Conference materials
UDC 538.975
DOI: https://doi.org/10.18721/JPM.163.181

Photo-assisted adsorption of enzyme molecules onto a surface-modified silicon substrate

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Abstract. In this work, the influence of illumination on the adsorption of enzyme molecules from an aqueous solution on a single-crystal silicon substrate with a layer of amorphous silicon (*a*-Si) is shown by atomic force microscopy. It was shown that the effect of illumination during the formation of an enzyme layer depends both on the type of Si conductivity and on the presence of an *a*-Si layer on the surface. The 2-beam interference pattern on the surface of the *n*-Si/*a*-Si structure, fabricated by illumination with a wavelength of 491 nm before the adsorption process, made it possible to fabricate ordered rows of the precipitated enzyme. This pattern not observed for *p*-Si/*a*-Si structure or bare substrate of single-crystal Si without the amorphous silicon layer. The developed technique is promising for the fabrication of multienzyme coatings for multiplex analysis using silicon transducer.

Keywords: atomic force microscopy, amorphous silicon, surface charge, enzyme, layer-by-layer adsorption

Funding: The research was carried out at the expense of the Russian Science Foundation grant No. 22-22-00194, https://rscf.ru/project/22-22-00194/.

Citation: Kozlowski A.V., Serdobintsev A.A., Stetsyura S.V., Photo-assisted adsorption of enzyme molecules onto a surface-modified silicon substrate, St. Petersburg State Polytechnical University Journal. Physics and Mathematics. 16 (3.1) (2023) 444–448. DOI: https://doi.org/10.18721/JPM.163.181

Материалы конференции
УДК 538.975
DOI: https://doi.org/10.18721/JPM.163.181

Фотоассистированная адсорбция молекул фермента на модифицированную поверхность кремниевой подложки

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Аннотация. В рамках данной работы методом атомно-силовой микроскопии показано влияние света на адсорбцию молекул ферментов из водного раствора на подложку из монокристаллического кремния со слоем аморфного кремния (*a*-Si). Показано, что эффект от освещения при формировании слоя ферментов зависит как от типа проводимости Si, так и от наличия на поверхности слоя *a*-Si. Двухлучевая интерференционная картина на поверхности структуры *n*-Si/*a*-Si, созданная светом с длиной волны 491 нм до процесса адсорбции, позволила создать упорядоченные ряды осажденного фермента. Это не удалось сделать на структурах *p*-Si/*a*-Si или подложке монокристаллического Si без слоя аморфного кремния. Разработанная методика перспективна для создания мультиферментных покрытий для мультиплексного анализа с помощью кремниевых трансдьюсеров.

Ключевые слова: атомно-силовая микроскопия, аморфный кремний, поверхностный заряд, фермент, послойная адсорбция

Финансирование: Исследование выполнено за счет гранта Российского научного фонда № 22-22-00194, https://rscf.ru/project/22-22-00194/.

Ссылка при цитировании: Козловский А.В., Сердобинцев А.А., Стецюра С.В. Фотоассистированная адсорбция молекул фермента на модифицированную поверхность кремниевой подложки // Научно-технические ведомости СПбГПУ. Физико-математические науки. 2023. Т. 16. № 3.1. С. 444–448. DOI: https://doi.org/10.18721/JPM.163.181

Introduction

Structures based on silicon and a buffer polyelectrolyte layer are promising for a potentiometric bio- and chemosensors, so-called electrolyte-insulator-semiconductor (EIS) sensor [1]. Since polyelectrolyte molecules in solution are ionized and have an effective charge, the layer-by-layer adsorption (LbL) technique developed by Decher [2] can be used to fabricate such structures. This technology also allows us to fabricate an enzymatic layer on the transducer surface [3]. Enzyme molecules are catalysts for specific chemical reactions in living systems and can be used as a sensitive layer in EIS-biosensors.

