*molecules*

MDPI

*Supplementary material*

# Enhanced Immobilization of Enzymes on Plasma Micro-Nano-textured Substrates and Microfluidics: Application to HRP

**Stefania Vorvi[1], Katerina Tsougeni[1], Angeliki Tserepi[1], Sotirios Kakabakos[2], Panagiota Petrou[2] and Evangelos Gogolides[1]**

[1] Institute of Nanoscience & Nanotechnology, NCSR "Demokritos", 15341 Aghia Paraskevi, Greece; svorvi@gmail.com (S.V.); k.tsougeni@gmail.com (K.T.); a.tserepi@inn.demokritos.gr (A.T.);
[2] Immunoassays/Immunosensors Lab, Institute of Nuclear & Radiological Sciences & Technology, Energy & Safety, NCSR "Demokritos", 15341 Aghia Paraskevi, Greece; skakab@rrp.demokritos.gr (S.K.)
* Correspondence: ypetrou@rrp.demokritos.gr (P.P), +30 210 6503819; e.gogolides@inn.demokritos.gr (E.G.), +30 210 650 3307

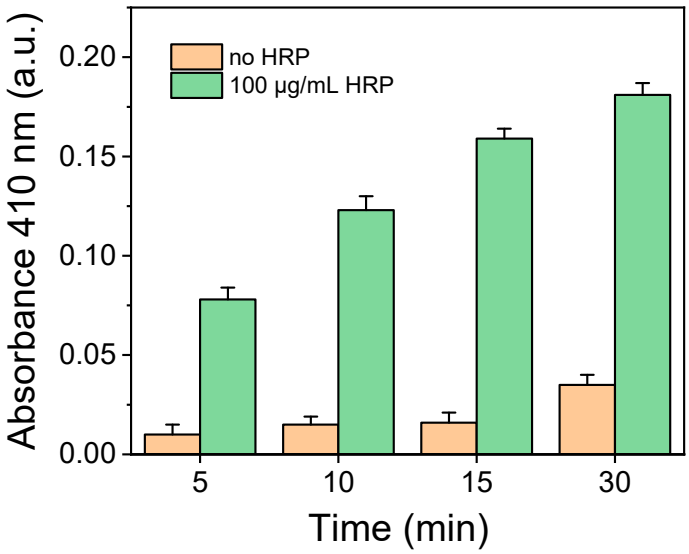



**Figure S1**. Absorbance values at 410 nm obtained from PMMA surfaces treated with oxygen plasma for 10 min not coated with HRP (orange columns) or coated with a 100 µg/mL HRP solution as a function of incubation duration with the DAB HRP substrate. Each column corresponds to mean value of triplicate measurements ± SD.

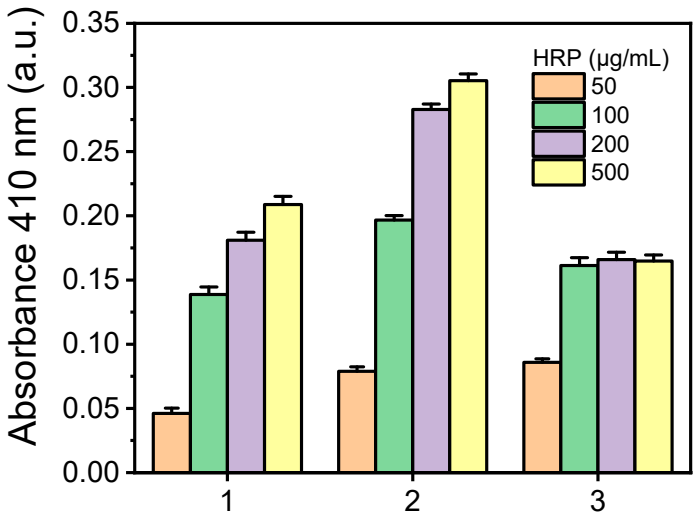



**Figure S2**. Absorbance values at 410 nm obtained from surfaces to which HRP has been immobilized on 10-min oxygen plasma-treated PMMA surfaces through adsorption (column 1), covalent binding (column 2) or affinity binding of biotinylated HRP on surfaces modified with a 0.5 mg/ml streptavidin solution. Each point corresponds to mean value of triplicate measurements ± SD.

