Eliminating Surface Adhesion Between Biomolecules and Silicon Surfaces

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Abstract

The adhesion of Bovine Serum Albumin (BSA) and the Q-β Bacteria phage to thin film silicon nitride surfaces was studied at different pH levels using spectro-fluorometer analysis. The range of pH values ran from pH 5 to pH 10, and the buffers for these solutions were zwitterionic. A pH value was determined where the molecules did not adhere to the silicon surface. It was also determined that using BSA prevented the phage from adhering at pH 9.

Introduction

Bio-molecules used in the development of single molecule detectors on optically integrated silicon chips have properties that cause them to adhere to the channel of the detector and thereby preventing detection in the apparatus.

When looking at the surface properties of proteins, viruses, and other bio-molecules, one can recognize that pH has a significant influence on the charge of the molecule. For example, molecules with amine residues become negatively charged at pH 9. Each molecule has an isoelectric point, which theoretically represents the pH value where the molecule has no net charge. Most of the liquid mediums used so far in the detecting apparatus were basic, and right around pH 9.

Here are the results of the experiment to determine the effects of pH on the adhesion of the molecules to the silicon surface. The results from this will also be used in determining the surface properties of the silicon.

Experimental

The following zwitterionic buffers were prepared and adjusted to the desired pH levels. These were mixed to a concentration of 20mM in 100mL of distilled water.

MES ~ pH 5 and pH 6

Hepes ~ pH 7

Tricine ~ pH 8

Bicine ~ pH 9

Ches ~ pH 10

For the silicon sample, the wafer used had a 112 nm film of silicon nitride. It was cleave to fit into a quartz cuvet of opening dimension 1 cm x 1 cm. The wafer was cut to 2 cm in length so there was as much surface area as possible for the laser to scan. Four pieces were cleaved, although the same piece was used in each set of trials. The wafers were also cleaned between each trial with concentrated nitric acid and rinsed with de-ionized water.

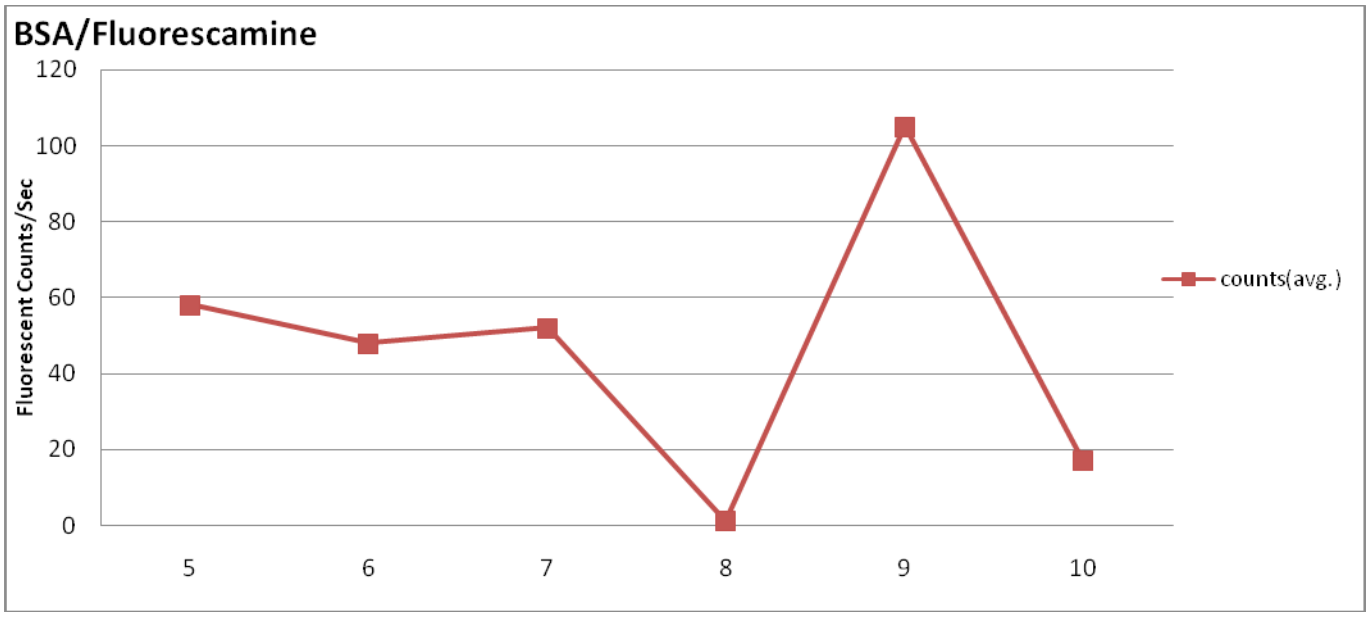
The molecules were prepared in the following way. For the BSA, a mixture of the protein was suspended in water at a concentration of 1 mg per 1 mL. An aliquot of this was then labeled with fluorescamine ($C_{17}H_{10}O_4$) in the following way. 4.5 mg of fluorescamine was suspended in 15 mL of acetone. 1 mL of BSA was place in a tube with 0.5 mL of a 0.2 M Borate buffer of pH 9. After 5 minutes this mixture was place on the vortex mixer and 0.5 mL of fluorescamine was added while the mixture was agitated. To this 2 mL of the zwitterionic buffer was added. This was repeated for each pH level from 5-10.

