Supplementary information for

TdT/Cas12a Cascade Amplification Biosensor for Sensitive ALP Activity Detection

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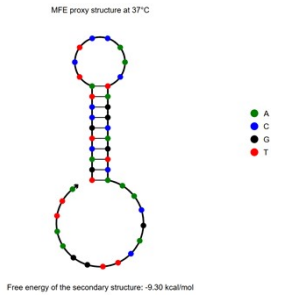
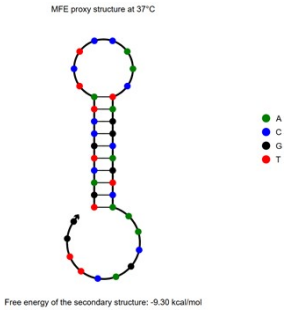
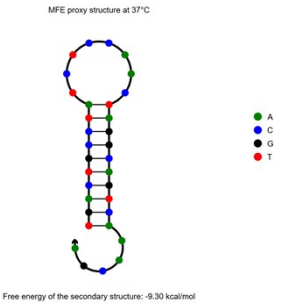
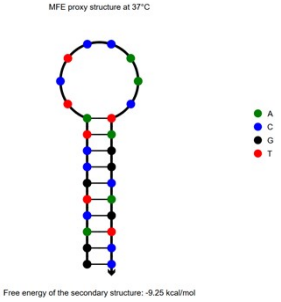
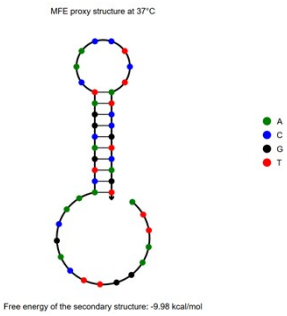
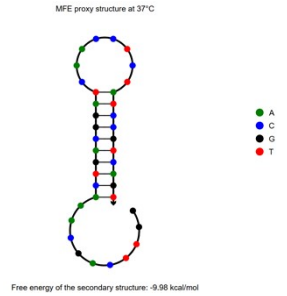
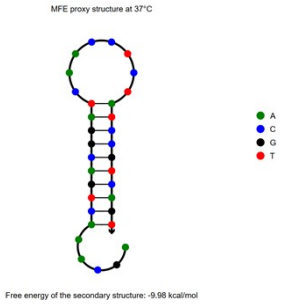
Table S1 Sequences of the oligonucleotides used in this work

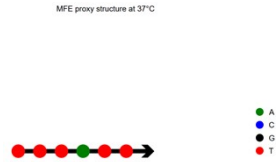
Name	Sequence (5'→ 3')	MFE structure
S001-15+nt	TgACTgCCTATCTCCAACTAggCAgTCAAACgACTTGGAATTA-PO4	
S002-10+nt	TgACTgCCTATCTCCAACTAggCAgTCAAACgACTTGG-PO4	
S003-5+nt	TgACTgCCTATCTCCAACTAggCAgTCAAACgA-PO4	
S004-0nt	GgACTgCCTATCTCCAACTAggCAgTCC-PO4	

S005-15-nt	ATT AAG GTT CAG CAA ACT GAC GGA TCA ACC TCT ATC CGT CAG T-PO4	
S006-10-nt	GGT TCA GCA AAC TGA CGG ATC AAC CTC TAT CCG TCA GT-PO4	
S007-5-nt	AGC AAA CTG ACG GAT CAA CCT CTA TCC GTC AGT-PO4	
S008-36nt	AGT GTA GAG CGT TAG AGT TTG GAA AGT ACA GTA AAG-PO4	 
S009-36nt	AGT GTA GAG CGT TAG AGT TTG GAA AGT ACA GTA AAG -OH	

S010-36nt

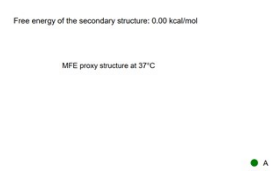
AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA -OH

Probe-1



TTT ATT

Probe-2



TTT ATT TAT T

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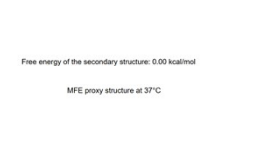

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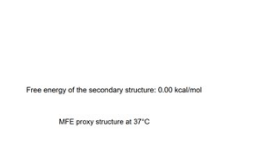
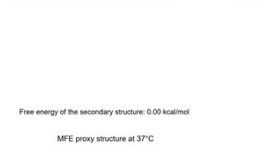


