Supplementary Material

Multiple scanning electrochemical microscopy mapping of tyrosinase in micro-contact printed fruit samples on polyvinylidene fluoride membrane

- Supporting Information -

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List of supplementary information:

SI-1. Microcontact-printed banana on PVDF membrane

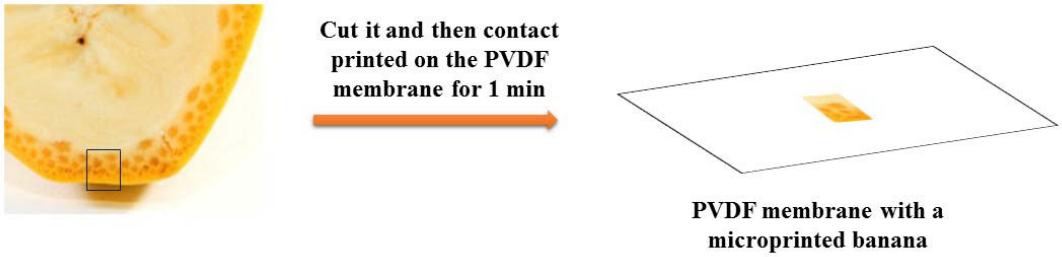

Fig. S1. Schematic representation for the preparation of microcontact-printed banana on polyvinylidene fluoride (PVDF) membranes. For more details please see the experimental part in the main manuscript.

SI-2. Protein tagging and silver staining of human fingerprints on PVDF membranes

Materials and methods

Protein inked human fingerprints were prepared by microcontact printing (μCP) as reported elsewhere [1]. Briefly, the finger of a volunteer was wetted with a BSA solution (2 g/L) and let it dry under a gently stream of nitrogen. Then the finger was pressed gently over a pre-wetted PVDF membrane for 60 s. After fingerprint application, the PVDF membrane was dried under a stream of nitrogen and subsequently labeled by silver staining or benzoquinone tagging as reported elsewhere [2]. The PVDF membrane was then washed extensively with water, dried, taped on a microscopic glass slide and finally scanned by SECM. In order to compare these two protein detection methods with the oxygen reduction method, after each SECM image was performed (*i.e.* over the benzoquinone or silver stained samples), a second consecutive image was run based on the oxygen reduction strategy. More specifically, for the benzoquinone tagged human fingerprint a solution of $K_3[Fe(CN)_6]$ 2 mM in KCl 100 mM was employed as redox mediator, and the microelectrode was biased at –0.2 V *vs* Ag-QRE. As a result, an increase on the current was observed when the redox mediator was recycled over the benzoquinone tagged fingerprints (*i.e.* thanks to the reduction of the benzoquinone adducts). As for the silver stained human fingerprint a solution of $K_3[IrCl_6]$ 2 mM in KNO_3 100 mM was employed, and the scanning microelectrode was biased at 0.8 V *vs* Ag-QRE in order to monitor the regeneration of the redox mediator over the silver nanoparticles deposited over the μCP fingerprint (*i.e.* thanks to the oxidation of the silver nanoparticles).

Results

Human fingerprints deposited on a PVDF membrane were labeled by silver staining and benzoquinone tagging, imaged by SECM and compared with the oxygen reduction method (**Fig. S2**). Details about the sample preparation can be found in the supporting information and elsewhere [1]. Briefly, the tip of a finger was covered with a protein containing ink. After drying, it was transferred onto a pre-wetted PVDF membrane by μCP. Silver staining or benzoquinone tagging protocols were applied as reported previously [2, 1]. Oxygen reduction SECM images were performed just after the respective SECM image for silver stained or benzoquinone tagged samples. **Fig. S2** shows the different SECM images obtained with each one of the employed protocols and its comparison with the oxygen reduction method. Indeed, a higher current contrast and therefore a better-defined human fingerprint is observed with the oxygen reduction method. For instance, the ridges and valleys in the human fingerprint can be recognized clearly, as well as the pores in the ridges. The latter confirms the fact that the oxygen reduction method is a very sensitive and label-free methodology for the indirect SECM detection of proteins immobilized on PVDF membranes. It is important to notice that the silver stained human fingerprint did not present a clear image due to background problems commonly encountered with low protein concentration (*e.g.* low protein blotting efficiency), over staining or sample contamination. Still the oxygen reduction image present a clearer and more defined human fingerprint SECM image.

