Electronic Supplementary Material (ESI) for Nanoscale Advances.


# Supporting information for the paper Universal control of protons concentration using electrochemically generated acid compatible with miniaturization

A brief comparison of the electrochemically generating platforms in literature was discussed in the introduction. These platforms mainly developed by Egeland et al[1] (R. D. Egeland, E. M. Southern, Nucleic Acids Res. 2005, 33, 1–7) and Maurer et al[2] (K. Maurer, J. Cooper, M. Caraballo, J. Crye, D. Suciu, A. Ghindilis, J. A. Leonetti, W. Wang, F. M. Rossi, A. G. Stö ver, C. Larson, H. Gao, K. Dill, A. McShea, PLoS One 2006, 1, 1–7.) were using Hydroquinone/benzoquinone pairs to generate the acid in situ by electrochemistry and photo-electrochemistry as well. In these cases, the HQ/Q pairs were solubilised in the medium, and not fixed to the surfaces. When the generation of protons occurs, the acid was generated in close proximity to electrodes. Regardless the quasi-reversibility of the processes, these techniques were limited by the fast diffusion of the protons and the limited range of achieved pH. Table S1 shows a comparison amongst them:

| Reference | pH control | medium | | limitations |
|---|---|---|---|---|
| **Egeland et al**[1] | Photo-electrochemistry | Hydroquinone/ benzoquinone | Boc deprotection microarrays | Limited by the diffusion And DNA synthesis due to the limited range acidity |
| **Maurer et al**[2] | electrochemistry | Hydroquinone, anthraquinone | Boc deprotection microarrays | Limited by the diffusion And DNA synthesis due to the limited range acidity |
| **Wang et al** [3] | electrochemistry | serial electrolytic cell for pH control | electrochemical reversibility of oxidation and reduction of hydrogen | Aqueous solutions, High voltages (possible degradation of electrodes) and high ph range from 7 to 12. (ΔpH=5) |
| **Frasconi et al**[4] | electrochemistry | 4-ATP | pH generation in aqueous | Complexity to create the AuNps composite matrix Δph =2 |
| **Balakrishnan et al**[5] | electrochemistry | 4-ATP | pH generation in aqueous | Large pH ranges from 7 to 1<br>Suitable for miniaturization and compatible with organic solvents. Less complexity in the choice of material and process.<br>No diffusion limitations |

