Investigation of Peptoid Thin Films and Their Potential Use in a Biosensor

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by Brian Lunt

Abstract

Peptoids are biomimetic polymers that combine the properties of proteins and plastics. This class of materials is predicted to play a significant role in several bio-electronic applications such as biosensors, drug discovery, and biomolecular recognition. In order to realize such potential, films of peptoids must first be reliably produced or transferred onto substrates of interest. This work reports the production of thin films of peptoids by way of various methods, including Gibbs monolayers, Layer-by-Layer multilayers, and peptoid nanosheet (bilayer) formation. Gibbs films were successfully transferred onto silanized silicon substrates using the Langmuir-Schaefer technique as verified by X-ray Photoelectron Spectroscopy and Atomic Force Microscopy. Gibbs films were also prepared from a peptoid-peptide hybrid polymer, containing a peptide sequence specifically recognized and phosphorylated by the enzyme Casein Kinase II (CK2). An Ion-Sensitive Field-Effect Transistor containing this peptoid-peptide hybrid film is being fabricated as a biosensor for detecting CK2 activity.

Acknowledgements

The author would like to tremendously thank Professor Ana Claudia Arias for her advice, encouragement, and support; Ron Zuckermann for his peptoid expertise and enthusiasm; Gloria Olivier for insightful discussions, for answering hundreds of questions, and for synthesizing the peptoids; Felippe Pavinatto for Langmuir trough assistance, useful discussions, and for converting me to team Corinthians; Mangesh Bangar for device fabrication assistance and quality discussions; Mary Gilles for allowing me to use her QCM; Sam Ho for obtaining the SEM images; Steve Ferreira for machine shop assistance; the Molecular Foundry at Lawrence Berkeley National Laboratory and the Users Program for permitting me the use of such excellent equipment and for the great view of the Bay; and the National Science Foundation Graduate Research Fellowship Program for their support.

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Introduction

Peptoids are synthetic, sequence-specific polymers that mimic the chemical structure of proteins [1]. In contrast to peptides, peptoids are composed of a specific sequence of modified glycine amino acids, whereby the substitution groups branch off from the backbone nitrogen atom rather than the alpha carbon. This class of materials combines the chemical and biological stability of conventionally synthesized polymers with the many interesting properties of proteins. For example, peptoids can exhibit chain folding and can be synthesized with specialized biological functions [2]. Additionally, these materials have demonstrated the ability to form secondary structures very similar to helices commonly found in proteins [3]. More recently, peptoids were found to form stable 2-D nanosheets less than 3 nm thick [4]. These sheets are unique due to their highly crystalline organic structure and their extremely high aspect ratio (area/thickness ratio > 10^9 nm).

Several applications are envisioned for these peptoid nanosheets and films such as sensing, filtering, templating growth for mineralization, and molecular recognition in biological systems [4]. In order to realize these applications, however, it is necessary first to reliably produce, transfer, or deposit thin films of solid peptoids on substrates. Here we investigated peptoid film formation and transfer through employing Layer-by-Layer, nanosheet, and Gibbs monolayer production techniques.

Peptoid Sequences

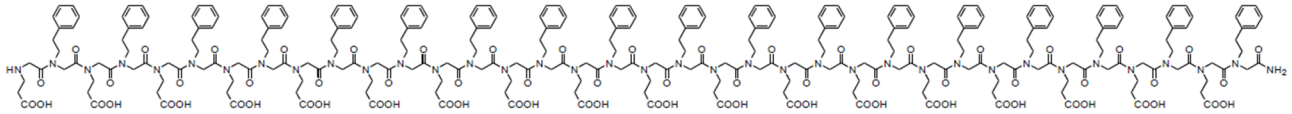
The six peptoids used in this work were synthesized by collaborators at the Molecular Foundry. The sequence, name, chemical formula, and molecular weight of each peptoid are shown in Table 1; the chemical structure of each is shown in Figure 1. Polyionic peptoid sequences were chosen for the LbL experiments. The anionic strand, composed of an alternating sequence of Phenethyl (P) and Carboxyethyl (C) residues, 36-residues in length [4], is referred to as "PC" (Figure 1A). The PC peptoid is negatively charged when in a neutral or high pH solution due to the deprotonated carboxyl groups. The positively charged strand, referred to as "PA" (Figure 1B), is also 36-residues in length, and consists of an alternating sequence of Phenethyl (P) and Aminoethyl (A) residues [4]. The PA peptoid is positively charged in a neutral or low pH solution due to the protonated amine groups. An analogue of the PA peptoid, known as "thiolated PA" (Figure 1C), was also studied as a means of covalently immobilizing peptoid onto a gold substrate. The thiolated peptoid is identical to PA except one of the amine groups is replaced by a thiol group. In addition to the polyionic PA and PC polymers, a single peptoid molecule that contains both PA and PC segments, referred to as "Block28" (Figure 1D) (due to the total number of monomers) [5], was also investigated. A thiolated version of this peptoid, referred to as "thiolated Block28" (Figure 1E), was also explored as a strategy for covalently attaching the peptoid onto a gold surface. Lastly, a hybrid peptoid/peptide sequence that contains an inner hydrophilic peptide sequence recognized and phosphorylated by the enzyme Casein Kinase II (CK2) was used to create a biologically-active peptoid film [5]. The sequence of the kinase-binding portion of the polymer is EEESGGE, where E = Glutamic Acid, S = Serine, and G = Glycine. As shown in Figure 1F, this peptide sequence is sandwiched between two amphiphilic peptoid regions. The hybrid sequence is referred to as "Loop12" because the twelve monomers sandwiched between the PA and PC segments are designed to fold into a loop when this polymer assembles at the air-water interface.

Table 1 – Peptoid Sequences, Names, Chemical Formulas, and Molecular Weights

Peptoid Sequence	Peptoid Name	Chemical Formula	Molecular Weight
$(Nce\text{-}Npe)_{18}$	PC	$C_{270}H_{327}N_{37}O_{72}$	5242.7
$(Nae\text{-}Npe)_{18}$	PA	$C_{252}H_{345}N_{55}O_{36}$	4720.8
$(Nae\text{-}Npe)_2\text{-}Nte\text{-}Npe\text{-}(Nae\text{-}Npe)_{15}$	Thiolated PA	$C_{252}H_{344}N_{54}O_{36}S$	4737.8
$(Nae\text{-}Npe)_7\text{-}(Nce\text{-}Npe)_7$	Block28	$C_{203}H_{262}N_{36}O_{42}$	3878.5
$(Nae\text{-}Npe)_2\text{-}Nte\text{-}Npe\text{-}(Nae\text{-}Npe)_4\text{-}(Nce\text{-}Npe)_7$	Thiolated Block28	$C_{203}H_{261}N_{35}O_{42}S$	3895.5
$(Nae\text{-}Npe)_7\text{-}Nme\text{-}\beta\text{-}Ala\text{-}EEESGGE\text{-}\beta\text{-}Ala\text{-}(Nme)_2\text{-}(Nce\text{-}Npe)_7$	Loop12	$C_{251}H_{338}N_{48}O_{66}$	5083.7

Key: Nce = N-(2-carboxyethyl) glycine), Npe = N-(2-phenylethyl) glycine, Nae = N-(2-aminoethyl) glycine, Nte = N-(2-thioethyl) glycine, Nme = N-(2-methoxyethyl) glycine, β-Ala = beta-Alanine, E = Glutamic Acid, S = Serine, and G = Glycine.

