Supporting Information

Molecular design for enhanced sensitivity in FRET aptasensor built on graphene oxide surface

Yuko Ueno,*[a] Kazuaki Furukawa,[b] Kota Matsuo [a], Suzuyo Inoue [a], Katsuyoshi Hayashi [a] and Hiroki Hibino[b]

[a] *NTT Microsystem Integration Laboratories, NTT Corporation, 3-1 Morinosato Wakamiya, Atsugi, Kanagawa, Japan. Fax: +81-46-240-4728; Tel: +81-46-240-3549; E-mail: ueno.yuko@lab.ntt.co.jp*

[b] *NTT Basic Research Laboratories, NTT Corporation*

Effect of 5' spacer

To examine the effect of introducing a 5' spacer, we prepared two 2×3 linear-array rGO aptasensors using T0/T0, T10/T0 and T10/T10 (chip 1) and T0/T0, T0/T10 and T10/T10 (chip 2) with the procedure shown in Fig. 2.

Figure S1a shows the fluorescence images obtained with the 2×3 linear-array sensors after injecting thrombin solution and water into the top and bottom microchannels, respectively. The three green lines in the top channel correspond to the positions where T0/T0, T10/T0 and T10/T10 (chip 1) and T0/T0, T0/T10 and T10/T10 (chip 2) were fixed, at left, centre, and right, respectively. The same probes were also fixed in the bottom channel, however, little fluorescence was observed. Figure S1b shows the average fluorescence intensity of the patterned area in the top microchannel. Since T0/T0 and T10/T10 were measured using both chips 1 and 2, the intensities for T0/T0 and T10/T10 were obtained by averaging the data for chips 1 and 2. By merging the data for chips 1 and 2, we can quantitatively compare the fluorescence intensities of four different probes. The fluorescence intensities were almost the same for T0/T0 and T10/T0 (without a 3' spacer), and T0/T10 and T10/T10 (with a 3' spacer), respectively.

The result indicates that with or without a 3' spacer, the 5' spacer has little effect on the fluorescence intensity.

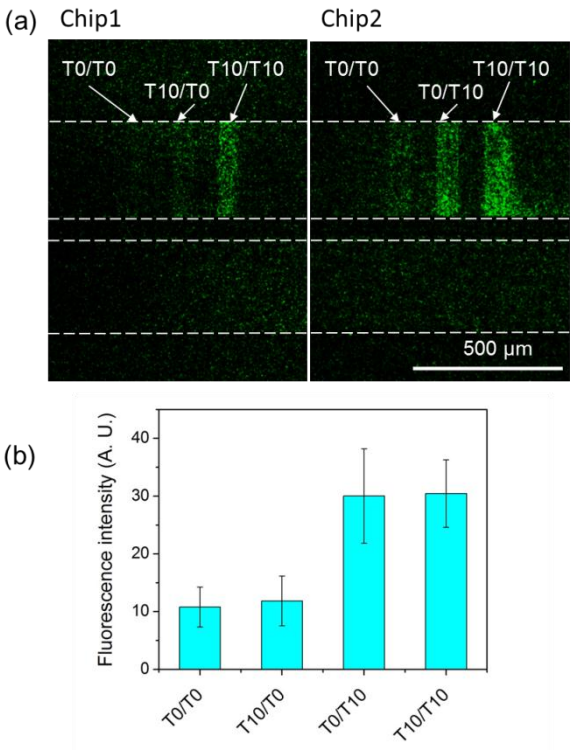

Fig. S1 (a) Fluorescence image of 2×3 multichannel linear-array aptasensors patterned with T0/T0, T10/T0 and T10/T10 (chip 1, left) and T0/T0, T0/T10 and T10/T10 (chip 2, right). Thrombin solution (100 μg/mL) and water were injected into the top and bottom microchannels. (b) Comparison of the average fluorescence intensities of the patterned area in the top microchannel. The error bars corresponds to the standard deviations. The data for chips 1 and 2 were merged.

/Estimated dependence of the fluorescence recovery efficiency on the spacer length

The FRET recovery estimation is based on the following equation

$$E_{FRET} = \frac{1}{1 + \left(\frac{d}{d_0}\right)^4}$$

where E_{FRET}, d and d_0, respectively, represent the FRET efficiency, the distance between a molecule and the GO surface, and the characteristic distance corresponding to the Förster radius. The efficiency E_{FRET} is related to the 4th power of the distance d between a molecule and a 2-dimensional sheet such as GO.

Figure S2 plots E_{FRET} vs. d at d_0 = 7.5 nm, indicating that the change in E_{FRET} is non-linear. When $d \ll d_0$, or d is less than 4 nm, which is the size of thrombin [12], the change in E_{FRET} in response to the small change in d, Δd, is more for the increase of d than for its decrease.

