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Lab on a Chip

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Supplementary Information

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5 **Title:** Open-tubular radially cyclical electric field-flow fractionation (OTR-
6 CyEIFFF): an on-line concentric distribution strategy for simultaneous separation of
7 microparticles

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14 **Contents:**

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25 **Part A: Chemicals and Instruments.**

26 **Chemicals.** Different polystyrene particles (0.3, 0.46, 0.8, 1.0 and 3.0 μm were negatively charged
27 and amino-group modified 0.1 μm was positively charged) used in this study were purchased from
28 Thermo Fisher Scientific, Inc. (Waltham, MA, USA) and Polysciences, Inc. (Warrington, PA,
29 USA), respectively. All the particle samples are typically supplied as a 10% suspension. The 0.3,
30 0.46, 0.8, 1.0, 3.0 and 0.1 μm were prepared by suspending the particles in deionized (DI) water
31 (after sonication for 30 min) and the concentration of particles were 0.01% w/w in DI water,
32 respectively. The injection volume was 2.0 μL . The silica precursor [tetraethoxyorthosilicate
33 (TEOS); 99.999%], a non-ionic surfactant F-127, Polyacrylonitrile (PAN, with an average
34 molecular weight of 150,000), 1-Butyl-3-methylimidazolium hexafluorophosphate, 1-Butyl-1-
35 methylpyrrolidine bis(trifluoromethanesulfonyl)imide, Methyltri-n-octylammonium bis
36 (trifluoromethanesulfonyl)imide, and Amyltriethylamine bis(trifluoromethanesulfonyl)imide were
37 purchased from Sigma-Aldrich (Bellefonte, PA, USA). The platinum wire was purchased from
38 Tianjin Lu Cheng metal processing factory (Tianjin, China). The stainless steel tube, the 3-way
39 connector and the PEEK screw head were purchased from Yuexu Technology Corporation
40 (Shanghai, China).

41 **Instruments.** Scanning electron microscopy (SEM) images were obtained by a Hitachi UHR S
42 5500 FE-SEM operating at an accelerating voltage of 30 kV. X-Ray diffraction (XRD) patterns
43 were obtained in reflection mode by using a Rigaku D/MAX-2200 Ultima equipped with Cu K_{α}
44 radiation at 30 kV and 40 mA. Transmission electron microscopy (TEM) images were obtained
45 using a JEOL JEM 3010 at an accelerating voltage of 300 kV. Cyclic voltammetry (CV) was
46 performed through an instrument obtained from Tianjin Lanli Chemical Electronic High

47 Technology Co., Ltd. The dip coater for modification was purchased from Shanghai Huotong
48 Experimental Instrument Co., Ltd. The UV detector used in this study was Agilent Technologies
49 1260 Infinity and the particles were detected by using UV absorption at 254 nm. The UV detector
50 was directly connected to the OTR-CyEIFFF column for online separation. AC electric field was
51 generated by using the Arbitrary Function Generator AFG-1205 signal generator purchased from
52 Guwei Electronic Co., Ltd (Suzhou, China). The syringe pump (0.10 A, 250 V) was purchased
53 from KD Scientific (Holliston, MA, USA). Particles injection was performed with a 5 μ L
54 Hamilton microliter syringe, while the mobile phase was injected by using a 5 mL syringe
55 (Hamilton, 1005RN) was used combined with the syringe pump.
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57 **Part B: Experimental Method and Characterization of Electrodes.**