A



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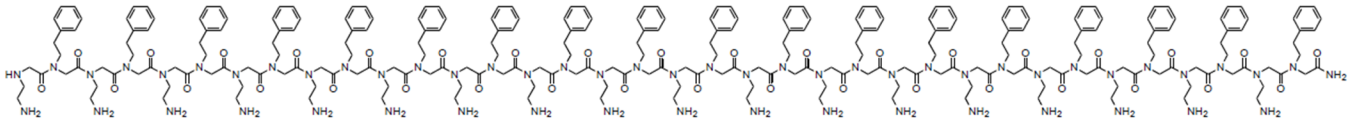

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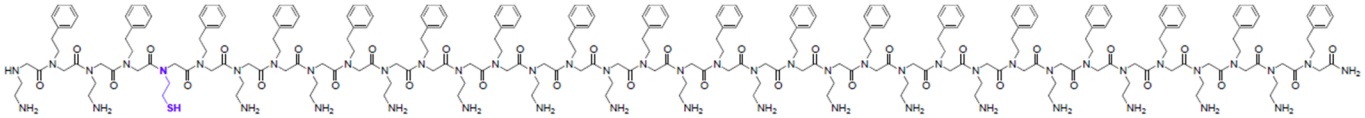

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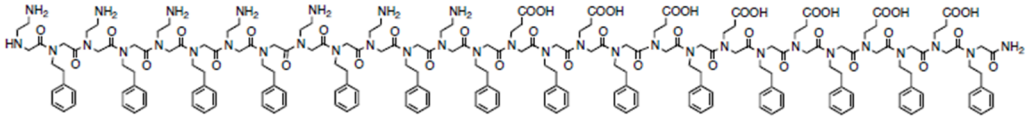

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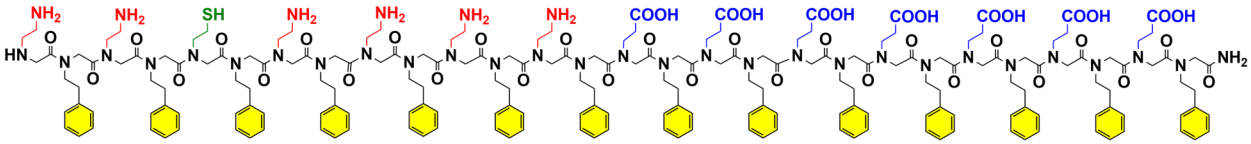

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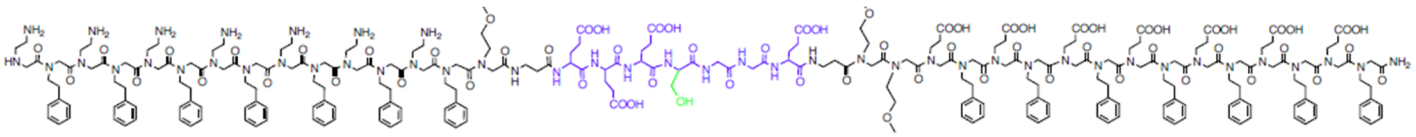

Figure 1. Chemical structures of the six peptoids used in this work. (A) PC, a polyanionic molecule composed of phenethyl and carboxyethyl groups. (B) PA, a polycationic molecule composed of phenethyl and aminoethyl groups. (C) Thiolated PA, identical to PA except one amine group is replaced by a thiol group. (D) Block28, a single molecule composed of both a PC and a PA segment. (E) Thiolated Block28, identical to Block28 except one amine group is replaced by a thiol group. (F) Loop12, a hybrid peptoid/peptide which contains an inner hydrophilic sequence that is recognized and phosphorylated by the enzyme Casein Kinase II.

Film Deposition Techniques

Three types of peptoid films were produced and studied: Layer-by-Layer multilayers, nanosheet bilayers, and Gibbs monolayers.

A. Layer-by-Layer Peptoid Films

One method of producing ultrathin nanostructured films is the Layer-by-Layer technique (LbL) [6]. This method entails depositing a film one layer at a time on a substrate. The substrate is subjected to a charged polyion, washed, subjected to an oppositely charged polyion, washed, and then this process may be repeated multiple times. This produces a thin film of oppositely charged layers. A Quartz Crystal Microbalance (QCM) system can be utilized to grow and analyze such films [7]. During operation, a quartz crystal, sandwiched between two electrodes, is driven into oscillation by applying a voltage across the electrodes. The resonance frequency of oscillation depends on the total mass of the sensor. As molecules flow over and deposit onto the sensor, the resonance frequency shifts. The Sauerbrey equation, shown below, provides the relationship between the change in mass of the growing film and the frequency change of the quartz sensor [8].

$$\Delta f = -\frac{2f_0^2}{A\sqrt{\rho_q \mu_q}}\Delta m \qquad \text{(Sauerbrey Equation)}$$

$$where \quad \Delta f = frequency\ change$$

$$f_0 = initial\ frequency$$

$$A = piezoelectrically\ active\ crystal\ area$$

$$\rho_q = density\ of\ quartz$$

$$\mu_q = shear\ modulus\ of\ quartz$$

$$\Delta m = change\ in\ mass\ of\ film$$

A single layer of positively-charged PA and a single layer of negatively-charged PC were deposited on a gold-coated QCM sensor using the LbL technique. The peptoids were flowed over the sensor at concentrations of 5 uM and at a flow rate of 50 uL/min. A washing step of water or 40 mM sodium hydroxide was performed after PA or PC were flowed, respectively. The deposition of each layer was verified by the frequency shift of the quartz crystal. The two layers adhered to the sensor regardless of which peptoid was deposited first (Figure 2). Additional layers did not adhere when the flow sequence was repeated. Based on the Sauerbrey equation, the film produced in which PA was deposited first had a PA film density of 159 ng/cm^2 and a PC density of 74 ng/cm^2 before the wash step. The PC-first, PA-second film had a PC film density of 112 ng/cm^2 before washing (71 ng/cm^2 after washing) and a PA density of 106 ng/cm^2. These film densities roughly equal the formation of a monolayer that covers an area between 0.59 and 0.94 cm^2, which is comparable to the area of the gold-

coated sensor. This calculation is based on the molecular weights of the peptoids and the estimated surface area that each molecule occupies in a film. Thus, the LbL method can be utilized to grow a thin, two-layer film of oppositely charged peptoids on a gold coated surface.

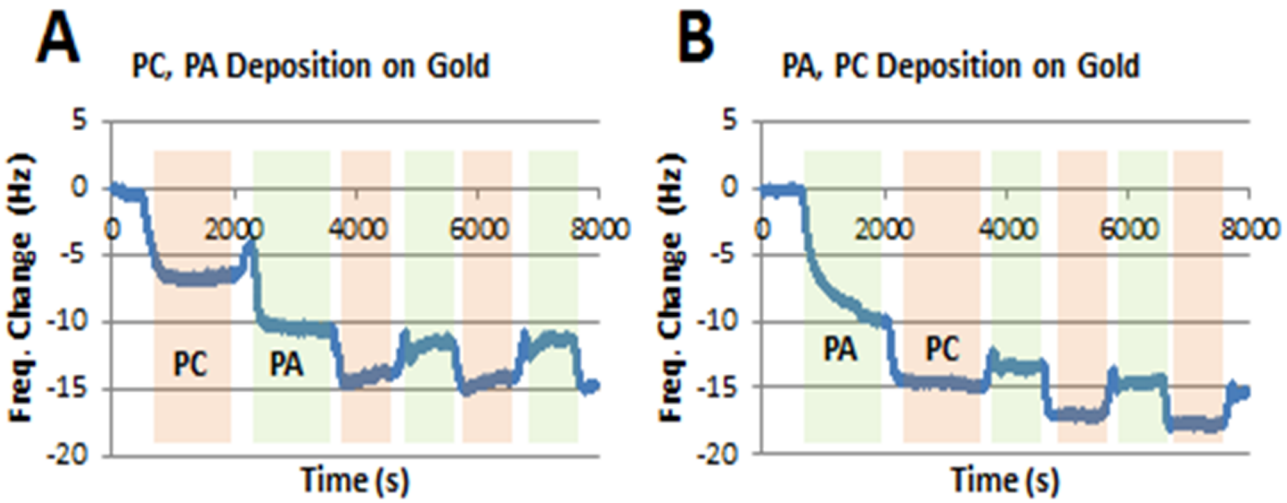

Figure 2. (A) Frequency changes in the QCM as PC and then PA are flowed (and repeated) over the gold-coated sensor. (B) Frequency changes as PA and then PC are flowed (and repeated). Orange and green frames represent the times when PC and PA were flowed, respectively.

B. Peptoid Nanosheets

Both PA and PC peptoids are amphiphilic; thus they are surfactants and, when in an aqueous solution, they migrate to the air-water interface and form a monolayer [9]. This monolayer consists of alternating strands of PA and PC due to the opposite charges on PA and PC at neutral pH. Each peptoid strand is oriented with hydrophobic moieties pointing out of the water and hydrophilic regions facing into the water. When the air-water interface containing a monolayer of PA and PC is compressed beyond a critical point the film collapses on itself into the aqueous phase and, as the hydrophobic phenyl rings orient themselves facing each other as to minimize exposure to water, nanosheets are formed (Figure 3). These sheets (peptoid bilayers) are exactly two monolayers thick, have thickness less than 3 nm, yet widths and lengths observed up to 1 mm, and remain stable in solution [9].