## SI-1 Fabrication of the electrochemical cell chip

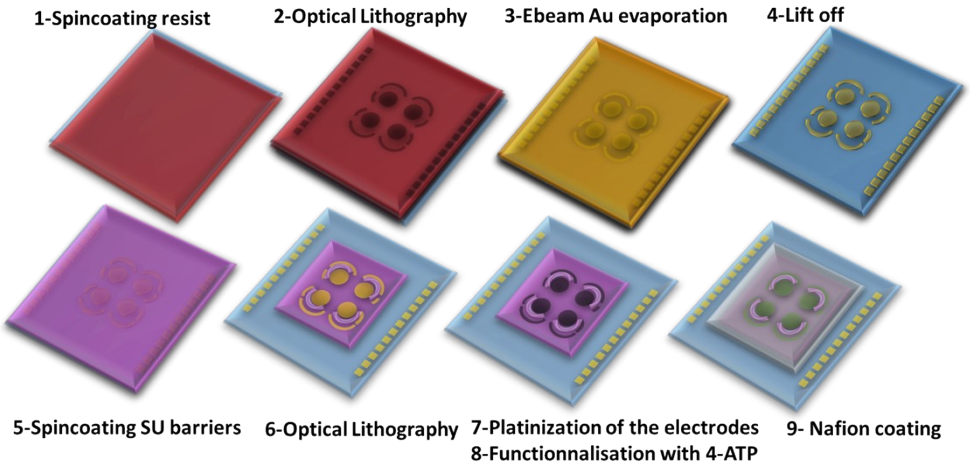


*Figure SI- 1: fabrication steps of the electrochemical Cell Chip.*

The electrochemical cell chip was fabricated on a 3*3 $cm^2$ Si Substrate with 50 nm oxide layer using maskless laser writer MLA 150 from Heidelberg for optical lithography. Two designs were made using KLayout CAD program. (1) The first design (M1) included the design of the electrodes with the contact pads. For the M1 design, the substrate was spincoated with LOR 3A (300nm) and S1813 (1.3um) photoresist. (2) Using the laser writer, the substrates were exposed with the design M1 with an exposure dose of 110 $mJ/cm^2$. Then the samples were developed in MF319 solution for 60 seconds, 10sec of DI water and the blow dried with $N_2$. (3) The photolithographed samples were placed on an e-beam evaporator PLASYS and 5 nm Chromium and 50 nm of Gold were evaporated. (4) The Lift-off process was carried out in a beaker containing 50 ml of acetone for 30 minutes and then in a beaker of RPG remover for 10 additional minutes. The photoresist S-1813 dissolves leaving the patterned electrodes. The sample was rinsed with distilled water and blow dried with nitrogen. (5) The evaporated sample was again spin coated with SU8 3010 epoxy resist and prebaked with a ramp from RT to 95 C for 1 minutes to obtain a uniform layer of 7.2 µm thickness. (6) The second design (M2) had the definition of the cell volumes with the diffusion barriers along with the opening for contact pads and electrodes. The substrate was then loaded into the laser writer for the second lithography of design M2 with an exposure dose of $1100mJ/cm^2$. The sample was then baked again from rt to 95 degrees and developed in a MICRO-CHEM SU8 solution for 1 minute 10 sec, 10 sec in IPA and dried with nitrogen.

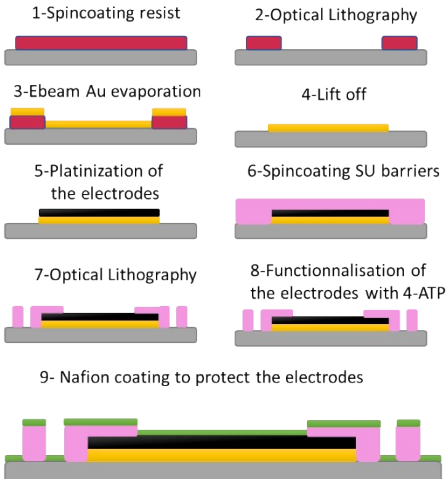


*Figure SI- 2: a cross-section view of the different layers of the devices.*

(7) the platinization of the electrodes takes place in a two beakers system connected with a KCl bridge (fig SI-2). Beaker 1 contains 40 mM of chloroplatinic acid and 100 µM lead acetate solution in distilled water, beaker 2 contains 1 M of phosphate buffer solution. A salt bridge was made with a filter paper strip that was immersed in potassium chloride solution, after removing the excess of electrolyte it was placed between the two beakers. The salt bridge was immersed in both liquids of the beakers. Using a clamp, the contact pad of the electrodes was connected to the potentiostat. The sample was immersed in Beaker 1 covering the electrode to be platinized. We used an Ag/AgCl reference electrode immersed in beaker 1. The Pt counter electrode was immersed in beaker 2. A cyclic voltammogram experiment was created using the potentiostat between the voltage range -0.2 V to 0.6 V at a scan rate of 30 mV/s for 5 cycles. the sample was washed with distilled water, ethanol and blow dried with nitrogen. (8) the chip was activated for 30 min in a UV-Ozone cleaner and immersed in a solution of 5% 4-ATP in absolute ethanol overnight. (9) Finally, the sample was spincoated with a solution of Nafion 117 and baked at 100 degrees for 30 min, then cleaned with acetone and blow dried with nitrogen. The Nafion membrane, while avoiding the possibility of cross reactions between 4ATP molecules and the carboxyfluorescein, it allows the selective transport of protons from the electrodes to the solution through its sulfonate channels.

