Electronic Supplementary Material (ESI) for Analyst.

Supporting Information for

APTAMERS vs. ANTIBODIES AS CAPTURE PROBES IN OPTICAL POROUS SILICON BIOSENSORS

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Table S1 Characterization results of the oxidized PSi nanostructures by spectroscopic liquid infiltration method (SLIM) (n=5).

Porosity (%)	Thickness (nm)	Pore diameter* (nm)
73±3	5500±200	35-65

*Average pore diameter was evaluated with HR-SEM

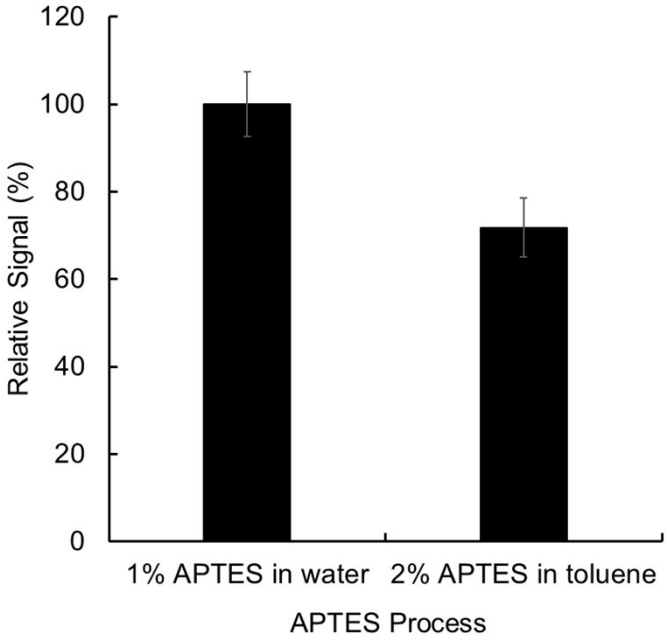

Figure S1. Comparison of the biosensing performance of the immunosensor upon exposure to 56 μM Tyrosinase, for two methods of APTES modification of the $PSiO_2$ film. The signal is normalized to the standard APTES method of the antibody immobilization process (1% APTES in water) (n≥3). Results indicate lower immunosensor performance, by 28%, upon APTES modification according to the aptamer immobilization process (2% APTES in toluene).

Table S2. A summary of the applied amount, number of moles cleaved, the immobilized percentage and surface density of the aptamers and oriented and unoriented antibodies within the $PSiO_2$ (n=3).

	Moles Applied (nmol)	Moles Cleaved (nmol)	Immobilization Percentage (%)	Surface Area (cm^2)	Surface Density (cm^{-2})
Aptamer	3.75	1.89±0.02	50.3±0.5%		$1.25 \cdot 10^{12}$
Oriented IgG	0.067	0.045±0.002	67±3%	910	$2.94 \cdot 10^{10}$
Unoriented IgG	0.067	0.0430±0.0006	64.5±0.8%		$2.83 \cdot 10^{10}$

The bioreceptor densities within the $PSiO_2$ were calculated by dividing the number of bioreceptor moles by the porous surface area. The latter was measured in a previous study [1] by nitrogen adsorption isotherms and application of BET (Brunauer-Emmett-Teller) model for a similar $PSiO_2$ nanostructure. Since the $PSiO_2$ utilized in the present study was characterized with a smaller layer thickness (5500 nm vs. 7880 nm), the surface area was corrected according to the layer thickness ratio of both nanostructures. Thus, the surface area value utilized for the calculations was 684 cm^2 STP cm^{-2} (expressed per unit area of $PSiO_2$ sample). The area of the $PSiO_2$ sample is 1.33 cm^2, resulting in a total surface area of 910 cm^2.

