Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information for

Nanopore sensitization based on double loop hybridization chain reaction and G-quadruplex

Yanru Li,[a,b] Chunmiao Yu,[a,b] Yesheng Wang,[a,b] Jin Yu,[a,b] Huaning Wang[a,b] and Bingling Li[*] [a, b]

[a] State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, Jilin, 130022, China.

[b] School of Applied Chemistry and Engineering, University of Science and Technology of China, Hefei, Anhui, 230026, China.

* Email: binglingli@ciac.ac.cn.

1. Chemicals and materials

All chemicals are of analytical grade and can be used without further purification.

1×TE, KCl, $MgSO_4$, 1×TAE are obtained from Sangon Biotech (Shanghai, China).

The PPIX is obtained from Sigma-Aldrich. All oligonucleotides are ordered from Sangon Biotech (Shanghai, China). Their sequences are listed in Table S1. All oligonucleotides are dissolved in H_2O as a stock solution at -20°C.

2. Experiments

2.1 Experiments of HCR and agarose gel electrophoresis

For DHCR, stock solutions of DL-H1, DL-H2, and I are diluted in H_2O to 10 μM. DL-H1, DL-H2, I are then annealed at 95°C for 5 min and cool down to 25°C at a rate of 0.1°C s^{-1} before use. To start reaction, these stock solutions are diluted in suitable concentration. The concentration ration between I and DL-H1, DL-H2 are 1:2, 1:5, 1:10. For 1:2 group, 4 μL of 3 μM DL-H1, 4 μL of 3 μM DL-H2, 2 μL of 3 μM I (only reaction group), 2 μL of 2 M $MgCl_2$,1 μL of 1 M KCl (only with K^+ group) are mixed together, finally 1×TE is added to form a 40 μL DHCR liquid. For 1:5 group: 4 μL of 3 μM DL-H1, 4 μL of 3 μM DL-H2, 0.8 μL of 3 μM I (only reaction group), 2 μL of 2 M $MgCl_2$, 1 μL of 1 M KCl (only with K^+ group) are mixed, finally 1×TE is added to form a 40 μL DHCR liquid. For 1:10 group, 4 μL of 3 μM DL-H1, 4 μL of 3 μM DL-H2, 0.4 μL of 3 μM I (only reaction group), 2 μL of 2 M $MgCl_2$, 1 μL of 1 M KCl (only with K^+ group) are mixed, finally 1×TE is added to form a 40 μL DHCR liquid.

For typical HCR, stock solutions of H1, H2, and I are diluted in H_2O to 10 μM. H1, H2, I are then annealed at 95°C for 5 min and cool down to 25°C at a rate of 0.1°C s^{-1} before use. To start reaction, these stock solutions are diluted in suitable concentration. The concentration ration between I and H1, H2 is 1:2. 4 μL of 3 μM H1, 4 μL of 3 μM H2, 2 μL of 3 μM I, 2 μL of 2 M $MgCl_2$,1 μL of 1 M KCl are mixed, finally 1×TE is added to form a 40 μL HCR liquid.

After the liquid is incubated at 37°C for 5 h, 7 μL of the reaction product is loaded into an agarose gel for electrophoresis detection. The 2% agarose gels contain 0.1 μL of GelRed per mL of gel volume and are prepared using 1×TAE buffer. The agarose gels are run at 120 V for 45 min and visualize under UV light.

2.2 PPIX enhanced fluorescence experiments

The PPIX is dissolved in DMSO and then stored at −4°C for subsequent use. A freshly prepared PPIX solution diluted with H_2O is added to the DNA solution, and the mixture is incubated for 1 h before a fluorescence test. The fluorescent analysis is performed in HCR liquid with a final concentration of 0.6 μM for PPIX and 0.3 μM for HCR products. Cytation-5 instruments are used to record the fluorescence emission spectra of 36 μL DNA−PPIX complexes from 580 to 660 nm, with an excitation wavelength of 410 nm.