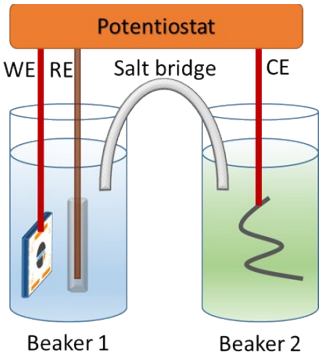


*Figure SI- 3: platinization setup.*

The optical set-up was made with an Olympus BX customized microscope, using a led source centered at 470nm (CoolLED-pE40000). The light was directed through a collimator and through a band pass filter, mounted with a dichroic mirror CROMA-49012 that has also a low pass filter at 510 nm for collection. The illuminated area was limited by a diaphragm limiting the field of view to ~ 50µm. The light was collected

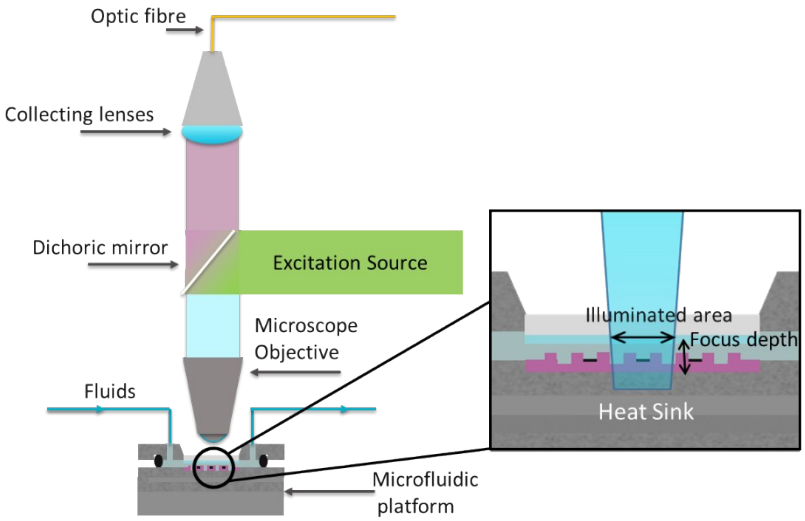


*Figure SI- 4: Schematic representation of the optical setup used to control and monitor the pH changes in the microfluidic platform.*

by a concave mirror, focusing the light from the field of view at the entrance of an optic fiber that sent the signal to a wide range spectrometer MAYA-S-VIS-NIR.

**Preparation for the fluorescence buffers**

The buffers solutions employed to solubilize FAM (0.5µM) during the experiments were (a) KCl (100mM) with a pH adjusted at 7 using KOH solution for the aqueous experiments with no tautomerization and (b) KCl (100mM) with no pH adjustment for tautomeric identification. These solutions were prepared using a MilliQ water (resistivity of 18.2 MΩ.cm at 25°C).
For the experiments in organic solvents, all the solutions used for pH monitoring, as well as acid titrations were prepared with the same ionic strength 10mM of $Bu_4PF_6$ (FAM 0.5Mm) in ACN.

**Fluorescence measurements**

Carboxyfluorescein (FAM) was used to monitor the pH changes inside the electrochemical cells. The fluorescence set-up was made with an Olympus BX customized microscope, using a led source centered at 470nm (CoolLED-pE40000), as described above. For the real time measurements, we followed the detection of FAM at obtaining the peak signal at 525nm using an integration time of 1s.

**SI-2. Calculation of Faradaic currents.**

The selection of the oxidation peak and the gaussian fitting to extract the total charge to calculate the protons concentration at each peak. Some of these concentrations are gathered in the following tables using the following equation:

Eq 1: $$pH = -\log\left[\frac{Q}{F.Vcell}\right]$$

where Q is equal to the integration of the area of the area of the $I$ vs $V$ Faradaic peak divided by the Scan rate:

