Supporting Information

Template-controlled rolling circle amplification for dual protein sensitive analysis

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Table S1. Oligonucleotide sequences used in the experiments.

Name	Sequence (5’ - 3’)
Apt 1	Biotin-AAAAACTGAATAAGGACTGCTTAGGATTGCGATGATTCAG
Tem 1	PO_4- ATGCTAAGAATACTACCACTGCTAAGAATACTACCTCGCAATCCTAAG
Primer 1	CTTAGCATCTTAGGATTGCG
Probe 1	FAM-GCTAAGAATACTACC
Apt 2	GCCTGTGGTGTTGGGGCGGGTGCGAAAAA-Biotin
Tem 2	PO_4- CCAACACCACAGGCTTACTGACTCCGTTCTGATTACTGACTCCGTTCT
Primer 2	TGTGGTGTTGGAGAACGGAG
Probe 2	Cy5-TTACTGACTCCGTTCT
MB[a]-1	CY5-ATGGATACCACAGGCTTACTGAATCCAT-BHQ2
MB-2	FAM-TACATTTACTACCTCGCAATCAATGTA-BHQ1

[a]For molecular beacon.

Table S2. The performance of different tau and AβO biosensors reported.

Technique	Biomarker	Recognition element	LOD	Ref
EIS	Tau	Tau protein	0.2-1 μM	1
EIS (SPCE)	Tau	Antibody	0.15 nM	2
Colorimetric	Tau	Aptamer	153 pg mL^{-1}	3
Fluorescence	AβO and Tau	Aptamer	20-50 pM	4
Electrochemical	AβO		0.45 nM	5
ELISA kit	AβO or Tau	Antibody	>1 ng mL^{-1}	Instructions
Fluorescence	AβO and Tau	Aptamer	10 pg mL^{-1} （~0.16 pM）	This work

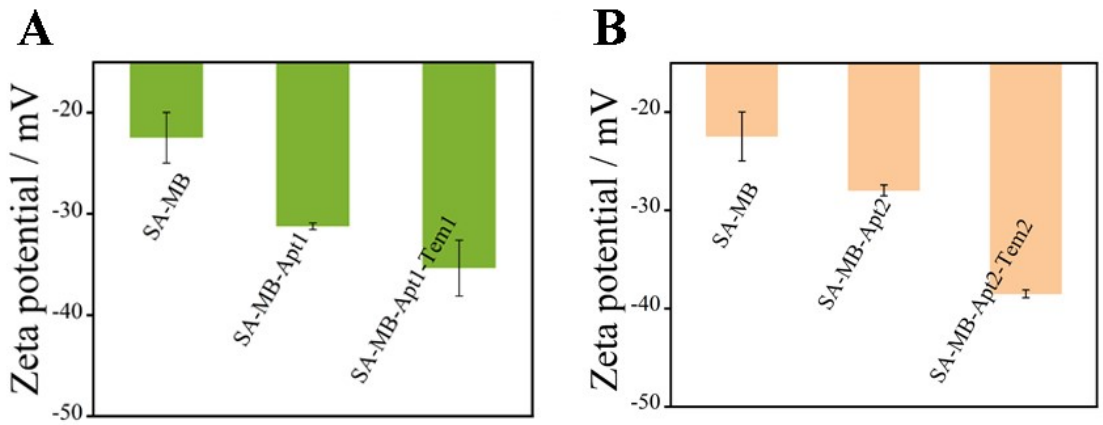

Figure S1. (A) Assembly of Apt1 and Tem1 composite probes on SA-MBs resulted in decreased potential. (B) Assembly of Apt2 and Tem2 composite probes on SA-MBs resulted in decreased potential.

