DNA-mediated synergistic proximity ligation for ultrasensitive electrochemiluminescence detection of microcystin-LR

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Materials and apparatus

Microcystin-LR (MC-LR), MC-RR, MC-YR, gonyautoxin 1/4 and nodularin were purchased from Qingdao Pribolab Bioengineering Co., Ltd. (Qingdao, China). Tris(2,2′-bipyridyl)dichlororuthenium(II) hexahydrate ($Ru(bpy)_3^{2+}$), Tris-(2-carboxyethyl) phosphine hydrochloride (TCEP), Tris(hydroxymethyl)aminomethane (Tris-HCl), Nafion (5% in a mixture of lower aliphatic alcohols and water), chloroauric acid ($HAuCl_4$) and 6-mercapto-1-hexanol (MCH) were purchased from Sigma-Aldrich (USA). Tri-n-propylamine (TPA) and sodium citrate were purchased from Sinopharm Chemical Reagent Co., Ltd. (China). 0.019 M NaH_2PO_4, 0.081 M Na_2HPO_4, and 0.10 M KCl made up 0.10 M phosphate buffer saline (PBS, pH 7.40). 50 mM NaCl and 5.0 mM $MgCl_2$ made up 10 mM Tris-HCl buffer (pH 7.4). 10 mM phosphate buffer (PB, pH 7.4) containing 1.9 mM NaH_2PO_4 and 8.1 mM Na_2HPO_4 was used as washing solution. Every chemical reagent was analytically graded and utilized exactly as supplied, and Millipore Milli-Q water (18.2 MΩ·cm) was used.

All electrochemical experiments were conducted on CHI 660f electrochemical workstation (Chenhua Instruments Co., China). The ECL measurements were performed using an HYZ-3002 ECL detector (Xi'an HeYongZhong Electronic Technology Co, Ltd., China) with photomultiplier tube biased at -500 V. A three-electrode system including a working electrode (modified glassy carbon electrode or Au electrode), a reference electrode (Ag/AgCl (saturated KCl) electrode), and a counter electrode (platinum wire) was employed. The UV-Vis spectroscopy was recorded with a spectrophotometer employed (UV-2450, Shimadzu Corporation, Japan). X-ray photoelectron spectroscopy (XPS) experiments were obtained from Kratos Analytical Axis Ultra photoelectron spectrometer (Kratos Analytical ltd, Japan).

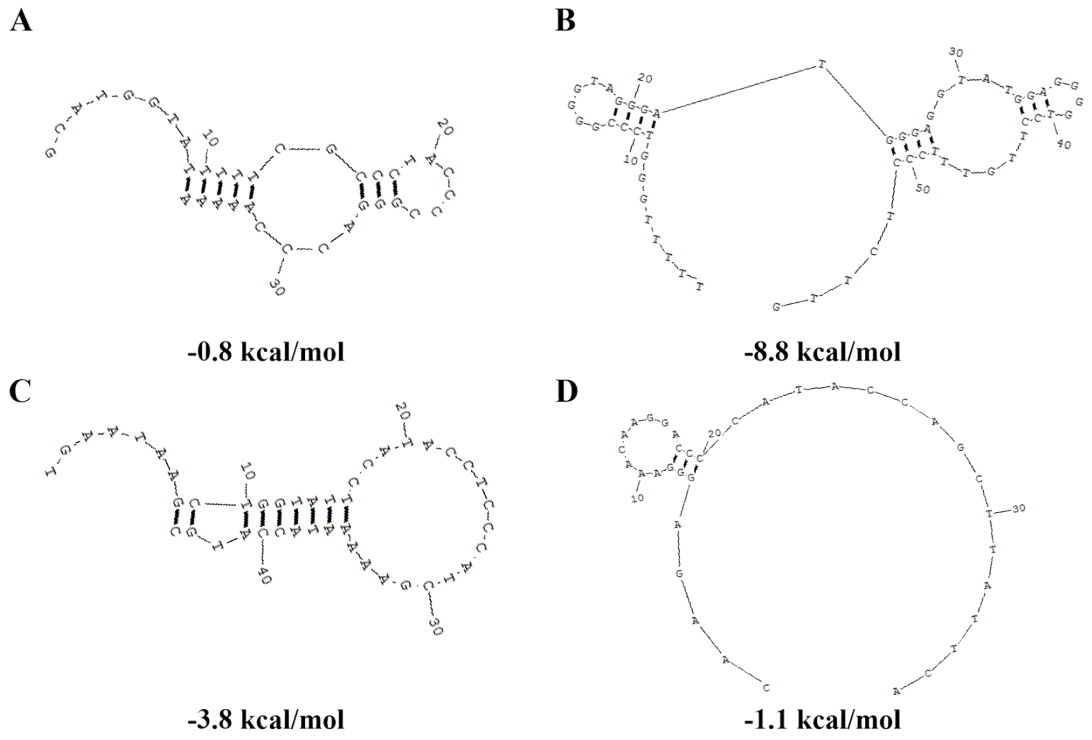

Fig. S1. Scheme of different ss-DNA self-hybridization: Capture DNA (A), MC-LR aptamer (B), DNA1 (C) and DNA2 (D).