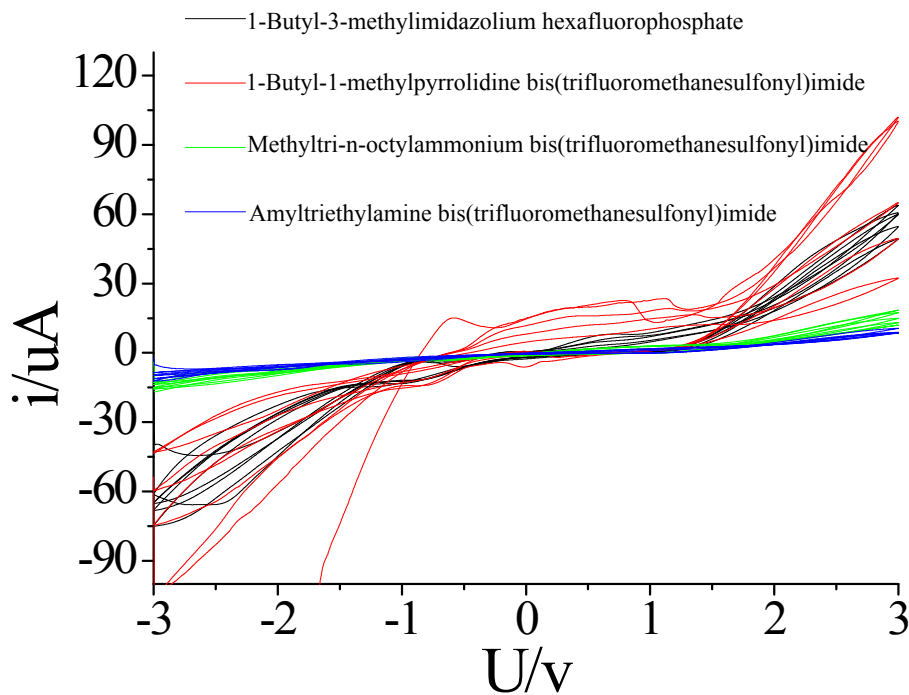
58 **Sample preparation.** The stainless steel tube ($D_{id}=0.75$ mm) and the platinum wire ($D_{id}=0.20$ mm)
59 were prepared as the external electrode and internal electrode, respectively. The platinum wire
60 was calcined at 600°C for 8 hours to oxidize the surface and prepared it for subsequent
61 modification with mesoporous silica materials (meso- SiO_2). Both the stainless steel tube and
62 platinum wire were successively sonicated with 1M NaOH, DI water, 1M HCl and DI water for 30,
63 15, 30 and 15 min, respectively. The sonication step was intended to remove the impurities on the
64 inner surface of the annular channel to ensure a uniformed modification of meso- SiO_2 on the
65 annular channel surface. Then, the platinum wire was blow dried. After that, the stainless steel
66 tube was immersed into the piranha solution ($\text{H}_2\text{SO}_4:\text{H}_2\text{O}_2 = 7:3$) for 60 min for hydroxylation.
67 The stainless steel tube was rinsed to $\text{pH}=7$ with DI water and dried under 80°C in the oven.

68 **Synthesis of mesoporous silica materials.** Briefly, 0.4 g F-127 was dissolved in 5 mL absolute
69 ethanol and stirred at 400 rpm for half an hour. After that, 1.5 g TEOS and 0.4 g 0.8 M HCl were
70 added drop by drop into the solution and was stirred (400 rpm) at 25°C for 20 h under a controlled
71 relative humidity of below 20%.

72 **Characterization of electrodes.** In order to increase the working voltage and the electrode, 1-
73 butyl-3-methylimidazolium hexafluorophosphate, 1-butyl-1-methylpyrrolidine
74 bis(trifluoromethanesulfonyl)imide, methyltri-n-octylammonium bis
75 (trifluoromethanesulfonyl)imide, and amyltriethylamine bis(trifluoromethanesulfonyl)imide were
76 investigated. Based on a series of assessments by cyclic voltammetry (Figure S-1), methyltri-n-
77 octylammonium bis(trifluoromethanesulfonyl)imide was eventually chosen to be modified into the
78 mesopores because it exhibited a wide electric window which indicated the absence of oxidation

79 and reduction reactions. Figure S-2 proved that the modified material did not peel off from the Pt
80 substrate, and the silica content remained unchanged after applying voltage, thus verifying the
81 stability of the Pt/meso-SiO₂/Ionic liquid electrode.