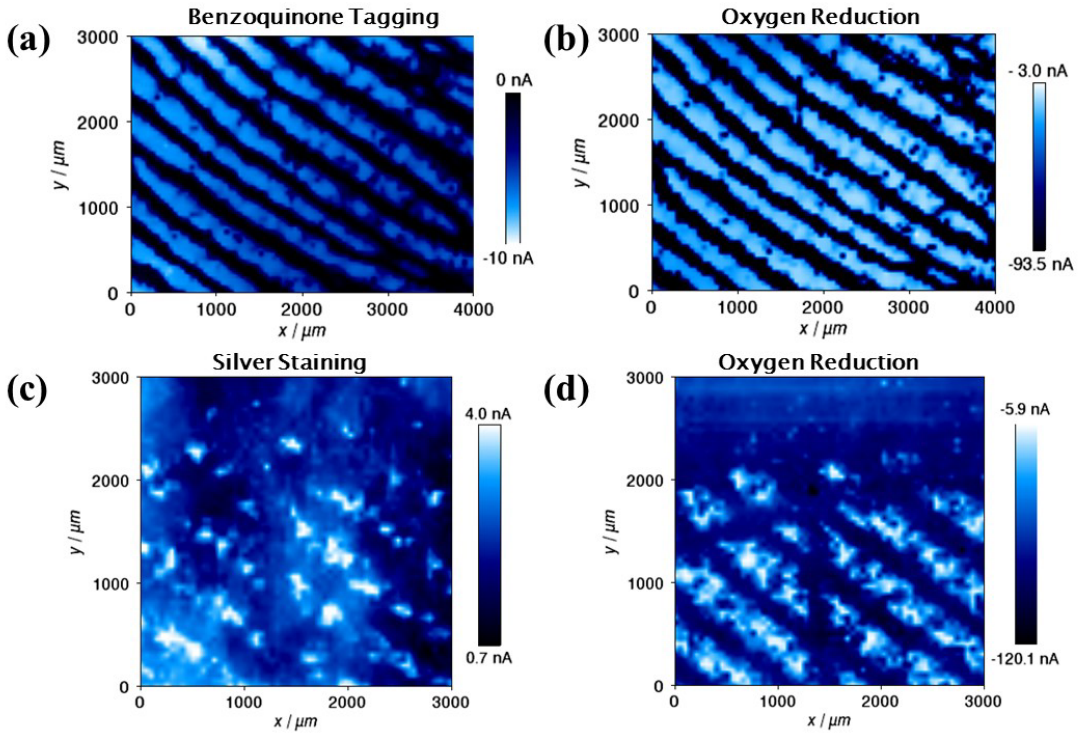

Fig. S2. Constant height image of human fingerprints on a PVDF membrane imaged by SECM coupled to a) benzoquinone tagging (electrode potential = E_T = –0.2 V), b) and d) oxygen reduction method (E_T = –0.8 V) and c) silver staining (E_T = 0.8 V). The scanned areas in a) and b), and in c) and d) are identical. Experimental conditions: d = 3 µm. step size = 50 µm, translation speed = 50 µm/s.

