Supplementary Information for the article titled:

Flexible and Efficient Eletrokinetic Stacking of DNA and Protein at HF Etched Porous Junction on Fused Silica Capillary

Zhi-Yong Wu,[a*] *Fang Fang,*[ab] *Yan-Qin He,*[a] *Ting-Ting Li,*[a] *Jing-Jing Li,*[a] *and LI Tian*[a]

[a] Research Center for Analytical Sciences, [b] Chemistry Department, Northeastern University, Shenyang 110819, PR China

[*]Correspondence: Zhi-Yong Wu, email: zywu_2008@yahoo.com.cn, Tel/Fax: +86 24 83687659

1. Sound indication of etching end

Fig.S2 is the circuit of the sound alarm system. When 45 V is applied, current over 100 nA will cause the system to alarm, indicating the time of conducting.

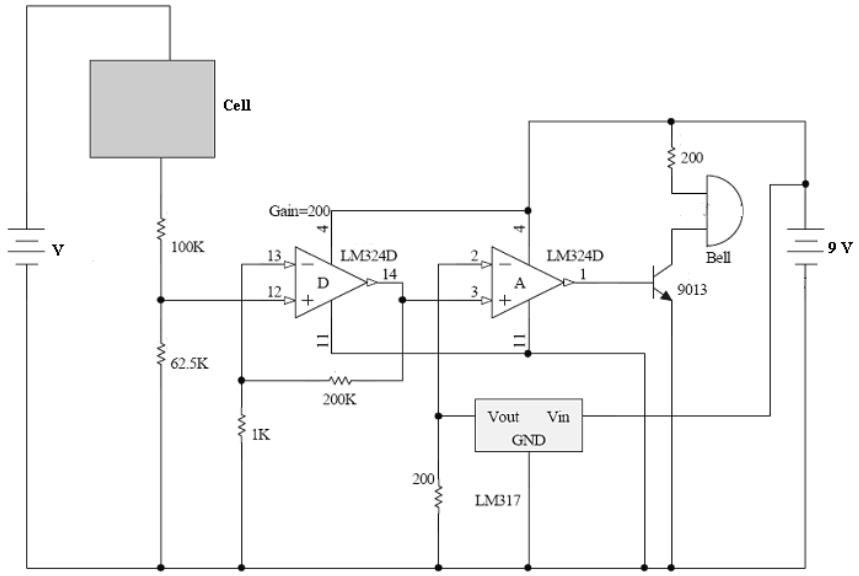


Figure S1. Circuit of the sound alarm system.

2. CCD imaging and LIF detection system

Figure S3 is a schematic of the homemade laser induced detection system. It is established based on an inverted fluorescent microscope. Blue diode laser (487 nm) was used for the laser induced detection of fluorescein, and green one (530 nm) for rhodamine 6G and rhodamine B. For CCD imaging, a Hg DC arc lamp was used as excitation light, with blue and green filter sets for fluorecein and rhodamine 6G fluorescent probes, respectively, and the pinhole was removed, and the PMT was replaced by the CCD.

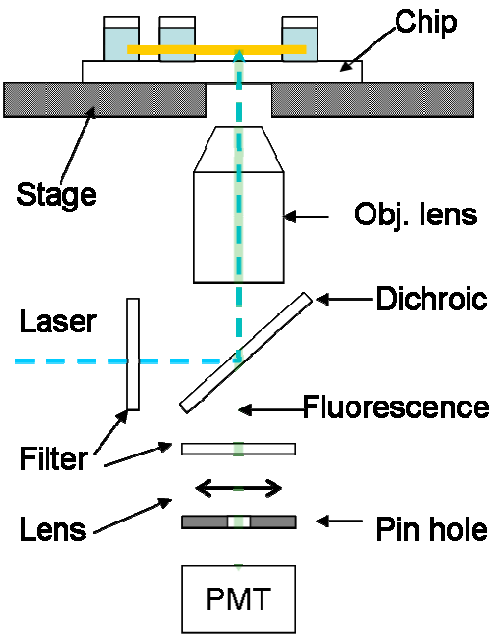


Figure S2. Schematic of the laser induced fluorescence detection system

3. ES Online with CE UV system

For online stacking and electrophoresis separation and detection of native DNA and protein, the system shown in Figure S3 was used. For protein stacking, positive voltage was applied between sample tube 2 and reservoir 4 with the junction by switch K to 7, and switch to 8 after manually the front sample tube was replaced with a running buffer tube for electrophoresis. For DNA, the same procedure was followed, but the polarity of the voltage was reversed.

