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DEPARTMENT OF CHEMISTRY

A NOVEL METHOD FOR PEGYLATION OF QUARTZ SLIDES

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ABSTRACT

Binding-based assays are valuable tools in studying proteins and nucleic acids, and are constantly used to determine both the structure and function of these molecules. An integral part of these assays is the medium on which the molecules are analyzed. The medium on which the assay is conducted is often a glass surface. The surface must be manipulated such that certain molecules bind the surface and others do not, allowing precise study of specific molecules that bind the surface. Such binding specificity is imperative for obtaining accurate results from assays. However, difficulty arises in creating such a surface that binds the desired compounds and sheds all others. Here, the problem addressed is creating a quick and efficient method to functionalize a glass microscope slide for DNA and histone H1 assays. A common method used for such assays is functionalization with polyethylene glycol (PEG). While this method is excellent for DNA assays, the histone H1 is highly positively charged and readily binds this surface, as the typical PEG surface concentration is often not sufficient to mask the negative charge on a quartz microscope slide completely. A novel method to increase the surface concentration of PEG conjugated to a microscope slide surface is tested here with the intent to minimize the affinity of H1 for the surface. Making this surface involves connecting biotin to the surface of a quartz slide, then using the biotin-binding protein StreptAvidin to tether biotinylated PEG to the biotinylated surface. This surface is shown to have a lesser affinity for H1 than a standard PEG surface, indicating a high potential for success in future experiments with other DNA binding proteins. This surface could allow for more accurate characterization of the properties of histone H1.

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Chapter 1

Background and Introduction

The expression of DNA is central to the development of organisms and to molecular biology as a whole. DNA expression is intimately connected to histone proteins, which associate with DNA to form chromatin and chromosomes. Hence the study of the association between DNA and histone proteins is critical to understanding DNA expression[1,2]. Histone H1 serves as a linker protein in the nucleosome and is critical to the condensation of chromatin[3,4,5]. However, its specific binding characteristics within the nucleosome are still under study[6]. Accurate study of H1 through DNA and nucleosome assays would allow characterization of H1's binding behavior, advancing knowledge regarding how it regulates gene expression.

The microarray is a valuable and well developed tool for studying nucleic acids. Microarrays have more recently been applied to the study of carbohydrates and proteins, and techniques for these studies are still being developed[7]. Often, microarrays are conducted through fluorescence, or fluorescence resonance energy transfer (FRET). During FRET, individual molecules are studied through the excitation of an associated fluorophore. An intrinsic problem with fluorescence-based assays is background fluorescence, created by components of the assay that are not specifically bound to the array surface[7]. This non-specific binding reduces the signal to noise ratio in fluorescence analysis. Thus a central issue in microarray technology is to create surfaces that minimize non-specific binding of assay molecules.

An ideal medium for conducting fluorescence experiments possesses surface chemistry that binds the molecule under study while shedding all others. This specificity maximizes signal to noise ratio and allows the accurate study of individual molecules. To date, many surfaces for DNA microarrays have been developed, which include both commercially available and custom-made surfaces. Commercial

production methods often utilize photolithography or inkjet processes[8]. Various types of custom-made assays exist, including aminosilane, poly-L-lysine, epoxide, membranes, and thin polymer film surfaces[7]. Of particular interest are polymer thin films, which utilize a hydrophilic surface polymer, such as polyethylene glycol (PEG), combined with specific reactive molecules to induce the binding of specific assay components. Some PEG surfaces are commercially available as well.

Protein assays are not as well-developed as DNA assays. This is in part due to the nature of proteins, whereby the natural structure and functionality must be maintained in an assay for the protein to be accurately studied[9]. Further, non-specific protein binding must be blocked as well. Despite the lack of diverse array techniques, success has been realized with protein arrays through the use of PEG surfaces[7].

Because of the overlap of PEG surfaces for DNA and protein assays, glass or quartz slides functionalized with a layer of PEG are often used in DNA and nucleosome assays. PEG itself has a low affinity for DNA, hence these surfaces are populated with specific binding sites designed to bind the DNA and/or proteins being studied. Here, the binding of DNA to a PEG surface is mediated through the biotin-binding protein Avidin. By sparsely populating a PEG molecule with biotin, Avidin proteins can strongly bind the surface at such sites. The subsequent addition of biotinylated DNA causes specific and high-affinity binding of DNA to a PEG surface. This technique allows only specific molecules to bind the surface, promoting accurate study of these molecules through fluorescence. Traditional PEG layers effectively block the non-specific binding of DNA, but they do not effectively block the non-specific binding of most DNA binding proteins that are typically positively charged, such as histone H1. The high affinity of traditional PEG surfaces for H1 makes studying H1 difficult, preventing many of its characteristics from being elucidated. This high affinity is due to the electrostatic attraction between the positive charges of H1 and the negative charges of quartz or glass surface that cannot be completely shielded by the PEG layer due to a low PEG coverage on the surface. Therefore, increasing the surface coverage of PEG on the surface would decrease the incidence of non-specific binding by H1.

The purpose of this research was to develop a quick and convenient method of functionalizing quartz slides for DNA and DNA-binding protein assays. Specifically, the method used here was designed to create a surface that shed histone H1 in addition to DNA by increasing the surface coverage of the PEG layer. Creating this improved PEG surface involves connecting biotin to the surface of a quartz slide, then using StreptAvidin to tether biotinylated PEG to the biotinylated surface. Surface biotinylation is much more efficient than direct PEGylation, thereby increasing the coverage of PEG on the surface and ultimately decreasing the amount of non-specific binding of H1. The efficacy of this surface was determined by comparing the binding rate of H1 on the novel surface with the binding rate of H1 on a traditional PEG surface. Successful creation of a surface that minimizes the non-specific binding of H1 would allow for characterization of the properties of H1 through FRET with little measurement error.

Chapter 2

Experimental

Preparation of Novel PEG Surface

All the chemicals used were purchased from Sigma-Aldrich (St. Louis, MO) unless noted otherwise.