For the Q-β phage, 15 μL of phage was added to 10 μL of the 0.2 Borate buffer and 7.5 μL of Alexa fluorescent dye. Approximately 5 μL was added to buffer was added to a tube, and 2 mL of the zwitterionic buffers was added to this.

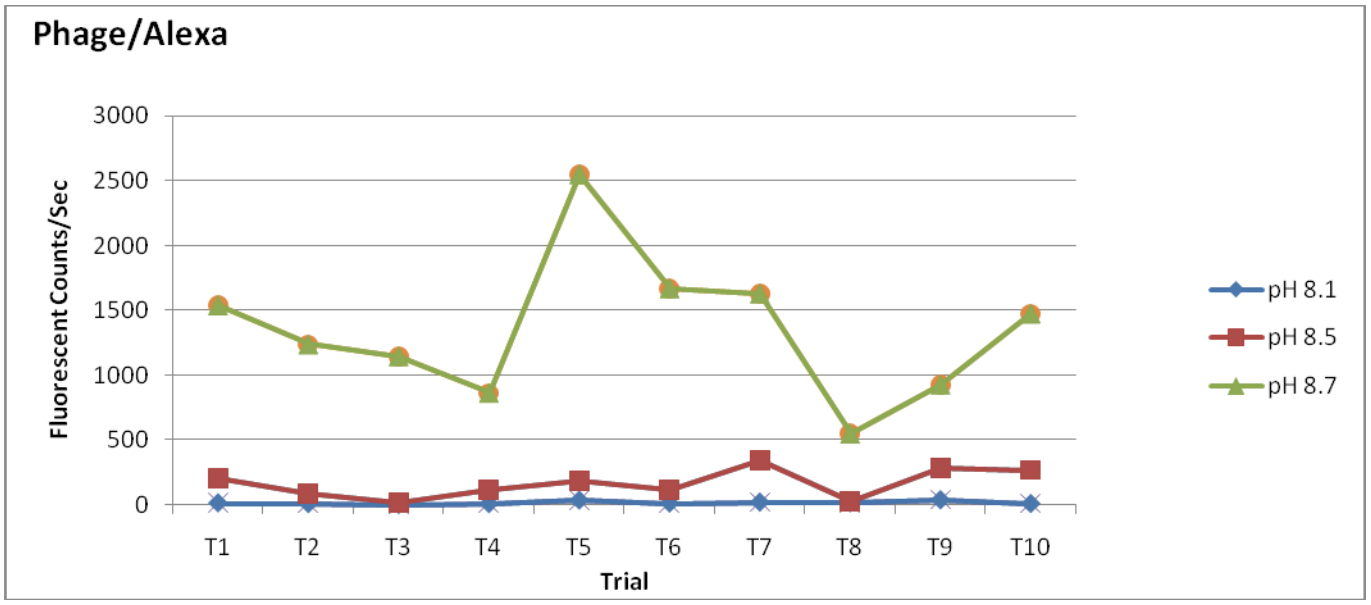
In each trial, the wafer was exposed to the solution for 5 minutes, rinsed in deionized water, and placed into the quartz cuvet. This was placed into the spectro fluorometer which was set at an excitation wavelength of 390 nm and emission wavelength of 483 nm for the BSA and an excitation wavelength of 633 nm and emission wavelength of 670 nm for the bacteria phage. Blanks were run with just the dye in a solution and no molecule to ensure that the dye itself was not interfering with the adhesion of the molecule or giving false positives.