An aqueous solution of the PA-PC sheets was prepared in a small vial by mixing 10 uM PA and 10 uM PC in 10 mM Tris buffer, pH 8. The vial was rotated overnight at one revolution per minute. This rotation speed was sufficient to allow the peptoids in solution time to migrate to the surface of the water and form enough of a monolayer film before being collapsed. Collapse occurred each time the vial was rotated due to the decrease in the liquid-air interface surface area; thus nanosheets were

formed at each vial rotation. The solution of PA-PC nanosheets was passed through the QCM flow cell. However, no adsorption of the nanosheets onto gold-coated sensors occurred as no frequency shift was observed during flow, even when the nanosheets (shown in Figure 3A) were prepared with thiolated PA (Figure 1C), in place of the PA peptoid (Figure 1B).

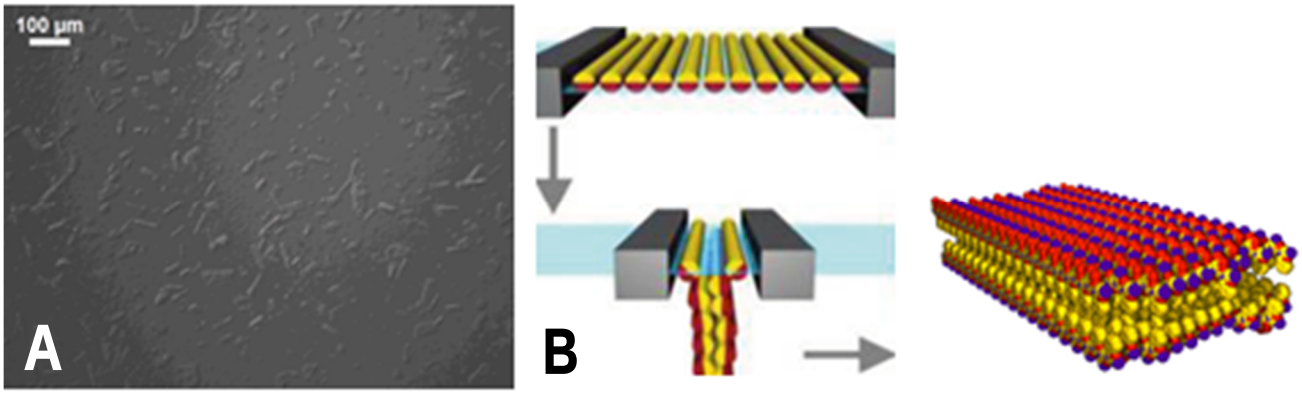

Figure 3. (A) Optical image of thiolated PA-PC nanosheets that flowed through the QCM, but did not adsorb on the gold-coated sensor. (B) PA-PC nanosheet formation. Color scheme: yellow = carbon, red = oxygen, blue = nitrogen [4]. A peptoid monolayer forms at the air-water interface with the hydrophobic moieties pointing out of the water. As the air-water interfacial area is reduced, the monolayer folds in on itself, with the hydrophobic groups burying themselves within the interior of the bilayer to avoid water exposure, thereby forming nanosheets in solution [9].

The binding of nanosheets to gold was also studied using gold nanoparticles. Thiolated Block28 nanosheets were prepared in solution and then 5 nm gold nanoparticles were added to the sheet solution. The nanosheet/nanoparticle solution was placed on a rocker at room temperature overnight and was tilted every 900 seconds in order to agitate the solution and keep it well mixed. Scanning Electron Microscopy images indicate that the gold nanoparticles adhered to the sheets, although complete coverage did not occur (Figure 4). These results demonstrate that the thiolated nanosheets are in fact capable of binding to gold. Perhaps the inability of sheets to bind to gold in the QCM study can be attributed to the constant flow of liquid over the gold sensor. Therefore, the conditions required for covalently immobilizing peptoid nanosheets onto a QCM sensor require further optimization. In view of these results, we opted to explore alternative methods of peptoid film deposition which do not involve fluid flow.

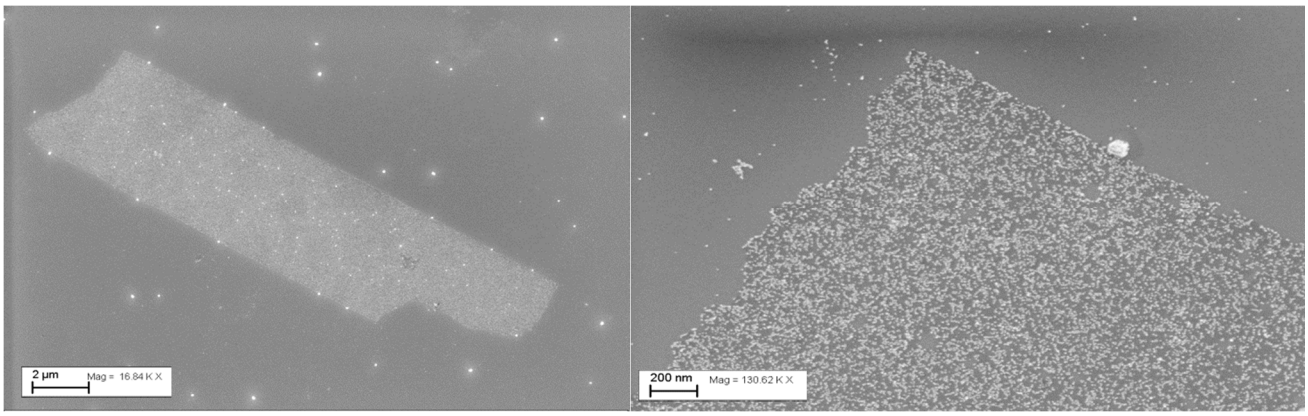

Figure 4. Scanning Electron Microscopy image of a thiolated Block28 peptoid nanosheet speckled with gold nanoparticles

C. Gibbs Monolayer Peptoid Films

Another technique utilized in this work was the production of Gibbs monolayer films. Such films are produced when water-soluble, amphiphilic surfactant molecules are deposited in a trough filled with an aqueous solution [10]. The amphiphilicity of the molecules causes them to migrate to the trough air-water interface and to orient themselves with their hydrophobic regions facing out of the water and their hydrophilic regions remaining in the water. This produces a monolayer which can be compressed into a packed film with the use of movable barriers on the surface of the trough. The available surface area (or "trough area") for the monolayer to occupy increases as the barriers open and decreases as the barriers close. Figure 5A shows a standard Langmuir trough with a Wilhelmy plate surface pressure sensor in the middle and two movable paddles or barriers on either side.

A Wilhelmy plate is often used to monitor the surface tension (and thus surface pressure) of the liquid in the trough [11]. Surface pressure measurements are critical in determining the monolayer properties of Gibbs films. Surface pressure can be expressed as the change in surface tension as a function of the area of water surface available to each molecule, or mathematically, $\pi = \gamma_0 - \gamma$, where pi is surface pressure and gamma is surface tension.

A common method for characterizing Gibbs films is the measurement of surface pressure (π) as a function of average area per molecule (A) [12]. This is performed by slowly compressing and opening the trough barriers at a constant temperature, thereby producing a plot of π vs. A, also known as an isotherm. Gibbs monolayers can exist in several phases depending on the surface pressure [13]. With no barrier compression, a Gibbs film is often gaseous, and as compression occurs, the film transitions to liquid-expanded to liquid-condensed, and finally to solid. A collapse point is often reached during compression and is signified by a stabilization of or decrease in the rate of change of π as compression increases (i.e., as A decreases). Collapse occurs when multilayers or vesicles form as the monolayer is compacted beyond its limits in terms of molecular packing.

Gibbs monolayers can be transferred to substrates via the Langmuir-Blodgett (LB) technique in which the substrate is slowly immersed orthogonal to the surface of the water, or via the Langmuir-Schaeffer (LS) technique (Figure 5B) in which the substrate is lowered parallel to the surface of the water and is raised once contact is made with the monolayer at the liquid surface [14]. A hydrophobic substrate is generally chosen for making LS transfers because the substrate, as it approaches the surface of the liquid, will interact with the hydrophobic side of the Gibbs film. This hydrophobic-hydrophobic interaction is desirable for effective film transfer and stability.

