Supporting Information for

Protein-induced fluorescence enhancement for a simple and universal detection of protein/small molecule interactions

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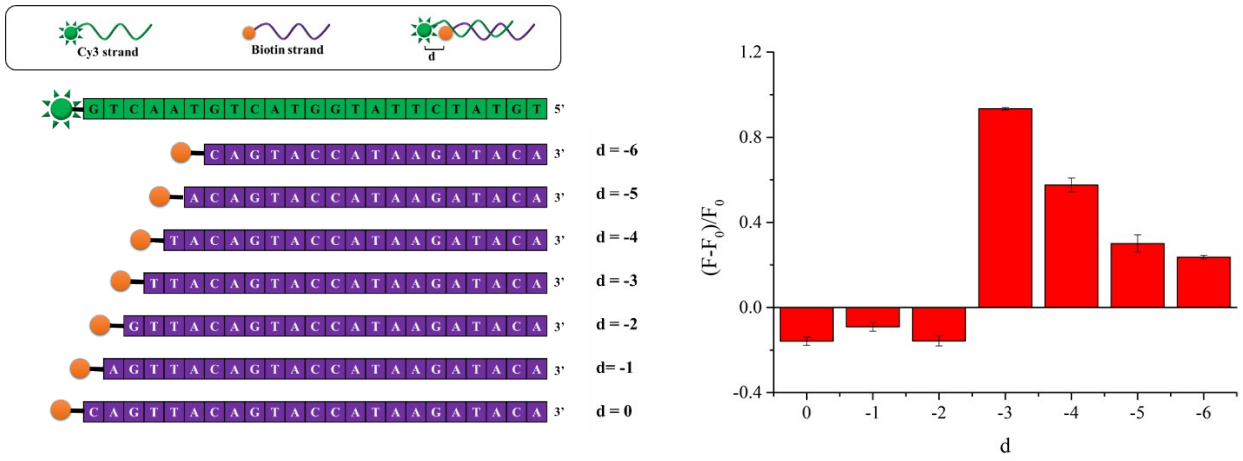
Table S1. Comparison of this method with previous fluorescent methods for protein/SM interactions.

Key principle	**Linear range**	**LOD**	**Analysis time**	**Limitations**	**Ref.**
FRET ($NaYF_4$ Nanocrystals)	-	4.8 nM	30 min	Target protein labeling, synthesis of SM-labeled nanomaterial	S1
FRET ($NaYF_4$:Yb,Tm UCNPs)	9 to 150 nM	3.8 nM	30 min	Synthesis of nanomaterial, multiple steps	S2
Fluorescence (Silver nanoclusters)	6 to 600 nM	2.6 nM	140 min	Synthesis of nanomaterial, use of enzyme, multiple steps	S3
Fluorescence (Copper nanoclusters)	0.5 to 1000 nM	0.1 nM	25 min	Synthesis of nanomaterial, use of enzyme, multiple steps	S4
PIFE (cyanine dyes)	0 to 150 nM	2.93 nM	10 min	-	This work

Table S2. DNA sequences employed in this work.

Strand name	DNA sequence (5' → 3')	Modification
3' Cy3-strand (CS_3)	TGT ATC TTA TGG TAC TGT AAC TG	3'-Cy3
BTN-strand (SS_{BTN})	TTA CAG TAC CAT AAG ATA CA	5'-Biotin
FA-strand (SS_{FA})	TTA CAG TAC CAT AAG ATA CA	5'-Folate

Figure S1. Optimization of the distance (d) between BTN and Cy3. ((F- F_0)/F_0) at different positions of BTN relative to Cy3, where F_0 and F represent the fluorescence intensities at 570 nm in the absence and presence of STV (500 nM), respectively. The reaction time and



temperature for STV/BTN interaction are 10 min and RT, respectively.

When d is -0, -1, and -2, the fluorescence signal is decreased. We assume that this reduction is attributed to the fluorescence resonance energy transfer (FRET) from Cy3 to protein based on the fluorescence quenching capabilities of streptavidin. In contrast, as d changes from -2 to -3, the significant fluorescence enhancement is observed, which is attributed to the PIFE that out-performs FRET in this range. These results are supported by the paper of Hwang et al. in which the authors found that the efficiency of PIFE is largely dependent on the distance between protein and fluorophore[S5]. In this paper, the efficiency of PIFE is

drastically increased from 2 base pair (bp) to 1 bp, clearly confirming that PIFE is quite sensitive to the very short distance (e.g., a single bp).