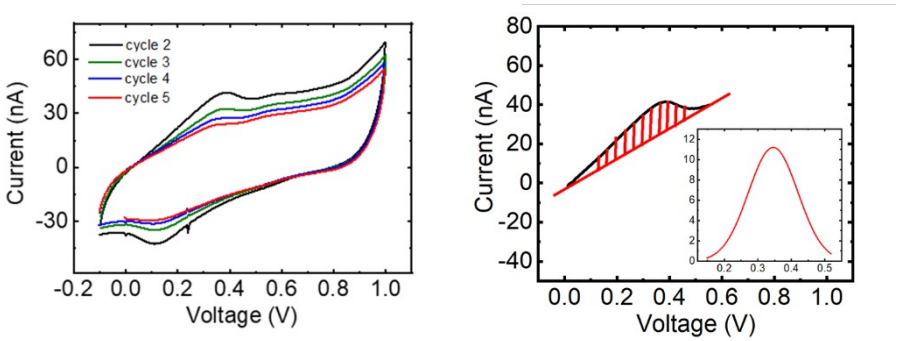


*Figure SI- 5: (a) 4 cycles of CV in ACN, (b), peak selection and gaussian fitting to extract the area of the peaks.*

$Q = \int I/SR$, $F$ is the faradaic constant=96 485, $V$ is the volume of the cell.

Figure SI-4 shows a typical CV obtained for the cycles 2 to 5 and the extraction of the Faradaic peak associated with the oxidation. The Gaussian peak in the inset was used to calculate the total charge using the method above described.

**SI-3 Monitoring acidity with FAM in aqueous solutions**

Table 1 gathers the data corresponding to the integration of the area of each of the oxidation and reduction peaks, the concentration of protons as well as the calculated pH based of the above cited formulas.

*Table 1: Calculation of the pH. proton concentrations using the peak area at every oxidation and reduction in aqueous solution.*

| Peak | (Peak area) | $[H^+]$ | pH* |
|---|---|---|---|
| **cycle 2 ox** | 8.25E-8 | 3.07E-03 | 2.51 |
| **cycle 2 red** | 9.48E-8 | 3.52E-03 | 2.45 |
| **cycle 3 ox** | 2.43E-9 | 4.03E-04 | 3.40 |
| **cycle 3 red** | 2.23E-9 | 3.70E-04 | 3.43 |
| **cycle 4 ox** | 2.51E-9 | 4.18E-04 | 3.37 |
| **cycle 4 red** | 1.85E-9 | 3.07E-04 | 3.51 |
| **cycle 5 ox** | 2.82E-9 | 4.68E-04 | 3.32 |
| **cycle 5 red** | 1.73E-9 | 2.87E-04 | 3.54 |
| **cycle 27 ox** | 3.78E-9 | 6.28E-04 | 3.20 |
| **cycle 27 red** | 4.82E-9 | 8.01E-04 | 3.09 |
| **cycle 28 ox** | 4.16E-9 | 6.90E-04 | 3.16 |
| **cycle 28 red** | 4.44E-9 | 7.38E-04 | 3.13 |
| **cycle 29 ox** | 3.67E-9 | 6.09E-04 | 3.21 |
| **cycle 29 red** | 3.92E-9 | 6.50E-04 | 3.18 |

During different experiments using different chips we observed a variety of surface functionalization in the electrodes that resulted in different dynamic ranges of pH obtained during the CV cycles. We also observed a small increase of the total area of the Faradaic peaks over the cycles and that the Q corresponding to the recovery of protons during the reduction was sometimes slightly less than the one corresponding to the proton release of the oxidation. This can be also attributed to contributions from residual electropolymerizing, which increases the slightly the number of redox molecules. This difference between the Faradaic currents at oxidation and reduction affected more the expressed equivalent pH corresponding the basic conditions than to the expression of the minimum pH achieved, which can be understood due to the logarithm dependence of the equivalent.

