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Lightly doped crystallized silicon (LDCS) film with coplanar electrodes for bio-sensing application through current-voltage, impedance and surface potential measurements: Detection of proteins and antibody

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Abstract:

A ~50nm-thick polycrystalline Si film (lightly doped crystallized silicon (LDCS)) doped with phosphorous atoms of 4.3 x 10^{17}/cm^3 was formed on quartz substrate using plasma enhanced CVD. Current-Voltage (I-V) and impedance as a function of frequency was studied to detect the proteins and antibody and are reported in this paper. From I-V measurements, the conductance was obtained and for without protein (WOP) and with the adsorption of protein A the values are 25 pS and 943 pS respectively. The temperature dependence of conductance is high for protein A when compared to LDCS film and the activation energy obtained from its temperature variation is 0.51 eV for protein A adsorption when compared to LDCS film (0.59 eV). From Cole-Cole plot, the total resistance of the LDCS film, after adsorption of SBP, protein A and antibody are 1.17 MΩ, 1.74 MΩ, 1.94 MΩ and 0.88 MΩ. LDCS film respectively. The LDCS film with coplanar electrodes can be used to detect significantly the proteins and antibody adsorption and this can be used to design a conductance or impedance based bio-sensing device. From KFM measurements, the value of surface potential for without and after adsorption of protein A (with concentration 10 mg/l) on p-Si(100) surface are 64 mV and 895 mV respectively.

Keywords: Lightly doped crystallized film, Impedance, Silica binding protein, protein A, Antibody, bio-sensing device.

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1. Introduction:

Over the many decades researchers have been carrying out research on the development of the biosensors for detection of bio-molecules like proteins, RNA's, DNA's, antibodies etc for the diagnosis of the health problems that are facing by humans. The detection of protein A, green fluorescent protein (GFP) and luciferace are also important as these proteins have strong affinity to antibodies produced in the body due to any kind of illness. These biosensors are usually fabricated using inorganic as the active sensing materials [1-3]. Of all inorganic materials silicon is semiconducting in nature and this material in thin film or nano wire form can be used to sense the bio-molecules with varying sensitivity. Since silicon is semiconducting material it has varying resistance or conductance depending up the type of form it is used for example in crystallized Si film, porous silicon, Si nano-wire etc [4-7] which makes it possible for the variety of bio-molecules to be detected. Silicon based devices are best suited because they have appropriate conductance in thin film or nano-wire form which makes it possible to detect the change in conductance due to diffusion of charge on proteins or any kind of bio-molecules in to the thin film or nano-wire of silicon. These bio-molecules or proteins can be detected by measuring the current through I-V measurements, resistance through impedance measurements or conductance through I-V measurements of this Silicon based material. In view of the above, lightly doped crystallized silicon (LDCS) film on quartz is used for the present investigation. The studies have been carried out to check whether lightly doped crystallized silicon films on quartz with resistance of the film around 1 MΩ laterally between two coplanar electrodes would be able to detect the adsorption of proteins and antibody. In the present study I-V and

impedance and Kelvin probe force microscopy (KFM) measurements have been carried out with and without proteins. The present study gave positive results and those results are presented and discussed in this paper.

2. Experimental procedure:

A ~50nm-thick lightly doped crystallized Si film doped with phosphorous atoms of 4.3 x $10^{17}/cm^3$ was formed on quartz substrate using plasma enhanced Chemical vapour deposition (CVD). The deposited films were crystallized using furnace at 600^{o}C for 24 hrs. The crystallization of films was inferred from the color change of the silicon film deposited. After crystallization, the surface was then treated with a 0.1% HF solution to form H-termination on the poly-Si surface in order to make it inert to not to form SiO_2 very easily if when exposed to air. For electric measurements, co-planar Al electrodes were evaporated on the films in a gap-cell structure with a size of 10 mm in gap and 5 mm in width. Si-tagged proteins or silicon binding protein (SBP) were dropped in between the co-planar electrodes. Si-I-V and impedance measurements were carried out using Agilent 1500 A semiconductor Device analyzer.

