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► **To cite this version:**

Viet Anh Pham Ba, Dong-Guk Cho, Seunghun Hong. Nafion-Radical Hybrid Films on Carbon Nanotube Transistors for Monitoring Antipsychotic Drug Effects on Stimulated Dopamine Release. ACS Applied Materials & Interfaces, 2019, 11 (10), pp.9716-9723. 10.1021/acsami.8b18752 . hal-02187852

HAL Id: hal-02187852

<https://hal.science/hal-02187852>

Submitted on 18 Jul 2019

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**Nafion-Radical Hybrid Films on Carbon Nanotube
Transistors for Monitoring Antipsychotic Drug Effects on
Stimulated Dopamine Release**

Journal:	<i>ACS Applied Materials & Interfaces</i>
Manuscript ID	am-2018-18752r.R1
Manuscript Type:	Article
Date Submitted by the Author:	07-Jan-2019
Complete List of Authors:	Pham Ba, Viet Anh; Seoul National University, Department of Physics and Astronomy Cho, Dong-guk; Seoul National University, Department of Physics and Astronomy Hong, Seunghun; Seoul National University, Physics

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6 Monitoring Antipsychotic Drug Effects on Stimulated Dopamine
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9 Release
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26 KEYWORDS: Dopamine, Pimozide, ABTS, Nafion, Carbon nanotube field-effect transistor
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3 **ABSTRACT:** We developed floating electrode-based carbon nanotube biosensors for the
4 monitoring of antipsychotic drug effects on the dopamine release from PC12 cells under
5 potassium stimulation. Here, carbon nanotube field-effect transistors with floating electrodes
6 were functionalized with ABTS[•] radicals by nafion films. This method allows us to build
7 selective biosensors for dopamine detection with a detection limit down to 10 nM even in the
8 presence of other neurotransmitters such as glutamate and acetylcholine, resulting from the
9 selective interaction between ABTS[•] radicals and dopamine. The sensors were also utilized to
10 monitor the real-time release of dopamine from PC12 cells upon the stimulation of high-
11 concentrated potassium solutions. Significantly, the antipsychotic effects of pimozide on the
12 dopamine release from potassium-stimulated PC12 cells could also be evaluated in a
13 concentration-dependent manner by using the sensors. The quantitative and real-time evaluation
14 capability of our strategy should provide a versatile tool for many biomedical studies and
15 applications.
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1. INTRODUCTION

Dopamine is one of important neurotransmitters, which regulates a wide variety of functions in the central nervous system. Dopamine dysfunctions in the nervous system are concerned various neurological symptoms such as Parkinson's disease and schizophrenia.^{1,2} It has been reported that the concentration changes of potassium ions (K^+) in the brain may induce neurological disorders including the release of neurotransmitters as dopamine.^{3,4} Therefore, the real-time monitoring of the K^+ -evoked dopamine release from neural cells should be important for the understanding of dopaminergic dysfunctions. Moreover, the primary therapy of schizophrenia is based on the regulation of dopaminergic functions by antipsychotic drugs. Previously, it was reported that antipsychotic drugs inhibited dopamine receptor activities and reduced K^+ -evoked dopamine release.^{2,5} Thus, one method to monitor the efficacy of antipsychotic drugs can be the monitoring of the dopamine release from drug-pretreated cells under K^+ -stimulation.

Conventional methods like high-performance liquid chromatography (HPLC), luminescent methods, and electrochemical techniques have been utilized to quantify dopamine release in living systems and evaluate the efficacy of antipsychotic drugs.⁴⁻¹³ However, these techniques have their own limitations. For example, HPLC can be complex and costly. Fluorescent methods require time-consuming preparation, and furthermore, the stability of dyes often degrade over time. For electrochemical methods, electrodes can be contaminated by the products of electrochemical reactions or agents in an ambient environment, resulting in the difficulty of data estimation. Moreover, the detection limit of common electrochemical techniques was only at a micromolar level.⁹⁻¹⁴ On the other hand, field-effect transistor (FET) devices have shown distinct advantages, and they have been extensively used to detect dopamine

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3 in various environments.¹⁵⁻¹⁸ However, they were not utilized to evaluate the efficacy of actual
4 antipsychotic drugs as well as real-time cellular measurements. Recently, many reports have
5 shown the successful development of carbon nanotube (CNT)-based FET devices for the
6 detection of biomaterials.¹⁹⁻²³ It indicates that a CNT-FET device functionalized with compatible
7 materials could be used as an effective tool for the biomaterial detection and drug screening.
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14 Herein, we report a nafion-radical hybrid film on a floating electrode-based carbon
15 nanotube sensor to monitor the effects of an antipsychotic drug, pimoziide, on the dopamine
16 release from living cells. In this work, a floating electrode-based CNT-FET device was coated by
17 a nafion film containing 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS^{*}) radicals
18 to build a selective biosensor for the detection of dopamine. In aqueous solution, ABTS^{*} radicals
19 can selectively react with dopamine, which was detected by underlying sensors for selective
20 sensing. This sensor can selectively monitor the variation of dopamine concentrations down to
21 10 nM level and distinguish dopamine from other neurotransmitters. We demonstrated the real-
22 time monitoring of K⁺-evoked dopamine release from PC12 cells using our sensors.
23 Significantly, the sensors were utilized to evaluate the effects of pimoziide on the dopamine
24 release of K⁺-stimulated PC12 cells. This is a selective and sensitive sensor which can be utilized
25 for versatile biological and medical applications.
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44 **2. RESULTS AND DISCUSSION**

