Abuse of amphetamine-type stimulants (ATS) is of increasing concern in many countries. Therefore, there is a large demand for the development of easy, fast, and cheap ATS detection methods. In this regard, we have developed a point-of-use, portable, wireless drug sensor with unprecedentedly high sensitivity toward ATS using a drug-specific host molecule, cucurbit[7]uril, and organic field-effect transistors. This work provides a viable methodology for the fabrication of highly sensitive and selective drug sensors.
Article

Point-of-Use Detection of Amphetamine-Type Stimulants with Host-Molecule-Functionalized Organic Transistors

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SUMMARY

In recent years, there has been a rapid increase in the abuse of amphetamine-type stimulants (ATS). One way to tackle this problem is to develop an easy, sensitive, rapid, and cheap ATS detection platform. Here, a strategy that synergistically combines the selectivity of supramolecular chemistry and the sensitivity of organic field-effect transistors is used as the basis of an ATS sensor. Cucurbit[7]uril derivatives that can selectively detect ATS have been synthesized and used as a functional material. The fabricated amperometric sensors exhibited unprecedented sensitivity toward ATS, with a detection limit of nanomolar concentrations in urine and picomolar concentrations in water or a physiologic buffer. The feasibility of this strategy was further demonstrated through the preparation of flexible and wearable devices with a wireless sensing platform. This sensing system offers rapid and sensitive detection of trace amounts of ATS in urine and other samples at the point of use.

INTRODUCTION

Amphetamine-type stimulants (ATS), a group of drugs whose principal members include amphetamine (1-phenylpropan-2-amine, speed) and methamphetamine (N-methyl-1-phenylpropan-2-amine, philopon), have been widely used for medical purposes for several decades. For example, it is known that amphetamine activates dopamine and norepinephrine neurotransmitter systems in the brain, and therefore it has been used for the treatment of attention deficit hyperactivity disorder (ADHD), narcolepsy, asthma, depression, and so on. However, frequent and long-term use of ATS may cause drug addiction and other serious side effects such as insomnia, hallucinations, delusions, mental illness, and violent tendencies, etc. Therefore, the use of ATS is restricted in many countries and allowed only for medical purposes with a doctor’s prescription. Despite such regulations and controls, however, there has been a pronounced increase in the abuse of ATS worldwide. ATS is particularly attractive to young people because it produces a sense of high energy, a release of social inhibitions, and feelings of cleverness, competence, and power. ATS is rapidly replacing heroin and cocaine among drug addicts because it is cheaper and more accessible than other illicit drugs; ATS was recently ranked the world’s second most used drug after cannabis. Accordingly, abuse of ATS is of increasing concern in many countries and a rapidly growing problem. In addition, the use of ATS through intravenous means is another emerging concern with widespread health issues particularly given its links to the spread of HIV and acquired immune deficiency syndrome. Therefore, the problems related to ATS abuse are not only an individual’s problem but also a community’s problem. As countermeasures against these
problems, there are strict regulations on the use of ATS by governments, and several methods have been developed to detect ATS use, including gas chromatography/mass spectrometry (GC/MS), liquid chromatography/mass spectrometry (LC/MS), immunoassays, molecularly imprinted polymer solid-phase extraction, and so on. However, these analysis methods usually require long operation time, sophisticated experimental procedures, and expensive equipment with well-trained professional operators; moreover, they are difficult to apply for on-site detection. Thus, there is a strong demand for an easy, sensitive, rapid, cheap, and portable ATS detection method.

Compared with the aforementioned conventional methods, supramolecular analytical chemistry approaches provide an alternative method for the detection of ATS and related synthetic drugs. In this approach, a set of synergistic weak interactions between a synthetic receptor and a target drug molecule enable the selective recognition of the drug. Although this approach offers an appealing method for the development of drug sensors by combining various physical and chemical tools, it has rarely been applied to drug sensing because of the lack of suitable synthetic receptors. As a recent example, it has been reported that cavitand-grafted silicon microcantilevers can be used for drug sensing in water. Even though this is a good example of how a synthetic receptor can be used for drug detection, there are still several issues that need to be improved, including the detection limit of the drugs, sensitivity in a complex biological mixture such as buffer or urine, on-site detection ability, and so on.

Sensors based on organic field-effect transistors (OFETs) show great promise as chemical or biological sensors because they have a number of advantages, including high sensitivity, low cost, simple fabrication, fast response, and flexible applications. Pristine OFET-based sensors typically exhibit low selectivity because of the lack of recognition units; therefore, highly selective detection with OFET-based sensors often requires chemical modification of specific receptors on the electroactive layer to selectively capture target analytes. Here, we report OFET sensors functionalized with cucurbit[7]uril (CB[7]) derivatives for the binding event of ATS, which show highly sensitive and selective sensing behavior for ATS in water and even in a physiological buffer solution and urine (Figure 1). In addition, we have also developed wireless sensors with a smartphone application which have great potential as portable or wearable sensors for on-site detection of ATS.

