Supporting Information (SI)

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

Giorgi Shtenberg[1], Naama Massad-Ivanir[2] and Ester Segal[2,3]*

[1]The Inter-Departmental Program of Biotechnology, [2]Department of Biotechnology and Food Engineering, [3]The Russell Berrie Nanotechnology Institute, Technion – Israel Institute of Technology, Haifa 32000, Israel

Corresponding Author

[*]E-mail: esegal@tx.technion.ac.il; phone: +972-4-8295071

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 $PSiO_2$.

2. Figure S-2. Relative EOT values at different steps throughout the enzyme (HRP) immobilization onto $PSiO_2$.

3. 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).

4. Figure S-4. The performance of HRP and Laccase-immobilized $PSiO_2$ biosensors to cumulative effect of heavy metals.

5. Figure S-5. Optical response of EDTA-treated Laccase-immobilized $PSiO_2$ to different copper ions concentrations.

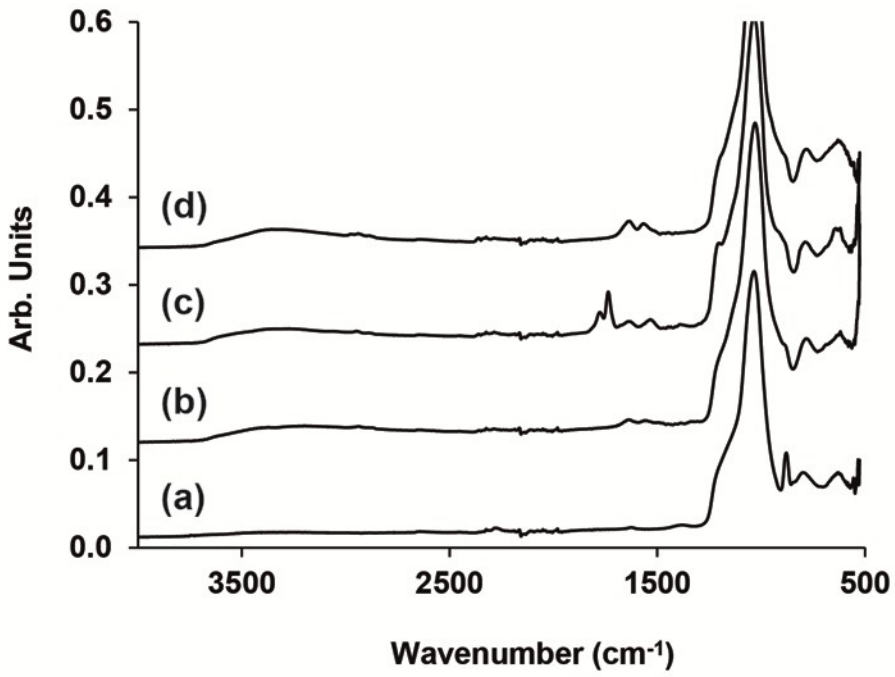

Figure S-1. ATR-FTIR spectra of the different functionalization followed for the preparation of HRP-immobilized $PSiO_2$: (a) Neat $PSiO_2$ surface; (b) APTES-modified surface; (c) DSC-modified surface and (d) enzyme-modified surface.

The neat $PSiO_2$ surface spectrum (trace a) shows a typical $-(O_ySiH_x)$ 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.

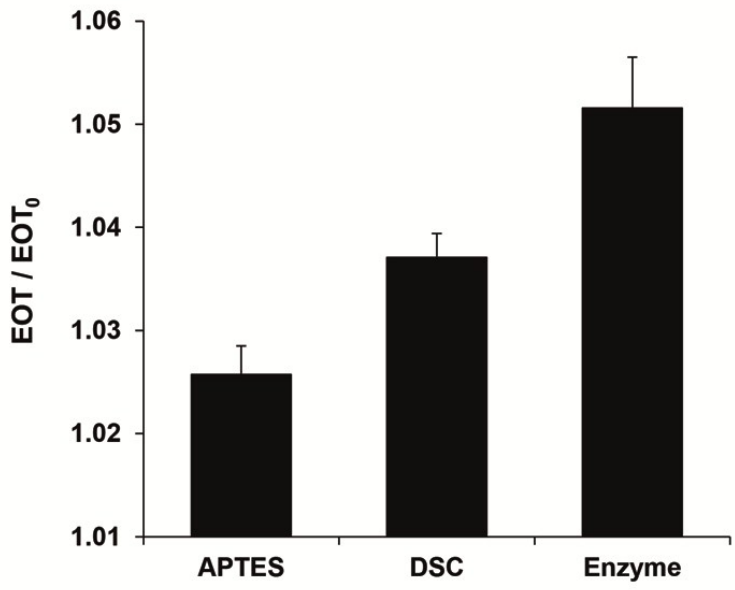

Figure S-2. Relative EOT values at different steps throughout the enzyme (HRP) immobilization onto $PSiO_2$.

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.

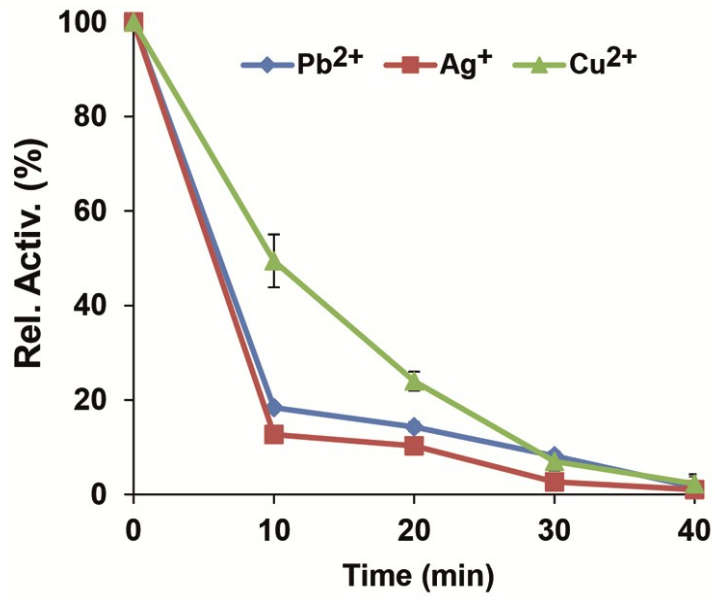

Figure S-3. The relative activity of HRP-immobilized $PSiO_2$ vs. incubation time of Pb^{2+}, Ag^{+} and Cu^{2+} ions at a constant concentration (100 μM).