45 **2.1. Characteristics of NRC sensors**

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49 Figure 1 illustrates the real-time monitoring procedure of dopamine release from living
50 PC12 cells by using a nafion-radical hybrid film-based carbon nanotube (NRC) sensor. First,
51 CNT-FET devices with floating electrodes were fabricated as described previously (Figure 1-
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3 i).^{19,20,23} Briefly, CNTs were selectively adsorbed on a SiO₂ substrate, followed by the fabrication
4 of source and drain electrodes (Pd/Au, 10 nm/30 nm) via a thermal deposition and a lift-off
5 process. After that, the source and drain electrodes were insulated by a photoresist-based
6 passivation layer to eliminate leakage currents during sensing experiments in an aqueous
7 environment. A nafion solution containing ABTS[•] radicals was directly dropped on the floating
8 electrodes-based CNT-FET device. Then, the device was dried in a nitrogen atmosphere to form
9 a stable and thin film upon the device. It was previously reported that negatively charged nafion
10 layers could attract positively charged molecules, and, thus, nafion has been used as a catalytic
11 layer to attract dopamine near a sensor surface.¹²⁻¹⁴ In our work, a nafion film not only acts as a
12 catalytic layer but also as a matrix holding ABTS[•] radicals to enhance the sensitivity of our
13 sensor. The redox reactions between dopamine and ABTS[•] radicals generate hydrogen ions (H⁺)
14 which can diffuse in the nafion film, increase the density of positive charges near the transducer
15 of CNT-FET sensors, and eventually change the electrical currents in the sensor. Using this
16 method, we could build NRC sensors for the selective detection of dopamine in aqueous
17 solutions (Figure 1-ii). It also should be mentioned that nafion has been extensively utilized as a
18 part of sensors operating in aqueous solutions with different chemical substances including
19 dopamine and PBS buffer, implying that the nafion was stable after our dopamine sensing
20 experiments in PBS buffer.¹²⁻¹⁴ For the measurements of dopamine release from living cells,
21 PC12 cells were cultured in a culture dish as reported previously (Figure 1-iii).²⁴ Then, a PC12
22 cell suspension was introduced on an NRC sensor using a pipette (Figure 1-iv), and the cells
23 were stimulated by high-concentrated K⁺ solutions for the release of dopamine (Figure 1-v). The
24 release of dopamine was monitored using our NRC sensors.
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3 The quality of an NRC sensor was checked by imaging the surface structure of the sensor.
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5 Figure 2a-i shows the optical image of an NRC sensor including five floating electrodes. The
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7 optical image was obtained by using an optical microscope (XY-MRT, Sunny) equipped with a
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9 CCD camera (SDC-415S, Samsung). The image shows well-defined electrodes with the $10\ \mu\text{m} \times$
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11 $60\ \mu\text{m}$ dimension for each floating electrode. Furthermore, the topography image (Figure 2a-ii)
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13 of an NRC sensor was taken by an atomic force microscope (AFM) system (MFP-3D, Asylum
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15 Research) in a tapping mode with a scan rate of 0.7 Hz. The AFM image shows a uniform nafion
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17 layer on the floating electrode area of the NRC sensor. The thickness of the nafion film could be
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19 estimated as $\sim 200\ \text{nm}$ using the AFM topography image on the boundary of the film (Figure S1
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21 in Supporting Information).
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26 To confirm the functionalization of an NRC sensor by ABTS^{\bullet} radicals, we performed the
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28 Raman scattering spectroscopy analysis on the devices *with* or *without* ABTS^{\bullet} radicals. Figure 2b
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30 shows the Raman spectra of an NRC sensor, a floating electrode-based CNT-FET device coated
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32 with a pure nafion layer *without* ABTS^{\bullet} radicals (NCF sensor), and a bare CNT-FET device with
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34 floating electrodes. Raman spectra were measured at CNT channel areas by using a Raman
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36 spectroscope (XperRam 200, Nanobase) in a single mode. The Raman spectrum (black line) of
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38 CNTs on a bare device exhibits a rather large G-band peak ($1588\ \text{cm}^{-1}$) as well as a much smaller
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40 D-band peak ($1314\ \text{cm}^{-1}$), indicating the small number of defects in used semiconducting
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42 CNTs.^{25,26} We also measured a UV-VIS-NIR spectrum from the bare CNT devices to evaluate
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44 the quality of the CNT channels in our sensors (Figure S2 in Supporting Information). The result
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46 clearly shows the peaks at wavelengths of 581 and 1012 nm, which can be attributed to (6,5)
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48 semiconducting CNTs as reported previously.²⁶ These results support the high quality of our
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50 semiconducting CNT channels in our devices. The Raman spectroscopy measurements of the
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3 devices on floating electrode areas are also shown in Supporting Information (Figure S3). The
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5 Raman spectra of the NRC sensor exhibit vibration bands at characteristic wavenumbers such as
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7 690, 862, 1046, 1201, 1255 and 1592 cm^{-1} which can be associated with ABTS[•] radicals as
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9 reported previously.^{27,28} It indicates the presence of ABTS[•] radicals in the nafion layer on the
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11 NRC sensor. This result clearly shows that our device was successfully functionalized with a
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13 nafion-radical hybrid film.
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17 The electrical characteristics of our NRC sensor was investigated by applying a liquid
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19 gate bias (V_{lg}) to the NRC sensor in a phosphate buffered saline solution. Figure 2c shows the
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21 typical liquid gating effect of an NRC sensor. Here, a gate bias was swept from -1 V to 1 V
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23 while a source-drain bias voltage was maintained at 0.1 V. The curve shows a decreasing source-
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25 drain current with an increasing gate bias, which is a typical behavior for p -type semiconductors.
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27 Importantly, the drastic change of source-drain currents in the small change of a gate bias voltage
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29 indicates the high sensitivity of the NRC sensor.
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35 **2.2. Discrimination of dopamine by NRC sensors**

