Supporting Information

Nanostructured Porous Si Optical Biosensors: Effect of Thermal Oxidation on Their Performance and Properties

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Freshly etched PSi nanostructures are *in situ* thermally oxidized at three temperatures (400, 600, and 800°C) in a TGA (thermal gravimetric analyzer). Figure S1 depicts the weight change of the PSi following oxidation. Oxide layer growth onto the PSi scaffolds is observed to result in a weight increase at elevated oxidation temperatures. For PSi oxidized at 800°C the highest weight change (0.19±0.01%) is obtained.

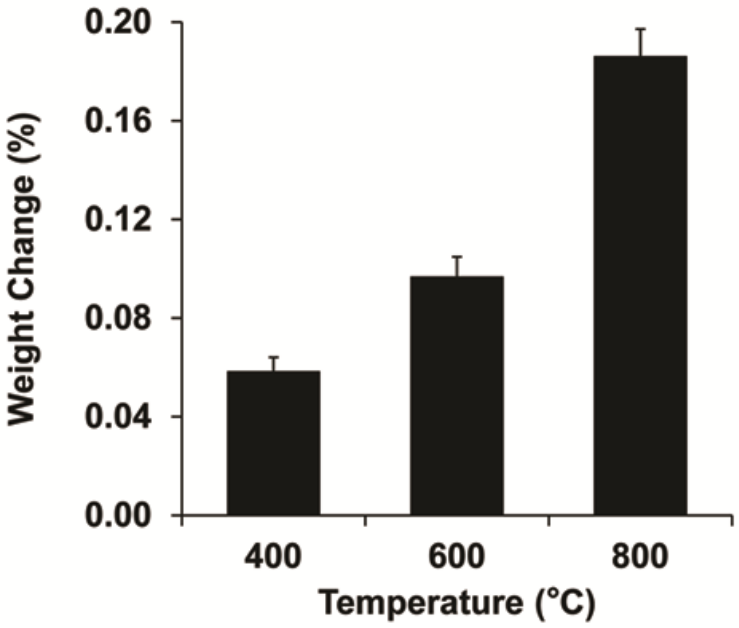

Figure S1. Weight change of PSi scaffolds following in situ thermal oxidization (at 400, 600, and 800°C) in a TGA.

The oxidation process is characterized by using attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy. Figure S2 depicts the ATR-FTIR spectra of the different surfaces oxidized at 400, 600 and 800°C. The absorbance of Si-O-Si stretching mode, –(O_ySiH_x) and Si-OH vibration modes are significantly increased with elevating the oxidation temperature, while the Si-H_x peak is observed to completely disappear.

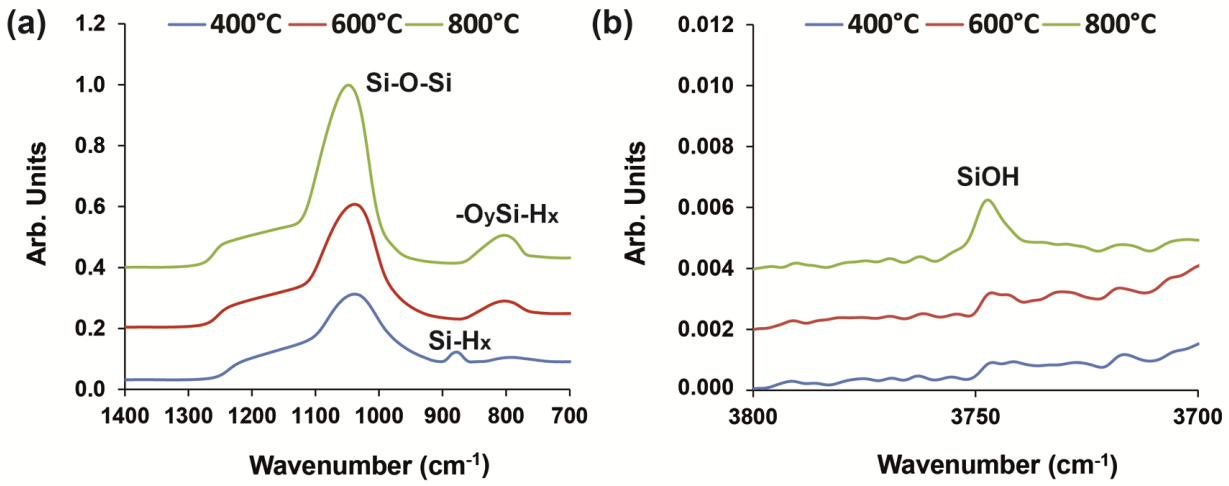

Figure S2. ATR-FTIR spectra of PSi films oxidized at different temperatures (400, 600, 800°C). Oxidation process is characterized by monitoring: **(a)** Si-O-Si, –(O_ySiH_x) and Si-H_x peaks. **(b)** Si-OH vibration mode.

