Supporting Information (SI)

Detection of Trace Heavy Metal Ions in Water by Nanostructured Porous Si Biosensors

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The supporting information contains:

1. Figure S-1. ATR-FTIR (Attenuated Total Reflection Fourier Transform Infrared) spectra of the different functionalization steps followed for the preparation of HRP-immobilized PSiO₂.

2. Figure S-2. Relative EOT values at different steps throughout the enzyme (HRP) immobilization onto PSiO₂.

3. Figure S-3. The relative activity of HRP-immobilized PSiO₂ vs. incubation time of Pb²⁺, Ag⁺ and Cu²⁺ ions at a constant concentration (100 µM).

4. Figure S-4. The performance of HRP and Laccase-immobilized PSiO₂ biosensors to cumulative effect of heavy metals.

5. Figure S-5. Optical response of EDTA-treated Laccase-immobilized PSiO₂ to different copper ions concentrations.
Figure S-1. ATR-FTIR spectra of the different functionalization followed for the preparation of HRP-immobilized PSiO₂: (a) Neat PSiO₂ surface; (b) APTES-modified surface; (c) DSC-modified surface and (d) enzyme-modified surface.

The neat PSiO₂ surface spectrum (trace a) shows a typical –(OₓSiHₓ) vibration mode at 803 cm⁻¹ and a peak at 1048 cm⁻¹ that is related to the Si-O-Si stretching mode. The APTES-modified surface spectrum (trace b) depicts two additional peaks; the 1641 cm⁻¹ is attributed to the bending band of prime amine and the 1555 cm⁻¹ to the bending band of protonated amines. The following synthetic step (DSC modification) is displayed trace c. Amide bands appear at 1634 cm⁻¹ (amide I) and 1529 cm⁻¹ (amide II), exhibiting a red shift compared to the bending bands of the APTES-modified surface. Two additional peaks appear at 1734 and 1776 cm⁻¹, are attributed to the asymmetric and symmetric stretching bands of succinimidyl ester, respectively.¹ Finally, for the enzyme-immobilized surface (trace d), amide bands appear at 1645 cm⁻¹ (amide I) and
1551 cm$^{-1}$ (amide II), showing a blue shift compared to the NHS species on the DSC-modified surface. These results verify that the enzyme molecules are conjugated to the PSiO$_2$ surface.

Figure S-2. Relative EOT values at different steps throughout the enzyme (HRP) immobilization onto PSiO$_2$.

The immobilization process includes three steps: (i) 3-Aminopropyl(triethoxyl)silane (APTES), modification, (ii) Bis (N-succinimidyl)carbonate (DSC) modification, (iii) HRP immobilization. The EOT values obtained after each step (APTES, DSC and enzyme) are normalized with respect to the EOT value of the neat PSiO$_2$ scaffold (termed as EOT$_0$). It is expected that the chemical modification of the porous nanostructure will induce a red shift in the EOT due to the increase in the value of $n$ upon attachment of the different species onto the pore walls$^2$. It should be noted that the PSiO$_2$ nanostructure is designed to allow proper infiltration of the biomolecules into the pores. Indeed, significant relative EOT changes are observed after each of the described functionalization steps, followed for enzyme immobilization.
Figure S-3. The relative activity of HRP-immobilized PSiO$_2$ vs. incubation time of Pb$^{2+}$, Ag$^+$ and Cu$^{2+}$ ions at a constant concentration (100 µM).

A specific enzymatic assay for HRP-immobilized PSiO$_2$ nanostructures is used in order to determine the time needed for complete inhibition of the immobilized enzyme by the different metal ions. Briefly, the modified surfaces are exposed with Ampliflu red solution, allowed to react, while the oxidation ability of the immobilized HRP is spectrophotometrically analyzed. The relative activity is compared to the HRP-immobilized PSiO$_2$ nanostructure (not treated with metals). The relative activity is observed to decrease with incubation time, and after 40 min a complete inhibition is attained for all metal ions.
Figure S-4. The performance of HRP and Laccase-immobilized PSiO₂ biosensors to cumulative effect of heavy metals. Pb²⁺, Ag⁺ and Cu²⁺ are mixed individually with a solution containing Mg²⁺, Zn²⁺, Ca²⁺, Fe²⁺, Na⁺, K⁺ (6 µM) while the inhibition of HRP or relative activity of Laccase are evaluated.
Figure S-5. Optical response of EDTA-treated Laccase-immobilized PSiO$_2$ to different copper ions concentrations. EDTA-treated Laccase-immobilized PSiO$_2$ is incubated with different standard copper ion solutions, followed by continuous cycling of 0.8 mM 1-naphthol in HEPES buffer (pH 8) through the flow cell. A control experiment for native Laccase (no EDTA-treatment), for defining maximal activity, is similarly obtained. The biosensor is fixed in a custom-made flow cell and the reflectivity spectra are recorded every 30 s.

REFERENCES