Electronic Supplementary Material (ESI) for Analyst.

Supplementary Information

A Sensitive and Microscale Method for Drug Screening Combining Affinity Probe and Single Molecule Fluorescence Correlation Spectroscopy

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The [P] value analysis

According to the reference,[1] substituting equations (17) and (18)into equation (16), we have

$$[P]^3 + \{ + \ + [A]_0 + [C]_0 - [P]_0\}[P]^2 K_d \ K_c$$
$$+ \{([C]_0 - [P]_0) + ([A]_0 - [P]_0) + \}[P] K_d \ K_c \ K_d \ K_c \quad (22)$$
$$- [P]_0 = 0 K_d \ K_c$$

Let

$$a = K_d + K_c + [A]_0 + [C]_0 - [P]_0$$
$$b = K_d ([C]_0 - [P]_0) + K_c ([A]_0 - [P]_0) + K_d K_c$$
$$c = -K_d K_c [P]_0$$

Then we obtained

$$[P]^3 + a[P]^2 + b[P] + c = 0 \quad (23)$$

By the substitution $[P] = u - (a/3)$, equation (23) becomes

$$u^3 - qu - r = 0 \quad (24)$$

where

$$q = \frac{a^2}{3} - b \ r = -\frac{2}{27}a^3 + \frac{1}{3}ab - c$$

The determination of equation (24) is given by

$$\Delta = \frac{r^2}{4} - \frac{q^3}{27} \quad (25)$$

Since $\Delta < 0$, the three real roots of equation (24) are given by

$$u_1 = \frac{2}{3}\sqrt{(a^2 - 3b)}\cos\frac{\theta}{3} \quad (26)$$

$$u_2 = \frac{2}{3}\sqrt{\left(a^2 - 3b\right)}\cos\frac{2\pi - \theta}{3} \tag{27}$$

$$u_3 = \frac{2}{3}\sqrt{\left(a^2 - 3b\right)}\cos\frac{2\pi + \theta}{3} \tag{28}$$

where

$$\theta = \arccos\frac{-2a^3 + 9ab - 27c}{2\sqrt{\left(a^2 - 3b\right)^3}}$$

According to the definition of u and the physical conditions of the problem proposed, it can be verified that u_1 expresses the unique physically meaningful root of equation (24), while u_2 and u_3 have no significance to our assay.[1] Thus, the proper root of equation (23) can then be written as:

$$[\mathrm{P}] = -\frac{a}{3} + \frac{2}{3}\sqrt{\left(a^2 - 3b\right)}\cos\frac{\theta}{3}$$

Synthesized of dasatinib derivative (4) connected with 6-aminohexanoic acid: To a solution of dasatinib (0.12g, 0.25 mM) dissolved in DMSO (2 mL) was added Boc-6-Ahx-OH (0.12 g, 0.5 mM), DEAD (0.12 mL, 0.75 mM) , PPh3 (0.2g, 0.75 mM). The reaction mixture was stirred at room temperature for 3h. Then, the reaction mixture was dissolved in dichloromethane (10 mL)and TFA (2 mL) added at room temperature for 1h for de-protection. The reaction mixture was purified by PSA 25-400 resin columns(Suzhou Nano-Micro Co., Ltd. China), eluted by aqueous methanol (containing0.05% TFA), and obtained 97 mg dasatinib derivative **4**.Molecular weight of compound **4** was analyzed byHR-ESI-MS(Waters Corp., Milford). Compound **4** was assigned the molecular formula ($C_{28}H_{37}ClN_8O_3S$) on the basis of HR-ESI-MS (*m/z* 601.248 ($[M+H]^+$， *m/z* 1201.487 ($[2M+H]^+$， *m/z* 301.118 ($[M+2H]^{2+}$)) and cleavage fragment peak (488.159), MS result see the Figure S2 (a).

Synthesis of Alexa 488-dasatinib (A): Labeling of the compound **4** with Alexa 488issimilar to the procedure described in the manufacturer's manual of labeling proteins. Briefly, approximately 2 mg/mL

of compound **4** was dissolved in a solution containing 0.1 M $NaHCO_3$ (pH 9.0). An appropriate amount of Alexa 488 was added. Alexa 488/compound **4** ratio was 3:1. The 500 μL mixture was incubated in a separate tube for 2 hr at room temperature under gentle stirring, and protected from light. The reaction mixture was purified by semi-prepared reversed-phase HPLC (Agilent 1100): C18column (5 μm, 10×250 mm, YMC), eluted by linear gradient from 20% to 60% using 0.05% trifluoroacetic acid (TFA) in CH_3CN for 20 min to afford linker **A**. Compound **A** was assigned the molecular formula ($C_{49}H_{49}ClN_{10}O_{13}S_3$) on the basis of HR-ESI-MS (*m/z* 1117.242 ($[M+H]^+$， *m/z* 559.114 ($[M+2H]^{2+}$)), MS result see the Figure S2 (b).