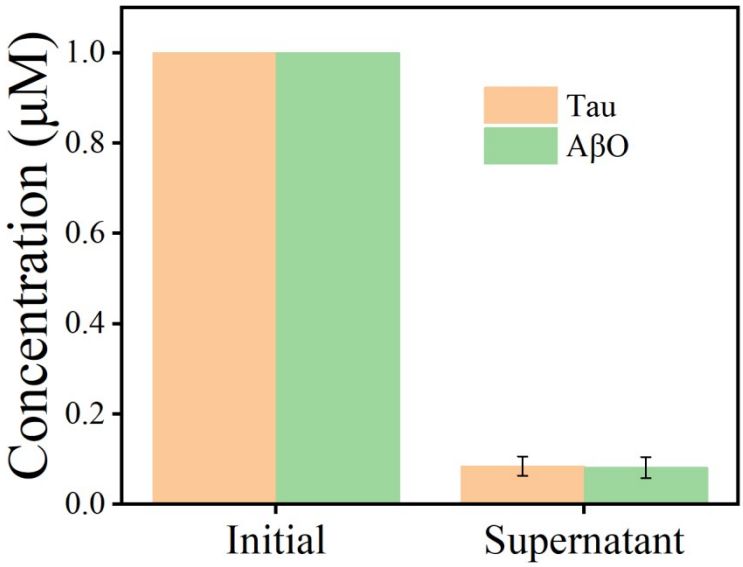

Figure S2. Incubate probe 1 and probe 2 (both concentrations are 1 μM) with 300μg/mL nanosheets separately, and compare the initial concentration of the probe with the concentration in the supernatant after centrifugation.

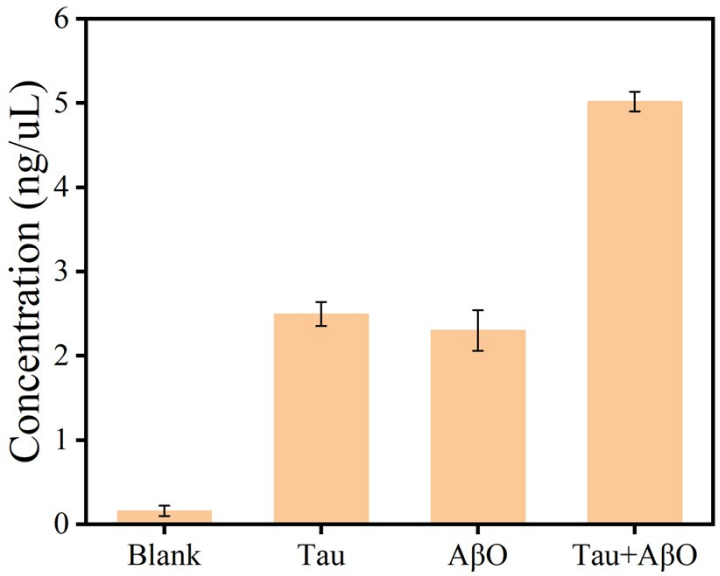

Figure S3. The concentration of the circular template in the magnetic separation supernatant before and after adding the target protein, n=4.