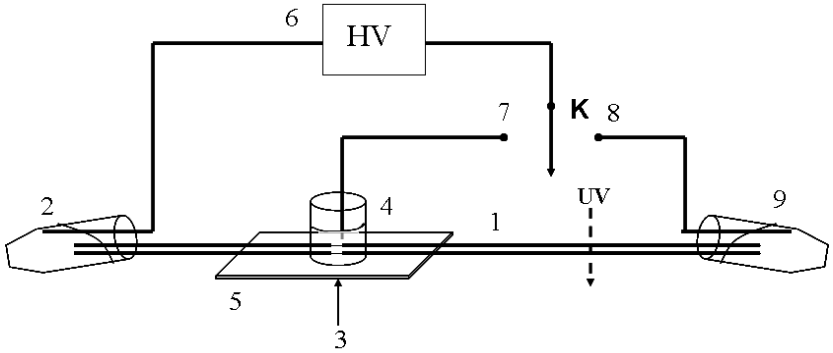


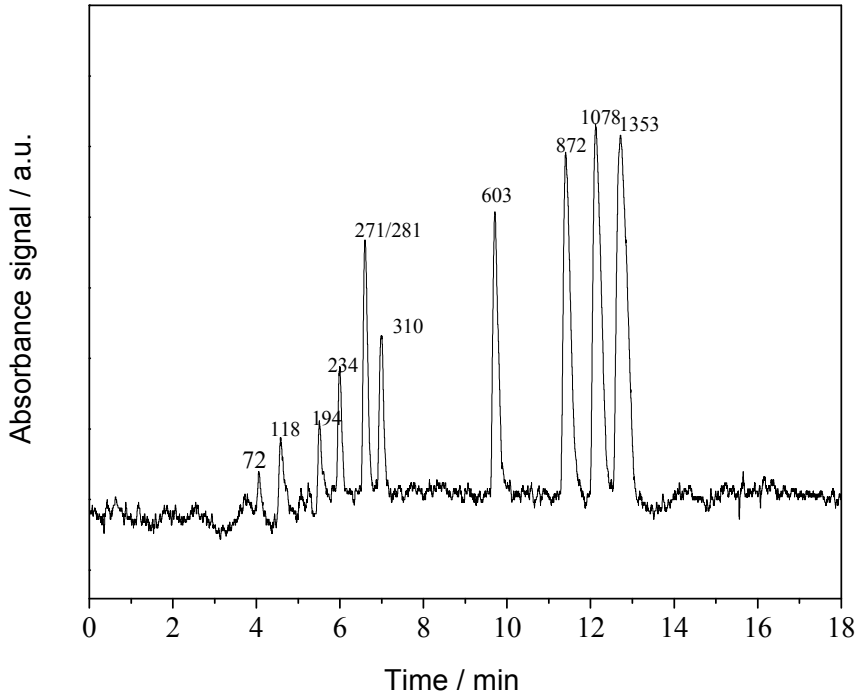
Figure S3. Schematic diagram of online ES and CE UV system 1-capillary; 2-inlet reservoir; 3-porous junction; 4-porous region reservoir; 5-glass slide; 6-high voltage power; 7-stacking

electrode; 8-outlet electrode; 9-outlet reservoir; K-switch

4. Optimization of DNA and protein separation

Dedicated treatment of the capillary was made for online ES and electrophoresis of DNA and protein. For DNA, the capillary was treated by HCl [Sanders J. C. et al., Anal. Chem., 2003, 75:986-994], and stabilized with the buffer of MES-Tris pH 6.18 condition. With this treatment, the marker was well separated, although the resolution was not very high due to absent of cooling and manual switching of tubes and voltage. This is good enough for demonstration of the ES online with CE. The separation and reproducibility are satisfactory for this work, as shown in Figure 4S.

For protein separation in low pH, depression of EOF and adsorption are critical. Surface chemical bonding of polyacrylamide (PA) was carried out following the procedure reported in [Ju, J.J.; Liu, S. R. Electrophoresis 2006, 27, 3764-3771], and the result was satisfactory as can be seen from the separation of two basic proteins (lysozyme and BAS) shown in Figure 5S. A human plasma protein sample was stacked and separated under this condition.