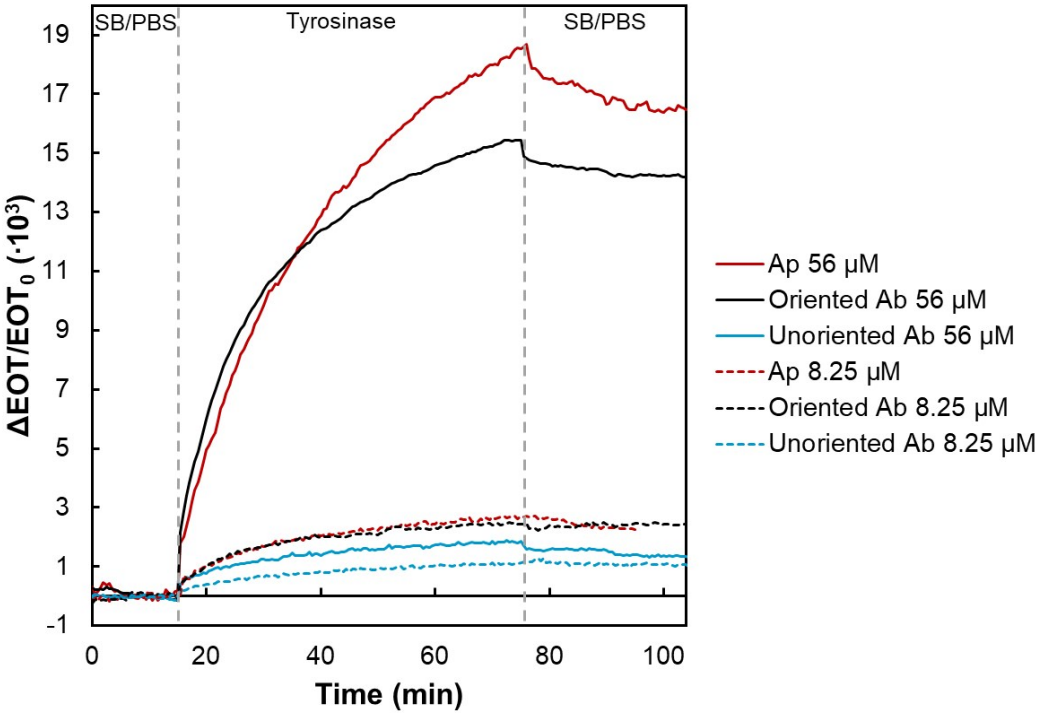

Figure S2. Real-time relative EOT changes for aptamer (Ap), oriented and unoriented antibody (Ab)-immobilized $PSiO_2$ upon exposure to 56 μM or 8.25 μM his-tagged tyrosinase (data represents an average of n = 3). SB denotes aptamer's selection buffer.

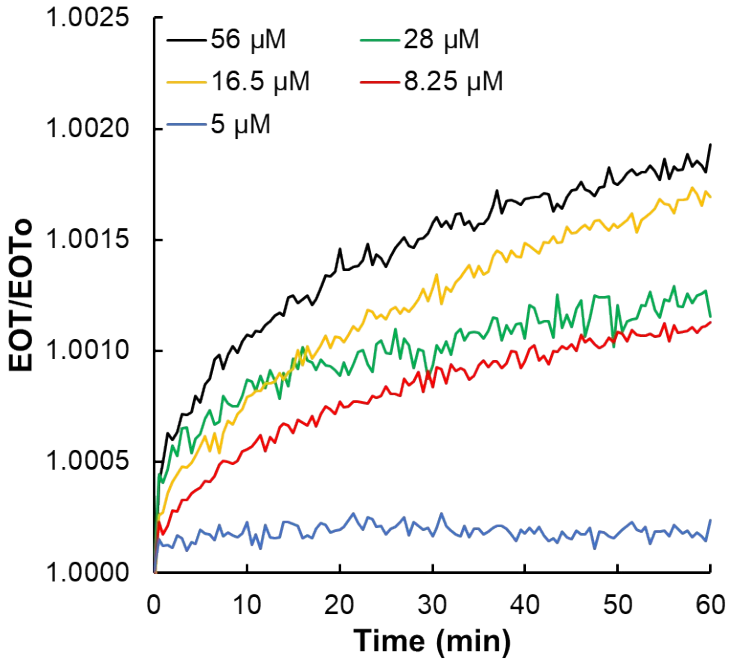

Figure S3. Relative EOT changes *vs.* time for randomly oriented antibody-biofunctionalized $PSiO_2$ upon exposure to different concentrations of his-tagged Tyrosinase (data represents an average of $n \geq 3$).

