Ph.D. Dissertation of Deokwon Seo

Sensitivity enhancement of colorimetric polydiacetylene-based biosensors towards highly sensitive real-time diagnostic method for clinical applications

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Abstract

Combining the data-oriented decision making and the desire for higher quality of life, there is an increasing interest in diagnosing and monitoring the state of one's health. In particular, a widespread pandemic in recent years has increased the necessity for fast and reliable screening methods. To meet the demand, various types of biosensors have been proposed and researched.

Polydiacetylene (PDA) is a type of conjugated polymer widely researched as biosensor materials for its low cost and expandable chemistry. Also, the unique optical properties (colorimetric change and fluorescence development) and lipid-bilayer cell membrane-like structure of self-assembled PDA liposomes allow interaction with diverse biomolecules, which are advantageous for biosensor applications. However, most of the developed PDA-based biosensors are yet far from commercial adoption because they have been developed based on pre-treated or purified target biomolecule, thereby questioning the fidelity in real applications. In addition, the sensitivity of PDA-based biosensors varies widely, limiting their applicability for the detection of trace amounts of analytes. Therefore, to improve a PDA-based sensor's commercial applicability, achieving higher selectivity as well as sensitivity is a must.

In this dissertation, a study on simple, sensitive, and rapid self-signaling PDA-based sensors that detect activated platelet, a specific target molecule, in a whole blood specimen without any pre-treatment is presented. Also, a universally applicable strategy to enhance the sensitivity of PDA sensors by pre-occupying the PDA liposome surface with artificial analytes (dummies) is reported, which can generate more effective stress to trigger the optical signals of the PDA backbone when biological target molecules are captured at the PDA liposome surface.

The necessity of a simple yet accurate platelet activation measurement has been increasing in clinical medicine to regulate a proper dose of antiplatelet drugs for patients having clinical outcomes in acute situations such as angina pectoris, stroke, or peripheral vascular disease or procedures involving angioplasty or coronary thrombolysis. A self-signaling PDA liposome microarray has been developed to detect activated platelets from whole blood samples in a single step. A specific antibody, 9F9 antibody, binding to platelet-bound fibrinogen was selected and conjugated to the PDA liposome microarray to quantify the fibrinogen-bound platelets. The developed PDA liposome−9F9 microarray generated an intense fluorescence signal when activated platelets in whole blood were introduced and also successfully distinguished the reduced platelet activation in the presence of Tirofiban, a model antiplatelet drug. The results of this single-step benchtop assay incorporate simple, sensitive, and rapid attributes that

can detect the extent of platelet activation prior to needed clinical procedures.

To improve the sensitivity, a new PDA sensor platform having pre-occupied artificial target molecules named as dummies is also developed. The dummy molecules generate repulsion force on the PDA sensor surface with analytes more effectively; thus, both device sensitivity and optical signal intensity are enhanced. It was discovered that the enhancement of sensory properties is maximized when the volumetric sizes of the analyte and dummy are close to each other. This dummy approach is also verified to be comparable with various PDA-based sensors. The dummy strategy was applied to the previously studied Neomycin and Surfactin detecting PDA sensors, and increased sensitivity of both sensors was observed. When the dummy strategy was combined with another sensitivity enhancement method based on phospholipid incorporation to make the PDA liposome more flexible, the combined effect enhanced the sensitivity by 16 times.

Keywords: polydiacetylene, fluorescence signal, platelet activation, artificial analyte, sensitivity enhancement

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Chapter 1. Introduction

1.1. Biosensor Development

A person living in a modern day society encounters numerous sensors everyday. Typically, a sensor's primary goal is to detect or measure changes in physical stimuli, to convert the information to signal or energy via a transducer, and finally to output a measurable signal. Generally speaking, there are two types of sensors: A physical sensor which measures physical quantities such as temperature and humidity, and a chemical sensor which detects and quantifies chemical analytes in the environment. A sensor can be integrated into functional devices. For example, an auto soap dispenser uses physical sensors to determine soap dispensing behavior and a chemical sensor is used within atmospheric alarm systems to alert dangerous atmospheric conditions. Living organisms also utilize more fascinating various types of sensors. A bat living in a dark cave possesses a sensing ability for ultrasound, which is a type of physical sensor. Dogs have a much more sensitive chemical sensor for smell than humans, which can be utilized to detect drugs and explosives in public places. Chemical sensors have a rich history starting from a gas analyzer in the early 1900s.[1] Since then, various chemical sensors have been developed and deployed. Biosensors have been considered as just a subset of chemical sensors until 1960s. Only in the 21st century, an explosive growth in research and industry made biosensor a separate field of study.

The concept of the biosensor was established after an oxygen electrode, which is also called Clark-type electrode, was invented by Leland C. Clark Jr. in 1962. The invention of the Clark-type electrode allowed other researchers to measure oxygen levels via electrochemical reaction. Furthermore, Leland C. Clark Jr. invented the first enzyme electrode by an enzyme immobilization method. The electrode, prepared by immobilizing glucose oxidase, could act as a sensor to measure dissolved oxygen concentration by glucose oxidation reaction.[2] At first, this type of sensor was referred to as bioelectrochemical sensor because of its electrochemical nature of the sensing mechanism. Overtime, sensing mechanisms other than electrochemical interaction have also been discovered, which gave birth to the term "biosensor." Today, there are various types of biosensors utilizing enzymes, antigen-antibody interaction, and microbial sensors.[3]

By nature, the close integration and collaboration between different fields of studies have made it difficult to clearly limit and define the field of the biosensor. In general, a biosensor satisfies one or more of the following criteria[4]:

1) The sensor's working principle involves interactions between biomolecules. If a sensor uses biomolecules such as enzymes, antibodies, antigens, or microbes, the sensor can be called a biosensor.

2) The sensor mimics a living organism's sensory organ. If a sensor aims to reproduce vision, sense of touch, smell, hearing, flavor, and any other sensory organs, the sensor can be called a biosensor.

3) The sensor is used to monitor vital signals. If a sensor is used to monitor a vital signal such as heart rate, blood, or any other body functions, the sensor can be called a biosensor.

1.1.1. Characteristics of Biosensor

A biosensor is a device that first detects a target matter's presence or concentration and outputs various forms of a signal by physical or chemical interaction with the target matter. Compared to a chemical sensor, a biosensor exhibits a few superior characteristics owing to the biomolecules.[5] The followings are such characteristics of a biosensor:

1. Selectivity

Selectivity is the most critical parameter of a biosensor. Selectivity is a sensor's ability to interact with a specific target in an environment containing other reactive molecules such as contaminants or metabolic byproducts. An example of a highly selective biosensor is the antigen-antibody sensor, where the antigens are used as the bioreceptors of the biosensor. When the biosensor is exposed to a buffer solution containing salts and the antibody, only the antibody reacts with the antigen. Since a biosensor's working environment often contains numerous types of chemicals, the selectivity must be considered first when constructing a biosensor.

2. Reproducibility

Reproducibility is the ability to output the same type and intensity of signal when exposed to the same type and level of the target matter on different occasions. In other words, when more than two measurements are conducted on the same type and level of the target matter, a sensor with high reproducibility will produce a signal corresponding to the actual value. Reproducibility of a sensor is related to the sensor's and the transducer's accuracy. A reproducible sensor is often also reliable.

3. Stability

Stability is the degree of response a sensor exhibits against the internal and external disturbance such as a change in temperature. Sensors with low stability can display erroneous output, which can result in misinterpretation of the analyte presence or concentration. It is highly important to utilize a stable sensor if a sensor requires prolonged incubation or long-term monitoring. A sensor's stability is often related to the affinity of the bioreceptor. Strong electrostatic or covalent bonding between the bioreceptor and the analyte often results in higher stability at a wider range of environmental fluctuation. Another factor to consider for stability is bioreceptor degradation over time.

4. Sensitivity

Sensitivity is a sensor's response to the change in analyte concentration. In particular, the smallest amount of analyte a sensor can detect is often called the limit of detection (LOD). Today's biosensing needs often require evaluating multiple analytes at very low concentrations, hence needing sensors with a low LOD. Therefore, it is important to develop a sensor with high sensitivity.

5. Detection range

Often called dynamic range or linearity, the detection range is the range of analyte concentration where the sensor signal output accurately corresponds in a linear manner.

6. Response time

Response time is the time needed to change the sensor's signal output from the value prior to sensing the change to the final value after sensing. The demand for shorter response time is increasing today because the response time is critical in some applications, such as some critical vital monitoring.

1.1.2. Applications of Biosensor

There are six characteristic areas of biosensor application: medical (clinical diagnosis), pharmaceutical, environmental, food, military, and academic research. Today, the highest demand for biosensors is from the medical field, and the trend is expected to continue in the future as well. In the medical field, a biosensor compatible with a human body over the long term can increase the patient's quality of life while offering minimum detection time. Such advantages can enable precise dosing of medicines with potential high toxicity and allow timely care for people with severe conditions. While the most well-known medical biosensor measures the level of glucose[6], sensors for other biomolecules such as lactic acid[7], cholesterol[8], and urea[9] are also available or under development.

Environmental biosensors often target critical molecules that can affect biological activities. For example, bio-oxygen demand of water, cyanide, phenol, heavy metals, pesticides, phosphorus compounds, and nitrogen compounds are all important indicators of an ecosystem.[10] Since detecting pollution can require a large quantity of sampling over a large landmass or a body of water, the economics of a sensor is also important. Therefore, a biosensor is expected to play a critical role in the future. For example, there is a continued effort to develop a biosensor with ultra-low LOD for endocrine disruptors such as dioxin.

In the food sector, biosensors can be applied to analyze food safety by detecting residual pesticides, antibiotic compounds, malicious germs, and

other toxins.[11] Typically, biosensors used in the food industry is less specific than those in other sectors.

In the military, biosensors are used to detect biochemical threats. Biosensors' short detection time is highly desirable under conditions requiring fast response, such as exposure to nerve agents, which can be lethal at merely low concentrations.[12]

In the pharmaceutical industry, biosensors are often used to detect specialty chemicals. In particular, the fermentation process uses biosensors.

Biosensors for academic research are prepared for very specific purposes, often at the cost of the size and ease-of-use. Typically, a biosensor for academic research is large in size and costly to produce. An example of a commercially available biosensor for academic research is surface plasmon resonance (SPR) biosensor, which is used to analyze the kinetics of biomolecules.[13]

1.2. Nano-Biotechnology

Biosensors, which have been applied in multiple fields, were advanced even further by integration with nanotechnology. Nanotechnology is an area of research to characterize and modify material properties as well as to synthesize new types of material from the molecular or atomic level. Nano-biotechnology, which is an integrated field between nanotechnology and biology, plays a critical role in explaining biological activities on a nano-

scale. Recent advancement in nano-biotechnology has contributed to new drug discovery, diagnostics, prognostics, and medical equipment by providing molecular-level insights into cellular activities.

Biosensors using nano-biotech have been developed with applications in biology, materials science, chemistry, electronics, mechanics, and medical studies. Because biosensors offer real-time analysis of biological or chemical molecules, biosensors have also been gaining interest as a subject of research. As a part of such research, nano-fabrication techniques are often used to prepare biosensors with high sensitivity and selectivity while maintaining a small size. Furthermore, molecular level detection and measurement can be offered by highly integrated non-invasive biosensors.

Typically, biological samples or chemical targets for biosensor analysis are acquired outside of a controlled laboratory environment. Therefore, it is also possible to integrate biosensors with micro-electro-mechanical systems (MEMS) or microfluidic devices to separate protein, gene, and other metabolites from easily accessible biological samples such as blood, urine, or saliva.[14] Advancing the frontier of such research could result in improved quality of life by offering early and accurate disease detection via an integrated biosensor system to separate, react, and quantify biological information.

In addition, integrating biosensors with other nanomaterial technology such as micro/macro-fluidics, functional material, self-assembled nanomaterials, and nanopatterning allowed the average population to easily

self-diagnose their health conditions which used to require well-trained professionals. Furthermore, integrated biosensors are also used to detect non-biological analytes for atmospheric and water pollution. In some cases, biosensors are also applied in food safety diagnosis and military applications.

Most biosensors' detection mechanism falls under the following three categories: electrochemical, mechanical, and optical. Below is a brief description of each type of biosensor:

1. Electrochemical detection

An electrochemical biosensor detects the physical and chemical change of receptor molecules, which are affixed on the sensor surface, when the receptor interacts with a specific analyte. In particular, highly sensitive small-size biosensor preparation has been researched by utilizing redox reactions to prepare amperometric, potentiometric, and conductometric sensors. In recent years, carbon nanotube (CNT) and graphene are also gaining interest because of their high conductivity and high surface area-volume ratio, which often leads to higher sensitivity. Research by Dr. Gorski's team at the University of Texas and Dr. Xiong's team at Xinyang Normal University are excellent examples of applying CNT[6] and graphene[15] to biosensor applications.

2. Mechanical detection

In mechanical biosensors, changes in mechanophysical parameters such as forces, motion, masses, etc., are quantified after the biomolecular interaction.[16] Mechanical biosensors can be classified into three main classes, quartz microbalance, surface acoustic waves, and nanomechanical systems. The mechanical biosensors consist of a cantilever probe, a mechanical transducer, and a processor. The cantilever probe is modified to detect the analyte, where its performance, such as protein binding capacity or detection limit, is a function of its shape/size and proportional to the mass of the cantilever.[17]

Mechanical transducers are grouped into two categories, namely displacement change based or piezoelectric based. The displacement-based transducer uses an optical detector that monitors a laser reflected from the surface of the cantilever, which changes its position based on the changed mass on it. The transducer with a piezoelectric crystal generates piezoelectricity against a stimulus, which is recorded for biosensing.

3. Optical detection

Biosensors with optical detection mechanisms have been widely applied to detect single molecules or to analyze single cells. To detect single molecules, methods such as surface enhanced Ramen (SERS), surface enhanced resonant Raman (SERRS), and surface plasmon resonance (SPR) have been developed. As an example, a biosensor developed by Dr. Corn’s

team at the University of Wisconsin can quantitatively detect the hybridization adsorption of short (18-base) unlabeled DNA oligonucleotides at low concentration.[18]

Another method of optical detection is to measure the changes in colorimetric and/or fluorometric signals when a conjugated polymer emits or absorbs light via interaction with the analyte. For example, Dr. Kim's team at the University of Michigan invented a new DNA detection method by bio-conjugating conjugated polymer electrode with detection DNA and utilizing the conjugated polymer electrode's optical antenna effect.[19]

1.3. Conjugated Polymer-Based Biosensor

As mentioned above, applying nanotechnology to biosensor research expanded the methods of sensor preparation. Today, a common goal of biosensor development is to establish a simple and inexpensive method, which offers an intuitive interpretation of the sensing results for untrained end-users.

Compared to its predecessor, small molecule sensors, conjugated polymer (CP) based biosensors exhibit a few advantages. A signal amplification effect is most notable advantage of a CP-based sensor over a small molecule sensor. When a small molecule sensor interacts with its analyte, only the specific small molecule that interacts with the target contricutes to the signal generation. On the other hand, a CP-based sensor

responds to analytes collectively because the individual sensory monomer units are wired throughout the conjugated backbone of the CP, resulting in amplified signal intensity, as demonstrated by the Swager group at MIT in 1995.[20] Today, active research is continued to further enhance CP-based sensor's signal by modifying functional groups, hydrophobicity/hydrophilicity, and electron density. With a deeper understanding of signal amplification mechanisms, sensor systems with higher sensitivity and selectivity are also being developed for medical, environmental, and pharmaceutical applications.

