

1 **Supplementary Information**

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3 **Fabrication of glass microchannels by xurography for electrophoresis**  
4 **applications**

5 Evandro Piccin,<sup>\*a</sup> Phillipe Pessoa de Santana,<sup>a</sup> Thiago Pinotti Segatto,<sup>b</sup> Emanuel Carrilho,<sup>b</sup> Renato Sousa  
6 Lima,<sup>b</sup> Nicolò Dossi,<sup>c</sup> Marcos Yassuo Kamogawa<sup>d</sup>, Angelo Luiz Gobbi<sup>e</sup> and Maria Helena Piazzeta<sup>e</sup>

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8 <sup>a</sup>*Departamento de Química, Instituto de Ciências Exatas, Universidade Federal de Minas Gerais, Belo*  
9 *Horizonte, MG, Brazil.*

10 <sup>b</sup>*Instituto de Química de São Carlos, Universidade de São Paulo, São Carlos, SP, Brazil.*

11 <sup>c</sup>*Department of Food Science, University of Udine, Udine, Italy.*

12 <sup>d</sup>*Escola Superior de Agricultura Luiz de Queiroz, Departamento de Química, Universidade de São Paulo,*  
13 *Piracicaba, SP, Brazil.*

14 <sup>e</sup>*Laboratório Nacional de Nanotecnologia, Centro Nacional de Pesquisa em Energia e Materiais,*  
15 *Campinas, SP, Brasil.*

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17 \*Corresponding author

18 Prof. Dr. Evandro Piccin

19 Universidade Federal de Minas Gerais - UFMG

20 Departamento de Química - DQ/ICEx

21 Avenida Antonio Carlos, 6627, Pampulha,

22 Belo Horizonte, MG, Brazil, CEP 31270-901

23 E-mail: [evandrop@ufmg.br](mailto:evandrop@ufmg.br)

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## 27 **1. Experimental**

### 28 *1.1. Materials and chemicals*

29 All the chemicals used were of analytical reagent grade quality and were employed as  
30 received. Sodium, lithium, and potassium chloride salts, fluorescein (FL), fluorescein  
31 isothiocyanate (FITC), sodium hydroxide, 2-(*N*-morpholino)ethanesulfonic acid (MES), L-  
32 histidine (His), and sodium tetraborate were purchased from Sigma-Aldrich (St. Louis, MO,  
33 USA). Hydrofluoric acid (HF) and Sylgard 184 silicone elastomer kit were obtained from Vetec  
34 (Rio de Janeiro, Brazil) and Dow Corning (Midland, MI, USA), respectively. Electrophoresis  
35 buffer and stock solutions (10.0 mmol L<sup>-1</sup>) of sodium, lithium, and potassium chloride were  
36 prepared in ultrapure water purified with a Milli-Q system (Millipore, Milford, MA). Standard  
37 solutions on desired concentrations were prepared daily by diluting appropriate volumes of the  
38 stock solutions in the electrophoresis buffer.

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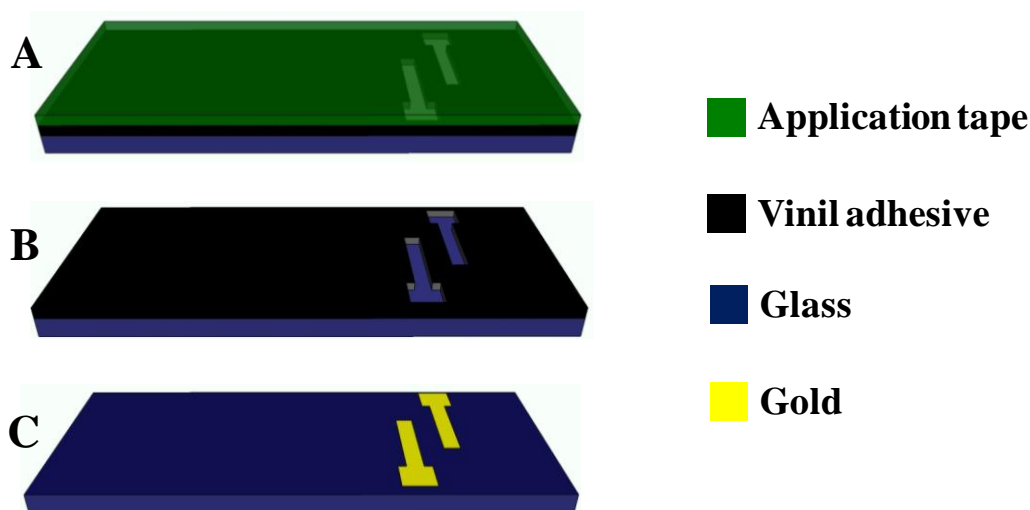
### 40 *1.2. Fabrication*

