with the same analyte solutions introduced in the same order was repeated. Specific detection of penicillin was achieved, and no signal was observed this time following urea injection (Figure 2c), demonstrating the successful reprogramming of the modularized FET biosensor and that the disconnection of the urease biorecognition module does not leave any cross-contamination. The integration and disintegration of biorecognition modules has been repeated over 35 times, and the recorded signal amplitudes are consistent between multiple switching, indicating the reproducibility and stability among module switches (Figure S3). This reprog-

rammability feature can also foster unique applications for the FET biosensor that involve an irreversible biorecognition process, such as detection of antibody–antigen binding kinetics, where regeneration of the devices can be conveniently achieved via replacement of fresh biorecognition modules. This discovery has extensively broadened our flexibility to design and fabricate both recognition and transducer modules independently for a specific tuning requirement and eventually developing a biosensor with multifunctionalities by integrating various kinds of modules.

In addition to the capability to customize and switch the bioreceptor encoding in the biorecognition modules, the structural and functional properties of the hydrogel micro-environment can also be independently fabricated and customized to meet various sensing requirements. For example, hydrogels with controlled porosities could allow us to control the diffusion dynamics²⁵ of analytes with different molecular weights; the tunable chemical properties of the hydrogels could also enable selective accessibility of molecules with certain charges or chemical affinity;²⁶ likewise a hydrogel capable of degrading or phase transition²⁷ could be utilized to make transient or switchable biosensors; and more. To prove our concept, we chose three commonly used hydrogel materials, PEGDA, gelatin methacryloyl (GelMA), and alginate, with significantly different porosity to demonstrate the modulation of the mass transport across the hydrogel matrices.^{25,28,29} Specifically, polymerized hydrogel “stamps” composed of PEG, gelatin, and alginate³⁰ were placed in a solution of 1 mM methylene blue (MW 320 g/mol) and collected after 0.5, 1, 3, 5, and 15 min for imaging. Figure 3a shows the distinct diffusion behavior of methylene blue into different hydrogel “stamps” from the solution interface over time. Quantitative analysis (Figure 3b) of the color changes was carried out by plotting the grayscale profiles horizontally across each panel from Figure 3a. It is clear to see that minimal changes in color occur after 5 min exposure of gelatin and alginate to methylene blue, at which condition the diffusion of methylene blue could be treated as saturation ($\sim 100\%$). Normalized by the saturation assumption, gelatin and alginate already exhibit 84% and 86% saturation after 3 min exposure to methylene blue, respectively. However, PEG hydrogel only reaches 56% saturation even after being exposed to methylene blue for 15 min. The different diffusion rate in these hydrogels can be attributed to the variation of the pore size between PEG, gelatin, and alginate. Cross-linking of small-molecular weight PEGDA (MW 575 g/mol) has been shown to have nanometer scale pore size,²⁵ as compared with micrometer for GelMA²⁸ and alginate,²⁹ while fine-tuning of the pore size can also be achieved by copolymerization of desirable porogens.^{31,32}

The difference in porosity has been utilized to tune and optimize sensing kinetics between analytes with distinct molecular weights, which has the potential to supplement additional selectivity when processing complex physiological fluids such as blood, saliva, urine, etc. PEG hydrogel-gate has been shown to eliminate absorption and detection of large molecular bovine serum albumin limited by its intrinsic small pore size.¹² Here, poly-L-lysine (PLL, MW $1.5\text{--}3.0 \times 10^5$ g/mol) was tested in parallel with FET biosensors integrated with gelatin and PEG modules, to evaluate the effect of hydrogel pore size on analyte accessibility. Simultaneous recording from gelatin-FET and PEG-FET biosensors was performed while 0.005% (w/v) PLL in 5 mM phosphate

