Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting Information

Sensitive and Homogenous Immunoassay of Fumonisin in Foods Using Single Molecule Fluorescence Correlation Spectroscopy

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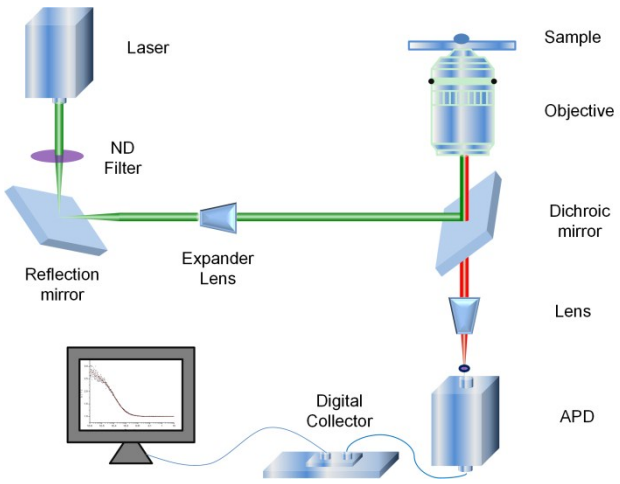

Fig. 1. Schematic diagram of FCS setup.

Preparation of Alexa 488-labeled FB_1.

The tracer was assigned the molecular formula ($C_{55}H_{71}N_3O_{25}S_2$) on the basis of HR-ESI-MS (*m/z* 1236.37 ([*M*-H]$^-$， *m/z* 617.67 ([*M*-2H]$^{2-}$)), MS result see fig. S2.

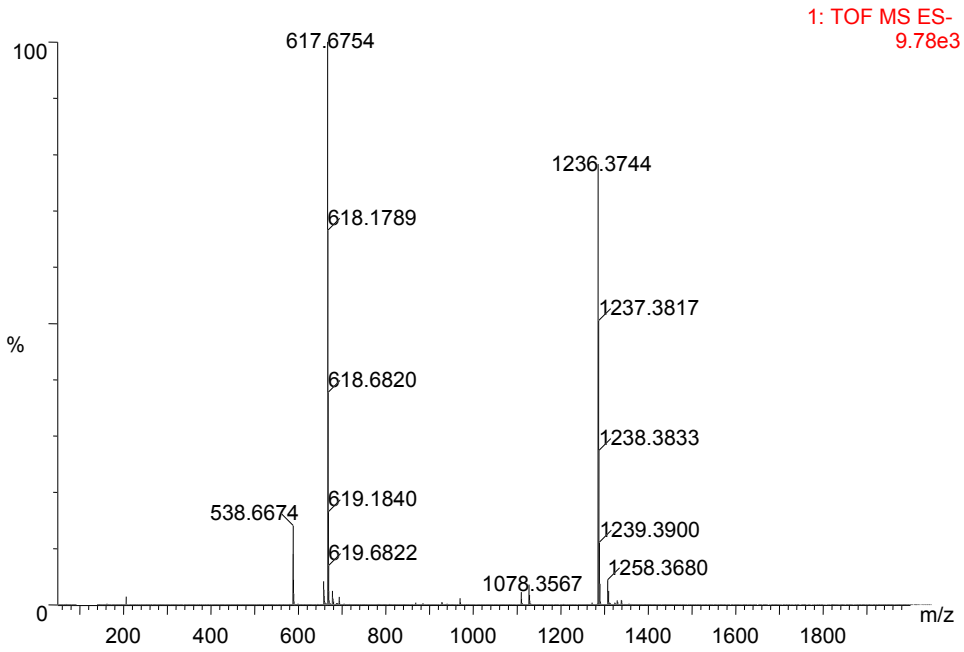

Fig. S2. MS results of Alexa 488–labeled FB_1

Determination of the FCS detection volume and the fraction Y.

