Electronic Supplementary Information

Protein-based mixed selector chiral monolithic stationary phase in capillary electrochromatography

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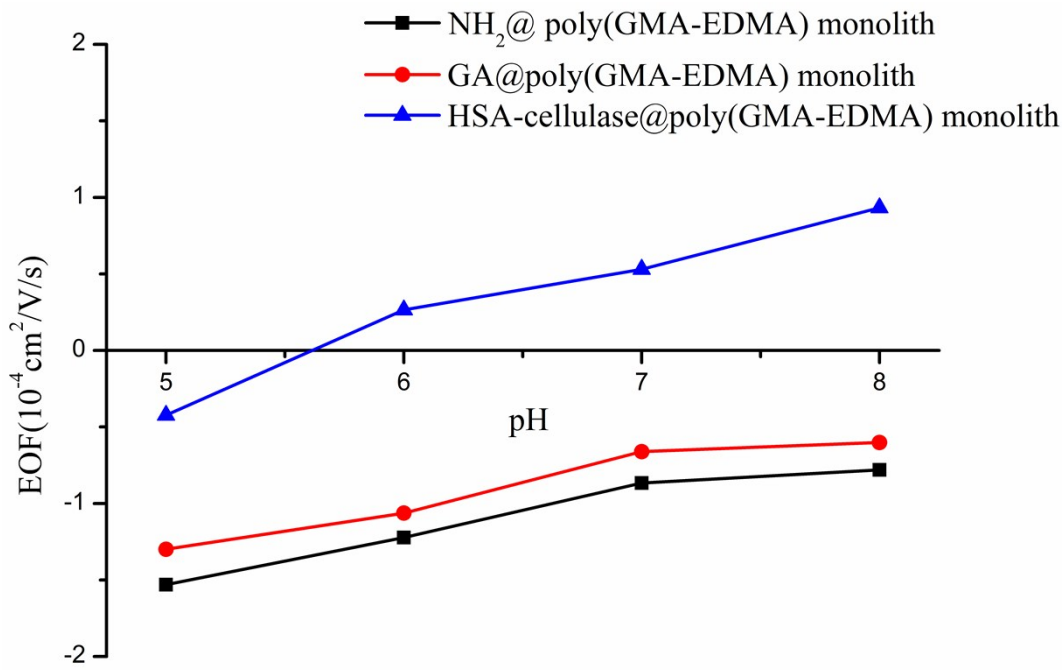

Fig. S1. The influence of buffer pH on the EOF mobility for different monolithic columns. Experimental conditions: running buffer, 10 mM phosphate buffer (pH 5.0-8.0); applied voltage, ±20 kV; injection, 10 kV x 3 s; detection wavelength, 210 nm; temperature, 20 °C; thiourea was used as EOF marker.

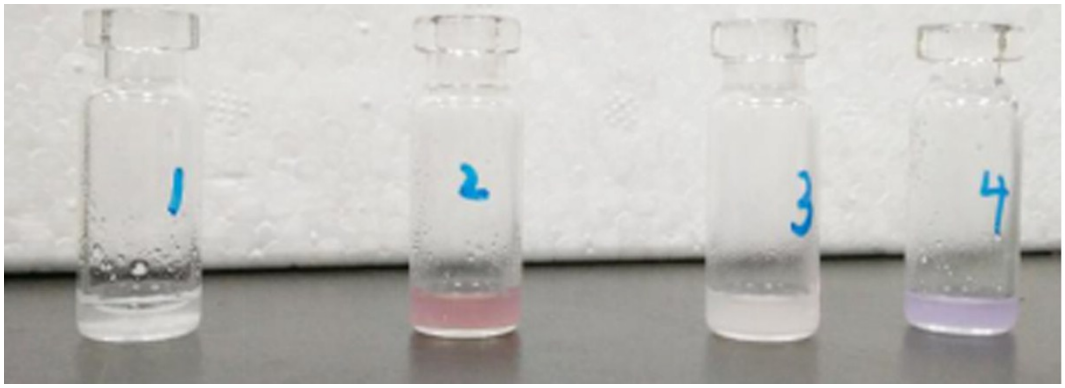

Fig. S2. Ninhydrin reaction with different monoliths. 1, poly(GMA-EDMA) monolith; 2, NH_2@poly(GMA-EDMA) monolith; 3, GA@poly(GMA-EDMA) monolith; 4, HAS-cellulase@poly(GMA-EDMA) monolith.