RESULTS
Specific Recognition of ATS by CB[7]
The formation of a stable 1:1 inclusion complex between ATS and CB[7] was established by nuclear magnetic resonance (NMR) and mass spectrometries. The $^1$H NMR spectra of ATS and a 1:1 mixture of ATS and CB[7] are shown in Figure 2, in which all the signals have been assigned by 2D NMR experiments, including correlation spectroscopy (COSY) and rotating-frame Overhauser effect spectroscopy (ROESY). As shown in Figure 2, $^1$H NMR spectroscopy showed drastic changes in proton resonance signals of ATS upon complexation with CB[7]. For example, when freshly dissolved amphetamine hydrochloride (1) in D$_2$O was treated with 1 equiv. of CB[7], most of the proton signals of 1 were shifted upfield, and only the methyl protons in N-methyl secondary amines were shifted downfield (Figures 2A and 2B). The COSY and ROESY data for the complex 1·CB[7] also further confirmed that 1 is buried deeply inside the CB[7] cavity. The cavity and carbonyl-laced portals of CB[7] direct the encapsulation of 1 through hydrophobic and ion-dipole interactions.
Similar NMR results were observed for methamphetamine hydrochloride (2) (Figures 2C and 2D). MALDI-TOF mass spectra also confirm the formation of 1:1 inclusion complex between ATS and CB[7] (Figures S1 and S2). In addition, quantitative measurements of the binding affinities between CB[7] and ATS were performed by isothermal titration calorimetry (ITC). ITC analyses revealed that CB[7] has high affinities for both ATS 1 and 2 with a 1:1 stoichiometry (K_a = 10^6 M) (Figures S3 and S4); for example, the binding constant between 1 and CB[7] was measured to be (1.2 ± 0.1) \times 10^6 M^{-1}. The formation of the inclusion complex is enthalpy driven (\Delta H^\circ = -41.5 ± 0.2 kJ/mol), and the unfavorable entropic contribution (\Delta S^\circ = -6.8 ± 0.3 kJ/mol) is apparently compensated by the large favorable enthalpic gain (Table S1). The enthalpy gain is a result of the hydrophobic interactions between the phenyl ring of the guest and the inner wall of the host cavity as well as strong ion-dipole interactions between ammonium groups of the guest and carbonyl-laced portals of the host. Release of high-energy water molecules from inside the cavity of CB[7] upon guest binding may also contribute to the high binding affinity.37,38 Finally, the stable inclusion complex formation was confirmed by single-crystal X-ray analyses. Single crystals of 1 or 2 complexed with CB[7] suitable for X-ray analysis were obtained from water by vapor diffusion, and the crystal structures of the complexes are depicted in Figures 2E and 2F, respectively (see also Figures S5 and S6). In both complexes, the benzyl residue of each guest molecule was encapsulated deep inside the CB[7] cavity, exhibiting complete inclusion of the guests, whereas the ammonium group was positioned near the carbonyl rim of CB[7]. The main planes passing through benzene ring of 1 and 2 are tilted by 55.2° and 45.0°, respectively, with reference to the main 7-fold symmetry axis of CB[7]. The orientations of 1 and 2 lead to ion-dipole interactions between CB[7] and ammonium groups (shortest N···O distances: 2.757 Å and 2.779 Å for 1·CB[7] and 2·CB[7], respectively).

**Design and Fabrication of Sensor Devices**

The unique recognition properties of CB[7] toward ATS prompted us to develop highly sensitive and selective ATS sensors with an OFET platform. To fabricate ATS sensors via functionalization of the surface of an organic semiconductor layer in an OFET device with CB[7], we synthesized CB[7] derivatives (allyloxyCB[7] 3 and phenylbutoxyCB[7] 4), which are soluble in methanol but not in water (see the
Therefore, the ATS-specific CB[7] derivatives can easily be deposited on top of a water-stable organic semiconductor 5,5-bis-(7-dodecyl-9H-fluoren-2-yl)-2,2-bithiophene (DDFTTF) layer by a spin-coating method using a methanol solution of the CB[7] derivatives without damaging the underlying organic semiconductor layer. The recognition properties of the CB[7] derivatives are essentially the same as CB[7] itself, because functionalization of the outer wall affects only its solubility and processability for the fabrication of the sensor device. Therefore, the DDFTTF layer functionalized with the film of CB[7] derivatives acts as a stable and selective sensing layer for ATS in an aqueous phase.