A specific enzymatic assay for HRP-immobilized $PSiO_2$ nanostructures [2] 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.

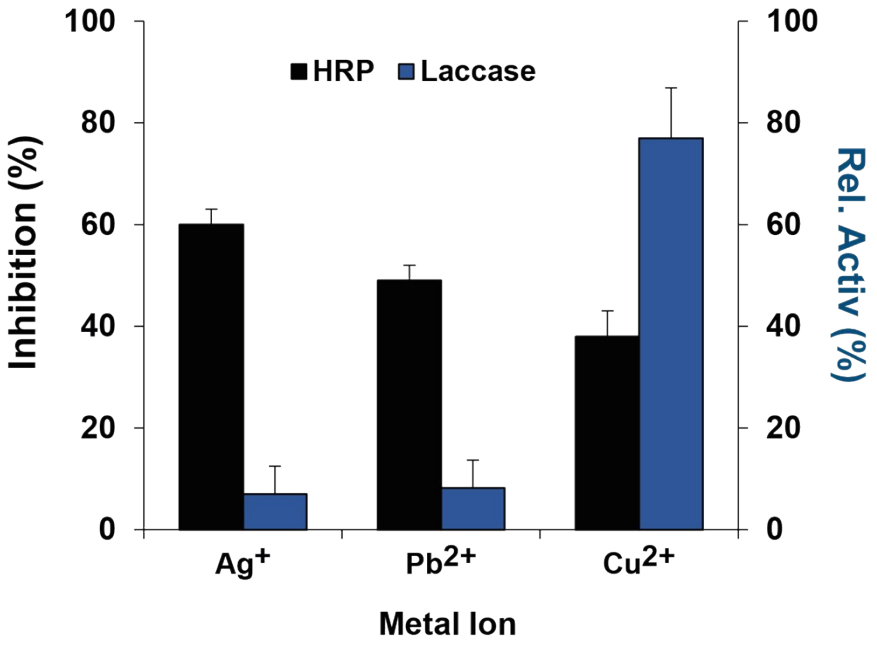

Figure S-4. The performance of HRP and Laccase-immobilized $PSiO_2$ biosensors to cumulative effect of heavy metals. Pb^{2+}, Ag^{+} and Cu^{2+} are mixed individually with a solution containing Mg^{2+}, Zn^{2+}, Ca^{2+}, Fe^{2+}, Na^{+}, K^{+} (6 μM) while the inhibition of HRP or relative activity of Laccase are evaluated.

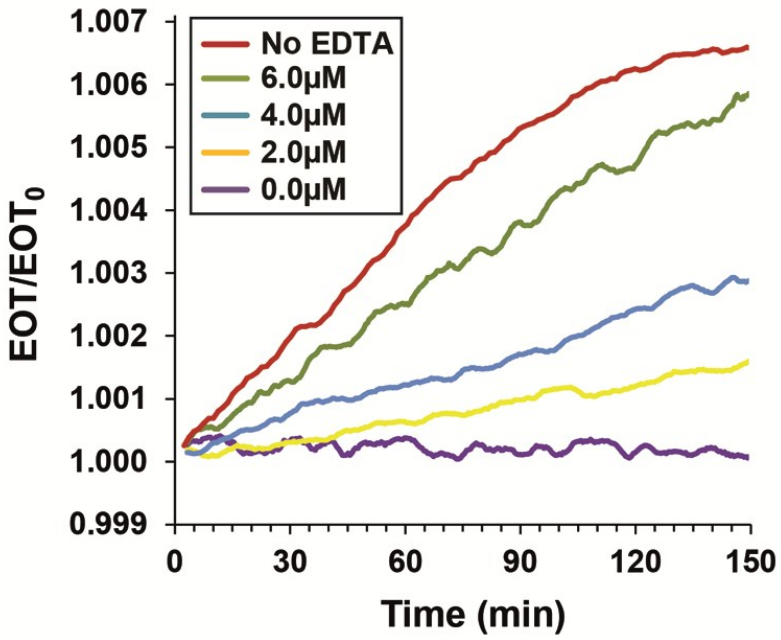

Figure S-5. Optical response of EDTA-treated Laccase-immobilized $PSiO_2$ to different copper ions concentrations. EDTA-treated Laccase-immobilized $PSiO_2$ is incubated with different standard copper ion solutions, followed by continuous cycling of 0.8 mM 1-naphthol in HEPES buffer (pH 8) through the 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

(1) Xia, B.; Xiao, S. J.; Guo, D. J.; Wang, J.; Chao, M.; Liu, H. B.; Pei, J.; Chen, Y. Q.; Tang, Y. C.; Liu, J. N. *J. Mater. Chem.* **2006**, *16*, 570-578.
(2) Shtenberg, G.; Massad-Ivanir, N.; Moscovitz, O.; Engin, S.; Sharon, M.; Fruk, L.; Segal, E. *Anal. Chem.* **2013**, *85*, 1951-1956.