Checker patterns with ~10×10 μm in size were fabricated on a p-Si(100) wafer by electron beam lithography and wet etching of ~10nm-thick SiO_2 grown at 1000 ºC. The surface was treated by a 0.1% HF solution to form surface Si-OH bonds on the SiO_2 pattern and to make H-termination on the bare Si surface. Protein A was adsorbed on to the checker pattern with adsorption time of 30 sec and subsequently rinsed with pure water for 10 sec at room temperature and then dried with nitrogen. Surface potential measurements were carried out using Nano Navi Dynamic force microscopy/Atomic force microscopy/Kelvin force microscopy (DFM/AFM/KFM) system. The surface measurements were carried on both SiO_2 and p-Si(100) surface to check whether proteins has affinity to SiO_2, other surface than Si or not.
The concentration of protein were prepared by using 20mM Hepes-NaOH (pH 7.5), 1mM Ethylene diamine tetra acidic acid, 1mM Dithiothreitol, 20% Gylcerol, 0.7 M NaCl) buffer solution.

3. Results and discussion:

Figure 1. Shows the schematic diagram of the lightly doped crystallized Si with coplanar electrodes which are under investigation. Figure 2(a & b) shows the variation of current with voltage for lightly doped crystallized silicon (LDCS) with coplanar electrodes and with the adsorption of proteins and without proteins (WOP) measured instantaneously after adsorption of proteins (i.e. after 0 months) and after 2 months of adsorption of proteins respectively. The adsorption of proteins after two months once it has been adsorbed has been studied to check whether they can be detected after such long time. The variation of current with voltage increases and is linear for LDCS film and after adsorption of proteins. The maximum current (I_{max}) at +10 V is 0.25 nA for LDCS film without proteins whereas after adsorption of protein A the I_{max} is 9.42 nA which is around 40 times higher than WOP sample. Similarly with the adsorption of green fluorescent protein (GFP) and luciferace, the value of I_{max} at +10 V is 2.21 nA and 3.70 nA. There is increase of slope with the adsorption of proteins on LDCS film. This result indicates that the proteins has some charge or ions and these charges or ions has the effect of enhancing the measured current with applied voltage and also increases their slope depending up on the charge of proteins. Higher is the charge on the proteins, higher is the diffusion/current and slope of I-V curve. The above result indicates that the charge on the proteins gives rise to noticeable change in current in LDSC film which in turn has nA level current at the highest applied voltage (10V) and the referred voltage is just for reference. This result indicates that LDSC sample with coplanar electrodes with nA level current between the electrodes with applied voltage can be used to easily detect the protein A, GFP and luciferace whose concentration is 5mg/l. From the slope of the current-voltage plots the conductance (G(S)) of the LDCS film and after attachment of proteins are estimated and are shown in Fig. 2c and Table 1. The conductance of the LDCS film without proteins is 25 pS and after the adsorption of protein A, GFP and luciferace are 843 pS, 221 pS and 371 pS respectively. There is decrease of conductance for the LDCS film for proteins which are measured after two months of adsorption time and these values are shown in Table 1. This may be due to drying of the adsorbed protein on LDCS film which in turn makes the diffusion of ions or charges to decrease leading to the decrease of conductance value.

Figure 3(a & b) shows the I-V plots at different temperatures for LDCS film without protein and with the adsorption of protein A respectively. These are measured to know the activation energy for conduction. With the increase of temperature the slope and the current at applied voltage increased indicating increase of channel conductivity. In the measured voltage range and temperature, the channel conductance is higher for with protein A adsorption. From the slope of the I-V curves the conductance is estimated and are plotted as function of log(G) vs. 1000/T as shown in Figure 3 c. The slope of log(G) vs. 1000/T (Arrhenius plot) gives the activation

energy for conduction and this is obtained from the equation of Arrhenius relation for conduction i.e. $G(T)=G_0*\exp(-E_{act}/(K_B*T))$. In this equation G_0 is the preterm conductance, E_{act} is the activation energy for conductance, K_B is the Boltzmann constant and T is the temperature in Kelvin. The activation energy for conductance for LDCS film without protein is 0.59 eV and with the adsorption of protein A is 0.51 eV. This indicates that the adsorption of protein A on LDCS film changes the activation energy of LDCS sample by the diffusion of charges on protein A in to the lightly doped crystallized silicon film. From the decrease in the activation energy from conduction it can be inferred that the charges or the ions on the protein A are negatively charged which leads to decrease in activation energy with the diffusion of these ions in to the LDCS film.