**SI-4 Monitoring acidity with FAM in organic solvents**

4 cycles of cyclic voltammetry are shown in fig SI-4. The selection of the oxidation peak and the gaussian fitting to extract the total charge to calculate the protons concentration at each peak. The extraction of the Faradaic currents was done using the same method above described. Table 2 reproduces some representative data of the Faradaic currents observed in these peaks.

$$pH = -\log\left[\frac{Q}{F.Vcell}\right]$$

*Table 2: Calculation of the pH, proton concentrations using the peak area at every oxidation and reduction in ACN.*

| Peak | Peak area | $[H^+]$ | pH* |
|---|---|---|---|
| cycle 2 ox | 2.38E-09 | 3.96E-04 | 3.40 |
| cycle 2 red | 3.31E-9 | 5.50E-04 | 3.26 |
| cycle 3 ox | 1.72E-9 | 2.86E-04 | 3.54 |
| cycle 3 red | 2.19E-9 | 3.64E-04 | 3.43 |
| cycle 4 ox | 1.75E-9 | 2.90E-04 | 3.53 |
| cycle 4 red | 1.70E-09 | 2.81E-04 | 3.55 |
| cycle 5 ox | 1.53E-9 | 2.55E-04 | 3.58 |
| cycle 5 red | 8.39E-10 | 1.39E-04 | 3.85 |

The behavior of the current was followed also for tenths of cycles with no significant differences in the behavior

**SI-5 Monitoring acidity with FAM in organic solvents**

After plotting the data of the calculated pH* vs the experimental normalized Fluorescence (black spots in Figure SI-5), an exponential growth fitting (shown in red) was applied to extract the equation:

$y=y_0+A1\exp((x-x_0)/t_1)$

where y is the new calculated pH for the titration using the fluorescence, $y_0$ is equal to 3.07844, A1 is equal to 4.884e-4, x, is the Fluorescence of FAM during the titration by the acid, $x_0$ is equal to 0.29149, and t1 is equal to 0.07608 leading to the equivalent of

pH=3.07844+(4.884e$^{-4}$)*exp((Fluorescence-0.29149)/ 0.07608)

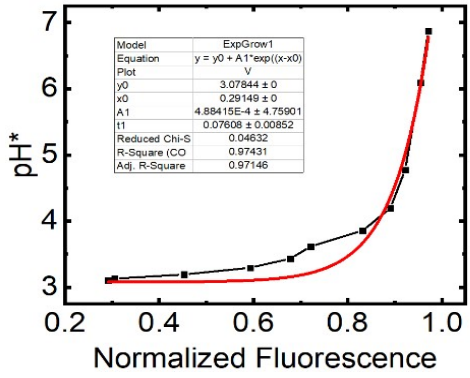


*Figure SI- 6:exponential growth of the curve ph*vs Normalized Fluorescence and the yielding equation.*

We used the exponential fitting to transform the fluorescence from the titration of the acids in the organic electrolyte (insets of figures 5 (d) and 5 (e) in the article and figure SI 7) into the proton concentration. Then we fitted the obtained values of $[H^+]^2$ vs the concentration of the titrated acids to obtain the dissociation constant using the formula described in the article. As shown in the graphs, we took the more linear part of the data free of effects of saturation.

**SI.6- Boc functionalization of glasses and characterization**

Three qualitative methods were used to characterize the glass functionalization with Aptes followed by Boc acid labile protecting groups:

First, the growth of the film was followed by contact angle measurements: 4uL of distilled water drop was deposited on the glass before and after each functionalization step. The glass completely hydrophilic before functionalization, had a contact angle of 56° after APTES layer, and became slightly hydrophobic 75° after the functionalization with the Boc-terminal amino-acid alkane.

The growth of the film was also tracked by X-ray photoelectron spectroscopy XPS by following the change of the N1s and C1s signals.