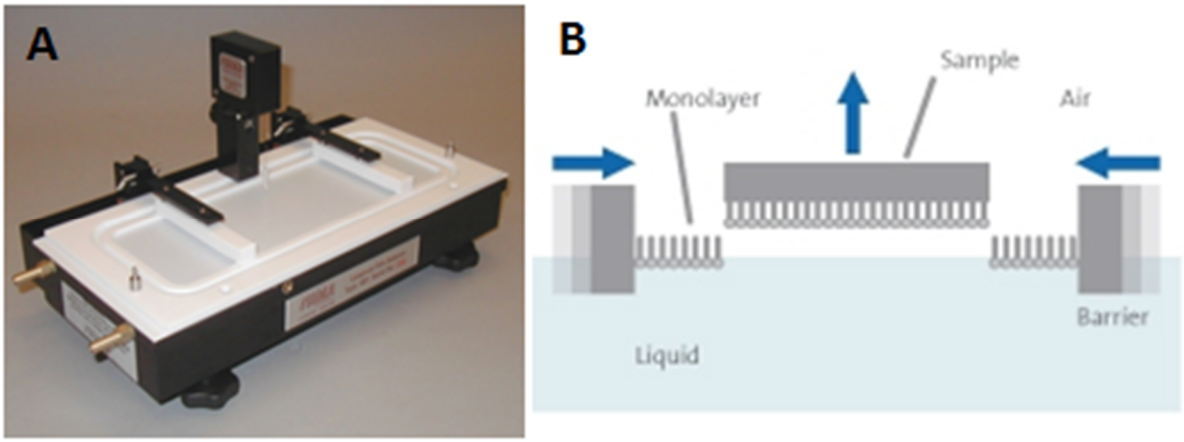

Figure 5. (A) A standard Langmuir trough containing a surface pressure sensor and two movable barriers on each side [15]. (B) Langmuir-Schaefer film transfer from a Langmuir trough [16]. The barriers move laterally to compress the Gibbs monolayer film to the desired surface pressure and then a substrate is lowered parallel to the monolayer. Once contact is made with the monolayer, the substrate is raised back up, transferring the monolayer from the trough to the substrate.

i. Block28 Gibbs films

In this study, a Langmuir trough was filled with 20 uM Block28 and the surface pressure was measured while the peptoids migrated to the air-water interface to form a monolayer (Figure 6A). After 1.5 hours the surface pressure approached equilibration at around 23 mN/m. An isotherm was obtained with 20 uM Block 28 which indicated that the collapse pressure occurs at approximately 41 mN/m (Figure 6B).

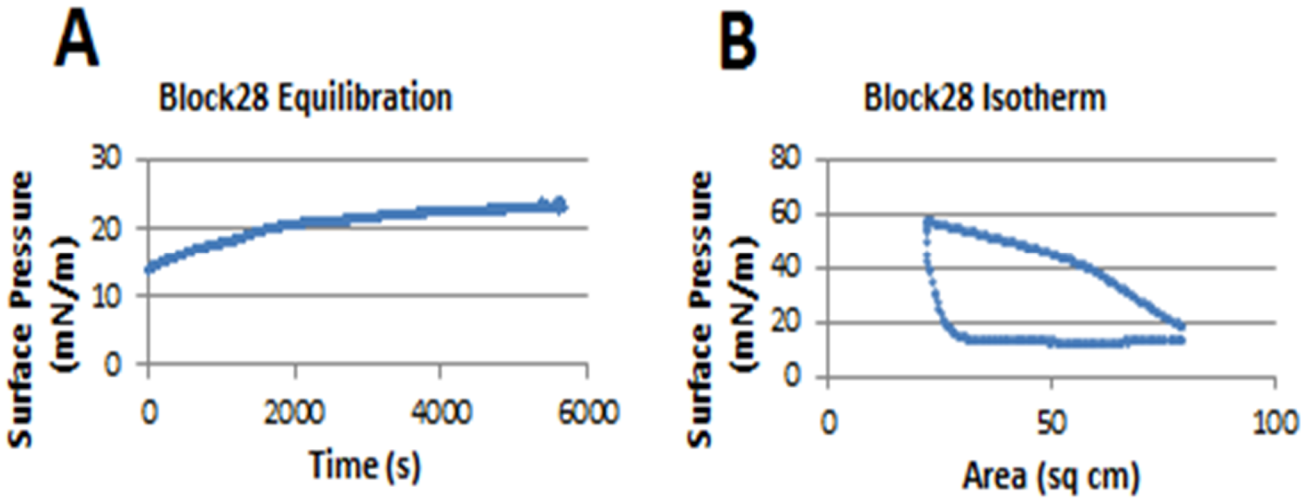

Figure 6. (A) Adsorption kinetic plot for 20 uM Block28. (B) Isotherm of 20 uM Block28.

Silicon was selected as a substrate to transfer the peptoid Gibbs films onto because of its flat surface and its common use as a substrate in fabricating electronic devices such as Field-Effect Transistors. Prior to film transfer silicon substrates were immersed overnight in a solution of trimethoxy (2-phenylethyl) silane in order to render the substrates hydrophobic [17]. This was determined necessary as silicon contains a hydrophilic native oxide. Since a Langmuir-Schaefer deposition involves bringing a substrate in contact with the hydrophobic portion of a Gibbs film at the air-water interface, it is important to ensure that the substrate is also hydrophobic in order to improve film adhesion and to minimize molecular reorientation during film transfer.

Langmuir-Schaefer depositions [18] were performed on silanized silicon at surface pressures between 27 (Figure 7) and 38 mN/m. This range was selected so as to ensure that the films were compressed (above their 23 mN/m equilibrium pressure) but not collapsed (occurring at 41 mN/m as shown in Figure 6B). As it is shown in Figure 7, prior to time = 40 s, the Block28 monolayer in the trough was at its equilibrium pressure of 23 mN/m. At 40 s the barriers were compressed until the pressure reached 27 mN/m. At 110 s the mechanical arm holding a silicon substrate began lowering the substrate towards the liquid surface. At approximately 142 s the substrate made contact at the liquid-air interface and then immediately the mechanical arm began removing the substrate from the liquid surface. The drop in surface pressure (going below 19 mN/m) immediately after substrate removal signifies the loss of surfactant from the air-water interface and is necessary for the peptoid monolayer to be transferred to the silicon. The surface pressure then begins to rise back to the set value of 27 mN/m due to the occurrence of two simultaneous events: 1) peptoid from the subphase begins to migrate to the surface to replace the transferred peptoid film by forming a new monolayer and 2) the barriers close somewhat in order to compress the remaining surfactants at the air-water interface. The latter of the two events is much more significant because the timescale for appreciable adsorption is much longer than the time required for the barriers to close.

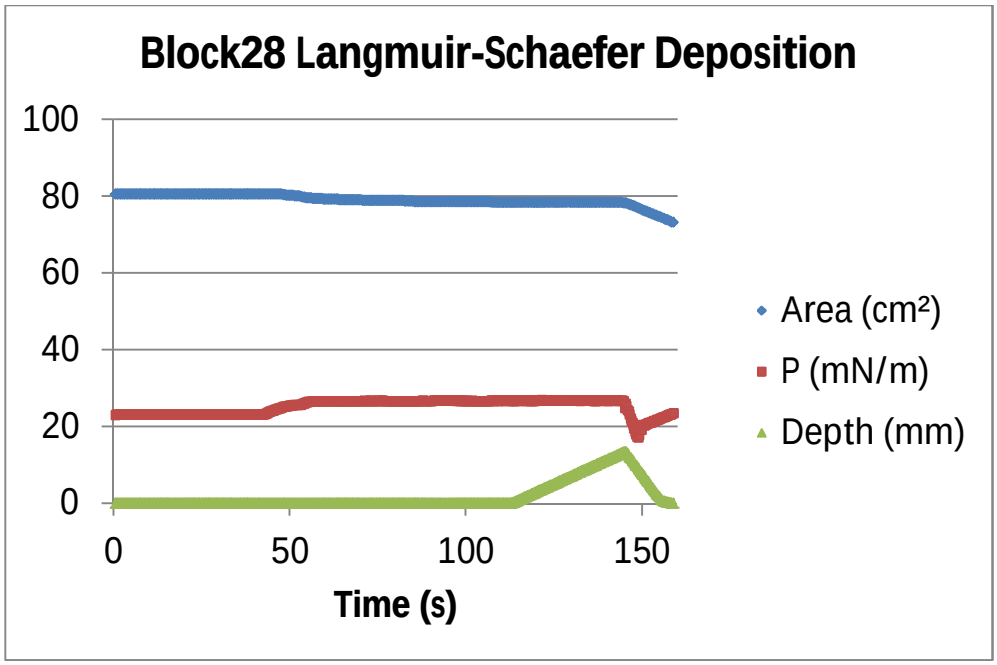

Figure 7. Langmuir trough data acquired while producing a Block28 Langmuir-Schaefer film on silanized silicon at a pressure of 27 mN/m. Blue (top) line represents trough area, red (middle) line represents surface pressure, and green (bottom) line represents mechanical dipping arm depth.

