Electronic Supplementary Information

WS_2 nanosheet-based nanosensor for ultrasensitive detection of small molecule-protein interaction via terminal protection of small molecule-linked DNA and Nt.BstNBI-assisted recycling amplification

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Optimization of assay conditions

In order to achieve the best sensing performance, a series of experimental parameters were investigated and optimized in this work.

It was found that the amount of WS_2 nanosheet used has a large influence on the fluorescence quenching efficiency. The fluorescence spectra of eight samples were measured in the presence of 0, 0.4, 0.6, 0.8, 1.2, 1.6, 2.0 and 2.4 μg/mL WS_2 nanosheet. As show in Fig. S4, The fluorescence intensity of FAM-labeled DNA at 521 nm dramatically decreased with the concentration of WS_2 nanosheet increased from 0 to 1.2 μg/mL, with a further increase the concentration of WS_2 nanosheet, the fluorescence intensity value at 521 nm of FAM-labeled DNA had no obvious change. The results indicated that when the concentration of the WS_2 nanosheet was up to 1.2 μg/mL, the fluorescence quenching of the dye FAM-labeled DNA reached the maximal value. As a result, 1.2 μg/mL WS_2 nanosheet was chosen for the optimized concentration for further experiments to reach the desired sensitivity and selectivity.

The kinetic behaviors of the FAM-labeled DNA and WS_2 nanosheet, as well as the FAM-labeled DNA-WS_2 complex with biotin-linked DNA, were studied by monitoring the fluorescence intensity as a function of time. As shown in Fig. S5 (curve a), it was observed that the adsorption of FAM-labeled DNA on the surface of WS_2 nanosheet was quite fast at room temperature. It reached equilibrium in about 20 min. Therefore, the incubation time of FAM-labeled probe and WS_2 nanosheet was 20 min in the following experiments. However, the formation and release of the dsDNA from WS_2 nanosheet was relatively slow, and reached equilibrium after 30 min (Fig. S5,

curve b).

We also investigated the fluorescence quenching efficiency (F_0−F/F_0) of WS_2 nanosheet to FAM-labeled DNA with different lengths. As shown in Fig. S6, a substantial increase in the fluorescence quenching efficiency of WS_2 nanosheet to FAM-labeled DNA was observed with the increasing base number of FAM-labeled DNA and experienced almost no obvious change when the length of FAM-labeled DNA between 15 and 23. In contrast, upon the addition of SA-linked DNA and Nt.BstNBI, the FAM-labeled DNA was cyclically cleaved by Nt.BstNBI, producing short FAM-linked oligonucleotide fragments. The fluorescence intensity of FAM was partially recovered because of the weak affinity of the short FAM-labeled oligonucleotide fragments to WS_2 nanosheet. However, with the increasing of the length of FAM-labeled DNA, the recovered fluorescence intensity decreased gradually. To fit the desired sensitivity, FAM-labeled DNA contains fifteen bases was used as the optimal length of FAM-labeled DNA for further experiments.

Meanwhile, the influence of the amount of Exo III and Exo III-catalyzed digest reaction time on the fluorescence intensity was also optimized. The results were shown in Fig. S7. The fluorescence intensity gradually decreased as the amount of Exo III increased, and reached to equilibrium when the Exo III concentration was up to 20 U. Therefore, the optimal amount of Exo III was chosen to be 20 U and used throughout subsequent experiments. Further, we studied the fluorescence signals at different digestion reaction times in the presence of 20 U Exo III. As shown in Fig. S8, it was found that the fluorescence intensity reached a plateau in 30 min. Hence, 30 min was

taken as the optimum digest reaction time for SA analysis.

Table S1 DNA oligonucleotides sequence used in this work

Oligonucleotides name	Sequences (5′ to 3′) Description
11-F (FAM-labeled DNA)	***GAG TCA ACA***↓***T***A -FAM
15-F (FAM-labeled DNA)	***GAG TCA ACA***↓***T***AT ATA-FAM
19-F (FAM-labeled DNA)	***GAG TCA ACA***↓***T***AT ATA TTT T-FAM
23-F (FAM-labeled DNA)	***GAG TCA ACA***↓***T***AT ATA TTT TTT TT-FAM
Biotin-linked DNA	GTC CC***A TGT TGA CTC***-Biotin

The italic bold letters between FAM-labeled DNA and Biotin-linked DNA show the complementary bases. The arrow indicates the nicking position.

