Supporting Information

METHOD

Device fabrication. We used ~150 µm thick Corning glass wafers as device substrate. Active electrode arrays were fabricated by first defining the electrode shape on the wafer surface via image reversal photolithography using PR5214 photoresist. Then, a ~200 nm thick indium-tin oxide (ITO) film was sputtered onto the photoresist structures, and the desired electrode patterns were formed by removing the sacrificial photoresist via a lift-off process. To complete the device structure a ~0.8 µm thick dielectric film of Parylene C was coated over the entire device surface by chemical vapour deposition. The bonding pads for external wire connections and the "solution biasing electrode" were exposed by scratching away the Parylene C film with a razor blade.

Protein labelling and fluorescence microscopy. BSA **(**albumin from bovine serum, Sigma) was labelled with a succinimidyl ester of tetramethylrhodamine (C-1171, Molecular Probes) at pH 8.3 in 0.1 M sodium bicarbonate buffer using a 12.5-fold stoichiometric excess of dye molecules. Free dye molecules were removed by repeated dialysis at pH 6.8 in BRB80 buffer. The final dye to protein ratio was found to be ~0.97, which was determined spectrophotometrically (UV-1601, Shimadzu) from the molar extinction coefficients of BSA and tetramethylrhodamine (TMR). An inverted fluorescence microscope (Axiovert 200, Zeiss) with a 40× (Plan Neofluar, Zeiss) or 100× (Achroplan, Zeiss) oil immersion objective and a 100 W mercury arc lamp was used for

fluorescence microscopy. Fluorescence signals were recorded using a digital CCD camera (ORCA ER II, Hamamatsu) and quantified off-line using Image J.

Protein surface density calibration. To relate the recorded fluorescence intensities to actual protein surface densities we calibrated the microscope system by recording the quantized photobleaching behavior of single dye molecules. A diluted TMR-BSA solution (~0.38 µg/ml) was coated onto a Parylene C surface and photobleached for ~250 seconds until step-wise decreases in the intensity of individual, diffraction-limited spots were observed. These step-wise intensity drops represented the intensity emitted from a single rhodamine dye molecule (3.3 ± 0.4 AU/dye). After taking into account the microscope and camera settings (e.g. numerical aperture, exposure time, pixel size, and excitation light intensity) we estimated that 1 AU of fluorescence corresponds to a surface density of ~3.1 ± 0.4 proteins/μm^2.

In addition, ITO has a slightly reduced transmission in the visible wavelength range (91.10% for the green (546 nm) fluorescence excitation light and 94.13% for the orange-red (580-650 nm) of the emitted fluorescence from TMR-labelled BSA) that should be considered when interpreting fluorescence images from the patterning devices. Thus, the ITO electrode layer effectively reduces the fluorescence signal of TMR to ~86% as compared to regions without ITO and appropriate corrections were applied to the intensity profiles and other quantitative data.

Electrowetting measurement. A sessile drop experiment setup was used to characterize the hydrophobicity of the device surface. We placed 10 µl de-ionized water droplets on electrode surfaces with composition identical to the actual device surface (~0.8 µm

Parylene C film on top of 200 nm thick ITO layer). The contact angle was measured from the shape of the droplet (captured via CCD camera through a microscope) at each bias voltage applied across the droplet and the electrode surface.