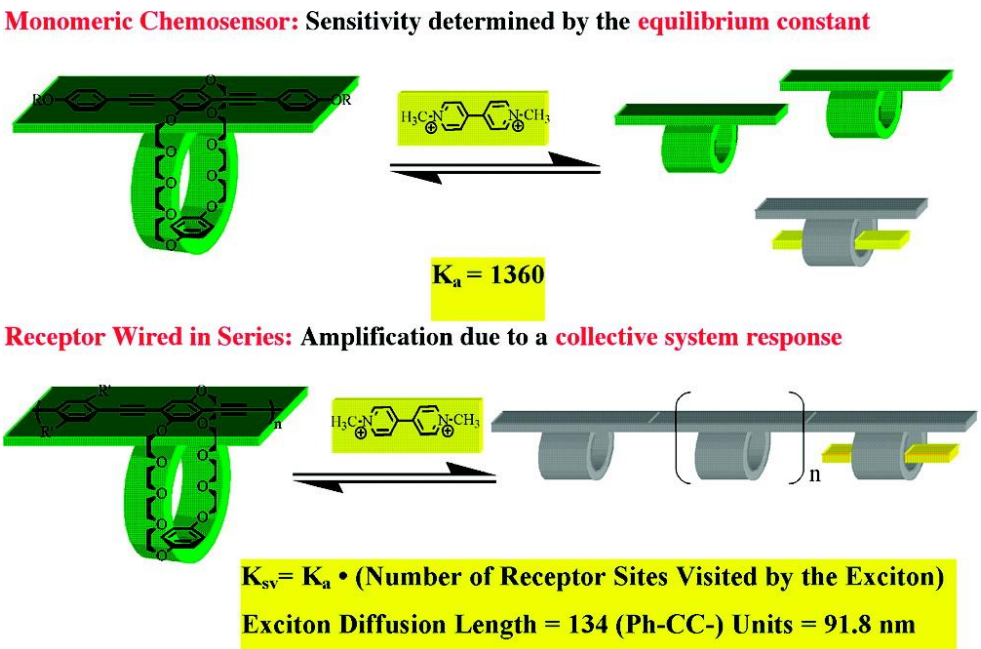


Figure 1.1. Demonstration of the amplified quenching in a CP. The determination of the Stern−Volmer quenching reveals the constant for binding of paraquat to a single cyclophane receptor (top). In the polymer, the larger (amplified) quenching constant reflects the fact that the quencher can be bound to any of the repeating units visited by the exciton (bottom).[21]

In addition to stronger signal strength, CP-based biosensors can also offer more intuitive signals such as a change in color. A CP backbone consists of overlapping p-orbitals because its backbone is constructed with alternating saturated/unsaturated bonds, which promotes SP1 or SP2 hybridized π-electron delocalization.[22] When the overlapped p-orbitals are disturbed via the change in intramolecular structure and/or intermolecular packing, the CP's optical properties (absorption, fluorescence) and conductivity can also change.[23-26] Utilizing such changes, a biosensor can be designed where receptor-analyte interaction triggers intra- or inter-molecular structure change, which can be detected by inspecting the fluorescence or the absorption spectrum.[27]

In addition, CP-based sensors can also be utilized with fluorescence quenchers. A fluorescence quencher reduces the fluorescence intensity of CP-based sensory molecules by binding to the fluorescent CP with strong electron affinity.[28, 29] Using this property, a fluorescence quencher can be used in two different ways in a biosensor design. The first type is a turn-on sensor, where pre-deployed fluorescence quencher molecules detach from the CP surface upon the analyte binding. The second type is a turn-off sensor, where the analyte binding causes the fluorescence quench of the CP.

Lastly, a molecular ruler can be prepared using Forster resonance energy transfer (FRET) exhibited by CP-based sensors.[30, 31] FRET is an energy transfer phenomenon between two fluorescent bodies with dissimilar

HOMO-LUMO energy gaps, caused by dipole-dipole interaction. Since FRET states that the energy transfer efficiency degrades by the 6th power of the donor to the acceptor separation distance, a molecular ruler can be devised. A molecular ruler sensor system is designed to allow a fluorescent body to approach the sensor body, which contains pre-labeled fluorescent molecules. When the target approaches the sensor, the observer can measure fluorescence induced via FRET to determine the presence or absence of the analyte.

1.4. Polydiacetylene-based Biosensor

The ease of preparation makes polydiacetylene (PDA) one of the most attractive CPs for biosensor applications. To prepare PDA, topochemical polymerization of diacetylene (DA) monomer is utilized. Topochemical polymerization is a method of inducing polymerization amongst tightly packed monomers by a light source. Typically, PDA synthesis is carried out in two steps. First, DA must be first arranged to a crystalline structure with 4.7-5.2A intermolecular distance, which is a characteristic of self-assembled solid DA molecules.[32-34] Following the dense packing of DA, 254 nm light is used to provide energy for photopolymerization between DA monomers. Upon exposure to the light source, the crystalline DA rearranges and forms ene-yne conjugated backbone of PDA. The resulting CP exhibits excellent dispersion within an aqueous solution, inheriting the amphiphilic nature of

its precursor monomer. Therefore, multiple self-assembled macrostructures of PDA can be prepared without complicated chemical modification to solubilize the monomer in an aqueous solution. When dispersed in an aqueous solution, PDA can form different macrostructures: self-assembled monolayer,[35] Langmuir films,[36] spherical colloids (liposomes/vesicles),[37, 38], and non-spherical colloids (nanowire, nanofiber, ribbons, sheets).[39-42]

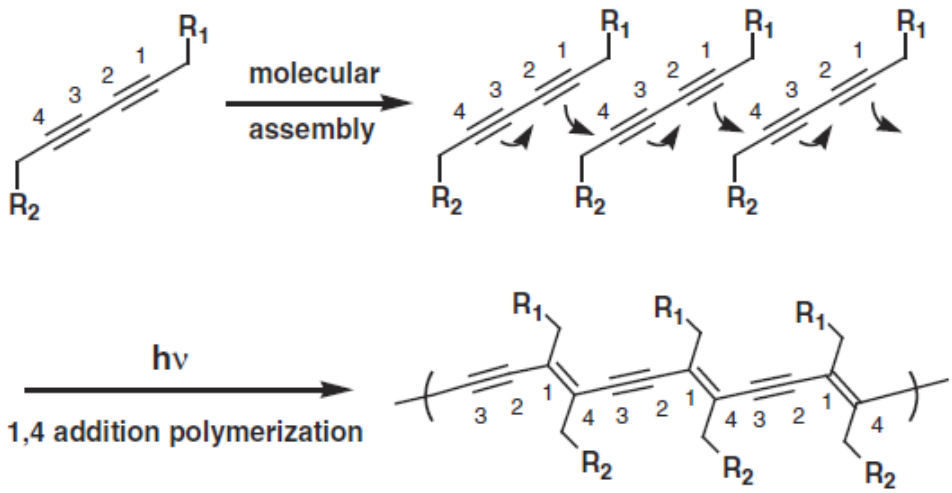


Figure 1.2. Schematic representation of topochemical photopolymerization of diacetylenes by UV light.[43] 2003 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

In addition to the ease of synthesis, the optical properties of PDA also promote the biosensor application of PDA. First, mechanochromism of PDA offers an intuitive self-signaling sensor behavior. Typically, PDA exhibits blue in color without any internal or external structural strain. When an

environment change induces PDA backbone disturbance and results in bandgap widening, the appearance color of PDA changes to red. Interestingly, the red phase PDA is also fluorescently emissive unlike the non-emissive blue phase, which is another easily measurable signal.[44] Owing to these attractive two optical properties, PDA-based sensors have been extensively studied in both film or self-assembled liposome structures of PDA. By designing PDA sensors for specific targets, PDA-based biosensors have been proven successful in detecting biological targets such as cholera toxin and *E. coli*.,[45] metal ions,[46] ligands,[47] and antigen.[48, 49]

1.4.1. Mechanism of Color Change

The most plausible mechanism of the PDA color change is that the external stimuli reduce the structural stress of the conjugated PDA backbone. When PDA is prepared by photopolymerizing tightly packed DA monomers, the carbon-carbon bonds on alkyne switch from SP to SP2 to accommodate conjugation. However, due to the tight packing, the bonding angle cannot change from 180° (SP) to 120° (SP2). Because of this mismatch, the resulting conjugated polymer backbone is under stress.[50] When external stimuli are introduced, the stimuli can provide energy to disrupt the tight packing and allow the PDA structure to rearrange and change color. Supporting the hypothesis, structures under higher intrinsic stress or weaker

hydrogen bonding between the head groups require smaller external energy to exhibit the color change.

It is also hypothesized that the mechanism of PDA backbone rearrangement can vary depending on the type of stimuli. For bulky biomolecules, it is generally hypothesized that binding large molecules on the surface cause repulsions among the bound molecules. The increased repulsion weakens intermolecular bonding between PDA head groups, which in turn allows PDA to release the accumulated structural stress.[51] Some other stimuli, such as a change in pH or temperature, may also promote stress release via a similar pathway. On the other hand, a surface covering by microorganisms may affect the backbone rearrangement via a different mechanism. Therefore, to design an optical sensor system with high sensitivity and selectivity, it is of critical importance to understand the interaction between PDA and the analyte.

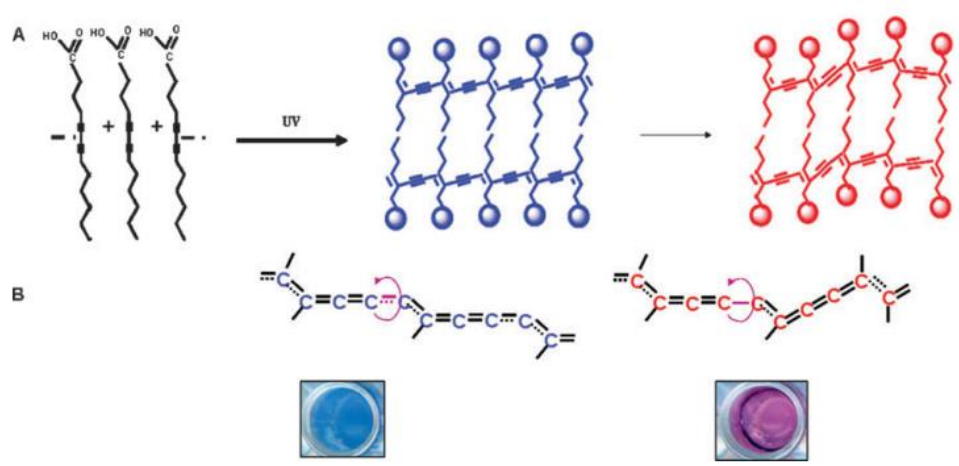


Figure 1.3. Structural features of polydiacetylene. (A) Creation of the polydiacetylene backbone from the diynoic acid monomers and induction of

the blue–red color transitions. (B) Structural transition within the PDA backbone leading to the blue–red change.[52] Copyright 2009 The Royal Society of Chemistry.

1.4.2. Colorimetric Property

The conjugated backbone of PDA produces two light absorption peaks at 630 nm and 550 nm. At its resting state, PDA normally appears blue due to the larger absorption of 630 nm. When exposed to external stimuli such as temperature,[53] pH,[54] ions,[55] solvents,[56] mechanical stress,[57] or presence of biomolecules,[58] the absorption at 550 nm increases while the absorption at 630 nm decreases.[59] Corresponding to the change in absorption, the color changes from blue to red to the naked eye. This change in color provides an intuitive indication of environmental disturbances, which has been applied to develop PDA-based colorimetric sensor systems.

1.4.3. Emissive Property

In addition to the intuitive colorimetric signal, another optical property of PDA, fluorescence emission, provides the potential to develop very sensitive sensor systems. When PDA is at its resting state (blue phase), fluorescence is not observed because the lowest excited energy state is in A_g symmetry with the ground state. On the other hand, PDA at its excited/stressed state (red phase) emits fluorescence because of B_u

symmetry between the lowest excited energy state and the ground state.[60] As the mechanism of fluorescence is not based on labeled dye, the magnitude can be weaker than traditional fluorescence labeling methods. However, this self-signaling behavior can be applied to develop label-free sensors with self-signaling capability.

1.4.4. Methods of Signaling

Multiple types of sensors have been developed by differentiating methods of stressing the conjugated backbone, which catalyzes PDA's unique optical properties. In particular, increasing PDA sensors' sensitivity to samples' unique physical properties and diversifying the target-sensor binding mechanism triggered PDA sensor application on bio-samples.

1. Physical condition sensitized system

Temperature is one of the primary variables that affect an organism's survival. For example, the temperature can be an indication of cell necrosis, bacteria multiplication, and food safety. Therefore, many types of temperature sensors have been developed, one of which uses PDA as its base. According to the research on thermochromism in a polydiacetylene crystal[61] carried out by Prodder Eckhardt at the University of Nebraska, the influx of thermal energy can adjust PDA backbone alignment. Since then, more research has followed to increase the effectiveness, such as allowing

the optical property change at low temperature for biosystems. Lowering the activation temperature was achieved in two main approaches. The first approach is to reduce the interaction between PDA's hydrophilic head, which results in a less tight self-assembled structure[62]. The second approach is to modify the hydrophobic tail's length to reduce the stress threshold for color change[63].

In addition to temperature, another critical parameter for a lifeform is suitable and stable pH. Within the human body, a pH can be an indicator of gastrointestinal disease[64] or cancer[65]. Therefore, a fast-acting, accurate biosensor for pH measurement is highly desired, which can be prepared by using PDA. Professor Kim at Hanyang University has successfully prepared such a sensor by utilizing carboxyl acid on PDA's head and stressing the backbone when the acid is deprotonated by Urease's enzymatic activity[66].

2. Biomolecules targeting sensor system

In addition to physical conditions, PDA backbone stress can be induced by chemically designing the DA unit's head group. By such a design, the head group is able to bind with specific target molecules. At the University of Michigan, Professor Kim's lab has demonstrated such application by detecting melamine with PDA[67]. Since melamine can induce serious health conditions such as acute kidney failure and serious renal problems, it is important to be able to detect the molecule at low concentration. To achieve good selectivity, Professor Kim's group used cyanuric acid to chemically

modify DA monomer's head group. The DA monomers were then used to fabricate PDA sensors, which formed the melamine-cyanurate complex when exposed to melamine. By using the stable complex formation, the sensor was able to detect melamine at a concentration as low as 0.5 ppm.

One advantage of using chemically modified DA for a sensor is its selectivity. For example, the Lee research group at Seoul National University was able to design a PDA sensor to detect bacteria[68]. In this sensor application, bacteria were detected indirectly by binding bacteria-produced Surfactin to chemically modified DA header groups. A unique advantage of this sensor is that different lengths of DA head unit, designed with a primary amine to utilize ionic interactions and van der Waals interaction, allow high selectivity to bacteria-produced Surfactin from other surfactant molecules.

It is also possible to utilize known selective interactions in developing PDA sensors. For example, antibody-antigen binding is highly selective and stable, which has been used to develop a PDA sensor for the Influenza A virus[58]. When a PDA surface is modified with M1 peptide antibody, the surface will bind with M1 peptide expressed on the surface of the influenza A virus. Capturing a large body such as a virus causes steric repulsion between the head groups and induces stress to the PDA backbone, causing the color change as a readable sensory signal.

Yet another application is to selectively detect inorganic ions via SS-DNA aptamer and potassium complex. Potassium is an essential ion in

which the extracellular potassium level in the bloodstream is an important component in diagnosing human health. To selectively detect potassium using PDA sensor, an ss-DNA aptamer with a Guanine-rich sequence was used to modify the head group[46]. The stress on the PDA backbone was induced when intramolecular hydrogen bonding between potassium and Guanine formed G-quadruplex.