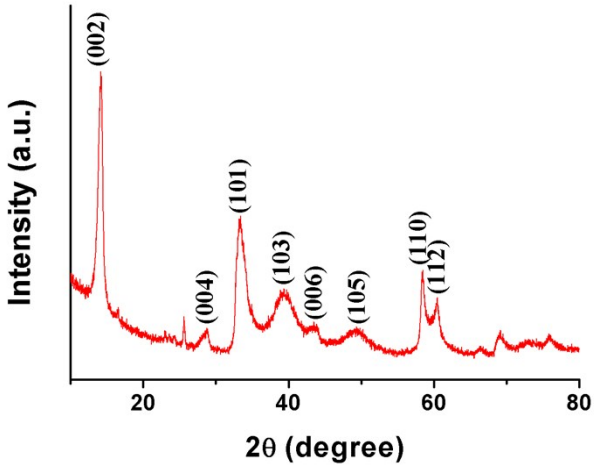

Fig. S1 XRD pattern of the as-synthesized layered WS_2 nanosheet.

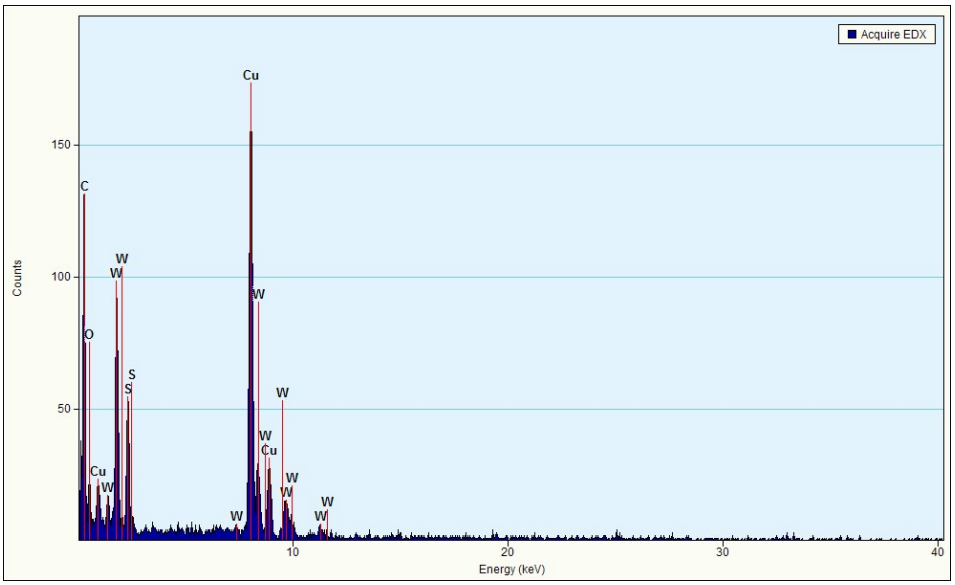

Fig. S2 EDX spectra of the as-synthesized layered WS_2 nanosheet.

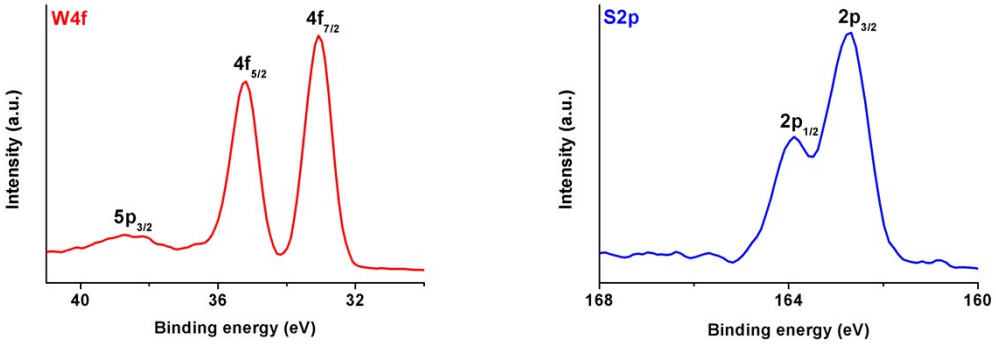

Fig. S3 The high-resolution XPS analysis for the W4f and S2p orbits of the as-synthesized layered WS_2 nanosheet.

