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Supporting Information

Study of Protein-Drug Interaction based on Solvent-Induced Protein Aggregation by Fluorescence Correlation Spectroscopy Method

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FCS System Setup

The diffusion behavior of probes in solution obtained by FCS.[1] The FCS setup is shown in the Fig. S1. The system selected a 488 nm laser (Coherent, USA) as excitation source. For the fluorescent dye-labelled protein sample, the excitation power was 100 μW, and for the recombinant fluorescent protein sample, the excitation power was 40 μW to avoid photobleaching. The laser was reflected by the dichroic mirror (505DRLP, Omega Optical, USA) into the objective lens (60×NA 1.2, Olympus, Japan), and focused on the sample solution on the cover glass. The generated signal was collected by the objective lens, entered the single photon counter (SPCM-AQR16, PerkinElmer EG&G, Canada) through the filter (530DF30, Omega Optical, USA) and pinhole, and then was recorded and processed by the digital correlator (Flex02-12D/C, Correlator.com, USA) to obtain the FCS curve. FCS data were nonlinearly fitted with the Microcal Origin 8.0 software.

Expression and Purification of Proteins DHFR and DHFR-EGFR. Constructs encoding DHFR and DHFR-EGFP (*Ec*DHFR) were cloned into pet28a vector with a C-terminal His6-tag. The plasmid was transformed into BL21 (DE3) *E. coli* cells.[2] Cells were grown to OD600 at 0.6 before induced by IPTG (100 μM) at 16 °C for 12 h. Cultured cells were harvested and resuspended in 20 mM Tris-HCl buffer (including 300 mM NaCl, 5 mM β-ME, 10% glycerol, 1 mM PMSF, and 5 mM imidazole, pH 7.9). Cells expressing recombinant proteins were lysed by sonication at 4 °C and then centrifuged for 30 min at 12,000 rpm. The supernatant was collected and loaded onto a 10 mL Ni-NTA column and washed with 20 mM Tris-HCl buffer (including 300 mM NaCl, 5 mM β-ME, 10% glycerol, 1 mM PMSF, and 50 mM imidazole, pH 7.9). The protein fractions were analyzed by 15% SDS-PAGE followed by staining with Coomassie Brilliant Blue. The protein was exchanged with PBS buffer by ultrafiltration at 4 °C, then aliquoted, frozen in liquid nitrogen, and stored at −80 °C.

TEM measurements. For the TEM measurements, 5 μL of the sample was dispersed on a hydrophilic treated carbon support film. The samples were washed three times and then dried naturally. The sample on carbon support film was stained with phosphotungstic acid and then tested. The sample was imaged with Talos F200C G2 TEM (Thermo Fisher Scientific, USA).

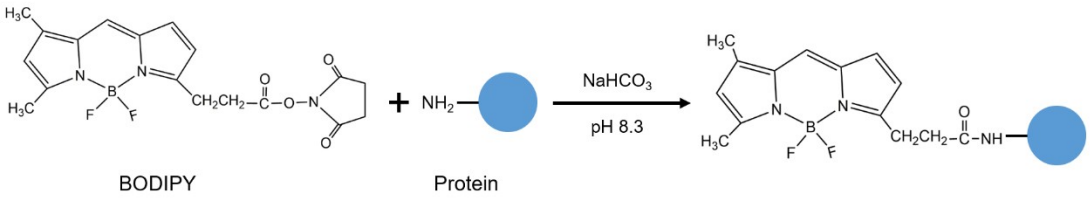

Fig. S1. Principle of fluorescent labelling reaction between the protein and BODIPY.

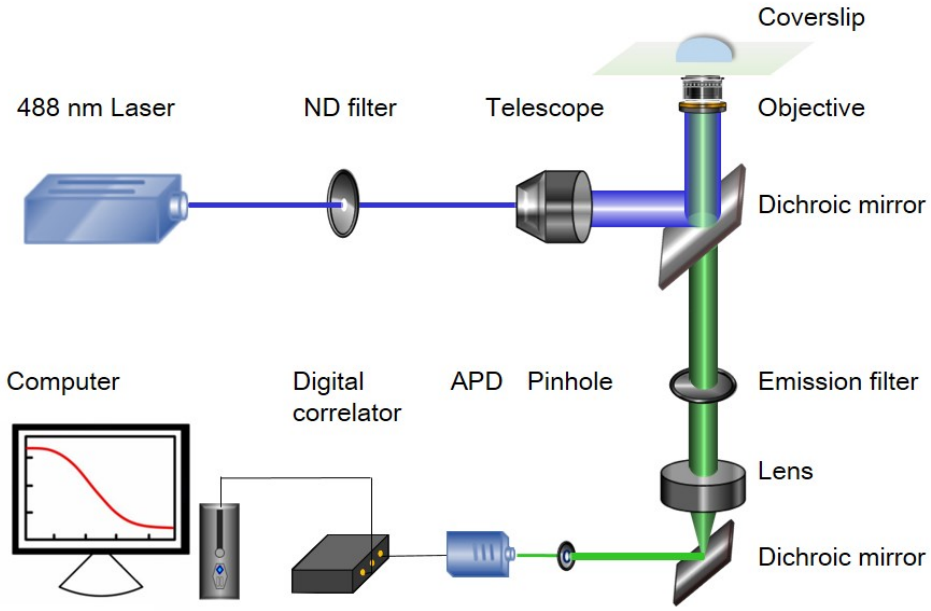

Fig. S2. FCS system for protein-drug interaction detection based on solvent-induced protein aggregation.

