Supporting Information

High Sensitive Detection of Organic Molecules on the basis of a Poly(*N*-isopropylacrylamide) Micro-assembly Formed by Plasmonic Optical Trapping.

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Experimental section

For the trapping target, poly(*N*-isopropylacrylamide) (PNIPAM) (Polymer Source Inc., Mw = 15.7×10^4) was dissolved in distilled and deionized water (polymer concentration: 3.5 wt%). For the analyte molecules, rhodamine B (RhB), acid red 88, crystal violet, pyrene, and *o*− and *p*−chlorophenol were purchased from Tokyo Chemical Industry Co., Ltd. The analytes except for pyrene were dissolved in polymer solutions with varying analyte concentrations. For pyrene, a dilute ethanol solution of pyrene was added to a polymer aqueous solution. The ethanol content was much low (0.5 % (v/v)), which hardly affected the micro-assembly formation of PNIPAM.[1] Gold nanopyramidal dimer arrays on a glass substrate were used for the plasmonic nanostructure.[2] The substrate was fabricated by an angle-resolved nanosphere lithography technique.[3,4] Upon resonant excitation of plasmon using 808 nm laser light, the electromagnetic field was estimated to have been enhanced by a factor of ~ 10^4 in $|\boldsymbol{E}|^2/|\boldsymbol{E_0}|^2$. Detailed properties of the plasmonic substrate have been reported elsewhere.[2]

The method requires 50 μl of the analyte/PNIPAM solution to be placed between a glass slide with holes (maximum depth ~ 300 μm) and the plasmonic substrate. Analytes in the polymer micro-assembly are detected using microspectroscopic systems as previously described.[2,5,6] Briefly, the light source for plasmon excitation is a continuous wave (cw) near-infrared (NIR) laser (λ = 808 nm). The laser light intensity is adjusted to 1.0 ~ 10 kW/cm^2 at the focal point. Fluorescence and Raman spectra are obtained using cw visible laser irradiation (λ = 375, 473, or 532 nm). These laser beams are coaxially introduced into an inverted microscope (NIKON, Ti-U), focusing onto the plasmonic nanostructures with an oil-immersion objective lens (× 100, *N.A.* = 1.40). The visible laser beams are tightly focused at the center of the NIR irradiation area. Raman/fluorescence signals are detected with a cooled CCD camera (Andor Tec.).

Fluorescence measurements of rhodamine B during near-infrared irradiation to plasmonic nanostructures

In order to investigate fluorescence of rhodamine B (RhB) via 2-photon absorption by plasmon, we carried out fluorescence measurements with varying near-infrared (NIR, λ = 808 nm) laser intensity (Figure S1). Figure S1(a) shows a reference spectrum of 1.0×10^{-5} mol/L RhB in 3.5 wt% poly(*N*-isopropylacrylamide) (PNIPAM) on the plasmonic nanostructure obtained only by visible laser excitation (λ = 473 nm) (it is obtained without NIR irradiation). Figure S1(b) – (f) show fluorescence spectra obtained by NIR laser excitation (1.0 – 11 kW/cm^2) without visible laser irradiation. The intensities were normalized at maximum fluorescence intensity in Figure S1(a). These figures clearly indicate that fluorescence via 2-photon absorption never occurred in our experimental conditions. It should be noted that 1-photon/ 2-photon fluorescence enhancements by plasmon are not easily observed since fluorescence quenching should take place competing with the enhancing.

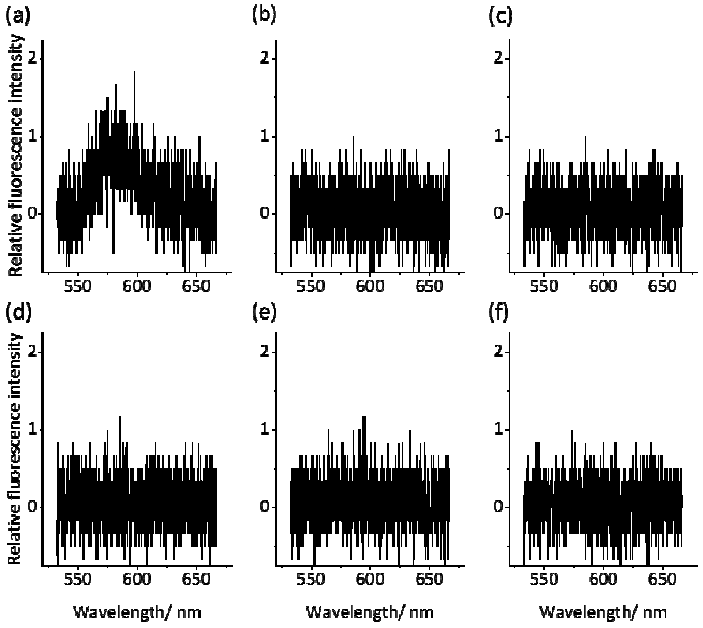

Figure S1 (a) Fluorescence spectrum of RhB excited by visible laser and (b-f) those with NIR laser excitation: (b)1.0, (c) 2.0, (d) 4.0, (e) 7.0, and (f) 11 kW/cm^2.

