Electronic Supplementary Information for

Pesticide vapor sensing using an aptamer, nanopore, and agarose gel on a chip

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Material and methods

Device setup and electrical measurement

The chamber used for the droplet contact method was constructed according to our previous reports[1, 2]. Double wells of 4-mm diameter, 3-mm depth were aligned and connected to an electrode at the bottom. We inserted a poly(methyl methacrylate) sheet of 75-μm thickness with a 600- or 700-μm pore to increase the stability of the lipid bilayer[1]. For the analysis of solubilized omethoate, dichlorvos, methamidophos, Phenylphosphonothioic acid O-ethyl O- (p-nitrophenyl) (EPN), and acetamiprid, 4.2 μL of a 20 mg/mL 1,2-diphytanoyl-sn-glycero-3-phosphocholine (DphPC) *n*-decane solution was applied in each well, and 21 μL of buffer A (2 mM KH_2PO_4, 0.8 mM K_2HPO_4, 1 M KCl, pH 7.4) was applied to the *trans*-side well. Another 21 μL of buffer with alpha-hemolysin (30 nM) and the DNA aptamer (10 μM) was applied to the *cis*-side well. For detection of vaporized omethoate, 0.1% (wt/vol) L03 agar (TakaraBio, Japan) was dissolved in buffer A and melted by microwave oven. The melted gel was cooled to room temperature, mixed with the DNA aptamers (10 μM) and alpha-hemolysin (3 nM), and then applied to the *cis*-side well before it solidified. To the *trans*-side well, 4.2 μL DphPC/*n*-decane, followed by 21 μL buffer A were applied. The ionic current was measured by a patch-clamp amplifier (Nihon Koden, CEZ-2400) and a digital data acquisition system (Axon Instruments, Digidata 1550), or multi-patch-clamp amplifier (JET-Bilayer, Tecella). The conditions for detection were as follows: 100 mV, low-pass filter set to 1 kHz, and a sampling period of 200 μs.

Optical and electron microscopic imaging

A digital microscope (Yashima Optical, YDZ-3F) system was used for imaging the lipid bilayer in the aperture. Scanning electron microscopy (JEOL, JCM-6000) was used to obtain an enlarged view of the separator in low-vacuum mode, by applying 15 kV to the electron beam.

Exposure to vaporized omethoate and analysis of absorption in the agarose gel

Vaporized omethoate was prepared by heating a 1–2 μL solution of omethoate for 30 min in a container. For the measurement of absorbed omethoate in the agarose gel, 50 μL of 0.1% (wt/vol) L03 agarose (TakaraBio, Japan) was dissolved in water by heating and then poured into a

plastic tube. In parallel, 50 μL of ultra-pure water was also placed in a tube and layered by 10 μL of *n*-decane. These tubes were placed in the container described above for 0, 5, and 10 min. Gas chromatography (GC2010, Shimadzu, Japan) was used for measurement of omethoate, with an Rxi-5Sil MS column (30 m, Shimadzu, Japan) and a flame photometric detector with the band-pass filter for phosphorus. The inlet temperature was 150°C and the detector temperature was 300°C. The column oven temperature was programmed to elevate from 100 to 200°C at 20°C/min, and then to 280°C at 10°C/min.

Evaluation of blockage signals

Blockages of the ionic current were evaluated according to methods described previously[3, 4], using a pCLAMP software (Molecular Devices, Sunnyvale, CA, USA). When the free DNA aptamer was applied, we observed two types of blocking signals, >80% blockage in a <510 ms retention time (Fig. S1a), and ~80% blockage over a wide range of retention times (~15,000 ms) (Fig. S1b). The observation of >80% blockage represents the smooth translocation of the DNA aptamer, with high blockage indicating penetration of the DNA aptamer through the transmembrane pore. By contrast, the observation of <80% blockage represents the congestion of the DNA aptamer in the internal cavity of the alpha-hemolysin structure[5]. As the congested DNA aptamer cannot translocate through the nanopore, it generally cannot be used for nanopore sensing. These congested DNA aptamers would leave the nanopore stochastically, which explains the variable retention times. When omethoate was applied along with the DNA aptamer, we observed the same signals as described above, as well as an additional type of signal, which was >80% blockage in a retention time of >510 ms (Fig. S1c). This signal represents the clogging of the DNA aptamer due to the bulky complex structure.