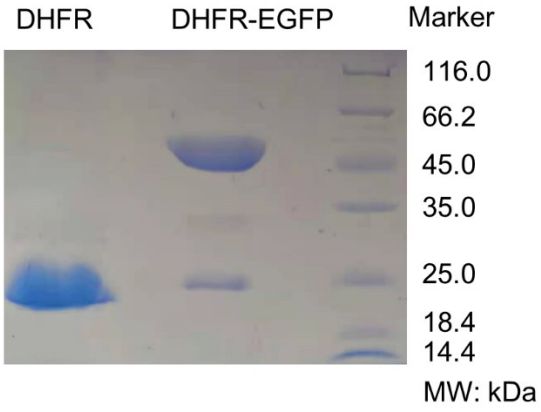

Fig. S3. The recombinant protein DHFR and DHFR-EGFP identified by SDS-PAGE and stained with Coomassie Brilliant Blue.

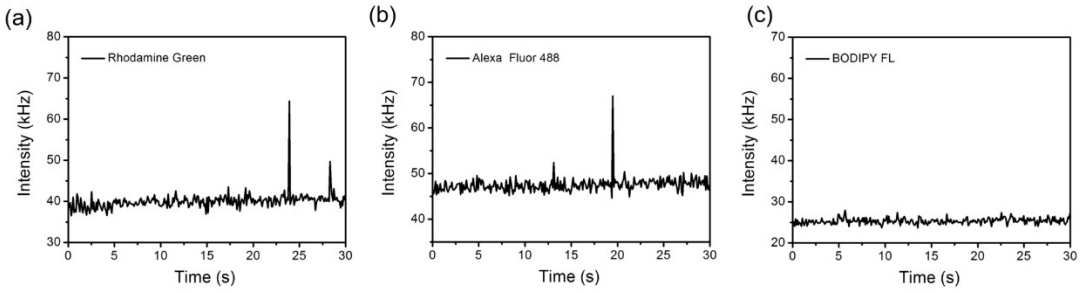

Fig. S4. Real-time fluorescence intensity of fluorescent dyes. (a) Rhodamine Green. (b) Alexa Flour 488. (c) BODIPY-FL. The concentrations of the three fluorescent dyes are all 20 nM.

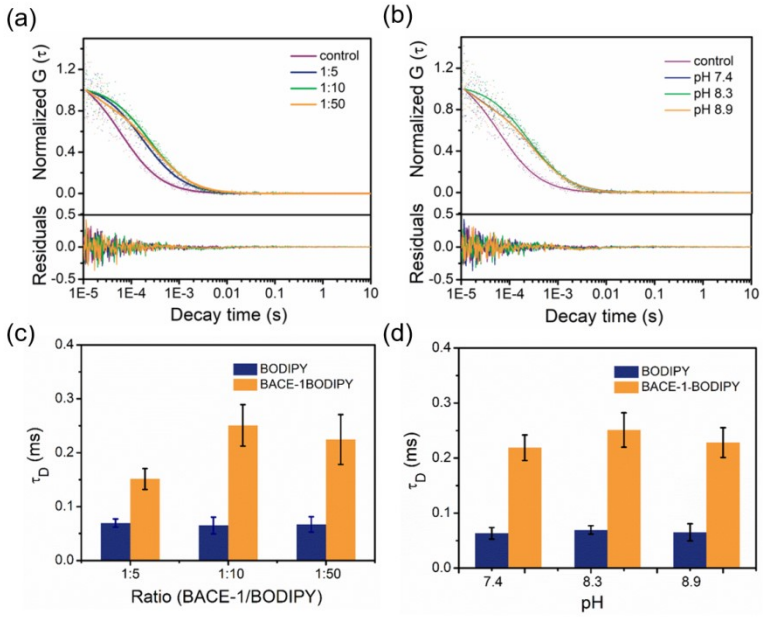

Fig. S5. Optimization of the conjugate reaction conditions between BACE-1 and BODIPY. (a) The normalized FCS curves and fitting residuals of BACE-1-BODIPY conjugates and BODIPY under different reaction ratios of BACE-1 and BODIPY. (b) The normalized FCS curves and fitting residuals of BACE-1-BODIPY conjugates and BODIPY under different pH values. (c) Comparison of the τ_D values of BODIPY and BACE-1-BODIPY conjugates under different reaction ratios of BACE-1 and BODIPY. (d) Comparison of the τ_D values of BODIPY and BACE-1-BODIPY conjugates under different pH. The concentration of BACE-1-BODIPY is 20 nM. The FCS measurement time was 30 s. Error bars show the standard deviations of three experiments.