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37 Figure 3a depicts a schematic diagram showing the sensing mechanism of an NRC sensor
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39 for dopamine molecules. Once dopamine molecules approach to the surface of an NRC sensor,
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41 redox reactions between dopamine molecules and ABTS[•] radicals occur (Scheme S1 in
42
43 Supporting Information).^{13,29,30} These redox reactions generate H^+ which can interact with the
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45 sulfonate groups of nafion, resulting in the increase of positive potentials in a nafion film on the
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47 NRC sensor. Here, nafion not only acts as an ion binder and a signal transporter, but also plays a
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49 role as an insulator to prevent the effects of an ambient environment on the sensor. Note that
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51 since CNT channels in our sensors exhibited p -type characteristics, the increase of positive
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3 potentials would cause the decrease of their conductance (Figure 2c). Thus, once a dopamine
4 solution is added on an NRC sensor, the increased positive potential in the nafion film upon the
5 device causes the decreased electrical conductance.
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10 Figure 3b shows the real-time measurement result of an electrical current in an NRC
11 sensor during the additions of dopamine solutions at various concentrations in the range of 10
12 nM to 100 μ M. The graph shows a sharp decrease in the source–drain current after the addition
13 of a dopamine solution, and then the electrical current gradually stabilizes. However, there were
14 no significant changes in an electrical current through a bare floating electrode-based CNT-FET
15 device during the additions of dopamine solutions (Figure S4 in Supporting Information). Note
16 that the NRC sensor exhibited current changes much larger than noise amplitudes even at 10 nM.
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18 These results indicate that our method can discriminate various concentration dopamine
19 solutions with the detection limit of 10 nM. As a control experiment, we measured the response
20 of an NCF sensor to dopamine (Figure S5 in Supporting Information). The results show
21 negligible current changes less than 3% by the dopamine with the concentrations up to 100 μ M,
22 which is much smaller than the signals by our NRC sensors with ABTS[•] radicals. The response
23 of the NCF sensor may be attributed to the electrostatic interactions between dopamine
24 molecules and nafion film, which may also cause unexpected effects on the responses of NRC
25 sensors to dopamine¹³⁻¹⁵. However, the control experiment results show that these effects are not
26 significant for dopamine concentrations up to 100 μ M. It also should be mentioned that the
27 response time of our sensor was ~25 seconds (Figure 3b), which was rather fast compared with
28 previous dopamine sensors including nafion layers (Table S1 in Supporting Information).
29 Previous works showed that the diffusion time of H⁺ in a nafion film depended on the film
30 thickness, which may delay sensor responses.³¹ However, in our case, the response time of the
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3 sensor was rather fast compared with previous works and enough for practical applications,
4 indicating the time delay by the diffusion in the nafion film was not a significant problem in our
5 sensors (Table S1 in Supporting Information). Moreover, the quick responses of the NRC sensor
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8 imply that our sensors can be used for real-time monitoring applications.
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12 Figure 3c shows the normalized conductance changes of our sensors exposed to
13 dopamine solutions with difference concentrations (C_{DA}). The dose-response curve indicated a
14 wide dynamic range (10 nM – 100 μ M) where our sensors can be used for monitoring dopamine
15 levels. Significantly, the equilibrium constant K_{eq} of redox reactions between ABTS \cdot radicals and
16 dopamine could be estimated by fitting data with Langmuir thermal equation given by^{19,32}
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$$|\Delta G/G_0| = \frac{|\Delta G/G_0|_{\max} \times C_{DA}}{1/K_{eq} + C_{DA}} \quad (1)$$

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28 where $|\Delta G/G_0|$ and $|\Delta G/G_0|_{\max}$ represent the absolute value of a relative conductance change and
29 its maximum absolute value. Based on this model, the value of K_{eq} was estimated as $10^{5.08 \pm 0.21}$.
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31 Previous reports showed the formal standard redox potential (E^0) values of ABTS and dopamine
32 are 0.47 V (vs Ag/AgCl) and 0.75 V (vs SHE), respectively.^{29,30} The equilibrium constant K_{eq} of
33 the redox reaction calculated based on these E^0 values is $10^{5.33}$ which is similar to the value
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41 estimated by our method.

42 To investigate the selectivity of our NRC sensor, we measured electrical currents through
43 an NRC sensor during the additions of other neurotransmitters such as glutamate and
44 acetylcholine which can be released with dopamine from stimulated cells.³³ Figure 3d shows the
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The graph indicates that only the addition of dopamine caused an electrical current decrease,
while the additions of glutamate and acetylcholine did not affect the electrical current in the

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3 NRC sensor. These results show that our NRC sensor could be used to selectively detect
4 dopamine even in a mixed solution. Note that the responses of NRC sensors come from the
5 interactions between ABTS[•] radicals and phenolic hydroxyl (OH) groups. Therefore, our sensors
6 can be applied to detect a broad range of biomolecules with phenolic OH groups such as
7 dopamine, uric acid, and ascorbic acid. In our work, *the NRC sensors were used to monitor*
8 *dopamine released from cells stimulated by K⁺*. In this case, K⁺-stimulated cells are reported to
9 release neurotransmitters such as dopamine, glutamate and acetylcholine, and dopamine is the
10 only chemical substance with phenolic OH groups.^{4,11,24,33} Thus, we performed the selective
11 sensing experiments with other neurotransmitters, showing that our method can be used to
12 selectively detect dopamine out of all neurotransmitters released from K⁺-stimulated cells
13 (Figure 3d).
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31 **2.3. Real-time monitoring of K⁺-evoked dopamine release from PC12 cells using NRC** 32 **sensors** 33