2.3 AFM sample preparation

Firstly, prepare mica sheets that match the number of samples, removing oxidation on the surface of mica sheets, dilute the reacted sample to the appropriate concentration and evenly laid on the surface of mica sheets, after 5

minutes, rinse 5-10 times with 100 μL of H_2O. Then blow dry with N_2 (5-6h).

2.4 Fabrication of nanopores and nanopore data collection and analysis

Conical glass nanopores are made of quartz glass capillaries (O.d: 1 mm I.d: 0.7 mm, QF100-70-10; Sutter Instrument Co.). All glass capillaries were thoroughly cleaned by immersing in freshly prepared piranha solution (3:1 98% H_2SO_4/30% H_2O_2) for 1 hour prior to experimental to remove organic impurities. The capillaries were then thoroughly rinsed with deionized water and vacuum dried at 70°C. The glass nanopores are then fabricated using a CO_2-laser-actuated pipette puller (model P-2000, Sutter Instrument Co.) with a oneline program containing the following parameters: HEAT=760, FIL=4, VEL=31, DEL=120, PUL=170. For DNA translocation, the nanopores are assembled into homemade horizontal-type glass cells. The cell acted as the cis reservoir, and the inner cavity of glass capillary nanopore acted as the trans reservoir. Two chlorinated silver electrodes are placed in each reservoir. The potential is applied to the electrode inside the nanopores. The DNA sample is added to the cis reservoir (outside of nanopore tip), which is set as the electrical ground. The ion currents are collected with a current amplifier Axopatch 200B (Molecular Devices) using a low-pass Bassel filter of 5 kHz and digitized with a DigiData 1550B digitizer (Molecular Devices) at a sample rate of 250 kHz. The current signal is processed using Clampfit 10.6 software (Molecular Devices) and Origin 2022 software.

3. Supporting Figures and Tables

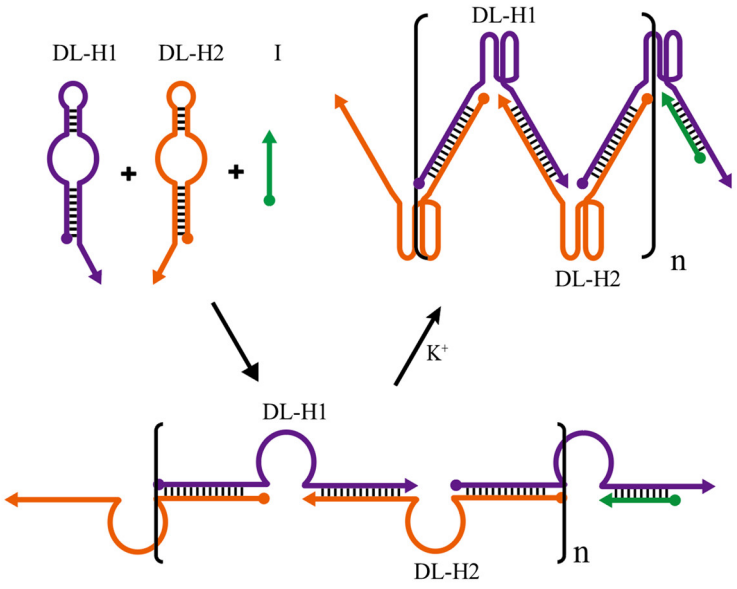

Figure S1. Schematic illustration of products for the double loop hybridization chain reaction.