Absorbance signal / a.u.
72
118
194
234
271/281
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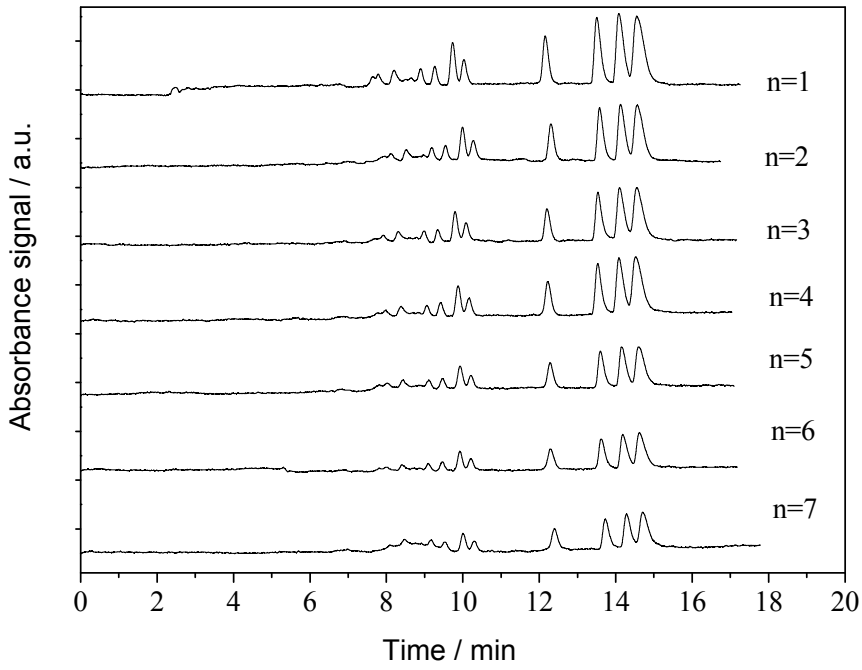
603
872
1078
1353
0
2
4
6
8
10
12
14
16
18
Time / min


Absorbance signal / a.u.
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n=3
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n=5
n=6
n=7
0
2
4
6
8
10
12
14
16
18
20
Time / min

Figure S4. Separation (top) and reproducibility (bottom) of ΦX174-HaeIII DNA marker in 80 mM pH 6.18 MES-Tris buffer with 0.8 % HEC, separation 110 V/cm, UV 256 nm.

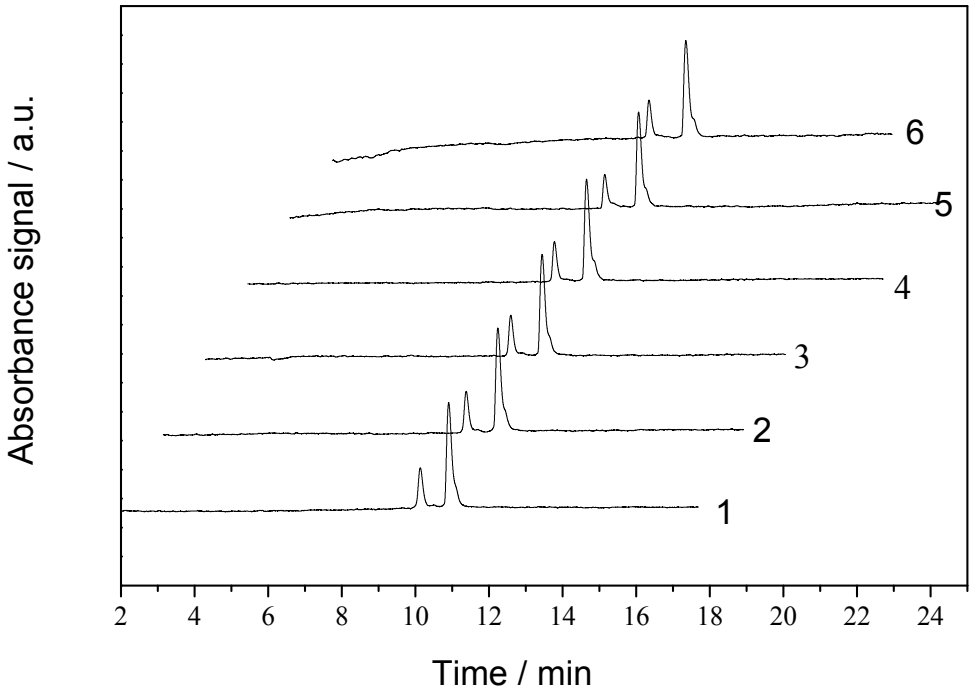

Figure S5. Separation of lysozyme and bovine serum albumin (BSA) with PA coated capillary, 50 mM pH 2.5 Tris-H_3PO_4 buffer with 4 % Dextran T2000, UV 214 nm detection.