ii. 50% Loop Gibbs films

After successful film transfers of Block28, Langmuir trough studies with Loop12 were performed. A trough was filled with 10 uM Block28 and 10 uM Loop12, a mixture referred to as "50% Loop." After 2.5 hours the surface pressure reached 30 mN/m (Figure 8A). An isotherm was obtained (Figure 8B) which reveals a collapse point that is not as pronounced as was with pure Block28 (Figure 6B). The collapse pressure of 10 uM Block28 and 10 uM Loop12 occurs roughly at 45 mN/m.

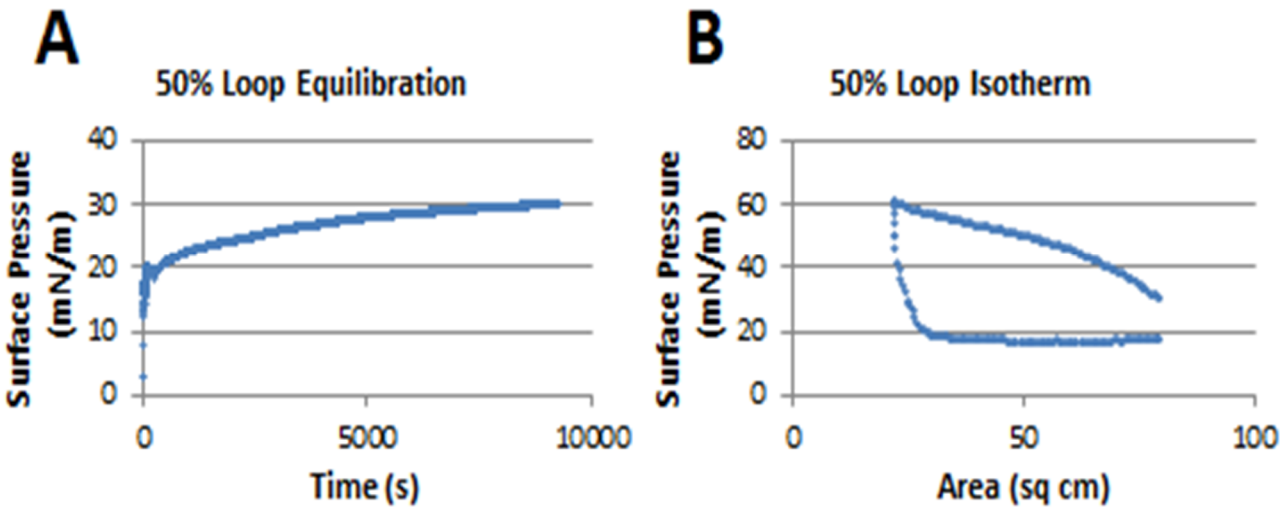

Figure 8. (A) Adsorption kinetics for 20 uM 50% Loop (10 uM Block28 and 10 uM Loop12). (B) Isotherm of 20 uM 50% Loop.

The peptide sequence inserted in the middle of the Loop12 sequence is hydrophilic, whereas the peptoid portion of the polymer chain (essentially Block28) is amphiphilic. When a Langmuir trough is filled with 50% Loop and given time to equilibrate, the water surface contains a monolayer of Loop12 and Block28 strands. As the barriers begin to compress the film, the hydrophilic peptide moieties will be submerged into the water as the peptoid "bookends" are pushed closer to each other while still remaining at the water surface due to their hydrophobicity. Hence, when 50% Loop is compressed sufficiently, it is expected that the monolayer will consist of mostly Block28-like structures at the air-water interface while the peptide sequence will be compressed into a loop oriented into the water (Figure 9). Loop12 was designed specifically in order that the peptide sequence (recognized and phosphorylated by CK2) would be exposed (pointing into the air) when transferred onto a hydrophobic substrate [5].

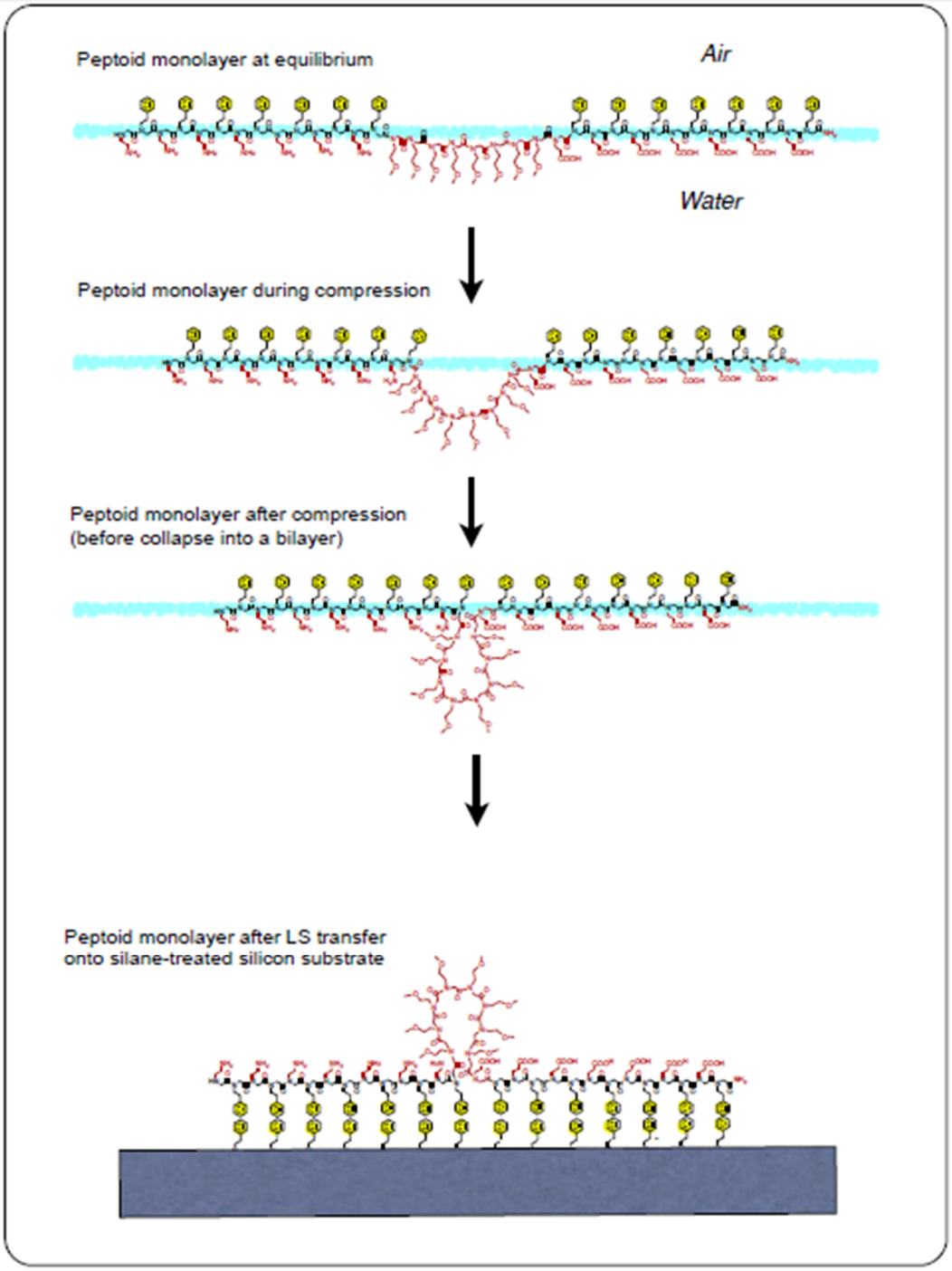

Figure 9. Peptoid loop monolayer formation in water and film structure following Langmuir-Schaefer (LS) deposition onto a silanized silicon substrate. A peptoid monolayer consisting of an inner hydrophilic sequence flanked by amphiphilic sequences forms at the air-water interface. As the monolayer is compressed the hydrophilic segment compacts into a loop domain submerged in the water. After LS film transfer the loop domains are exposed, pointing into the air. Note: this figure shows a loop peptoid with a slightly different composition than Loop12, but the overall concept and mechanism is the same [5].