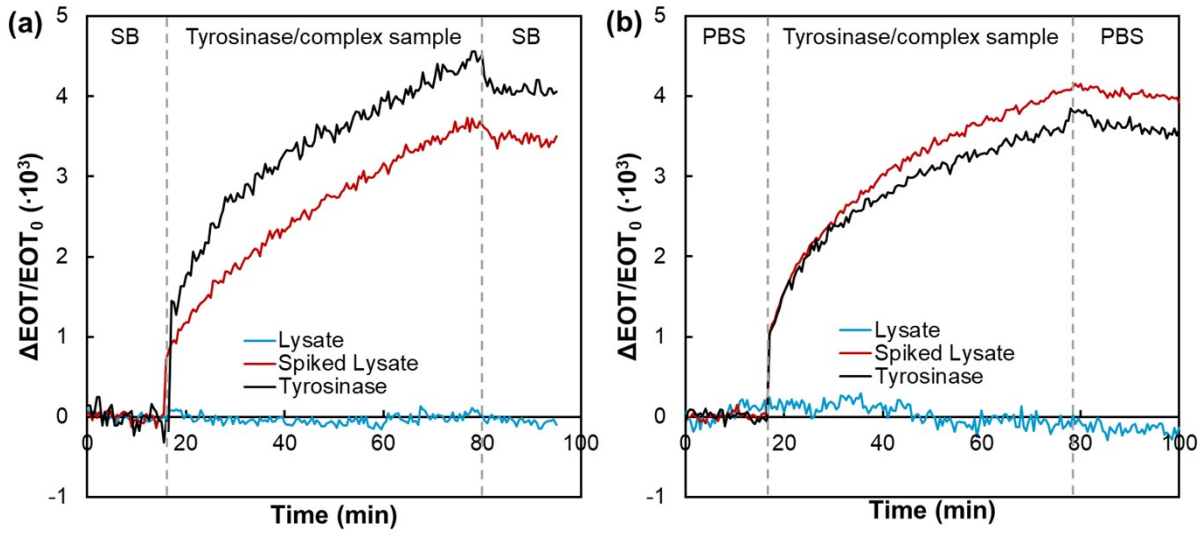

Figure S4. Characteristic relative EOT changes *vs.* time for the (a) aptasensor and (b) oriented-immunosensor upon exposure to neat bacterial lysate, bacterial lysate spiked with 16.5 µM tyrosinase and 16.5 µM tyrosinase in a buffer. SB denotes aptamer's selection buffer.

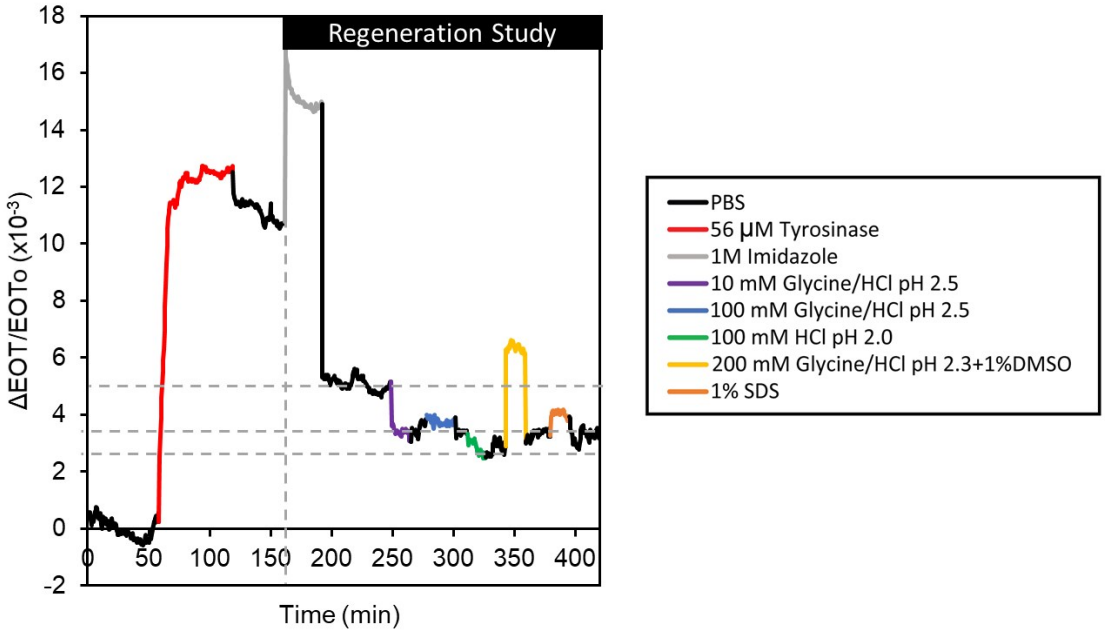

Figure S5. Relative EOT changes *vs.* time upon exposure of the oriented antibody-biofunctionalized $PSiO_2$ to 56 µM Tyrosinase, followed by washing with PBS and exposure to different regeneration solutions. Although complete regeneration to initial PBS baseline is not achieved, 1 M imidazole, 10 mM glycine/HCl pH 2.5 and 100 mM HCl pH 2.0 have the most significant regeneration effect.