5. Temperature on etching rate

Etching rate depends very much on the temperature, as shown in Figure 6S. The capillary is 100 μm id, the wall is 85 μm. It took more than 4 h at 19 °C, but less than 3 h at 26 °C to become ion conductive. To avoid over etching, a sound alarm system was designed for timely cleaning procedure.

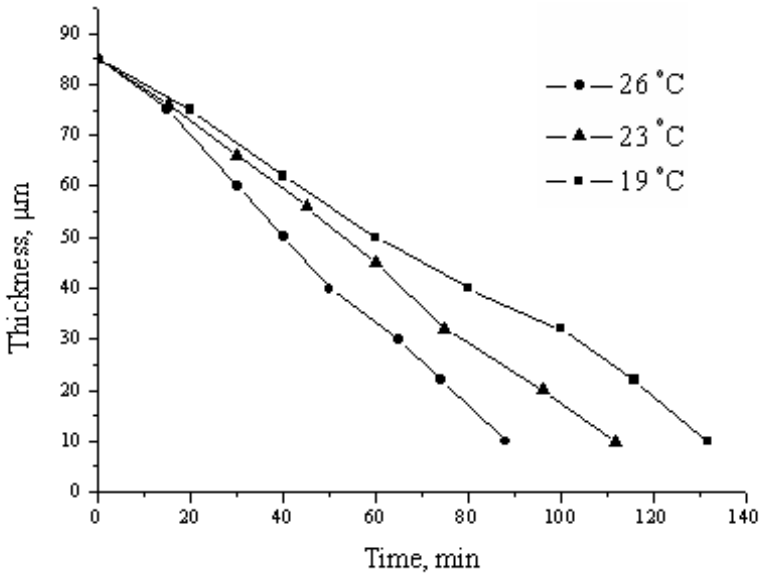

Figure S6. The etching rate of different temperature with 40 % HF, 100 μm id, 375 μm od. Fused silica capillary

6. Auto-stop etching principle

Auto-stop etching principle is schematically shown with Figure 7S. Before penetrating pores are formed, the current remain at the background level. An abrupt current increase indicate the time of penetrating. At the same time EOF is initiated in the direction from inside to outside, forcing the etchant out of the newly formed pores, thus the pore size is maintained and more time allowed for post cleaning procedure.

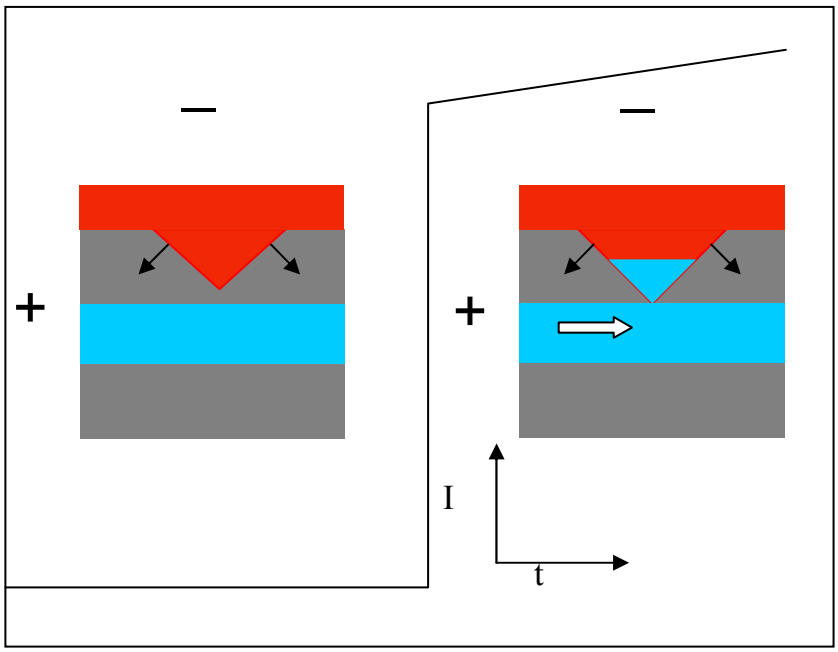

Figure S7. Schematic of auto-stop etching principle. By the time of becoming ion conductive, the EOF automatically forces the HF etchant out of the pores, and the etching is automatically stopped.