TTT ATT TAT TTT ATT

Probe-5



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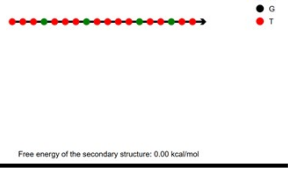


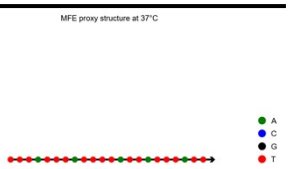
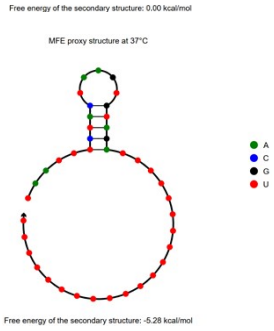
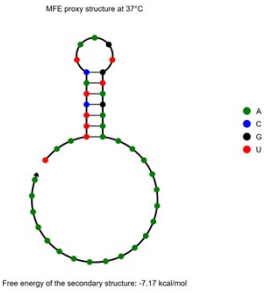
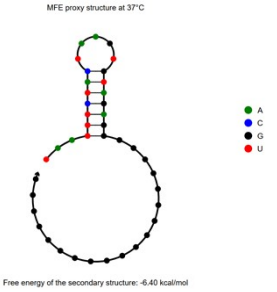
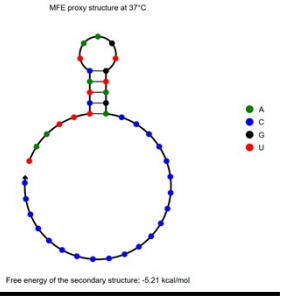
Probe-6	TTT ATT TAT TTT ATT ATT TAT T	
crRNA-1	UAA UUU CUA CUA AGU GUA GAU UUU UUU UUU UUU UUU UUU UU	
crRNA-2	UAAUUUCUACUAAGUGUAGAAAAAAAAAAAAA AAAAAAAAA	
crRNA-3	UAAUUUCUACUAAGUGUAGAGGGGGGGGGGG GGGGGGGGG	
crRNA-4	UAAUUUCUACUAAGUGUAGACCCCCCCCCCCCC CCCCCCCC	

Table S2 Comparison with other recent methods for ALP detection [2]

Strategy		Materials or enzymes	Selectivity			LOD (U/L)	Refs.
			Non-related proteins	Nucleases	Other enzymes		
Dephosphorylation induces the fluorescence change of foreign labels	Based on noble metal nanomaterials	AgNPLs	ND	ND	p-APP	0.0011	[22]
		Agnanoshells and Aunanobipyramids	ND	ND	Glucose Phosphate	0.085	[23]
		AgNPs	ND	ND	NaBH4，ascorbic acid	0.003	[24]
		AgNPs，AAP,TMB	ND	ND	ND	0.037	[25]
		AuNCs	ND	ND	ND	0.1	[26]
		AuNCs	ND	ND	Gox, Galactosidase, Thrombin, Pepsin, Trypsin	0.002	[27]
		AuNCs and KMnO4	Glutathione, Cysteine	ND	Lysozyme, Trypsin, Thrombin	0.002	[28]
	Based on carbon nanomaterials	CQDs, Ce^{3+}, ATP	BSA, IgG	DNase	Thrombin, AChE	1.4	[29]
		CQDs, AA2P	ND	ND	ND	0.45	[30]
		N-GQDs, AA2P	ND	ND	PPase, Thrombin, GOx, Trypsin, Lysozyme, Gal, PP1	0.07	[31]
	Based on phosphate labelled DNA	TdT, hemin/G-quadruplex DNAzyme	BSA	ND	Lysozyme, Pepsin	0.03	[32]
		TdT, SYBR	ND	ND	Lysozyme, Pepsin,	0.025	[33]

		Green I			GOx, Trypsin		
		λ exo	BSA	ND	Lysozyme, UDG, hOGG1	3	[34]
		MB, Exo III, Klenow fragment polymerase, Nb.BbvCl	BSA, CHT	Dpn I	UDG, Dam	0.37	[35]
		λ exo	BSA	DNase I, T7 exo, Exo III	UDG	0.014	[36]
		UCNPs, AgNCs	ND	ND	Lysozyme, HRP	0.0018	[37]
		KF, Nb.BbvCl	BSA, Streptavidin	ND	Lysozyme, Hemoglobin	0.001	[38]
		CdTe/CdS QDs	HSA, BSA	ND	Pepsin, Acetylcholine esterase, Trypsin, GOx	0.34	[39]
		CuInS2 QDs	ND	ND	HRP, LDH, Trypsin, Pepsin, GOx, Urase	0.0036	[40]
	Based on organic molecules	7-MC-3-COOH, Eu^{3+}, AMP	ND	ND	HRP, GA, LP	0.35	[41]
		4-aminophenyl phosphate (APP), Diethanolamine (DEA)	ND	ND	ND	0.037	[42]
		DQM-ALP	ND	ND	Acetylcholinesterase, Butyrylcholinesterase, Carboxylesterase	0.15	[43]