We are investigating glucose oxidase (GO_x) as a model enzyme. GO_x releases electrons during interaction with glucose. Its leads to equivalent change of silicon surface charge. Multilayer multi-enzyme coatings can be fabricated by LbL adsorption [4]. However, EIS-sensors detect changes in charge in solution only within the order of the Debye screening length from the surface [5]. The electrostatic coupling between an ionized enzyme molecule and the transducer strongly depends on the ionic strength of the solution as well as the distance between the charge of the molecule and the transducer surface. Thus, there are problems of multiplexed assays of different chemical analytes as well as signal amplification.

In this work we suggest another immobilization method of enzyme molecules by means of photo-assisted adsorption and an additional nanolayer of amorphous silicon (*a*-Si) on a single-crystal Si substrate (*c*-Si). Previously, an effect of light on GO_x molecules adsorption onto a silicon substrate was demonstrated [4], i.e. illumination during adsorption and Si conductivity type can control the adsorption of GO_x molecules. However, the previously used method did not allow one to localize the enzyme adsorption to regions of submicron width. In this study, we attempted to solve this problem by illuminating the substrate before immersing it in the enzyme solution. This makes it possible to reduce the scattering of the laser beam by the enzyme solution. In order for the illumination effect (changed surface potential of the silicon structure in the illumination region) to be preserved for the time of enzyme adsorption, *a*-Si was deposited on the *c*-Si surface. It is known [6] that the electron mobility of *a*-Si is 100 times less than their mobility in a single crystal. Therefore, the developed new algorithm for photo-assisted enzyme adsorption can make it possible to fabricate ordered structures of enzyme molecules using well-known photolithography setups without photoresist. Also, this approach can lead to the fabrication of an ordered multi-enzyme monolayer and solve the problem of signal attenuation due to the Debye screening length.

Materials and Methods

The experiments were performed with (100)-oriented single-crystalline Si wafers of *n*- and *p*-type. Prior to experiments, the substrates were boiled in peroxide–ammonia solution ($NH_4OH/H_2O_2/H_2O$ = 1:1:4 vol.) at 75 °C and rinsed in deionized water. According to [7], ammonia solution removes native oxide from the silicon surface, while H_2O_2, on the contrary, produces oxidation of silicon. As a result, this treatment produces "renewal" of the native oxide layer. A layer of amorphous silicon (*a*-Si) was deposited by direct current magnetron sputtering method (Angstrom Nexdep, Angstrom Engineering). The thickness of *a*-Si layer was 100 nm.

Then, using a system of mirrors, half wave plate and a polarizer, two linearly horizontally polarized beams were created (Fig. 1, *a*), which produce an interference pattern with a grating period of 2 μm on the silicon structure surface. We used a laser with wavelength of 491 nm and an intensity value of 10 mW/cm^2.

After Irradiation for 60 min, the samples were placed the enzyme solution. The deposition time was 10 min. The glucose oxidase (GO_x, type X-S, Sigma Aldrich Co.) enzyme was used. GOx molecules were dissolved in deionized water to a concentration of 0.1 mg/mL. After GO_x adsorption, the samples were rinsed in deionized water and dried in nitrogen.

The obtained samples were studied by atomic force microscopy (AFM) by NTEGRA Spectra (NT-MDT Spectrum Instruments) before and after enzyme adsorption. The Kelvin probe technique was used to measure the contact potential difference (CPD) using a device "ΔΦ" (Bespoke GmbH, Germany) with a gold grid.