The bottom-gate/top-contact transistor-based sensors were fabricated to demonstrate highly sensitive chemical detection, using a CB[7]-functionalized semiconductor layer. The DDFTTF thin film (~15 nm thickness) was deposited with thermal evaporation onto n-octadecylmethoxysilane (OTS)-treated SiO$_2$/Si substrates as the active layer for the OFET sensor device. The gold source and drain electrodes (~40 nm thickness) in the channel area were covered with a SiO passivation layer (~20 nm thickness) as reported previously (see also Experimental Procedures). The ATS-specific receptor, 3, was dissolved in methanol (5 mg mL$^{-1}$) and spin coated on top of the semiconductor. The corresponding device structure is shown in Figure 3A. The electrical characteristics of OFETs with and without 3 measured

Figure 2. Characterization of Host-Guest Complexes between ATS and CB[7]

$^1$H NMR spectra of (A) 1•CB[7], (B) 1, (C) 2•CB[7], and (D) 2 in D$_2$O. The signals marked with asterisks belong to CB[7]. The NMR spectra showed drastic changes in the proton resonance signals of ATS upon complexation with CB[7]. X-ray single-crystal structures of (E) 1•CB[7] and (F) 2•CB[7]. Both host-guest complexes exhibit complete inclusion of the guests with the benzene ring deeply buried inside the CB[7] cavity, and the ammonium group of the guests positioned near one of the carbonyl rims of CB[7]. For clarity, the H atoms of CB[7] have been omitted.
in the saturation regime exhibited similar average field-effect mobilities ($\mu_{\text{FET}}$) of $\sim 0.02 \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ (Figure 3B). The DDFTTF OFETs with 3 showed slightly increased off current with easier turn on in the forward sweep most likely a result of the doping effects after the solution processing of 3,24,39 compared with the pristine DDFTTF OFETs, but still exhibited a high on/off current ratio ($I_{\text{on}}/I_{\text{off}}$) of more than $10^5$ in ambient conditions. The linear regime operation also showed obvious field-effect behaviors (Figure 3C), which were stable enough to achieve signal amplification and a fast response in the sensing conditions.

To investigate the surface morphological characteristics of DDFTTF films and 3-functionalized DDFTTF films, tapping-mode atomic force microscopy (AFM) experiments were conducted. The thermal evaporation of 15-nm-thick DDFTTF film resulted in planar surfaces over a large area as shown in Figures S7A and S7B. The annealed DDFTTF thin films showed polycrystalline grains and distinct grain boundaries with a root-mean-square roughness of 1.7 nm. The spin-coated 3 layer covered the DDFTTF layer fully and uniformly (Figures S7C and S7D), which is crucial for the fabrication of highly sensitive sensors. In addition, the 3-coated film was well adsorbed onto the DDFTTF thin films via hydrophobic interactions between 3 and DDFTTF. The cross-sectional AFM analysis revealed that the thickness of the 3 layer was 3.7 nm.

**ATS Sensing Experiments**

A sensing system for the detection of liquid analytes was prepared by placing a polydimethylsiloxane (PDMS) mold container onto the sensor device (Figure 4A). Sensing
experiments were performed under ambient conditions by monitoring the output current as a function of time. \( V_{DS} \) and \( V_{GS} \) were fixed at \(-2\) V and \(-60\) V, respectively. Prior to detecting the analytes, a baseline current was estimated with deionized (DI) water. After stabilizing the output current, one drop of solution (\( \sim 15\) μL) containing ATS was injected into the mounted PDMS container. The sensing signals were normalized by dividing the drain current by the baseline current \( (I_D/I_{D\text{-BASE}}) \).