SI-3. Optimization of the SECM tyrosinase activity detection

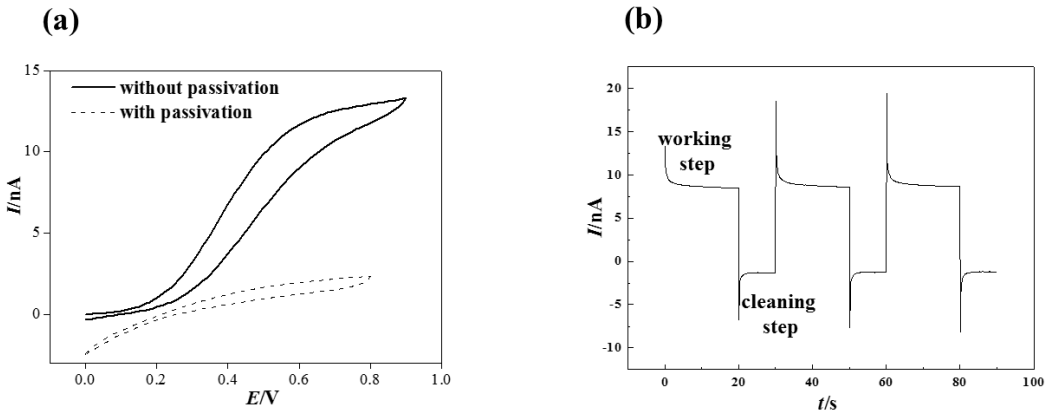

Fig. S3 (a) Cyclic voltammetry performed before and after 2.5 h of biasing a Pt microelectrode at 0.7 V in the presence of L-3,4-dihydroxyphenylalanine (L-DOPA) 2 mM. (b) Chronoamperometry of a solution of L-DOPA 2 mM in phosphate buffer 50 mM. For cyclic voltammetry: scan rate = 50 mV/s. For chronoamperometry: 5 cleaning cycles were performed by applying a potential of 0.7 V for 20 seconds and –0.8 V for 10 seconds. The measured currents at the plateau during the working steps (0.7 V) are nearly stable and reproducible after the cleaning cycles. Experimental conditions: Pt UME (r_T = 12.5 µm, RG = 9-10).

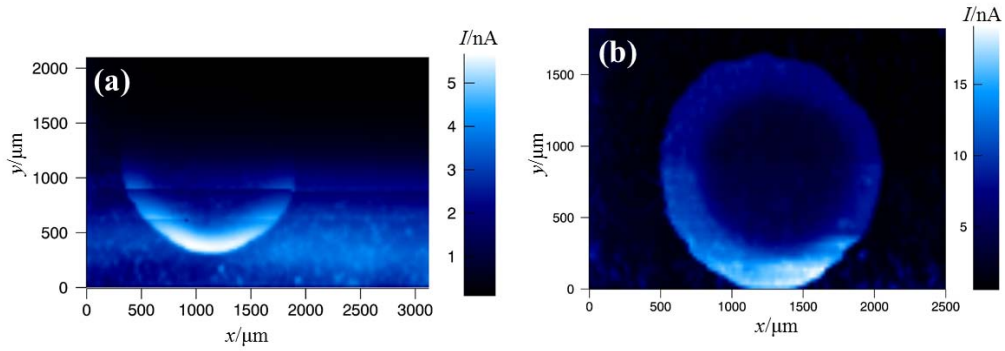

Fig. S4 SECM image of a tyrosinase spot on a PVDF membrane in 50 mM phosphate buffer (a) without and (b) with electrochemical cleaning. Experimental conditions: Forward E_t = 0.7 V, reverse E_t = –0.8 V, Pt UME (r_T = 12.5 µm, RG = 9-10), step size = 25 µm, translation speed = 20 µm/s, d = 5-7 µm.

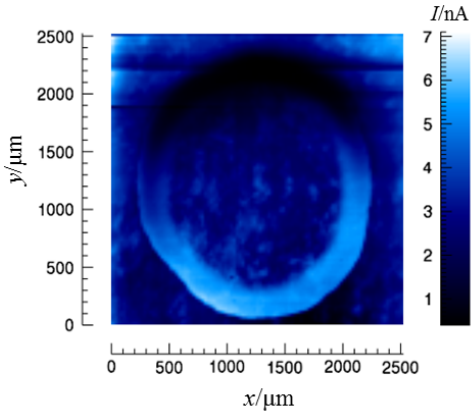

Fig. S5 Constant height SECM image of an immobilized tyrosinase spot over a PVDF membrane in 2 mM of 4-methyl catechol and 50 mM phosphate buffer, pH 6.0. Experimental conditions: Forward scan E_T = 0.7 V, reverse scan E_T = –0.8 V, Pt UME (r_T = 12.5 µm, RG = 9-10), step size = 25 µm, translation speed = 20 µm/s, d = 7 µm.