Langmuir-Schaefer depositions of 50% Loop were performed on silanized silicon at surface pressures between 35 and 40 mN/m (Figure 10). This range was chosen to ensure that the films were close to but below collapse such that a peptoid monolayer functionalized with peptide loops would be formed without collapsing the monolayer into nanosheets. The decrease in surface pressure (and the subsequent decrease in trough area in order to raise the surface pressure back up to the set value of 40 mN/m) after the substrate contacts the film at time = 210 seconds attests to the transfer of material from the trough.

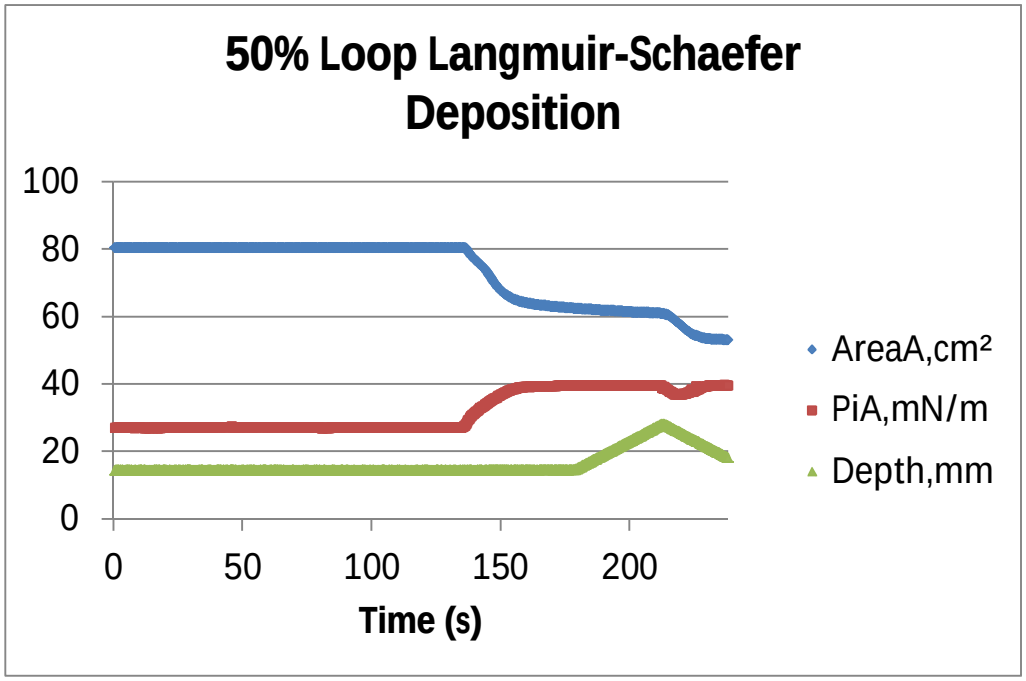

Figure 10. Langmuir trough data acquired while producing a 50% Loop Langmuir-Schaefer film on silanized silicon at a pressure of 40 mN/m. Blue (top) line represents trough area, red (middle) line represents surface pressure, and green (bottom) line represents mechanical dipping arm depth.

Film Characterization

Contact angle measurements were performed on bare silicon and on the trimethoxy (2-phenylethyl) silanized silicon substrates to determine the effectiveness of the treatment. A single water drop on silicon receiving no silane treatment fully wetted the substrate, indicating that the bare silicon (with a native silicon dioxide layer) was quite hydrophilic, as expected. With the goal of transferring peptoid films onto silicon such that the polar residues (particularly the biologically-active loop domain of Loop12) would be oriented away from the substrate, it was necessary to render the silicon substrate hydrophobic. This would permit the hydrophobic side of each Gibbs film to adhere to the substrates during Langmuir-Schaefer film transfers (Figure 9). The average advancing (θ_{adv}) and receding (θ_{rec})

contact angle was 86.3 degrees and 75.4 degrees, respectively, for the silane-treated silicon substrates. The observed contact angle hysteresis (θ_{adv} - θ_{rec}) of 11 degrees is quite low and suggests that the silane treatment was successful in producing a uniform, defect-free, hydrophobic surface coating sufficient for Langmuir-Schaefer transfers [19].

The thickness of Langmuir-Schaefer monolayer films was measured using Atomic Force Microscopy. Block28 and 50% loop films measured on average 3.5 and 5 nm, respectively. The thicker 50% loop films are consistent with peptide loops having been successfully deposited onto the substrate. Measurements were obtained by making a 2 square micron scratch in the films and then imaging the height profile inside and outside of the scratch (Figure 11).

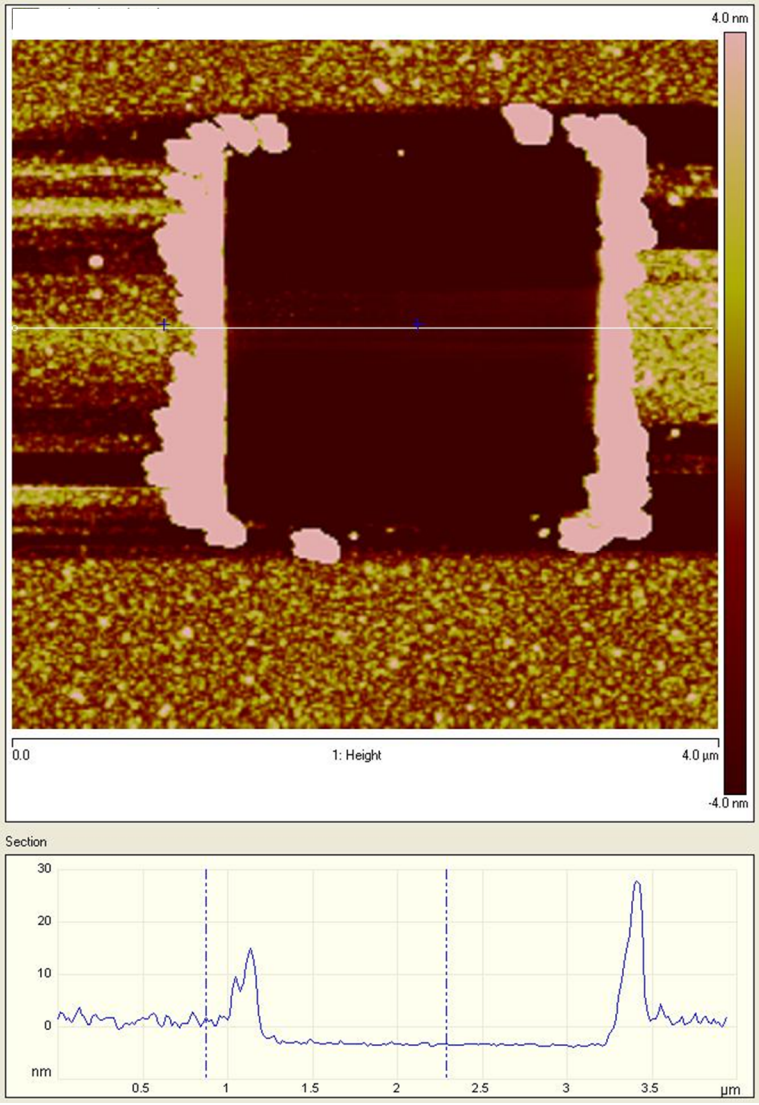

Figure 11. AFM image of the 50% Loop film containing a 2x2 um scratch. The height profile indicates a film height of 5 nm.