7. LIF calibration for fluorescein probe

Figure 7S is the calibration curve of fluorescein by the LIF system. Stacking assays were performed under the same conditions.

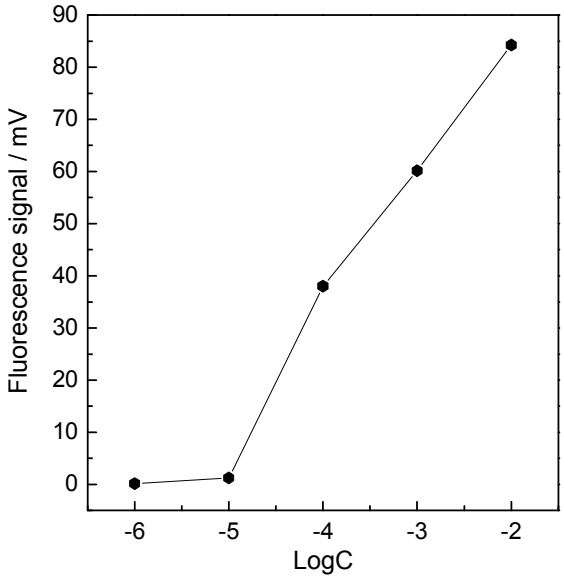

Figure S8. Calibration of fluorescein sodium concentration in mole/L, blue diode laser 487 nm, PMT 700 V, capillary id. 100 um. The signal was read by feeding the standard solutions in the capillary.

8. Type I ES of negatively charged fluorescent probe

In this test, the sample was loaded in the whole capillary, and stacked inside the capillary at the junction. After stacking, the stacked zone was transferred by EOF to the LIF detection window, and detected as peaks. This stacking process could be performed repeatedly by automatic programming of the voltages by the computer without any mechanical operations.

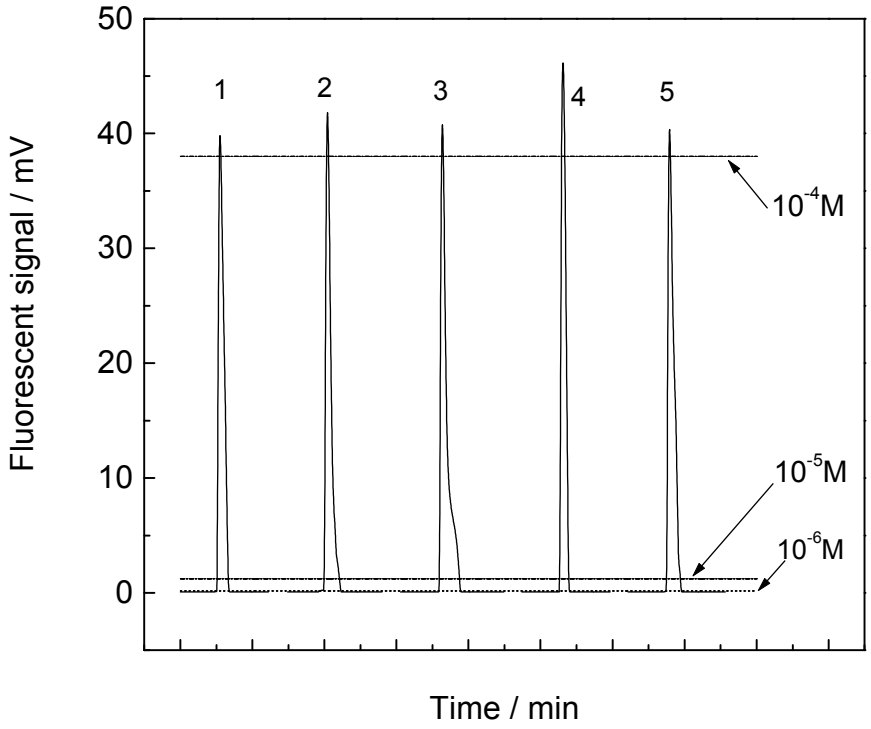

Figure S9. The first peak of 5 ES of fluorescein with renewal of the probe solution. The first peak of 5 tests was showed. Type I ES 50 V for 400 s, 1 μM fluorescein in 1 mM pH 9.24 Tris-HCl, capillary: 7 cm with the junction at the center and a LIF detection window between the junction and end reservoir.