Relative fluorescence intensity of rhodamine B dependent on PNIPAM concentration

Figure S2(a) and (b) show temporal profiles of relative fluorescence intensity (fluorescence intensity normalized at that without plasmon excitation, corresponding to fluorescence enhancement factor) during plasmonic optical trapping (POT) of PNIPAM with different concentration ((a) 0.1 wt% and (b) 10 wt%). At 0.1 wt% of PNIPAM solution (Fig. S2(a)), relative fluorescence intensity gradually increased with plasmon excitation time due to slow growth of PNIPAM micro-assembly formed by POT. On the other hand, at 10 wt% (Fig. S2(b)), relative fluorescence intensity rapidly reached a maximum in a few seconds. The relative fluorescence intensity reached to 10 within one second. Thus, we found that the velocity of the relative fluorescence intensity to be constant increased with PNIPAM concentration.

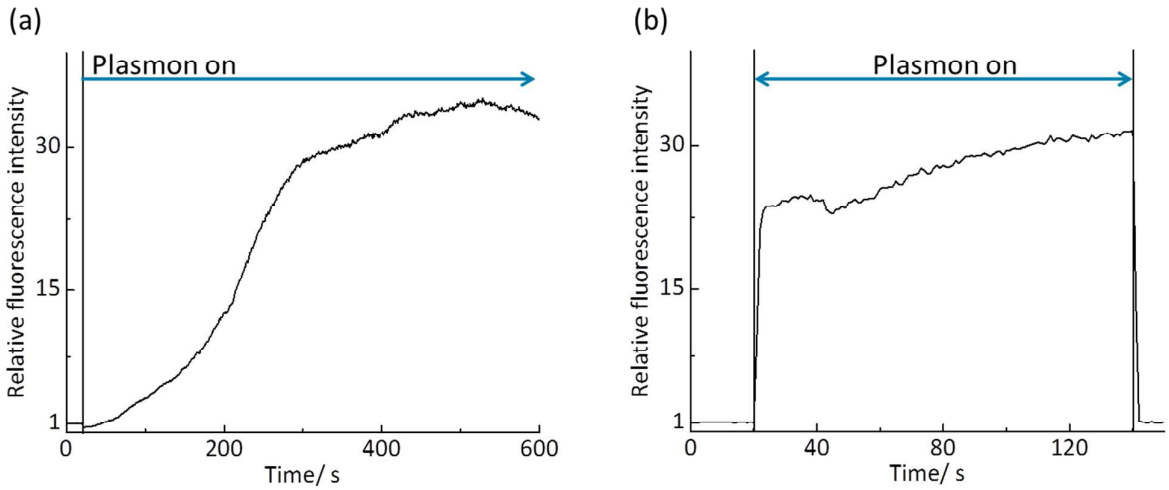

Figure S2 Temporal profiles of relative fluorescence intensity for RhB (1.0×10^{-5} mol/L) during plasmon excitation in (a) 0.1 wt% and (b) 10 wt% PNIPAM solution. Plasmon excitation intensity was 4.0 kW/cm^2.

Fluorescence detection of rhodamine B extracted into a PNIPAM micro-assembly (Mw = 4.8 × 10^4)

We used a PNIPAM with Mw = 4.8 × 10^4 (Polymer Source Inc.) only in this section, whose molecular weight was smaller than that used in other experiments (Mw = 15.7×10^4). Figure S3 shows relative fluorescence intensity of RhB extracted into the PNIPAM micro-assembly formed by POT, as a function of excitation intensity of plasmon. The fluorescence intensity increased with the increase of plasmon excitation intensity. This result would be caused by the increment of the number of trapped PNIPAM with an increase in plasmon excitation intensity. Since an enhanced optical force decreases with the size of nanoparticles, higher plasmon excitation intensity (20 kW/cm^2) was required for stable trapping of the smaller sized PNIPAM.[7] Increasing the number of trapped PNIPAM with plasmon excitation intensity, RhB molecules extracted into the micro-assembly also increased. The fluorescence intensity with PNIPAM (Mw = 4.8×10^4) became higher than that with the other PNIPAM (Mw = 15.7×10^4).