SI-4. Elucidation of the SECM detection mechanism of the tyrosinase activity

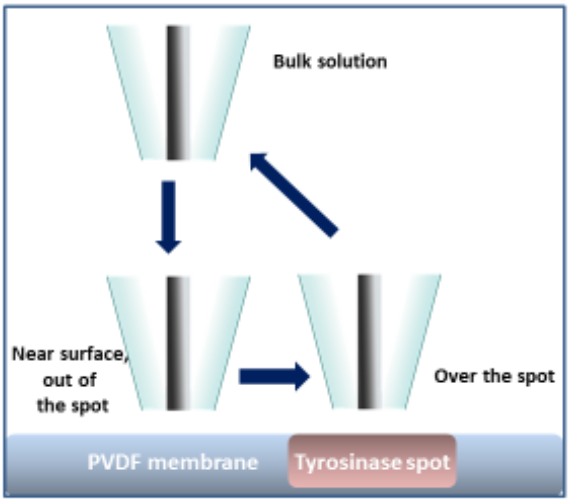

Fig. S6 Locations where the three differential pulse voltammetry (DPV) experiments were performed. For more information see the main manuscript.

Electrostatic-spray ionization (ESTASI) methodology

ESTASI was used for mass spectrometry (MS) analysis (ESTASI-MS) as described previously [3]. Briefly, the PVDF membrane was placed on top of a PET substrate (120 µm thick) that separated the sample from a vertically oriented disk electrode. Above the PVDF membrane an ion transfer capillary of a linear ion trap mass spectrometer (LTQ Velos, Thermo scientific) was located. The ion transfer capillary was a self-designed "L-shaped" ion transfer capillary instead of the original linear one of the MS instrument. The modification was carried out in order to place the PVDF membrane horizontally for MS analysis. The PVDF membrane was moved in *x*, *y*, *z* directions by a positioning system to be able to analyze different tyrosinase-free or -bound PVDF membrane regions, which were covered by droplets of the sample solutions such as 2 mM L-DOPA in 50 mM phosphate buffer or simply phosphate buffer. A pulsed high voltage (HV, from 0 V to 8 kV, frequency 20 Hz) was generated using amplifying voltage square wave pulses (0 to 8 V) by a high voltage amplifier (10HVA24-P1, HVP High Voltage Products GmbH, Martinsried/Planegg,

Germany), and applied on the electrode to induce ESTASI against the ion transfer capillary that was always grounded. The electrospray ionization (ESI) voltage of the internal power of the MS instrument was always set as 0. The enhanced ion trap scanning rate of 10000 m/z per second was used. The other parameters were optimized for the selected analytes. All MS analyses were performed under the positive scanning mode.

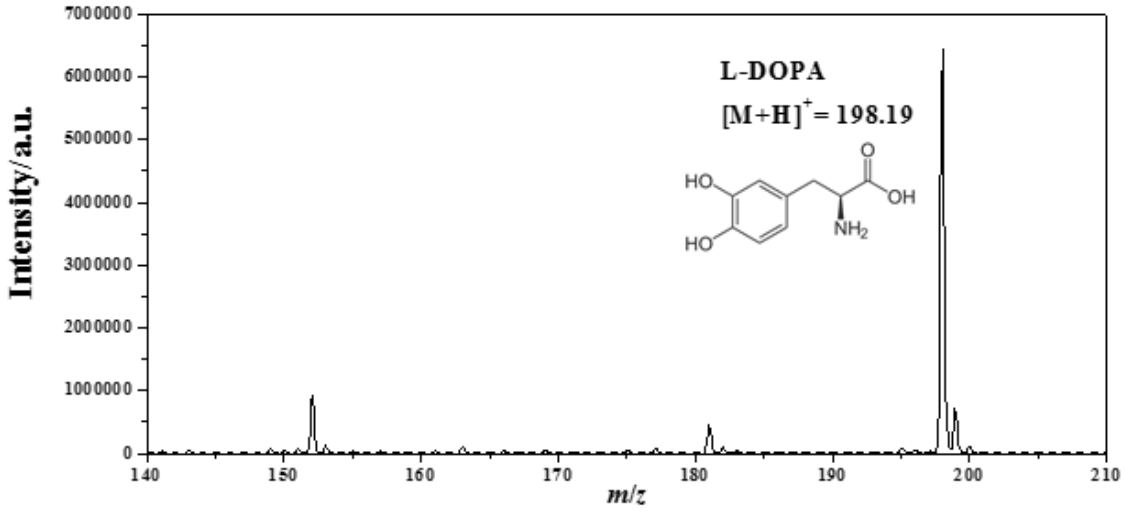

Fig. S7 Mass spectrum of L-DOPA.