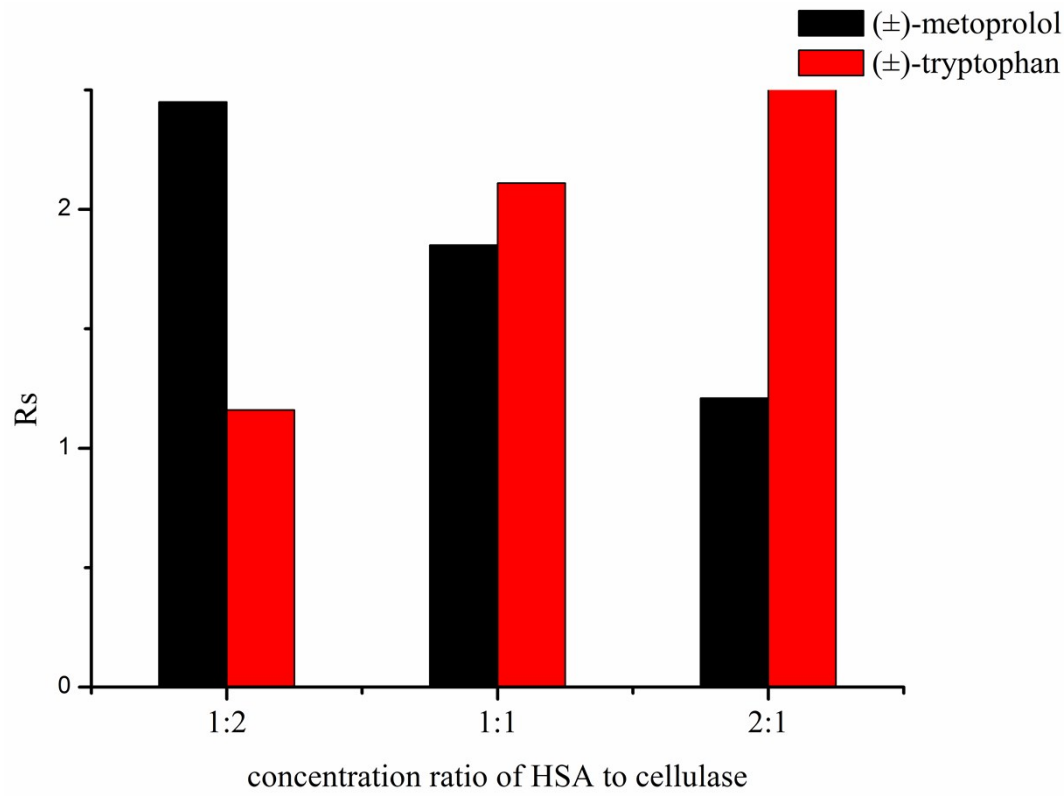

Fig. S3. Effect of concentration ratio of HSA to cellulase on enantioseparation of (±)-metoprolol and (±)-tryptophan on HSA-cellulase@poly(GMA-EDMA) monolith. Experimental conditions: samples, 0.7 mg mL^{-1} (±)-tryptophan, 50 μg mL^{-1} (±)-metoprolol; running buffer, 10 mmol L^{-1} phosphate buffer (pH 7.0, containing 10% 2-propanol for (±)-metoprolol, without 2-propanol for (±)-tryptophan); applied voltage, 10 kV for (±)-metoprolol and 15 kV for (±)-tryptophan; injection, 10 kV x 2 s for (±)-metoprolol and 10 kV x 1 s for (±)-tryptophan; detection wavelength, 225 nm for (±)-metoprolol and 215 nm for (±)-tryptophan ; temperature, 20 ℃.

Measurement of immobilized protein amount by Bradford assay

The amount of immobilized protein on HSA-cellulase@poly(GMA-EDMA) monolithic columns was determined by Bradford assay, according to the previous report[1] with minor modifications. Briefly, the column with a length of 10 cm was first chopped into small pieces and then immersed into 1 mL of 100 mmol L^{-1} NaOH for 2 h at room temperature to cleave protein completely. Mixed protein standard solutions (the concentration ratio of HSA to

cellulase was 1:1) were prepared by dissolving the mixed proteins in 100 mmol L^{-1} NaOH in the concentration range of 10-120 μg mL^{-1}. A volume of 1 mL of each protein standard or the cleaved protein solution was mixed with 1 mL of Bradford reagent, respectively. After each mixture was incubated at room temperature for 5 min, the absorbance was measured with a spectrophotometer at 595 nm. Standard curve was shown in Figure S4. The results indicated that 33.5, 55.5 and 68.1 μg of proteins were immobilized on 10 cm length HSA-cellulase@poly(GMA-EDMA) monolith prepared with 50 μm, 75 μm and 100 μm inner diameter capillaries, respectively. That is, the amount of immobilized proteins per centimeter was 3.35, 5.55 and 6.81, respectively.