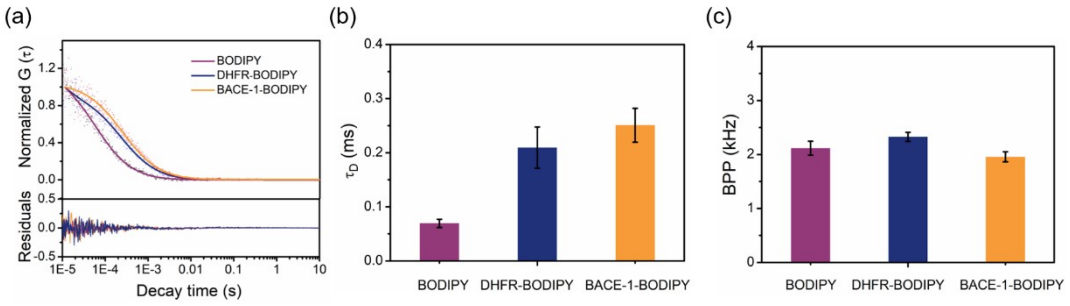

Fig. S6. (a) The normalized FCS curves and fitting residuals of BODIPY, BACE-1-BODIPY conjugates and DHFR-BODIPY conjugates. (b) The τ_D values of BODIPY, BACE-1-BODIPY conjugates and DHFR-BODIPY conjugates. (c) The brightness per particle (BPP) values obtained by FCS. The concentrations of BODIPY, DHFR-BODIPY and BACE-1-BODIPY are all 20 nM. The laser intensity is 100 μW. Error bars show the standard deviations of three experiments.

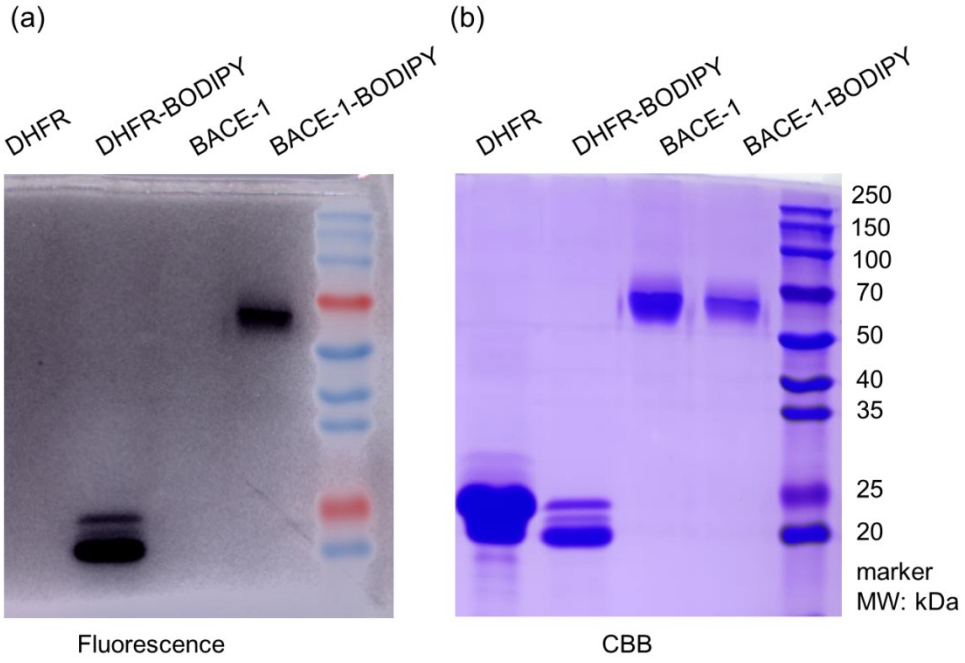

Fig. S7. DHFR, DHFR-BODIPY conjugates, BACE-1, and BACE-1-BODIPY conjugates were analyzed by SDS–PAGE. The fluorescence of protein was visualized by gel fluorescence scanning imaging, followed by Coomassie Brilliant Blue.