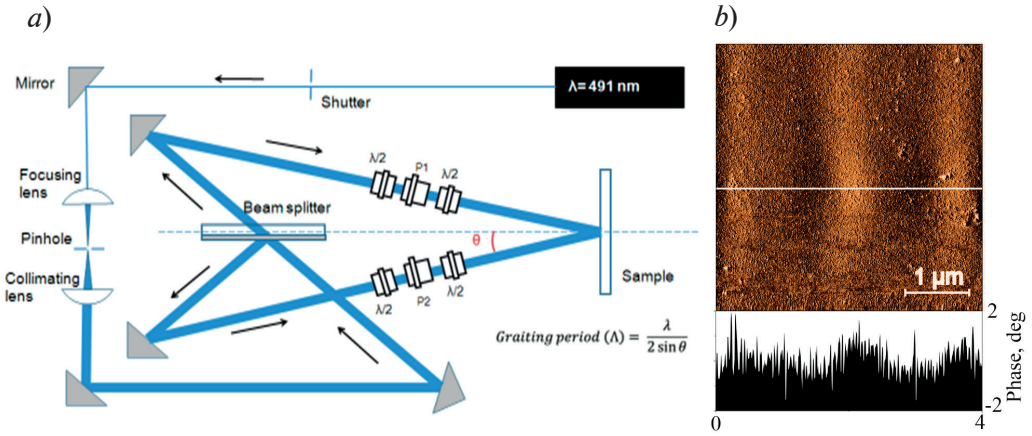

Fig. 1. Experimental setup (*a*) and phase contrast image (*b*) of the *n*-Si/*a*-Si/GO_x structures surface irradiated for 1 hour until the GO_x adsorption with an interference pattern at an intensity of 10 mW/cm^2. λ/2 – half wave plates; P1, P2 – polarizers

Results and Discussion

Fig. 2, *a* shows that charge relaxation in the structures free of *a*-Si layer proceeds rapidly and the initial values of surface potential φ are almost "instantaneously" restored. As expected, φ variations in *c*-Si/*a*-Si structures (Fig. 2, *b*) are more "delayed" upon the light switching both on and off. However, the CPD curve as a function of time for *p*-Si/*a*-Si structure initially exhibits sharp "jumps" of φ with subsequent slow decrease (Fig. 2, *b*, region I) or increase (Fig. 2, *b*, region II). In contrast, no sharp jump of φ is observed in *n*-Si/*a*-Si structures and their illuminance leads to a continuous decrease in φ as from the moment the light is turned on (Fig. 2, *b*, region I). In about 1 *h* after the light switch-off, the degree of φ relaxation does not

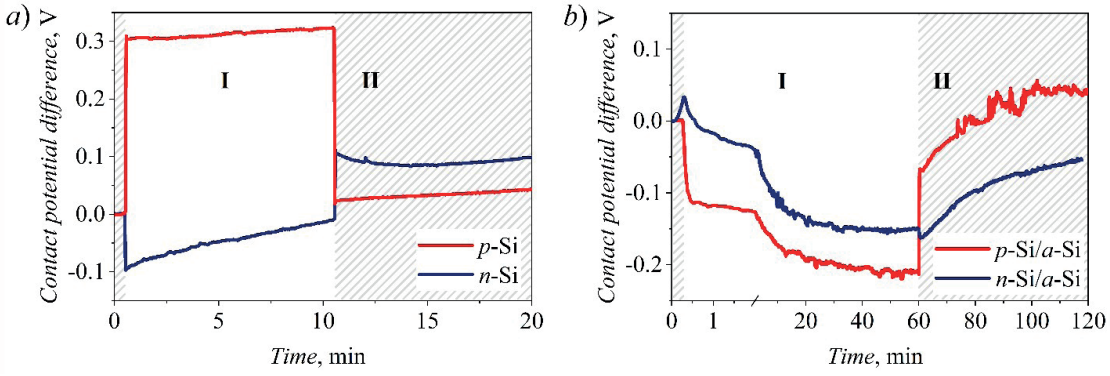

Fig. 2. Contact potential difference measurements as a function of time for structures based on *n*-Si and *p*-Si (*a*) without *a*-Si layer and (*b*) with *a*-Si layer. Region I corresponds to illumination, while region II shows measurements in dark

exceed 50% of the initial value. That is, φ relaxation for 10–15 min (i.e., for a time sufficient for GO_x adsorption) amounts to only about 15–16%.

Fig. 1, *b* illustrate phase contrast after 1 hour irradiation at intensity of 10 mW/cm^2 using an interference pattern and subsequent GO_x adsorption from the aqueous solution. It can be seen that in the phase contrast image there is a change in the morphology of the *n*-Si/*a*-Si/GO_x structure corresponding to the interference pattern period, transmitted by the grating period.