Figure 4a shows the variation of real part of impedance (Z') with frequency with the adsorption of proteins. There is no significant change in real part of impedance with frequency for with and without proteins. In this case the proteins are silica binding protein (SBP), protein A, and antibody. The real part of impedance gives information about the space charge effects related to electrodes. Since there is no change in real part of impedance with frequency for with and without proteins and antibody indicating that the space charge effects are negligibly small. Whereas there is change in peak frequency i.e resonance frequency with and without adsorption of proteins as shown in Figure 4b in imaginary part of impedance (Z"). The imaginary part of the impedance gives the information about the type of charges and their relaxation over all. The relaxation frequencies are tabulated in Table 2. The relaxation frequency for SBP, SBP/protein A is lower when compared to LDCS film (WOP) and it is higher for antibody indicating the type of the charges. This can be further elucidated from the Cole-Cole plots. Figure 4c shows the Cole-Cole plot for the above proteins. It can be seen that there is extended semicircle with varying chord indicating the change in total resistance after the adsorption of proteins as shown in Table 2. The chord represents the total resistance of the film after adsorption of proteins. Firstly the total resistance of the LDCS film have lower value (1.17 MΩ) and it increases with the adsorption of SBP (1.74 MΩ) and after adsorption of protein A on to this SBP it further increases to 1.94 MΩ as shown in Figure 5. Adsorption of antibody on to the SBP/Protein A decreases the total resistance of LDCS film to 0.88 MΩ. This indicates that the type of charge is opposite on the SBP, protein A is different from that of antibody. The above results indicate that detection of antibody through impedance measurements can be useful to design a device for bio-sensing application and detection of antibody is important to know the disease and to diagnose it. The above studies are preliminary and further studies are needed to know the variation with concentration and type of antibodies in particular and their number density and to standardize for different kind of antibodies related to different kind of illness in humans and for diagnosing process. The other studies can be C-V measurements and density functional theory studies.

The alternate methods to measure the bimolecular and chemical species reported by other researcher are by nano wire based transistors [8]. But this requires complex method to prepare nano wires. The present study is the simple way to do so and is cost effective.

Figure 6(a-e) shows the surface potential images taken on checker pattern of SiO_2 and p-Si(100) surfaces for without protein (WOP) and with different concentration of protein A. The surface potential is the work function difference between the Rh tip and the sample surface under investigation [9] which is measured using Kelvin Probe Force Microscopy (KPFM). Significant change in surface potential images were observed with the increase of concentration and the value of surface potential increases with the increase of concentration and these values are shown in Figure 7 and Table 3. The value of surface potential on SiO_2 and p-Si(100) surface for protein A with concentration 50 mg/l are 805 mV and 895 mV respectively where as for without protein A adsorbed on to the surfaces results in surface potential of 45 mV and 64 mV respectively for SiO_2 and p-Si(100) surfaces on checker pattern. These results indicates that surface potential measurements using Kelvin probe microscopy is one tool to detect the adsorption of proteins on SiO_2 and p-Si(100) surfaces and this is extension of the studies which we have carried out and reported by us earlier [10].

4. Conclusions:

Lightly doped crystallized silicon film was formed on the quartz substrate. This film with coplanar electrodes was used to study the I-V and impedance measurements to detect the adsorption of proteins on LDCS film. I-V measurements indicates that there is 40 times enhancement in the channel current with voltage with the adsorption of Protein A. The temperature dependence of conductance is high for protein A when compared to LDCS film and the activation energy obtained from its temperature variation is 0.51 eV for protein A adsorption when compared to LDCS film (0.59 eV). From Cole-Cole plot, the total resistance of the LDCS sample have lower value (1.17 MΩ) and it increases with the adsorption of SBP (1.74 MΩ) and after adsorption of protein A on to this SBP if further increases to 1.94 MΩ. Adsorption of antibody on to the SBP/Protein A decreases the

total resistance of LDCS sample to 0.88 MΩ. From KFM measurements, the value of surface potential on SiO_2 and p-Si(100) surface for protein A with concentration 50 mg/l are 805 mV and 895 mV respectively where as for without protein A adsorbed surfaces results in surface potential of 45 mV and 64 mV respectively for SiO2 and p-Si(100) surfaces on checker pattern. This result indicates that LDCS film with coplanar electrodes can be used to detect the proteins and antibody adsorption significantly and this can be used to design a conductance or impedance based bio-sensing device.