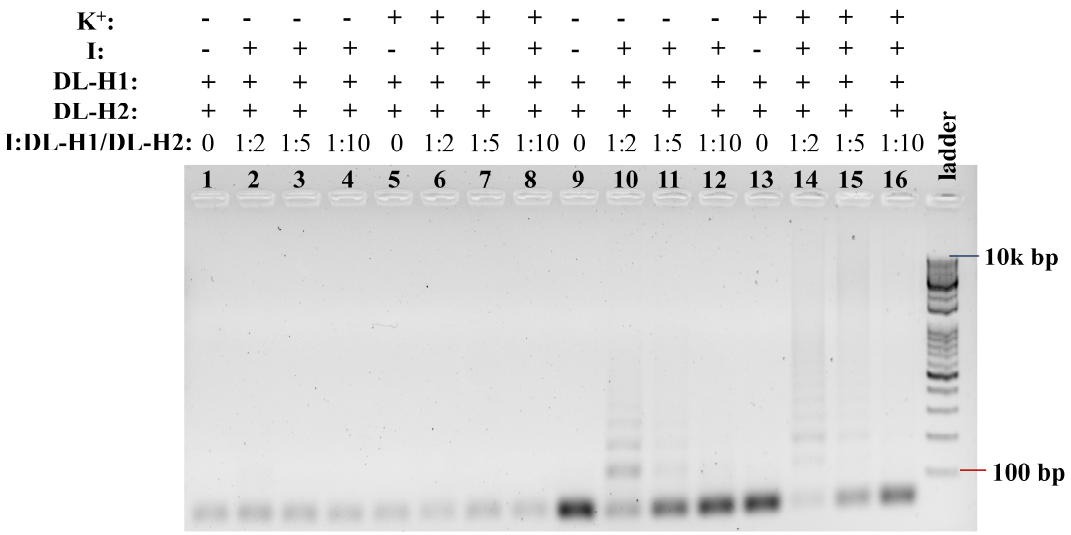

Figure S2. **Agarose gel electrophoresis characterization of DHCR.** The final concentration of hairpins for 1-8 are 100 nM. The final concentration of hairpins for 9-16 are 300 nM.