FIGURES

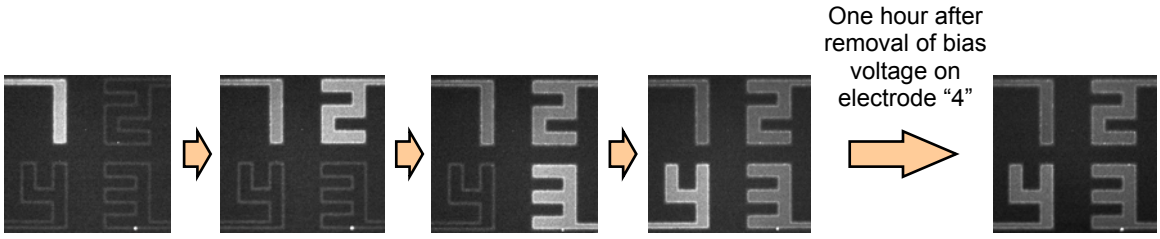

Figure S1. An alternative way to sequentially pattern proteins. Bias voltages are applied only during the protein adsorption onto the electrodes. After forming a monolayer of Pluronic copolymers on the entire device surface, patterning was carried out as follow: electrode “1” was turned on with 60 V bias and exposed to TMR-BSA for 3 minutes to allow proteins to be adsorbed onto the pattern. The bias to the patterning electrode was then turned off and unbound proteins were washed away with 1 ml buffer. This procedure was then repeated for electrodes “2” through “4”. Unlike the sequential patterning procedure presented in Fig. 3, the bias on each pattern was turned off before unbound proteins were washed away and before activating the next pattern. To reduce the possible photobleaching effect on fluorescence intensity measurements, data were taken from equivalent patterns from device regions that have not been directly exposed by the excitation light. Following the four consecutive patterning steps, the fluorescence signals (fluorescence intensity above the background) emitted from the protein patterns compared to their initial signal values declined to 75.9% ± 11.8%, 84.1% ± 13.7%, and 90.6% ± 8.6% on electrodes “1”, “2”, and “3”, respectively. This reduction of the protein surface density is consistent with the observations reported in Figure S2 and indicates the dissociation of a fraction of reversibly (weakly) bound BSA proteins on the patterns, which were washed away during successive patterning steps. As shown above, one hour after the last image in the patterning sequence was taken, the protein patterns remained distinguishable, as indicated by the fluorescence signals retained on the electrodes: 73.0% ± 26.5% on electrode “1”, 80.9% ± 29.5% on electrode “2”, 87.2% ± 29.7% on electrode “3”, and 96.2% ± 31.5% on electrode “4”. Note, about 5 minutes were required for each patterning step (3 minutes wait for protein adsorption, ~1 minute for loading and washing the chamber, and ~1 minute for observation); therefore, about 20 minutes have elapsed between the patterning of electrode “1” and “4”.

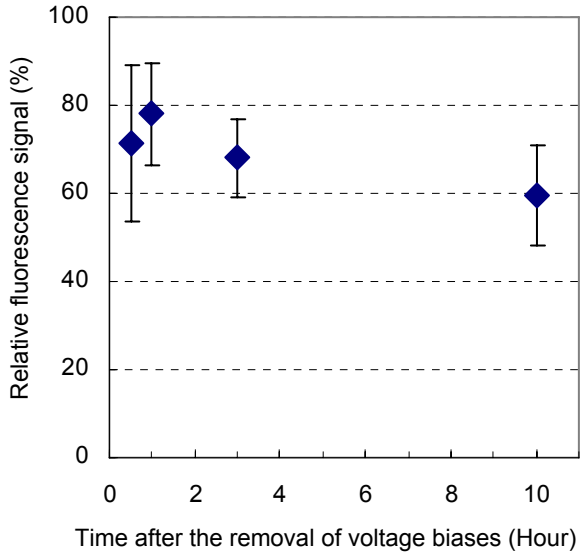

Figure S2. After the removal of control bias voltages, the protein patterns remained intact. Fluorescence signals (fluorescence intensity above the background) emitted from the protein patterns were normalized to the average initial fluorescence signals at time 0 (right after the voltage biases are removed). Each data point (mean ± standard deviation) was obtained from a different device and was averaged over six patterns on the device. After the electrical bias was turned off, the device chamber was sealed with wax to prevent evaporation of the solution from the chamber. While the device chambers were kept in the dark to reduce photobleaching, some limited exposure to scattered light during device mounting and focusing on neighboring patterns can not be ruled out completely. Thus, the data represent a lower limit of the bound proteins. Initially**,** the fluorescence signal dropped rapidly from its reference value but appeared to stabilize at about 60% after several hours. These measurements suggest that a portion of weakly bound BSA molecules are released into solution, leaving irreversibly bound BSA on the device surface. The data show that fluorescence signals from protein patterns are distinguishable even after 10 hours and that the protein patterns could possibly be used even after electrical connections were removed.