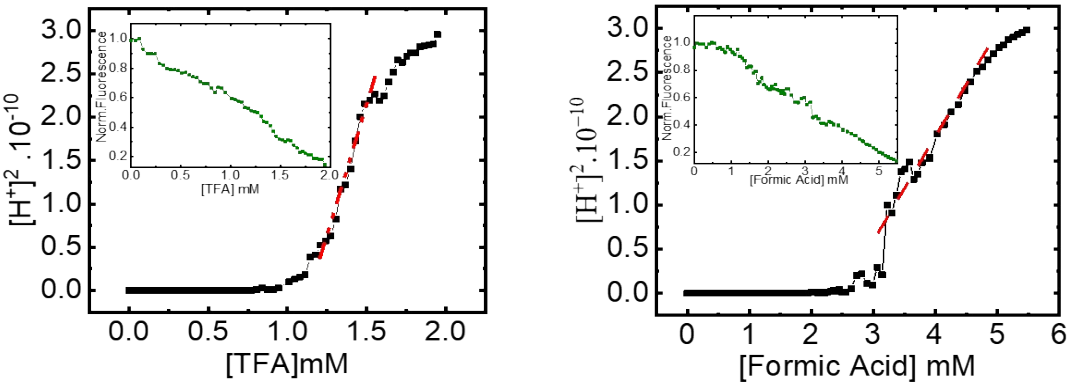


*Figure SI- 7: (a) transformation of the normalized fluorescence into $[H^+]^2$ Vs TFA acid concentration in black, the slope of the corresponding curve in dashed red inset in green the titration curve of TFA acid, Fluorescence vs the concentration, (b) transformation of the normalized fluorescence into $[H^+]^2$ Vs Formic acid concentration in black, the slope of the corresponding curve in dashed red inset in green the titration curve of Formic acid, Fluorescence vs the concentration*

| Sample | Name | Position | %At Conc | N/C |
|---|---|---|---|---|
| **Glass-** | O 1s | 532.0 | 56.0 | 0.156 |

| | | | | |
|---|---|---|---|---|
| **APTES** | N 1s | 398.8 | 1.7 | |
| | C 1s | 284.8 | 10.9 | |
| | Si 2p | 103.2 | 31.4 | |
| **Glass-BOC** | O 1s | 532.1 | 46.7 | 0.123 |
| | N 1s | 399.8 | 2.7 | |
| | C 1s | 284.6 | 21.6 | |
| | Si 2p | 103.3 | 28.1 | |
| | P 2s | 191.6 | 0.9 | |


*Figure SI- 8: water contact angle measures on glass samples (a) bare sample, (b) ATPES functionalized, (c) boc functionalized glass.*

Another test was done by dipping a bare glass as well as a boc-functionalized one in TFA 50% in DCM for an hour, washing and dipping them again in a solution of Rhodamine B isothiocyanate in DMF for an additional hour. Then these glasses were cleaned with ethanol and the fluorescence of each one was measured. The bare glasses showed no fluorescence after contact with the dye while the functionalized one has a fully coverage of rhodamine as showed in fig SI-9.

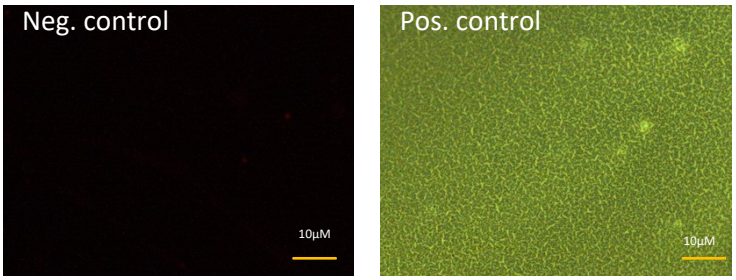


*Figure SI- 9: positive and negative control of boc protected glasses.*

References:

[1] R. D. Egeland, E. M. Southern, *Nucleic Acids Res.* **2005**, *33*, 1–7.

[2] K. Maurer, J. Cooper, M. Caraballo, J. Crye, D. Suciu, A. Ghindilis, J. A. Leonetti, W. Wang, F. M. Rossi, A. G. Stö ver, C. Larson, H. Gao, K. Dill, A. McShea, *PLoS One* **2006**, *1*.

[3] Y. C. Wang, C. Bin Lin, J. J. Su, Y. M. Ru, Q. Wu, Z. Bin Chen, B. W. Mao, Z. W. Tian, *Anal. Chem.* **2011**, *83*, 4930–4935.

[4] M. Frasconi, R. Tel-Vered, J. Elbaz, I. Willner, *J. Am. Chem. Soc.* **2010**, *132*, 2029–2036.

[5] D. Balakrishnan, G. Lamblin, J. S. Thomann, A. Van Den Berg, W. Olthuis, C. Pascual-García, *Nano Lett.* **2018**, *18*, 2807–2815.