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# Microfluidic free-flow electrophoresis chips with an integrated fluorescent sensor layer for real time pH imaging in isoelectric focusing†

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## Supplementary Information

### 10 Chemicals and materials

OEG-DA (MW ~258), OEG-DA (MW ~575) (termed polyethyleneglycol diacrylate by Sigma), 2,2-dimethoxy-2-phenylacetophenone (DMPA), (3-methacryloyloxypropyl) trichlorosilane (TPM), trichloromethane, acetone, PEG (20) sorbitan monolaurate (Tween 20), ampholyte pH 3-10 (40%), sodium bicarbonate, fluorescent IEF marker mix for CE and gel electrophoresis, Sephadex G 25, phosphate buffered saline (PBS), bovine serum albumin (BSA), conalbumin (type 1: 15 from chicken egg white), and (hydroxypropyl)methyl cellulose (HPMC) were purchased from Sigma-Aldrich (Steinheim, Germany). Boric acid, orthophosphoric acid, and acetic acid were acquired from Merck (Darmstadt, Germany) and ethanol, methanol, n-heptane, and sulphoric acid from Roth (Karlsruhe, Germany). 2,7-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein-dextran conjugate (BCECF-dextran) was purchased from Life Technologies GmbH (Darmstadt, Germany) and sodium hydroxide from Reinhaus-Chemie (Essen, Germany). Chromeo P503 was obtained from Universität 20 Regensburg, Germany. Britton-Robinson buffer (BRB) consisted of boric acid, orthophosphoric acid, and acetic acid, each 0.02 M. The pH values were adjusted by titration with 1 mM NaOH. Bicarbonate buffer (BCB) was made of 50 mM sodium bicarbonate in water (pH 9).

### Microchip fabrication

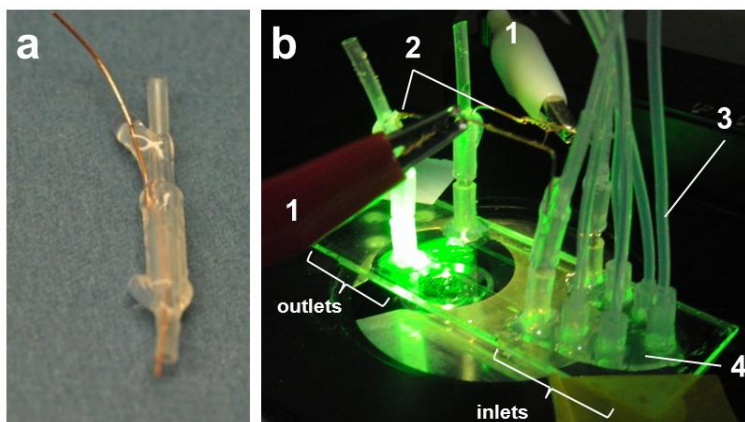
25 To create the lids, holes were powder blasted into microscope glass slides (Menzel, Braunschweig, Germany) at the inlet and outlet positions. Glass surfaces were covalently modified with TPM using a protocol after Revzin et al.<sup>1</sup> and Brzoska et al.<sup>2</sup> Clean microscope glass slides were dried and treated for 2 min. in a 5 mM solution of TPM in n-heptane and trichloromethane (3:1, w/w). The slides were then rinsed with n-hexane and water. The success of the surface modification was verified by contact angle measurements of water droplets on dry glass slides.

30 For the first polymerization step, PEG hydrogel precursor consisting of 60 % (w/w) OEG-DA (MW: 575), 38 % (w/w) water, 1 % (w/w) DMPA as photoinitiator and 1 % (w/w) BCECF-dextran was prepared. 60 µL of this mixture were evenly spread on a TPM-coated glass slide. A glass slide (not treated with TPM) was used as lid, and carefully lowered onto the prepolymer solution avoiding bubble formation. The assembly was placed in an MJB4 laboratory mask aligner (SÜSS MicroTec AG, Munich, Germany) equipped with a mercury arc lamp (15 mW\*cm<sup>-2</sup> at 365 nm) and was illuminated for 35 1.3 s. The polymer was bonded to the TPM treated surface and untreated slides were removed. In a second step, 80 µL of a prepolymer mixture containing 99 % (w/w) OEG-DA (MW: 258) and 1% (w/w) DMPA were distributed onto the cured

polymer and a TPM-treated lid with powder blasted holes was carefully put on. Subsequently, a light-impermeable mask (offset print, 3600 DPI, DTP-System-Studio, Leipzig, Germany) was aligned onto the lid. The chip was placed in the mask aligner and illuminated for 1.5 s. The uncured prepolymer resin was removed by reduced pressure and the chip was carefully flushed with water. Structural imperfections e.g. in the partitioning walls that occurred occasionally could be removed by introducing prepolymer mixture of 99 % PEG-DA (MW: 258) and 1 % DMPA. Desired areas were illuminated for one second with UV light through a small aperture and a 20x objective on an IX70 microscope to create small PEG-DA plugs and repair the imperfections.