Fluorescein-labeled HRP is immobilized onto three $PSiO_2$ nanostructures (oxidized at 400, 600, 800°C). The samples are studied by CLSM and the fluorescence intensity is calculated by image analysis of the CLSM data using the Imaris software. Figure S3 summarizes the mean fluorescence intensity values resulting from the fluorescein-labeled HRP and the photoluminescence (PL) of the $PSiO_2$ scaffolds. Both signal are observed to increase with oxidation temperature.

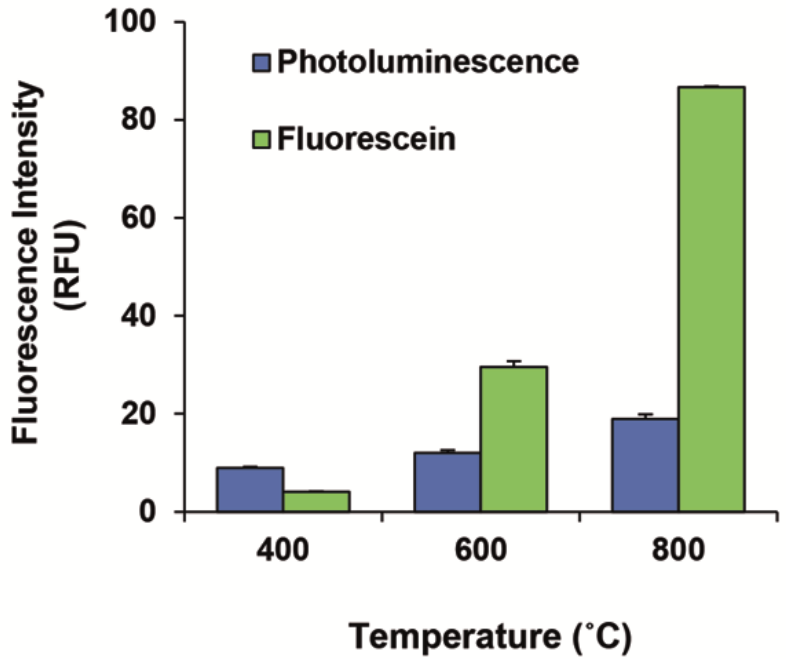

Figure S3. Fluorescence and photoluminescence intensity values obtained following fluorescein-HRP (FTC-HRP) immobilization onto $PSiO_2$ scaffolds oxidized at different temperatures.

Figure S4 summarize the results of optical stability experiments presented in Figures 6, S5, S6 and S7. The results are presented as the maximal rate of relative EOT change with time, calculated from the slopes of the relative EOT vs. time plots.

