Supplementary Information for

Screen-printed endotoxin sensor based on amperometry using novel *p*-aminophenol conjugated substrate for Limulus amebocyte lysate protease reaction

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1. Explanation for exchanging Toxipet Plate to Stripwell Plate after incubation for GC measurements

As described in section 2.4, we transferred the sample from a Toxipet Plate to a Stripwell Plate after incubation for GC measurements according to the following reason. The Stripwell is preferable for use as an electrochemical measurement cell containing 100–300 µL solutions because it can be easily split into small segments. However, as described below, yellow color development of ES-24S solutions proceeds in the Stripwell without endotoxin, which is probably due to the hydrolysis of LGR-pNA induced by the material of the well. The same phenomenon should potentially occur with LGR-pAP. Therefore, we used the Toxipet Plate instead of the Stripwell for the reaction of ES-24S to avoid the increase of background signal by non-endotoxin-induced hydrolysis of LGR-pAP. Then, we transferred the sample to a Stripwell for the easy electrochemical measurement setup. Although the cause of this phenomenon is unknown, similar problem may occur in other *in vitro* protease assays.

The experiment to check the non-endotoxin-induced color development with Stripwell was conducted as below. We prepared seven ES-24S test vials without endotoxin by adding 200 µL of assay buffer and 200 µL of endotoxin free water in each test vial containing freeze-dried LAL reagents. Then, we transferred half of the solution of each vial (200 µL) to the Stripwell at time = 0 min. All seven test vials with the remaining 200 µL of reaction solution and the Stripwells were incubated at 37 °C. At predetermined times (time = 0, 30, 60, 120, 150, and 180 min), the solution in a test vial and a Stripwell were exchanged and incubation was resumed. After the last solution exchange at 180 min, each of the solutions in the 7 test vials was transferred to a new well of the Stripwell. The yellow color development of 14 samples (7 samples were exchanged from test vial to Stripwell and 7 samples were exchanged from Stripwell to test vial) were measured by a plate reader (BioRad Model 680 Microplate Reader, BioRad, USA) as a function of absorbance at 405 nm.

Figure S1 shows the result of this experiment. As shown in Fig. S1, the yellow color development increased with incubation time in the Stripwell. This color development was not induced by proteases activated with endotoxin but induced for some other reasons associated with the well material because the solutions exchanged from the Stripwell to the test vial showed no further color development (Fig. S1, closed circle).

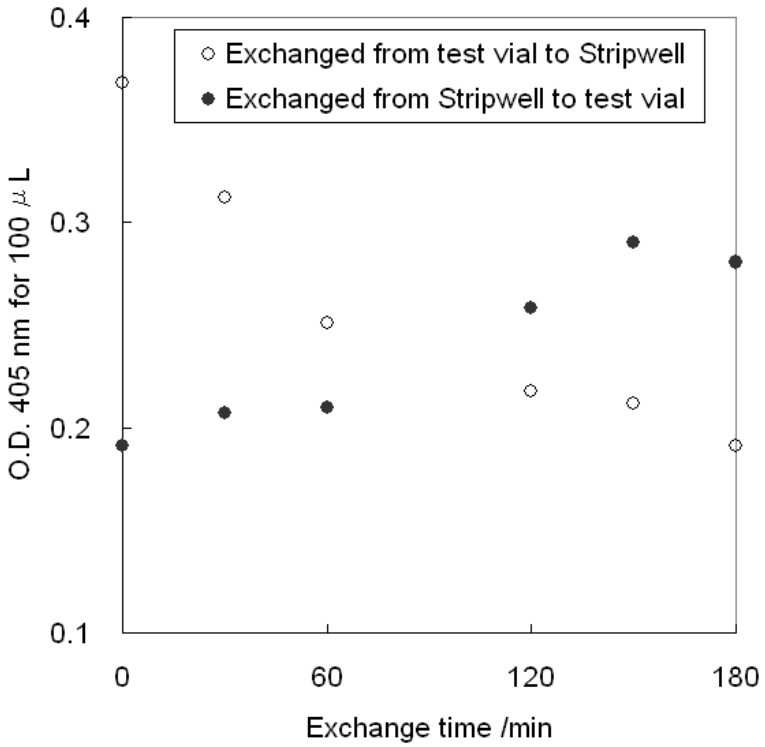

Figure S1. Yellow color development depending on the incubation time in the Stripwell for the ES-24S sample solutions without endotoxin. Half of the sample solution prepared in an ES-24S vial was incubated in the vial (open circle) and the other half was incubated in the Stripwell (closed circle). The solutions were exchanged with each other after 0, 30, 60, 120, 150, or 180 min of incubation. After the total 180 min incubation, 405 nm absorbance was measured for each 100-μL sample.