Figure S2. Optimization of the reaction time and temperature. (a) The degree of fluorescence increase ($(F-F_0)/F_0$) at different reaction times for STV/BTN interaction. The distance and reaction temperature for STV/BTN interaction are -3 and RT, respectively. (b) ($(F-F_0)/F_0$) at different reaction temperatures for STV/BTN interaction. The distance and reaction time for STV/BTN interaction are -3 and 10 min, respectively. F_0 and F represent the fluorescence intensities at 570 nm in the absence and presence of STV (500 nM), respectively.

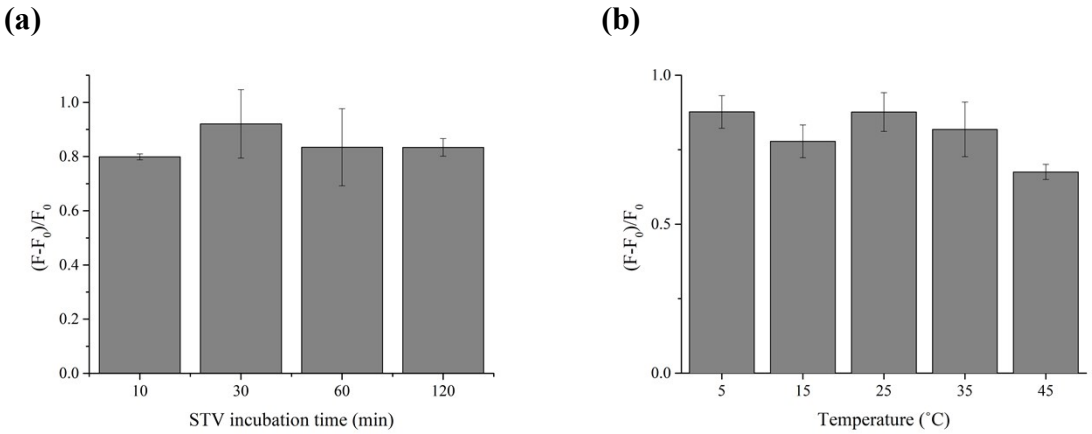
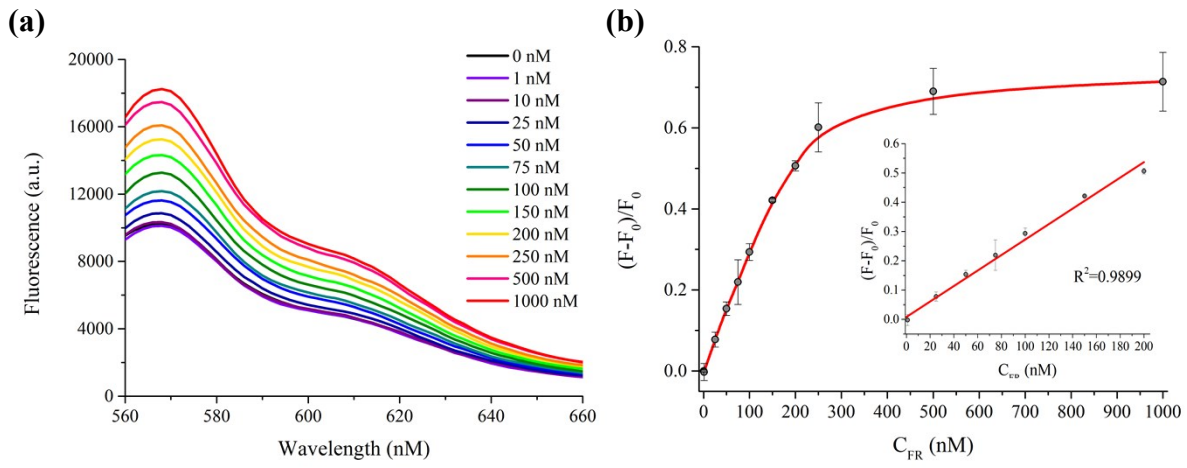
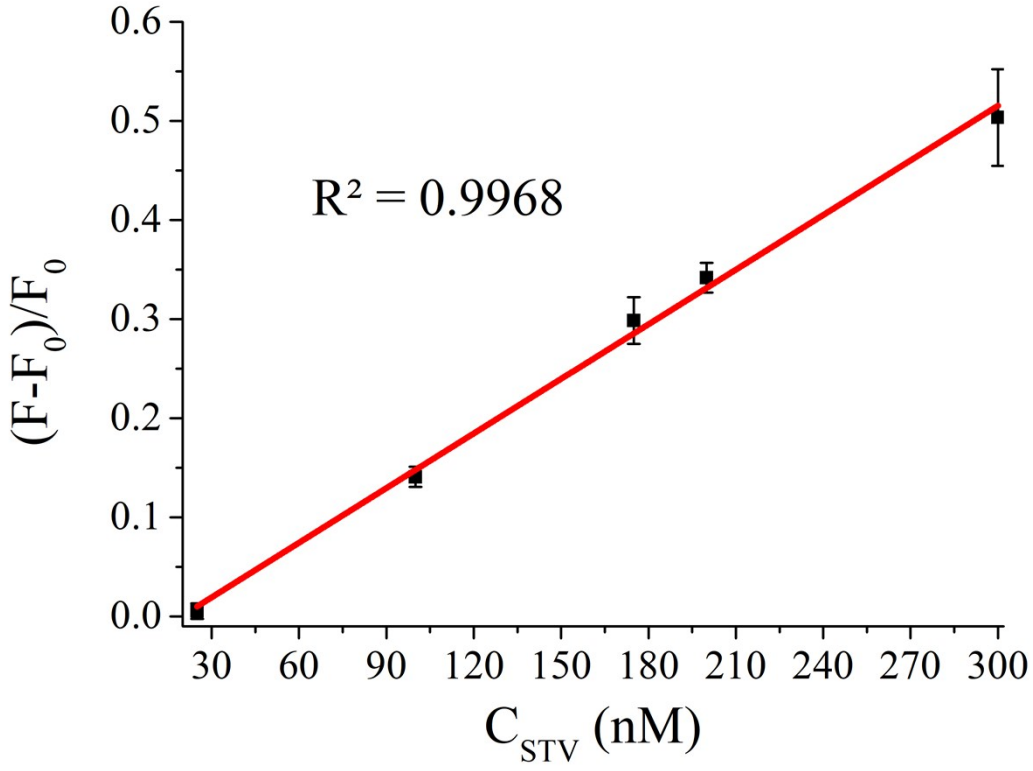