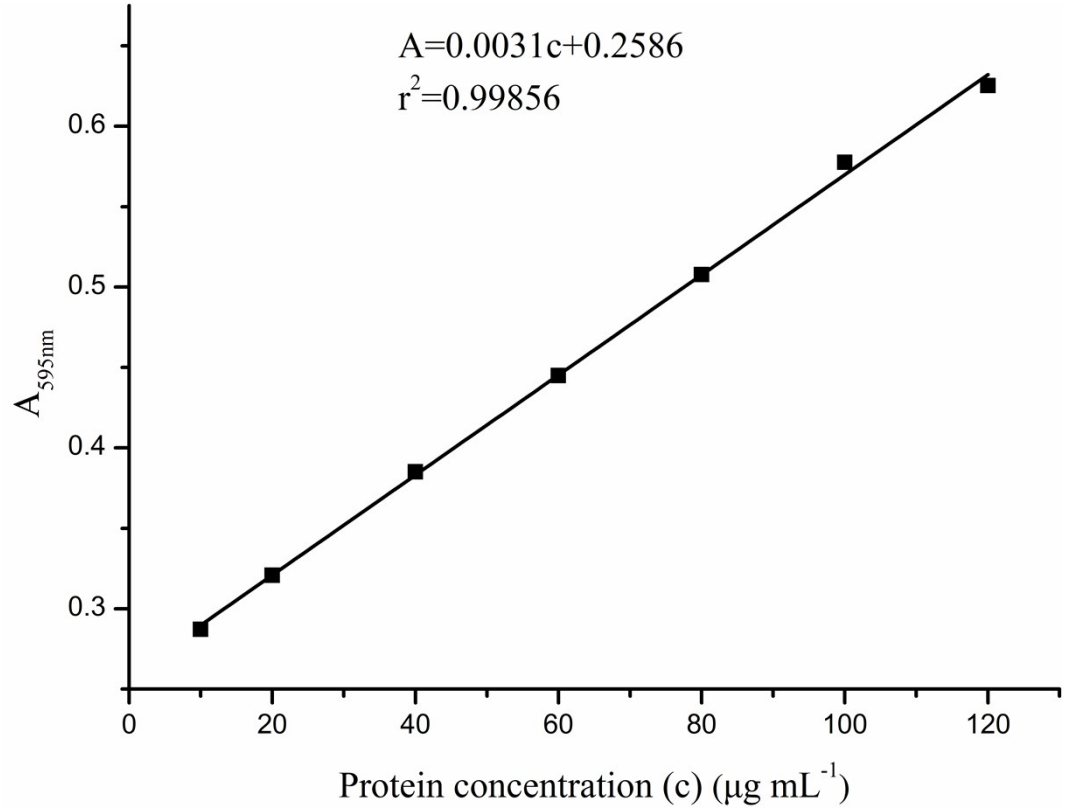

Figure S4: Standard curve for Bradford assay

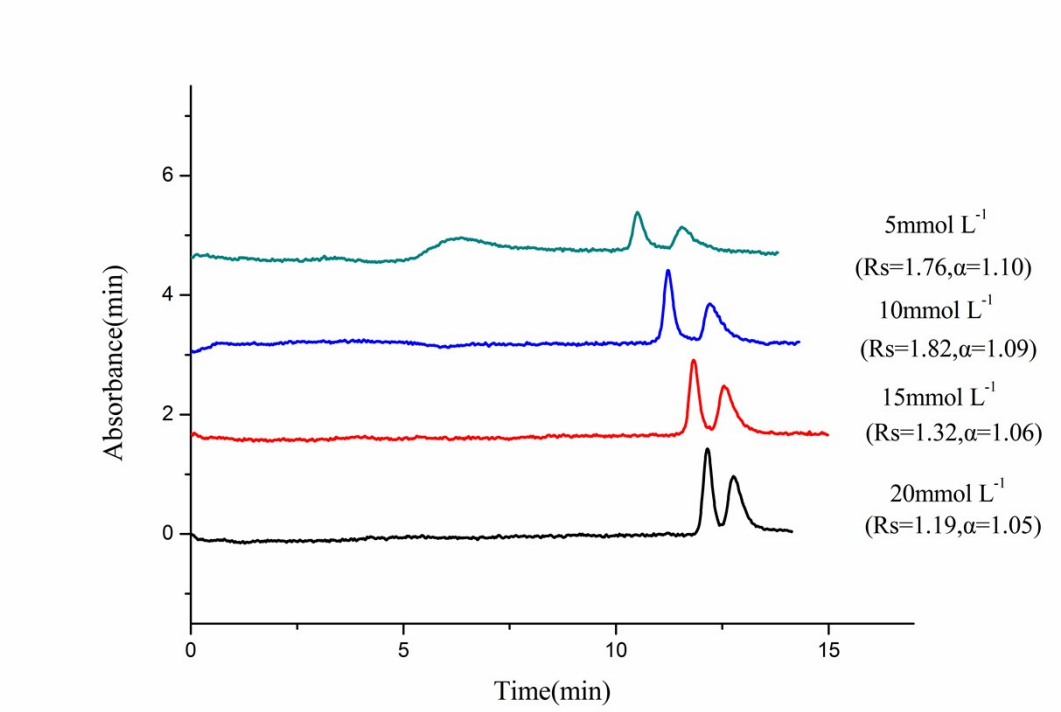

Fig. S5. Effect of buffer concentration on enantioseparation of (±)-metoprolol on HSA-cellulase@poly(GMA-EDMA) monolith. Experimental conditions: 50 μg mL^{-1} (±)-metoprolol; running buffer, different concentration of phosphate buffer (pH 7.0, containing 10% 2-propanol); applied voltage, 10 kV; injection, 10 kV x 2 s; detection wavelength, 225 nm; temperature, 20 ℃.

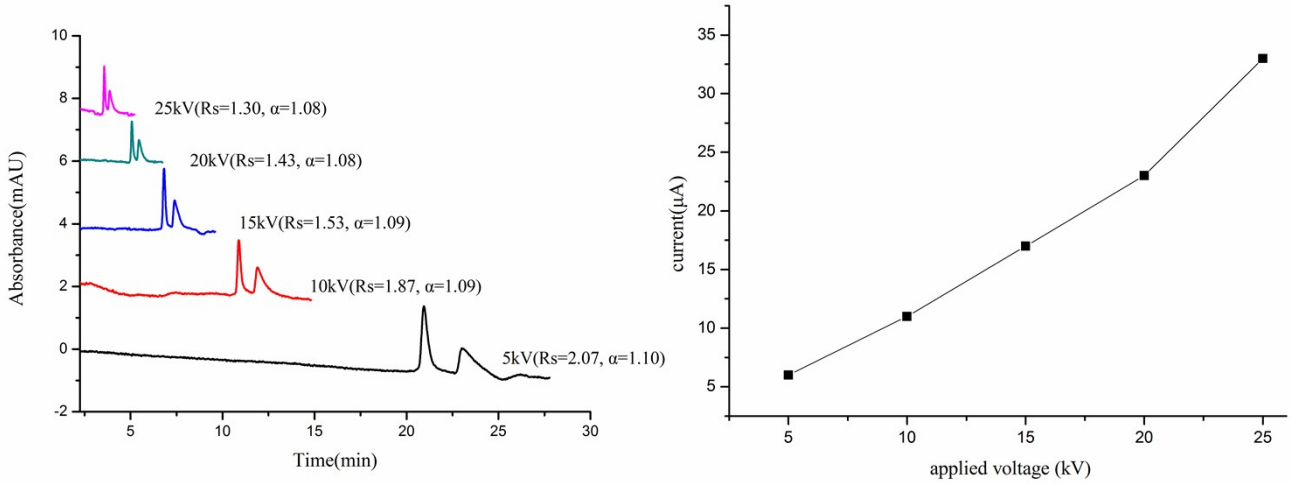

Fig. S6. Effect of applied voltage on enantioseparation of (±)-metoprolol on HSA-cellulase@poly(GMA-EDMA) monolith and the disproportionate increase in current with voltage. Experimental conditions: 50 μg mL^{-1} (±)-metoprolol; running buffer, 10 mmol L^{-1} phosphate buffer (pH 7.0, containing 10% 2-propanol); applied voltage, 5-25 kV; other conditions are the same as Fig. S5.