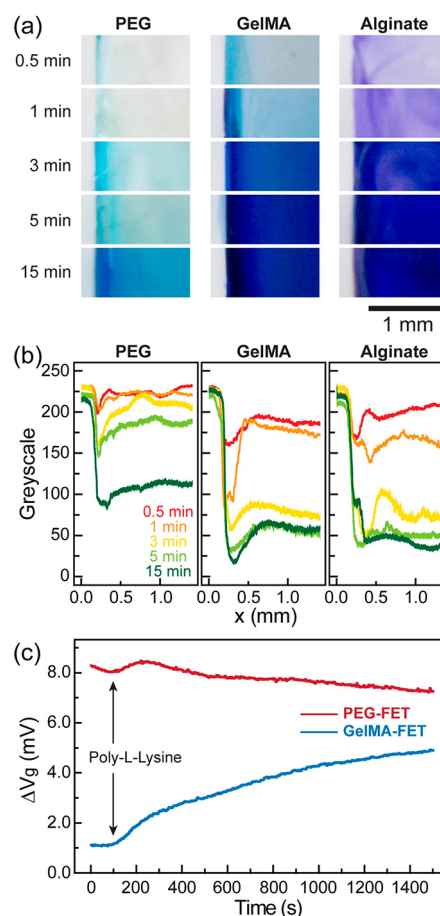


Figure 3. Material versatility. (a) Photographs showing diffusion of methylene blue into PEG, gelatin, and alginate hydrogels for 0.5, 1, 3, 5, and 15 min. The vertical white/blue color transition aligned to approximately one-sixth of each panels indicates the boundary of hydrogels. (b) Quantitative analysis of grayscale profiles across each of the panels in (a), indicating time-dependent diffusion of methylene blue into PEG, gelatin, and alginate hydrogels. All the traces are averaged from five horizontal cross sections evenly distributed. (c) Real-time recording from FET biosensors integrated with receptor module composed of PEG (red trace) and gelatin (blue trace), respectively. Arrow indicates the time point of solution switching to phosphate buffer containing 0.005% (w/v) poly L-lysine.

buffer²³ was introduced. An approximate 4 mV signal was observed due to the nonspecific binding of positively charged PLL only from gelatin-FET device whereas no signal was detected from the PEG-FET (Figure 3c). Moving forward, selectivity with different requirements can be achieved by more sophisticated structural and compositional modulation of the receptor module backbone.^{33,34}

Stemming from the modular design, we have demonstrated the capability to independently customize the biorecognition modules with precise control over both active (bioreceptors) and passive (microenvironment) components. The design and processing is inherently compatible with additive manufacturing techniques, such as 3D printing, to construct a customizable interface for module integration, low cost and high material efficiency, ease of fabrication and mass production.³⁵ As shown schematically in Figure 4a, the biorecognition modules adopted in our tests could be mass produced by 3D printing in batches, followed by photopolymerization of hydrogels in a large array. Specifically, 3D printing was first