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Conflict of interest: The authors declare that they have no conflict of interest.

References:

[1]. Kim Y I,Park T S, Kang J H, Lee M C, Kim J T and Park J H et al 2006 *Sens. and Actuators B* **119** 592
[2]. Jong-in Hahm and Charles M. Lieber 2004 *Nano Lett.* **4** 51
[3]. Yi Cui, Qingqiao Wei, Hongkun Park and Charles M. Liebe 2001 *Science* **293** 1289
[4]. Andrea M. Rossi, Lili Wang, Vytas Reipa and Thomas E. Murphy 2007 *Biosens. Bioelectron.* **23** 741
[5]. Inkyu Park, Zhiyong Li, Xuema Li, Albert P. Pisano and R. Stanley Williams 2007 *Biosen. Bioelectron.* **22** 2065
[6]. Toshiya SAKATA, Masao KAMAHORI and Yuji MIYAHARA 2005 *Jpn. J. Appl. Phys.* **44** 2854
[7]. Toshiya Sakata, Izumi Makino, Sayaka Kita and Yuji Miyahara 2008 *Microelectron. Eng.* **85** 1337
[8]. Yi Cui,Qingqiao Wei, Hongkun Park,Charles M. Lieber, 2001 *Science* **293** 1289
[9]. Wikipedia contributors. (2022, July 11). Kelvin probe force microscope. In *Wikipedia, The Free Encyclopedia*. Retrieved 11:40, August 24, 2022, from https://en.wikipedia.org/w/index.php?title=Kelvin_probe_force_microscope&oldid=1097495861
[10]. Mahboob S, Makihara K, Ohta A, Higashi S, Hata Y and Kuroda A et al 2009 *ECS Trans.* **19** 35

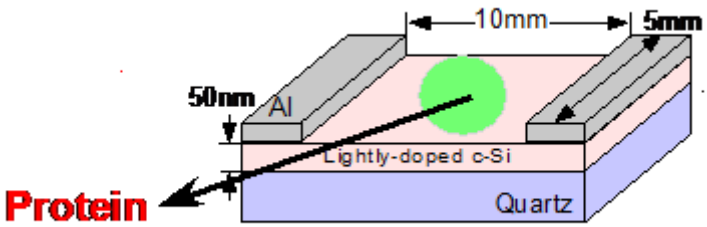

Figure 1. Schematic diagram of the lightly doped crystallized Si with coplanar electrodes.

