Supporting Information

Ultrasensitive electrochemical detection of Parvovirus B19 DNA by combining CRISPR-Cas12a and multivalent framework nucleic acids

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Table S1. Oligonucleotides used in the present work.

Name	Sequence（5' - 3'）
A1	CCCTGTACTGGCTAGGAATTCACGTTTTAATCTGGGCTTTGGGTTAAGAAACTCCCCG
B1	CGGTGATGCGCCTCCAGCGCGGGGAGTTTCTTAACCCTTTCCGACTTACAAGAGCCGG
C1	CCCATGAGAATAATACCGCCGATTTACGTCAGTCCGGTTTCCCACACGGGACGGCAGGC
D1	GCCCAGATTAAAACGTGAATTCCTAGCCAGTACAGGGTTTCCGGACTGACGTAAATCGG
A2	CGCTGGAGGCGCATCACCGTTTGCGTATGTGTTCTGTGCGGCCTGCCGTCCCGTGTGGG
B2	GCGAGACTCAGGTGGTGCCTTTGGCATTCGACCAGGAGATATCGCGTTCAGCTATGCCC
C2	CGCACAGAACACATACGCTTTGGGCATAGCTGAACGCGATATCTCCTGGTCGAATGCC
D2	CGGTATTATTCTCATGGGTTTGGCACCACCTGAGTCTCGCCCGGCTCTTGTAAGTCGG
A1-SH	SH-CCCTGTACTGGCTAGGAATTCACGTTTTAATCTGGGCTTTGGGTTAAGAAACTCCCCG
B1-SH	SH-CGGTGATGCGCCTCCAGCGCGGGGAGTTTCTTAACCCTTTCCGACTTAC

	AAGAGCCGG
C1-SH	SH-CCCATGAGAATAATACCGCCGATTTACGTCAGTCCGGTTTCCCACACGGGACGGCAGGC
D1+22-bio	bio-ACCACCACCACCAAAAAAAAAAGCCCAGATTAAAACGTGAATTCCTAGCCAGTACAGGGTTTCCGGACTGACGTAAATCGG
A2+22-bio	CGCTGGAGGCGCATCACCGTTTGCGTATGTGTTCTGTGCGGCCTGCCGTCCCGTGTGGGAAAAAAAAAAACCACCACCACCA-bio
B2+22-bio	GCGAGACTCAGGTGGTGCCTTTGGCATTCGACCAGGAGATATCGCGTTCAGCTATGCCCAAAAAAAAAAACCACCACCACCA-bio
C2+22-bio	CGCACAGAACACATACGCTTTGGGCATAGCTGAACGCGATATCTCCTGGTCGAATGCCAA AAAAAAAACCACCACCACCA-bio
PB19	CATTATTAAGTCCACTATTGTGGAAGCTGCAAAAGCTATT
crRNA-PB19	UAAUUUCUACUAAGUGUAGAUCAGCUUCCACAAUAGUGGAC
PB-mis1	CATTATTAAGTCCACTATTGTGGAAGCAGCAAAAGCTATT
PB-mis2	CATTATTAAGTCCACTATTGTGGAAGCAGCAATAGCTATT
PB-mis3	CATTATTAAGTCCACTATTGTGTAAGCAGCAAAAGCTATT
FQ-ssDNA	FAM-TTATT-BHQ
H9N2	AACCAGGCCAGACATTGCGAGTAAGATCC
HSV-1	CCGCCGAACTGAGCAGACACCCGCGC
HAdV	TGCACCAGACCCGGGCTCAGGTACTCCGA

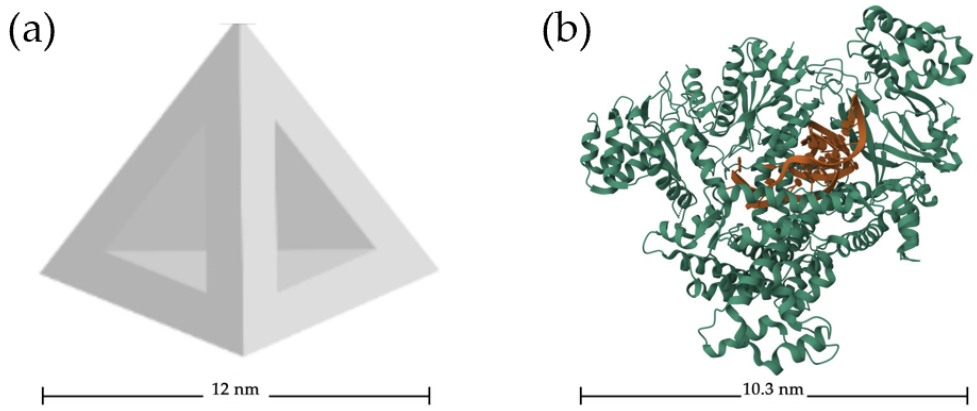

Figure S1. Schematic illustration of the sizes of (a) TDNs and (b) Cas12a-crRNA.