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35 Figure 4a depicts the mechanism for the responses of an NRC sensor to dopamine
36 molecules released from a PC12 cell. Once the PC12 cell is stimulated by a high-concentrated K⁺
37 solution, the depolarization of the plasma membrane may induce the influx of calcium ions
38 (Ca²⁺) into the cell through voltage-dependent calcium channels (VDCCs) and the release of
39 intracellular Ca²⁺ stores. The increase of intracellular Ca²⁺ evokes the neurotransmitter release
40 from the cell. Besides, it was reported that stimulations by high-concentrated K⁺ solutions
41 preferentially induced the release of dopamine by exocytosis.^{4,11,24} After that, released dopamine
42 molecules diffuse to a nafion film and interact with ABTS[•] radicals in the nafion film on an NRC
43 sensor. This interaction generates H⁺ which will diffuse in the nafion film, leading to the
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3 decrease of the electrical current as described previously (Figure 3a). Thus, our NRC sensor
4 could be used to quantitatively monitor dopamine release from living cells. Furthermore, in our
5 experiments, PC12 cells can be stored during the sensing processes without being cultured on an
6 NRC sensor, indicating rather simple sensing processes compared with previous works.¹⁷
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12 Figure 4b shows the real-time changes of electrical currents in an NRC sensor by
13 dopamine release from PC12 cells. Here, the PC12 cells were stimulated by high-concentrated
14 K^+ solutions (containing 2.5 mM Ca^{2+}) to release dopamine by exocytosis. The graph shows that
15 the addition of a cell suspension did not affect the electrical currents of the NRC sensor. In
16 contrast, the additions of high-concentrated K^+ solutions caused the decrease of its electrical
17 current, indicating that PC12 cells released a significant amount of dopamine to the buffer
18 solution. We also measured the electrical current changes of NRC sensors in the absence of
19 PC12 cells during the additions of different K^+ solutions, which did not show any significant
20 current changes (Figure S6 in Supporting Information). These results clearly show that our
21 sensors can selectively monitor the K^+ -evoked dopamine release from live cells.
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36 Figure 4c shows a correlation between the concentrations of released dopamine and the
37 concentrations of K^+ solutions. The dose-response curves of our sensors (Figure 3c) were used to
38 calculate the concentration of dopamine released from PC12 cells. The dopamine release was
39 increased significantly when the concentration of K^+ solutions increased from 20 mM to 160
40 mM, presumably due to the increase of membrane depolarization as reported previously.⁴ This
41 result shows that the concentration change of extracellular K^+ may influence neurological
42 functions such as membrane potential maintenance and neurotransmitter transport.^{3,4} Previous
43 works showed that the sensor response could be significantly delayed by the diffusion time of
44 target molecules in solution and in nafion layers.³¹ However, in our experiment, the response
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3 time was quite quick enough for practical applications. Presumably, due to the small volume of
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5 our working solution ($\sim 10 \mu\text{L}$), it did not take a long time for released dopamine to reach the
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7 sensor surface. Also, H^+ generated during the sensing process can diffuse very quickly inside the
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9 nafion film with a rather small thickness of $\sim 200 \text{ nm}$, resulting in the quick response of our
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11 sensors. On the other hand, it was previously reported that the concentration of a ABTS \cdot radical
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13 solution ($\text{pH} = 7.4$) decreased by 10% after 60 min exposure to ambient air, which may cause
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15 instability of signals if the sensing experiments last for a long time period.³⁴ However, the rather
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17 fast response time of our sensors allowed us to finish most of the sensing experiments in less
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19 than ten minutes (Figure 4b). Thus, the effect by the degradation of ABTS \cdot radicals is expected
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21 to be minimal in our experiments. Importantly, these results imply that our method can be used
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23 to quantitatively monitor the real-time release of biomaterials in living systems. Moreover, these
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25 results show that our method based on CNT FET devices exhibited several advantages over
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27 conventional methods (Table S1 in Supporting Information). For example, our NRC sensors
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29 exhibited a higher sensitivity and faster responses than most of previous sensors and, thus, can be
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31 utilized for the measurement of real samples such as cells.
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40 **2.4. Monitoring antipsychotic effects on K^+ -evoked dopamine release from PC12 cells by** 41 **NRC sensors** 42 43

44 Since the abnormal changes of dopamine levels in nervous cells is involved in
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46 neurological diseases, the therapeutic methods have been suggested using dopaminergic drugs.
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48 For example, pimozide is an antipsychotic, which has been marketed to care for patients with
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50 schizophrenia.³⁵ Since the mechanism of dopamine release from PC12 cells is similar to that
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52 from neurons, PC12 cells have been extensively used for studies regarding antipsychotics.^{5,36}
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3 Figure 5a illustrates the mechanism of the effect by pimoziide for the synthesis and accumulation
4 of dopamine in the cytosols of PC12 cells. Once pimoziide molecules bind to dopamine D2
5 receptors (D2Rs) on a plasma membrane, their antagonistic activity inhibits the production of
6 dopamine and the transport of dopamine vesicles.^{5,37} Therefore, pimoziide can induce the
7 reduction of dopamine release from neurons and PC12 cells. Thus, by measuring the reduction of
8 dopamine release with antipsychotic drugs via our NRC sensors, we can quantitatively evaluate
9 the effects of antipsychotic drugs on the dopamine release.
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19 Figure 5b shows the dependence of K⁺-evoked dopamine release from PC12 cells on the
20 concentrations of pimoziide. In these experiments, PC12 cells were pretreated with pimoziide with
21 different concentrations of 0.01 – 10 μ M for 24 h, followed by the stimulation of 80 mM K⁺
22 solutions. Post-stimulated solutions were then harvested and introduced onto our NRC sensors to
23 evaluate the released dopamine levels. The responses show that PC12 cells expressed decreasing
24 dopamine release with higher concentrations of pimoziide. It clearly shows that pimoziide acted as
25 an antagonist on D2Rs, resulting in the reduced K⁺-evoked dopamine release from PC12 cells.
26 Also, these results are consistent with reported results.⁵ This indicates that our NRC sensor could
27 be used as a reliable tool to monitor the effect of dopaminergic drugs as well as other biomedical
28 applications.
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45 3. CONCLUSIONS