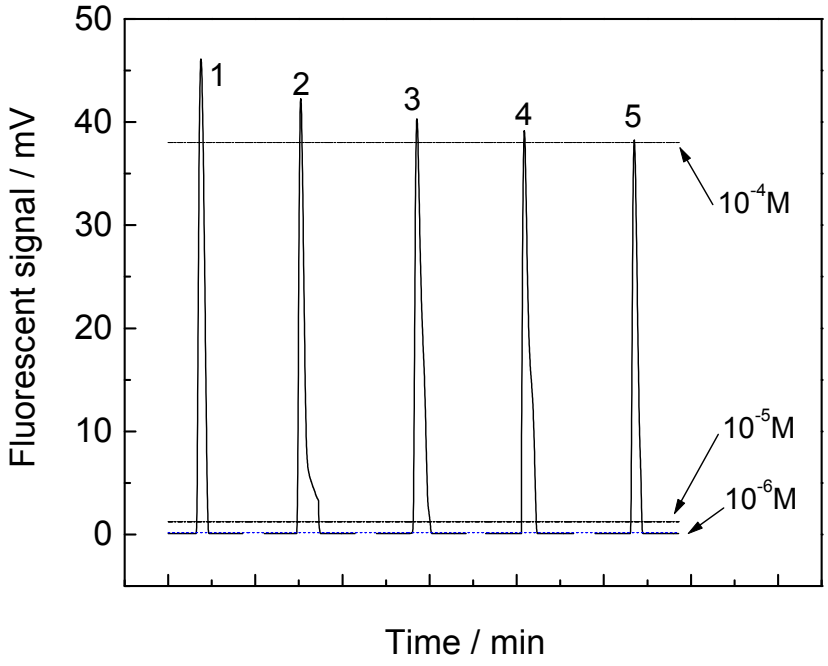

Figure S10. 5 consecutive ES without renewal of the sample solution. other condition the same as Figure S8.

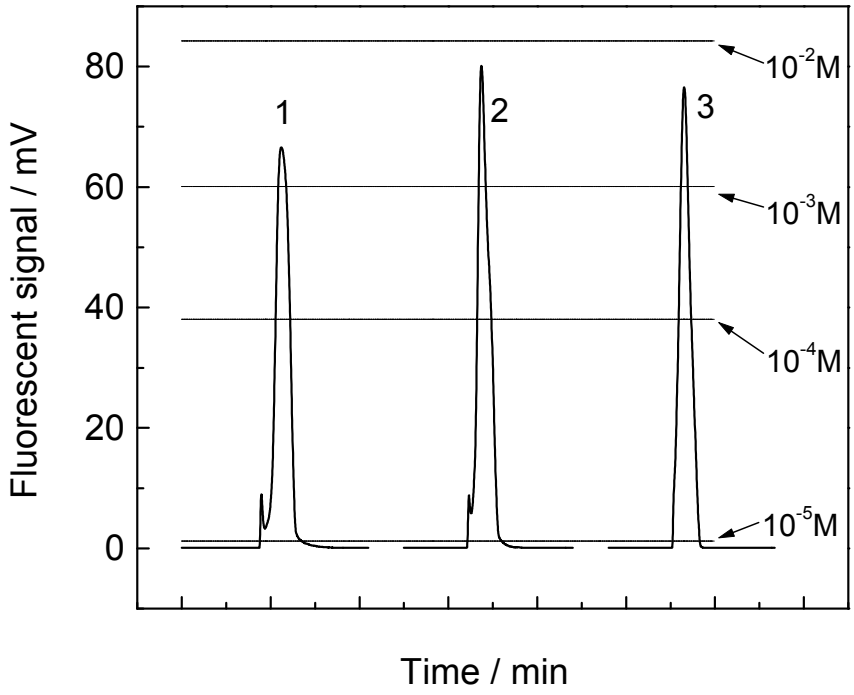

Figure S11. Repeated Type I ES of 1 pM fluorescein. The ES was performed with the same capillary chip, and the probe solution was renewed between the test. The first peak was used for the tests. Stacking: 50 V 1200 sec. Other conditions the same as Figure 6 in the main test.

9. Type II ES of positively charged species and LIF detection

Given the stacking voltage, the peak of rhodamine 6G increases by stacking time and voltage, as shown in Figure 10S and Figure 11S. Stacking voltage over 100 V may result in damage to the junction. This type II ES assay was implemented with a capillary chip with both fracture and junction.