The procedure for fabrication of PDMS/glass chip with micro-wells: The PDMS/glass chip with micro-wells was fabricated according to the procedure described in the reference.[2] Briefly, the coverslip was soaked by piranha solution (sulfuric acid:hydrogen peroxide=3:1) for 3 h, and then, cleaned by ultrapure water. An appropriate amount of PDMS precursor polymer and curing agent (Dow Corning) in a mass ratio of 10:1 were weighted, and casting to a tray for slides produced after degassed in vacuum, then baked for 2 hr at 60 ^oC. Carefully peel off the PDMS sheet from the slides, punched with a 1.9 mm diameter punching bear, and irradiated under UV light for 1 hr. The PDMS to the microscope slide was sealed by baking for 2 hr at 80 ^oC, and PDMS/glass chip with 36 micro-wells was obtained. The photos of PDMS/glasschip with micro-wells were shown in Figure S5.

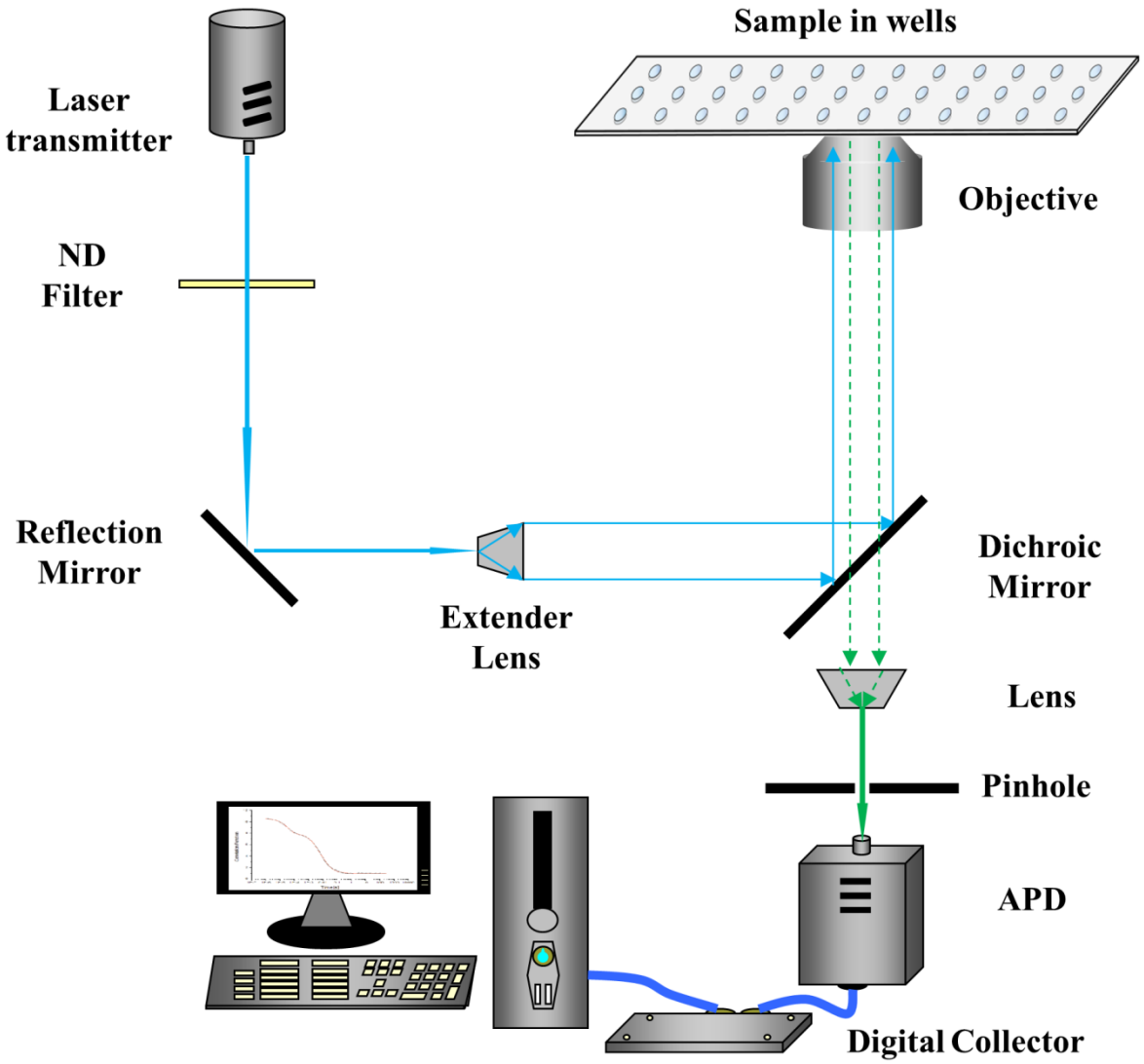

Figure S1. Schematic diagram of FCS setup.

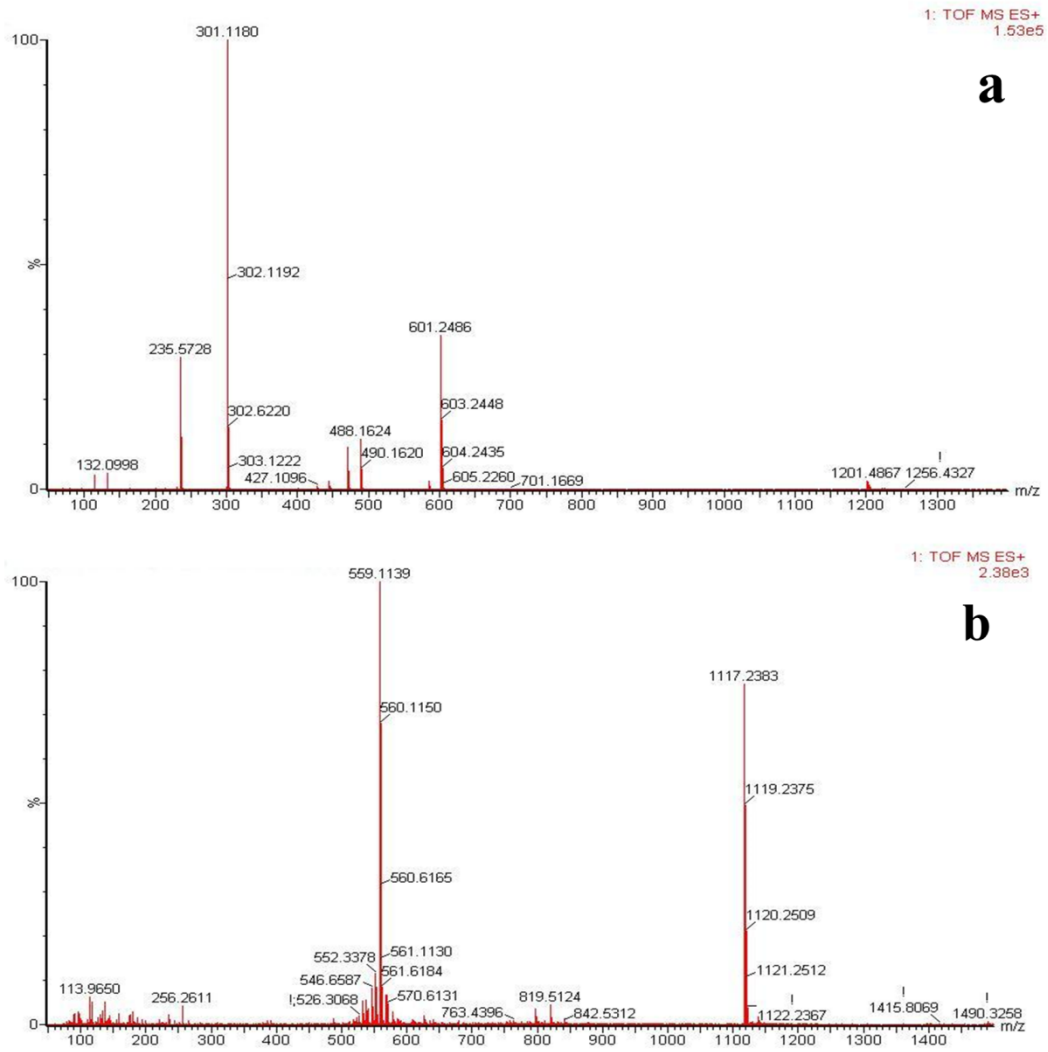

Figure S2. MS results of compound **4** (a) and **A** (b).