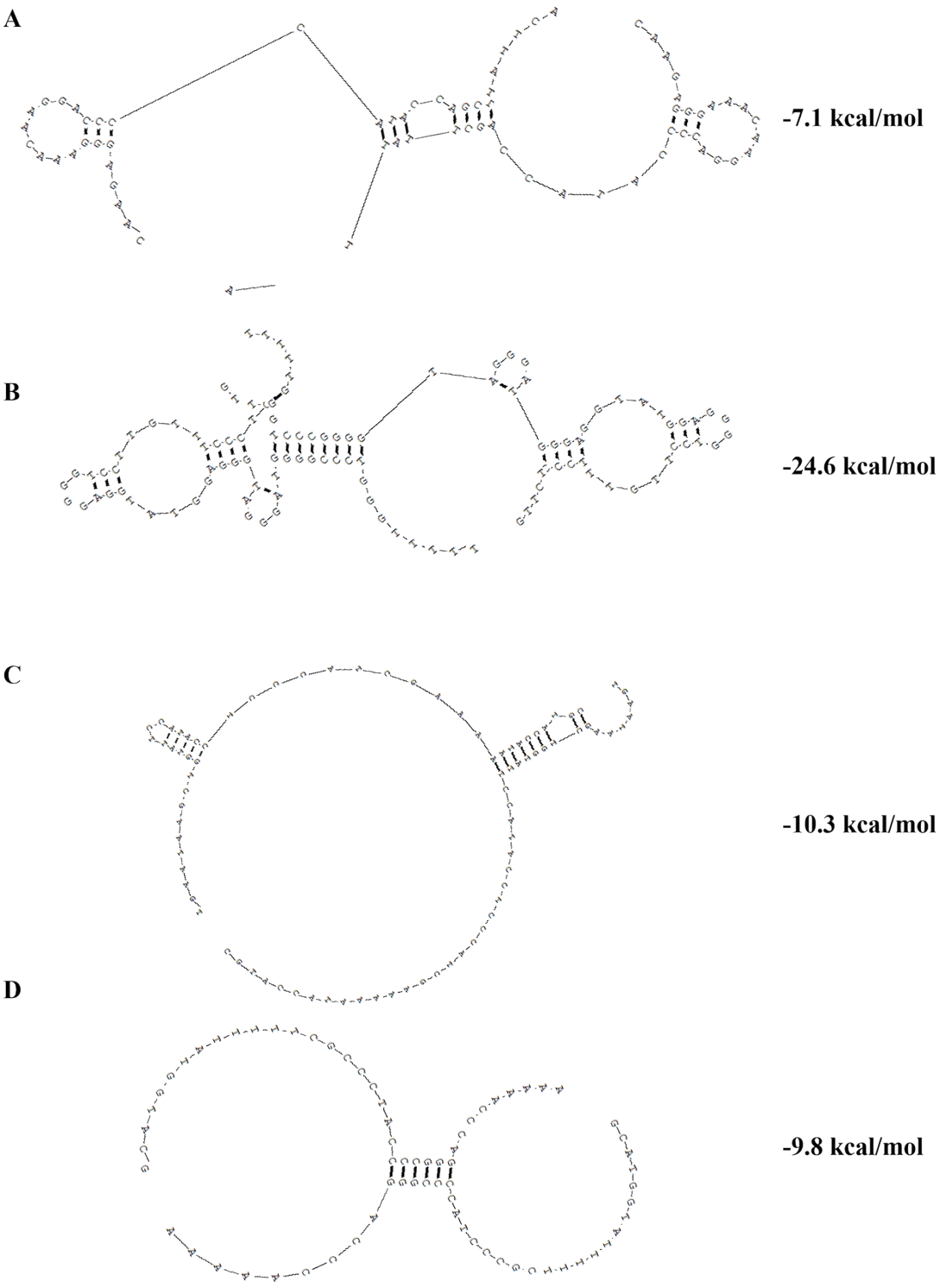

Fig. S2. Scheme of hybridization of different ss-DNA probe: two Capture DNAs (A), two MC-LR aptamers (B), two DNA1s (C) and two DNA2s (D).