Figure S3. General applicability of the proposed strategy. (a) Fluorescence emission spectra and (b) the degree of fluorescence increase ($(F-F_0)/F_0$) in the presence of FR at varying concentrations, where F_0 and F represent the fluorescence intensities at 570 nm in the absence and presence of FR, respectively. The inset in (b) shows the linear relationship between $(F-F_0)/F_0$ and FR concentration (C_{FR}) in the range from 1 nM to 200 nM.



The fluorescence intensities increased with increasing concentrations of FR an excellent linear relationship (R^2=0.9899) was established in the range from 1 to 200 nM by the linear equation, $(F-F_0)/F_0 = 0.0026 \times C_{FA}/nM + 0.0081$. LOD was calculated to be 3.00 nM, which is comparable or higher than those from previous fluorescent methods.

Figure S4. The linear relationship between the F_{570} and STV concentration in human serum. The plot of F_{570} *vs*. STV concentration (C_{STV}) shows an excellent linear relationship ($(F-F_0)/F_0$ = 0.0018 x C_{STV}/nM - 0.0371) in the range of from 25 nM to 300 nM.



S1. D. Tu, L. Liu, Q. Ju, Y. Liu, H. Zhu, R. Li, X. Chen, *Angew. Chemie - Int. Ed.* 2011, **50**, 6306–6310.

S2. S. Zhang, J. Wang, W. Xu, B. Chen, W. Yu, L. Xu, H. Song, *J. Lumin.,* 2014, **147**, 278–283.

S3. Y. He, B. Jiao, *Microchim. Acta*, 2016, 3183–3189.

S4. H.B. Wang, H.D. Zhang, Y. Chen, Y.M. Liu, Biosens. Bioelectron, 2015, 74, 581–586.

S5. H. Hwang, H. Kim and S. Myong, Proc. Natl. Acad. Sci. USA, 2011, 108, 7414–7418.