Result/Discussion

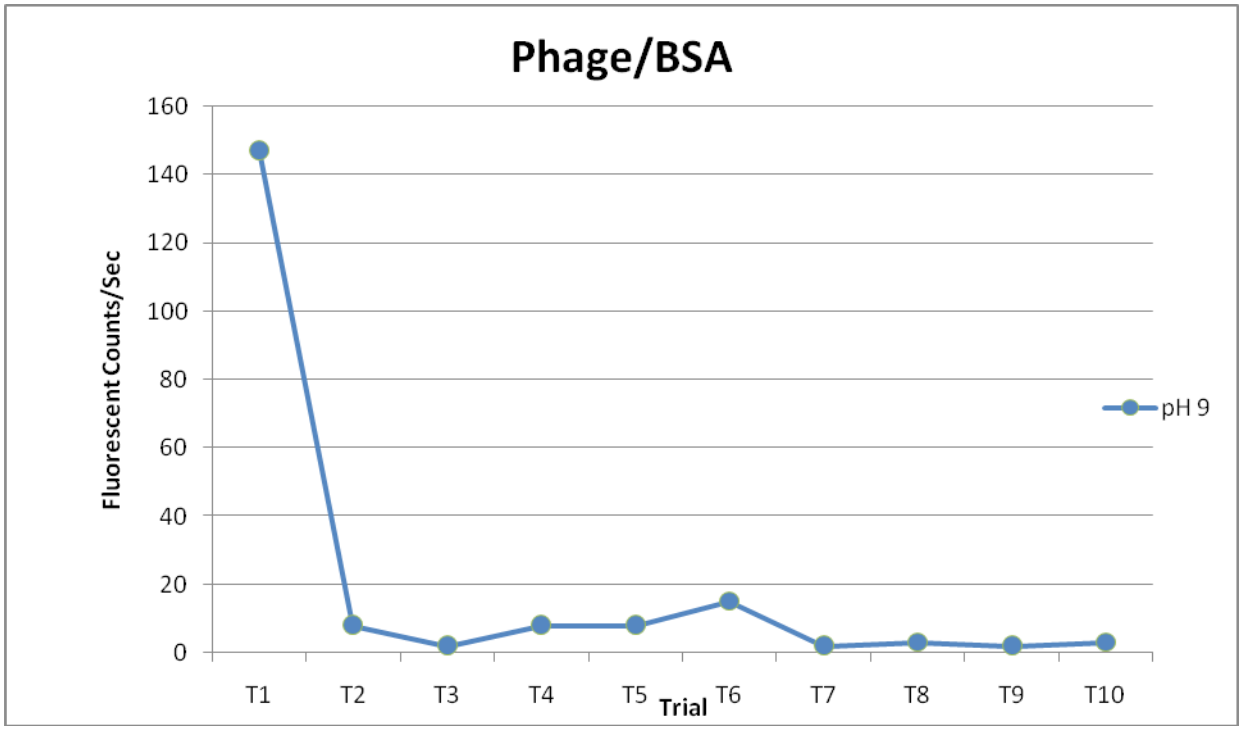
From this experiment the following data was obtained. For BSA, the count is very low around pH 8. This trend is very similar to that of the Q-β phage.



For this reason, in the study of the Q-β phage we focused on the range from pH 8 to pH 9.The results show that even as the pH increases slightly, adhesion greatly increases.



Since many of the liquid mediums used to transport molecules through the detecting apparatus are near pH 9, testing was done on a mixture of untagged BSA and tagged bacteria phage. The results show that the BSA has apparently stronger interactions with the silicon surface, blocking the phage from adhering to the surface.



From this data we can conclude that the molecules do not adhere to the silicon surface, and hopefully these results will also allow us to model similar molecule behavior. Work must still be done on viruses, and the data from this experiment will help in determining the properties of the silicon itself.