Lastly, a sensor for an antibiotic molecule, neomycin, was also developed using phosphatidylinositol 4,5-bisphosphate (PIP2) phospholipid as the chemical receptors on DA monomers[69]. In this sensor, neomycin's side effect mechanism was used. In normal use, neomycin works as antibiotics by binding to PIP2 on the cell membrane and preventing the degradation of PIP2, thus inhibiting 1,4,5-triphosphate (IP3) related signal cascade. To develop a sensor for neomycin, a PDA sensor's surface was decorated with PIP2.

1.4.5. PDA Sensors Developed through the Dissertation Works

In the following chapters, this dissertation reports the effort and results of developing highly selective and sensitive self-signaling PDA sensors. The first prepared sensor emphasizes selectivity, which allows activated platelets to be detected within a whole blood sample containing multiple biomolecule species. The second sensor focuses on sensitivity by introducing a new signal enhancement method. In this sensor, the sensor's response to the analyte is enhanced by pre-occupying the PDA surface with

“dummy” target molecules. The predisposition allows the sensor to exhibit higher sensitivity to the actual targets.

Chapter 2.

Polydiacetylene Liposome Microarray toward Facile Measurement of Platelet Activation in Whole Blood

Adapted from Deokwon Seo, Terry C. Major, Do Hyun Kang, Sungbaek Seo, Kangwon Lee, Robert H. Bartlett, Jinsang Kim "Polydiacetylene Liposome Microarray toward Facile Measurement of Platelet Activation in Whole Blood" ***ACS Sensors*** **2021*, 6,* 3170.**

2.1. Purpose of Research

The degree of platelet activation is a key parameter to be monitored during clinical treatment of vascular diseases or blood-related illnesses, because a proper dose of antiplatelet drugs is needed to avoid malicious side effects. Therefore, a sensor that allows simple and continuous measurement of platelet activation is highly desired in both clinical treatment and self-diagnostics. However, previously reported PDA-based optical biosensors are not able to meet the need due to a shortcoming, where non-target components of the whole blood sample interfere with accurate measurement.

Overcoming these shortcomings, we developed a PDA sensor that allows single-step platelet activation detection by applying liposome

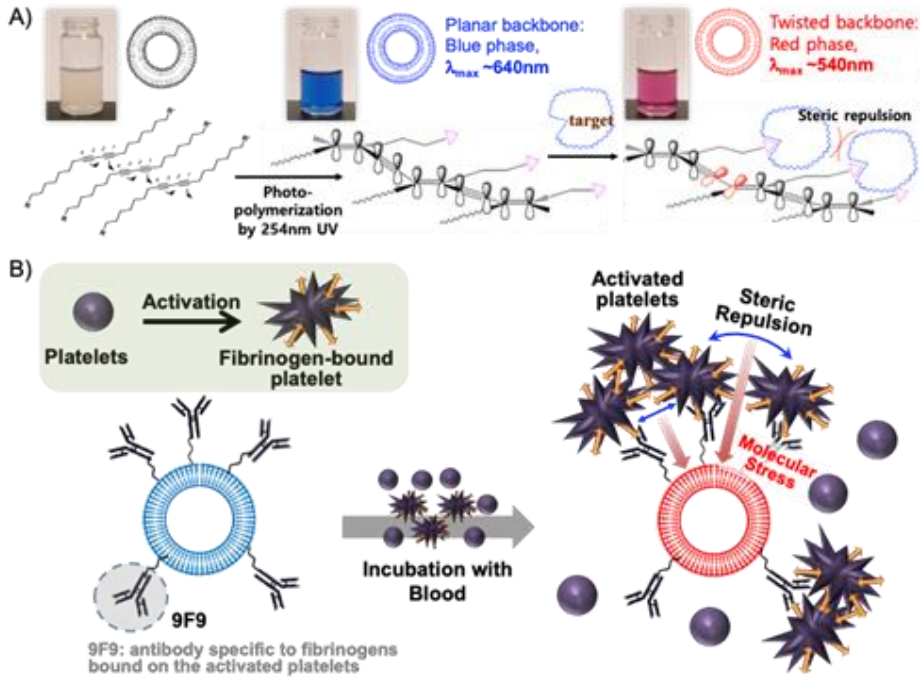
microarray. The sensor uses specific binding between the 9F9 antibody and fibrinogen, which specifically binds with activated platelets, to measure the degree of platelet activation within whole blood samples. Following, the sensor was applied to measure anti-platelet drug concentration within whole blood.

2.2. Introduction

Heart disease and its related symptoms affect hundreds of thousands of Americans each year. According to the Center for Disease Control and Prevention, heart disease remains the leading cause of death in the United States. For many patients, taking antiplatelet medication, Aspirin, Plavix, and Tirofiban, has been a commonly recommended practice to help prevent heart attacks and strokes. This family of antiplatelet drugs is commonly used to prevent blood clotting in patients with cardiovascular conditions such as myocardial infarction, stroke, and prosthetic heart valve issues. However, medicating antiplatelet drugs is often hazardous since it can lead to haphazard bleeding upon accident or surgery, which may require an emergency operation. In addition, many medical symptoms such as sepsis, malignancies, extracorporeal circulation, and dialysis may cause abnormal platelet activity. Continuous monitoring of platelet function from patients with cardiac records is critical as many patients with the record may adjust dosage arbitrarily or take multiple medications from other diseases.

However, monitoring of platelet function has been crudely implemented by either measuring bleeding time from a wound or by analyzing blood samples using complicated methods such as aggregometry,[70] cytometry,[71] or atomic force microscope (AFM).[72] Although the latter provides accurate results, it complicates the monitoring too much for the patients since it requires access to a laboratory, too long examination time, and costs. Consequently, patients are often simply advised to take the standard dose without accurate and vital monitoring of platelets; a practice that may endanger the lives of the patients.

Polydiacetylene (PDA) is a unique sensory material that changes its optical properties in response to molecular stress produced by steric repulsion between the captured analytes on adjacent receptors, providing useful self-signaling optical detection as illustrated in Scheme 2.1A.[73-75] Upon exposure to various molecular stresses, the color of PDA is changed from blue to red through the distortion of its conjugated yne-ene main chain and a consequential bandgap change. Furthermore, the converted red-phase PDA also emits red fluorescence, while the original blue-phase PDA has no fluorescence emission. Through such a dual colorimetric and fluorometric self-signaling property, the PDA has been used to conveniently and sensitively detect various molecular stresses generated by heat,[62, 76, 77] humidity,[78, 79] and binding of metal ions,[51, 80, 81] chemicals,[67, 69, 82-86] or biomolecules[58, 87-92].



Scheme 2.1. Schematic illustration of (A) PDA liposome self-assembly, photopolymerization, and stimuli-responsive property, (B) platelet activation assay based on sensory PDA liposome-9F9 antibody.

We previously reported a highly-sensitive and selective, and generic biosensor platform using PDA materials.[51, 58, 69, 78, 80, 87, 93] We have developed an amphiphilic PDA monomer having an epoxy headgroup and self-assembled them into the liposome form.[51] Then, the resulting PDA-epoxy liposomes were efficiently tethered to amine-functionalized substrates by amine-epoxy chemistry, rendering practical microarray-type of PDA liposome biosensors. Subsequently, various amine-containing bioreceptors (i.e., DNA aptamers, peptides, antibodies) can be bound to the epoxy head groups of the immobilized PDA liposome surface followed by photopolymerization with a 254 nm UV light to form blue PDA liposomes,

which are sensitive to molecular stress produced by various external stimuli. The prepared sensory PDA microarray can be readily used for selective recognition of target molecules such as mercury ion,[51] bovine viral diarrhea virus antibody [87], and influenza viruses[58], which successfully produces intense optical signals based on the recognition-induced molecular stress. Moreover, the signal intensity increases gradually with increasing amounts of target analytes and saturates as the receptors are occupied by analytes, allowing quantitative analysis provided that a correlation curve is established.[51, 58, 67, 69, 80, 83, 87, 93] We rationally applied this sensory PDA platform to the development of an activated platelet detection system by employing a specific antibody, 9F9 antibody, to provide selectivity since 9F9 antibody is known to have specific interaction with platelet-bound fibrinogen (Scheme 2.1B).[94, 95] The developed PDA liposome-9F9 microarray produced an intense fluorescence signal when the platelets in whole blood were activated and also could distinguish reduced platelet activation when varying amounts of an antiplatelet drug, Tirofiban, were added to blood samples by the signal intensity change. The presented results of this single-step bench-top assay suggest a potentially practical test that processes a large number of samples economically and derives test results in a timeframe meaningful for clinical outcomes in acute situations such as angina pectoris, stroke, peripheral vascular disease or procedures involving angioplasty or coronary thrombolysis. The assay incorporates simple, sensitive and rapid attributes that can detect the extent of platelet activation.

The developed PDA liposome assay allows 1) a rapid and simple measurement using whole blood samples without the need for expensive and complicated blood cell separation equipment as well as the need for additional in vitro platelet stimulation with exogenous agonists such as ADP or collagen and 2) a sensitive assessment of various degrees of platelet activation especially the determination of platelet function of patients on low dose aspirin or of patients on antiplatelet drugs.

2.3. Experimental section

Materials. A PDA monomer, 10,12-pentacosadiynoic acid (PCDA)-epoxy was synthesized as described in our previously published results.[51] A phospholipid, 1,2-dimyristoyl-sn-glycero-3-phosphate (DMPA) was ordered from Avanti Polar Lipids. Unlabeled FITC-9F9 Antibody, Collagen, and Tirofiban were purchased from Biocytex, Chrono-log, and Sigma-Aldrich, respectively. The chemicals such as solvents, buffers, and blocking agents were also purchased from Sigma-Aldrich.

PDA-Epoxy Liposome Assembly. The PDA liposomes consisting of PCDA-epoxy and DMPA lipids were assembled by the following injection method.[51] PCDA-epoxy and DMPA were co-dissolved (4:1 molar ratio) in the 0.1 ml of tetrahydrofuran/water mixture (9:1 v/v) and the lipid solution was injected to the 20 ml of 5 mM HEPES buffer at pH 8. The total lipid

concentration in the final aqueous solution was 0.5 mM. The liposome solution was probe-sonicated with 120W for 10 mins and was filtrated through a 0.8 μM cellulose acetate syringe filter. The filtrate was stored at 5℃ before use.

PDA Liposome Microarray Fabrication. The surface of the glass was treated with 3-aminopropyltriethoxysilan to make amine functionality following the literature process.[58] A slight modification was made when immobilizing PDA liposome on the resulting amine-modified glass substrate and the details are as follows. Glass slides were washed with chloroform, acetone, and 2-propanol for 15 mins each. The cleaned glass slides were then sonicated in sulfuric acid containing NOCHROMIX for 2 hours. After a thorough rinse with deionized water and air dry, the glass slides were placed in a UV/Ozone cleaning apparatus and treated for 30 mins. The glass slides were then stirred in a 2 wt% 3-aminopropyltriethoxysilane in anhydrous toluene solution on an orbital shaker for 6 hrs in a glove box at 70° C and afterward baked at 115 ° C for 30 mins. The glass slides were sonicated in toluene, toluene: methanol (1:1), and methanol for 15 mins each to remove any unbound silane reagent.

To fabricate the PDA liposome Microarray, the liposomes were covalently immobilized on amine-modified glass slides through the amine-epoxy chemistry. 0.5 mM PDA-epoxy liposome solution was spotted on amine-coated glass slides with a manual microarrayer (VP 475, V&P

scientific, INC) and was incubated at 5℃ for 24 hrs to prevent fast drying of the spotted solution. After removing the unbound liposomes by rinsing with 5 mM of pH 8 HEPES buffer, the PDA liposome-spotted slides were incubated with 0.06 mg/ml of 9F9 antibody in 5 mM of pH 8 HEPES buffer for 1 hr. After removing the unbound antibodies by rinsing with 5 mM of pH 8 HEPES buffer. The polymerization of the immobilized PDA-epoxy liposomes on the slides was carried out by illuminating 254 nm UV (1 mW/cm2) for 1 minute. The resulting PDA liposome microarray was used for the subsequent detection analysis.

Detection of Activated Platelet with PDA Liposome Microarray. Samples were taken from healthy blood donors who had abstained from taking aspirin for two weeks before sampling. All donor consent was obtained according to GT IRB H15258. Blood samples were taken by median venipuncture into sodium citrate tube (BD Vacutainer) and were used immediately. Platelet-free plasma was obtained from the supernatant after 15-min centrifugation of the blood at 1500 G and was used immediately. For the subsequent experiment described in this paper, 4 ml of collagen solution (1mg/ml) and 2 ml of various concentration of Tirofiban solution were added to the 94 ml of blood samples, and the mixture was loaded immediately on the PDA liposome microarray. After 20 mins of incubation, fluorescence microscope images were obtained on Olympus BX 71 microscope equipped with a mercury lamp and a cut-off filter of 540 nm

excitation and 600 nm emission. For fluorescence signal intensity measurement, the fabricated PDA liposome-9F9 microarray was placed on top of a 96-well plate and the combined substrates were inserted into a plate reader. Fluorescence emission intensities at 634 nm of total 120 microarray spots from three different devices were individually measured by using the excitation wavelength of 548 nm.

Fluorescence-activated Cell Sorting Analysis. Platelets diluted from whole blood (1:100) were treated with Tirofiban (0-10 nM), GPIIb/IIIa fibrinogen receptor inhibitor, and incubated for 20 min at room temperature. In a separate tube, 1 µl 10% DMSO was added to serve as a vehicle control (i.e., 0 nM Tirofiban). Collagen (40 µg/ml) was then added (4 µl) and incubated for 2 min at room temperature in all tubes. Platelet activation was measured by using 20 µl of the anti-9F9 antibody-FITC and 10 µl of the constitutive platelet marker, anti-CD61-PE (Phycierythrin). As a control for the anti-9F9 antibody, 10 µl of the isotype control, anti-mouse IgG1-FITC, was used with 10 µl of anti-CD61-PE in a separate tube. The antibodies were incubated at room temperature in the dark for 15 minutes. After the incubation, 700 µl of 1% formalin/dPBS was added to each tube and stored at 4oC for up to 24 hrs post fixing until ready to run the fluorescence-activated cell sorting (FACS) analysis on a FACSCalibur flow cytometer (Beckton Dickenson San Jose, CA). CellQuest software (Beckton Dickenson San Jose, CA) was employed for the data analysis.