Quartz slides were obtained and ten holes were drilled in each slide as shown in Figure 1.

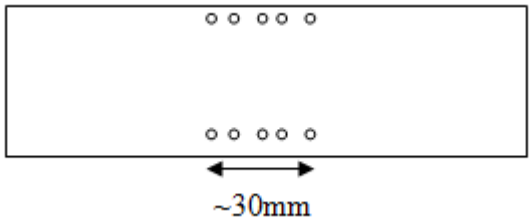

Figure 1: Diagram of holes drilled in quartz slides

The quartz slides, along with an equal number of coverslips, were then sonicated in Nanopure™ water (18.2 MΩ) for 10 minutes at room temperature. The slides and coverslips were then cleaned and activated using a solution of Nochromix in 18 M sulfuric acid. The slides and coverslips were left in the acid overnight. The slides and coverslips were then washed with Nanopure water 6 times. The coverslips were dried in an oven for 15 minutes at 100˚C. The slides were washed 3 times in 100% ethanol to remove residual water. The slides were then incubated in a solution of 99:1 ethanol: (3-aminopropyl)trithoxysilane (APTES) for 10 minutes at room temperature. This silanization step created a uniform surface of amine groups for further functionalization, shown in Figure 2.

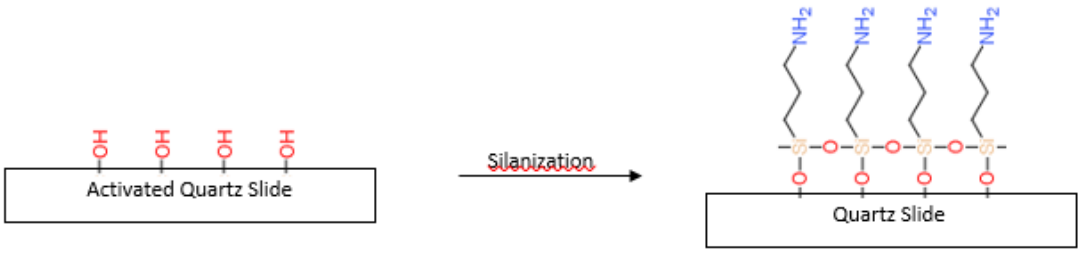

Figure 2: Silanization of quartz slides

The slides were again washed 3 times in 100% ethanol. The slides were then dried with a stream of nitrogen gas and placed in an oven for 15 minutes at 100˚C. Once cooled, the slides were ready to be functionalized. Biotin was attached to the surface by incubating 2 mg/mL NHS-biotin (ThermoFisher Scientific Inc.) in DMSO overnight, as seen in Figure 3.

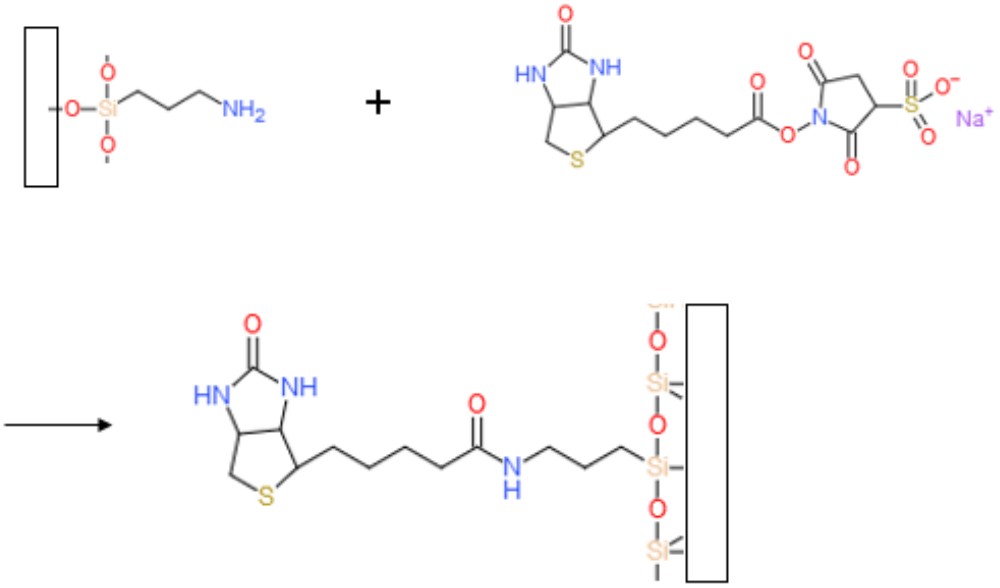

Figure 3: Attachment of biotin to the silanized quartz surface

This was accomplished by adding 100 µL of NHS-biotin dropwise to the slide, then carefully placing the coverslip on the slide. Placing the coverslip over the NHS-biotin solution caused it to be evenly distributed over the entire surface. After incubation, both sides of the coverslips and slides were

then rinsed with ethanol from a squirt bottle for 10 seconds to remove residual NHS-biotin. The same process was done with water, and the slides and coverslips were then dried using a stream of nitrogen gas. Once dry, the slides could be functionalized with the biotin-binding protein StreptAvidin (ThermoFisher Scientific Inc.), seen in Figure 4.