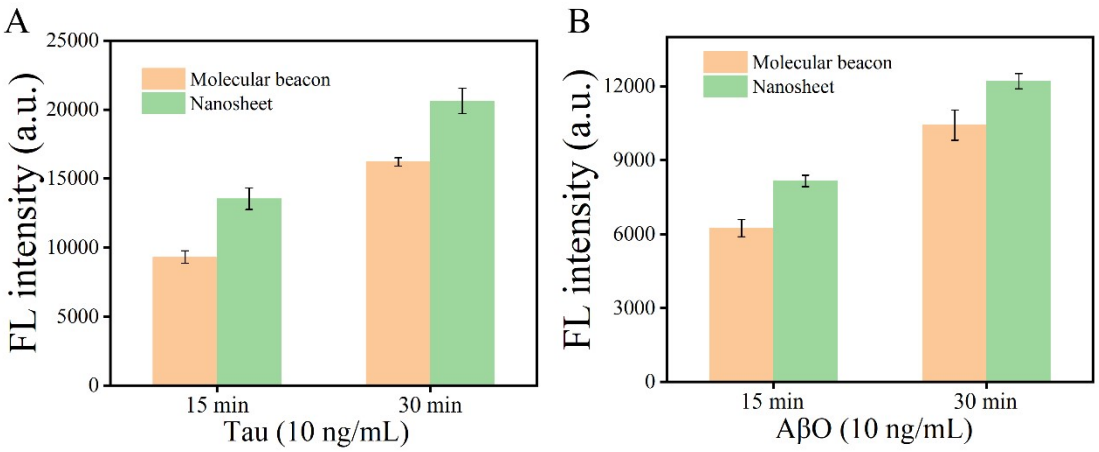

Figure S4. Comparison of reaction rates using self-quenching molecular beacons and nanosheets to output fluorescence signals, n=4.

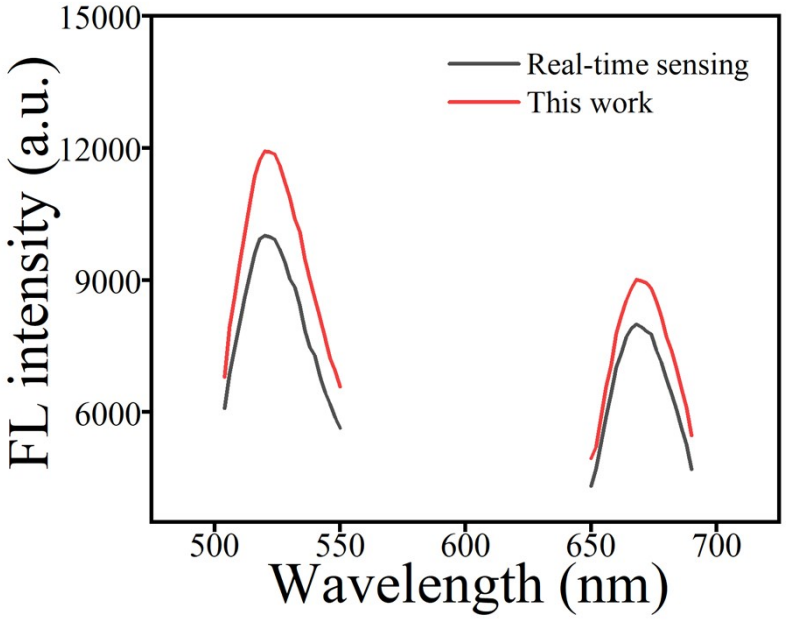

Figure S5. Real-time sensing: adding nanosheets/probe complex before RCA; This work: adding nanosheets/probe complex after stopping RCA. The fluorescence signal intensity of non real-time sensing was higher than that of real-time fluorescence sensing.

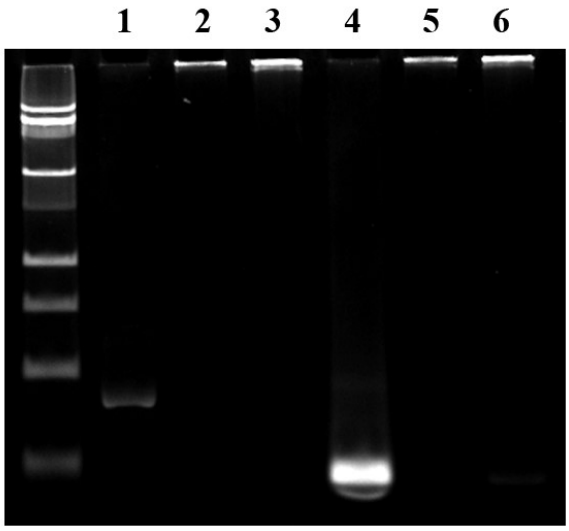

Figure S6. Hybridization of RCA products with DNA probes. Lanes 1 to 6 are: Probe 1; Tau-RCA products; Probe 1+Tau-RCA products; Probe 2; Probe 2+AβO-RCA products.