#### 41 *1.2.1. C<sup>4</sup>D electrodes*

42 Gold planar electrodes for C<sup>4</sup>D were also constructed using masks made by xurography.  
43 Fig. S1 schematically illustrates some steps of the C<sup>4</sup>D electrodes fabrication procedure.  
44 Similarly to the microchannels fabrication procedure, the C<sup>4</sup>D electrodes layout was drawn using  
45 a graphic editor and transferred to vinyl adhesives using a cutting plotter. The negative mask  
46 obtained after removing the undesired portions from the release liner was covered by an  
47 application tape and transferred to the surface of a borosilicate glass slide (45 × 100 × 2.0 mm)  
48 (Fig. S1-A). The application tape was removed and the surface of the glass slide containing the  
49 vinyl adhesive attached was sputtered with a 20 nm adhesion layer of titanium followed by 150

50 nm of gold (Fig. S1-B). Finally, the vinyl adhesive was removed, leaving the planar C<sup>4</sup>D  
51 electrodes fabricated on the glass surface (Fig. S1-C).

52



54 **Fig. S1.** Schematic illustration of the xurographic fabrication of planar electrodes for C<sup>4</sup>D. A,  
55 vinyl adhesive with electrodes layout transferred to the surface of the glass slide using an  
56 application tape. B, removal of application tape, leaving adhesive mask attached to the glass  
57 surfaces. C, gold electrodes for C<sup>4</sup>D produced by sputtering.

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### 59 1.2.2. Sealing

60 The sealing process followed the method described in detail by Segato *et al.*<sup>1</sup> Access  
61 holes were drilled on the glass slide containing the microchannels in order to create reservoir  
62 ports. We used a drilling machine (Dremel 395JU, Boch Corporation, Gerlingen, Germany) and  
63 a 1-mm diamond drill bit. For the microfluidic devices utilized in microchip-C<sup>4</sup>D experiments,  
64 the glass slide containing the C<sup>4</sup>D electrodes was spin-coated (3000 rpm for 10 s) with a 50- $\mu$ m  
65 thick PDMS layer. PDMS was prepared with a 10:1 mixture of Sylgard 184 prepolymer and  
66 curing agent, and degassed under vacuum. Before sealing, the PDMS layer was cured for 5 min

67 at 100 °C in a hot plate and oxidized for 1 min in oxygen plasma cleaner (PLAB SE80, Plasma  
68 Technology, Wrington, England) together with the glass slide containing the microchannels.  
69 Immediately after removing from the plasma cleaner, the substrates were aligned by means the  
70 separation channel and the C<sup>4</sup>D electrodes, and brought into contact to form an irreversible seal.  
71 For the microfluidic devices utilized in microchip-LIF experiments, the same sealing procedure  
72 was performed using a similar borosilicate glass slide (45 × 100 × 2.0 mm) but without the  
73 incorporation of any electrodes.

74

### 75 *1.3. Electrophoresis procedure*

76 Microfluidic devices utilized for electrophoresis experiments were constructed with a  
77 simple cross-channel layout. The separation channel was 50 mm in length, while the injection  
78 channel was 15 mm long. The intersection of the separation and injection channel was located 5  
79 mm from the buffer reservoir, yielding a separation channel with an effective length of 45 mm.  
80 The channels fabricated by xurography had a maximum depth of 20 μm and widths of 140 and  
81 220 μm at bottom and top, respectively. Pipette tips were cut and fixed in each drilled access  
82 holes of the glass microchip to serve as reservoir (100 μL). Platinum wires were placed into each  
83 reservoir and were used as electrical contacts for the high-voltage power supply CZE 1000R  
84 (Spellman, Hauppauge, NY) controlled by a computer equipped with a National Instrument (NI)  
85 interface model USB-6009.