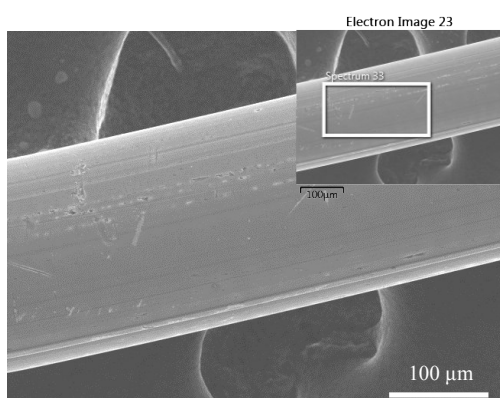
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84 **Figure S-1.** The electrochemical window of four different ionic liquids analyzed by cyclic
 85 voltammetry. Experimental conditions were as follows: Reference electrode was Ag/AgCl, counter
 86 electrode was platinum, working electrode was platinum wire, scan range was in between -3 and 3 V,
 87 scan rate was 0.5 V s⁻¹ and the four selected types of ionic liquids were used as electrolytes.

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Element	Wt%	Atomic %
O	35.52	75.08
Si	13.33	16.06
Pt	51.15	8.87
Total:	100	100

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90 **Figure S-2.** Characterization of the Pt/meso-SiO₂/ionic liquid by SEM-EDS after applying voltage.

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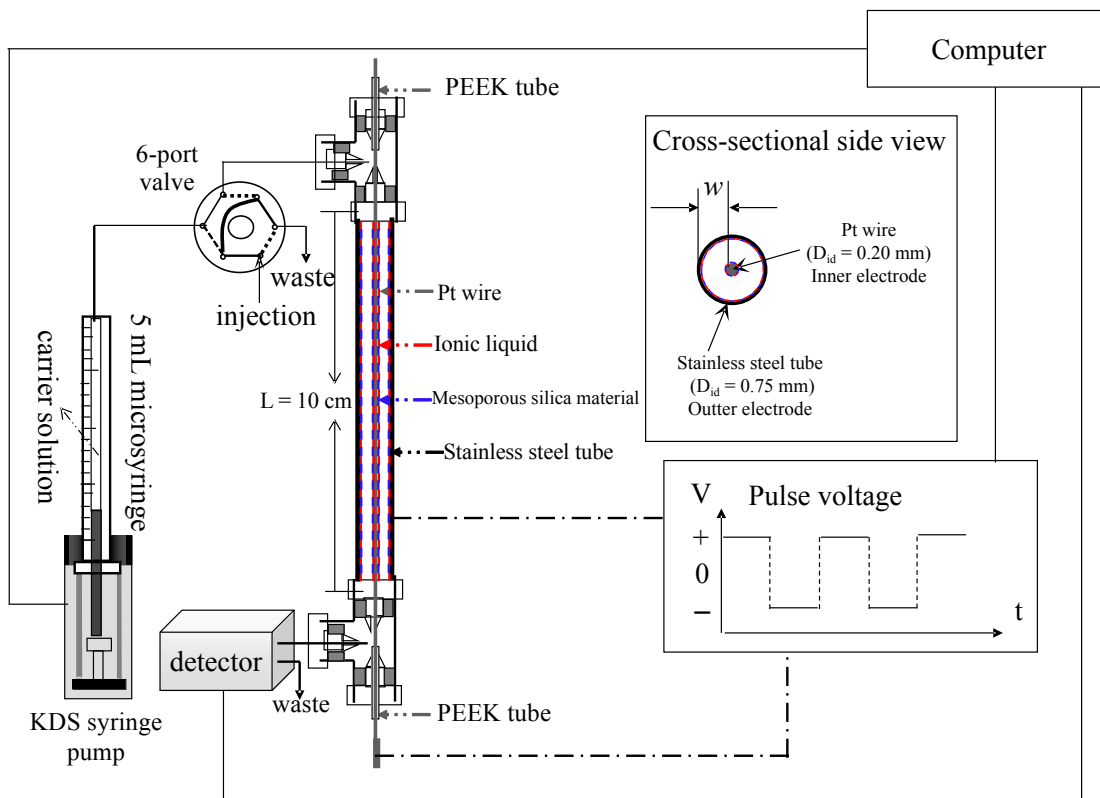
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93 **Part C: Device fabrication**