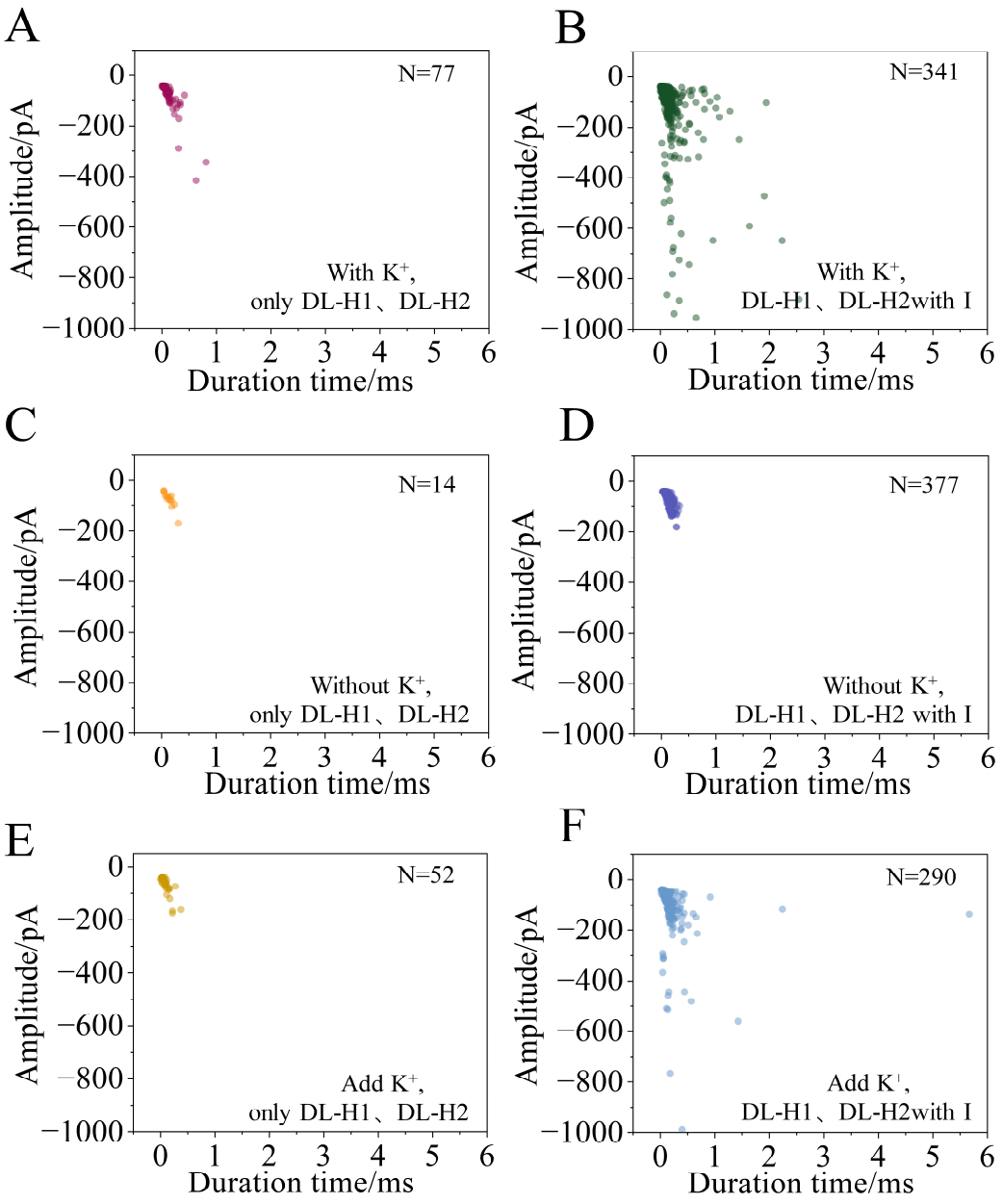

Figure S3. The scatter plots of nanopore data for DHCR. (A-B) The negative control (NC, only DL-H1, DL-H2) and the positive control (PC, DL-H1, DL-H2 with I) with K^+; (C-D) The negative control (NC, only DL-H1, DL-H2) and the positive control (PC, DL-H1, DL-H2 with I) without K^+; (E-F) The negative control (NC, only DL-H1, DL-H2) and the positive control (PC, DL-H1, DL-H2 with I) with K^+ added in the later stage of the reaction.

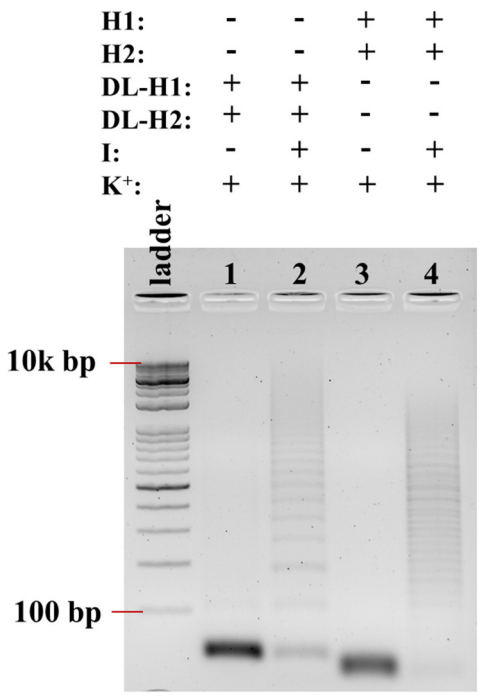

Figure S4. Comparison of DHCR and typical HCR by 2% agarose gel electrophoresis. 1-2 are DHCR and 3-4 are typical HCR.