2. Determination of diffusion coefficient of pAP for the COMSOL simulation

We determined the diffusion coefficient of pAP used for the COMSOL simulation by fitting the experimentally obtained amperogram with a GC electrode to the COMSOL simulation. The experimental curve was obtained according to the method described in section 2.4. After a 60 min incubation of a 1:1 mixture of ES-24S reagents containing 1.0 mM LGR-pAP and 1000 EU/L endotoxin, PSCA was conducted with a GC electrode. The geometry used for simulation is shown as an inset of Fig. S2.

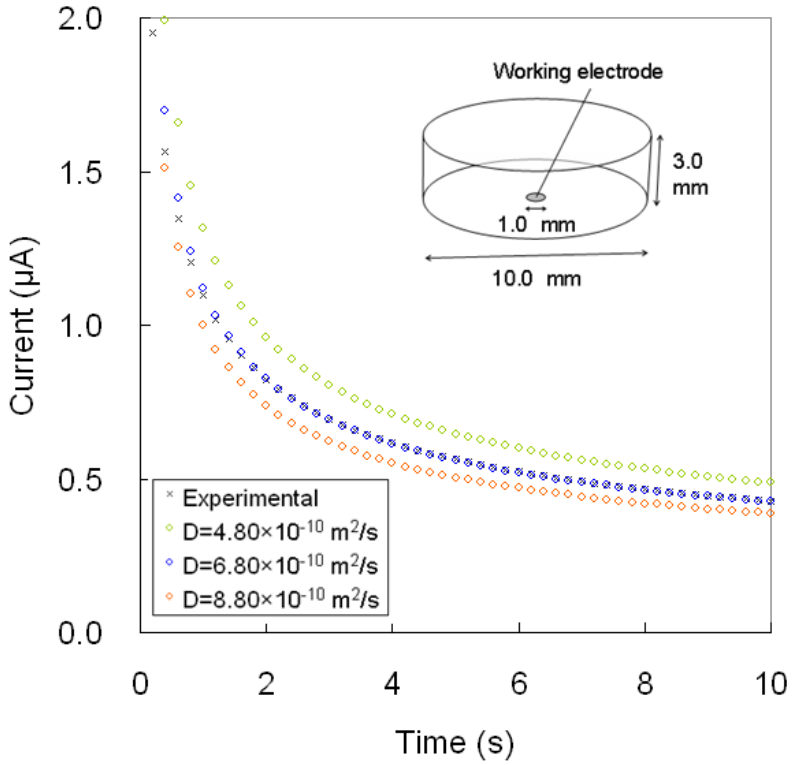

Figure S2. Curve fitting of the experimental data to the COMSOL simulation to determine the diffusion coefficient of pAP.

3. Effect of the height of the reaction chamber on simulated calibration line for the chip sensor employing currents at various times after the potential step as signals.

We simulated calibration lines with COMSOL Multiphysics by employing signals that occur 1–20 s after the potential step. The conditions for the simulation were same as described in section 2.6 and Fig. 7A. As shown in Fig. S3, by employing a current at 2 s after the potential step as a signal, the height of the reaction chamber (> 0.075 mm) does not significantly affect the signal.