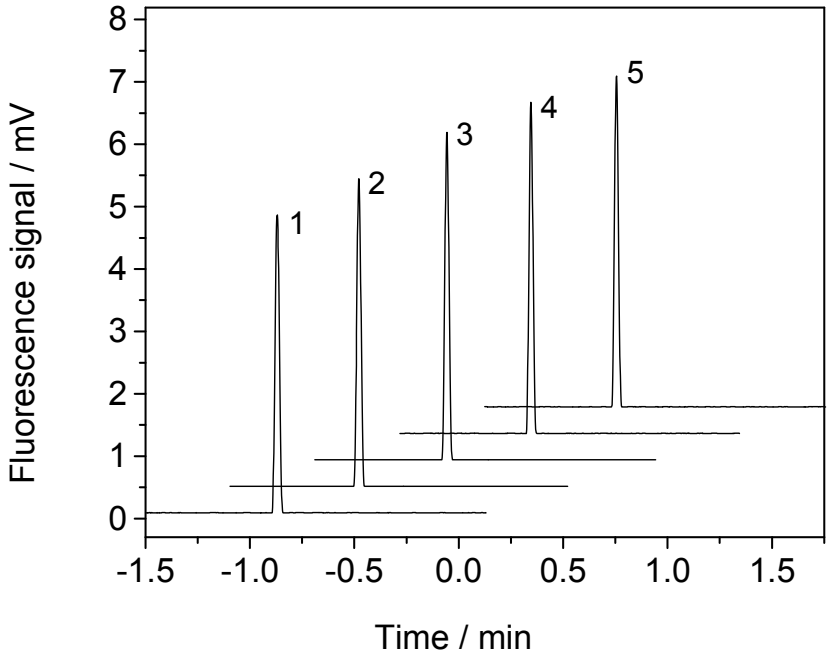

Figure S12. Reproducibility of type II ES CZE LIF of rhodamine 6G.

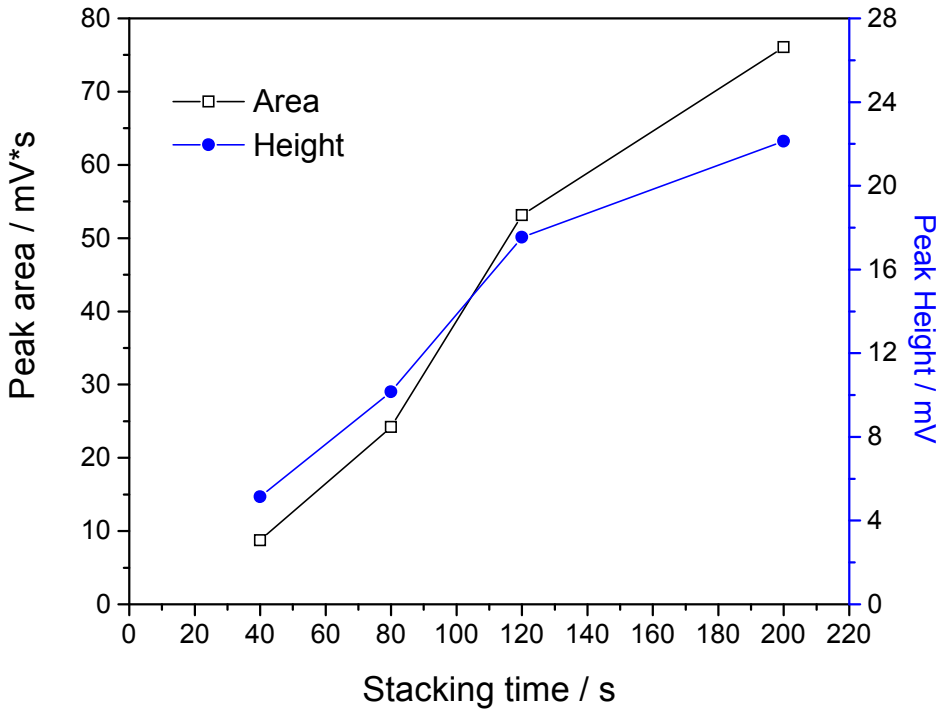

Figure S13. Peak height and area over stacking time. Type II ES CZE LIF of rhodamine 6G, given stacking voltage 50 V.