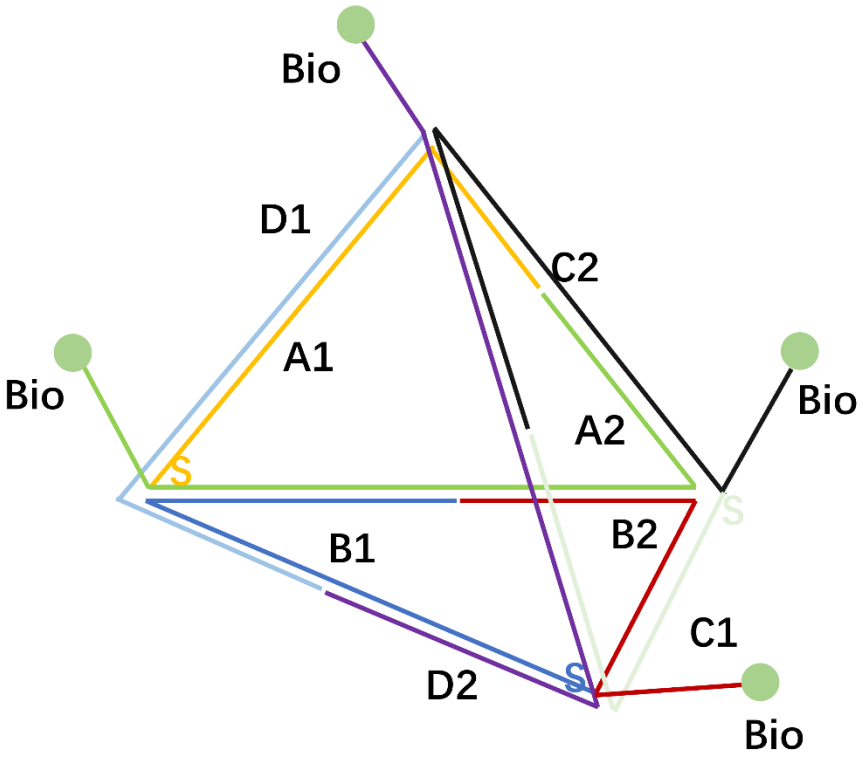

Figure S2. Schematic of the tetrahedral DNA nanostructure (TDN) illustrating the exact routing of the eight DNA strands (A1-D2) and the placement of the ssDNA reporter probes

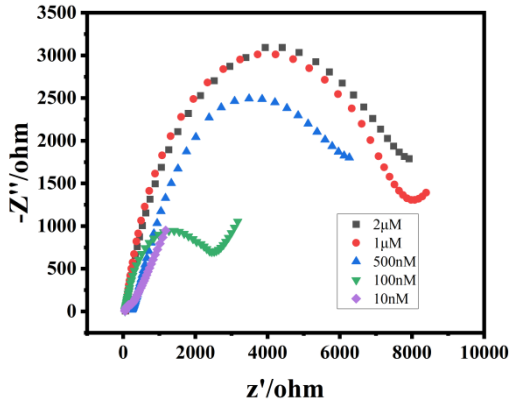

Figure S3. The electrochemical impedance spectra of gold electrodes assembled with different concentrations of TDNs.

As for the electrochemical impedance spectroscopy (EIS) characterization of TDN immobilization on the gold electrode, our strategy involved using an excess concentration of TDNs to achieve the densest possible monolayer. We prepared a TDN solution at a concentration of 1 μM and, after thorough washing to remove any unbound TDNs, considered the surface saturated. Figure S2 presents the impedance data, which clearly shows that at a TDN concentration of 1 μM, the impedance reaches its maximum value. This indicates that this concentration is sufficient for effective surface functionalization. Importantly, increasing the TDN concentration beyond 1 μM did not lead to a significant change in impedance, confirming that the gold electrode surface was fully covered with TDNs at this concentration.