94 **Design and Fabrication of the micro-device.** After modifying the ionic liquid and meso-SiO₂
95 onto the stainless steel tube and platinum wire, both ends of Pt wire were coated with 10% PAN in
96 DMF solution and blow dried under nitrogen gas to form a PAN film. Then the Pt was placed in
97 an oven for 3 h at 180°C. When the Pt wire is returned to room temperature, the Pt wire was
98 inserted into the stainless steel tube, and the stainless steel tee was connected to both ends of the
99 Pt wire. It must be ensured that the PAN film on both ends of the Pt wire did not come into
100 contact with the stainless steel tee in the tee. In order to seal the separation column securely and to
101 ensure that the Pt wire was positioned at the center of the stainless steel tube, both ends of the Pt
102 wire were inserted into the PEEK tube. As shown in Figure 2B, first, the Pt wire was fixed by the
103 stainless steel tee with a PEEK joint. Then, the Pt wire was straightened to ensure it was parallelly
104 positioned at the center of the stainless steel tube and it was attached to the tee with a PEEK joint
105 at the same time. The resistance between the two electrodes was measured by a multimeter, and
106 the measurement was assured to be infinite, indicating the successful preparation of the separation
107 column. The width of separation channel width between the platinum wire and the stainless steel
108 tube is 0.275 mm. The equipment included a pump, a sample injection valve with a 5- μ L sample
109 loop, an annular separation channel, power supply, and a UV detector equipped with a 1.7- μ L
110 flow cell. The KDS syringe pump was connected to the sample injection valve, and carrier
111 solutions were supplied to the separation system using a 5 mL syringe (Hamilton, 1005RN). A
112 three-way connector was used as a coupler between the annular separation channel and the sample
113 injection valve (or UV detector). Power supplies were clipped on the platinum wire and stainless
114 steel tube, respectively. The UV detector was connected to a computer, which was used to record

115 the data signals. All connecting tubes were of 0.15-mm-I.D. Teflon tubes were used to minimize
116 the dead volume of the system. A schematic diagram of the OTR-CyEIFFF device is shown in
117 Figure S-3.

118 **Microchip preparation.** In order to observe the particles motion under different electric
119 parameters in the OTR-CyEIFFF system, the transparent cycloolefin copolymer (COC) material
120 was used to fabricate the microchip according to the Ref S-1. The fabrication process was shown
121 in Figure S-4B. In brief, a circular channel was fabricated by clamping two COC plates and a rod
122 template altogether and calcined at 147°C for 25 min. Then a platinum wire was embed at the edge
123 of the circular channel as the external electrode by hot pressing method at 147°C for 10 min.
124 Finally, a straightly-stretched platinum wire (red) was embed at the center of the circular channel
125 as the internal electrode under the 137°C for 10 min to seal the two COC plates. When particles
126 were injected into this system, the power supply applied the desired electrical parameters through
127 the outer electrode (blue) and the inner electrode (red) as indicated in Figure S-4A. A cross section
128 of this system was shown in Figure S-4C. The space between inner electrode and the outer
129 electrode was observed by using a microscope. A theoretical motion of particles in this system
130 was shown in Figure S-4D, in this case, the flow velocity distribution is the same as in OTR-
131 CyEIFFF. All the platinum wires utilized in this part were modified with ionic liquid and meso-
132 SiO₂.