If assuming the small observation volume as a three-dimensional Gaussian profile, the autocorrelation function has the following form[1-3]:

$$G(\tau) = \frac{1}{N} \cdot \left(1 + \frac{Te^{-\tau/\tau_{triplet}}}{1-T}\right) \cdot \frac{1}{\left(1 + \left(\tau/\tau_D\right)\right)} \cdot \frac{1}{\sqrt{1 + \left(\omega_0/z_0\right)^2 \cdot \tau/\tau_D}}$$

(1)

where N is the number of fluorescent molecules in the small observation volume, τ_D is the characteristic diffusion time of fluorescent molecules, ω_0 and z_0 are the lateral and axial radius of the volume and T is the fraction of fluorescent molecules in the triplet state with a lifetime $\tau_{triplet}$. ω_0 and z_0 were measured by using 1.6 nM Rhodamine Green solution, assuming its diffusion coefficient of $D = 2.8\times10^{-6}$ cm^2 s^{-1} in water. The diffusion time τ_D of Rhodamine Green (50 μs) and the ratio of ω_0 to z_0 (0.17) can be obtained from Eq. (2), and τ_D is related to the diffusion coefficient, D.

$$\tau_D = \frac{\omega_0^2}{4D} \qquad (2)$$

As a result, the obtained ω_0 and z_0 were about 0.24 and 1.43 μm, thus the detection volume ($V_0 = \pi^{3/2}\omega_0^2 z_0$) was calculated to be about 0.44 fL.

The above autocorrelation function can be modified as the following two-component model. The equation of two-component model:

$$G(\tau) = \frac{1}{N}\left[1 - T + T\exp\left(\frac{-\tau}{\tau_T}\right)\right]\left[\frac{1-Y}{\left(1+\frac{\tau}{\tau_{free}}\right)\sqrt{1+\frac{\omega_o^2}{z_o^2}\frac{\tau}{\tau_{free}}}} + \frac{Y}{\left(1+\frac{\tau}{\tau_{bound}}\right)\sqrt{1+\frac{\omega_o^2}{z_o^2}\frac{\tau}{\tau_{bound}}}}\right]$$

(3)

Where τ_{free} and τ_{bound} are characteristic diffusion times of free A^* and binding complex A^*B. Due to different diffusion times between the Alexa 488–labeled FB_1 probe and the Alexa 488–labeled FB_1-antibody complex, the fraction Y can be measured by two-component fitting procedure without separation of the free Alexa 488–labeled FB_1 probe and the Alexa 488–labeled FB_1-antibody complex by fixing

τ_{free} and τ_{bound} as 91.3 and 216.0 μs, respectively. Therefore, the fraction Y can be measured by two-component fitting procedure by non-linearly fitting of FCS curves.

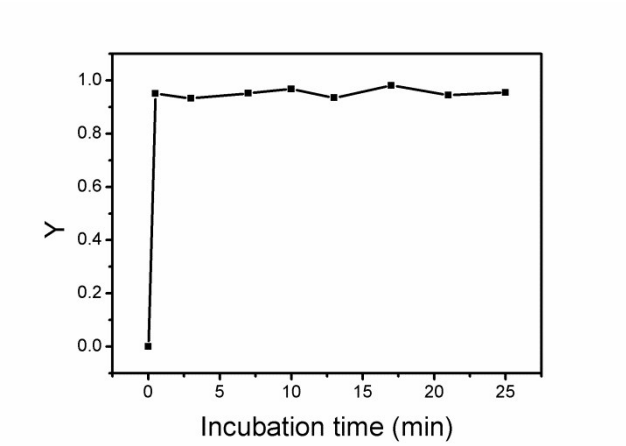

Fig. S3. The binding rate curve. 3.3 nM Alexa 488–labeled FB_1 was mixed with 33.0 nM FB_1–antibody in 10 mM PBS, incubated at 37 ^oC, after incubation for 0.5, 3, 7, 10, 13, 17, 21 and 25 min and measured by FCS, respectively.