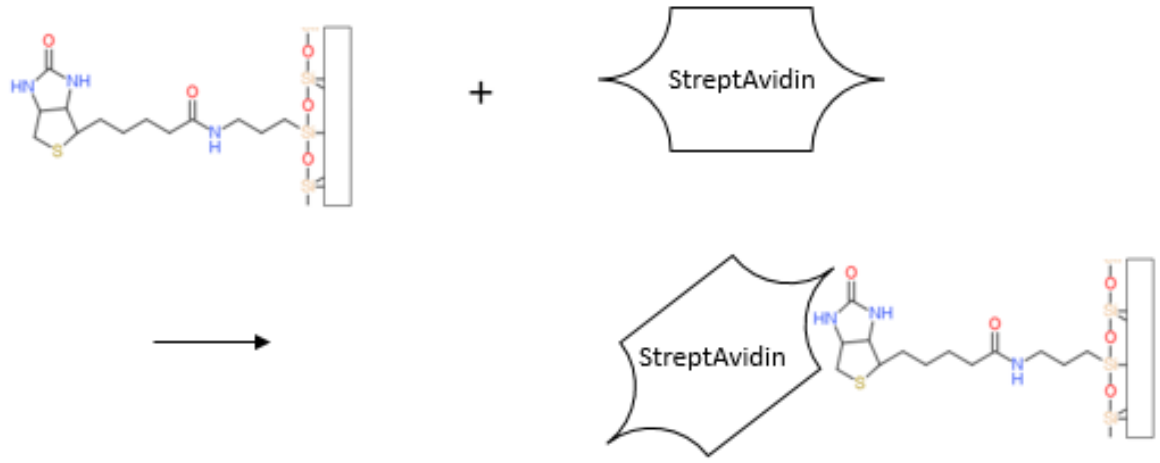

Figure 4: Binding of StreptAvidin to biotinylated surface

This functionalization was done in a manner similar to that used for the NHS-biotin. 100 µL of 1 µg/mL StreptAvidin in 1X PBS buffer (10 mM Na_2HPO_4, 137 mM NaCl, and 2.7 mM KCl) was added to each slide, and a coverslip was carefully placed over each. The slides were then incubated for 45 minutes in a humidity chamber at room temperature.

The slides were then washed in 1X PBS buffer solution. To avoid streaking, the slides were removed from the 1X PBS solution, then immediately swirled in Nanopure water for 2 seconds, then dried with a stream of nitrogen gas. Once dry, the slides were functionalized with polyethylene glycol (PEG). This was accomplished by adding 100 µL of 1 mg/mL 100:1 biotin-PEG: biotin-PEG-biotin (Laysan Bio, Inc. Arab, AL) in 1X PBS to each slide, then carefully placing a coverslip over each. The substrates were allowed to incubate for 45 minutes at room temperature in a humidity chamber. This step

is seen in Figures 5 and 6. It should be noted in Figures 5 and 6 that multiple biotin-PEG molecules can attach to each StreptAvidin protein, not just one.

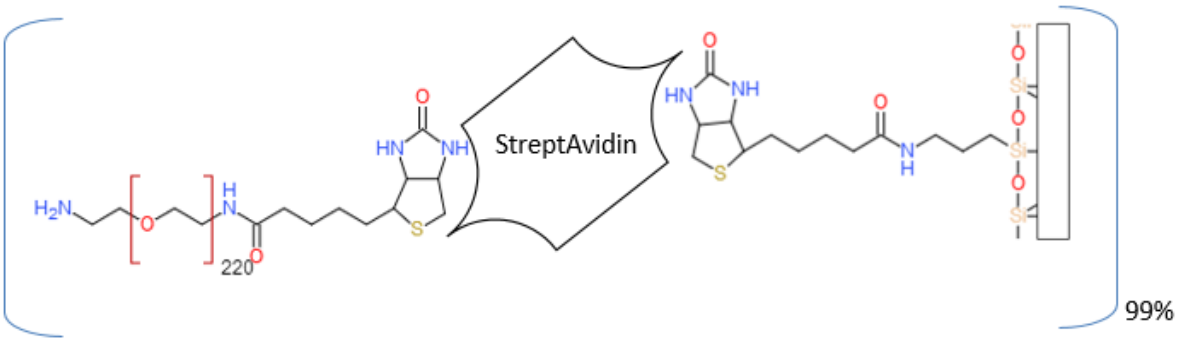

Figure 5: Structure of biotin-PEG

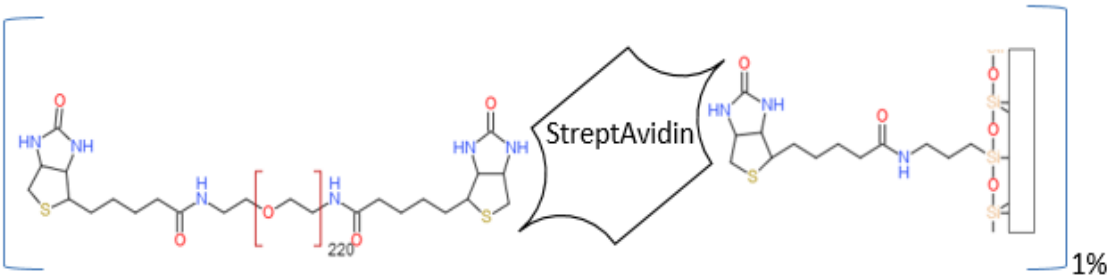

Figure 6: Structure of biotin-PEG-biotin

The slides and coverslips were then removed from the chamber, separated, and washed in 1X PBS. The same procedure for drying the slides and coverslips was again observed. The slides and coverslips were removed from the buffer, swirled in water, and then dried with a stream of nitrogen. This completed the functionalization of the slides.

To prepare the slides for use, five channels were created on each one. This was done by placing thin strips of double-sided tape between each of the five sets of holes. A coverslip was then placed over the tape and firmly pressed into place. The ends of the channels were sealed using epoxy. This configuration allowed the injection of protein solutions into the channels via a micropipette. This configuration of the slides can be seen in Figure 7.