Cell populations were identified for data collection by their log forward scatter (FSC) and log side scatter (SSC) light profiles. For each sample, 5,000 total events were collected in a region (R1) gated on platelets within the FSC versus SSC profile (Figures 2. 4A, C, E). Mean fluorescence intensity (MFI) of the anti-9F9 immunostaining was quantitated by a FITC (FL1) versus PE (FL2) log plot analysis. The platelets from region R1 were plotted and a gated region R2 was drawn to separate the platelets from debris on the FL2 axis (Figures 2.4B, D, and F). At this point all tubes were run on the cytometer noting the MFI change in the platelet cloud in region R2. The adjusted MFI was expressed as the geometric mean channel fluorescence minus the appropriate isotype control. The platelet reactivity index [42] was calculated by the following equation:

$$PRI=[(MFI_{c(T1)}-MFI_{C(T2)})/MFI_{C[T1]}]\times 100 \quad (1)$$

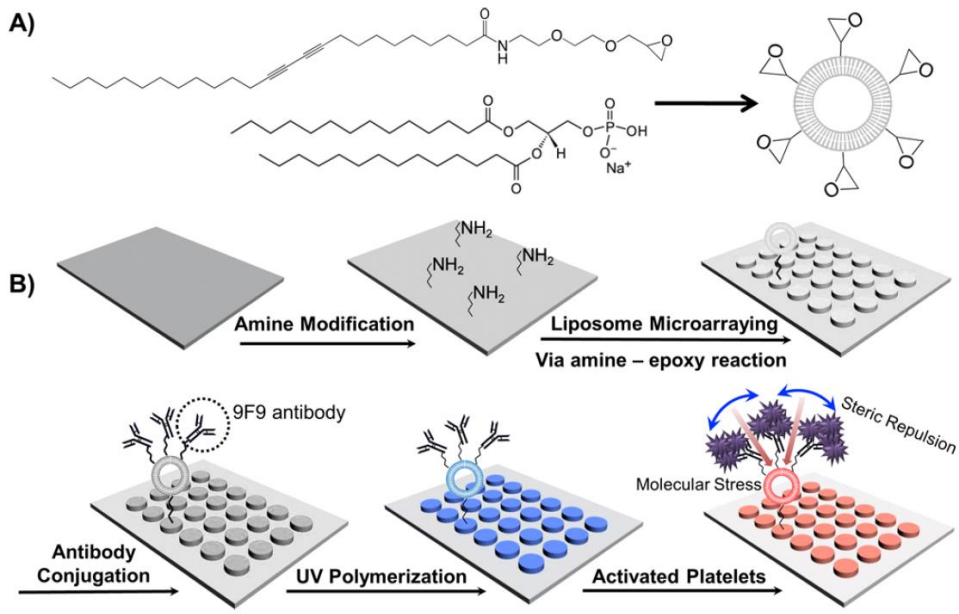
where, MFIc(T1) is the adjusted MFI for the collagen plus vehicle control tube, and the MFIc(T2) is the adjusted MFI for the collagen plus each concentration of Tirofiban tubes.

2.4. RESULTS AND DISCUSSION

The overall fabrication procedure of the developed PDA liposome-9F9 antibody microarray is schematically illustrated in Scheme 2.2. We specifically aimed for solid-state biosensors to directly use a whole blood

sample without pre-treatment or pre-separation. The predominant reason for pretreating blood samples lies in the fact that whole blood is comprised of non-specific components such as red blood cells, white blood cells, and inactivated platelets that may interfere with the optical detection through colorimetric change. To circumvent this problem, a separation process is usually required – centrifugation or filtration, especially as seem in the solution-type detection system. However, this makes the sensor cumbersome to use and significantly lengthens the time for detection or data acquisition.

We constructed PDA liposome by using (10,12-pentacosadiynoic acid)-epoxy (PCDA-epoxy) and 1,2-dimyristoyl-sn-glycero-3-phosphate (DMPA) as shown in Scheme 2.2A. PDCA-epoxy monomer was rationally designed for the following two reasons. First, the epoxy group allows the epoxy-amine chemistry between the PDA liposome and the amine-modified glass substrate that enables stable and covalent immobilization of PDA liposome onto the glass substrate.51, 58 This was further beneficial for binding of 9F9 antibodies to the PDA liposome surface as we can use the same epoxy-amine chemistry between the epoxy group on the PDA-epoxy and the amine groups of the antibody. A phospholipid DMPA, another constituent for self-assembling PDA liposome, was used to alter the self-assembly of the PDA monomer and to prevent high-order packing. Such insertion of phospholipid increased the flexibility of the liposome bilayer, which led to increased device sensitivity.[58, 69, 87]



Scheme 2.2. (A) Chemical structure of PDA-epoxy and DMPA, (B) Schematic illustration of the PDA liposome microarray fabrication procedure for detecting platelet activation.

We immobilized PDA liposome on the glass substrate to develop the solid-type sensor as described in the experimental section. To confirm the successful immobilization of PDA-epoxy liposomes onto the amine-modified glass substrate, we incubated a PDA-epoxy liposome solution on the amine substrate, photopolymerized, and applied heat, the most straightforward external stimulus, to the immobilized PDA liposomes at 150 ℃ for 3 mins, which would effectively distort the resulting conjugated PDA backbone and produce red fluorescence. We could observe the saturated fluorescence intensity of red-phase PDA by using a fluorescence microscope (Figure 2.1A). There was only 3.45% of the average difference

in fluorescence intensity across 36 devices, indicating the robust and homogenous immobilization of the PDA liposome onto the glass substrate as well as the reproducibility of our developed devices.

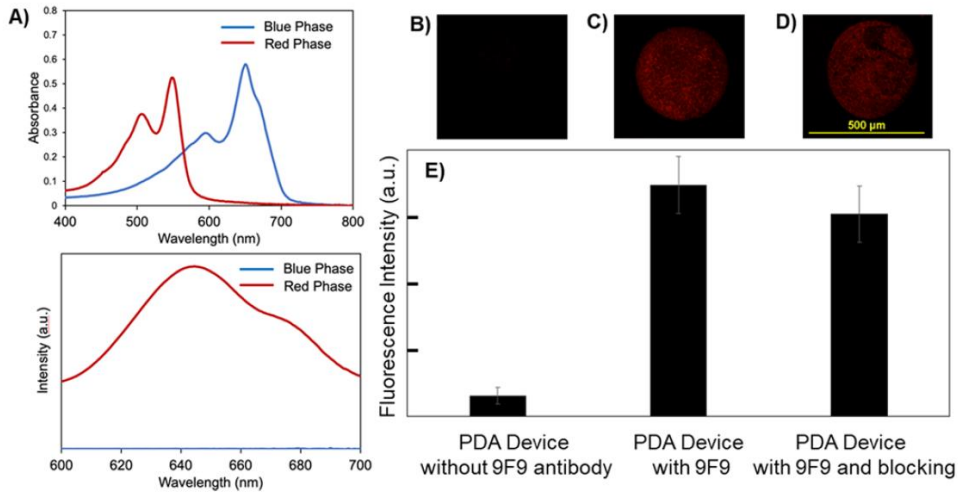


Figure 2.1. (A) Absorption and emission spectra of the blue and red phases of PCDA-epoxy liposome tethered on an amine-functionalized glass substrate. Fluorescence microscopy images of (B) a negative control PDA device without antibody incubation, a PDA microarray device having 9F9 antibodies without (C) and with (D) ethanolamine blocking, (E) fluorescence emission intensity of the PDA liposome of each sample arrays.

9F9 antibodies were subsequently tethered to the immobilized PDA liposome layer on the amine substrates, followed by blocking any unreacted epoxy groups of the PDA layer with ethanolamine. Further testing was implemented to confirm this blocking does not affect the efficacy of the antibody before applying whole blood samples on the developed PDA liposome-9F9 microarray. As shown in the Figure 2.1B, upon incubating

with the whole blood samples, no signal was detected from the PDA microarray device without having 9F9 antibodies but simply blocked with ethanolamine. On the contrary, as shown in Figure 2.1D, antibody-incubated and ethanolamine-blocked devices emitted red fluorescence signal from the samples. The device having 9F9 antibodies without enthanolmaine blocking (Figure 2.1C) showed 13.72% higher fluorescence signal intensity than the ethanolamine-blocked device (Figure 2.1E). We believe that the non-specific binding of non-targeted blood components to unreacted epoxy groups resulted in an additional random background signal.

The two major receptors on the platelet surface, integrin α2β1 and GPVI, are used for interaction with collagen, which activates the platelet. Additionally, adenosine diphosphate (ADP) and epinephrine activate platelets which are mediated by 3 purinergic receptors (P2Y1, P2Y12, and P2X1) and the alpha2 adrenergic receptor, respectively. Platelet activation then leads to the increased affinity of the GPIIb/IIIa to plasma fibrinogen molecules leading to platelet aggregation.[96, 97] Our PDA microarray device was developed to selectively measure activated platelets since the 9F9 antibody tethered on the PDA surface selectively interacts with fibrinogen bound to the activated platelets. In order to ensure specific measurement for activated platelets, we conducted a control experiment comparing whole blood samples with and without 40 mg/ml of collagen. As seen in Figure 2.2, the PDA device with added collagen displayed a red fluorescence intensity of 2.8 times greater than the device without it. The significant increase in

the fluorescence signal of the sample with collagen confirmed that our PDA liposome-9F9 microarray device detected specifically for activated platelets and was unaffected by the number of total platelets present in the samples.

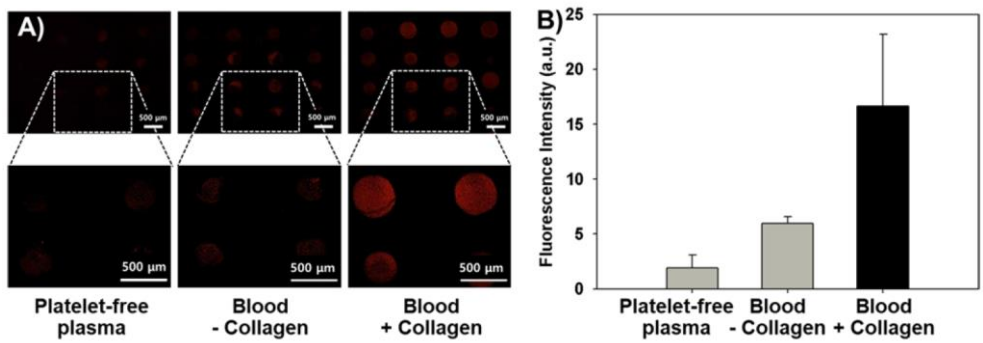


Figure 2.2. (A) Fluorescence microscope images and (B) fluorescence emission intensity of PDA liposome arrays after 20-min incubation at room temperature. Scale bars are 500 μm.

As shown from the results above, the devised PDA microarray device is specific to the activated platelets. Due to its specificity, we further investigated whether our device can be used to determine the appropriate dosage of antiplatelet drugs for individual patients and their personal healthcare providers. Tirofiban is an inhibitor of platelet-activated coagulation which acts by inhibiting GPIIb/IIIa. It was incubated in the blood samples for 20 mins. The results showed that the Tirofiban concentration was inversely related to the fluorescence signal intensity of the PDA microarray device (Figure 2.3). As the concentration of incubated

Tirofiban increases in the sample, the signal decreases. The limit of detection of Tirofiban in the sample was measured to be 0.16 μM.

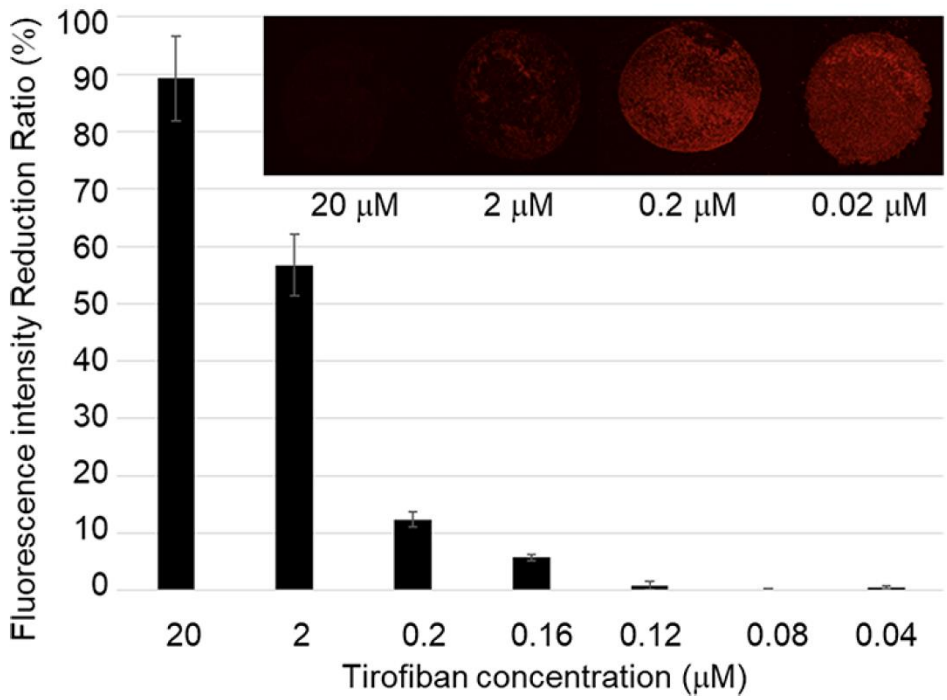


Figure 2.3. Fluorescence intensity reduction ratio of PDA liposome-9F9 microarray devices after 20-min incubation with Tirofiban and whole blood. (inset) Fluorescence microscopy images.

The 20-mins incubation time of the anticoagulation drug was selected to obtain the optimal signal. We incubated 100 ml of the blood samples with various Tirofiban concentrations to 9 mm diameter silicon isolator of the PDA microarray device and could find that the red fluorescence signal for all samples significantly increased when Tirofiban was incubated for more than 30 minutes. After 50 min, even the blood sample having 20 ml of Tirofiban showed a similar signal intensity to samples without Tirofiban.

Altogether, we realized that in the open-air condition with longer than 30-mins incubation time, signal intensities from both Tirofiban-treated group and non-treated group showed a similar level of coagulation from activated platelets. Therefore, we concluded that the anticoagulation drug should be incubated for 20 mins for the best reliable and reproducible result to be presented in the PDA microarray device.

To confirm the results from the PDA liposome-9F9 microarray, a modified flow cytometric assay was conducted using the same anti-human fibrinogen antibody, 9F9, as in the PDA liposome-9F9 complex as described in the Experimental Section.[98] Figure 2.4 shows the flow cytometric analysis (A-F) of the inhibition of collagen and epinephrine stimulated human platelets by Tirofiban, collagen concentration responses on 9F9 antibody expression (G), and the Tirofiban's inhibition of 40 mg/ml collagen and 10 mM epinephrine-stimulated human platelets (H). This stimulation of platelets elicited a maximum platelet activation response.

The results of the modified flow cytometric assay confirmed the results of the PDA liposome-9F9 microarray. The level of Tirofiban inhibition directly affects the measured fluorescence intensity of FACS analysis since the fluorescence intensity is determined by the level of bound F9F antibody-FITC conjugate to platelets. Less bound F9F conjugate to platelets means an increase in the inhibition of platelet activation.

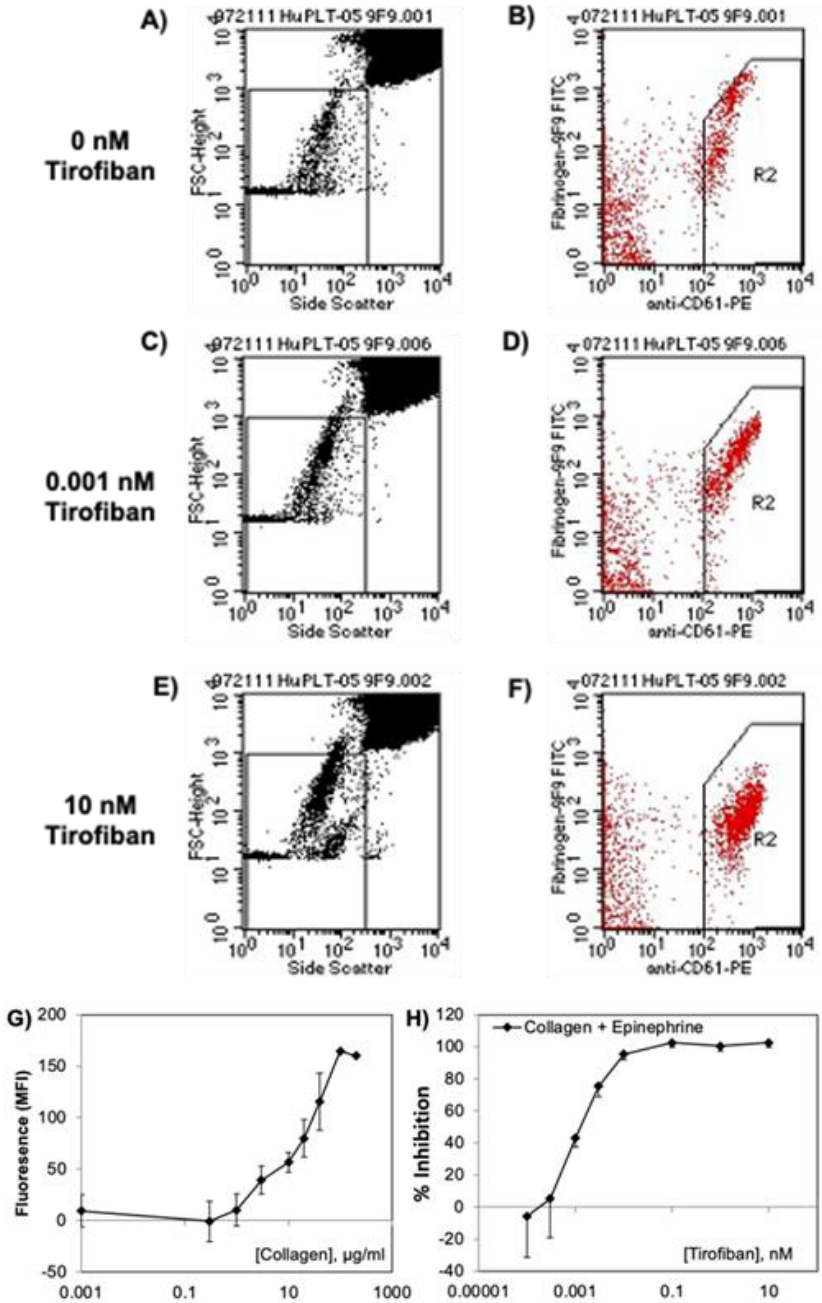


Figure 2.4. (A-F) FACS analysis of the FITC-9F9 antibody in human whole blood, (G) Flow cytometric analysis of collagen concentration response on 9F9 antibody expression. MFI= maximum fluorescence intensity, (H) concentration response of GPIIb/IIIa inhibitor, Tirofiban, on collagen and epinephrine stimulated human platelets.