Optimization of the ratio of FB_1-antibody to Alexa 488–labeled FB_1.

Fig. S4a. shows that the optimal concentration of the FB_1–antibody added to 33.0 nM Alexa 488–labeled FB_1 solution was 82.5 nM; Fig. S4b shows that the optimal concentration of the FB_1–antibody added to 13.0 nM Alexa 488–labeled FB_1 solution was 66.0 nM; Fig. S4c shows that the optimal concentration of the FB_1-antibody added to 6.1 nM Alexa 488–labeled FB_1 solution was 33.0 nM; Fig. S4d shows that the optimal concentration of the FB_1-antibody added to 3.2 nM Alexa 488–labeled FB_1 solution was 33.0 nM.

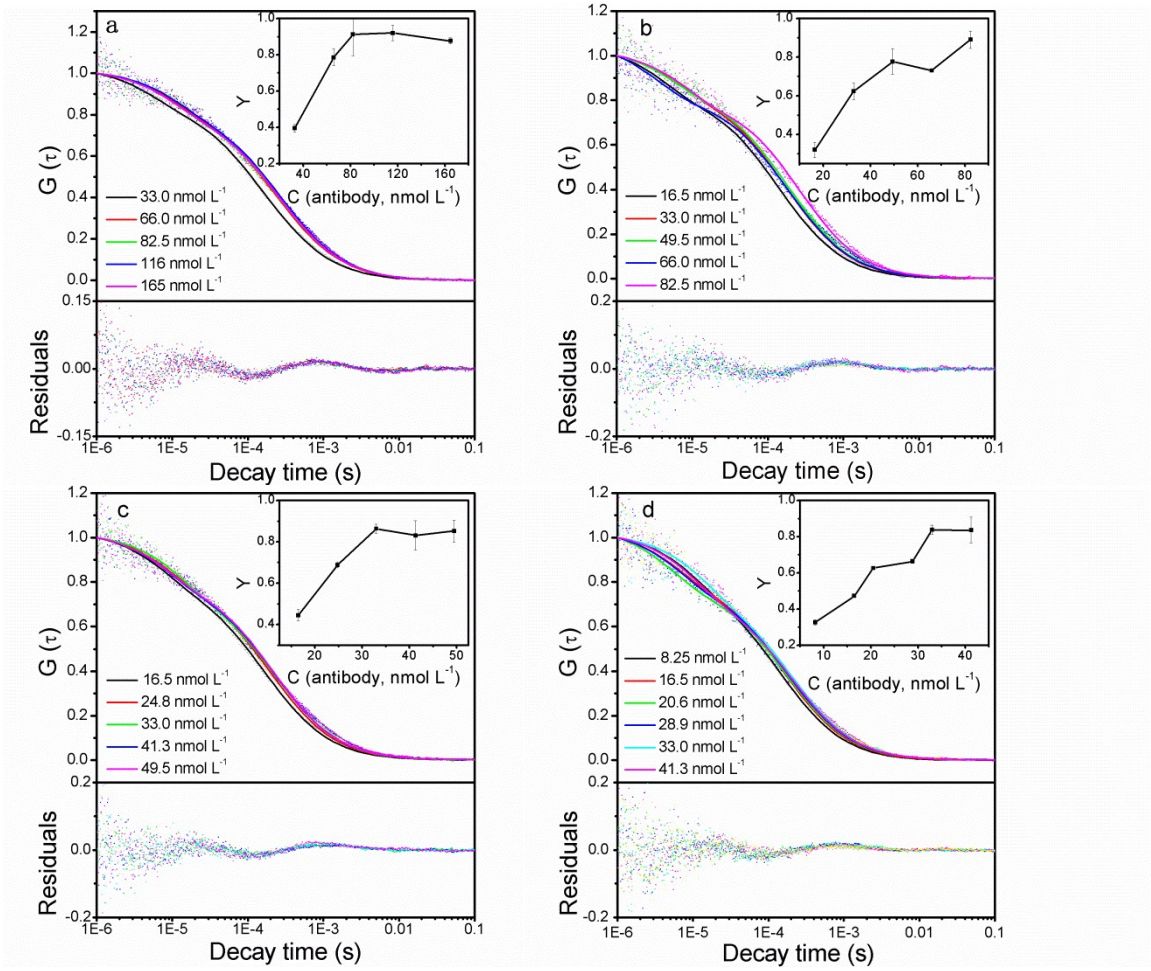

Fig. S4. (a) Normalized fluorescence correlation curves and their fitting curves of 33.0 nM Alexa 488–labeled FB_1 and its binding complex at different concentration of FB_1-antibody, the fitting residuals and the relations between the bound ratio of Alexa 488–labeled FB_1 to FB_1–antibody (Y) and FB_1–antibody concentration (C). (b) Normalized fluorescence correlation curves and their fitting curves of 13.0 nM Alexa 488–labeled FB_1 and its binding complex at different concentration of FB_1–antibody, the fitting residuals and the relations between the bound ratio of Alexa 488–labeled FB_1 to FB_1–antibody (Y) and FB_1–antibody concentration (C). (c) Normalized fluorescence correlation curves and their fitting curves of 6.1 nM Alexa 488–labeled FB_1 and its binding complex at different concentration of FB_1–antibody, the fitting residuals and