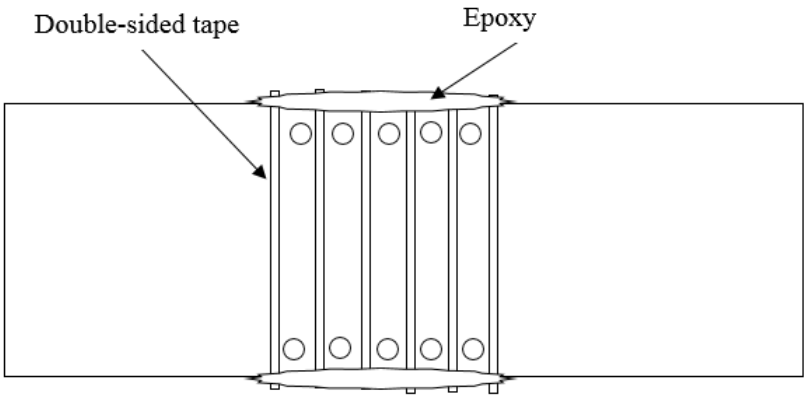

Figure 7: Quartz slides with channels

New Surface Evaluation

With the channels complete, the surface could be evaluated using total internal reflection (TIR) fluorescence (TIRF) microscopy. The first substrate tested on the surface was DNA tethered to biotin (DNA+bio). The protocol for loading biotinylated DNA (IDT, Coralville, IA) was as follows: 50 µL of 1 mg/mL StreptAvidin was injected into a channel with a micropipette via one of the holes drilled in the slide. Because each channel held 7 µL, the excess solution was absorbed with a lab wipe as it exited the opposite end of the channel through the other drilled hole. The injected StreptAvidin solution was then allowed to incubate for 5 minutes. After incubation, StreptAvidin that had not bound the surface was removed by injecting 50 µL of 1X PBS. 50 µL of 20 pM DNA+bio tagged with the fluorophore Cy5 (GE

Healthcare Life Sciences) was then injected into the channel and allowed to incubate for five minutes. A solution of 1 mM 50:50 PCA and PCD was then injected to remove excess DNA.

Once the DNA was injected the slide could be imaged using TIR microscopy. Both sides of the slide were cleaned with methanol. The slide was then loaded into the microscope such that total internal reflection of a red laser (Cube 635nm by Coherent Inc., Santa Clara, CA) was obtained in the channel containing the DNA. Using a CCD camera (Ixon 897 by Andor Technology, Belfast, NIR), several movies of the excited fluorophores were recorded at different locations in the channel. These movies were then analyzed via software (homebuilt by the Lee lab) to count the number of fluorophores present in each movie. Figure 8 below illustrates the emission spectrum of Cy5. Figure 9 is a diagram of the microscope setup and Figure 10 is a closer diagrammatic view of the quartz slide.

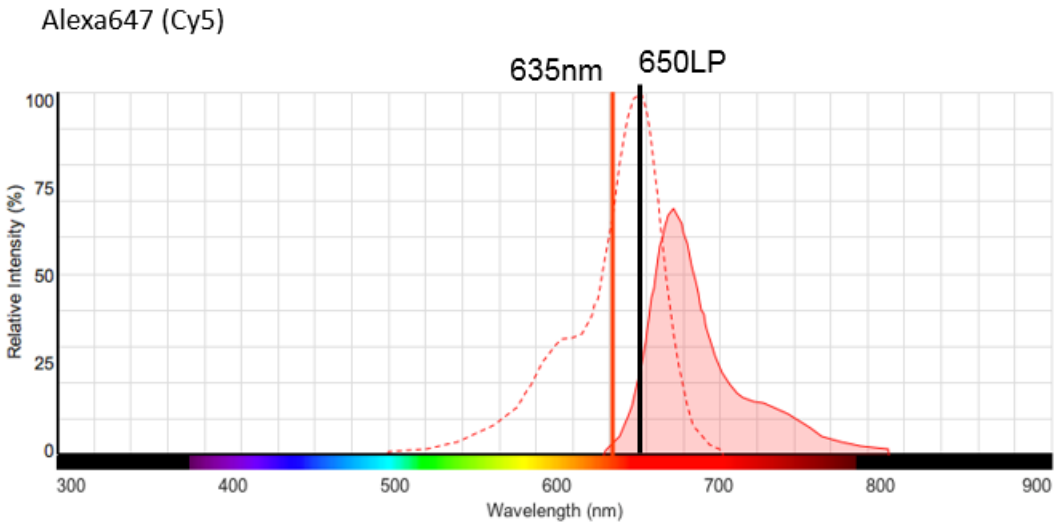

Figure 8: Absorption and emission spectrum of Cy5[10]