X-ray Photoelectron Spectroscopy (XPS) was performed on bare silicon, silanized silicon, Block28 on silanized silicon, and 50% Loop on silanized silicon. The results of this study are shown in Table 2. The bare silicon substrate shows a composition of silicon and oxygen as one would expect, although it also shows the presence of carbon and a trace amount of sulphur (Figure 12). The 164 eV peak is attributed to sulphur, while both the 99 and 151 eV peaks are attributed to silicon (2p3 and 2s orbitals). The carbon may be due to a layer of environment hydrocarbons on the surface of the silicon. The oxygen is expected due to the native silicon dioxide layer which forms naturally on silicon. Silanized silicon shows the presence of silicon, oxygen, and carbon as one would expect (Figure 13).

Table 2 – Elemental Composition of the Silicon Substrate, Silanized Silicon, and the Peptoid Films

Film	% Carbon	% Nitrogen	% Oxygen	% Silicon	% Sulphur
Silicon	32.5	-	44.5	19.8	3.1
Silanized Silicon	44.5	-	32.7	22.8	-
Block28	54.0	6.2	28.0	11.8	-
50% Loop	68.5	7.1	24.4	-	-

The Block28 film on silanized silicon shows a composition of oxygen, carbon, silicon, and nitrogen as expected (Figure 14). The presence of nitrogen on the Block28 substrate and the absence of nitrogen on the silanized substrate indicate peptoid film transfer. In addition, the increase in carbon content (due to the large carbon content in peptoids) and the decrease in silicon content (XPS measures the atomic composition of the top few nanometers of a film [20]; thus a drop in silicon content may be due to the presence of a non-silicon containing film on top of it) support the claim of film transfer.

The 50% Loop film on silanized silicon shows a composition of oxygen, carbon, and nitrogen as one would expect (Figure 15). The presence of nitrogen and the large carbon content are again indicative of the presence of peptoids on the substrate. The complete lack of silicon in the spectra can be attributed to the thickness of the film (5 nm), which is further indicative of peptoid film transfer. Furthermore, it can be concluded that the 50% Loop films (which contain the protruding peptide loop) are thicker than the Block28 films because silicon was found in the spectra of the Block28, whereas no silicon was found in the 50% Loop spectra. This is explicable if the Block28 film was not sufficiently thick to prevent the X-ray from penetrating through the film and into the silicon enough to emit photoelectrons from the silicon atoms. The absence of silicon in the 50% Loop film spectra can be attributed to the film being thick enough to prevent the penetration into and excitation of electrons within the underlying silicon substrate. Thus, the XPS data indicates that peptoids were transferred on to the silanized silicon substrates during the Langmuir-Schaefer depositions of Block28 and 50% Loop and that the transferred 50% Loop films are indeed thicker than the Block28 films.

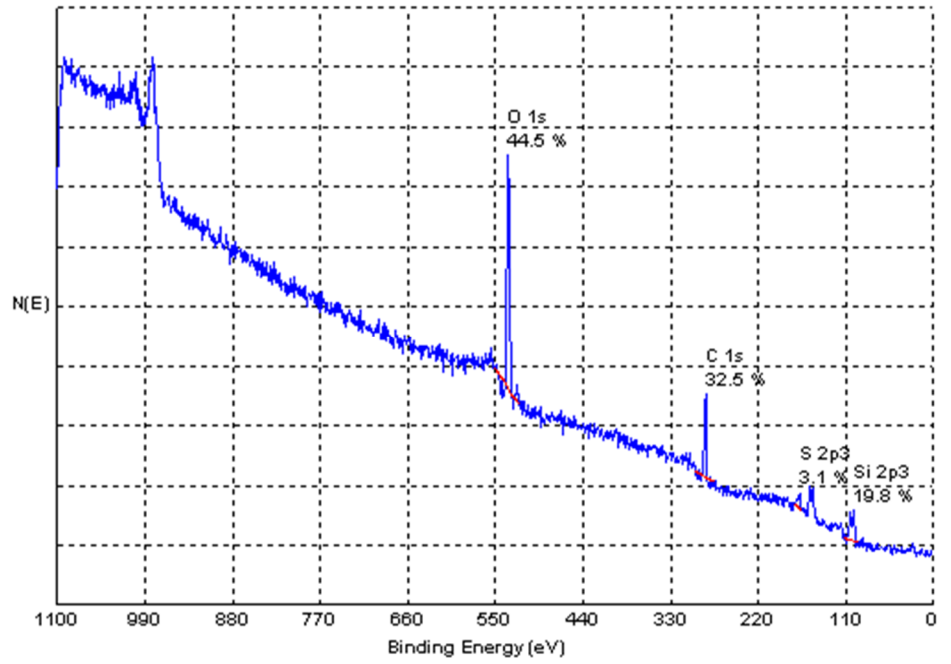

Figure 12. XPS spectra of bare silicon

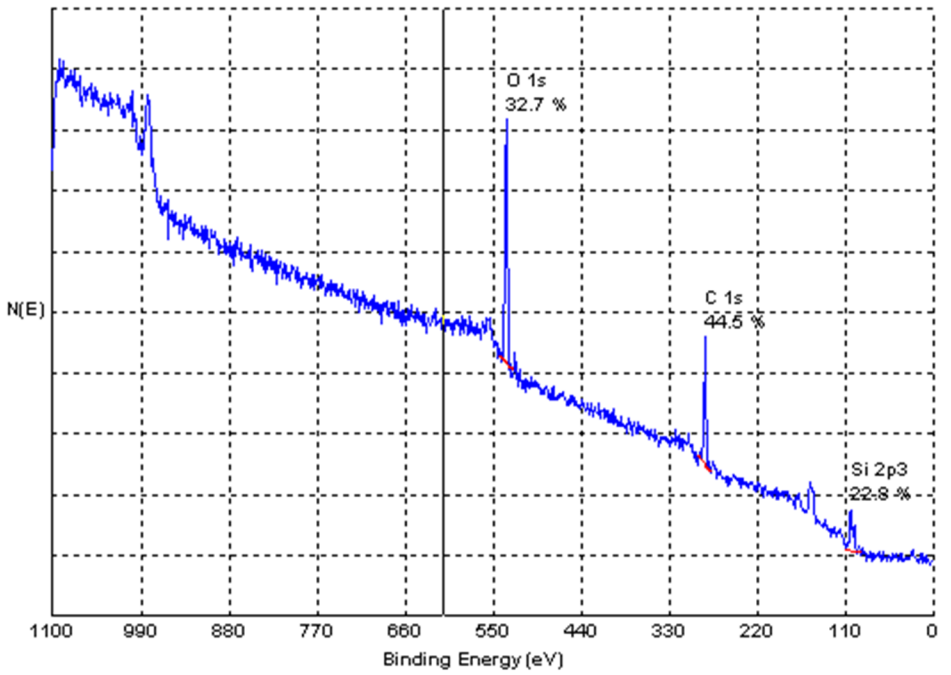

Figure 13. XPS spectra of trimethoxy (2-phenylethyl) silanized silicon

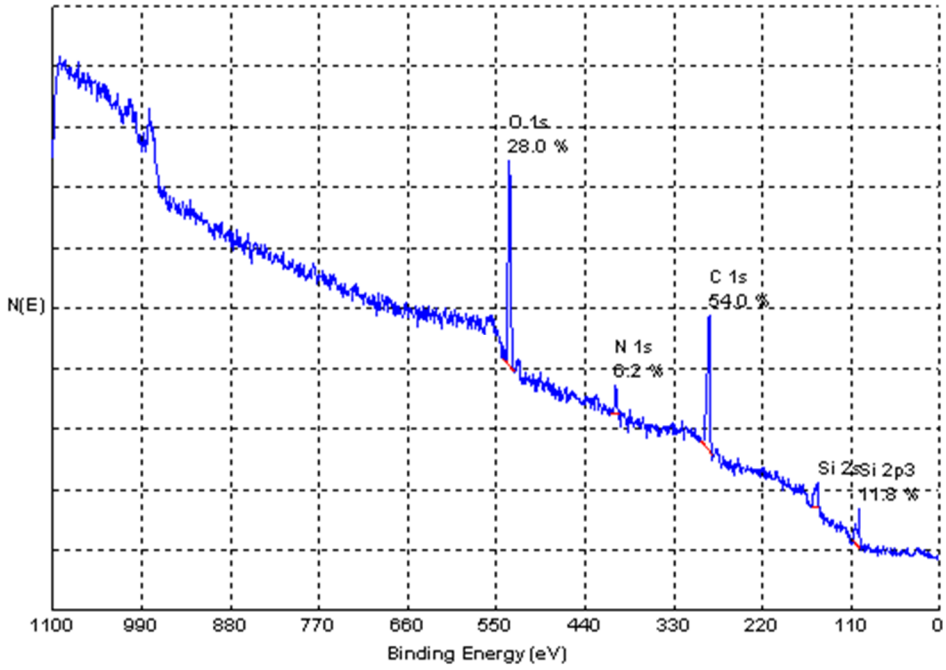

Figure 14. XPS spectra of a Langmuir-Schaefer Block 28 film deposited at 27 mN/m on silanized silicon