The real-time liquid-phase sensing responses of the OFET-based sensors functionalized with 3 toward ATS are shown in Figure 4. The sensor showed an enhanced drain current after injection of the analyte solution. In p-channel OFET-based sensors, hole charge-transport tends to be increased by the binding event to a receptor.
with a positively charged species because of the electron-withdrawing effects into the channel region, which leads to the increase in the density of hole carriers at the channel region. This can be induced by charge-dipole interactions between the carbonyl group of 3 and the positively charged ammonium group of the analytes. Surprisingly, the 3-functionalized OFET sensor showed linear responses in the ATS concentration ranges studied, and the detection limit was as low as picomolar, as shown in Figures 4B–4D. To the best of our knowledge, this result is the highest sensitivity toward ATS in amperometric sensors. In addition to the sensing of ATS, we also monitored changes in the drain current of the sensors with and without the 3 layer, as a control experiment (Figure S8). When the devices were exposed to 1 pM 1, the sensor with the 3 layer exhibited a high and stable sensing signal for 1, whereas the sensor without the 3 layer showed negligible and decreasing responses upon addition of 1. As another control experiment, we firstly blocked the portal of 3 on the sensor device surface by treatment with (dimethylaminomethyl) ferrocene, which is well known to strongly bind to the CB[7] molecule and therefore is expected to hamper drug sensing. As expected, the blocked sensor device showed almost no sensing response to 1 (Figure 4D). These two control experiments clearly show that the sensing event is highly dependent upon 3 in the device platform. These results also indicate that the introduction of the ATS-specific 3 layer significantly improves the sensitivity of the sensors through selective binding of ATS on the device surface.

In addition to the sensing of 1, Figure 4C shows that 2 can also be detected; 2 is another representative ATS but more critical in terms of side effects, including addiction and increased health risks. As expected, the 3-functionalized sensor can also detect 2 in low concentrations with a similar picomolar detection limit, and positive sensing behavior, in which the drain current is enhanced after binding events with the analytes, was also observed (Figure S9).

To further investigate the applicability of our sensing platform in real biological systems, we tested the sensors toward PBS systems (pH 7.4, 0.01 M), containing sodium chloride (137 mM) and potassium chloride (2.7 mM) (Figure 4E). The sensing tests were performed with 4-functionalized OFET devices because these sensors exhibited better results than those with 3-functionalized surfaces in PBS, possibly because of the improved stability of the spin-coated film. In PBS solution, the OFET sensors also showed stable and concentration-dependent sensing results at various concentrations (1 pM to 1 μM) of 1, albeit with slightly reduced signals, probably because of the interfering cations (Na+ and K+).

Drugs tend to be excreted from the body via urine and sweat, either unchanged or broken down into specific metabolites. Therefore, drug testing of urine has been the most common method for drug detection. To check the feasibility of our sensors in urine, we first performed drug sensing experiments using synthetic urine, which is a non-biological urine with constituents that mimic human urine. Prior to injection of the analytes, baseline tests were performed using synthetic urine. The 4-functionalized OFET sensors exhibited minimal sensing signals after continuous injections of only synthetic urine solution, then ATS solutions with various concentrations were prepared in the synthetic urine solution and sensing experiments were performed. As shown in Figure S10, the sensors certainly detected 1 at concentrations as low as sub-nanomolar. Based on these results, sensing experiments with real urine samples were performed according to the same procedure as the synthetic urine tests, and the sensing results are shown in Figure 4F. The sensors also showed stable sensing of 1, albeit with reduced sensitivity (1 nM) probably because of the
interfering ions and various metabolites in the real urine. These results imply that our sensors can be used for the detection of ATS in real urine samples with high sensitivity.

Flexible and Wireless Sensor Devices
The OFET-based flexible and wearable sensors are highly promising for use in portable devices and health care systems, such as bio-signal measurements and drug addiction therapies. As shown in Figure 5A, we also fabricated flexible drug sensors using an indium tin oxide (ITO)-coated polyethylene naphthalate (PEN) substrate and an aluminum oxide (Al₂O₃) transparent gate dielectric. A 100-nm-thick Al₂O₃ dielectric layer was deposited onto the ITO-coated PEN substrate via a radio frequency magnetron sputtering technique, and a photograph of the fabricated flexible sensor is shown in the inset of Figure 5B. The transfer characteristics of the 4-functionalized DDFTTF OFET-based sensors exhibited ample electrical performance (Figure S11). The results of the sensing experiments for 1 showed performances similar to those of the aforementioned Si wafer-based sensors. Using the 4-decorated flexible sensor platform, the selective detection of 1 in DI water at a concentration as low as 1 pM was realized under relatively low-voltage operating conditions (V_DS = −1.5 V and V_GS = −10 V) (Figure 5B). These results provide the first demonstration of rapid ATS sensing with synthetic receptor-molecule-functionalized flexible OFET-type sensors.
More importantly, portable and miniaturized sensors with wireless communication systems can be utilized for on-site real-time detection of analytes. Organic-transistor-based sensors are highly suitable for the low-cost fabrication of state-of-the-art wearable sensors. We successfully developed wireless OFET prototype sensors connected to a smartphone application, as shown in Figure 5C. A custom-built printed circuit board (Kong Tech), including the antenna, was connected to gold wires connecting its three terminal electrodes using conductive copper tape. The digitized sensing current could be transmitted to the Android application via wireless Bluetooth communication. This system could potentially be installed for bracelet-type wearable OFET sensors (Figure 5D). The 4-functionalized OFET wireless sensors also exhibited an increase in the output current when the device was exposed to a 1 pM aqueous solution of 1 (Figure S12 and Movie S1). Notably, stable and repeatable sensing signals were obtained at such low concentrations (~1 pM 1) (Figure 5E and Movie S2), demonstrating the great potential of these OFET-based sensors for use in on-site, real-time, and portable drug sensors with high sensitivity and accuracy.