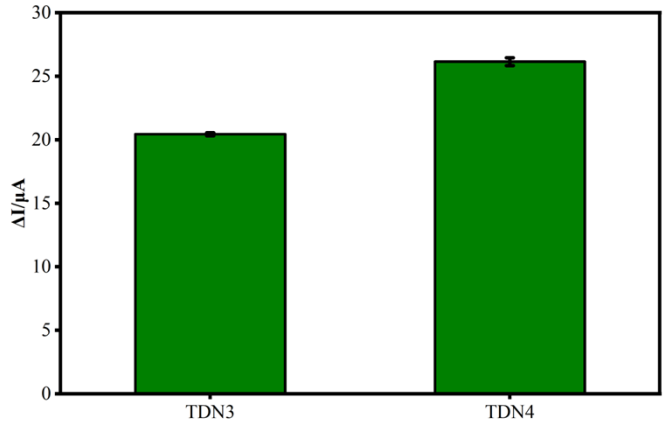

Figure S4. Tetravalent TDN (TDN4) yields a significantly higher signal change compared to the trivalent TDN (TDN3).

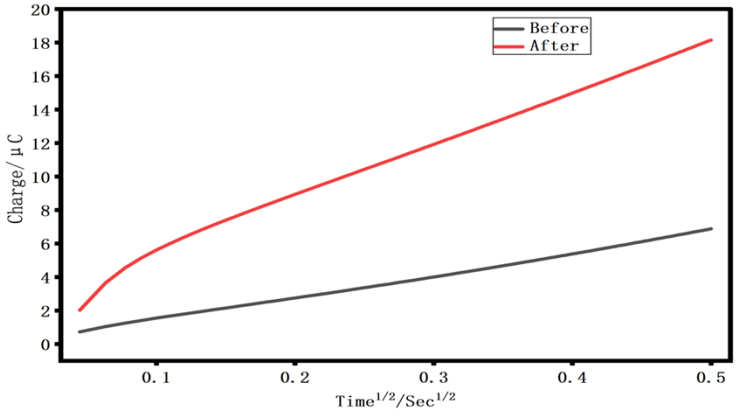

Figure S5.. The Chronocoulometric curves of TDNs before and after the addition of $Ru(NH3)6^{3+}$.

Table S2. Comparison of this method with other methods.

Analytical Methods	Target	LOD	Ref.
Dual-Functional Tetrahedron Multivalent Aptamer Assisted Amplification-Free CRISPR/Cas12a Assay	Salmonella	7 cfu mL–1	[1]
Framework-Hotspot Enhanced Trans Cleavage of CRISPR-Cas12a for Clinical Samples Detection	DNA	100 fM	[2]
Mesoporous Nanozyme-Enhanced DNA Tetrahedron Electrochemiluminescent Biosensor	microRNA	273.20 aM	[3]
Sensitive Detection of Salmonella Based on CRISPR-Cas12a and the Tetrahedral DNA Nanostructure-Mediated Hyperbranched Hybridization Chain Reaction	Salmonella	8 CFU/mL	[4]
CRISPR/Cas12a Powered DNA Framework-Supported Electrochemical Biosensing Platform	DNA	100 fM	[5]
Ultrasensitive electrochemical detection of Parvovirus B19 DNA by combining CRISPR-Cas12a and multivalent frame-work nucleic acid	DNA	2.19 fM	This work

Reference

[1] Qiao Z, Xue L, Sun M, et al. Dual-Functional Tetrahedron Multivalent Aptamer Assisted Amplification-Free CRISPR/Cas12a Assay for Sensitive Detection of Salmonella [J]. Journal of Agricultural and Food Chemistry, 2024, 72(1) : 857-864.

[2] Li F Q, Li J R, Yang W Q, et al. Framework-Hotspot Enhanced Trans Cleavage of CRISPR-Cas12a for Clinical Samples Detection [J]. Angewandte Chemie-International Edition, 2023, 62(32).

[3] Shen B, Li L, Liu C, et al. Mesoporous Nanozyme-Enhanced DNA Tetrahedron Electrochemiluminescent Biosensor with Three-Dimensional Walking Nanomotor-Mediated CRISPR/Cas12a for Ultrasensitive Detection of Exosomal microRNA [J]. Analytical Chemistry, 2023, 95(9) : 4486-4495.

[4] Cai Q, Shi H, Sun M, et al. Sensitive Detection of Salmonella Based on CRISPR-Cas12a and the Tetrahedral DNA Nanostructure-Mediated Hyperbranched Hybridization Chain Reaction [J]. Journal of Agricultural and Food Chemistry, 2022, 70(51) : 16382-16389.

[5] Su J, Ke Y Q, Maboyi N, et al. CRISPR/Cas12a Powered DNA Framework-Supported Electrochemical Biosensing Platform for Ultrasensitive Nucleic Acid Analysis [J]. Small Methods, 2021, 5(12).