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134 **Figure S-3.** A schematic diagram of the open-tubular radially cyclical electric field-flow

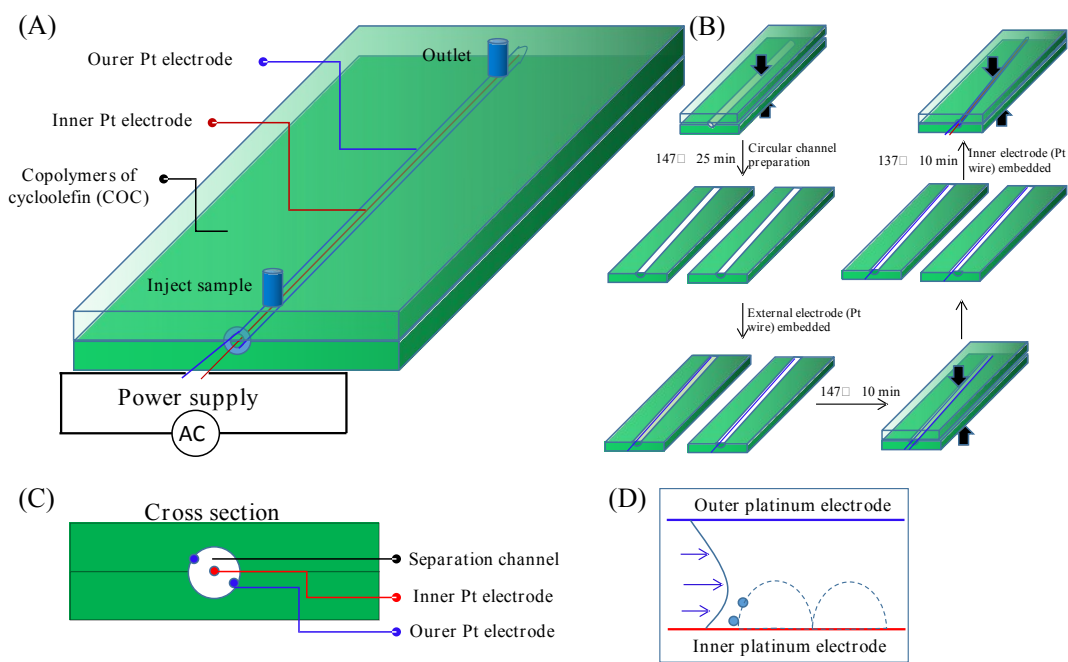
135 fractionation (OTR-CyEIFFF) separation system.

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142 **Figure S-4.** (A) represents the microchip system of the OTR-CyEIFFF. (B) represents the

143 chip fabrication procedures by hot pressing method. (C) represents the cross-sectional view of the

144 separation channel. The platinum wire (red) as the internal electrode was located at the center of

145 the channel, and the platinum wire (blue) as the external electrode was embedded at the edge of

146 the circular channel. (D) depicts the zig-zag motion of particles under a parabolic velocity profile

147 within the separation channel between the internal Pt electrode (red) and the external Pt electrode

148 (blue).

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154 ^{S-1} L. Martynova, L. E. Locascio and M. Gaitan, Fabrication of plastic microfluid channels by imprinting methods,

155 *Anal. Chem.*, 1997, **69** (23):4783.

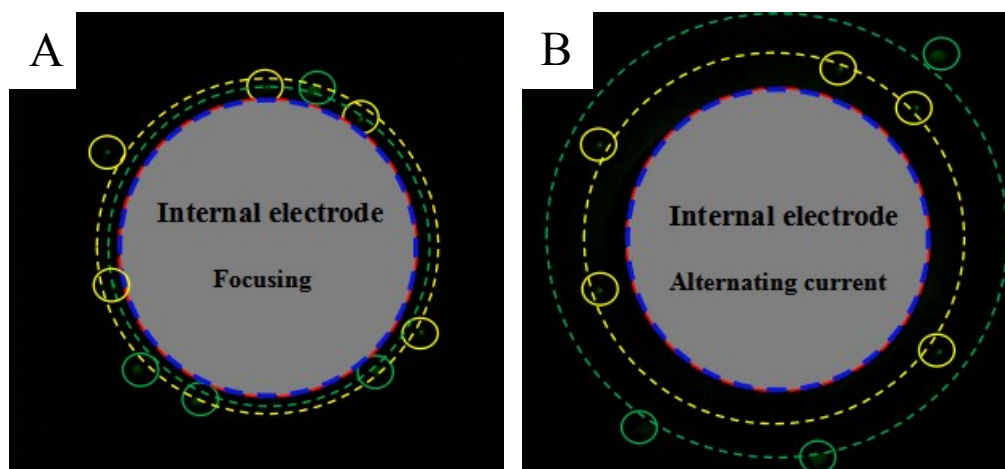
156 **Part D: Annulus distribution of particles.**