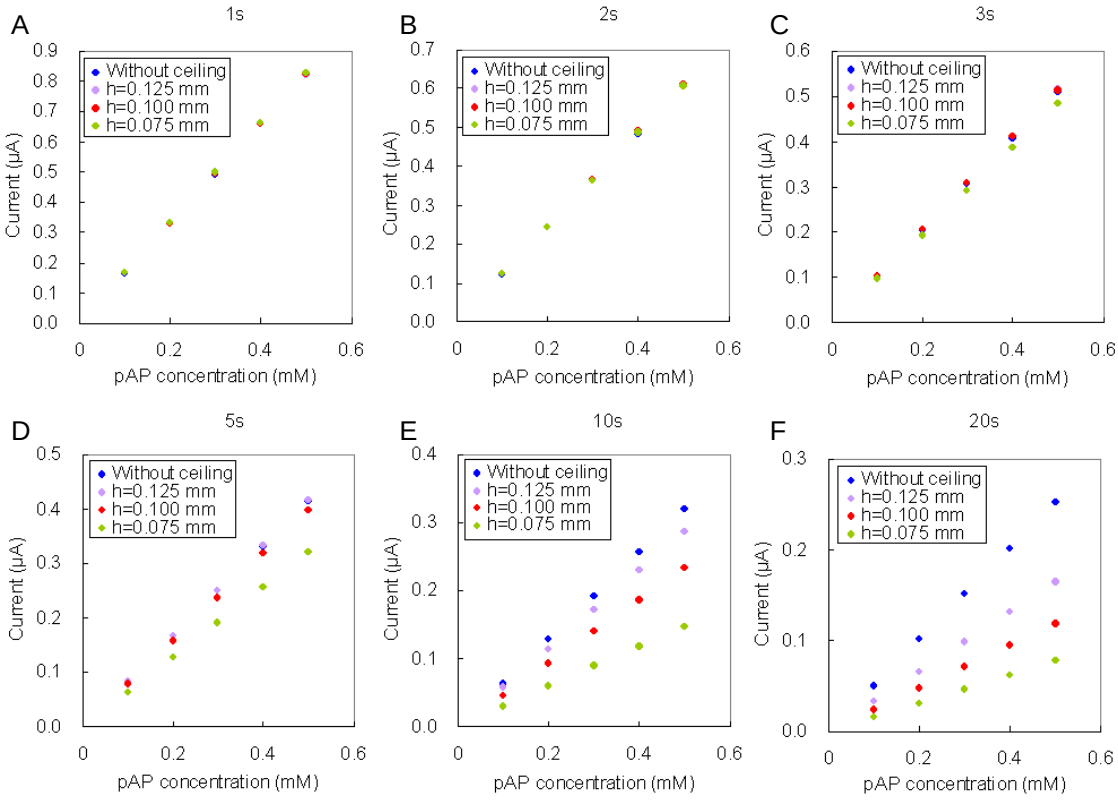

Figure S3. Simulated calibration lines for the chip sensor with various heights of the reaction chamber (h = 3.0 mm (free diffusion without ceiling), 0.125 mm, 0.100 mm and 0.075 mm in Fig. 7A). Currents at 1s (A), 2 s (B), 3 s (C), 4 s (D), 5 s (E), and 6 s (F) after the potential step serving as the signal.

4. Comparison of experimental data and simulated curve for amperometry with the chip sensor.

We compared the chip sensor's experimentally obtained amperogram to simulated curves. The experimental curve was obtained according to the method descried in section 2.4. After a 60-min incubation of a 1:1 mixture solution of ES-24S reagents containing 1.0 mM LGR-pAP and 1000 EU/L endotoxin, a 10 µL portion of the reacted solution was placed onto a chip sensor, then a PSCA scan was obtained. PSCA was performed with the potential step from the 0.0 V to 0.4 V. The conditions for simulation were the same as described in section 2.6 and Fig. 7A.

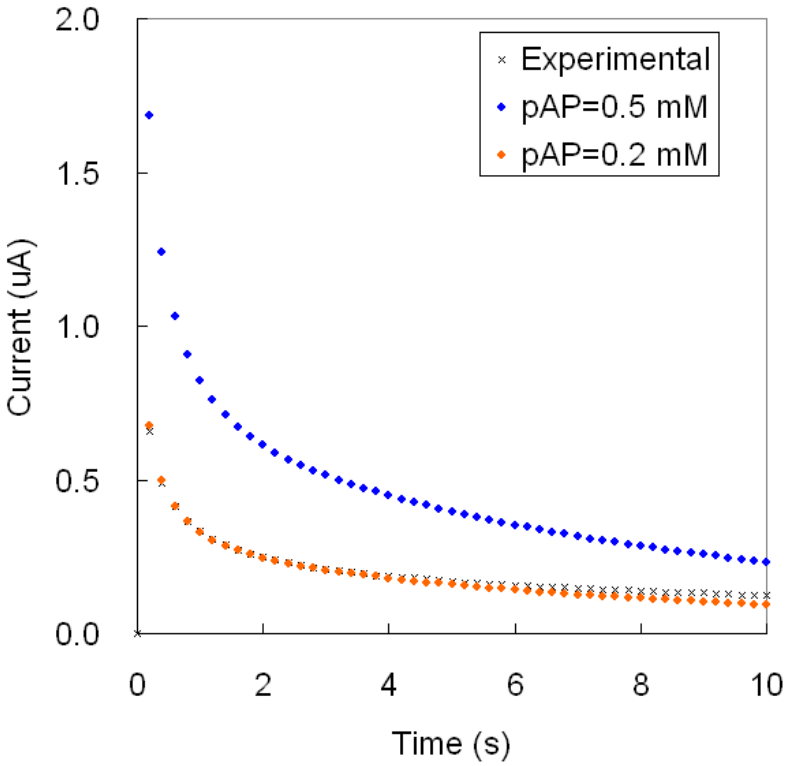

Figure S4. Comparison of experimental data and simulated curve for amperometry with the chip sensor.