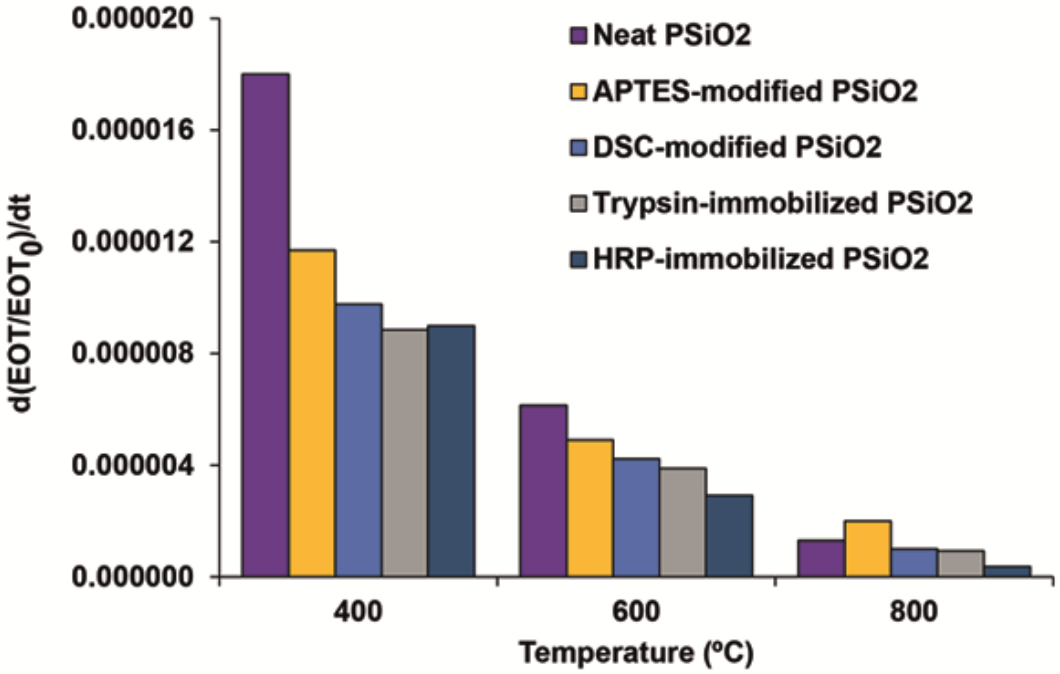

Figure S4. Maximal rate of relative EOT change with time of the neat $PSiO_2$, APTES-modified $PSiO_2$, DSC-modified $PSiO_2$ and enzyme-immobilized $PSiO_2$ (oxidized at three different temperatures 400, 600, 800°C) upon exposure to aqueous solution flow conditions. The nanostructures are continuously washed with 0.1 M HEPES buffer solution.

APTES-modified $PSiO_2$, DSC-modified $PSiO_2$ and Trypsin-immobilized $PSiO_2$ are fixed in a flow cell setup and are exposed to an aqueous solution of HEPES buffer (pH 8), while their optical properties are monitored in real time. Figures S5, S6 and S7 depict the changes in the relative EOT as a function of time of these modified nanostructures.

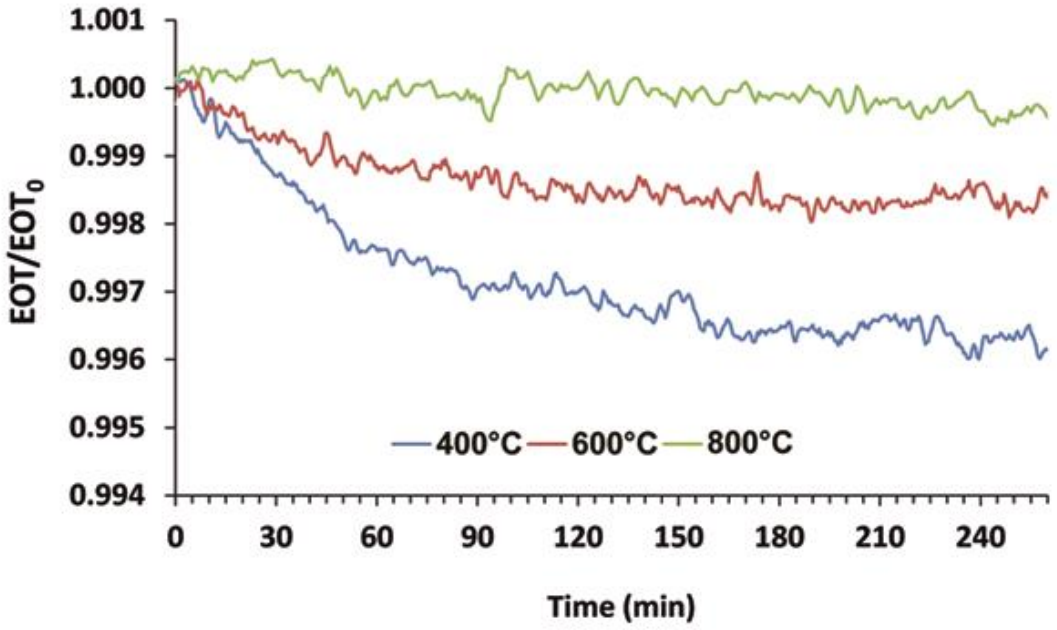

Figure S5. Optical response, expressed in terms of the relative EOT, of APTES-modified $PSiO_2$ (oxidized at three different temperatures 400, 600, 800°C) films vs. exposure time to aqueous solution flow conditions. The nanostructures are continuously washed with 0.1 M HEPES buffer solution (pH 8) at 0.5 mL min^{-1}. The samples are fixed in a custom made cell, and the reflectivity spectra are recorded every 30 s.