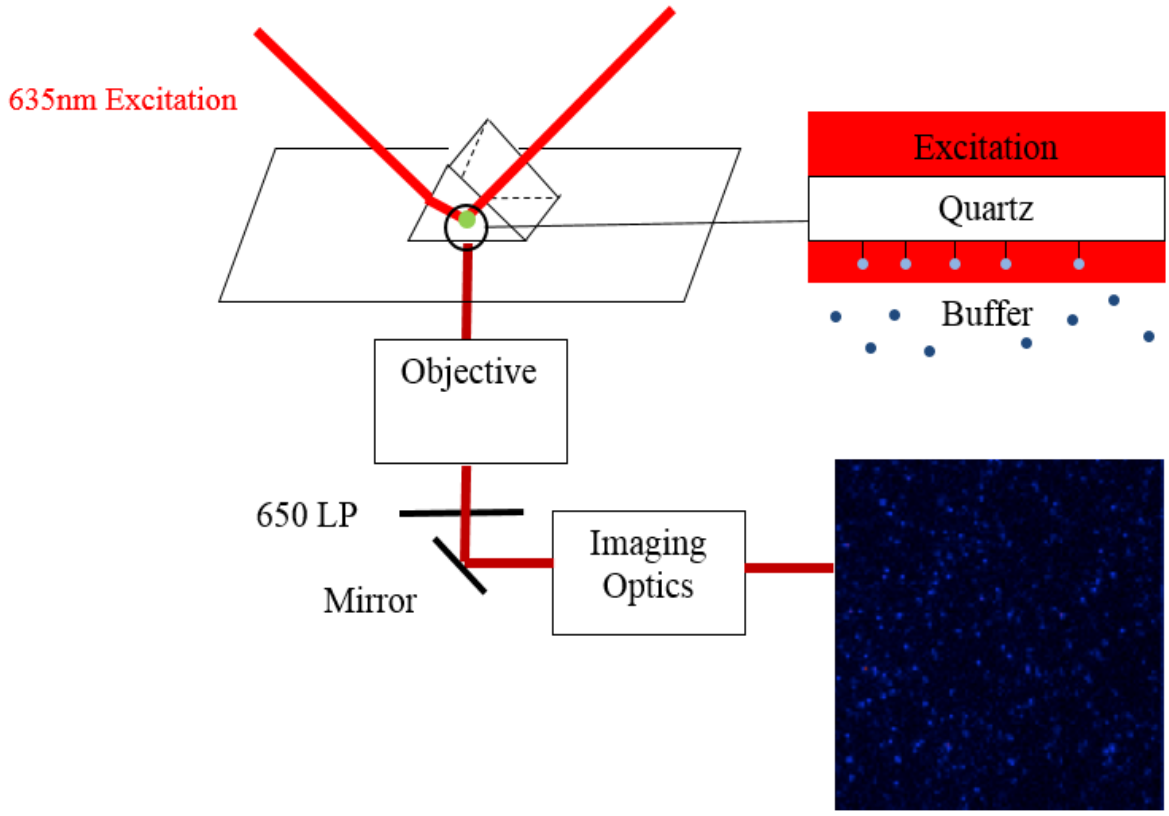

Figure 9: Diagram of TIR microscope setup

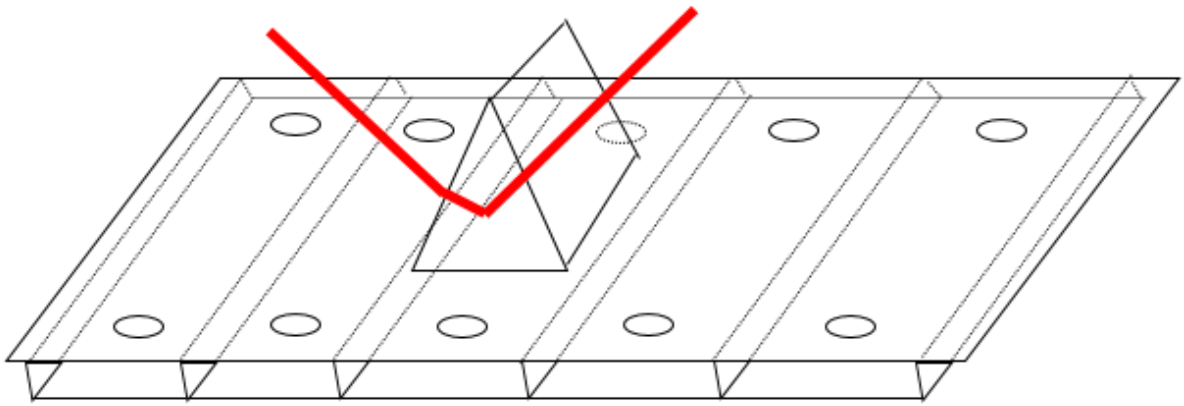

Figure 10: Closer view of quartz slide within microscope setup

The entire process of loading and viewing the channel was then repeated for 100 pM and 500 pM DNA concentrations. These measurements established the positive control. To establish the negative control, DNA without a bound biotin (DNA-bio) was injected into the channels and measured under the microscope. This was conducted in a manner identical to that used for the DNA+bio, except StreptAvidin was not injected into the channels. Rather, the DNA-bio was injected, allowed to incubate, then PCA and PCD were injected and the slide was imaged in the microscope. The concentrations of DNA-bio were also identical, at 20 pM, 100 pM, and 500 pM.

With the positive and negative controls established, the surface was tested for its efficacy in preventing the binding of nucleosome protein H1. The loading and viewing of the slide was accomplished in a manner identical to that used for DNA-bio. No StreptAvidin was used in the H1 channels. The concentrations of H1 were 20 pM, 100 pM, and 500 pM. Collecting the H1 images concluded data collection and allowed the quality of the surface to be analyzed.

Preparation of Standard Surface

For comparative purposes, slides were prepared using a standard method. Holes were drilled in 5 quartz slides in a manner identical to that used before. The slides and coverslips were then sonicated in 1 M potassium hydroxide solution for 15 minutes at room temperature. After being rinsed with Nanopure™ water, the quartz slides and coverslips were etched in Nochromix and 18 M sulfuric acid overnight. The slides were then washed three times with ethanol and incubated in a solution of 99:1 ethanol: (3-aminopropyl)trithoxysilane (APTES) for 10 minutes at room temperature. The slides and coverslips were then rinsed in Nanopure™ water 7 times and dried with a stream of nitrogen. The slides were then incubated for 45 minutes with 35:1 SVA-PEG:SVA-PEG-Bio. This was accomplished by dispensing 50 µL of the solution on the slide, then carefully placing a coverslip over the drop of solution. Channels were

then created on the slides in a manner identical to that seen above. The slides were then tested in the TIR microscope in a manner identical to that used for the novel surface.

Chapter 3

Results

Below are tables and figures describing the results from the previously described experiments.

Traditional PEG Layer

Table 1: Numbers of molecules on traditional PEG slides at varying concentrations

Solution	Concentration (pM)	Number of molecules	Std. Error	# of Movies
DNA (+bio)	20	10.4	2.43	5
DNA (+bio)	100	70.8	4.57	6
DNA (+bio)	500	83.0	6.91	5
DNA (-bio)	20	12.9	2.06	8
DNA (-bio)	100	15.4	1.40	5
DNA (-bio)	500	24.5	5.42	6
H1	20	13.5	5.39	6
H1	100	54.8	3.21	6
H1	500	79.7	5.14	6