In order to identify irregularities in the AFM image corresponding to molecules of the adsorbed enzyme, GO_x molecules were also adsorbed from an aqueous solution onto a freshly cleaved mica surface, which was exposed to a laser pattern similar in time and intensity. According to the AFM images (Fig. 3), the deposition of enzyme molecules significantly increases the roughness of mica surface. In this case, the irregularities height is comparable to the size of the GO_x molecule [8]. It should be noted that after GO_x deposition on a mica, the aggregations with diameter of about 40 nm appear. This lateral dimension does not correspond to the true size of the enzyme molecule. This is due to the "expansion" effect. The AFM images of GO_x molecules with four different tip radius were simulate in [9]. As the tip radius increases, the observed lateral dimensions exceed the real size of the molecule significantly and there is no possibility to resolve neither the structural details nor the individual GO_x monomers. The calculated images shown in [9] are consistent with the experimental AFM images, giving an estimated tip radius of about 10 nm. Such processing of the AFM image made it possible to recognize individual GO_x molecules in the AFM image (Fig. 3, *a*). In contrast to the image in Fig. 1, *b*, there are no irregularities in Fig. 3, *b* that correspond to the interference pattern of illumination with a period of 2 μm.

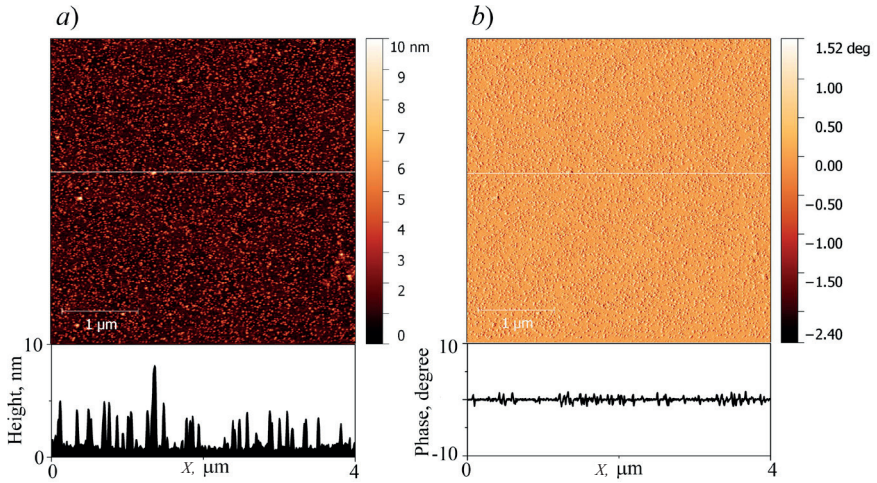

Fig. 3. AFM-image (*a*) and phase contrast (*b*) of GO_x molecules deposited onto freshly cleaved mica surface

Conclusion

Thus, within the framework of this work, the influence of light on the adsorption of enzyme molecules from an aqueous solution onto a single-crystal silicon substrate with an amorphous silicon layer was demonstrated by AFM. It was shown that the photo-memory effect observed for *n*-Si/*a*-Si structures. Thus, the 2-beam interference pattern on the surface of the *n*-Si/*a*-Si structure before the GO_x adsorption process leads to ordered rows after GO_x deposition. This effect is not an artifact of the AFM-image or the *a*-Si crystallization and it could not be detected in the case of *p*-Si/*a*-Si structures, or bare *p*-Si and *n*-Si. Thus, experiments with illumination through a mask are necessary. In the future, it is necessary to search for the optimal values of the ionic strength and pH of the solution during the GO_x adsorption onto the *a*-Si surface in order to improve the adsorbing properties of illuminated areas and increase the contrast of AFM images. After optimization, the developed approach can enable the production of multi-enzyme coatings for multiplex analysis.

Acknowledgments

This study was funded by the Russian Science Foundation (project No. 22-22-00194, https://rscf.ru/en/project/22-22-00194/).

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Received 10.07.2023. Approved after reviewing 17.07.2023. Accepted 18.07.2023.