		Antibodies, p-nitrophenyl phosphate, calcein, Ce^{3+}	ND	ND	ND	0.023	[44]
	Based on specific DNAzyme	Zn^{2+}-dependent DNAzyme	HSA, BSA, SA, IgG	ND	Lysozyme	5.2	[45]
Fluorescence difference between S–P and S		reactive p-hydroxybenzyl group	HSA, BSA	ND	Pepsin, Trypsin, Esterase, Lysozyme	1.09	[46]
		Fluorogens with aggregation-induced emission	BSA	ND	Lysozyme, Trypsin, Papain, Pepsin	0.2	[47]
Dephosphorylation induces the fluorescence change of foreign labels	Based on phosphate labelled DNA	TdT, Endo IV	BSA	DNase I, T7 exo, Exo III	UDG	0.0043	[2]
Dephosphorylation induces the fluorescence change of foreign labels	Based on phosphate labelled DNA	TdT, Cas12a	BSA	T7 exo, Endo IV	UDG, hAAG	**0.0017**	**This work**

ND: no data; AA2P: ascorbic acid 2-phosphate; AChE: acetyl cholinesterase; AgNCs: silver nanoclusters; ALP: alkaline phosphatase; AuNCs: gold nanoclusters; CHT: chymotrypsin; CQDs: carbon quantum dots; CuNPs: copper nanoparticles; Dam: DNA adenine methylation methyltransferase; Dpn I: methylation-sensitive restriction endonuclease; Endo IV: endonuclease IV; Exo I: exonuclease I; Exo III: exonuclease III; GA: gangliosides; Gal: galactosidase; GOx: glucose Oxidase; GQDs: graphene quantum dots; hOGG1: human 8-oxoguanine DNA glycosylase; HRP: horseradish Peroxidase; HSA: human serum albumin; LDH: lactate dehydrogenase; PP1: protein phosphatase 1; PPase: pyrophosphatase; QDs: quantum dots; TdT: terminal deoxynucleotidyl transferase; UDG: uracil glycocasylase; UCNPs: upconversion nanoparticles; hAAG：3-methyladenine DNA glycosylase,. p-APP：dephosphorylates p-aminophenol phosphate, TMB: 5,5′-tetramethylbenzidine; AAP: ascorbic acid phosphate; BSA: bovine serum albumin, Cas12a: CRISPR-associated endonuclease 12a.

Table S3 The Fluorescence Increase Rate of 0.1 U/L ALP in the TdT/Cas12a Biosensor with 25 Repeats.

Test	1	2	3	4	5	Mean（Increase Rate）	Standard deviation	Intraday RSD (%)
Day 1	6.37	6.38	6.33	6.26	6.09	6.286	0.1067	1.697
Day 2	6.52	6.77	7.26	6.91	7.31	6.954	0.2982	4.288
Day 3	7.06	7.1	6.86	7.25	6.31	6.916	0.3276	4.736
Day 4	7.49	7.96	7.77	8.16	7.35	7.746	0.2967	3.830
Day 5	6.47	6.38	7.02	7.18	7.09	6.828	0.3341	4.894