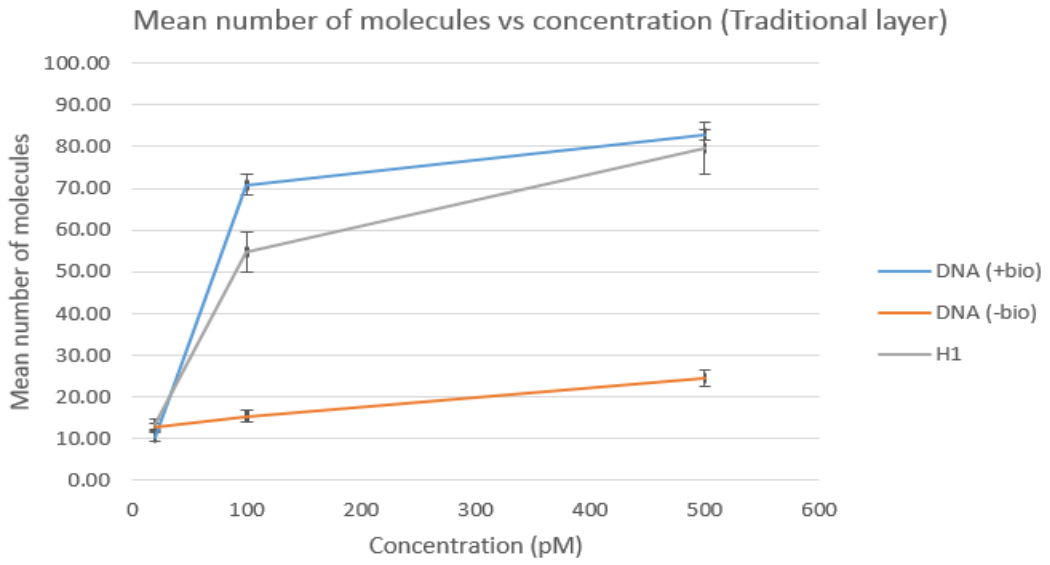

Figure 11: Graph of number of molecules vs concentration for traditional PEG layer

Table 2: Ratios of specific to non-specific binding for traditional PEG surface

Concentration	Ratio $\frac{DNA\ (+bio)}{DNA(-bio)}$	Ratio $\frac{DNA\ (+bio)}{H1}$
20 pM	0.81	0.77
100 pM	4.60	1.29
500 pM	3.39	1.04

New PEG surface

Table 3: Numbers of molecules on new PEG slides at varying concentrations

Solution	Concentration (pM)	Number of molecules	Std.Error	# of Movies
DNA (+bio)	20	27.0	2.43	10
DNA (+bio)	100	92.0	4.57	8
DNA (+bio)	500	86.9	6.91	9
DNA (-bio)	20	28.8	2.06	8
DNA (-bio)	100	23.8	1.40	6
DNA (-bio)	500	65.3	5.42	12
H1	20	63.6	5.39	8
H1	100	47.3	3.21	8
H1	500	61.5	5.14	10

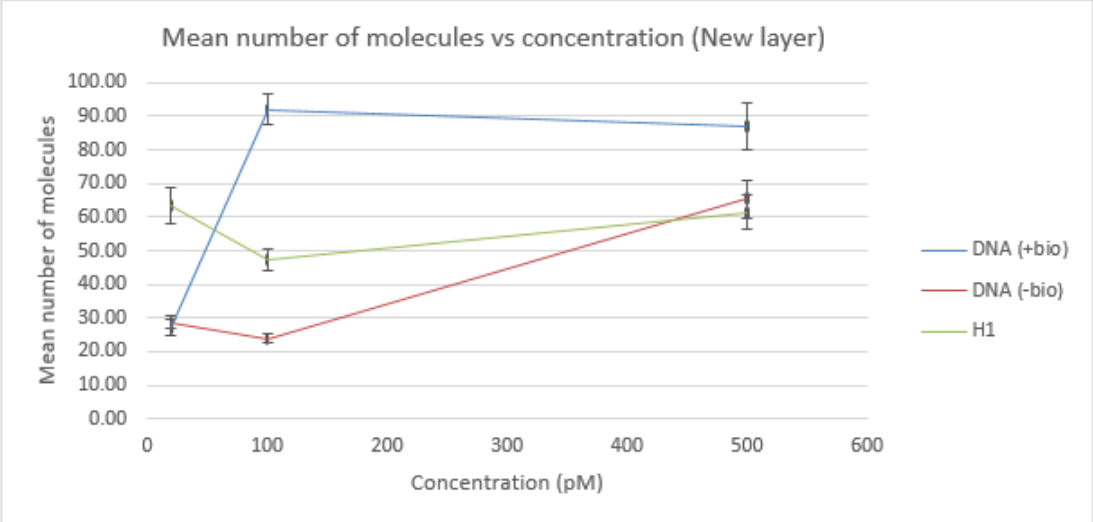

Figure 12: Graph of mean number of molecules on the new PEG surface versus the concentration of the molecules

Table 4: Ratios of specific to non-specific binding for the new PEG surface

Concentration	Ratio $\frac{DNA\ (+bio)}{DNA(-bio)}$	Ratio $\frac{DNA\ (+bio)}{H1}$
20 pM	0.94	0.42
100 pM	3.86	1.95
500 pM	1.32	1.41

New PEG Surface (Corrected)

Table 5: Corrected numbers of molecules on new PEG slides at varying concentrations

Solution	Concentration (pM)	Number of molecules	Std. Error	# of Movies
DNA (+bio)	20	16.0	2.43	10
DNA (+bio)	100	81.0	4.57	8
DNA (+bio)	500	75.8	6.91	9
DNA (-bio)	20	17.8	2.06	8
DNA (-bio)	100	12.8	1.40	6
DNA (-bio)	500	54.3	5.42	12
H1	20	52.6	5.39	8
H1	100	36.1	3.21	8
H1	500	50.5	5.14	10