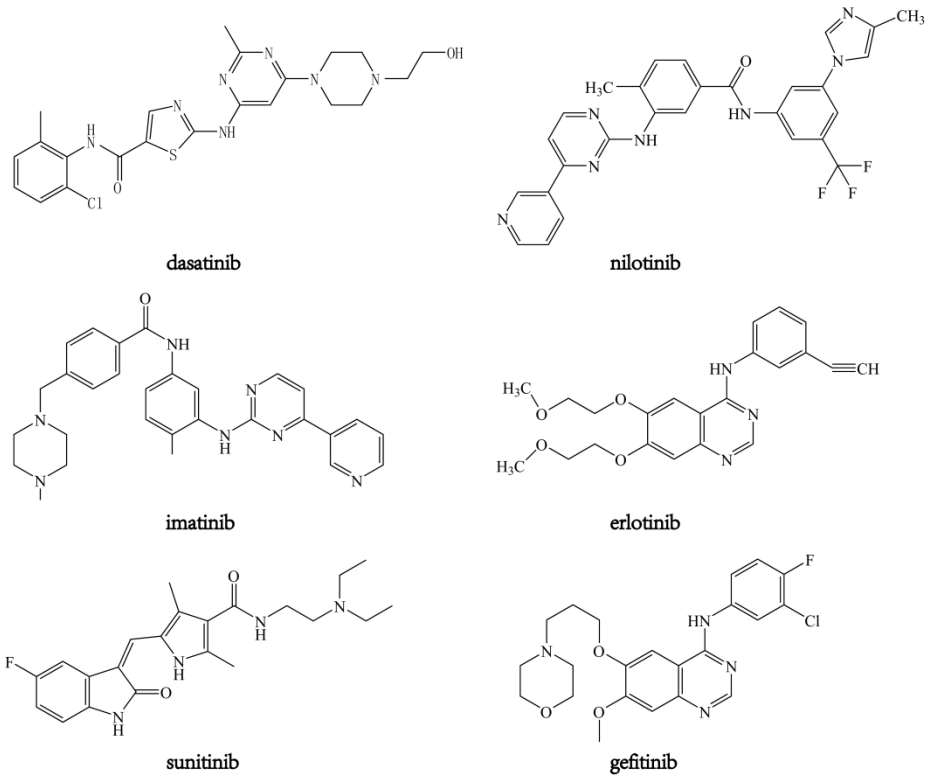

Figure S3. The structures of six known CML drugs.