86 Prior to use, the microchannels were rinsed with 1.0 M and 0.10 M sodium hydroxide for  
87 20 min each and then with ultrapure water for 5 min. The electrophoresis running buffers were  
88 20 mmol L<sup>-1</sup> MES/ 20 mmol L<sup>-1</sup> His solution at pH 6.1 (for electrophoresis-C<sup>4</sup>D experiments)  
89 and 25 mmol L<sup>-1</sup> borate solution at pH 9.0 (for electrophoresis-LIF experiments). Buffer and

90 sample reservoirs in the microchip were filled with the electrophoresis buffer and the standard  
91 solutions dissolved in the corresponding buffer, respectively. Electrokinetic injections were  
92 accomplished by applying a high voltage potential to the injection reservoir for 10 s with the  
93 sample waste reservoir grounded, and all other reservoir floating. Separation was carried out by  
94 applying a high voltage potential to the running buffer reservoir while the detection reservoir was  
95 grounded; all other reservoirs floating. All injection and separation parameters were set using a  
96 program written in LabVIEW (National Instruments).

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#### 98 *1.4. C<sup>4</sup>D*

99 C<sup>4</sup>D measurements were performed using a home-made system described previously.<sup>1,2</sup>

100 A function generator (CFG-250, Tektronix, Plano, TX) was used to provide a sinusoidal  
101 excitation signal of 6.0 V<sub>pp</sub> (peak-to-peak) to the detector cell. An optimized excitation  
102 frequency of 90 kHz was used for all experiments. The detection cell consisted of two electrodes  
103 (1.0 mm width × 16 mm length) positioned in an antiparallel configuration and separated by a  
104 1.0 mm gap. Data acquisition was performed in a program written in LabVIEW and the  
105 electropherograms were recorded using a time resolution of 50 ms. All measurements were  
106 carried out in a Faraday cage in order to reduce electrical noise pickups.

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#### 108 *1.5. LIF*

109 LIF detection was performed using a confocal compact system (IS Biotech, Porto Alegre,  
110 RS, Brazil) equipped with a 488 nm argon ion laser beam (1-50 mW output power)  
111 (LaserPhysics, Salt Lake City, UT, USA). The resulting fluorescence (selected by a dichroic

112 mirror 488/520 nm) was focused on a photomultiplier (Hamamatsu, Japan) and the signal sent to  
113 a NI interface and monitored in real time using a program written in LabVIEW.

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### 115 *1.6. Measurements of electroosmotic flow (EOF)*

116 EOF was measured by the current monitoring method.<sup>3</sup> This was done using phosphate  
117 buffers in pH 7. Briefly, the inlet/outlet reservoirs and the fluidic channel (20  $\mu\text{m}$  deep  $\times$  220  
118  $\mu\text{m}$  wide (top)  $\times$  50 mm length) were filled with a 20 mmol L<sup>-1</sup> phosphate buffer, and the  
119 contents of the inlet reservoir were then replaced with a buffer of reduced ionic strength (10  
120 mmol L<sup>-1</sup> phosphate buffer). The current in the channel was monitored after an electric field of  
121 250 V cm<sup>-1</sup> was applied to the channel. The current decreased gradually until it reached a  
122 constant level when the contents of the entire channel were replaced with the lower ionic  
123 strength buffer. The time required for the current to reach a constant level was recorded, and the  
124 EOF velocity ( $v_{\text{EOF}}$ ) was calculated by dividing the channel length by the buffer replacement  
125 time. The mobility of the EOF ( $\mu_{\text{EOF}}$ ) was then calculated dividing the  $v_{\text{EOF}}$  by the applied  
126 electric field,  $\mu_{\text{EOF}} = v_{\text{EOF}}/E$ .<sup>4</sup>

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