Flow cytometry analysis data shown in Figure 2.4G demonstrates that 9F9 antibody expression increases as the concentration of collagen increases, supporting the results shown in Figure 2.2. As shown in Figure 2.4H, the increase in Tirofiban concentration resulted in an increase in the % inhibition of platelet activation via collagen stimulation. The Tirofiban inhibited collagen with IC50 values of about 0.0015 nM. Therefore, direct detection of platelet activation using the specific antibody, 9F9, to platelet-bound fibrinogen allows the determination of the extent of platelet activation under most conditions, including contact activation in extracorporeal circulations antiplatelet drug effects, and hereditary platelet diseases. Similarly, as shown in Figure 2.3, the PDA liposome-9F9 complex produced weaker fluorescence when the concentration of Tirofiban was increased. Thus, the PDA liposome-9F9 assay provides a simple single-step measurement procedure compared to the complex flow cytometric method to assess the real-time functional state of circulating platelets.

2.5. Conclusion

We devised a PDA-based microarray sensor device as effective tool to specifically determine the level of activated platelet in whole blood samples. 9F9 antibodies that are specific for fibrinogen bound to the activated platelets are tethered at the PDA liposome surface to provide selective recognition of activated platelet. The fluorescence signal intensity inversely

related to the amount of Tirofiban, an inhibitor of platelet-activated coagulation, added to whole blood samples, implying possible determination of appropriate dosage of antiplatelet drugs. Without any special separation process or pretreatment of the whole sample, patients as well as their healthcare providers can conveniently monitor the level of activated platelet as a reference for appropriate anti-platelet drug dosage.

Chapter 3.

Amplifying the sensitivity of Polydiacetylene sensors: The Dummy molecule approach

Adapted from Deokwon Seo, Ramin Ansari, Kangwon Lee, John Kieffer, Jinsang Kim "Amplifying the Sensitivity of Polydiacetylene Sensors: the Dummy Molecule Approach" ***ACS Applied Materials & Interfaces* 2022, *14*, 14561.**

3.1. Purpose of Research

Recent advances in medical science revealed the relationship between trace biomolecules and human health, which increases the need for a simple detection method for trace biomolecules. Unfortunately, PDA-based biosensor application in this area may be limited because PDA-based sensors often require an analyte concentration high enough to influence the sensor's structural stability. To overcome such limits, there have been studies focused on reducing the sensor's structural stability hence decreasing the amount of external stimuli needed. However, such an approach also makes the sensor become less robust against other environmental parameters. Here, a new method of enhancing the PDA-based sensor's sensitivity is proposed. The method involves pre-disposing

artificial target molecules on the sensor surface, thereby decreasing the number of actual target molecules needed to activate the sensor. Depending on the level and type of the pre-disposed artificial target, the sensor can be tuned for specific analyte types and concentrations. The new method is also synergistic with another signal-enhancing method, which greatly improves the signal intensity and reduces the limit of detection.

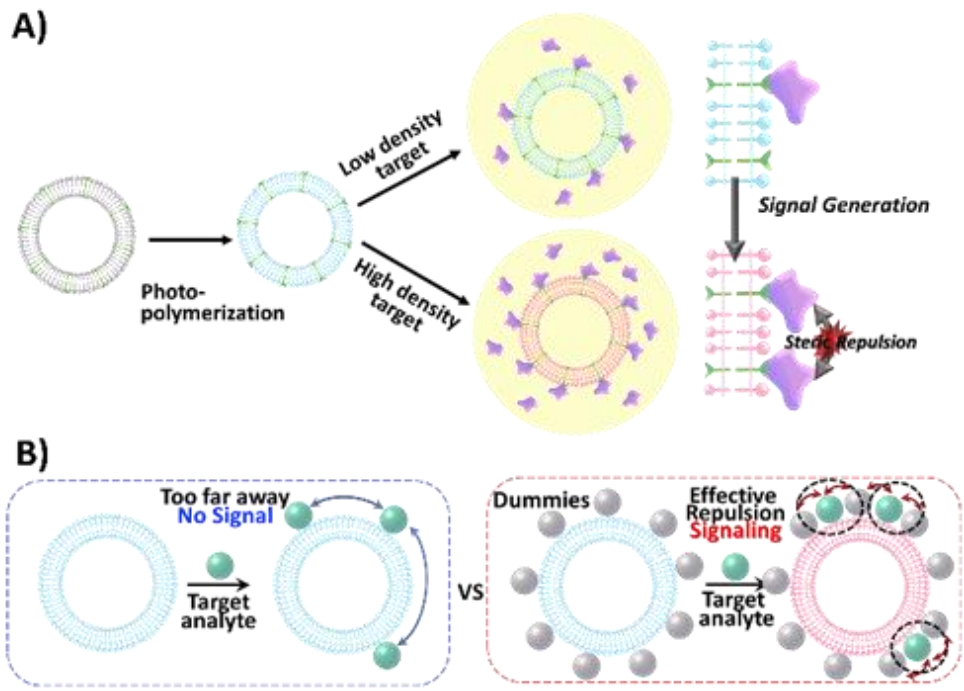
3.2. Introduction

Polydiacetylenes (PDAs) have gained much attention for their rapid and convenient colorimetric detection scheme for chemical and biosensing applications. The detectable optical property change as a sensory signal is originated from the distorted conjugated yne-ene main chain of PDA molecules induced by steric repulsion among the receptor-target complexes formed at the PDA surface via specific recognition events.[73-75] The distorted PDA backbone widens the bandgap of PDA and causes its absorption max change from 650 nm to 540 nm, which appears as the color change from blue to red. In addition to the appearance of color change, the red phase is fluorescently emissive at 530 nm. This unique self-signaling property combined with rational receptor design for specificity enables PDA to be a convenient and universally applicable optical sensor platform. PDA sensors have shown fast colorimetric and fluorometric dual signaling to various

external stimuli such as chemicals,[67, 69, 83-86, 99] biomolecules,[58, 87-92] light,[100] heat,[62, 76, 77] and humidity.[78, 79]

The convenient optical detection scheme and fast response time make PDA an attractive material for biomedical sensors to meet today's needs for rapid and equipment-free self-diagnosis at home. In recent years, COVID-19 pandemic has amplified such needs at multiple levels from self-assessment for individuals to mass screening for the larger public. PDA biosensors have already been demonstrated for selective detection of Influenza A virus,[58] bacteria,[68] antibiotics,[69] and activated platelet.[101] However, the detection limit ranges widely from millimolar to nanomolar concentration, implying the need for sensitivity enhancement and reducing the limit of detection (LOD). We previously reported an effective strategy to achieve a lower LOD and higher sensitivity by co-assembling a lipid, 1,2-dimyristoyl-sn-glycero-3-phosphate (DMPA), within the PDA liposome, which adds more fluidity to the self-assembled PDA liposomes making them more responsive to the molecular stress.[87] This lowered threshold stress results in a reduced number of the minimum target molecules needed for the signal generation.

We investigated a new strategy to enhance the sensitivity of the PDA sensors by analyzing the fundamental reasons for the wide range of LOD. It is plausible that LOD largely depends on the relative size of target molecules to the receptor size because the sensory signal is generated by the steric repulsion among the formed receptor-target complexes.



Scheme 3.1. A) Schematic illustration of the working mechanism of PDA liposome-based sensor, and B) enhanced signal generating mechanism by means of dummies.

Therefore, as we previously demonstrated, if the target molecule is smaller than the receptor, no effective steric repulsion is anticipated and consequentially no signal would be generated.[58] Likewise, a more critical origin of the intrinsic sensitivity limitation of PDA sensors can be identified by analyzing the signal generation mechanism. The required steric repulsion for signal generation arises only if adjacent receptors are occupied by target molecules, which is unlikely, however, until a certain amount of target molecules are available as schematically illustrated in Scheme 3.1A. We

envisioned that preoccupying some of receptors with artificial target molecules named “dummy” would make otherwise required target bindings to adjacent receptors unnecessary. Here, we systematically investigated the optimum number density and size of dummy molecules for signal amplification by using phosphatidylinositol 4,5-bisphosphate (PIP2) and Neomycin as a model receptor and target pair. We also examined the general applicability of the dummy strategy by implementing the concept to a Surfactin detecting PDA system. We further demonstrated synergistic enhancement of PDA sensitivity by combining the dummy strategy with the lipid insertion method.

3.3. Experimental Section

Materials. 1,2-dimyristoyl-sn-glycero-3-phosphate (DMPA) and L-α-phosphatidylinositol-4,5- bisphosphate (PIP2) were ordered from Avanti Polar Lipids. 2-Hydroxymethyl-18-crown-6, α-cyclodextrin, β-cyclodextrin, and γ-cyclodextrin were purchased from Sigma-Aldrich. Other chemical reagents were purchased from Sigma-Aldrich and directly used without further purification.

Fabrication of polydiacetylene liposome. The injection method was used to prepare the PDA liposomes. Constituent components of PDA liposome (PCDA, PCDA-EDA, PCDA-EDEA, PIP2, and DMPA) were dissolved in 300 mM DMSO at the molar ratios specified in Table 3.1 (their

chemical structures are in Figure 3.1B, 3.4A, and 3.8). The DMSO solution was injected to 20 mL water followed by sonication at 90 ℃ by means of a 120 W probe sonicator for liposome self-assembly. After 20 minutes of sonication of the aqueous liposome solution at 0.5 mM the solution was filtered through 0.8 mm cellulose acetate syringe filter. The filtrate was stored overnight at 5℃ prior to using it for further experiment.

	PCDA	PDA-EDA	PDA-EDEA	PIP2	DMPA
Neomycin detection (without DMPA)	8	1	-	1	-
Neomycin detection (with DMPA)	6	1	-	1	2
Surfactin Detection	-	4	4	-	2

Table 3.1. Mole ratio of PCDA: PDA-EDA: PDA-EDEA: PIP2: DMPA in PDA liposomes

For the post attachment approach of dummy molecules, 5℃ overnight-incubated liposome solution was first UV-irradiated (1mW/cm2, 254 nm) for 30 seconds for ene-yne conjugated backbone formation, which was confirmed by blue color generation as well as UV-absorption at 650 nm. To the 380 ml of photopolymerized PDA liposome solution, 20 ml of aqueous solution of epoxy-functionalized dummy molecules at varying concentrations was then added and incubated for 12 hours at room temperature. PDA microarrays were fabricated by microarraying the resulting PDA solution on amine-functionalized glass substrates.

Optical signal analysis of polydiacetylene liposomes. Aqueous solution of target molecules was added to the prepared PDA liposome microarrays. After 20 minutes of incubation followed by stringent rinsing, chromatic change in the PDA microarrays was examined with an optical fluorescence microscope (Olympus DP71). The quantitative absorbance and fluorescence of the PDA microarrays were measured using a spectrophotometer (Varian Cary 50 UV−vis spectrophotometer) and a fluorescence microplate reader (Horiba PTI QuantaMaster 400).

Computational calculation. First, we performed quantum mechanical calculations using Gaussian 16[102] for the molecules investigated in this work. We endeavored to better understand the molecular shape and volume of the molecules. We used B3LYP functional with 6-31G(d,p) basis set. To incorporate the solvent effects, we used the Polarizable Continuum Model (PCM) using the integral equation formalism variant (IEF-PCM).[103] All IEF-PCM calculations were performed using default settings of Gaussian 16 program package. All geometries and free energies were calculated at 298.15 K. The optimized ground state geometries were obtained, followed by an analysis of normal modes of atomic motion to confirm the stability of the optimized structures. Then the molecular volumes were calculated as implemented in Gaussian 16.

The optimized structures obtained from quantum mechanical calculations were then used to assess the similarity between molecules. We used the USR (Ultrafast Shape Recognition)[104] function of Open Drug

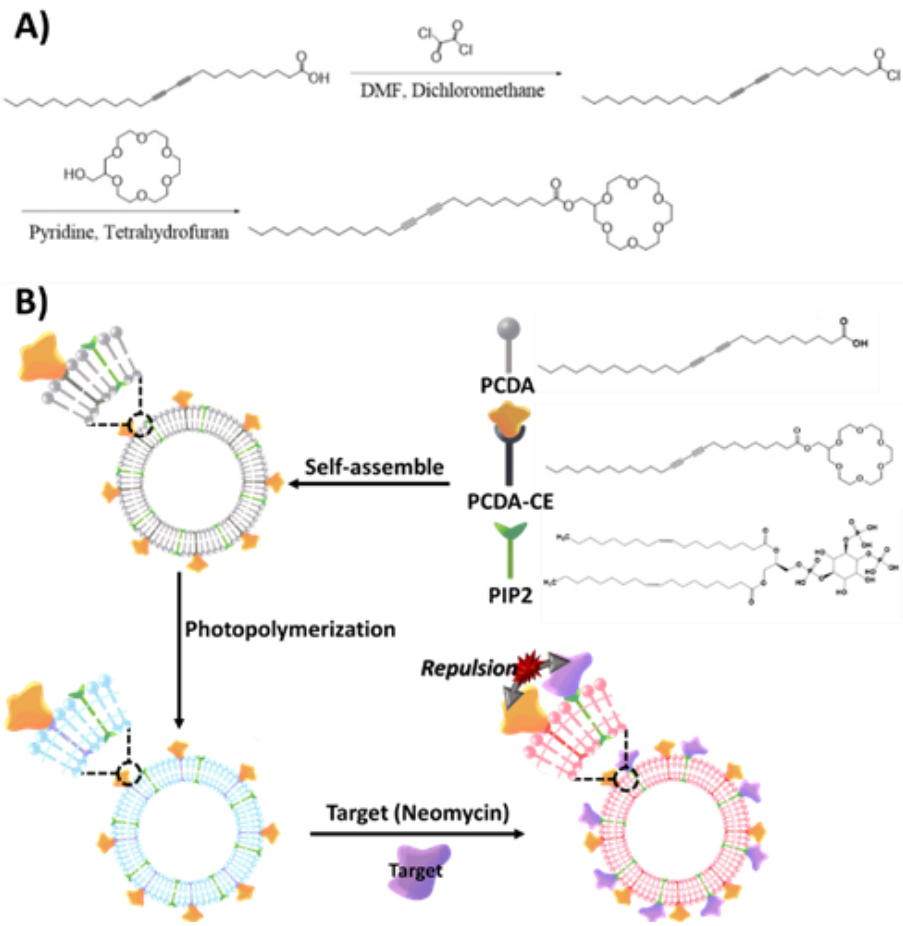
Discovery Toolkit (oddt)[105] for molecular shape comparison. USR accurately describes the shape of a molecule via a vector of geometrical descriptors. The comparison of molecular shape via these USR descriptors has been rigorously shown to be highly effective and efficient.