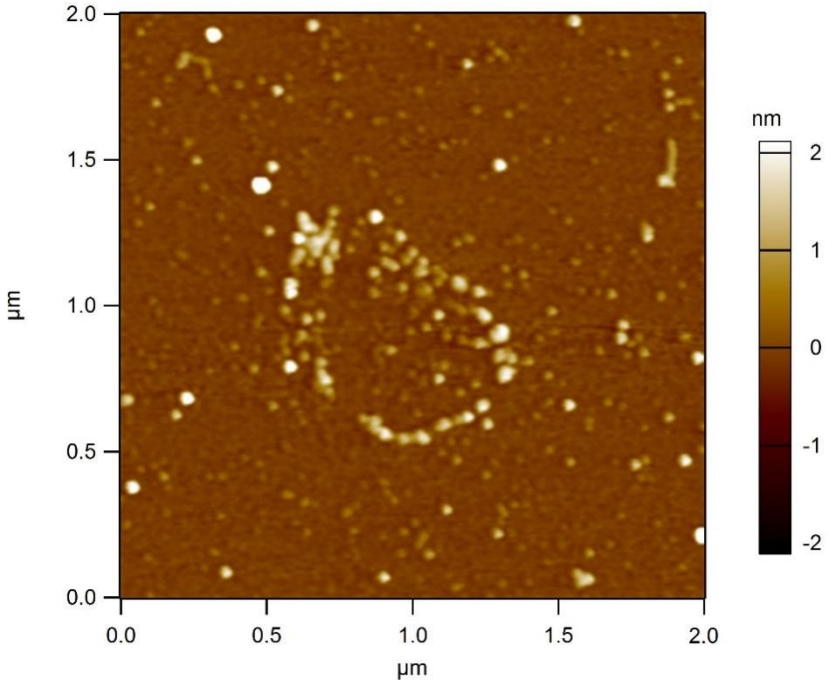

Figure S7. The phase image of atomic force microscope (AFM) shows that when there is no target protein, rolling circle amplification (RCA) products with long DNA chains are not produced.

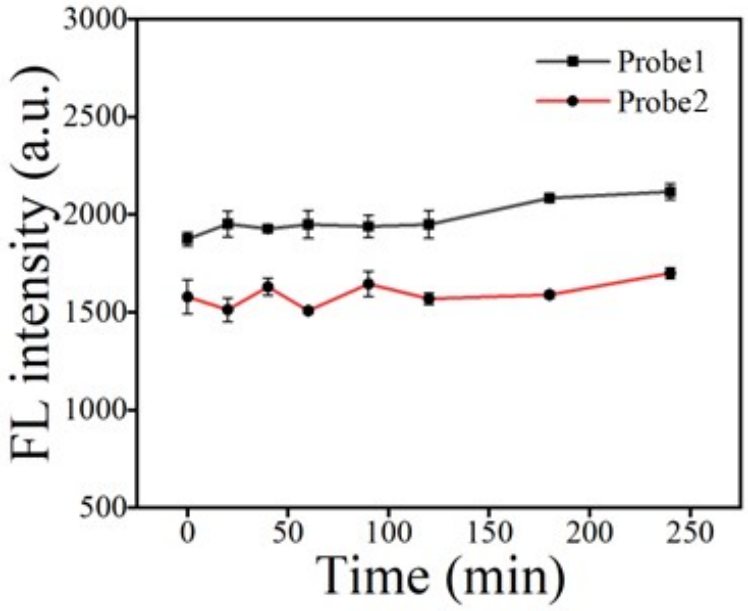

Figure S8. Fluorescence intensity of nanosheets/probes complex at 0, 20, 40, 60, 90, 120, 180, and 240 min in 37 ℃.