Table S1. Effect of 2-propanol content on enantioseparation of (±)-metoprolol on HSA-cellulase@poly(GMA-EDMA) monolith

concentration of 2-propanol	μ_{eo}（$10^{-4}cm^2/(s\cdot V)$)	t_1 (min)	t_2 (min)	n_1 (plates/m)	n_2 (plates/m)	Rs	α
0	0.53	7.771	8.267	55410	12790	1.06	1.06
2%	0.50	8.314	8.885	54902	14522	1.18	1.07
6%	0.44	9.391	10.121	57049	15688	1.37	1.08
10%	0.42	10.559	11.552	62263	24546	1.93	1.09
14%	0.32	12.070	13.265	72351	7688	1.38	1.10

Thiourea was used as the EOF marker. Experimental conditions: 50 μg mL^{-1} (±)-metoprolol; running buffer, 10 mmol L^{-1} phosphate buffer (pH 7.0, containing different proportions of 2-propanol); applied voltage, 10 kV for (±)-metoprolol and 20 kV for thiourea; injection, 10 kV x 2 s for (±)-metoprolol and 10 kV x 1 s for thiourea ; detection wavelength, 225 nm for (±)-metoprolol and 210 nm for thiourea; temperature, 20 °C.

References:

1. J. Ma, Z. Liang, X. Qiao, Q. Deng, D. Tao, L. Zhang and Y. Zhang, *Anal. Chem.* 2008, **80**, 2949-2956.