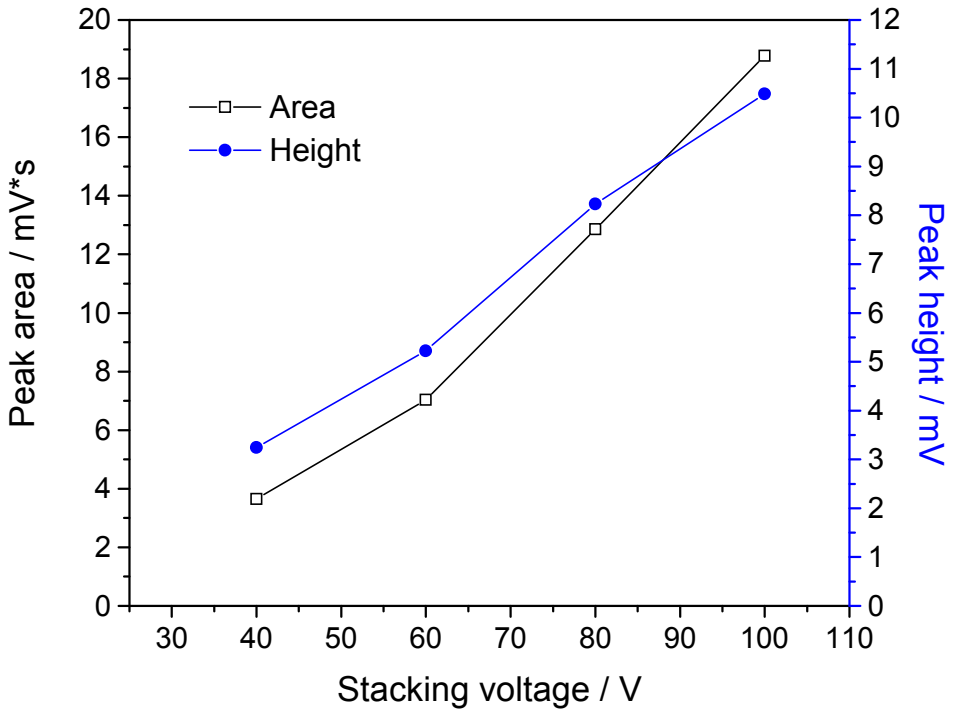

Figure S14. Peak height and area over stacking voltage. Type II ES CZE LIF of rhodamine 6G, given the stacking time of 60 s.

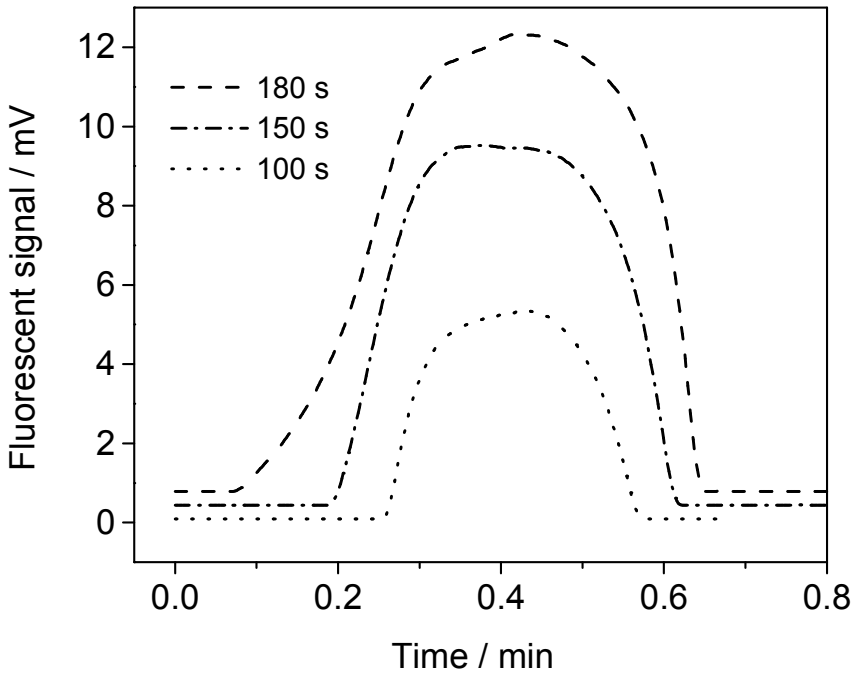

Figure S15. Peak profile of neutral probe (rhodamine B) generated by the type II ES, 80 V was applied between the fracture and the junction. Other conditions the same as Figure 7 in the main text.