3.4. RESULTS AND DISCUSSION

The limit of detection (LOD) is the minimum concentration of target molecules required for a sensor to exhibit a detectable response. For PDA-based sensors, the LOD is dependent on the number of available target molecules as well as the target's molecular properties, because the sensors' working mechanism requires the conjugated PDA backbone to be strained by the steric repulsion among the captured target molecules at the PDA liposome surface. However, in some cases, the molecule of interest to detect is at a lower concentration than the LOD of PDA-based sensors, limiting the PDA sensors' practical applicability. In this study, we explored a strategy of decreasing the LOD by preoccupying the PDA liposome surface with designed dummy molecules (Scheme 3.1B).

The sensor performance was compared between PDA liposomes with and without the preoccupied dummy molecules. Neomycin was selected as the model target since we had previously developed a PDA sensor built on the well-studied specific interaction between Neomycin and PIP2.[69, 106] As the first dummy molecule for the proof of concept study, 18-crown-6-

ether [10] was chosen considering that CE has a similar solubility in water, does not interact with Neomycin, and has a similar flexible structure to Neomycin. We later additionally studied three more different dummy molecules. To control the number of CE at the PDA liposome surface, initially we synthesized the CE-tethered diacetylene monomer (PCDA-CE) by attaching CE to 10,21-pentacosadiynoic acid (PCDA)[107] (Figure 3.1A) and prepared PDA liposomes having varying ratios of PCDA, PCDA-CE, and PIP2 by controlling their mixing ratio (Figure 3.1B and Table 3.2).



C)

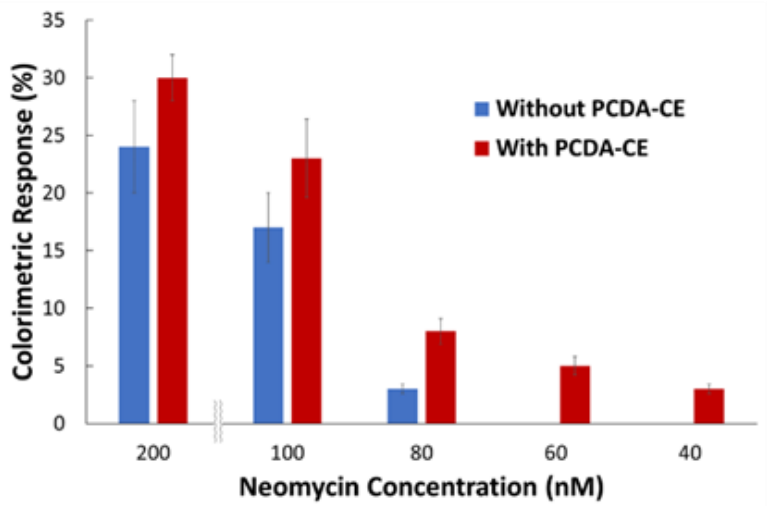


Figure 3.1. A) Synthesis of PCDA-CE, B) Schematic illustration of the PDA liposome-based Neomycin detection mechanism by the designed liposome including PCDA-CE dummy monomer. C) Colorimetric response of PDA liposome solution after 20 min incubation with Neomycin.

	PCDA	PCDA-CE	PIP2
0 % Dummy	9	0	1
5 % Dummy	8.5	0.5	1
10 % Dummy	8	1	1

Table 3.2. Mole ratio of PCDA : PCDA-CE : PIP2

However, we found that this preparation method caused an undesired consequence of reduced signal intensity because the liposome self-assembly was disrupted by the dummy molecule's bulky headgroup. As shown in Figure 3.2, as the amount of PCDA-CE increases in the mixture of PCDA-CE and PCDA, rapid reduction in the blue absorption intensity was

observed, indicating unstable liposome packing and consequentially hindered photopolymerization. Nevertheless, the resulting PDA having the mixing ratio of PCDA:PDA-CE:PIP2 =8:1:1 exhibited a lower LOD of 40 nM Neomycin compared to the control PDA liposome's 80 nM LOD without the dummy (Figure 3.1C).

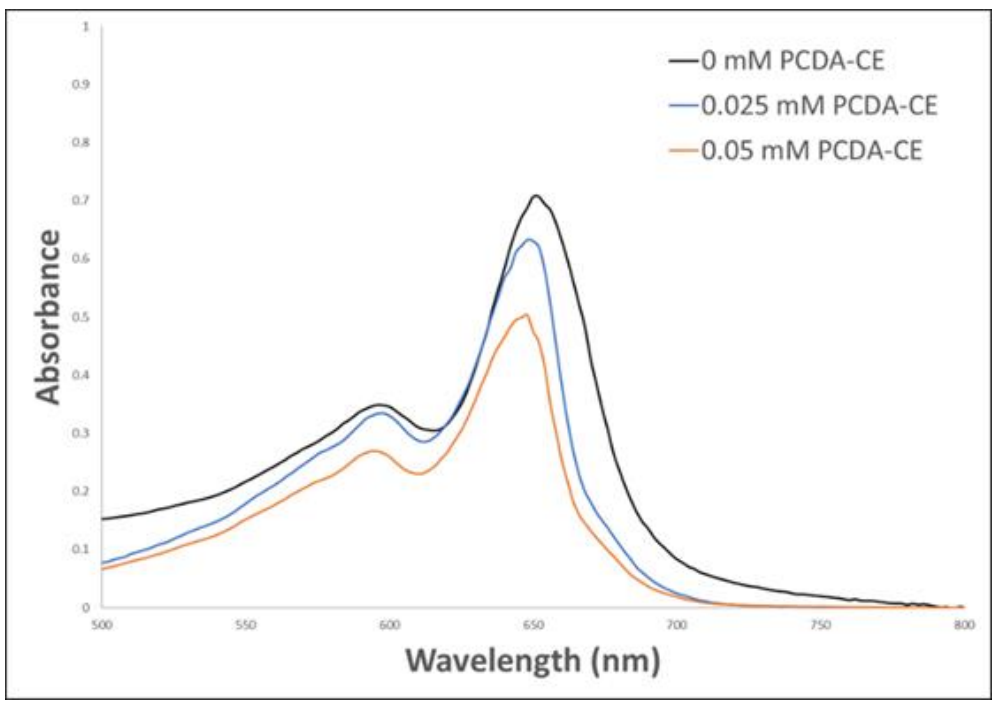


Figure 3.2. Absorption spectra of PDA solution with different PCDA-CE ratios.

However, because we could not systematically study the dummy effect due to the self-assembly issue, we decided to attach the dummy molecules to pre-assembled PDA liposomes for further studies (Figure 3.3A).

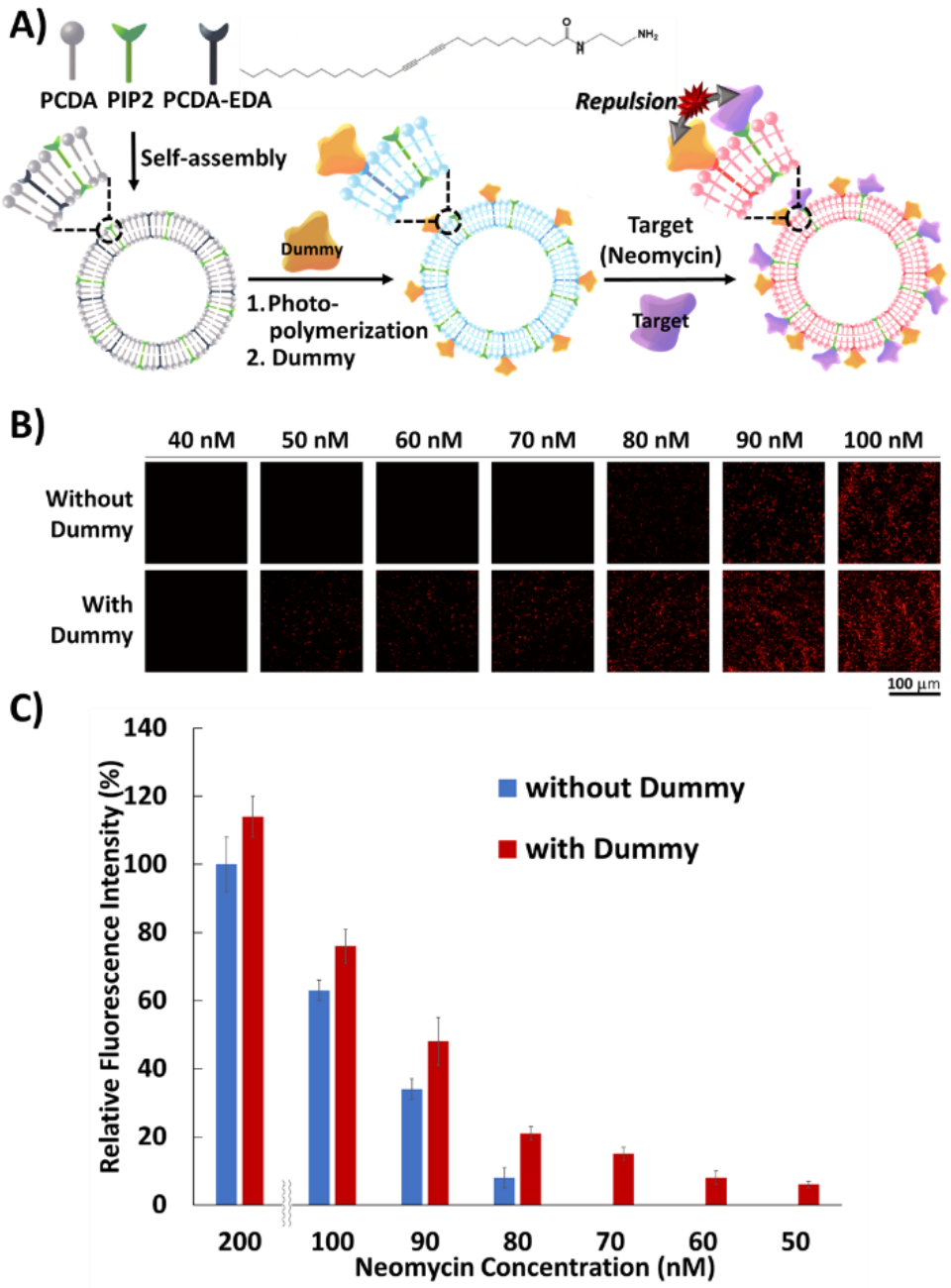


Figure 3.3. A) Schematic illustration of the PDA liposome having the pre-occupied dummy molecules for Neomycin detection with enhanced sensitivity, B) Fluorescent microscope images, C) Relative fluorescence intensity comparison between the PDA liposome with and without the CE dummy at the surface.

To attach the CE molecules to self-assembled PDA liposomes, the amine-epoxy reaction was utilized. First, N-(2-aminoethyl)pentacosa-10,12-diynamide (PCDA-EDA) monomer was prepared by reacting ethylene diamine with PCDA to introduce amine functionality.[68] The PCDA-EDA was then self-assembled with PCDA and PIP2 into liposomes at the molar ratio of PCDA:PCDA-EDA:PIP2 = 8:1:1. An epoxy functional group was introduced to CE by reacting CE with epibromohydrin[101] (Figure 3.4) and the resulting epoxy-functionalized CE was tethered to the diacetylene liposome having PCDA-EDA to prepare PDA liposomes having preoccupied dummy molecules (Figure 3.3A)

When the dummy molecules are attached to the surface of the PDA liposome, they can also generate optical signals if they occupy the surface too densely. In this case, the signals from the dummy themselves become noise background signal for the PDA sensor. Therefore, the dummy concentration should be in the range in which the dummy themselves are not generating optical signal. To avoid such a background signal, we performed titration in order to identify a suitable dummy concentration range before applying the system to the Neomycin detection. We tested four different dummy molecules (Figure 3.4A) to find that to ensure no background signal for all four chosen dummy molecules, the highest dummy concentration to react with pre-assembled PDA liposomes should be 1 mM (Figure 3.4B).

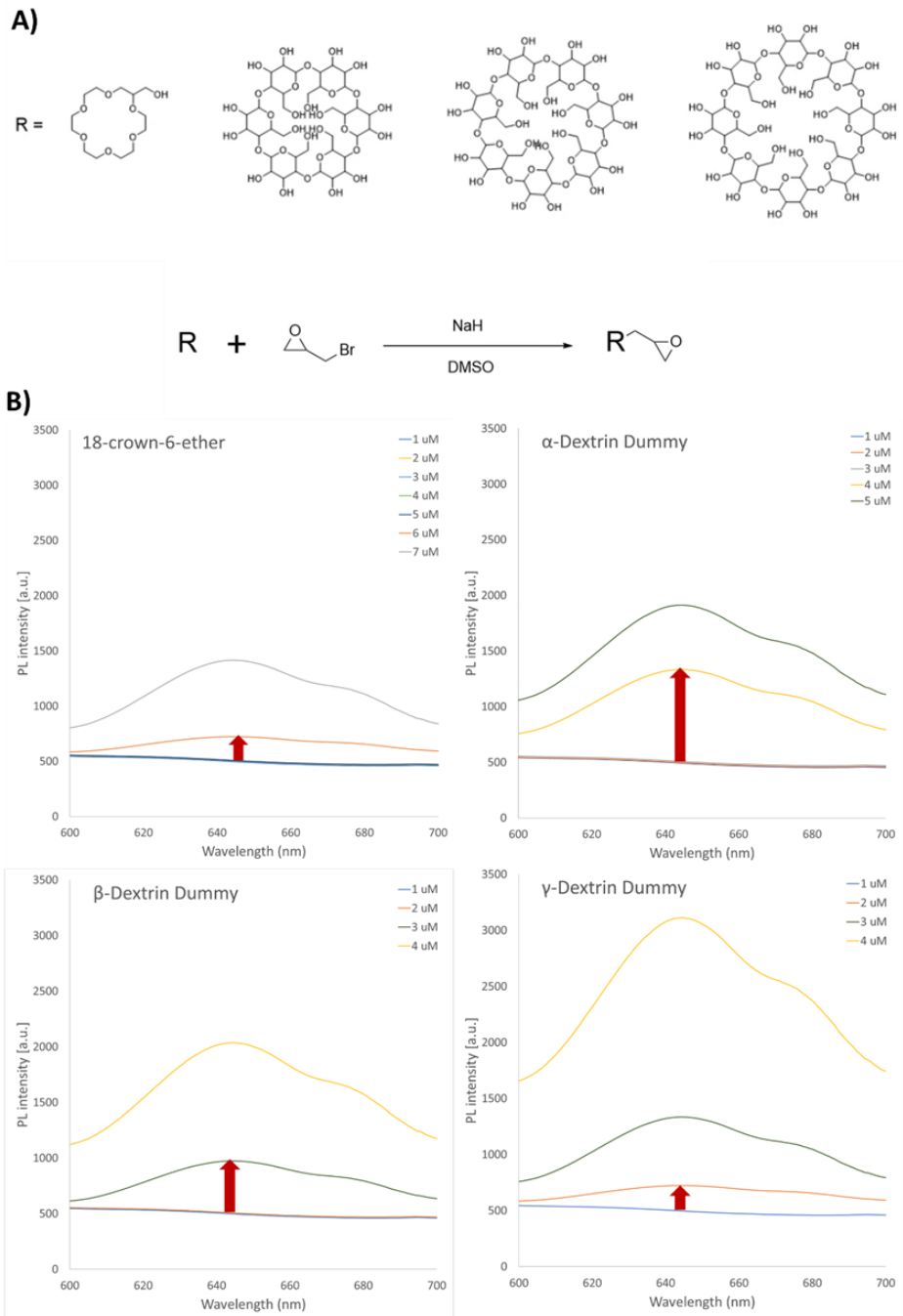


Figure 3.4. A) Synthesis of epoxy-functionalized dummies, B) Emission spectra of PDA liposome incubated with epoxy functionalized dummy molecules.