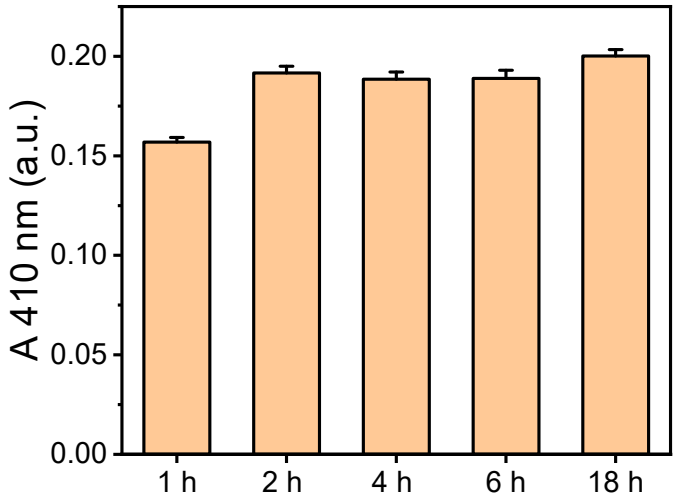



**Figure S3.** Absorbance values at 410 nm obtained from 10-min oxygen plasma treated PMMA versus the duration of incubation with a 200 µg/mL HRP solution. All surfaces have been incubated for 15 min with the precipitating DAB HRP solution. Each point corresponds to mean value of triplicate measurements ± standard deviation.

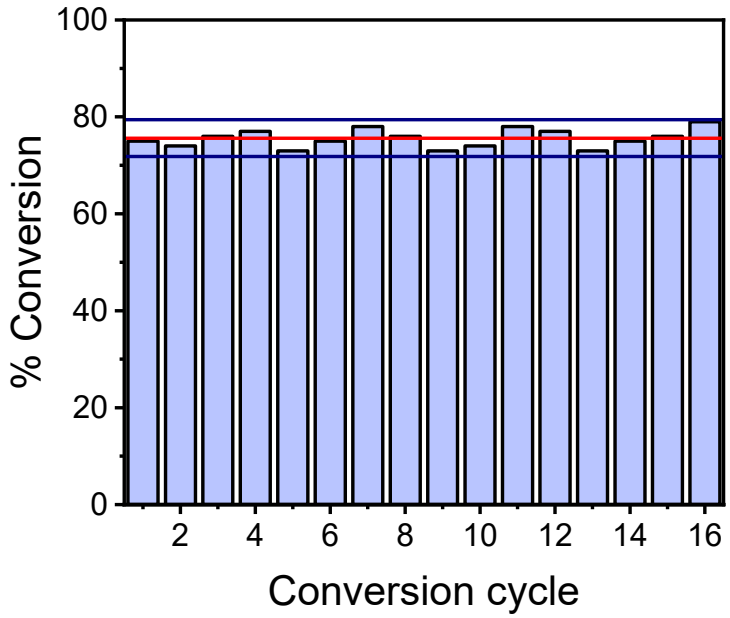



**Figure S4**. Values of %conversion of 4-phenylazophenol obtained from an HRP-modified oxygen-plasma micro/nanotextured PMMA microreactor upon 16 sequential injections of 4-phenylazophenol/$H_2O_2$ mixtures. The red line corresponds to the mean of the 16 values and the blue lines to mean±2SD of the 16 values.

