Supporting information

Automatic and Integrated Micro-Enzyme Assay (AIμEA) Platform for Highly-sensitive Thrombin Analysis via Engineered Fluorescence Protein-functionalized Monolithic Capillary Column

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Additional Experimental Section

Comparison of the micro-reaction system and the bulky reaction

The amount of the recombinant GFP immobilized on the monolithic matrix was calculated to be ~43.56 pmol/cm (~1.18 μg/cm), according to the absorbance decrease at 488 nm (ε =56000 $M^{-1}cm^{-1}$)[1] of the GFP solution after rinse through the monolith. High concentration EDTA rinsing released all GFP anchored on the monolith, whose fluorescent intensity was detected by CE-LIF. Then, the amount of the thrombin catalytic product, the cleaved GFP reporter, was calculated according to the ratio of the cleaved GFP to the total anchored GFP. As shown in **Figure S2A**, 50 mM EDTA rinsing at 5 psi for 90 s induced strong signal with the fluorescent intensity up to 7.26×10^7 au (arbitrary unit), while little fluorescence was detected with more EDTA rinsing (data not shown), confirming that the anchored GFP was completely eluted in the process. The fluorescent intensity of the GFP reporter released by 0.05 pmol thrombin for 10 min to 120 min increased from 1.23×10^6 au to 1.10×10^7 au, which were 1.69 % to 15.15 % of total GFP anchored on the AIμEA platform. Therefore, 0.74 pmol to 6.60 pmol GFP reporter was estimated under that condition.

For convenient comparison, the amount of thrombin and the recombinant GFP in the 50 μL bulky reaction was kept constant with that in the micro-column reaction. The catalytic product was also analyzed by CE-LIF. Considering that His-tag has many positive charges but limited molecular weight, cleaving it would have obvious effect on charge of the protein but little effect on its molecular mass. Hence, the

substrate (the recombinant GFP) and the product (the GFP reporter) can be separated by CE analysis under positive high voltage (peak 1 and peak 2 in **Figure S2B**). The amount of the product was estimated by the fluorescent intensity ratio of the product (peak 2 in **Figure S2B**) to total GFP in the reaction (~43.56 pmol, peak 1 and peak 2 in **Figure S2B**). The fluorescent intensity of the GFP reporter digested by 0.05 pmol thrombin for 10 min to 120 min slowly increased from 0 to 1120 au. That means 0 to 0.45 % of the total GFP, equal to 0 pmol to less than 0.2 pmol substrate, were digested by thrombin in that certain time in the bulky reaction.

Regeneration procedure

For the AIμEA platform regeneration, the capillary column was firstly rinsed with 50 mM EDTA for 30 min at room temperature through a syringe pump, followed by water and the phosphate buffer washing, to elute out Ni^{2+} as well as the immobilized recombinant GFP protein. Then the micro-reaction column was regenerated through re-chelation of 100 mM Ni^{2+} with NTA carboxylate group for 1.5 h, and then 12.84 μM recombinant GFP rinsing for 15 min.

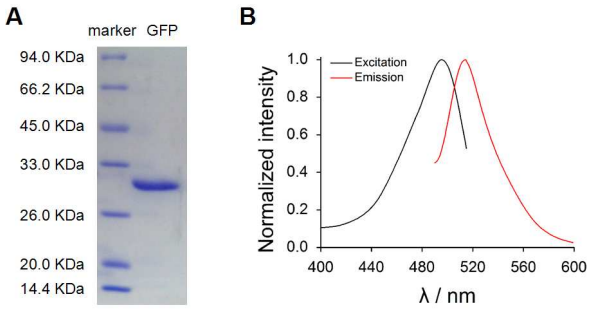

Figure S-1. Characterization of the recombinant GFP protein through (A) SDS-PAGE and (B) fluorescence spectra.

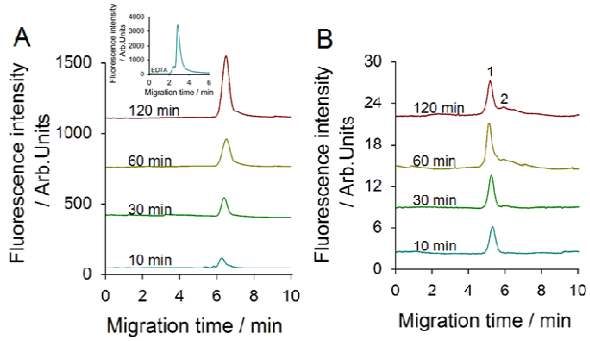

Figure S-2 (A) Electropherograms of the thrombin catalyzed on-line micro-reaction in the AIμEA platform from 10 min to 120 min with CE-LIF. 100 nM Thrombin was injected with 0.5 psi for 20 s, on-line reacted for certain time, and then followed by electrophoresis at +10 kV. Inset shows the electropherogram of the GFP reporter in the AIμEA platform eluted by 50 mM EDTA at 0.5 psi for 90 s with CE-LIF analysis. (B) CE-LIF analysis of the thrombin catalyzed cleavage of the recombinant GFP in the bulky reaction. Bulky mixture after reaction for 10 min to 120 min was separately injected at 0.5 psi for 20 s and electrophoretic separated at +10 kV. Peak 1: the recombinant GFP; Peak 2: the GFP reporter.

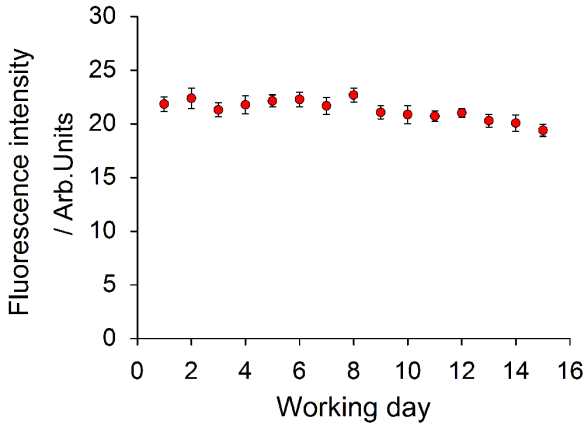

Figure S-3. Working life of the AIμEA column estimated through 5 nM thrombin cleaving for 5 times each day.

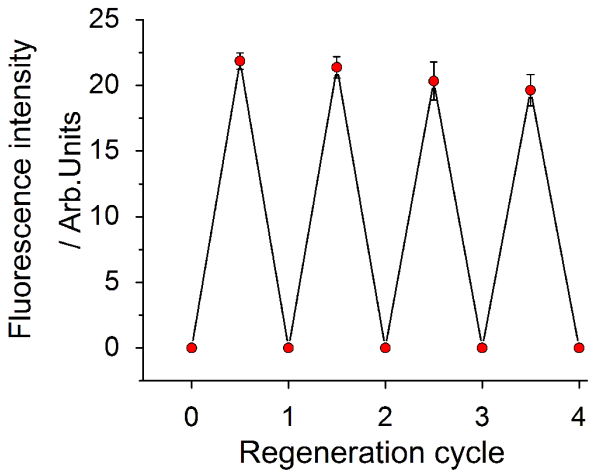

Figure S-4. Regeneration of the AIμEA column, with the stability estimated by 5 nM thrombin digestion for 5 times during each regeneration cycle.

Reference:

(1) Tsien R.Y. *Annu Rev Biochem*, 1998, *67*, 509-544.