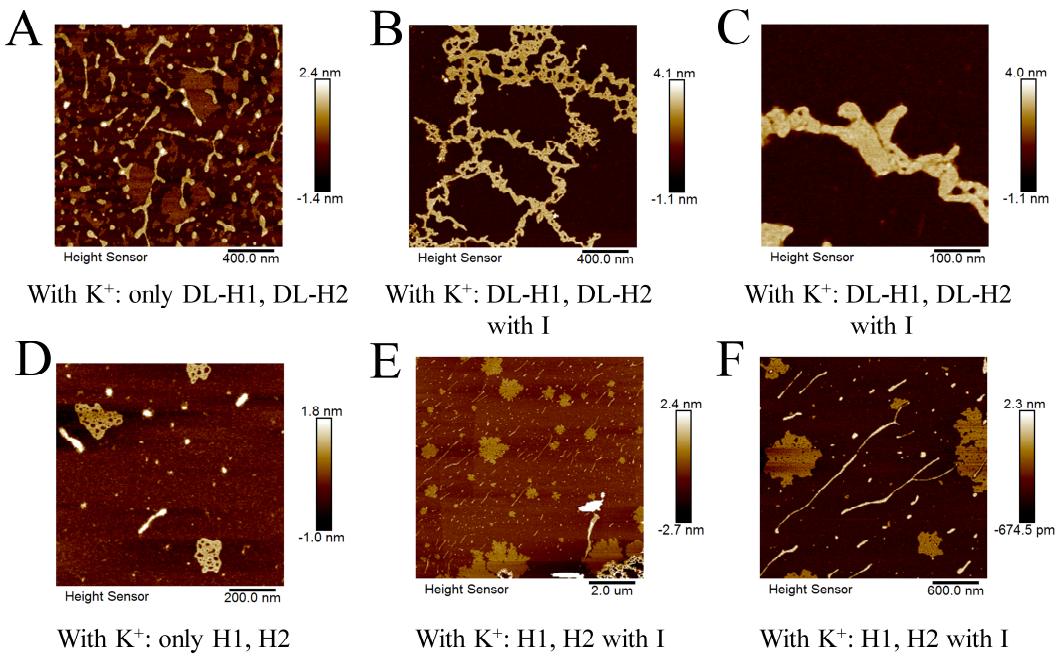

Figure S5. AFM characterization of DHCR and typical HCR. (A) The negative control (NC, only DL-H1, DL-H2) with K^+ of DHCR. (B-C) The positive control (PC, DL-H1, DL-H2 with I) with K^+ of DHCR. (D) The negative control (NC, only H1, H2) with K^+ of typical HCR. (E-F) The positive control (PC, H1, H2 with I) with K^+ of typical HCR.

Name	Sequence 5'-3'	Label and notes
Sequences for DHCR reaction		
I	[TAGCTTATCAGACTGATGTTGA}	
DL-H1	[ATCAGACTGATGTTGAGTGTCACCGCCCTCAGGAGGGCGGGTTGGGTCAACATCAGTCTGATAAGCTAGGCGGGAG}	
DL-H2	[GGGCGGTGACACTCAACATCAGTCTGATGGGTTGGGCGGGAGGGACTCCCGCCTAGCTTATCAGACTGATGTTGAG}	
Sequences for typical HCR reaction		
I	[AGTCTAGGATTCGGCGTGGGTTAA}	
H1	[TTAACCCACGCCGAATCCTAGACTCAAAGTAGTCTAGGATTCGGCGTG}	
H2	[AGTCTAGGATTCGGCGTGGGTTAACACGCCGAATCCTAGACTACTTTG}	

Table S1. **Oligonucleotides used in this paper.** All the sequences were purified with high-pressure liquid chromatography.

Reference:

(1). Walker, G. T.; Fraiser, M. S.; Schram, J. L.; Little, M. C.; Nadeau, J. G.; Malinowski, D. P. *Nucleic Acids Res*., **1992**, *20*, 1691-1696.

(2). C. Yu, Y. Wang, R. Wu and B. Li, *ACS Appl. Mater. Interfaces*, **2023**, *15*, 23602-23612.

(3). X. Peng, J. Yang, W. Liang, Y. Sun, X. Mei, G. Zhang, R. Yuan and Y. Li, *Sens. Actuators, B,* **2022**, *369,*132327.

(4). Z. Zhu, R. Wu, B. Li, *Chem. Sci.,* **2019**, *10*, 1953-1961.

(5). R. Wu, Z. Zhu, X. Xu, C. Yu and B. Li, *Nanoscale,* **2019**, *11*, 10339-10347.