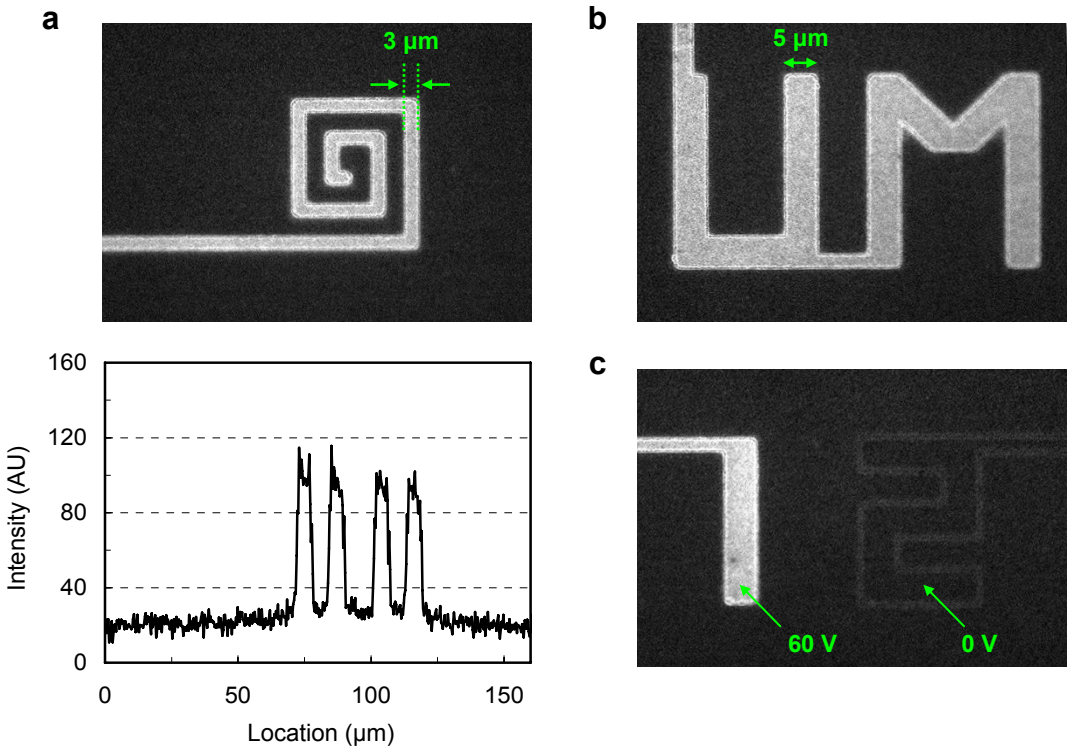

Figure S3. Voltage-controlled protein patterning of casein. To test the versatility of our technique, we conducted comparative patterning experiments with fluorescently-labeled casein (TMR) protein molecules following the methodology established for BSA protein molecules and characterized the resulting protein patterns with fluorescence microscopy. The same procedure used for pattering BSA shown in Fig. 2 was repeated here to produce casein protein patterns shown. In general, the patterned casein protein molecules form the same high resolution patterns, such as swirl and "UM" patterns as shown in (a) and (b). To demonstrate programmability shown in (c), pattern "1" was activated with a 60 V bias while the pattern "2" was inactivated with a 0 V bias. The detail of the patterning procedure is as follow: all device surfaces were first pre-coated with Pluronic copolymers by immersing the surface in 2.5 mg/ml Pluronic solution for 10 minutes. To pattern proteins, a 60 V bias was applied to a selected patterning electrode and 300 µl of ~1 µg/ml casein in 1 mM K-PIPES buffer (pH 6.8) was introduced into the microfluidics chamber. Casein was allowed to be adsorbed onto the surface for 3 minutes before unbound proteins are washed away with 2 ml of 1 mM K-PIPES buffer (pH 6.8). The ability to pattern casein similar to BSA supports the general potential of this technique for protein patterning.