Therefore, after self-assembly and photopolymerization, the PDA liposomes were incubated with 1 mM epoxidized dummy molecule for 12 hours. The resulting liposome solutions were then exposed to various concentrations of Neomycin, the target molecule. While the LOD of the PDA liposome without the dummy was 80 nM, that of the PDA having CE dummy was lower at 50 nM (Figures 3.3B and 3C). Moreover, 2.6 times stronger fluorescent intensity was observed at 80 nM Neomycin concentration, implying that the sensitivity was also increased by preoccupying the liposome surface with CE dummy molecules (Figure 3.3C).

After confirming the improved sensor performance by the dummy approach, we investigated the size effect of dummy molecules since our previous results showed that a larger target molecule can induce more effective steric repulsion and consequentially better LOD than a smaller target.[58] Following the same logic, larger and compatible dummy candidates were selected for Neomycin by surveying molecules that satisfy the above-mentioned criteria (solubility, reactivity, and structure). After the initial screening, their molecular size was computationally calculated. α-cyclodextrin was chosen considering that it is significantly larger than CE but similar to Neomycin (Table 3.3). To attach the α-cyclodextrin dummy to the PDA surface the pre-determined 1 mM concentration of α-cyclodextrin having epoxy functionality was used.

	Molar Volume (r_{Bohr}^3/mol) (in Water)	**USR* Shape Comparison** (Neomycin)
Neomycin	4644.698	1.000
18-crown-6	2255.047	0.179
α-Cyclodextrin	6272.443	0.397
β-Cyclodextrin	7493.048	0.294
γ-Cyclodextrin	8648.770	0.182

Table 3.3. Summary of calculated molar volume and USR* shape comparison of Neomycin and dummy molecules

As shown in Figure 3.5A, the PDA liposomes with α-cyclodextrin dummy showed higher signal intensity and lower LOD for Neomycin detection compared to the PDA liposomes having CE dummy. We further investigated the size effect by additionally testing β-cyclodextrin and γ-cyclodextrin dummies which are larger than α-cyclodextrin (Table 3.3). Interestingly, while the PDA liposomes having β-cyclodextrin dummy showed about the same sensitivity and signal intensity as the PDA liposomes with α-cyclodextrin dummy, the PDA liposomes with γ-cyclodextrin performed much worse as it showed lower sensitivity and signal intensity (Figure 3.5B and 5C). It is plausible that the much larger γ-cyclodextrin might sterically hinder the interaction between PIP2 and Neomycin or might be too large to be pushed effectively by Neomycin. These results imply that a dummy molecule having a similar form factor to the target molecule is the more effective one for sensitivity enhancement.

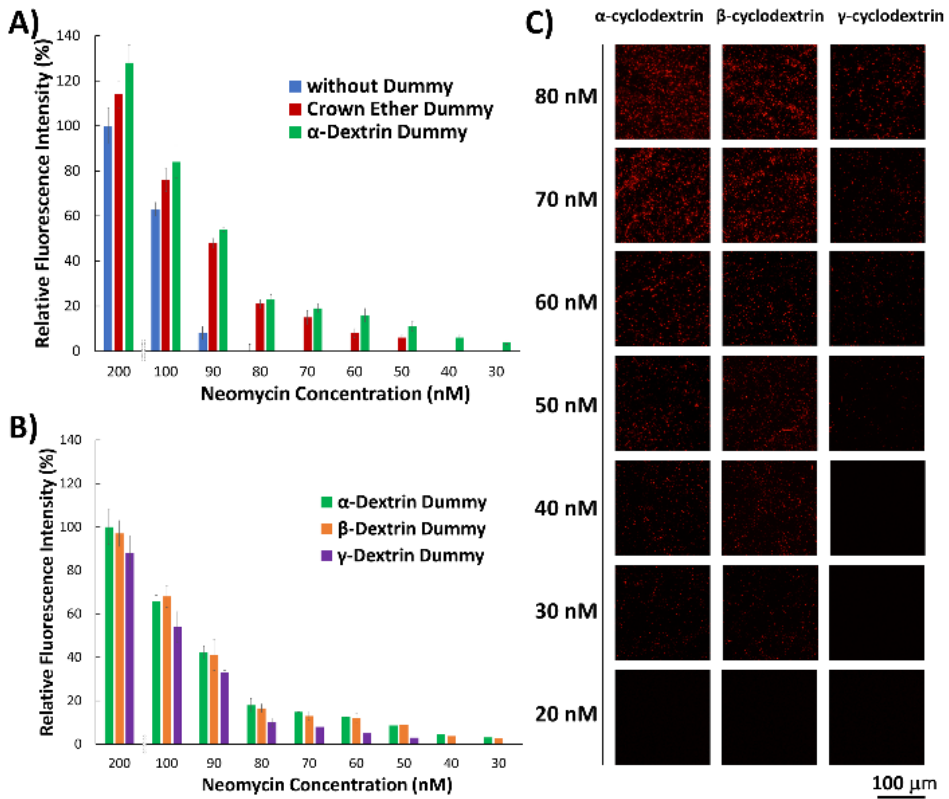
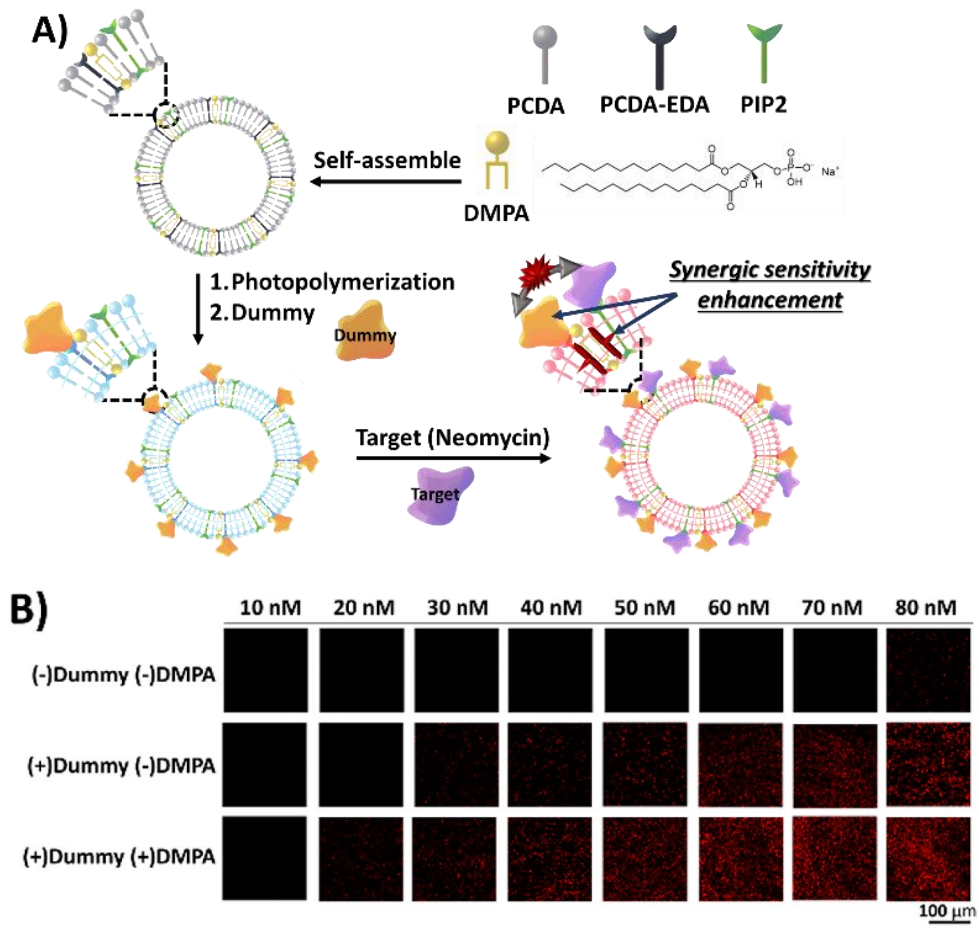


Figure 3.5. A) Relative fluorescence intensity comparison among the PDA liposome without dummy, with CE, and with α-cyclodextrin dummy, B) Relative fluorescence intensity, C) fluorescence images comparison among the series of cyclodextrin dummies

We investigated a feasible synergic sensitivity enhancement effect by combining the dummy concept with the DMPA-based strategy in which co-assembled phospholipids add more fluidity to the self-assembled PDA liposomes making them more responsive to the molecular stress.[87] Previously, by adapting this phospholipid-based enhancement strategy in PDA sensors we achieved higher sensitivity in the detection of Neomycin,[69] bovine viral diarrhea virus (BVDV),[87] and platelet activation.[101] First, a

liposome solution containing DMPA was prepared at the mole ratio of PCDA:PCDA-EDA:DMPA:PIP2 = 6:1:2:1 followed by α-cyclodextrin attachment to the resulting PDA liposome surface by using 1 mM α-cyclodextrin (Figure 3.6A). The prepared PDA liposomes did not show red fluorescence upon photopolymerization, confirming that the distance between the tethered α-cyclodextrin is large enough not to produce steric repulsion among them and background signal, and were used for the sensitivity study.



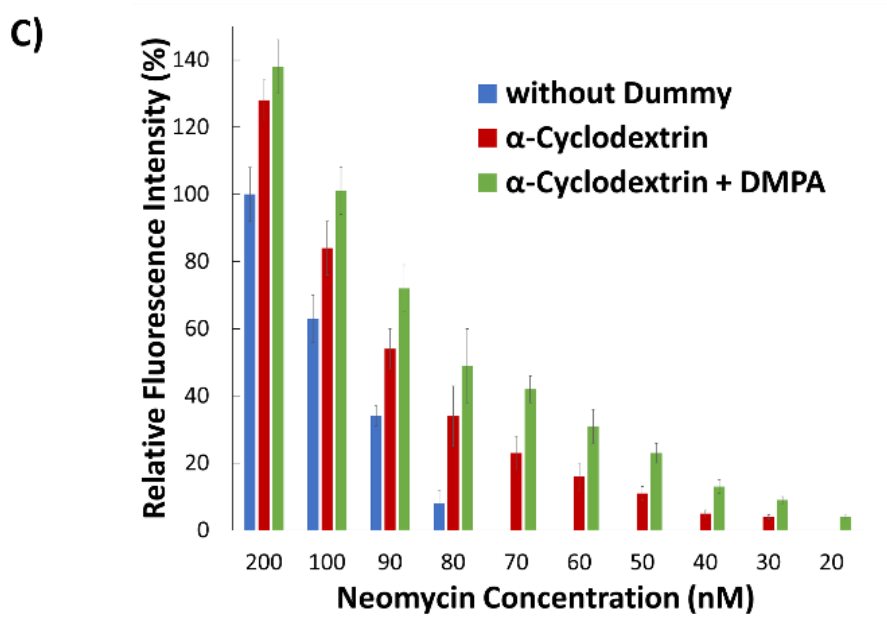


Figure 3.6. A) Schematic illustration of the PDA liposome self-assembled with DMPA and having pre-occupied dummy molecules for Neomycin detection with enhanced sensitivity. B) Fluorescent microscope images, C) Relative fluorescence intensity comparison among the PDA liposome without dummy, with α-cyclodextrin dummy, and with DMPA as well as α-cyclodextrin dummy.

As shown in Figure 3.6B, the synergy between the two sensitivity enhancement strategies further improved the LOD for Neomycin from 80 nM to 20 nM. Furthermore, the signal intensity increased at each level of target concentration (Figure 3.6C).

The PDA liposome sensor performance was further optimized to achieve the highest signal intensity and lowest LOD for Neomycin by adjusting the amount of α -cyclodextrin. The maximum amount of α-cyclodextrin that can be added without disruption the liposome packing

tuned out to be 3.4 mM. Above 3.4 mM, red fluorescence was observed right after the photopolymerization and even prior to Neomycin introduction, implying that the PDA packing was already distorted by excessive steric repulsion between the surface-bound α-cyclodextrin. As shown in Figure 3.7, when 3.4 mM of α-cyclodextrin dummy was used, higher signal intensity was observed consistently throughout the entire target concentration range. The LOD was as low as 7 nM.

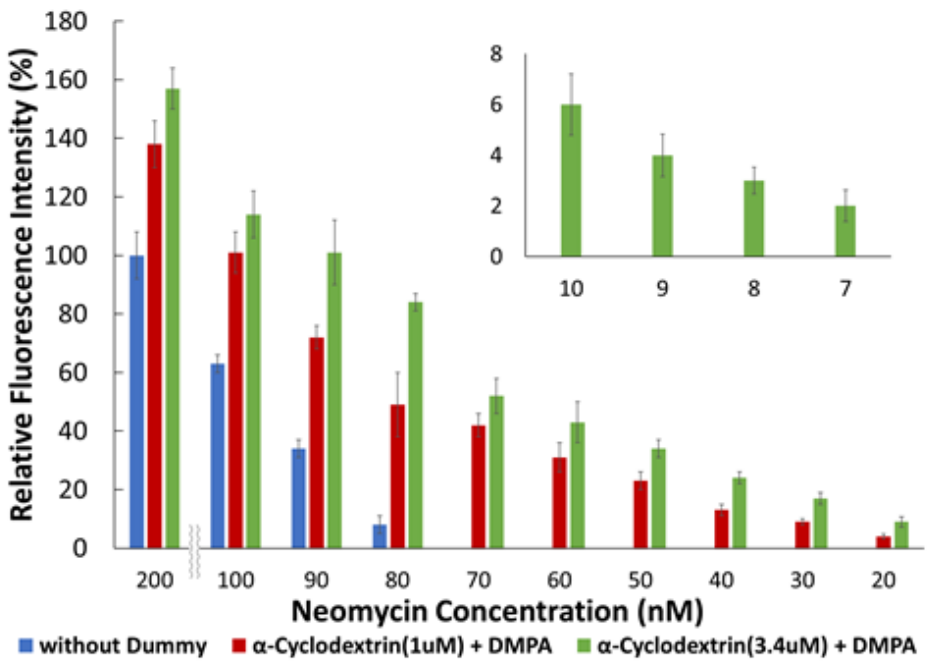


Figure 3.7. Relative fluorescence intensity comparison among the PDA liposome without dummy, with 1 mM α-cyclodextrin dummy and DMPA, and with 3.4 mM α-cyclodextrin dummy and DMPA.

Finally, we examined if the dummy strategy is generally applicable to other PDA detection scenarios by applying the dummy idea to a previously developed PDA sensor for Surfactin detection. The composition of the PDA

liposome was PCDA-EDA:PCDA-EDEA:DMPA = 4:4:2.(Figure 3.8) The PCDA-EDA was used as the receptor for Surfactin binding through Coulombic interactions[68] as well as the functional group for attaching epoxy-functionalized cyclodextrins.