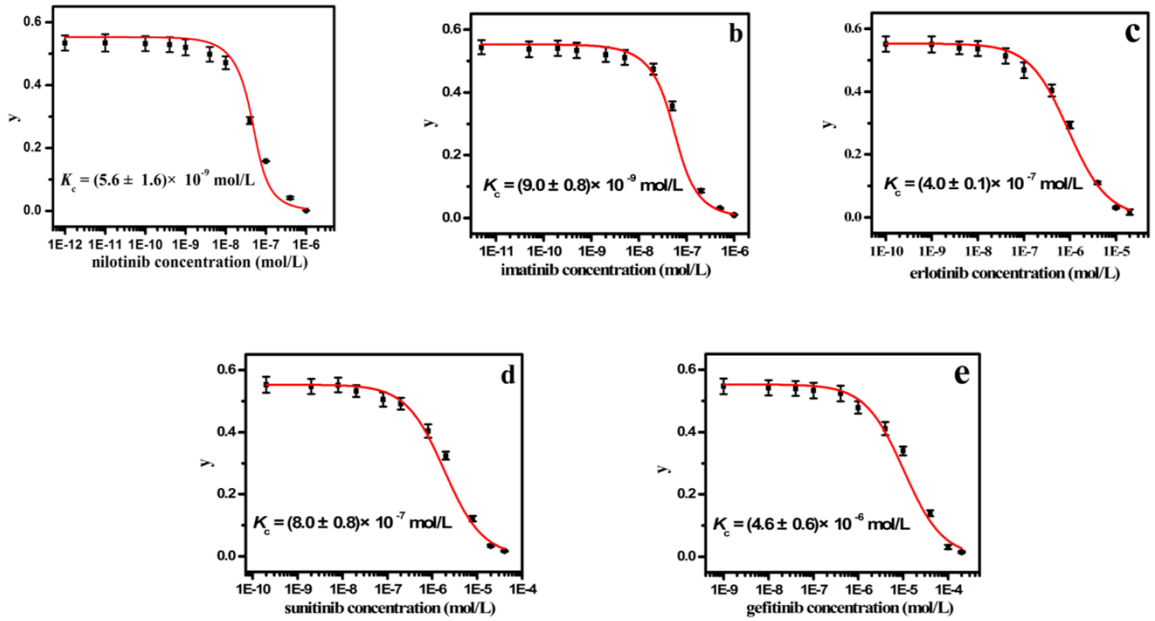

Figure S4. Titration curves of known CML drugs binding to ABL1 obtained from FCS measurements. The

known CML drugs are nilotinib (a), imatinib (b), erlotinib (c), sunitinib (d) and gefitinib (e), respectively. The error bars represent the standard deviation of 3 time measurements.

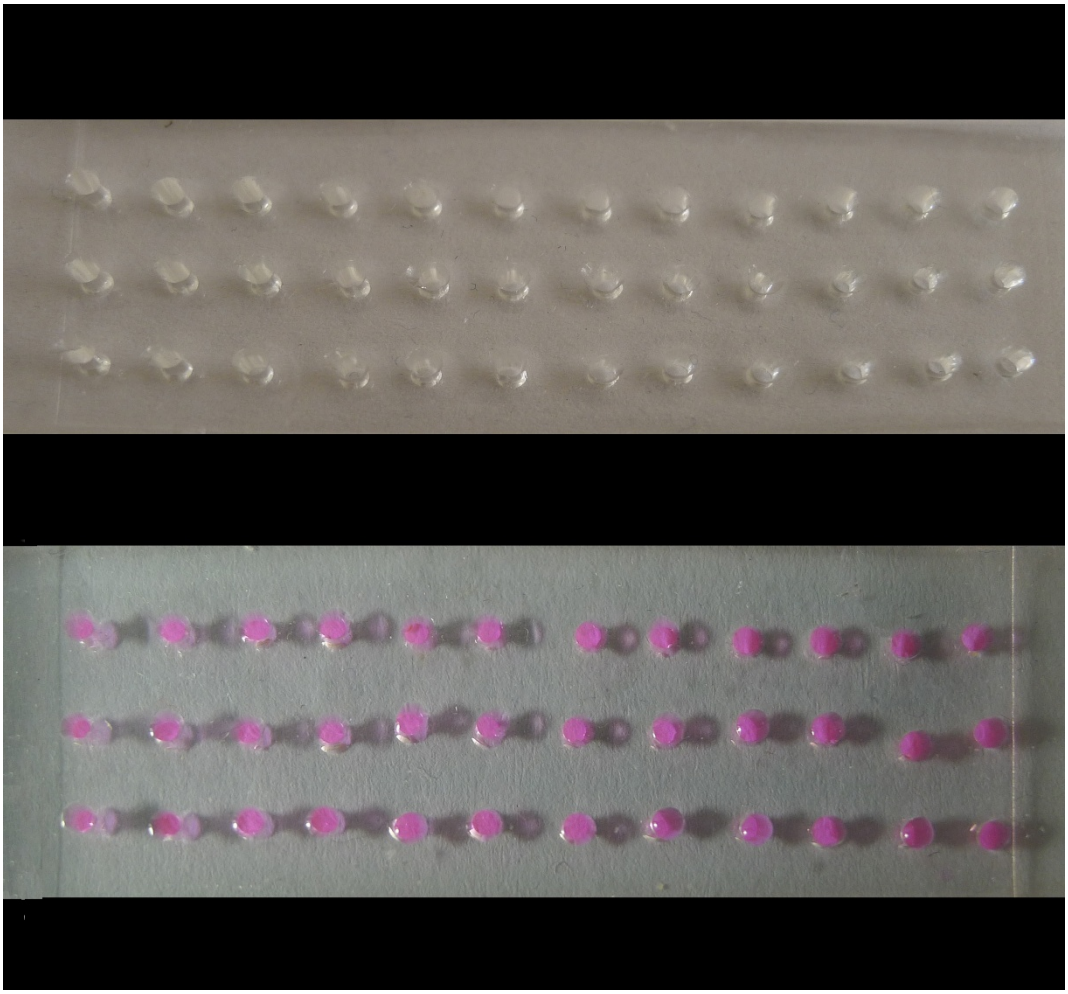

Figure S5. The photos of PDMS/glasschip with micro-wells, each micro-well was filled with 2 μL 2.0 $\times 10^{-5}$ mol/L Rhodamine B (purple) in below photo.