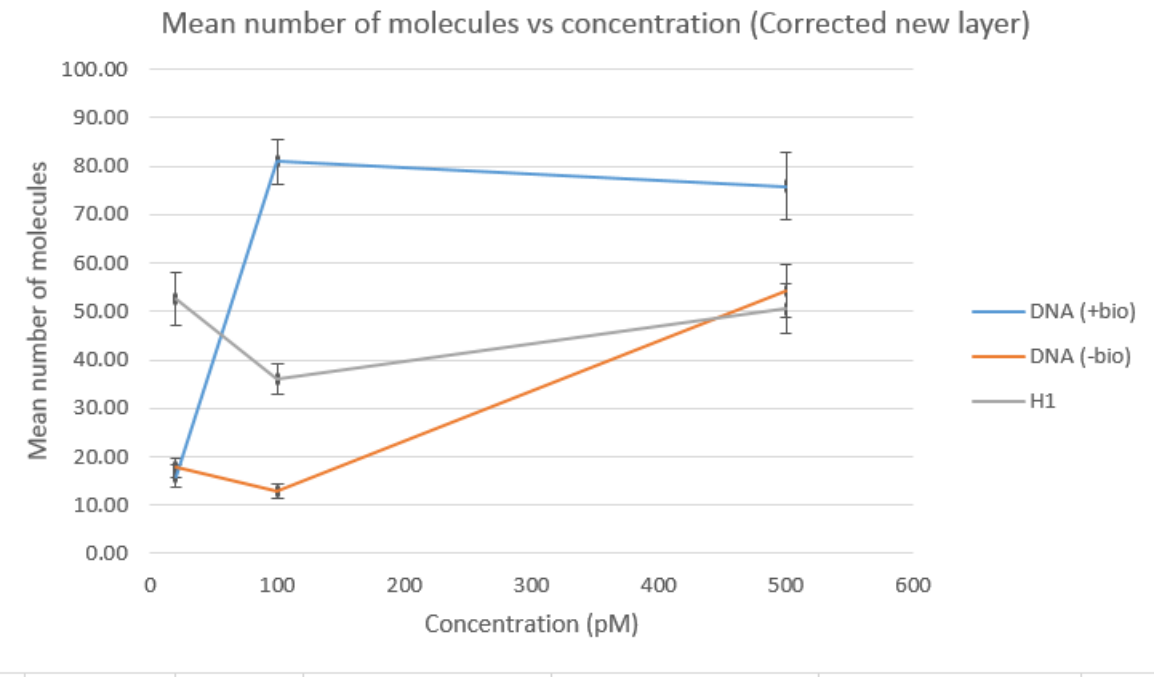

Figure 13: Graph of mean number of molecules on the new PEG surface versus the corrected concentration of the molecules

Table 6: Corrected ratios of specific to non-specific binding for the new PEG surface

Concentration	Ratio $\frac{DNA\ (+bio)}{DNA(-bio)}$	Ratio $\frac{DNA\ (+bio)}{H1}$
20 pM	0.90	0.30
100 pM	6.31	2.24
500 pM	1.39	1.50

Statistical Analysis

Table 7: Results of t-tests based on experimental results

Solution	Concentration (pM)	Traditional PEG # of Molecules	New PEG # of Molecules	p-value (two tailed)
DNA (+bio)	100	70.8	81.0	0.078
DNA (+bio)	500	83.0	75.8	0.400
DNA (-bio)	100	15.4	12.8	0.244
DNA (-bio)	500	24.5	54.3	0.0002
H1	100	54.8	36.1	0.01
H1	500	79.7	50.5	0.004

Chapter 4

Discussion

Each PEG surface contains a small percentage of biotinylated PEG. The exposed biotin on the surface mediates the binding of proteins and DNA to the surface when preparing a microarray. When creating a microarray on one of these surfaces, the biotin-binding protein StreptAvidin is used, which has a strong affinity and selectivity for biotin. Injecting a solution of StreptAvidin into the channels causes the StreptAvidin protein to bind the exposed biotin, tethering it to the surface. Because StreptAvidin has multiple biotin-binding sites, injecting biotinylated DNA and proteins after injecting the StreptAvidin causes these molecules to bind StreptAvidin, tethering them to the surface with a high affinity. The microarray created by tethering these proteins to the surface can then be viewed through a TIR microscope to characterize their interactions.

When characterizing the interactions of proteins and DNA, specific binding to the surface is critical for the accurate study of these proteins. Thus, ideally, no proteins other than those tethered to biotin should bind the surface during the experiment. Hence an ideal surface will have a low affinity for any and all proteins that are not tethered to biotin. The undesirable binding of non-biotinylated proteins and DNA is known as non-specific binding. The traditional PEG layer described previously sufficiently blocks the non-specific binding of DNA, because the negative charge of DNA is naturally repelled by the negative charge of the quartz surface. However, the traditional PEG has been shown to not sufficiently block the non-specific binding of the histone H1. Thus, this surface is not suitable for microarrays of H1, making it difficult to elucidate its binding properties.

The novel surface developed here was theorized to be able to block the non-specific binding of both DNA and H1. By blocking the non-specific binding of H1, precise microarrays can be created,

allowing accurate characterization of H1. It was theorized that the greater coverage of the PEG molecules on the surface by the novel method would shield the negative charge of the quartz from the positive charge of H1 more effectively than a conventional PEG surface, resulting in a better performance in blocking non-specific binding.

In order to determine the efficacy of the novel surface in preventing non-specific interactions, the results of the new surface and the old surface must be compared. As can be seen in Figure 11, on the traditional surface, the binding of biotinylated DNA is much greater than the binding of non-biotinylated DNA. This result is expected because the traditional PEG layer is known to shed DNA. However, the traditional surface does not block the non-specific interaction of H1. This is shown by the large number of H1 molecules at each concentration, which is nearly as large as the number of biotinylated DNA molecules at each concentration. This result was also expected for the traditional layer since it is known to poorly block the binding of H1. As can be seen in Figure 12, the new PEG layer shows a much greater affinity for biotinylated DNA than for non-biotinylated DNA, indicating that it is also effective in preventing the non-specific binding of DNA. Further, the new PEG layer also shows a greater affinity for biotinylated DNA than for H1, indicating that it is more effecting in preventing the non-specific interaction of H1 than is the traditional PEG layer.