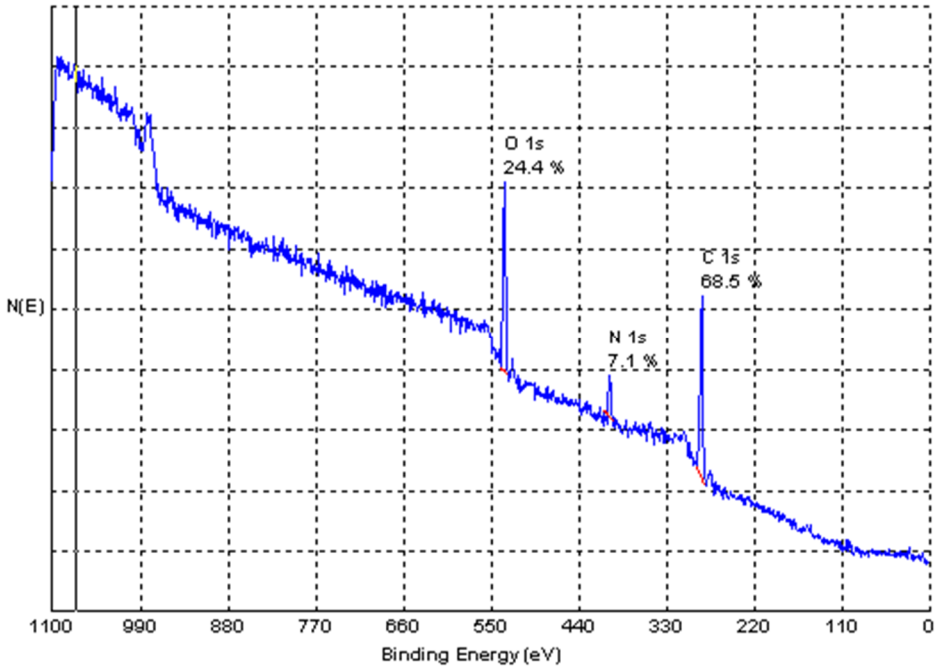

Figure 15. XPS spectra of a Langmuir-Schaefer 50% Loop film deposited at 40mN/m on silanized silicon

Next Step: Biosensor Device Fabrication

A biological sensor can be defined as a device that detects the presence of an analyte by transducing a biological interaction into a signal that is then processed. Individuals with diabetes are in need of reliable blood glucose sensors [21], agencies responsible for counter bioterrorist efforts need tools for airborne bacteria sensing [22], and food producers need dependable sensors for drug residues in food (i.e., growth factors or antibiotics in meat and honey) [23].

One of the many types of biosensor architectures is an ISFET (Ion-Sensitive Field-Effect Transistor) (Figure 16). An ISFET sensor is designed to transduce a biological interaction into an electronic signal. The device consists of a substrate containing source and drain electrodes separated by a semiconductor. A passivating dielectric covers the semiconductor and is exposed to a solution. A gate electrode (often a Ag/AgCl reference electrode) is suspended in the solution. As with a typical MOSFET (Metal Oxide Semiconductor Field-Effect Transistor), when potential is applied to the gate electrode, the electric field produced causes a change in the conductivity of the semiconductor. Thus, with a source-drain bias applied, the gate electrode can turn the drain current on or off as well as modulate it. Since the medium between the semiconductor and the gate is liquid, analytes and molecules of interest can be administered in the ISFET. The semiconductor surface (or insulating layer above the semiconductor) can be functionalized rendering it possible for reactions to take place at the surface of the device. If such reactions result in a charge build up (or decrease) then the electric field experienced by the semiconductor is altered, leading to a change in the drain current. Hence, one can directly correlate changes in drain current with biological interactions/reactions [24].

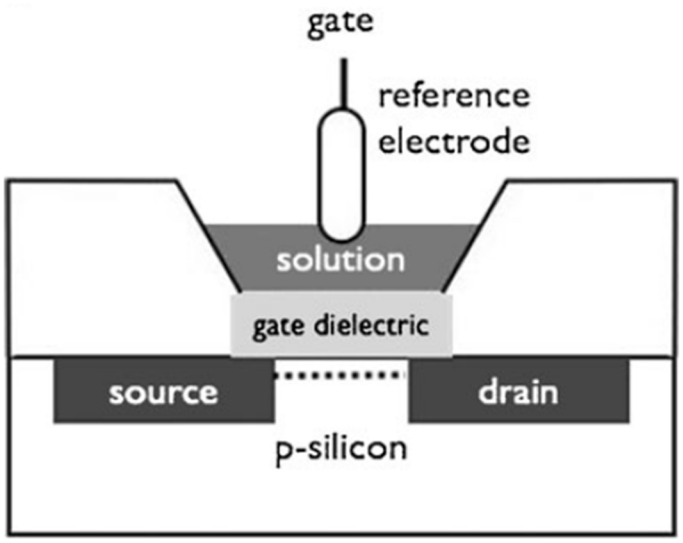

Figure 16. Typical device structure of an Ion-Sensitive Field-Effect Transistor [24]. The current that flows between the source and drain is modulated by reactions taking place in the solution.

Freeman et al. produced an ISFET biosensor capable of detecting the activity of CK2 at a concentration of less than 0.002 units [25] (a unit is defined as the amount of the enzyme that catalyzes the conversion of 1 micro mole of substrate per minute at room temperature). Casein Kinase II is a protein kinase known to be involved in cell cycle, transcriptional control, apoptosis, and signal transduction [26]. The neurons of individuals with Alzheimer's disease have been found to have reduced amount and reduced activity of CK2, whereas individuals with various types of cancer have higher amounts of CK2 [27]. Casein Kinase II operates in cells by transferring a phosphate group from Adenosine Triphosphate (ATP) onto specific proteins, a process known as phosphorylation. Freeman produced an ISFET using a silicon substrate and an aluminum oxide passivation layer covering the source and drain. The aluminum oxide was functionalized with a specific peptide sequence recognized and known to be phosphorylated by CK2 in the presence of ATP.

Similar to the ISFET device produced by Freeman et al., the next stage of this work is the production of a CK2-sensing ISFET that utilizes peptoids. At the time of the submission of this report the biosensor ISFET device is in the fabrication process. Photolithography is being used to pattern source and drain electrodes on silicon. A silicon dioxide passivation layer is deposited via Plasma Enhanced Chemical Vapor Deposition. This oxide layer is silanized and then Loop films are transferred. The device will be placed in a microfluidic chamber with an inlet, outlet, and a port for the gate electrode. A second fabrication method is underway in which source and drain electrodes are evaporated and photolithographically defined on an oxide wafer and an organic semiconductor is spin-coated on top of the source and drains. Peptide loop-displaying peptoid films are then Langmuir-Schaefer deposited on top of the semiconductor wafer.

Conclusion

Peptoids show great promise as a platform and material for biosensing. However, no reports of a peptoid-containing electronic biosensor have been published in the literature as of yet. This work is a step toward realizing such a device. Peptoid films were produced using various methods, including Layer-by-Layer and Gibbs monolayer. Two peptoid Gibbs films, Block28 and 50% Loop, were transferred using the Langmuir-Schaefer technique onto silanized silicon. Characterization methods attested to the successful transfer of these films. The 50% Loop film will soon be incorporated in an Ion-Sensitive Field-Effect Transistor device in order to detect the activity of the enzyme Casein Kinase II.

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