Supporting Information

Simultaneous Electrokinetic Concentration and Separation of Proteins on a Paper-based Analytical Device

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Figure S1: Photo of the smartphone-based imaging system. The smartphone was fixed to the eyepiece tube of the stereomicroscope by an attaching clamp. A white LED light was used to provide a stable light condition for quantitative analysis. We also surrounded the stereomicroscope using a piece of shade cloth to avoid the interference of ambient light (not shown).



Figure S2: (a) Fabrication of paper discs by a simple 'press' step. We placed a circular plastic mold (6 mm in inner diameter) on the glass fiber paper followed by pressing it and the disk can be easily obtained thanks to the fragility of the glass fiber paper. (b) For calibration test, two paper discs were placed on a hydrophobic paper strip to avoid capillary penetration effect. The bottom paper strip was treated by a commercial hydrophobic reagent obtained from local supermarket. For each test, 7 μ L BGE was loaded in the left disk and 7 μ L standard solution in right disk. (c) and (d) are the calibration curves corresponding to the brilliant blue and amaranth. The intensity of each dye is corrected by subtracting the intensity value of the BGE. Additionally, each concentration was repeated four times. The pictures of the dyes in different concentrations are shown below the curves.



Figure S3: Data processing was performed with ImageJ software developed by the National Institutes of Health. After opening the image, the region of interest was selected. Then the image was inverted and the region of each stacking band was selected through the threshold tool (Image>Adjust>Color threshold). The mean gray value of the band was measured for quantitative analysis. This tool also helps us to exclude the overlap zone of the two bands. Note that each band intensity was corrected by subtracting the value of the BGE zone near the band.

— 0 s	
5 s	
10 s	
20 s	
30 s	
	5 mm

Figure S4: Paper-based FASS for sample concentration and separation with low sample consumption. 150 μ L BGE (50 mM Tris-HCl with 0.4 % HEC) was loaded to the anode reservoir, then 150 μ L deionized water was loaded to the cathode reservoir. After applying a 300 V voltage for 60 s, 5 μ L sample (10 μ g mL⁻¹ brilliant blue and 10 μ g mL⁻¹) was added to the paper channel close to the cathode reservoir and this time point was regarded as t = 0 s. A stacking band was quickly developed within 5 s, and the two dyes were successfully separated within 30 s. This method can effectively decrease the sample consumption, which is also rapid and easy to operate.