The efficacy of preventing non-specific interactions can also be judged by determining the ratio of specific to non-specific binding. As seen in Tables 2 and 4, the ratio of specific to non-specific binding is poor for both surfaces at the low concentration of 20 pM. This low ratio is likely caused by a relative lack of specific binding by biotinylated DNA due to the low concentration combined with a baseline level of non-specific binding for both DNA and H1. Hence, these 20 pM measurements were excluded from further analysis. As seen in Table 2, the binding ratio of biotinylated DNA to non-biotinylated DNA is high for the traditional PEG layer. However, the number of H1 molecules that bound the surface is very close to the number of biotinylated DNA molecules that bound the surface, which is manifested in

binding ratios that are close to 1. This result was expected of the traditional PEG layer since it is ineffective at blocking the non-specific binding of H1.

Upon initial review, the new PEG layer appears to be less effective at preventing the non-specific binding of DNA. This is evidenced by the biotinylated DNA to non-biotinylated DNA ratios seen in Table 4 that are lower than those found in Table 2. Further, the new PEG layer appears to be minimally more effective in preventing the non-specific interaction of H1, again evidenced by the ratios found in Table 4 versus those found in Table 2. However, it was noted that the results from the new PEG surface had a higher baseline number of molecules than did the traditional surface. That is, even at low concentrations, the new PEG surface showed a far greater number of molecules than did the traditional PEG surface. It was suspected that the greater number of steps involved in preparing the new PEG surface cause a greater amount of contamination on the surface, leading to a greater baseline value. These contaminants can be bleached with a UV lamp before use, and therefore should be subtracted from the analysis. Thus, a blank slide was loaded in the microscope and viewed to determine the amount of contamination present on the slide. The average number of molecules found in the resulting images was then subtracted from the average number of molecules found in the images of the new PEG slides. These results are termed “corrected” and can be seen in Tables 5 and 6 and in Figure 13. Analysis of these corrected results indicates that the new PEG layer is equally effective in preventing the non-specific binding of DNA to the surface as the traditional PEG layer and more effective in preventing the non-specific binding of H1 than the traditional PEG layer. As seen in Table 4, the ratios of biotinylated DNA to non-biotinylated DNA for the traditional PEG layer at 100 pM and 500 pM are 4.60 and 3.39, respectively. As seen in table 6, the corresponding corrected ratios for the new PEG layer are 6.31 and 1.39, respectively. From these ratios it is difficult to conclude that either layer is more effective in blocking the non-specific binding of DNA. However, the consistently low binding of DNA-bio on the traditional surface seen in Figure 11 suggests that the traditional surface is more effect in blocking the non-specific binding of DNA. This may be due to the greater coverage of the quartz surface by the novel

PEG layer. This greater coverage may have shielded the DNA from the negative charge of quartz, thereby increasing the non-specific binding. For H1, the ratios of specific binding by biotinylated DNA to non-specific binding by H1 on the traditional surface at 100 pM and 500 pM, are 1.29 and 1.04, respectively. On the new surface, the corresponding corrected ratios are 2.24 and 1.50, respectively. While this surface may not be quite as effective at blocking DNA as the traditional layer, it is more effective at blocking H1. Thus it can be concluded from these ratios that this novel method of functionalizing a slide with PEG produces a surface that repels DNA while simultaneously repelling H1 more effectively than a traditional PEG layer.

A statistical analysis of the mean number of molecules bound to each surface confirms these results. A two sample t-test indicates that the novel PEG surface binds a similar number of biotinylated DNA molecules as the standard surface. At 500 pM, there is no statistically significant difference in binding rates for the two surfaces, and the p-value at 100 pM is 0.078, indicating that the difference in binding rates is not significant. Overall, it can be concluded that the two surfaces generally behave similarly toward biotinylated DNA. This result was expected, since the specific bind sites on each of these surfaces were the same and had similar concentrations.

With regard to non-biotinylated DNA, the statistical analysis indicates that the novel PEG layer is not as effective as is the traditional layer in blocking the non-specific binding of DNA. Again, this might be a result of a greater surface coverage of PEG screening the negative charges of the surface. At 100 pM, and the p-value is 0.224, indicating no significant difference between the two layers. However, there is a difference at 500 pM, with a p-value of 0.0002. This indicates that the traditional surface more effectively blocks the non-specific binding of DNA. Despite the relative lack of blocking conferred by the novel PEG layer, the previously discussed specific to non-specific binding ratios indicate that the novel layer may still block non-specific binding enough to be useful for DNA experiments.

For the H1 experiments, the statistical analysis indicates that the novel PEG layer is more effective at higher concentrations. At both 100 pM and 500 pM, the novel layer binds fewer molecules at

a statistically significant level. The novel PEG layer shows a relatively constant number of bound H1 molecules across the concentrations, while the number of H1 molecules on the traditional PEG layer increases with concentration. These trends explain the trends in statistical significance, and drawn from the aforementioned ratios. The novel layer is not quite as effective in blocking the non-specific binding of DNA, but is still effective enough to be used in experiments. The novel layer is also more effective in blocking the non-specific binding of H1.

Chapter 5

Conclusions

From the above analysis it can be concluded that this novel PEG layer blocks the non-specific binding of H1 more effectively than a traditional PEG layer while simultaneously blocking the non-specific binding of DNA. This experiment does not definitively prove the efficacy of this novel layer, but it shows a high potential for success in assays of DNA and H1. Further testing is merited on this new surface to determine if it is indeed suited for FRET experiments, but the current evidence indicates that it could be used to characterize the properties of H1. Additionally, this method of slide preparation is quick and convenient, allowing for adjustments and variations to be made to continually improve this layer. Thus the purpose of this project was completed by developing a simple method for functionalizing a quartz slide that simultaneously blocks non-specific interactions with both DNA and the nucleosome protein.

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EXPERIENCE

Penn State Hershey Medical Center — **Hershey, PA**
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