46 We successfully demonstrated a biocompatible, simple, and flexible method for the effect
47 evaluation of antipsychotic drugs on dopamine release from PC12 cells upon the stimulation of
48 high-concentrated K⁺ solutions. In this strategy, we modified floating electrode-based CNT-FET
49 devices by a thin nafion film containing ABTS[•] radicals to build NRC sensors for dopamine
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3 detection. Using NRC sensors, we could distinguish dopamine in aqueous solutions from the
4 concentration of 10 nM and in the presence of other neurotransmitters, indicating the sensitivity
5 and selectivity of our method. In particular, the dopamine release from PC12 cells stimulated by
6 high-concentrated K^+ solutions could be quantitatively monitored by our NRC sensors.
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8 Moreover, the antipsychotic effects of pimoziide on the K^+ -evoked dopamine release from PC12
9 cells were also evaluated in a concentration-dependent manner. The biocompatible capability of
10 our method should provide a vital tool and open up various developments in biosensor research
11 and biomedical applications.
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24 **4. EXPERIMENTAL SECTION**

25 **4.1. Materials**

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28 Semiconducting single-walled carbon nanotubes (CNTs), dopamine hydrochloride,
29 nafion, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt, potassium
30 persulfate, L-glutamic acid monosodium salt hydrate, acetylcholine chloride, pimoziide and other
31 chemical reagents were purchased from Sigma-Aldrich and used as received. CNTs had the
32 diameter of 0.7 – 1.1 nm and the length of 300 – 2300 nm. PC12 cells were purchased from the
33 Korean Cell Line Bank (KCLB).
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44 **4.2. Fabrication of CNT-FET Devices**

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47 First, CNTs were dispersed in 1,2-dichlorobenzene via an ultrasonication for 5 h. The
48 concentration of a CNT solution was 0.05 mg/mL. Then, an octadecyltrichlorosilane self-
49 assembled monolayer with nonpolar terminal groups was patterned on SiO_2 substrate (3000 Å).
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51 For the selective adsorption of CNTs, the substrate was placed in the CNT solution for 10 sec
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3 and rinsed with 1,2-dichlorobenzene. The dimension of CNT network channels was 3 μm in
4 width and 170 μm in length. Afterward, metal electrodes (Pd/Au 10 nm/30 nm) were fabricated
5 using thermal evaporation and lift-off processes. Our devices had five floating electrodes, and
6 the dimension of each floating electrode was 60 μm in width and 10 μm in length. Lastly, source
7 and drain electrodes were passivated by a photoresist layer (DNR) to eliminate leakage currents
8 during electrical measurements in an aqueous environment.
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19 **4.3. Preparation of NRC Sensors**

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21 A nafion solution (0.5% in ethanol) containing 1.4 mM ABTS and 0.49 mM $\text{K}_2\text{S}_2\text{O}_8$ was
22 mixed in an ultrasonic bath for 30 min to form a coating solution. Afterward, 2 μL of the coating
23 solution containing ABTS $^{\bullet}$ radicals was directly dropped on a floating electrodes-based CNT-
24 FET device. Then, the device was dried in a nitrogen atmosphere at room temperature to form a
25 stable and thin film upon the device. Using this method, we could build NRC sensors for the
26 selective detection of dopamine in aqueous solutions (Figure 1-ii).
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38 **4.4. Surface Imaging Procedures of NRC Sensors**

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40 The optical image of an NRC sensor was taken by a microscope (XY-MRT, Sunny)
41 equipped with a 20 \times objective lens and a CCD camera (SDC-415S, Samsung). The topography
42 of a sensor surface was measured by using an AFM system (MFP-3D, Asylum Research) in a
43 tapping mode with a scan rate of 0.7 Hz. Raman scattering spectra were obtained in a
44 backscattering configuration using a Raman microprobe (XperRam 200, Nanobase) in a single
45 mode with the spectral resolution of 4 cm^{-1} . A 532 nm solid laser was used as an excitation
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3 source with the laser power of ~ 2 mW and the beam diameter of ~ 50 μm on the surface of
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5 samples.
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10 **4.5. Measurements of the Liquid Gating Effect of NRC Sensors**

11
12 A gold electrode was utilized to apply a liquid gate bias (V_{lg}) on an NRC sensor through a
13 phosphate buffered saline (PBS) solution. For liquid gating effect measurements, a gate bias was
14 swept from -1 to 1 V while a source-drain bias was maintained with 0.1 V. The source-drain
15 current of the sensor was measured by a semiconductor characterization system (Keithley, 4200,
16 USA).
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26 **4.6. Sensing Measurements**

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28 For sensing measurements, an NRC sensor was connected to a Keithley 4200
29 semiconductor analyzer, and a source-drain bias voltage of 0.1 V was maintained during the
30 measurements. The 9 μL droplet of a PBS solution was placed on the sensing region of the NRC
31 sensor, and a source-drain current was monitored in response to the additions of different target
32 materials. In our experiments, the conductance change over the original conductance of sensors
33 $\Delta G/G_0$ was used as a sensor signal.
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45 **4.7. Measurements of Dopamine Release from PC12 Cells**

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47 For the measurements of dopamine release from living cells, PC12 cells were cultured in
48 a culture dish as reported previously (Figure 1-iii).²⁴ Briefly, PC12 cells were cultured in RPMI
49 1640 Medium (A10491-01, Gibco) supplemented with horse serum (10% v/v), fetal bovine
50 serum (5% v/v), penicillin (100 U/mL), and streptomycin (100 $\mu\text{g}/\text{mL}$) at 37 $^\circ\text{C}$ in a 5% CO_2
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3 atmosphere. Before electrical measurements, PC12 cells were washed three times with a PBS
4 solution (DPBS 17-512, Lonza). Then, PC12 cell suspensions in the buffer solution with the
5 density of 1.3×10^6 cells/mL were loaded on an NRC sensor (Figure 1-iv). After that, the cells
6 stimulated by high-concentrated K^+ solutions (containing 2.5 mM Ca^{2+}) for dopamine release
7 which was monitored using our NRC sensors (Figure 1-v). The powders of calcium chloride and
8 potassium chloride were dissolved in the PBS solution to prepare stimulated solutions containing
9 2.5 mM Ca^{2+} and the different concentrations of K^+ .
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22 **4.8. Evaluation of antipsychotic drug**