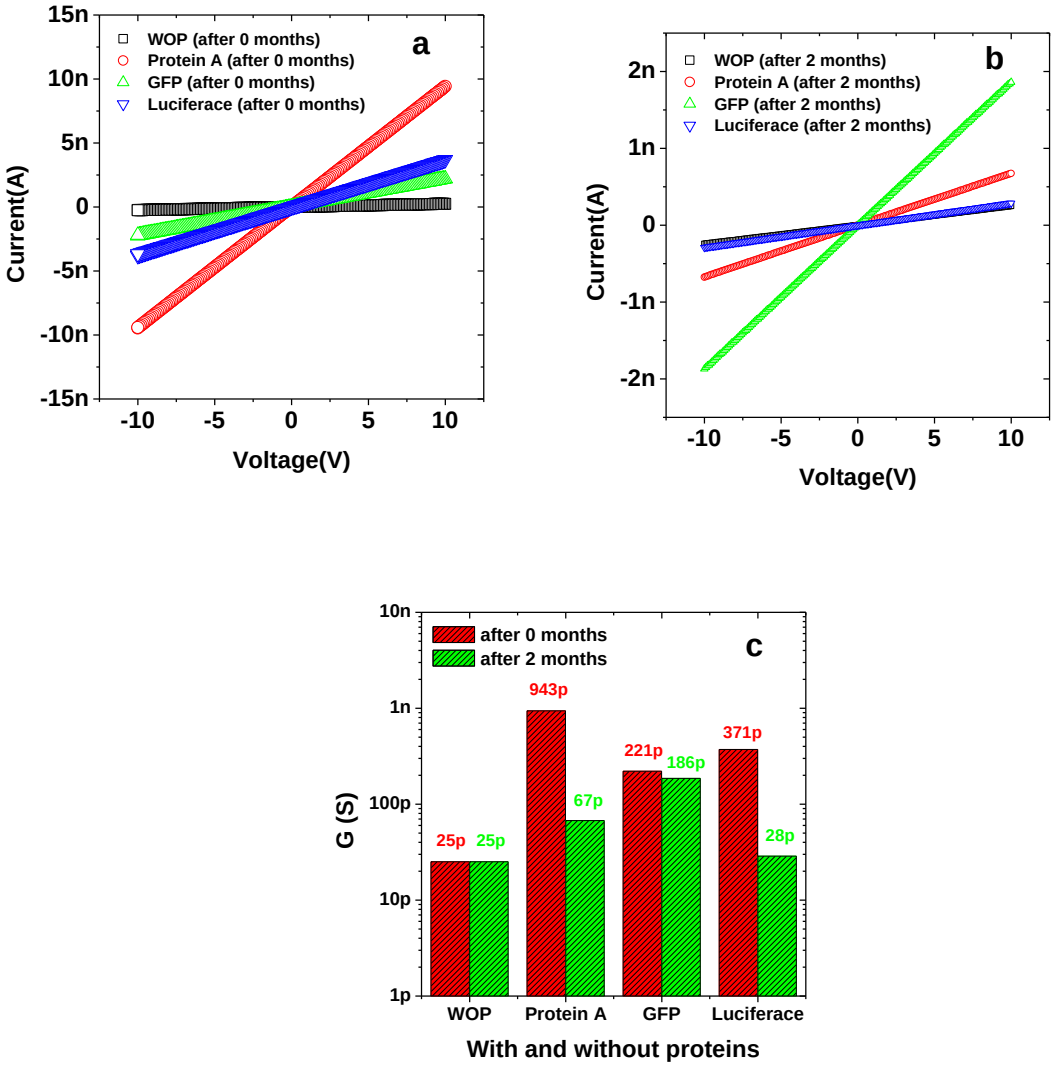

Figure 2. (a) Current vs. Voltage plot for with and without proteins on lightly doped crystallized silicon film which were measured instantaneously after adsorption of proteins (b) Current vs. Voltage plots after 2 months of adsorption time of proteins and (c) Conductance plots for with and without proteins.