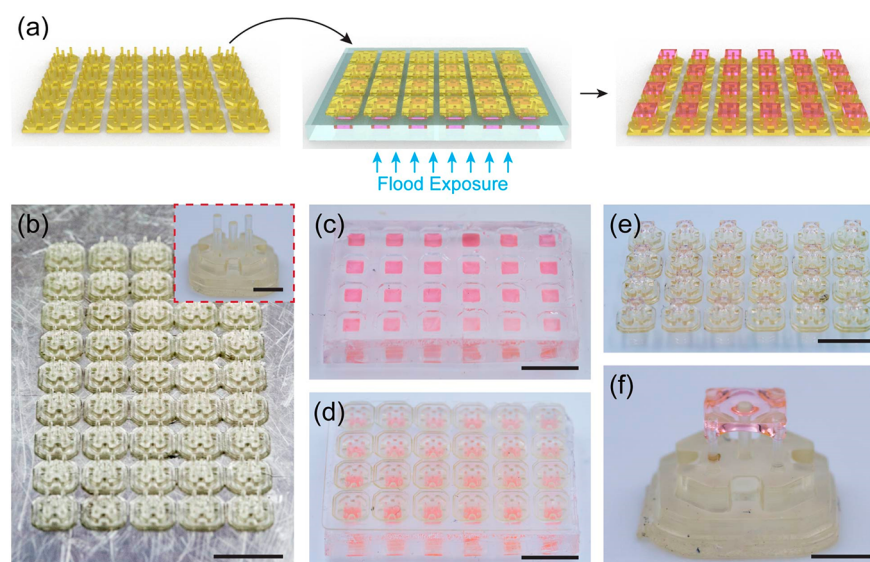


Figure 4. Additive manufacturing. (a) Schematic showing the process of mass fabrication of an array of hydrogel receptor modules. (b) Photograph of “stamps” used for picking up thin hydrogels and interface with FET modules via standard sockets, printed en masse on a stereolithography 3D printer building plate. Scale bar, 10 mm. Inset, a high-magnification photograph of an individual “stamp”. Scale bar, 2 mm. (c) Photograph of hydrogel monomer solution in PDMS wells. Scale bar, 10 mm. (d) Photograph of an array of 3D-printed “stamp” placed into PDMS wells. Scale bar, 10 mm. (e) Photograph of an array of fabricated receptor modules. Scale bar, 10 mm. (f) A high-magnification photograph of an individual receptor module, highlighting the thin hydrogel held by several pillars on the “stamp” and standard socket used for interface with the FET transducers. Scale bar, 2 mm.

used to fabricate the “stamps” designed with (1) standardized LEGO-like docking structure to facilitate the “plug-and-play” integration to FET module attached with complementary structure, and (2) an array of pillars to pick up the hydrogel elements (Figure 4b). After 3D printing, the “stamps” were cleaned with ethanol and UV-cured for 30 min. Polydimethylsiloxane (PDMS) mold casted with the same pitches was made to create mini-wells holding hydrogel monomer solution (Figure 4c) and then interfaced with the pillar side of the “stamps” (Figure 4d). Following the light exposure of 1 min, the array of “stamps” could be detached from the PDMS wells with a thin piece of hydrogel elements picked up by the pillar structure, and the “stamp” modules with hydrogel elements could be easily integrated with the FET devices or stored for later use (Figure 4e,f). 3D printing allows for low-cost, high-throughput fabrication, and compared with that of traditional functionalization methods, the polymerization step requires as little as 4 μL of monomer solution to fabricate a hydrogel-equipped “stamp” capable of covering $2 \times 2 \text{ mm}^2$ and can make 100% use of the bioreceptor molecules. This generic strategy is not limited to photopolymerization and can be customized for different categories of material processing such as gelation of gelatin and agarose through temperature-mediated phase transition.³⁶ This strategy should also not be limited to enzyme-based biosensors but can be expanded to other biorecognition elements such as single-stranded DNA, antibodies, aptamers, and so on. These methods may require additional optimization of the hydrogel microenvironment for optimal signal transduction. Significantly, granted by the simple “plug-and-play” integration and standardized interface, a large pool of biorecognition modules fabricated by different bioreceptors and matrix materials can be preformulated and stored as bioactive “cartridges” for potential customizable assembly of modularized FET biosensors upon usage, showing its great potential for a low-cost personalized multifunctional point-of-care biosensor tool kit.

In conclusion, we outline a novel and general strategy to fabricate a modularized FET biosensor consisted of independently customized hydrogel “stamps” as biorecognition modules and graphene FETs as transducer modules. The modular design has been exploited to enable real-time, label-free detection of penicillin G and urea with penicillinase- and urease-encoded PEG hydrogel as biorecognition modules, respectively. Additionally, the modular design allows us to freely assemble and disassemble FET transducer module with a variety of biorecognition modules composed of different hydrogel materials, different bioreceptors, and so on, to achieve a number of different purposes in sensing tests. The modularization also offers standardized preparation of biorecognition modules for customizability, low cost, and mass production. The modularized design and facile integration of hydrogel “stamps” and graphene FETs has demonstrated functional integration of biorecognition modules and transducer modules in biochemical sensing and could lead to broadly functional integration of biological (in biomaterials) and electronic (in solid-state) transducers for many new possibilities in hybrid device development.^{37–39} We anticipate this strategy will lead to improvements in FET biosensors for applications such as point-of-care diagnostics and personalized medicine.^{40–42}

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.nanolett.9b02939.

Additional information including figures showing liquid gate measurement, concentration-dependent sensing, and reproducibility test (PDF)

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Notes

The authors declare no competing financial interest.

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- (20) The 3D-printed fluidic chamber was printed using Wanhao Duplicator 7 Desktop 3D Printer with Wanhao 3D-Printer UV Resin—Clear. After printing, the chamber was washed with ethanol and UV cured for 30 min. The chamber consists of several features for insertion of inlet/outlet tubing, mounting Ag/AgCl reference electrode, and standard docking stations for aligning hydrogel stamp on top of FET device.
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