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24 For the evaluation of antipsychotic drug effects, PC12 cells were cultured in 35×10 mm
25 culture dishes for 2 days. The cells were further incubated in complete growth medium
26 containing pimozide with different concentrations of 0.01 – 10 μ M for 24 h. After a medium
27 washout, the cells were stimulated by 80 mM K^+ solutions (containing 2.5 mM Ca^{2+}).
28 Subsequently, the solutions were harvested and introduced onto NRC sensors to evaluate the
29 levels of dopamine release.
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40 **ASSOCIATED CONTENT**

41 **Supporting Information**

42 Scheme for the interaction between dopamine molecules and ABTS \cdot radicals; AFM topography
43 image on the boundary of the nafion film; Additional data for the qualitative evaluation of
44 sensors such as UV-VIS-NIR and Raman spectra; Data about control experiments.
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Notes

The authors declare no competing financial interest.

ACKNOWLEDGEMENTS

This work was supported by Bio Nano Health–Guard Research Center funded by the Ministry of Science and ICT (MSIT) of Korea as Global Frontier Project (No. 2013M3A6B2078961). SH also acknowledges the support from the MSIT (Nos. 2014M3A7B4051591, 2017R1A2B2006808) and the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement No 682286).

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Figures

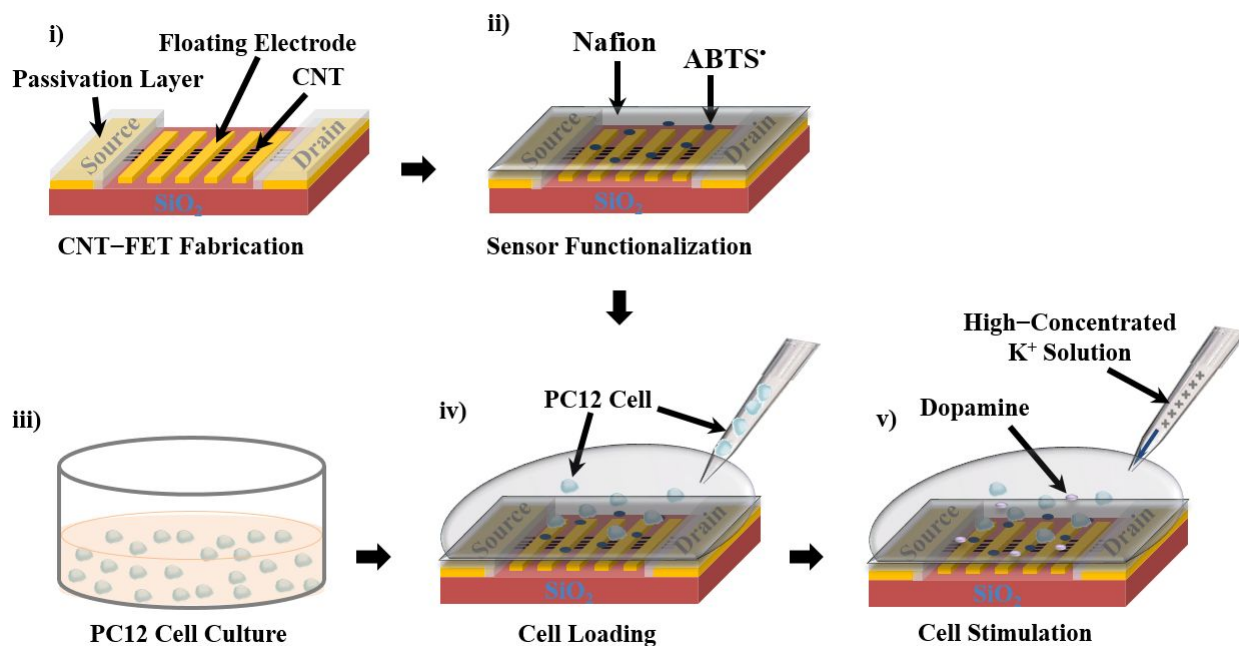


Figure 1. Schematic diagram depicting preparatory processes for the detection of dopamine by using an NRC sensor: (i) fabrication of a conventional CNT-FET device with floating electrodes; (ii) functionalization of the device by a nafion-radical hybrid film to build an NRC sensor; (iii) culturing of PC12 cells in RPMI 1640 medium; (iv) transferring of the cells into the sensor surface; (v) stimulating of the cells by a high-concentrated K⁺ solution and measuring of dopamine release by using the sensor. This sensor can selectively respond to dopamine released from living PC12 cells. The drawing is not to scale.