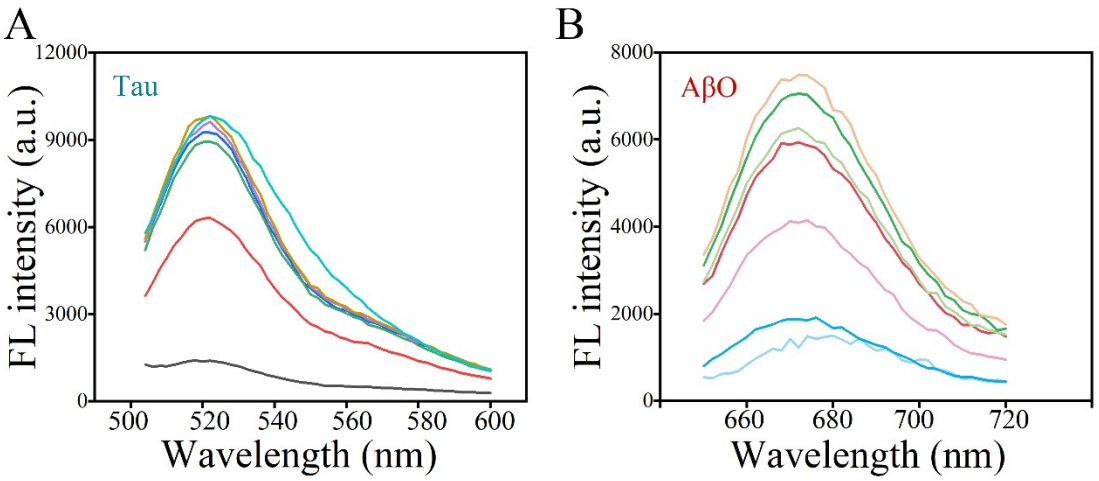

Figure S9. Time-dependent fluorescence spectrum of nanosheets loaded with FAM- and Cy5-labeled probes co-incubated with the RCA products.

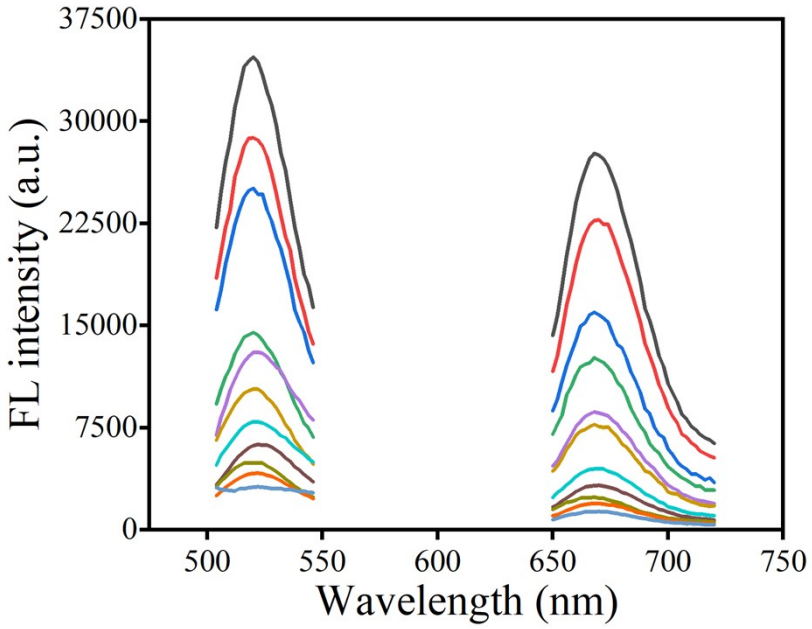

Figure S10. Signal response fluorescence spectra of targets with different concentrations (0, 0.001, 0.01, 0.1, 0.3, 0.5, 0.8, 1, 10, 100 and 1000 ng/mL).

