Electronic Supplementary Material (ESI) for Environmental Science: Nano.

Exploring the Entropy-Driven Amplification Reaction and Trans-Cleavage Activity of CRISPR-Cas12a for the Development of an Electrochemiluminescence Biosensor for the Detection of SARS-CoV-2 RdRp Gene in Real Samples and Environmental Surveillance

Kai Zhang,[a] Zhenqiang Fan,[a] Yuedi Ding,[a] Sha Zhu,[c] Minhao Xie,[a] Nan Hao*[b]*

[a]NHC Key Laboratory of Nuclear Medicine, Jiangsu Key Laboratory of Molecular Nuclear Medicine, Jiangsu Institute of Nuclear Medicine, Wuxi, Jiangsu, China 214063

[b]School of Chemistry and Chemical Engineering, Jiangsu University, Zhenjiang, Jiangsu, 212013, PR China

[c]Department of Oncology, The Affiliated Wuxi No.2 People's Hospital of Nanjing Medical University, Wuxi, 214000, PR China

* Corresponding authors.

E-mail addresses: zhangkai@jsinm.org (K. Zhang), hn@ujs.edu.cn (N. Hao)

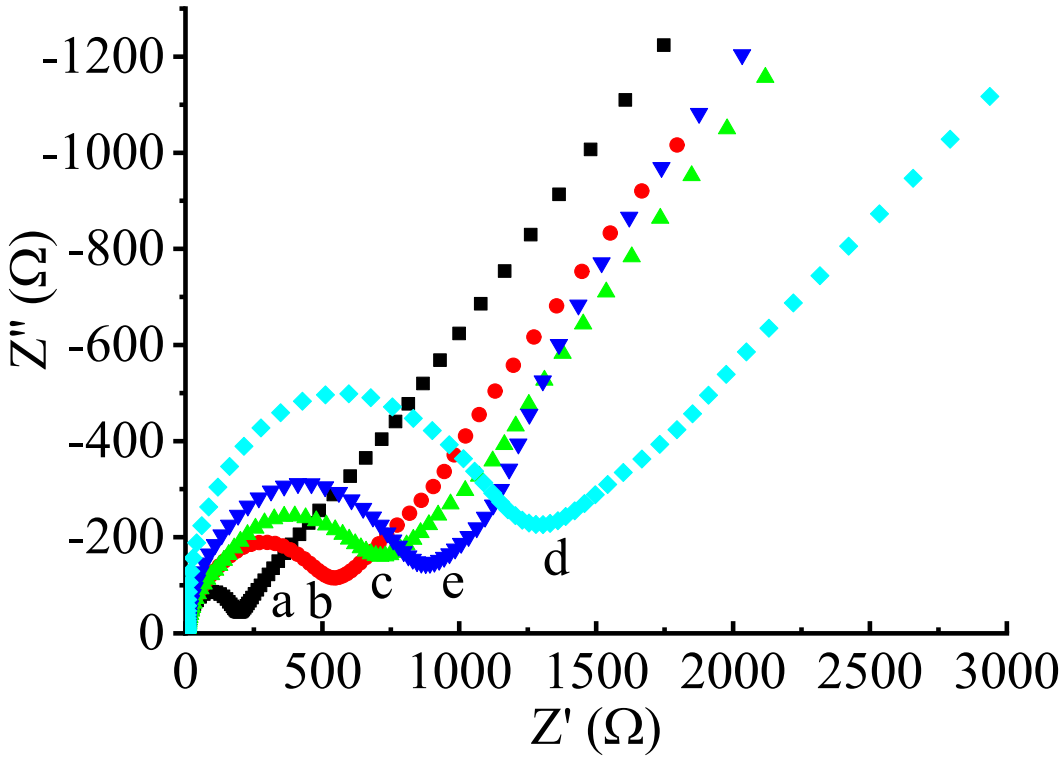

Figure S1. EIS of the biosensor at different stages of modification: (a) bare GCE; (b) GOAu-Ru modified GCE. (c) DNA7 and GOAu-Ru modified GCE (d) MCH, DNA7 and GOAu-Ru modified GCE. (e) CRISPR-Cas12a/gRNA complex treated biosensor.

The electrochemical impedance spectroscopy (EIS), as it is an effective method to probe the interfacial properties of surface-modified electrodes, was also used to characterize the interfacial changes during the preparation of CRISPR-Cas12a-based biosensors, and the results were shown in Figure S1. The impedance diagram of the bare glassy carbon electrode can be seen that the native impedance semicircle diameter is less than 200 (curve a), indicating that the bare glassy carbon electrode has excellent conductivity and low impedance. When GOAu-Ru was modified on the electrode surface and becomes a film, the impedance of the modified electrode increases significantly (curve b), which is mainly due to the dense GOAu-Ru film hindering electron transfer, thus leading to a larger impedance of the sensor. When DNA7 was assembled on the electrode surface through the Au-S bond, the impedance of the biosensor increased significantly (curve c). It indicates that DNA7 similarly impedes the electron transfer. When MCH was modified on the AuNP surface, the impedance

value further increased (curve d), indicating that MCH sealed the electrode surface. When the electrode was incubated with the Cas-12a/gRNA complex, the impedance of the biosensor decreased significantly (curve e), indicating that DNA7 was cleaved by the CRISPR-Cas12a/gRNA complex, resulting in an increase in the rate of electron transfer. Thus, this data further indicates the successful assembly of the target sensor.