the relations between the bound ratio of Alexa 488–labeled FB_1 to FB_1–antibody (Y) and FB_1-antibody concentration (C). (d) Normalized fluorescence correlation curves and their fitting curves of 3.2 nM Alexa 488–labeled FB_1 and its binding complex at different concentration of FB_1–antibody, the fitting residuals and the relations between the bound ratio of Alexa 488–labeled FB_1 to FB_1–antibody (Y) and FB_1–antibody concentration (C). The reaction and detection buffer were 10 mM PBS buffer (containing 0.1 mg mL^{-1} BSA, pH 7.3).

Optimization of the working curve.

Fig. S5a shows the working curve of 70.0 nM Alexa 488–labeled FB_1. It has a linear ranger from 20.0 μg L^{-1} to 110.0 μg L^{-1}. The detection limit is 20.0 μg L^{-1} for FB_1. Fig. 5b shows the working curve of 33.0 nM Alexa 488–labeled FB_1, the linear ranger from 20.0 μg L^{-1} to 100.0 μg L^{-1}. Fig. 5c shows the working curve of 13.0 nM Alexa 488–labeled FB_1, the linear ranger from 10.0 μg L^{-1} to 60.0 μg L^{-1}. Fig. 5d shows the working curve of 6.1 nM Alexa 488–labeled FB_1, the linear ranger from 5.0 μg L^{-1} to 35.0 μg L^{-1}.

