Glucose level determination with a multi-enzymatic cascade reaction in a functionalized glass chip

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Electronic Supplementary Information

1. Materials and Equipment.

On flat substrates (glass microscope slides) the thickness of PHEMA layers at various functionalization steps was measured using atomic force microscopy (AFM). Samples were measured both in air and in fluid by employing a Dimension Icon atomic force microscope with a Nanoscope V Controller (Bruker AXS, Germany) in tapping mode. The Scan AsystTM imaging mode was employed, a Bruker-proprietary imaging mode which automatically and continuously monitors the quality of the image and self-optimizes all imaging parameters by using an algorithm operating in Peak Force Tapping™. This mode permits the use of reduced imaging forces, protecting both fragile probes and samples, and increases image resolution. For measurements in air, high resolution Rotated-Tapping Mode-etched silicon probes (RTESP) with 8 nm radius of curvature, resonant frequency around 300 kHz and a nominal spring constant of 50 N/m were employed. AFM measurements in fluid were performed by wetting the film with a solution of phosphate buffer (pH=7.5, 10 mM), and use of Scan Asyst Fluid probes fabricated in Silicon Nitride, with nominal curvature radius less than 10 nm and a nominal spring constant of 0.7 N/m. All the AFM images were analysed with the free software Gwiddion, and the only correction was the background subtraction by a plane levelling, assuming the scratched region, which is uncovered by the polymer film, as reference value. The film thickness was calculated as the average of a set of 512 horizontally-levelled sampling profiles.

On oxidized silicon based samples, FTIR spectra were recorded using a Shimadzu IRPrestige 21 spectrometer. Spectra of brushes were taken in transmission mode using a bare silicon wafer as background.

2. Polymer brushes: immobilization and functionalization on oxidized silicon and glass slides

Microscope glass slides and oxidized silicon samples (for FTIR spectroscopy) were immersed in a Piranha solution (H_2SO_4: H_2O_2 3:1) for 10 min, copiously rinsed with Milli-Q water and then dried with a stream of nitrogen. Subsequently, the samples were incubated in a solution of 0.2% of 2-bromo-2-methyl-propionic acid 3-trichlorosylanyl-propyl ester in toluene over the night.[1] A solution of 20 mL 2-hydroxyethyl methacrylate (HEMA) and 20 mL water was degassed by bubbling through dry nitrogen (N2) for 30 min and transferred in a schlenk tube where it was stored under argon. Copper(I) chloride (0.110 g), copper(II) bromide (0.072 g) and 2.2'-dipyridyl (0.488g) were added. To dissolve the solid, the mixture was stirred for 10 min (while degassing), which yielded a dark brown solution. The solution was then sonicated till complete dissolution of the solid and subsequently transferred with a cannula in the schlenk tube containing glass/oxidized silicon samples. After polymerization (90 minutes at room temperature, in the dark), the samples were removed and washed with methanol and MilliQ water. The PHEMA polymer films were treated (18 h, 60 ºC) with a solution of succinic anhydride (100 mg) and triethylamine (100 µL) in 2 ml of dry tetrahydrofuran (THF). Subsequently, they were rinsed with THF and Milli-Q water and dried with a stream of nitrogen. One mL of a water solution containing 13 mg of n-hydroxysuccinimide (NHS) and 75 mg of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC)) was poured on the films and left to react for 1h. The films were rinsed with water and dried with a stream of nitrogen. Finally, a solution of phosphate buffer containing GOx and HRP (1 mg/mL) was poured on the polymer films functionalized with n-hydroxysuccinimide and incubated for 8h.

3. AFM-imaging

AFM-images of the polymer film at the surfaces after each step of the functionalization sequence are given in Figure 1. In Figure 2 AFM-images are shown of PHEMA-SA-NHS, PHMEA-GOx, PHEMA-HRP and PHEMA-GOx+HRP in liquid solution (buffer).

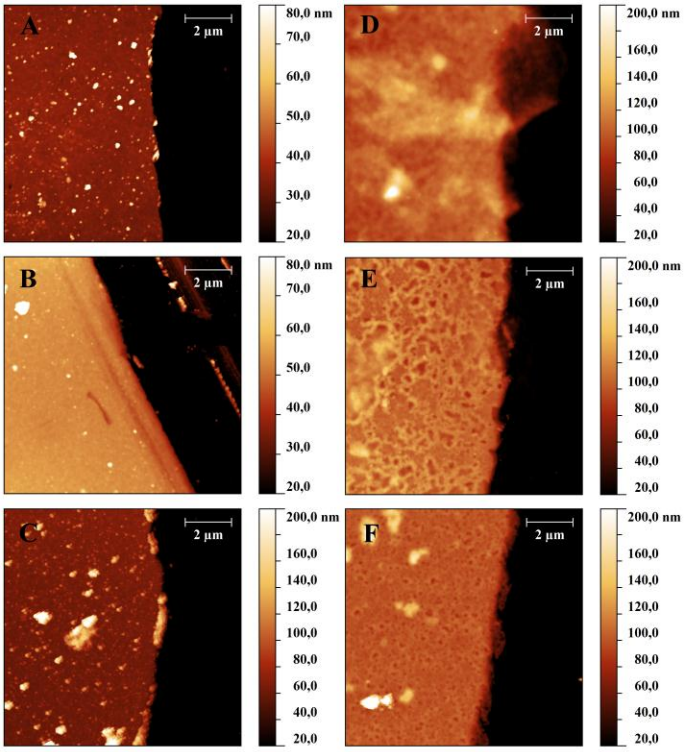

Figure 1: AFM images of the dry polymer film at each step of the functionalization sequence (90 min polymerization time) **A)** PHEMA, **B)** PHEMA after reaction with succinic anhydride (PHEMA-SA), **C)** PHEMA-SA after reaction with n-hydroxysuccinimmide (PHEMA-SA-NHS) **D)** PHEMA with immobilized HRP (PHEMA-HRP), **E)** PHEMA with immobilized GOx (PHEMA-GOx), **F)** PHEMA with co-immobilized GOx and HRP (PHEMA-GOx+HRP).