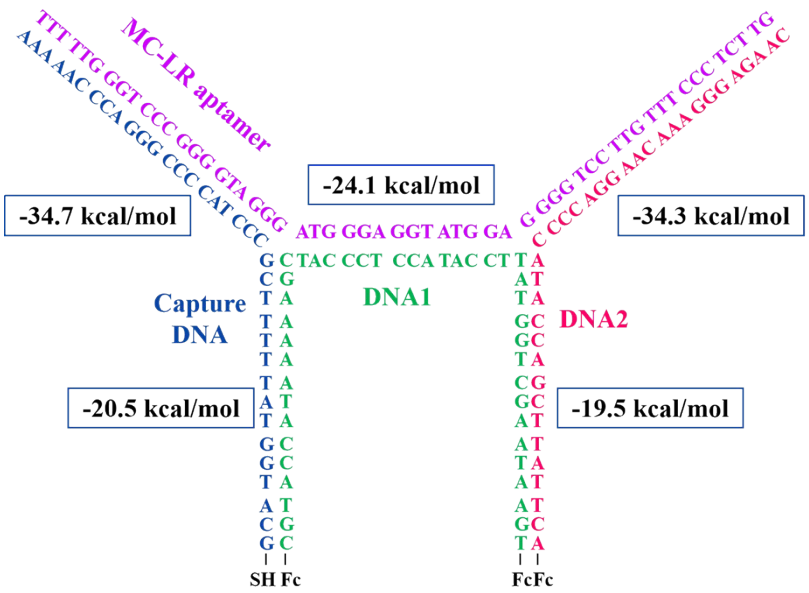

Fig. S3. Scheme of hybridization of Capture DNA-MC-LR aptamer, Capture DNA-DNA1, MC-LR aptamer-DNA1, MC-LR aptamer-DNA2 and DNA1-DNA2.

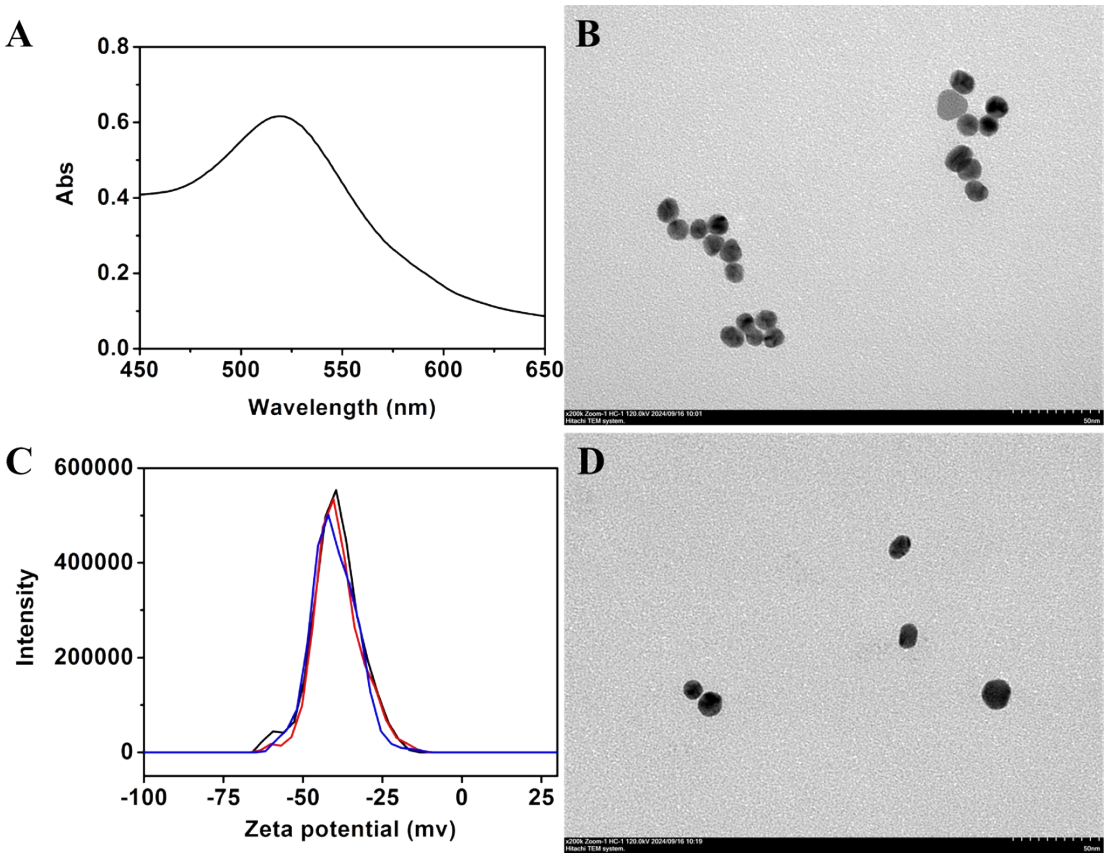

Fig. S4. UV-Vis spectrum (A), TEM image (B) and zeta potential (C) from 3 different samples of AuNPs and TEM image of Nafion-AuNPs (D).