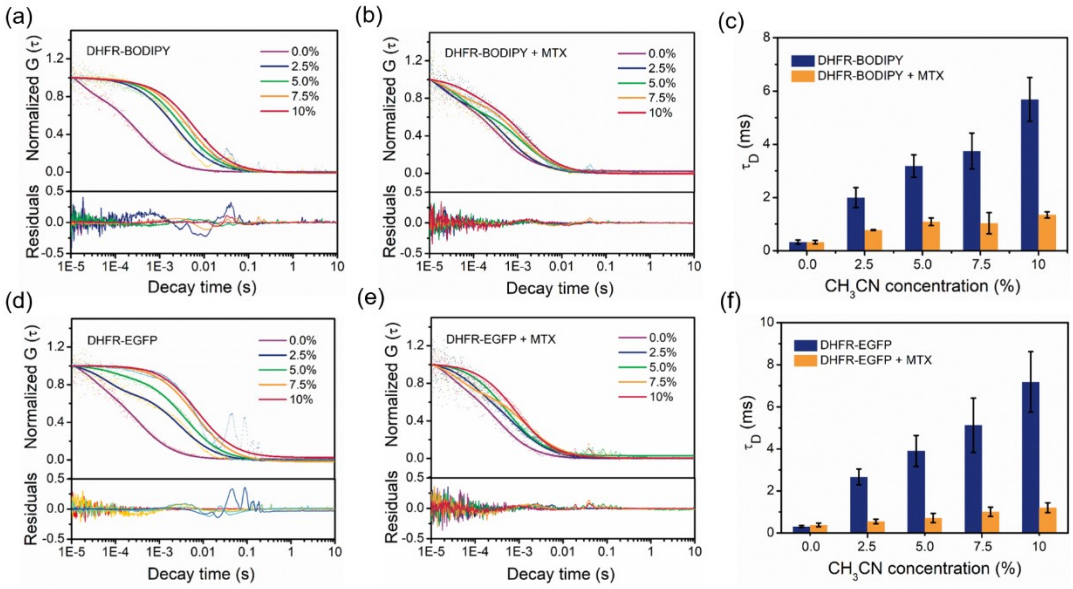

Fig. S8. Comparison of the aggregation behavior of DHFR-EGFP with DHFR-BODIPY conjugates in different volume fractions of acetonitrile in control and drug-treated group. The normalized FCS curves and fitted residuals of DHFR-BODIPY conjugates in (a) control group, (b) drug-treated group. (c) Comparison of the τ_D values of DHFR-BODIPY conjugates in the control and the drug-treated groups. The normalized FCS curves and fitted residuals of DHFR-EGFP in (d) control group, (e) drug-treated group. (f) Comparison of the τ_D values of DHFR-EGFP in the control and the drug-treated groups. The concentration of DHFR-BODIPY conjugates and DHFR-EGFP are both 50 nM. The concentration of MTX is 1 μM. Error bars show the standard deviations of three experiments.

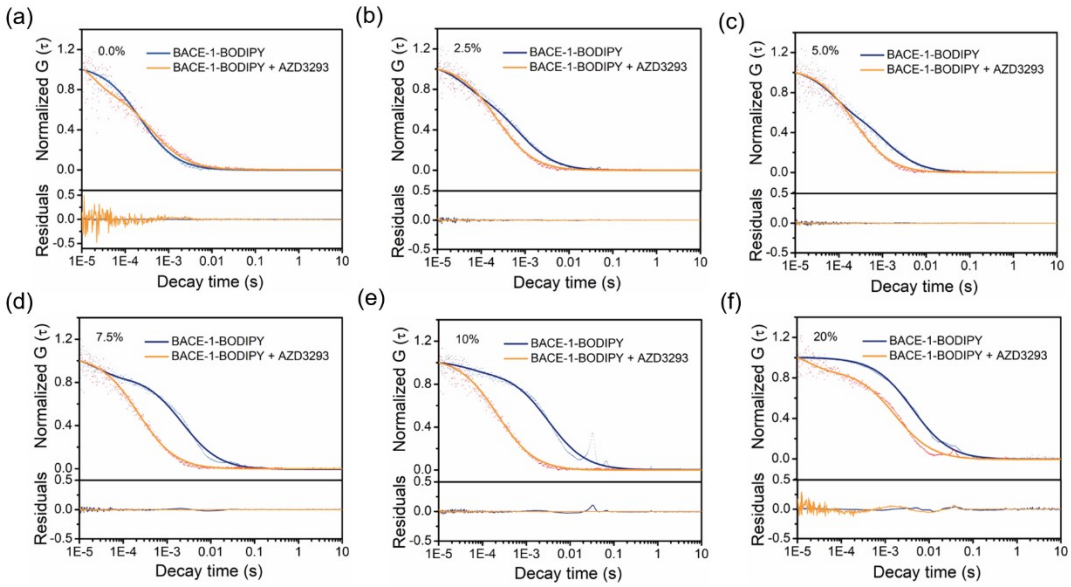

Fig. S9. The normalized FCS curves and fitted residuals of BACE-1-BODIPY in different volume fractions of acetonitrile for the control and the drug-treated groups. The volume fractions of acetonitrile is (a) 0%, (b) 2.5%, (c) 5.0%, (d) 7.5%, (e) 10.0%, (f) 20.0%, respectively. The concentration of BACE-1-BODIPY is 20 nM. The concentration of AZD3293 is 1 μM.