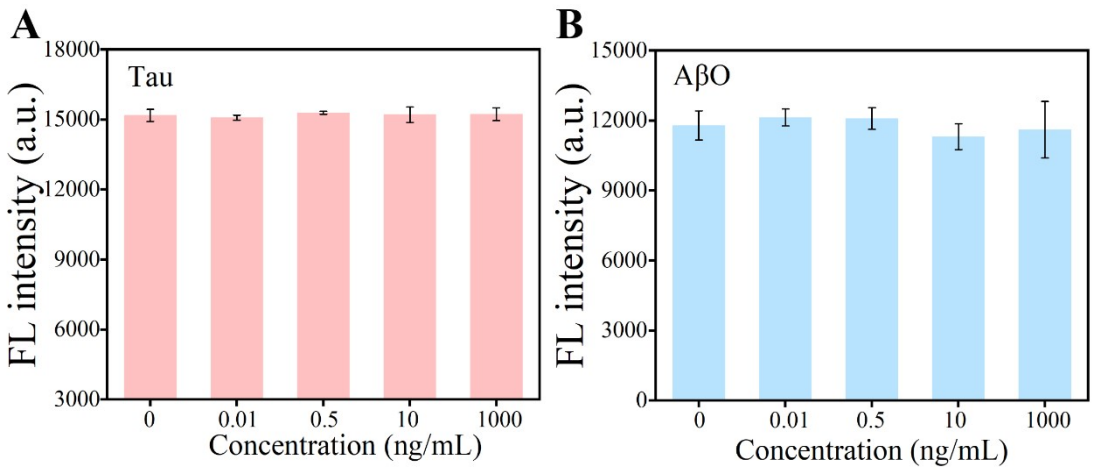

Figure S11. (A) When the concentration of Tau is fixed at 1ng/mL, adding AβO with concentrations of 0, 0.01, 0.5, 10 and 1000 ng/mL respectively, the fluorescence signal intensity generated by Tau. (B) When the concentration of AβO is fixed at 1ng/mL, adding Tau with concentrations of 0, 0.01, 0.5, 10 and 1000 ng/mL respectively, the fluorescence signal intensity generated by AβO.

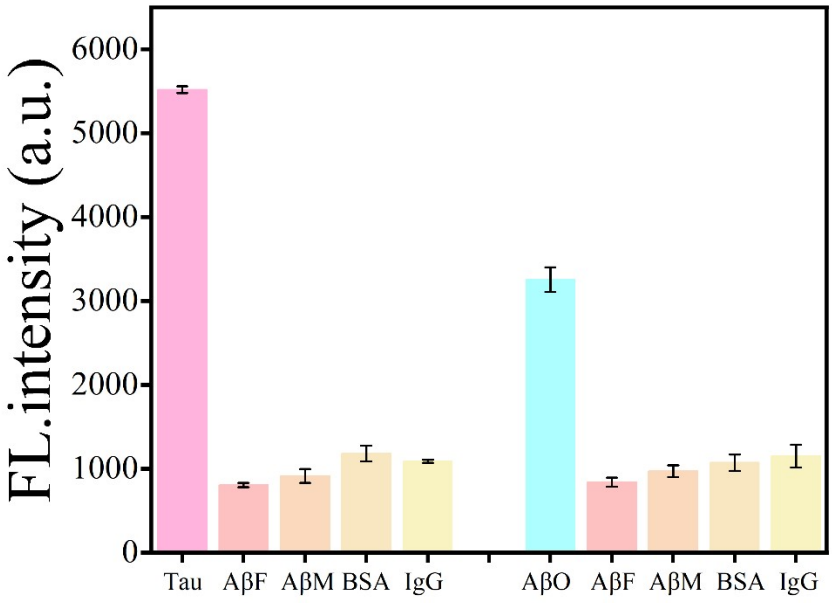

Figure S12. Selectivity of the proposed method. Peak values of fluorescence spectrum scanning by detecting 10 ng/mL target proteins (Tau and AβO) and 100 ng/mL several other proteins.

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