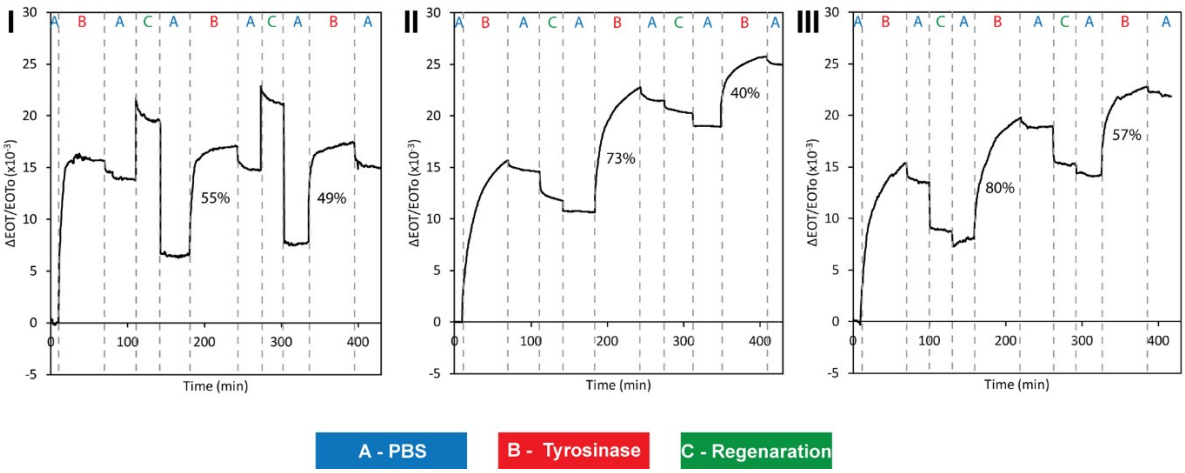

Figure S6. Relative EOT changes *vs.* time upon exposure of the oriented antibody-biofunctionalized $PSiO_2$ to 56 µM Tyrosinase in three consecutive biosensing cycles, utilizing a regeneration solution of (I) 1M imidazole, (II) 100 mM HCl pH 2.0 and (III) 10 mM glycine/HCl pH 2.5. Percentages represent biosensing signal (calculated after exposure to 56 µM Tyrosinase and wash with PBS) of the second and third cycles out of the first cycle.

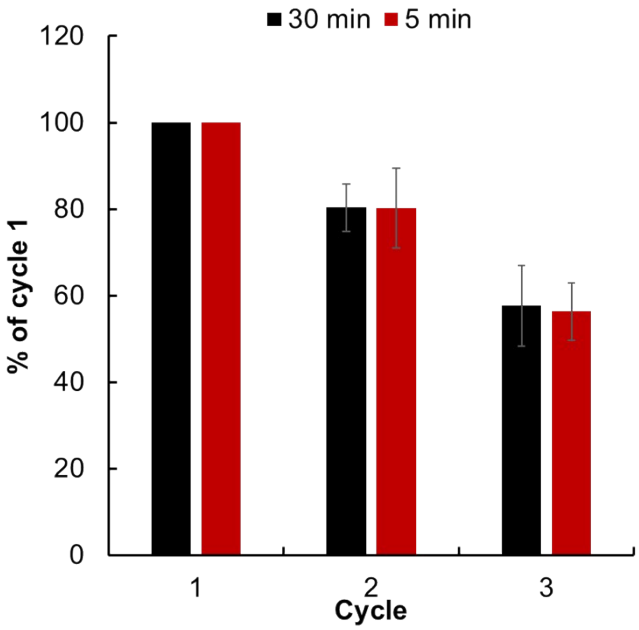

Figure S7. Comparison of a 30-min and a 5-min exposure time of the immunosensor to a regeneration solution of 10 mM glycine/HCl pH 2.5, presented as the relative signal for each biosensing cycle (presented as % of the EOT signal collected in the first biosensing cycle) (n≥3). Both regeneration periods result in similar regeneration performance.

References

1. Massad-Ivanir, N.; Friedman, T.; Nahor, A.; Eichler, S.; Bonanno, L. M.; Sa'ar, A.; Segal, E., Hydrogels synthesized in electrochemically machined porous Si hosts: effect of nano-scale confinement on polymer properties. *Soft Matter* **2012,** *8* (35), 9166-9176.