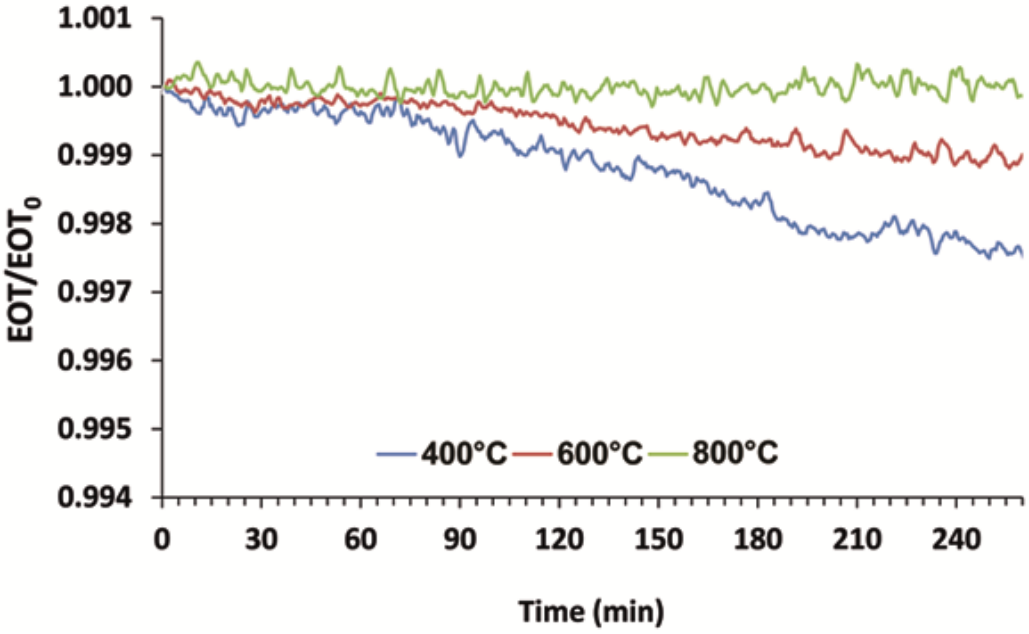

Figure S6. Optical response, expressed in terms of the relative EOT, of DSC-modified $PSiO_2$ (oxidized at three different temperatures 400, 600, 800°C) films vs. exposure time to aqueous solution flow conditions. The nanostructures are continuously washed with 0.1 M HEPES buffer solution (pH 8) at 0.5 mL min^{-1}. The samples are fixed in a custom made cell, and the reflectivity spectra are recorded every 30 s.

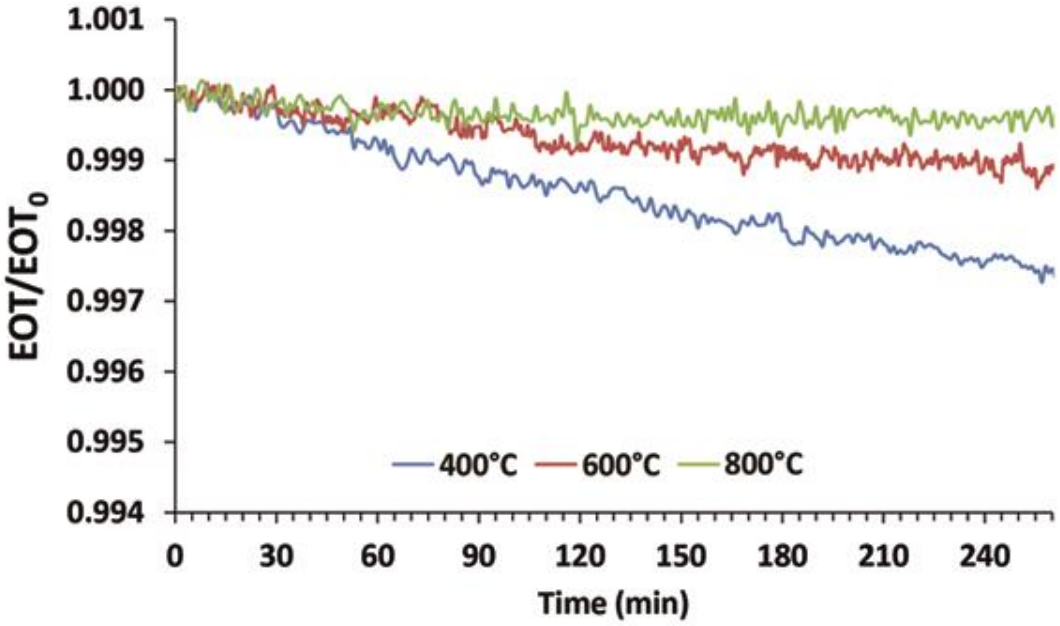

Figure S7. Optical response, expressed in terms of the relative EOT, of trypsin-immobilized $PSiO_2$ (oxidized at three different temperatures 400, 600, 800°C) films vs. exposure time to aqueous solution flow conditions. The nanostructures are continuously washed with 0.1 M HEPES buffer solution (pH 8) at 0.5 mL min^{-1}. The samples are fixed in a custom made cell, and the reflectivity spectra are recorded every 30 s.