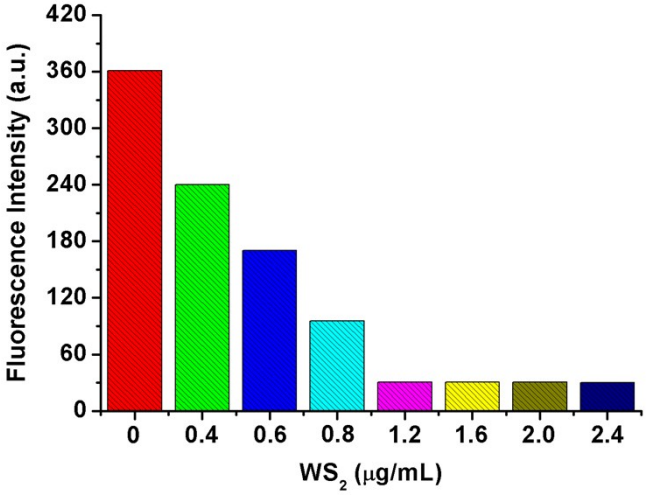

Fig. S4 Fluorescence intensity of FAM-labeled DNA after addition of WS_2 nanosheet with different concentrations. Concentration: FAM-labeled DNA, 60 nM; WS_2, 0, 0.4, 0.6, 0.8, 1.2, 1.6, 2.0 and 2.4 μg/mL, Excitation wavelength: 480 nm.

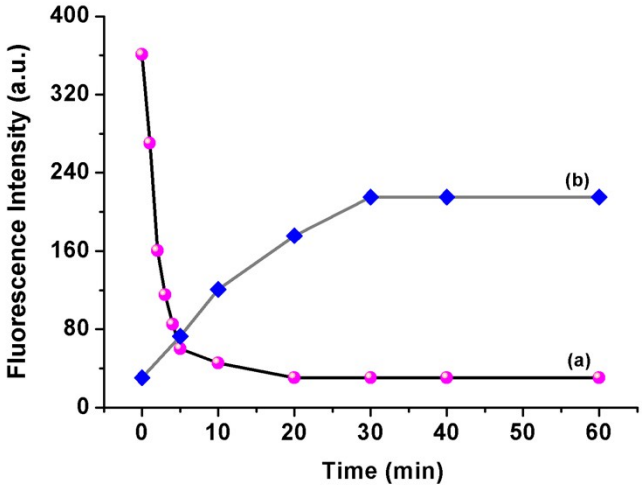

Fig. S5 (a) Fluorescence quenching of FAM-labeled DNA (60 nM) in Tris-HCl buffer by WS_2 nanosheet (1.2 μg/mL) as a function of time, and (b) Fluorescence restoration of FAM-labeled DNA (60 nM) in WS_2 nanosheet (1.2 μg/mL) solution by biotin-linked DNA as a function of time. Excitation wavelength: 480 nm.

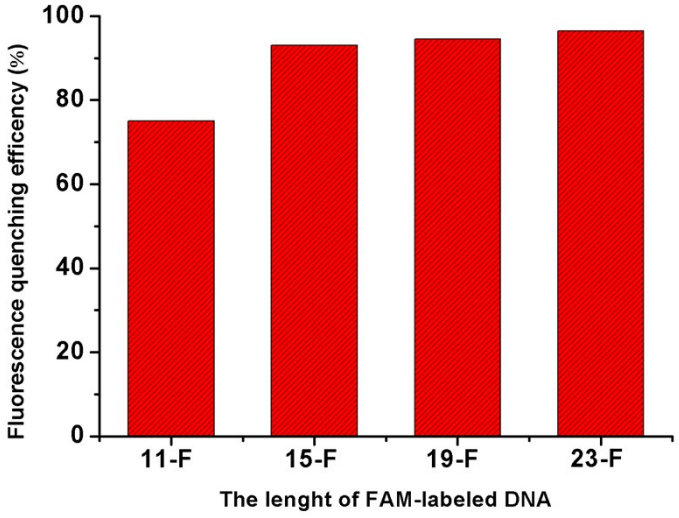

Fig. S6 Comparison of the fluorescence quenching efficiencies (F_0-F/F_0, F and F_0 are the fluorescence intensities in the absence and presence of WS_2 nanosheet, respectively) of WS_2 nanosheet to FAM-labeled DNA with different lengths. The 11-F, 15-F, 19-F and 23-F indicate FAM-labeled DNA containing 11, 15, 19 and 23 bases, respectively. Concentration: FAM-labeled DNA, 60 nM; WS_2, 1.2 μg/mL.