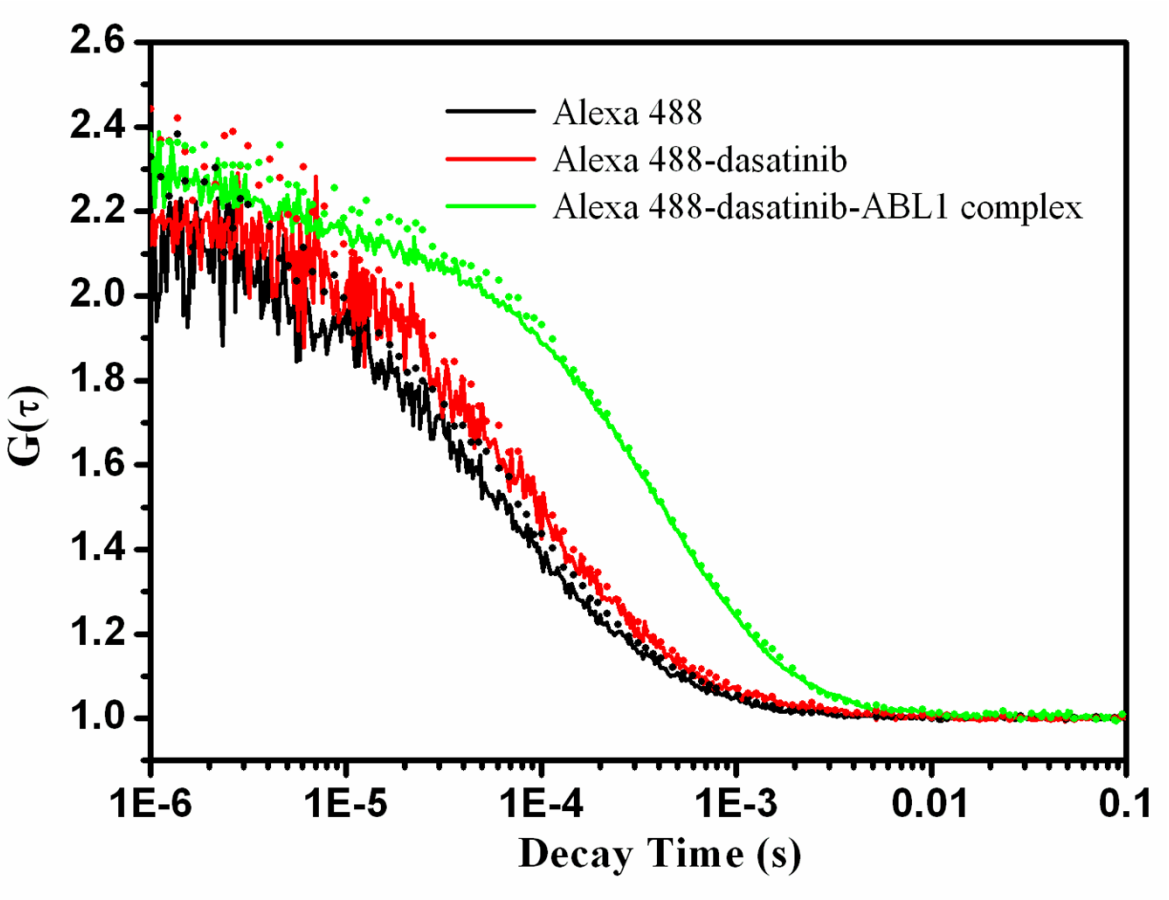

Figure S6. The auto correlation curves of Alexa 488 (black), Alexa 488-dasatinib (red) and Alexa 488-dasatinib-ABL1 protein complex (green) measured on a homemade chip with micro-wells (solid line) and a coverslip (small dots). The laser intensity is 80 μW, and the concentration of each sample is 1 nM.

Supplementary Information references

(1) Wang, Z.-X. *FEBS letters* **1995**, *360*, 111.
(2) Chen, L.; Ren, J.; Bi, R.; Chen, D. *Electrophoresis* **2004**, *25*, 914.