Electronic Supplementary Material (ESI) for RSC Advances.

Electronic Supporting Information

Fabrication of bioactive polypyrrole microelectrodes on insulating surfaces by surface guided biocatalytical polymerization.

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Direct current electrical measurements:

Direct current electrical measurements were conducted on polypyrrole thin films grown on silanized (pyrrole-silane) SiO_2 surfaces, which were confined in the space between two square gold electrodes (Figure S1). Electrical conductivity was calculated by two-contact measurements, measuring the resistance value obtained by applying a sweep potential from -10 V to +10 V using a 6430 Sub-Femtoamp Remote SourceMeter with a 6430's Remote PreAmp for a very sensitive bi-directional amplification, from Keithley Instruments Inc. and LabTracer 2.0 (ver. 2.8) software from National Instruments Corporation.

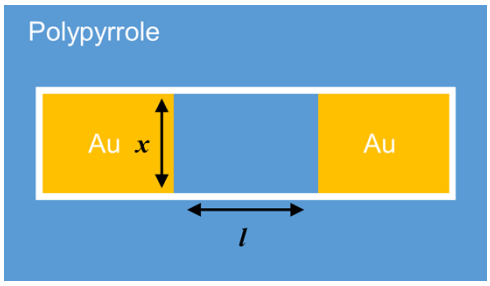

Figure S1. Schematics of two-contact measurement configuration.

Controlled biotin release:

Controlled biotin release experiments were conducted by cyclic voltammetry in a three-electrode electrochemical cell, with a platinum counter electrode and a Ag/AgCl-NaCl (3M) reference electrode. Working electrodes consisted on polypyrrole films grown onto silanized (pyrrole-silane) SiO_2 surfaces containing metallic electrodes. Such working electrodes were mounted on polyvinyl chloride (PVC) substrates with large printed copper electrodes. The copper electrodes were soldered to the microelectrodes on the substrate. Subsequently, the whole system was passivated with teflon tape containing circular openings, eluding the interference of the external contacts and solderings with the measuring solution as shown in Figure S2.

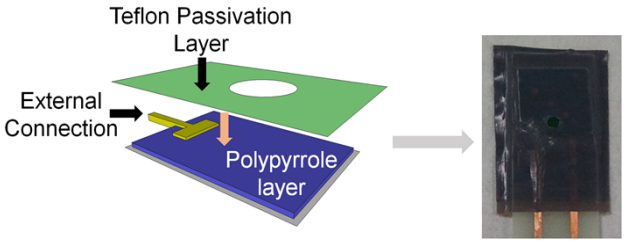

Figure S2: Passivation step of polypyrrole working electrodes. Teflon covers external electrical pads and defines the electrochemical working area.

Fabrication Yield:

The following analysis for the fabrication yield corresponds to microelectrodes of polypyrrole of 5 µm in width. The width histogram for the fabrication of polypyrrole lines, revealed a 77% of yield, considering for calculations only the polypyrrole electrodes with 5.0 ± 0.2 µm widths.

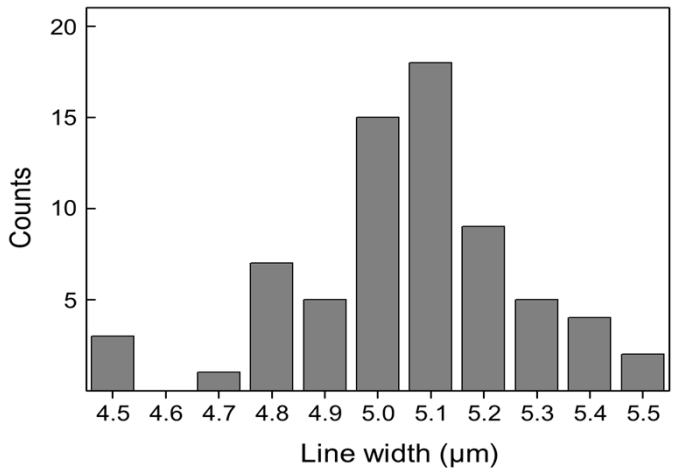

Figure S3. Yield histogram for biocatalytical polypyrrole microelectrodes of 5 µm width. Measurements revealed a 77% of fabrication yield for the microelectrodes.

Thickness analysis:

Polymerization experiments at various growth times were conducted and characterized by AFM. Three series of experiments where performed taking at least three measurements per sample. AFM profiles taken from scan areas of 3µm x 3µm, at different times during the polymer growth, revealed an increase of the thickness within the first hour, which reached a plateau of 75 nm after 60 minutes of reaction.