### Microfluidic connections

For contact of the microfluidic chip to syringe pumps, silicone tubes Versilic (inner diameter 2 mm, Roth, Karlsruhe, Germany) were attached on to powder blasted holes of the lids with Elastosil E43 (Wacker Chemie, München, Germany). The flow in the separation area was generated by neMESYS syringe pumps (cetoni, Korbussen, Germany). For the analyte and ampholyte flow 1 mL glass syringes by ILS (Stuetzerbach, Germany) were used, for the anodic and cationic support 2.5 mL glass syringes. The flow in the electrode channels was generated by a PHD 22/2000 (Harvard Apparatus, March-Hugstetten, Germany) syringe pump using 5 mL Omnifix syringes (B. Braun, Melsungen, Germany). The pumps were connected with polytetrafluoroethylene (PTFE) tubes (1.58 mm outer diameter \* 0.5 mm inner diameter, Supelco, Bellefonte, PA, USA), which could be connected to the silicone tubing via a short tube as adapter. Electric contact was established by external electrodes, as described in Suppl. Ref. 3. In brief, a copper wire was pinched into a tube and fixed with Elastosil E43. A short piece of silicone tube, used as a sleeve, was pulled over the pinching hole and also fixed with silicone glue. This leads to an electrical contact from outside of the tube to the inside where a solution can be pumped through (Suppl. Fig. 1). An electric field was applied by a HCL 35-6500 power supply (FuG Elektronik, Rosenheim, Germany).



Suppl. Fig. 1: (a) External electrodes: copper wire pinched into tube and fixed with silicone glue. (b) Experimental setup:  $\mu$ FFIEF chip in chip holder on microscopic stage. Connection to electrical power supply (1), external electrodes (2), tubes containing analyte and support solutions (3) and fluidic connectors (4).

### Microscopic observation

Experiments were monitored on an IX71 or IX70 fluorescence microscope (Olympus, Hamburg, Germany) using a fluorescein filter set ( $\lambda_{\text{ex}}$ : 460–490 nm, dichroic mirror: 500 nm,  $\lambda_{\text{em}}$ : 520 nm, Olympus), a UV filter set ( $\lambda_{\text{ex}}$ : 330–385 nm, dichroic mirror: 400 nm,  $\lambda_{\text{em}}$ : 420 nm, Olympus), and a Cy3 filter set ( $\lambda_{\text{ex}}$ : 510–550 nm, dichroic mirror: 570 nm Olympus,

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$\lambda_{em}$ : > 590 nm (Edge basic 594 LP, Semrock, from AHF Analysentechnik, Tübingen, Germany). For microscopic observation a Nikon D90 CCD camera was used, the integration time was set to 2 s. Leaching, bleaching, and response time experiments were observed with a photomultiplier tube (H9306-04, Hamamatsu, Hamamatsu City, Japan) connected to the microscope with an applied acceleration voltage of 500 V.

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### Sensor layer characterization

For bleaching experiments a chip was filled with 20 mM NaOH and constantly illuminated with a 2x objective (2x Plan N, NA = 0.06, Olympus). To record possible leaching of the pH probe, all fluidic contacts, except for one inlet and one outlet, were sealed and the chip was connected to a Harvard syringe pump filled with 20 mM NaOH. The applied flow rate of 50  $\mu\text{L}\cdot\text{min}^{-1}$  corresponded to a linear velocity of  $4.5\text{ mm}\cdot\text{s}^{-1}$ . For leaching experiments the chips was illuminated for only short time periods to avoid bleaching. For pH calibration Britton-Robinson buffers, of the same conductivity ( $\chi = 690\text{ V}\cdot\text{cm}^{-1}$ ) as used in IEF experiments were used and obtained via dilution with ddH<sub>2</sub>O. 5 min. after the chips were filled with buffer, measurements of the fluorescence intensity was started. The microchips were dried subsequently.

### 15 Protein labelling

For protein separation BSA and conalbumin were labeled with Chromeo P503. 5  $\mu\text{L}$  of a  $10\text{ mg}\cdot\text{mL}^{-1}$  solution of P503 were added to 3 mg protein in 1 mL bicarbonate buffer 50 mM, pH 9. After 30 min the conjugates were separated on a Sephadex G 25 medium column using PBS (150 mM, pH 7.4) as eluent.

### 20 Free flow isoelectric focusing

In  $\mu\text{FFIEF}$  analytes are continuously separated via a constant hydrodynamic flow induced through syringe pumps and an orthogonally applied electric field. The displayed images are representative snapshots of these separations. Sample solutions were injected via the centre inlet and focused by flanked ampholyte flows, and anodic and cathodic support streams. Anodic support and cathodic support and the electrode solutions consisted of 20 mM NaOH or 20 mM H<sub>2</sub>SO<sub>4</sub> respectively. A mixture of 2 % ampholyte pH 3-10 with 0.1% Tween 20 in ddH<sub>2</sub>O was used for IEF. Prior to connection of the syringe pumps, the microchip was completely filled with ampholyte solution. Line scan evaluations and profile plots were obtained using ImageJ software (version 1.46r, US National Institutes of Health, Bethesda, MD, USA).

For pI marker separation, linear velocity over the separation area was  $0.8\text{ mm}\cdot\text{s}^{-1}$  and the applied potential was 300 V (550  $\mu\text{A}$ ). For protein separation, voltage was increased to 400 V (1.2 mA), which lead to higher gas bubble formation. Flow rates of support streams as well as electrode streams were increased to a linear velocity of  $4.6\text{ mm}\cdot\text{s}^{-1}$  for better bubble removal. During protein FFIEF separation there was unsteadiness in the analyte flow caused by the higher applied current leading to errors when recording the sensor layer channel and the analyte channels consecutively with a delay of 2 - 3 s. Therefore sensor layer images were recorded with a color CCD and the green and red channel were separated digitally. The green channel was used for sensor layer evaluation and the red channel for detection of analyte position. An analyte image

was recorded afterwards and the shift of electrophoretic separation was calculated. In the results presented here a shift of 169  $\mu\text{m}$  to the right was detected by comparison of green and red channels.

s Supplementary references:

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