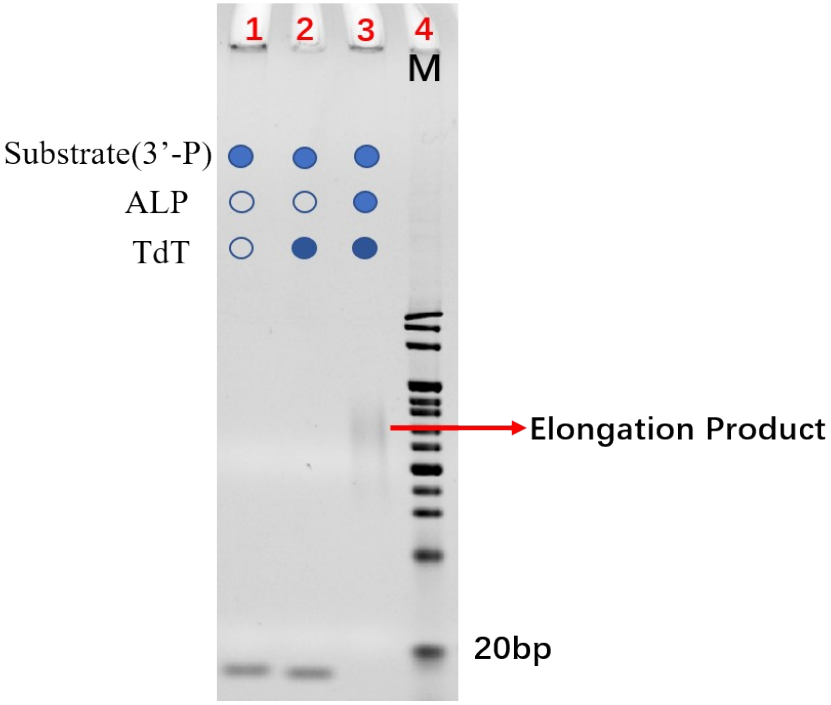


Figure S1 Native PAGE analysis of the ALP-triggered TdT elongation process. Lane 1: The original 3'-phosphorylated (3'-PO_4) ssDNA substrate (S008, 100 nM). Lane 2: Substrate S008 (100 nM) incubated with TdT (0.5 U) and dATP (1 mM). Lane 3: Substrate S008 (100 nM) incubated with ALP (0.25 U/L), followed by further incubation with TdT (0.5 U) and dATP (1 mM). The gel was stained with GelRed for visualization.

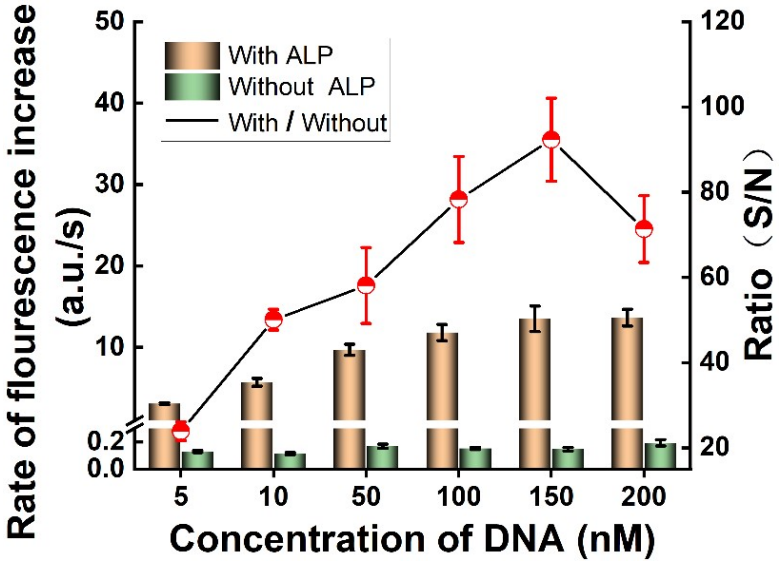


Figure S2 Fluorescence responses of the TdT/Cas12a biosensor under different concentration of 3'-phosphate-ended substrates.

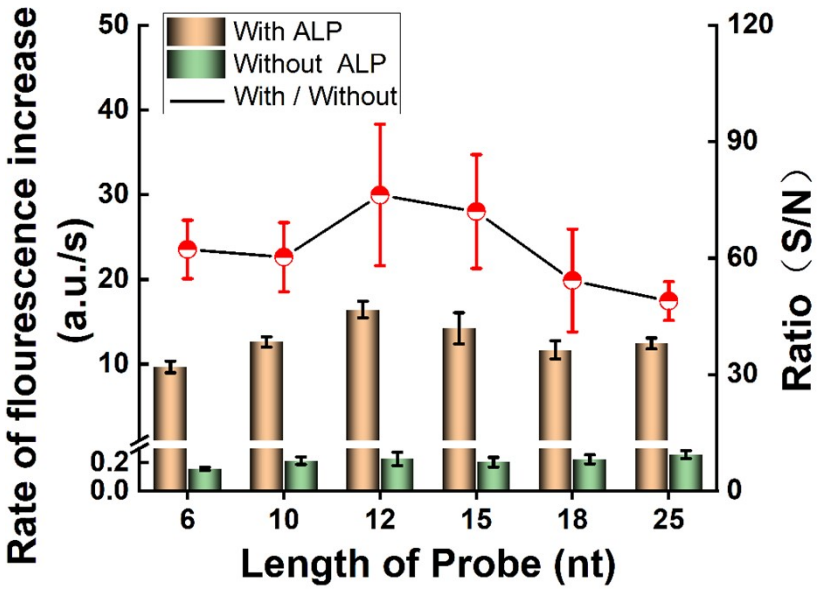


Figure S3 Fluorescence responses of the TdT/Cas12a biosensor under different length of probe.

