Electronic Supplementary Information

One-tube B7-H3 detection based on isothermal exponential amplification and dendritic hybridization chain reaction

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Stability of the one-tube B7-H3 assay

The protein-to-DNA signal transducer (anti-B7-H3 antibody conjugated DNA1/DNA3 duplex) and other reaction components were stored at 4 °C for a period from 0 day to 15 days.

Atomic force microscopy (AFM) imaging

Assembly products were diluted in 1× TAE/Mg2+ buffer and deposited onto freshly cleaved mica (Ted Pella, USA) for 3 min. Samples were then rinsed with deionized water and dried at room temperature. AFM imaging experiments were performed in the air in tapping mode on a Dimension 3100 AFM (Veeco, USA). Silicon probes FS-1500AuD (Santa Barbara.CA). The image background was flattened by Nanoscope IIIa software.

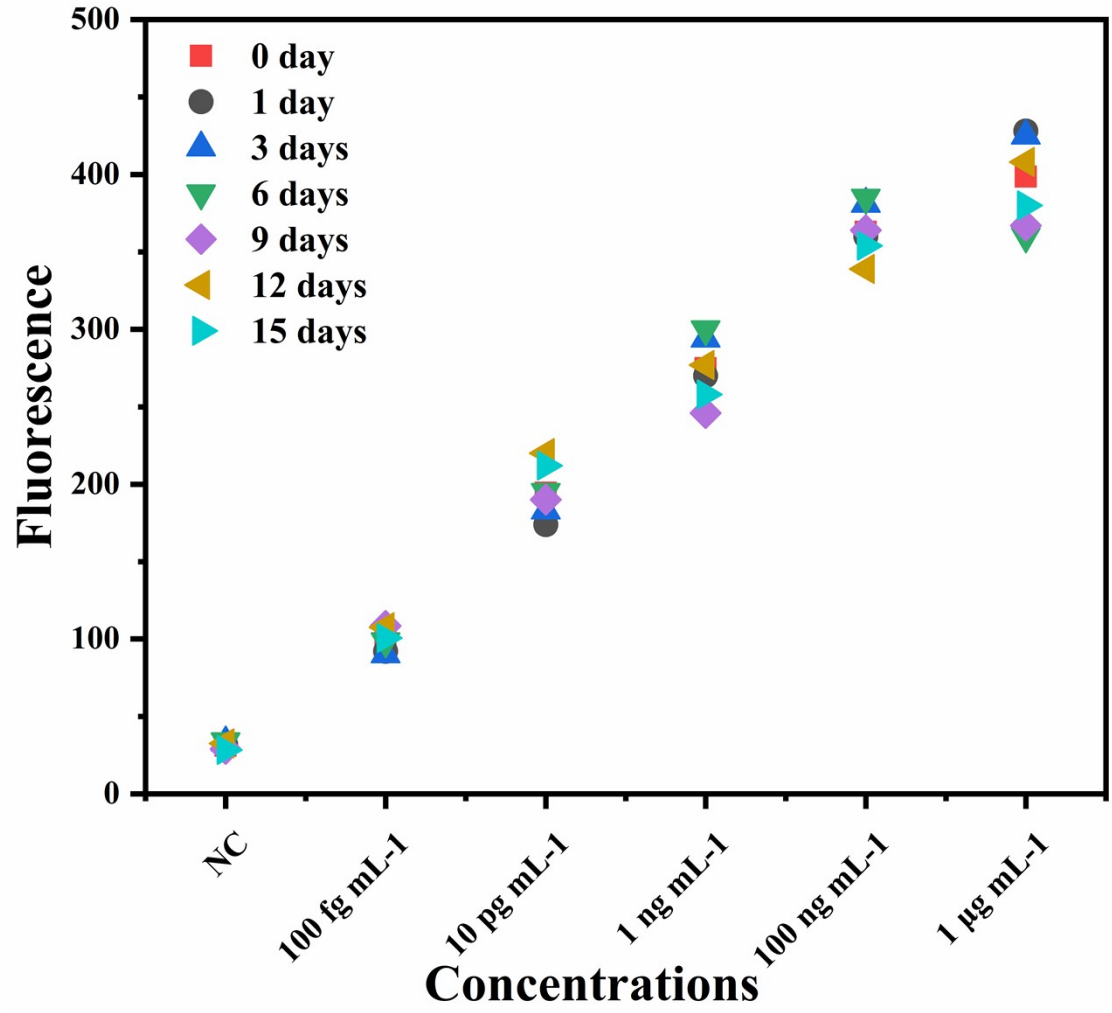

Fig. S1 The stability of the one-tube B7-H3 assay. The fluorescence response of the construction of protein-to-DNA signal transducer at different storage periods with various concentrations of B7-H3.

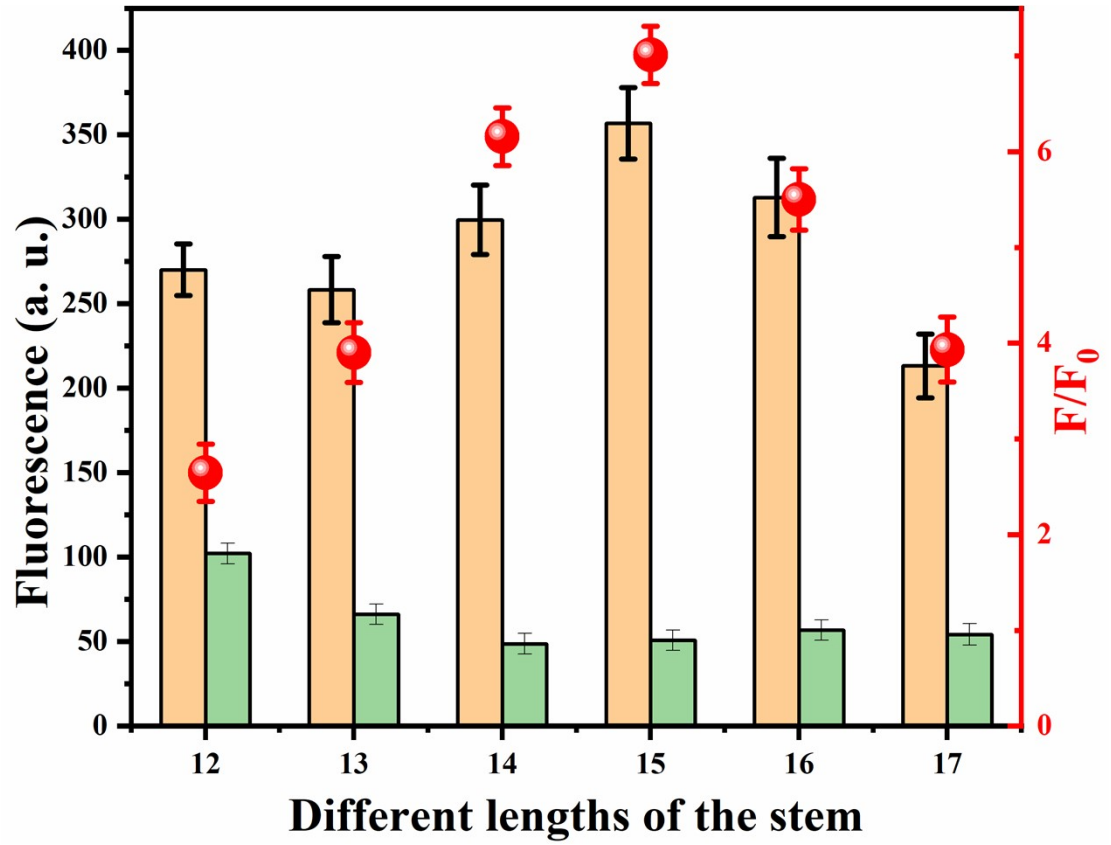

Fig. S2 Optimization of different lengths of the stem in DH2 and DH3.