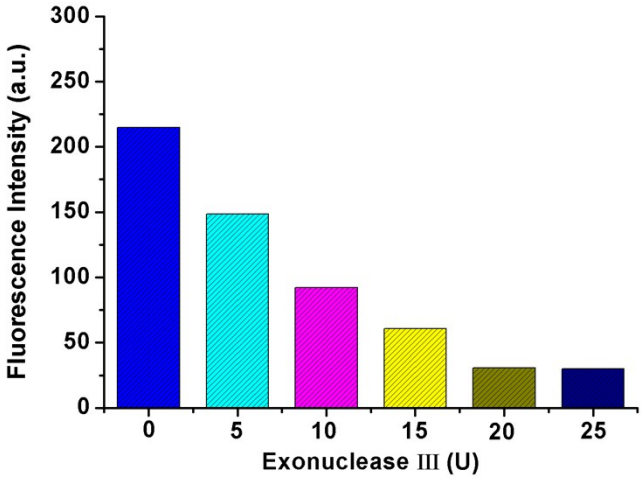

Fig. S7 Fluorescence intensity of FAM-labeled DNA upon the addition of different concentrations of Exo III. Concentration: FAM-labeled DNA, 60 nM; biotin-linked DNA, 600 nM; WS_2, 1.2 μg/mL.

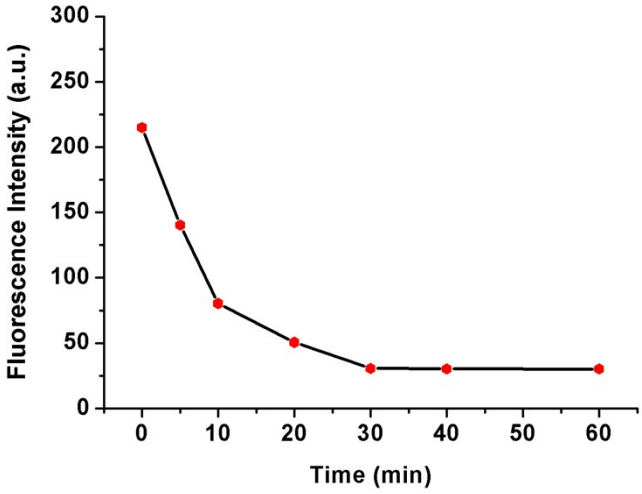

Fig. S8 Fluorescence intensity versus different reaction time of Exo III. Concentration: FAM-labeled DNA, 60 nM; biotin-linked DNA, 600 nM; Exo III, 20 U; WS_2, 1.2 μg/mL. Excitation wavelength: 480 nm.

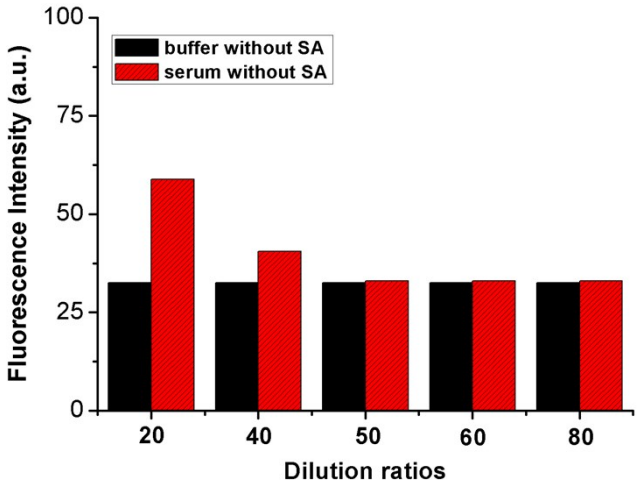

Fig. S9 Fluorescence intensity obtained from the testing of buffer solution (control) and different dilution ratios of serum samples without SA.

Table S2 Comparison of the performance of those reported approaches for the detection of SA.

Method	Labeling number	Dynamic range	Detection Limit	Ref.
FL	One (Biotin)	0.15-1.5 nM	94.8 pM	1a
FL with microfluidic	Two (Biotin, Cy3)	---------	1 pM	13
FL	One (Biotin)	0.1-200 nM	20 pM	15
EL with RCA	Two (SH, Biotin, P)	1 pM-500 nM	0.4 pM	28a
EL	Two (SH, Biotin)	10 pM-50 nM; 100 nM-1μM	10 pM	28b
FL	Two (Biotin, FAM)	0-9.1nM	10.2 pM	28c
FL	Three(Biotin, Rox, BHQ)	8.3 fM-83.3 pM	0.8 fM	28d
FL	Two (Biotin, FAM)	7.6 pM-30 nM	5.3 pM	This work

FL: Fluorometric method; EL: Electrometric method; RCA: Rolling circle amplification.