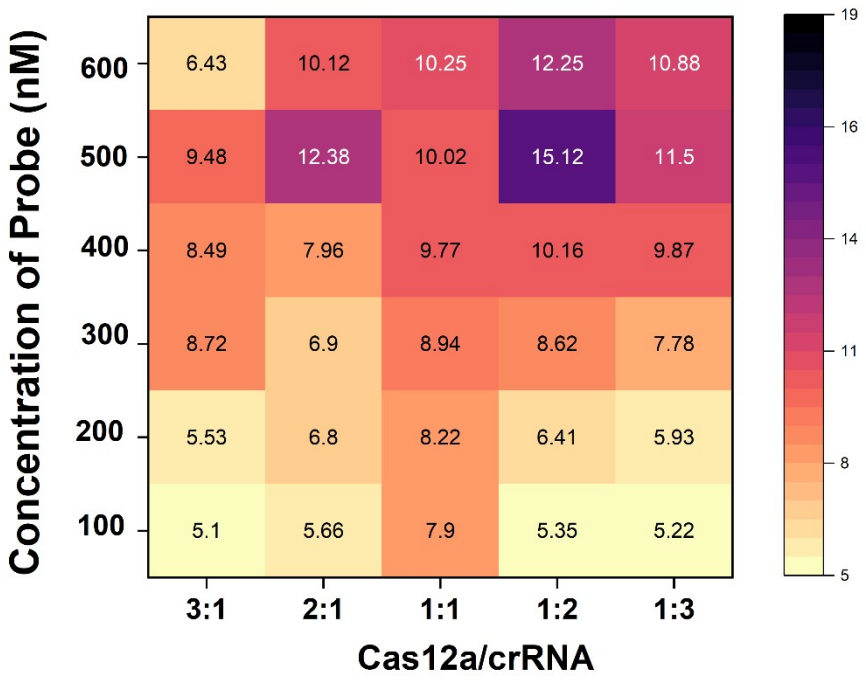


Figure S4 Optimization of reaction conditions using S010 as the substrate strand with crRNA by monitoring the fluorescence rise rate.

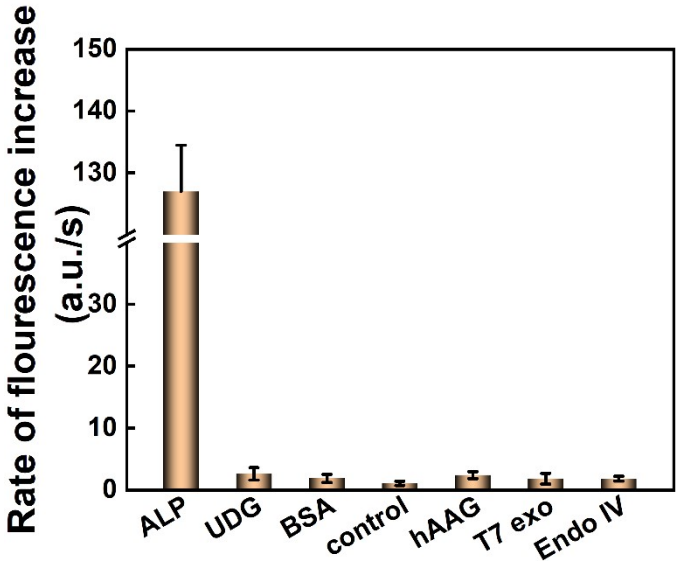


Figure S5 Selectivity evaluation of the TdT/Cas12a biosensor by testing possible interference from other enzymes or proteins, including ALP (1 U/L), UDG (uracil DNA glycosylase, 10 U/L), Endo IV (endonuclease IV, 10 U/L), hAAG (3-methyladenine DNA glycosylase, 10 U/L), T7 exonuclease (T7 exo, 10 U/L), and BSA (bovine serum albumin, 0.1 mg/mL). The control group was a blank sample with enzyme-free water.