157 We simulated the electric field intensity and the velocity distribution of mobile phase in
158 order to further elucidate the particle movement inside the annular channel. According to
159 COMSOL simulation in Figure 3, the intensity of electric field decreases from the center to the
160 annular wall. It signifies that the particles tend to move in a zig-zag motion at the inner electrode
161 surface under different electric parameters. The maximum flow velocity is located near the center
162 part of the microchannel and such a formation of laminar flow is beneficial for particles to form a
163 conical arrangement within the annular separation channel. To further verify the particle
164 movement, an annular microchip that contained two platinum wires modified with meso-SiO₂ and
165 ionic liquid as the inner electrode and outer electrode were prepared.

166 Without an electric field, all particles were eluted directly from the annular channel resulted
167 in low retention time and resolution. Before the application of AC electric field on both electrodes,
168 the mobile phase was halted and the electrical parameter were adjusted to 1.5 V, 0.1 Hz and duty
169 cycle value of 99% to focus the zig-zag motion 1 μm (yellow dashed line) and 5 μm (~~pink~~ blue
170 dashed line) polystyrene particles at the internal electrode (Figure S-5A). Particles can move back-
171 and-forth under different electric field parameters between the two electrode surfaces. While
172 under the AC electric parameters (2.0V, 1.0 Hz and duty cycle value of 50%), the two different
173 sizes of PS formed an annular distribution at different annuli according to the size difference
174 (Figure S-5B). When a mobile phase was added, the particles located at different annuli will form
175 a conical distribution due to the parabolic profile of the mobile phase. The mobile phase flow and
176 the AC electric field together applied in this system. As a result, particles will be separated based
177 on the different forces being exerted and the different flow velocities in the mobile phase.

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181 **Figure S-5.** (A) represents the electrofocusing of the 1 μm (yellow dashed line) and 5 μm (green

182 dashed line) polystyrene particles under electrical parameters of 1.5 V, 0.1 Hz and duty cycle

183 value of 99%. (B) represents the formation of annular distribution of different particles under the

184 electrical parameters of 2.0V, 1.0 Hz and duty cycle value of 50%.

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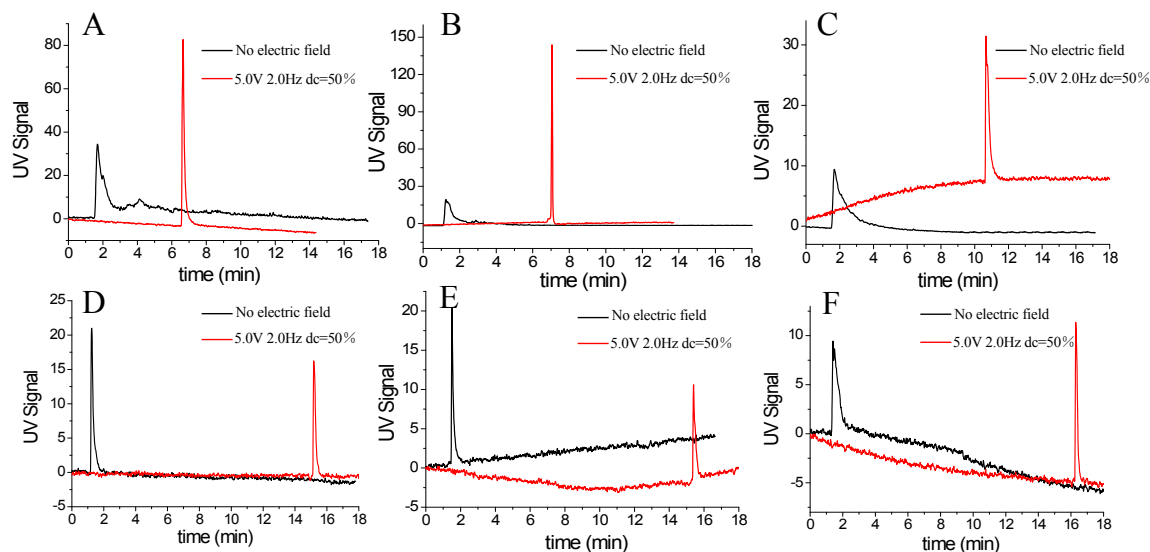
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191 **Part E: Recovery and purity.**