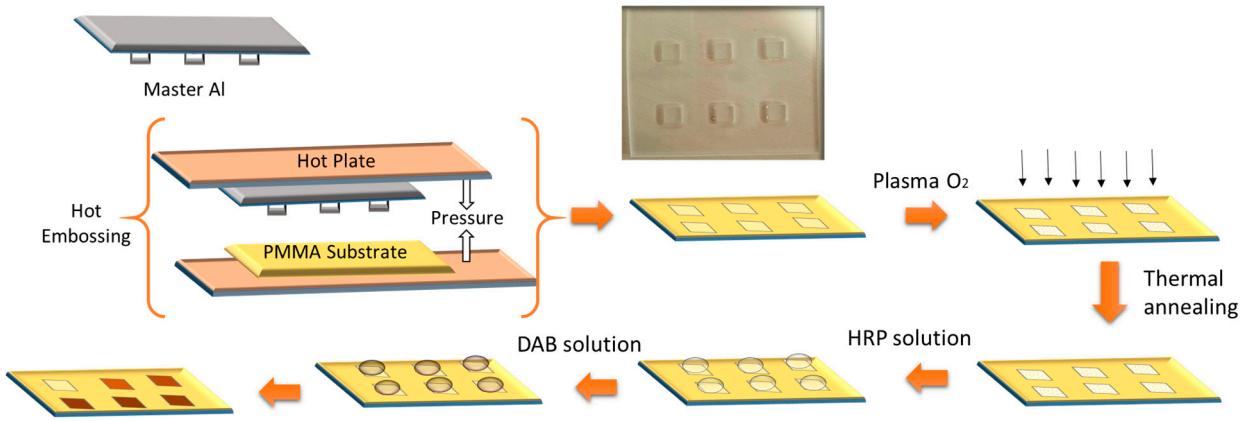
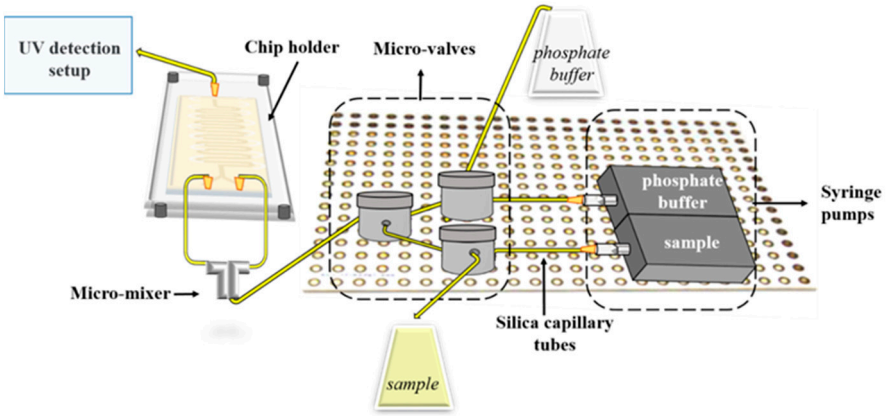
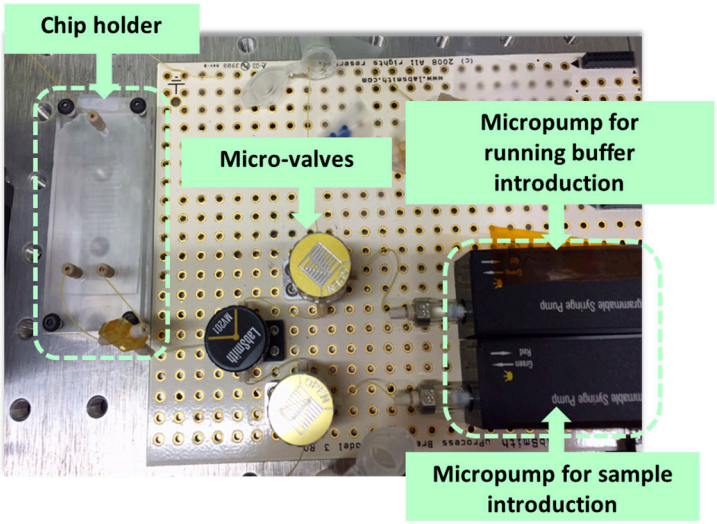



**Figure S5**. Schematic of the procedure for fabrication of open PMMA oxygen plasma nanotextured surfaces and of the procedure for HRP immobilization and detection on them.




(a)




(b)

**Figure S6**. (a) Schematic of pumps' and valves' connection to the chip placed on a dedicated holder. (b) Image of the chip placed on the dedicated holder and connected to the valves and pumps that regulate the flow of the running buffer and the sample.