Designing the DNA aptamer

The sequence of the DNA aptamer was designed by combination of the omethoate-binding sequence[6] and 27 bases of a cytosine homopolymer (C chain) at the 3' teriminus (5'-AAGCTTTTTTGACTCACTGCAGCGATTCTTGATCGCCACGGTCTGAGGAAAGAGCCCCCCCCCCCCCCCCCCCCCCCCCCC-3'). In this manner, when the DNA aptamer forms a complex with omethoate, the omethoate-binding domain forms a secondary structure, whereas the C chain is maintained in the single-stranded form. Following the applied voltage, the C chain is guided to translocate through the nanopore, and the omethoate-binding domain will halt the translocation, and generate the "clogging" signals. This DNA aptamer could form a secondary structure (Fig. S2) without application of omethoate (ΔG = -6.72 kcal/mol), which is assumed to be the reason for the relatively slow velocity

of translocation (Fig. 3a-d).

Fitting analysis

We verified the results of the correlation between duration time for omethoate detection and omethoate concentration (Fig. 3f) by fitting the data with the Poisson distribution. As the DNA aptamer-omethoate complex (λ: Dashed line in Figure 3f) can be calculated by DNA aptamer (10 μM), omethoate (5 nM – 1 mM), and the constant dissociation value (Kd = 2 μM)[6], the probability of the translocating number (k) of the DNA aptamer complex in a constant nanopore region can be described according to the following formula:

$$P(k) = \frac{(\lambda^k\ e^{-a\lambda})}{k!},$$

where *a* represents the coefficient number of translocation of the complex through the nanopore. Therefore, the probability that the complex does not translocate (k=0), can be written as

$$P(0) = e^{-a\lambda}.$$

The detection of the omethoate signal requires the translocation of at least one aptamer complex; thus, the probability of translocation of the DNA aptamer complex through the nanopore is

$$P(\geq 1) = 1 - e^{-a\lambda}.$$

Finally, the reciprocal of this value represents the duration required for omethoate signal detection.

We found that the fitting curve correlated with our experimental results, implying the stochasticity of the translocation of aptamer-omethoate complex.

Figures

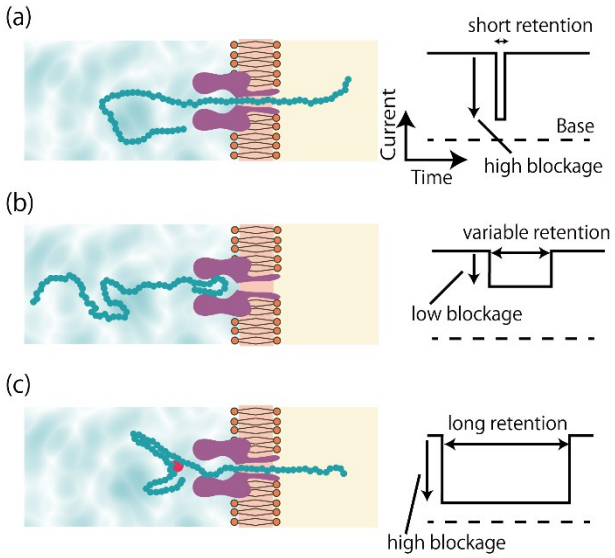

Fig. S1. Blocking of the ionic current observed by DNA translocation through the nanopore could be categorized in three types. The left column shows the conceptual scheme of the state of the DNA aptamer, and the right column shows the recording pattern of ionic current in each blocking event. (a) Smooth translocation and (b) congestion were the typical patterns when the free DNA aptamer was applied, as observed with previously reported nanopore sensing systems[7, 8]. (c) When the complex of the DNA aptamer and omethoate was applied, cloggings were observed. Unlike previous reports utilizing the DNA aptamer[9], our result showed the stochastic dissociation of the DNA aptamer and omethoate complex; thus, the blocking was not permanently observed.