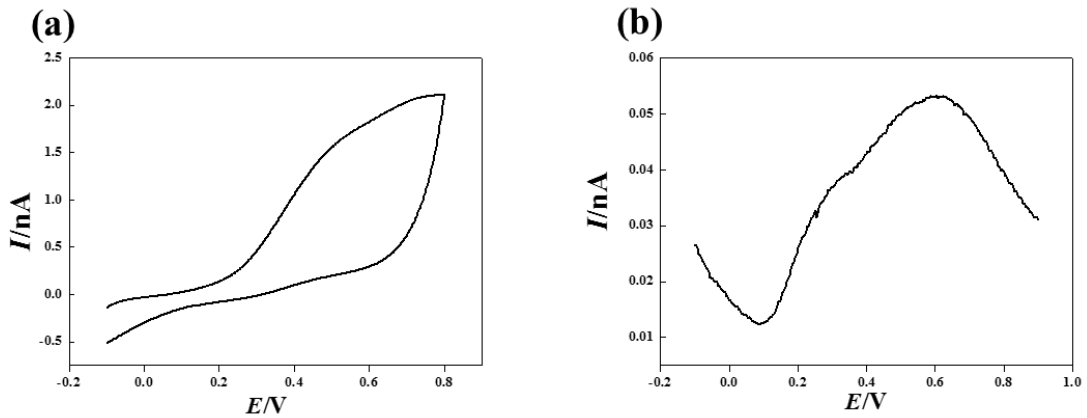

Fig. S8 (a) Cyclic voltammetry and (b) DPV of a solution of 1 mM 5,6-dihydroxyindole (DHI) at a Pt UME (r_T = 12.5 μm, RG = 9-10). For cyclic voltammetry: scan rate = 50 mV/s. For DPV: E_{step} = 4 mV, pulse amplitude = 10 mV, pulse time = 200 ms, scan rate = 4 mV/s.

SI-5. Cyclic voltammetry of a 3,3',5,5'-tetramethylbenzidine (TMB_{red}) solution

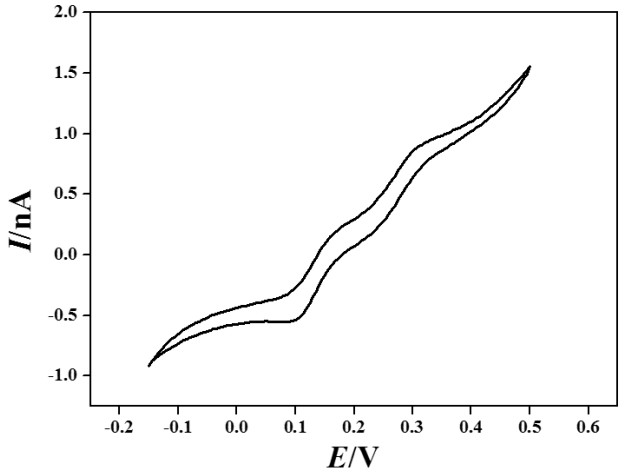

Fig. S9 Cyclic voltammetry of a solution of TMB_{ox} at a Pt UME (r_T = 12.5 μm, RG = 9-10) with a Ag wire as QRE and a Pt wire as counter electrode. Scan rate = 50 mV/s.

References

[1] F. Cortés-Salazar, J.-M. Busnel, F. Li, H.H. Girault, Adsorbed protein detection by scanning electrochemical microscopy, J. Electroanal. Chem. 635 (2009) 69-74.

[2] M. Zhang, H.H. Girault, Fingerprint imaging by scanning electrochemical microscopy, Electrochem. Commun. 9 (2007) 1778-82.

[3] L. Qiao, R. Sartor, N. Gasilova, Y. Lu, E. Tobolkina, B. Liu, H.H. Girault, Electrostatic-spray ionization mass spectrometry, Anal. Chem. 84 (2012) 7422-30.