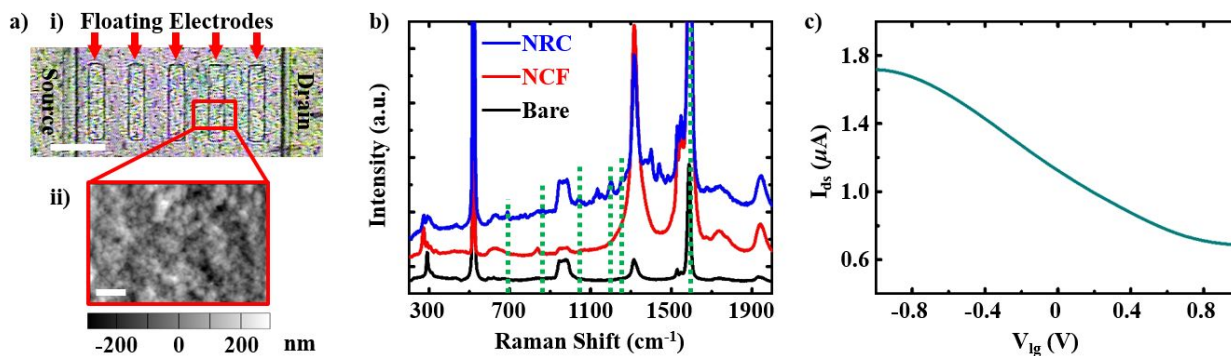


Figure 2. Characterization of a nafion-radical hybrid film on a floating electrode-based carbon nanotube sensor. (a) Optical image (i) and AFM topography image (ii) of an NRC sensor which has five floating electrodes. Each individual floating electrode had the dimension of $10 \mu\text{m} \times 60 \mu\text{m}$. The AFM image taken in a tapping mode shows the uniform surface of a sensing area. The scale bars represent $40 \mu\text{m}$ (i) and $5 \mu\text{m}$ (ii). (b) Raman spectra of the CNT regions of an NRC sensor (marked by “NRC”) and two different CNT-FET devices: a floating electrode-based CNT-FET device coated with a nafion layer (marked by “NCF”) and a bare floating electrode-based CNT-FET device (marked “Bare”). The Raman spectrum of the NRC sensor exhibits characteristic peaks of ABTS[•] radicals at the wavelengths of 690, 862, 1046, 1201, 1255 and 1592 cm^{-1} (marked by green dotted lines). (c) Liquid gating effect of an NRC sensor measured in a phosphate buffer. A gate bias was swept from -1 V to 1 V while a source-drain bias voltage was maintained at 0.1 V .

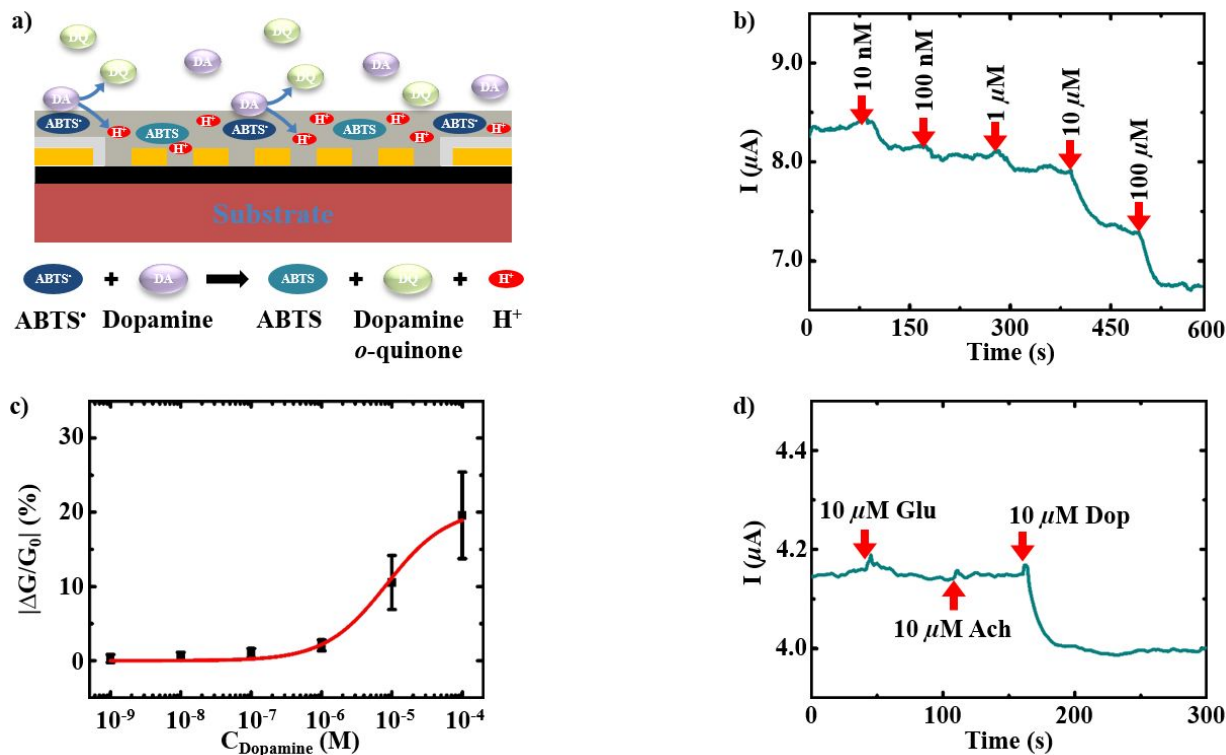


Figure 3. Responses of NRC sensors to the presence of dopamine solutions. (a) Mechanism of dopamine sensing using an NRC sensor. Once dopamine molecules react with ABTS^{•+} radicals in a nafion film on the sensor, the formation of hydrogen ions (H⁺) induces a positive field effect on the underlying CNTs. (b) Real-time electrical current measurement of an NRC sensor during the additions of dopamine solutions with different concentrations ranging from 1 nM to 100 μM . A source-drain bias voltage of 0.1 V was maintained during electrical measurements. (c) Dose-dependent responses of NRC sensors to various concentrations of dopamine. We repeated sensing measurements for five different NRC sensors to confirm the reliability. Data are expressed as means \pm SEM (standard error of the mean), ($n = 5$). (d) Real-time responses of an NRC sensor to different neurotransmitters such as Glutamate (Glu), Acetylcholine (Ach), and Dopamine (Dop). The drastic change of currents was observed only when a dopamine solution was added.