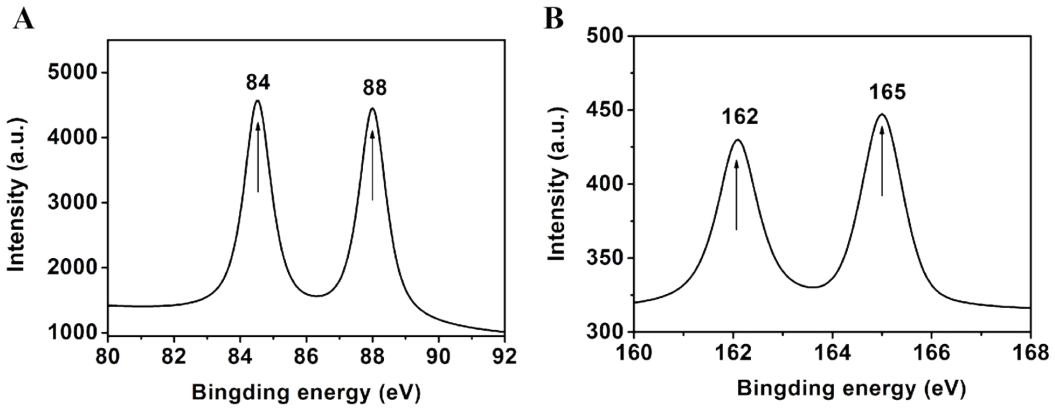

Fig. S5. High resolution XPS spectra for (A) Au 4f region and (B) S 2p regions collected from Capture DNA/Nafion/AuNPs film.

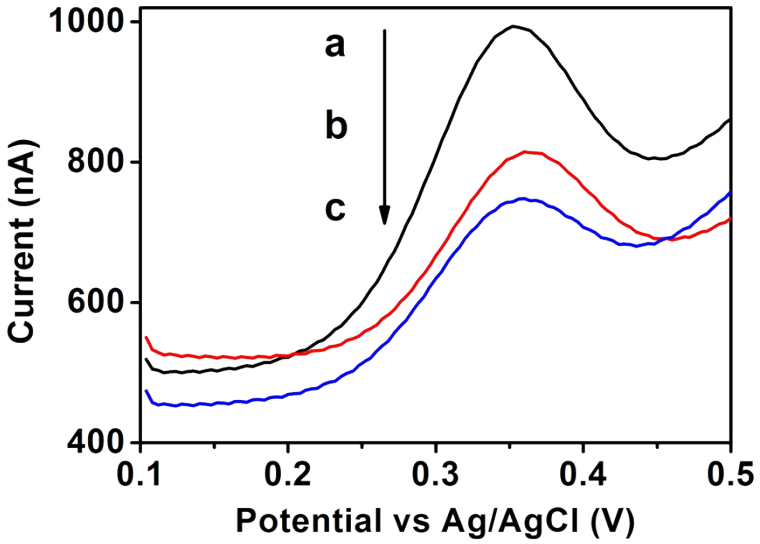

Fig. S6. Square wave voltammetric curves of the PLA-based ECL aptasensor (Capture DNA/Au) before (a) and after incubation with 0.05 ng/mL (b) and 0.5 ng/mL (c) MC-LR. The measurement conditions: 0.10 M PBS containing 50 mM TPA (pH 7.4). Init E: 0.1 V; Final E: 0.5 V; Incr E: 0.004 V; Amplitude: 0.05 V; Frequency: 15 Hz.