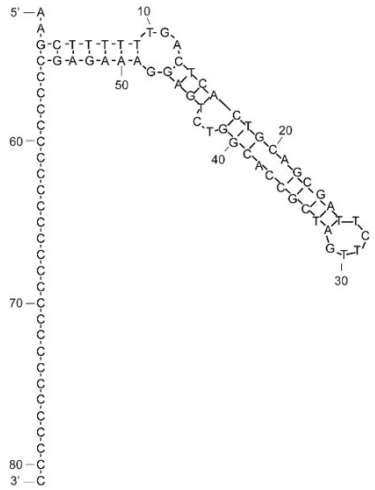

Fig. S2. Secondary structure predicted by the *mfold Web Server* (URL: http://unafold.rna.albany.edu/?q=mfold/dna-folding-form). The sequence of the DNA aptamer was input, and showed the possible formation of the indicated secondary structure.

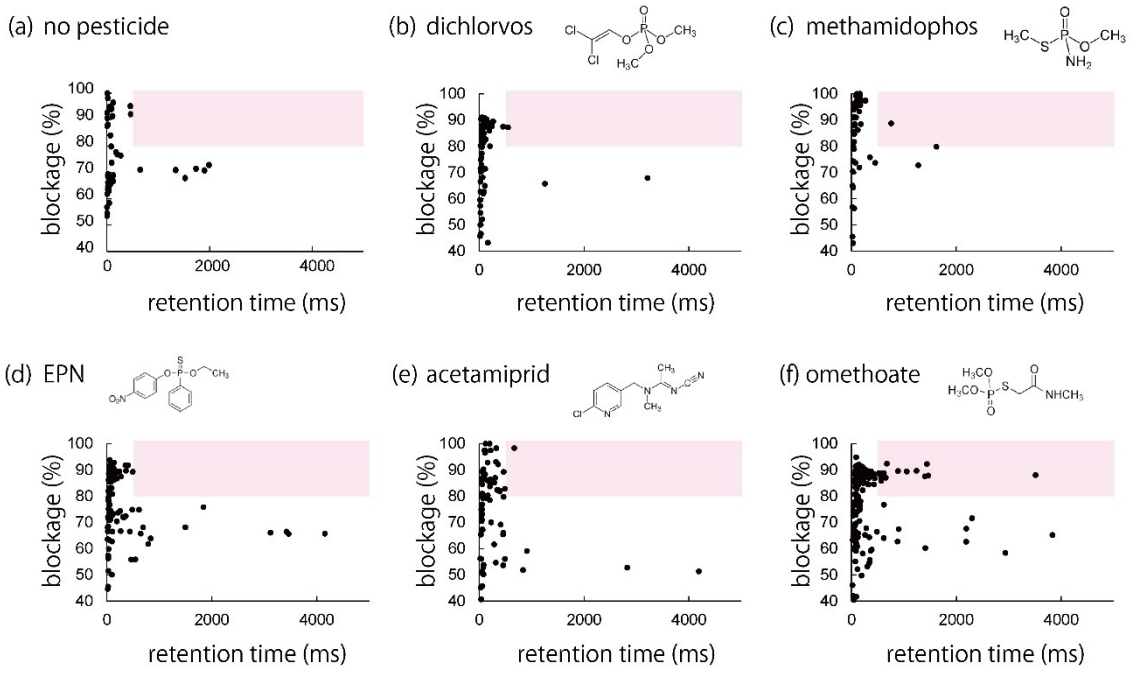

Fig. S3. Blockage ratio against retention time, obtained by applying (a) no pesticide, (b) 100 µM dichlorvos (F.W. 220.98), (c) 100 µM methamidophos (F.W. 141.13), (d) 100 µM EPN (F.W. 323.303), (e) 100 µM acetamiprid (F.W. 222.67), and (f) 5 µM omethoate (F.W. 213.193). Chemical structures are shown in top of each plot. The dots within the shaded area correspond to the criteria zone (>80% blockage, >510 ms retention).

References

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