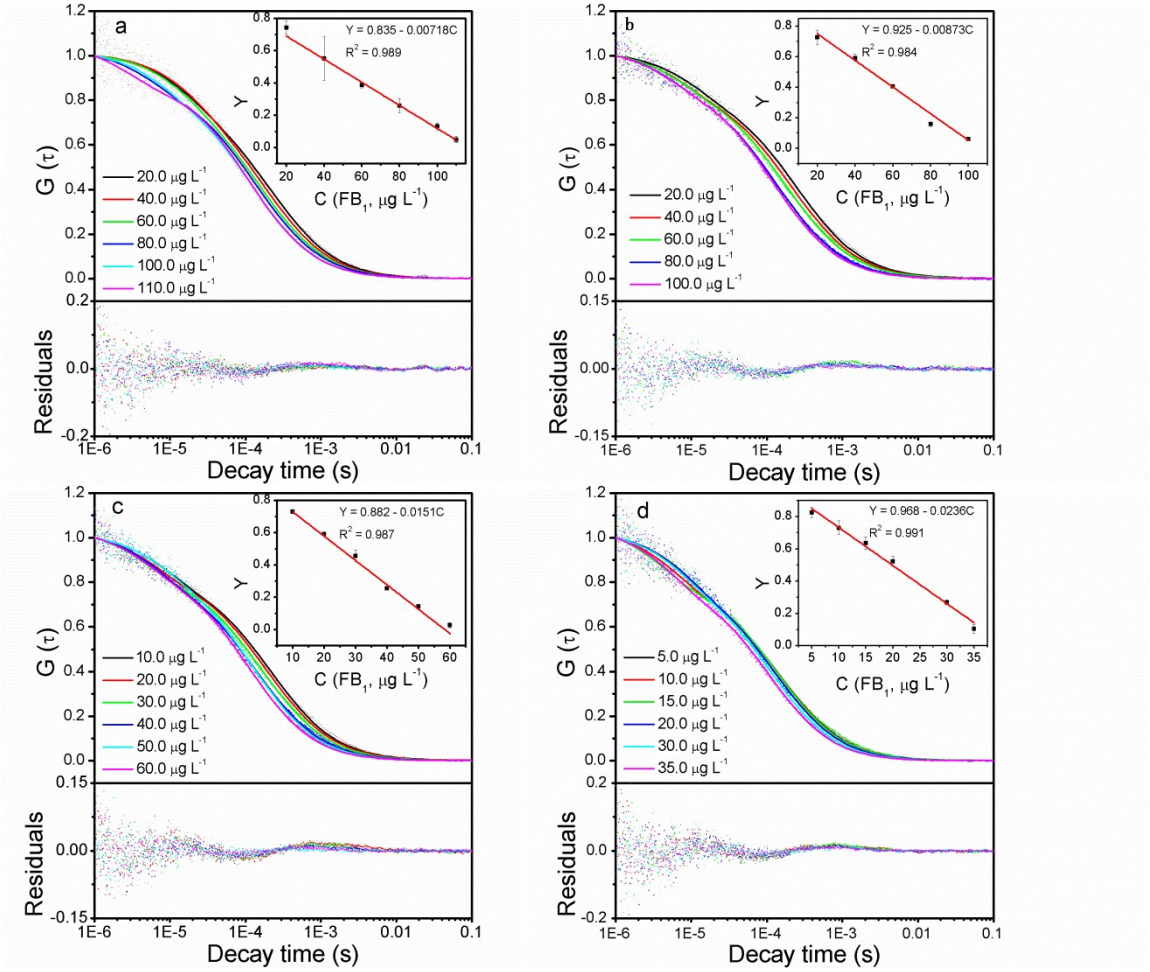

Fig. S5. (a) Normalized fluorescence correlation curves and their fitting curves of 70.0 nM Alexa 488–labeled FB_1 and its binding complex at different concentration of FB_1, the fitting residuals and the relations between the bound ratio of Alexa 488–labeled FB_1 to FB_1–antibody (Y) and FB_1 concentration (C). (b) Normalized fluorescence correlation curves and their fitting curves of 33.0 nM Alexa 488–labeled FB_1 and its binding complex at different concentration of FB_1, the fitting residuals and the relations between the bound ratio of Alexa 488–labeled FB_1 to FB_1–antibody (Y) and FB_1 concentration (C). (c) Normalized fluorescence correlation curves and their fitting curves of 13 nM Alexa 488–labeled FB_1 and its binding complex at different concentration of FB_1, the fitting residuals and the relations between the bound ratio

of Alexa 488–labeled FB_1 to FB_1–antibody (Y) and FB_1 concentration (C). (d) Normalized fluorescence correlation curves and their fitting curves of 6.1 nM Alexa 488–labeled FB_1 and its binding complex at different concentration of FB_1, the fitting residuals and the relations between the bound ratio of Alexa 488–labeled FB_1 to FB_1–antibody (Y) and FB_1 concentration (C). The reaction and detection buffer were 10 mM PBS buffer (containing 0.1 mg mL^{-1} BSA, pH 7.3).

References

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