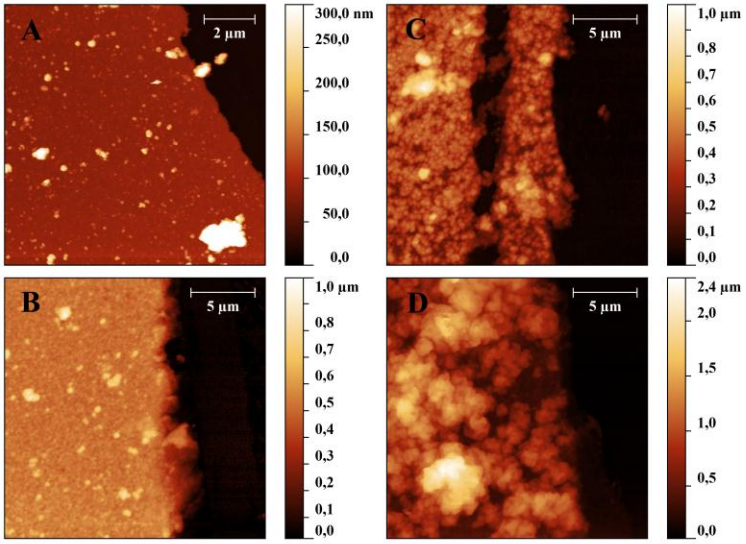

Figure 2: AFM images of the polymer film in fluid: **A)** PHEMA-SA-NHS, **B)** PHEMA-HRP, **C)** PHEMA-GOx, **D)** PHEMA-GOx+HRP.

4. FTIR-spectroscopy

FTIR analyses of the brush layer at various stages of the functionalization procedure are given in Figure 3. PHEMA (Figure 3a) shows a spectrum with two major absorption bands at 1700 and 3500 cm^{-1}, which arise from the stretching of the carbonyl and from the hydroxyl (OH) groups, respectively. As can be seen in Figure 3b, following functionalization with SA, the OH-related band disappears, confirming the reaction of the hydroxyl groups and the introduction of carboxylic acid functions along the brush structure.[2] Carboxylic acid moieties react with NHS in the presence of EDC, forming NHS-ester groups, which display two characteristic absorption peaks at 1710 cm^{-1} and 1720 cm^{-1} (Figure 3c). After reaction with HRP and GOx, the FT-IR spectrum (Figure 3d) shows 2 peaks at 1700-1600 cm^{-1} and 1600-1500 cm^{-1} that correspond to amide I and II vibration[2], confirming the successful immobilization of the HRP and GOx enzymes.

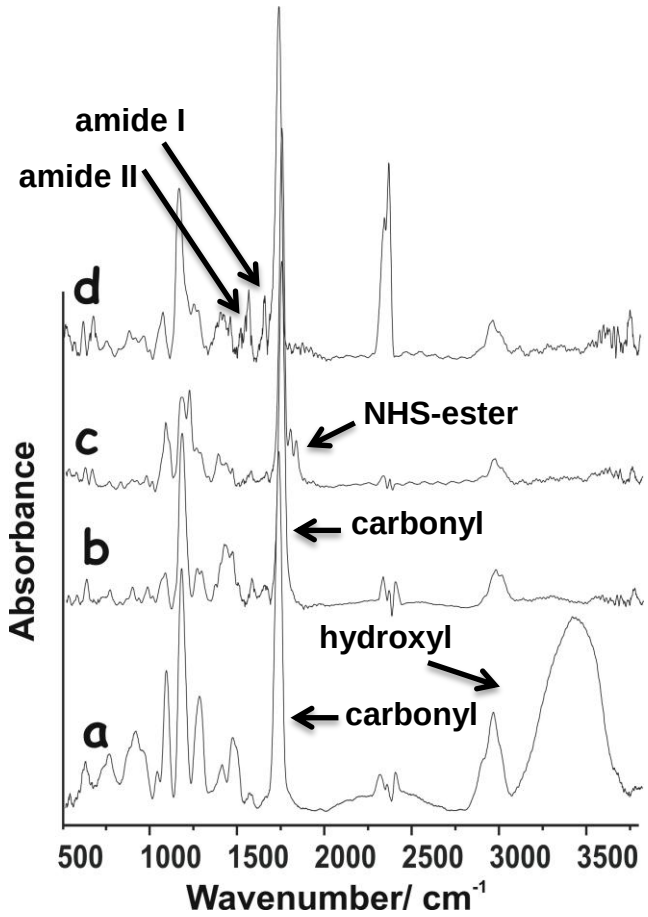

Figure 3: FTIR spectra of **a)** PHEMA, **b)** PHEMA-SA, **c)** PHEMA-SA-NHS, **d)** PHEMA-GOx+HRP.

[**1**] F. Costantini, W. P. Bula, R. Salvio, J. Huskens, H. Gardeniers, D. N. Reinhoudt and W. Verboom, *J. Am. Chem. Soc.* **2009**, *131*, 1650
[**2**] E. M. Benetti, C. Acikgoz, X. F. Sui, B. Vratzov, M. A. Hempenius, J. Huskens and G. J. Vancso, *Adv. Funct. Mater.* **2011**, *21*, 2088-2095.