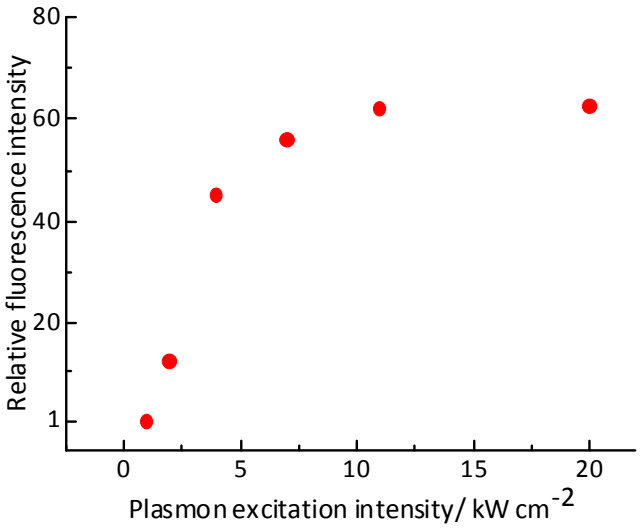

Figure S3 Plasmon excitation intensity dependence of the fluorescence intensity. Rhodamine B (1.0 × 10^{-5} mol/L) was dissolved in a 3.5 wt% PNIPAM (Mw = 4.8 × 10^4) aqueous solution. The fluorescence intensities are normalized to that without plasmon excitation.

Plasmon excitation intensity dependence of relative fluorescence intensity at different concentration of rhodamine B

Figure S4 shows relative fluorescence intensity dependent on RhB concentration at 4.0 (black) and 7.0 kW/cm^2 (red) in 3.5 wt% PNIPAM. We successfully demonstrated fluorescence detection of RhB extracted into PNIPAM micro-assembly irrespective of RhB concentration, since the volume of PNIPAM micro-assembly formed by POT (~ pL order) was quite smaller than that of aqueous solutions (50 μL). Here we estimated the local concentration of RhB in a PNIPAM micro-assembly at 4.0 kW/cm^2 of plasmon excitation. We assumed that the molecular absorption coefficient and fluorescence quantum yield of RhB in a PNIPAM micro-assembly were regarded as those in ethanol. We estimated the concentration: 1.0×10^{-7} (1.0×10^{-8} mol/L in solution), 9.8×10^{-7} (1.0×10^{-7} mol/L), and 1.1×10^{-4} mol/L (1.0×10^{-5} mol/L), respectively. The concentration in a PNIPAM micro-assembly became ca. 10 times higher than that in water. This simple estimation was in good agreement with the condensation ratio reported by Katayama group (3.4 times).[8]

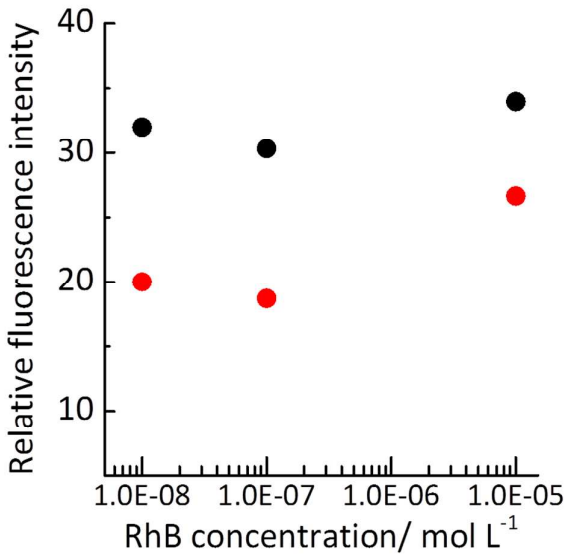

Figure S4 RhB concentration dependence of relative fluorescence intensity at (black) 4.0 and (red) 7.0 kW/cm^2 of plasmon excitation intensity. PNIPAM concentration is 3.5 wt%.

References

(1) Saitoh, T.; Yoshida, Y.; Matsudo, T.; Fujiwara, S.; Dobashi, A.; Iwaki, K.; Suzuki, Y.; Matsubara, C. *Anal. Chem.* **1999**, *71*, 4506–4512.

(2) Tsuboi, Y.; Shoji, T.; Kitamura, N.; Takase, M.; Murakoshi, K.; Mizumoto, Y.; Ishihara, H. *J. Phys. Chem. Lett.* **2010**, *1*, 2327–2333.

(3) Hulteen, J. C. *J. Vac. Sci. Technol. A* **1995**, *13*, 1553–1558.

(4) Nagasawa, F.; Takase, M.; Murakoshi, K. *J. Phys. Chem. Lett.* **2014**, *5*, 14–19.

(5) Shoji, T.; Saitoh, J.; Kitamura, N.; Nagasawa, F.; Murakoshi, K.; Yamauchi, H.; Ito, S.; Miyasaka, H.; Ishihara, H.; Tsuboi, Y. *J. Am. Chem. Soc.* **2013**, *135*, 6643–6648.

(6) Toshimitsu, M.; Matsumura, Y.; Shoji, T.; Kitamura, N.; Takase, M.; Murakoshi, K.; Yamauchi, H.; Ito, S.; Miyasaka, H.; Nobuhiro, A.; Mizumoto, Y.; Ishihara, H.; Tsuboi, Y. *J. Phys. Chem. C* **2012**, *116*, 14610–14618.

(7) Shoji, T.; Tsuboi, Y. *Opt. Rev.* **2015**, *22*, 137–142.

(8) Inoue, H.; Yamamoto, T.; Kuwahara, S.; Katayama, K. *Chem. Asian J.* **2013**, *8*, 108–112.