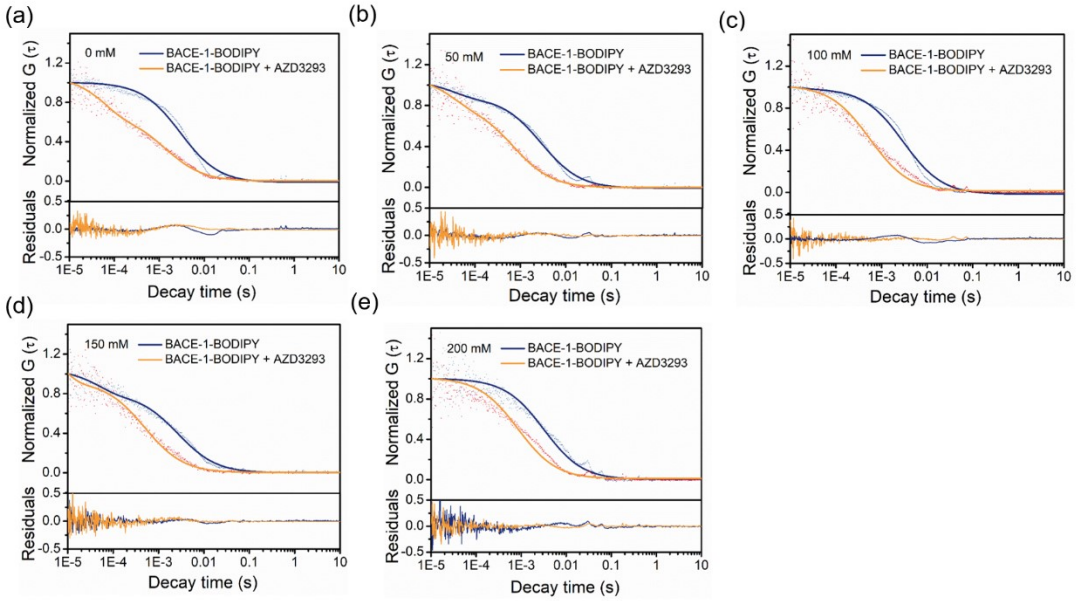

Fig. S10. The normalized FCS curves and fitted residuals of BACE-1-BODIPY in different concentration of NaCl for the control and the drug-treated groups. The concentration of NaCl is (a) 0 mM, (b) 50 mM, (c) 100 mM, (d) 150 mM, (e) 200 mM, respectively. The concentration of BACE-1-BODIPY is 20 nM. The concentration of AZD3293 is 1 μM.

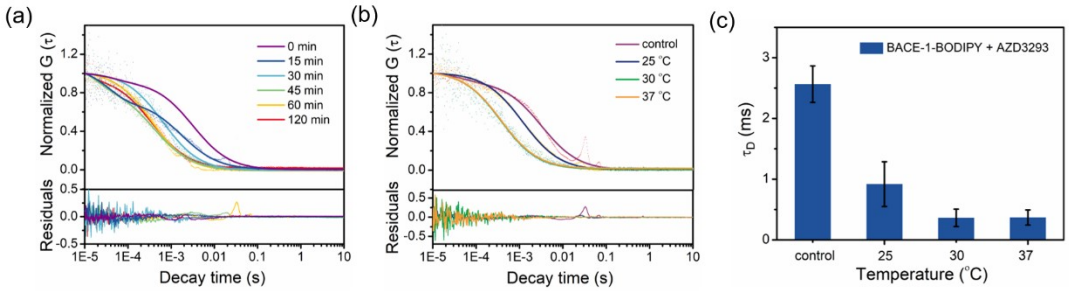

Fig. S11. Optimization of reaction conditions for AZD3293 binding to BACE-1-BODIPY. (a) The normalized FCS curves and fitted residuals of AZD3293 combined with BACE-1 at different time. (b) The normalized FCS curves and fitted residuals of AZD3293 combined with BACE-1 at different temperatures. (c) The value of τ_D depends on the temperature of AZD3293 binding to BACE-1-BODIPY. The concentration of BACE-1-BODIPY is 20 nM. The concentration of AZD3293 is 1 μM.

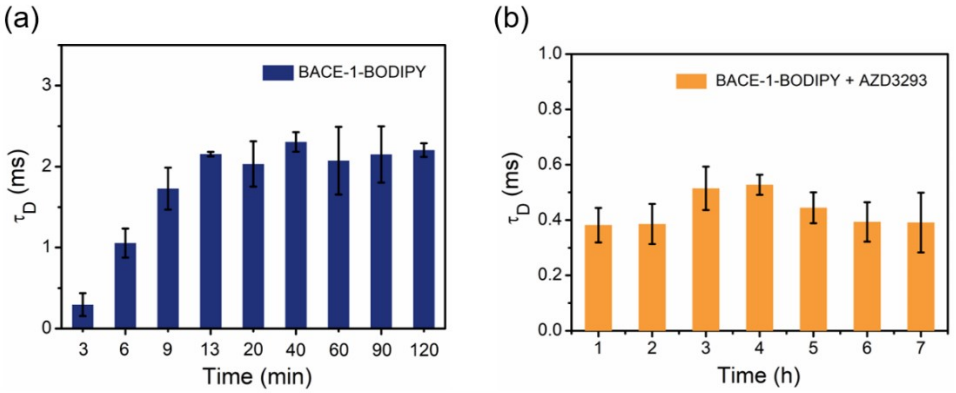

Fig. S12. (a) The τ_D values of BACE-1-BODIPY with the reaction time of acetonitrile-induced aggregation. (b) The τ_D values with the reaction time of acetonitrile-induced aggregation after AZD3293 binding to BACE-1-BODIPY. The concentration of BACE-1-BODIPY is 20 nM. The concentration of AZD3293 is 1 μM. Error bars show the standard deviations of three experiments.