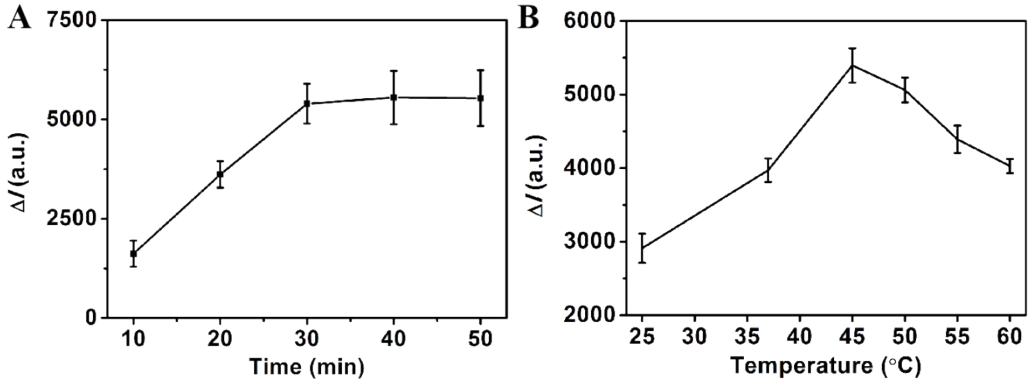

Fig. S7. Effects of binding time (A) and hybridization temperature (B) of the PLA-based ECL aptasensor for 0.1 ng/mL MC-LR on the ECL intensities. $\Delta I = I_S - I_0$, where I_S and I_0 were signal and background intensity, respectively.

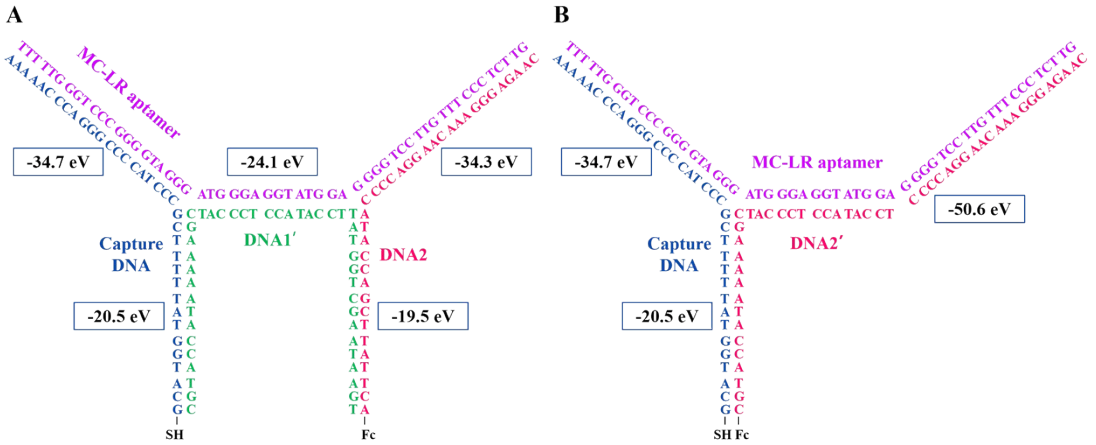

Fig. S8. Scheme of hybridization among Capture DNA, MC-LR aptamer, DNA1′ and DNA2 (A), Capture DNA, MC-LR aptamer and DNA2′ (B).

Table S1. Analytical performance for aptasensors or immunosensors based on different methods for MC-LR detection.

Method	Linear range	Detection of limit (pg/mL)	Ref
Colormetric aptasensor	4.0-10000 ng/L	3.0	[1]
Colormetric aptasensor	10-150 ng/mL	380	[2]
Colormetric aptasensor	1-200 ng/mL	849	[3]
Colormetric aptasensor	1-50 ng/mL	2500	[4]
Surface-enhanced Raman scattering immunosensor	0.01-100 μg/L	14	[5]
Surface-enhanced Raman scattering immunosensor	1.0-500 ng/mL	32	[6]
Fluorescent aptasensor	5-100 ppb	1.9 ppb	[7]

	(5-100 pg/mL)	(1.9 pg/mL)	
Fluorescent aptasensor	0.01-50 ng/mL	2	[8]
Fluorescent aptasensor	0.01-1000 μg/L	4.8	[9]
Chemiluminescent immunosensor	0.23-190 μg/L	30	[10]
Chemiluminescent immunosensor	0.062-0.65 μg/L	32	[11]
Chemiluminescent immunosensor	0.02- 200 μg/L	6	[12]
Electrochemical aptasensor	0.01-50 ng/mL 0.1-50 ng/mL	6 45	[13]
Electrochemical aptasensor	0.1-1000 ng/mL	4	[14]
Electrochemical immunosensor	0.05-25000 ng/mL	17	[15]
Electrochemical immunosensor	0.01- 20 μg/L	5	[16]
ECL immunosensor	0.01-50 μg/L	2.8	[17]
ECL immunosensor	0.005-100 μg/L	3.2	[18]
ECL aptasensor	0.01-50 ng/mL 0.01-50 ng/mL	5.9 1.2	[19]
ECL aptasensor	0.005-5.0 ng/mL	1.4	This Work

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