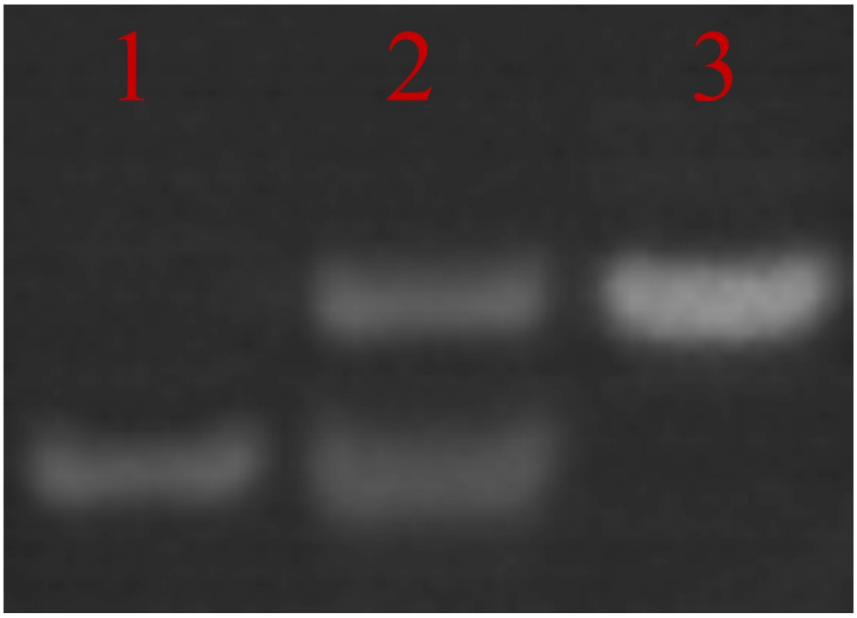

Figure S2. Polyacrylamide gel electrophoresis (PAGE) experiments to verify entropy-driven reactions. Lane 1: DNA5/DNA6 duplex, Lane 2: DNA5/DNA6 duplex+DNA2 with a ratio of 1:0.5, Lane 3: DNA5/DNA6 duplex+DNA2 with a ratio of 1:1.

Table S1: The nucleic acid sequences used in this work. The double underlined nucleic acid bases in the DNA2, DNA5 and DNA6 sequences can form a triple-helix structure. The yellow portions of DNA5 and DNA6 are PAM structure.

Name	Sequence (5'-3')
SARS-CoV-2 RdRp gene	CAGGTGGAACCTCATCAGGAGATGC
SARS-COV RdRp gene	CCAGGTGGAACATCATCCGGTGATGC
mismatched DNA1	CAGGTGGGTGCTCATCAGGAGATGC
mismatched DNA2	CAGGTGGAACCTCATCAGGCGGTCC
DNA1	CCACATACATCATATTCCCTCAGGTGGAACCTCATCAGG
DNA2	CTTTCCTACACCTACGTCTCCC***TTCCTTTCTCCTTCTTT***
DNA3	GCATCTCCTGATGAGGTTCCACCTGAGGGAAAGAAGGAG AAAGGAAGGGAGACGTAGG
DNA4	CCTACGTCTCCC*TTCCTTTCTCCTTCTTT*CCCTCAGGTGGAA CCTCATCAGGAGATGC
DNA5	**CGATTTTCTTCCTCTTTCCTTGCATAGTCTCA**
DNA6	**TGAGACTATGCAAGGAAAGAGGAAGAAAATCG**
gRNA	UAAUUU**CUAC**UAAGU**GUAG**AUUUCCUCUUUCCUUGCA UAGU
DNA7	SH-TTTTTTTTTTAGCTCTCATTTTTTGAC-Fc

Table S2: The percentages of various different elements in GOAu-Ru

Element	Line Type	k factor	Absorption Correction	Wt%	Wt% Sigma
C	K series	2.50675	1.00	75.26	0.54
N	K series	3.14061	1.00	7.09	0.37
O	K series	1.86867	1.00	3.30	0.18
Ru	L series	1.74537	1.00	6.41	0.33
Au	L series	2.72091	1.00	7.94	0.35
Total:				100.00	

Table S3: Comparison of different methods for SARS-COV-2.

Method	Target	LOD	Reference
Colorimetric method	N gene	43 nM	(Moitra et al. 2020)
Dual-Functional Plasmonic Photothermal	RdRp gene	0.22 pM	(Qiu et al. 2020)
ECL	RdRp gene	2.67 fM	(Fan et al. 2021)
CRISPR-Cas12a based ECL biosensor	RdRp gene	32.80 aM	This work

References:

Fan, Z., Yao, B., Ding, Y., Zhao, J., Xie, M., Zhang, K., 2021. Biosens. Bioelectron. 178, 113015.
Moitra, P., Alafeef, M., Dighe, K., Frieman, M.B., Pan, D., 2020. ACS Nano 14(6), 7617-7627.
Qiu, G., Gai, Z., Tao, Y., Schmitt, J., KullakUblick, G.A., Wang, J., 2020. ACS Nano 14(5), 5268-5277.