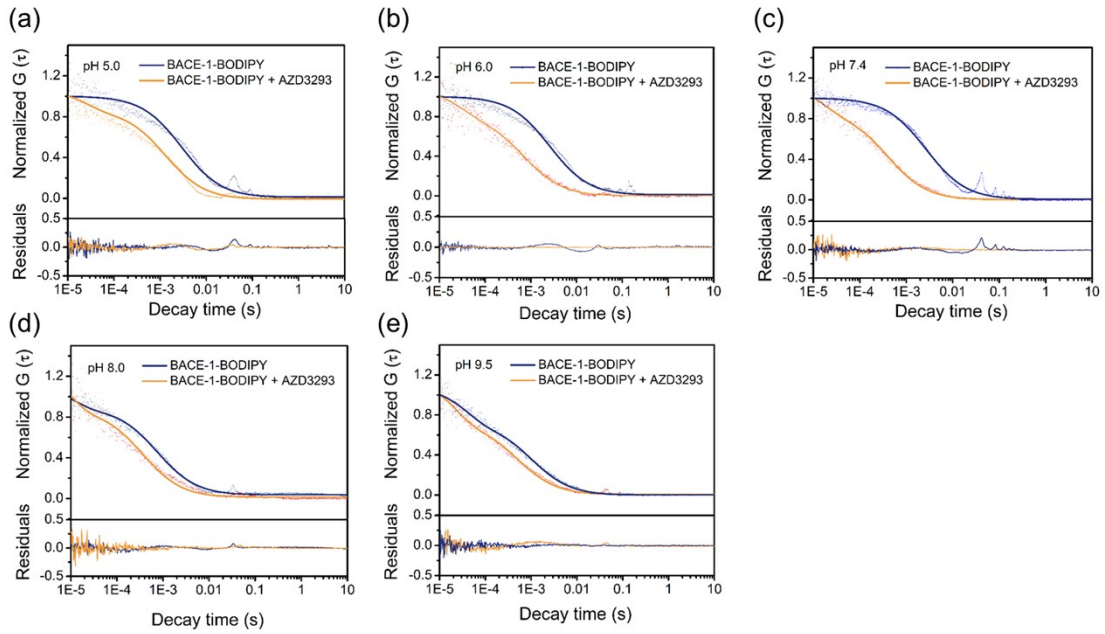

Fig. S13. The normalized FCS curves and fitted residuals of BACE-1-BODIPY in different pH for the control and the drug-treated groups. The pH is (a) 5.0, (b) 6.0, (c) 7.4, (d) 8.0, and (e) 9.5, respectively. The concentration of BACE-1-BODIPY is 20 nM. The concentration of AZD3293 is 1 μM.

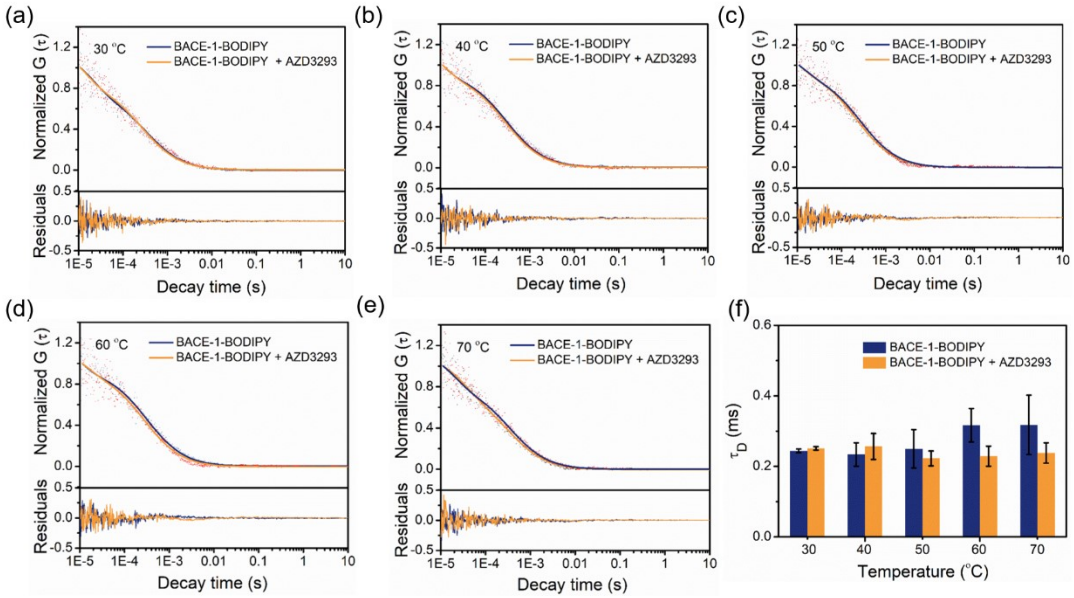

Fig. S14. The normalized FCS curves and fitted residuals of BACE-1-BODIPY at different temperature (a) 30°C, (b) 40°C, (c) 50°C, (d) 60°C, (e) 70°C for the control and the drug-treated groups, respectively. (f) Comparison of the τ_D values of BACE-1-BODIPY in the above temperature. The concentration of BACE-1-BODIPY is 20 nM. The concentration of AZD3293 is 1 μM. Error bars show the standard deviations of three experiments.