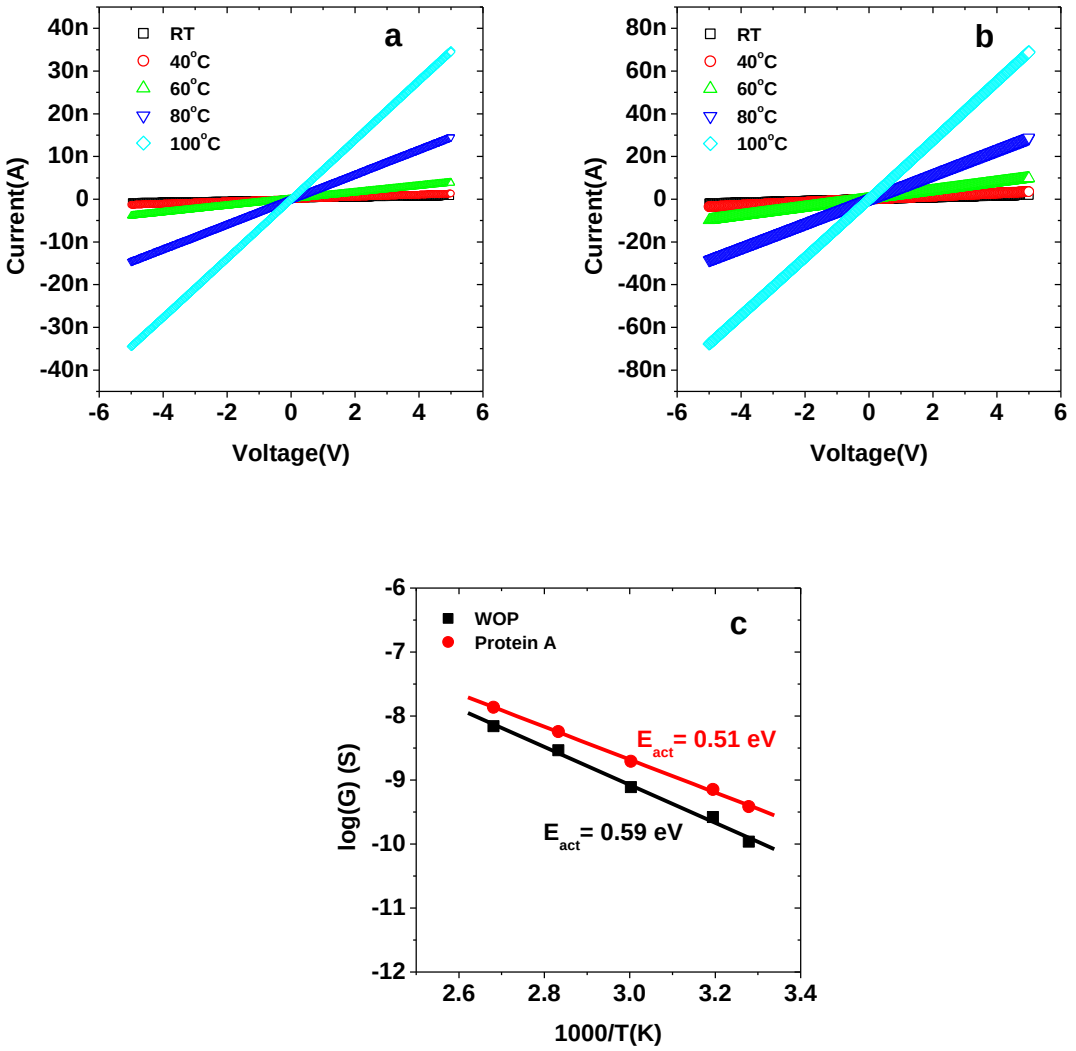

Figure 3. (a & b) Current vs Voltage plots at different temperatures with Protein A adsorption on lightly doped crystallized silicon film and (c) log(G) vs. 1000/T for lightly doped crystallized silicon film with and without protein A.