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193 **Figure S-6.** UV chromatograms showed the detection of six types of particles with different sizes

194 under separation mode II. (A), (B), (C), (D), (E) and (F) respectively represent the 0.3, 0.46, 0.8,

195 1.0, 3.0 and amino-group modified 0.1 μm polystyrene particles were directly eluted from the

196 separation system without an electric field (black line) and were retained for different times when

197 an electric field was applied under separation mode II (red line). The column length is 13 cm.

198 Electrical parameters were 5.0 V, 2.0 Hz and dc=50%. The flow rate of the mobile phase (water)

199 was remained constant at 1.0 mL h⁻¹.

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Table S-1. The recovery for particles of different sizes under separation mode II.

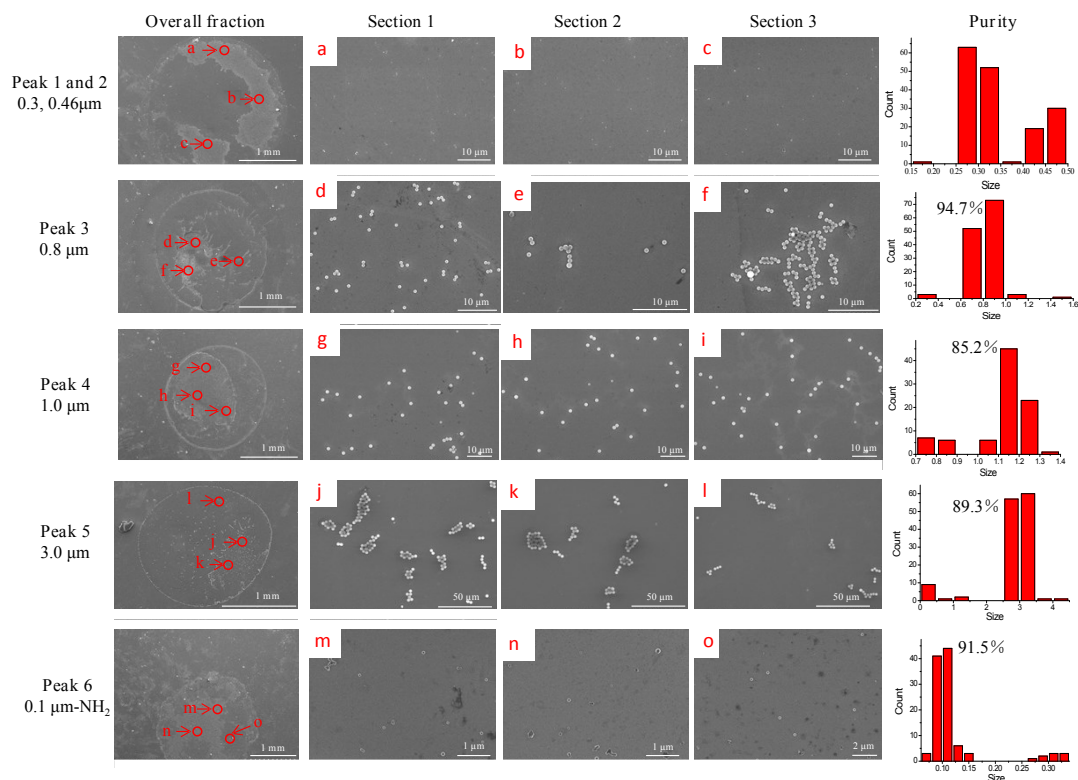
		Retention time (min)	Peak width	Peak intensity	Peak area	Recovery (%)
0.3 μm PS	Without E	1.5	0.4752	33.6	958.7	82.1
	With E	6.2	0.1229	85.9	787.0	
0.46 μm PS	Without E	1.5	0.5299	20.8	630.1	92.0
	With E	6.3	0.0665	145.3	579.4	
0.8 μm PS	Without E	1.5	0.6947	9.6	399.0	82.3
	With E	10.3	0.2213	24.0	328.3	
1.0 μm PS	Without E	1.5	0.1495	21.3	225.4	78.0
	With E	14.8	0.1346	17.2	178.0	
3.0 μm PS	Without E	1.5	0.0996	19.8	141.8	83.0
	With E	15.1	0.1265	12.4	117.7	
0.1 μm PS-NH₂	Without E	1.5	0.3327	9.3	185.3	79.2
	With E	16.1	0.1195	16.6	146.8	