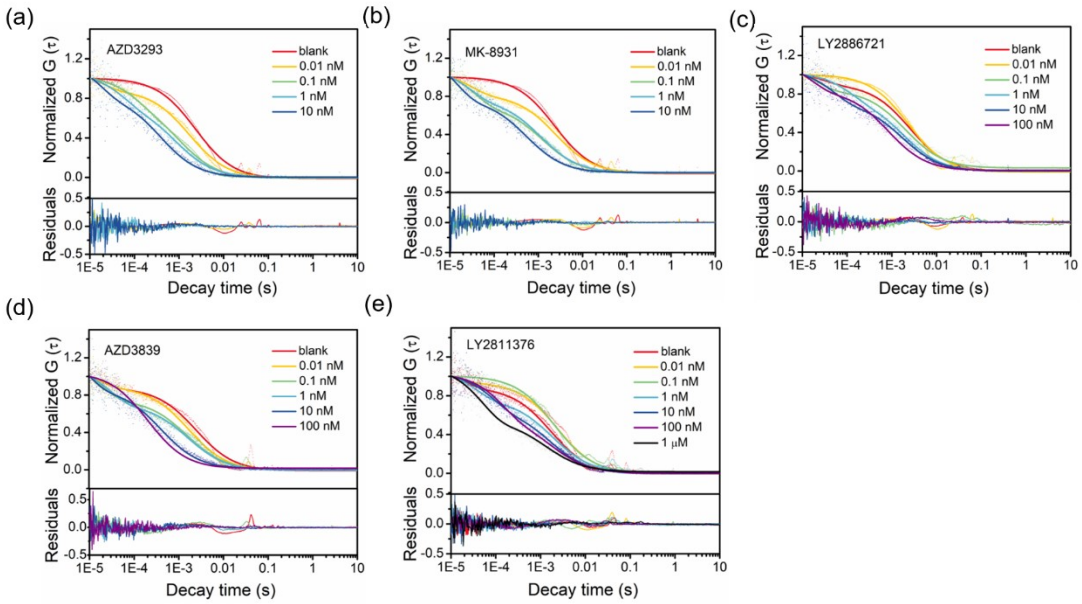

Fig. S15. Inhibitory effect of different drugs on BACE-1-BODIPY aggregation. Normalized FCS curves and fitted residuals for different drugs binding with BACE-1-BODIPY. The drug is (a) AZD3293, (b) MK-8931, (c) LY2886721, (d) AZD3839, and (e) LY2811376, respectively. The concentration of BACE-1-BODIPY is 20 nM.

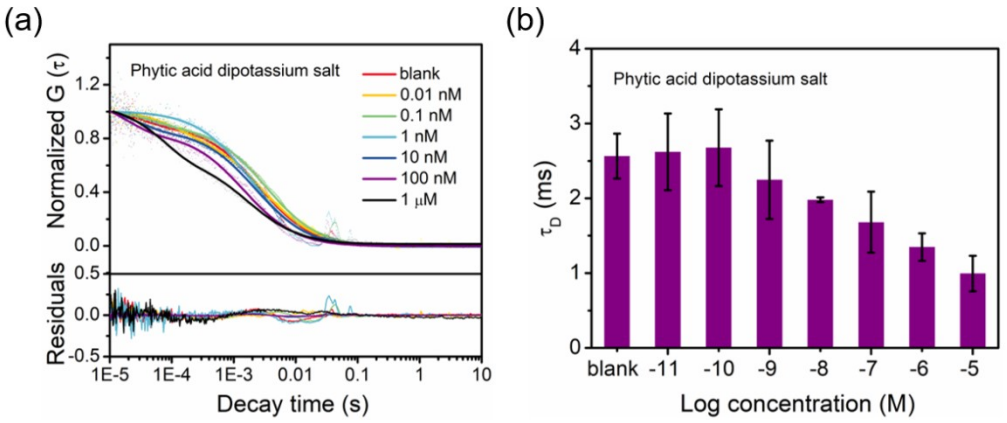

Fig. S16. Inhibitory effect of Phytic acid dipotassium salt on BACE-1-BODIPY aggregation. (a) The normalized FCS curves and fitted residuals for Phytic acid dipotassium salt binding with BACE-1-BODIPY. (b) The τ_D values under different concentrations of Phytic acid dipotassium salt combined with BACE-1-BODIPY. The concentration of BACE-1-BODIPY is 20 nM. Error bars show the standard deviations of three experiments.

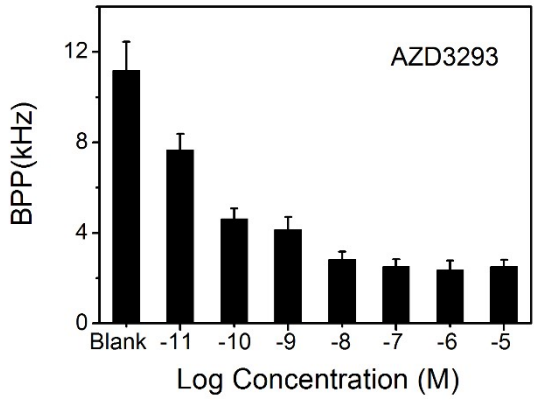

Fig. S17. The BPP values under different concentrations of AZD3293 combined with BACE-1. The concentration of BACE-1-BODIPY is 20 nM. Error bars show the standard deviations of three experiments.