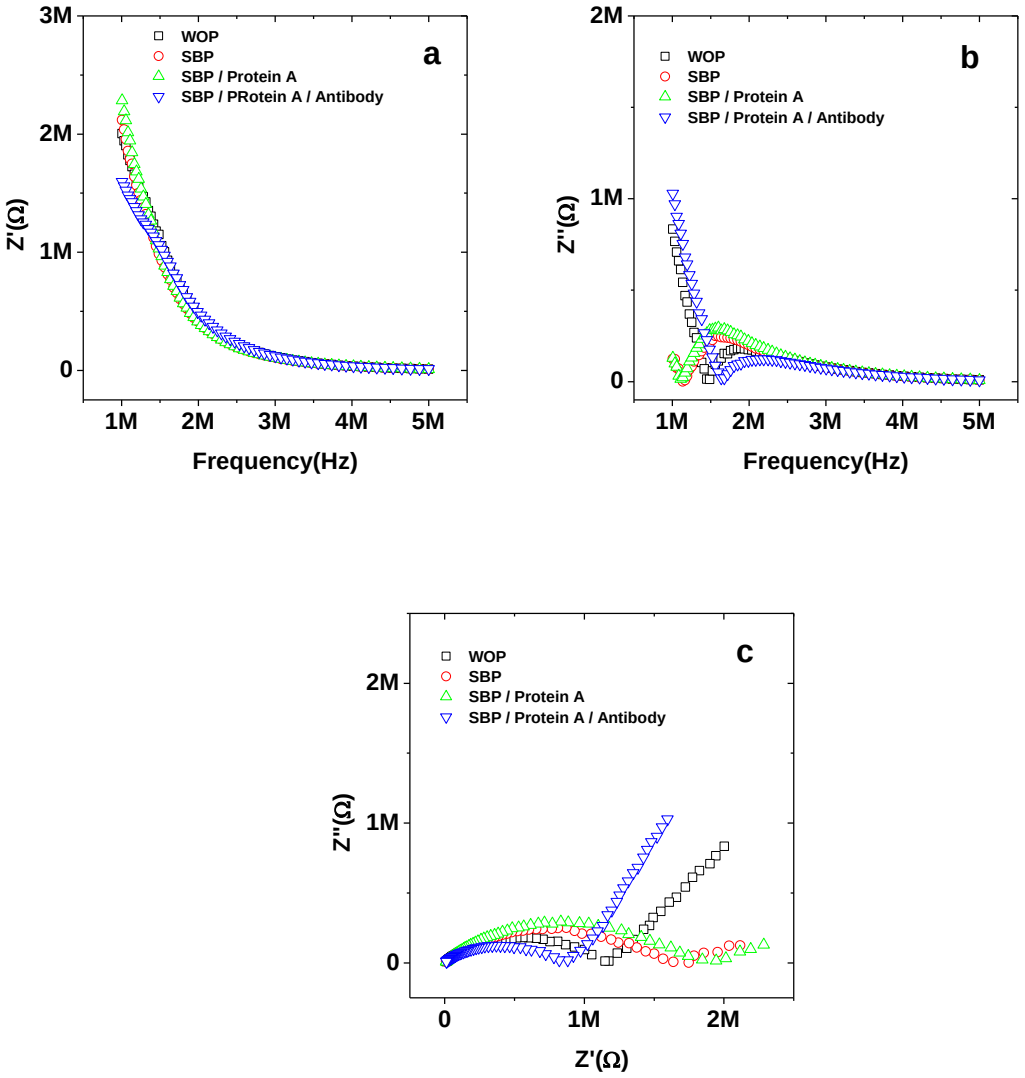

Figure 4. (a) Z' vs. Frequency (b) Z" vs. Frequency and (c) Z" vs. Z' (Cole-Cole) plots for with and without proteins and antibody on lightly doped crystallized silicon.

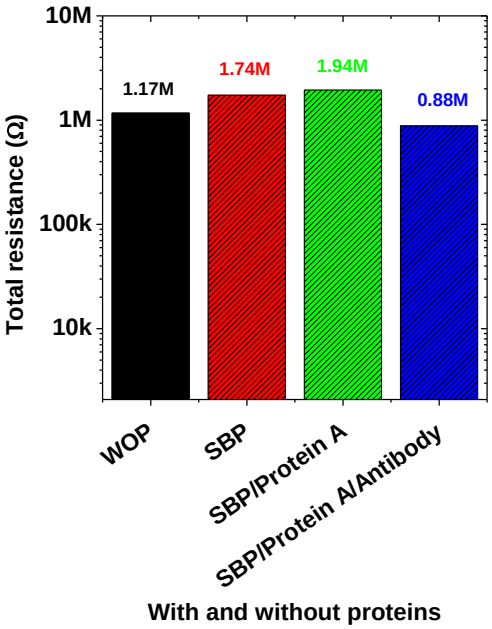

Figure 5. Total resistance for with and without proteins and antibody obtained from the Cole-Cole plots (Z" vs. Z' plots).