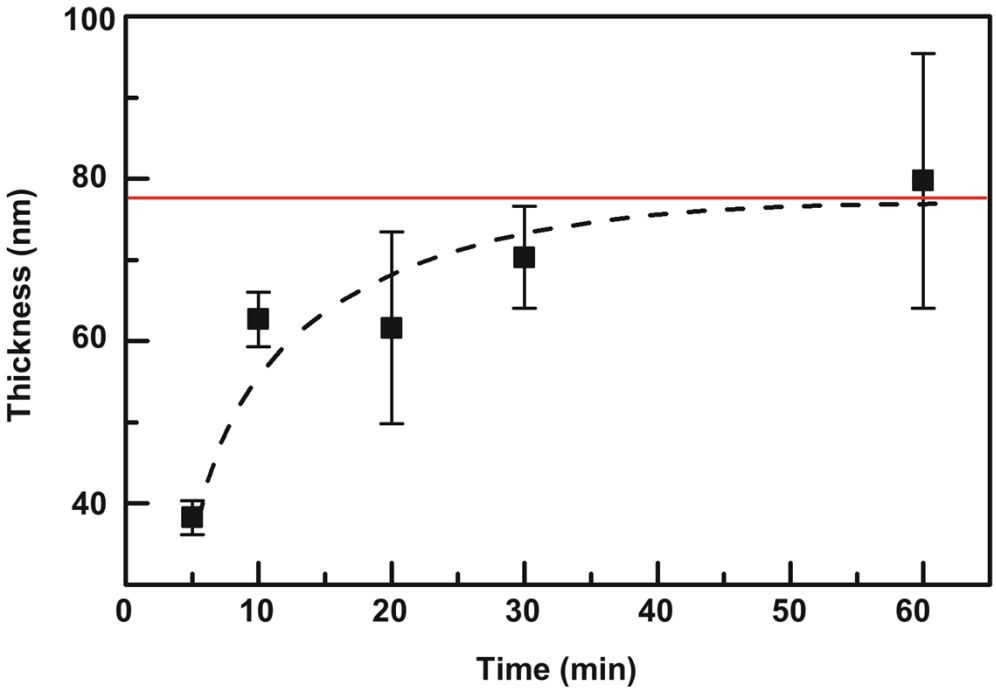

Figure S4. Plot of the thickness (measured with Atomic Force Microscopy, AFM) at different time-points, of biocatalytically generated polypyrrole films grown on pyrrole-silane monolayers on insulating SiO_2. Dashed line is an eye guide. Solid red line indicates the thickness plateau at 75 nm approx.

X-ray photoelectron spectroscopy (XPS) analysis of biotin-containing polypyrrole electrodes:

High-resolution spectrum was recorded for the core-level peaks of S 2p and compared to that obtained when polypyrrole was grown in the absence of biotin. In both cases, S 2p signal showed two strong components centered at 165.1 and 162.0 eV, respectively. The oxidized sulfur species ($-SO_3H$) at 165 eV is most likely to correlate with the use of ABTS as dopant, and the peak centered at 162 eV can be attributed to C-S-C and C-SH species. In the absence of biotin, the peak at 162 eV could be due to the contribution of the enzyme HRP that is also entrapped in the polymer structure during the polymerization process.[1,2] In that case the peak area ratio between the two sulfur components at 162 and 165 eV resulted in 0.90. Due to the presence of biotin, the contribution of the 162 eV peak increases leading to a ratio of 1.14.

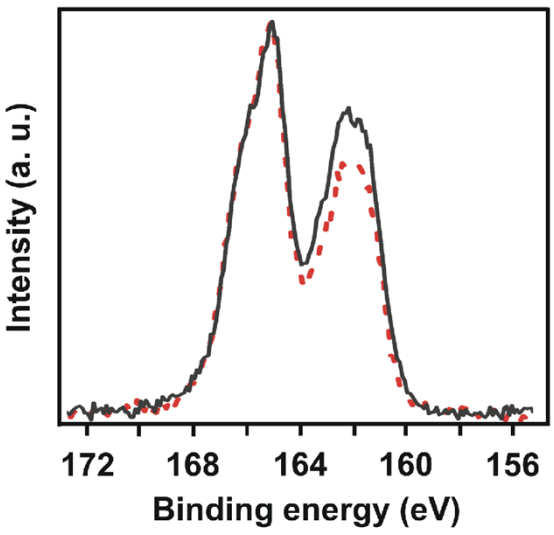

Figure S5. S 2p core-level XPS analysis of biocatalytically synthesized polypyrrole in the presence (solid black line) and in the absence of biotin (dashed red line), showing the increase in the 162.0 eV peak due to the contribution of sulfur from biotin.

References:

1 A. Ramanavicius, A. Kausaite, A. Ramanaviciene, J. Acaite and A. Malinauskas, *Synth. Met.*, 2006, **156**, 409–413.

2 X. Cui, C. M. Li, J. Zang, Q. Zhou, Y. Gan, H. Bao, J. Guo, V. S. Lee and S. M. Moochhala, *J. Phys. Chem. C*, 2007, **111**, 2025–2031.