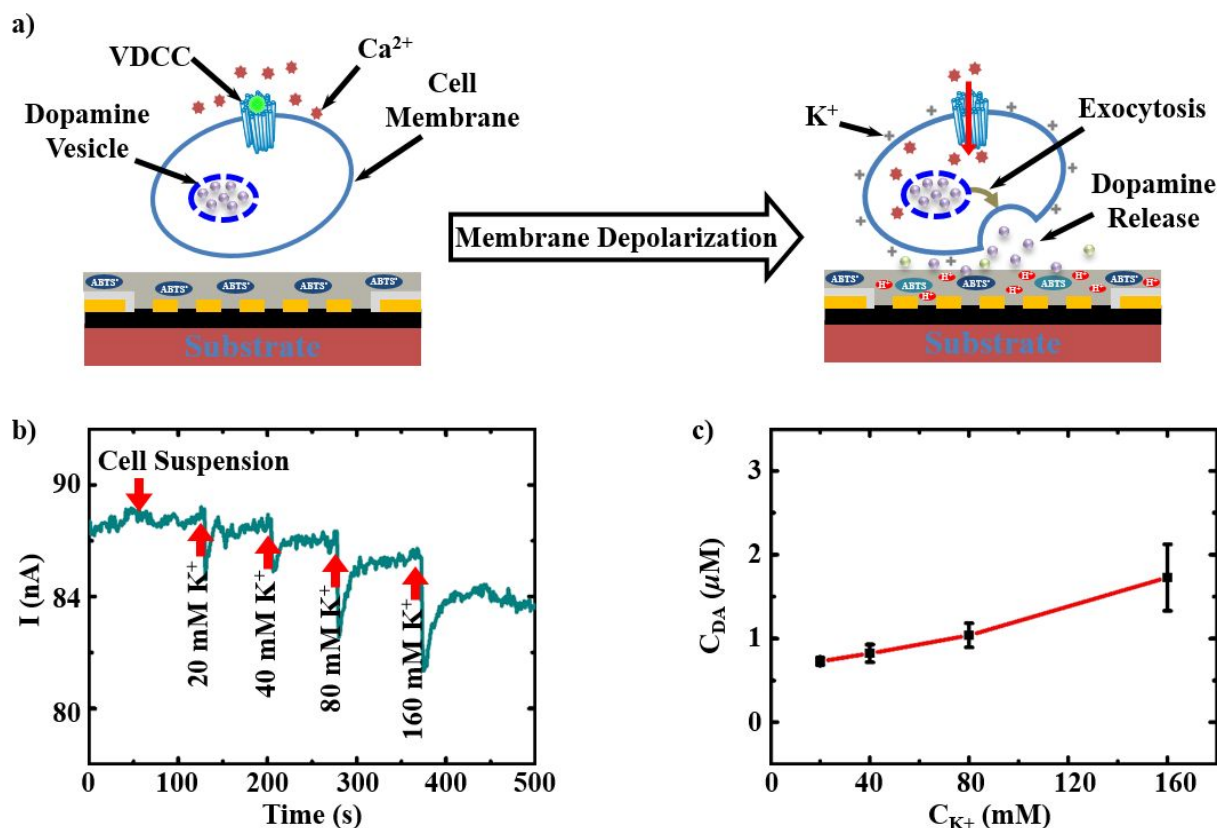
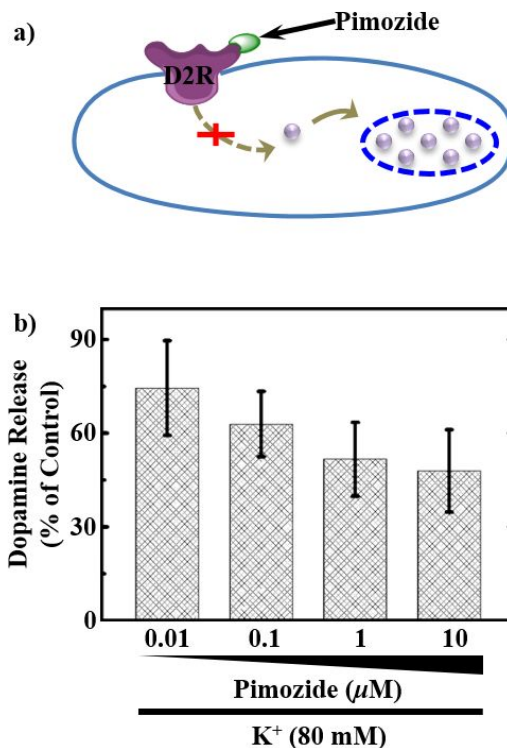


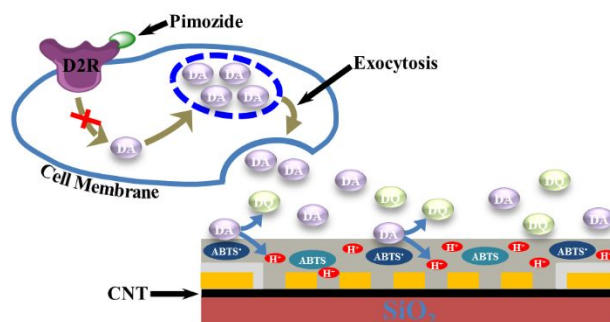
Figure 4. Quantitative monitoring of dopamine release from living PC12 cells under the stimulation of K⁺ solutions. (a) Schematic diagram showing the mechanism of dopamine release from PC12 cells stimulated by a high-concentrated K⁺ solution and the real-time monitoring of the dopamine release by an NRC sensor. (b) Real-time current changes measured by an NRC sensor during the additions of a cell suspension solution and K⁺ solutions at various concentrations. The significant reduction of currents was observed on the additions of K⁺ solutions containing 2.5 mM Ca²⁺. (c) Dose-response curve for the stimulation of different K⁺ solutions on dopamine release from PC12 cells. The concentrations of dopamine were calculated from the relative conductance changes of sensors by using the Langmuir thermal equation (eq 1). Data are expressed as means \pm SEM (n = 5).



28 **Figure 5.** Evaluation of the effects of pimoziide on K^+ -evoked dopamine release from PC12 cells.

29 (a) Schematic drawing depicting the mechanism about the effect of pimoziide on the dopamine
30 synthesis in PC12 cells. (b) K^+ -evoked dopamine release from PC12 cells pretreated with
31 pimoziide. The cells were pretreated with pimoziide at different concentrations in the range of
32 0.01 – 10 μM for 24 h, followed by the stimulation of 80 mM K^+ solutions. Pimoziide reduced the
33 production and secretion of dopamine in the cells. The bars indicate means \pm SEM.
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TOC Graphic



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