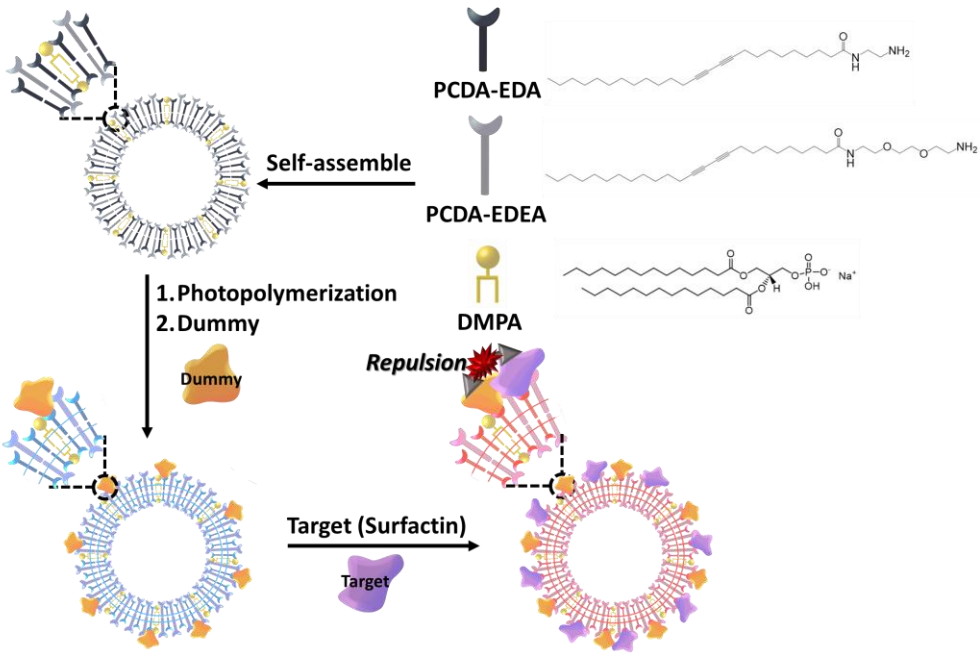


Figure 3.8. Schematic illustration of the PDA liposome having the pre-occupied dummy molecules for Surfactin detection with enhanced sensitivity

As shown in Figure 3.9A, a similar sensitivity enhancement effect was observed, except for that γ-cyclodextrin exhibited the best performance (22% increased signal intensity and 4 nM LOD) whereas α-cyclodextrin was the most efficient one for Neomycin detection. As discussed above, in this case it was also true that a dummy molecule having a similar form factor works best. As one can see from Table 3.4, γ-cyclodextrin has the closest

molar volume to Surfactin and showed the best enhancement result. The results strongly suggest that the dummy strategy, when combined with the lipid insertion strategy, provides one order of magnitude improved LOD and that when the form factor of the dummy matches well with that of the target, the largest sensitivity enhancement can be achieved.

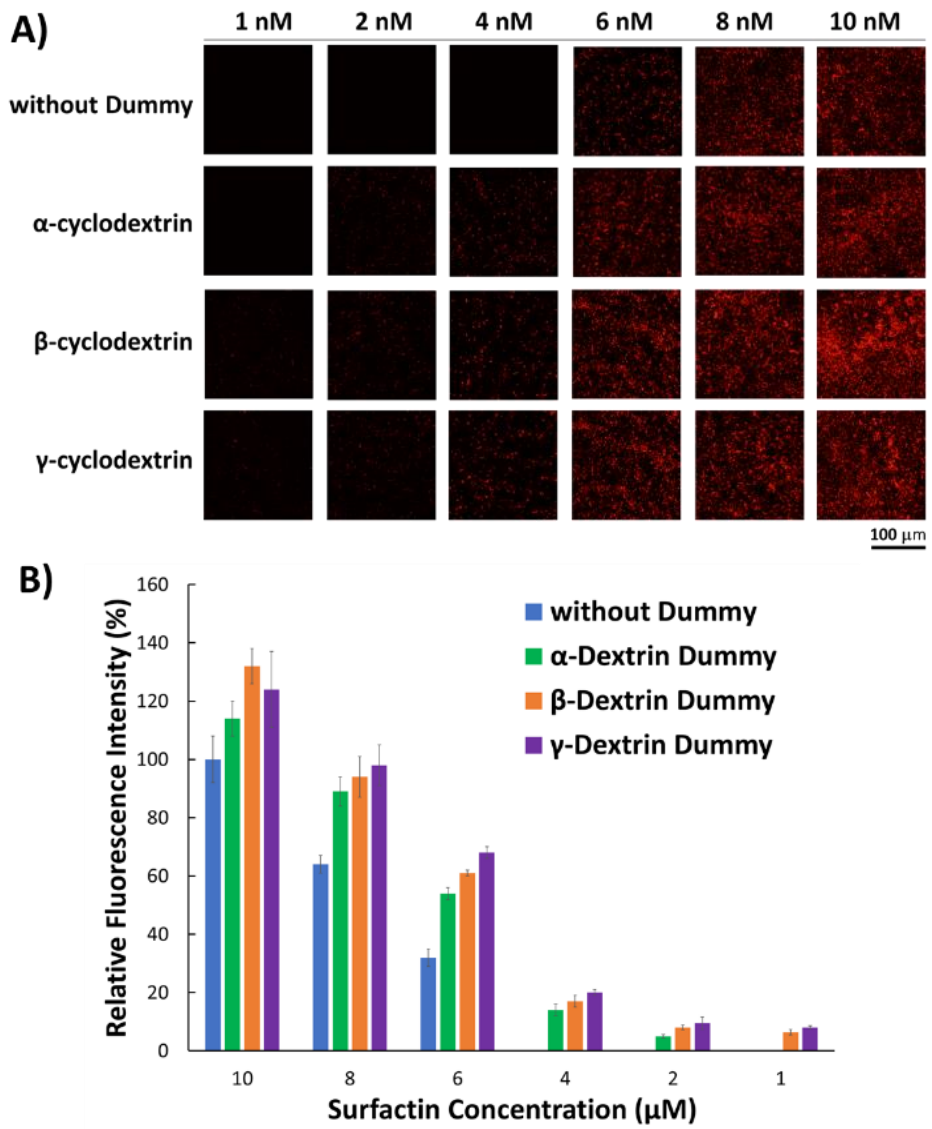


Figure 3.9. A) Fluorescent microscope images, B) Relative fluorescence intensity comparison among the PDA liposomes having the series of cyclodextrin dummies.

	Molar Volume (r_{Bohr}^3/mol) (in Water)	**USR* Shape Comparison** (Surfactin)
Surfactin	9880.917	1.000
18-crown-6	2255.047	0.0571
α-Cyclodextrin	6272.443	0.0581
β-Cyclodextrin	7493.048	0.0586
γ-Cyclodextrin	8648.770	0.0594

Table 3.4. Summary of calculated molar volume and USR* shape comparison of Surfactin and dummy molecules

3.5. Conclusion

We report a universally applicable strategy to improve the sensitivity and detection limit of PDA-based sensors by preoccupying the PDA liposome surface with artificial target molecules. By using Neomycin as a model target, and 18-crown-6-ether, α-cyclodextrin, β-cyclodextrin, and γ-cyclodextrin as the dummy, we found that a dummy having a similar form factor to the target molecule provides the largest signal enhancement effect. We applied the same dummy approach to a PDA liposome system for Surfactin detection and observed the same signal enhancement and improved LOD, which testifies that the dummy approach is generally applicable to the PDA sensory system. We also combined the dummy approach with another signal amplification strategy, the lipid insertion

approach, and achieved a synergic effect to accomplish one order of magnitude improved LOD.

Chapter 4. Conclusion and Outlook

4.1. Research Summary

In this dissertation research, simple, sensitive, and rapid self-signaling PDA-based sensors that detect specific target molecules in a biological sample have been studied. The biosensors prepared as a part of this dissertation demonstrated improved selectivity and sensitivity. First, a clinically applicable PDA-based sensor with excellent selectivity was developed for the detection of activated platelet in whole blood samples. A generally applicable sensitivity enhancement strategy has also been established, providing the potential to further expand the PDA sensor applications. The outcomes of this dissertation research advance PDA-based self-signaling sensors one step closer to commercially viable, robust sensor systems which offer simple administration and intuitive interpretation.

Chapter 2 illustrates the effort to increase PDA-based sensors' selectivity within a complex bio-sample such as whole blood containing various types of cells, nutrients, salts, and metabolites toward clinically viable colorimetric sensors. To achieve the goal, I researched a self-signaling polydiacetylene (PDA) liposome microarray to detect activated platelets from whole blood samples in a single step. A specific antibody, 9F9 antibody, to platelet-bound fibrinogen was identified and conjugated to the PDA liposome microarray to quantify the fibrinogen-bound platelets.

The developed PDA liposome−9F9 microarray generated an intense fluorescence signal when activated platelets in whole blood were introduced and also successfully distinguished the reduced platelet activation in the presence of Tirofiban, a model antiplatelet drug. The results of this single-step benchtop assay incorporate simple, sensitive, and rapid attributes that can detect the extent of platelet activation prior to needed clinical procedures.

In Chapter 3, a novel strategy to enhance the sensitivity of PDA sensors is described. The self-signaling feature of PDA sensors stems from steric repulsion-induced bandgap change of the conjugated PDA backbone at the interaction events of surface-bound receptors and target analytes. Therefore, unless adjacent receptors are occupied by target analytes, no sensory signal can be anticipated. Because of this intrinsic limitation of PDA-based sensors' working mechanism, detecting trace elements or biomolecules has been a challenge. To overcome the sensitivity limitation, a new generally applicable strategy to improve the detection limit of PDA sensor platforms has been investigated. In this approach, optimized numbers of receptors are pre-occupied with artificial target molecules, dummies, so that the dummy molecules can generate repulsion force on the PDA sensor surface with a much smaller number of analytes, enhancing the sensitivity and amplifying the optical signal. It was discovered that when the volumetric size of the dummy matches the size of the analyte, a maximum sensitivity enhancement effect can be achieved. The dummy approach was

applied to the previously studied Neomycin and Surfactin detecting PDA sensors and increasing the sensitivity of both sensors, demonstrating its applicability to the general PDA-based sensor platform. This dummy method could also be combined with another previously developed sensitivity improvement method for synergic sensitivity enhancement and demonstrated a 16-time better detection limit.

4.2. Future Consideration

One of the keys to developing the highly selective activated platelet sensor was to find and apply the very specific interaction between the fibrinogen-activated platelet complex and the 9F9 antibody. Similarly, other specific interactions can be devised to expand PDA sensor applications. For example, low-cost robust PDA biosensors would be particularly useful for diagnosing fast-spreading pandemics such as severe acute respiratory syndrome (SARS) and coronavirus disease 2019 (COVID-19). In a similar manner, low-cost PDA biosensors having the convenient equipment-free detection feature for infectious diseases amongst domesticated animals can also be prepared for farmers without resources to be trained for complicated administration and diagnosis.

When it comes to sensitivity, expanded investigation on the selection criteria for artificial targets, such as physical and chemical properties as well as the number density, is highly desirable. Establishing such selection

criteria can not only contribute to further improving the sensitivity of existing PDA based sensors but also enables rapid development of future PDA sensors for newly discovered biomolecules.

Numerous PDA sensors have been studied, and various fabrication strategies have also been developed to utilize PDA sensors for various purposes. However, there are still many hurdles to overcome in commercializing the PDA sensors. It is essential to provide accurate information to the end-users. However, the recognition ability can vary depending on the environment in which the sensors are used and the analytes exist. Another challenging factor is that the PDA sensors have to be photopolymerized before applying to analyte samples because during the storage many environmental variables such as temperature, humidity, and physical and chemical stimulus can trigger a false alarm, raising the shelflife issue. Therefore, rapidly recognizing and delivering information while ensuring accuracy is the core element for commercialization. In order to solve these potential problems, further research on sensor packaging and stable sensor platform should also be carried out.

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요약 (국문초록)

Polydiacetylene(PDA) 공액 고분자는 독특한 광학적 특성으로 인해 다양한 센서 제작에 사용된다. 특히, 공액 고분자와 결합 가능한 다양한 생체 분자들 간의 상호작용으로 인한 색변화 및 형광 발현 효과는 이 PDA 기반 센서를 통해 다양한 생체 분자를 검출할 수 있도록 하였다. 하지만 현재까지 개발된 PDA 기반 센서에는 몇 가지 결점이 존재한다. 땀, 체액, 혈액 등 전처리 및 분리 과정을 거치지 않은 생체 샘플을 PDA 기반 센서에 사용하였을 경우, 다양한 불특정 생체 분자들에 의해 광학적 특성이 간섭 받을 수 있다. 또한, 센서를 통해 표적물질을 검출하기 위해서는 많은 양의 표적물질이 PDA 센서 표면에 결합되어야 하지만 낮은 농도의 표적물질을 포함하는 샘플의 경우 검출이 어렵다는 단점이 존재한다.

본 논문에서는 전처리 과정없이 생체 샘플에서 간단하며 빠르고 정확하게 표적물질을 검출할 수 있는 PDA기반 센서를 제작하는 방식에 대해 설명할 것이다. 또한, 인위적으로 만들어진 표적물질을 이용하여 PDA 기반 센서의 민감도를 증가시켜 보다 정확하게 표적물질을 검출할 수 있는 센서 제작 방식에 대해서 설명할 것이다.

혈소판의 활성도를 간단하게 측정하는 방식을 개발하는 것은 임상의학에서 중요한 화제이다. 특히 여러가지 혈액 및 혈관 질병에 의해 항혈소판제를 투약하는 환자의 경우, 혈소판 활성도 판단은 환자의 신체 상태를 확인하는데 아주 중요한 요소이다. 전처리 과정없이 혈액 샘플을 사용하여 혈소판 활성도를 측정하기 위해 피브리노겐과 결합한 혈소판만을 특정하여 결합할 수 있는 9F9 항체를 PDA 기반 마이크로어레이 센서에 접목시켜 혈소판의 활성도를 측정하는 연구를 실행했다. 특히, 항혈소판제 중 하나인 Tirofiban을 이용, 혈소판 활성도에 따른 PDA 센서의 형광신호를 측정하여 정량적인 혈소판 활성도를 확인할 수 있다.

Polydiacetylene 공액 고분자는 그 광학적 특성에 의하여 다양한 센서 제작에 사용된다. 하지만 이 공액 고분자 기반 센서 표면에 표적물질이 일정 수 이상 결합하지 못할 경우, 결합한 표적물질 간의 반발력에 의한 광학특성 변화가 신호로 사용되는 이 PDA기반 센서로는 표적물질을 확인할 수 없다는 단점이 존재한다. 따라서 본 연구는 인공 표적물질(더미)을 PDA 기반 센서 표면에 결합하는 방식을 통해 표적물질과 추가적인 반발력을 생성하여 센서의 민감도를 증가시키는

방식을 고안하였다. 특히, 더미 물질이 PDA 센서 표면에서 표적물질과 3차원적 공간 점유도가 비슷할 경우, 센서의 민감도 증가가 가장 크다는 것을 밝혀냈다. 또한 기존에 연구되었던 PDA 기반 Neomycin과 Surfactin 센서에 동일한 더미 물질을 적용한 결과, 센서의 민감도 뿐만 아니라 형광신호가 증폭되는 결과를 확인했다. 더욱이 기존 PDA 기반 센서의 민감도를 증가시키기 위해 연구되었던 PDA-인지질 초거대 분자 활용법에 이 더미 시스템을 접목시킨 센서로 Neomycin을 검출해 본 결과 약 16배의 민감도 증가를 확인하였다.

주요어 : Polydiacetylene, 혈소판 활성도, 형광신호, 인공 표적물질, 민감도

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