Table S1 Oligonucleotide sequences of different lengths of the stem in DH2 and DH3

Oligonucleotide	Sequences (5’ to 3’)
D-HCR-H2-12	TTT GAT ACG GCT GTG TCG ACCATG CGA CAC AGC CGT
D-HCR-H3-12	CGA CAC AGC CGT ATC AAA CGA CAC AGC CGT ATC AAA GCT GTG TCG ACG GCT GTG TCG CAT GGT
D-HCR-H2-13	TTT GAT ACG GCT GTG TCG T ACCA TG A CGA CAC AGC CGT
D-HCR-H3-13	A CGA CAC AGC CGT ATC AAA A CGA CAC AGC CGT ATC AAA GCT GTG TCG T ACG GCT GTG TCG T CAT GGT
D-HCR-H2-14	TTT GAT ACG GCT GTG TCG TA ACCA TG TA CGA CAC AGC CGT
D-HCR-H3-14	TA CGA CAC AGC CGT ATC AAA TA CGA CAC AGC CGT ATC AAA GCT

	GTG TCG TA ACG GCT GTG TCG TA CAT GGT
D-HCR-H2-15	TTT GAT ACG GCT GTG TCG TAG ACCA TG CTA CGA CAC AGC CGT
D-HCR-H3-15	CTA CGA CAC AGC CGT ATC AAA CTA CGA CAC AGC CGT ATC AAA GCT GTG TCG TAG ACG GCT GTG TCG TAG CAT GGT
D-HCR-H2-16	TTT GAT ACG GCT GTG TCG TAGG ACCA TG CCTA CGA CAC AGC CGT
D-HCR-H3-16	CCTA CGA CAC AGC CGT ATC AAA CCTA CGA CAC AGC CGT ATC AAA GCT GTG TCG TAGG ACG GCT GTG TCG TAGG CAT GGT
D-HCR-H2-17	TTT GAT ACG GCT GTG TCG TAGGC ACCA TG GCCTA CGA CAC AGC CGT
D-HCR-H3-17	GCCTA CGA CAC AGC CGT ATC AAA GCCTA CGA CAC AGC CGT ATC AAA GCT GTG TCG TAGGC ACG GCT GTG TCG TAGGC CAT GGT

Table S2 Comparison of our approach with other protein assays.

Methods	Strategy	LOD	Assay time (h)	Linear range	Ref
Fluorescence	Dropcast Single Molecule Assays	19 aM	2	-	[1]
Electrochemiluminescence	Dual-Polar Electrochemiluminescence from Au 25 Nanoclusters	0.43 pg mL^{-1}	2	1 pg/mL - 1 ng/mL	[2]
Electrochemical ELISA	Protein Biosensing in Undiluted Serum Using a Polypyrrole-Based Platform	78 pg mL^{-1}	2	100 pg/mL–100 ng/m	[3]

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Electrochemical	peptide inhibitor-based biosensing platform	0.03 ng mL^{-1}	2		[4]
Lateral flow assay	protein-to-DNA transducer-based approaches	0.74 fM	2	1 fM-100 nM	[5]

Table S3 Spiking test in human serum and saliva samples.

Sample	Spiked	Found	Recovery	RSD
serum	**100 ng /mL**	**106.9 ng /mL**	**106.9%**	**2.62%**
serum	**1 ng /mL**	**1.054 ng /mL**	**105.4%**	**4.64%**
serum	**10 pg /mL**	**9.057pg /mL**	**90.57%**	**8.20%**
saliva	**100 ng /mL**	**89.42ng /mL**	**89.42%**	**3.32%**
saliva	**1 ng /mL**	**1.096 ng /mL**	**109.6%**	**3.25%**
saliva	**10 pg /mL**	**11.22 pg /mL**	**112.2%**	**8.28%**

All the data in the table represents the average of three measurements

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[4] V. Vanova, K. Mitrevska, V. Milosavljevic, D. Hynek, L. Richtera, V. Adam, Peptide-based electrochemical biosensors utilized for protein detection, Biosensors & bioelectronics, 180 (2021) 113087.

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