The designed probe consists of an aptamer part and a spacer part as shown in Figure S3. There are two parameters d_{T0} and R_g for the fluorescence recovery efficiency (E_{re}) estimation. The distance d_{T0} could be unique when the biomolecular probe is tethered to a GO surface. However, d_{T0} is not determined at this stage, and thus we consider d_{T0} to be a parameter. The parameter d_{T0} is the dye-to-GO surface distance at the aptamer-thrombin complex formation. As it is known that the thrombin aptamer has a G quartet structure for a complex formation, we assumed that d_{T0} would be in the 2.0 to 3.5 nm range. This is less than the thrombin size of 4 nm. The parameter R_g is a gyration radius determined by N under the self-avoiding random walk model.

For the numerical calculations the dye is assumed to distribute equally on the surface of a hemisphere of radius R_g (Figure S3). If $R_g > d_{T0}$, part of the hemisphere overlaps the substrate area, which is unrealistic. In the case, we set E_{FRET} = 1 because the dye could stay in the vicinity of the GO surface.

We calculated E_{FRET} in 0.5 nm steps and then averaged the results to obtain the average efficiency for different d_{T0} and R_g values. Finally, we subtracted the averaged efficiency from 1 to give E_{re}, the fluorescence recovery efficiency shown in Table 1.

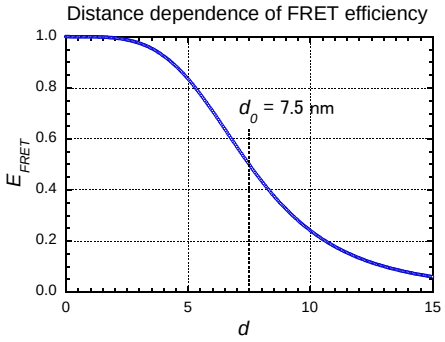

Fig. S2 Plot of E_{FRET} vs. d with d_0 = 7.5 nm.

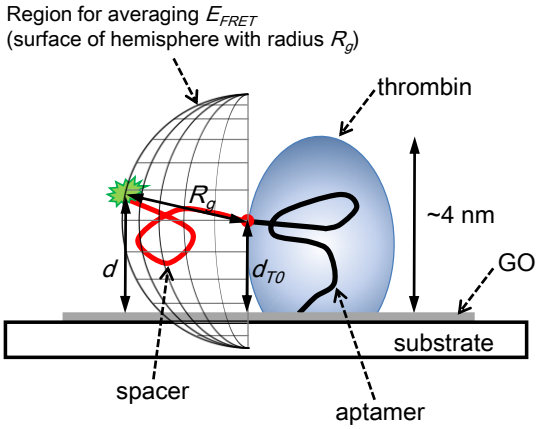

Fig. S3 Calculation model for estimating average E_{FRET} at different d_{T0} and R_g values.

Detection limit using T0/T20

We evaluated the detection limit for thrombin by using an on-chip dual-channel aptasensor prepared with a T0/T20 probe. We prepared the sensor without patterning. Figure S4 shows the fluorescence image around the border of the two microchannels obtained by using a water-immersion objective lens Plan Apo 40× WLSM. The fluorescence in the top channel, which was filled with the thrombin solution (~1 nM, 37 ng/mL), was brighter than that in the bottom channel, which was filled with water. The result demonstrates that we successfully detected ~1 nM (37 ng/mL) of thrombin, which is in the *in vivo* concentration range during blood clotting [11].

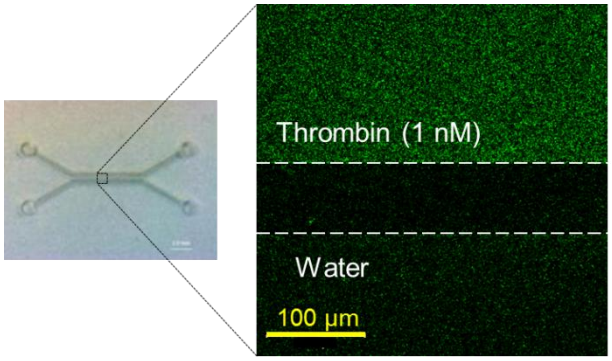

Fig. S4 Fluorescence image of the dual-channel aptasensor prepared with a T0/T20 probe after injecting thrombin solution (~1 nM, 37 ng/mL) and water into the top and bottom channels obtained in an area including two whole microchannels.

Reference

12 S. Rinker, Y. Ke, Y. Liu, R. Chhabra, and H. Yan, *Nat. Nanotechnol.*, **2008**, 3, 418.