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214 **Figure S-7.** Five fractions were obtained under separation mode II. The peak numbers correspond
 215 to the elution sequence in separation mode II. Each fraction was detected by SEM and more than
 216 eight different positions were observed. The three representative positions were represented in red
 217 circles. The total numbers of particles were analyzed by using the software ImageJ. Each fraction
 218 showed more than 100 particles to provide a good representativeness of the separation results. The
 219 purity of the OTR-CyEIFFF system was analyzed based on statistical approach, and the purities
 220 were in the range of 85.2-94.7% except for peak 1 and 2.

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222 **Part F: Supplementary videos**

223 This section contains detailed information on all the supplementary videos. The size of the
224 particles in these videos was larger than 2 μm in order to observe the particle movement clearly.
225 To simulate the OTR-CyEIFFF system, different electrical parameters were applied. The 2.0 μm
226 and 3.0 μm FITC PS were used under separation mode I in Video S-1. As only one side of the
227 electrodes play a part in separation mode I, Video S-1 only focused on one side of the electrodes.
228 From the video, it is obvious that particles with different sizes migrated in different speeds and
229 trajectories, which was finally separated. Video S-2 showed the separation of 3.0 μm and 5.0 μm
230 FITC PS under separation mode II. A higher migration speed of 3.0 μm particles than the 5.0 μm
231 FITC PS was observed from the video. All the particles in these videos were observed under
232 dynamic flow of the mobile phase.

233 **Video S-1.** The separation of 2.0 μm and 3.0 μm FITC PS under separation mode I. The electrical
234 parameters were $U=3.0\text{ Vpp}$, $f=4.0\text{ Hz}$ and $dc=75\%$. Mobile phase was DI water, particles were
235 suspended in DI water and the constant dynamic flow of the mobile phase at 1.0 mL h^{-1} was
236 regulated by a peristaltic pump.

237 **Video S-2.** The trajectories of 3.0 μm and 5.0 μm FITC PS under separation mode II. The
238 electrical parameters were $U=5.0\text{ Vpp}$, $f=2.0\text{ Hz}$ and $dc=50\%$. Mobile phase was DI water,
239 particles were suspended in DI water and the constant dynamic flow of the mobile phase at 1.0 mL
240 h^{-1} was regulated by a peristaltic pump.

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243 **Part G: Repeatability assessment**

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Table S-2. Repeatability of intra-annular channel

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		Parallel-1	Parallel-2	Parallel-3	Parallel-4	Parallel-5	Average	RSD (%)
Retention time	Without E	3.72	3.69	3.75	3.73	3.7	3.72	0.6
(min)	With E	6.28	6.35	6.41	6.32	6.29	6.33	0.8

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Table S-3. Repeatability of inter-annular channel

	Column 1	Column 2	Column 3	Column 4	Column 5	Average	RSD (%)
Retention time	6.5	5.93	6.16	6.89	6.19	6.33	5.9
(min)							

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