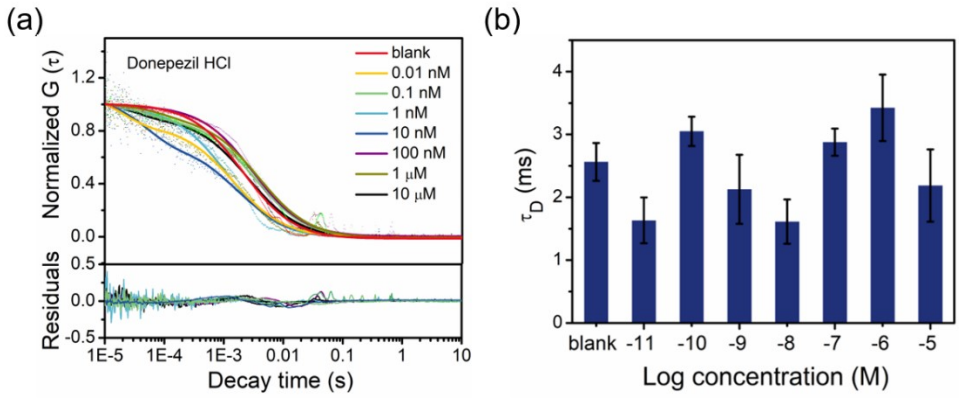

Fig. S18. Inhibitory effect of Donepezil HCl on BACE-1-BODIPY aggregation. (a). The normalized FCS curves and fitted residuals for Donepezil HCl binding with BACE-1-BODIPY (b) The τ_D values under different concentrations of Donepezil HCl combined with BACE-1. The concentration of BACE-1-BODIPY is 20 nM. Error bars show the standard deviations of three experiments.

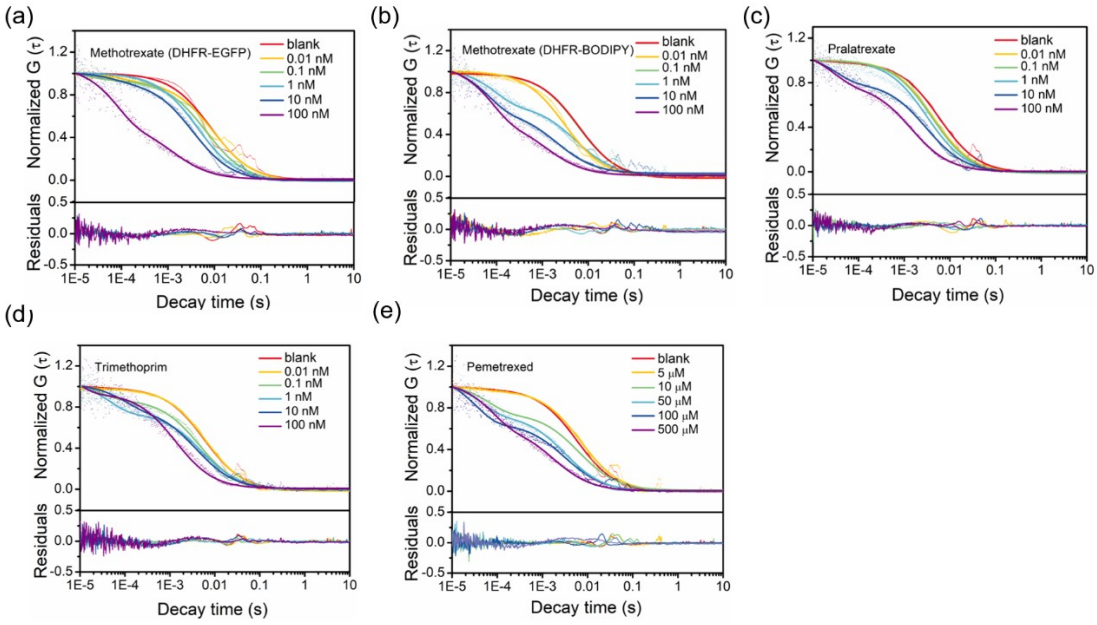

Fig. S19. Inhibitory effect of different drugs on DHFR aggregation. The normalized FCS curves and fitted residuals for different drug binding with DHFR. The drug is (a) Methotrexate, the fluorescent-labelled probe is DHFR-EGFP, (b) Methotrexate, (c) Pralatrexate, (d) Trimethoprim, (e) Pemetrexed, the fluorescent probes used for the latter four drugs are all DHFR- BODIPY. The concentrations of DHFR-EGFP and DHFR-BODIPY are both 50 nM.

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2 H. Goto, S. Okuda, A. Mizukami, H. Mori, N. Sasaki, D. Kurihara and T. Higashiyama, *Plant Cell Physiol.*, 2011, **52**, 49-58.