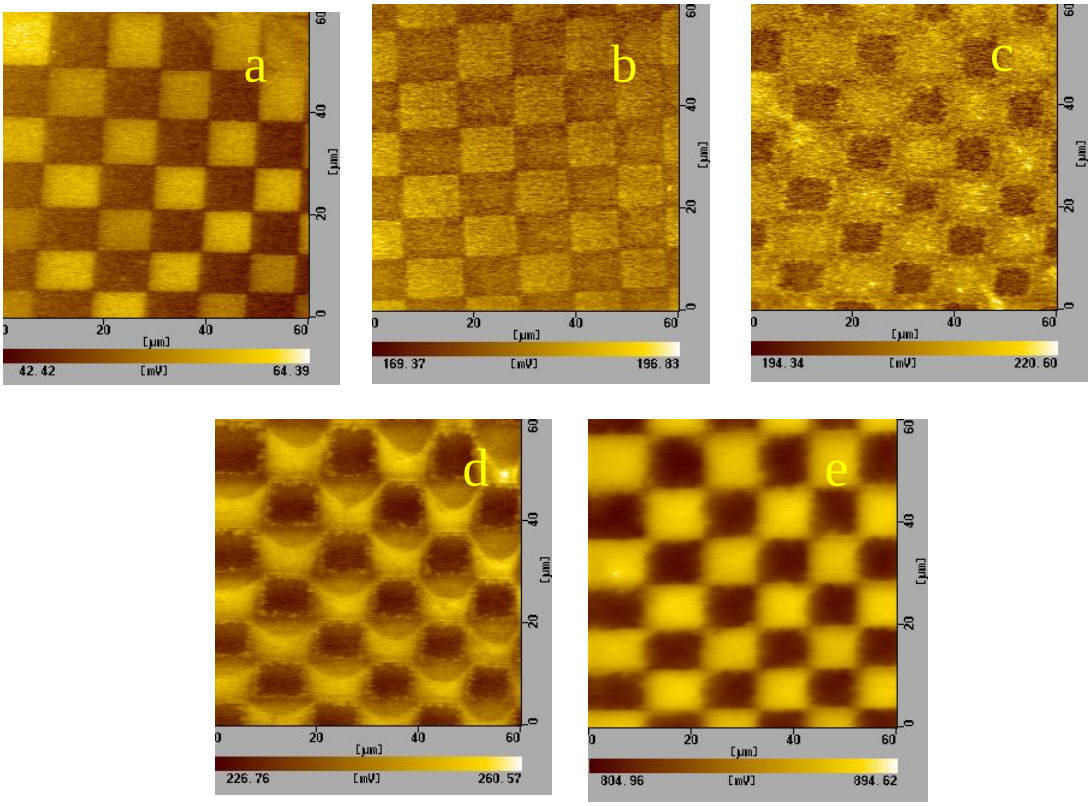

Figure 6(a-e). Surface potential images for without protein (WOP), 1 mg/l, 5 mg/l, 10 mg/l and 50 mg/l concentration of Protein A respectively on checker pattern formed by Si and SiO_2 surface.

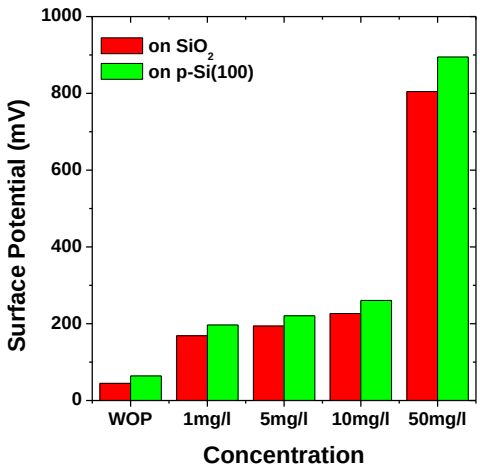

Figure 7. Surface potential vs. Concentration of Protein A on P-Si(100) surface and SiO_2 surface.

Table 1. Parameter values obtained from I-V measurements.

Proteins	Parameters			
	After 0 months		After 2 months	
	I_{max} (at +10 V)	G	I_{max} (at +10 V)	G
WOP	0.25nA	25 pS	0.25 nA	25 pS
Protein A	9.42 nA	943 pS	0.67 nA	67 pS
GFP	2.21 nA	221 pS	1.86 nA	186 pS
Luciferace	3.70 nA	371 pS	0.29 nA	28 pS

Table 2. Parameter values obtained from Impedance measurements.

Proteins	Parameters	
	ω_{max}(MHz)	Total resistance (MΩ)
WOP	1.89	1.17
SBP	1.56	1.74
Protein A	1.59	1.94
Antibody	2.19	0.88

Table 3. Parameter values obtained from Surface potential measurements on checker pattern using Kelvin probe force microscopy (KFM).

Concentration of protein A	Surface potential (mV)	
	On SiO_2	On p-Si(100)
WOP	45	64
1 mg/l	169	197
5 mg/l	194	221
10 mg/l	227	261
50 mg/l	805	895