DISCUSSION

In summary, we have developed highly sensitive OFET-based sensors that can selectively detect ATS in water, PBS buffer, and urine. To develop ATS sensors, we first established that CB[7] can specifically recognize ATS and then functionalized the surface of a water-stable organic semiconductor DDFTTF layer with CB[7] derivatives, i.e., 3 and 4. The OFET sensors prepared on a rigid Si wafer or a flexible plastic substrate showed a detection limit for ATS as low as 1 pM in DI water and 1 nM in real urine, as a result of the synergistic effects of the selectivity of supramolecular analytical chemistry and the sensitivity of the OFET sensor platform. Furthermore, we successfully demonstrated a portable and miniaturized sensor with a wireless communication system that could be utilized for rapid point-of-use detection of ATS. This work provides a low-cost, simple, and viable methodology for the fabrication of highly sensitive and selective ATS sensors, and may open an avenue to new opportunities to replace current drug detection methods.

EXPERIMENTAL PROCEDURES

Detailed experimental procedures are provided in the Supplemental Information.

Synthesis of CB[7] Derivatives

Cucurbit[7]uril (CB[7]) was purchased from CBTech (www.cbtech.co.kr). HydroxyCB [7] and allyloxyCB[7] (3) were synthesized based on a reported method. PhenylbutoxyCB[7] (4) was synthesized by following the procedure for the synthesis of 3 with appropriate modifications. For experimental details, see Supplemental Experimental Procedures.

Fabrication of DDFTTF-Based OFET Sensors

OFET-based sensors were fabricated with a heavily n-doped silicon wafer (<0.004 Ω cm) covered with a thermally grown 300-nm-thick oxide layer (SiO₂, C₉ = 10 nF cm⁻²). The SiO₂ surface was treated with OTS according to the previously reported method. The DDFTTF thin films (~15 nm nominal thickness) were thermally deposited onto the OTS-treated SiO₂/Si wafer at 0.1–0.2 Å s⁻¹ under a base pressure below 5.0 x 10⁻⁶ torr. During evaporation, the optimal substrate temperature for DDFTTF deposition was 105 °C. The films were annealed at 150°C for 30 min in a nitrogen atmosphere. Gold contacts (~40 nm thickness) were thermally evaporated onto the DDFTTF films to form the source and drain with a channel width (W) and a
channel length (L) of 9,000 μm and 50 μm, respectively. A silicon monoxide (SiO, ~20 nm thickness) layer was thermally deposited onto the electrodes except for the probe contact area. The SiO layer was used as a passivation layer of source-drain electrodes for sensor operation in liquid solutions. Then, the solution-processable CB[7] derivatives were dissolved in methanol (5 mg mL⁻¹), and the film was spin-coated onto the underlying film at 7,000 rpm for 30 s. The films were dried in a vacuum oven at 60°C overnight and annealed at 150°C for 30 min to remove the residual solvent.

ACCESSION NUMBERS
The 1·CB[7] and 2·CB[7] structures reported in this article have been deposited in the Cambridge Crystallographic Data Center under accession numbers CCDC: 1542694, 1543082.

SUPPLEMENTAL INFORMATION
Supplemental Information includes Supplemental Experimental Procedures, 12 figures, 1 table, and 2 movies and can be found with this article online at http://dx.doi.org/10.1016/j.chempr.2017.08.015.

AUTHOR CONTRIBUTIONS
I.H., J.H.O., and K.K. conceived and designed the experiments. Y.J. and E.J. synthesized compound 3 and 4, and performed characterization of host-guest complexes. M.J., H.K., and S.J.L. fabricated all the devices and analyzed the electrical characteristics of the organic transistors and sensors. J.Y.K., I.-C.H., and Y.K. carried out the single-crystal X-ray analyses and Y.-H.K. performed the NMR analyses. I.H. and K.K. supervised the syntheses of CB[7] derivatives. J.H.O. supervised the device experiments. Y.J., M.J., I.H., J.H.O., and K.K. wrote the manuscript. All authors discussed the results and commented on the manuscript.

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REFERENCES AND NOTES


